

Application of atmospheric-pressure argon plasma jet for bread mold decontamination

P Thonglor¹ and P Amnuaycheewa²

¹ Department of Industrial Physics and Medical Instrumentation, King Mongkut's University of Technology North Bangkok, Bangkok, 10800, Thailand

² Department of Agro-Industrial, Food, and Environmental Technology, King Mongkut's University of Technology North Bangkok, Bangkok, 10800, Thailand

E-mail: panakamond@kmutnb.ac.th

Abstract. Atmospheric-pressure argon plasma (APAP) is a promising non-thermal technology for microbial control and prevention minimally affecting quality of foods. Effect of APAP jet on the growth of bread molds, including two *Aspergillus* sp., *Rhizopus stolonifer*, and *Penicillium roqueforti*, isolated from white bread were investigated. The molds were isolated, verified, cultured to fully grown on potato dextrose agar (PDA), and subsequently treated with APAP jet using plasma generating power at 24 W for 5, 10, and 20 min, respectively. The inhibition of mold growth was investigated by comparing fungal dry weights and the effect on fungal cell structure was observed using compound light microscope. The results indicated that the 20-min treatment time is most effective in retarding the growth of the three bread molds. However, this level of generating power did not lead to destruction of the cellular structures for all the four fungi. Plasma generating power and treatment time are significant parameters determining the success of bread mold decontamination and further investigation on real bread matrix is needed.

1. Introduction

Microorganisms can grow under various environmental conditions and are widely distributed in nature. They play an important role in both food production and in food spoilage and poisoning. Many types of molds are found in foods and many strains produce mycotoxins which have been implicated in foodborne intoxication. For examples, 1) *Aspergillus flavus* can produce aflatoxin which has been reported to be associated with many human diseases such as hepatitis, liver cancer, stunted growth, and delayed development, 2) *Rhizopus stolonifer*, a black mold, cause spoilage of bread, and 3) *Penicillium* cause spoilage of bread, grain, fruit and meat.

Molds are important spoilage microorganisms in a number of low-moisture foods, especially bread and other bakery products. Various food preservation schemes are used to reduce the contamination and the health hazard. Since certain chemical food preservatives can be harmful to consumer [1], to avoid residual chemicals in food, the application of low-pressure plasma has been developed to be an alternative non-thermal technology to reduce the microbial contamination. The technology appears to be applicable for surface disinfection since cold plasma can generate energetic charged particle, radical species and high energy photons to decontaminate molds growth on the surface of food [2]. For instance, the growth of *A. flavus* on brown rice cereal bars was inhibited after exposed to cold atmospheric pressure [3]. On the same hand, the efficacy of atmospheric pressure plasma was confirmed on sliced cheese and ham for inactivation of *L. monocytogenes* [4] and for decontamination on fresh products such as chicken skin, pork, fruit and vegetables

slices [5-8]. In this study, the effect of low-pressure plasma technology on bread mold growth was observed using the APAP jet and four bread mold species.

2. Materials and Methods

2.1. Experimental setup

The APAP jet was used in this study, as shown in **Figure 1**. Briefly, the plasma source, generated using a tungsten rod as powered electrode, was covered with a 2 mm thick Pyrex glass tube of 6 mm diameter. The electric power was supplied to the discharge using a high frequency of 43 kHz. Plasma was generated using argon at atmospheric pressure with gas flow rate of 0.5 L/min. The power was maintained at 24W and the trialed treatment times were 5, 10, and 20 min for this study. During the treatment, the argon plasma jet was positioned 1 cm (distance from the end of nozzle) above the samples.

