

# Effect of Non-Thermal Plasma-Activated Water on Fruit Decay and Quality in Postharvest Chinese Bayberries

Ruonan Ma<sup>1</sup> · Shuang Yu<sup>1</sup> · Ying Tian<sup>1</sup> · Kaile Wang<sup>1</sup> · Chongde Sun<sup>3</sup> · Xian Li<sup>3</sup> · Jue Zhang<sup>1,2</sup> · Kunsong Chen<sup>3</sup> · Jing Fang<sup>1,2</sup>

Received: 14 December 2015 / Accepted: 20 June 2016  
© Springer Science+Business Media New York 2016

**Abstract** Recently, non-thermal plasma-activated water (PAW) became a relatively new concept developed in the food industry. The effects of PAW on fruit decay, microbial loads, and quality of postharvest Chinese bayberry were investigated. Chinese bayberries were treated by PAW for 0.5, 2, or 5 min and then stored at 3 °C for 8 days. Experimental results show that all PAW treatments could reduce fruit decay by around 50 % compared to control at the end of storage. There was no dose-effect relationship between PAW treatment time and fruit decay. Meanwhile, a 0.5-min PAW treatment could remarkably decrease microbial population on Chinese bayberries during storage, and the maximum reductions reached around 1.1 log CFU/g both for bacteria and fungi at the end day of storage. Scanning electron microscopy results reveal that PAW could significantly change the morphology of microbial cells on Chinese bayberries. Moreover, physicochemical properties analysis of PAW demonstrates that the microbial inactivation of PAW is mainly attributed to the combined action of high oxidation reduction potential and low pH. Additionally, PAW-treated fruits exhibited markedly higher firmness, color index of red grapes, and total soluble solids than the control did at the eighth day. These results indicate that PAW might be a

promising strategy to control fruit decay and maintain quality of Chinese bayberry during postharvest storage.

**Keywords** Chinese bayberry · Non-thermal plasma-activated water · Fruit decay · Microbial loads · Fruit quality · Reactive oxygen species

## Introduction

Chinese bayberry (*Myrica rubra* Seib & Zucc.), a subtropical fruit native to China, is grown commercially in eastern and southern China. The fruit consists of capsule-like cellules termed flesh segments and is noted for its attractive red to purple color and appealing flavor (Wang et al. 2010b). Meanwhile, Chinese bayberry is also a good source of natural antioxidants, such as anthocyanins, flavonoids, and other phenolic compounds (Bao et al. 2005), which could inhibit oxidation of human low-density lipoprotein (LDL) and liposome (Heinonen et al. 1998), thus making it potentially effective in reducing the incidence and mortality rates of cancer, cardiovascular disorders, and other degenerative diseases caused by oxidative stress (Ames et al. 1993). However, Chinese bayberry is a highly perishable soft fruit, susceptible to microbiological decay, mechanical injury, physiological deterioration, and water loss, resulting in a postharvest life to 1–2 days under ambient temperature, which limits its marketing and causes severe postharvest loss (Wang et al. 2009). Therefore, there is an urgent need to prolong the postharvest storage of Chinese bayberry and preserve fruit quality.

Especially, microbiological decay is the main cause of the rapid and intensive postharvest deterioration of Chinese bayberry, with the major decay organisms being *Saccharomyces* spp., *Candida* spp., *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., and *Fusarium* spp. Hence, the common traditional methods employed to extend the shelf-life of Chinese

✉ Jue Zhang  
zhangjue@pku.edu.cn

<sup>1</sup> Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, People's Republic of China

<sup>2</sup> College of Engineering, Peking University, Beijing 100871, People's Republic of China

<sup>3</sup> China Laboratory of Fruit Quality Biology, Zhejiang Provincial Key Laboratory of Horticultural Plant Integrative Biology, The State Agriculture Ministry Laboratory of Horticultural Plant Growth, Development and Quality Improvement, Zhejiang University, Zijingang Campus, Hangzhou 310058, People's Republic of China

bayberry are based on application of synthetic fungicides (Zheng et al. 2008). However, increasing public concerns related to development of pathogen resistance to many currently used fungicides and potentially harmful effects on the environment and human health have encouraged the rapid development of alternative effective approaches to control microbial contamination of Chinese bayberries during storage.

Non-thermal plasma has been widely considered to be an effective method for sterilization (Moreau et al. 2008; Niemira 2012; Pankaja et al. 2014; Selcuk et al. 2008; Wan et al. 2009), which is an ionized gas, consisting of charged particles, electric fields, ultraviolet photons, and reactive species (Laroussi 2005). Among those, reactive oxygen species (ROS) are considered as the major bactericidal agent in plasma inactivation (Ma et al. 2012; Ma et al. 2013; Graves 2012). Recently, it has been realized that the active compositions in non-thermal plasma could react with water to generate plasma-activated water (PAW) with outstanding antibacterial ability, which could efficiently inactivate microbial cells, including *Hafnia alvei*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* (Burlica et al. 2010; Kamgang-Youbi et al. 2009; Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013). It is generally agreed that the bactericidal activity of PAW derives from the combined action of a high positive oxidation reduction potential (ORP) and low pH (Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013). Furthermore, our previous study has shown that PAW has the potential to inactivate *S. aureus* inoculated on strawberries without inducing significant change in the color, firmness, and pH of the strawberries (Ma et al. 2015). However, to the best of our knowledge, there is no report so far on the application of PAW to inactivate the indigenous microorganisms of postharvest Chinese bayberries.

The present work explores the effect of PAW on the fruit natural decay, microbial contamination, and quality attributes of Chinese bayberries during postharvest storage. The microbial inactivation of PAW against fungi and bacteria on Chinese bayberries was assessed by colony-forming unit (CFU) count and further verified by scanning electron microscope (SEM). Moreover, with respect to the fruit quality, parameters of firmness, color index of red grapes (CIRG), and total soluble solids (TSS) were evaluated. Additionally, the ORP, electrical conductivity, and pH of PAW were recorded to estimate its physicochemical properties.

