

Efficacy of DBD Plasma Generator with Different Shapes and Materials of Electrodes for Reducing the Microbial Contamination of Herb Powder[†]

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Abstract

This paper studies the efficacy of a dielectric barrier discharge (DBD) plasma generator with circular and square electrodes with stainless steel and copper materials in the reduction of microbial contamination in herbs. Four types of electrodes are used: copper and stainless-steel square electrodes of the size 110×110 mm² and circular electrodes with a 94 mm diameter. The voltage supplied to plasma generator varies between 5 - 15 kV and the frequency is between 1 - 7 kHz. Licorice (*Glycyrrhiza glabra* Linn.) and sappan tree (*Ceasalpinia sappan* Linn.) herb powders were packed in polyethylene bags of the size 89×89 mm² and tested for microbial contamination before and after the plasma irradiation. The samples were treated with plasma for 5, 10, 15, 20, 25 and 30 min. The results indicated that plasma treatment of 30 min is the most effective time for reducing in the aerobic plate count and yeast and mold count of licorice herb powder. This plasma treatment condition effectively reduced initial the aerobic plate count and yeast and mold count from 8.00×10^4 to 1.30×10^3 CFU/g and 4.20×10^2 to 8.0×10 CFU/g, respectively. *Escherichia coli* were not founded in the control sample and plasma treatment samples (MPN/g < 3.0). The results revealed that plasma treatment reduced microbial contamination to acceptable levels in herb seasoning as indicated in TCP 1380/2550. In sappan tree powder, plasma irradiation also effectively reduced artificially contaminated of *Bacillus subtilis* in sappan herb.

Keywords: Dielectric Barrier Discharge (DBD) plasma, licorice, sappan tree, herb powder, microbial contamination

Introduction

This research investigates the efficacy of the dielectric barrier discharge (DBD) plasma for reduction of microbial contamination in herbs. The sterilization technique can be applied for medical disinfection. In order to develop a new knowledge database in food disinfection tools correctly and safely, the first commercial ozone generator device was proposed by Ernst Werner Von Siemens in 1857 with the general names DBD plasma [1]. It is widely known as a “barrier discharge” or a “silent discharge” which was proposed by Andrews and Tait in 1860 [2]. Kogelschatz and group showed plasmas which were generated between electrodes and many types of shapes such as planar and cylindrical shapes in 1997 [3] because the shape and structure of the electrodes have effects on the characteristics of the plasma generated [4,5]. In 2012 Wang *et al.* studied how the different electrode shapes affect the electrical

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properties [6]. Moreover gas types which are used as an intermediary between the electrodes were also studied [7,8]. In recent years, Okazaki *et al.* studied the appearance of electrical properties in different types of gas. The use of argon, oxygen and nitrogen at atmospheric pressure and 50 Hz frequency showed that different types of intermediary gas affect the electrical characteristics which are important in sterilization [9,10]. The benefit of the DBD plasma is applied in the industry environment and medicine, such as the production of ozone in killing bacteria. Many research studies have been made to bring the plasma applications to agriculture, such as the disinfection of agricultural crops and food, and the acceleration of germination of seed and growth by studying the effects of the seed through the plasma by Bosenia *et al.* in 2010 [11]. Jai *et al.* studied efficiency of a machine using DBD plasma for killing *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The disinfection discharged by 50 mm equals size area \times 20 mm air gap size equal to 1 mm aluminum, thickness 1 mm, is the dielectric material by *E. coli* testing sterilization, which is coated on glass slide off by using poly-L-lysine as a negative pulse discharge levels where adhesion 1 kHz and 11 kV. In the period of 0, 10, 30, 50 and 70 s, respectively. Three-repeat testing found the time 70 seconds are most effective in breaking down the levels of *E. coli* infection 99.99 % plot graph to find out the results of the D-Value period in reducing the infection from the initial fuel quantity in 90 %, equivalent to 15.2 s [12].

