CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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EFFECT OF ULTRAVIOLET (UV-C) LIGHT AND GASEOUS OZONE ON MICROBIAL AND COLOR QUALITIES OF WHOLE BLACK PEPPER SEEDS (*PIPER NIGRUM* L.)

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https://doi.org/10.34302/crpjfst/2022.14.2.10

Article history:
Received:
23 May 2021
Accepted:
1 April 2022

Keywords:
Ultraviolet light;
Ozone;
Black pepper;
Decontamination;
Escherichia coli.

ABSTRACT

Microbial contamination of spices, especially black pepper, may sometimes reach as high as 8 log cfu/g, which can cause a major problem in the quality and safety of foods they are added. This study aimed to explore the potential of ultraviolet C (UV-C) light and ozone for decontamination of black pepper seeds.

Ozone (15 ppm for 1 h) and UV-C (28.8 J/cm^2) treatments were applied alone, in succession, or simultaneously to whole black pepper seed with a laboratory scale fluidized bed UV-C system. Total aerobic mesophilic bacteria (TAMB) count (in uninoculated seeds), *Escherichia coli* (*E. coli*) count (in inoculated seeds) and color (L*, a* and b*) of black pepper were evaluated.

Ozone and UV-C treatments alone caused 0.41- and 0.77-log reduction in the initial TAMB count (6.97 log cfu/g), respectively. TAMB decreased by 0.74- and 0.66-log upon successive and simultaneous treatments, respectively. Thus, the combined treatments did not have any additive or synergistic effects on TAMB. Both ozone and UV-C treatments alone resulted a 0.8-log reduction in the initial *E. coli* count of 6.3-log. The combined treatments caused an additive effect on inactivation of *E. coli* in black pepper seeds. The successive and simultaneous treatments caused 1.7- and 1.4-log reductions in the *E. coli*, respectively. None of the treatments affected the color (L*, a* and b* values) significantly. In conclusion, the individual treatment has potential for reducing the natural contamination level on the seeds, and the combined treatments may have further potential towards reducing specific microbial contaminations.

1.Introduction

Black pepper (*Piper nigrum* L.) is one of the most widely used spice to enhance the flavour of foods. It is a tropical, perennial climbing plant belonging to the family Piperaceae. The black pepper seeds are produced from berries that have just started to yellow. The berries are generally blanched by immersing them in hot water at 90 ºC for up to 10 minutes. Then, they are dried to reduce moisture content below 12% and to develop the dark, wrinkled layer (Zachariah, 2000). Although these steps reduce microbial load, black pepper, like other herbs and spices, generally contain a high level of microbial load up to 6-8 log cfu/g, which may include pathogenic and/or spoilage organisms. Spices may be contaminated by microorganisms because of poor hygienic conditions under which they are cultivated, harvested, transported and stored. High microbial load of spices may

cause a risk to consumer health if they are added to high moisture foods which are eaten raw or not further processed (Schweiggert et al., 2007; Farkas and Mohácsi-Farkas, 2014). Therefore, appropriate decontamination methods should be applied to spices to food quality and safety.

Fumigation, steam sterilization and irradiation are the most common methods for microbial inactivation in spices (Tainter and Grenis, 2001). Fumigation with ethylene oxide is an oldest decontamination method applied to spices. However, it is not allowed in many countries due its carcinogenic effects (Fowles et al., 2001). Steam sterilization is a chemical-free process but it is associated with color degradation and a reduction in volatile oil content. Additionally, steam sterilization increases the moisture content of spices and requires additional drying step to avoid microbial growth (Schweiggert et al., 2007). Irradiation is another commercial decontamination method, which is approved by many authorities such as Food and Drug Administration, World Health Organisation, and the Codex Alimentarius Commission (Sadecka, 2007). But it has some drawbacks such as formation of oxidative compounds, high costs of installation, and consumer's negative perception (Sharma and Demirci, 2003). Hence, there is a need for finding effective, easily applicable and nontoxic alternative methods to decontaminate spices. Several research studies investigating various technologies to reduce the microbial load of herbs and spices have been reported (Keith et al., 1997; Staack et al., 2008; Eliasson et al., 2014; Hertwig et al., 2015; Nicorescu et al., 2013; Kim et al., 2014; Erdoğdu and Ekiz 2011; Erdoğdu and Ekiz 2013; Cheon et al., 2015).