## Materials and Methods

### Plasma Device and PAW Generation

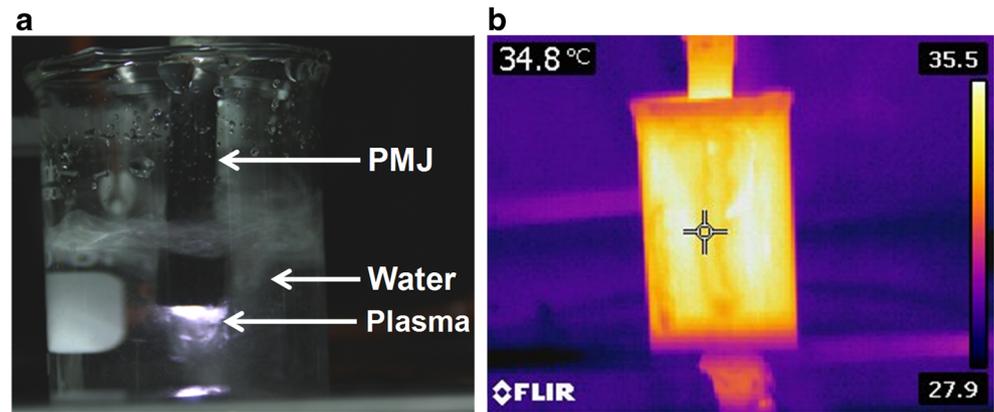
An air non-thermal plasma jet was used to generate PAW. As shown in Fig. 1a (photograph of plasma jet) and Fig. 2 (schematic diagram of plasma jet), the non-thermal plasma device

is designed based on a dielectric barrier structure with a hollow electrode (HEDBS) structure. The whole system, mainly consisting of copper electrodes and quartz dielectric, is set at the end of a quartz tube with the inlet diameter of 1.5 mm. Air with 260 L/h gas flow rate is injected into the quartz tube as the working gas, and the high-voltage electrode is connected to a power source with 20 kHz. Homogeneous plasma is generated in the discharge gap of 0.5 mm and a plasma jet reaching lengths of up to 7 mm long is ejected through the end outlet of 0.5 mm. A detailed description of the working principle of HEDBS has been introduced by Yu et al. (2015). PAW was generated by plasma discharge under the water, and the distance between the end of the plasma jet and the water surface was 3 cm. Sterile distilled water of 1600 ml was activated for 25 min to obtain PAW solution in this study. A thermal imaging camera (FLIR E50, USA) was employed to measure the temperature values of PAW during its generation. The mean temperature of the non-thermal plasma under water surface is 34.8 °C (Fig. 1b).

### Plant Materials and PAW Treatment

Chinese bayberry (*M. rubra* Sieb. & Zucc. cv. Dongkui) fruits were hand-harvested at mature stage from a commercial orchard in Xianju County, Zhejiang Province, China, in July, and transported within 4 h to the laboratory of Zhejiang University, Hangzhou. Chinese bayberries were selected for uniform size and color, as well as without mechanical damage. As shown in Fig. 2 (a schematic diagram of the experimental arrangement), the Chinese bayberries were randomly divided into four groups, and then soaked in a 2000-ml sterile glass beaker containing 1600 ml PAW solution for 0.5, 2, and 5 min, respectively, defined as PAW-0.5, PAW-2, and PAW-5 for simplified description. Based on that PAW was liquid solution, the group of fruits immersed into 1600-ml sterile deionized water was served as the control. Following the treatments, the fruits were placed individually on sterile polyethylene (PE) food wrap films and dried by air inside a biosafety hood for 90 min at room temperature (25 °C), and subsequently stored in a freezer with  $3 \pm 1$  °C temperatures and  $85 \pm 5$  % relative humidity for 8 days. There were 150 fruits (about 3 kg) for each treatment group, comprising 3 replicates with 50 fruits per replicate. Fruits from each treatment group were randomly selected initially (day 0) and at 2-day intervals during storage for decay and microbiological analysis. For microbiological analysis, fruits without any treatments were used to estimate the initial population of microorganisms attached on Chinese bayberries. Meanwhile, fruits without any treatments (day 0) and with PAW or water treatments at 2-day intervals during storage were analyzed for fruit quality parameters (firmness, CIRG, and TSS).

**Fig. 1** **a** A photograph of PAW generation and **b** the thermographic measurement of plasma discharge under water during PAW generation



### Physicochemical Properties Measurement

The ORP, electrical conductivity, pH of PAW were immediately measured after PAW generation. PAW after 0.5, 2, and 5 min of treatment without fruits (defined as PAW-A, PAW-B, and PAW-C for simplified description, respectively) were also measured. ORP, pH, and temperature were measured by a multimeter pH and Redox (Mettler-Toledo, Switzerland). Electrical conductivity was measured with an electric conductivity meter (DDB-303A).

### Fruit Decay

The symptom of decay in Chinese bayberry during storage is visible mold growth on the fruit surface. Fruit decay was visually estimated by using 50 fruits of each replicate during storage. Fruit with visible mold growth covering about 2 % of the surface affected was considered decayed in this study. The decay incidence was calculated based on the number of decayed fruit divided by the total number of fruit for each treatment using the following formula: Decay Incidence% =  $N_0 / N \times 100$  %, where  $N$

was the total number of measured fruit and  $N_0$  was the number of decayed fruit.

