Experimental study on sterilization of food with self-igniting plasma formed from liquid using plasma-based ion implantation

Koji Kakugawa, Manami Hosotani, Miyo Arikado, Tomonori Tabe, Kenta Fukutomi, Yoshinobu Tsuchiya, Takeshi Tanaka

We attempted to sterilize food using plasma-based ion implantation with self-ignited plasma formed from liquid. Based on sterilization experiments, hydrogen peroxide was selected to generate self-ignited plasma. The most effective pulse voltage was determined to be −10 kV. However, for pulse width, the optimum condition differed for every sample. Finally, the mortality under the optimum condition of each sample was 98.7% from rice bran, 80.5% from coriander, and 81.4% from cumin. Through this study, it was clarified that self-ignited plasma generated from hydrogen peroxide was able to increase the sterilization effect.

Keywords – food sterilization, hydrogen peroxide, plasma-based ion implantation, optimum conditions, self-igniting plasma.

Експериментално проучване за стерилизация на храна със самозапалима плазма, образувана от течност, използваща плазмена йонна имплантация (Кожи Какугава, Манами Хосотани, Миайо Арикадо, Томонори Табе, Кента фукутоми, Йошинобу Тсучия, Такеши Танака). *Направен е опит да се стерилизира храна, като се използва плазмена йонна имплантация със самовъзпламеняваща се плазма, образувана от течност. На базата на стерилизационни експерименти за генерирането на самозапалима се плазма, беше избран водороден пероксид. Беше установено, че най-ефективното пулсово напрежение е 10 kV. В същото време оптималната продължителност на пулса варира в зависимост от пробата. Накрая бе установено, че смъртността при оптимални условия за всяка една проба е: 98.7% за оризовите трици, 80.5% при кориандъра и 81.4% при кимиона. Чрез проведеното изследване беше потвърдено, че самозапалимата плазма генерирана от водороден пероксид успешно повишава стерилизационният ефект.*

Introduction

The main purpose of sterilization is to kill harmful microorganisms. In general, food products are sterilized by moist heating (boiling or retort treatment) to eliminate spore-forming bacteria. However, the excessive thermal conditions needed to ensure safety against microbial spoilage also degrade the quality of the food products. Thus, it would be useful to develop a low-temperature sterilization method.

Plasma-based ion implantation (PBII) can be applied for sterilization of three-dimensional (3D) targets. It is a low-temperature, cost-efficient technique for sterilizing surfaces, such as those of medical equipment and containers, as well as and powdery foods [1 - 3]. PBII can be used to sterilize (3D) objects quickly without using toxic gases. However, the sterilization performance of conventional PBII where the target is immersed in a radio frequency burst of inductively coupled plasma generated externally is somewhat limited; certain areas may be shadowed from exposure owing to the shape of the object, and the ion penetration depth may not be adequate for complete sterilization. These shortcomings can be overcome with self-ignited plasma, which can be generated around the workpiece as a transient ion sheath by applying a pulsed DC voltage to the target. In this process, known as energetic ion-assisted mixing and deposition (EIAMAD) [4], energetic ions are accelerated in the gap between the sputtering cathode and surface of the substrate, whereas in conventional beam-line ion-beam enhanced deposition, the substrate must be manipulated to achieve coverage and the ion beam requires rastering [4]. Our group previously reported that thermotolerant spores of *Bacillus subtilis* were reduced from 10^7 to 10^2 cfu/mL after a 10 min exposure [5]. Our group also reported that the

sterilization effect might be improved by generating self-ignited plasma of vapor from water in the chamber [6]. Our goal is to develop a new sterilizing apparatus for food that sterilizes under low-temperature conditions.

In this study, we attempted to evaluate the efficacy of PBII as a food sterilization method. Actual foods are more difficult to sterilize than foods sterilized under model conditions. We verified the sterilization effect of PBII using the self-ignited plasma generated from various liquids.

Materials and Methods

Rice bran, coriander, and cumin were separately employed as the food materials for sterilization. These samples were powdered using Wonder Crusher WC-3L (OSAKA CHEMICAL CO., LTD.) before sterilization. Each powdered sample (0.1 g) was suspended in 1 mL of phosphate buffer (pH 7.2). This suspension was applied to a sterile glass dish (15 mm × 90 mm) and was dried on a glass dish.

Fig. 1 shows a schematic diagram of the SIP PBII apparatus. No external rf excitation source was employed in the proposed procedure. The treatment chamber is electrically grounded and has dimensions of 200 mm in height and 150 mm in diameter.

