

Application of electron beam irradiation for improving the microbial quality of processed laver products and luminescence detection of irradiated lavers

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Abstract The laver (*Porphyra* spp.) is normally processed in three kinds of products: dried laver (DL), roasted laver (RL), and seasoned roasted laver (SL). This work evaluated the effects of electron beam (E-beam) irradiation at different doses (0, 1, 4, 7, and 10 kGy) on microbiological and physicochemical qualities and detection characteristics of irradiated samples by luminescence analysis. E-beam irradiation resulted in dose-dependent microbial reductions, showing that 1 kGy destroyed initial coliforms ($< 2.35 \log$ CFU/g) to undetectable levels (< 10 CFU/g), while 7 kGy (approved dose for seaweed in Korea Food Code) reduced total aerobic bacteria ($3.72\text{--}6.33 \log$ CFU/g) and yeasts and molds ($2.05\text{--}4.98 \log$ CFU/g) by about 2 log cycles. Chlorophyll content remained unaffected in irradiated samples as compared to control; however, carotenoids content and Hunter's *b* values (degree of yellowness) showed a tendency to decrease in a dose-dependent manner ($p < 0.05$). However, E-beam irradiation less than 7 kGy did not significantly affect sensory properties of the processed laver products. Irradiated laver products (DL, RL, and SL) could be screened and detected by analyzing photostimulated luminescence and thermoluminescence, respectively, from the non-irradiated ones. The overall results indicated that E-beam irradiation is effective for ensuring the improved microbial quality ($< 4 \log$ CFU/g) for the exporting processed laver products without apparent quality changes.

Keywords E-beam irradiation · Luminescence detection · Microbial quality · Processed laver products

Introduction

Laver (*Porphyra* spp.) is an edible seaweed found all over the world, including Asia. As a eukaryote, it belongs to the Rhodophyta division and its further biological classification is as follows: Bangiaceae (family), Bangiales (order), and class Rhodophyceae. It grows in cold and shallow seawater and is mainly produced in Korea, China, Japan, and Taiwan [1, 2]. Fresh laver produced in the sea contains high moisture content ($> 90\%$), and its quality may be easily deteriorated. Therefore, further processing of fresh laver involves drying to obtain dried laver. Dried laver is used in the form of paper-like thin-layered products termed as “*Gim*” in Korea, “*Zicai*” in China, and “*Nori*” in Japan. In terms of its nutritional value, laver contains about 10% moisture content, 40% carbohydrates, 10% dietary minerals (especially iron and iodine), 30–40% proteins, and 2% lipids. The nutrient profile of dried laver products may vary depending on the collection area and season [2]. Dried laver is employed as an important food ingredient in food processing industry in Korea and mostly used as a raw material for roasted (without oil and salt) and seasoned roasted (roasted with oil and salt) laver. In Korea, most export-quality laver are made in the form of dried and seasoned roasted laver [1].

Dried and roasted lavers are often used as a raw material for laver roll (*Kimbab*, steamed rice and various vegetables in roasted laver) in Korea. *Kimbab* is a popular ready-to-eat food and may be used as a take-out food item for outdoor purposes; it is usually served with *danmuji*

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(Korean yellow pickled radish) and *kimchi* (a traditional Korean side dish made from fermented and salted vegetables, mostly nappa cabbage and Korean radishes with seasonings, including ginger, garlic, and Korean spices). However, dried laver displays high level of microbial contamination and a higher bacterial count of about 10^6 colony-forming units (CFU)/g [3]. Moreover, there is a high possibility of secondary contamination during the drying process. Laver is susceptible to microbial contamination, owing to the water-borne microorganisms present in seawater during the growth season [4]. Therefore, various studies are carried out to reduce the microbial population from dried laver using various treatments such as heat treatment, ultraviolet and gamma irradiation, and light pulse treatment [5–8]. The Korean Food Code [9] defines limits for food-poisoning bacteria and cadmium in laver and specifies the minimum levels for various parameters of seasoning laver (limited to the oil-treated laver only), such as the amount of tar pigment, acid value, and peroxide value. However, no regulation exists for the total aerobic bacterial count of processed laver products. Laver produced in Korea and exported to foreign countries may be rejected if it exceeds the standard; therefore, compliance with the standards of the importing countries is mandatory to ensure the export of processed laver products from Korea. In China, in particular, dried laver products must comply with the standards of seaweed products and should not exceed 30,000 CFU/g for total aerobic bacterial count, 30 most probable number (MPN)/100 g for *Escherichia coli*, and 300 CFU/g for pathogenic bacteria (*Salmonella* spp., *Vibrio* spp., *Staphylococcus aureus*, and *Shigella* spp.) [10].

Food irradiation has been approved, as it offers several technological advantages, including improvement in the microbial quality and reduction in the post-harvest losses during storage [11]. Irradiation technology is globally practiced in more than 55 countries for commercial applications; however, the general applicability of this technology requires international consensus. Moreover, food irradiation has also been approved by international organizations [Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and World Health Organization (WHO)], and WHO guarantees the safety of food items treated with certain irradiation source at specific doses. In Korea, food irradiation has been licensed since 1987 and 28 items, including seaweed food (maximum dose of 7 kGy), are currently approved. Different radiation sources such as gamma rays, electron beam (E-beam), and X-rays employed for food irradiation treatment may offer different technological advantages, including germination inhibition, sterilization, insect control, compliance with quarantine regulation, and maturity control [9, 11]. Various international and national

regulatory bodies have enforced mandatory requirement of labeling of irradiated foods. This necessitates the need of reliable and effective detection methods to gain acceptance of irradiated foods and regulate international trade on a global scale. Various analytical methods used for the detection of irradiated foods include photostimulated luminescence (PSL), thermoluminescence (TL), electron spin resonance (ESR), and gas chromatography–mass spectrometry for hydrocarbons [12]. Among these, two luminescence-based techniques, PSL and TL, have been adopted for screening and confirmatory purposes, respectively, by the European Committee for Standardization (CEN). These techniques may be employed for the successful identification of irradiated foods, especially food products with sufficient inorganic silicates [13, 14]. Different factors such as the quantity and quantity of the isolated inorganic minerals determine the reliability of these luminescence techniques, whereas the adverse processing conditions may affect the sensitivity of the luminescence properties.

