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Radiosensitivity of microorganisms in *Saengshik* products and irradiation effects on the sensorial properties

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ABSTRACT

Saengshik products usually include powders of plant-derived foods that are normally non-heated and thus have inherent limitations in microbial control. Here, we investigated the microbial reduction and sensorial properties of two types of *Saengshik* products commercially available in Korean markets after electron-beam and gamma-ray irradiation (0, 1, 3, 5, and 10 kGy). The initial microbial loads in the products were 6–7 log colony-forming units (CFU)/g of total aerobic bacteria (TAB), 4–5 log CFU/g of yeasts and molds (YM), and ≤ 3 log CFU/g of coliforms. Radiosensitivities (D_{10} values) of TAB, YM, and the indicator pathogens *Clostridium perfringens* and *Bacillus cereus* were 1.21–1.86 kGy, 1.03–1.97 kGy, 0.42–0.48 kGy and 0.58–0.68 kGy, respectively, regardless of the radiation source or product type. Radiation 5D values for both pathogens were 2.10–3.40 kGy, which is the dose needed to achieve the recommended 5-log reduction. The radiation sensitivity (D_{10} and 5D values) was higher in *B. cereus* than in *C. perfringens* for both samples. Irradiation up to 10 kGy induced negligible changes in sensory scores for the *Saengshik* samples ($p > 0.05$). An electronic nose effectively distinguished the flavor profiles of irradiated products between 10 kGy and ≤ 5 kGy approved by the Korean Food Code for *Saengshik* ingredients. These results indicate that 5 kGy irradiation with electron-beam or gamma-ray was sufficient for achieving microbial control in powdered *Saengshik* products.

1. Introduction

According to the Korean Food Standards Codex (2016), *Saengshik* is a formulation of vegetal materials, including dried grains, beans, vegetables, fruits, and mushrooms. The product is available in various forms, such as powders, granules, bars, pastes, gels, and liquids. *Saengshik* intake has shown improvements in hyperlipidemia (Park and Han, 2003), and *Saengshik* has been reported to contain antioxidant compounds (Chen et al., 2007) as well as to show efficiency in the amelioration of cancer (Zhou et al., 2003) and diabetes (Kim et al., 2004) conditions. Moreover, *Saengshik* provides better health effects than its constituent single ingredients through the synergistic effects of various phytochemicals (Liu, 2003). For consumption, *Saengshik* is processed non-thermally to preserve the digestive enzymes and biologically active substances that are eliminated in heat-processed foods (Hwang, 2002). The recognition of the safety, health, and convenience aspects related to the consumption of minimally processed foods has increased the popularity of *Saengshik* as a meal substitute. Usually, *Saengshik* is marketed in broad assortments to ensure a good balance of nutrients, so it provides sufficient amounts of protein, carbohydrates, vitamins, minerals, chlorophyll and other nutrients. From several years ago, in Korea, the *Saengshik* industry started to grow very quickly. In addition, the 'Korea *Saengshik* Association' was founded in 2002. It devotes itself to popularizing *Saengshik* in many foreign countries, as well as in Korea. It is generally prepared by mixing a powder in any liquid or beverage before consumption (Chung and Han, 2003). This form of *Saengshik* is very popular with patients recovering from illness. To them it is convenient and easy to eat, like a porridge, and satisfies them because of its high nutritional value. Considering these facts, *Saengshik* is as an ideal food. Until now, over 30 food companies such as Pulmuone, Daesang Co., Erom Life Co., CJ, Ohaengsaengshik Co., etc., are producing *Saengshik* in Korea.

