Effect of atmospheric pressure floating-electrode dielectric-barrier discharge (FE-DBD) plasma on microbiological and chemical properties of *Nigella sativa* L.

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Abstract

Introduction: Recently, atmospheric pressure non-thermal plasma has been used as a new method for decontamination of medicinal plants. The aim of this research was to investigate the effects of atmospheric pressure floating-electrode dielectric-barrier discharge (FE-DBD) on *Nigella sativa (N. sativa)* which has many therapeutic properties.

Materials and methods: *N. sativa* seeds were exposed to atmospheric pressure FE-DBD plasma for 15, 30 and 40 min. and total microbial counting of the seeds was performed. Antioxidant activity and total phenol were measured to evaluate the chemical properties changes of *N. sativa* seeds under the exposure of plasma. Fatty acid analysis of the extracted oil from *N. sativa* was determined using gas-liquid chromatography in this research before and after the exposure to cold plasma.

Results: The results showed that the density of microorganisms significantly decreased at all three exposure times compared to the control ($P<0.05$) and eliminate total microorganisms at 40 min of exposure. There was no significant change in the amount of total phenolic compounds and antioxidant activity before and after plasma exposure. Linoleic acid and oleic acid were decreased under the exposure of FE-DBD plasma for 40 min which indicate that cold plasma can lead to the oxidation of unsaturated fatty acids. The ratio of unsaturated fatty acids to saturated fatty acids were significantly decreased ($P<0.05$).

Conclusion: In conclusion, exposure of cumin seeds to FE-DBD plasma can effectively reduce or eliminate microorganisms. On the other hand, cold plasma treatment brings about some biochemical changes. Total phenol content increased and antioxidant activity was decreased slightly. The unsaturated fatty acid contents of black cumin seeds samples decreased with FE-DBD plasma exposure at effective time for decontamination.

Keywords: *Nigella sativa* seed, FE-DBD plasma, Decontamination, Antioxidant activity

Introduction

One concern in the food industry is the risk of transmitting pathogenic bacteria through food. Reports indicate that food poisoning can lead to death (1). Food products can transmit bacteria, parasites and viruses that

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can cause human diseases (2). *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*) and *Salmonella* have been recognized as pathogens in many countries (3). Fresh products known as pathogens due to contaminated with *E. coli*, *L. monocytogenes* and *Salmonella* has included lettuce, spinach, radish, alfalfa sprouts, tomatoes, peppers, cantaloupe, strawberries, and fruit and vegetable salads (3).

In addition to fresh food products, spices can also be contaminated with bacteria. Spices are used in food products because of their aroma, color and therapeutic properties. If the bacteria in the spices are high, they cause poisoning and rapid spoilage of food. Epiphytic micro flora that grow on some plants or environmental micro flora can be the source of pathogenic microorganisms on the surface of the plant and, if not eliminated, lead to food spoilage. Among spices, *Nigella sativa* (*N. sativa*) is considered as a spice that is in addition to the flavor food has many therapeutic properties (4). Reports indicate that *N. sativa* extracts have therapeutic properties, including anti-inflammatory (5), antitumor (6), anti-diabetic (7). *N. sativa* seeds contain essential oil, calcium, phosphorus, zinc, potassium, iron, copper and vitamin E, A, C and B_6 (8). Pathogenic bacteria such as *E. coli*, *Salmonella*, *Clostridium perfringens*, *Bacillus cereus* can contaminate the surface of the *N. sativa* (9, 10).

Due to toxic foodborne illnesses more efficient decontamination methods is necessary (3, 11, 12). Common methods such as the heat treatment and chmical solutions used to sterilize the surface of spices are often time consuming and harmful (13). Recently, atmospheric low temperature plasma technology has been used as an alternative method to surface sterilization and disinfection process. Plasma is made of ionised gas. Various gas such as argon, helium, nitrogen and compressed air could be genarated plasma (14). Air bacterial inactivation time is shorter in both vegetative cells and spors by cold plasma method (15). Cold plasma technique as a new method of sterilization is effective in killing a large number of microorganisms (16, 17). Studies show that the effect of cold plasma on bacteria is more than heat and chemical technique (18).

