Ph.D. Dissertation of Agriculture

Characteristics of food irradiated by 7.5 MeV X-ray

7.5 MeV 엑스선 조사처리 식품의 특성 규명에 관한 연구

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Abstract

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Food irradiation, which exploits the microbicidal effect of ionizing radiation, is used in many countries to improve the microbiological safety and to extend the shelf-life of food. Among different ionizing radiations including X-rays, gamma rays, and electron-beams (e-beams), 7.5 MeV X-rays generated when electrons with 7.5 MeV energy collide with a metal target have been newly suggested as an alternative food pasteurization process to overcome the application limitation from disadvantages of conventional irradiation sources such as low energy efficiency of 5 MeV X-rays, low consumer acceptance for gamma rays, and low penetration power of e-beams. To evaluate the availability of food irradiation using 7.5 MeV X-rays,

characteristics of 7.5 MeV X-ray-irradiated foods must be investigated. Therefore, this study was conducted to evaluate the microbiological, physicochemical, and toxicological characteristics of model foods (red pepper powder, chicken breast meat, and ground beef) irradiated with 7.5 MeV X-rays, which have greater conversion efficiency than 5 MeV X-rays and higher penetration power than both gamma rays and e-beams.

The D_{10}-values, which are the irradiation doses required achieving a decimal reduction in the initial bacterial population, of 7.5 MeV X-rays were ranged from 0.11 to 0.21 kGy for pathogens including *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus* suspended in a buffer solution and 0.22–0.41 kGy for beef-inoculated bacteria. Moreover, no significant differences in bactericidal efficiency of 7.5 MeV X-rays, gamma rays, and 10 MeV e-beams were observed for the bacterial strains inoculated on ground beef. In addition, 7.5 MeV X-ray irradiation showed the inactivation efficiency of 3 log reductions in total aerobic bacteria in red pepper powder irradiated at approximately 6 kGy. The total aerobic bacteria in chicken breast meat and ground beef were below the detection limit of 1 log CFU/g when they were irradiated at more than 4 kGy.

In the bacterial reverse-mutagen, *in vitro* chromosomal aberration, and *in vivo* micronucleus assays, the irradiated chicken breast meat with 7.5 MeV X-rays at 30 kGy exhibited dose-independent responses similar to those shown by the negative control. These results suggested that the irradiated samples were not genotoxic. In

acute and sub-chronic toxicity studies, mortality or any abnormal clinical signs of ICR mice were not observed during the test periods. Several hematological and serum biochemical parameters of ICR mice showed significant differences from the values in the control group; however, those values were within the normal range for hematological and serum biochemical parameters of ICR mice. No specific toxic effects were observed in male and female ICR mice upon single oral administration of X-ray-irradiated (30 kGy) chicken breast at up to 2000 mg/kg body weight. Furthermore, daily intake of X-ray-irradiated chicken breast at 2500 mg/kg body weight for 90 days did not cause in any toxicological effects on the male or female mice. Therefore, these results revealed that chicken breast irradiated with 7.5 MeV X-rays at 30 kGy was not toxic to mice under the tested conditions.

Red pepper powder samples X-ray-irradiated at more than 8 kGy exhibited significantly more off-odor than did non-irradiated sample; however, there was no significant difference between non-irradiated and irradiated samples at less than 7 kGy, which is the maximum irradiation dose permitted in Korea. Characteristics of red pepper powder irradiated with 7.5 MeV X-rays including color, contents of capsaicinoids and capsanthin, and organoleptic properties except for off-odor exhibited no significant change upon an absorbed dose ($p < 0.05$). 2-thiobabituroinic acid reactive substance values of chicken breast meat and ground beef irradiated with 7.5 MeV X-rays increased as absorbed dose increased, whereas pH of meats did not exhibited significant changes, regardless of absorbed dose. Therefore, it is considered that 7.5 MeV X-ray-irradiated model foods at less than

the upper dose limit did not exhibit quality deterioration induced by irradiation.

The photo-stimulated luminescence photon counts for a minute (PCs/60 s) of red pepper powder irradiated at a dose less than 2 kGy were 700–5000 PCs/60 s, indicating the need for further confirmative analysis. In contrast, all samples irradiated at more than 4 kGy were correctly identified as irradiated (> 5000 PCs/60 s). In thermoluminescence (TL) analysis, TL ratios (TL_1/TL_2), which were calculated from the TL signal intensity of silicate mineral separated from red pepper powder irradiated with 7.5 MeV X-rays at 0.49–9.27 kGy (TL_1) and the TL signal intensity of the TL_1 mineral normalized with gamma rays at 1 kGy (TL_2), of all the irradiated samples were over 0.1, resulting in positive results of all irradiated samples with 7.5 MeV at various doses. In X-ray-irradiated chicken breast meat, specific radiolytic hydrocarbons such as $C_{16:2}$ and $C_{17:1}$, which are used as markers for irradiated meat, were detected in the samples irradiated at > 4 kGy. Furthermore, 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone, unique radiolytic products derived from lipids, were detected in all samples 7.5 MeV X-ray-irradiated at various doses and increased with a function of absorbed dose, whereas these were not detected in the non-irradiated sample.

From the result, 7.5 MeV X-ray irradiation can be used as a food pasteurization process not providing the physicochemical quality deterioration induced by irradiation within the allowed upper limit dose. Moreover, it is elucidated that 7.5 MeV X-ray-irradiated food is toxicologically safe and can be supervised by using physical and chemical identification methods.

Key words: X-ray, Gamma ray, Electron-beam, Bactericidal efficiency, Food quality, Identification of irradiated food, Toxicological safety

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Chapter I. Literature review

I-1. Food irradiation by X-ray

Food irradiation is a physical treatment, in which the food is subjected to a defined dose of ionizing radiation to extend the shelf life and inactivate harmful organisms (Arvanitoyannis & Tserkezou, 2010). Decades of research have conclusively shown that food irradiation can have myriad beneficial applications, including the insect disinfestation of fruits and grains, inhibition of sprouting in potatoes and onions, the delayed ripening of fresh fruits and vegetables, and the enhanced safety and sterilization of fresh and frozen meat products, seafood, and eggs (Diehl, 2002; Kume et al., 2009b).

Food irradiation technology has been permitted for more than 250 food items in 56 countries, with 400000 tons of food irradiated globally in 2005. China accounted for 36% of the total global irradiated food, which included the irradiation of 80000 tons of garlic to inhibit its germination, and 52000 tons of dried vegetables and spices. In the US, a total of 92000 tons of food was irradiated, including 80000 tons of spices, 8000 tons of ground beef and chicken meat, and 4000 tons of fruits and vegetables. In addition, Ukraine irradiated 70000 tons of wheat and barley using an electron accelerator. While the EU has shown a decrease in food irradiation due to the enforcement of regulations on labeling violations, food irradiation in Asian countries has increased (Kume et al., 2009a).

Irradiation sources for food treatment include the following: gamma rays from radionuclides ^{60}Co or ^{137}Cs; electron-beams (e-beams) generated from machine sources operated at or below a nominal energy level of 10 MeV; and X-rays generated from e-beam machines operated at below a nominal energy level of 5 MeV (Code of Federal Regulations, 1986; Codex Alimentarius Commission, 2003). The US Food and Drug Administration has approved an increase of the limit for e-beam energy used for generating X-rays to 7.5 MeV in response to a petition from IBA (Code of Federal Regulations, 2004).

X-rays are electromagnetic radiation with a wide energy spectrum, and generated by colliding accelerated electrons with a dense material (target) such as tantalum or tungsten in a process known as bremsstrahlung-conversion (Koch & Motz, 1959; Miller, 2005). The efficiency for converting electron beam power to X-ray power increases with the energy of the incident electrons and with the atomic number of the target material. Increasing the energy improves also the X-ray penetration; however, higher energy of incident e-beams than the threshold energy of target material can induce radioactivity of the material by emission of neutrons (Grégoire et al., 2003). Because the threshold energies of tungsten, tantalum, and gold are 6.19, 7.58, and 8.07 MeV, respectively, tantalum or gold is used as a target material for 7.5 MeV X-rays, whereas the maximum energy of tungsten for generating X-rays is 5 MeV (Cleland & Stichelbaut, 2013). In a tantalum target, the conversion efficiency from incident electron beam power to emitted X-ray powder in the forward direction is about 8–9% at 5 MeV and 12–13% at 7.5 MeV (Meissner et al.,

2000). The broad energy spectrum of bremsstrahlung photons emitted from tantalum target extends from a few tens of keV up to the maximum energy of the incident electrons. However, the peak of the photon energy spectrum occurs at about 0.3 MeV in the electron energy range from 5.0 to 10 MeV (Seltzer et al., 1983).

X-rays have a deep penetration power comparable to gamma rays from ^{60}Co sources, with the added advantage of using an electronic source that stops radiating when switched off (Lazurik et al., 2007). A bremsstrahlung radiation from a 5.0 MeV electron beam penetrates slightly more than the gamma radiation (Seltzer & Berger, 1987). This greater penetration is partly caused by the higher photon energy of X-rays and the angular distribution. The narrow angular distribution of X-rays at above 5 MeV increases the penetration in materials because the most intense zone of the emitted radiation is perpendicular to the surface of the irradiated products, whereas gamma radiation has a wide angular distribution (Meissner et al., 2000). The optimum thickness of material irradiated with 7.5 MeV X-rays is approximately 38 g/cm^2, whereas those of 10 MeV e-beams, gamma rays, and 5.0 MeV X-rays are 8, 31, and 34 g/cm^2, respectively (Cleland & Stichelbaut, 2013).

Since the first discovery by Wilhelm Conrad Rőntgen in 1895, X-rays have been mainly used for medical radiography, scanning for security, and analyzing instruments such as X-ray diffractometer. For these applications, low-energy X-rays at below a few hundreds of keV were used. As the energy of electrons for generating X-rays increased for keV to MeV, sterilization of packaged food and

medical devices was proposed. Although the greater penetration, improved conversion efficiency, and radiological safety, the commercial application of 7.5 MeV X-rays is still scarce, owing to the economic issues such as lower throughput than e-beam irradiation and higher cost than gamma irradiation. However, recent comparisons have shown that the capital and electric power costs for electron accelerators with energies of 5–7 MeV equipped with X-ray targets can be lower than the capital and source replenishment costs for ^{60}Co source loadings greater than 6 MCi based on the recent prices of ^{60}Co at $2.5 US per curie and average cost of electric power at 12 cents per kilowatt hour (Cleland & Stichelbaut, 2007). IBA has reported that cost of energy for sterilizing 1 m^3 of products is 8.6 € for 7.5 MeV X-ray irradiation, 9.1 € for gamma irradiation, and 4.1 € for e-beam irradiation, based on the same throughput at 64,000 m^3/year (Mullier, 2015).

Different characteristics of X-rays with gamma rays and e-beams are summarized in Table 1. Gamma rays are a type of electromagnetic radiation emitted from radioactive isotopes such as ^{60}Co and ^{137}Cs (Woods & Pikaev, 1994). This type of radiation has the highest frequency and energy with the shortest wavelength, and can easily remove an orbital electron from an atom in the irradiated medium (Laughlin et al., 1989). This ionizing property imparts significant damage to a living cell and thus gamma radiation is often used to inactivate living organisms in food. ^{137}Cs is not suitable for commercial use due to its lower energy compared to ^{60}Co, and is used only in small hospital units to treat blood before transfusion for the prevention of Graft-versus-host disease (Ehlermann, 2005). Food irradiation using

^{60}Co is the preferred method by most processors, as its deeper penetration enables the administration of treatment to entire industrial pallets. The main characteristics of gamma irradiation include the treatment of food with photons of well-defined energies (1.17 and 1.33 MeV) emitted from ^{60}Co sources, and the irradiation of large amounts of food in bulk through its high penetration power. However, gamma irradiation also presents a number of disadvantages. These include the low consumer acceptance through fear of the radioisotope, and high construction costs due to the requirement for shielding (World Health Organization, 1988). However, despite of its disadvantages, gamma irradiation is used predominantly in food industries to inactivate microorganisms in spices, dried vegetables, and meat products (Kume et al., 2009a).

E-beam irradiation uses electrons accelerated in an electric field to a velocity close to the speed of light. High-energetic electrons are particulate ionizing radiation. As electrons have a significantly larger cross section than photons, they generally do not penetrate the product beyond a few centimeters, depending on product density (Bhat et al., 2012). The maximum energy of e-beams approved for food irradiation is 10 MeV, because higher energetic e-beams than 10 MeV can result in the subject becoming radioactive. The optimum penetration depth of such 10 MeV e-beams is approximately 8 g/cm^2 with double sided irradiation. However, the use of e-beam irradiation is growing in the food irradiation market, as food can be treated quickly with e-beams at high dose rates. For example, frozen food such as meat and seafood can be treated with e-beam without any quality deterioration

induced from thawing, which is proceeded during gamma irradiation process (Brown, 2015).

Conclusively, food irradiation by 7.5 MeV X-rays has a few advantages compared to gamma and e-beam irradiation. Similarly with gamma irradiation, food product in bulk can be irradiated with 7.5 MeV X-rays, due to the highest penetration depth. The recent development of high-energy and high-power electron accelerators has made X-ray processing a practical alternative to gamma-ray processing in applications requiring greater penetration than can be provided by energetic electron beams. Moreover, the increased conversion efficiency makes 7.5 MeV X-ray irradiation competitive to gamma irradiation in terms of economic. The abilities to turn the radiation source on and off and to control the X-ray intensity are attractive features of an accelerator facility. Despite of the advantages, characterization studies and commercial application of 7.5 MeV X-ray irradiation to food commodities are still scarce. Therefore, further studies to evaluate microbiological, physicochemical, and toxicological characteristics of irradiated food by 7.5 MeV X-rays are needed.

Table 1. Characteristics of three irradiation sources for food treatment

Characteristics	Irradiation sources		
	X-ray	Gamma-ray	E-beam
Radiation source	Electric machine	Radionuclide	Electric machine
Energy type	Photon	Photon	Electron (particle)
Maximum energy	7.5 MeV	1.33 MeV	10 MeV
Dose rate	24 kGy/h	1 kGy/h	8000 kGy/h
Penetration depth (double sided)	38 g/cm^2	31 g/cm^2	8 g/cm^2

I-2. Bacterial inactivation by X-ray irradiation

When an atom in material is exposed to X-rays, energy transactions occur between the incident photons and the orbiting electrons. These interactions result in a series of energy transfer from X-rays to material, then ionization occurs when the energy level sufficiently increases to produce ions by the removal of an orbiting electron (Wilkinson& Gould, 1998). Activity generated by the photon of X-rays exhibited two absorption processes in the irradiated material, Compton scattering and photoelectric absorption (Miller, 2005). When using low-energy photons, photoelectric absorption is generally occurred as all of the photon's energy transferred to the electrons in material. Compton scattering occurs at higher energy levels, where a portion of the photon energy is absorbed by the encountered electron (Pizzarello & Witcofski, 1975).

The inactivation mechanism by X-ray can be explained by a combination of direct and indirect effects. The nucleic acid molecules may be ionized or excited by direct absorption of radiation energy, so initiating the chain of events that leads to biological change and to cell death if the change is serious enough. If a cell or an organism is exposed to radiation, direct damage to DNA occurs by chemical transformation of its components such as purine and pyrimidine bases, often resulting in the destruction of the DNA double helix or its phosphodiester linkages (Zerial et al., 1978). This is the so-called direct effect of radiation, which is the dominant process when dry spores of spore-forming microorganisms are irradiated.

Alternatively, indirect effect of radiation is the damage caused by radicals formed

through the absorption of energy into other molecules such as water in the cell. The hydroxyl radical ($OH^{\cdot}$) in particular is responsible for approximately 90% of damage to DNA, and is known to have the biggest influence on the radiation sensitivity of organisms. In addition, diverse damaging effects can occur with cells, including changes in cell components, such as protein, fat and carbohydrates. Such damage can result in chromosomal aberration, delay in cell division, metabolic suppression of carbohydrate and amino acids, and inactivation of enzymes. When living organisms are exposed to radiation, both direct and indirect effects occur simultaneously. With regard to the biological effects of radiation exposure, direct effects account for approximately 25%, while indirect effects account for 75% of the damage (Moosekian et al., 2012).

Viruses, bacterial spores, vegetative bacteria, fungi, and insects exhibit different sensitivity to radiation. The molecular weight of DNA influences the radio-sensitivity of organisms. The DNA in nuclei of the cells of insects represents a target much larger than the genome of bacteria. Not surprisingly, bacteria are less radiation sensitive than insects. Another factor influencing radiation effects is the structural arrangement of the DNA in the cell. During the cell division, the normally double-stranded DNA of bacteria separates, and by means of a polymerase new chains of DNA are assembled along the template. The double-stranded form DNA is much less sensitive to radiation than in the single-stranded form. The radiation sensitivity of a cell is proportional to cell proliferation and inversely proportional to cell differentiation. Therefore, radiation sensitivity is high when the metabolic rate

is high, when cell division is active, or when an individual is young.

However, results from studies on the radiation sensitivity of microorganisms suggest that the response of a microorganism to radiation largely depends on external environment, as this also has an influence on the survival of the microorganism (Molins, 2001). This implies that the external environment, including factors such as irradiation temperature, oxygen presence, and water activity of food have an influence on the physical and chemical characteristics of cells, thus causing differences in radiation sensitivity. These radiation sensitivity differences among similar groups of microorganisms are correlated to their inherent diversity with respect to the chemical and physical structure as well as their capacity to recover from radiation injuries.

I-3. Physicochemical change by X-ray irradiation

Radiation loses energy through interaction with materials, while the materials receive energy, generating ionization and excitation in materials. As a result of ionization and excitation, ions, electrons, excitation states, and radicals are formed. These generate new active species by interacting with one another or reacting with the surrounding molecules, and are known as reactive intermediated. Through the reaction of these intermediates, stable final products are eventually created. For example, when water is irradiated, ionization and excitation result in the generation of H_2O^+ and e^- under 10^{-12} s. After 10^{-6} s, radicals such as $H^\cdot$ and $OH^\cdot$, hydrated electrons, and the acidic radical H_3O^+ are generated. In pure water, a molecule of hydrogen (H_2) and peroxide (HOOH) are generated as the final stable products. If the water also contains a solute, these species can react with the solute.

When food is irradiated, the chemical changes may arise from the direct action of radiation on the carbohydrates, proteins, fats, and other compounds in the food, or by the indirect action of reactive intermediates formed by the radiolysis of water. In terms of food quality, changes in major components such as carbohydrates, proteins, and lipids may affect the organoleptic characteristics of the food. For example, the irradiation of sugar in an aqueous environment changes its optical rotation and often causes browning. Moreover, the degree of degradation in sugars in solution is proportional to the radiation dose, and can affect the sweet taste of the food following irradiation. Irradiation also leads to the degradation of polysaccharides such as starch, cellulose, and pectin, resulting in changes to texture and viscosity by

complex mechanisms. In addition, the production of hydrogen sulfide and methyl mercaptan, induced by the irradiation of sulfur-containing amino acids, can lead to off-odors and off-flavors (Simic, 1983). Furthermore, the irradiation may alter the viscosity of proteins, owing to the degradation and aggregation of proteins, while the production of radiolytic compounds from lipids can affect the flavor of food.

A number of physical and chemical methods based on the physicochemical changes of food components or silicate minerals in food by irradiation are used for identification of irradiated food. Among the physical techniques, photostimulated luminescence (PSL) and thermoluminescence (TL) measurements using the luminescence properties of contaminated minerals in food materials are promising methods. PSL is a rapid, simple, and inexpensive screening method, while TL analysis is one of the most reliable and sensitive methods for the detection of irradiated food (Bayram & Delincée, 2004; Chauhan et al., 2009; Sanderson et al., 1998). Luminescence is the emission of light when trapped energy is liberated by the addition of chemicals, heat, or light. The TL process is based on electrons in the excited state returning to the ground state when thermally stimulated (Heide & Bogl, 1987). When a substance exhibiting TL is exposed to ionizing radiation, electron-hole pairs are produced, and some electrons (or holes) may become trapped at certain sites in the material. They remain in these traps until sufficient thermal energy is acquired to escape. As the material is heated, electrons are released from the traps, and light is emitted as they recombine with holes. The intensity of the emitted light can be measured as a function of temperature to give a so-called glow

curve, which is characteristic of the examined substance. The TL phenomenon is not unique to irradiation, but if the TL response following irradiation is significantly greater than the background signal, and the fading (i.e. the decrease in the TL signal) is low over a period of weeks and months, then TL measurements may be suitable for determining whether foodstuffs have been irradiated. The PSL method was then developed to resolve the practical limitations of silicate TL methods as the standard TL method requires a physical separation of the minerals from the food matrix. The requirement for careful laboratory preparation of TL samples and access to a calibration source of ionizing radiation have limited the widespread use of TL for routine commercial or enforcement testing (Sanderson et al., 1989). In contrast, PSL employs light rather than heat as a stimulus for releasing the trapped energy induced by radiation in solid materials. This has overcome the need for full mineral separation and for providing radiation-specific stimulation schemes appropriate for biogenic materials. However, it should be noted that PSL analysis has been used as a screening method, owing to a lower sensitivity than the TL method.

Most of the volatile products in food induced by irradiation originate from the lipid fraction; therefore, measurement of radiolytic products from food lipids could perform the basis for chemical methods to identify irradiated foods. Only two hydrocarbons are formed in relatively large quantities (Nawar & Balboni, 1970). One has a carbon atom less than the parent fatty acid, and results from cleavage at the carbon-carbon bond alpha to the carbonyl group; the other has two carbon atoms less and one extra double bond, and results from cleavage beta to the carbonyl

(Nawar et al., 1990). Nawar & Balboni (1970) reported that six specific radiolytic hydrocarbons, which are tetradecadiene ($C_{14:1}$), pentadecane ($C_{15:0}$), hexadecane ($C_{16:1}$), heptadecane ($C_{17:0}$), hexadecadiene ($C_{16:2}$), and heptadecene ($C_{17:1}$) produced from palmitic, stearic, and oleic acids, were detected in irradiated pork. A linear relationship between irradiation dose and each of these compounds has also been demonstrated (Morehouse & Ku, 1991).

