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Effect of Oxygen on Decontamination of Cumin Seeds by Atmospheric Pressure Dielectric Barrier Discharge Plasma

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ABSTRACT: We studied the effect of oxygen on decontamination of cumin seeds by atmospheric pressure dielectric barrier discharge (DBD) plasma. A peak-to-peak 15-kV radio-frequency power supply at 17-kHz frequency was used to generate Ar and Ar-O_2 plasmas between two circular electrodes. Electrodes were covered by mica plates with a 5-mm gap between each plate. Seeds were exposed to plasma until they were completely decontaminated. To determine total bacteria content, the seeds were cultured using a pour-plate method on a nutrient agar medium. To evaluate bacterial destruction, concentrations of double-stranded DNA and protein were determined spectrophotometrically. Results indicate that the surviving microorganism density decreased significantly after exposure of Ar and Ar-O_2 plasma in a time-dependent manner. Complete elimination of total bacteria was obtained after 20 and 40 min of exposure for Ar-O_2 and Ar plasma, respectively. Our research shows that atmospheric pressure DBD plasma using Ar or Ar-O_2 gas may be a suitable alternative method for decontamination of cumin seeds, without resulting in any detrimental effects on food quality of seeds.

KEY WORDS: cumin seed, dielectric barrier discharge plasma, decontamination, culturing method

I. INTRODUCTION

Spices and aromatic herbs, which bring a world of flavors, aromas, and colors to food, are widely used in different cuisines. However, spices and herbs are often contaminated with high levels of bacteria, molds, and yeasts. The origin of microorganisms that are present on the surface of the plant may be epiphytic microflora, growing on specific varieties of plants, or microflora of the plant environment, such as soil, water, and air. Pathogenic microorganisms on the surface or inside plant tissue, if left untreated, can lead to rapid spoilage of the products that they are supposed to enhance.

Cumin seed (*Cuminum cyminum* L.) is one of the most valuable and widely used spices, because of its distinctive flavor, aroma, and medicinal and therapeutic properties.[1] Cumin seeds can be contaminated with toxigenic molds and bacterias such as *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, and *Bacillus cereus*, potentially creating a public health risk and problems during transportation.[2,3] The importance of this spice makes necessary the use of a suitable decontamination process that does not change in the spice's quality.

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Conventional sterilization techniques, such as fumigation with ethylene oxide, irradiation with ionizing radiation, and treatment with superheated steam, are examples of applicable processes that are used for spice decontamination.[4] However, with ethylene oxide treatment, toxic residues may remain after the process.[5] Irradiation using ionizing radiation and exposure to gamma and X-rays decontaminate spices powerfully but can lead to oxidation of most aromatic components of the spices, making unwanted changes to this class of materials.[6] Overheating may lead to the loss of flavor and color components of the spice.

Nutrient analysis of cumin seeds indicates that they contain proteins, fats, and vitamins including folate, niacin, pyridoxine, riboflavin, thiamin, and vitamins C, A, D, and K.[7] Irradiation with ionizing radiation such as gamma radiation and overheating can change the structure of these molecules and affect the quality of the fatty acids and vitamins. A sterilization method that has recently been considered for decontamination is the use of low-temperature plasma at atmospheric pressure. Cold sterilization techniques are already used to decontaminate a wide variety of heat-sensitive instruments and materials in modern medical practice.[8] Nonthermal plasma is an effective source of active species, free radicals, negative and positive ions, excited atoms, molecules, and ultraviolet (UV) radiation, which are able to deactivate and kill bacteria, viruses, and other microorganisms without any significant temperature effects.[9–11] This characteristic suggests the possibility of using this type of plasma for treatment of heat-sensitive materials.[12] Nonthermal atmospheric pressure discharges, particularly in the air, are very effective in deactivation of microorganisms.[13] Dielectric barrier discharges (DBDs) are convenient plasma sources for the generation of nonthermal plasma at atmospheric pressure. It has been demonstrated that DBD is an effective tool, particularly in the destruction of resistant microorganisms such as *Bacillus subtilis* (spores), *Bacillus anthracis* (anthrax spores), and *Deinococcus radiodurans* (microorganisms that survive strong radiation of nuclear materials). The range of applications of plasma sterilization at atmospheric pressure is wide, from medical instruments and spacecraft to different food products.[14]

Recently, atmospheric pressure plasma was used to decontaminate spices, herbs, seeds, and dehydrated vegetable substances.[15] Plasma discharge may be preferable for surface decontamination of spices such as cumin seeds without producing unwanted quality damage. In our experimental research, we applied atmospheric pressure DBD plasma for microbial decontamination of cumin seeds.

