

Research Article

Development of Decontamination Treatment Techniques for Dry Powder Foods by Atmospheric-Pressure Nonequilibrium DC Pulse Discharge Plasma Jet

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Dry powder food ingredients imported to Japan contain large amounts of viable bacteria and coliform bacteria, and we need a simple, low-cost, dry nonthermal decontamination method without spoiling nutrients, color, fragrance, and flavor. In this study, it is shown that the decontamination performance against viable bacteria and coliform bacteria is proportional to the plasma irradiation time when OH and O₃ radicals are incident on the dry powder food ingredients placed in an atmospheric-pressure nonequilibrium DC pulse discharge Ar + O₂ mixture gas plasma jet. Our study revealed that there is a correlation between the plasma irradiation time and DC pulse frequency increase and the decontamination effect on the general bacterial count.

1. Introduction

Introduced in 1991 by the Japanese government, the FOSHU (Foods for Specified Health Use) system [1–5] expanded the food market including supplements and Chinese herbal medicine. The supply of food ingredients including supplements and Chinese herbal medicine greatly depends on both domestic production and importation. The companies taking part in FOSHU market are lately experiencing problems—especially food poisoning caused by orally transmitted bacteria—with large amounts of viable bacteria and coliform bacteria in the dry powder food ingredients imported from abroad. As a means to sterilize viable bacteria and coliform bacteria, the facilities for producing FOSHU

adopt hot air process or steam process [6–8], which causes, due to heat or steam, an adverse effect on nutrients, color, fragrance, and flavor. To solve this problem, a simple, low-cost, dry nonthermal decontamination method is needed without affecting the qualities such as nutrients, color, fragrance, and flavor in the facilities producing FOSHU using dry powder food ingredients containing viable bacteria and coliform bacteria.

To this, the authors have succeeded in introducing the use of the laser-induced fluorescence method [9–12]—which is often said to be difficult—to observe the OH and O₃ radicals emitted from oxygen gas using atmospheric-pressure nonequilibrium discharge plasma. Based on the observations of radicals in this research, it was found that the

existence of OH radicals was verified at a position of 45 mm downstream from the plasma torch nozzle [13]. The techniques using OH radicals, attracting attention as sterilization technology, have often been reported in the medical field [14–17]. Also, Cheng et al. worked on the plasma using equivalent total oxidation potential, which is based on the oxidation potential of the role of reactive oxygen nitrogen species. To evaluate the feasibility of the equivalent total oxidation potential dose, a fitting model is developed, and the bacterial reduction factor is selected as the indicator of plasma's biological effects in the latest research [18].

In this study, to sterilize viable bacteria and coliform bacteria without spoiling nutrients, color, fragrance, and flavor, a new methodology using atmospheric-pressure nonequilibrium DC pulse discharge plasma is proposed for use at low temperatures so as to perform the irradiation of OH radicals to dry powder food ingredients. Therefore, it is shown that the decontamination effect against viable bacteria and coliform bacteria in the dry powder food ingredients increases proportionally to the irradiation time and pulse frequency of the plasma irradiated using an atmospheric-pressure nonequilibrium DC pulse discharge Ar + O₂ mixture gas plasma jet generated by high-frequency DC pulsed power supply.

2. Experimental Setup

Figure 1 shows a schematic of the experimental setup. As shown in the figure, plasma was generated using a DC pulse power source for plasma generation (PHF-1K-W; Haiden Laboratory Inc.) and atmospheric-pressure nonequilibrium DC pulse discharge plasma jet electrodes (PJ-6K; Haiden Laboratory Inc.) with a 1.0–10.0 kHz pulse frequency and 1.0 kW plasma power. The gas mixture of argon (5.0 L/min) and oxygen (0.1–1.0 L/min) was used for the plasma. The atmospheric-pressure nonequilibrium DC pulse discharge plasma jet electrodes can generate dielectric barrier electric discharge over a relatively large area of the upstream part of the torch and efficiently generate active species. In addition, arc discharge is generated in the downstream torch where the dielectric is not present.

