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Sterilization treatment of bacterial spores contaminated spices by Atmospheric Plasma

大気圧プラズマを利用した香辛料の殺菌

Y. Takemura[1)], T. Fujiyama[2)], H. Matsuura[2)], M. Furuta[2)]
武村祐一朗, 藤山貴友, 松浦寛人, 古田雅一

[1]*Graduated School of Sciences and Engineering, Kinki University*
3-4-1 Kowakae, Higashiosaka City, Osaka 577-8502, Japan
近畿大学大学院エレクトロニクス系工学専攻 〒577-8502 大阪府東大阪市小若江3-4-1
[1]*Radiation Research Center, Osaka Prefecture University*
1-1 Gakuen-cho, Nakaku, Sakai, Osaka 599-8531, Japan
大阪府立大学放射線研究センター 〒599-8531 大阪府堺市中区学園町1-2

The microbial contamination of imported spices has been causing problems in the food industry. It has been reported that microbial spores of *Bacillus* species are the common contaminants in many spices. We performed the inactivation processing of the spices by using atmospheric pressure plasma to achieve efficient inactivation of microorganisms for thermosensitive materials like spices. The microorganisms attached to the spice could be sterilized by 5-minute treatment of atmospheric plasma.

1. Introduction

Spices are widely utilized in various food products for their flavor and aroma. The contamination by microorganisms of imported spices has been causing problems in food industries. It has been reported that microbial spores of Bacillus species, such as *B. subtilis* and *B. pumilus*, are the common contaminants in many spices (10^5 - 10^8 spores per gram)[1]. The most popular methods for spice decontamination are superheated-steam treatment, thermal inactivation, ethylene oxide fumigation, irradiation with ^{60}Co gamma rays and electron beams. However, these decontamination methods may have drawbacks, such as color loss and flavor changes by heat deterioration of ingredients of spices with, adverse effect on human health by residual ethylene oxide within the fumigated spices, taking a long time for public acceptance of the ^{60}Co-gamma and electron irradiation method for the food decontamination although they are recognized as the most effective method among the 3 methods. Therefore, more safe and flexible sterilization technologies are required.

In this research, to avoid these adverse effects, we performed the sterilization processing of the spices by using atmospheric pressure plasma to achieve efficient inactivation of microorganisms for thermosensitive materials like spices.

2. Experimental setup

A schematic of our plasma experimental systems of direct discharge system (a) and barrier discharge system (b) is shown in Fig. 1.

2.1 *APPJ of direct discharge system*

The plasma experimental system is an atmospheric pressure plasma jet source, which consists of a conical inner electrode and a grounded outer electrode with a nozzle of 5 mm diameter. The inner electrode is coupled to a stepped high-frequency pulse current power supply (about 2.5 kW, and 16.20 kHz), through a high-voltage transformer[2].

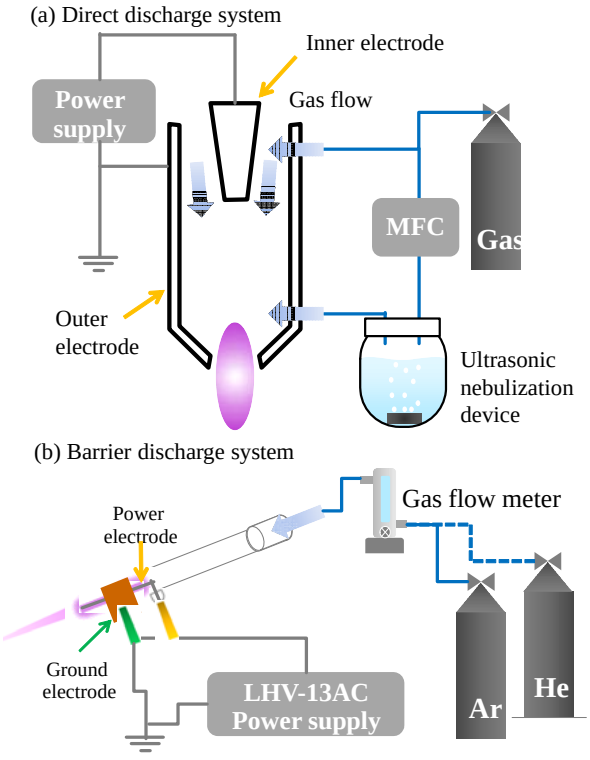

Fig.1. Schematic of the germicidal treatment system

The working gas is divided into two lines, and the main line is connected through the two electrodes and the sub line including water vapor with an ultrasonic nebulizer device is connected with nozzle.