II. MATERIALS AND METHODS

A. Plasma System

In this study, we used a radio-frequency (RF) power supply and discharge chamber to produce low-temperature atmospheric pressure plasma, as is shown in Fig. 1. Plasma was generated between two aluminum electrodes covered with 1-mm mica as an insulating dielectric barrier. The electrode geometry was disc to disc, with an adjustable interelectrode gap. Atmospheric pressure DBD plasma was generated by an RF discharge

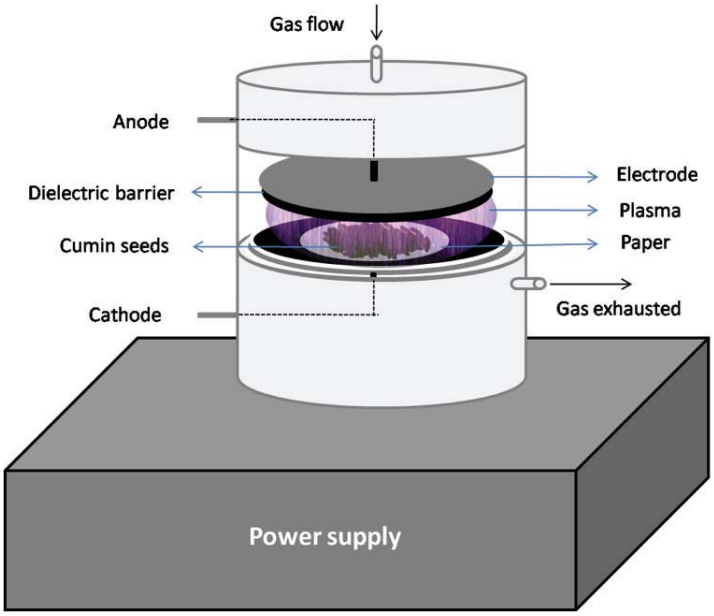

FIG. 1: Schematic of DBD generator system with power supply

of 15 kV at a 17-kHz frequency between two parallel-plane electrodes (the diameters of the top and bottom electrodes were 10 and 8 cm, respectively). Inside the DBD chamber, argon and argon-oxygen (80% Ar, 20% O_2) gases were fed from the top and exhausted from the bottom of the discharge chamber. During the experiment, a very low-rate needle valve was open, so the amount of gas flow was very small (measured on a scale of milliliters pre minute). To produce uniform plasma, we adjusted the distance between the two electrodes to 5 mm.

B. Exposing Protocol

We spread 1 g of cumin seeds in a single layer onto a round paper with a diameter of 6 cm and exposed the seeds to the DBD plasma for different amounts of times. To evaluate the colonies, at the end of the each time point the cumin seeds were mixed with 9 mL sterile normal saline in a test tube and vortexed vigorously. A dilution of 10^{-3} supernatant was selected for culture. We cultured 1 mL of each dilution using a pour-plate method on a nutrient agar medium. We counted the number of colonies after 24 h of incubation at 37°C.

C. Gram Staining

To identify Gram-negative and -positive bacteria, we used Gram staining. Gram-positive bacteria have a thick outer coating of peptidoglycan, whereas Gram-negative bacteria have a thin layer of peptidoglycan located between two other cell membranes.[16]

D. DNA and Protein Measurement

To evaluate bacterial destruction, concentrations of double-stranded DNA and protein in the supernatant of cumin seeds, suspended in normal saline, were determined using a T80 UV-Visible-NIR spectrophotometer (PG Instruments Ltd., London, UK). DNA has a maximum absorbance peak at 260 nm, and protein has a peak at 280 nm. The wavelength of 320 nm was used as a background correction. DNA and protein concentrations were calculated using the Warburg and Christians factors preset in the instrument based on wavelength (260 and 280 nm).[17] To determine DNA and protein concentrations after exposing, 1 g cumin seeds was mixed vigorously for 1 min in 9 mL of normal saline, and the supernatant was analyzed using the spectrophotometer.

