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Purevdorj, Dambadarjaa
Laboratory of Food Process Engineering, Facultry of Agriculture, Kyushu University

Igura, Noriyuki
Laboratory of Food Process Engineering, Facultry of Agriculture, Kyushu University

Shimoda, Mitsuya
Laboratory of Food Process Engineering, Facultry of Agriculture, Kyushu University

Hayakawa, Isao
Laboratory of Food Process Engineering, Facultry of Agriculture, Kyushu University

他

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Survival of Vegetative Pathogens under Argon Plasma Treatments

Dambadarjaa PUREVDORJ *, Noriyuki IGURA *, Mitsuya SHIMODA *, Isao HAYAKAWA * and Osamu ARIYADA **.

* Laboratory of Food Process Engineering, Faculty of Agriculture, Kyushu University 6–10–1, Fukuoka 812–8581, Japan.
** ARIOS Inc, Musashino 3–2–11, Akishima shi , Tokyo 1960021, Japan.
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Argon plasma sensitivities of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* cells were studied by plasma treatments generated in the low gas pressure of 50 Pa. The different plasmas were induced at 2.63 and 4.21 w/cm^3 microwave power densities to have the low temperature distributions below 60 °C. Certain external factors that may affect the pathogen survival under plasma processing such as the extent of bio–indicator loads and the nature of bio–indicator carriers was examined. The increase of microwave power density of the plasma treatments caused significant reductions in the pathogen survival, and thereby correlated with the increases in cell injury. Among the pathogens subjected to the plasmas, the gram–positive *S. aureus* cells were the most resistant and the plasma sensitivity was followed by the gram–negative *E. coli* and *S. typhimurium* cells. Survival of the *E. coli* cells was lower when the extent of bio–indicator loads was larger, and its survival was affected most strongly on paper followed by propylene and glass bio–indicator carriers.

INTRODUCTION

Although estimates vary widely, there is a common agreement that food–borne illness is a serious problem in the human society. Globally, people not only demanding more food but also a safe supply of food and the world globalization has increased the risk of spreading food–borne illness internationally. Food manufactures, companies dealing with the retail market of food supply and consumers have symbiotic relationship. Safe food is paramount to both. Errors made in the manufacturing, transporting, storing and in serving food can enable harmful microorganisms to survive and cause risks to health and even life itself. Physical and chemical methods are commonly implemented in the cold sterilization practices of the food industry. Plastic packaging materials and PET drink bottles used for variety purposes in the food and pharmaceutical industry are thermo–sensitive and require proper sterilization procedures. For example: ionization radiation is widely used in the industries, but propylene materials became degraded during the sterilization process. Peracetic acid or hydrogen peroxide is commonly used for sterilization of PET drink bottles. However, the residues left on the sterilized packaging materials are highly toxic and associated with health problem of employers (Young, 1997). Soon or later, these methods will be gradually replaced with new alternative ones. New approaches such as the low temperature gas plasma sterilization have been already

* Corresponding author. Tel. : +81–92–642–3017; Fax : +81–92–642–3015
E–mail address : purevdor@agr.kyushu–u.ac.jp

emerged (Boucher, 1985; Nelson and Berger, 1989). Montie *et al.* (2000) have studied inactivation of various microorganisms at different environments using one atmosphere uniform glow discharge plasma. The plasma treatments were conducted in a room temperature at atmospheric pressure in air and hypothesized that the vulnerability of cell membranes was due to reactive oxygen species. It is a promising technology in that it is fast and it does not leave toxic residuals on the processed objects (Koulik *et al.*, 1999). Eventually, these various approaches will merge in such a way as to lead to new methods of spore control. For instance, in the disinfection and sterilization of such heat sensitive materials as PET and plastic packaging used in the food industry (Purevdorj *et al.*, 2001b).

Consequently, the objective of this study was to investigate plasma sensitivities of the *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* cells by low temperature argon plasma induced at different microwave power densities in low gas pressure. Another objective was to examine external factors such as the extent of bio–indicator loads and the nature of bio–indicator carriers, which may affect the pathogen survival under the plasma treatment conditions.

MATERIAL AND METHODS

Appartus

The design and gas plasma set up principle of the gas plasma sterilization technique have been reported elsewhere (Purevdorj *et al.*, 2001a, b), and here is shortly described for completeness. In order to generate the gas discharge plasma, a gas was injected into crystal tube located on the top of sterilization chamber and excited under 2.45 GHz frequency microwave energy inputs. Then, it was diffused into the cylindrical sterilization chamber (ϕ250×220 mm), where the bio–indicator holder was positioned at the center and there, the plasma inactivation of the bio–indicators was taken place. The distance from the plasma generation point to the bio–indicator holder was 270 mm. The argon plasmas were generated at the 2.63 and 4.21 w/cm^3 MWPD in low gas pressure of 50 Pa to have the low temperature distribution below 60 °C.