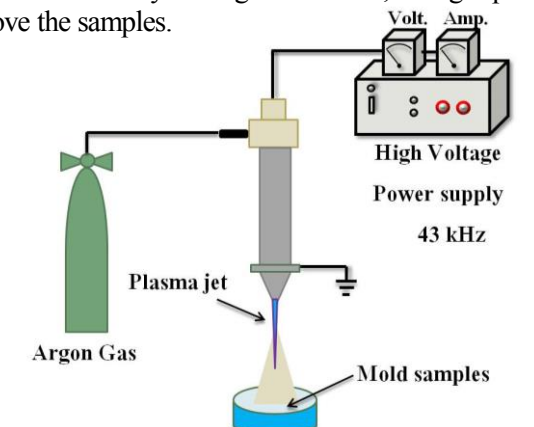


Figure 1. Schematic diagram of the APAP jet setup

2.2. Mold preparation, plasma treatment, and determination of fungal biomass

Four fungal strains were used in this study. *Aspergillus flavus* and *Aspergillus niger* were identified from white bread and whole bread, purchased from local markets (Bangkok, Thailand), *Penicillium roqueforti* TISTR3511, and *Rhizopus stolonifer* TISTR3376 were obtained from the Thailand Institute of Scientific and Technological Research (Pathum Thani, Thailand). Preparation of the molds for plasma treatment was conducted as followed [9]. Briefly, all the molds were cultured on potato dextrose agar (PDA) medium and incubated to fully growth at room temperature for about 7 days. Spores were collected by flooding the surface of the PDA medium using 5 mL sterile saline solution (NaCl, 8.5 g/L water) containing Tween 80 (0.1% v/v). Aliquot (1 mL) of the fungal spore suspension was then spread onto the surface of a new PDA in petri dish of 5 mm diameter and used for plasma treatment.

The plasma treatments were conducted for 5, 10, or 20 min then all the plates were incubated at room temperature for 3 days. Controls were the plates without plasma treatment incubating under the same condition. At the end of the incubation period, each fungal biomass was collected using the sterile saline solution, filtered through Whatman No.4 filter paper (GF/C glass microfiber; Whatman, US), washed with copious amount of sterile distilled water to remove salt and Tween 80, air-dried and weighed to determine percent reduction of fungal dry weight. For each of the four molds, the experiments were conducted in triplicate for each of the three treatment times and the results were expressed as mean \pm SD. In addition to the determination of fungal dry weight, at the end of the 3-day incubation period, the molds were observed for their structures using a compound light microscope (Olympus CX21FS1, Tokyo, Japan) to compare with those without the APAP jet treatment.

3. Results and discussions

In order to determine the effect of the treatments on mold growth, we used the 3-day incubation period after the plasma treatments to allow the treated molds to grow, and thus difference in the final fungal biomass weight would reflect different level of mold decontamination. **Figure 2.** showed the fungal spores on PDA media treated using different treatment times after 3-day incubation. As seen in the

figures, no noticeable difference was observed in term of morphological appearance. These indicated that all the molds were not completely removed upon treating with the plasma even for 20 min, and the remaining spores gained the opportunity to recover/grow during the 3-day incubation period.

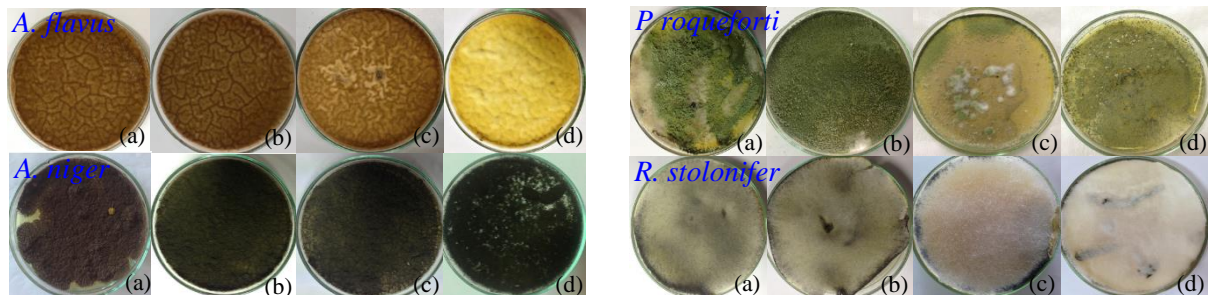


Figure 2. Fungal growth on PDA media at 3 days without plasma treatment (a), and APAP jet treatment at 24W for 5 (b), 10 (c), and 20 min (d), respectively.