Here, we investigated the applicability of E-beam irradiation for improving the microbial quality of processed laver products and its effects on the physicochemical quality. In addition, we evaluated CEN protocols for their effectiveness for the identification of E-beam-irradiated processed laver products by detecting luminescence characteristics.

Materials and methods

Sample preparation, E-beam irradiation, and storage

Samples comprising of three types of processed laver products: dried laver (DL), roasted laver (RL), and seasoned roasted laver (SL), were used in this experiment. Nine lots of three types of processed laver products manufactured by three different companies were purchased from local supermarkets located in different regions of Daegu, Korea. All the samples packaged in form of polypropylene package (100 sheets, approximately 200 g) were coded according to their types of processing. Table 1 shows the samples in coded notation along with their moisture contents. The samples were E-beam-irradiated (0, 1, 4, 7 and 10 kGy) with applied dose rate of 2.1 kGy/h at room temperature using high-energy linear accelerator (10 MeV, EB Tech Co., Ltd., Daejeon, Korea) located in Daejeon, Korea. The absorbed doses were verified using a ceric-cereus and cellulose triacetate (CTA) dosimeters. All samples were kept at room temperature. In order to carry out physicochemical analyses, individually coded laver products were cut into pieces and mixed well.

Table 1 List of laver products used in the analysis

| No. | Processed type | Companies | Moisture content (%) | Coded name |
|------|------------------------|-----------|----------------------|------------|
| DL-1 | Dried laver | A | 9.11 ± 0.34 | DL |
| DL-2 | Dried laver | B | | |
| DL-3 | Dried laver | C | | |
| RL-1 | Roasted laver | A | 5.56 ± 0.38 | RL |
| RL-2 | Roasted laver | B | | |
| RL-3 | Roasted laver | C | | |
| SL-1 | Seasoned roasted laver | A | 3.78 ± 0.37 | SL |
| SL-2 | Seasoned roasted laver | B | | |
| SL-3 | Seasoned roasted laver | C | | |

Microbiological analysis

Microbial counts, including total aerobic bacteria, yeasts and molds, and coliforms, were performed for all the laver samples. Mixtures comprising 5 g of each laver sample and 45 mL of peptone water (sterile) were prepared. Dilutions of each mixture were plated on a medium specific for total aerobic bacteria (plate count agar, PCA), yeasts and molds (potato dextrose agar acidified with tartaric acid, PDA), and coliforms (deoxycholate agar, DCA) (Difco, Sparks, MD, USA). The samples were incubated for 24–48 h at 35 and 37 °C for the counting of total aerobic bacteria and coliforms, respectively, while yeasts and molds were enumerated after 3 days of incubation at 30 °C.

Measurement of Hunter's color values

Colorimeter (CR-200; Minolta, Osaka, Japan) was used for the measurement of Hunter's color values, *L* (lightness/darkness), *a* (redness/greenness), and *b* (yellowness/blueness). All the measurements were recorded from three random locations on the packaged surface of the samples. The results were reported as mean values. The overall color difference (ΔE) was calculated from the obtained Hunter's color values. The *L*, *a*, and *b* values of the standard plate were 97.66, – 0.36, and 1.92, respectively.

Determination of chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents of laver were determined according to the method described by Lee et al. [15]. Chlorophyll was extracted with acetone and methanol (1:1, v/v) (Duksan Pure Chemicals, Gyeonggi, Korea), partitioned into diethyl ether (Duksan Pure Chemicals, Gyeonggi, Korea), and dehydrated with sodium sulfate (Na₂SO₄). Carotenoid analysis was performed by the extraction of carotenoids via saponification with potassium hydroxide (KOH), followed by their transfer to the diethyl ether layer and spectrophotometric quantification.

Chlorophyll and carotenoid contents were quantified by measuring the absorbance at 663 and 447 nm wavelengths, respectively, using an ultraviolet–visible spectrophotometer (Optizen 2120UV, Mecasys, Daejeon, Korea) and the equations developed by Mecbeth [16].

Photostimulated luminescence (PSL) analysis of for the screening of irradiated samples

We performed PSL analysis using a PPSL Irradiated Food Screening System (serial 0021, SURRC; Scottish Universities Research and Reactor Center, Glasgow, UK). The standard EN 13751 [13] recommendation was followed for sample preparation, measurement of photon counts (PCs), and interpretation of the results. Samples were sliced into uniform thickness and placed in a 50-mm-wide disposable petri dish (Bibby Sterilin type 122, Bibby Scientific Ltd., Staffordshire, UK) in the form of a uniform thick layer without any specific sample treatment. Measurements were taken by employing the sample chamber under passive light conditions. Radiation-induced PSL signals emitting per second from the irradiated samples were automatically accumulated by a personal computer for up to 60 s. After every 10 negative results, blank trials were carried out and the same was repeated after a positive result. PSL photon counts less than 700 counts/60 s were referred to as negative (non-irradiated) and counts greater than 5000 counts/60 s were recorded to be positive (irradiated). The standard EN 13751 [13] recommends calibrated PSL measurements to estimate PSL sensitivity of the sample in cases of doubtful results. After initial PSL measurements, all samples were subjected to a calibration radiation dose of 1 kGy and then processed for the second PSL measurements of calibrated PSL. For re-irradiation, all samples were taken to the EB tech Co. (Daejeon, Korea) and treated with E-beam using high-energy linear accelerator (10 MeV) at 1 kGy with an applied dose rate of 2.1 kGy/h at room temperature. The absorbed doses were verified using a ceric-cereus and CTA dosimeters.