Microorganisms

Escherichia coli IFO 3972, *Staphylococcus aureus* IFO 13276 and *Salmonella typhimurium* IFO 13245 used as the bio–indicator microorganisms were obtained from the Institute for Fermentation Osaka (Osaka, Japan).

Bio–indicator preparation

Microscope slide glass, antibiotic test paper discs (ref # 10321262, Scheicher and Schuell) and polypropylene test tube caps were used as the bio–indicator carriers. The *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* cells were cultivated in a nutrient broth adjusted to pH 7.0 (Eiken chemical Co., Tokyo, Japan) with shaking 15 h at the temperature of 30 °C. Then, the vegetative cells were harvested and the bio–indicators were prepared according to the procedure followed in the previous study (Purevdorj *et al.*, 2001a). Inoculated area of bio–indicator loads on the bio–indicator carriers was 9 mm in diameter, except the bio–indicator carriers used for deter-

ination of the extent of bio–indicator load effect.

Evaluation of the survivors

The viable cells after the plasma treatments were recovered aseptically in a phosphate buffered physiological saline (0.9% sodium chloride) by scraping and ultra–sonication for 3 min with sterile glass beads (ϕ 0.6 mm). Then 0.1 ml of the cell suspension was inoculated on a nutrient agar (Eiken chemical Co., Tokyo, Japan). The survivors were counted as colony forming units per each bio–indicator (CFU/carrier) after incubation for 48 h at temperature of 30 °C. Injury of *E. coli* cells that occurred during the plasma processing were detected by incubation of the survivors on the same nutrient media but supplied to have additionally 4% sodium chloride.

Statistical analysis

All experiments were repeated four times and the data presented as the means and standard error of the mean log No/N.

RESULTS AND DISCUSSION

Argon plasma inactivation of different vegetative pathogens

Inactivation of the different vegetative pathogens deposited on polypropylene bio–indicator carriers by 2.63 w/cm^3 MWPD argon plasma treatment is shown in Figure 1. Among the pathogens subjected to the plasma, the gram–positive *S. aureus* was the most resistant and the plasma sensitivity was followed by the gram–negative *E. coli* and *S. typhimurium* cells. This different plasma sensitivity was probably related to the cell structure of the vegetative pathogens and due to the higher agitation of the *S. aureus*

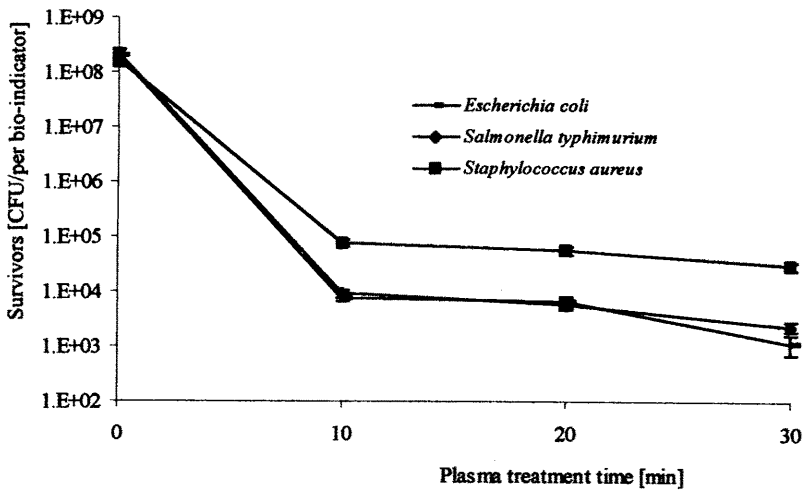

Fig. 1. Inactivation of vegetative pathogens by 2.63 w/cm^3 MWPD argon plasma

cells to form more cell clumps on the bio–indicator carriers. It can be seen that the plasma inactivation was much faster in the beginning stage and followed by slower ones, despite the bacterial genus. In other words, the plasma inactivation was a two–stage process. Similar inactivation profile was observed by Hury *et al.* (1998) and Purevdorj *et al.* (2001a, b), when different *Bacillus* spores and *E. coli* cells were subjected to gas plasma treatments in low gas pressure. It was speculated that this inactivation mechanism was related to etching effect of the plasma processing. The eroded or dead microorganisms and the leaked cytoplasmic materials would create a new barrier over the other live cells and the sputtering process slows (Purevdorj *et al.*, 2001b).

