International Food Research Journal 20(2): 947-951 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro and *in vivo* antifungal activities of various gas species under plasma jet treatment against brown rice cereal spoilage molds

[1]Suhem, K., [1,4*]Matan, N., [2,4]Nisoa, M. and [3,4]Matan, N.

[1]Food Science and Technology, School of Agricultural Technology, Walailak University, Nakhon Si Thammarat 80160, Thailand
[2]Plasma Technology for Agricultural Applications Research Laboratory, School of Science, Walailak University, Nakhon Si Thammarat 80160, Thailand
[3]School of Engineering and Resources, Walailak University Nakhon Si Thammarat 80160, Thailand
[4]ThEP Center, CHE, 328 Si Ayutthaya Rd., Bangkok 10400, Thailand

Article history

Received: 17 July 2012
Received in revised form: 24 August 2012
Accepted: 25 August 2012

Keywords

In vitro
in vivo
various gas species
antifungal activities

Abstract

The objective of this study was to compare the effectiveness of various gas species (argon, water vapor and air) of plasma on *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Rhizopus* sp., the causal agents of mold spoilage in brown rice cereal. Antifungal activities of plasma were obtained from various gas species at 0 W (control), 20 W, and 40 W under plasma discharge and were investigated against mycelium and spore germination of all molds. Argon plasma at 40 W was found to be the most effective agent to inhibit the growth of all molds and to reduce spore germination for *in vitro* test. Furthermore, *in vivo* assays determined that Ar plasma at 40 W was the most efficient agent against mold spoilage on brown rice cereal. The results indicated that Ar plasma provided good protection against mold on brown rice cereal for at least 19 days under storage conditions at 25°C and 100%RH. This study showed that Ar plasma is a potential and promising antifungal agent that could be used as a fungicide to protect brown rice cereal against mold spoilage.

Introduction

Brown rice, also known as hulled rice, contains aspartic acid, arginine, leucine, glutamic acid (Zhang *et al*., 2011) and B complex vitamins (B1, B2, B3, B5, B6 and B12) (Kyritsi *et al*., 2011). Because of its high nutritional value, the numbers of brown rice products in the world market have increased every year. However, brown rice is not considered a good option for table rice because of its color and hard texture. On the other hand, it is used as the main ingredient in various types of healthy foods such as breakfast cereals and brown rice beverages (Ohtsubo *et al*., 2005). Brown rice cereal is a good choice for the breakfast menu because it contains sources of energy, amino acids, vitamins and high fiber. Unfortunately, brown rice cereal is easily contaminated by molds. Fungi like *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp., and *Penicillium* sp. (Suhem *et al*., 2012) can be found on brown rice cereal. In an attempt to reduce the growth of molds on brown rice cereal, plasma jet treatment was employed in this work.

Plasma consists of partially ionized gases also known as the highly energized fourth state of matter. It contains ions, electrons, and reactive neutral species (radicals, and excited atoms and molecules). Because of its cold and energetic charged particles, chemically active radicals and high energy photons to get rid of contaminations on the surface of food and packaging without damaging their qualities, plasma was reported to be used to inhibit mold growth on nutritional value products (Ehlbeck *et al*., 2011). Lee *et al*. (2011) reported that input gas used with an N_2+O_2 mixture in an atmospheric pressure plasma (APP) jet most effectively reduced *L. monocytogenes* inoculated onto sliced chicken breast and ham. Also, plasmas in argon jets can kill endospores of *Bacillus atrophaeus* very effectively (Uhm and Hong, 2011). However, the application of plasma jet treatment to improve food safety is still very limited. Therefore, the objective of this study was to evaluate the efficacy of various gas species in plasma jet treatment used to inactivate mold on brown rice cereal.

*Corresponding author.
Email: *nnarumol@wu.ac.th*

Materials and Methods

RF plasma jet apparatus

The RF plasma jet system developed by the Plasma Technology for Agricultural Applications Research Laboratory of Walailak University in the Nakhon Si Thammarat province of Thailand shown in Figure 1 was used in this study. Air, water vapor and argon gas were used as discharge gases. A working gas was introduced to a flexible tube with a 6 mm inner diameter and a thickness of 2 mm. The plasma jet was induced by high voltage and high frequency RF power supply. The power and frequency of the RF can be adjusted between 0 – 40 W and 20 kHz - 600 kHz, respectively.