E. Statistical Analysis

All statistical analyses were performed with SPSS statistical software, version 16.0 (SPSS, Chicago, IL). Data were expressed as mean ± standard deviation (SD). $p < 0.05$ was considered to be statistically significant. A comparison of data between control and exposed samples was examined using the one-way analysis of variance test.

III. RESULTS

Table 1 indicates the density of surviving microorganisms of cumin seeds after exposure to Ar plasma at 10, 20, 30, and 40 min, compared to the control. The results show that the density of surviving microorganisms decreased parallel to the increase in time of exposure. The decrease was significant after 20, 30, and 40 min ($p < 0.05$); complete decontamination occurred at 40 min.

TABLE 1: Average of surviving microorganisms in cumin seeds after exposure to Ar plasma, compared to control

Exposure time (min)	Surviving microorganism (colony forming unit per milliliter)
0	$14.7 \times 10^3 \pm 747$
10	$13.3 \times 10^3 \pm 706$
20	$12.3 \times 10^3 \pm 1189^*$
30	$11.7 \times 10^3 \pm 624^*$
40	$0 \pm 0^*$

Data are represented as mean ± SD obtained from five separate experiments.
*$p < 0.05$.

The effect of Ar-O_2 plasma on the sterilization of cumin seeds at different times of exposure is shown in Table 2. The results indicate that the density of surviving microorganisms decreased significantly at 5, 10, and 15 min ($p < 0.05$); complete decontamination occurred after a 2-min exposure. Survival curves of cumin microorganisms based on data shown in Tables 1 and 2 are plotted in Fig. 2.

TABLE 2: Average surviving microorganisms in cumin seeds after exposure to Ar-O_2 plasma, compared to control

Exposure time (min)	Surviving microorganism (colony forming unit per milliliter)
0	$14.7 \times 10^3 \pm 747$
5	$12.3 \times 10^3 \pm 818^*$
10	$11.5 \times 10^3 \pm 118^*$
15	$10.5 \times 10^3 \pm 624^*$
20	$0 \pm 0^*$

Data are represented as mean ± SD obtained from five separate experiments.
*$p < 0.05$.

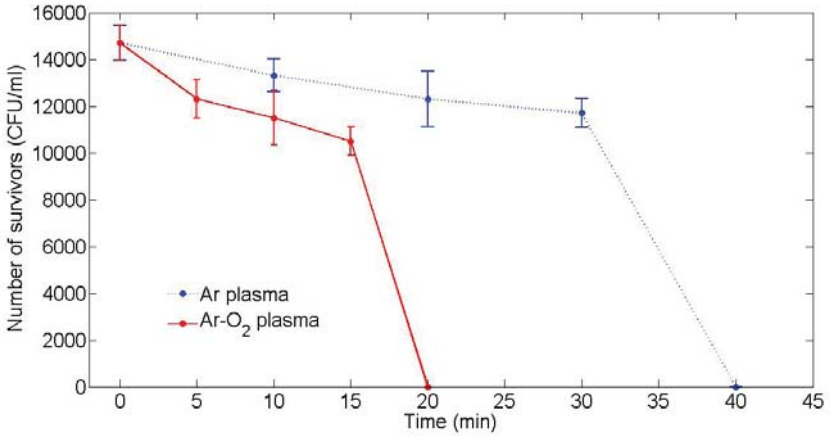

FIG. 2: Survivor curves of cumin bacteria

Table 3 shows the concentrations of DNA and protein after treatment with Ar plasma for 10, 20, 30, and 40 minutes, compared to the control. DNA and protein concentrations were calculated from absorbance at 260 and 280 nm, respectively. It can be seen that concentrations of DNA and protein significantly increased ($p < 0.05$) after exposure at 30 and 40 min, compared to control samples.