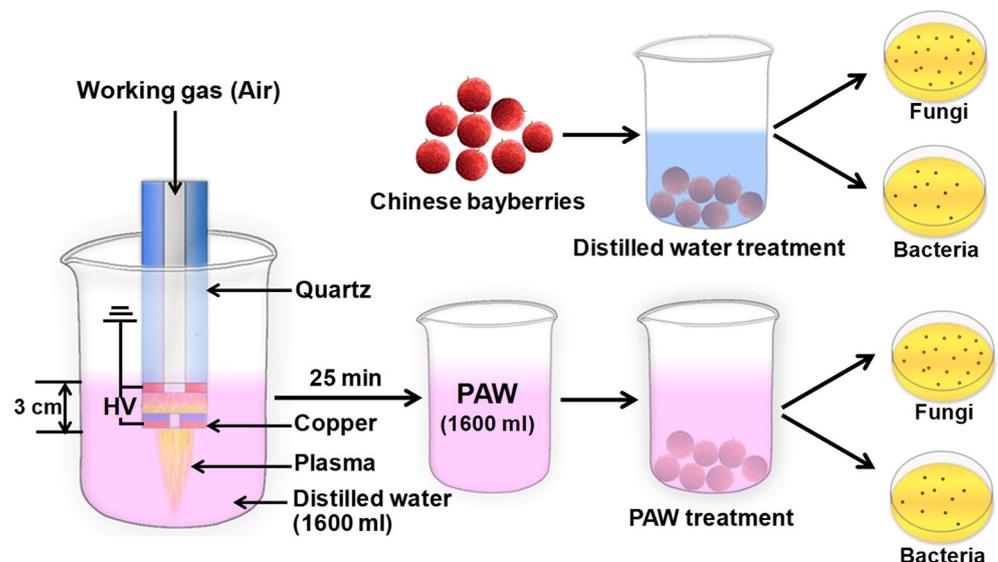
### Microbiological Analysis

The antimicrobial ability of PAW is evaluated via colony count assay. Three replicates of 15 Chinese bayberries from each treatment group were homogenized by aseptically transferring into separate sterile stomacher bags with 200 ml of sterile 0.1 M phosphate-buffered saline (PBS, pH 7.4) and hand rubbing for 5 min. Tenfold serial dilutions of 100- $\mu$ l homogenate were spread uniformly on Luria-Bertani (LB) agar and potato dextrose agar (PDA) plates, and then incubated at 37 °C and at 30 °C for a subsequent CFU counting of bacteria and fungi, respectively. The microbial counts were expressed as  $\log_{10}$  CFU/g sample.

### Scanning Electron Microscopy

After treatments, small square pieces of Chinese bayberries were cut and placed in sterile Petri dishes. The samples were

**Fig. 2** A schematic diagram of the experimental arrangement, including PAW generation and PAW treatment of Chinese bayberries. Chinese bayberries treated by sterile distilled water were served as the control group



fixed with 2.5 % glutaraldehyde in 0.1 M PBS (pH 7.2) at 4 °C overnight, and then washed twice for 15 min in the same phosphate buffer prior to dehydration in a series of increasing ethanol concentrations (20, 40, 60, 80 and 100 % v/v<sup>-1</sup> ethanol in water; 15 min in each solution). Subsequently, they were dried by critical point drying with liquid CO<sub>2</sub>. Following this, all the samples were mounted on aluminum stubs with double-sided carbon sticky-tape, sputtered with gold in a vacuum evaporator, and visualized with SEM (S-4800, HITACHI, Japan) at 25 kV.

### Fruit Quality Analysis

#### Firmness

Fruit firmness was measured on 15 fruits per treatment, consisting of 5 fruits from each of the 3 replicates. Firmness was determined on each fruit at two paired surfaces at 180° separations using a TA-XT2i texture analyzer (Stable Micro Systems, England) fitted with a 5-mm diameter probe. The rate of penetration was 1 mm s<sup>-1</sup> with a final penetration depth of 4 mm and data are expressed in newtons (N).

#### Fruit Surface Color

Fruit surface color was measured by three replicates of 15 Chinese bayberries from each treatment group with a reflectance spectrophotometer (TC-P2A). Standard illuminant D65 was used as reference and observer 10°.  $L^*$ ,  $a^*$ , and  $b^*$  values were recorded and the intensity of the hue was quantified by hue angle ( $H^\circ$ ) and chroma ( $C$ ). Hue angle was calculated as  $H^\circ = \arctan(b^*/a^*)$ ; chroma was calculated as  $C = (a^{*2} + b^{*2})^{0.5}$ . The color index of red grapes (CIRG) =  $(180 - H^\circ) / (L^* + C)$  was used to detect the color differences of Chinese bayberries, which also have a color range from white, pink to dark violet similar to that of red grapes (Zhang et al. 2005). Two readings were taken from the opposite cheeks of each fruit.

#### Total Soluble Solids

Three replicates of 15 fruits per treatment were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed for TSS. TSS was determined at 25 °C using an Atago digital refractometer (model PR-101, Tokyo, Japan).

### Statistical Analysis

All data were obtained from three replicate experiments with a completely randomized design. Values from all experiments were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS statistical package 17.0 (SPSS Inc., USA). An analysis of variance

(ANOVA) was conducted to compare the effects of different treatments on the decay incidence and microbial populations of Chinese bayberries, and significant differences between mean values were identified by Duncan's multiple range test with a confidence level at  $P \leq 0.05$ . Moreover, Duncan's multiple range test was also applied to compare the values of physicochemical properties (ORP, electrical conductivity, and pH) among different groups. Additionally, the paired-sample  $t$  test was applied to compare the values of fruit qualities (firmness, CIRG, and TSS) between PAW and the control group. The significant difference is expressed as \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

## Results and Discussion

### Evaluation of the Physicochemical Properties of PAW

ORP is considered as an important factor in water disinfection due to affecting microbial inactivation as high ORP can damage the outer and inner membrane of microbial cells, which also reveals the ability of the disinfectant to oxidize, the concentration of oxidizers, and their activity or strength. In Table 1, compared to control (269 mV), the ORP of PAW markedly increased to 510 mV ( $P \leq 0.05$ ). The result indicated that a large amount of ROS was generated in PAW, which has been considered to play a critical role in the PAW inactivation process (Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013). Moreover, hydroxyl radical (OH·) and nitric oxide radical (NO·) were proved to exist in PAW by electron spin resonance (ESR) diagnosis in our previous work (Zhang et al. 2016). Meanwhile, the signal intensity of OH· and NO· in PAW increased with the plasma inactivation time, indicating that radicals could accumulate in PAW.

Electrical conductivity is an important indicator to determine the level of active ions that existed in PAW. In Table 1, after 25 min of plasma activation, the electrical conductivity of water increased dramatically from 2.77 to 121.33 μS/cm ( $P \leq 0.05$ ). The result was consistent with our previous work (Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013), demonstrating that many active ions were accumulated in PAW, which may be various ROS and other reactive chemical species derived from chemical reaction between water molecules and plasma electrons (Maeda et al. 2003). Therefore, the behavior of the change in electrical conductivity might relate to the inactivation behavior as well.