Thermoluminescence (TL) analysis for the identification of irradiated samples

We followed the standard method of EN 1788 [15] to perform TL analysis. Density separation procedure was used to separate minerals from each sample (100 g). Clean stainless-steel disks were used for the deposition of isolated minerals, and these disks were placed overnight in a dry oven at 50 °C. TL reader (Harshaw 4500, Thermo Fisher Scientific Inc. Waltham, MA, USA) was used to record TL measurements at a temperature range of 50–400 °C with heating at a rate of 6 °C/s. First glow curve (TL₁) was obtained from the first readout measurement taken on extracted minerals. Normalization of TL response was carried out by re-irradiating the samples at 1 kGy dose as described earlier. Once again, the disks were subjected to overnight storage at 50 °C in dry oven. Measurements were repeated to obtain the second glow curve (TL₂). Throughout the procedure, full-process blanks were prepared and analyzed alongside the laver samples. Each sample was classified (non-irradiated or irradiated) on the basis of TL glow curve shape, intensity, temperature range of TL peak maxima, and TL ratio (TL₁/TL₂). The differentiation between the irradiated and non-irradiated laver samples was performed based on two decisive factors: (1) TL₁ shape and (2) TL ratio (TL₁/TL₂). Evaluation of TL ratio was performed over the temperature range of 150–250 °C. Based on TL shape and ratio, samples were regarded as irradiated if they had a TL ratio > 0.1, relatively higher TL intensity, and TL peak before 250 °C.

Sensory evaluation

Sensory evaluation for all laver samples was performed by 10 trained panelists. Hedonic scales of 7 points were employed for scoring the sensory attributes by the panelist following the guidelines reported by Meilgaard et al. [17]. Panelists recorded the sensory scores for different sensory attributes such as color, flavor, odor, taste, and overall acceptability. During the sensory evaluation, panelists used mineral water, unsalted crackers, and expectorant cups to neutralize and rinse their palates for rational assessment. On the descriptive scale, “0” was regarded as the lowest and “7” was scored as the highest score value.

Statistical analysis

All measurements were taken in triplicate ($n = 3$), and the results were represented as mean values \pm standard deviation. Microsoft Excel version 2007 (Microsoft, Redmond, WA, USA) and Origin 8.0 (OriginLab, Northampton, MA, USA) were used for data analysis. Analysis of variance was

carried out, and data significance was analyzed at level of $p < 0.05$ using Duncan’s multiple-range test.

Results and discussion

Microbial reduction by E-beam irradiation

All three types of laver samples from different manufacturing companies demonstrated variation in the level of microbial contamination (Fig. 1A–C). The sterilization effect following irradiation treatment varies with the type, number, and physical characteristics of microorganisms existing in the sample, the chemical composition and applied dose of sterilization, as well as the environmental factors and storage conditions under which the samples are stored. To elucidate the effects of E-beam on microbial growth, the number of bacteria, yeasts, molds, and coliforms was counted and expressed as log CFU/g (Fig. 1).

Total aerobic bacterial counts

The average total aerobic bacterial counts were 6.33, 4.33, and 3.72 log CFU/g for the control non-irradiated DL, RL, and SL, respectively. Maximum contamination with aerobic bacteria was reported for DL, while the number of aerobic bacteria decreased in non-irradiated RL and SL by 2.00 and 2.61 log CFU/g, respectively, owing to the heating effect during thermal treatment. Overall, the E-beam irradiation treatment at a low dose of 1–4 kGy resulted in lower microbial reductions, excluding coliforms, in all the three types of lavers (DL, RL, and SL). In comparison with the control samples, DL and RL showed a slight reduction in total aerobic bacteria at 1 kGy, while SL showed no significant change in the total aerobic bacterial count at 1 kGy. Microbial reduction was observed in all irradiated laver samples in a dose-dependent manner at applied doses from 4 to 10 kGy. However, no significant decrease was reported in total aerobic bacteria in RL samples treated at 4 kGy as compared with those treated at 1 kGy dose. However, the total aerobic bacteria decreased considerably in DL and SL samples treated at 4 kGy as compared with samples from control (0 kGy) and 1 kGy groups (Fig. 1A).

Yeast and mold counts

Yeast and mold counts in control (0 kGy) samples were 4.98, 2.33, and 2.05 log CFU/g for DL, RL, and SL, respectively. Yeast and mold counts were the highest for non-irradiated DL (5.00 log CFU/g), followed by RL (2.65 log CFU/g) and SL (2.93 log CFU/g). The coliform count was only 2.35 log CFU/g for the non-irradiated DL, whereas both RL and SL showed no detectable coliform

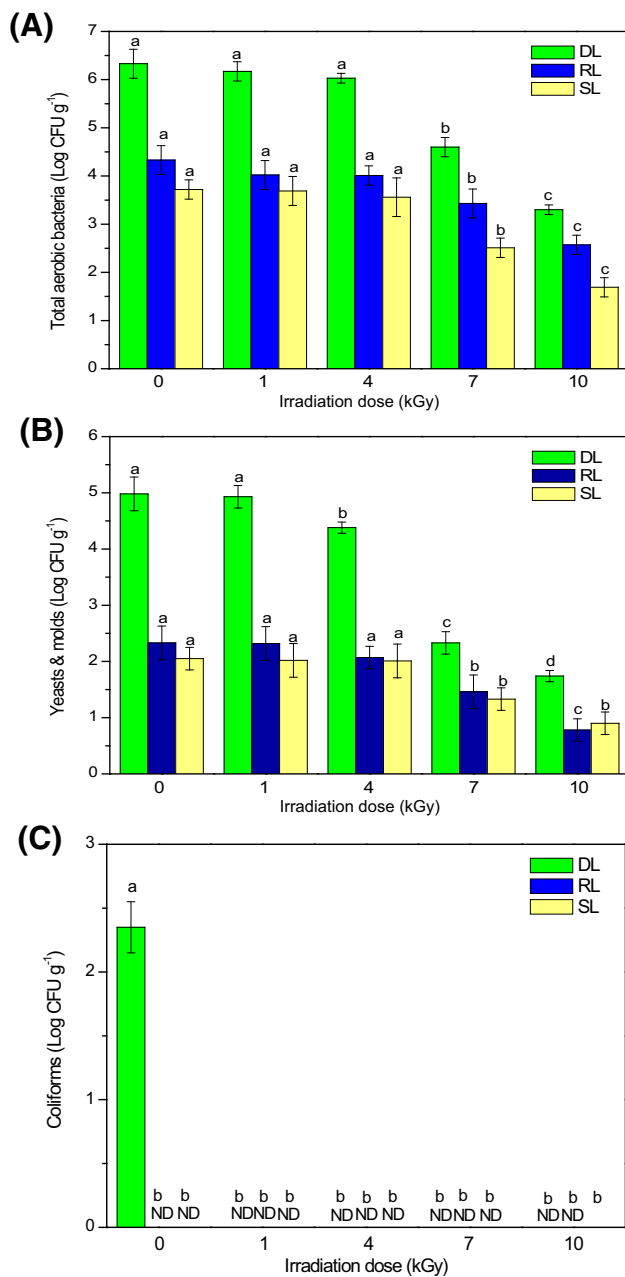


Fig. 1 Total aerobic bacteria (A), yeasts and molds (B), and coliform (C) counts of E-beam-irradiated dried processed laver products (ND \leq 10 CFU/g)