In vitro testing

Four strains of the molds *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp. and *Penicillium* sp. were identified from brown rice cereal surfaces. Codes refer to strains held in the culture collection of the Cellulose Protection Technology Laboratory of Walailak University. Spores of test molds were obtained from mycelia grown on a malt extract agar (MEA; Merck, Thailand) medium at 30°C for 7 days, and were collected by flooding the surface of the plates with ~5 ml of sterile saline solution (NaCl, 8.5 g l^{-1} water) containing Tween 80 (0.1% v v^{-1}). The viability of all strains was checked by using quantitative colony counts at 10^5 CFU ml^{-1}.

Aliquots (0.1 ml) of the mold spore suspensions were spread on the surface of malt extract agar (MEA, Merck, Thailand). Inoculated plates were left at room temperature for approximately 30 min to allow inoculums to be fully absorbed by the agar. The lids of Petri dishes were removed and three sets of experiments were acted as the inoculated agar surface was exposed to various plasma gas species (argon, water vapor and air) at 0 W (control), 20 W, and 40 W for 10 min. The plates were then incubated for 72 hours at 25°C and colonies were counted. The percentage of stain and mold (based on a control) for each surface was calculated as (A/B)x100, where A is the total colony count for the mold at plasma treatment, and B is the total colony count for the mold at the control.

In vivo testing

Khai Mod Rin (NSRC950013) brown rice, a local rice grown in the Nakhon Si Thammarat province, was selected for this study and it was obtained from the Nakhon Si Thammarat Rice Research Center. It was harvested in December 2010. A 300g sample of brown rice was washed and soaked in a steam for 10 min and then was cooked in an automatic rice cooker

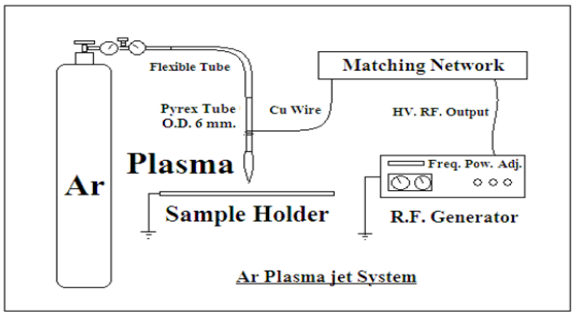

Figure 1. Schematic diagram of the low temperature atmospheric plasma jet

(SR-DG 182, Panasonic Management (Thailand) Co., Ltd., Thailand). After that, it was removed from the cooker and put into a stainless template that was 5 cm wide by 5 cm long by 0.5 cm deep. The cooked brown rice was dried in a tray dryer at 60°C followed by frying at 190°C for 20 s. Lastly, after the temperature of the samples decreased to room temperature, the brown rice cereal was put in plastic bags.

For the test, 5 ml of each mold spore was inoculated onto the upper surfaces of the brown rice cereal by spray inoculation with a hand-held spray bottle. This was done in a biological safety cabinet. The suspension culture was left on the brown rice cereal surfaces for 8 hrs. Then the upper surface sample was irradiated with the argon, water vapor and air plasma at 0 W (control), 20 W, and 40 W for 10 min at a distance of 10 cm from the jet nozzle. The brown rice cereal was then placed on a glass rod on top of moistened filter papers in sterile Petri dishes to maintain a high humidity (~100%RH). The Petri dishes were incubated at 25°C and 80%RH in an environmental chamber (Binder, Germany) until mold growth was observed. The time period needed for the initiation of mold growth on the cereal surface was recorded.

Effect of the plasma jet treatment on brown rice cereal surface mold

After plasma exposing for 10 min at 40 W using water vaper, argon or air gas, the mold growth on the surface of the brown rice cereals was ready to be studied. The control was done in the same way but without plasma treating. Storage time was recorded and the mold growth on the surface was observed under the storage condition at 25°C and 100%RH.

