

## Physicochemical and Microbiological Qualities of Steamed and Irradiated Ground Black Pepper (*Piper nigrum* L.)

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The effects of steam and irradiation treatments on the physicochemical properties (moisture content, pH, extractable yield, reducing sugar, soluble pigment, antioxidant activity, piperine, Hunter's color, and sensory attributes) and microbiological quality (total aerobic bacteria, coliforms, and yeasts and molds) of ground black pepper stored at refrigerated and room temperatures for 6 months were compared and evaluated. Irradiation resulted in a higher microbial reduction in pepper, with minimal effects on the proximate composition, functional components, color, and sensory attributes of the spice. Steamed peppers appeared darker, and a considerable decrease in the piperine content was observed after treatment and storage. This study illustrates that irradiation is a better decontamination method than steam treatment in eliminating microorganisms without apparently affecting the quality of the powdered spice. Storage at 4 °C enhanced the microbial quality and minimized the loss of piperine content in ground black peppers.

**KEYWORDS:** Black pepper; irradiation; microbiological quality; physicochemical property; steam treatment; storage

### INTRODUCTION

Black pepper is among the most widely consumed food spices in the world due to its distinctive flavor, color, and aroma. Added at any stage of the cooking process or as a table condiment, ground black pepper became popular in cuisine worldwide because of its characteristic pungent taste. However, peppers are often contaminated with high levels of molds, yeasts, and bacteria (1–3) due to improper handling and contaminated surroundings (4), which then results in a rapid spoilage of the foods that they are supposed to enhance. The high temperature and humidity in tropical countries where peppers are usually grown may also favor the development of microorganisms in fruits and seeds (5, 6). Mycological growth in peppers creates serious problems because of the mycotoxin-producing potential of molds. High levels of aflatoxin B<sub>1</sub>, a highly toxic secondary metabolic product of *Aspergillus flavus* and *Aspergillus parasiticus*, were found in powdered pepper (5, 7). Pathogenic bacteria, such as *Salmonella* sp. and *Bacillus cereus*, were also reported to contaminate ground black pepper and other dried

spices (6, 8, 9). The contaminated spices, when added to foods that do not undergo further cooking, such as processed meat, can result in serious foodborne illness.

Fumigation with ethylene oxide is one of the oldest decontamination methods for disinfecting spices. It has been proven to significantly reduce microbial population in spices (10). However, because of the carcinogenic properties of ethylene oxide (11–13), its safety standards have grown increasingly more restricted in recent years, and many countries have suspended its use (4). Because the use of ethylene oxide has been banned, alternative methods, such as steam treatment and irradiation, have gained popularity in the spice industry.

Steam treatment involves the application of high-temperature steam for a short period of time to whole spices prior to grinding (4) to destroy pathogens and reduce the total bacteria in the produce. It was reported that treatment of paprika with superheated steam resulted in a reduced number of microorganisms (14). Steam-treated pepper is readily accepted by consumers worldwide, as the process does not involve the use of chemicals. However, application of steam to spices is associated with color degradation, a decrease in volatile oil content, and an increase in moisture content of the spices, which leads to a decreased shelf life (1, 14).

Irradiation with ionizing energies is another method for disinfecting ground peppers. It has become an economically

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**Table 1.** Chemical Properties<sup>a</sup> of Steamed and Irradiated Ground Black Peppers Stored at 4 and 20 °C for 6 Months

parameter	month	refrigerated temperature (4 °C)			room temperature (20 °C)		
		control	steamed	irradiated	control	steamed	irradiated
moisture content (%)	0	13.88 ± 0.25 ax	10.23 ± 0.24 cx	12.84 ± 0.04 bx			
	6	11.91 ± 0.57 ay	10.29 ± 0.83 bx	10.86 ± 0.66 aby	10.91 ± 0.38 ab	9.97 ± 0.65 b	10.32 ± 0.42 b
pH	0	6.35 ± 0.11 by	6.41 ± 0.05 by	6.77 ± 0.02 ax			
	6	6.63 ± 0.05 bx	6.92 ± 0.01 ax	6.56 ± 0.01 cy	6.48 ± 0.06 d	6.93 ± 0.01 a	6.63 ± 0.01 b
yield (%)	0	0.20 ± 0.01 by	0.15 ± 0.00 cy	0.22 ± 0.01 ay			
	6	0.25 ± 0.02 ax	0.19 ± 0.01 bx	0.26 ± 0.02 ax	0.25 ± 0.02 a	0.18 ± 0.01 b	0.27 ± 0.02 a
total reducing sugar (% d.b.)	0	1.96 ± 0.01 ax	0.77 ± 0.15 by	2.06 ± 0.05 ax			
	6	1.72 ± 0.16 bx	1.19 ± 0.04 cx	2.18 ± 0.11 ax	1.61 ± 0.10 b	1.03 ± 0.12 c	1.75 ± 0.30 b
total soluble pigment (O.D. 420 nm)	0	0.27 ± 0.00 by	0.26 ± 0.00 cy	0.30 ± 0.00 ay			
	6	0.66 ± 0.01 cx	0.62 ± 0.01 dx	0.70 ± 0.02 bx	0.67 ± 0.01 c	0.63 ± 0.00 d	0.75 ± 0.02 a