counts. Yeast and mold counts were slightly reduced at a low dose of 1 kGy in all three types of lavers. The yeast and mold counts showed decreasing tendencies with an increase in the applied doses from 1 to 10 kGy. The highest dose of 10 kGy resulted in about 2–3 log CFU/g reduction as compared with the non-irradiated samples (0 kGy). A significant reduction in yeasts and molds was observed at a dose of 7 kGy and above, and the degree of microbial reduction was higher in DL as compared to RL and SL

(Fig. 1B). A dose of more than 7 kGy was found to be effective for significant microbial reduction.

Coliforms count

Coliforms showed the highest sensitivity to E-beam irradiation at 1 kGy, and no detectable coliforms were found in DL samples treated at 1 kGy dose as compared with the untreated control (Fig. 1C).

The above results suggest that the use of the E-beam for sterilization was more effective for DL than for RL and SL. DL showed higher degree of microbial contamination, and E-beam was more effective in reducing microbial load in DL as compared to RL and SL. The microbial reduction in DL may be maximized with the combination of E-beam irradiation and other methods such as heat treatment. Kim et al. [18] reported a significant decrease in the number of total aerobic bacteria following E-beam irradiation of dried laver (*Porphyra tenera*) at 7 kGy as compared with the untreated control. Waje et al. [19] have found similar results, wherein E-beam irradiation at 3 kGy was effective for the reduction in total aerobic microbes below the threshold levels in seed sprouts.

Approved dose of maximum 7 kGy is required for microbial decontamination of seaweed foods in Korea, and hence, seaweed foods should be labeled [9]. Processed laver products are exported to other countries (e.g., China), and total aerobic bacterial counts must be in compliance with the standards specified by China ($\leq 3 \times 10^4$ CFU/g) [10], respectively. The E-beam irradiation treatment can effectively reduce the total aerobic bacterial count below 4 log in processed laver products.

Effects of E-beam irradiation on Hunter's color values

Hunter's color scale comprises *L* (degree of lightness), *a* (degree of redness), and *b* (degree of yellowness) values and enables uniform color measurements at visible wavelengths. The measured Hunter's color values of the laver samples are listed in Table 2. The *L* value showed a decreasing tendency in a dose-dependent manner for DL and RL samples; however, no significant change was observed in the *L* value at a dose as high as 10 kGy as compared with the control sample. SL showed an increase in *L* values with the corresponding increase in the applied doses from 1 to 10 kGy. The degree of redness increased in DL samples with a corresponding increase in the applied doses. On the contrary, irradiated samples of RL and SL showed decreasing tendencies of *a* values as compared with the control (0 kGy). All irradiated laver samples demonstrated a decrease in the *b* value with an increase in the applied dose as compared with the control. A small

Table 2 Hunter's color values of E-beam-irradiated processed laver products

| Sample | Hunter's parameter ¹ | Irradiation dose (kGy) | | | | |
|--------|---------------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| | | 0 | 1 | 4 | 7 | 10 |
| DL | L^* | 41.40 ± 1.25 ^{a2} | 40.71 ± 0.29 ^{ab} | 39.79 ± 1.11 ^{bc} | 39.05 ± 1.48 ^{n.s.} | 38.62 ± 0.44 ^{n.s.} |
| | a^* | 0.16 ± 0.03 ^{n.s.} | 0.20 ± 0.06 ^{n.s.} | 0.22 ± 0.06 ^{bc} | 0.30 ± 0.10 ^{ab} | 0.31 ± 0.06 ^a |
| | b^* | 3.16 ± 0.12 ^{n.s.} | 3.10 ± 0.20 ^{n.s.} | 3.00 ± 0.22 ^{ab} | 2.75 ± 0.21 ^{bc} | 2.70 ± 0.21 ^c |
| | ΔE | 0.00 ^e | 0.69 ^d | 1.62 ^c | 2.39 ^b | 2.82 ^a |
| RL | L^* | 40.46 ± 1.57 ^a | 39.54 ± 1.17 ^{ab} | 39.14 ± 1.27 ^{ab} | 38.57 ± 1.13 ^b | 38.49 ± 0.64 ^b |
| | a^* | -0.82 ± 0.19 ^d | -0.62 ± 0.08 ^c | -0.30 ± 0.14 ^b | -0.17 ± 0.07 ^{ab} | -0.04 ± 0.04 ^a |
| | b^* | 3.41 ± 0.28 ^a | 2.95 ± 0.29 ^b | 2.48 ± 0.20 ^c | 2.33 ± 0.30 ^{cd} | 2.01 ± 0.18 ^d |
| | ΔE | 0.00 ^e | 1.04 ^d | 1.70 ^c | 2.27 ^b | 2.53 ^a |
| SL | L^* | 40.94 ± 0.50 ^b | 42.07 ± 0.95 ^{n.s.} | 42.21 ± 1.11 ^{n.s.} | 42.31 ± 1.19 ^{n.s.} | 42.58 ± 1.09 ^a |
| | a^* | -2.75 ± 0.11 ^b | -2.56 ± 0.11 ^{n.s.} | -2.53 ± 0.14 ^{n.s.} | -2.51 ± 0.08 ^{n.s.} | -2.47 ± 0.18 ^{n.s.} |
| | b^* | 6.81 ± 0.23 ^{n.s.} | 6.61 ± 0.27 ^{n.s.} | 6.54 ± 0.26 ^{n.s.} | 6.50 ± 0.25 ^{n.s.} | 6.46 ± 0.28 ^{n.s.} |
| | ΔE | 0.00 ^e | 1.17 ^d | 1.32 ^c | 1.43 ^b | 1.70 ^a |

n.s. (nonsignificant)

