

Original Research Article

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Effect of Non-Thermal Dielectric Barrier Discharge Plasma on Decontamination of Cumin Seeds

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ABSTRACT

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Cumin (*Cuminum cyminum L.*) seed is one of the widely used spices because of its distinct flavour, aroma, medicinal and therapeutic properties. Cumin seeds are reported to contaminate with toxigenic molds and bacteria such as *E. coli*, *Salmonella*, *C. perfringens* and *B. cereus*, potentially creating a public health risk. The importance of this spice makes necessary the use of a suitable decontamination process that does not change in the spice's quality. Cold atmospheric plasma has potential in the food manufacturing sector to decontaminate microorganisms, thereby improving food safety without loss of physicochemical or sensory properties. The efficacy of the Dielectric Barrier Discharge Atmospheric Cold Plasma (DBD-ACP) for surface decontamination of cumin seeds was evaluated. Cumin seeds spiked with known pathogens were exposed to different plasma levels for different exposure time. Bacterial log reduction was evaluated by comparing it with the control sample. Using the DBD-ACP system around 2 to 3 log reduction (CFU/g) has been achieved after 24 min exposure at 5 kV.

Introduction

Spices bring a world of aromas, flavors and colors to food, are widely used in different cuisines. It also delivers digestion stimulating action, antioxidant potential, hypolipidemic effect, antidiabetic influence, anti-inflammatory properties, antimutagenic and anticarcinogenic potential (Rajamani *et al.*, 2005; Srinivasan, 2005). Cumin seed (*Cuminumcyminum L.*) is one of the most valuable and widely used spices, because of its unique flavors, aroma, and therapeutic and medicinal properties (Mathew, 2005). It

contains fats, proteins and various vitamins including thiamin, pyridoxine, riboflavin, folate, niacin, etc. Along with this, important functional component present in cumin is cuminaldehyde. Different important pharmacological properties of the essential oil of the cumin have been exploited like antimicrobial activity (Hanafy and Hatem 1991), antioxidant activity (Burits and Bucar 2000), and antitumor effect (Salomi and others 1992). Cumin seeds can be contaminated with the pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, and *Bacillus cereus*,

potentially creating a public health risk and problems during transportation (Al-Jassir, 1992; Banerjee, 2003). Therefore, several decontamination technologies have been developed to reduce microbial loads of from the surface of the spices. Ethylene oxide has been proven to considerably reduce the microbial population (Toofanian and Stegeman, 1988). However, apart from aroma and color alterations, volatile compounds are lost because of the low pressure that is necessary to remove the sanitizing agent; furthermore, ethylene oxide is generally considered a carcinogen and mutagen, (Farkas and Adrassy, 1988; Vajdi and Pereira, 1973). Irradiation, including the application of gamma rays, electron beam and X-rays, is an effective method for spice decontamination (Nieto-Sandoval, Almela, Fernandez-Lopez, and Munoz, 2000). Even though ionizing radiation has proven to be an environmentally clean, and energy effective method, it is rarely used because of its poor consumer acceptance. Moreover, modifications in antioxidative properties and sensorial have been observed and it may cause the formation of low-molecular-weight non-volatile or volatile radiolysis products originating from the packaging material (Goulas, Riganakos, and Konotminas, 2004).

High hydrostatic pressure is also one of the popular non-thermal decontamination technologies. In this method pressure ranging from 100 to 1000 MPa provides microbiologically safe and shelf-stable fruits and vegetable products (Guerrero-Beltran, Barbosa-Canovas, and Swanson, 2005; Manas and Pagan, 2005). But, the inactivation of microorganisms is strongly dependent on water activity. Spice samples with water activities below 0.66 showed less reduction in the microbial count (Butz, Heinisch, and Tauscher, 1994). Therefore, high-pressure treatment is an unsuitable sanitation method in spice production.