<sup>a</sup> Values represent means ± SD, *n* = 3. Means followed by the same letters within the row (a–d) and within the column per parameter (x and y) are not significantly different (*p* < 0.05).

viable alternative to fumigation. Irradiation of dried spices is widely recognized and is now legally accepted in at least 51 countries with a maximum overall average of 10 kGy (15). In some countries, such as Australia, New Zealand and the United States, up to a 30 kGy dose is permitted. Irradiation at 10 kGy was found to be effective in destroying bacteria and molds without affecting the quality attributes of different spices (16–19). This study was conducted to compare and evaluate the effects of steam treatment and irradiation on the physicochemical properties and microbiological quality of ground black pepper. The effect of storage time and temperature on the quality of spice was also assessed.

## MATERIALS AND METHODS

**Sample Preparation, Treatment, and Storage.** Black peppercorns were divided into three lots (~1 kg each). One lot was steamed under the commercial conditions of a batch type at 1020 mbar and about 100 °C for 16 min using a steam sterilizer (DEBAC, Bucher, France). The steamed, whole peppercorns were processed into powder form using a sterile laboratory Waring blender (Dynamic Corp. of America, New Hartford, CT) and sieved in a testing sieve (60 mesh, 250 μm) (Chung Gye Industrial Mfg., Co., Korea) in a clean bench. The second lot was also powdered, packed in polyethylene bags, and irradiated at 10 kGy using a Co-60 γ-irradiator (Kaeri, Cheongseup, Korea) with a dose rate of 2.5 kGy/h. An absorbed dose was verified using a ceric/cerous dosimeter (Bruker Instruments, Rheinstetten, Germany). The third lot, which served as the control, was powdered and packed in polyethylene bags. All samples were stored at 4 °C and analyzed for physicochemical and microbiological qualities. Samples from each lot were subdivided into two. The first half was kept refrigerated at 4 °C, and the other half was kept at 20 °C. All analyses were repeated after 6 months of storage.

**Determination of Moisture Content.** The moisture content of steamed or irradiated ground black pepper was determined using an Infrared Moisture Determination Balance (FD-240, Japan). Approximately 1 g of each sample was used to determine the % moisture.

**Determination of pH.** Pepper extracts were prepared by mixing 2 g of powder samples with 80 mL of distilled water in a shaker-incubator for 3 h at 200 rpm. The mixture was centrifuged, and the supernatant was filtered (Whatman #41). The pH was determined using a pH meter (Thermo Scientific Orion Star Series, United States).

**Determination of Extractable Yield Content.** A 2 mL sample extract was placed in an aluminum disk having a predetermined constant weight and dried in an oven at 105 °C until a constant weight was obtained.

**Analysis of Total Reducing Sugar Content.** The total reducing sugar contents of the powder samples were determined using the modified Somogyi method (20).

**Analysis of Total Soluble Pigment Content.** One gram of sample was mixed with 80 mL of distilled water for 3 h at 200 rpm. The mixture was centrifuged, and the supernatant was filtered (Whatman

#41). The extract was placed in a 100 mL volumetric flask and filled up with distilled water. Absorbance of the extract was measured at 420 nm using a UV–vis spectrophotometer (Optizen 2120UV, Korea).

**Measurement of Antioxidant Activity by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Method.** One gram of sample was added with 50 mL of ethanol (50%) and mixed overnight. After centrifugation, the ethanol extract was added with DPPH solution, and the absorbance was measured at 517 nm using a UV–vis spectrophotometer following the method of Blois (21).

**Analysis of Piperine Content.** The piperine content of ground black pepper was determined based from the AOAC method (22).

**Determination of Hunter's Color Values.** The Hunter's color *L* (lightness), *a* (redness), and *b* (yellowness) values were determined using a colorimeter (Minolta CR-200, Japan). Powdered samples were spread on a piece of paper, and measurements were taken at three random locations. Averages were reported. A numerical total color difference ( $\Delta E$ ) was calculated from the Hunter values obtained. The *L*, *a*, and *b* values of the standard plate were 97.66, –0.36, and 1.92, respectively.