Ionization is the most important element in the plasma process whitch is as a result of other factors such as reaction rate, mean free path and electron energy distribution (19). One of the reasons that plasma affects on microbial cells, is the interaction of ions with cells. The oxidative effects on the cell surface of microbs is associated with the reactive species in plasma. Inactivation of bacteria by plasma results from the destruction of DNA by active species whitch produced by plasma. Our previous studies have shown that plasma causes DNA damage in microorganisms of cumin seeds (20).

In our experimental research, we investigate the microbial decontamination of the seeds of *N. sativa* by employing atmospheric pressure FE-DBD plasma and evaluation of the plasma effects on Chemical Properties of *N. sativa* seeds.

Materials and Methods

The device used to produce FE DBD plasma is made from a power supply and an electrical discharge chamber. Plasma is formed between the power electrode which covered by a quart's layer and the target surface which *N. sativa* seeds were placed.

One gram of *N. sativa* seeds was spread on the glass slide as a single layer and was exposed to the plasma for different times. In order to evaluate the colonies, at the end of each time point the seeds of *N. sativa* were vigorously mixed in 9 ml normal saline in a test tube. The supernatant then diluted to 10^{-1} to 10^{-4} and cultured in nutrient agar medium

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for 24 hours at 37°C using pour plate method and colonies were counted.

Determination of phenolic compounds

Folin-Cioculteu reagent as a reactive material was used to measure the total phenolic compounds of *N. sativa* samples. The phosphotungstic acid present in the reagent is used as a reducing agent that rapidly reduces the oxidized hydroxyphenol group and eventually forms a blue color with a maximum absorption at 765 nm. 0.025 mL of samples were mixed with Folin-Cioculteu reagent and after 10 min, 0.375 mL of 20% sodium carbonate were added and after mixing was kept at ambient temperature and away from light for 2 h. Absorbance was measured at 765 nm. Gallic acid was used as standard and the results were calculated based on mg of Gallic Acid Equivalents (GAE)/kg of dry matter (21).

Antioxidant activity

Antioxidant activity of N. *sativa* extracts was evaluated using ABTS (2,2-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) method. In order to evaluate the antioxidant activity, ABTS (7mM) was dissolved in water and in exposure to potassium persulfate (2.45 mM) at room temperature for 12-16 h in dark, cause to generate free radicals. $ABTS^{\bullet+}$ solution was dissolved in 5 mM phosphate buffer (pH 7.4), to obtain absorbance 0.7±0.2 at 734 nm. The absorbance of the mixture of samples and $ABTS^{\bullet+}$ was determined at 25ºC for 5 min at 734 nm. The inhibitory potential of free radicals was calculated by the following equation:
Free radical inhibitory = [(ABTS Absorption - Sample Absorption) / ABTS Absorption] ×100. The results were expressed as the antioxidant capacity of Trolox equivalent (22).

Gas chromatography and fatty acid measurement of *N. sativa*

All chemical compounds including a commercially available standard mixture of 37 fatty acid methyl esters, individual standards of fatty acid methyl esters, nonmethylated heptadecanoic acid (C17:0) (as internal standard) and boron trifluoride-methanol (BF3-methanol) were purchased from Sigma (Sigma Chemical Co., MO, USA) Butylated hydroxyl toluene and all other reagents, chemical compounds and solvents were obtained from Merck (Merck Co., Germany).