Results and Discussion

Antifungal activities of plasma on spore germination

The results of the exposures of *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp. and *Penicillium* sp. spores to various gas species (argon, water vapor and

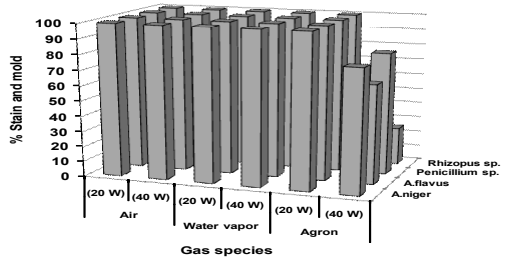

Figure 2. Percentage of stain and mold (based on control) on MEA treated with various plasma gas species after incubation at 25°C for 72 h

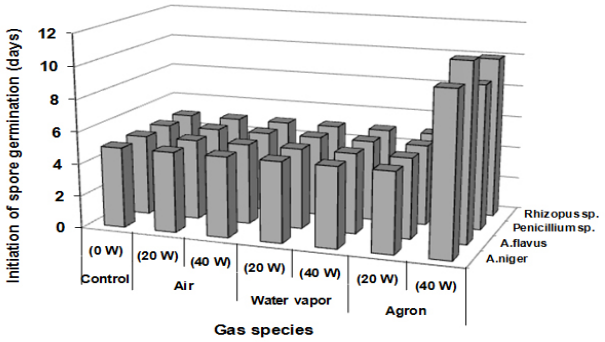

Figure 3. Initiation days of spore germination on brown rice cereal treated with various plasma gas species after incubation at 25°C 100% RH

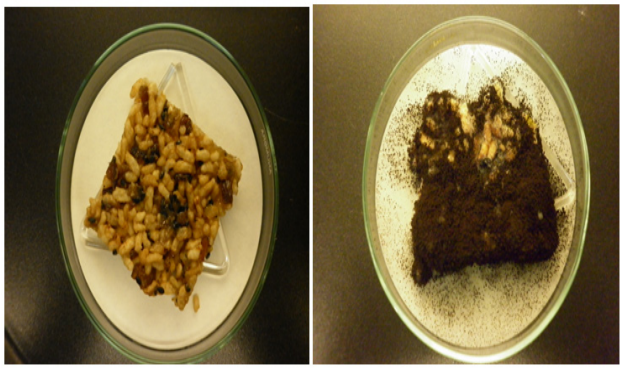

Figure 4. Spore germination of *Aspergillus niger* on brown rice cereal at 18 days (a) with Ar plasma jet treatment at 40 W for 10 min (b) without Ar plasma

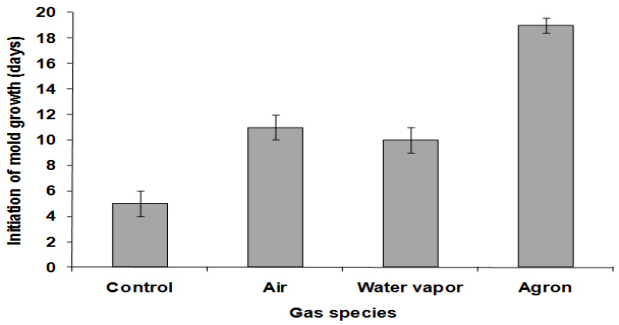

Figure 5. Initiation days of mold growth on brown rice cereal treated with various plasma gas species at 40 W after incubation at 25°C 100% RH

air) of plasma discharge at 20 W and 40 W for 10 min are shown in Figure 2. After treating with Argon plasma for 10 min at 40 W, the percentage of stain and mold was reduced from 80% to 25%. *Rhizopus* sp. showed the greatest reduction and was followed by *A. flavus*, *A.niger* and *Penicillium* sp. spores. No reduction was found when using steam and air gas of plasma both at 20 W and 40 W.

