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Article

Microbial Reduction of Bitter Melon *(Momordica charantia L.)* and Chan Khao (*Tarenna hoaensis Pitard*) Herb Powder by Dielectric Barrier Discharge Plasma for Food Sanitary

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Abstract. The influence of air plasma treatment on microbial inactivation contaminants in bitter melon (*Momordica charantia L.)* and Chan Khao (*Tarenna hoaensis Pitard*) powders, obtained from the local retail market in Nakhonnayok, has been investigated. The dielectric barrier discharge (DBD) air plasma model has been designed for in-package treatment by using a neon transformer. From the microbial analysis, there were high amounts of total aerobic plate count (TPC) as well as total yeast and mold counts (TYM) contaminants in both samples. It could be confirmed that the DBD air plasma treatment has a positive effect on microorganism reduction. Moreover, the color of air plasma-treated powders has still been chiefly preserved after the treatment process. The kinetics microbial inactivation process in this study has been found to be a single-slope survivor curve since the reduction rate is linear and increased with treatment time. The TPC and TYM concentration in the plasma-treated samples could be decreased to microbiologically qualify for the acceptance criteria of herbal drug preparations as indicated in the Thai Pharmacopoeia Volume I and II Supplement 2005 standard (TP Supplement 2005).

Keywords: Non-thermal plasma, air discharge, food preservation, microorganism inactivation.

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1. Introduction

Thai herbal seasoning is one of the most famous ingredients globally due to its unique tastes and smells as well as nutritional benefits [1]. There is a wide range of Thai herbal seasoning production from in-house manufacturing in the commercial industry. Generally, most consumers prefer purchasing these products in retail shops, which provide various affordable priced products from in-house manufacturing. However, some of those products have not qualified for the standards in manufacturing. During the drying or preparing processes of herbal powder preparation, bacterial contamination and fungal growth may arise. These pathogenic bacteria and mold can grow and produce toxins in the products, resulting in foodborne illnesses. These illnesses would primarily be due to the contamination of chemical substances and microorganisms in food consumption [2].

Microbial food contamination is one of the main factors that result in various sicknesses such as food poisoning, diarrhea, meningitis, and enteric infections. Moreover, long-term consumption of microbial contaminated food could result in cancer and fatal health consequences [2]. The most common microorganisms that result in foodborne illness are *Bacillus cereus*, *Escherichia coli, Salmonella, and Staphylococcus aureus,* including viruses, parasites, yeast, and mold. Therefore, food pasteurization is an unavoidable process that should be included in all food production. Several pasteurization techniques include ohmic heating, high-pressure processing, ultrasonic technology, pulsed electric field processing, and non-thermal plasma treatment [1]–[4].

The non-thermal plasma technique has recently become attractive among food sterilization techniques due to its various advantages, such as non-thermal processing, shortened processing time, and environmental-friendly processes. Various energetic short-life beneficial reactive oxygen and nitrogen species (ROS and RNS) produced during air plasma generation have played a crucial role in microbial inactivation. Examples of some of these useful radicals include singlet oxygen (O), ozone (O_3), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-), nitric oxide (NO) and peroxynitrite ($ONOO^-$), and radicals of hydrogen ($H^\cdot$) and oxygen ($O^\cdot$) [5]–[12]. A fast pasteurization process could be achieved regarding these reactive species resulting in nutritional contents, appearance, and taste preservation. One of the most famous non-thermal plasma models applied in thermal sensitive material treatment is dielectric barrier discharge (DBD) plasma. The unique configuration of the DBD plasma model is that at least one dielectric layer has been inserted between model electrodes. This benefit causes the sample treated by DBD plasma to not be in contact with the high voltage electrode. Besides, the dielectric layer of DBD plasma would prevent arc discharge development [13], [14].