Figure 2 shows the oscillogram of the voltage waveforms (pulse frequency: 10 kHz and input power: 300 W) with argon (5.0 L/min) and oxygen (0.1–1.0 L/min) gas. The system first generates silent discharge using the high-frequency component (rising edge) of a single pulse applied by the DC pulse power source and then generates an arc discharge when the pulse voltage reaches a peak; since the voltage is applied as a pulse wave, the discharge time is as small as several μ s, and electromagnetic pumping is weak compared with the DC pulse discharge plasma jet [19]. The plasma torch nozzle is made up of a titanium rod (4.0 mm OD \times 10 mm length) at the center of body and is covered with an SUS pipe (36 mm OD \times 30 mm ID \times 87 mm length). A quartz tube (26 mm OD \times 24 mm ID \times 87 mm length) is placed between the titanium rod and the SUS pipe at the upper part of plasma torch nozzle. The plasma gas flow into the torch is controlled at 5.0 L/min, and at the nozzle exit, the speed is increased by means of a small diameter; active

species generated by silent discharge in the upstream part of the torch efficiently jets out with the gas force [20] and the electromagnetic pumping action [21]. The heating of the electrodes is prevented, and the plasma temperature is kept relatively low because the plasma is supplied by intermittently applying the voltage and the plasma gas flow rate is relatively high [22, 23].

The plasma jet electrodes used in this experiment use argon gas, which is noble gas, to stably generate plasma. Due to the structure of the electrodes, we cannot introduce oxygen gas at the flow rate exceeding 20% of the flow rate of the noble gas. As a preparation of this experiment, we set a stage of aluminium sheet at the distance of 50 mm at the downstream section of the nozzle tip of the electrode. The measurement of the temperature on the stage substrate using type-E thermocouples revealed the substrate temperature around 30°C. At the same time, we installed an ultraviolet lamp at the same height as the tip of the electrode nozzle to verify the decontamination and disinfection effect of the light. However, we observed no change in the general viable bacteria count, and coliform bacteria verification test showed simply positive with no significant effect by the light.

Figure 3 shows the fine powder of mulberry leaves, which is used as dry powder food ingredients in the experiment. The general viable bacteria count in 1.0 g of the mulberry leaves used in the experiment is 3.6×10^5 (g/counts), showing positive result in the coliform bacteria verification test. Generally, the general bacterial count—the count of mesophilic aerobic bacteria such as *Salmonella* and enterohemorrhagic *E. coli* O157 [24–27], which grow under certain conditions—is generally used as a representative index of the level of microbial food contamination for the comprehensive evaluation of safety, storage stability, and hygienic handling of food. In addition, coliform bacteria are non-spore-forming Gram-negative bacilli—without specifying the types of bacteria—covering all types of aerobic or facultative anaerobes that break lactose into acid and gas [28].

Note that the fine powder of mulberry leaves, used in dietary supplements such as green juice, contains carotene, calcium, vitamin B1, and iron, rich with protein, minerals, and nutrients such as dietary fibers and has an effect to improve blood cholesterol levels and neutral fat [29]. In the experiment, the fine powder of mulberry leaves (20 g) was put in a beaker of 50 mL and placed at the distance of 15 mm from the nozzle tip so that the surface of fine powder is not scattered by the plasma gas coming from the plasma electrode. Hence, we were previously able to conduct surface treatment of polymer film using atmospheric-pressure nonequilibrium DC pulse discharge plasma jet at a position of 35 mm from the plasma torch nozzle using laser-induced fluorescence method, which made it possible to confirm the effect of surface treatment based on the influence of radicals [30]. The plasma irradiation time was set to one minute, five minutes, and ten minutes, and the fine powder was stirred every two minutes so that the plasma was irradiated on the whole area of the fine powder. The plasma irradiation time on the