Fatty acid extraction and methyl esters preparation

Total lipid extract of *N. sativa* before and after the exposure to cold plasma were carried out according to the Folch method (23). As described by Emami Razavi et al. (24). Fatty acid methyl esters (FAMEs) were prepared according to the method of Morrison and Smith, using BF3/methanol (25). Fatty acid methyl esters were dissolved in 200 µl hexane and fatty acid composition was identified by gas liquid chromatography. Younglin GC–FID system model Acme 6000M (Young Lin Co., Korea) equipped with a FID detector and SP-2560 fused silica capillary column 100 m × 0.25 mm × 0.2 µm film thickness (Supelco Co., PA, USA) were used for gas chromatography (GC) analyses. The temperature of injector and detector was held at 260°C, and the flame was maintained with 300 ml/min air and 40 ml/min H2. Helium whit a flow of 28 ml/min was used as the detector auxiliary gas and a flow rate of 18 cm/s with constant flow compensation as the carrier gas. Sample injections of 1 µl with split ratio of 17:1 were performed for the analysis. The total analysis time was 45 min. The oven temperature was programmed from 140 to 245°C at a rate of 4°C/min after an initial time hold of 5 min and final time hold of 15 min.

Statistical analysis

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Statistical analyses were performed with the SPSS software version 16.0 (SPSS, Chicago, IL, USA). Comparison of data between control and exposed samples was examined by k independent samples using kuruskal-wallis H test. Data were expressed as mean ± standard deviations of five repeat. *P <0.05* was considered statistically significant.

Results

Black cumin seed samples that have been exposed to FE-DBD plasma for 15, 30 and 45 min, and the control sample were examined to determine their microbiological properties, phenolic compounds, antioxidant activity and free fatty acid compositions.

Table 1 is shown the effect of FE-DBD plasma on density of surviving microorganisms at 15, 30 and 40 min. As the results show, Increasing the time of plasma exposure results in the reduction of microorganism's density and this decrease is significant after 15, 30 and 40 min (*P <0.05*).

Table1. Microbial count (CFU g) of *N. sativa* seeds after the exposure to FE DBD plasma for 15, 30 and 40 min in comparison with control.

	Time of exposure to FE-DBD plasma			
	0 min	15 min	30 min	40 min
Total count	$1.3 \times 10^4 \pm 1.8$	$9.2 \times 10^3 \pm 1.1$ **	$1.3 \times 10^2 \pm 5.1$ **	0±0 **
E.coli count	$6.3 \times 10^2 \pm 6.9$	$2.1 \times 10^2 \pm 4.3$ **	$1.6 \times 10 \pm 8.7$ **	0±0 **
B. cereus count	$6.8 \times 10^2 \pm 8.7$	$4.1 \times 10^2 \pm 5.7$ **	$3.8 \times 10 \pm 8.7$ **	0±0 **

** P <0.001

Exposure to FE-DBD plasma for 40 min totally eliminated the microorganisms from cumin seeds. The effect of plasma on total phenolic compounds and antioxidant activity of *N. sativa* seeds at different times of exposure is shown in Table 2. The results indicate that plasma treatment have no significant effect on the antioxidant activity of *N. sativa* seeds, while the phenolic content was decreased, although it is not significant.

Table 2. Percentage inhibition of ABTS radical scavenging activity and Phenolic content of *N. Sativa* seeds before and after the exposure to FE DBD plasma.

	Control	40 min plasma treatment
Total Phenol	49.86±0.32	50.45±0.21
Antioxidant Activity	5.63 ± 0.49	4.16 ± 0.85

Table 3 shows the comparison between the fatty acid composition of *N. sativa* before and after the exposure of seeds to FE-DBD plasma for 40 min. Compositions and differences related to FE-DBD plasma exposure on palmitic, stearic, oleic, linoleic and other fatty acids, total saturated and total mono and polyunsaturated fatty acids were statistically analyzed. As the results indicate FE-DBD plasma has no significant effect on the percent of MUSFAs or PUSFAs to the total fatty acids although they show the slight decrease. The percent of LA to total fatty acid is significantly decreased (P <0.05) under the exposure of FEDBD plasma (55.803 ± 0.43) in comparison with control (56.574 ± 0.51). Oleic acid is also decreased under the exposure (24.571 ± 0.49) when compared with control (24.894 ± 0.43) but this decrease is not significant. Decrease in unsaturated fatty acids (USFAs) lead to an increase in percent of saturated fatty acids (SFAs) especially palmitic acid (11.173 ± 0.31 v 10.625 ± 0.29, P <0.05). The ratio of USFAs: SFAs (MUSFA: SFA and PUSFA: SFA) is also decreased significantly (P <0.05 and P <0.01, respectively).