¹ L^* : Degree of lightness (white +100 ↔ 0 black)

a^* : Degree of redness (red +100 ↔ - 80 green)

b^* : Degree of yellowness (yellow +70 ↔ - 80 blue)

ΔE : Overall color difference ($\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$)

²Values are mean ± SD ($n = 10$)

^{a-c}Values within the same column with different superscript letters are significantly different at $p < 0.05$

change was observed in the overall color difference (ΔE) of DL and RL at 1 kGy, and this change markedly increased with higher doses of irradiation. At 4 and 7 kGy, the increase in ΔE values of DL and RL was considerably noticeable as compared with control samples. SL showed increases in ΔE values, and the highest value of 1.70 was observed for SL samples treated at 10 kGy; however, the increase in ΔE values was higher in DL and RL samples as compared with SL samples. Thus, E-beam irradiation failed to exert any significant impact on the color properties of all laver samples. Our results are in agreement with the previously published report by Ko et al. [20], wherein no significant color change was observed in whole black pepper powder and commercial Sunsik irradiated at 2–16 kGy doses.

Effects of irradiation on chlorophyll and carotenoid contents

Chlorophyll content

Dried laver is used as a food colorant and garnishing agent in *Kimbab* and *sushi*. The color properties are attributed to the presence of photosynthetic pigments such as chlorophyll and carotenoids [21]. The effects of irradiation on chlorophyll and carotenoid contents of the samples are shown in Fig. 2. The chlorophyll content of non-irradiated laver was in the range of 552.32–772.69 mg/100 g and in

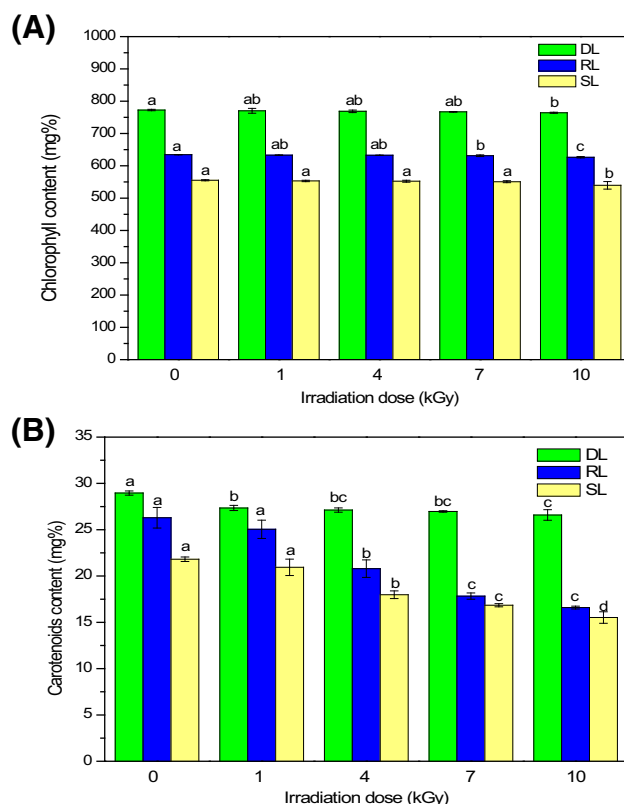


Fig. 2 Chlorophyll content (mg %, DW) (A) and carotenoids content (mg %, DW) (B) of E-beam-irradiated dried processed laver products

the following order: DL > RL > SL. No significant change in the chlorophyll content was observed for DL samples even at high doses of irradiation (10 kGy). Overall, the chlorophyll content showed a stable trend in all irradiated samples as compared with the control samples. However, a small but nonsignificant ($p < 0.05$) decrease in the chlorophyll content was observed for both RL and SL at a high dose of 10 kGy. The carotenoid content of the unexposed laver samples ranged from 21.81 to 28.96 mg/100 g and decreased with the addition of the processing as chlorophyll. No significant difference was observed in the amount of carotenoid in DL samples subjected to irradiation at concentrations as high as 10 kGy; however, the carotenoid content slightly decreased in RL and SL samples irradiated at a dose of 4 kGy or more. The stable characteristics of chlorophyll in the irradiated laver samples resulted in the maintenance of the green color. A previous study observed no significant variation in the chlorophyll content of Chinese cabbage sprouts upon exposure to E-beam irradiation at different doses; the chlorophyll content remained stable in both irradiated and non-irradiated sprout samples during storage [22].

Carotenoid content

The carotenoid content in laver samples was sensitive to E-beam irradiation; a significant decrease in the carotenoid content was observed for DL samples even at 1 kGy dose as compared with the non-irradiated control (0 kGy). All the irradiated DL samples showed similar carotenoid content, which remained unaffected at different doses of irradiation. The carotenoid content of RL samples decreased following irradiation treatment in a dose-dependent manner as compared with the untreated control (0 kGy). A significant decrease in carotenoid content was observed in RL samples treated at 4 and 7 kGy ($p < 0.05$) as compared with the untreated control and those treated at 1 kGy dose. The carotenoid content was similar between samples treated at 7 and 10 kGy, indicating that the higher dose of 10 kGy failed to exert any significant effect on the carotenoid content of the irradiated laver samples. Similar results have been reported by Kwon et al. [22], wherein irradiation treatment at 3 kGy dose considerably decreased the carotenoid content of Chinese cabbage seeds.

Photostimulated luminescence (PSL) screening results of E-beam-irradiated lavers

Food materials are composed of inorganic particles (contaminating dust) such as silicates, which store energy, and this stored energy may be used to measure the luminescence properties in response to optical stimulation [12]. Food materials may be categorized based on the irradiation

status through the potential application of radiation-induced characteristics. In general, PSL technique may be used for the rapid screening of samples on a large scale without any need for sample pretreatment [13, 14]. The results of PSL analysis of irradiated or non-irradiated laver samples are tabulated in Table 3. Of the total nine samples of three different laver types, the non-irradiated control samples showed PCs in the range of lower threshold value (380–461 PCs) (T_1 : < 700 PCs, negative), thereby highlighting the non-irradiated status of all control samples. On the other hand, DL and RL samples irradiated with 1 kGy dose showed PC values in the intermediate range [700–5000 PCs; DL (1123 PCs) and RL (779 PCs)]. PC values were reported to be above intermediate range for all other laver samples. In comparison with DL and RL samples, SL samples showed a significant rise in PC values, which increased with the corresponding increase in the radiation dose from 1 to 10 kGy. PSL is a screening technique, and more sophisticated analytical techniques are required for the absolute confirmation of the irradiation status of the samples. However, PSL analysis can discriminate samples effectively based on their irradiation history, as indicated with higher PC values. Previous studies have reported the reliability of PSL as a screening technique for dried vegetables (exposed to irradiation at 3 kGy or higher doses) [23] and teas (exposed to a dose of 5 and 10 kGy) [24]. Different confirmatory techniques such as TL and ESR may be used for better discrimination of radiation-treated samples with intermediate results or very low sensitivity to irradiation treatment [25].