Ozone is a potent, broad-spectrum antimicrobial agent against a wide variety of microorganisms (Khadre et al., 2001). Because of its high reactivity, high penetration power, short contact time and generally recognized as safe (GRAS) status it can be employed under various forms for decontaminating foods (Patil et al., 2014). Potential of gaseous ozone has been shown for decontamination of herbs and spices such as black pepper (Emer et al., 2008), flaked red peppers (Akbas and Ozdemir, 2008), oregano (Torlak et al., 2013), sumac, cumin and pepper (Hemmati et al., 2017).

UV-C radiation is another effective non-thermal technology generally used for various food surfaces and liquid products. It has lethal effect on microorganisms by forming pyrimidine dimers, disrupting the DNA, which stops its ability to reproduce (Gómez-López et al., 2012). Process parameters, microbial characteristics and product parameters are the main factors affecting microbial resistance to UV-C light (Gayán et al., 2014). The use of UV-C light is limited with food surfaces due to its poor penetrative capacity (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Thus, effective exposure of the surfaces to UV-C is critical for its efficiency in microbial inactivation. This needs new design approaches to make the product surfaces exposed to UV-C effectively. Moreover, hurdle approach to have UV-C combined with other nonthermal methods can be an effective way to enhance microbial inactivation in the products. A lab scale fluidized bed UV-C system was built and tested for decontaminating thyme (*Thymus vulgaris* L.) in our earlier works (Dogu-Baykut et al., 2014; Dogu-Baykut and Gunes, 2019). A limited but significant reduction (up to 1.8-log) in natural microbial load of the samples were observed in these studies. Combination of UV-C and ozone may have improved potential for microbial decontamination of dehyrated herbs and spices. To our knowledge there is no information on effectiveness of such combination on decontamination of powdered spices. The present study was aimed to explore the potentials of combined uses of ozone and UV-C for decontamination of black pepper seeds.

2. Materials and methods

2.1. Chemicals and materials

Unsterilized and dried whole black pepper seeds were obtained from a local company (Kadioglu Spice Co. Inc., Mersin, Turkey). Black pepper seeds, which were separated for use in bacterial inoculation studies, were

weighed to polyethylene (PE) packages and sterilized by gamma rays from a ^{60}Co source at a commercial irradiation facility (Gamma-Pak Sterilization Co., Cerkezkoy-Tekirdag, Turkey) at room temperature.

Sodium chloride was obtained from Riedel-de Haen (Seelze, Germany). Plate count agar (PCA), Chromocult Tryptone Bile X-glucuronide (TBX) Agar, tryptic soy broth (TSB), and buffered peptone water (BPW) was purchased from Merck (Darmstadt, Germany). Peptone was from Oxoid (Basingstoke, Hampshire, UK).

2.2. Preparation of bacterial suspension

A loop of *E. coli* (ATCC 25922) was taken from slant medium and inoculated into TSB broth and incubated at 37℃ for 18 h. Bacterial cells were separated by centrifugation at 5000 rpm for 5 min. The cells were centrifuged for the second time by adding equal volume of TSB and then resuspended in BPW buffer. A stock suspension of *E. coli* with 10^9 cfu/ml in BPW was prepared using McFarland standard (BioMerieux, Durham, NC, USA).

2.3. Inoculation of bacteria

Black pepper samples weighed in a beaker and then the *E. coli* suspension was added into the beaker with a 1:1 ratio (w/w). Intermittent mixing was provided during 5 min dipping period at room temperature to have homogeneous inoculation. The inoculated seeds were separated from the bacterial suspension by filtering through a sterile cheese cloth and placed as a single layer into an open sterile container. The seeds were first incubated at 25 ℃ and 90% relative humidity for 24 h for acclimation of the bacteria on the seeds. Then, the seeds were dried at 30 ℃ and 30% relative humidity for 24 h to achieve their initial moisture and water activity (a_w) levels prior to UV-C and ozone treatments.

2.4. Determination of moisture/relative humidity/water activity

The moisture content of the samples was determined by infrared moisture analyzer (IR35, Denver Instruments, Fisher Scientific, USA). A 2 g. of sample was weighed into the measuring cup and analyzed at 106 ℃.