**Sensory Evaluation.** The peppers were evaluated for color, odor, pungent taste, and overall acceptability by 15 trained judges. Approximately 2 g of black pepper in a disposable Petri dish was given to each panelist. All samples were coded with three-digit numbers. The panelists were instructed to record their rating using a five-point hedonic scale (5 = like extremely and 0 = dislike extremely for color, odor, and overall acceptability; and 5 = extremely strong and 0 = extremely weak for pungent taste) (23). Odor and pungent taste evaluation were performed under ordinary light conditions using a pepper powder fusion, which was prepared by dissolving 1 g of the powder into 100 mL of water (60 °C).

**Microbiological Analysis.** All samples were analyzed for the total aerobic bacteria, yeasts and molds, and coliform counts. Five grams of pepper powder was mixed with 45 mL of sterile peptone water. Subsequent dilutions were prepared and plated on plate count agar for the total aerobic bacteria, potato dextrose agar (acidified with 10% tartaric acid) for yeasts and molds, and desoxycholate agar for coliforms. Microbial counting was performed 24–48 h after incubation at 30 and 37 °C for total aerobic bacteria and coliforms, respectively. Yeast and mold colonies were counted 3 days after incubation at 30 °C.

**Statistical Analysis.** Results from the measurements (*n* = 3) were analyzed statistically using the Statistical Analysis System for Windows V8. Analysis of variance and Duncan's multiple range test were employed.

## RESULTS AND DISCUSSION

**Chemical Properties.** The chemical properties of steamed or irradiated ground black pepper are presented in **Table 1**. The moisture content of steamed pepper was significantly lower than that of the control and irradiated ones. While it is believed that steam treatment could cause a significant increase in the moisture level of spices, it is likely that the re-evaporation stage during

**Table 2.** Functional Components<sup>a</sup> of Steamed and Irradiated Ground Black Peppers Stored at 4 and 20 °C for 6 Months

parameter	month	refrigerated temperature (4 °C)			room temperature (20 °C)		
		control	steamed	irradiated	control	steamed	irradiated
electron-donating ability (% d.b.)	0	86.67 ± 0.64 ay	80.02 ± 0.38 by	80.07 ± 0.59 by			
	6	89.36 ± 0.46 ax	82.43 ± 0.71 dx	87.86 ± 0.24 bx	89.07 ± 0.60 a	85.09 ± 0.36 c	85.38 ± 0.98 c
piperine (% d.b.)	0	12.36 ± 0.03 ax	9.30 ± 0.08 cx	11.10 ± 0.07 bx			
	6	9.69 ± 0.36 ay	6.67 ± 0.03 ey	9.04 ± 0.19 by	7.83 ± 0.07 d	5.75 ± 0.06 f	8.48 ± 0.06 c

<sup>a</sup> Values represent means ± SD, *n* = 3. Means followed by the same letters within the row (a–f) and within the column per parameter (x and y) are not significantly different (*p* < 0.05).

**Table 3.** Hunter's Color Values<sup>a</sup> of Steamed and Irradiated Ground Black Peppers Stored at 4 and 20 °C for 6 Months

Hunter's parameter <sup>b</sup>	month	refrigerated temperature (4 °C)			room temperature (20 °C)		
		control	steamed	irradiated	control	steamed	irradiated
<i>L</i>	0	55.52 ± 0.47 ax	52.70 ± 0.30 by	54.79 ± 0.37 ay			
	6	56.35 ± 0.67 bx	54.13 ± 0.19 cx	56.00 ± 0.22 bx	57.43 ± 0.31 a	54.42 ± 0.09 c	56.14 ± 0.22 b
<i>a</i>	0	2.41 ± 0.04 by	2.84 ± 0.14 ay	2.45 ± 0.07 by			
	6	2.63 ± 0.12 cx	3.24 ± 0.06 ax	2.64 ± 0.09 cx	2.14 ± 0.23 d	3.03 ± 0.06 b	2.66 ± 0.02 c
<i>b</i>	0	13.22 ± 0.18 ax	9.97 ± 0.03 bx	12.78 ± 0.38 ax			
	6	11.25 ± 0.34 ay	8.88 ± 0.13 by	11.44 ± 0.07 ay	11.40 ± 0.37 a	8.94 ± 0.07 b	11.24 ± 0.13 a
$\Delta E$	0	0.00	2.08	0.60			
	6	1.26	0.53	0.87	2.31	0.23	1.05