TABLE 3: DNA and protein concentration after exposure to Ar plasma, compared to control

Exposure time (min)	A_1 (260 nm)	DNA (microgram per gram of cumin)	A_2 (280 nm)	Protein (microgram per gram of cumin)
0	0.22 ± 0.03	95.83 ± 14.04	0.21 ± 0.04	203.78 ± 32.7
10	0.23 ± 0.07	100.96 ± 15.21	0.21 ± 0.05	244.94 ± 36.4
20	0.28 ± 0.06	127.15 ± 16.5	0.26 ± 0.07	256.75 ± 33.38
30	0.31 ± 0.04	139.11 ± 15.5*	0.30 ± 0.06	299.48 ± 32.63*
40	0.39 ± 0.05	173.62 ± 17.17*	0.38 ± 0.05	373.48 ± 31.55*

Data are represented as mean ± SD obtained from five separate experiments.
*$p < 0.05$.

The DNA and protein concentration in cumin seeds after exposure to Ar-O_2 plasma for 5, 10, 15, and 20 min, compared to control is shown in Table 4. Results indicate that the concentration of DNA and protein increased significantly after 15 and 20 min of exposure ($p < 0.05$).

TABLE 4: Measurement of DNA and protein concentration after exposure to Ar-O_2 plasma, compared to control

Exposure time (min)	A_1 (260 nm)	DNA (microgram per gram of cumin)	A_2 (280 nm)	Protein (microgram per gram of cumin)
0	0.22 ± 0.03	95.83 ± 14.04	0.21 ± 0.04	203.78 ± 32.7
5	0.24 ± 0.04	107.09 ± 14.3	0.23 ± 0.05	227.81 ± 32.7
10	0.27 ± 0.06	121.48 ± 13.5	0.26 ± 0.04	257.48 ± 32.28
15	0.31 ± 0.04	138.11 ± 14.03*	0.31 ± 0.06	304.41 ± 30.15*
20	0.38 ± 0.06	166.95 ± 15.09*	0.38 ± 0.05	372.87 ± 31.5*

Data are represented as mean ± SD obtained from six separate experiments.
*$p < 0.05$.

IV. DISCUSSION

We investigated the decontamination effects of atmospheric pressure DBD plasma on cumin seeds in this study. Our results indicate that after exposure of DBD plasma, the density of surviving microorganisms decreased to acceptable levels.

Plasma sterilization is quite a complicated process that is determined by multiple plasma species and factors, including charged and excited species, reactive neutrals, and UV radiation. Several components can impact the destruction process, including type of bacteria, medium, and exposure; number of cell layers, contribution of UV, and operating gas mixture. Argon and argon-oxygen gas were used to produce plasma in this research. Our results indicate that the addition of oxygen to argon gas enhances the decontaminative effects of plasma. The addition of oxygen to a working gas leads to an increase in reactive oxygen species in plasma and accelerates the etching process.

Figure 2 indicates that the decontamination process can be analyzed in three steps. At the first step, shown by a short line with the sharp negative slope, the destruction of microorganisms starts with UV irradiation of the genetic material of the microorganisms. At this step, the negative slope of the curve of Ar-O_2 plasma is sharper due to the larger intensity of UV photons in Ar-O_2 plasma, compared to Ar plasma. At the second step, the negative slope of the curve decreases. This step may be described as occurring after the erosion of microorganism through intrinsic photodesorption. The chemical bonds of microorganism material may be broken by the energy of UV photons. A larger number of free radicals in Ar-O_2 plasma accelerates this process. The sharpest negative slope can be found at the third step of the survival curve. Here, the remnants of microorganisms react with reactive species of plasma, etching occurs, and microorganisms become completely destroyed.[18] Figure 2 shows that oxygen may noticeably accelerate the reactions in the first and second steps of treatment. Without oxygen, the slope of the lines in steps 2 and 3 in the

survival curve is smaller. Thus, the time required for the decontamination process to reach the final step increases. The third step occurs similarly in both cases, but with the presence of oxygen in the plasma, the third step begins sooner. This step of decontamination occurs steeply. The same procedure was reported by Moisan et al.[19] and Vrajova et al.[20] This third step in the decontamination process is steeper than the first two steps. This can be explained by shadowing effects of the spore protecting the underlying layers. as a result of the layer-by-layer protection produced by DNA, the first two steps of decontamination occur more slowly than the third step. At the third step, UV and active radicals attack the DNA of the decontaminators directly and no spore layer is present to offer protection.