Thermoluminescence detection results of E-beam-irradiated lavers

Thermoluminescence technique has been extensively used as per the European standard [14] and involves separation of contaminating inorganic minerals existing in foods as silicate minerals (environmental contaminants) through density separation method. In particular, silicate minerals such as feldspar and quartz store energy by charge trapping. This energy is released in a controlled manner in response to the heating of the isolated silicate minerals, giving rise to TL intensities because both feldspar and quartz exhibit well-defined radiation-specific TL characteristics. This property may be employed for effective discrimination of irradiated food samples from their non-irradiated counterparts. TL technique has been successfully employed for the characterization of the irradiation status of various foodstuffs such as teas, sesame seeds, shellfish, shrimps, and dried anchovies [14, 24, 26]. TL analysis was performed after isolation of silicate minerals by density separation method to confirm PSL screening results. The results of TL analysis of the three laver types are shown in

Table 3 Photostimulated luminescence properties of processed laver of different kinds according to the E-beam irradiation

| Sample | Irradiation dose (kGy) | | | | |
|--------|----------------------------------------------|----------------------------|-------------------------------|-------------------------------|-------------------------------|
| | 0 | 1 | 4 | 7 | 10 |
| DL | 461.33 ± 50.90 ^a (-) ^b | 1122.67 ± 456.58 (M) | 7471.33 ± 4501.28 (+) | 6555.33 ± 1614.56 (+) | 7196.00 ± 1573.70 (+) |
| RL | 380.00 ± 17.35 (-) | 779.33 ± 18.90 (M) | 5583.33 ± 823.60 (+) | 8363.00 ± 3187.90 (+) | 6711.67 ± 1203.76 (+) |
| SL | 391.67 ± 51.07 (-) | 346,898.33 ± 99,605.57 (+) | 1,393,983.67 ± 782,895.68 (+) | 1,431,237.33 ± 987,747.62 (+) | 1,482,790.33 ± 261,331.95 (+) |

Photon counts: PCs/60 s

^aMean ± SD (n = 5)^bThreshold value: $T_1 = 700$, $T_2 = 5000$, (-) < T_1 , T_1 < (M) > T_2 , (+) > T_2

Fig. 3. Similar results were observed for all samples regardless of the sample type and form. The results provide a clear discrimination between irradiated and non-irradiated samples, and TL intensities of TL glow curves showed the clear difference based on the applied irradiation dose. As shown in Fig. 3, TL glow curve intensities and shapes were used for the interpretation of the results. All non-irradiated control samples displayed weak TL glow curves at maximum peak temperatures after 350 °C. TL signals obtained for non-irradiated control samples after 300 °C could be related to natural radiations, which are employed in archeological studies for dating. On the other hand, irradiated samples displayed TL glow curves with distinctive shapes in the peak temperature range of 180–220 °C. Electron traps were produced (a process termed as ionization) in the mineral lattice of irradiated samples as a consequence of the exposure to ionizing irradiation. All samples showed TL glow curves of similar shapes, but a significant difference was observed in the intensity of TL glow curves depending on the laver sample type. The highest TL intensity was observed for SL samples, followed by DL and RL samples. These differences may be associated with the variability in the mineral composition of different sample types [27, 28]. TL glow characteristics are mainly defined by the presence of feldspar and quartz; however, TL glow curve characteristics may be affected by their relative abundance [14, 29]. Conclusive evidences may be achieved for the irradiation history of the sample by determining two key factors—intensity and shape of TL glow curve. Furthermore, the analytical reliability of this technique may be ascertained by calculating TL ratio following a normalization step (re-irradiation at a normalization dose of 1 kGy) [14]. The main dependence of TL results lies with quality and quantity of the minerals present on TL disks. After normalization step of the already measured (TL₁) TL signal, the re-irradiated TL disks were reused to obtain the second (TL₂) TL glow curve. TL ratios (TL₁/TL₂) of laver samples listed in Table 4 reveal that the non-irradiated control samples demonstrated TL ratios of < 0.1, while the irradiated samples showed TL ratios of > 0.1. This observation is in line with that reported by Ahn et al. [29], wherein non-irradiated samples showed TL ratios of < 0.1 with weak TL glow curves, while the irradiated samples with well-defined glow curves had TL ratios of > 0.1.

Sensory properties of E-beam-irradiated processed laver products

Sensory analysis was performed to evaluate the effects of E-beam irradiation on sensory profiles of three processed laver types. Table 5 shows the results of the sensory properties of processed laver products (DL, RL, and SL) in

control and irradiated samples. DL samples irradiated at 1, 4, and 7 kGy doses failed to show any significant changes in sensory attributes as compared to their untreated control samples, whereas those samples irradiated at a higher dose of 10 kGy showed a remarkable decrease in odor attribute as compared with the untreated control (0 kGy). The irradiated RL samples showed no significant difference as compared with the control (0 kGy) in terms of sensory attributes such as color, flavor, odor, taste, and overall acceptability. A small but nonsignificant change in sensory

attributes was observed for samples treated at 7 and 10 kGy. A similar trend was observed for SL samples, and the applied irradiation doses failed to affect their sensory attributes (Table 5). An et al. [30] reported similar observation for duck meat samples irradiated at a dose of 4.5 kGy.