Sterilization is a physical or chemical process that impairs or eliminates potentially harmful microbes, especially bacteria. In herbs and spices, the high levels of microbial contamination such as bacteria and fungi reported by many of studies [13,14]. Bacterial contamination may come from soil, insects, animals and fungal growth may occur during the drying process or during storage. These biological food contaminations can cause foodborne illness. From recent studies about plasma system, the results show that the utilization of plasma can significantly reduce the population of microbes in variety of food products [13,15-17]. However, the decontamination of herbs by using plasma has rarely been described [13].

The aim of this study is to evaluate the effect of DBD plasma on reduction of microbial contamination of herb powder. This paper is organized as follows; brief descriptions of the setup, the characteristics of DBD plasma, and sample preparation are given in the next section. Then, the microbial analysis method is described, followed by the results and discussions.

Materials and methods

Set up for study of characteristics of DBD plasma

The DBD plasma generator is used with an AC high voltage power supply. The main structure of the plasma consists of two plate electrodes, 2 dielectric plates, and a generator. The gap between the two dielectrics is 1, 2 and 3 mm. The AC power supply was used with voltage up to ± 15 kV, 470 nF capacitance, and the 1 - 7 kHz frequency. When a high voltage is run through the electrodes, an electric field ionizes the air around the top electrode and creates plasma. The plasma power can be obtained by measuring the voltage across the capacitor and the voltage supplied to the electrode. As shown in **Figure 1**, the voltage measured by the coupler can be calculated from the charge and capacitance using the equation $V = Q/C$. The charge as a function of the applied voltage can be plotted as the discharge Lissajous figures [18,19].

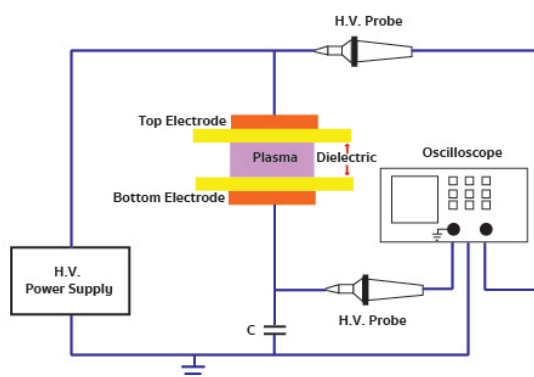


Figure 1 Diagram shows the structure of the main generator, consisting of 2 electrodes separated by at least one layer of dielectric material and an air gap.

Electrode materials

Four types of electrodes are used: stainless steel [20] and copper [21] square electrodes of the size $110 \times 110 \text{ mm}^2$ and the circular electrodes with a 94 mm diameter. The selection of such high temperature and humidity resistant materials is based on the aim to decontaminate or sterilize the samples. The materials are required to have high temperature resistant, anti-scaling, high durability under plasma treatment. The surface must be smooth and easy to clean. The general parts of the device are made of stainless steel, but such material is a poor conductor of electricity. The plasma power generated using copper and stainless-steel electrodes are compared in this study.

Sample preparation

Five grams of each sample of sappan tree (*Ceasalpinia sappan* Linn. or Fang in Thai) and licorice (*Ocimum tenuiflorum* Linn. or Cha Eam in Thai) powders were packed in sealed polyethylene (PE) bags of size $89 \times 89 \text{ mm}^2$. In sappan tree samples, artificial contamination of *Bacillus subtilis* which is the most important contaminate in herb was added in the powder at level of $3.50 \times 10^2 \text{ CFU/g}$ of dry sample (**Figure 2**). For the microbial reduction test of plasma, a total of 42 packets were prepared for each type of herb and 6 packets were placed inside the device for each treatment.

Experimental setup

The DBD plasma generator consisted of circular stainless-steel electrode setup at the parameters of frequency 5 kHz, voltage 16 kV, gap 1 mm was set up to test of microbial reduction in herb powder. The 5 g of sample was placed between the gap and treated by plasma. The plasma treatment time was varied from 0, 5, 10, 15, 20, 25 and 30 min. A total of 6 packets was run in each treatment.



Sappan tree (*Ceasalpinia sappan* Linn.)



Licorice (*Ocimum tenuiflorum* Linn.)

Figure 2 Herb powders; sappan tree [22,23] and licorice [24].