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Discussion

In this experimental research, antimicrobial properties of FE-DBD plasma and its effect on chemical characterization of *N. sativa* seeds were investigated. The results showed that the microorganisms in *N. sativa* seeds including *E. coli*, as gram- negative bacterium, and *B. sereius*, as gram- positive bacterium, decreased significantly with plasma treatment. Phenolic content of *N. sativa* shows a slight decrease and antioxidant activity didn't change following the exposure to cold plasma. The ratio of total USFA: SFAs especially the ratio of oleic acid and linoleic acid to palmitic acid is decreased after the exposure of *N. sativa* oil to cold plasma.

Table 3. Comparison of fatty acid composition of *N. sativa* seeds before and after the exposure to FE DBD plasma.

Fatty acids	Normal	DBD exposure	p value
SFA	14.662 ± 0.37	15.906 ± 0.44	<0.05
C10:0 (pentadecanoic acid)	0.816 ± 0.08	0.969 ± 0.09	NS
C12:0 (lauric acid)	0.125 ± 0.08	0.148 ± 0.08	NS
C14:0 (myristic acid)	0.731 ± 0.07	0.868 ± 0.09	NS
C16:0 (palmitic acid)	10.625 ± 0.29	11.173 ± 0.31	<0.05
C18:0 (stearic acid)	0.377 ± 0.09	0.448 ± 0.09	NS
C20:0 (arachidic acid)	0.283 ± 0.06	0.337 ± 0.08	NS
C22:0 (behenic acid)	0.637 ± 0.07	0.756 ± 0.09	NS
C24:0 (lignoceric acid)	1.068 ± 0.09	1.207 ± 0.08	NS
MUFA	28.295 ± 0.43	27.854 ± 0.41	NS
C14:1 (myristoleic acid)	0.183 ± 0.07	0.177 ± 0.06	NS
C16:1 (palmitoleic acid)	0.174 ± 0.06	0.169 ± 0.08	NS
C18:1n7 (vaccinic acid)	1.291 ± 0.09	1.236 ± 0.09	NS
C18:1n9c (oleic acid)	24.894 ± 0.43	24.571 ± 0.49	NS
C20:1n9 (vaccinic acid)	1.753 ± 0.08	1.701 ± 0.08	NS
PUFA	57.043 ± 0.59	56.240 ± 0.47	NS
C18:2n6 (LA)	56.574 ± 0.51	55.803 ± 0.43	<0.05
C18:3n3 (ALA)	0.195 ± 0.07	0.179 ± 0.06	NS
C20:3n3 (ETE)	0.036 ± 0.08	0.038 ± 0.09	NS
C20:3n6 (DGLA)	0.051 ± 0.07	0.049 ± 0.07	NS
C20:4n6 (AA)	0.069 ± 0.04	0.054 ± 0.06	NS
C20:5n3 (EPA)	0.071 ± 0.05	0.072 ± 0.06	NS
C22:6n3 (DHA)	0.043 ± 0.04	0.045 ± 0.05	NS
USFA:SFA	5.820 ± 0.14	5.287 ± 0.12	<0.01
MUSFA:SFA	1.929 ± 0.08	1.751 ± 0.07	<0.05
PUSFA:SFA	3.890 ± 0.09	3.535 ± 0.08	<0.01

ALA: α-linolenic acid, ETE: eicosatrienoic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, LA: linoleic acid, DGLA: di-homo gamma linolenic acid, AA: arachidonic acid, SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: mono-unsaturated fatty acids, PUFA: poly-unsaturated fatty acids. Data are expressed as mean ± SE and indicated the percent of fatty acid to total fatty acids.

Peroxidation of membrane lipid in gram negative bacteria could happen under the exposure of plasma. On the other hand, important chemical bonds in the cell wall of Gram-positive bacteria are broken by active species of plasma. Thus, plasma will lead to the leakage of contents of the cell such as potassium, nucleic acid and proteins by disrupting the outer layer of the bacteria cells (26). Previous studies have shown the morphological change and disrupted bacterial cell walls and the leakage of cell content by plasma treatment (27).