External factors affecting the pathogen survival under argon plasma treatments

The *E. coli* cells were deposited on glass bio–indicator carriers with the different extent of bio–indicator loads (9 and 20 mm in the diameter). Survival of the *E. coli* cells was much lower, when the extent of bio–indicator loads was larger under the 2.63 w/cm^3 MWPD argon plasma treatments (see Table 1). The reason was explained that, when the bio–indicator loads was spread more widely, the bacterial cells had a less chance to form multi–layers in cell bio–mass and more chance to encounter the electron and ion bombardment. However, survival of the *E. coli* cells by the 2.63 w/cm^3 MWPD argon plasma treatments was most strongly affected on the paper followed by propylene and glass bio–indicator carriers (see Table 2). It was assumed that the specific surface area on the paper bio–indicator carriers was larger owing to its fine fiber structure. Diffusion of

Table 1. Effect of the extent of bio–indicator loads on the survival *Escherichia coli* cells by argon plasma treatment

Inoculation extent in diameter [mm]	Log No/N
9	3.84±0.11*
20	5.89±0.07

* –the values corresponds to the standard error of the mean Log No/N
No–approximately 1.7×10^8 cells were inoculated on the glass bio–indicator carriers
N–the number of cells recovered after the 2.63 w/cm^3 MWPD argon plasma treatment of 10 min

Table 2. Effect of the bio–indicator carrier nature on the survival of *Escherichia coli* cells by argon plasma treatment

Nature of the bio–indicator carriers	Log No/N
Paper	4.66±0.06 *
Polypropylene	4.24±0.04
Glass	3.95±0.08

* –the values corresponds to the standard error of the mean Log No/N
No–approximately 1.7×10^8 cells were inoculated on the bio–indicator carriers
N–the number of cells recovered after the 2.63 w/cm^3 MWPD argon plasma treatment of 10 min

plasma metastables into a certain depth of the bio–indicators was expectable to inactivate the pathogen cells. Consequently, the pathogen inactivation log No/N was higher on the paper bio–indicator carriers than the propylene and glass bio–indicator carriers due to the larger specific surface area of the paper bio–indicator carriers.

Cell injury due to the increased MWPD of the argon plasma

If cell injury was related to the cell membrane, it became sensitive to sodium chloride content present in the nutrient media. The *Escherichia coli* cells were deposited on polypropylene bio–indicator carriers and subjected to 2.63 and 4.21 w/cm^3 MWPD plasma treatments. In order to find out the correlation between uninjured and injured cells among the survivors, the cells after the plasma treatments were inoculated on nutrient media with or without 4% sodium chloride supplement. The increase of microwave power density of the plasma treatments significantly caused reductions in the pathogen survival, and thereby correlated with the increases in the cell injury (see Table 3). Cell injury was intensified, when the MWPD of the plasma treatments was increased. This finding suggests that the inactivation effect of the plasma treatments was related to the plasma etching process and when the process was intensified the injury of cell membrane was also greatly enhanced.

Table 3. Injury of *Escherichia coli* cells by argon plasma treatments generated at the different microwave power densities

Cells counted as	MWPD of the argon plasma treatments	
	2.63 w/cm^3 Log No/N	4.21 w/cm^3 Log No/N
Uninjured	4.30±0.04*	5.16±0.03
Injured	5.39±0.06	6.25±0.05

*–the values corresponds to the standard error of the mean Log No/N
No– approximately 2×10^8 cells were inoculated on the polypropylene bio–indicator carriers
N–the number of cells recovered after the different MWPD argon plasma treatments of 10 min and were counted respectively on the different nutrient media

CONCLUSIONS

When the vegetative pathogens were subjected to the low temperature argon plasma, the gram–positive *Staphylococcus aureus* cells were the most resistant and then the plasma sensitivity was followed by the gram–negative *Escherichia coli* and *Salmonella typhimurium* cells. Increases in the microwave power density of the plasma treatments caused significant reductions in the pathogen survival, and thereby correlated with increases in the cell injury. The pathogen survival was affected by external factors such as the extent of bio–indicator loads and the nature of bio–indicator carriers under the argon plasma treatments. Pathogen survival was lower, when the extent of bio–indicator loads was larger and its survival was affected most strongly on the paper followed by

propylene and glass bio–indicator carriers.

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