In the inoculation study, the relative humidity of the environment was measured with a data logger (HOBO U12-013 Temp/RH/2 External Data Logger, Onset Computer Corporation, Bourne, MA, USA) in every 5 minutes.

The a_w of the samples was determined by Lab master a_w device (Novasina, Lachen, Switzerland). After placing the samples into the sample holder, a_w values were measured at 25 ℃.

2.5. UV-C and ozone treatments

The experimental UV-C and ozone system is schematically shown in Figure 1. The UV-C part of the combined system consisted of a quartz glass cylindrical tube (10 cm x 100 cm) with four UV lamps emitting 254 nm light (GHO36T5L/4P, Atlantic Ultraviolet Inc., Hauppauge, NY, USA) installed around it. This system was connected to an air pump with a pipe (100 cm × 10 cm). Air velocity in the treatment area was set approximately to 8m/s to keep the seeds fluidized in the system. Ozone gas was generated using a laboratory scale ozone generator (Model No. H-50, HessMachines International, PA, ABD) and fed through a 6 mm tubing connected to the pipe carrying the air to the system (Fig.1) Ozone concentration was measured continuously by an ozone monitor (Model 106-M, 2B Technologies Co., USA) in the system.

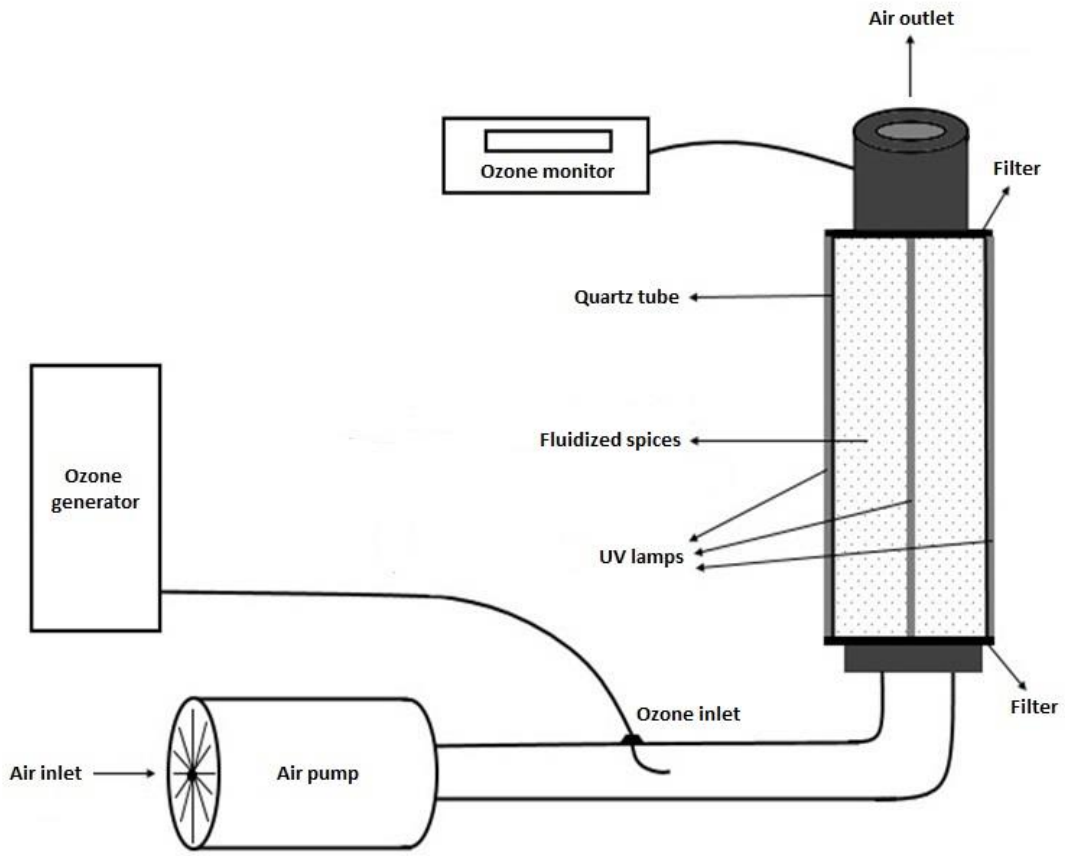

Figure 1. Schematic diagram of the combined UV-C and ozone system.

