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ORIGINAL PAPER

A Novel Approach to Enhance Blueberry Quality During Storage Using Cold Plasma at Atmospheric Air Pressure

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Abstract

Blueberry is an important health food, as it contains vitamins, anthocyanins, and antioxidative enzymes. However, post-harvest life of this fruit is very short, and its quality (in terms of microbial growth, antioxidant value, and decay rate) deteriorates rapidly during storage. In this work, air cold plasma at atmospheric pressure was evaluated as a pre-treatment approach for prolonging the shelf life and improving the quality of blueberries. After plasma treatment for 10 min, the number of bacteria and fungi decreased by 93.0% and 25.8%, respectively, which might be due to the increases in DNA damage and guanine oxidation. Accordingly, the blueberry decay rates reduced by 17.7%, 14.3%, and 5.2% in the plasma treatment groups of 6, 8, and 10 min, respectively, after 20 days of storage. Interestingly, the contents of sugar, vitamin C, and total anthocyanin as well as the superoxide dismutase activity level showed the maximum increases of 1.5-fold, 1.5-fold, 2.2-fold, and 79.3%, respectively, following different plasma discharge treatments and storage times compared with those in the control groups. Furthermore, in the treated samples, the degradation times for these four parameters to decrease to control levels were longer compared to the samples without treatment. These results indicated that air cold plasma at atmospheric pressure has excellent potential as a method for enhancing the quality and shelf life of fresh produce in the food industry.

Keywords Cold plasma · Blueberry · Enhanced quality · Microbial inactivation · Increased antioxidant value

Introduction

Blueberry (*Vaccinium* spp.), also named "gold berry," has been categorized as one of five major health foods by the United Nations Food and Agriculture Organization (FAO) due to its high nutritional and medicinal value (Wang et al. 2017). The important nutrient components of blueberries include vitamins, anthocyanins, and antioxidative enzymes. However, the post-harvest life of this fruit is very short at ambient temperatures because of microbial-induced decay and mechanical damage, along with moisture and nutrient loss. These problems limit the geographical access to blueberry markets and the quantity of sales.

Various methods have been applied to manage post-harvest decay and prolong shelf life of fresh blueberries in the food industry. Low temperature is used to inhibit the growth of microorganisms, activity of enzymes, and purely chemical reactions in the commercial blueberry production industry (Erkmen and Bozoglu 2016). Gamma irradiation treatments at certain doses are able to weaken the respiration intensity, lower the ethylene production, and increase the activities of lipoxygenase (LOX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Chen et al. 2017). Coating technology has been demonstrated to efficiently prevent yeast and mold growth, and improve the firmness, antiradical activity, color, aroma, and texture of blueberries (Mannozzi et al. 2018). However, there are still several drawbacks in the different treatment methods. Low-temperature storage requires costly equipment and cannot efficiently provide rapid, uniform, and stable cooling (Ambaw et al. 2016), thereby affecting the post-harvest quality of fruits. Irradiation technology has the problem of dose limit. At a high dose, irradiation may cause the loss of some micronutrients (Amit et al. 2017). The films in the coating method for preservation present some problems, such as instability, easy degradation, and

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high hydrophilic character (Olivas and Barbosacanovas 2009). Therefore, the development of an innovative approach for enhancing food safety and quality, without compromising the nutritional value or characteristics of blueberries, would provide significant economic and nutritional advantages for the food industry.

Cold plasma at atmospheric pressure is a promising and green processing technology with potential applications in many industries. The most appealing property of cold plasma is the high efficiency of microbial inactivation, thus making this technology ideal for applications in the food industry (Bourke et al. 2018; Ekezie et al. 2017; Mir et al. 2016). Originally, cold plasma was applied as a powerful tool for surface decontamination of foodstuffs and food packaging materials (Dasan et al. 2017; Sen and Mutlu 2013) and to improve the functional properties of food packaging materials (Dong et al. 2017a; Mir et al. 2016; Muhammad et al. 2018). Subsequently, atmospheric cold plasma treatment has demonstrated a significant antimicrobial efficacy on fresh produce such as various fruits, fruit juices, nectars, meats, and vegetables, which were incubated with various kinds of exogenetic microbes (Dasan and Boyaci 2018; Segura-Ponce et al. 2018; Ziuzina et al. 2014) or from which the spoilage bacteria were isolated (Wang et al. 2016). Lacombe et al. (2015) have reported that atmospheric air cold plasma jet directly and effectively killed the native yeast and mold on fresh blueberries. Dielectric barrier discharge (DBD) air plasma has also been used to efficiently inactivate the background microflora (aerobic mesophilic bacteria, yeast, and mold) on strawberries that were contained in a polypropylene (PP) package and placed between two circular aluminum plate electrodes (Misra et al. 2014b). It has not been reported that the DBD air plasma was directly used to kill microbes on blueberries. Inactivation mechanisms of microbes by atmospheric cold plasma have been investigated at the levels of membrane, protein, and/or DNA (Dasan et al. 2017; Dezest et al. 2017; Joshi et al. 2011; Sen and Mutlu 2013; Xu et al. 2018). However, the exact mechanism of inactivation still needs to be further explored.

To promote the commercial application of atmospheric cold plasma as a sustainable and safe processing technology, it is essential to investigate the effects of plasma treatment on the quality of fresh produce and the interactions between plasma and nutrients in food. Most of the previous studies indicated that product quality parameters, such as color, vitamin C, polyphenols, and anthocyanin, showed no significant changes after cold plasma sterilization (Mandal et al. 2018; Misra et al. 2014b; Pankaj et al. 2018; Puligundla et al. 2017), or even had some negative effects (Pankaj et al. 2018). Reduction in ascorbic acid (vitamin C) content was observed in fresh produce treated by cold plasma (Mandal et al. 2018; Rodriguez et al. 2017; Xu et al. 2017). Decreased anthocyanin content has been also reported in the treated blueberries in the study about evaluating the efficacy of microbial inactivation and agrochemical degradation on blueberries after cold plasma treatment, including plasma jet and DBD treatment (Lacombe et al. 2015; Sarangapani et al. 2017). Muhammad et al. have explored the influence of nonthermal plasma on food microstructure and found that the nonthermal plasma promoted oxidation, depolymerization, cross-linking, addition, ablation, or/and abstraction of functionality in the molecular chains of starch, protein, and lipid. These changes resulted in the alterations of their rheological behaviors, while there was little or no alteration in food quality (Muhammad et al. 2018). Very few studies have investigated the possibility of applying cold plasma to enhance the quality of liquid foods. After cold plasma treatment of fruit juice, it was found that sucrose content increased while glucose and fructose contents decreased (Rodriguez et al. 2017), ascorbic acid (Sarangapani et al. 2017) and anthocyanin (Kovacevic et al. 2016) showed increased contents, and no changes were observed in vitamin C content (Paixao et al. 2018). Until now, it has not been reported whether the nutrient contents (total sugar, vitamin C, and total anthocyanin) and antioxidant enzyme activity (SOD) in blueberry fruits could be improved by atmospheric air DBD plasma enhancement treatment under certain discharge parameters.

