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# Effect of plasma activated water on the postharvest quality of button mushrooms, *Agaricus bisporus*

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ABSTRACT

Non-thermal plasma is a new approach to improving microbiological safety while maintaining the sensory attributes of the treated foods. Recent research has reported that plasma activated water (PAW) can also efficiently inactivate a wide variety of microorganisms. This study invested the effects of plasma-activated water soaking on the postharvest preservation of button mushrooms (*Agaricus bisporus*) over seven days of storage at 20 °C. Plasma activated water reduced the microbial counts by 1.5 log and 0.5 log for bacteria and fungi during storage, respectively. Furthermore, the corresponding physicochemical and biological properties were assessed between plasma activated water soaking groups and control groups. The results for firmness, respiration rate and relative electrical conductivity suggested that plasma activated water soaking can delay mushroom softening. Meanwhile, no significant change was observed in the color, pH, or antioxidant properties of *A. bisporus* treated with plasma activated water. Thus, plasma activated water soaking is a promising method for postharvest fresh-keeping of *A. bisporus*.



## 1. Introduction

China is not only the largest button mushroom *(Agaricus bisporus*) producer in the world but the most potent exporter, whose value has risen to $335 million in 2011 (Wang, Chen, Yang, & Wang, 2015). Compared with vegetables, button mushrooms are an outstanding source of mineral elements (potassium, phosphorus, copper and zinc), vitamins (B2, niacin, and folates), and several essential amino acids (Mattila et al., 2001). However, the short shelf life of button mushroom has become the biggest limitation of their industrial development. Harvested button mushrooms experience browning, cap opening, stipe elongation, weight loss, cap diameter increases, textural changes and sporulation after 1–3 days of storage at ambient temperature (Burton & Twyning, 1989) or 5–7 days at 0–2 °C (Gormley, 1975). The fresh-keeping methods for button mushrooms have been studied extensively by many researchers, including chemical treatments (tyrosinase inhibitors (Nerya et al., 2006), sodium metabisulfite (Brennan, Le Port, Pulvirenti, & Gormley, 1999), citric acid (Brennan, Le Port, & Gormley, 2000), hydrogen peroxide (Sapers, Miller, Pilizota, & Kamp, 2001), and glycine betaine (Wang et al., 2015)), physical treatments (refrigeration, coatings, and modified-atmosphere packaging (Li & Zhang, 2013); dehydration (Singh, Sodhi, Singh, & Khanna, 2012); and irradiation techniques (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012), and combinations thereof (Alikhani-Koupaei, Mazlumzadeh, Sharifani, & Adibian, 2014).

All of these methods increase mushroom shelf life but also have their own drawbacks, such as safety considerations, decreased nutritive value, discoloration, textural changes, contamination by pathogenic microorganisms, off-flavors, high energy consumption, and unsuitability on the industrial scale (Rico, Martin-Diana, Barat, & Barry-Ryan, 2007).

Non-thermal plasma is a novel approach for microbial inactivation in the food industry. It involves exposing food to ionizing

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radiation (such as charged particles, electric fields, ultraviolet (UV) photons, or reactive species) to disinfect the microbes while ensuring the safety of the products. Previous work has demonstrated that non-thermal plasmas can efficiently disinfect a wide range of microorganisms, including bacteria, fungi, viruses, bacterial spores and biofilms (Kolb et al., 2008; Pan et al., 2013). Reactive oxygen species (ROS) are the major bactericidal agents, which cause damage to DNA and proteins in microbial cells (Joshi et al., 2011). Non-thermal plasma is also known to be highly effective in reducing harmful bacteria and eliminating toxins in fruits, vegetables, and various meat-stuffs, while preserving the fresh taste, aroma, texture, wholesomeness, and nutritional content of food (Baier et al., 2013; Pankaj et al., 2014).

In addition to the non-thermal plasmas mentioned above, plasma-activated water (PAW) can also inactivate certain types of microbes (Oehmigen et al., 2010). To date, research on the impact of pH and $H_2O_2$ on the progress of PAW disinfection has highlighted the importance of ROS in PAW solutions (Naitali, Kamgang-Youbi, Herry, Bellon-Fontaine, & Brisset, 2010; Oehmigen et al., 2010). The species generated in the fluid are stable for an extended period of time and are partially responsible for the long-lasting antimicrobial properties. Traditional chemical sanitizers, which are currently widely used in the food industry, have increased public health concerns about the risk of carcinogenic organic compound formation, whereas PAW is more environmentally-friendly and cost-effective. However, to our knowledge, there are very few studies on the use of PAW to enhance the shelf life of button mushrooms.