The main point of this study was to concentrate on the effect of various gases of plasma on the inhibition of fungi *in vitro* conditions. Very few studies have been conducted with various plasma gases to show the fungicidal properties of plasma against mold spoilage. Wang *et al*. (2003) demonstrated that argon gas could be used to replace helium to produce stable plasma discharge with a wide range of chemistry. Hury *et al*. (1998) reported that oxygen-based plasma (oxygen atoms and oxygen-containing radicals present in the plasma) could be used to destroy microorganisms. Similarly, Gweon *et al*. 2009 found that plasma of helium and oxygen gas in combination showed higher sterilization effect to *Escherichia coli* than plasma generated from helium gas alone. Yang *et al*. (2009) indicated that Ar plasma can effectively sterilize *Pseudomonas aeruginosa* in a short time. In addition, cracking of cell walls or cell membrane was observed after the plasma treatment. An air microplasma jet was reported to effectively treat yeast infection on skin (Kolb *et al*., 2008). Most research on the sterilization effect of Ar plasma at atmospheric pressure reported what active species such as radicals, electrons, ions and UV light could generate during the plasma treatment (Moisan *et al*., 2002; Lee *et al*., 2005). Moreover, plasma generation of UV radiation occurs in the range of 10 - 290 nm and those wavelengths above 200 nm, at a fluence of several mW s cm^{-2}, are responsible for microcidal effects (Laroussi, 2005). On the other hand, UV-C radiation (254 nm) was demonstrated to inhibit mold spores (Cia *et al*., 2007). Therefore, the reduction of mold spores after Ar plasma in this experiment could be explained according to the effects from the active species of plasma. This, however, warrants further study.

Antifungal activities of plasma on brown rice cereal

Growth of mold spores on the brown rice cereal after being irradiated by various gas species (argon, water vapor and air) of plasma at 20 W and 40 W for 10 min are shown in Figure 3. The initiation of the spore germination on brown rice cereal was recorded after 5 days of storage for steam and air of plasma at 20 W and 40 W treatments. The result was not different from the initiation day of the control treatment. Only Ar plasma at 40 W could extend the spore germination from 5 days to between 9 and 11 days (Figure 4). Therefore, the results confirm that Ar

plasma at 40 W can be used to inhibit mold growth on brown rice cereal. Using the maximum power of plasma in this experiment (40 W) for 10 min could not completely kill the mold spores. Higher power and exposure time are needed for more experiments. However, the possibility of using plasma to reduce mold growth and shelf-life extension was confirmed.

In vivo inhibitory activity of the most efficient Ar plasma was also investigated in a real food system such as sliced chicken breast and ham (Lee *et al*., 2011). The ability of plasma to work at low temperatures has opened up the possibility of using for the treatment on heat-sensitive food (Gurol *et al*., 2012). However, investigation of toxic molecules on food after plasma treatment needs to be performed to meet food safety standards. Therefore, using plasma on food products is noted to be observed by many countries.

Shelf-life evaluation

Initiation day for mold growth on untreated brown rice cereal (control) was only 5 days of storage. The shelf-life results of brown rice cereal treated with the air, water vapor or argon gas of the plasma at 40 W are shown in Figure 5. The shelf-life of the brown rice cereal after argon, air and water vapor plasma treating were extended for up to 19 days, 11 days, and 10 days respectively. The differences in shelf-life observed in this study could possibly be caused by the effect of various gas types of plasma. While, air and water vapor gas plasma were ineffective in reducing mold spore spoilage in this experiment, they could extend the shelf-life of brown rice cereal by up to 10 to 11 days. Therefore, this work has shown the potential for using air, water vapor and Ar plasma to extend the shelf-life of brown rice cereal under severe conditions. Optimization of expose time, power and gas plasma to reduce mold growth in food products needs to be studied in the future.

Conclusions

Among various plasma gases studied, Ar plasma has the best efficacy and was capable of preventing the growth of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Rhizopus* sp. spores on MEA and on brown rice cereal. Higher power of Ar plasma jet was more effective in inhibiting those molds than that at the lower power. The Ar plasma jet treatment at 40W with the exposure time of 10 min was capable of extending shelf-life of brown rice cereal for up to 19 days under storage conditions at 25°C and 100%RH. Higher power and longer exposure time of plasma treatment to further extend shelf-life of brown rice cereal should be explored in the future. Effects of plasma treatment on nutritional value and consumer acceptability of the cereal products should also be investigated. Finally, mechanisms underpinning antifungal activities of the Ar plasma jet treatment should be studied using spectroscopic techniques.