<sup>a</sup> Values represent means ± SD, *n* = 3. Means followed by the same letters within the row (a–d) and within the column per parameter (x and y) are not significantly different (*p* < 0.05). <sup>b</sup> Hunter's parameters: *L*, degree of whiteness (white + 100 → 0 black); *a*, degree of redness (red + 100 → -80 green); *b*, degree of yellowness (yellow + 70 → -80 blue); and  $\Delta E$ , overall color difference ( $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ ).

the steaming process in this study sufficiently removed the adsorbed water on the surface of the spice, thus maintaining the low moisture content. Storage for 6 months resulted in reduced moisture levels in both control and irradiated peppers, whereas storage temperature had no considerable effects on the moisture content of the spices. A slightly higher pH was initially observed in irradiated black pepper, but it became lower after storage. The decrease in pH during storage was probably due to an increased amount of organic acids released during irradiation treatment. Steamed pepper exhibited low amounts of extractable yield, reducing sugar, and soluble pigment. Kispeter et al. (24) previously reported that application of saturated steam in spice paprika resulted in an enhanced loss of pigment content. Steam treatment involves the application of high-temperature steam, in which some of the chemical components of spices are degraded and diffused into the vapor during the process (4, 14). Irradiated black pepper, on the other hand, exhibited slightly higher amounts of extractable yield and soluble pigment than that of the untreated samples, which were fairly stable during storage regardless of the storage temperature. This may be due to the structural alteration during irradiation, which resulted in an increased extractability of some compounds in pepper (4, 18, 25).

**Functional Components.** The DPPH scavenging activity of pepper decreased considerably after both treatments (Table 2). However, a significant increase in the antioxidant activity of all black pepper samples was observed after storage. These results are in accordance with the findings of Suhaj et al. (26) wherein irradiation at 5–30 kGy significantly decreased the DPPH radical-scavenging activity and reducing power of ground black pepper extracts; however, a 4–9% increase of the DPPH scavenging activity was observed in both irradiated and non-irradiated peppers after 5 months of storage. This was attributed to the increase of dry matter content during storage of the spice due to the decrease in moisture content.

Irradiation slightly decreased the piperine content of black pepper, but steam treatment resulted in a considerable reduction of the compound. Studies have shown that heat processing caused a significant loss in the piperine content of black pepper

due to the heat-induced chemical alterations in the pungent component of the spice (25). Storage of black pepper at room temperature for 6 months resulted in a lower piperine content of all of the samples, indicating the importance of keeping ground black peppers under refrigeration. Storage of spices under high temperature and humid conditions could promote microbial growth and metabolic activities of the endogenous deteriorative enzymes, which are not completely inactivated during treatments. This may lead to water loss, deterioration of flavor and color, softening and decay, and losses of valuable compounds such as piperine (4).

**Hunter's Color Values.** Steam treatment caused discoloration in black pepper, whereas irradiation did not significantly change the color of the spice. The steamed pepper exhibited reduced *L* and *b* values (Table 3), indicating that the sample became darker. Furthermore, the degree of redness became more intense (higher *a* value) after steam treatment. In studying the effect of thermal treatment on the color properties of paprika, Almela et al. (14) noted that high temperature promotes color degradation as the paprika became darker. While storage temperature did not affect the color of the pepper samples, prolonged storage resulted in the darkening of the samples as indicated by the increase in *a* and *b* values. The overall changes in color ( $\Delta E$ , NBS) of ground black pepper revealed that irradiation slightly influenced (0.60) the color of the spice powder, but steam treatment resulted in a noticeable color difference (2.08), which became slight (0.53) after storage (27). Similarly, Kispeter et al. (24) reported that steamed paprika showed a significantly higher color change than that of the irradiated sample. A noticeable color difference (2.31) was also observed in untreated peppers stored under room temperature.

**Sensory Attributes.** No significant effect among treatments was observed in the sensory attributes of the samples (Table 4). However, after 6 months, the sensory scores for color and odor became lower in steamed pepper as compared to the control samples regardless of the storage temperature. Such a difference in odor may be due to the disappearance of some volatiles in the pepper after steam treatment (1), which became more