Regarding pH measurement, compared to control (6.06), the pH value of PAW significantly decreased to 3.57 after 25 min of plasma activation ( $P \leq 0.05$ ), indicating that plasma discharge can lead to the acidification of water. Moreover, the antimicrobial activity of PAW was considered to result from the combined action of high positive ORP and low pH for the reason that low pH is favorable for the reactive species to

**Table 1** Physicochemical properties (ORP, conductivity, and pH) of control (water) and PAW, as well as PAW after 0.5, 2, and 5 min treatment without fruits (defined as PAW-A, PAW-B, and PAW-C for simplified description)

Treatment	ORP (mV)	Conductivity ( $\mu\text{S}/\text{cm}$ )	pH
Control	269 $\pm$ 7.51 a	2.77 $\pm$ 0.25 a	6.06 $\pm$ 0.13 a
PAW	510 $\pm$ 4.04 b	121.33 $\pm$ 1.53 b	3.57 $\pm$ 0.06 b
PAW-A	512 $\pm$ 4.16 b	123.00 $\pm$ 2.65 b	3.60 $\pm$ 0.00 b
PAW-B	511 $\pm$ 4.58 b	123.00 $\pm$ 2.00 b	3.53 $\pm$ 0.06 b
PAW-C	511 $\pm$ 3.06 b	125.00 $\pm$ 2.00 b	3.53 $\pm$ 0.12 b

Data were expressed as the mean  $\pm$  standard deviation of measurements made on three replicate experiments ( $n = 3$ ). Values in the same column with different letters were significantly different ( $P \leq 0.05$ ) according to Duncan's multiple range test

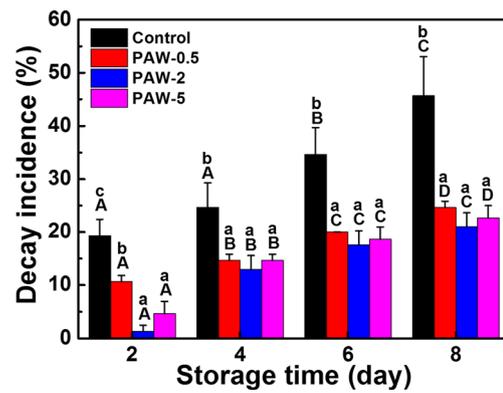
penetrate cell walls; on the other hand, the presence of reactive species reduces the resistance of bacteria to acidic environment (Oehmigen et al. 2010).

The physicochemical properties of PAW during 0.5, 2, and 5 min of treatment without fruits (defined as PAW-A, PAW-B, and PAW-C for simplified description, respectively) were also measured. As shown in Table 1, the ORP, conductivity, and pH of PAW remained essentially unchanged after 0.5, 2, and 5 min of treatment without fruits. There was no significant difference among PAW, PAW-A, PAW-B, and PAW-C in ORP, conductivity, and pH ( $P > 0.5$ ), indicating that the physicochemical properties of PAW were stable during these short treatment times.

### Effects of PAW on Fruit Decay

As shown in Fig. 3, all PAW treatments in this study significantly ( $P \leq 0.05$ ) inhibited fruit decay compared to control throughout the storage period. The decay incidence of all the groups was 0 % on day 0 (data not shown), and then the decay incidence increased with the storage time for all groups. Moreover, compared with the PAW-0.5 treatment, the decay incidence of PAW-2 and PAW-5 increased more rapidly from the second day to the eighth day. At the end of the storage, the decay incidences of PAW-0.5, PAW-2, and PAW-5 were only 24.67, 21.05, and 22.67 %, respectively, markedly ( $P \leq 0.05$ ) lower than that of control (45.67 %). Furthermore, there was no significant ( $P > 0.05$ ) difference in decay incidence among all PAW treatments during storage except for day 2. On day 2, the decay incidence of PAW-2 and PAW-5 was 1.33 and 4.67 %, respectively, significantly ( $P \leq 0.05$ ) lower than that of PAW-0.5 (10.67 %). Among these treatments, PAW-2 was the most effective in controlling fruit decay during the 8-day storage. Thus, there was no dose-dependent effect of PAW treatment time on fruit decay in Chinese bayberries.

Based on the results, PAW could effectively control the fruit decay of Chinese bayberries, which is comparable to



**Fig. 3** Effects of PAW treatments on decay incidence of Chinese bayberry during storage at 3 °C for 8 days. Chinese bayberry was treated by PAW for 0.5, 2, and 5 min, respectively, defined as PAW-0.5, 2, and 5 for short. Vertical bars represent the mean  $\pm$  standard deviation of measurements made on three replicates with 50 fruits per replicate ( $n = 3$ ). Different lowercase letters (a–c) on the bars for the same storage time indicate significant differences ( $P \leq 0.05$ ) among different treatments. Different capital letters (A–D) on the bars for the same treatment indicate significant differences among different storage times ( $P \leq 0.05$ ) according to Duncan's multiple range test

other postharvest treatments of Chinese bayberries, such as high oxygen atmosphere, hot air, ethanol vapor, methyl jasmonate, essential oils, and hypobaric storage (Chen et al. 2013; Jin et al. 2012; Wang et al. 2009; Wang et al. 2010a; Wang et al. 2010b; Yang et al. 2009; Zhang et al. 2007; Zheng et al. 2008). Moreover, PAW was considered to be a short-time, uniform, and fully contacted postharvest treatment for Chinese bayberry. However, unexpectedly, there was no dose-effect relationship between PAW treatment time and decay incidence, which may be a result of the following two aspects. On one hand, it has been reported that prolonged PAW treatment time could lead to higher inactivation efficiency (Zhang et al. 2013); consequently, the decay incidence of PAW-2 and PAW-5 was lower than that of PAW-0.5 after the first 2 days of storage. On the other hand, longer PAW treatment time might also induce oxidative stress of Chinese bayberries, thereby accelerating the ripening and senescence process of the fruit, which would be more susceptible to pathogen infection (Cantu et al. 2008; Cantu et al. 2009), and hence leading to a more rapid increase of decay incidence during the following 6 days of storage. Meanwhile, due to the complex structure of Chinese bayberries, the liquid embedded in the cracks and crevices is difficult to completely evaporate during the drying process after liquid treatment of Chinese bayberries. Thus, longer PAW treatment time may result in more liquid residues in Chinese bayberries, which have the potential to spread contaminants and cause osmotic damage (Zhang et al. 2007), accordingly increasing the decay incidence. The specific mechanisms responsible for the observed effects are unclear at this time and will be investigated in future studies.