In summary, mostly processed lavers are susceptible to high microbial contamination during growth season, owing to the presence of water-borne microorganisms and processing operations such as drying. In this study, the effects

Fig. 3 TL glow curves of dried processed laver of different kinds according to the E-beam irradiation. *DL* dried laver, *RL* roasted laver, *SL* seasoned roasted lavers

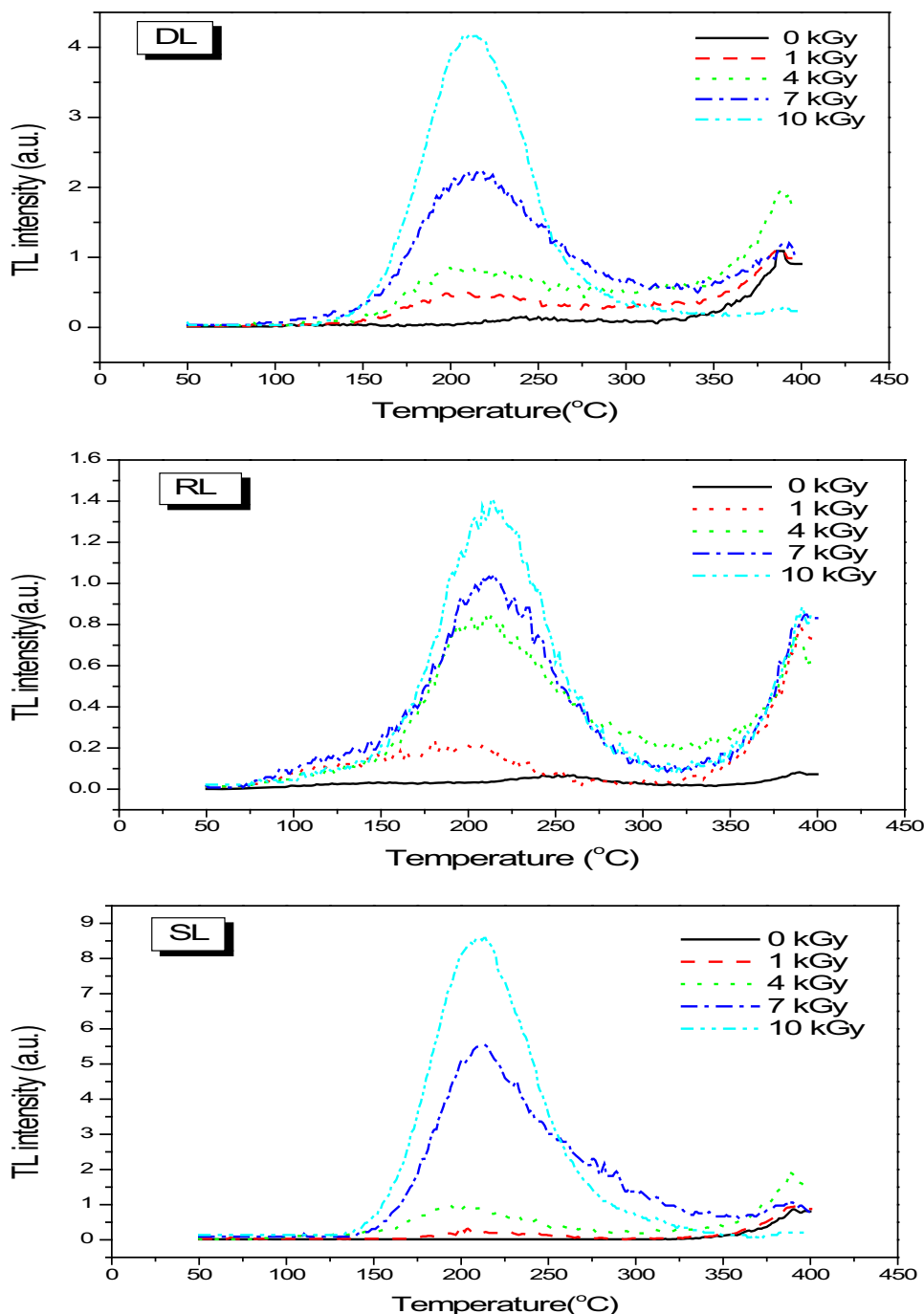


Table 4 TL ratio (TL₁/TL₂) of minerals separated from processed laver products

| Sample | Irradiation dose (kGy) | TL ₁ ^a (first measured) | TL ₂ ^b (second measured) | TL ratio ^c (TL ₁ /TL ₂) |
|--------|------------------------|-----------------------------------------------|------------------------------------------------|-----------------------------------------------------------|
| DL | 0 | 1.076 ± 0.032 ^d | 216.96 ± 21.578 | 0.005 ± 0.004 (–) |
| | 1 | 1.299 ± 0.121 | 2.748 ± 0.456 | 0.473 ± 0.120 (+) |
| | 4 | 21.042 ± 2.321 | 118.350 ± 48.745 | 0.178 ± 0.047 (+) |
| | 7 | 25.683 ± 5.647 | 210.280 ± 47.268 | 0.122 ± 0.052 (+) |
| | 10 | 187.730 ± 32.154 | 39.870 ± 2.476 | 4.709 ± 0.908 (+) |
| RL | 0 | 6.563 ± 0.856 | 92.910 ± 10.734 | 0.072 ± 0.017 (–) |
| | 1 | 37.484 ± 12.850 | 68.845 ± 22.465 | 0.534 ± 0.009 (+) |
| | 4 | 101.755 ± 17.685 | 128.305 ± 25.010 | 0.795 ± 0.017 (+) |
| | 7 | 211.700 ± 6.930 | 198.105 ± 3.528 | 1.098 ± 0.116 (+) |
| | 10 | 145.890 ± 8.188 | 70.330 ± 5.020 | 2.075 ± 0.032 (+) |
| SL | 0 | 1.913 ± 0.180 | 71.810 ± 3.748 | 0.027 ± 0.004 (–) |
| | 1 | 23.545 ± 2.170 | 66.025 ± 6.371 | 0.357 ± 0.002 (+) |
| | 4 | 64.360 ± 5.176 | 154.775 ± 15.804 | 0.416 ± 0.009 (+) |
| | 7 | 83.965 ± 9.567 | 89.595 ± 9.327 | 0.937 ± 0.009 (+) |
| | 10 | 320.800 ± 13.435 | 164.270 ± 11.837 | 1.955 ± 0.059 (+) |