The plasma decontamination mechanism is performed in three stages. The first stage is the decontamination by ultraviolet radiation. Ultraviolet radiation damages genetic material, at this stage the rate of bacterial

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killing is very high. Secondly, the UV radiation is absorbed by the bacteria and the chemical bonds of the bacterial wall break down. The reaction of the remaining microorganisms with the active species of plasma and completely destroyed of microorganisms is occurred in the third stage. Various factors such as the type of bacteria, the type of plasma, bacterial cell layers' number, the intermediate in which the bacteria are located, etc. are involved in killing the bacteria.

Phenolic compounds in natural sources are the most important compounds that have antioxidant activity and effectively act as hydrogen and electron donor and prevent the formation of free radicals by oxidative reactions (28).

The results of this study showed a very small decrease in phenolic content, which was not significant, indicating that the phenolic content was maintained during the cold plasma process. Herceg et al. reported that plasma treatment increased phenolic compounds in pomegranate juice depending on treatment time and sample size. Their studies showed that when pomegranate juice is treated with cold plasma, active chemical species, charged particles, and ultraviolet photons are produced that have enough electrical energy to break the covalent bonds and stimulate several chemical reactions. Which may break down the cell membrane and improve the hydrolysis and depolymerization of phenolic compounds (29). In contrast, application of 70 ° C significantly decreased total phenol content. Phenol is a heat-sensitive compound that is reduced by the heat treatment process, but the cold plasma process is a non-thermal method that preserves more phenolic content (30).

Our results showed that FE-DBD plasma had no significant effect on *N. sativa* seeds antioxidant activity. Recent studies shown that DBD plasma treatment does not significantly affect the antioxidant activity of red chicory (*Cichorium intybus* L.) and fresh-cut kiwifruit extract (31, 32). Kim et al., found that antioxidant activity of dried laver did not change following the exposure of corona discharge plasma (33). Unchanged rate of antioxidant activity by plasma treatment can be due to the reaction of antioxidant in foods with plasma free radicals. However, in this mechanism, plasma species concentration, rates of recombination, rates of the antioxidant versus free radical reaction, the free radicals' diffusivity into the food matrix control the extent of reaction.

The action of radicals on lipids is well-known to induce oxidation and formation of primary and secondary oxidation products. Because of this property cold plasma has been used for its rapid esterification of waste frying oils to produce biodiesel (34). The accelerated oxidation of lipids using a plasma source has been demonstrated for fish oils (35). The plasma chemical species-induced oxidation is not a significant problem in fruits and vegetable, because they are a poor source of fats and oils. However, lipid oxidation could be problematic when treating grains and flours and could be responsible for off-odours.

Fatty acid analysis of the extracted oil from *N. sativa* was determined using gas- liquid chromatography in this research before and after the exposure to cold plasma. The major USFAs of *N. sativa* are linoleic acid (56.5%) and oleic acid (24.9%) and the predominant saturated fatty acid is palmitic acid (10.6%). Decrease of linoleic acid and oleic acid following the exposure to FEDBD plasma fore 40 min in our research indicate that cold plasma can lead to the oxidation of unsaturated fatty acids. This decrease is significant, especially when the ratio of total USFA: SFAs is considered. By the way, the magnitude of this reduction in USFAs is negligible at all. The effects of FE-DBD plasma on fatty acid composition of cumin

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seeds obtained in this study is comparable with the study on the effects of gamma irradiation on cumin seeds by Muhammet Arici et al. (36). They reported that along with the increase in the dose of irradiation, the percentages of unsaturated fatty acids decreased, while trans fatty acid levels increased.

Conclusion

In conclusion, exposure of cumin seeds to FE-DBD plasma can effectively reduce or eliminate microorganisms. On the other hand, cold plasma treatment brings about some biochemical changes. Total phenol content increased and antioxidant activity is decreased. The unsaturated fatty acid contents of black cumin seeds samples decreased with FE-DBD plasma exposure at effective time for decontamination.

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