Each treatment was performed with 10 g sample loaded to the UV system. UV-C and ozone treatments were applied alone (1 h), in succession (1 h ozone after 1 h UV-C) or simultaneously (ozone and UV-C together for 1 h). The ozone application was applied at a concentration of 15 ppm. UV-C intensity was measured with a portable digital radiometer, which measures UV radiation at 254 nm and fitted with a UVX-25 sensor (UVP Inc., Upland, CA, USA), and the average UV intensity was determined as 8 mW/cm^2 in the system. The UV dose was calculated as 28.8 J/cm^2 for 1 h exposure. The control samples were treated for 2 h in the same air flow without ozone and UV-C. The experiment was repeated three times for each treatment.

2.6. Microbial analysis

Pour plate technique was used in microbial analyses (ICMSF 1978). Homogenized samples were prepared by mixing 10 g black pepper seeds with 90 ml of peptone water (0.1%) at medium speed for 2 min using a stomacher (AESAP1068-Easymix, AES Chemunex, Combourg, France). The homogenized samples were diluted in peptone water as needed. The PCA plates used for enumeration of total aerobic mesophilic bacteria were incubated at 37 ℃ for 48 h. TBX plates used for enumeration of *E. coli* were incubated at 44 ℃ for 24 h. The colonies on agar plates were counted and expressed as log cfu/g sample.

2.7. Color analysis

Three color parameters L*, a* and b* of the black pepper samples after UV-C and ozone treatments were measured with a chromameter (Model CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). The instrument was calibrated using a standard white calibration plate (CR-A43, Konica Minolta Sensing Inc., Tokyo,

Japan) prior to analysis. Color values were measured on each sample (10 g) at three different positions and averaged.

The total color difference (ΔE) with reference to the control samples was calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$$

2.8. Statistical analysis

Each treatment was applied in triplicate. Analysis of variance (ANOVA) was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Multiple comparisons of the treatments were done by Duncan's multiple-range test.

3. Results and discussions

3.1. Effect of UV-C and ozone treatments on TAMB

In the preliminary tests, ozone concentration less than 15 ppm did not cause a significant inactivation of total aerobic mesophilic bacteria (TAMB) on black pepper seeds. For this reason only 15 ppm ozone, which was the maximum level achieved in our system, were applied in our study.

Table 1 shows the effect of UV-C and ozone treatments on microbial quality of black pepper samples as assessed by TAMB counts. Initial TAMB count of untreated sample was 6.97 log cfu/g. The UV-C treatment (28.8 J/cm^2) alone decreased the TAMB count of black pepper seeds by 0.77-log ($p<0.05$). The ozone treatment (15 ppm for 1 h) alone caused a 0.41-log reduction in the TAMB count in the samples ($p<0.05$). Although each of the UV-C and ozone treatments resulted in significant reduction in TAMB count of black pepper seeds, inclusion of ozone to UV-C in succession or simultaneously did not have any additive or synergistic effects on inactivation of TAMB ($p>0.05$). TAMB decreased by 0.74- and 0.66-log upon successive and simultaneous treatments, respectively.

We observed a 0.4- and 1.8-log reduction in TAMB count in thyme with 25.7 and 205.6 J/cm^2 UV-C using the same system in our previous work, respectively (Dogu-Baykut and Gunes, 2019). Erdoğdu and Ekiz (2011) reported that UV-C treatment at 37.8 J/cm^2 reduced the TAMB counts of cumin seeds by 0.6-log. Hidaka and Kubata (2006) studied on wheat grain and reported that 6.3 h is required for 1-log reduction in the number of TAMB at a UV-C intensity of 9.7 mW/cm^2 on the recirculating grain sterilization equipment. Different levels of microbial inactivation on dehydrated seeds and herbs by UV-C reported in literature is certainly due to differences in the UV-C system used, food product, and state of microbial flora on the samples.