Acknowledgments

This study was supported by the Thailand Center of Excellence in Physics through the Plasma Agricultural Applications Laboratory and the Walailak University Fund. The authors would like to thank Mr. Thammanoon Srinuam and Mr. Chalerm Tehsanu for their help on the plasma experimental setup and Ms. Saifon Phothisuwan for her help identifying mold species.

References

Cia, P., Pascholati, S. F., Benato, E. A., Camili, E. C. and Santos, C. A. 2007. Effects of gamma and UV-C irradiation on the postharvest control of papaya anthracnose. Postharvest Biology and Technology 43 (3): 366-373.

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, Th., Brandenburg, R., von dem Hagen3, T. and Weltmann, K.-D. 2011. Low temperature atmospheric pressure plasma sources for microbial decontamination. Journal of Physics D: Applied Physics (44): 1-18.

Gurol, C., Ekinci, F.Y., Aslan, N. and Korachi M. 2012. Low temperature plasma for decontamination of *E. coli* in milk. International Journal of Food Microbiology 157(1): 1-5.

Gweon, G., Kim, D.B., Moon, S.Y. and Cheo, W. 2009. *Escherichia coli* deactivation study controlling the atmospheric pressure plasma discharge conditions. Current Applied Physics 9: 625–628.

Hury, S., Vidal, D.R., Desor, F., Pelletier, J. and Lagarde, T. 1998. A parametric study of the destruction efficiency of Bacillus spores in low pressure oxygen-based plasmas. Letters in Applied Microbiology 26: 417–42

Kolb, J.F., Mohamed, A.-A.H., Price, R.O., Swanson, R.J., Bowman, A., Chiavarini, R.L., Stacey, M., and Schonenbach, K.H. 2008. Cold atmospheric pressure air plasma jet for medical applications. Applied Physics Letter 92:241501

Kyritsi, A., Tzia, C. and Karathanos, V. T. 2011. Vitamin fortified rice grain using spraying and soaking methods. LWT-Food Science and Technology 44 (1): 312-320.

Laroussi, M. 2005. Low temperature plasma-based sterilization: overview and state-of-art. Plasma Processes and Polymers 2: 391–400.

Lee, H. J., Jung, H., Choe, W., Ham, J. S., Lee, J. H. and Jo, C. 2011. Inactivation of *Listeria monocytogenes* on agar and processed meat surfaces by atmospheric pressure plasma jets. Food Microbiology 28 (8): 1468-1471.

Lee, K.Y., Park, B. J., Lee, D. H., Lee, I.S., Hyun, S. O.,

Chung, K.H. and Park, J.C. 2005. Sterilization of *Escherichia coli* and MRSA using microwave-induced argon plasma at atmospheric pressure. Surface and Coatings Technology 193 (1–3): 35-38.

Moisan, M., Barbeau, J., Crevier, M., Pelletier, J., Philip, N. and Saoudi, B. 2002. Plasma sterilization. Methods and mechanisms. Pure Applied Chemistry 74: 349-358.

Ohtsubo, K., Suzuki, K., Yasui, Y. and Kasumi, T. 2005. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. Journal of Food Composition and Analysis 18: 303–316.

Suhem, K., Matan, Na., Nisoa, M. and Matan, Ni. 2012. Application of cold plasmas to extent shelf-life of brown rice cereal for primary school children. Walailak University (Unpublished).

Uhm, H. S. and Hong, Y. C. 2011. Various microplasma jets and their sterilization of microbes. Thin Solid Films 519 (20): 6974-6980.

Wang, S., Schulz-von der Garhen, V. and Dobele, H.F. 2003. Discharge comparison of nonequilibrium atmospheric pressure Ar/O_2 and He/O_2 plasma jets. Applied Physics Letter 83(16): 3272.

Yang, L., Chen, J. and Gao, J. 2009. Low temperature argon plasma sterilization effect on *Pseudomonas aeruginosa* and its mechanisms. Journal of Electrostatics 67(4): 646-651.

Zhang, B., Rong, Z. Q., Shi, Y., Wu, J. G. and Shi, C. H. 2011. Prediction of the amino acid composition in brown rice using different sample status by near-infrared reflectance spectroscopy. Food Chemistry 127 (1): 275-281.