Numerous sterilization methods have been used for the decontamination of different food products. All the decontamination methods can be categorized into two types based on the process operate at a specific temperature that is the thermal and non-thermal decontamination method. Thermal methods are extensively used for sterilization or to achieve a predetermined reduction in viable microorganisms associated with a particular food product. But, the most important limiting factor of the thermal decontamination method that the nutritional and sensory characteristics of a food product can be changed as many component cannot withstand higher temperature. Thus, for the decontamination of the food product, a more promising method is the non-thermal decontamination method. It has potential applications for the decontamination of raw produce, nuts, minimally processed foods and packaging materials. Recently, it has been started to use in the decontamination of herbs, spices, seeds, and dehydrated vegetable substances without producing unwanted quality damage (Schluter, 2013). These non-thermal gas discharges comprise highly energetic plasma species including charged particles, chemically reactive metastable species, free radicals and UV photons, all of which can impart energies as high as 10 eV and which are capable of breaking molecular bonds and are therefore lethal for microorganisms (Hermann *et al.*, 1999; Montie *et al.*, 2000; Laroussi and Leipold 2004).

Two classes of plasma, namely thermal and Non-thermal Plasma (NTP) can be distinguished based on conditions in which they are generated. Plasma generation at atmospheric pressure includes Corona discharge, Dielectric Barrier Discharges (DBD), Radio-frequency plasmas (RFP) and the gliding arc discharge. For deactivation of microorganisms from the air, Non-thermal atmospheric pressure is effective technology.

The advent of electrically generating non-thermal gas plasmas at ambient conditions rather than under vacuum conditions offers a potentially new process for ensuring the microbiological safety of a range of products (Eliasson and Kogelschatz 1991). Cold sterilization techniques are already used to decontaminate a wide variety of heat-sensitive instruments and materials in modern medical practice (Moisan, 2002). It has numerous advantages over more conventional methods such as low process operational costs, short treatment time at low temperatures, nontoxic nature, significant reduction of water consumption throughout disinfection processes, and its application for a wide variety of goods (Song *et al.*, 2009; Chiang *et al.*, 2010; Korachi *et al.*, 2010).

The term “plasma” refers to a partially or wholly ionized gas composed essentially of photons, ions and free electrons as well as atoms in their fundamental or excited states possessing a net neutral charge. Plasma generated at atmospheric pressure between two parallel electrodes, at least one of which is covered by a dielectric layer is called Dielectric Barrier Discharge - Atmospheric Cold Plasma. The DBD system consists of a plasma chamber containing a pair of electrodes separated by an insulating dielectric barrier. The schematic diagram of the plasma system is shown in Fig. 1. The material is fed into the plasma chamber, passes from the electrode pair at where it is exposed to the plasma (Fig. 2) and finally treated material is collected from the other end of the chamber. 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 (Fridman, 2008).

Non-thermal plasma is a new concept in food processing technology and reports available are scanty in India. To explore this novel physical decontamination technology to increase the shelf life of farm produce, a laboratory study has been carried out using cumin seed spiked with the different bacterial cultures.

Materials and Methods

Preparation of inoculums

Bacterial pure culture (*E. coli*, *S. typhi*, *E. aerogenes* and *S. aureus*) were inoculated in nutrient broth and incubated at 37°C for 24 h. Activated cultures were streaked onto the nutrient agar plate using a sterile wire loop and incubated at 37°C for 24 h. A single colony was then transferred and inoculated in sterile liquid broth. It was then grown overnight in an incubator at 37°C for about 24 h.

Dielectric Barrier Discharge (DBD) cold plasma treatment

Cold plasma treatment for pure bacterial cultures: The efficacy of the DBD non-thermal plasma for pure bacterial cultures (*E. coli*, *S. typhi*, *E. aerogenes* and *S. aureus*) was evaluated. Pure bacterial cultures were exposed to different plasma levels for different exposure time.

Cold plasma treatment for spiked cumin seeds

Spiked sample preparation: 5 g Cumin seeds were taken in the sterile petri plate. Activated pure cultures (*E. coli*/*S. typhi*/*E. aerogenes*/*S. aureus*) were inoculated onto the cumin seeds using a micropipette and then

mixing was carried out by vigorously shaking of plates. The spiked samples were allowed to dry for about 15 min in laminar airflow. Then the spiked samples were divided into two parts, one for control and another for treatments.