^aIntegrated TL₁ intensity at 150–250 °C^bIntegrated TL₂ intensity at 150–250 °C^cTL₁ intensity/TL₂ intensity^dMean ± SD (*n* = 3)**Table 5** Changes in sensory scores of processed laver according to E-beam irradiation

| Sample ¹ | Sensory properties ² | Irradiation dose (kGy) | | | | |
|---------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | | 0 | 1 | 4 | 7 | 10 |
| DL | Color | 4.00 ± 0.00 ^{n.s.} | 4.40 ± 1.71 ^{n.s.} | 4.30 ± 1.06 ^{n.s.} | 3.90 ± 0.99 ^{n.s.} | 3.90 ± 1.10 ^{n.s.} |
| | Flavor | 4.00 ± 0.00 ^{n.s.} | 4.30 ± 1.06 ^{n.s.} | 4.30 ± 0.67 ^{n.s.} | 3.80 ± 0.32 ^{n.s.} | 3.70 ± 0.25 ^{n.s.} |
| | Odor | 4.00 ± 0.00 ^{n.s.} | 3.90 ± 0.91 ^{n.s.} | 3.90 ± 1.10 ^{n.s.} | 3.40 ± 0.71 ^{n.s.} | 2.60 ± 1.07 ^{n.s.} |
| | Taste | 4.00 ± 0.00 ^{n.s.} | 4.10 ± 0.60 ^{n.s.} | 4.80 ± 0.79 ^{n.s.} | 4.20 ± 0.79 ^{n.s.} | 3.80 ± 1.03 ^{n.s.} |
| | Overall acceptability | 4.00 ± 0.00 ^{n.s.} | 4.30 ± 0.49 ^{n.s.} | 4.30 ± 0.82 ^{n.s.} | 4.20 ± 0.92 ^{n.s.} | 3.50 ± 0.53 ^{n.s.} |
| RL | Color | 4.00 ± 0.00 ^{n.s.} | 4.60 ± 1.07 ^{n.s.} | 4.30 ± 0.82 ^{n.s.} | 4.30 ± 1.06 ^{n.s.} | 4.60 ± 0.84 ^{n.s.} |
| | Flavor | 4.00 ± 0.00 ^{n.s.} | 4.40 ± 0.84 ^{n.s.} | 4.50 ± 0.65 ^{n.s.} | 4.20 ± 0.48 ^{n.s.} | 3.50 ± 0.85 ^{n.s.} |
| | Odor | 4.00 ± 0.00 ^{n.s.} | 4.30 ± 0.82 ^{n.s.} | 4.00 ± 0.76 ^{n.s.} | 4.40 ± 0.51 ^{n.s.} | 3.60 ± 0.97 ^{n.s.} |
| | Taste | 4.00 ± 0.00 ^b | 4.10 ± 0.45 ^{n.s.} | 4.40 ± 0.26 ^{n.s.} | 4.90 ± 0.52 ^a | 4.70 ± 0.57 ^{n.s.} |
| | Overall acceptability | 4.00 ± 0.00 ^{n.s.} | 4.80 ± 0.14 ^{n.s.} | 4.50 ± 0.72 ^{n.s.} | 4.80 ± 0.40 ^{n.s.} | 4.40 ± 0.70 ^{n.s.} |
| SL | Color | 4.00 ± 0.00 ^{n.s.} | 4.20 ± 1.62 ^{n.s.} | 4.30 ± 0.48 ^{n.s.} | 4.10 ± 0.88 ^{n.s.} | 4.70 ± 1.06 ^{n.s.} |
| | Flavor | 4.00 ± 0.00 ^b | 5.30 ± 0.34 ^a | 4.60 ± 0.43 ^{ab} | 4.30 ± 0.49 ^{n.s.} | 4.20 ± 0.48 ^{n.s.} |
| | Odor | 4.00 ± 0.00 ^{n.s.} | 4.60 ± 1.07 ^{n.s.} | 4.10 ± 0.45 ^{n.s.} | 5.00 ± 0.56 ^{n.s.} | 4.20 ± 1.10 ^{n.s.} |
| | Taste | 4.00 ± 0.00 ^{n.s.} | 5.10 ± 0.29 ^a | 3.70 ± 0.67 ^{n.s.} | 4.30 ± 0.25 ^{n.s.} | 4.00 ± 0.25 ^{n.s.} |
| | Overall acceptability | 4.00 ± 0.00 ^{n.s.} | 5.00 ± 0.56 ^{n.s.} | 4.30 ± 0.48 ^{n.s.} | 4.40 ± 0.70 ^{n.s.} | 4.20 ± 1.03 ^{n.s.} |

n.s. (nonsignificant)¹DL dried laver, RL roasted laver, SL seasoned roasted laver²Sensory evaluation was conducted by 15 panelists using a 7-point hedonic scale (7 = very good, 1 = very bad)^{a,b}Values within the same column with different superscript letters are significantly different at *p* < 0.05

of E-beam irradiation at different doses including 7 kGy (approved by Korean Food Code for seaweed food) were evaluated on microbial quality (total aerobic bacteria, yeasts and molds, and coliforms) and physicochemical attributes (Hunter's color values, chlorophyll and

carotenoids contents, and sensory properties). E-beam irradiation resulted in a dose-dependent reduction in the microbial counts; 1 kGy dose reduced coliforms (< 2.35 log CFU/g) to undetectable levels (< 10 CFU/g), while 7 kGy dose reduced total aerobic bacterial counts and

yeasts and molds by about 2 log cycles, thereby ensuring the microbial levels up to less than 4 log CFU/g for the exporting processed laver products without coliforms. Chlorophyll content remained unaffected in irradiated samples as compared to control; however, carotenoids content and Hunter's *b* values (degree of yellowness) showed tendencies to decrease in dose-dependent manner ($p < 0.05$). Nevertheless, E-beam irradiation induced no significant effects on the sensory properties of processed laver products. Irradiated laver products (DL, RL, and SL) could be clearly screened and detected from the non-irradiated counterparts using PSL and TL analyses. Thus, E-beam irradiation is found to be effective for securing the microbial quality of processed laver products for exporting without affecting the sensory properties.

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