Therefore, these results provide valuable guidance for the treatment time of PAW applied to preserve fresh produce. The

factors including the beneficial effect of inactivating pathogens on fresh produce, the negative effects of oxidative stress induced by PAW as well as the spread of contaminants and osmotic damage owing to its liquid property, and the structure of fresh produce should all be considered to confirm the optimum PAW treatment parameter. In this study, given that no significant difference of fruit decay existed among all the PAW treatments at the end of storage and the convenience of practical application in the food industry, 0.5 min was regarded as the optimal PAW treatment time and used in the following experiments.

### Effects of PAW on microbial loads of Chinese bayberries

Microorganisms, such as pathogenic bacteria and fungi, distributed on fruit surface, are usually regarded as a serious hazard to human health and the main reason leading to the postharvest deterioration of fruits (Wang et al. 2011). Thus, CFU assay was employed to evaluate the postharvest contamination of Chinese bayberries after water and PAW treatment (Fig. 4). At harvest, the populations of fungi and bacteria on Chinese bayberries without any treatments were 5.97 and 5.47 log CFU/g, respectively (data not shown). At day 0, the total fungal and bacterial counts on water-treated fruits (control) were 5.95 and 5.37 CFU/g, which had no significant difference ( $P > 0.05$ ) with fruits without any treatments, indicating that water treatment almost had no effect on the reduction of microorganisms on Chinese bayberries. The similar results were also found in other studies with water treatment (Liao et al. 2010; Ma et al. 2015). PAW achieved 0.4 log reduction for fungi and a higher reduction of 0.8 log for bacteria at day 0. Moreover, during the 8-day storage, the microbial populations of the control increased significantly ( $P \leq 0.05$ ) compared to that of PAW-treated fruits, which only had a slight gradual increase. Meanwhile, the microbial counts of the control were remarkably ( $P \leq 0.05$ ) higher than that of PAW-treated fruits throughout the storage period, and the maximum reductions of microbial population reached around 1.1 log CFU/g both for bacteria and fungi at the end of storage, demonstrating that PAW could effectively inhibit the microbial contamination on Chinese bayberries and ensure the safety of fruits. These findings were consistent with our previous study on PAW inactivation of *S. aureus* on strawberries (Ma et al. 2015).

The antimicrobial effects of PAW on Chinese bayberries were well confirmed in this study. Recently, many researchers have focused on the sterilization mechanisms of PAW (Burlica et al. 2010; Kamgang-Youbi et al. 2009; Ma et al. 2015; Tian et al. 2015; Zhang et al. 2013). Based on previous studies, it has been revealed that ROS in PAW could cause oxidative stress in microbial cells by accumulation of intracellular ROS, consequently leading to a drop in the membrane potential, a breach in membrane integrity, as well as damage of cell internal components and structure, and eventually cell death.

Moreover, the disinfection efficacy of PAW was evidenced to be closely dependent on the ROS level in PAW.

### Effect of PAW on the Morphological Changes of Microorganisms on Chinese Bayberries

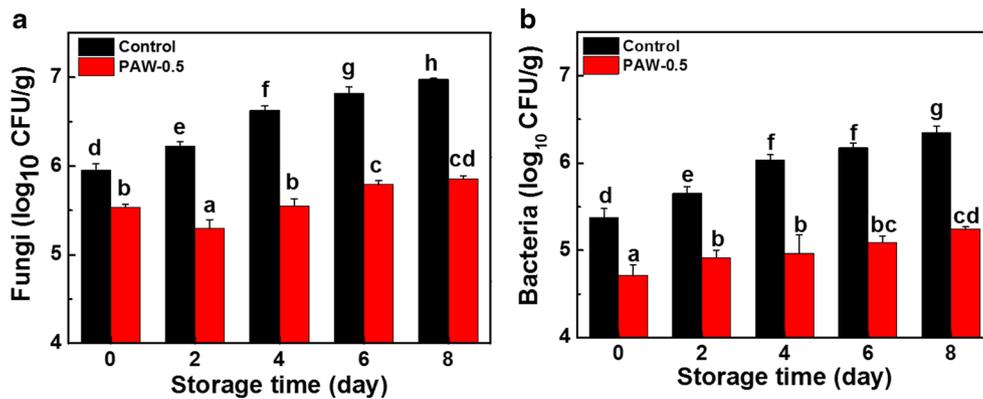
The morphological changes of microorganisms on Chinese bayberries after PAW treatment were observed with SEM. In Fig. 5b, there was a variety of normal microbial cells with smooth surface on water-treated Chinese bayberries (control). After PAW treatment, the microbial cells exhibited an irregular shape and there was much more debris on Chinese bayberries (Fig. 5d). Moreover, the cell surface of water-treated Chinese bayberries (control) appeared relatively homogeneous, without surface sculpturing (Fig. 5a), which was very similar to that of PAW-treated fruits (Fig. 5c), indicating that PAW treatment could significantly affect the morphology of microbial cells on Chinese bayberries without causing notable change in the cell surface and structure of Chinese bayberries.

### Effects of PAW on Fruit Firmness

Firmness is one of the most common physical parameters used to assess the quality of fruits. Chinese bayberry fruits softened rapidly during storage, attributed greatly to its short postharvest life and susceptibility to fungal contamination. As shown in Fig. 6, throughout the storage, the firmness loss of the control fruit was significantly greater than that of the PAW-treated fruit, indicating that PAW could effectively delay fruit softening. The similar results were also found in our previous work (Ma et al. 2015), demonstrating that PAW could maintain the firmness of strawberries during storage of 4 days. The firmness of control decreased from 2.84 to 1.51 after the 8-day storage, while the firmness of PAW-treated fruits almost kept unchanged. Especially, on the sixth and eighth day, PAW treatment had a remarkably higher firmness values than did the control ( $P < 0.001$ ), which may be mostly due to the low counts of microorganism on Chinese bayberries by PAW inactivation.