Microbial analysis

The microbial study was done to enumerate the aerobic plate count, yeast and mold count, coliform, *E. coli* and *Clostridium perfringens* before and after 5 days of treatment. Ten grams of herb powder sample were collected with sterile technique. The aerobic plate count were done by pouring samples in plate count agar (Hi-Media, India) and incubated at 35 ± 1 °C (BAM Online, 2001) [25]. The yeast and molds count were enumerated by pouring samples in potatoes dextrose agar (Hi-Media, India) (BAM Online, 2001) [26]. Coliforms (presumptive) and *E. coli* were enumerated by the 3-tube Most Probable Number (MPN) procedure (BAM Online, 2002) [27]. *C. perfringens* were detected in TSC Agar (Tryptose Sulphite Cycloserine Agar) (Merck, German) and followed up by the procedure (BAM Online, 2001) [28].

Statistical analysis

The measurements were repeated 3 times ($n = 3$) and presented as means \pm log CFU/g from 7 independent experiments determined by one-way ANOVA with Duncan's new multiple range -post hoc test. A *p*-value of less than 0.05 was considered to be statistically significant.

Results and discussion

DBD plasma generator with different electrode shapes and materials, voltages, and frequencies

The highest plasma power was achieved at the following parameters: frequency 5 kHz, voltage 16 kV, gap 1 mm, circular stainless-steel electrode. In **Figure 3**, at the frequency range 1 - 2 kHz, no plasma appeared. The plasma power was high in the frequency range 3 - 7 kHz and increased when the voltage is applied. The results show that the voltage could not reach 15 kV due to the RLC circuit limitation. The frequency change resulted in a different total resistance of the system. The electrodes have different resonance frequencies.

DBD plasma treatment on reduction of microbial contamination

The results showed that the microbial contamination in untreated plasma licorice powder exhibited high level of the aerobic plate count, yeast and mold count and coliform bacteria. The high contamination level of yeast and mold count and coliform were also found in sappan tree powder. This microbial level exceeds the accepted level in seasoning herb as indicated in the standard (TCPS 1380-2550: total aerobic plate count $< 1.0 \times 10^6$ CFU/g, yeast and mold count $< 1.0 \times 10^2$ CFU/g, *E. coli* < 3 MPN/g). Plasma are an efficient source of electrons, ions, electric field and free radicals, depending on the discharge conditions. In this study, plasma treatment times at 5 min produced from circular stainless-steel electrode at the frequency 5 kHz, voltage 16 kV, gap 1 mm. significantly reduced microbial level in sappan tree and licorice powder samples as compared to the standard samples. The treatment time of 30 min was the most effective in reducing yeast and mold count in sappan tree (0.94 log) and licorice powder (0.73 log) (**Table 1**). Plasma treatment time at 5 - 30 min reduced the bacteria count of the licorice and artificially contaminated of the sappan tree powder by more than 1.6 log. Aerobic plate count and yeast and mold count at different treatment times are shown in **Figure 4**. However, these conditions treatment could not perfect eliminate coliform bacteria in licorice, due to the highly contamination level in the initial samples. There is only a limited effect of active plasma on the surface of herb powder due to the presence of the packing cover of the sealed PE packets.