The objective of the current study was to investigate the possible use of air DBD plasma at atmospheric pressure for enhancing the quality and shelf life of blueberries. The efficacy of cold plasma treatment in the reduction of potentially spoiling microorganisms on the surface of blueberries was evaluated along with the potential mechanism of microbial inactivation at the level of DNA. Additionally, the effects of plasma treatment on nutritional components of blueberries, including sugar, vitamin C, and total anthocyanin content as well as SOD activity, were analyzed. The results from this study could provide useful information concerning the possible use of pre-treatment with air cold plasma at atmospheric pressure to improve blueberry quality. Moreover, blueberry samples were directly exposed in an organic glass reaction chamber with a DBD plasma device in this study, which is different from the earlier reports in which the samples were placed between two electrodes of plasma discharge (Misra et al. 2014a, b; Ziuzina et al. 2014). The results of this work suggest the feasibility of using cold plasma enhancement technology in warehouse storage of fresh produce.

Materials and Methods

Fruit

Bluecrop fruits of high-bush blueberry were plucked from a blueberry plantation in Jinpu New Area of Dalian, China, and used immediately in this study. The blueberries were screened for uniformity in size, color, and maturity. Defective fruits

(crushed, cracked, or immature) were excluded. The selected blueberries were randomly separated into six groups of 1050 blueberries each for further use, and each group was further divided into three groups of 350 each for testing in triplicate.

Sample Preparations of Plasmid DNA and Guanine

Plasmid DNA of pUC19 (2686 base pairs, Takara Bio Inc., Japan) was diluted in Tris-EDTA (TE) buffer to a final concentration of 0.25 mg mL^{-1}. Guanine (Sigma-Aldrich Inc., Germany) was dissolved in TE buffer for a final concentration of 0.50 mg mL^{-1}. The samples (40 μL for plasmid DNA or 500 μL for guanine) were deposited onto a cleaned glass slide (that had been soaked in 2% HCl solution for 12 h, then soaked in water for 12 h, and finally washed with water several times) and exposed to plasma discharge, as outlined below. All exposed samples were re-collected with the micropipette and the exposed guanine sample solutions were diluted to a final volume of 1 mL with TE buffer for further analyses.

DBD Plasma Treatment

The air DBD plasma discharge system at atmospheric pressure (Nanjing Su Man Electronics Co., Ltd., China) for sample treatment was set up as illustrated in Fig. 1. The apparatus consisted of a power supply (CTP-2000 K, Nanjing Su Man Electronics Co., Ltd., China) with a generator, an amplifier, and a reactor chamber. The reactor chamber consisted of a pair of circular aluminum plate electrodes covered with quartz glass. There was a 3-mm discharge gap between the upper electrode and the lower electrode. The latter was connected to the ground by an aluminum base.

The weighed fruits within each group were placed in four glass culture plates of 9-cm diameter and then placed in the reactor chamber. The plasma discharge was generated at input voltage of 36 V and current of 1.8 A. The exposure times were set at 0, 2, 4, 6, 8, and 10 min. Untreated fruits were used as the control sample. Samples from different groups were placed respectively in preservative boxes with breathable holes for 0, 4, 8, 12, 16, or 20 days in the laboratory at 25 °C with a relative humidity of 50%. In Chinese supermarkets, fresh blueberries are normally sold at room temperature. Therefore, room temperature storage was directly evaluated after plasma treatment in this paper. After the storage period at room temperature, the samples from different storage days were collected and then stored at − 20 °C until further analyses.

The sample suspensions of DNA and guanine were treated by DBD plasma as previously described (Dong et al. 2017b). To avoid the direct and rapid degradation of samples after exposure to cold plasma at input voltage of 36 V and current of 1.8 A, plasma discharge was generated at lower input voltage of 26 V and current of 1.1 A, and exposure times were set at 0, 15, 30, 45, 60, 75, 90, 105, and 120 s for plasmid samples, whereas guanine samples were exposed for 0, 60, and 120 s. Untreated samples were used as controls.