Cold plasma treatment of spiked sample:

The efficacy of the DBD non-thermal plasma for surface decontamination of spiked cumin seed was evaluated. Spiked cumin seeds were exposed to different plasma levels for different exposure time.

Microbial analysis: Microbiological quantification was carried out for control (plasma untreated) and test (plasma-treated) samples both for pure culture samples and spiked samples separately. Microbial enumeration was carried out through the spread plate technique after serial dilutions. Then the plates were incubated at 37°C for 24 h in an incubator. After incubation, samples were enumerated and the microbiological counts were expressed as log₁₀ CFU/g. The difference between the test and control

samples was represented as microbial log reduction (log₁₀ CFU/g).

Results and Discussion

Effect of non-thermal DBD plasma on *E. coli* in cumin seeds: Efficacy of cold plasma on a pure culture of *E. coli* and cumin seed spiked with *E. coli* were evaluated at 2 kV, 3.5 kV and 5 kV for 8, 16 and 24 min exposure time.

Maximum reduction of 3.2 log CFU/g and 3.28 log CFU/g was observed after 24 min exposure at 5 kV in pure culture of *E. coli* and cumin seed spiked with the *E. coli* respectively. The effect of DBD plasma on *E. coli* culture as well as spiked in cumin seeds was found almost similar at 5 kV for 24 min. The effect of DBD plasma on decontamination rate was found increasing with increasing voltage and exposure time. The effect of DBD plasma treatment on *E. coli* showed a significant reduction of around 3 log (Table 1).

Table.1 Effect of DBD plasma treatment on *E. coli*

Plasma Voltage, KV (V)	Exposure Time, min (T)	<i>E.coli</i> spiked in cumin seeds			<i>E. coli</i> pure culture		
		Log reduction (log CFU/g)			Log reduction (log CFU/ml)		
2	8	0.89			0.25		
2	16	1.17			0.93		
2	24	1.61			1.53		
3.5	8	1.29			0.51		
3.5	16	1.70			1.07		
3.5	24	2.49			2.08		
5	8	2.26			0.69		
5	16	2.76			2.04		
5	24	3.28			3.20		
		T	V	T × V	T	V	T × V
SEm		0.06	0.06	0.11	0.06	0.06	0.11
CD (0.05)		0.19	0.19	0.33	0.19	0.19	0.33
CV%		2.26			2.37		

Table.2 Effect of DBD treatment on *S. typhi*

Plasma Voltage, KV (V)	Exposure Time, min (T)	<i>S. typhi</i> spiked in cumin seeds			<i>S. typhi</i> pure culture		
		Log reduction (log CFU/g)			Log reduction (log CFU/ml)		
2	8	0.89			0.40		
2	16	1.20			0.74		
2	24	1.49			1.05		
3.5	8	1.16			0.84		
3.5	16	1.67			1.15		
3.5	24	2.21			1.58		
5	8	2.02			1.13		
5	16	2.71			1.71		
5	24	3.30			2.25		
		T	V	T × V	T	V	T × V
SEm		0.08	0.08	0.15	0.07	0.07	0.12
CD (0.05)		0.26	0.26	NS	0.20	0.20	0.41
CV%		4.46			2.48		

Table.3 Effect of DBD treatment on *E. aerogenes*

Plasma Voltage, V (V)	Exposure Time, min (T)	<i>E. aerogenes</i> spiked in cumin seeds			<i>E. aerogenes</i> pure culture		
		Log reduction (log CFU/g)			Log reduction (log CFU/ml)		
2	8	0.81			0.28		
2	16	1.09			0.59		
2	24	1.32			0.92		
3.5	8	1.05			0.43		
3.5	16	1.52			0.94		
3.5	24	1.90			1.35		
5	8	1.32			0.64		
5	16	1.87			1.99		
5	24	2.28			3.12		
		T	V	T × V	T	V	T × V
SEm		0.05	0.05	0.10	0.05	0.05	0.10
CD (0.05)		0.17	0.17	NS	0.17	0.17	0.30
CV%		2.30			2.14		