Limited accessibility of UV-C light to the microorganisms in the lower layers on the surface of foods is the main difficulty in UV light studies. So, hurdle strategies that combine UV light with other novel processing techniques were used in some studies to achieve the necessary microbial reduction. Erdoğdu and Ekiz (2011, 2013) combined UV-C and far infrared radiation (FIR) to decontaminate cumin and black pepper seeds. They obtained additional 1.65-log reduction when cumin seeds exposed to the UV-C light (10.5 mW/cm^2) for 2 h following 5.6 min FIR treatment at 200 ºC. Under the same conditions, combined UV-C and FIR treatments were also used for microbial inactivation of black pepper. However, synergistic effect of UV-C with FIR treatment was not observed. The authors stated that this may be due to surface topography or higher initial microbial count of black pepper seeds.

In the literature, the results of the studies searching decontamination potential of gaseous ozone vary according to the sample type, concentration of ozone gas and exposure time. Surface area is also important factor during ozonation because ground samples require a higher ozone concentration and longer exposure than the whole grain samples to achieve similar microbial inactivation (Akbas and Ozdemir, 2008). Dhillon et al. (2010) designed a fluidized bed system to decontaminate durum wheat grain with gaseous ozone. They found that application of gaseous ozone at 6 ppm for 14 min did not affect aerobic plate count (APC) and yeast and mold count (YMC). In another study conducted by Torlak et al. (2013), gaseous ozone treatment on dried oregano at 2.8 ppm for 30 min did not

significantly reduce initial levels of APC and YMC on oregano, however, ozone treatment at 5.3 ppm for 30 min significantly reduced levels of APC and YMC by 0.5- to 0.4-log, respectively.

In our study, we also obtained a statistically significant decrease of 0.41-log in TAMB count on black pepper after treating with 15 ppm ozone for 1 h. Furthermore, we combined ozone with UV-C treatment. It was expected that ozone-damaged cells could be inactivated by UV-C more effectively, and this combination could have a synergistic effect, but this was not observed in our work. This may be associated with limited penetration of UV-C and ozone to the surface contamination which probably contain biofilm. The inactivated microbial cells by one of the treatments on the most outer surface probably shaded the viable cells in the inner parts against the next treatment.

Table 1. Effect of UV-C and ozone treatments on the TAMB (log cfu/g) of black pepper.

Application method	Application	TAMB	Reduction
Control	2 h air alone	6.97 ± 0.07^{a}	-
Ozone (15 ppm)	1 h	6.56 ± 0.17^{b}	0.41 ± 0.17^{a}
UV-C (28.8 J/cm^2)	1 h	6.20 ± 0.19^{c}	0.77 ± 0.19^{b}
Ozone → UV-C (in succession)	1 h UV-C after 1 h ozone	6.23 ± 0.13^{c}	0.74 ± 0.13^{b}
Ozone + UV-C (in simultaneous)	1 h UV-C and ozone together	6.31 ± 0.02^{c}	0.66 ± 0.02^{ab}

Data represent mean values (n = 3) ± standard deviations
Means within a column having the same letter are not significantly different (p>0.05).

3.2. Effect of UV-C and ozone treatments on *E. coli*

Properties of samples during inoculation steps is shown in Table 2. After inoculation, the samples were stored at 25 ºC and 90% RH for 24 h to stabilize the bacterial population in the samples. In our preliminary studies it was observed that the number of the inoculated bacteria on the sample decreased rapidly when no conditioning at high relative humidity was applied after inoculation. Thus, with the applied inoculation process, a stable *E. coli* count (6.82 log cfu/g) was achieved prior to UV-C/ozone treatments.

Each of the UV-C and ozone treatments caused a 0.8-log reduction in the initial *E. coli* count of 6.3-log, as shown in Table 3. The successive and simultaneous treatments caused 1.67- and 1.38-log reductions in the *E. coli*, respectively. Thus, UV-C and ozone had additive effect on inactivation of inoculated *E. coli* as opposed to TAMB. This may be associated with presence of biofilms of natural flora (TAMB) and lack of biofilms of *E. coli* in freshly inoculated samples. It is known that biofilms make microorganisms more resistant to environmental conditions (Srey et al., 2013).