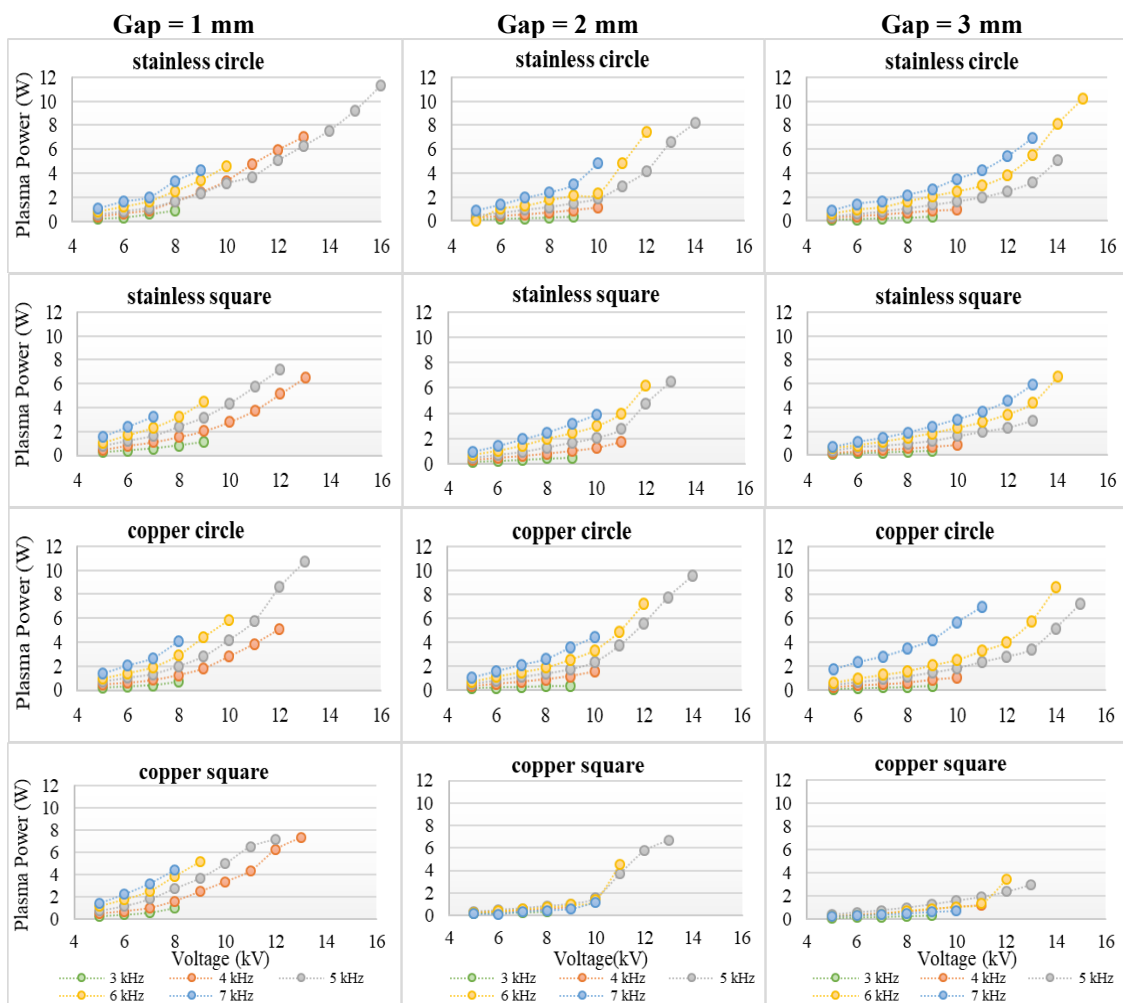


Figure 3 Plasma power vs. voltage graphs for different types of electrodes: stainless steel & circular (1st row), stainless steel & square (2nd row), copper & circular (3rd row), copper & square (4th row) and gap distance between electrodes are 1 (left col.), 2 (center col.) and 3 mm (right col.).

Table 1 Reduction of microbial contamination in sappan tree and licorice herb powder after DBD plasma treatment.

Herb	Times (min)	APC (log CFU/g)	YM (log CFU/g)	CP (log CFU/g)	Coliform (MPN/g)	EC (MPN/g)
Sappan tree	0	3.48±0.11 ^a	2.53±0.28 ^a	0	3.6	< 3
	5	2.85±0.15 ^b	1.84±0.12 ^b	0	< 3	< 3
	10	2.89±0.16 ^b	1.75±0.08 ^b	0	< 3	< 3
	15	2.77±0.10 ^b	1.69±0.09 ^b	0	< 3	< 3
	20	2.83±0.18 ^b	1.80±0.14 ^b	0	< 3	< 3
	25	2.83±0.18 ^b	1.77±0.12 ^b	0	< 3	< 3
	30	2.81±0.05 ^b	1.59±0.16 ^b	0	< 3	< 3
Licorice	0	4.71±0.45 ^a	2.61±0.12 ^a	0	460-1,100	< 3
	5	3.62±0.11 ^b	2.18±0.35 ^{bc}	0	38-150	< 3
	10	3.59±0.58 ^b	2.09±0.02 ^b	0	23	< 3
	15	3.85±0.16 ^b	2.19±0.12 ^b	0	43	< 3
	20	3.10±0.14 ^b	2.26±0.04 ^b	0	9.2-27	< 3
	25	3.03±0.47 ^b	2.16±0.23 ^b	0	9.2-11	< 3
	30	3.04±0.06 ^b	1.88±0.03 ^c	0	3.6-11	< 3