### Effects of PAW on Fruit Color

Color is also a critical quality attribute in the consumer acceptability of fresh fruits. As an attractive fruit, Chinese bayberry has wide diversity in fruit color during storage, ranging from pink to black or purple. Moreover, there is a good relationship between the CIRG values and visual perception for pink, red, and violet colors of Chinese bayberries (Zhang et al. 2005). As shown in Fig. 7, with the storage time extending, the CIRG value of the control had a slight gradual increase (from 8.2 to 9.6), while PAW treatment had a remarkable increase (from 8.2 to 12.2). Moreover, at the end of storage, the CIRG values of PAW treatment were significantly higher ( $P < 0.001$ ) than



**Fig. 4** The antimicrobial effect of PAW against **a** fungi and **b** bacteria on Chinese bayberry during storage at 3 °C for 8 days. Vertical bars represent the mean  $\pm$  standard deviation of measurements made on three replicates with five samples per replicate ( $n = 15$ ). Bars labeled

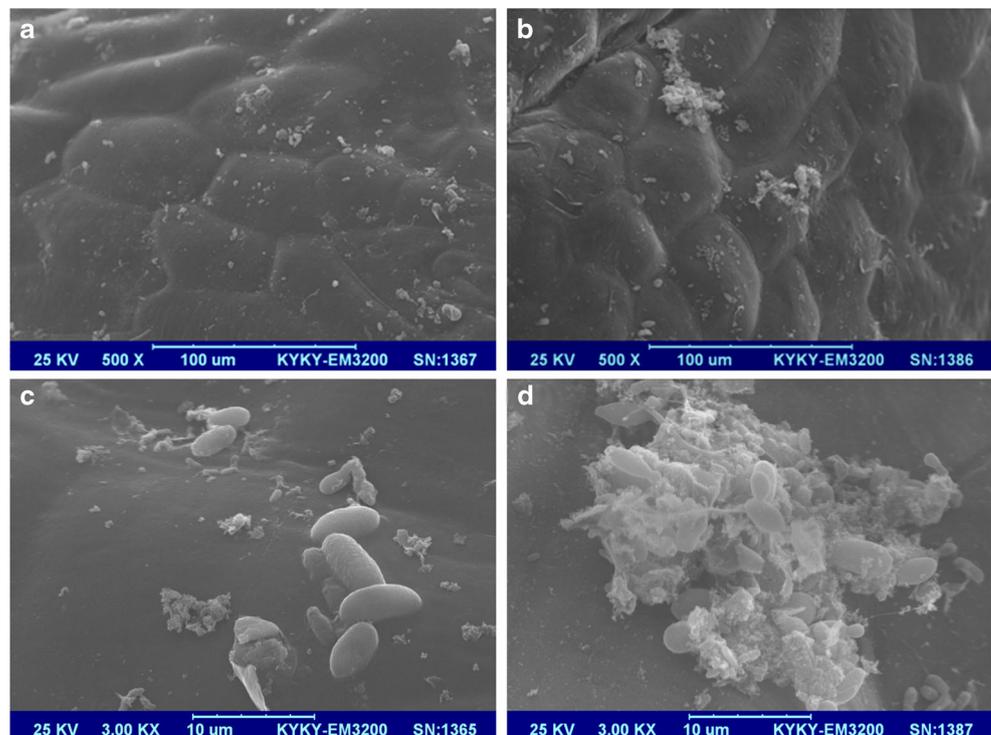
with different letters (*a–h*) across the treatments represent a significant difference in the microbial population ( $P \leq 0.05$ ) according to Duncan's multiple range test

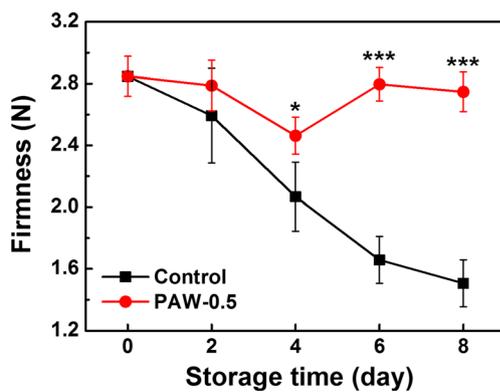
that of the control. The result indicated that PAW treatment could promote the process of color changing from red or violet to dark violet in Chinese bayberries during storage.

The color of Chinese bayberry is due to the presence of anthocyanins, which is one of the most broadly distributed water-soluble pigment groups in the plant world. Anthocyanins provide colors ranging from salmon-pink to red and violet to nearly black in a variety of plants (Abdel-Aal and Hucl 2003; Gao and Mazza 1994). Besides their food colorant roles, anthocyanins are also important antioxidants, showing a significant positive relationship with the antioxidant activity of Chinese bayberries (Bao et al. 2005).

Moreover, there is increasing evidence showing that the levels of anthocyanins in Chinese bayberries can be elevated to control the ROS level by postharvest treatment with high oxygen atmosphere, which could cause oxidative stress in Chinese bayberries through production of ROS, such as superoxide, hydrogen peroxide, and the hydroxyl radical (Yang et al. 2009). Similar to high oxygen treatment, PAW with high ORP, possessed a strong oxidizing strength, which could also generate large amounts of ROS. It is hypothesized that ROS in PAW could cause oxidative stress to Chinese bayberries. As a defense response, Chinese bayberries would enhance the antioxidant capacity through producing much more antioxidants

**Fig. 5** SEM micrographs of Chinese bayberries after water treatment (control) at magnifications of **a**  $\times 500$  and **b**  $\times 3000$ , as well as Chinese bayberries after PAW-0.5 treatment at magnifications of **c**  $\times 500$  and **d**  $\times 3000$



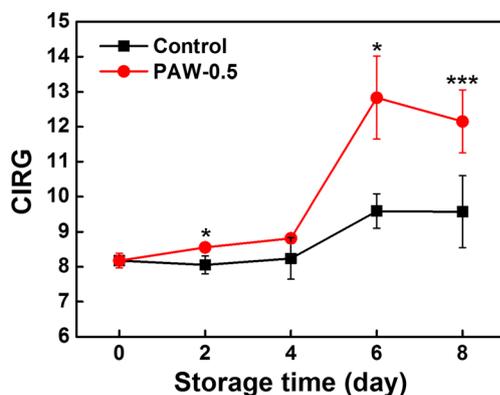


**Fig. 6** Effects of PAW treatment on firmness of Chinese bayberry during storage at 3 °C for 8 days. Values are the mean  $\pm$  standard deviation of measurements made on three replicates with five samples per replicate ( $n = 15$ ). The significant difference between PAW-treated samples and the control at the same storage time is expressed as \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  according to paired-sample  $t$  test