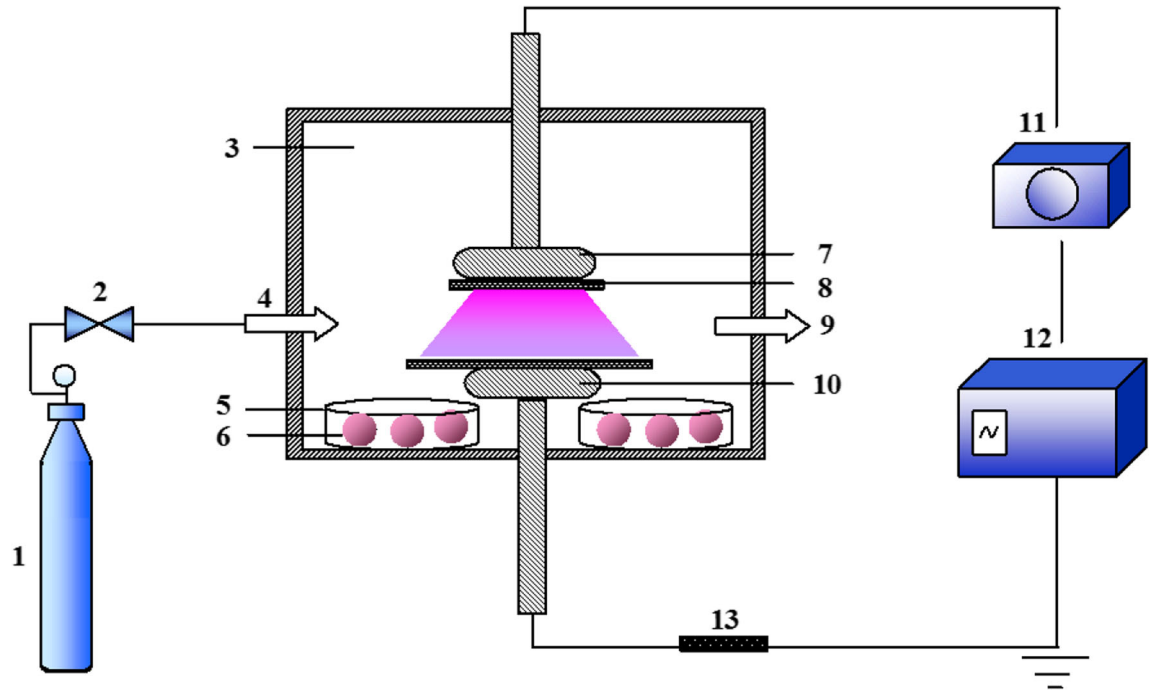

Fig. 1 Schematic diagram illustrating the apparatus for air DBD plasma discharge at atmospheric pressure. 1, air cylinder; 2, mass flow controller; 3, organic glass reaction chamber; 4, gas inlet; 5, glass plate; 6, blueberry sample; 7, upper electrode; 8, quartz dielectric; 9, gas exhaust; 10, bottom electrode; 11, power amplifiers; 12, power generator; 13, resistor

The Numbers of Bacteria and Fungi

Beef extract peptone agar medium (HuaYueYang Biotechnology, Co., Ltd., Beijing, China) was used for bacterial growth. Potato dextrose agar medium (Beijing Solarbio Science & Technology Co., Ltd., China) was used for yeast and mold growth.

Three blueberries were randomly selected from each group and then weighed. The three-blueberry sample from each group was added to 10 mL aseptic physiological saline (0.85% NaCl) and the mixture was shaken for 30 min. Tenfold serial dilutions were prepared, and 100 μL of each diluted solution was spread in triplicate onto plate agar medium and incubated for 24 h at 37 °C. Flat colony counting method was used to count the microbial number. The bacteria and fungi counts were ultimately interpreted as the number of colony-forming units (CFU) per gram of fresh fruit weight ($\log_{10}$ CFU $g^{-1}{}_{FW}$).

DNA Damage and Guanine Structure Analyses

The DNA damage induced by plasma exposure was determined qualitatively and quantitatively by electrophoresis technique using a gel set (LiuYi Biotechnology Co., Ltd., Beijing, China). The samples were electrophoresed on 1% agarose gel, pre-stained with a fluorescent dye (Goldview Nucleic Acid Gel Stain, 10,000 × concentration in DMSO, YEASEN Co., Ltd., China). The power source of electrophoresis kit was set at 100 V in 1 × TAE running buffer. After the electrophoresis, fluorescent bands on the gel corresponding to three different conformations (undamaged supercoiled DNA, open circular form containing a single-stand break, and full-length linear DNA in which double-stand breaks occurred) were visualized by a gel imager (Bio-Rad Laboratories, Inc., America) upon UV light exposure. The gel images were analyzed, and the intensity of each band was measured (Quantity One, 1-D Analysis Software, Bio-Rad Laboratories, Inc., USA) to determine the amount of each form of DNA present in the sample. The ratio of fluorescence intensity of each band or DNA conformation to the total fluorescence within each irradiated or control sample was measured and averaged from three independent replicates that were treated under the same conditions. The plasma treatment–induced damage level was expressed as a fraction of the total plasmid DNA in the solution.

Two controls included in the experiment were an untreated sample in which the DNA was loaded directly into the gel, and an air-exposed control in which the DNA sample was placed onto a cover slip of glass and exposed to air for 120 s prior to loading on the gel.

Fourier-transform infrared spectroscopy in attenuated total reflectance mode (ATR-FTIR) for the characterization of guanine was conducted using an FTIR reflectance spectrophotometer (Bruker Daltonics Inc., USA) over a range of 4000–1000 cm^{-1} at a resolution of 4 cm^{-1} in the transmission mode.

Fruit Decay Incidence

The fruits were assessed for symptoms of any visible rot, fungal growth, or/and bacterial lesions on the surface, and the amounts of decay were recorded after 0, 4, 8, 12, 16, and 20 days of storage at 25 °C. Decay rate was expressed as a percentage according to Eq. 1:

$$\text{Decay rate}\ (\%) = \frac{\text{number of decayed fruits}}{\text{total number of fruits}} \times 100 \qquad (1)$$

Measurement of Sugar and Vitamin C

Frozen blueberries were peeled and weighed before homogenization in 20 mL of 2% oxalic acid solution, followed by clarification by centrifugation at 10,000×*g* for 20 min. The supernatant was collected and used to determine the amount of reducing sugar and vitamin C content in each sample.

The reducing sugar content was measured using the 3,5-dinitrosalicylic acid colorimetric assay (Wood et al. 2012). DNS reagent containing 3,5-dinitrosalicylic acid (10 g L^{-1}), sodium potassium tartrate (30 g L^{-1}), and NaOH (16 g L^{-1}) was stored in darkness at room temperature. Each reaction mixture contained 35 μL of the above supernatant, 3 mL DNS solution, and 1.965 mL Milli-Q water. The glass tubes were sealed with parafilm (Bemis, USA). The resulting solutions were heated in a thermocycler (HerryTech, China) at 100 °C for 5 min and quantified at 540 nm with UV-visible spectrophotometer (UV 2102C, Unico, USA). The reducing sugar content was calculated by D-glucose calibration curves. The results were expressed as gram of reducing sugar per kilogram of fresh fruit weight (g $kg^{-1}{}_{FW}$).

The vitamin C content was determined by the 2,6-dichloroindophenol titrimetric method (Yang et al. 2010). Briefly, 0.3 mL of the above supernatant was mixed with 20 mL of a solution of glacial acetic acid (3%) and metaphosphoric acid (8.0%). The mixture was then titrated with 2,6-dichloroindophenol solution (0.025%) until the solution was pink for 10 s. The ascorbic acid content of sample was calculated on the basis of the standard curve of ascorbic acid and expressed as milligram of ascorbic acid per kilogram of fresh fruit weight (mg $kg^{-1}{}_{FW}$).