In this study, the influence of PAW on the postharvest quality of the button mushroom, *A. bisporus*, was evaluated. The antimicrobial efficacy of PAW was examined based on the colony forming unit (CFU) count. Optical emission spectroscopy (OES) was employed to detect the major excited reactive species in PAW. In addition, the quality (color, firmness, pH, respiration rate, weight loss, relative electrical conductivity, malondialdehyde (MDA) and vitamin C contents, and superoxide dismutase (SOD) activity) of the treated button mushrooms was assessed.

## 2. Materials and methods

### 2.1. Sample preparation

Button mushrooms (*A. bisporus*) were obtained from a commercial button mushroom cultivation facility in Beijing, China. The mushrooms were carefully collected at the closed cap stage with a pilei diameter of approximately 5 cm. The button mushrooms were transported to the laboratory within an hour after harvest and stored at 4 °C and 90% RH for 24 h to reduce the respiration rate.

### 2.2. Plasma device and PAW generation

A single-electrode, non-thermal, atmospheric-pressure plasma jet was used to generate the PAW. Fig. 1a and b shows a photograph of PAW generation and the thermographic measurement of plasma near the water surface. The non-thermal plasma jet device consists of a quartz tube, a 1-mΩ resistor, and an outer copper foil that surrounds the quartz tube. The outer copper foil, as a single electrode, is connected to a 10-kHz, sinusoidal, high-voltage source with an 18-kV peak-to-peak voltage. Premixed argon and oxygen (98% Ar and 2% $O_2$ per volume, referred to as $Ar/O_2$) are used as the working gas at a flow rate of 5 L/min.

The PAW solution was produced by plasma discharge above the water surface. The distance between the terminal of the plasma jet and the liquid level was approximately 10 mm, and 500 mL of sterile distilled water were activated by plasma for 20 min to obtain the PAW (Fig. 1a) in our study. A thermal imaging camera (FLIR E50, USA) was employed to measure the temperature values during PAW generation. The mean temperature of the non-thermal plasma near the water surface was 32.7 °C (Fig. 1b).

### 2.3. PAW treatment

The mushrooms were randomly divided into three treatment groups and two control groups. The button mushrooms were immersed in 500 mL of PAW for 5, 10, and 15 min, defined as PAW-5, -10 and -15 for a simplified description. All of the experiments compared PAW-treated mushrooms with those that had received no treatment (dry control), defined as the control group, and with those that had been immersed in sterile distilled water for 15 min (wet control), defined as the water group, to explore the effects of the soaking step on the reduction of microorganisms on the mushrooms. Button mushrooms were screened for uniform size, maturity, and the absence of mechanical damage. The mushrooms (400 ± 10 g) were randomly selected for each treatment. After treatment, the samples were immediately analyzed and then stored in an environmental incubator at 20 ± 2 °C and 70 ± 5% relative humidity for seven days. Thirty replicates were included in each treatment group, and three replicates from each treatment group were subsequently randomly selected and analyzed for physicochemical parameters every other day.

### 2.4. Optical emission spectroscopy

Optical emission spectroscopy (OES) on an AvaSpec-2048-8 Fiber Optic Spectrometer (Avantes, USA) was employed to identify the major excited reactive species during PAW generation by the $Ar/O_2$ plasma. One end of the fiber optic cable was used to acquire the light signals at the bottom of the water container (quartz tube) at a distance of approximately 5 mm from the exit nozzle. The dispersed emission spectra were recorded by a 2048-pixel charge-coupled device (CCD) detector array.

### 2.5. Physicochemical properties measurement

ORP, electrical conductivity, and pH of the PAW were all measured immediately after PAW generation. ORP and pH were measured by a pH and redox multimeter (Mettler-Toledo, Switzerland). Electrical conductivity was measured with an electrical conductivity meter (DDB-303A).

### 2.6. Colony count assay

A sterile knife was used to peel the cap off of the button mushrooms. The skin (2 ± 0.1 g) was placed into a glass homogenizer with 10 mL of sterile water for homogenization. Ten-fold serial dilutions of 100 μL of homogenate were spread uniformly on lysogeny broth (LB) and potato dextrose agar (PDA) culture media, which were then incubated at 37 °C and at 30 °C to evaluate aerobic bacteria, as well as yeasts and molds, respectively. Microbial counts were expressed as $\log_{10}$ CFU/g.

### 2.7. Button mushroom quality analysis

#### 2.7.1. pH, relative electrical conductivity, and texture measurements

One button mushroom was homogenized with 10 mL of sterile water for 3 min. The pH of the homogenate was measured using a pH meter (Mettler-Toledo, Switzerland). Each analysis was performed in triplicate.

One button mushroom was randomly selected, and the *A. bisporus* fruit bodies were treated with a hole punch. One gram