In this paper, the study of the influence of DBD plasma treatment on the inactivation of microorganisms contaminated in Thai seasoning powder has been proposed. Two popular Thai herbal powders obtained from the local retail shop, bitter melon *(Momordica charantia L.)* (MC) and Chan Khao (*Tarenna hoaensis Pitard*), have been evaluated at this time. These two herbs could be effectively applied for medical treatment. It has been reported that MC has potential for blood glucose modulation, which is helpful in diabetes treatment; moreover, it has also shown positive effects on the treatment of cancer, blood diseases, ulcers, urinary discharges, asthma, and others [15]–[17]. Chan Khao is highly prized for its medicinal applications and used as an ingredient in many Ayurvedic and Siddha formulations in India, which possesses antipyretic activity in Thai traditional medicine [18], [19]. The DBD air plasma model has been economically and compactly designed for in-package treatment, which would enable applications used in in-house manufacturing. The characteristics of DBD plasma generated by a low-cost neon transformer have also been discussed. The microbial disinfection efficiency of plasma treatment at different treatment times has been analyzed. The electrical characteristics of DBD air plasma, and the color of the plasma-treated samples have also been investigated.

2. Materials and Experimental Procedures

2.1. Plasma Model and Experimental Setup

The schematic drawing of the dielectric barrier discharge (DBD) plasma model configuration and experimental setup have been illustrated in Fig. 1. The DBD plasma model has been composed of two conductive electrodes; the upper electrode was a 9.4Ø cm circular copper plate, and the lower electrode was an 11×11cm^2 copper plate. The upper electrode was hooked onto the acrylic board and hung on the exterior frame. High-frequency high voltage (125W commercial neon transformer, Topneon, TPN-1520A) was supplied to the upper electrode through the 150 kΩ ballast resistor in series while the lower electrode was connected to another power-supplied terminal and grounded. A 12×12cm^2 acrylic sheet with a thickness of 1 mm, as a dielectric material, was attached to the lower and upper surfaces of the upper and lower electrodes. The space between the electrode units could be adjusted by the laboratory scissor jack placed under the acrylic board under the lower electrode. The discharge waveforms of voltage (V_d) and current (I_d), monitored by an oscilloscope (SIGLENT, SDS2304), were measured across the DBD plasma model by a high voltage probe (Pintek HVP-39pro), and measured by a current probe (Tektronix, A621) connected between the lower electrode and ground, respectively. An optical fiber detector of a CCD spectrometer (Newport 71SI00087) was placed between the gap of two electrode units to investigate the optical mission spectroscopy (OES).

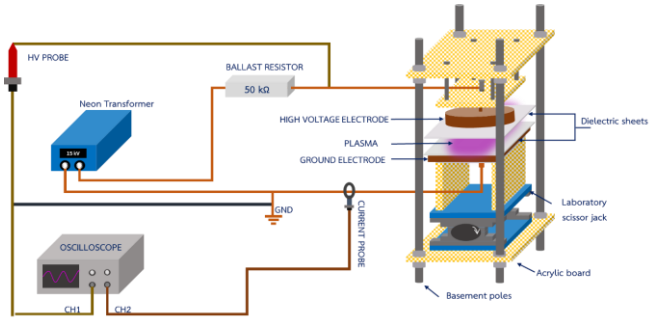


Fig. 1. The schematic drawing of DBD plasma model.

2.2. Plasma Treatment Process

The samples consist of bitter melon powder *(Momordica charantia* L.) and Chan Khao powder (*Tarenna hoaensis Pitard*) obtained from the retail market in Ongkarak district, Nakhonnayok province, Thailand. Five grams of herb powder were packed and sealed in an 80×80 mm² polyethylene (PE) bag. At this condition, the thickness of the bag was 1 mm. For the effect of plasma on microbial reduction, a total of 48 packets were prepared for each type of herb. The herb samples were treated with the plasma for 60, 120, and 180 seconds. A total of 12 packets were run in each treatment. The control sample was prepared in the same condition without plasma treatment. All of the samples were kept at ambient temperature (25±3 ^{0}C).

2.3. Bacteriological Analysis

After the treatment process, microbiological analysis was performed using a quantitative method at the microbiology laboratory of the Thailand Institute of Nuclear Technology (Public Organization). All the stages involved culture medium preparation and inoculation. The reference standard cultures were performed before sample analysis. Working standards were cultured on an agar plate medium such as tryptic soy agar (TSA) (Merck, German) and incubated at 35±1 ^{0}C for 24-48 hours. This technique is used to check the purity of cultures, and should be tested by streaking for single colonies. The followed-up procedure was performed by inoculating the single colony into trypticase soy broth (TSB) (Merck, German) and incubated at 35±1 ^{0}C for 24-72 hours. When the sample analysis was determined, the diluting method was used for preparing microbiological cultures at a concentration of 100-1,000 CFU/g.