Total Anthocyanin Content

The determination of total anthocyanin content was performed as previously described (Li and Wu 2013). Briefly, the weight

of fruits in each group was weighed. Extraction was performed by treatment for 24 h in the dark with 30 mL of 80:20 ($v\ v^{-1}$) methanol:water solution containing 0.1 mL L^{-1} acetic acid. After centrifugation at 10,000×g for 30 min at room temperature, the supernatant volume was measured and total anthocyanin content was determined using a pH differential method. Each extracted sample was diluted in either 0.025 M potassium chloride buffer (pH 1.0) or 0.4 M sodium acetate buffer (pH 4.5) using a dilution factor of 6. The absorbance of each solution was measured at 510 nm and 700 nm, and the absorbance value (A) of the diluted sample was calculated according to the following Eq. 2:

$$A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \quad (2)$$

The monomeric anthocyanin pigment concentration in the original sample was expressed in equivalents of cyanidin-3-glucoside according to Eq. 3:

$$\text{Anthocyanin concentration}\left(\text{mg L}^{-1}\right) = (A \times MW \times DF \times 1000)\ \varepsilon \times 1 \quad (3)$$

where MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (6), and ε is the molar absorptivity for cyanidin-3-glucoside (26,900 $M^{-1}{\cdot}cm^{-1}$). The results were expressed as gram of total anthocyanin per kilogram of fresh fruit weight (g $kg^{-1}{}_{FW}$).

Assay of Superoxide Dismutase Activity

Superoxide dismutase (SOD) was extracted and assayed as previously described (Zhou et al. 2014). Briefly, the blueberries were weighed and then extracted for 10 min using 2.5 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 0.1% ($w\ v^{-1}$) polyvinylpyrrolidone at 4 °C. The protein concentration of the extract solution was measured using the Bradford assay (Bradford 1976) and a bovine serum albumin dilution series was used to generate a standard curve. The extract solution was centrifuged for 30 min at 12,000×g, and the supernatant was collected for determination of SOD activity using a SOD assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radical. The results were expressed as unit of SOD activity per gram of fresh fruit weight (U $g^{-1}{}_{FW}$).

Statistical Analysis

Each experiment was performed in triplicate, and one-factor analysis of variance (one-way ANOVA) test (Origin 7.0) was applied to evaluate whether the difference between plasma-treated samples and the controls was statistically significant (Dong et al., 2017b). Statistical significance was considered at the level of $^{a}p < 0.05$, $^{b}p < 0.01$, or $^{c}p < 0.001$.

Results and Discussion

Effect of Cold Plasma on the Amount of Bacteria and Fungi on Blueberries

Microbial growth is one of the main causes of decay during the storage of blueberries. Thus, the inactivation or removal of microbes is an important method of fruit preservation. We therefore tested the effect of cold plasma treatment on the total number of native bacteria and fungi (yeast and mold) populations on the surface of fresh blueberries. For the untreated samples (0 min), the bacteria count was 2.16 $\log_{10}$ CFU $g^{-1}{}_{FW}$ and the fungi count was 2.25 $\log_{10}$ CFU $g^{-1}{}_{FW}$ (Fig. 2a). After 10 min of treatment, the total bacteria count showed a decrease of 2.01 $\log_{10}$ CFU $g^{-1}{}_{FW}$ and the fungi count decreased by 0.58 $\log_{10}$ CFU $g^{-1}{}_{FW}$. These results indicated that air DBD plasma at atmospheric pressure as a post-harvest pretreatment method possessed the ability of rapidly killing microorganisms on the surface of blueberries.

Although the results of this work and previous work (Akan et al. 2006; Mastanaiah et al. 2013) demonstrated that cold plasma effectively killed both bacteria and fungi, the treatment was less effective in inhibiting fungal growth compared to bacterial growth. This may be due to the fact that the cell walls of fungi are much thicker and more complex than those of bacteria, thus making fungi more difficult to inactivate than bacteria (Lee et al. 2006; Korachi et al. 2009). In addition, the spores generated by the fungi possess unique protective structures, such as their spore coats, inner membrane, and the small acid-soluble proteins (SASP). Thus, they are more resistant than the vegetative cells against various physical and chemical agents (Ekezie et al. 2017; Puligundla and Mok 2018; Tseng et al. 2012). This may also enhance fungi resistance to plasma inactivation.

Effect of Cold Plasma on Characteristics of Plasmid DNA and Guanine

Damage of genetic materials is one of the causes of microbial death. Therefore, structural forms of plasmid DNA and guanine of DNA base following DBD plasma treatment were analyzed. Initially, the untreated plasmid DNA was predominantly supercoiled, but following plasma treatment, the formations of single- and double-strand breaks led to opened circular DNA and linear DNA, respectively (Fig. 3a). As treatment time increased, the relative concentration of supercoiled DNA decreased, and the relative concentrations of broken forms increased (Fig. 3b). All control samples exhibited low levels of DNA damage (less than 5%). Comparison of the untreated

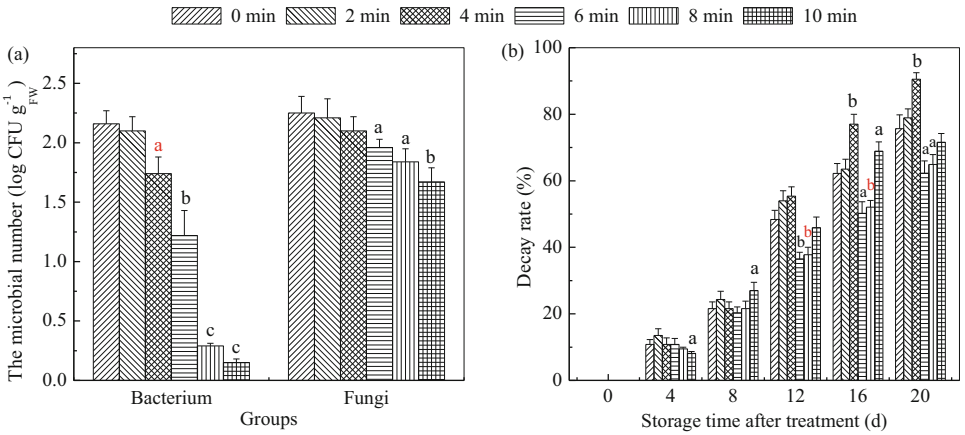

Fig. 2 (**a**) Effect of cold plasma exposure on microbial number of blueberries. (**b**) Effect of cold plasma exposure on decay rate of blueberries. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$. No decay was observed on the day of plasma treatment (storage day 0). Vertical bars represent the standard errors of the means ($n = 3$)

control sample with the air-exposed control sample was used to determine the level of damage induced by air exposure. Minimal damage was observed in these two control samples, which allowed us to attribute plasma as the main contributor to the observed DNA damage. These results indicated that DNA damage occurred following plasma treatment and that this damage was correlated with plasma exposure time, which was consistent with data reported by Han et al. (2014).