However, because of its unheated manufacturing process, *Saengshik* has an inherent limitation in microbial control, in addition to the unsanitary handling of pulverizing equipment and insufficient washing and sterilization of raw materials (Chang et al., 2004). Moreover, non-heated and nutrition-dense foods are particularly prone to microbial growth, especially *Clostridium perfringens* and *Bacillus cereus* in the case of *Saengshik*. According to the Korean Food Standards Codex (2016), *Saengshik* products should have $< 1 \times 10^2$ colony forming units (CFU)/g of mesophilic bacteria, no *Escherichia coli*, $< 10^2$ CFU/g of *C. perfringens*, and $< 10^3$ CFU/g of *B. cereus*. Among the available food

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decontamination methods, irradiation is a popular physical technique that is comparable to thermal pasteurization. In this method, the food is exposed to one of the three types of ionizing energy such as γ-rays, machine-generated electrons, or X-rays to remove any pathogens (Farkas, 2006). Use of ionizing radiation has been effective for the inhibition of microbial proliferation during the processing and storage of food. Low doses of irradiation can decontaminate the sample from microorganisms without changing the quality and nutritional characteristics. The Korean government has approved electron-beam (E-beam) and gamma-ray (γ-ray) irradiation and determined the maximum dose for 26 food groups, including bulbs, roots, tubers, fruits, vegetables, cereals, spices, and dried foods of animal origin. Considering the non-heated characteristics of *Saengshik* ingredients, which are mostly composed of powdered cereals and legumes, irradiation treatment with electron-beam and gamma-ray up to a dose of 5 kGy is currently allowed for their microbial control in the Korean Food Standards Codex (2016). The US Food and Drug Administration (2016) has also authorized food irradiation with 1 kGy to eliminate insects and inhibit the maturation of foods, 3 kGy to inhibit foodborne pathogens, 4.5 kGy and 7 kGy for meat stored at 4 °C or −20 °C, respectively, 10 kGy to reduce the microbial load of dried products, and 30 kGy for powdered aromatic substances. Previous studies on *Saengshik* reported the incidence and impurity level in handling during various processing methods as well as its nutraceutical effects (Bang et al., 2007). The Korean Atomic Energy Research Institute (KAERI) reported the presence of 4 CFU/g of mesophilic aerobes, negative coliforms, yeasts, and molds in 1 g of irradiated *Saengshik* prepared in the form of a non-cooked nutrition bar as food for astronauts (Song et al., 2012). However, to date there is no report on the effects of different irradiation sources on the pathogens levels in *Saengshik*.

Accordingly, the objective of this work was to investigate the microbial reduction and sensorial properties of two types of *Saengshik* products (SP and SCP) that are commercially available in Korean markets after electron-beam and gamma-ray irradiation. The radiosensitivities (D10 and 5D values) of *C. perfringens* and *B. cereus* detected in *Saengshik* products were determined, along with the effects of irradiation on Hunter's color parameters, flavor profiles based on electronic nose (e-nose) measurement, and sensory scores.

2. Materials and methods

2.1. Saengshik samples

The two representative *Saengshik* samples, *Saengshik* product (SP – refers to a product with at least 80% of unprocessed raw materials), and *Saengshik* containing product (SCP – refers to a product with at least 50% of unprocessed raw materials) were purchased in their original polyethylene packs (30 g) from three different supermarkets (n = 3) in two major cities (Daegu and Seoul) of Korea.

As per the label information in the product package, SP contains brown rice, barley, wheat, rice, sorghum, millet, perilla seeds, black sesame, soybean, adzuki beans, water parsley, yacon, carrot, pumpkin, onion, cabbage, chives, white radish, potato, sweet potato, laver, sea mustard, kelp, green laver, balloon flower, kudzu, *Cordyceps militaris*, milk vetch root, yam, burdock, lotus root, taro, Chinese matrimony vine, *Ligusticum acutilobum*, *Eucommia* bark, omija, shiitake, *Ganoderme luisant*, *Coriolus versicolor*, mugwort, pine needles, *Codonopsis lanceolata*, ginger, citron, Japanese apricot, persimmon, apple, cactus, *Saururus chinensis* Baill, chlorella, red ginseng, royal jelly.