**Table 4.** Sensory Properties<sup>a</sup> of Steamed and Irradiated Ground Black Peppers Stored at 4 and 20 °C for 6 Months

sensory parameter	month	refrigerated temperature (4 °C)			room temperature (20 °C)		
		control	steamed	irradiated	control	steamed	irradiated
color	0	3.5 ± 0.5 ax	3.1 ± 1.1 ax	3.6 ± 0.5 ax			
	6	3.6 ± 0.7 ax	2.4 ± 1.1 bx	2.9 ± 0.5 by	3.7 ± 0.8 a	2.7 ± 1.1 b	3.9 ± 0.8 a
odor	0	3.3 ± 0.7 ax	2.9 ± 1.2 ax	3.2 ± 0.9 ax			
	6	3.5 ± 1.0 ax	2.7 ± 0.9 cx	3.0 ± 0.8 abcx	3.6 ± 0.5 a	2.8 ± 1.1 bc	3.5 ± 0.8 ab
pungent taste	0	3.3 ± 1.2 ax	2.9 ± 1.3 ax	3.1 ± 1.0 ax			
	6	3.7 ± 0.7 ax	3.4 ± 1.1 ax	3.3 ± 0.6 ax	3.4 ± 1.1 a	3.5 ± 1.0 a	3.0 ± 0.7 a
overall acceptability	0	3.3 ± 0.7 ax	3.1 ± 1.1 ax	3.4 ± 0.6 ax			
	6	3.6 ± 0.8 abx	2.8 ± 0.7 cx	3.1 ± 0.6 bcx	3.8 ± 0.8 a	2.9 ± 1.1 c	3.3 ± 0.9 abc

<sup>a</sup> Sensory evaluation was conducted by 15 panelists using a five-point hedonic scale (5 = like extremely and 0 = dislike extremely for color, odor, and overall acceptability; and 5 = extremely strong and 0 = extremely weak for pungent taste). Means followed by the same letters within the row (a–c) and within the column per parameter (x and y) are not significantly different ( $p < 0.05$ ).

**Table 5.** Microbial Count (CFU/g)<sup>a</sup> of Steamed and Irradiated Ground Black Peppers Stored at 4 and 20 °C for 6 Months

microorganism	month	refrigerated temperature (4 °C)			room temperature (20 °C)		
		control	steamed	irradiated	control	steamed	irradiated
total plate count	0	$2.8 \times 10^6$	$7.0 \times 10^3$	$3.5 \times 10^2$			
	6	$6.4 \times 10^6$	$6.7 \times 10^3$	$2.1 \times 10^3$	$3.3 \times 10^6$	$1.2 \times 10^4$	$3.6 \times 10^3$
yeasts and molds	0	$5.2 \times 10^3$	$2.5 \times 10^1$	$7.5 \times 10^0$			
	6	$1.6 \times 10^2$	<10	<10	$5.0 \times 10^1$	<10	<10
coliforms	0	$2.1 \times 10^5$	$1.5 \times 10^1$	$5.0 \times 10^0$			
	6	$8.4 \times 10^3$	<10	<10	$9.8 \times 10^2$	<10	<10

<sup>a</sup> Values represent means,  $n = 3$ .

pronounced after storage. The overall acceptability of steam-treated peppers significantly decreased, suggesting that prolonged storage affects the sensory quality of the steamed samples.

**Microbiological Quality.** The untreated black pepper contained relatively high aerobic bacteria ( $10^6$  CFU/g) (Table 5), but treatment with steam and irradiation reduced the population by 3 and 4 logs, respectively. A similar study also obtained a 3-log reduction in the population of aerobic mesophilic bacteria in black pepper after steam treatment (1). Likewise, Oularbi et al. (3) reported that irradiation at 6 kGy reduced the microbial contamination by 4 logs in black and red peppers. Steaming and irradiation resulted in 2- and 3-log reductions, respectively, in the yeast and mold counts. The control samples exhibited high coliform counts ( $10^5$  CFU/g), but the population decreased by 4 and 5 logs after steam and irradiation treatments, respectively. The results illustrate that irradiation is a better alternative method than steam treatment in disinfecting spice powders. No considerable change on the population of aerobic bacteria was observed in control and steamed peppers after storage at refrigerated temperature, but the counts slightly increased (1 log) in steamed and irradiated samples stored at room temperature. On the other hand, the yeasts and molds and coliform counts further decreased to nondetectable levels after storage. Untreated peppers also exhibited a decrease in the counts after storage. These results showed that storage could further enhance the microbial quality of treated peppers since the injured microbial cells, as a result of steaming and irradiation, were unable to repair and proliferate over time.

Results of this study demonstrated that irradiation is a better decontamination method than steam treatment in disinfecting ground black pepper. While steam treatment reduced the microbial population in the pepper, application of high temperature could cause significant loss of color and flavor in the spice. On the other hand,  $\gamma$ -irradiation at 10 kGy is sufficient to eliminate microorganisms, causing only slight changes in the physicochemical properties of black pepper. To improve the

microbiological quality and minimize the loss of piperine content, ground black peppers must be kept in refrigerated conditions.

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