To assess whether air cold plasma treatment at atmospheric pressure would chemically alter the base structure in DNA, the influence of plasma exposure on the FTIR spectral characteristics of guanine was explored, as illustrated in Fig. 3c. The spectral bands were assigned based on previous data (Colarusso et al. 1997; Wang et al. 2001). The following characteristic absorption peaks were related to the chemical structure of guanine: an N–H stretching vibration peak at 3238.25

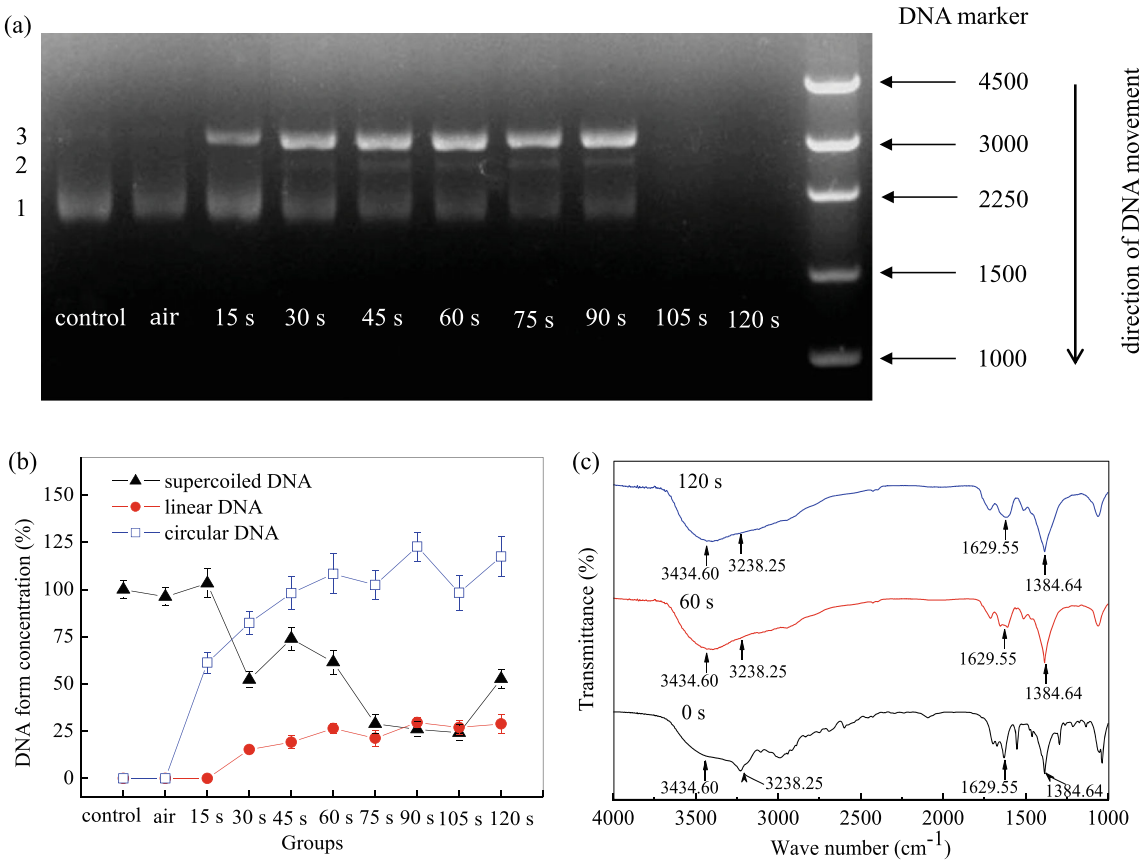

Fig. 3 (**a**) Plasmid DNA conformation analysis following DBD plasma exposure. A representative agarose gel electrophoresis image of the control and plasma-exposed DNA samples is shown. 1, supercoiled plasmid DNA; 2, linear plasmid DNA; 3, circular plasmid DNA. (**b**) Relative quantification of DNA conformations following plasma treatment. (**c**) Effects of cold plasma exposure on guanine structure. ATR-FTIR spectra of guanine over the range of 4000–1000 cm^{-1}. The experiment was repeated, so a representative spectrum is shown

cm^{-1}, a C=O stretching vibration peak at 1629.55 cm^{-1}, and a C–H deformation vibration peak at 1384.64 cm^{-1} (Colarusso et al. 1997; Wang et al. 2001). After plasma treatment, the N–H stretching vibration peak at 3238.25 cm^{-1} disappeared, the C=O stretching vibration peak at 1629.55 cm^{-1} decreased, and the C–H deformation vibration peak at 1384.64 cm^{-1} increased. A new absorption peak at 3434.60 cm^{-1} was assigned to the O–H stretching vibration peak. These results indicated that air cold plasma treatment at atmospheric pressure chemically altered guanine structure. Namely, cold plasma damaged C=O bond and degraded N–H bond, while it promoted C–H bond formation and generation of new O–H bond.

The new O–H bond formed by treatment of cold plasma was likely due to oxidation of guanine by interaction with ROS. Primary and secondary reactive species, such as hydroxyl radicals (·OH) and peroxynitrite (NO_3^-), are generated in gaseous phase, gaseous-aqueous interface, and aqueous phase of plasma (Dong et al. 2016; Lukes et al. 2014). Guanine is the most easily oxidized component of DNA, and it readily interacts with ·OH or $OONO^-$. Consequently, 8-hydroxy-7,8-dihydro-7-yl radical (8-OHdG) is formed by the initial ·OH addition at the C8 of guanine (Fig. 4a, Cadet et al. 1997; Guo et al. 2016). Moreover, the adduct 5-hydroxy-4-nitrosoxy-4,5-dihydro-2′-deoxyguanosine (73) may be produced by the homolytic addition reaction of NO_3^- on the purine ring of 2′-deoxyguanosine (dGuo, 42) (Fig. 4b, Cadet et al. 1997). The above two reactions resulted in the new OH stretching vibrational peak (3434.60 cm^{-1}) and the increased C–H deformation vibration peak (1384.64 cm^{-1}), as observed in the ATR-FTIR spectra after plasma exposure (Fig. 3c). Correspondingly, the intact supercoiled structure of plasmid DNA could relax into a circular shape or into a linear shape owing to the damage of guanine (Cadet et al. 1997; Guo et al. 2016) as shown in Fig. 4c.