Mean with difference small letters within column attribute are significantly different ($p < 0.05$). APC: Aerobic plate count, YM: Yeast and mold count, CP: *Clostridium perfringens*, EC: *Escherichia coli*.

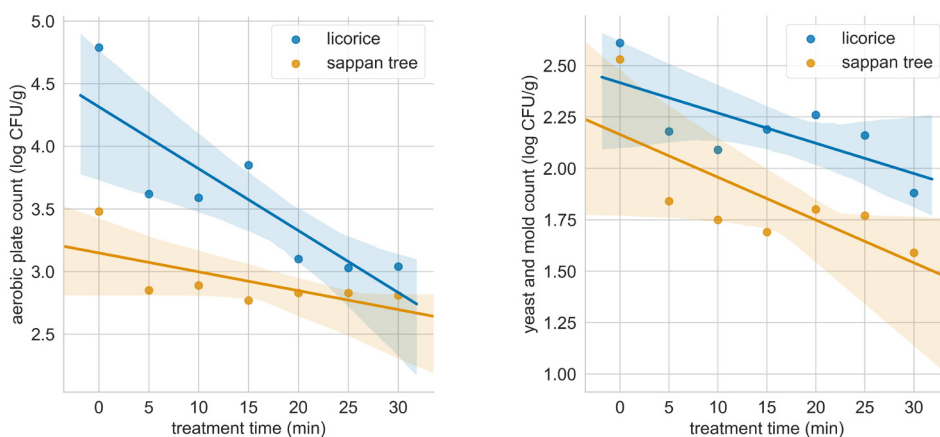


Figure 4 Aerobic plate count (left) and yeast and mold count (right) against the plasma treatment time for the herb samples. The bands show the 95 % confidence interval.

The results in this study have similar findings compared to the work by Hertwig *et al.* [29] that reports the efficacy of a non-thermal remote plasma application for 3 different types of herbs and spices (pepper seeds, crushed oregano and paprika powder) with various surface-to-volume ratios were treated with plasma processed air, the inactivation of their native microbial flora was examined. The remote plasma treatment reduced the native microbial flora of the pepper seeds and the paprika powder by more than 3 log after 60 min treatment time. Takemura *et al.* [30] performed inactivation processing using an atmospheric pressure plasma jet to achieve efficient inactivation of microorganisms for spices. Based on sterility tests, the microorganisms attached to the spice could be sterilized by 5 min treatment with an argon⁺ carbon dioxide atmospheric plasma jet. This present study revealed that efficacy of plasma treatment on inactivation of microbes in dry foods such as spices and herbs depend on plasma treatment time and plasma chemical reactions following breakdown.

Conclusions

A dielectric barrier discharge consisting of 2 electrodes with at least one layer of insulating material in between them is used to test microbial contamination reduction. For the purpose of reducing microbial contamination in this experiment, the circular stainless-steel electrode setup with 16 kV voltage at 5 kHz frequency power supply, and 1 mm of gap distance has been shown to generate the highest plasma power. The efficacy of plasma decontamination of herbs depends on the material type and shape of the electrodes, frequency range, applied voltage and gap size. For sappan tree powder, 5 min plasma treatment at the highest plasma power could be effective in eliminating coliform bacteria and significantly reduce artificially contaminated *B. subtilis* as well as yeast and mold count. At the 5 - 10 min treatment interval (5 - 10 min), plasma also significantly reduced natural contaminants of aerobic bacteria, yeast and mold and coliform bacteria in licorice powder. Thirty min treatment was the most effective time in reducing yeast and mold count. However, these conditions treatment could not perfectly eliminate coliform bacteria because of the high level of natural contamination.

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