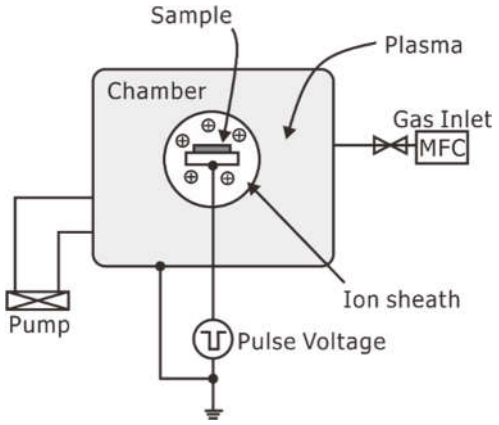

Fig. 1. Schematic diagram of the experimental apparatus

In the experiment, the sample fixed glass plate was placed on a stainless-steel electrode, which was supported by an insulated stainless-steel rod at the center of the vacuum chamber as shown in Fig. 2.

Three black circles indicate the location where the liquid was spotted for generating the self-ignited plasma. Distilled water (H_2O), oxygen-saturated water (oxy H_2O), oxygen-nanobubble water (nano H_2O), hydrogen peroxide (H_2O_2), and sodium perchlorate (NaClO) were used as liquids for plasma generation. Oxygen-saturated water was prepared by blowing high purity oxygen with vigorous stirring into distilled water. Oxygen-nanobubble water was prepared using nanofresher NF-WP0.4 (NANOX CO., LTD.). The dissolved oxygen concentration of the oxygen-saturated water was 38.7 mg/L and that of the oxygen-nanobubble water was 33.0 mg/L.

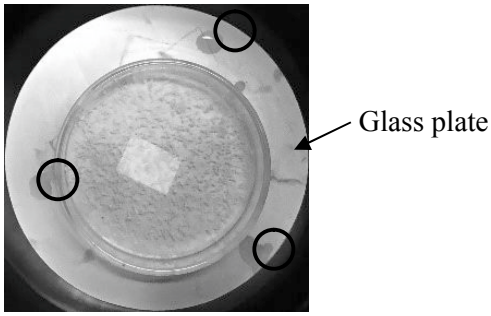

Fig. 2. Stage of sterilization apparatus

A pulsed DC voltage was employed to simplify the sterilization apparatus. A diffusion pump was used to evacuate the chamber to a base pressure of 30 Pa. A summary of the experimental sterilization condition is shown in Table 1.

Table 1

Setting of sterilization apparatus

Items	Value
Supplied Gas	O_2
Gas pressure [Pa]	1
Frequency [kHz]	1
Pulse width [μs]	5
Pulse voltage [kV]	−8
Exposure time [min]	10

After exposure to plasma and ion bombardment, 100 μL of phosphate buffer (pH 7.2) was added to the treated glass plates. After removal using a pipette, the suspension was added to 900 μL of phosphate buffer (pH 7.2). A dilution series was made using phosphate buffer, and 100 μL of the diluted sample solution was inoculated onto empty plates. Standard Method Agar "Nissui" was used to measure the number of general viable bacteria. After pouring molten agar, all plates were mixed well and incubated at 35º C for 48 h. After incubation under an adequate condition for each medium, colony-forming units were counted to determine the numbers of survivors. All data are expressed as the mean ± standard deviation of experiments that were performed in triplicate.

Results and Discussion

Our group previously raised the possibility that generating self-ignited plasma of vapor from water improved sterilization. In this study, we used five liquids: H_2O, oxy H_2O, nano H_2O, H_2O_2, and NaClO.

We conducted preliminary experiments to determine the amount of liquid necessary for sterilization. The amount of each liquid was increased from 10 – 30 μL, but a significant increase in sterilization effect was not confirmed (data not shown). Furthermore, the addition of > 30 μL of hydrogen peroxide for than 1 min was necessary to bring a predetermined degree of vacuum in the chamber. Thus, we decided that the required amount of liquid was 30 μL. Next, rice bran was sterilized using each liquid to determine which liquid produced the best sterilization effect. The results are shown in Fig. 3.

Unlike previous reports, the condition with water had a greater number of survivors than the condition without water [6]. Moreover, we expected that the amount of oxygen plasma would increase by the use of oxy H_2O and nano H_2O.