Effect of Cold Plasma on Decay Rate of Blueberries

Spoilage could be generated by microorganisms on the surface of blueberries, which directly influences the quality of the fruit. Thus, the decay rate is used as an important standard to

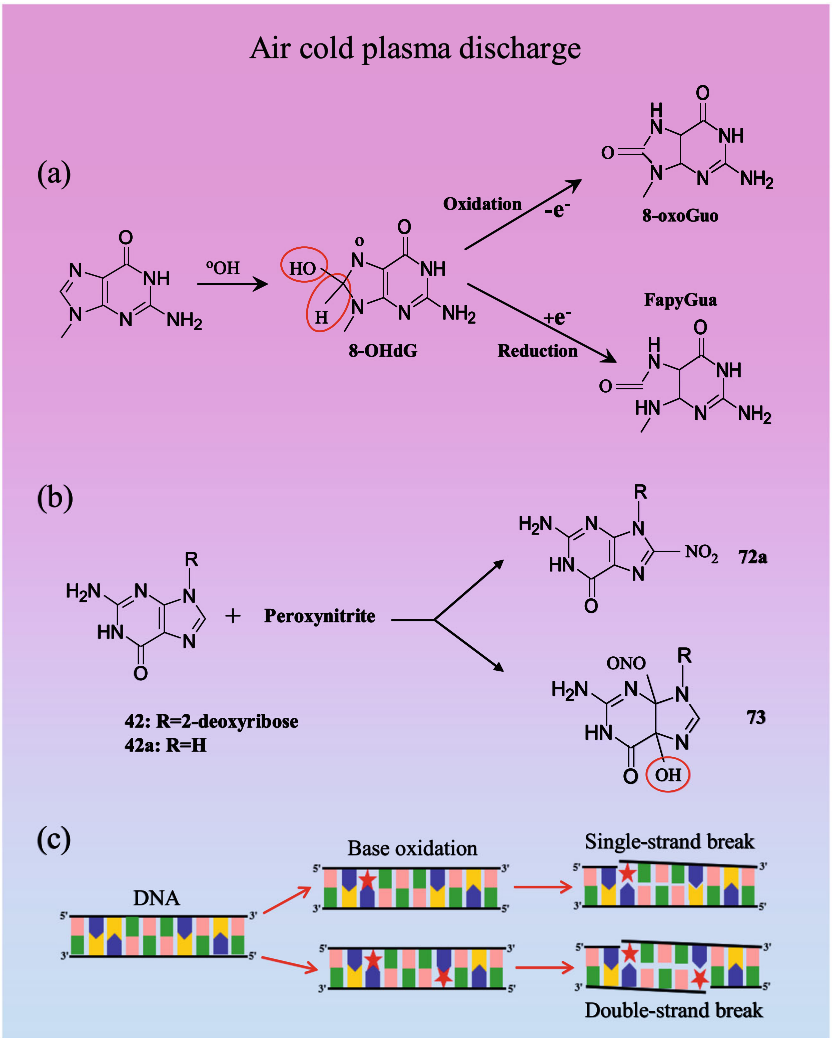

Fig. 4 (**a**) Reaction of guanine with OH· radicals. (**b**) Reaction of guanine with peroxynitrite (NO_3^-). 42a: guanine, 42: 2′-deoxyguanosine, 72a: 8-nitroguanine, 73: 5-hydroxy-4-nitosoxy-4,5-dihydro-2′-deoxyguanosine (Cadet et al. 1997). (**c**) Schematic diagram of DNA strand breaks. The newly formed bands of O–H and C–H are marked with red circles

measure the effects of fruit preservation treatments. As shown in Fig. 2a, microbial number on the blueberries could be reduced by cold plasma exposure. However, it is not certain whether the spoilage of blueberries could be inhibited due to the decrease in microbial amount after cold plasma exposure. To test the effect of cold plasma treatment on blueberry decay, the blueberries were treated by cold plasma for different times and then stored at 25 °C for up to 20 days. Figure 2b illustrates the fruit decay rate at 25 °C during the 20-day storage period following treatment. Decay was first observed after 4 days of storage, and the decay rate decreased with treatment time for each treatment (Fig. 2b). The decay rates for the 6-min and 8-min treated groups were lower than those of the control groups after 12 to 20 days of storage. These results indicated that DBD plasma decreased the decay rate. Additionally, these results demonstrated that the decay could be inhibited to a greater extent under certain discharge parameters. Damages to DNA and guanine would be expected to inhibit microbial survival, finally decreasing the fruit decay rate. This might extend the shelf life of blueberries.

Effect of Cold Plasma on Sugar Content of Blueberries

Sugar content in blueberries influences not only the sweetness but also the synthesis of other metabolites such as acids, carotenoids, aromatic substances and other nutrient components (Shi et al. 2016). To test the effect of cold plasma treatment on sugar content, the changes of reducing sugar content in blueberries were investigated after plasma treatment and during storage. As illustrated in Fig. 5a, the sugar content of blueberry fruits in all groups increased at first and then decreased during the storage period. At the original storage period (0 day), the sugar concentrations of 6- to 10-min treatment groups were 51.6%, 82.2%, and 61.5% higher compared to that of the control group, respectively. While the sugar content of the control reached a maximum on the fourth day of storage and then decreased, the sugar contents in the groups treated for 6–10 min exhibited a slow increase before they decreased. The group treated with plasma for 8 min showed the maximum sugar content (54.7 g kg^{-1}_{FW}) on the 12th day after plasma treatment. These results indicated that treatment with air DBD plasma at atmospheric pressure increased the sugar content and delayed the onset of the sugar content decrease during storage at ambient temperature. The time at which the sugar content began to decrease was correlated with plasma treatment time. These results are consistent with a previous study on Chinese bayberry fruit (Shi et al. 2016).