2.2 *APPJ of barrier discharge system*

Plasma source used in[3] consists of a glass tube with two ring electrodes[4]. Working gas is supplied through a glass tube, and constantly controlled by flow-meter (Kofloc, Model RK1710). Electric power is applied by a commercial power source (Loggy Electronics Ltd, LHV-13AC). In order to obtain not only helium but also argon plasma jet, high voltage electrode of new plasma source was changed to a needle set at the center of the glass tube. Due to field concentration effect, discharge between electrodes starts at about 2 kV and plasma jet is ejected at about 3 kV.

3. Experimental result

Plasma-irradiated spices (black pepper seeds, laurel leaves, peppermint leaves and sage leaves) were soaked in the nutrient-rich medium (LB Broth) and incubated for 24 hours at 37°C. Figure 2 shows the results of the incubation of untreated spices ([a]: black pepper) and Ar plasma-irradiated spices at 5 min ([b]: black pepper, [c]: laurel leaves, [d]: peppermint leaves, and [e]: sage leaves). Although the medium containing untreated spices (all kind of spices) became crowded, the spices plasma-irradiated for 5 min was still transparent. Therefore, the microorganisms attached with the spices could be sterilized by atmospheric plasma.

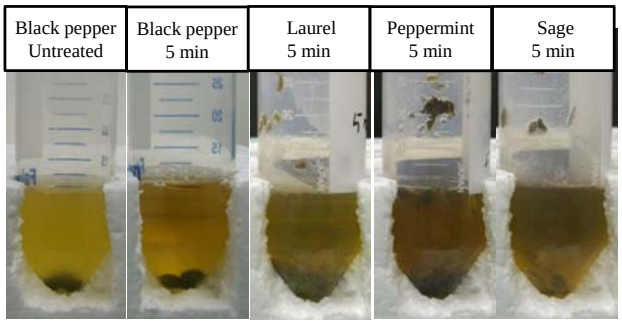

Fig.2. Photograph of sterility tests in LB broth

Calorimetry is the technique of measuring metabolic heat produced by bacterial propagation, and the growth pattern of bacteria can be evaluated quantitatively. The method further provides information on the bacteriostatic and bactericidal effects of various chemicals.

A multiplex isothermal batch calorimeter containing 24 calorimetric units was used to detect the heat evolution during growth under the conditions mentioned above. The plasma-irradiated spices were added into sterile glass vials containing 5 ml of the appropriate medium and sealed tightly.

The vials placed in the calorimeter produced heat during growth of contaminated microorganism of the black pepper samples and the heat was detected as a signal and recorded as a function of incubation time. The obtained g(t) curve was converted into the actual heat evolution (f(t) curves) , according to the following equation as reported previously[5].

$$f(t) = g(t) + K\int f(t)dt \qquad (1)$$

where K is the heat conduction constant of the apparatus. Figure 3 shows actual heat evolution of untreated spices and plasma-irradiated spices. The exposure time was 3 min and the exposure distance was 10 mm. The heat evolution from plasma-treated samples showed clear retardation compared with untreated samples.

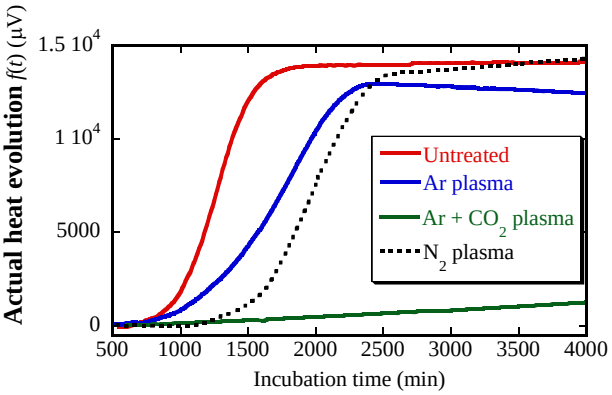

Fig.3. Comparison of actual heat evolution as a function of incubation time.

4. Summary

From the results of calorimetry, the growth rate constants of plasma-irradiated spices was the smaller than that of untreated spices, and the retardation in growth of plasma-irradiated spices was the larger than that of untreated spices. Therefore, the microorganisms attached with the spice could be inactivated by atmospheric plasma.

References

[1] L. J. Muhamad, H. Ito, H. Watanabe and N. Tamuram Agric. Biol. Chem., 50, 2 (1986)
[2] Y. Takemura, N. Yamaguchi, and T. Hara, Jpn. J. Appl. Phys. 47 (2008).
[3] H. Matsuura et al., Proc. ISPlasma2013 (Aichi), P2001 (2013).
[4] Y. Onishi et al., Proc. ISPlasma2014 (Nagoya), 03pP02 (2014)
[5] S. Wirkner, et al., Radiat. Phys. Chem. 63(2002)