There are some studies in literature which examined the potential of ultraviolet light and ozone for inactivation of *E. coli* O157:H7 in poultry chiller water and on blueberries (Ngadi et al., 2004; Kim and Hung, 2012). Similar to our results, combined treatments of ozone (4000 ppm for 1 min) and UV-C (0.95 J/cm^2) achieved additional reduction on *E. coli* O157:H7 count on blueberry samples compared to the UV-C or the ozone alone (Kim and Hung, 2012). The UV-C treatment with an intensity of 7.95 mW/cm^2 for 2 min (0.95 J/cm^2) alone caused a 2.2-log reduction in *E. coli* O157:H7 count on the blueberries while the combined treatment caused a 3.7-log reduction on the bacterial count. Similarly, Ngadi et al. (2004) found an additive effect of UV-C (7 J/cm^2) and ozone (1 mg/ml for 30 s) treatments on inactivation of *E. coli* O157:H7 in poultry chiller water when they are applied together.

Table 2. Change of water activity, moisture and *E. coli* count of black pepper seeds during the inoculation with *E. coli* (ATCC 25922)

	Initial sample	**Upon inoculation**	**After 24 h of holding at 25 ºC and 90% RH**	**After drying for 24 h at 30 ºC and 30% RH**
a_w	0.54±0.01	0.94±0.01	0.93±0.01	0.53±0.02
Moisture (%)	3.42±0.13	16.41±0.87	18.02±1.63	3.40±0.12
E. coli count (log cfu/g)	-	7.16±0.12	7.54±0.16	6.82±0.14

Data represent mean values (n = 3) ± standard deviations.

Table 3. Effect of UV-C and ozone treatments on the *E. coli* count (log cfu/g) of inoculated black pepper seeds.

Application method	**Application**	***E. coli***	**Reduction**
Control	2 h air alone	6.35±0.04[a]	-
Ozon (15 ppm)	1 h	5.53±0.09[b]	0.82±0.09[a]
UV-C (28.8 J/cm^2)	1 h	5.52±0.34[b]	0.83±0.34[a]
Ozon → UV-C (in succession)	1 h UV-C after 1 h ozone	4.68±0.22[c]	1.67±0.22[b]
Ozon + UV-C (in simultaneous)	1 h UV-C and ozone together	4.97±0.14[c]	1.38±0.14[b]

Data represent mean values (n = 3) ± standard deviations
Means within a column having the same letter are not significantly different (p>0.05).