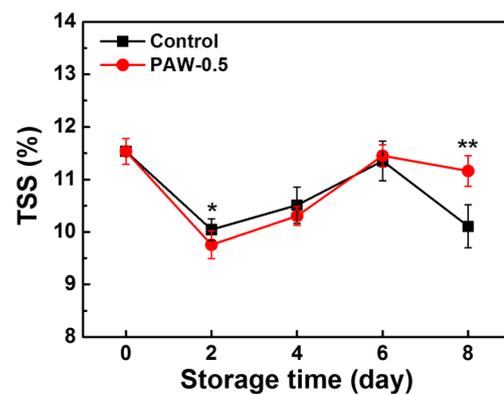
and increasing the activity of antioxidant enzymes to scavenge excessive ROS, ultimately protecting the fruits from oxidative stress. Therefore, the anthocyanin contents of Chinese bayberries could be improved by PAW, consequently leading to a higher CIRG value compared to the control. Additionally, in our previous work (Xu et al. 2016), vitamin C, also an important antioxidant, was observed to increase during the 7-day storage in the PAW-treated button mushrooms. More studies of investigating the effects of PAW on the antioxidative enzymes and antioxidants in Chinese bayberries would be conducted in future work.

### Effects of PAW on Fruit TSS

In Fig. 8, the TSS value of Chinese bayberries on day 0 was 11.54 %. After the storage of 8 days, the TSS value of the control decreased to 10.11 %, while that of the PAW-treated



**Fig. 7** Effects of PAW treatment on CIRG values of Chinese bayberry during storage at 3 °C for 8 days. Values are the mean  $\pm$  standard deviation of measurements made on three replicates with five samples per replicate ( $n = 15$ ). The significant difference between PAW-treated samples and control at the same storage time is expressed as \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  according to paired-sample  $t$  test



**Fig. 8** Effect of PAW treatment on TSS of Chinese bayberry during storage at 3 °C for 8 days. Values are the mean  $\pm$  standard deviation of measurements made on three replicates with five samples per replicate ( $n = 15$ ). The significant difference between PAW-treated samples and control at the same storage time is expressed as \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  according to paired-sample  $t$  test

fruits decreased to 11.45 %. There was a significant difference ( $P < 0.01$ ) between them. As the main substrates of respiratory metabolism, sugars and acids are consumed, causing corresponding changes in TSS of fruits during storage (Chen et al. 2013). Thus, high contents of TSS in PAW-treated fruits after the 8-day storage may be due to the inhibition of PAW treatment on the respiratory rate of Chinese bayberries, which consequently decreased the consumption of sugars and acids during storage.

### Conclusions

This study demonstrates that PAW has the potential to control fruit decay and microbial contamination as well as maintain fruit quality of postharvest Chinese bayberry during 8-day storage. All PAW treatments could decrease fruit decay by around 50 % compared to the control at the end of storage. There was no dose-effect relationship between PAW treatment time (0.5, 2, and 5 min) and fruit decay, which provides valuable guidelines for the application time of PAW to preserve fresh produces. Moreover, PAW had antimicrobial effects against natural microorganisms on Chinese bayberries due to the combined action of its high ORP and low pH, and the maximum reductions of microbial population reached around 1.1 log CFU/g both for bacteria and fungi at the end of storage. With respect to the fruit quality, PAW could maintain the firmness and TSS contents of Chinese bayberries, as well as increase the CIRG value during postharvest storage. Based on these results, PAW can be a new promising alternative of traditional sanitizer to control the fruit decay and preserve the fruit quality of postharvest Chinese berries. Future studies will focus on exploring the fresh-keeping effect of PAW on other produces, investigating PAW effect on the antioxidant ability of produces, determining nutritional and chemical

changes of food, as well as developing appropriate plasma device for a large-scale food industrial application.