The microbial study was done to enumerate the aerobic plate count, yeast and mold count, coliform bacteria, fecal coliform, and *Escherichia coli.* (*E. coli*). Pathogens such as *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*) were also investigated. Herb powder samples were collected with sterile technique at 10 g. The total aerobic plate count was done by pouring samples in plate count agar (Hi-Media, India) and incubated at 35±1^{0}C for 24-48 hours [20]. The total yeast and mold count was enumerated by pouring the samples in potato dextrose agar (Hi-Media, India) and incubated at 25±1^{0}C for 5-7 days (Bacteriological Analytical Manual, 2001). Coliforms, fecal coliform bacteria, and *E. coli* were counted by the three-tube most probable number (MPN) procedure and followed up by the identification procedure [21]. *E. coli* TISTR 117 was used as a positive control, and *Salmonella typhimurium* TISTR 292 (ATCC 13311) was used as a negative control. *S. aureus* was enumerated in trypticase soy broth (Merck, German) containing 10% sodium chloride and 1% sodium pyruvate by MPN method and followed up by the procedure for identification and confirmation of *S. aureus* [20]. The working culture of *S. aureus* TISTR 885 (ATCC 13150) was used as a positive control, and *Staphylococcus epidermidis* TISTR 518 (ATCC 14990) was used as a negative control. *B. cereus* was enumerated by spreading dilution samples on Mannitol-egg yolk polymyxin agar (Merck, German) with a modified method and incubated at 35±1^{0}C for 24 hours, after which the positive colony was confirmed (Bacteriological Analytical Manual, 2020). The purified working culture of *B. cereus* identifying from dry food was used as a positive control. and *B. subtilis* TISTR 008 (ATCC 6633) was use as negative control. The measurement was performed repeatedly three times (n=3) for the statical analysis.

2.4. Effects on Color

After plasma treatment, all samples were kept at ambient temperature (25±3^{0}C). The measurement for color characteristics was conducted after 45 days of storage. The samples were measured the color in Hunter *L**, *a**, *b** color system by Hunter *L* a* b** colorimeter (Chroma Meter Konica Minolta CR-300, Osaka, Japan), where *L** referred to darkness to lightness, a* green to red and b* yellow to blue color parameters. The average of *L*, a**, and *b** were calculated, and the results were compared with the control samples within the groups. The results (*L*, a**, and *b**) are the mean of 6 replication.

2.4. Statistical Analysis

The measurements were performed repeatedly three times (n = 3) and presented as means ±SD Log CFU/g from 4 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 statistically significant by SPSS statistical software version 19.0 (SPSS Inc.; Chicago, IL, USA).

3. Experimental Results

3.1. Electrical and Optical Emission Characteristics of DBD Plasma

To archive the uniform DBD plasma using a neon transformer, the DBD plasma model was developed from our previous work [4]. The upper electrode was designed as a circular shape with a diameter smaller than the size of

the square counter electrode. Both dielectric layers have a larger size than both electrodes to prevent the arc-flash discharge. The gap between both electrode units had been optimally adjusted for the most uniform plasma. The optimum gap distance for uniform plasma generation at this time was 2 mm. Fig. 2 presents the image of uniform DBD air plasma treatment on the in-package sample. Numerous air micro discharges (the purple color area) have been generated uniformly, covering all overlapping areas between high voltage electrodes. Therefore, it could be implied that the sample had been thoroughly treated by air plasma.

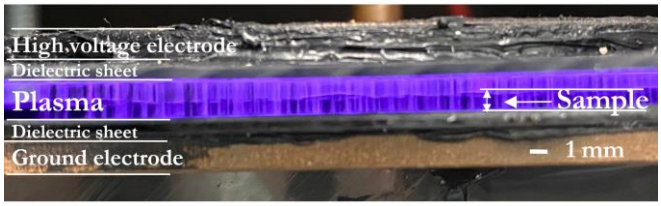


Fig. 2. DBD air plasma characteristic.