2-Alkylcyclobutanones (2-ACBs) are cyclic compounds containing four carbon rings and produced by disruption of fatty acids when fat in food are irradiated. 2-ACBs have the same number of carbons as the precursor fatty acid, and an n-4 alkyl group side chain, which is formed by electron loss of the oxygen molecule in the fatty acid/triglyceride carbonyl group through ionization and consecutive rearrangement, is linked to the second carbon of the cyclic carbon ring (LeTellier & Nawar, 1972). Following extraction from food, these products can be detected and quantified using gas chromatography and mass spectrometry. 2-ACBs have not been detected in foods that have been heated, microwaved, UV irradiated, or subjected to high pressure processing, or ultrasonic waves (Crews et al., 2012); therefore, 2-ACBs are considered as unique radiolytic products that can serve as a marker to confirm whether fat-containing foods have been irradiated (CEN, 2003b). Contrary to a previous theory, it was reported that 2-ACBs exist naturally in food (Variyar et al., 2008). However, two researches recently reconfirmed the uniqueness of 2-ACBs in irradiated nutmeg (Chen et al., 2012) and cashew nuts, nutmeg, apricot kernel, and pine nuts (Leung et al., 2013).

I-4. Safety of irradiated food by 7.5 MeV X-ray

In order to evaluate the safety of irradiated food with 7.5 MeV X-rays, induced radioactivity in red meat was compared with natural radioactivity and background exposure (Grégoire et al., 2003). The induced radioactivity in X-ray-irradiated meat was significantly lower than the natural radioactivity in food. Corresponding annual dose is several orders of magnitude lower than the environmental background (1500 times lower in the worst case). Moreover, it is considered that intake of food irradiated with X-rays generated by electrons with nominal energy as high as 7.5 MeV is trivial.

In the history of food preservation, food irradiation is perhaps the most studied food processing technology in terms of toxicological safety. The wholesomeness of irradiated food has been carefully evaluated in an unprecedented number of studies for more than 50 years. At an international level, the need to consider the wholesomeness of irradiated foods was emphasized at a meeting sponsored by FAO, IAEA, and WHO in Brussels in 1961. The appropriate studies required to ascertain the wholesomeness were discussed by a Joint FAO/IAEA/WHO Expert Committee on Food Irradiation (JECFI) in Rome in 1964. Taking as a premise that the irradiation of food resulted in the production of radiolytic products in the foods, the Committee adopted the view that these products represented additions to the food. It therefore concluded that the establishment of the safety of irradiated foods should follow procedures similar to those generally used for evaluating the safety of food additives, and should be pursued on a food-by-food basis (World Health

Organization, 1966). A subsequent meeting was convened to assess the wholesomeness of irradiated wheat, potatoes, and onions in Geneva in 1969. The next Joint Expert Committee, which convened in 1976, reviewed a large number of animal studies on various irradiated foods. The committee also reviewed the results of radiation chemistry studies on the major components of food; it noted that many of the radiolytic products identified were present in food treated by heat and other processes, and considered that the health hazards from the concentrations found in irradiated foods were probably negligible (World Health Organization, 1977).

In 1980, the JECFI reviewed numerous microbiological, nutritional, and toxicological studies of irradiated foods and concluded that "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological problems" (World Health Organization, 1981). In 1999, the joint FAO/IAEA/WHO Study Group on High-Dose Irradiation concluded that "irradiation to high doses is essentially analogous to conventional thermal processing, such as canning of low-acid foods, in that it eliminates biological hazards from foodstuffs intended for human consumption, but does not result in the formation of physical or chemical entities that could constitute a hazard." (World Health Organization, 1999).

I-5. Food irradiation in Korea

Food irradiation studies were commenced by the Radiation Research Institute in Agriculture in Korea in 1966 to extend the shelf life of garlic, strawberries, and sweet potatoes. In addition, since the 1980s, the Korea Atomic Energy Research Institute has conducted various application studies on food irradiation. The food irradiation business was then established based on the Presidential decree 11717 in 1985, and the Gyeong-in Regional Korea Food and Drug Administration revised the Food Sanitation Act to permit food irradiation in 1986. Through 5 revisions in 1987, 1988, 1991, 1995, and 2004, gamma irradiation at various doses less than 10 kGy was permitted for 26 food groups, based on the appropriate purposes. The regulations for labeling and detection methods for irradiated food were subsequently added to the Korean Food Code in 2008 and 2010, respectively. In July 2012, electron-beam irradiation at less than 10 MeV was permitted for the same food groups as for gamma irradiation (Table 2). In this context, approval on X-ray food irradiation is needed because the lack of legislation on X-ray food irradiation in Korea can cause international conflict when food irradiated with X-ray is imported.

Only two gamma irradiation facilities (Greenpia technology Co. and Soya greentech Co.) are currently available for food irradiation. Dried agricultural products, such as spices or dried vegetables for the ingredients of processed foods are mainly treated by gamma irradiation, and it is estimated that approximately 5394 tons were treated in 2005. Since 2010, however, food-labeling regulations

have been initiated for all irradiated foods and ingredients, which may rapidly decrease the industrial use of irradiation. Low consumer acceptance of irradiated foods in Korea has also become an obstacle making food irradiation technology a widely accepted method for the treatment of foods. Therefore, education or promotion programs to convey correct information on food irradiation to consumers are needed.

Table 2. List of food items permitted for gamma and e-beam irradiation in Korea

Food item	Maximum dose (kGy)	Purpose	Permission year
Potato Onion, Garlic	0.15	Sprout inhibition	1987
Chestnut	0.25	Sprout inhibition	
Mushroom (fresh and dried)	1.00	Disinfestation, controlling ripening	
Egg powder Cereals (grain and powder) Legumes (grain and powder) Starch as ingredient of food products	5.00	Pasteurization	1991/ 2004
Dried meat powder Dried fish and shellfish powder Soybean paste powder Red pepper paste powder, Soy sauce powder, Dried vegetables Dried yeast Aloe powder Ginseng products (including red ginseng) Enzyme food Algae powder	7.00	Pasteurization	1991/ 1995/ 2004
Dried spice Composite seasoning products Sauces Powdered tea Leached tea Patients' diets	10.00	Pasteurization	1995/ 2004

Chapter II. Bacterial inactivation characteristics of 7.5 MeV X-ray irradiation

II-1. Introduction

Food can be treated to extend its shelf life and inactivate bacteria and insects only with the three types of ionizing radiation such as X-rays, gamma rays, and e-beams (Farkas & Farkas, 2011). Among these, high-energy X-rays generated by using electrons with more than 5 MeV energy offer considerable promise for commercial application (Cleland & Stichelbaut, 2013). This is because they have higher penetration power than e-beams and better consumer acceptance than gamma rays generated from radionuclide sources (Fan & Sommers, 2013).

Dried vegetables, chicken meat, and ground beef are often contaminated with foodborne pathogens such as *Bacillus cereus*, *Escherichia coli* O157:H7, and *Salmonella enterica*, which can cause food spoilage and foodborne outbreaks (Banerjee & Sarkar, 2003). Among the food decontamination processes, irradiation is recommended, because the process is effective in inactivation of foodborne bacteria after packaging and a non-thermal pasteurization process, which can prevent food from thermal changes (Farkas, 1998; Farkas & Andrassy, 1988). Therefore, large amount of dried vegetables and raw meats are irradiated annually in many countries to reduce bacterial populations in the foods (Kume et al., 2009a).

However, studies and practical applications of food irradiation using high-energy X-rays have been scarcely reported. Therefore, this study was conducted to evaluate the inactivation characteristics of bacteria in model foods (red pepper powder, chicken breast meat, and ground beef) irradiated with 7.5 MeV X-rays, which have greater conversion efficiency of electric energy to radiation than 5 MeV X-rays and higher penetration power than both gamma rays and e-beams. Furthermore, to consider the feasibility of using 7.5 MeV X-rays for commercial sterilization of food products, the inactivation characteristics of 7.5 MeV X-rays were compared with those of gamma rays and e-beams, which are currently used for commercial sterilization of food.

II-2. Material and methods

II-2-1. Test strains and sample preparation

In this study, four species of pathogenic bacterial strains (2 gram-negative: *E. coli* and *Salmonella* Typhimurium and 2 gram-positive: *Listeria monocytogenes* and *Staphylococcus aureus*) were investigated. Lyophilized *E. coli* KCCM 40406, *S.* Typhimurium KCTC 1925, *L. monocytogenes* KCCM 40307, and *S. aureus* KCCM 11335 were obtained from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). Each strain was cultivated in Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD) at 37°C for 18 h. The cultures of each strain were transferred twice at 24-h intervals in 10 mL of TSB in screw-cap test tube by using an inoculation loop. One hundred microliters of the fresh culture was aseptically transferred to 100 mL of TSB in a 250-mL Erlenmeyer flask. The bacterial growth curve of each bacterial strain was obtained by measuring the optical density of each respective culture (incubated at 37°C in a shaking incubator, 1200 rpm) at 600 nm using a Libra S70 spectrophotometer (Biochrom Ltd., Cambridge, UK) (Fig. 1). Cells in the late exponential growth phase were harvested by incubating *S.* Typhimurium and *S. aureus* for 6 h, *E. coli* for 7 h, and *L. monocytogenes* for 14 h at 37°C. The cultured broth of each strain was separated into four 50-mL centrifuge tubes (25 mL of culture in a tube), and the cells were harvested by centrifugation (3000 × *g* for 10 min at 4°C). The pellet in each centrifuge tube was washed twice with 25 mL of

sterile phosphate-buffered saline (PBS; Lonza, Walkersvile, MD) and re-suspended in 25 mL of PBS. All cell suspensions were collected in a 250 mL Erlenmeyer flask for a final concentration of approximately 10^8–10^9 colony forming units (CFU) per mL. The final culture suspensions of each strain were used to inoculate raw ground beef, or 1 mL of the suspension was transferred aseptically to individual microcentrifuge tubes, in order to evaluate bacterial cell viability.

For inoculation of pathogens, a 10 g portion of the beef was placed in a sterile filter bag (BagFilter®, Interscience, Rockland, MA), which was then packed in an oxygen-impermeable nylon bag (2 mL O_2/m^2/24 h at 0°C, 0.09 mm thickness; Sunkyung Co., Ltd., Seoul, Korea). These were subjected to gamma irradiation at 30 kGy in order to inactivate all microorganisms in the sample 1 day before inoculation. The test culture suspension (0.1 mL) was aseptically inoculated onto five spots of the sterile ground beef; this was blended manually for 5 min and formed in a size (length × width × height = 10 × 12 × 0.5 cm) within the bag. The inoculated samples were stored overnight in a refrigerator (4°C) prior to irradiation.

Red pepper powders, chicken breast meat, and ground beef (2.38 mm grind) were purchased from a local market in Jeongeup, Korea. The samples (100 g) were immediately placed in sterilized oxygen-impermeable nylon/polyethylene bags (20 × 30 cm, thickness: 0.07 mm; Sunkyung Co. Ltd., Seoul, Korea) and packaged to a thickness of 3.0 cm to minimize the variation in penetration depth among radiation sources. The packaged samples were stored overnight in a refrigerator (4°C) until irradiation.

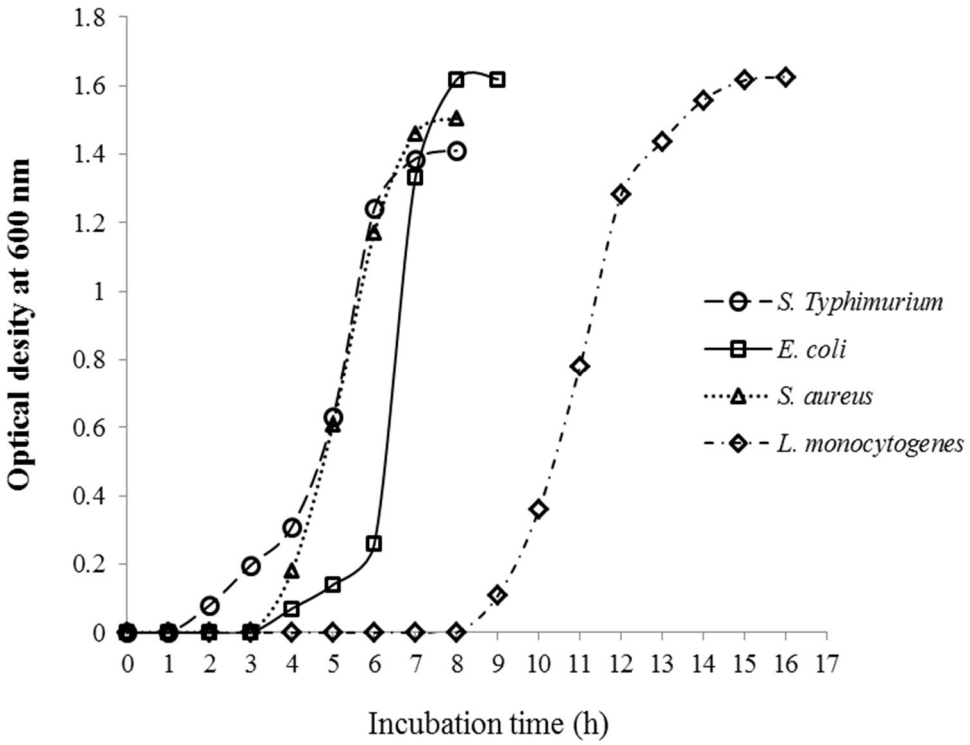


Fig. 1. Growth curve of different bacteria incubated in tryptic soy broth at 37°C.

II-2-2. Irradiation of samples

Five bacterial suspension or ground beef samples were simultaneously irradiated at targeted irradiation doses (0.1–1.0 kGy), whereas the packaged model food samples were irradiated at a nominal dose of 2, 4, 6, 8, or 10 kGy using one of the irradiation sources. The three irradiation sources were treated on the same day for all samples.

7.5 MeV X-ray and e-beam irradiations were conducted using a linear electron accelerator, which delivers e-beams with a well-defined energy in the range of 5–10 MeV, at EB-Tech Co. (Daejeon, Korea) at an ambient temperature of approximately 22°C. X-rays were produced using 7.5 MeV e-beams, whereas e-beams at 10 MeV were employed for e-beam irradiation. Gamma irradiation was performed using a ^{60}Co gamma irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 8.8 PBq and the photon energies of gamma rays from ^{60}Co were 1.17 and 1.33 MeV. The applied dose rates were 24 kGy/h for X-ray irradiation, 8 MGy/h for e-beam irradiation, and 1 kGy/h for gamma irradiation. Geometrically, all samples were irradiated in a perpendicular direction to the incident radiation. The irradiated samples were immediately stored at 4°C and subjected to microbiological analysis.

II-2-3. Dosimetry

The actual doses absorbed by each sample and dose distribution across the samples were determined by using a 5 mm-diameter alanine dosimeter (Batch No.: T020604, Bruker BioSpin GmbH, Rheinstetten, Germany). Three dosimeters were placed in the bottom, middle, and top of an empty microcentrifuge tube and the space was filled with tissue paper. The tube containing 3 dosimeters was packaged in a polyethylene bag together with 5 PBS suspension samples. The bag was stored in a refrigerator until irradiation treatment. For dosimetry of inoculated beef and model food samples, a dosimeter was packaged in a polyethylene bag to prevent absorbing moisture, and 3 dosimeters were attached to the top of a sample for each irradiation dose. The samples with dosimeters were also stored in a refrigerator until irradiation.

Following irradiation, the dosimeters separated from samples were stored overnight at room temperature (approximately 25°C). The absorbed dose was measured within 24–48 h subsequent to irradiation using an electron paramagnetic resonance analyzer (e-scan^{TM} alanine dosimeter reader, Bruker BioSpin GmbH, Rheinstetten, Germany) according to international standards (ISO/ASTM 51607:2004). The alanine dosimeter reader was calibrated using reference standard dosimeters provided from UK National Physics Laboratory according to an international standard on 30th April, 2015 (ISO/ASTM 51261, 2002). The dose uniformity ratios of all the type of radiation at various doses were less than 1.2.

The combined standard uncertainty of the reader was 2.2%, which was calculated

by estimation of the uncertainty Type A and Type B in the absorbed dose measured with the alanine-EPR dosimetry system, according to the international guideline (ISO/IEC Guide 98-3, 2008). The expanded uncertainties of measured doses at a given dose for e-beam, gamma, and X-ray irradiation were calculated from the final combined standard uncertainty multiplied by a coverage factor $k = 2$, corresponding to an interval with a 95% level of confidence. The final combined standard uncertainty included reproducibility of the dose measurement (n=3) and uncertainties derived from establishing the traceability of the dosimetry system. In this study, absorbed dose of sample was expressed as a mean value ± the expended uncertainty.

II-2-4. Microbiological analysis

A series of decimal dilutions of the cell suspensions was prepared with sterile PBS, and each diluent (0.1 mL) was spread on tryptic soy agar culture (TSA, Difco Laboratories, Detroit, MI) plates. Ten grams of each of the ground beef samples were blended with 90 mL of sterile saline using a stomacher (Bag Mixer 400, Interscience Ind., St. Nom, France) for 1 min. A series of decimal dilutions was prepared with sterile PBS, and a 0.1 mL aliquot was spread on TSA. For model foods, a 10 g of samples was aseptically removed from the packages and placed into a stomacher bag (Nasco, Ft. Atkinson, WI, USA) with 90 mL of 0.1% buffered peptone water. Samples were then stomached for 1 min and serial dilutions were prepared. A series of decimal dilutions was prepared with sterile PBS, and a 0.1 mL

aliquot was spread on TSA. Plates were then incubated at 37°C for 24–48 h in an incubation chamber. Viable cells were enumerated based on dilutions exhibiting colony forming units (CFU) per plate. All subsequent bacterial counts were expressed as the mean value (log CFU/mL or CFU/g) with a standard deviation per absorbed dose.

II-2-5. Statistical analysis

The D_{10}-vales of bacteria were determined by calculating the reciprocal of the slope subsequent to fitting the survival data against the absorbed dose by simple linear regression. The significant differences among the mean values were identified by Duncan's multiple range test using the Statistical Package for Social Sciences (IBM, Armonk, NY, USA) with a confidence level of $p < 0.05$.

II-3. Results

II-3-1. Inactivation of foodborne pathogens by 7.5 MeV X-ray

The viable cell counts of foodborne pathogens (*E. coli*, *S.* Typhimurium, *L. monocytogenes*, and *S. aureus*) persisting in the PBS suspension or in the inoculated ground beef followed by irradiation with 7.5MeV X-rays at various absorbed doses are shown in Tables 3–6 and the D_{10}-values are summarized in Table 7.

Initial counts of the pathogens were approximately 8–9 log CFU/mL for cell suspensions and 6–7 log CFU/g for ground beef; the bacterial cells viability decreased significantly with the increase in absorbed dose ($p < 0.05$). The D_{10}-values of the pathogens suspended in PBS for 7.5 MeV X-rays were ranged from 0.11 to 0.21 kGy, while those values of cells inoculated in ground beef were 0.22–0.41 kGy. In striking contrast to those of suspensions, the D_{10}-values of treated beef-inoculated bacteria were approximately 2 times higher.

In comparison with those of 7.5 MeV X-rays, the D_{10}-values of gamma rays generated from ^{60}Co source and 10 MeV e-beams showed no significant differences in bactericidal efficiency (Fig. 2 and Table 8).

Table 3. Viable cell count of *Escherichia coli* irradiated by 7.5 MeV X-rays at various doses

Medium	Absorbed dose (Gy)	Viable cell count (log CFU/mL)
Phosphate-buffered saline	0	8.16 ± 0.03^{F}
	94 ± 7	6.85 ± 0.56^{E}
	249 ± 23	5.75 ± 0.14^{D}
	332 ± 21	4.86 ± 0.06^{C}
	437 ± 32	3.71 ± 0.14^{B}
	557 ± 41	3.14 ± 0.09^{A}
Medium	Absorbed dose (Gy)	Viable cell count (log CFU/g)
Ground beef	0	7.26 ± 0.11^{E}
	194 ± 11	7.01 ± 0.14^{E}
	402 ± 24	5.75 ± 0.10^{D}
	683 ± 35	4.87 ± 0.43^{C}
	855 ± 44	3.49 ± 0.36^{B}
	1139 ± 64	2.30 ± 0.48^{A}

Absorbed dose and viable cell count are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=5).

$^{A-F}$Means followed by different letters within the column of a medium are significantly different ($p < 0.05$).

Table 4. Viable cell count of *Salmonella* Typhimurium irradiated by 7.5 MeV X-rays at various doses

Medium	Absorbed dose (Gy)	Viable cell count (log CFU/mL)
Phosphate-buffered saline	0	8.82 ± 0.16^{E}
	232 ± 17	7.51 ± 0.07^{D}
	366 ± 32	6.39 ± 0.41^{CD}
	575 ± 30	5.61 ± 0.39^{C}
	829 ± 45	4.20 ± 0.37^{B}
	944 ± 59	3.60 ± 0.09^{A}
Medium	Absorbed dose (Gy)	Viable cell count (log CFU/g)
Ground beef	0	6.90 ± 0.21^{D}
	233 ± 30	6.11 ± 0.17^{C}
	408 ± 28	5.95 ± 0.15^{BC}
	649 ± 49	5.20 ± 0.70^{B}
	863 ± 51	4.67 ± 0.69^{AB}
	1019 ± 81	4.47 ± 0.18^{A}

Absorbed dose and viable cell count are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=5).

$^{A-E}$Means followed by different letters within the column of a medium are significantly different ($p < 0.05$).