SCP contains brown rice, barley, sorghum, adlay, black bean, corn, buckwheat, millet, red bean, carrot, *Angelica utilis* Makino, kelp, kale, burdock, lotus root, water parsley, cabbage, pumpkin, green tea, mandarin, mugwort, shiitake, apple, pine needles, chicory, royal jelly, Aloe vera. Details on the remaining components are undisclosed by the company to protect the distinct taste of their products. The moisture content of 1 g each of SP and SCP determined using an infrared moisture determination balance (FD-240, Kett Electric Laboratory, Tokyo, Japan) was 5% and 3%, respectively. As per the product information given by the manufacturer, carbohydrate, protein, fat, and fiber contents of SP and SCP are 10%, 7%, 3%, 20% and 10%, 5%, 2%, 16%, respectively. The packed samples were kept at 6 ± 2 °C during transportation and prior to analysis.

2.2. Analysis of microbial loads

The levels of total mesophilic aerobic bacteria, yeasts and molds, coliforms, *C. perfringens*, and *B. cereus* in *Saengshik* were detected in the products before and after irradiation. Ten grams of each sample was taken aseptically and pummeled with 90 mL of sterile peptone water for 90 s using a stomacher (BA 7021, Seward Medical Ltd., UK). Successive dilutions of the samples were poured onto plate count agar, potato dextrose agar (PDA) acidified with 10% tartaric acid, and desoxycholate agar (DIFCO, USA) plates to determine the total mesophilic, fungi, and coliforms counts, respectively. The mesophilic and coliforms counts were recorded after incubation for 24–48 h at 30 °C and 37 °C, respectively. The PDA plates were observed for yeasts and molds after incubation at 25 °C for 48 h.

2.2.1. C. perfringens culture and detection

C. perfringens present in the samples was cultured under anaerobic conditions in cooked meat broth for 18–24 h at 35–37 °C. The culture from the enrichment broth was streaked on *C. perfrigens* agar (Oxoid, UK) containing egg yolk and 200 μg/mL kanamycin. The plates were incubated in an anaerobic environment for 24 h at 35 °C. Yellowish colonies with bumpy rings (2 mm diameter) appeared on the *C. perfrigens* agar and colonies with grayish turbid rings were observed on tryptose sulfite cycloserine agar (Oxoid, UK). The confirmatory test was conducted using the Korean Food Standards Codex (2013).

2.2.2. B. cereus culture and detection

Ten grams of sample was homogenized with 90 mL phosphate-buffered saline, and one loopful of this was streaked onto mannitol egg yolk polymyxin agar (Oxoid, UK) with 10% egg yolk emulsion and *B. cereus* selective supplement. After incubation for 24 h at 30 °C, presumptive rough and dry colonies in a bright pink background were observed on nutrient agar (NA). Colonies from the NA plate were subjected to β-hemolysis and motility tests. The presence of *B. cereus* was confirmed using the API CHB/E kit (Biomerieux, USA).

2.3. Samples for the determination of radiation D-values

Fifty grams of each *Saengshik* sample was vacuum-packaged and heat-sealed in stomacher filter bags (Bagpage). A gamma-ray dose of 30 kGy using a Cobalt-60 irradiator (Korean Atomic Energy Research Institute, Jeongup, South Korea) was applied to remove the background microflora. All samples were kept refrigerated at 4–8 °C until use.

2.4. Culture preparation and bacterial inoculation

The pathogens *C. perfringens* ATCC 13124 and *B. cereus* KCCM 11341 were received from the Korean Culture Center of Microorganisms (Seoul, South Korea) and maintained at 4 °C in tryptic soy agar (TSA; DIFCO, USA). Prior to inoculation, the pathogens were cultured individually in 50 mL TSB at 150 rpm for 18 h at 35 °C (*Clostridium*) or 30 °C (*Bacillus*). The initial population level was 10^8–10^9 CFU/mL. The cultures (0.5 mL) were added to 50 g of the sample, and then the *Saengshik* samples were dried in a desiccator for 24 h at room temperature (30 ± 2 °C) to reach the standard moisture content of the product. The initial level of the pathogens in samples was 10^6–10^7 CFU/g.