The electrical characteristics of DBD plasma have been depicted in Fig. 3a). The discharge voltage and current waveforms were out of phase around 90 degrees regarding a capacitive plasma load. The primary frequency of the applied voltage was around 18 kHz. The average voltage across the plasma model was 6.36 kV_{rms}. It could be observed that there were many micro discharges generated during the rising and falling edge of the voltage waveform with a micro discharge frequency around 256 kHz. The maximum peak discharge current of micro discharge was up to 158 mA, approximately [7], [22]. For the 180 seconds treatment, the temperature between electrodes was no higher than 48° Celsius; therefore, the samples were not be damaged by heat.

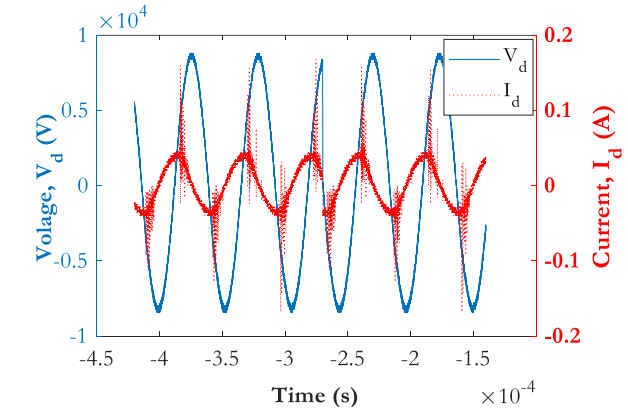
The optical emission spectroscopy (OES) of DBD air plasma is illustrated in Fig. 3c. The emission lines of the hydroxyl ion (OH^-) radical (306-315 nm) and oxygen excited atoms (777 and 844 nm) were noticed. Additionally, dominant detected peaks (300−400 nm) corresponding to the N_2 second positive system were observed [9], [23]–[27]. These radicals have influenced an essential role in generating various beneficial reactive oxygen and nitrogen species (RONS) products such as nitrogen oxide (NO), nitrogen dioxide (NO_2), OH^-, O_3, and H_2O_2, which strongly affect microbial inactivation.

3.2. Influence of DBD Air Plasma Treatment on Microbial Inactivation

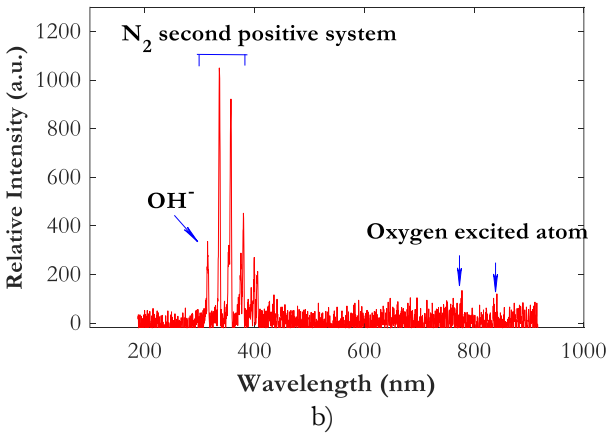
Regarding the bacteriological analysis, it was found that the control group of bitter melon powder carried certain high amounts of total aerobic plate count (TPC), and total yeast and mold count (TYM) at the level of 6.80×10^5 CFU/g, and 1.10×10^2 CFU/g, respectively, which exceeded the acceptance criteria for microbiological quality of herbal drug preparations as indicated in the Thai Pharmacopoeia Volume I and II Supplement 2005 standard (TP Supplement 2005): TPC < 5.0×10^5 CFU/g, TYM < 5.0×10^3 CFU/g) as shown in Table 1. The TPC was found on a medium level of natural bacterial contamination for the control group of Chan Khao powder, while TYM was found at a low level. The TPC and TYM reduction trend when the treatment time was increased has been illustrated in Fig. 4.

The experimental results and statistical analysis revealed that DBD plasma treatment for 180 seconds could effectively improve microbial inactivation of bitter melon powder. TPC was also significantly decreased, resulting in meeting microbial criteria standard satisfaction. DBD plasma treatment at 180 seconds had also significantly reduced the TPC and eliminated TYM from 10-50 CFU/g to < 10 CFU/g in Chan Khao powder. The inactivation efficiency of both samples increases with an increase in treatment time. However, it could be noticed that all-detected microorganisms could be reduced below the standard within the first 60 s plasma treatments in the case of Chan Khao powder, which was faster than that of bitter melon powder.