Table 5. Viable cell count of *Listeria monocytogenes* irradiated by 7.5 MeV X-rays at various doses

Medium	Absorbed dose (Gy)	Viable cell count (log CFU/mL)
Phosphate-buffered saline	0	9.01 ± 0.16^{D}
	228 ± 21	8.16 ± 0.54^{C}
	373 ± 19	7.27 ± 0.38^{B}
	556 ± 44	6.43 ± 0.49^{AB}
	837 ± 51	5.94 ± 0.74^{A}
	1103 ± 56	5.89 ± 0.29^{A}
Medium	Absorbed dose (Gy)	Viable cell count (log CFU/g)
Ground beef	0	7.56 ± 0.27^{D}
	249 ± 25	6.80 ± 0.24^{C}
	437 ± 42	6.42 ± 0.14^{B}
	658 ± 35	6.05 ± 0.46^{AB}
	923 ± 47	5.53 ± 0.50^{A}
	1134 ± 84	4.78 ± 0.93^{A}

Absorbed dose and viable cell count are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=5).

$^{A-D}$Means followed by different letters within the column of a medium are significantly different ($p < 0.05$).

Table 6. Viable cell count of *Staphylococcus aureus* irradiated by 7.5 MeV X-rays at various doses

Medium	Absorbed dose (Gy)	Viable cell count (log CFU/mL)
Phosphate-buffered saline	0	8.38 ± 0.18^{E}
	224 ± 19	7.79 ± 0.24^{D}
	473 ± 34	5.78 ± 0.38^{C}
	665 ± 41	4.23 ± 0.28^{B}
	912 ± 47	2.30 ± 0.41^{A}
	1151 ± 67	2.14 ± 0.37^{A}
Medium	Absorbed dose (Gy)	Viable cell count (log CFU/g)
Ground beef	0	7.34 ± 0.16^{C}
	228 ± 21	7.17 ± 0.17^{C}
	373 ± 29	6.27 ± 0.30^{BC}
	556 ± 37	5.76 ± 0.52^{B}
	837 ± 56	4.14 ± 0.40^{AB}
	1103 ± 52	3.34 ± 0.45^{A}

Absorbed dose and viable cell count are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=5).

$^{A-E}$Means followed by different letters within the column of a medium are significantly different ($p < 0.05$).

Table 7. Radiation sensitivity of foodborne pathogens for 7.5 MeV X-rays

Medium	D_{10}-value (kGy)			
	Escherichia coli	*Salmonella* Typhimurium	*Staphylococcus aureus*	*Listeria monocytogenes*
Phosphate-buffered saline	0.11	0.18	0.14	0.21
Ground beef	0.22	0.41	0.25	0.41

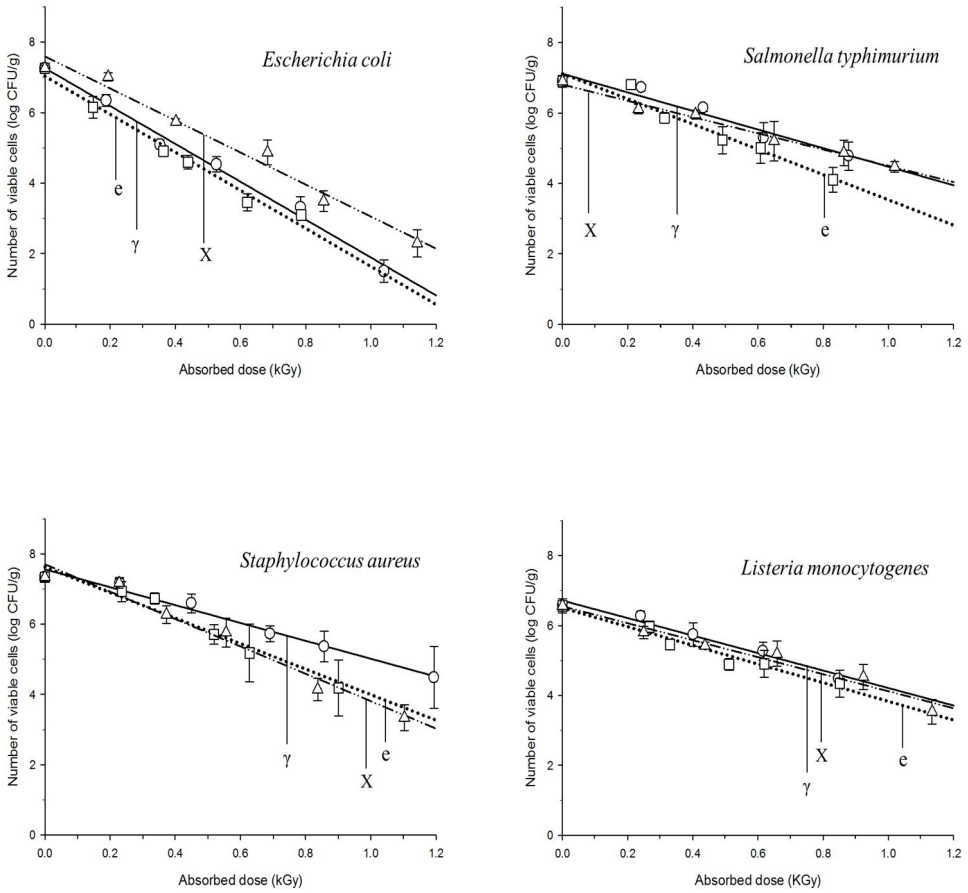


Fig. 2. Viability of bacterial pathogens inoculated on ground beef following treatment with different types of radiation.

Table 8. D_{10}-values (kGy) for four strains inoculated on ground beef by radiation type

Radiation type	*Escherichia coli*	*Salmonella* Typhimurium	*Staphylococcus aureus*	*Listeria monocytogenes*
7.5 MeV X-rays	0.22	0.41	0.25	0.41
Gamma-rays	0.18	0.35	0.37	0.40
10 MeV e-beams	0.18	0.30	0.27	0.37

II-3-2. Radiation sensitivity of bacteria in model foods for 7.5 MeV X-ray

The data presented in Table 9 show that the total aerobic bacteria count (TAB) of red pepper powder was significantly reduced as radiation dose increased ($p < 0.05$). The initial TAB of the non-irradiated sample was 5.27 log CFU/g, whereas the TABs in samples irradiated with 7.5 MeV X-rays were significantly reduced by 2 log values at a dose of 6 kGy ($p < 0.05$). No growth of TAB was observed in samples irradiated with a dose of 10 kGy. The D_{10} value of bacteria in red pepper powder for 7.5 MeV X-rays was 2.76 kGy.

TABs in chicken breast meat irradiated with 7.5 MeV X-rays at various doses during storage at 30°C for 7 days are shown in Table 10. The initial population in non-irradiated chicken meat was 4.84 log CFU/g, whereas viable cells were not observed in any irradiated samples. On day 3, a sharp increase in TAB of X-ray-irradiated samples at less than 8 kGy was observed; however, TAB in 10-kGy sample was not observed. On day 7, TAB in 10-kGy sample was 6.92 log CFU/g, indicating an X-ray irradiation at a dose of 10 kGy did not sterilize the TAB in chicken breast meat.

TABs in ground beef irradiated with 7.5 MeV X-rays at various doses during storage at 30°C for 7 days are shown in Table 11. The initial population in non-irradiated ground beef was 4.24 log CFU/g and viable cells were not observed in any irradiated samples. After 3-day storage at 30°C, however, TABs in X-ray-

irradiated samples at less than 10 kGy were greater than 3 log CFU/g. This result indicated that an X-ray irradiation at a dose of 10 kGy did not also sterilize the TAB in ground beef.

Table 9. Total aerobic bacteria count in red pepper powder irradiated by 7.5 MeV X-rays at various doses

Sample	Absorbed dose (kGy)	Total aerobic bacteria count (log CFU/g)
Red pepper powder	0	5.27 ± 0.16^{D}
	2.13 ± 0.07	4.15 ± 0.12^{C}
	4.27 ± 0.12	4.26 ± 0.12^{C}
	6.01 ± 0.15	2.87 ± 0.19^{B}
	8.45 ± 0.23	2.00 ± 0.12^{A}
	10.37 ± 0.38	ND[1)]
D_{10}-value (kGy)	2.76	

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed ± standard deviation (n=3).

[A-D]Means followed by different letters within a column are significantly different ($p < 0.05$).

[1)]Not detected within a detection limit of 1 log CFU/g.

Table 10. Total aerobic bacteria count in chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	Total aerobic bacteria count (log CFU/g)		
	0 day	3 day	7 day
0	4.84 ± 0.59^{B}	–[1)]	–
1.92 ± 0.11	2.81 ± 0.63^{A}	–	–
4.04 ± 0.23	ND[2)]	7.20 ± 0.49^{B}	–
5.89 ± 0.41	ND	6.12 ± 0.36^{A}	–
8.39 ± 0.25	ND	5.32 ± 0.64^{A}	7.72 ± 0.23^{NS}
10.20 ± 0.34	ND	ND	6.92 ± 0.53

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Bacterial counts are expressed as mean of triplicates ± standard deviation.

[1)]Bar indicates no examination due to the spoilage of sample.

[A-B]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

Table 11. Total aerobic bacteria count in ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	Total aerobic bacteria count (log CFU/g)		
	0 day	3 day	7 day
0	4.24 ± 0.69	–[1)]	–
2.27 ± 0.10	ND	–	–
4.15 ± 0.21	ND	–	–
6.21 ± 0.37	ND	4.67 ± 0.35[B]	–
8.32 ± 0.22	ND	3.41 ± 1.06[AB]	7.29 ± 0.95[NS]
10.09 ± 0.63	ND	3.25 ± 0.29[A]	7.13 ± 0.74

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Bacterial counts are expressed as mean of triplicates ± standard deviation.

[1)]Bar indicates no examination due to the spoilage of sample.

[A-B]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

II-4. Discussion

This study was conducted to evaluate inactivation characteristics of pathogens and bacteria in food for 7.5 MeV X-rays. The D_{10}-values of 7.5 MeV X-rays for foodborne pathogens were 0.11–0.21 kGy, when they are suspended in PBS. For ground beef, the D_{10}-values of 7.5 MeV X-rays for the pathogens were 0.22–0.41 kGy. Furthermore, X-ray irradiation at a dose of 2 kGy reduced approximately 1, 2, and 4 log levels of TAB in red pepper powder, chicken breast meat, and ground beef, respectively. Several studies have reported that the D_{10}-values of low-energy X-rays with < 150 keV energies for various bacteria in food were ranged from 0.39 to 1.30 (Mahmoud, 2009a; 2009b; 2010a; 2010b; Mahmoud et al., 2010).

No significant differences were evident between the bactericidal efficiencies of the three irradiation sources for *E. coli* and *L. monocytogenes* inoculated on ground beef within the maximum uncertainty (10%) of absorbed dose in this study. Moreover, it was reasonable that the efficiency of the three irradiation sources were similar for *S.* Typhimurium and *S. aureus*, because the responses to the irradiation sources were overlapped with each other. The overlapped variation in viable bacterial counts in ground beef samples can be explained by interference by the food matrix. The radiation sensitivity of the microorganisms could be varied as a result of the complexity of the food matrix, in which components compete with microorganisms for ionization by radiation (Thayer et al., 1995). This result was consistent with the results of a study by Tallentire & Miller (2015), in which no differences in the inactivation effectiveness of 5–7 MeV X-rays, gamma-rays, and

10 MeV e-beams were observed for bacterial spores mounted on wet filters within the measurement uncertainty of the absorbed dose. It is important note that the ground beef samples used in the present study were too thin (5 mm) to enable an accurate prediction of irradiation effects in commercial food products, which are generally treated in bulk. Therefore, further studies on commercial food products are currently ongoing using substantive irradiation practices in order to provide more realistic information regarding the inactivation efficiency of the three irradiation sources. Despite the model food study, the similar bactericidal efficiencies of the three irradiation sources in this study implied that 7.5 MeV X-rays may be an effective irradiation source for food pasteurization. Moreover, food irradiation by 7.5 MeV X-rays has a few advantages including increased conversion efficiency and penetration depth compared to gamma and e-beam irradiation. Recent advancement in X-ray machine development is allowing X-ray irradiation to actively compete with gamma and e-beam irradiation as a microbial reduction strategy for foods. In addition, better consumer acceptance for X-rays than gamma rays generated from radionuclides may contribute to the extended use of X-ray irradiation for food pasteurization.

Chapter III. Toxicological safety of irradiated chicken breast meat by 7.5 MeV X-ray

III-1. Introduction

Since the 1960s, there have been many studies related to the safety of irradiated food. Most provide only a small link in the chain of evidence; some provide a major body of evidence, such as a study using 135 t of chicken meat (Thayer, 1994). A Joint Expert Committee on Food Irradiation concluded that irradiation of food up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or microbiological problems (World Health Organization, 1981). Since 1981, several other international agencies, such as US Food and Drug Administration, Health Canada, and the European Food Safety Authority, have reviewed safety issues again and endorsed food irradiation as a safe process.

Despite these international reviews, some critics still question the safety of food irradiation (PC, 2003). Moreover, toxicological studies of X-ray-irradiated food have scarcely been reported. Therefore, the present study evaluated the genotoxicity, acute toxicity, and sub-chronic toxicity (90 days) of irradiated chicken breast by 7.5 MeV X-rays at 30 kGy.

III-2. Materials and Methods

III-2-1. Genotoxicity test

III-2-1-1. Sample preparation and irradiation

Chicken breast meat was purchased from a local market in Jeongeup city, Korea. The meat was ground by a homogenator, and 1 kg of meat was packed in an oxygen-impermeable nylon bag (2 mL O_2/m^2/24 h at 0 °C, 0.09 mm thickness; Sunkyung Co., Ltd., Seoul, Korea). The ground chicken beast was stored overnight in a refrigerator (4°C) until X-ray irradiation.

X-ray irradiation was performed with a linear electron accelerator, which delivers e-beams with a nominal energy of 7.5 MeV, at EB-Tech Co. (Daejeon, Korea) at an ambient temperature of approximately 20°C. The applied dose rate was 24 kGy/h. The ground chicken meat was irradiated at a nominal dose of 30 kGy. The actual absorbed dose was determined by using a 5 mm-diameter alanine dosimeter (Batch No.: T020604, Bruker BioSpin GmbH, Rheinstetten, Germany) and was measured within 24–48 h after irradiation using an electron paramagnetic resonance analyzer (e-scan^{TM} alanine dosimeter reader, Bruker BioSpin GmbH, Rheinstetten, Germany) according to ISO/ASTM 51607:2004. The actual dose was within 5% of the target dose.

The irradiated chicken breast was then dried using a freeze dryer (Model SFDSF12, Samwon Freezing Co., Seoul, Korea) and the powder was sieved using a 25 mesh sieve shaker (Chung Gye Industrial Mfg., Co., Gyeonggi, Korea). The

powder was subjected to further toxicological analysis.

III-2-1-2. Ames test

A bacterial reverse mutation assay was performed according to the recommendations of Maron & Ames (1983) and the principles of Organization for Economic Cooperation and Development (OECD) guideline No. 471 (OECD, 1997) to evaluate the mutagenicity of X-ray-irradiated (30 kGy) chicken breast with or without S9 metabolic activation system from rat liver (Aroclor 1254, Sigma, Spain), using the following four histidine-auxotrophic *Salmonella* Typhimurium strains: TA98, TA100, TA1535, and TA1537. All strains were provided by the Korea National Institute of Health and evaluated for maintenance of genetic markers such as histidine requirement, deep rough (rfa) characteristic, UV sensitivity (uvrB mutation), and ampicillin- or tetracycline-resistance by the R-factor prior to the study. The strains were inoculated in the broth media (Nutrient broth No. 2, Oxoid Co., Hampshire, England) and cultured for 10 h at 37°C with a continuous agitation at 200 rpm (Vision Scientific Co., Incheon, Korea) to use cells under exponential stage.

Serial dilution of extracted solution of ground chicken meat with an organic solvent (ethanol : chloroform = 1 : 1, v/v) were prepared in dimethyl sulfoxide (DMSO, Aldrich Chemical Co., USA) to deliver the required concentration at a constant volume. The doses tested were 40, 200, 1000, and 5000 μg sample/plate.

Sodium azide (SA), 4-nitroquinoline-1-oxide (4-NQO), acridine ICR-191 (ICR-191), benzo[α]pyrene (B[α]P), and 2-aminoanthracene (2-AA) were dissolved in deionized distilled water (DDW) or DMSO and used as a positive control for all strains tested. The activity of the S9 mix was confirmed from the induced mutagenesis by using 2-AA.

The assay tubes were pre-incubated at 37°C for 30 min before plating onto minimal agar. An aliquot of 0.1 mL of the cell culture (2×10^9 CFU/mL), 0.1 mL of the sterilized test sample suspension, and 0.5 mL of the S9 mixture or DDW were mixed. The mixture was transferred to 2.5 mL of top agar containing histidine-biotin (kept warm at 45 ± 2°C) and poured onto minimal glucose agar plates. After the agar had solidified, these plates were incubated for 48 h at 37°C, and then the number of revertant colonies was counted. Mutagenicity was evaluated according to the method proposed by Maron & Ames (1983), who suggested it to be positive when the number of revertant colonies was more than double relative to the negative control and when this increase was dose-dependent.

III-2-1-3. *In vitro* chromosomal aberration test

The chromosomal aberration test was performed according to the OECD guideline for the testing of chemicals NO. 473 (OECD, 1997). A Chinese hamster lung fibroblast cell line (CHL) derived from a newborn female was used for chromosomal aberration test of X-ray-irradiated (30 kGy) chicken meat. The cells

were maintained in Eagle's minimum essential medium (Gibco, 410–1100) supplemented with 5% fetal bovine serum (FSB). The modal chromosome number was 25, and the doubling time was 15–17 h.

Serial dilutions of extracted solution of ground chicken meat with an organic solvent (ethanol:chloroform = 1:1, v/v) were prepared in DMSO to deliver the required concentration at a constant volume. The doses tested were 625, 1250, 2500, and 5000 μg sample/mL. B[α]P dissolved in DMSO in the presence of S9 mixture and ethyl methane sulfonate (EMS) without S9 mixture were used as positive control.

Cells (approximately 5×10^5 cells/60-mm plastic petri dish) were treated with a test substance together with liver S9 (20%, v/v) for 6 h followed by additional cultivation with fresh medium for another 16 h. Chromosomal preparations were made as follows; Colcemid (final concentration 0.05 μg/mL) was added to the culture 1 h before cell harvesting. The cells were then collected by trypsinization, and incubated in 0.075 M KCl hypotonic solution for 20 min at 37°C. The cells were then fixed 3 times with ice-cold 3:1 methanol-acetic acid. The fixed cell suspension was placed on a clean glass slide, air-dried, and stained with Giemsa solution. The number of cells with chromosomal aberrations and the types of aberrations were recorded based on the observation of 100 well spread metaphase cells per dose. The incidence of polyploid cells among the 100 metaphase cells was also recorded. Solvent treated cells served as a negative control.

III-2-1-4. Micronucleus test

Male ICR mice aged 5 weeks were purchased from Jung-Ang Lab. animal, Inc. (Seoul, Korea); they were used for experiments at 6 weeks of age, after acclimatization for 1 week. Animals were fed commercial pellets and tap water ad libitum throughout the acclimatization and experiment periods. The test substance was administrated to mice by oral gavage twice with a 24 h interval to mice at dose levels of 250, 500, 1000, and 2000 mg/kg body weight (b.w.). Smear preparations were made with bone marrow cells 24 h after the last oral treatment of test substance, according to the methods described by Schmid (1975). Two thousand bone marrow polychromatic erythrocytes (PCE) per animal were observed, and the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) were recorded.

III-2-2. Acute oral toxicity test

III-2-2-1. Animals and housing conditions

Male and female Crj:CD-1 (ICR) mice, 5 week old, were purchased from Jung-Ang Lab. animal, Inc. (Seoul, Korea) and used after 1 week of acclimatization. On the day before study initiation, mice were allocated to 3 groups of each sex, for a total of 6 groups (10 mice/group each sex). During this period, and throughout the experiment, the mice were housed individually in plastic case (278 × 420 × 200 mm) with cedar chip bedding and were maintained under controlled temperature

conditions (23 ± 3°C) and relative humidity (60 ± 10%), with a 12-h light/dark cycle.

III-2-2-2. Oral administration of sample

An acute, one-day oral toxicity study was conducted in accordance with the OECD Guideline No. 423 (OECD, 2001). The powdered sample (non-irradiated or X-ray-irradiated at 30 kGy) dissolved in distilled water to a certain concentration and an aliquot of the total administration concentration at 1000 or 2000 mg/kg were delivered to mice via oral gavage (10 mL/kg b.w.). Control groups were administered water equally.

III-2-2-3. Clinical observation and tissue preparation

Clinical signs and mortality of the mice were observed daily for 14 days. Body weight was recorded on day 1, 3, 7, and 14. On day 15 after 1 day of fasting, the mice were killed by decapitation. Blood was collected for hematology and biochemical analysis. Gross observations were performed at autopsy, and recorded. At terminal sacrifice, the following organs (the liver, spleen, kidney (left and right), testis/ovary, lung, and heart) from each mouse were weighed.

III-2-2-4. Hematology analysis

Blood for hematology was collected into tubes treated with EDTA dipotassium.

Hematological estimations were carried out using an automatic analyzer (Sysmex F820; Symex Co., Ltd., Hyogo, Japan) for white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MONO), eosinophil (EOS), basophil (BASO), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet (PLT), and mean plasma volume (MPV).

III-2-2-5. Serum biochemistry analysis

The following clinical chemistry measurements were performed on sera obtained by centrifugation of the aforementioned blood samples: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total bilirubin (T-BIL), total cholesterol (T-CHO), triglyceride (TG), and total protein (TP). All parameters were determined using an automatic analyzer, model 7070 (Hitachi Ltd., Tokyo, Japan).