Stress response exists in all biological systems. Air cold plasma, as an environment stress, might induce stress response of bio-systems. At the original storage period (0 day), the sugar concentrations of 6- to 10-min groups were evidently increased after plasma treatment. In the flesh of berries,

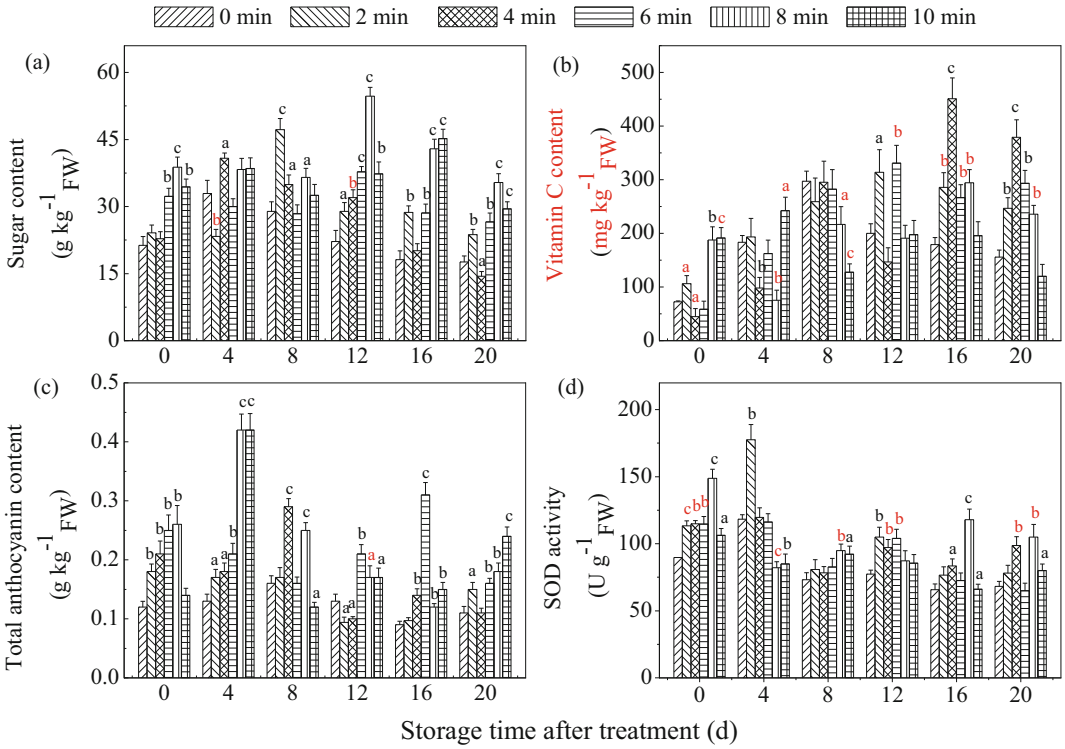

Fig. 5 (**a**) Effect of cold plasma exposure on sugar content in blueberries. (**b**) Effect of cold plasma exposure on vitamin C (ascorbic acid) content in blueberries. (**c**) Effect of cold plasma exposure on total anthocyanin content in blueberries. (**d**) Effect of cold plasma exposure on SOD activity in blueberries. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$. Vertical bars represent the standard errors of the means ($n = 3$)

including blueberries, phosphoenolpyruvate carboxykinase (PEPCK) regulates gluconeogenesis in response to various environmental stressors, and high PEPCK expression leads to high sugar content during ripening (Huang et al. 2015). In our previous study, air cold plasma at atmospheric pressure could significantly improve key enzyme activity in *Klebsiella pneumoniae,* such as glycerol dehydrogenase, glycerol dehydratase, and 1,3-PD oxidoreductase (Dong et al. 2015). In germinating brown rice, α-amylase activity was significantly higher in treated groups than in controls under the conditions of 3 kV plasma exposure for 10 min (Chen et al. 2016). Air cold plasma is a kind of environmental stress. With plasma discharge, the RONS were continually accumulated. These accumulated RONS might induce PEPCK expression to promote the sugar generation in blueberries of the 6-, 8-, and 10-min treatment groups at the original storage period, as well as the sugar generation of 8-min cold plasma treatment group at all storage periods. Thus, the sugar contents of these groups were higher than those of the control.

Effect of Cold Plasma on Vitamin C Content of Blueberries

Vitamin C is an important nutritional component of blueberry fruit and acts as an antioxidant (Englard and Seifter 1986; Oroian and Escriche 2015). To assess whether air cold plasma treatment at atmospheric pressure would promote vitamin C formation, the effect of plasma treatment on vitamin C content in blueberries was investigated. As presented in Fig. 5b, vitamin C contents of blueberries in the treatment groups increased initially and subsequently decreased during storage. After 16 and 20 days of storage, vitamin C contents in 2- to 8-min treatment groups were higher than those in the control groups. The maximum vitamin C content reached 451.1 mg kg^{-1}_{FW} in the 4-min treatment group after 16 days of storage, which represented a 1.5-fold increase compared with the control. The vitamin C content in the control group reached the maximum value after 8 days of storage. The results indicated that DBD plasma increased the vitamin C content in blueberry fruits and lengthened the preservation period for this vitamin. Moreover, the change in vitamin C content in blueberries correlated with plasma discharge time.

It has been reported that NO was one of the excited species generated by cold plasma, which could regulate the ascorbate-glutathione cycle and increased the dehydroascorbate reductase activity. Dehydroascorbic acid is an oxidized form of ascorbic acid, which could be converted to ascorbic acid by dehydroascorbic acid reductase (Hou et al. 2019). Therefore, the alterations of vitamin C content might be due to the rate of regeneration of ascorbic acid. When the rate of regeneration of ascorbic acid by the ascorbate-glutathione cycle was greater than the rate of decay of ascorbic acid through its reaction with other plasma generated reaction species, the vitamin C contents were increased. While the rate of decay exceeded the rate of regeneration, the vitamin C contents were reduced (Fig. 5b).

Effect of Cold Plasma on Total Anthocyanin Content of Blueberries

Anthocyanin is another important functional component in blueberry fruits (Norberto et al. 2013; Syamaladevi et al. 2012). To test the influence of air cold plasma treatment at atmospheric pressure on total anthocyanin content, the changes in total anthocyanin content in blueberries were explored after plasma treatment and during storage. As shown in Fig. 5c, the total anthocyanin contents in blueberries within the treated groups increased to different extents after plasma treatment. After 4 days of storage, the total anthocyanin contents of 8- and 10-min groups reached the maximum of 0.42 g kg^{-1}_{FW}. During storage, the anthocyanin contents in 6- and 8-min treatment groups were always higher than those in the control groups. The results indicated that DBD plasma treatment in these groups improved the total anthocyanin content in blueberries, and that this enhancement effect continued for a prolonged period (up to the 20 days measured).