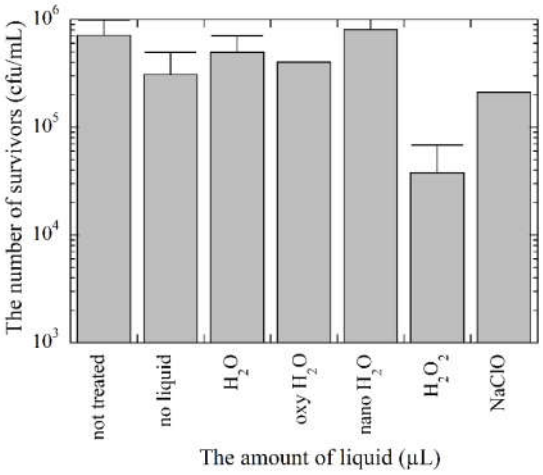

Fig. 3. The effect of various liquids on the sterilization of rice bran.

However, an improvement in sterilization was not observed under these conditions. The amount of water to be used in this experiment was decided based on the restriction of the degree of vacuum, and it was less than the amount of that in previous experiments. For this reason, we think that sufficient sterilization was not acquired. Both H_2O_2 and NaClO are the commonly used disinfectants. Among these liquids, the sterilization effect of hydrogen peroxide was the strongest. Therefore, subsequent experiments were conducted using hydrogen peroxide.

The next experiment was conducted using coriander powder and cumin powder in addition to rice bran. These food materials are known to have microorganisms living in them. We conducted an experiment to examine the effect of pulse voltage. Pulse voltage was varied from −10 to −6 kV. The other experimental conditions were based on the parameters mentioned in Table 1. The results are shown in Fig. 4.

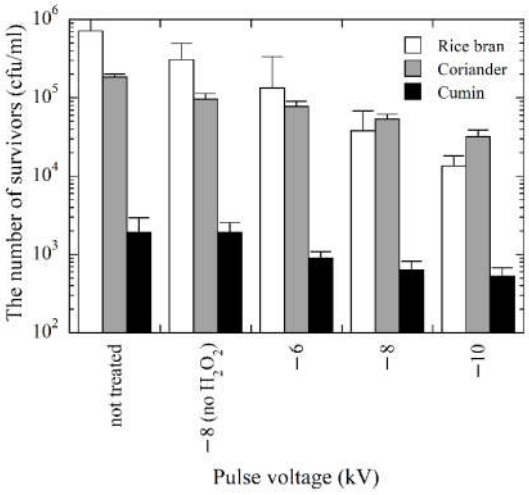

Fig. 4. The effect of pulse voltage (kV) on general living bacteria.

As shown in Fig. 4, the number of survivors decreased when the pulse voltage increased. The efficiency of sterilization increased almost exponentially with voltage. Additionally, the sterilization effect of the −6 kV treatment with hydrogen peroxide was greater than that of the −8 kV treatment without hydrogen peroxide.

In the current apparatus, we cannot provide an applied voltage over −10 kV. No experiment to further increase the pulse voltage was conducted owing to limitations in the specifications of the apparatus.

As described above, the pulse voltage was set to −10 kV in the next experiment. Pulse width was varied from 5 to 15 μs. The results of the experiment are shown in Fig. 5.

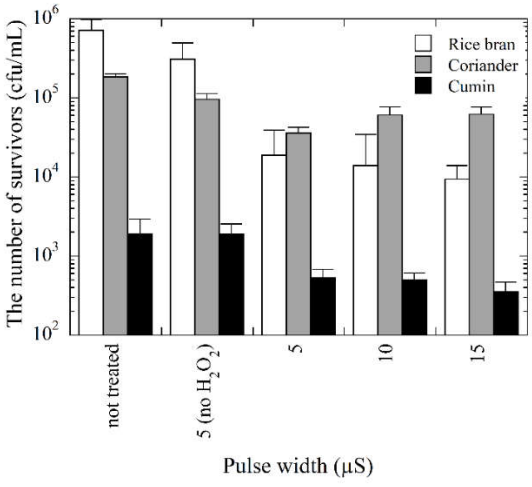

Fig. 5. The effect of pulse width (μs) on general living bacteria

As shown in Fig. 5, we did not observe a common trend among all samples. In previous experiments with *Geobacillus stearothermophilus*, we confirmed that pulse width affected the effect of sterilization [7]. In this experiment, we used foods, which have irregular surfaces; microorganisms are thought to live on these irregular surfaces. Therefore, the accelerated ions cannot easily collide with microorganisms on the surface of food materials. It is considered that the adjustment range of the pulse width, which was decided based on limitations in the specifications of the apparatus, is too narrow to obtain effective results.