III-2-3. Sub-acute (90 days) oral toxicity test

Male and female Crj:CD-1 (ICR) mice aged 4 weeks were purchased from Jung-Ang Lab. animal, Inc. (Seoul, Korea) and used for the test after a 1 week acclimatization period. During this period, and throughout the experiment, the mice

were housed individually in plastic case (278 × 420 × 200 mm) with cedar chip bedding, and were kept under controlled temperature conditions (23 ± 3°C) and relative humidity (60 ± 10%), with a 12-h light/dark cycle. On the day before study initiation, mice were allocated to 4 groups of each sex, for a total of 8 groups (12 mice/group each sex).

Male or female mice in control group were fed for 13 weeks a standard feed AIN-76, whereas other groups received the modified AIN-76 feed including freeze-dried and non-irradiated or chicken breast meat irradiated with X-rays at 30 kGy (Table 12). Proximate composition of freeze-dried chicken meat was 4.26% carbohydrates, 55.80 proteins, and 34.61% lipids in dry base. The daily intake of chicken meat that corresponds to the nutritional benefits provided by the standard diet was determined to be 2.5%, whereas the amount of cornstarch, casein, and soybean oil was decreased to formulate a diet with identical caloric content as the standard feed.

During the experimental period, clinical signs and mortality of the mice were observed daily. Body weight and food intakes were measured weekly throughout the study. At the end of administration, the mice were killed by decapitation. Blood was collected for hematology and biochemical analysis. Gross observations were made at autopsy, and recorded. At terminal sacrifice, the following organs (the liver, spleen, kidney (left and right), testis/ovary, lung, and heart) from each mouse were weighed. Hematological and serum biochemical analyses of the sacrificed mice were carried out according to the methods previously described in acute toxicity test.

At terminal sacrifice, the fresh organs (liver and lung) were fixed in 10%

phosphate-buffered formalin and embedded in paraffin wax. The paraffin wax-embedded samples were cut into 7-μm sections, stained with hematoxylin and eosin, and then examined using light microscopy (×250) (Opticphot-2, Nikon, Tokyo, Japan) for histopathological observation.

III-2-4. Statistical analysis

Data were analyzed statistically using IBM® SPSS Statistics 21 software for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance and Duncan's multiple range tests at $p < 0.05$ were used to compare the differences among mean values.

Table 12. Formula of experimental diets

Ingredient	AIN-76	Chicken meat	
		Non-irradiated	30 kGy
Casein	200	186	186
AIN-76 mineral mix	35	35	35
AIN-76 vitamin mix	10	10	10
DL-methionine	3	3	3
Cellulose	50	50	50
Cornstarch	550	548	548
Sucrose	100	100	100
Corn oil	50	41	41
Choline bitartrate	2	2	2
Chicken powder	-	25	25
Total (g)	1000	1000	1000

III-3. Results

III-3-1. Genotoxicity of irradiated chicken breast meat by 7.5 MeV X-ray

The bacterial reverse-mutation result of X-ray-irradiated (30 kGy) chicken breast meat using *S.* Typhimurium strain is shown in Table 13. The numbers of revertant colonies for the 4 tested strains exhibited independency at doses up to 5000 μg/plate of extracted sample from X-ray-irradiated chicken meat. Thus, negative results were obtained at all tested dose levels, because all the revertant numbers were 2-fold lower than those of negative control (DMSO).

Result of the chromosomal aberration assay is shown in Table 14. On the basis of the preliminary experiments, applied concentrations from 625 to 5000 μg were established at a common ratio of 2 in the absence or presence of the S9 mix after 24 h of treatment. The numbers of normal chromosome were comparable to that observed for the negative control (DMSO) and were independent of dose and the presence of S9 mix, indicating negative results. On the other hand, EMS and B[α]P used as the positive control induced 36 and 22% chromosomal aberrations in CHL cells, respectively, indicating their mutagenic potential, because a positive decision is made when the number of aberrations is over 10%.

Frequency of micronuclei in marrow of mice fed X-ray-irradiated chicken breast meat is shown in Table 15. No toxicological signs were observed in any of the treated mice (data not shown). Statistically significant increases were also not

observed in the frequency of the MNPCEs at doses up to 2000 mg/kg b.w./day. Moreover, the proportion of PCEs in total erythrocytes showed no significant differences between the test group and the vehicle-control group (DMSO). On the other hand, the positive control CPA clearly induced MNPCEs. Thus, X-ray-irradiated (30 kGy) chicken breast meat did not induce micronuclei in mice.

Table 13. *Salmonella* Typhimurium reversion assay with X-ray-irradiated chicken meat at 30 kGy

Test compound[1)]	Presence of S9	Conc. (μg/plate)	No. of His+ revertant per plate			
			TA 98	TA 100	TA 1535	TA 1537
DMSO	+	0	39±8	177±16	12±2	12±2
Chicken meat	+	40	38±6	162±17	9±3	14±3
	+	200	42±8	154±14	11±1	10±2
	+	1000	35±9	172±12	12±1	12±2
	+	5000	40±7	168±17	11±2	10±3
B[α]P	+	1.0	218±26*	-	-	-
2-AA	+	1.0	-	648±35*	103±12*	218±27*
DMSO	-	0	28±6	167±16	13±3	7±2
Chicken meat	-	40	25±3	171±12	15±6	8±3
	-	200	26±4	174±16	13±2	10±2
	-	1000	22±6	168±14	14±3	11±3
	-	5000	27±4	157±16	12±2	8±2
SA	-	0.5	-[2)]	412±13*	319±16*	-
4NQO	-	05	532±27*	-	-	-
ICR-191	-	0.5	-	-	-	135±28*

Each value represents the mean ± SD of three plates and expressed of revertant colonies per plate.

[1)]Dimethyl sulfoxide (DMSO) was used a negative control and sodium azide (SA), 4-nitroquinoline N-oxide (4NQO), acridine mutagen ICR-191 (ICR-191), Benzo[α]pyrene (B[α]p), and 2-Aminoanthracene (2-AA) were used as positive controls for the corresponding strains.

[2)]Not examined.

*Significantly different from the control ($p < 0.05$).

Table 14. Chromosomal aberration test on X-ray-irradiated chicken meat at 30 kGy using a Chinese hamster lung cell line

Test compound[1)]	Presence of S9	Conc. (μg/mL)	G[2)]	CD	CX	TD	TX	Other	Nor	Total
DMSO	+	0	0	0.5	1	0	0	0.5	98	100
Chicken meat	+	625	0	1	1	0	0	0	98	100
	+	1250	0	0.5	0.5	0	1	0	98	100
	+	2500	0	0.5	1	0.5	1	0	97	100
	+	5000	0	1	0	0.5	0	0.5	98	100
B(α)p	+	20	0	2	3	4	13	0	78	100
DMSO	-	0	0	0.5	1	0	0	0.5	98	100
Chicken meat	-	625	0	1	1	0	0.5	0.5	97	100
	-	1250	0	0.5	0	0.5	1	0	98	100
	-	2500	0	0	1	1	0	0	98	100
	-	5000	0	1	0.5	1	0	0.5	97	100
EMS	-	800	1	1	1	4	29	0	64	100

Number of findings of mean aberrant metaphases. 100 metaphases were examined per culture.

[1)]DMSO, dimethyl sulfoxide (negative control); B(α)p, Benzo(α)pyrene (positive control); EMS, ethyl methanesulfonate (positive control)

[2)]G, gaps (chromatid type + chromosome type); CD, chromosome type deletions; CX, chromosome type exchanges; TD, chromatid type deletions; TX, chromatid type exchanges; Other, metaphases with more than 10 aberrations (including gaps) or with chromosomes fragmentation; Nor, normal.

Table 15. Frequency of micronuclei from marrow in mice treated with X-ray-irradiated chicken meat at 30 kGy

Test compound[1)]	Dose (mg/kg b.w.)	MNPCE/2,000 PCE[2)]	PCE:RBC Ratio[3)]
DMSO	0	2.3 ± 1.3	0.31 ± 0.03
Chicken meat	250	1.8 ± 1.4	0.29 ± 0.03
	500	2.5 ± 1.6	0.32 ± 0.03
	1000	3.8 ± 1.2	0.31 ± 0.02
	2000	2.8 ± 1.1	0.33 ± 0.03
CPA	70	98.2 ± 27.4*	0.24 ± 0.03

Each value represents the mean ± S.D. (n=10).

[1)]Dimethyl sulfoxide (DMSO) was used as negative control and cyclophosphamide·H_2O (CPA) was used as positive control.

[1)]MNPCE: micronucleated polychromatic erythrocyte, PCE: polychromatic erythrocyte.

[2)]RBC: red blood cells (polychromatic erythrocyte + normochromatic erythrocyte).

*Significantly different from the control at $p < 0.05$.

III-3-2. Acute toxicity of irradiated chicken breast meat by 7.5 MeV X-ray

All tested mice survived and no abnormal clinical and necropsy signs were observed in any laboratory animals in all dose groups during the experimental period of 14 days (Tables 16–19). An approximate lethal dose of X-ray-irradiated chicken meat was assumed more than 2000 mg/kg. Normal increase in body weight of both male and female mice (10.66–11.28 g for male, 6.58–8.00 g for female) was observed, and the change in body weight gain was not significantly different at different doses (Tables 20 and 21).

The hematological data of mice after 14 days since the oral administration is shown in Tables 22 and 23. LYM for male mice significantly increased, but MONO for female mice significantly decreased at increasing treatment doses. Other hematological parameters exhibited no significant difference with at increasing dosed. Moreover, no significant differences were observed in serum biochemical parameters, regardless of the treatment dose (Tables 24 and 25).

Table 16. Mortality of ICR mice administered during acute toxicity test for X-ray-irradiated chicken meat at 30 kGy

Gender	Day	Treatment dose (mg/kg b.w.)	Frequency[1)]
Male	0	0	0/10
		1000	0/10
		2000	0/10
	1-13	0	0/10
		1000	0/10
		2000	0/10
	14	0	0/10
		1000	0/10
		2000	0/10
Female	0	0	0/10
		1000	0/10
		2000	0/10
	1-13	0	0/10
		1000	0/10
		2000	0/10
	14	0	0/10
		1000	0/10
		2000	0/10

[1)] Number of dead animals/total animals.

Table 17. Clinical signs of male ICR mouse during acute toxicity test for X-ray-irradiated chicken meat at 30 kGy

Day	Clinical sign	Treatment dose (mg/kg b.w.)	Frequency[1)]
0	Normal	0	10/10
		1000	10/10
		2000	10/10
	Soft stool	0	0/10
		1000	0/10
		2000	0/10
	Diarrhea	0	0/10
		1000	0/10
		2000	0/10
	Solid perineal region	0	0/10
		1000	0/10
		2000	0/10
1-13	Normal	0	10/10
		1000	10/10
		2000	10/10
14	Normal	0	10/10
		1000	10/10
		2000	10/10
	Terminal sacrifice	0	10/10
		1000	10/10
		2000	10/10

[1)]Number of animals observed/total number of animals.

Table 18. Clinical signs of female ICR mouse during acute toxicity test for X-ray-irradiated chicken meat at 30 kGy

Day	Clinical sign	Treatment dose (mg/kg b.w.)	Frequency[1)]
0	Normal	0	10/10
		1000	10/10
		2000	10/10
	Soft stool	0	0/10
		1000	0/10
		2000	0/10
	Diarrhea	0	0/10
		1000	0/10
		2000	0/10
	Solid perineal region	0	0/10
		1000	0/10
		2000	0/10
1-13	Normal	0	10/10
		1000	10/10
		2000	10/10
14	Normal	0	10/10
		1000	10/10
		2000	10/10
	Terminal sacrifice	0	10/10
		1000	10/10
		2000	10/10

[1)]Number of animals observed/total number of animals.

Table 19. Necropsy findings of ICR mice administered with X-ray-irradiated chicken meat (30 kGy) at various doses

Gender	Treatment dose (mg/kg b.w.)	Findings	Frequency[1)]
Male	0	No gross findings	10/10
	1000	No gross findings	10/10
	2000	No gross findings	10/10
Female	0	No gross findings	10/10
	1000	No gross findings	10/10
	2000	No gross findings	10/10

[1)]Number of animals observed/total number of animals.

Table 20. Body weight change of male ICR mice during acute toxicity test for X-ray-irradiated chicken meat at 30 kGy

Day	Treatment dose (mg/kg b.w.)	Body weight (g)
0	0	28.40±0.48[NS]
	1000	28.43±1.39
	2000	28.20±1.08
1	0	32.96±1.23[NS]
	1000	33.33±1.67
	2000	33.63±1.50
3	0	35.26±1.75[NS]
	1000	35.48±1.71
	2000	34.83±1.30
7	0	37.16±2.95[NS]
	1000	37.25±1.92
	2000	37.13±1.59
14	0	39.06±4.51[NS]
	1000	39.18±1.51
	2000	39.48±1.86

Values are expressed as mean ± S.D. (n=10).

[NS]No significant difference within a column ($p < 0.05$).

Table 21. Body weight change of female ICR mice during acute toxicity test for X-ray-irradiated chicken meat at 30 kGy

Day	Treatment dose (mg/kg b.w.)	Body weight (g)
0	0	24.26±0.92^{NS}
	1000	24.03±1.14
	2000	23.80±0.94
1	0	28.62±0.93^{NS}
	1000	27.08±1.02
	2000	26.90±0.92
3	0	28.58±0.88^{NS}
	1000	28.65±1.16
	2000	28.4±1.57
7	0	29.02±0.63^{NS}
	1000	29.10±1.89
	2000	29.03±1.76
14	0	32.26±1.24^{NS}
	1000	30.63±2.06
	2000	30.3±81.96

Values are expressed as mean ± S.D. (n=10).

^{NS}No significant difference within a column ($p < 0.05$).

Table 22. Hematological test of male ICR mice administered with X-ray-irradiated chicken (30 kGy) at various doses

Treatment dose (mg/kg b.w.)	WBC[1)] (K/μL)	NEU (K/μL)	LYM (K/μL)	MONO (K/μL)	EOS (K/μL)
0	5.52±0.73[2)]	1.39±0.34	3.38±0.74[A]	0.13±0.09	0.02±0.02
1000	4.01±1.68	0.53±0.28	3.28±1.44[A]	0.10±0.08	0.01±0.01
2000	7.64±2.50	1.19±0.92	6.47±1.36[B]	0.16±0.11	0.03±0.02

Treatment dose (mg/kg b.w.)	BASO (K/μL)	RBC (M/μL)	HB (g/dL)	HCT (%)	MCV (fL)
0	0.01±0.01	10.70±0.47	14.85±0.70	61.40±3.65	58.35±1.40
1000	0.01±0.01	10.45±0.83	14.00±1.22	55.25±3.99	54.90±2.55
2000	0.01±0.01	10.95±0.20	15.23±0.85	60.95±4.30	55.15±3.22

Treatment dose (mg/kg b.w.)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/μL)	MPV (fL)
0	14.10±0.18	24.18±0.40	15.25±0.66	398.51±61.50	4.97±0.12
1000	13.90±0.18	25.33±1.18	15.55±0.59	450.30±147.32	4.80±0.24
2000	14.05±0.58	25.45±0.48	15.95±0.42	548.74±149.01	4.70±0.01

[1)]White blood cell (WBC), Neutrophil (NEU), Lymphocyte (LYM), Monocyte (MONO), Eosinophil (EOS), Basophil (BASO), Red blood cell (RBC), Hemoglobin (HB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width (RDW), Platelet (PLT), and Mean plasma volume (MPV).

[2)]Values are expressed as mean ± S.D. (n=10).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

Table 23. Hematological test of female ICR mice administered with X-ray-irradiated chicken (30 kGy) at various doses

Treatment dose (mg/kg b.w.)	WBC[1)] (K/μL)	NEU (K/μL)	LYM (K/μL)	MONO (K/μL)	EOS (K/μL)
0	2.24±0.88[2)]	0.33±0.11	1.70±0.67	0.10±0.03[B]	0.02±0.01
1000	1.91±0.71	0.26±0.20	1.55±0.51	0.09±0.03[AB]	0.01±0.01
2000	2.09±0.56	0.34±0.09	1.68±0.48	0.04±0.02[A]	0.02±0.01
Treatment dose (mg/kg b.w.)	BASO (K/μL)	RBC (M/μL)	HB (g/dL)	HCT (%)	MCV (fL)
0	0.01±0.01	10.09±0.25	14.38±0.35	59.57±3.06	57.60±1.19
1000	0.01±0.01	9.70±0.56	14.15±0.78	54.10±3.34	57.80±2.35
2000	0.01±0.01	10.31±0.08	14.63±0.19	59.00±1.21	57.60±0.84
Treatment dose (mg/kg b.w.)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/μL)	MPV (fL)
0	14.17±0.21	25.13±1.05	15.33±0.46	598.72±16.34	5.03±0.19
1000	14.58±0.50	25.28±0.88	15.40±0.62	567.08±57.71	4.58±0.87
2000	14.20±0.08	24.63±0.33	15.15±0.37	630.01±89.34	5.10±0.29

[1)]White blood cell (WBC), Neutrophil (NEU), Lymphocyte (LYM), Monocyte (MONO), Eosinophil (EOS), Basophil (BASO), Red blood cell (RBC), Hemoglobin (HB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width (RDW), Platelet (PLT), and Mean plasma volume (MPV).

[2)]Values are expressed as mean ± S.D. (n=10).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

Table 24. Serum biochemistry test of male ICR mice administered with X-ray-irradiated chicken (30 kGy) at various doses

Treatment dose (mg/kg b.w.)	ALB[1)] (g/dL)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
0	3.43±0.32[2)]	329.35±94.52	55.54±27.15	209.48±76.37
1000	3.72±0.36	320.76±55.40	55.53±6.91	137.84±57.62
2000	3.44±0.35	325.13±69.45	44.00±17.53	149.53±56.32

Treatment dose (mg/kg b.w.)	BUN (mg/dL)	CRE (mg/dL)	GLU (mg/dL)	T-BIL (mg/dL)
0	23.16±4.16	0.38±0.05	110.46±30.88	0.12±0.03
1000	20.24±3.42	0.39±0.06	97.31±30.57	0.11±0.03
2000	23.62±3.43	0.38±0.05	109.31±27.40	0.13±0.02

Treatment dose (mg/kg b.w.)	T-CHO (mg/dL)	TG (mg/dL)	TP (g/dL)
0	147.11±21.42	197.31±23.12	4.62±0.48
1000	150.58±21.71	172.44±47.19	4.63±0.35
2000	145.19±39.09	163.72±50.01	4.72±0.21

[1)]Albumin (ALB), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Creatinine (CRE), Glucose (GLU), Total bilirubin (T-BIL), Total cholesterol (T-CHO), Triglyceride (TG), and Total protein (TP).

[2)]Values are expressed as mean ± S.D. (n=10).

Table 25. Serum biochemistry test of female ICR mice administered with X-ray-irradiated chicken (30 kGy) at various doses

Treatment dose (mg/kg b.w.)	ALB[1)] (g/dL)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
0	3.48±0.10[2)]	332.26±42.38	36.62±2.25	119.35±16.50
1000	3.47±0.15	301.89±95.06	49.52±10.17	123.23±39.19
2000	3.29±0.38	290.25±4.38	37.23±0.99	92.04±13.94

Treatment dose (mg/kg b.w.)	BUN (mg/dL)	CRE (mg/dL)	GLU (mg/dL)	T-BIL (mg/dL)
0	17.24±0.50	0.37±0.02	100.01±19.75	0.09±0.01
1000	18.12±3.52	0.36±0.02	121.69±16.65	0.08±0.01
2000	15.95±2.14	0.32±0.04	94.19±18.17	0.08±0.02

Treatment dose (mg/kg b.w.)	T-CHO (mg/dL)	TG (mg/dL)	TP (g/dL)
0	103.41±15.93	163.07±29.45	4.91±0.08
1000	96.72±18.06	180.89±32.31	4.70±0.20
2000	102.36±25.95	197.86±22.03	4.47±0.60

[1)]Albumin (ALB), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Creatinine (CRE), Glucose (GLU), Total bilirubin (T-BIL), Total cholesterol (T-CHO), Triglyceride (TG), and Total protein (TP).

[2)]Values are expressed as mean ± S.D. (n=10).

III-3-3. Sub-chronic toxicity of irradiated chicken breast meat by 7.5 MeV X-ray

No deaths and abnormal clinical signs were observed in any laboratory animals of control, 0 kGy and 30 kGy groups during the experimental period of 13 weeks (Tables 26–28). Normal increase in body weight of both male and female mice (0.10–0.14 g/day for male, 0.15–0.19 g/day for female) was observed, and there was no significant difference in body weight and food consumption among groups of control, 0 kGy and 30 kGy (Table 29).

Relative organ weights in control, 0 kGy and 30 kGy groups showed no significant difference for the liver (male, 3.09–3.87%; female, 3.89–4.12%), spleen (male, 0.24–0.32%; female, 0.31–0.40%), left kidney (male, 0.75–0.92%; female, 0.51–0.60%), right kidney (male, 0.64–0.79%; female, 0.50–0.58%), heart (male, 0.43–0.63%; female, 0.39–0.42%), and lung (male, 0.46–0.54%; female, 0.49–0.54%) at week 13 ($p < 0.05$) (Table 30).

The hematological data of mice after the 13-week administration is shown in Tables 31 and 32. PLT and MPV of male mice in 0 and 30 kGy groups were significantly lower than those of the control group. Moreover, MPV of female mice in 0 and 30 kGy groups was lower than that of the control group, whereas MCH and MCHC in both groups were significantly higher than those of the control group. No significant difference was observed for other tested parameters among the 3 groups, regardless of gender ($p < 0.05$).