Table.4 Effect of DBD treatment on *S. aureus*

Plasma Voltage, KV (V)	Exposure Time, min (T)	<i>S. aureus</i> spiked in cumin seeds			<i>S. aureus</i> pure culture		
		Log reduction (log CFU/g)			Log reduction (log CFU/ml)		
2	8	0.89			0.45		
2	16	0.94			0.93		
2	24	1.00			1.41		
3.5	8	1.01			0.71		
3.5	16	1.25			1.31		
3.5	24	1.58			1.81		
5	8	1.19			0.92		
5	16	1.76			1.45		
5	24	2.37			2.15		
		T	V	T × V	T	V	T × V
SEm		0.09	0.09	0.16	0.09	0.09	0.16
CD (0.05)		0.28	0.28	NS	0.28	0.28	0.57
CV%		3.46			3.46		

Fig.1 Schematic diagram of DBD plasma system

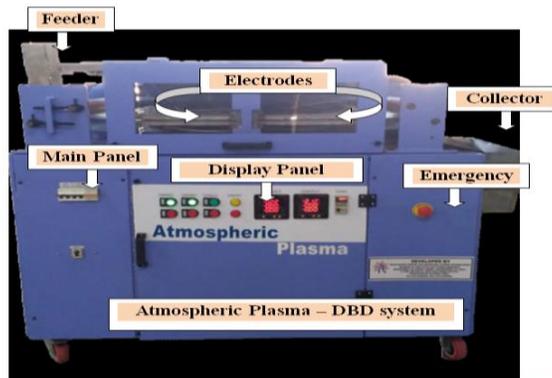
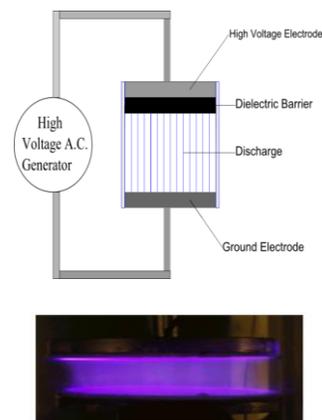
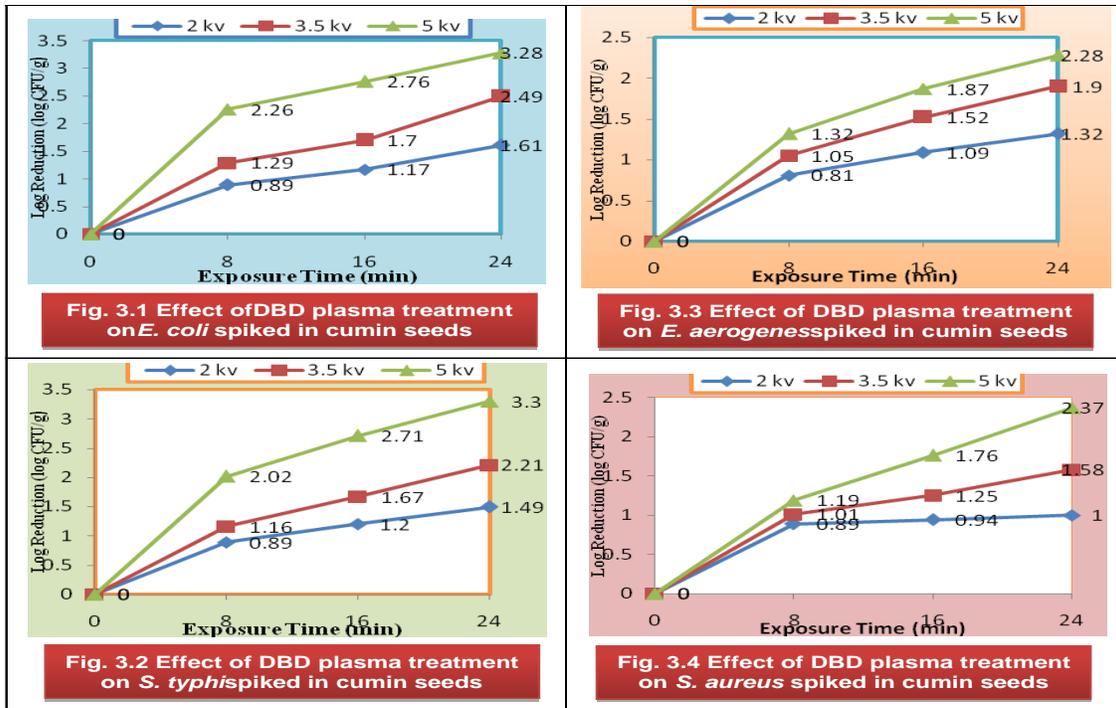