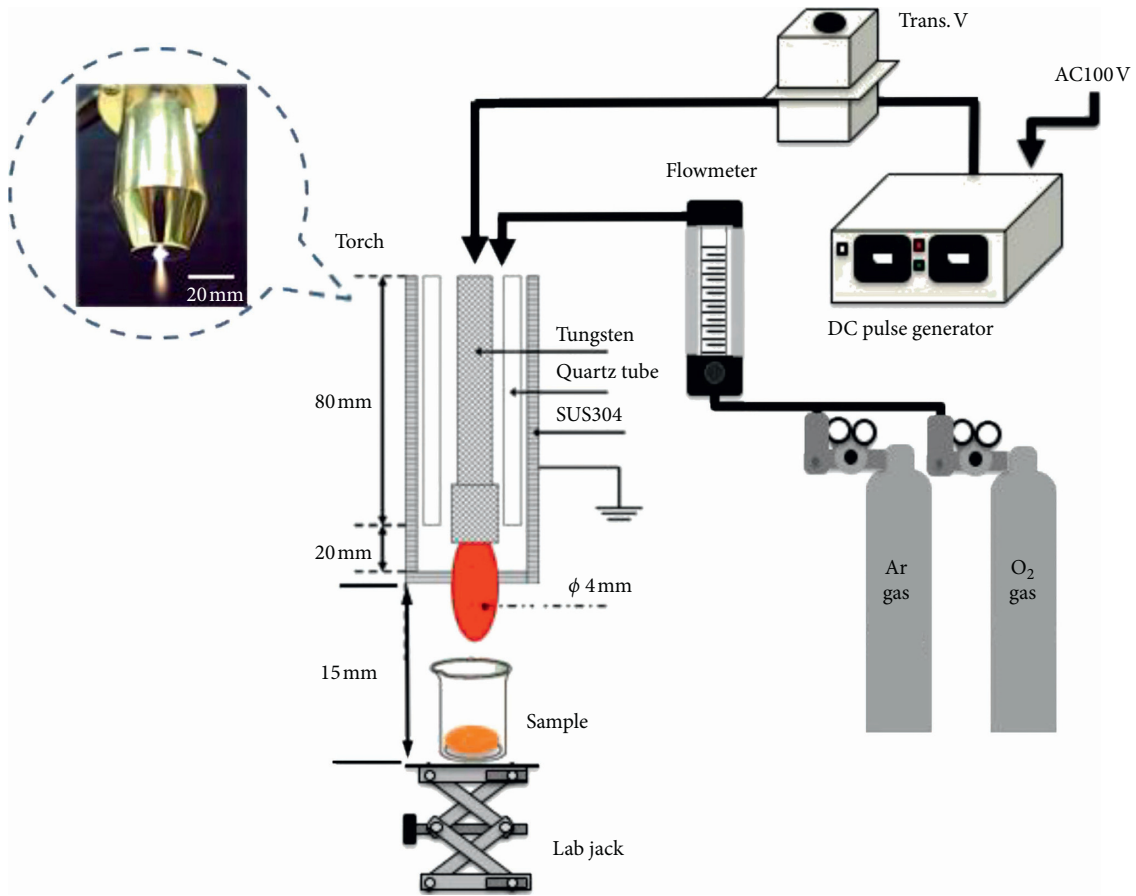


FIGURE 1: Schematic of the experimental setup.

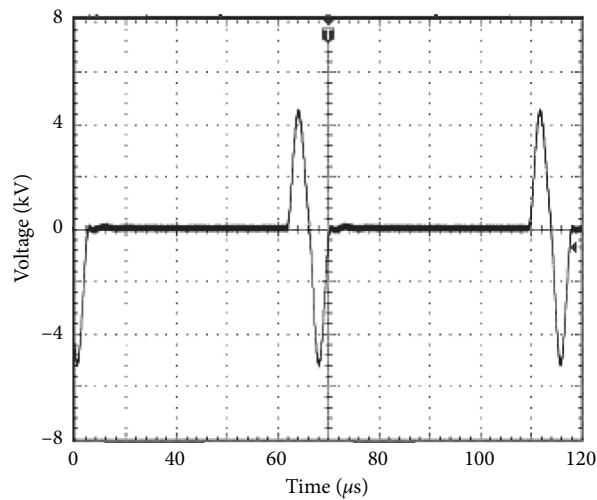


FIGURE 2: The oscillogram of the voltage waveforms (pulse frequency: 10 kHz and plasma power 300 W).

fine powder of mulberry leaves was between 1 and 10 minutes. For plasma irradiation time of more than 2 minutes, we mixed the mulberry leaves' fine powder for about 5 seconds every 2 minutes since the powder diameter is very small at 0.5 mm or smaller, so that the plasma will be irradiated uniformly over the mulberry

leaves' fine powder in the beaker. The number of general bacteria and coliform bacteria was counted by the 3M™ Petrifilm™ (3M Company) method [31] with 100-time diluted solution using 3M™ Petrifilm™ AC medium for general bacteria and 3M™ Petrifilm™ CC medium for coliform bacteria.



FIGURE 3: Picture of dry food powder (in beaker).

3. Results and Discussion

Figure 4 shows the distribution of general bacteria per gram of fine powder of mulberry leaves in the 3M™ Petrifilm™ AC medium evaluated by the 3M™ Petrifilm™ method in the plasma processing time window on 10 kHz pulse frequency, and a gas mixture of Ar gas with a steady flow rate of 5.0 L/min and O₂ gas with a flow rate 1.0 L/min is used as the plasma gas, and 10 kHz of pulse frequency and 100 W plasma power were preferred. The figure clearly shows that the red dot colonies in the film medium decrease with the plasma irradiation time.