Serum biochemical data of mice are given in Tables 33 and 34. ALT of male mice in 0 and 30 kGy groups were significantly different from those of the control group, whereas no significant difference was observed between 0 and 30 kGy groups. Furthermore, ALP and T-CHO of female mice in 0 and 30 kGy groups also exhibited differences relative to the control group with no significant difference between the two treatment groups (0 and 30 kGy). Other parameters showed no significant difference among the 3 groups ($p < 0.05$).

Histopathological examinations of the organs such as the liver and kidney showed no detectable abnormalities (Figs. 3 and 4). Microscopic examination of the internal organs did not reveal any alterations; damage to cell morphology (tissue atrophy, necrosis, exfoliation, and inflammation) of cellular appearances was unremarkable in both 0 and 30 kGy groups relative to the control group, regardless of gender.

Table 26. Mortality of ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Gender	Week	Group	Frequency[1)]
Male	0	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	1-12	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	13	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
Female	0	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	1-12	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	13	Control	0/12
		0 kGy	0/12
		30 kGy	0/12

[1)]Number of dead animals/total number of animals

Table 27. Clinical signs of male ICR mice administered with X-ray-irradiated chicken at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Week	Clinical sign	Treatment dose (mg/kg b.w.)	Frequency[1)]
1	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
2-12	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
	Loss of fur	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	Compound-colored stool	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
13	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
	Terminal sacrifice	Control	12/12
		0 kGy	12/12
		30 kGy	12/12

[1)]Number of animals observed for the specific clinical signs/total number of animals.

Table 28. Clinical signs of female ICR mice administered with X-ray-irradiated chicken at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Week	Clinical sign	Treatment dose (mg/kg b.w.)	Frequency[1)]
1	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
2-12	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
	Loss of fur	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
	Compound-colored stool	Control	0/12
		0 kGy	0/12
		30 kGy	0/12
13	Normal	Control	12/12
		0 kGy	12/12
		30 kGy	12/12
	Terminal sacrifice	Control	12/12
		0 kGy	12/12
		30 kGy	12/12

[1)]Number of animals observed for the specific clinical signs/total number of animals.

Table 29. Body weight change and food consumption of ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg bw/day) for 90 days

Gender	Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g/day)	Food consumption (g/day)
Male	Control	37.33±4.54^{NS}	47.80±4.54^{NS}	0.10±0.03^{NS}	4.82±0.81^{NS}
	0 kGy	37.97±3.45	49.25±3.45	0.14±0.07	4.41±0.12
	30 kGy	37.30±2.67	50.09±1.88	0.14±0.02	4.50±0.17
Female	Control	30.25±3.31^{NS}	41.90±5.09^{NS}	0.19±0.15^{NS}	3.76±0.26^{NS}
	0 kGy	29.34±2.17	40.53±1.48	0.15±0.08	3.75±0.15
	30 kGy	29.12±2.27	40.10±1.26	0.15±0.06	3.88±0.27

Values are expressed as mean±S.D (n=12).

^{NS}No significant difference within a column ($p < 0.05$).

Table 30. Relative organ weights (%) of male ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Gender	Group	Liver	Spleen	Left kidney
Male	Control	3.09±0.56^{NS}	0.26±0.06^{NS}	0.92±0.10^{NS}
	0 kGy	3.72±0.43	0.32±0.12	0.83±0.16
	30 kGy	3.87±0.46	0.24±0.06	0.75±0.08
	Group	Right kidney	Heart	Lung
	Control	0.79±0.05^{NS}	0.63±0.17^{NS}	0.54±0.08^{NS}
	0 kGy	0.67±0.17	0.43±0.06	0.49±0.07
	30 kGy	0.64±0.11	0.45±0.05	0.46±0.09
Female	Group	Liver	Spleen	Left kidney
	Control	4.12±0.41^{NS}	0.40±0.14^{NS}	0.51±0.06^{NS}
	0 kGy	4.03±0.42	0.31±0.06	0.56±0.11
	30 kGy	3.89±0.44	0.35±0.10	0.60±0.08
	Group	Right kidney	Heart	Lung
	Control	0.50±0.09^{NS}	0.39±0.03^{NS}	0.49±0.14^{NS}
	0 kGy	0.56±0.09	0.40±0.08	0.51±0.11
	30 kGy	0.58±0.08	0.42±0.05	0.54±0.09

Relative organ weights were expressed as the percentage of organ weights (mean ± S.D) to body weights (n=12).

^{NS}No significant difference within a row ($p < 0.05$).

Table 31. Hematological test of male ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Group	WBC[1)] (K/μL)	NEU (K/μL)	LYM (K/μL)	MONO (K/μL)	EOS (K/μL)
Control	2.26±0.88[2)]	0.52±0.13	1.69±0.70	0.05±0.03	0.01±0.01
0 kGy	4.28±1.30	1.38±0.91	2.50±1.15	0.10±0.07	0.03±0.03
30 kGy	4.10±0.81	0.93±0.55	2.63±0.67	0.15±0.08	0.02±0.01

Group	BASO (K/μL)	RBC (M/μL)	HB (g/dL)	HCT (%)	MCV (fL)
Control	0.01±0.01	9.26±2.05	11.47±2.36	53.40±2.40	52.03±1.52
0 kGy	0.01±0.01	9.99±2.65	14.58±1.77	55.72±8.48	54.17±3.06
30 kGy	0.01±0.01	10.34±0.61	14.23±0.84	53.13±3.30	51.25±1.64

Group	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/μL)	MPV (fL)
Control	12.40±0.56	23.87±1.17	16.20±0.52	936.01±179.93[B]	5.23±0.21[B]
0 kGy	13.89±1.58	25.20±1.93	17.98±2.88	687.31±143.64[A]	4.83±0.17[A]
30 kGy	14.06±0.40	25.72±1.42	16.71±1.02	668.83±72.29[A]	4.72±0.28[A]

[1)]White blood cell (WBC), Neutrophil (NEU), Lymphocyte (LYM), Monocyte (MONO), Eosinophil (EOS), Basophil (BASO), Red blood cell (RBC), Hemoglobin (HB), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width (RDW), Platelet (PLT), and Mean plasma volume (MPV).

[2)]Values are expressed as mean ± S.D. (n=12).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

Table 32. Hematological test of female ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Group	WBC[1)] (K/μL)	NEU (K/μL)	LYM (K/μL)	MONO (K/μL)	EOS (K/μL)
Control	3.30±1.69[2)]	0.85±0.46	2.01±1.14	0.14±0.10	0.01±0.01
0 kGy	3.89±0.79	0.81±0.24	2.46±0.73	0.14±0.08	0.01±0.01
30 kGy	2.27±0.70	0.52±0.16	1.48±0.69	0.16±0.11	0.02±0.01

Group	BASO (K/μL)	RBC (M/μL)	HB (g/dL)	HCT (%)	MCV (fL)
Control	0.03±0.01	11.06±0.81	12.73±1.23	52.73±5.59	51.30±3.50
0 kGy	0.01±0.01	10.94±0.54	14.79±1.24	53.40±7.65	52.58±1.86
30 kGy	0.01±0.01	10.21±0.75	12.89±2.43	51.41±4.04	52.90±3.01

Group	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (K/μL)	MPV (fL)
Control	11.53±0.46^{A}	23.10±0.88^{A}	17.78±0.60	640.31±102.52	5.40±0.29^{B}
0 kGy	13.69±0.67^{B}	26.06±1.25^{B}	16.68±0.98	737.23±58.92	4.48±0.42^{A}
30 kGy	14.15±1.25^{B}	26.88±1.75^{B}	17.13±0.88	755.75±105.56	4.83±0.27^{A}

[1)]White blood cell (WBC), Neutrophil (NEU), Lymphocyte (LYM), Monocyte (MONO), Eosinophil (EOS), Basophil (BASO), Red blood cell (RBC), Hemoglobin (Hb), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width (RDW), Platelet (PLT), and Mean plasma volume (MPV).

[2)]Values are expressed as mean ± S.D (n=12).

[A-B]Means followed by different letters within a row are significantly different ($p < 0.05$).

Table 33. Serum biochemistry test of male ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Group	ALB[1)] (g/dL)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Control	2.91±0.60[2)]	183.32±94.87	126.21±10.6[B]	195.25±26.30
0 kGy	3.44±0.27	126.01±35.61	44.98±7.76[A]	165.22±20.39
30 kGy	3.49±0.38	126.38±31.24	46.37±10.62[A]	174.39±21.51

Group	BUN (mg/dL)	CRE (mg/dL)	GLU (mg/dL)	T-BIL (mg/dL)
Control	28.89±2.44	0.37±0.06	62.55±39.29	0.16±0.05
0 kGy	26.99±2.57	0.42±0.04	115.0±29.8	0.17±0.04
30 kGy	25.76±3.85	0.38±0.05	113.9±28.4	0.18±0.05

Group	T-CHO (mg/dL)	TG (mg/dL)	TP (g/dL)
Control	102.03±34.23	100.71±27.63	5.09±0.45
0 kGy	134.46±25.52	89.80±14.73	5.15±0.38
30 kGy	137.18±29.67	90.26±13.85	5.26±0.39

[1)]Albumin (ALB), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Total cholesterol (T-CHO), Creatinine (CRE), Glucose (GLU), Total bilirubin (T-BIL), Triglyceride (TG), and Total protein (TP).

[2)]Values are expressed as mean ± S.D. (n=12).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

Table 34. Serum biochemistry test of female ICR mice administered with X-ray-irradiated chicken meat at 0 and 30 kGy (2500 mg/ kg b.w./day) for 90 days

Group	ALB[1)] (g/dL)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Control	3.91±0.65[2)]	258.20±99.51[B]	50.17±23.73	149.74±42.50
0 kGy	3.36±0.30	168.42±40.73[A]	44.10±11.84	175.53±57.01
30 kGy	3.49±0.22	169.56±38.41[A]	46.77±11.72	183.09±51.27
Group	BUN (mg/dL)	CRE (mg/dL)	GLU (mg/dL)	T-BIL (mg/dL)
Control	17.38±2.68	0.45±0.02	108.67±17.6	0.10±0.01
0 kGy	19.62±4.35	0.41±0.03	118.83±17.56	0.11±0.03
30 kGy	18.96±3.63	0.42±0.04	112.63±15.41	0.11±0.02
Group	T-CHO (mg/dL)	TG (mg/dL)	TP (g/dL)	
Control	124.71±22.21[B]	109.58±33.89	5.33±0.51	
0 kGy	83.44±14.67[A]	90.82±18.31	4.74±0.34	
30 kGy	85.99±19.99[A]	79.27±18.02	4.78±0.18	

[1)]Albumin (ALB), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), Total cholesterol (T-CHO), Creatinine (CRE), Glucose (GLU), Total bilirubin (T-BIL), Triglyceride (TG), and Total protein (TP).

[2)]Values are expressed as mean ± S.D (n=12).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

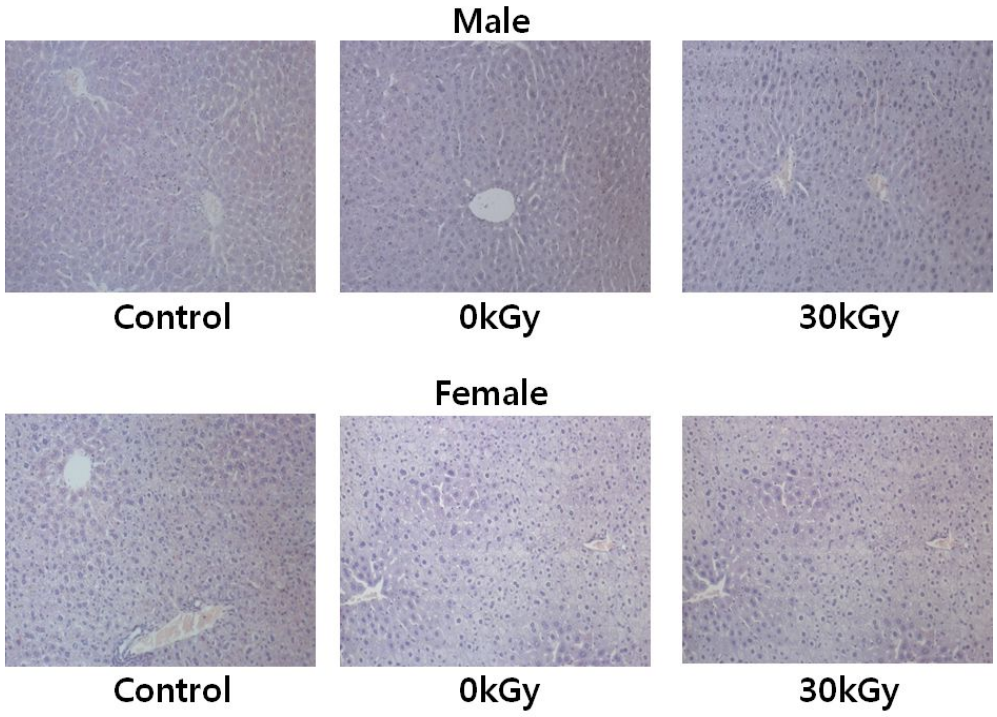


Fig. 3. Histopathological examination of the liver of ICR mice administered with X-ray-irradiated chicken meat at 30 kGy for 90 days, ×250.

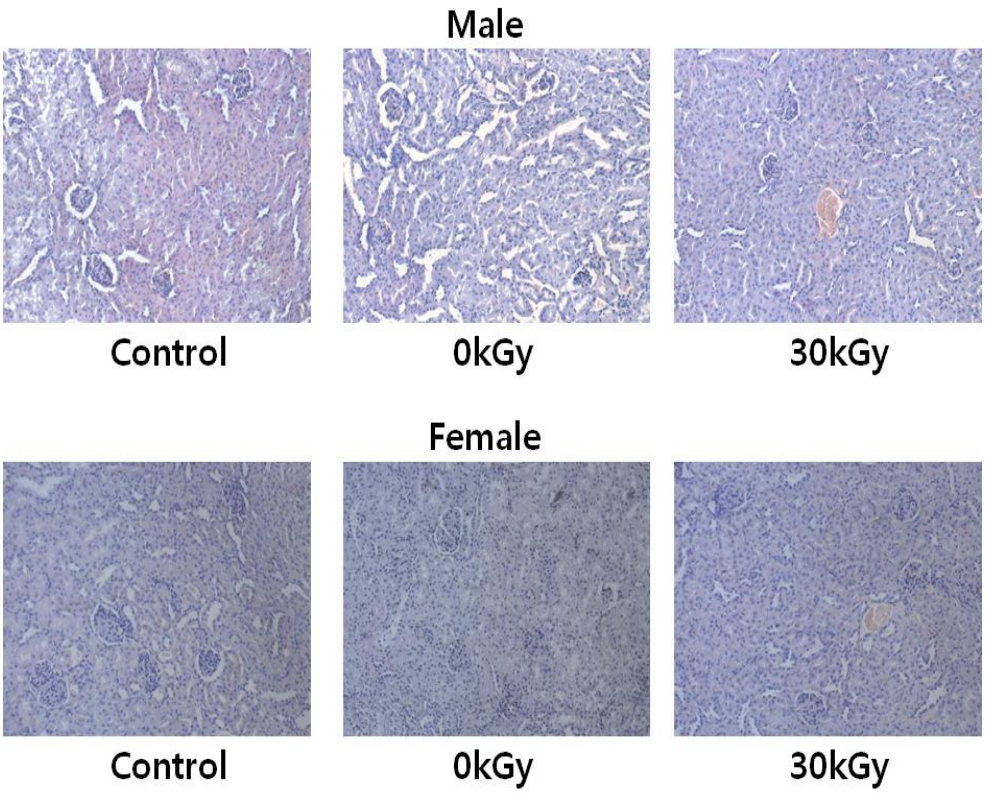


Fig. 4. Histopathological examination of the kidney of ICR mice administered with X-ray-irradiated chicken meat at 30 kGy for 90 days, ×250.

III-4. Discussion

In this study, genotoxicity, acute, and sub-chronic toxicity of X-ray-irradiated chicken meat at 30 kGy were evaluated. The sample was not found to be genotoxic since the bacterial reverse-mutagen, *in vitro* chromosomal aberration, and *in vivo* micronucleus assay exhibited dose-independent and similar responses to those of negative control. Acute and sub-chronic toxicity studies showed no significant changes in mortality and abnormal clinical signs. Several hematological and serum biochemical parameters showed dose-dependency or significant difference relative to the control group, but these changes were within normal range. No special toxic effect of X-ray-irradiated (30 kGy) chicken meat on male and female ICR mice was observed up to 2000 mg/kg b.w. of the single oral administration. Furthermore, 2500 mg/kg b.w. of daily intake of X-ray-irradiated (30 kGy) chicken meat for 90 days did not exhibit any toxicological effects on male and female mice. Moreover, the daily treatment dose (2500 mg/kg b.w.) of chicken meat powder irradiated with 7.5 MeV X-rays at 30 kGy to a mouse corresponds to 8300 mg of raw chicken meat, which is approximately 15 times higher than the average daily intake of chicken meat (530 mg/ kg b.w./day) consumed by Korean people (Ministry of Agriculture, Food and Rural Affairs, 2013). Our result is in agreement with those of previous toxicological studies, wherein no toxic effects of gamma-irradiated food at more than 25 kGy were observed, such as chicken-breast meat, ice-cream, and porridge, and *bulgogi* (Korean seasoned beef) (Jeon et al., 2013; Park et al., 2011; Yin et al., 2012; Zhu et al., 2012).

Many sub-chronic studies on safety have been conducted in various laboratory animals such as rat, mice, chickens, dogs, pigs, and monkeys. These studies examined the toxicological safety and nutritional adequacy of a variety of dietary items and complete laboratory diets treated with high-dose irradiation of > 10 kGy. The majority of these studies did not report toxic effects in laboratory animals after consumption of high-dose irradiated foods (Thayer, 1994). Additionally, no irradiation-related increases in tumors occurred in any of the studies that involved administering irradiated (> 10 kGy) food or diets containing the irradiated food to rodents (WHO, 1981). Chronic studies in dogs, conducted for 2–4 years, reported no adverse findings attributable to high-dose irradiated food such as fruit, vegetables, tuna, meat, and processed meat products (Blood et al., 1966). In a non-human primate study wherein high-dose irradiated peaches (27.9 and 55.8 kGy) were fed to rhesus monkeys for 2 years, no adverse findings were reported (WHO, 1999).

Based on the results on genotoxicity, acute toxicity and 90-days sub-chronic toxicity tests, 7.5 MeV X-ray-irradiated chicken breast meat at 30 kGy seems to be non-toxic to human.

Chapter IV. Physicochemical quality characteristics of irradiated food by 7.5 MeV X-ray

IV-1. Introduction

Food irradiation is conducted to improve the microbiological safety and extending the shelf-life of food exploiting the microbicidal effect of ionizing radiations including X-rays, gamma rays, and e-beams (Arvanitoyannis, 2013). The nucleic acid molecules may be ionized or excited by direct absorption of radiation energy, so initiating the chain of events that leads to biological change and to cell death if the change is serious enough. Alternatively, microorganisms can be damaged by radicals formed through the absorption of energy into other molecules such as water in the cell (Grecz et al., 1983).

In addition, major food components such as proteins, carbohydrates, and lipids can be also affected by ionizing radiation (Molins, 2001). Radiation loses energy through interaction with materials, while the materials receive energy, generating ionization and excitation in materials. When food is irradiated, the chemical changes may arise from the direct action of radiation on the carbohydrates, proteins, fats, and other compounds in the food, or by the indirect action of reactive intermediates formed by the radiolysis of water (Elias & Cohen, 1977). In terms of food quality, changes in major components such as carbohydrates, proteins, and

lipids may affect the organoleptic characteristics of the food. For example, the irradiation of sugar in an aqueous environment changes its optical rotation and often causes browning. Moreover, the degree of degradation in sugars in solution is proportional to the radiation dose, and can affect the sweet taste of the food following irradiation. Irradiation also leads to the degradation of polysaccharides such as starch, cellulose, and pectin, resulting in changes to texture and viscosity by complex mechanisms (Diehl et al., 1978). In addition, the production of hydrogen sulfide and methyl mercaptan, induced by the irradiation of sulfur-containing amino acids, can lead to off-odors and off-flavors (Simic, 1983). Furthermore, the irradiation may alter the viscosity of proteins, owing to the degradation and aggregation of proteins, while the production of radiolytic compounds from lipids can affect the flavor of food (Ahn et al., 2000; Fan & Sommers, 2013).

However, previous studies evaluating qualities of irradiated food have mostly involved in gamma rays and e-beams, and quality evaluation of X-ray-irradiated food has been scarcely reported. Therefore, this study was conducted to evaluate quality characteristics of model foods (red pepper powder, chicken breast meat, and ground beef) irradiated with 7.5 MeV X-rays.

IV-2. Materials and Methods

IV-2-1. Preparation of samples

Red pepper powders, chicken breast meat, and ground beef (2.38 mm grind) were purchased from a local market in Jeongeup, Korea. The samples (100 g) were immediately placed in sterilized oxygen-impermeable nylon/polyethylene bags (20 × 30 cm, thickness: 0.07 mm; Sunkyung Co. Ltd., Seoul, Korea) and packaged to a thickness of 3.0 cm to minimize the variation in penetration depth among radiation sources. The packaged samples were stored overnight in a refrigerator (4°C) until irradiation.

IV-2-2. Irradiation of samples

The packaged samples were irradiated with a nominal dose of 0 (control), 2, 4, 6, 8, or 10 kGy using one of the ionizing radiations. 7.5 MeV X-ray and e-beam irradiations were conducted using a linear electron accelerator, which delivers e-beams with a well-defined energy in the range of 5–10 MeV, at EB-Tech Co. (Daejeon, Korea) at an ambient temperature of approximately 22°C. X-rays were produced using 7.5 MeV e-beams, whereas e-beams at 10 MeV were employed for e-beam irradiation. Gamma irradiation was performed using a ^{60}Co gamma irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 8.8 PBq and the photon energies of gamma

rays from ^{60}Co were 1.17 and 1.33 MeV. The applied dose rates were 24 kGy/h for X-ray irradiation, 8 MGy/h for e-beam irradiation, and 1 kGy/h for gamma irradiation. Geometrically, all samples were irradiated in a perpendicular direction to the incident radiation. The irradiated samples were immediately stored at 4°C.