In this study, the total anthocyanin contents in blueberry fruits in most of the treated groups were significantly higher compared to the control (Fig. 5c). In order to explain this phenomenon, some microscopic and physiological characteristics need to be considered. In fruits and vegetables, epidermal cell wall forms a barrier against various damages, and pretreatment process of cold plasma can reduce its protective ability by etching of fruit surface (Medina-Meza et al. 2016). The structure of exposed cell envelope was deformed, disrupted, or partially lost, resulting in extensive electroporation (Mir et al. 2016). Thus, anthocyanin in the treated group was more easily extracted than that in the control. This pattern differed from the earlier reports in which the total anthocyanin levels of most of the treated groups exhibited significant decrease after cold plasma exposure (Lacombe et al. 2015; Sarangapani et al. 2017). This discrepancy may have been due to differences in the plasma discharge mode and discharge parameters. The plasma discharge mode used in our study was dielectric barrier discharge with 19.7 kHz of discharge frequency, 12.6 kV of discharge voltage, 6.48-W input power consumption, 3-mm discharge distance between upper electrode and bottom electrode, and 0 to 10 min of discharge time with 2-min interval time. On the other hand, the plasma discharge mode in the study by Lacombe et al. (2015) was plasma jet at 47 kHz of pulse frequency, 549 W of power consumption, 7.5 cm of discharge distance between plasma jet apparatus and the sample jar, and 0 to 120 s of discharge time with 15-s interval time. Although the plasma mode of DBD in the report by Sarangapani et al. (2017) was the same as that in our study, their discharge parameters were 50 Hz of discharge frequency, 60 and 80 kV of discharge voltage, and 0, 1, and

5 min of discharge time. The different types and input parameters of plasma discharge yield different types and concentrations of reactive species (Aboubakr et al. 2015), which have varying degrees of effect on cell envelope. As a result, the total anthocyanin contents of treated fruits in this study were different from those in the references reported by Lacombe et al. and Sarangapani et al.

Effect of Cold Plasma on SOD Activity of Blueberries

SOD is considered to be one of the most important antioxidant enzymes that help fruit tissues scavenge endogenous ROS during cold storage (Yan et al. 2012). While SOD activity can be increased by short-term UV-B radiation (Claudio et al. 2016), it is unknown whether air cold plasma treatment at atmospheric pressure induces an increase in SOD activity. Therefore, the changes in SOD activity in blueberries from plasma-treated groups were tested after plasma exposure and during storage. As demonstrated in Fig. 5d, SOD activity levels in the treated blueberry fruits significantly increased. In particular, SOD activity after 8 min of plasma exposure was 65.5% higher compared to the control. During storage, SOD activities in most of the treated groups were enhanced. By the fourth day of storage, the SOD activity in fruits exposed to plasma for 2 min was 50% higher than that in the control. After 16 days of storage, the maximum increase in SOD activity was seen in the fruits treated for 8 min, which was 79.3% higher than that of the control. These results indicated that air DBD plasma at atmospheric pressure could improve SOD activity in blueberry fruits.

After cold plasma treatment for 8 min, the maximum increase in SOD activity (118.0 U g^{-1}_{FW}) was 79.3% in the fruits stored for 16 days compared with that of the control (Fig. 5d). SOD serves as the first line of defense against endogenous ROS and acts by breaking down the toxic superoxide radicals into oxygen and hydrogen peroxide (Jaikua et al. 2016; Zhou et al. 2014). The generation of various exogenous ROS by DBD plasma increases ROS production in vivo, which induces SOD activity in treated fruits (Li et al. 2017). An increase in SOD activity could enhance the elimination of superoxide radicals in vivo (Zhou et al. 2014). The elimination of ROS might inhibit the aging cycle. Therefore, air cold plasma treatment can not only improve the SOD activity, but also prolong the shelf life of blueberry fruit.

Several previous studies have reported that decay rate, shelf life, and/or nutrient content of various fruits could be ameliorated by certain pre-treatment methods for sterilization and pesticide degradation (Chen et al. 2017; Lires et al. 2018; Mannozzi et al. 2018; Sarangapani et al. 2017; Shi et al. 2016; Zhou et al. 2014). However, it has not been reported that blueberry qualities during storage could be enhanced after the pre-treatment of air DBD plasma, especially the increased nutrient contents of total sugar, vitamin C, anthocyanin, and SOD activity in blueberry fruits. Hence, this is a valuable finding of this work. Air plasma could generate various reactive species, especially reactive oxygen species (ROS) and reactive nitrogen species (RNS). These short-lived gaseous reactive species are capable of penetrating cell wall into the cytoplasm of plants and inducing the formation of long-lived aqueous species, such as H^+, nitrate, nitrite, H_2O_2, and O_3 (Liu et al. 2016). These aqueous reactive species could interact with DNA, amino acids, and secondary structure of protein, resulting in alterations of gene expression and protein conformation, which might improve or deactivate the enzyme activity (Pankaj et al. 2018). Air cold plasma could increase or decrease the activities of key metabolic enzymes in microbial cells, thus enhancing the concentration of target product (Dong et al. 2015). It is also reported that ROS induced by environmental stress could promote increases in ascorbic acid, polyphenols, flavonoids, and antioxidant activity in plants (Sarker and Oba 2018). Therefore, the nutrient levels in blueberry fruits treated by air cold plasma still could increase or decrease during storage. Furthermore, a patent on this finding has already been granted by the State Intellectual Property Office of China (Dong and Yang 2017). However, further process optimization will be necessary for the complimentary benefits of microbial safety, shelf life extension, and fruit quality improvement and for the feasibility of scale-up of this technology for fresh produce processing. It has been reported that cold plasma pre-treatment alters the microstructure of cereal components and enhances the seed germination and seedling growth of cereal (Mir et al. 2016; Mitra et al. 2014; Muhammad et al. 2018). Recently, three federal agencies, Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) in the USA are considering the approval of an application of a novel dielectric barrier discharge (DBD) direct plasma treatment for whole wheat grain with the claim of spoilage reduction (Bourke et al. 2018). Therefore, cold plasma is evidently becoming a promising technology for safety and sustainability in agriculture and food production.

Conclusion

Enhancement of blueberry quality with air cold plasma was investigated in this study. Microbial viability on the blueberries significantly decreased due to oxidation of guanine and damage of DNA structure after plasma treatment. As a result, the decay rate of blueberries presented a notable reduction. Moreover, sugar, vitamin C, total anthocyanin content, and SOD activity of treated blueberries were remarkably enhanced under certain parameters of cold plasma discharge. Importantly, this enhancement effect continued for the several days after the treatment. Thus, the nutrient contents of blueberries can be improved and also preserved for longer by this

treatment method. From the above findings, it can be concluded that air cold plasma at atmospheric pressure has excellent potential as a useful pre-treatment technology for enhancing the quality of fresh produce in food industry.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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