Figure 5 shows the relationship between the general bacterial count per gram of fine powder of mulberry leaves in the 3M™ Petrifilm™ AC medium and the plasma irradiation time window on 10 kHz pulse frequency, and a gas mixture of Ar gas with a steady flow rate of 5.0 L/min and O₂ gas with a flow rate 1.0 L/min is used as the plasma gas and also the distribution of coliform bacteria per gram of fine powder of mulberry leaves in the 3M™ Petrifilm™ CC medium over the entire plasma processing time evaluated by the 3M™ Petrifilm™ method. In Figure 5, a 10 kHz of pulse frequency and 100 W plasma power is used. It also shows that the general bacterial count is 1.8×10^5 (g) if the plasma processing time is one minute and 8.0×10^4 (g) in ten minutes, indicating that half of the bacteria are killed.

Figure 6 shows the relation between changes in the DC pulse frequency and oxygen ratio in the plasma gas obtained with 3M™ Petrifilm™ method and the general bacterial count per 1.0 g of fine mulberry leaf powder inspected with the 3M™ Petrifilm™ AC medium, keeping the gas flow rate of Ar constant (5.0 L/min) and that of O₂ in the range from 0.1 to 1.0 L/min, in which the input power was 100 W and the plasma treatment time was 10.0 min. From Figure 6, the

general bacterial count was 1.8×10^4 (g) at the DC pulse frequency of 1.0 kHz and it decreased to 8.0×10^4 (g) at the DC pulse frequency of 5.0 kHz, almost half of the count at 1.0 kHz, which confirms the sterilization effect. At the same time, as the oxygen ratio in the plasma gas increases, the general bacterial count decreased, confirming the enhanced sterilization effect.

In the atmospheric-pressure nonequilibrium discharge plasma processing of the fine powder of mulberry leaves, the general bacteria and coliform bacteria are sterilized because during the process that the Ar + O₂ mixture gas mixture in the atmospheric-pressure nonequilibrium discharge plasma reacts with the electrons emitted from the plasma or the atmospheric moisture, oxygen and water molecules are detached to produce oxygen atoms and, as discussed in earlier reports, OH and O₃ radicals are created with a strong oxidizing and decontaminating power.

Plasma treatment of the fine mulberry leaf powder reduced the number of general bacteria counts and contributed to the negative result of coliform bacteria verification test. This revealed that the plasma treatment has the decontamination effect. The major factor is the electrons emitted in the plasma and oxygen radicals derived from oxygen gas reacting with vapor in the air to detach oxygen molecules and water molecules to generate oxygen atoms. This produces ozone and OH radicals with strong oxidizing power and high sterilization effect. We believe that these radicals react with the general bacteria and coliform bacteria adhered to fine mulberry leaves' powder to produce decontamination and disinfection effect.

The chemical reaction formulas (1)–(4) [32, 33] are shown for the creation of O₃ in the atmospheric-pressure nonequilibrium discharge DC pulse plasma jet, where *M* is any neutral:

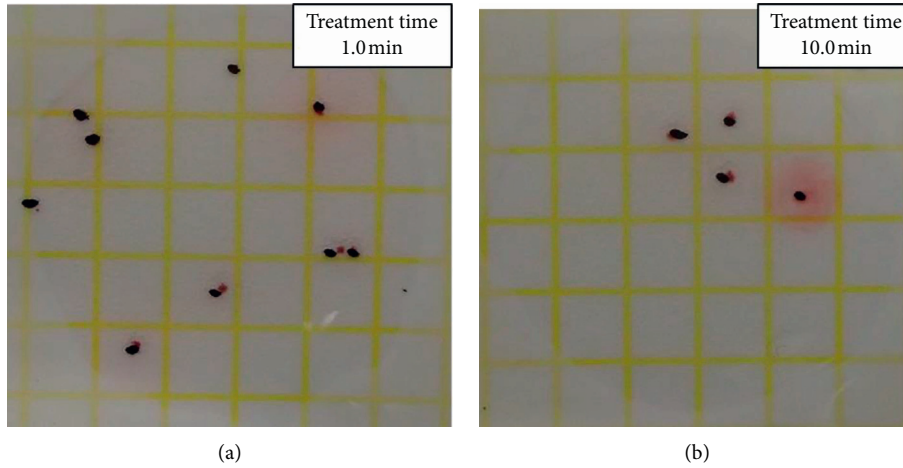


FIGURE 4: Picture of 3M™ Petrifilm™ AC Staph Express Count Plate (bacteria per gram) in mulberry. Treatment time: (a) 1.0 min; (b) 10.0 min.