IV-2-3. Dosimetry

The actual dose absorbed by each sample and dose distribution across the samples were determined by using a 5 mm-diameter alanine dosimeter (Batch No.: T020604, Bruker BioSpin GmbH, Rheinstetten, Germany). For dosimetry of model food samples, a dosimeter was packaged in a polyethylene bag (15 × 15 mm) to prevent absorbing moisture, and 3 dosimeters were attached to the top of a sample for each irradiation dose. The samples with dosimeters were also stored in a refrigerator until irradiation.

After irradiation, the dosimeters separated from samples were stored overnight at room temperature of approximately 25°C and measured within 24–48 h after irradiation using an electron paramagnetic resonance analyzer (e-scan^{TM} alanine dosimeter reader, Bruker BioSpin GmbH, Rheinstetten, Germany) (ISO/ASTM 51607:2004). The alanine dosimeter reader was calibrated using reference standard dosimeters provided from National Physics Laboratory, UK, according to an international standard on 30th April, 2015 (ISO/ASTM 51261, 2002). In this study, absorbed dose of sample was expressed as a mean value ± the expended uncertainty with a 95% level of confidence.

IV-2-4. Hunter's color values

To quantify the color of samples in terms of Hunter's *L** (lightness), *a** (redness), and *b** (yellowness), each sample was placed in a petri dish (5 cm in diameter) and measured using a spectrophotometer (Konica Minolta CM-5, Tokyo, Japan). For each treatment, five measurements along the equatorial area of ten samples were obtained. The color difference (ΔE^*_{ab}) was calculated using the following equation:

$$\Delta E^*_{ab} = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

IV-2-5. Analysis of capsaicinoids and capsanthin

Irradiated red pepper powder (1 g) was mixed with 10 mL of methanol in a vial with a screw-cap and extracted in a shaking incubator (180 rpm) at 30°C for 4 h. After centrifugation (1000 × *g*, 10 min, 4°C), the extracts were filtered through a 0.45-mm syringe filter, and an aliquot (10 μL) of the filtrate was injected directly into the high-performance liquid chromatography system (HPLC). The HPLC system consisted of a dual pump and a UV detector set at 280 nm (Agilent Technologies 1200 series, Santa, Clara, CA, USA). The column was Nova-Pak C18 (4 mm, 150 × 3.9 mm inner diameter; Waters, Milford, MA, USA). The isocratic mobile phase was a mixture of methanol/water (60:40 v/v) with a flow rate of 0.8 mL/min.

For capsanthin analysis, red pepper powder (30 mg) was extracted with 4 mL of

diethyl ether/methanol (50:50, v/v) in a shaking incubator (180 rpm) at 30°C for 4 h until colorless extracts were obtained. After centrifugation (1000 × *g*, 4°C) for 10 min, the supernatants were treated with 20% KOH in methanol for 1 h at room temperature for saponification. One milliliter of 10% NaCl was added to separate the phases. The layer of diethyl ether was passed through anhydrous sodium sulfate and dried completely under a stream of nitrogen. The residue was dissolved in 1 mL of acetone, filtered through a 0.45-mm syringe filter, and injected (10 mL) into the HPLC system coupled with a UV detector with a wavelength set at 450 nm. A Nova-Pak C18 was used and acetonitrile/2-propanol/ethyl acetate (80:10:10, v/v) was used at a flow rate of 0.8 mL/min. As a standard compound, the synthetic chemicals capsaicin, dihydrocapsaicin, and capsanthin were purchased from Sigma Aldrich (St. Louis, MO, USA).

IV-2-6. pH and TBARS analysis

For pH determination, meat (10 g) was homogenized with chilled distilled water (50 mL) and measured with a digital pH meter (Thermo Scientific Co., Pittsburgh, PA).

Lipid oxidation was assessed using the TBA method as described by Tarladgis et al. (1960) with minor modifications. A 10-g sample was blended with 50 mL distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 mL of distilled water, which was added to the same distillation flask containing 2.5 mL 4N HCl and a few drops of an

antifoam agent (KMK-73, Shin-Etsu Silicone Co., Ltd., Korea). The mixture was distilled and 50 mL distillate was collected. Five milliliters of 0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA-reagent) was added to test tube containing 5 mL of the distillate and mixed well. The tubes were capped and heated in a boiling water bath for 30 min to develop chromogen and cooled to room temperature. Absorbance was measured at 538 nm against a blank prepared with 5 mL distilled water and 5 mL TBA-reagent using a UV/vis spectrophotometer (Optizen 2120 UV plus, Mecasys Co. Ltd., Korea). TBA values were calculated as mg MDA/kg meat.

IV-2-7. Sensory evaluation

Sensory evaluation was carried out for each sample immediately after irradiation with X-rays, gamma-rays, or e-beams by ten trained panelists including members of the Team for Radiation Food Science and Biotechnology at the Atomic Energy Research Institute. Both irradiated (2, 4, 6, 8, and 10 kGy) and non-irradiated samples were provided to panelists along with an explanation of the purpose of irradiation. According to the method described by Civille & Szczesniak (1973), a sensory test was administered to panelists using a 7-point scale, where “7” indicates that the panelist extremely liked the sample and “1” indicates that the panelist extremely disliked the sample. Off-flavor was evaluated on a scale from 1 to 7, where 7 indicates very strong and 1 indicates no off-flavor. The samples were placed on a white plastic dish and labeled randomly with three-digit numerical

codes.

IV-2-8. Statistical analysis

Data were statistically analyzed using IBM® SPSS Statistics 21 software for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance and Duncan's multiple range tests at $p < 0.05$ were used to compare differences among mean values.

IV-3. Results

IV-3-1. Quality characteristics of irradiated red pepper powder by 7.5 MeV X-ray

Color measurement of red pepper powder irradiated by 7.5 MeV X-rays at various doses was shown in Table 35. Lightness, redness, and yellowness of samples irradiated at more than 2 kGy did not show significant difference from those of non-irradiated control sample. Moreover, the ΔE^* values indicating the differences of irradiated sample at a given dose in color with non-irradiated sample were less than 1.2. According to the evaluation classification suggested by Young & Whittle (1985), the result was interpreted as all irradiated samples showed a slight difference in color from that of non-irradiated sample, because ΔE^* value from 0.5 to 1.5 represented a slight difference between two samples.

The concentration of capsaicinoids such as capsaicin and dihydrocapsaicin and capsanthin, which show the largest contribution to pungency and color of red pepper powder, of irradiated red pepper powder by 7.5 MeV X-rays at various doses is shown in Table 36. The concentration of capsaicinoids in the samples irradiated by 7.5 MeV X-rays ranged from 7.03 to 7.70 μg of capsaicin and 1.73 to 1.82 μg of dihydrocapsaicin per 100 mg of sample. However, no significant difference in contents of capsaicin and dihydrocapsaicin was observed between irradiated samples and non-irradiated sample. Furthermore, the concentration of capsanthin ranged from 5.23 to 6.30 μg/100 mg. and there were no significant differences in

individual capsanthin contents of red pepper powder after irradiation, regardless of the absorbed dose ($p < 0.05$)

Organoleptic evaluation in terms of intensity of pungent flavor and off-odor and preference to color and overall acceptance of red pepper powder is shown in Table 37. Most panelists could not distinguish the pungent odor between the non-irradiated and irradiated samples. Moreover, no differences in the preference to color and overall acceptance of red pepper powder irradiated with 7.5 MeV X-rays up to 10 kGy were observed. However, the intensities of off-odor in X-ray-irradiated samples at more than 8 kGy were significantly higher than those of non-irradiated samples ($p < 0.05$). In comparison of the off-odor intensity, no significant difference among the samples irradiated with various irradiation sources at 6 kGy was observed (Fig. 5).

Table 35. Color measurement of red pepper powder irradiated by 7.5 MeV X-rays at various doses

Absorbed dose (kGy)	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)	Difference index (ΔE^*)
0	45.3 ± 0.2[NS]	17.3 ± 1.6[NS]	10.1 ± 0.4[NS]	
2.35 ± 0.11	45.3 ± 0.2	17.7 ± 0.7	10. 3± 0.6	0.8[1)]
4.52 ± 0.29	45.7 ± 0.1	18.2 ± 0.1	10.7 ± 0.2	0.6
6.03 ± 0.27	45.4 ± 0.3	17.6 ± 0.9	10.3 ± 0.6	1.2
8.16 ± 0.36	45.3 ± 0.4	17.7 ± 0.3	10.4 ± 0.2	0.9
10.27 ± 0.54	45.5 ± 0.1	18.1 ± 0.2	10.7 ± 0.2	0.6

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

[1)]The difference index of color from non-irradiated sample is calculated from the square root of the sum of $(\Delta L^*)^2$, $(\Delta a^*)^2$, and $(\Delta b^*)^2$.

Table 36. Contents of capsaicinoids and capsanthin in red pepper powder irradiated by 7.5 MeV X-rays at various doses

Absorbed dose (kGy)	Capsaicin (μg/100 mg)	Dihydrocapsaicin (μg/100 mg)	Capsanthin (μg/100 mg)
0	7.03 ± 0.12^{NS}	3.40 ± 0.10^{NS}	6.00 ± 0.72^{NS}
2.35 ± 0.11	7.17 ± 0.06	3.27 ± 0.06	6.20 ± 0.66
4.52 ± 0.29	7.17 ± 0.21	3.30 ± 0.10	6.30 ± 0.90
6.03 ± 0.27	7.70 ± 0.36	3.50 ± 0.17	6.00 ± 0.75
8.16 ± 0.36	7.63 ± 0.15	3.50 ± 0.10	5.23 ± 0.38
10.27 ± 0.54	7.57 ± 0.06	3.43 ± 0.06	5.47 ± 0.40

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

^{NS}No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

Table 37. Organoleptic evaluation of red pepper powder irradiated by 7.5 MeV X-rays at various doses

Absorbed dose (kGy)	Pungent flavor[1)]	Off-odor	Color	Overall acceptance
0	6.0 ± 0.4^{NS}	1.6 ± 0.3^{A}	6.1 ± 0.5^{NS}	6.0 ± 0.4^{NS}
2.35 ± 0.11	6.0 ± 0.6	2.0 ± 0.3^{AB}	6.0 ± 0.3	6.0 ± 0.6
4.52 ± 0.29	5.8 ± 0.8	2.4 ± 0.4^{ABC}	6.2 ± 0.4	5.7 ± 0.7
6.03 ± 0.27	5.6 ± 0.5	2.3 ± 0.4^{AB}	6.2 ± 0.7	5.7 ± 0.7
8.16 ± 0.36	5.7 ± 0.4	2.9 ± 0.3^{B}	6.3 ± 0.8	5.5 ± 0.5
10.27 ± 0.54	5.9 ± 0.9	3.1 ± 0.5^{C}	6.1 ± 0.4	5.0 ± 1.0

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)] The intensities of pungent flavor and off-odor were evaluated on a scale from 1 to 7, where "1" indicates no off-flavor and "7" indicates very strong, and the preferences for color and overall acceptance were evaluated using a 7-point scale, where "7" means the panelist liked the sample extremely and "1" means the panelist disliked the sample extremely.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

[A-C]Values followed by the different letters within a column are significantly ($p < 0.05$).

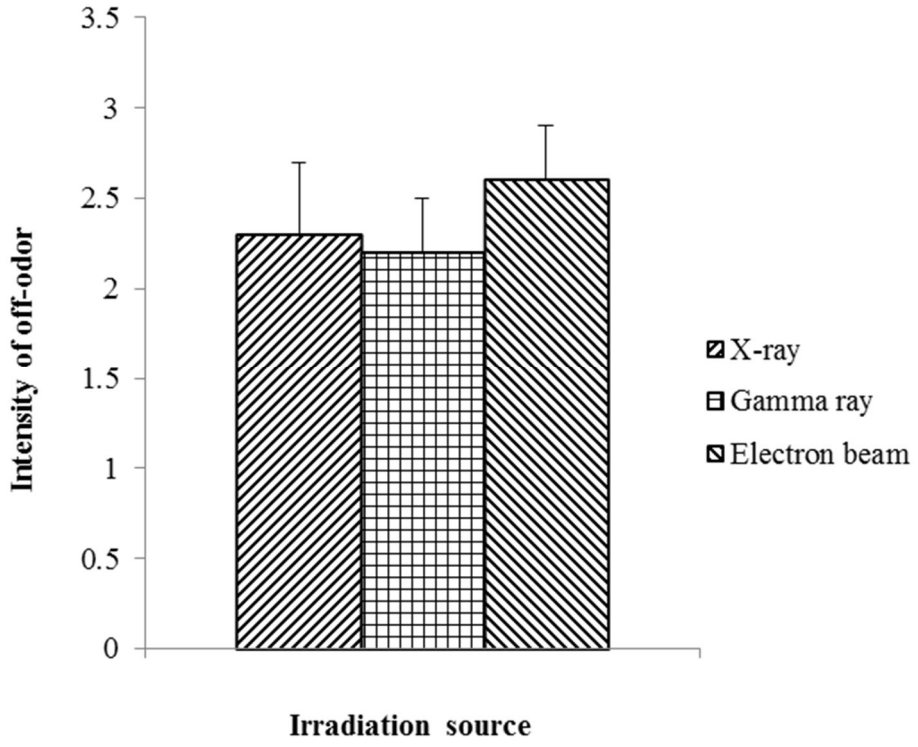


Fig. 5. Organoleptic comparison in terms of off-odor intensities of red pepper powder irradiated by 7.5 MeV X-rays, gamma rays, or 10 MeV e-beams at 6 kGy. The intensities of off-odor were evaluated on a scale from 1 to 7, where "1" indicates no off-flavor and "7" indicates very strong.

IV-3-2. Quality characteristics of irradiated chicken breast meat by 7.5 MeV X-ray

The physicochemical characteristics of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days are shown in Tables 38–42. No significant differences in the pH of chicken meat were observed between non-irradiated and irradiated samples during the storage periods ($p < 0.05$). The TBARS value of X-ray-irradiated chicken breast meat increased proportionally to an absorbed dose and the values of samples X-ray-irradiated at more than 2 kGy were significantly higher than that of non-irradiated sample.

The L^* value (lightness) and a^* value (redness) of all the irradiated chicken meat increased with the increased absorbed dose, and redness of all samples irradiated with 7.5 MeV X-rays were significantly higher than those of non-irradiated samples. Moreover, the redness was sustained during storage at 30°C for 7 days. In addition, there was no significant difference in the b^* (yellowness) values of irradiated samples from those of non-irradiated samples during the storage period.

Moreover, there was no significant difference in quality characteristics such as lipid oxidation and color among the samples irradiated with various ionizing radiations at 2 kGy (Figs. 6 and 7).

Table 38. pH of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	pH		
	0 day	3 day	7 day
0	5.93 ± 0.21[NS]	–[1)]	–
2.28 ± 0.09	5.85 ± 0.15	–	–
4.37 ± 0.11	5.79 ± 0.11	5.79 ± 0.14[NS]	–
6.28 ± 0.22	5.69 ± 0.14	5.75 ± 0.15	–
7.96 ± 0.32	5.71 ± 0.16	5.64 ± 0.11	5.76 ± 0.19[NS]
10.14 ± 0.49	5.72 ± 0.11	5.65 ± 0.19	5.78 ± 0.23

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

Table 39. TBARS values of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	TBARS value (mg of malondialdehyde/kg)		
	0 day	3 day	7 day
0	0.17 ± 0.02^{A}	–[1)]	–
2.28 ± 0.09	0.28 ± 0.02^{B}	–	–
4.37 ± 0.11	0.37 ± 0.02^{C}	0.34 ± 0.01^{A}	–
6.28 ± 0.22	0.43 ± 0.04^{CD}	0.46 ± 0.05^{B}	–
7.96 ± 0.32	0.46 ± 0.02^{D}	0.45 ± 0.04^{B}	0.46 ± 0.02^{NS}
10.14 ± 0.49	0.42 ± 0.02^{D}	0.46 ± 0.03^{B}	0.47 ± 0.01

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[1)]Bar indicates no examination due to the spoilage of sample.

[A-D]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

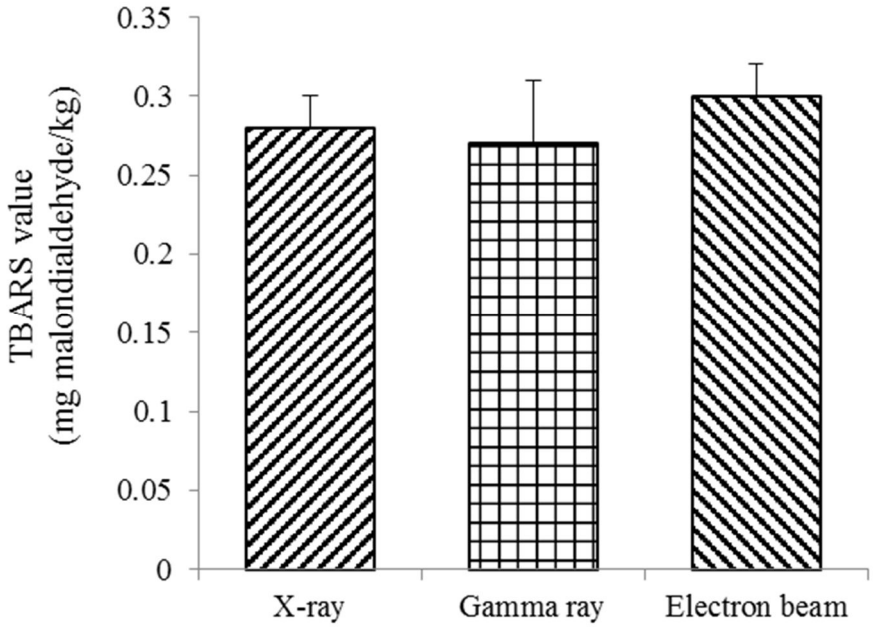


Fig. 6. Comparison in TBARS value of chicken breast meat irradiated by 7.5 MeV X-rays, gamma rays, or 10 MeV e-beams at 2 kGy.

Table 40. Lightness (L^* value) of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	L^* value		
	0 day	3 day	7 day
0	59.81 ± 0.53^{A}	–[1)]	–
2.28 ± 0.09	61.03 ± 0.34^{B}	–	–
4.37 ± 0.11	62.04 ± 0.59^{C}	61.34 ± 0.85^{NS}	–
6.28 ± 0.22	62.36 ± 0.67^{C}	61.73 ± 0.67	–
7.96 ± 0.32	61.74 ± 0.65^{BC}	60.76 ± 0.82	60.85 ± 0.87^{NS}
10.14 ± 0.49	62.16 ± 0.48^{C}	61.84 ± 0.76	61.97 ± 0.58

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[A-C]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

Table 41. Redness (a^* value) of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	a^* value		
	0 day	3 day	7 day
0	4.03 ± 0.63^{A}	–[1)]	–
2.28 ± 0.09	6.00 ± 0.49^{B}	–	–
4.37 ± 0.11	6.44 ± 0.56^{BC}	5.86 ± 0.65^{NS}	–
6.28 ± 0.22	6.33 ± 0.85^{BC}	6.12 ± 0.89	–
7.96 ± 0.32	6.89 ± 0.33^{C}	5.64 ± 0.74	5.79 ± 0.66^{NS}
10.14 ± 0.49	6.55 ± 0.72^{BC}	5.48 ± 0.68	5.67 ± 0.53

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[A-C]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

Table 42. Yellowness (b^* value) of chicken breast meat irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	b^* value		
	0 day	3 day	7 day
0	14.60 ± 0.74[NS]	–[1)]	–
2.28 ± 0.09	13.36 ± 0.89	–	–
4.37 ± 0.11	14.19 ± 0.74	13.93 ± 0.79[NS]	–
6.28 ± 0.22	13.94 ± 0.49	14.43 ± 0.75	–
7.96 ± 0.32	13.26 ± 0.84	13.23 ± 0.84	13.48 ± 0.37[NS]
10.14 ± 0.49	13.59 ± 0.65	13.50 ± 0.87	13.36 ± 0.82

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column by Duncan's multiple range test ($p < 0.05$).

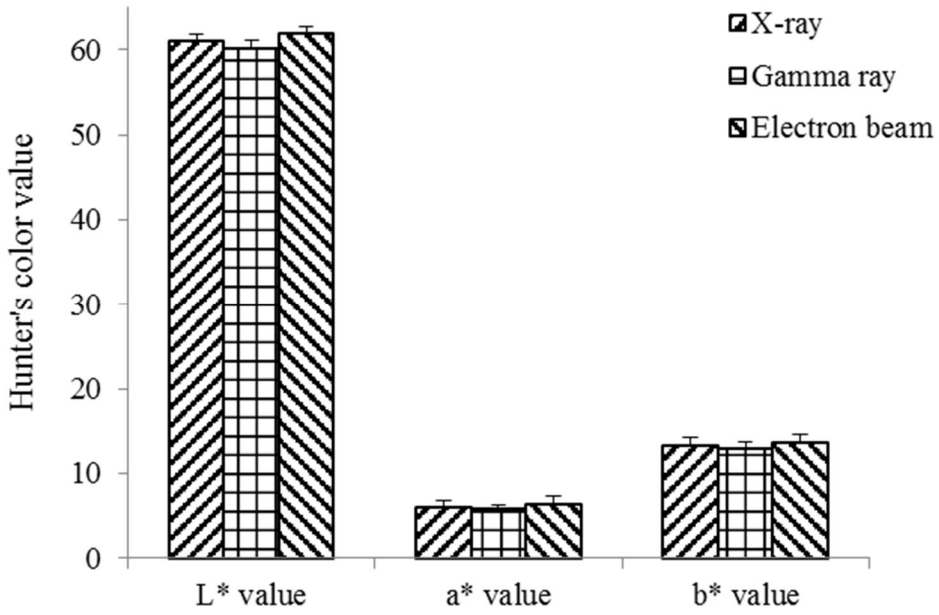


Fig. 7. Comparison in color of chicken breast meat irradiated by 7.5 MeV X-rays, gamma rays, or 10 MeV e-beams at 2 kGy.