Bacillus, a Gram-positive bacterium, is the most likely occurring microorganisms in cumin seeds. When bacteria are of the Gram-positive type, they are able to form spores, which are highly resistive states of cells. The genetic material inside spores is protected by several surrounding coats that are made up of proteins. These proteins are susceptible to chemical attack by reactive neutral species.[21] Therefore, it is expected that the integrity of the walls, coats, and membranes of the cells and microorganisms will be greatly compromised by reactive neutral species generated in air plasma. The morphological changes in *E. coli* cells treated with atmospheric plasma at 75 W for 2 min, as observed under an electron microscope by Hong et al., clearly revealed that the treated cells had severe cytoplasmic deformations and leakage of the bacterial chromosome.[22] These observations demonstrate the loss of viability of bacterial cells after plasma treatment. It is well established that electroporation of membranes is induced by pulsed electric fields. It appears that plasma acts along similar lines by inducing perforations in the membranes of microorganisms.[23–25]

In this research, the concentrations of DNA and protein in cumin suspensions were determined to evaluate the wall destruction of bacteria caused by plasma radiation. Our results indicate that Ar-O_2 plasma, in comparison to Ar plasma, is more effective in destructing the bacterial wall and improving the bactericidal effects of plasma.

The combined effect of UVC light and far infrared (FIR) radiation on the quality and decontamination of cumin seeds was evaluated by Erdoğdu et al.[26] Although combined UVC and FIR treatments reduced the microbial numbers of the cumin seeds to acceptable levels, the temperature used for decontamination reached 300ºC. In another study, the effect of gamma radiation on microbiological and oil properties of black cumin was evaluated by Arici et al.[27] These results indicated that the microbial count of the samples decreased as the dose of irradiation increased. It was observed that total bacterial count as well as total yeast and mold counts decreased to an undetectable limit. However, gamma radiation produced many unwanted effects on cumin's biochemical properties. Both the free fatty acid and peroxide values of the samples increased, whereas oil contents, iodine numbers, refraction index, and Rancimat values decreased. Evaluation of the composition of fatty acids also indicated that the levels of trans fatty acids increased, but the percentages of unsaturated fatty acids decreased.[27]

Using atmospheric pressure DBD plasma for decontamination of cumin seeds may be a suitable alternative for FIR and gamma irradiation, because of the low-temperature

and low-penetration power of plasma. Selcuk et al.[28] successfully decontaminated the seeds of tomato, wheat (*Triticum durum*), beans, chickpeas, soybeans, barley, oats, rye, lentils (*Lens culinaris*) and corn contaminated with *Aspergillus parasiticus* and *Penicillium* sp. to <1% of the initial count by plasma, depending on treatment times. Treatment times varied from 30 s to 30 min. This group used a custom-designed batch-type low-pressure cold plasma prototype unit operating under vacuum, using air and SF6 gases. The results suggest that in practical terms, after plasma treatment the food qualities of wheat and beans were unaffected or only marginally affected. It is worth mentioning that the seeds were found to be viable after plasma processing.[28]

V. CONCLUSIONS

Atmospheric plasma technology is an emerging disinfection method that offers an exciting complementary or alternative, novel nonthermal approach for reducing microbial populations on raw or fresh produce surface and packaging materials. Our research indicates that atmospheric pressure DBD plasma using Ar or Ar-O_2 gas may be a suitable alternative method for decontamination of cumin seeds without producing any detrimental effects on quality of the seeds. Cold plasma technology could be use on a large scale for decontaminating food and agriculture products using various energy sources and methods. This technology is increasingly finding acceptance among food processors for surface sterilization. The effect of cold plasma on the sensitive constituents of foods, mainly lipids, vitamins, etc., must still be addressed. Once a successful outcome is achieved, the technology will find wider applications and adaptation in food industries.