Fig.2 Cold plasma generations between two electrodes





Effect of Non-Thermal DBD Plasma on *S. typhi* in cumin seeds: Efficacy of cold plasma on a pure culture of *S. typhi* and cumin seed spiked with *S. typhi* were evaluated at 2 kV, 3.5 kV and 5 kV for 8, 16 and 24 min exposure time (Table 2).

Maximum reduction of 2.25 log CFU/g and 3.30 log CFU/g was observed after 24 min exposure at 5 kV in pure culture of *S. typhi* and cumin seed spiked with the *S. typhi* respectively. The effect of DBD on decontamination rate was found increasing with increasing voltage and exposure time. The effect of DBD plasma treatment on *S. typhi* showed a significant reduction of around 2 - 3 log.

Effect of Non-Thermal DBD Plasma on *E. aerogenes* in cumin seeds: Efficacy of cold plasma on a pure culture of *E. aerogenes* and cumin seed spiked with *E. aerogenes* was evaluated at 2 kV, 3.5 kV and 5 kV for 8, 16 and 24 min exposure time (Table 3).

Maximum reduction of 3.12 log CFU/g and 2.28 log CFU/g was observed after 24 min exposure at 5 kV in pure culture of *E. aerogenes* and cumin seed spiked with the *E. aerogenes* respectively. The effect of DBD plasma treatment on *E. aerogenes* showed significant reduction of around 2 - 3 log.

Effect of Non-Thermal DBD Plasma on *S. aureus* in cumin seeds: Efficacy of cold plasma on a pure culture of *S. aureus* and cumin seed spiked with *S. aureus* was evaluated at 2 kV, 3.5 kV and 5 kV for 8, 16 and 24 min exposure time (Table 4).

Maximum reduction of 2.15 log CFU/g and 2.37 log CFU/g was observed after 24 min exposure at 5 kV in pure culture of *S. aureus* and cumin seed spiked with the *S. aureus* respectively. The effect of DBD plasma treatment on *S. aureus* showed a significant reduction of around 2 log.

In conclusion the dielectric Barrier Discharge - Atmospheric Cold Plasma (DBD-ACP)

technology has been emerging as a promising non-thermal decontamination technology which can be used for the reduction of surface microbial load with maintaining the sensory and nutritional quality of the product. This technology is increasingly finding acceptance among food processors for surface decontamination in many countries for the fruits, vegetables and spices. The effect of cold plasma treatment on cumin seeds spiked with pure microbial culture showed that upon microbial exposure to 5 kV cold plasma for 24 min, around 3.3, 3.3, 2.3 and 2.4 log reduction (CFU/g) were observed in *E. coli*, *S. typhi*, *E. aerogenes* and *S. aureus* respectively. Data revealed that DBD-ACP system of 5 kV can be effectively used for the reduction of microbial load of around 2 – 3 log from the surface. For more promising data more research needs to be undergone using this technology with different commodities and different microorganisms. This technology can become one of the reliable most efficient non-thermal microbial decontamination technologies in the upcoming era.

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