IV-3-3. Quality characteristics of irradiated ground beef by 7.5 MeV X-ray

The physicochemical characteristics of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days are shown in Tables 43–47. No significant differences in the pH of ground beef were observed between non-irradiated and irradiated samples during the storage periods ($p < 0.05$). The TBARS value of X-ray-irradiated ground beef increased significantly when the samples were irradiated at more than 4 kGy; however, there was no significant change in the TBARS value during the storage at 30°C for 7 days ($p < 0.05$).

The L^* value (lightness), a^* value (redness), and b^* value (yellowness) of all the irradiated ground beef up to 10 kGy exhibited no significant difference from those of non-irradiated samples during the storage period ($p < 0.05$).

Table 43. pH of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	pH		
	0 day	3 day	7 day
0	5.97 ± 0.15[NS]	–[1)]	–
2.27 ± 0.10	5.76 ± 0.23	–	–
4.15 ± 0.21	5.83 ± 0.26	–	–
6.21 ± 0.37	5.82 ± 0.17	5.84 ± 0.19[NS]	–
8.32 ± 0.22	5.95 ± 0.28	5.97 ± 0.28	5.83 ± 0.25[NS]
10.09 ± 0.63	5.83 ± 0.11	5.91 ± 0.17	5.78 ± 0.34

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

Table 44. TBARS values of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	TBARS value (mg of malondialdehyde/kg)		
	0 day	3 day	7 day
0	1.36 ± 0.11^{A}	–[1)]	–
2.27 ± 0.10	1.42 ± 0.11^{AB}	–	–
4.15 ± 0.21	1.87 ± 0.10^{B}	–	–
6.21 ± 0.37	1.88 ± 0.09^{B}	1.96 ± 0.09^{NS}	–
8.32 ± 0.22	1.85 ± 0.08^{B}	1.98 ± 0.11	1.93 ± 0.09^{NS}
10.09 ± 0.63	1.84 ± 0.13^{B}	1.95 ± 0.09	2.07 ± 0.08

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[1)]Bar indicates no examination due to the spoilage of sample.

[A-B]Values followed by the different letters within a column are significantly different by Duncan's multiple range test ($p < 0.05$).

[NS]No significant difference within a column ($p < 0.05$).

Table 45. Lightness (L^* value) of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	L^* value		
	0 day	3 day	7 day
0	44.88 ± 0.79[NS]	–[1)]	–
2.27 ± 0.10	44.68 ± 0.83	–	–
4.15 ± 0.21	45.69 ± 0.94	–	–
6.21 ± 0.37	45.08 ± 0.68	43.89 ± 0.65[NS]	–
8.32 ± 0.22	44.81 ± 0.92	43.84 ± 0.58	43.96 ± 0.79[NS]
10.09 ± 0.63	44.24 ± 0.89	43.49 ± 0.83	45.82 ± 0.87

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

Table 46. Redness (a^* value) of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	a^* value		
	0 day	3 day	7 day
0	8.72 ± 1.13[NS]	–[1)]	–
2.27 ± 0.10	9.48 ± 0.86	–	–
4.15 ± 0.21	8.35 ± 0.94	–	–
6.21 ± 0.37	8.90 ± 0.81	9.14 ± 0.58[NS]	–
8.32 ± 0.22	9.05 ± 0.97	10.31 ± 0.97	10.49 ± 0.64[NS]
10.09 ± 0.63	9.53 ± 1.21	10.58 ± 0.83	10.95 ± 0.87

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

Table 47. Yellowness (b^* value) of ground beef irradiated by 7.5 MeV X-rays at various doses during storage at 30°C for 7 days

Absorbed dose (kGy)	b^* value		
	0 day	3 day	7 day
0	10.31 ± 0.75[NS]	–[1)]	–
2.27 ± 0.10	10.36 ± 0.83	–	–
4.15 ± 0.21	9.95 ± 0.66	–	–
6.21 ± 0.37	9.86 ± 0.73	9.53 ± 0.82[NS]	–
8.32 ± 0.22	9.90 ± 0.82	9.57 ± 0.93	9.69 ± 0.64[NS]
10.09 ± 0.63	9.73 ± 0.74	9.53 ± 0.44	9.62 ± 0.39

Absorbed dose are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=10).

[1)]Bar indicates no examination due to the spoilage of sample.

[NS]No significant difference within a column and row by Duncan's multiple range test ($p < 0.05$).

IV-4. Discussion

The levels of capsanthin and capsaicinoids in red pepper powder, which are related to redness and pungency, were not affected by irradiation at doses of up to 10 kGy. It has been reported that capsaicinoids are relatively stable under gamma and e-beam irradiation for doses of up to 15 kGy (Byun et al., 1996; Lee et al., 2000). Lee et al., (2004) reported that gamma irradiation did not affect the amount of capsanthin or the redness of red pepper powder. Color stability of red pepper powder against radiation exposure did not differ with respect to irradiation dose or radiation source. In the organoleptic evaluation of red pepper powder by trained panelists, no organoleptic parameters except for off-odor showed differences from non-irradiated red pepper powder samples, regardless of the absorbed dose. There was no significant difference in off-odor among the samples irradiated with various irradiation sources at 6 kGy. Generally, it has been recognized that the development of radiolytic off-odor are greatly associated with the acceleration of lipid oxidation (Champagne & Nawar, 1969) and production of sulfur volatile compounds, such as methyl mercaptan, sulfur dioxide and dimethyl trisulfide (Jo & Ahn, 2000; Patterson & Stevenson, 1995; Shahidi et al., 1987). Kim et al. (2005) reported that hexanal, which is the major component associated with lipid oxidation, and dimethyl disulfide in gamma-irradiated red pepper powder significantly increased as a function of absorbed dose.

The increased TBARS value of X-ray-irradiated chicken breast meat and ground beef upon the increased absorbed dose was consistent with previous results of

irradiated meat (Ahn & Nam, 2004). Lipid oxidation of irradiated meat and meat products is generated from the formation of free radicals from water within meat, primarily hydroxyl radicals (Thakur & Singh, 1994). Moreover, the redness of X-ray-irradiated chicken breast meat was significantly higher than non-irradiated samples. Ginger et al. (1955) revealed that ionizing radiation caused the formation of a pink color in metmyoglobin extracts and the formation of metmyoglobin in oxymyoglobin extracts. Bagorogoza et al. (2001) reported that the increased redness of chicken breast meat was due to the production of carboxymyoglobin in the meat during irradiation, which has been reported to be responsible for the development of pink color in poultry meat (Lyon and Lyon, 1989). Moreover, it is clear that the meat figment formed on irradiation is unstable in air because the exterior goes brown while the interior remains pink (Millar et al., 2000). However, these changes in quality of meat were resulted from the change of components in food induced by irradiation depends on the irradiation dose but irradiation sources.

In conclusion, 7.5 MeV X-ray irradiation caused no significant difference in quality compared to gamma and e-beam irradiation, and X-ray-irradiated food at a maximum dose permitted by a national legislation did not exhibited significant difference in quality characteristics from those of non-irradiated samples. This indicates that X-ray irradiation, which is preferred by consumers, can be used an alternative to gamma irradiation using radioisotopes, because there is no significant difference in quality of food irradiated with various ionizing radiations (Cember & Johnson, 2009).

Chapter V. Identification characteristics of irradiated food by 7.5 MeV X-ray

V-1. Introduction

To identify irradiated red pepper powder, photostimulated luminescence (PSL) and thermoluminescence (TL) methods described in the European standards EN 13751:2002 and EN 1788:2001 are used internationally as screening and reference methods, respectively (Bayram & Delincee, 2004; Chauhan et al., 2009; Sanderson et al., 1989). However, both of the standards were established solely through interlaboratory studies on gamma-irradiated foods followed by re-irradiation with gamma rays at 1 kGy. Therefore, the acceptability of the TL method for the detection of food samples irradiated with X-rays or e-beams needs to be confirmed. Moreover, the silicate minerals separated from food sample should be re-irradiated at a defined dose after the TL measurement (TL_1), because the content of compounds in the silicate minerals affecting the TL intensity is immeasurable. This re-irradiation step is called “normalization” and the TL intensity of the normalized minerals is known as the second TL intensity (TL_2). In all the reference studies used in EN 1788:2001, the mineral samples are normalized with gamma rays at 1 kGy except for potatoes, resulting in a TL ratio (TL_1/TL_2) criterion of > 0.1 for positive identification of

irradiation treatment. However, the use of e-beam and X-ray irradiation for normalization is not mentioned in the standard.

In addition, the detection methods of them using gas chromatography (GC) is suitable for irradiated food containing lipids such as meats, because specific radiolytic hydrocarbons and 2-alkylcyclobutanones are found in the highest concentrations and are absent or present at a low level in non-irradiated samples (Nawar et al., 1990; Morehouse & Ku, 1991; Stevenson et al., 1993). Numerous studies to verify the reliability of various detection methods for irradiated food have been mainly performed to gamma- and e-beam-irradiated foods, because both ionizing irradiations are available in commercial use of food sterilization. However, the detection properties of food irradiated with 7.5 MeV X-rays have been scarcely reported.

In this study, the detection properties of red pepper powder, chicken breast meat, and ground beef irradiated with 7.5 MeV at various doses were evaluated by using PSL, TL, and GC analyses.

V-2. Materials and Methods

V-2-1. Preparation of samples

Red pepper powders, chicken breast meat, and ground beef (2.38 mm grind) were purchased from a local market in Jeongeup, Korea. The samples (100 g) were immediately placed in sterilized oxygen-impermeable nylon/polyethylene bags (20 × 30 cm, thickness: 0.07 mm; Sunkyung Co. Ltd., Seoul, Korea) and packaged to a thickness of 3.0 cm to minimize the variation in penetration depth among radiation sources. Three packages of the sample were prepared for each irradiation dose of a designated radiation type.

Pure quartz and K-feldspar powders (purity of 99.9%) were provided by Risø National Laboratory, Technical University of Denmark (Roskilde, Denmark). Each mineral powder sample was sieved using a nylon cloth with pore size of 125 μm. Aliquots of 2.0 mg were placed on aluminum discs (diameter: 6mm) and fixed with silicon. Five discs of each pure mineral sample were stuck in a disposable petri dish with a diameter of 90 mm using a double-sided tape and irradiated at a designated dose with different types of radiation.

V-2-2. Irradiation of samples

The packaged samples were irradiated with a nominal dose of 0 (control), 2, 4, 6, 8, or 10 kGy using one of the ionizing radiations, whereas the pure quartz and K-feldspar powders were irradiated at different doses ranging from 0.2 to 1.0 kGy

using one of the three designated radiation types. 7.5 MeV X-ray and e-beam irradiations were conducted using a linear electron accelerator, which delivers e-beams with a well-defined energy in the range of 5–10 MeV, at EB-Tech Co. (Daejeon, Korea) at an ambient temperature of approximately 22°C. X-rays were produced using 7.5 MeV e-beams, whereas e-beams at 10 MeV were employed for e-beam irradiation. Gamma irradiation was performed using a ^{60}Co gamma irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 8.8 PBq and the photon energies of gamma rays from ^{60}Co were 1.17 and 1.33 MeV. The applied dose rates were 24 kGy/h for X-ray irradiation, 8 MGy/h for e-beam irradiation, and 1 kGy/h for gamma irradiation. Geometrically, all samples were irradiated in a perpendicular direction to the incident radiation. The irradiated samples were immediately stored at 4°C.

V-2-3. Dosimetry

The actual dose absorbed by each sample was determined by using a 5 mm-diameter alanine dosimeter (Bruker Instruments, Rheinstetten, Germany). The dosimeters were attached to the top and bottom in the middle of the package bag containing red pepper powder and two dosimeters were placed next to the discs in the disposable petri dish to measure an absorbed dose of mineral samples. After irradiation, the dosimeters were measured using an Electron Paramagnetic Resonance analyzer (EMS-104, Bruker Instruments Inc., Billerica, MA), which was

calibrated against an International Standard set by the International Atomic Energy Agency (Vienna, Austria). The average dose of the duplicates in a given dose was used as an absorbed dose for each sample.

After irradiation, the dosimeters separated from samples were stored overnight at room temperature of approximately 25°C and measured within 24–48 h after irradiation using an electron paramagnetic resonance analyzer (e-scan^{TM} alanine dosimeter reader, Bruker BioSpin GmbH, Rheinstetten, Germany) (ISO/ASTM 51607:2004). The alanine dosimeter reader was calibrated using reference standard dosimeters provided from National Physics Laboratory, UK, according to an international standard on 30th April, 2015 (ISO/ASTM 51261, 2002). In this study, absorbed dose of sample was expressed as a mean value ± the expended uncertainty with a 95% level of confidence.

V-2-4. Photostimulated luminescence analysis

PSL photon counts (PCs) of the non-irradiated control and irradiated red pepper powder samples were measured 24 h after irradiation, as described by EN 13751 (CEN, 2009) using a SURRC Pulsed PSL system (Scottish Universities Research and Reactor Centre, Glasgow, UK), which comprised of a control unit, sample chamber, and detector. Briefly, 5 g of sample (less than 50 mesh) was put in a disposable petri dish (Bibby Sterilin type 122, Glasgow, UK) with a diameter of 50 mm. All the preparation, handling, measurement and storage operations of the samples were carried out under subdued ambient light. The PSL PCs were recorded

in the measuring mode and were expressed as PCs/60 s. The accumulated PCs were interpreted with respect to two thresholds, namely the lower threshold (T_1 = 700 counts/60 s) and upper threshold (T_2 = 5000 counts/60 s). Samples exhibiting a signal less than T_1 were classified as non-irradiated samples, whereas those with a signal greater than T_2 were deemed to be irradiated samples. Samples with signal levels between the two thresholds were classified as intermediate samples requiring further investigation.

V-2-5. Thermoluminescence analysis

The TL signal intensities of the quartz, K-feldspar, and mineral samples separated from red pepper powder irradiated with three different types of radiation were analyzed by a TLD system instrument (Harshaw TLD-4500, Dreieich, Germany) under nitrogen flush (99.99%). For separating the minerals from red pepper powder, about 100 g of samples were used to collect an aliquot of silicates following the procedure prescribed in the CEN Standard EN 1788 (CEN, 2001). The isolated minerals were carefully deposited on clean aluminum discs (diameter: 6 mm) and kept overnight at 50°C in a laboratory oven. The discs were then heated from 50 to 350°C at a scan rate of 5°C/s. After the first glow (TL_1) measurement, the samples were annealed at 350°C for 5 s to completely remove the TL characteristics. To normalize the TL response, the second glow (TL_2) was obtained from the TL_1 tested minerals that were re-irradiated with e-beams, gamma rays, and low or high-energy X-rays at a dose of 1 kGy. Re-irradiation of the discs with e-beams, gamma rays,

and high-energy X-rays was performed under the identical condition with the first irradiation, whereas low-energy X-ray irradiation was carried out using a cabinet type X-ray machine (CP-160, Faxitron X-ray LLC, Lincolnhire, IL, USA) operating at 160 kV and 10 mA with a dose rate of 0.4 kGy/h.

The first and second glow curves (TL_1 and TL_2) were then compared. To verify the reliability of the detection results from TL_1, the TL ratios (integrated intensity of TL_1/TL_2 between 150 and 250°C) were calculated for both the irradiated and non-irradiated samples, with the threshold value set at less than 0.1 for non-irradiated samples and more than 0.1 for irradiated samples.

V-2-6. Hydrocarbons and 2-ACBs analyses

Extraction, purification, and analysis of hydrocarbons and 2-ACBs in meat samples were carried out by EN 1784:2003 and 1785 (CEN, 2003a; 2003b), respectively. Briefly, a 10 g of sample was extracted with 100 mL of n-hexane using Soxhlet apparatuses. A column was prepared by filling it with inactivated Florisil (40 g) and sodium sulfate (approx. 3g) on top with n-hexane. The extract (equivalent to 200 mg fat) was loaded on the column, and then was washed with n-hexane (150 mL). The eluent was used for hydrocarbon analysis. 2-ACBs were elutes with 1% diethyl ether/hexane (150 mL) with flow rate of approximately 2 mL/min. The eluent was evaporated at 45°C, and solvent was removed by nitrogen blowing resulting in 1mL of final volume. 2-ACBs was analyzed using an Agilent 6980N gas chromatograph (Agilent, Palo Alto, CA, USA) coupled with an Agilent

5973 inert mass selective detector. Splitless injections of 1 μL of solution were made into a DB-5MS capillary column, 30 m × 250 μm i.d., 0.25 μm film thickness (J&W Scientific, Folson, CA, USA). Following injection the oven was held at 55°C for 1 min and then the temperature was raised at 15°C/min to 300°C. The injector was held at 280°C. Helium was employed as the carrier gas at a constant flow of 1 mL/min. The MS was operated in electron impact mode with scan mode for hydrocarbons or selected ion monitoring mode measuring m/z 98 for 2-dodecylcylobutanone and 2-tetradecylcyclobutanone.

V-2-7. Statistical analysis

Data were statistically analyzed using IBM® SPSS Statistics 21 software for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance and Duncan's multiple range tests at $p < 0.05$ were used to compare differences among mean values.

V-3. Results

V-3-1. Identification properties of irradiated red pepper powder by 7.5 MeV X-ray

The PSL PCs of the red pepper powder irradiated by 7.5 MeV X-rays at different doses are presented in Table 48. The PCs of non-irradiated samples were 264 ± 45 counts/ 60 s, which were less than the lower threshold value (700 counts/60 s), clearly indicating a negative result. On the other hand, the samples irradiated at less than 1.42 kGy yielded intermediate PCs between 700 and 5000 counts/60 s. All the samples irradiated at more than 4 kGy were correctly identified as irradiated with the PCs greater than the upper threshold value of 5000 counts/60 s.

The first glow curves (TL_1) of red pepper powder irradiated with 7.5 MeV X-rays at different doses are presented in Fig. 8. The TL signal intensity of the irradiated samples increased with increase in dose. Moreover, all the samples showed a typical well-defined peak with a maximum at approximately 190°C.

The TL ratios of red pepper powder irradiated with 7.5 MeV X-rays at different doses followed by normalization with the identical ionizing radiations at 1 kGy are given in Table 49. The TL ratios of non-irradiated samples were 0.001, indicating a negative result. However, the TL ratios of irradiated samples at 0.49–9.27 kGy significantly increased as a function of absorbed dose; in addition, all the ratios were greater than 0.1 resulting in the positive response.

Table 48. Photo-stimulated luminescence analysis of red pepper powder irradiated by 7.5 MeV X-rays at various doses

Absorbed dose (kGy)	Photon count/60 s	Decision
0	264 ± 45^{A}	Negative[1)]
0.49 ± 0.03	1484 ± 210^{B}	Intermediate
0.76 ± 0.04	2728 ± 680^{C}	Intermediate
1.42 ± 0.07	3392 ± 916^{C}	Intermediate
3.93 ± 0.20	6908 ± 958^{E}	Positive
5.26 ± 0.26	7732 ± 401^{E}	Positive
7.66 ± 0.38	6804 ± 929^{DE}	Positive
9.27 ± 0.46	5855 ± 287^{D}	Positive

Absorbed doses are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3).

[1)]Decision is drawn as negative when the PSL PCs/60 s are lower than the lower threshold value (700 counts/60 s, T_1); positive when the PSL PCs/60 s are higher than the upper threshold value (5000 counts/ 60 s, T_2); intermediate when the PSL PCs/60 s are between T_1 and T_2.

[A-E]Means followed by different letters within a column are significantly different ($p < 0.05$).

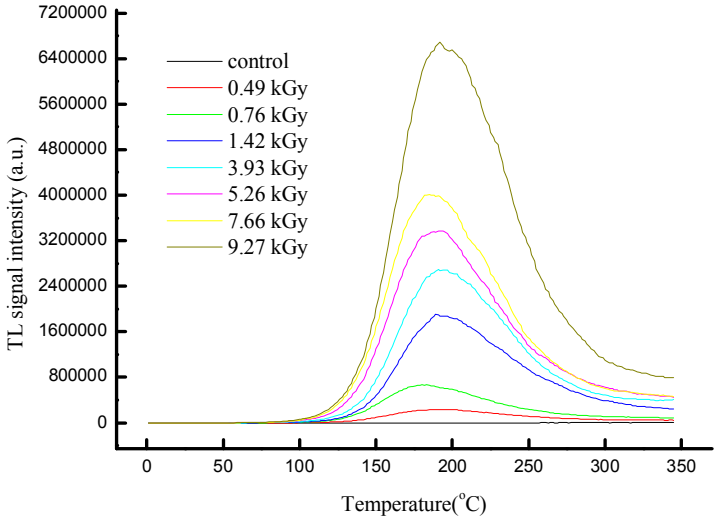


Fig. 8. The first glow curves of red pepper powder irradiated by 7.5 MeV X-rays at different doses.

Table 49. Thermoluminescence analysis of red pepper powder irradiated by 7.5 MeV X-rays at various doses

Absorbed dose (kGy)	TL ratio (TL_1/TL_2) normalized with gamma rays at 1 kGy	Decision[1)]
0	0.001 ± 0.001^{A}	Negative
0.49 ± 0.03	0.122 ± 0.005^{B}	Positive
0.76 ± 0.04	0.405 ± 0.060^{C}	Positive
1.42 ± 0.07	0.714 ± 0.027^{D}	Positive
3.93 ± 0.20	0.965 ± 0.021^{E}	Positive
5.26 ± 0.26	0.977 ± 0.043^{E}	Positive
7.66 ± 0.38	1.014 ± 0.065^{E}	Positive
9.27 ± 0.46	1.271 ± 0.103^{F}	Positive

Absorbed doses are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3).