The microbial analysis of coliform bacteria, fecal coliform bacteria, *E. Coli*, *B. cereus,* and *S. aureus*, the foodborne pathogenic bacteria commonly found in herbal seasoning powder, contamination in the samples was also investigated. The analysis results show that those mentioned microorganisms had not been found in both samples. (Table 1.).



a)



b)

Fig. 3. Characteristics of a) electrical waveforms, and b) optical emission spectra of DBD air plasma.

3.3 Effect on Color

The effects of plasma treatment on the color of herb powders are shown in Table 2. Colors are considered one of the most significant properties of food. The poor color quality of food has not been favorable to the customer attraction. In this study, the investigation of color characteristics was surveyed by a color system of Hunter L^* a^* b^*. In bitter melon, the results showed that DBD air plasma treatment at 60-180 seconds did not affect on L^* value. However, a minor effect on color a and b values have been found in this case. A significantly slight increase in greenness and yellowness was found after 60-180 seconds of treatment. In contrast, the color of Chan Khao powder has been preserved for all experimental conditions.

Table 1. Microbial inactivation of herb powders by DBD plasma at various treatment times (s).

	Microbe/treatment time (s)*			
	0	60	120	180
Bitter Melon (*M. charantia*)				
TPC	5.81±0.16^{a}	5.50±0.09^{ab}	5.37±0.01^{ab}	5.03±0.32^{c}
TYM	2.01±0.17^{a}	1.37±0.41^{a}	1.46±0.45^{a}	1.59±0.11^{a}
BC	< 10	< 10	< 10	< 10
CL	< 3.0	< 3.0	< 3.0	< 3.0
EC	< 3.0	< 3.0	< 3.0	< 3.0
SA	< 3.0	< 3.0	< 3.0	< 3.0
Chan Khao (*T. hoaensis*)				
TPC	3.29±0.36^{a}	2.55±0.15^{b}	2.66±0.10^{b}	2.85±0.15^{b}
TYM	1.23±0.4^{a}	0.67±0.57^{ab}	0.67±0.57^{ab}	0.00^{b}
BC	< 10	< 10	< 10	< 10
CL	< 3.0	< 3.0	< 3.0	< 3.0
EC	< 3.0	< 3.0	< 3.0	< 3.0
SA	< 3.0	< 3.0	< 3.0	< 3.0

TPC: Total aerobic plate count (mean±SD log CFU/g); TYM: Total yeast and mold count (mean±SD log CFU/g). Values with difference letters (a, b, c) within the row differ significantly (p< 0.05). BC: *B. cereus* (CFU/g). CL: Coliform bacteria (MPN/g). EC: *E. coli* (MPN/g). SA: *S. aureus* (MPN/g).

Table 2. Comparison of color in Hunter L^* a^* b^* unit of the control herb sample and plasma treatment groups.

	Average colour /treatment time (seconds)*			
	0	60	120	180
Bitter Melon (*M. charantia*)				
L	48.87±0.71^{a}	49.67±0.12^{a}	49.42±1.17^{a}	49.23±0.78^{a}
a	-2.22±0.10^{a}	-2.37±0.03^{b}	-2.32±0.04^{b}	-2.31±0.09^{b}
b	13.51±0.29^{a}	13.96±0.16^{b}	13.88±0.25^{b}	14.04±0.39^{b}
Chan Khao (*T. hoaensis*)				
L	73.60±0.70^{ab}	74.67±0.89^{b}	73.61±1.09^{ab}	73.23±1.00^{a}
a	1.06±0.03^{a}	1.06±0.03^{a}	1.00±0.07^{a}	1.03±0.09^{a}
b	15.98+0.22^{a}	16.13±0.12^{a}	15.86±0.26^{a}	15.99±0.30^{a}

Values with difference letters (a, b, c) within the row differ significantly (p< 0.05).