**Acknowledgments** This work was supported by the Peking University Biomed-X Foundation.

## References

- Abdel-Aal, E. M., & Hucl, P. (2003). Composition and stability of anthocyanins in blue-grained wheat. *Journal of Agricultural and Food Chemistry*, *51*, 2174–2180.
- Ames, B. M., Shigena, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 7915–7922.
- Bao, J. S., Cai, Y., Sun, M., Wang, G. Y., & Corke, H. (2005). Anthocyanins, flavonols and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*, *53*, 2327–2332.
- Burlica, R., Grim, R. G., Shih, K. Y., Balkwill, D., & Locke, B. R. (2010). Bacteria inactivation using low power pulsed gliding arc discharges with water spray. *Plasma Processes and Polymers*, *7*, 640–649.
- Cantu, D., Vicente, A. R., Greve, L. C., Dewey, F. M., Bennett, A. B., Labavitch, J. M., & Powell, A. L. T. (2008). The intersection between cell wall disassembly, ripening, and fruit susceptibility to *Botrytis cinerea*. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 859–864.
- Cantu, D., Blanco-Ulate, B., Yang, L., Labavitch, J. M., Bennett, A. B., & Powell, A. T. (2009). Ripening-regulated susceptibility of tomato fruit to *Botrytis cinerea* requires NOR but not RIN or ethylene. *Plant Physiology*, *150*, 1434–1449.
- Chen, H. J., Yang, H. L., Gao, H. Y., Long, J., Tao, F., Fang, X. J., & Jiang, Y. M. (2013). Effect of hypobaric storage on quality, antioxidant enzyme and antioxidant capability of the Chinese bayberry fruits. *Chemistry Central Journal*, *7*, 1–7.
- Gao, L., & Mazza, G. (1994). Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *Journal of Food Science*, *59*, 1057–1059.
- Graves, D. B. (2012). The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology. *Journal of Physics D: Applied Physics*, *45*, 263001–263042.
- Jin, P., Wu, X., Xu, F., Wang, X. L., Wang, J., & Zheng, Y. H. (2012). Enhancing antioxidant capacity and reducing decay of Chinese bayberries by essential oils. *Journal of Agricultural and Food Chemistry*, *60*, 3769–3775.
- Kamgang-Youbi, G., Herry, J. M., Meylheuc, T., Brisset, J. L., Bellon-Fontaine, M. N., Doubla, A., & Naïtali, M. (2009). Microbial inactivation using plasma-activated water obtained by gliding electric discharges. *Letters in Applied Microbiology*, *48*, 13–18.
- Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry*, *46*, 4107–4112.
- Laroussi, M. (2005). Low temperature plasma-based sterilization: overview and state-of-the-art. *Plasma Processes and Polymers*, *2*, 391–400.
- Liao, C. H., Cooke, P. H., & Niemira, B. A. (2010). Localization, growth, and inactivation of *Salmonella Saintpaul* on Jalapeño peppers. *Journal of Food Science*, *75*, M377–M382.
- Ma, R. N., Feng, H. Q., Li, F. T., Liang, Y. D., Zhang, Q., Zhu, W. D., Zhang, J., Becker, K. H., & Fang, J. (2012). An evaluation of antioxidative protection for cells against atmospheric pressure cold plasma treatment. *Applied Physics Letters*, *100*, 123701–123704.
- Ma, R. N., Feng, H. Q., Liang, Y. D., Zhang, Q., Tian, Y., Su, B., Zhang, J., & Fang, J. (2013). Atmospheric pressure cold plasma leads to apoptosis in *Saccharomyces cerevisiae* by accumulating intracellular reactive oxygen species and calcium. *Journal of Physics D: Applied Physics*, *46*, 285401–285408.
- Ma, R. N., Wang, G. M., Tian, Y., Wang, K. L., Zhang, J., & Fang, J. (2015). Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. *Journal of Hazardous Materials*, *300*, 643–651.
- Maeda, Y., Igura, N., Shimoda, M., & Hayakawa, I. (2003). Bactericidal effect of atmospheric gas plasma on *Escherichia coli* K12. *International Journal of Food Science and Technology*, *38*, 889–892.
- Moreau, M., Orange, N., & Feuilleley, M. G. J. (2008). Non-thermal plasma technologies: new tools for bio-decontamination. *Biotechnology Advances*, *26*, 610–617.
- Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, *3*, 125–142.
- Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., & Weltmann, K. D. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, *7*, 250–257.
- Pankaja, S. K., Bueno-Ferrera, C., Misraa, N. N., Milosavljevi, V., O'Donnellb, C. P., Bourkea, P., Keenera, K. M., & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science and Technology*, *35*, 5–17.
- Selcuk, M., Oksuz, L., & Basaran, P. (2008). Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillium* spp. by cold plasma treatment. *Bioresource Technology*, *99*, 5104–5109.
- Tian, Y., Ma, R. N., Zhang, Q., Feng, H. Q., Liang, Y. D., Zhang, J., & Fang, J. (2015). Assessment of the physicochemical properties and biological effects of water activated by non-thermal plasma above and beneath the water surface. *Plasma Processes and Polymers*, *12*, 439–449.
- Wan, J., Coventry, J., Swiergon, P., Sanguansri, P., & Versteeg, C. (2009). Advances in innovative processing technologies for microbial inactivation and enhancement of food safety—pulsed electric field and low-temperature plasma. *Trends in Food Science and Technology*, *20*, 414–424.
- Wang, K. T., Jin, P., Cao, S. F., Shang, H. T., Yang, Z. F., & Zheng, Y. H. (2009). Methyl jasmonate reduces decay and enhances antioxidant capacity in Chinese bayberries. *Journal of Agricultural and Food Chemistry*, *57*, 5809–5815.
- Wang, K. T., Cao, S. F., Jin, P., Rui, H. J., & Zheng, Y. H. (2010a). Effect of hot air treatment on postharvest mold decay in Chinese bayberry fruit and the possible mechanisms. *International Journal of Food Microbiology*, *141*, 11–16.
- Wang, K. T., Jin, P., Shang, H. T., & Zheng, Y. H. (2010b). Effect of methyl jasmonate in combination with ethanol treatment on postharvest decay and antioxidant capacity in Chinese bayberries. *Journal of Agricultural and Food Chemistry*, *58*, 9597–9604.
- Wang, K. T., Jin, P., Tang, S. S., Shang, H. T., Rui, H. J., Di, H. T., Cai, Y., & Zheng, Y. H. (2011). Improved control of postharvest decay in Chinese bayberries by a combination treatment of ethanol vapor with hot air. *Food Control*, *22*, 82–87.
- Xu, Y. Y., Tian, Y., Ma, R. N., Liu, Q. H., & Zhang, J. (2016). Effect of plasma activated water on the postharvest quality of button mushrooms. *Agaricus bisporus*. *Food Chem.*, *197*, 436–444.
- Yang, Z. F., Zheng, Y. H., & Cao, S. F. (2009). Effect of high oxygen atmosphere storage on quality, antioxidant enzymes, and DPPH-radical scavenging activity of Chinese bayberry fruit. *Journal of Agricultural and Food Chemistry*, *57*, 176–181.
- Yu, S., Chen, Q., Liu, J., Wang, K., Sun, S., Jiang, Z., Zhang, J., & Fang, J. (2015). Dielectric barrier structure with hollow electrodes and its recoil effect. *Applied Physics Letters*, *106*, 244101–244104.
- Zhang, W. S., Chen, K. S., Zhang, B., Sun, C. D., Cai, C., Zhou, C. H., Xua, W. P., Zhang, W. Q., & Ferguson, I. B. (2005). Postharvest responses of Chinese bayberry fruit. *Postharvest Biology and Technology*, *37*, 241–251.

- Zhang, W. S., Li, X., Wang, X. X., Wang, G. Y., Zheng, J. T., Abeysinghe, D. C., Ferguson, I. B., & Chen, K. S. (2007). Ethanol vapour treatment alleviates postharvest decay and maintains fruit quality in Chinese bayberry. *Postharvest Biology and Technology*, *46*, 195–198.
- Zhang, Q., Liang, Y. D., Feng, H. Q., Ma, R. N., Tian, Y., Zhang, J., & Fang, J. (2013). A study of oxidative stress induced by non-thermal plasma-activated water for bacterial damage. *Applied Physics Letters*, *102*, 203701–203704.
- Zhang, Q., Ma, R. N., Tian, Y., Su, B., Wang, K. L., Yu, S., Zhang, J., & Fang, J. (2016). Sterilization efficiency of a novel electrochemical disinfectant against *Staphylococcus aureus*. *Environmental Science & Technology*, *50*, 3184–3192.
- Zheng, Y. H., Yang, Z. F., & Chen, X. H. (2008). Effect of high oxygen atmospheres on fruit decay and quality in Chinese bayberries, strawberries and blueberries. *Food Control*, *19*, 470–474.