[1)]Decision is drawn as positive when the TL ratio is higher than 0.1.

[A-F]Means followed by different letters within a column are significantly different ($p < 0.05$).

Comparing the types of ionizing radiation used for normalization, the TL ratios of the non-irradiated samples ranged from 0.001 to 0.002. The TL ratios of X-ray-irradiated samples at 0.49 kGy were over 0.1 except for the samples normalized with 160 keV X-rays at 1 kGy (Table 50). However, all samples exhibited TL ratios more than 0.1, when X-ray-irradiated samples at 0.76 kGy were normalized with the various irradiation sources.

To figure out the reason of false decision on X-ray irradiated samples at 0.49 kGy followed by normalization with 160 keV X-rays at 1 kGy, pure silicate minerals (99.9%) such as quartz and K-feldspar irradiated with various irradiation sources at 1 kGy are analyzed by TL methods (Fig. 9). For quartz, the TL integral intensities of e-beam-irradiated samples exhibited the lowest values, whereas those of samples irradiated with 160 keV X-rays at 1 kGy were the highest among those of samples treated by different irradiation sources. No significant difference was observed between gamma- and 7.5 MeV X-ray-irradiated samples ($p < 0.05$). A similar tendency was also observed for the irradiated K-feldspar samples.

Table 50. TL analysis of red pepper powder irradiated by 7.5 MeV X-rays followed by normalization with different types of ionizing radiation at 1 kGy

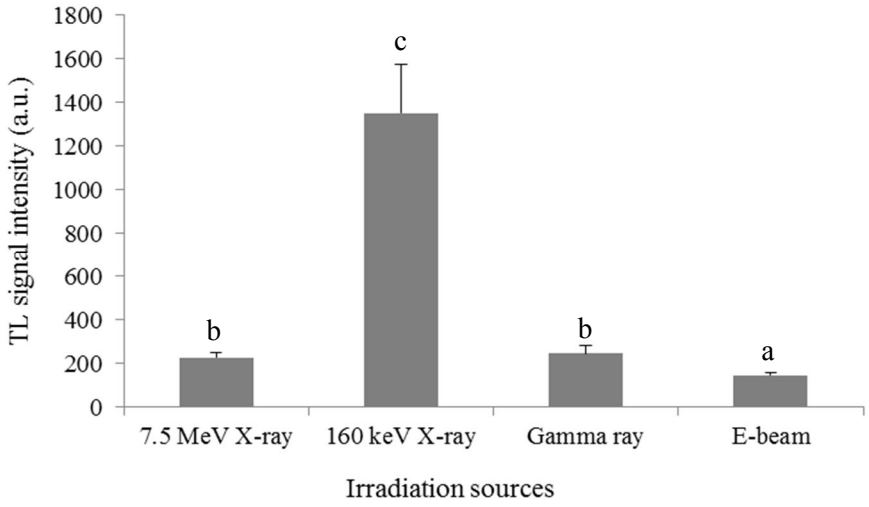
Absorbed dose (kGy)	Radiation type for normalization (1 kGy)	TL ratio (TL_1/TL_2)	Decision[1)]
0	7.5 MeV X-ray	0.001 ± 0.001^{NS}	Negative
	160 keV X-ray	0.001±0.001	Negative
	Gamm ray	0.001±0.001	Negative
	E-beam	0.002±0.001	Negative
0.49 ± 0.03	7.5 MeV X-ray	0.142 ± 0.003^{B}	Positive
	160 keV X-ray	0.057 ± 0.003^{A}	Negative
	Gamm ray	0.122 ± 0.005^{B}	Positive
	E-beam	0.163 ± 0.002^{C}	Positive
0.76 ± 0.04	7.5 MeV X-ray	0.456 ± 0.025^{B}	Positive
	160 keV X-ray	0.180 ± 0.015^{A}	Positive
	Gamm ray	0.405 ± 0.060^{B}	Positive
	E-beam	0.517 ± 0.036^{B}	Positive

Absorbed doses are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3).

[A-F]Means followed by different letters within a column are significantly different ($p < 0.05$).

[1)]Decision is drawn as positive when the TL ratio is higher than 0.1.

(A)



(B)

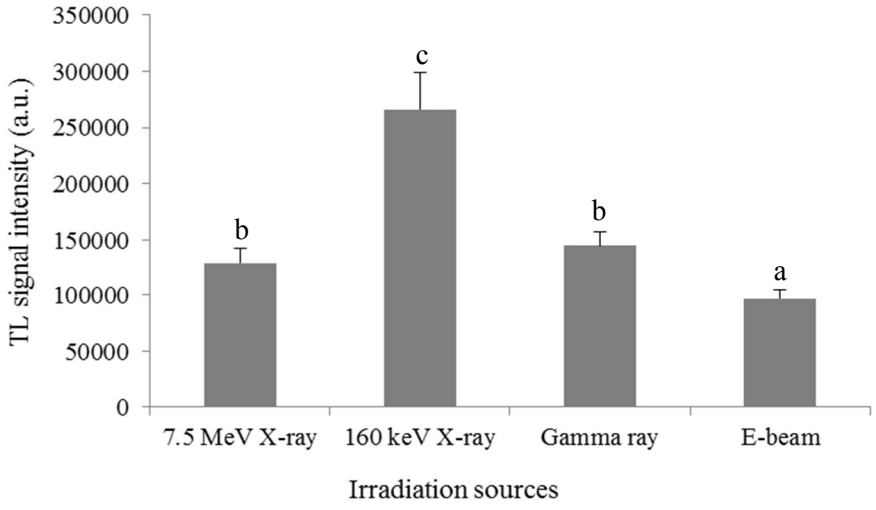


Fig. 9. TL signal intensity of quartz (A) and K-feldspar (B) irradiated by 7.5 MeV and 160 keV X-rays, gamma rays, and 10 MeV e-beams at 1 kGy. [a-c]Means followed by different letters are significantly different ($p < 0.05$).

V-3-2. Identification properties of irradiated meats by 7.5 MeV X-ray

The GC chromatogram of extracts from chicken breast meat irradiated by 7.5 MeV X-rays at various doses are shown in Fig. 10 and Table 51. The specific radiolytic hydrocarbons including 1-tetradecene ($C_{14:1}$), pentadecane ($C_{15:0}$), 1-hexadecene ($C_{16:1}$), 1,7-hexadecadiene ($C_{16:2}$), and 8-heptadecene ($C_{17:1}$) were detected in X-ray-irradiated samples at 2–10 kGy, whereas the peaks were not observed in non-irradiated samples. Among these, the concentrations of $C_{16:2}$ and $C_{17:1}$, which are used as marker hydrocarbons for detection of irradiated meats, were 39.71–43.33 μg/g lipid in irradiated samples at more than 4 kGy and 40.63–43.58 μg/g lipid in irradiated samples at more than 6 kGy.

The concentrations of 2-dodecylcycloburanon and 2-tetradecylcyclobutanone of ground beef irradiated with 7.5 MeV X-rays at various doses are shown in Table 52. The both compounds were not observed in non-irradiated samples, whereas they increased linearly to the increase of absorbed dose. The concentration of 2-dodecylcyclobutanone in 2-kGy samples was 1.90 μg/g lipid, and that was increased to 6.85 μg/g lipid in 10-kGy sample. In addition, the concentration of 2-tetradecylcyclobutanone was increased from 0.70 to 2.21 μg/g lipid as absorbed dose increased from 2.27 to 10.09 kGy.

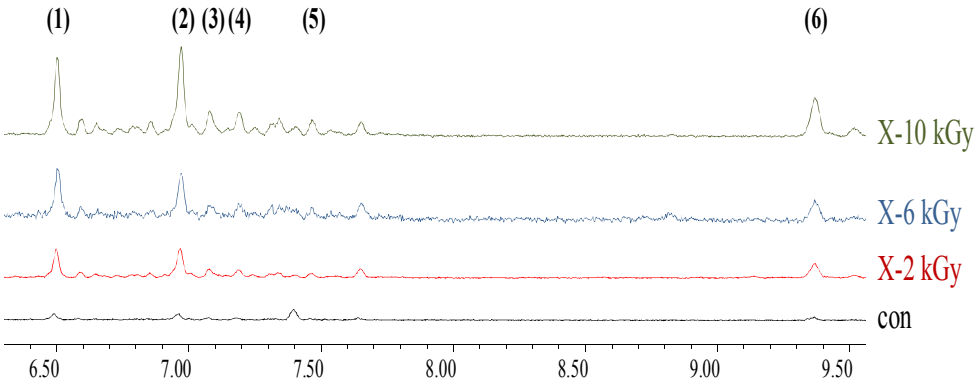


Fig. 10. Chromatogram of hydrocarbons detected from chicken breast meat irradiated by 7.5 MeV X-ray. (1), 1-tetradecene ($C_{14:1}$); (2), pentadecane ($C_{15:0}$); (3), 1,7-hexadecadiene ($C_{16:2}$); (4), 1-hexadecene ($C_{16:1}$); (5), 8-heptadecene ($C_{17:1}$).

Table 51. Concentration of hydrocarbons detected from irradiated chicken breast meat by 7.5 MeV X-ray at various doses

Absorbed dose (kGy)	Concentration of hydrocarbon (μg/g lipid)		
	1,7-hexadecadiene ($C_{16:2}$)	8-heptadecene ($C_{17:1}$)	Decision
0	ND [1)]	ND	Negative
2.28 ± 0.09	ND	ND	Negative
4.37 ± 0.11	39.71 ± 1.59	ND	Positive
6.28 ± 0.22	41.78 ± 3.04	40.64 ± 1.33[A]	Positive
7.96 ± 0.32	40.06 ± 0.35	41.39 ± 0.52[A]	Positive
10.14 ± 0.49	43.33 ± 2.97	43.58 ± 0.70[B]	Positive

Absorbed doses are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

[1)]Not detected.

Table 52. Concentration of 2-alkylcyclobutanones in ground beef irradiated by 7.5 MeV X-ray at various doses

Absorbed dose (kGy)	Concentration of 2-alkylcyclobutanones (μg/g lipid)		
	2-dodecylcyclobutanone	2-tetradecylcyclobutanone	Decision
0	ND[1)]	ND	Negative
2.27 ± 0.10	1.90±0.14[A]	0.70±0.09[A]	Positive
4.15 ± 0.21	3.25±0.21[B]	1.10±0.13[B]	Positive
6.21 ± 0.37	4.23±0.20[C]	1.43±0.02[C]	Positive
8.32 ± 0.22	6.05±0.11[D]	1.66±0.04[D]	Positive
10.09 ± 0.63	6.85±0.50[E]	2.21±0.21[E]	Positive

Absorbed doses are expressed as mean ± the expended uncertainty at a 95% confidence interval (n=3). Measurements are expressed as mean ± standard deviation (n=3).

[A-B]Means followed by different letters within a column are significantly different ($p < 0.05$).

[1)]Not detected.

V-4. Discussion

PSL analysis has been used as a screening method owing to the convenience of the process since it does not need further sample preparation. However, this technique exhibits low sensitivity. In the previous studies, red pepper powder samples gamma-irradiated at more than 1 kGy were clearly identified as irradiated (Ahn et al., 2013; Alberti et al., 2007; Choi et al., 2004). However, the uncertain results observed in this study for the samples irradiated with 7.5 MeV X-rays at less than 2 kGy appear to be caused by low amount or quality of the minerals, necessitating an additional confirmatory step is needed for the intermediate samples. Sanderson et al. (1998) found that a low PSL sensitivity of the accompanying minerals of very clean samples or blends with only a low amount of irradiated material might hinder the successful application of the PSL analysis method. Moreover, the dose independency for the PSL signals can be explained the inequivalent amount of silicate minerals for each sample (Anderle, 1997).

For TL analysis, it was clearly observed that different TL intensity of pure quartz, K-feldspar, and minerals separated from red pepper powder upon various irradiation sources. The TL integral intensities of all samples irradiated with 160 keV X-rays were the highest among the samples irradiated with all the four types of ionizing radiation, whereas the e-beam-irradiated samples exhibited the lowest TL integral intensities at an equivalent dose. Unfortunately, the reason for the different signal intensities for various irradiation sources has not been reported. However, Soika & Delincée (2000) reported that the glow curve of quartz induced by gamma rays

exhibited a higher TL intensity than those obtained from accelerated electrons. The result in this study is consistent with the previous study. A possible explanation for this observation is that the high-charged particle energy of the accelerated electrons may be trapped to a lesser extent in minerals than the photon energy of gamma rays and X-rays, owing to higher dose rate and radiant energy of e-beams than those of photons. In contrast, low-energy X-rays appeared to be trapped in the minerals to a considerably greater extent than the other types of ionizing radiation with higher radiant energy.

Minerals separated from the food matrix should be normalized by re-irradiation at 1 kGy after TL_1 measurement to determine the TL ratio, which provides reliability in detecting irradiated samples (Ahn et al., 2012). However, the normalization step used for TL analysis in some of the food control laboratories gives rise to some problems because the laboratory may not have suitable irradiation facilities for the second normalizing irradiation. In this case, they would have to send their discs containing the isolated silicate minerals to the facilities, which may lead to delayed analysis and the cost for re-irradiation may be a burden to the laboratories.

In this context, a cabinet X-ray irradiator is advantageous compared to the existing high-energy irradiation facilities from the points of view of installation and operational costs as well as radiation safety management associated with the radioactive sources used for gamma irradiation (Moosekian et al., 2012). In this study, it was observed that the TL ratios of samples varied depending on the irradiation sources used for normalization, with e-beam and low-energy X-ray

irradiation exhibiting the lowest and highest TL signal intensity, respectively. This result implies that a dose of 1 kGy for normalization should be adjusted based on the TL intensities of each irradiation sources. If the minerals separated from the red pepper powder were re-irradiated with low-energy X-rays at one-third of the normalization dose conventionally used for gamma re-irradiation, the TL ratios could be similar with those of gamma re-irradiated samples. In this context, the cabinet X-ray irradiator generating low-energy X-rays can be used as an alternative radiation type for normalization, relieving the existing problems related with the normalization step. To verify the suggestion, however, interlaboratory comparison studies on the TL characteristics of various types of food irradiated with various irradiation sources must be conducted in the future.

For identification of irradiated chicken breast meat and ground beef by 7.5 MeV X-rays, analysis of 2-ACBs was preferred because all irradiated meats were correctly identified, whereas the indicate markers for hydrocarbons such $C_{16:2}$ and $C_{17:1}$ were detected in samples at more than 4 kGy. Therefore, 7.5 MeV X-ray-irradiated food can be supervised by using physical and chemical identification methods.

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국문초록

7.5 MeV 엑스선 조사처리 식품의 특성 규명에 관한 연구

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식품조사(food irradiation)는 이온화 방사선의 살균효과를 이용하여 식품의 미생물학적 안전성 향상 및 저장기한 연장을 목적으로 국제적으로 상용화되고 있는 식품 가공 공정이다. 식품조사에 사용되는 이온화 방사선에는 엑스선, 감마선 및 전자선이 있으며, 이중 전자선가속기에서 발생하는 7.5 MeV의 전자를 금속물질에 충돌시킬 때 생성되는 엑스선(이하 7.5 MeV 엑스선)은 5 MeV 엑스선의 낮은 에너지 효율, 감마선의 낮은 소비자 수용도 및 전자선의 낮은 투과력 등 기존 조사선원들의 적용한계를 극복할 수 있는 대안으로 새롭게

제안되었다. 7.5 MeV 엑스선 조사가 식품살균 기술로의 사용 가능여부를 판단하기 위하여, 해당 조사선원으로 조사 처리한 식품의 특성 규명이 필요하다. 따라서 본 연구에서는 식품의 미생물학적 안전성 확보를 위해 사용되는 7.5 MeV 엑스선 조사처리 식품의 특성을 규명하고자 모델식품(고춧가루, 닭 가슴살 및 분쇄 우육)의 미생물 살균효과, 독성학적 안전성, 이화학적 품질 특성 및 조사여부 확인 연구를 수행하였다.

7.5 MeV 엑스선의 D_{10}값(초기균수의 90% 살균에 필요한 선량)은 인산완충용액에 현탁된 *Escherichia coli, Salmonella* Typhimurium, *Listeria monocytogenes* 및 *Staphylococcus aureus*에 대하여 0.11–0.21 kGy 및 분쇄우육에 접종된 병원균에 대하여 0.22–0.41 kGy의 범위를 나타내었다. 분쇄우육에 접종된 병원균에 대한 7.5 MeV 엑스선의 살균효과를 감마선 및 전자선과 비교한 결과 7.5 MeV 엑스선 조사는 코발트 선원에서 발생하는 감마선 및 10 MeV 전자선과 유사한 살균 효과를 나타내었다. 또한 고춧가루의 전체 호기성균의 경우 6 kGy의 엑스선 조사에 의해 약 3 log CFU가 사멸되었으며, 닭 가슴살 및 분쇄우육에 대하여 4 kGy 이상의 선량으로 조사 처리 시 총균수를 1 log CFU/g의 검출한계 이하로 감소시킬 수 있었다.

7.5 MeV 엑스선을 이용하여 30 kGy의 흡수선량으로 조사 처리한

닭 가슴살의 유전독성, 급성 및 아급성 독성 평가를 수행한 결과, 복귀돌연변이원성, 염색체 이상 및 소핵 시험 모두 음성 대조군과 유사한 수준의 반응을 나타내어 유전독성은 없는 것으로 확인되었다. 급성 및 아급성 독성 평가에서 실험 기간 중 사망동물이나 병리학적 이상 증상은 발견되지 않았다. 혈액학적과 혈청생화학 특성치 중 일부 평가항목에서 대조군에 비해 시료를 섭취한 실험군에서 유의적인 차이가 관찰되었으나, 모두 정상 범위에 해당하였다. 즉, 수컷과 암컷 ICR 마우스 모두에 대하여 최고 2000 mg/kg body weight의 농도에서 특이적인 급성독성을 나타내지 않았으며, 매일 2500 mg/kg body weight의 농도로 90일간 구강 투여한 마우스에 있어 어떠한 독성학적 징후를 관찰하지 못하였으므로 7.5 MeV 엑스선을 이용하여 30 kGy의 흡수선량으로 처리한 닭 가슴살은 본 시험조건 하에서 유전독성, 급성 독성 및 아급성 독성이 없는 것으로 판단하였다.

7.5 MeV 엑스선으로 조사처리(2−10 kGy)한 고춧가루의 품질 평가 결과 흡수선량의 증가에 따라 이취의 강도가 증가하는 경향을 나타내었으나, 식품공전에서 허용하는 최대 선량인 7 kGy 이하의 선량으로 조사 처리된 시료들은 비조사구와 유의적인 차이를 나타내지 않았다($p < 0.05$). 이취의 강도를 제외한 고춧가루의 관능학적 품질, 색도, capsaicinoids와 capsanthin 함량은 흡수선량의 증가에 따른

유의적인 차이를 나타내지 않았다($p < 0.05$). 7.5 MeV 엑스선으로 조사처리한 닭 가슴살과 분쇄 우육의 2-thiobabituroinic acid reactive substance값은 흡수선량이 증가할수록 증가하는 경향을 나타내었으나 pH는 흡수선량의 증가에 따른 유의적 차이는 나타나지 않았다($p < 0.05$). 따라서, 7.5 MeV 엑스선 조사는 각 모델식품에 대한 최대 허용 선량 이하에서 품질의 저하를 일으키지 않는 것으로 판단하였다.

광자극발광법(PSL법)을 이용하여 7.5 MeV 엑스선으로 조사처리한 고춧가루의 조사여부 확인한 결과, 2 kGy 이하의 선량으로 조사 처리된 시료의 PSL photon counts/60 s (PCs/60 s)는 700-5000 PCs/60 s 사이의 중간값을 나타내어 조사여부 판별을 위해서는 추가적인 확인시험이 필요하였으며, 4 kGy 이상의 흡수선량으로 조사 처리된 시료들은 모두 5000 PCs/60 s 이상의 값을 나타내어 조사된 것을 확인할 수 있었다. 열발광법(TL법)을 이용하여 7.5 MeV 엑스선으로 조사처리한 고춧가루의 조사 여부 확인 결과, 다양한 흡수선량(0.49-9.27 kGy)으로 조사 처리된 시료의 TL ratio가 모두 0.1 이상으로 나타나 조사 여부 확인이 가능하였다. 7.5 MeV 엑스선 조사 처리(2-10 kGy)된 계육의 hydrocarbon 분석 결과, 4 kGy 이상의 흡수선량으로 조사된 시료들에서 식육의 조사처리 판별 마커인 $C_{16:2}$와 $C_{17:1}$가 검출되었다. 7.5 MeV 엑스선 조사 처리된 우육의 2-

alkylcyclobutanones 분석결과, 흡수선량(2-10 kGy)의 증가에 따라 비조사구에서는 검출되지 않은 2-dodecylcyclobutanone과 2-tetradecylcyclobutanone 함량이 비례적으로 증가하여 조사여부 확인이 모두 가능하였다.

결론적으로 모델식품의 특성 규명을 통해 7.5 MeV 엑스선 식품조사는 품질의 저하 없이 미생물학적으로 안전성을 부여하기 위한 식품 살균 공정으로 활용이 가능할 것으로 사료된다. 또한, 7.5 MeV 엑스선 조사처리 식품은 독성학적으로 안전하며, 조사 여부 확인 시험을 통한 관리가 가능함을 확인하였다.

키워드 : 엑스선, 전자선, 감마선, 살균효과, 품질특성, 조사 여부 확인법, 독성학적 안전성

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