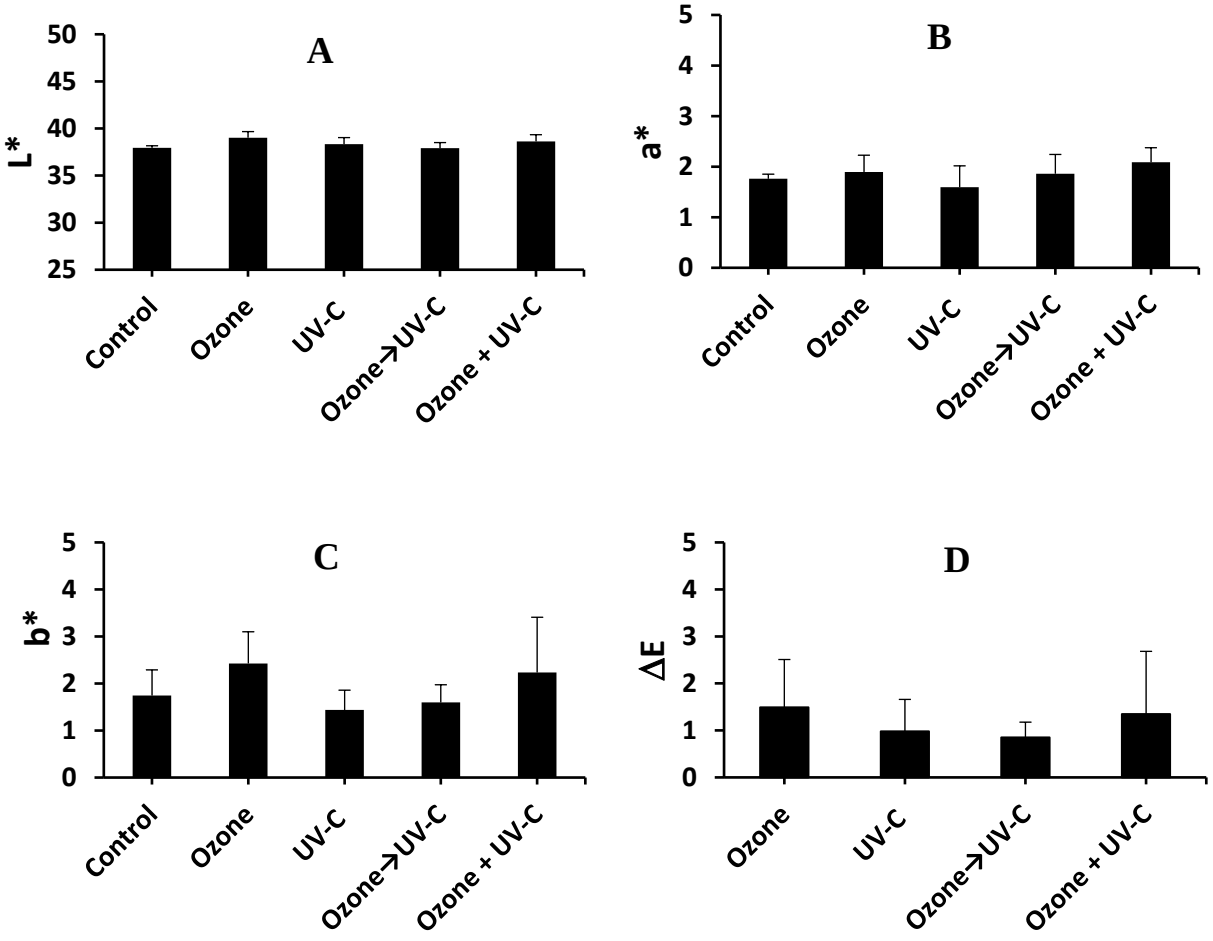

Figure 2. Effect of UV-C and ozone treatments on redness (a), yellowness (b), lightness (c), and total color difference (d) of black pepper. Error bars represent standard deviations of the mean (n = 3). Ozone: 15 ppm for 1 hr; UV-C: 28.8 J/cm^2; Ozone→UV-C: combined treatment applied in succession; Ozone+UV-C: combined treatment applied simultaneously.

3.3. Effect of UV-C and ozone treatments on color values

Black pepper seeds have black, bright color. The loss of color during decontamination processes is important factor for consumer acceptance. The change in L *, a *, b * and total color values of black pepper seeds with UV-C and ozone applications are shown in Figure 2.

The initial L*, a* and b* values of black pepper seeds were measured as 37.96, 1.76 and 1.74, respectively. It was found that the use of the ozone and the UV-C applications individually or together did not cause a significant change in the color values of black pepper ($p>0.05$). Total color changes on the samples were not affected by any of the treatments either ($p>0.05$). The highest total color change (ΔE values) was only 1.49 in the ozone treated samples (Fig. 2). Virtanen et al. (2014) reported that ΔE values below 2 indicates a small difference and cannot be distinguished by an uneducated eye.

Erdoğdu and Ekiz (2013) did not observe a change in Hunter color values of black pepper subsequent to UV-C treatment at 75.6 J/cm^2. Akbas and Ozdemir (2006, 2008) found that flaked red pepper and ground pistachios, which exposed to ozone concentrations at 7 and 9 ppm, had slight, but significantly lower scores for appearance than the samples exposed to ≤5 ppm by panelists on sensory panels. But no significant changes on appearance were obtained by panelists for pistachio kernels. In another study, oregano samples treated with ozone gas at a concentration of 5.3 ppm for 120 min were graded significantly lower than the samples treated at 2.8 ppm for 120 min (Torlak et al., 2013).

4. Conclusions

The UV-C (28.8 J/cm^2) and the ozone treatments (15 ppm for 1 h) caused significant reductions in TAMB and *E. coli* counts (up to 0.8-log) in black pepper seeds. While the combination of these treatments did not have any additive or synergistic effects on TAMB, further inactivation of freshly inoculated *E. coli* on the seeds (up to 1.7-log) was observed. The applied treatments did not adversely affect the color of the samples.

Thus, each of the UV-C (28.8 J/cm^2) and the ozone treatments (15 ppm for 1 h) has some potential for reducing the natural contamination level on black pepper seeds, but the combined treatment may have further potential towards inactivation of specific microorganisms. Increased UV-C and ozone intensities may further increase the inactivation levels and thus should be investigated in further studies.

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Acknowledgements

This research was financially supported by the grant from Istanbul Technical University Scientific Research Projects Department (ITU BAP Project No: 36019).