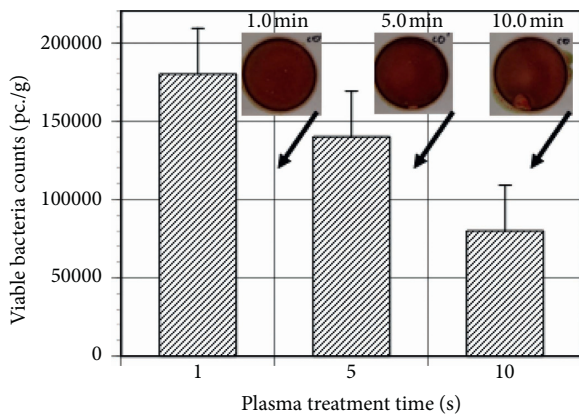


FIGURE 5: Relationship between plasma treatment time and variety bacteria count and 3M™ Petrifilm™ CC Staph Express Count Plate (in mulberry).

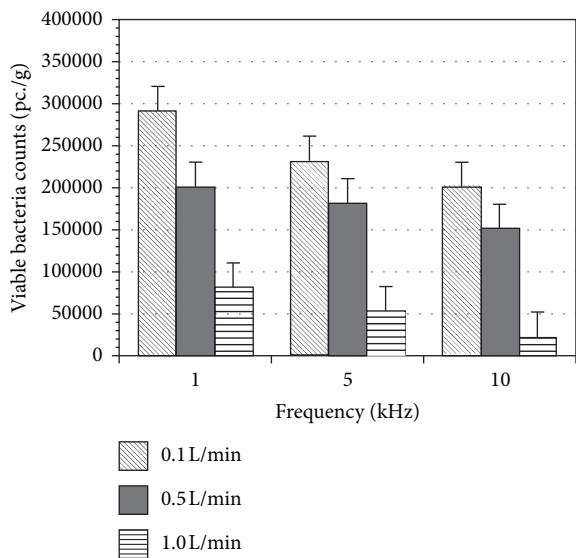
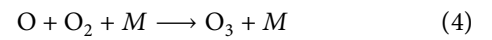
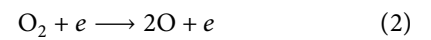
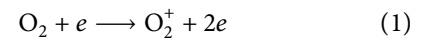
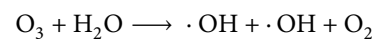
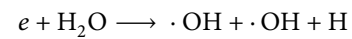
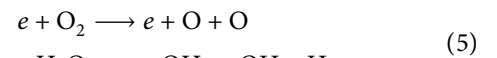
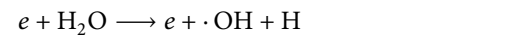


FIGURE 6: Relationship between DC pulse frequency and variety bacteria count and oxygen gas ratio in Staph Express Count Plate on 3M™ Petrifilm™ CC (in mulberry).



Although short-lived, OH radicals with a strong oxidizing and decontaminating power are continually generated as far as ozone and water molecules exist in the atmosphere. We assume that they greatly contribute to the decontamination and inactivation of bacteria. The mechanism of the creation of OH radicals is shown as follows [34]:



4. Conclusion

This study confirmed that using atmospheric-pressure nonequilibrium DC pulse discharge with Ar + O₂ mixture plasma gas and irradiating the plasma on the dry powder food improved the decontamination processing capacity on general bacterial count with the bacterial count reduced to 50% when the irradiation time was increased by 10. It also revealed that increasing the plasma irradiation time, pulse frequency, and the oxygen gas ratio in the plasma gas further improved the decontamination processing capacity on general bacterial count. The decontamination effect on coliform bacteria was also confirmed. The results suggest that OH and O₃ radicals with a strong oxidizing and decontaminating power, created by the reaction between plasma radicals and atmospheric moisture, have a great decontamination effect against both general bacteria and coliform

bacteria. O₃ and other active species generated in the reacting space play major roles during the degradation process. The atmospheric-pressure nonequilibrium DC pulse discharge plasma process was analyzed. The radical species (OH, O₃, etc.) were produced in the gas phase and transferred to the air, where they reacted with the substances in it.

In the future, in pursuit of superior performance on dry powder food ingredients, we will examine the plasma gas species and irradiation techniques to complete the dry nonthermal decontamination method using an atmospheric-pressure nonequilibrium DC pulse discharge plasma jet.

Data Availability

The datasets during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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