REFERENCES

1. Mathew AG, Pushpanath S. Indian Spices. India: Dee Bee Info Publications; 2005.
2. Al-Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem. 1992;45(4):239–42.
3. Banerjee M, Sarkar PK. Microbiological quality of some retail spices in India. Food Res Int. 2003;36(5):469–74.
4. Schweiggert U, Carle R, Schieber A. Conventional and alternative processes for spice production–a review. Trends Food Sci Technol. 2007;18(5):260–8.
5. Sospedra I, Soriano JM, Mañes J. Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Foods Human Nutr. 2010;65(4):364–8.
6. SádECká J. Irradiation of spices: a review. Czech J Food Sci. 2007;25(5):231–42.
7. Hepburn FN. The USDA National Nutrient Data Bank. Am J Clin Nutr. 1982;35(5):1297–301.
8. Moisan M, Barbeau J, Crevier M-C, Pelletier J, Philip N, Saoudi B. Plasma sterilization: methods and mechanisms. Pure Appl Chem. 2002;74(3):349–58.
9. Gaunt LF, Beggs CB, Georghiou GE. Bactericidal action of the reactive species produced by gas-discharge nonthermal plasma at atmospheric pressure: a review. IEEE Trans Plasma Sci. 2006;34(4): 1257–69.
10. Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. IEEE Trans Plasma Sci. 2002;30(4):1409–15.
11. Moisan M, Barbeau J, Moreau S, Pelletier J, Tabrizian M, Yahia LH. Low-temperature sterilization

using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms. Int J Pharm. 2001;226(1):1–21.
12. Kogelschatz U. Atmospheric-pressure plasma technology. Plasma Phys Controlled Fusion. 2004;46(12B):B63.
13. Napartovich A, Deryugin A, Kochetov I. Discharge production of the singlet delta oxygen for an iodine laser. J Phys D Appl Phys. 2001;34(12):1827.
14. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. Plasma Proc Polymers. 2008;5(6):503–33.
15. Schlüter O, Ehlbeck J, Hertel C, Habermeyer M, Roth A, Engel KH, Holzhauser T, Knorr D. Opinion on the use of plasma processes for treatment of foods. Mol Nutr Rood Res. 2013;57(5):920–7. doi: 10.1002/mnfr.201300039.
16. Costerton J, Ingram J, Cheng K. Structure and function of the cell envelope of gram-negative bacteria. Bacteriol Rev. 1974;38(1):87.
17. Manchester K. Use of UV methods for measurement of protein and nucleic acid concentrations. Biotechniques. 1996;20(6):968.
18. Salarieh S, Dorranian D. Sterilization of turmeric by atmospheric pressure dielectric barrier discharge plasma. Plasma Sci Technol. 2013;15(11):1122.
19. Moisan M, Barbeau J, Crevier M, Pelletier J, Philip N, Saoudi B. Plasma sterilization: methods and mechanisms. Pure Appl Chem. 2002;74(3):349–58.
20. Vrajova J, Chalupova L, Novotny O, Cech J, Kreme F, Stahel P. Removal of paper microbial contamination by atmospheric pressure DBD discharge. Eur Phys J D. 2009;54:233–7.
21. Kayes MM, Critzer FJ, Kelly-Wintenberg K, Roth JR, Montie TC, Golden DA. Inactivation of foodborne pathogens using a one atmosphere uniform glow discharge plasma. Foodborne Pathog Dis. 2007;4(1):50–9.
22. Hong Y, Kang J, Lee H, Uhm H, Moon E, Park Y. Sterilization effect of atmospheric plasma on Escherichia coli and Bacillus subtilis endospores. Lett Appl Microbiol. 2009;48(1):33–7.
23. Pothakamury UR, Monsalve-Gonzalez A, Barbosa-Cánovas GV, Swanson BG. Inactivation of Escherichia coli and Staphylococcus aureus in model foods by pulsed electric field technology. Food Res Int. 1995;28(2):167–71.
24. Sale A, Hamilton W. Effects of high electric fields on microorganisms: I. Killing of bacteria and yeasts. Biochim Biophys Acta. 1967;148(3):781–8.
25. Wouters PC, Smelt JP. Inactivation of microorganisms with pulsed electric fields: potential for food preservation. Food Biotechnol. 1997;11(3):193–229.
26. Erdoğdu SB, Ekiz Hİ. Effect of ultraviolet and far infrared radiation on microbial decontamination and quality of cumin seeds. J Food Sci. 2011;76(5):M284–92.
27. Arici M, Arslan Colak F, Gecgel Ü. Effect of gamma radiation on microbiological and oil properties of black cumin (Nigella sativa L.). Grasas y Aceites. 2007;58(4):339–43.
28. Selcuk M, Oksuz L, Basaran P. Decontamination of grains and legumes infected with Aspergillus spp. and Penicillum spp. by cold plasma treatment. Biores. Technol. 2008;99(11):5104–9.