Conclusions

We tried to sterilize rice bran, coriander, and cumin using self-igniting plasma formed from hydrogen peroxide with PBII apparatus. The mortalities obtained in this study are as follows: rice bran, 98.7% (pulse voltage of 10 kV, pulse width of 15 μs), coriander, 80.5% (pulse voltage of 10 kV, pulse width of 5 μs), and cumin, 81.4% (pulse voltage of 10 kV, pulse width of 15 μs). Through this study, it was clarified that self-ignited plasma generated from hydrogen peroxide was able to increase the sterilization effect. Although the mortality of coriander or that of cumin is low, this is a problem because of the kind of microorganisms that live on the surfaces of food materials. Although these data are not shown, we identified the microorganisms that grew on an agar plate after sterilization. Most microorganisms that were detected from sterilized coriander or sterilized cumin were heat-resistant spore-forming bacteria, such as *B. subtilis* and *B. licheniformis*. We should improve our apparatus to sterilize food samples containing these microorganisms. It is necessary to increase the capacity of the exhaust pump; by doing so, we can increase the amount of hydrogen peroxide to raise the sterilization efficiency. Moreover, it is thought that an increase in pulse voltage will also improve the sterilization efficiency. However, altering these parameters may cause damage to food materials. In future experiments, we would like to improve our apparatus and determine the optimum sterilization conditions for food materials.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number JP16K07758.

REFERENCES

[1] Jacobs, P. T., S.-M. Lin, in: R.L. Clough, S.W. Shalaby (Eds.). Irradiation of polymers: fundamentals and technological applications, American Chemical Society, Washington, DC, 1996, pp. 216-239.

[2] Kelly-Wintenberg, K., A. Hodge, T. C. Montie, L. Deleanu, D. Sherman, J. Reece Roth, P. Tsai, L. Wadsworth. Use of a one atmosphere uniform glow discharge plasma to kill a broad spectrum of microorganisms. Journal of Vacuum Science & Technology A 17, 1999, pp.1539-1544.

[3] Moreau, S., M. Moisan, M. Tabrizian, J. Barbeau, J. Pelletier, A. Ricard, L.H. Yahia. Using the flowing afterglow of a plasma to inactivate *Bacillus subtilis* spores: Influence of the operating conditions. Journal of Applied Physics, Vol. 88, 2000, pp.1166-1174.

[4] Malik, S. M., R. P. Fetherston, J. R. Conrad. Development of an energetic ion assisted mixing and deposition process for TiNx and diamondlike carbon films, using a co-axial geometry in plasma source ion implantation. Journal of Vacuum Science & Technology A 15, 1997, pp.2875-2879.

[5] Kakugawa, K., M. Kubo, H. Noguchi, K. Shimono, N. Fujimura, Y. Tshuchiya, T. Tanaka. Spore-Forming Bacteria Sterilization Using Plasma-Based Ion Implantation. ELECTROTECHNICA & ELECTRONICA Vol. 49, No. 5-6, 2014, pp.343-347.

[6] Tanaka, T., I. Wakamoto, N. Takagi. Plasma generation of liquid material (H_2O) using by PBII (in Japanese). The papers of Technical Meeting on Plasma Science and Technology, IEE Japan, 2007, pp.31-34.

[7] Kakugawa, K., A. Yamashiro, K. Saitoh, T. Mizutane, T. Kano, T. Tanaka. The effect of pulse width on the sterilization of *Bacillus stearothermophilus* spores using plasma-based ion implantation. In Proceeding of 4th Asia-Pacific Conference on Engineering and Applied Sciences, Bali, 2017, pp.84-90.

Prof. PhD Koji Kakugawa - *Department of Food Science and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Tel: +81-82-921-6472

e-mail: k.kakugawa.db@it-hiroshima.ac.jp

Student Manami Hosonotani - *Department of Food Science and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Student Miyo Arikado - *Department of Food Science and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Student Tomonori TABE - *Department of Electronics and Computer Engineering, Faculty of Engineering, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Student Kenta Fukutomi *- Department of Electronics and Computer Engineering, Faculty of Engineering, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Prof. PhD Yoshinobu Tsuchiya *- Department of Food Science and Biotechnology, Faculty of Life Sciences, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Tel: +81-82-921-9464

e-mail: y.tsuchiya.d2@it-hiroshima.ac.jp

Prof. PhD Takeshi TANAKA *- Department of Electronics and Computer Engineering, Faculty of Engineering, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, 731-5139, Hiroshima, Japan*

Tel.: +81-82-921-4256

e-mail: t.tanaka.wy@it-hiroshima.ac.jp