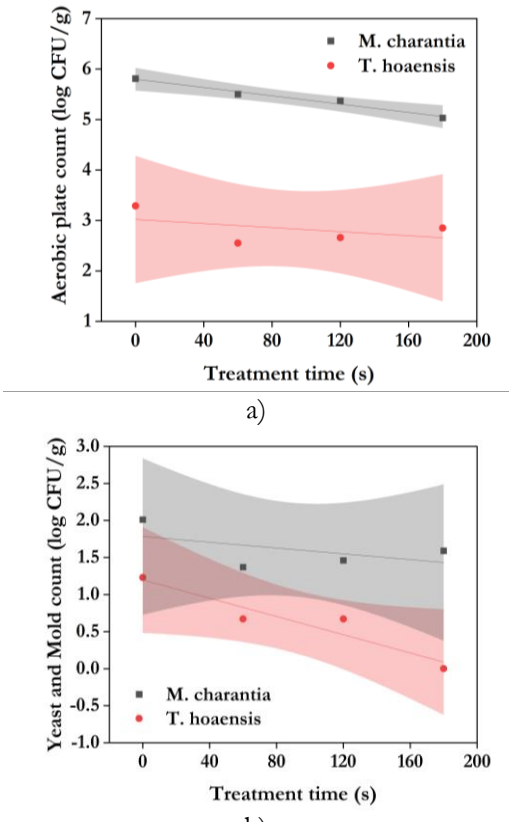


Fig. 4. Reduction of a) total aerobic plate count, and b) total yeast and mold count at various treatment times.

4. Discussion

Concerning the experimental results and microbial analysis, it could be confirmed that DBD air plasma has significantly enhanced the detected microorganism inactivation in this study (TCP and TYM). The kinetics of the TCP and TYM inactivation process in this study could be classified in a single-slope survivor curve since the microorganism reduction rate are linear, as shown in Fig. 4 [4], [28]–[32]. The mechanism of microbial destruction by air plasma could be discussed as follows. The microbial inactivation process could possibly be due to three main factors, which are UV radiation [33], [34], charged particles [35], and energetic active radicals [28], [36]. UV radiation in the air DBD plasma would contribute a secondary role in the destruction process by reaction with the nucleic acids in microorganism cells. The charged particles generated during plasma generation would affect the microorganism cell instability resulting in electroporation and electrostatic disruption of microbial cells [37], [38]. The essential RONS (especially OH^- and NO radicals, NO_2, H_2O_2, O_3, and atomic oxygen) seemed to be the most crucial factor contributing to microorganism inactivation of non-thermal air plasma [23],

[38]–[40]. Those RONS have directly interacted with the microorganism cells causing the etching process on the cell membranes. This process would let the RONS bio-chemically react with the macromolecules within cells resulting in lesions and destruction of chemical bonds and molecules of microorganism cells.

Regarding the experimental results and economic perspective of the proposed method, the operating cost is comparatively low since the treatment time is very short compared to other thermal pasteurization techniques [3], [4], [11], [31], [38], [41]. At the most effective treatment time of this study, the energy consumption was just 0.00625 units (kW/h). Moreover, the model configuration is simple and easy to be operated. Therefore, it could be confirmed that the proposed DBD treatment technique can be practically utilized for food sanitary and microorganism inactivation. Even though there is no report about the threat caused in plasma-treated food, the effect of DBD air plasma on other physical and chemical analyses, such as taste, grain texture, and nutrition, should be investigated and clarified to attract consumer trust, which would be the further study of this research.

5. Conclusions

Atmospheric non-thermal dielectric barrier discharge (DBD) air plasma has been proposed for microbial inactivation contaminated in bitter melon and Chan Khao powders from in-house manufacturing for promoting the health benefits of herb seasonings. High amounts of total aerobic plate count (TPC) and total yeast and mold count (TYM) contaminants from the microbial analysis were found in both samples. The proposed DBD model was suitable for the in-package treatment. At this time, the neon transformer was used as the high voltage source for low-cost DBD plasma generation. The herb samples were treated by DBD air plasma at different times, ranging from 0, 60, 120, and 180 seconds. From the experimental results, it could be confirmed that the air plasma could significantly decrease the TPC and TYM contamination in samples to satisfy the TP Supplement 2005 standard. Moreover, the color characteristics of the herb after air DBD treatment could be mostly preserved. The increase of treatment time could enhance the microorganism inactivation efficiency. It was found that the microorganism reduction rate in Chan Khao powder was faster than that of bitter melon powder. In this case, only 60-second treatments could reduce all-detected microorganisms to meet the standard. The study could be confirmed that DBD air plasma could be a novel technique for food sterilization and preservation. Moreover, the proposed DBD model could easily be also flexibly and economically applied to in-house manufacturing.

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