Generally, the inhibitory effect of the APAP treatment on microbial growth is in proportion to the degree of the generating power and the treatment duration which is the higher the generating power and/or the longer the treatment time, the more the microbial inhibition [2,3]. The percent reduction was calculated from fungal dried biomass in comparison to the control and illustrated as a function of treatment time. As shown in **Figure 3.**, all the mold growths decreased proportionally to increase of the APAP treatment time. *A. flavus* appeared to be the most sensitive mold which showed the greatest reduction at 83% with a maximum exposure time after 3-days storage. While, *A. niger*, *R. stolonifera*, and *P. roqueforti* spores were inactivated by APAP jet approximately 75%, 71%, and 48%, respectively. These results appear to be useful to predict the exposure time to completely disinfect the mold spores. On the other, all the results were completely different when compared the 5-min treatment time with the 20-min treatment time. We concluded that greater fungal spore viability and activity was inhibited, as a result of the longer exposure to the APAP jet treatment.

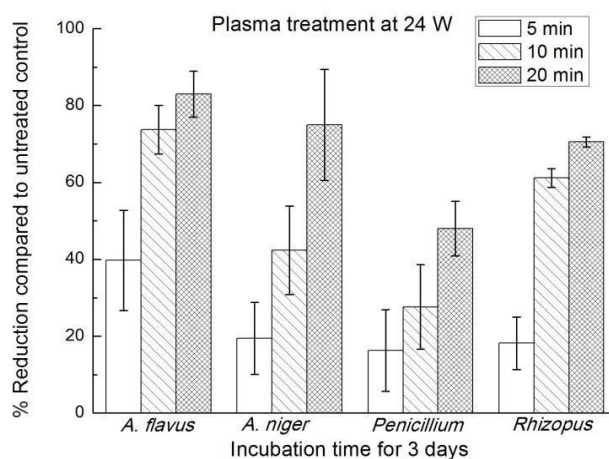


Figure 3. The percent reduction of mold growth on PDA after the APAP treatment at 24W.

In addition, the physical change of spore was observed by comparing between before and after the APAP treatment as shown in **Figure 4.** The APAP treated organisms exhibited no significant structural difference compared to the control when observed under microscope. These indicated that the maximum treatment time (20 min) at 24W could not completely inhibit the mold spores. However, the 20-min APAP jet treatment appears to be the most effective to reduce the mold growth and to extend the shelf life. It should be noted that the exposure time depends on the type of microorganism and size of the cell [3]. For example, in the fungi required a longer plasma treatment time than the bacteria because the cell wall of fungi are larger and stronger than bacteria. Therefore, the effect of exposure time for plasma treatment should be studied in the future work. Although, there is no percent reduction or log reduction limit set for mold for bread or baked products. In some countries, the total number of yeast and mold count in bread product must not exceed 10,000 CFU/g [10]. In other word, the ultimate goal for mold decontamination is to reduce to less than 10,000 CFU/g. Nevertheless, in our current study on the 5 mm diameter PDA petri dish, even the

fully grown mold for all the four molds is less than 1,000 CFU/g. In our future experiment, we would separately inoculate an equivalent of 10,000 CFU/g of the four bread molds on bread and treat with the optimized APAP treatment conditions to evaluate the % reduction level and product acceptability.

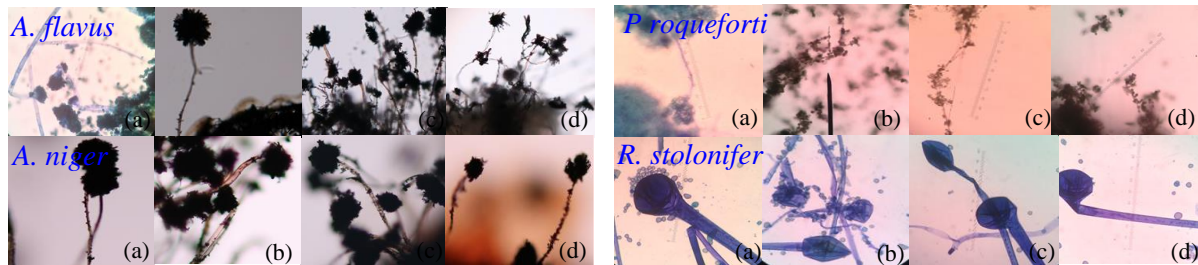


Figure 4. Microscopic view of mold spores before plasma treatment (a), and after APAP jet treatment for 5, 10, and 20 min, respectively (40× magnification).

4. Conclusions

The results shown that APAP jet treatment on four fungi, consisting of *A. niger*, *A. flavus*, *R. stolonifer*, and *P.roqueforti* on PDA media can increase the decontaminating effect. The best efficiency to decontaminate the mold growth is with the power of 24 W and with the exposure time of 20 min. In addition, *A. flavus* was the most sensitive to the APAP jet treatment, while *P. roqueforti* was the least sensitive. In conclusion, this research showed the potential of using APAP jet to decontaminate bread mold under the optimized time and power condition. Based on the optimal conditions obtained in this study, we plan to determine the inhibitory effect of the APAP jet on the four bread molds inoculated on real breads (whole wheat and white bread) to investigate the possibility of apply this technology to bread industry.

Acknowledgements

The authors would like to thank Assoc.Prof.Dr. Dheerawan Boonyawan for his guidance and Dr. Pansak Kerdtongmee for helping in the plasma experimental setup and Ms.Nichakan Saetae and Ms.Wanpen Noimuang for their help on identifying mold species. This research was funded by King Mongkut's University of Technology North Bangkok. Contact no. KMUTNB-GEN-59_29.

References

- [1] Razzaghi-Abyaneh, Mehdi, et al 2009 Chemical composition and antiaflatoxigenic activity of *Carum carvi* L., *Thymus vulgaris* and *Citrus aurantifolia* essential oils *Food Control*, **20** (11) 1018-1024
- [2] Suhem K, Matan N, et al 2013 *In vitro* and *in vivo* antifungal activities of various gas species under plasma jet treatment against brown rice cereal spoilage molds *Int. Food Res. J.* **20**(2) 947-951
- [3] Suhem K, Matan N, et al 2013 Inhibition of *Aspergillus flavus* on agar media and brown rice cereal bars using cold atmospheric plasma treatment *Int. J. Food Microbiol.* **161** 107-111
- [4] Song HP, Kim B, et al 2009 Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes* *Food Microbiol.* **26**(4):432-6
- [5] Noriega E, Shama G, et al 2011 Cold atmospheric gas plasma disinfection of chicken meat and chicken skin contaminated with *Listeria innocua* *Food Microbiol.* **28**(7):1293-300
- [6] Wang R, Nian W, et al 2012 Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation. *The European Physical Journal D EPJD* **66**(10):1-7
- [7] Bermúdez-Aguirre D, Wemlinger E, et al 2013 Effect of atmospheric pressure cold plasma (APCP) on the inactivation of *Escherichia coli* in fresh produce *Food Control* **34**(1):149-57
- [8] Zuzina D, Patil S, et al 2014 Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce *Food Microbiol.* **42**:109-16
- [9] Deeyai P, Amnuaycheewa P, et al 2016 Effect of atmospheric pressure argon plasma jet on the growth of bread molds *Key Eng. Mat.* 675:744
- [10] GCC Standardization organization (GSO) 1998 Microbiological criteria for food stuffs *Fiinal draft*