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Impact of cold atmospheric pressure plasma processing on storage of blueberries

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Abstract

The current study aimed at investigating the impact of nitrogen (N)-generated cold atmospheric pressure plasma (CAPP) treatment on blueberries focusing on the overall impact on berry quality and microbial load along a storage period of 10 days. Blueberries were treated for 0 (control), 5, and 10 min. Assessment of fruit quality (°Bx, ascorbic acid, anthocyanins, titratable acidity, elasticity, and color parameters) and microbial analysis was performed. Results showed that CAPP treatment was more effective in inhibiting bacterial growth than fungal growth and during the subsequent storage, the quality parameters did not differ significantly from the control, under the same conditions. The study supports N-generated CAPP as a disinfection technique to reduce microbial load in blueberries without significantly impacting most quality parameters.

Practical applications

Over the last decades, foodborne illness outbreaks around the world have been associated with berries. For that reason, due to the increasing consumption of berries it is paramount to study technologies that can eliminate pathogens responsible for such outbreaks. Cold atmospheric pressure plasma (CAPP) can be a promising technology to be used as an alternative to traditional decontamination methods of food. In this context, this study explored the effect and efficiency of this novel technology on reduction of native microflora and its impact on the physical and chemical properties of blueberries treated by nitrogen (N)-generated CAPP with subsequent storage of 10 days. Results of this work confirmed that such technology has high potential application for decontamination of berries without significantly impacting most quality parameters and thereby can be a potential technology for industrial applications.

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1 | INTRODUCTION

Over the past decades consumer awareness about the undeniable nutritional value of blueberries (*Vaccinium* spp.) has grown considerably, resulting in a remarkable increase in blueberry consumption (Lacombe et al., 2017; Quansah et al., 2019). Approximately 237,000 and 100,000 tons of blueberries were cultivated in the United States and Europe in 2017, these corresponds to 39.6% and 16.8% of the global blueberry production, respectively (FAOSTAT, 2019). Blueberry fruits are rich in anthocyanins, which have antioxidant and antiaging properties (Liu et al., 2019). Despite their high nutritional value, blueberries can also be carriers of undesirable microbial loads, as they are prone to contamination with pathogenic or spoilage microorganisms on the field, during harvesting, processing, packaging, storage and/or distribution. Moreover, it is common practice that blueberries destined to the fresh market do not undergo any pretreatment. Consequently, several foodborne epidemics have been related to the consumption of fresh berries (FAO/WHO, 2008). This presses for an effective decontamination process of the blueberries after harvest. However, the adoption of decontamination process for fruits such as blueberries should take into account the sensitivity of the product, as conditions of elevated temperature or mechanical stress could severely decrease both the nutritional and the organoleptic quality of the berries. In that direction, cold atmospheric pressure plasma (CAPP) process has been suggested as a promising technology to improve the microbial quality of berries (Bovi, Fröhling, Pathak, Valdramidis, & Schlüter, 2019).

CAPP refers to the non-equilibrium plasma generated at near-ambient temperatures and pressure (Han et al., 2016). Moreover, CAPP is an emerging and innovative technology with high potential for food surface microbial load reduction (Schnabel, Niquet, Schlüter, Gniffke, & Ehlbeck, 2015). Apart from decontamination, CAPP technology can also affect enzymatic activity (Bußler, Ehlbeck, & Schlüter, 2017; Pankaj, Misra, & Cullen, 2013) and degradation of chemical compounds (Misra, Pankaj, et al., 2014; Sarangapani, O'Toole, Cullen, & Bourke, 2017). In addition, it can be applied for the decontamination of the wastewater produced by washing fruits and vegetables (Ouf, Mohamed, & El-Sayed, 2016), and for processing food waste (Sarangapani, Patange, Bourke, Keener, & Cullen, 2018). The efficacy of CAPP depends on the employed feed of the plasma gas, the apparatus used for the plasma generation, and the characteristics of the product under treatment. Inherent parameters like water activity, components' concentration, and geometry of the product can also largely affect the degree of microbial inactivation and the quality of the final product (Niemira, 2012).

Very few studies have reported the use of CAPP technology for treating blueberries. These few studies aimed at inactivating native microbiota (Bogdanov et al., 2018; Dong & Yang, 2019; Lacombe et al., 2015), *Norovirus* surrogates Tulane virus and murine norovirus (Lacombe et al., 2017), and degrading residual pesticides (Sarangapani et al., 2017). Moreover, most of these studies only evaluated the effect of CAPP immediately after treatment; nonetheless, it is of great importance to investigate what is the effect of CAPP in the longer term. In this context, the objective of the present study was to investigate the effect of nitrogen (N)-generated CAPP on blueberries (*Vaccinium corymbosum*) directly after treatment and during storage of 10 days, with respect to various parameters, such as microbial load, physical (color and texture), and chemical properties (total soluble solids [TSS], titratable acidity [TA], anthocyanins, and ascorbic acid concentration).

Highlights

- Blueberries were treated with cold atmosphere pressure plasma (CAPP)
- CAPP was more effective in inhibiting bacterial growth than fungal growth
- CAPP treated blueberries tended to be slightly softer than the untreated samples
- CAPP treatment did not significantly impact most quality parameters

2 | MATERIALS AND METHODS

2.1 | Materials

Blueberries were obtained from a local supermarket (REWE, Potsdam, Germany). The blueberries were transported to the Freshness Laboratory, Department of Horticultural Engineering, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany and were carefully sorted and the damaged, overripe, and poor quality fruit were discarded in order to obtain uniform samples.

2.2 | Plasma treatment

For the CAPP treatment, a diffuse coplanar surface barrier discharge 400 (DCSBD) plasma unit (CEPLANT, R&D Centre for Low-Cost Plasma and Nanotechnology Surface Modifications, Masaryk University, Brno, Czech Republic) was used. This system consisted of a chamber, where the treatment takes place, and a generator, in which plasma is generated (Hertwig, Reineke, Rauh, & Schlüter, 2017). The blueberries were placed on top of a net inside the chamber between the two DCSBD ceramic plates (200 × 80 mm) and treated from both sides. The distance of the plates was set to 1 cm from each side of the samples (Figure 1). The DCSBD plasma was generated by applying 300 W which generated a high number of micro discharges of approximately 0.3-mm homogeneous plasma layer. Dielectric insulating oil was used in a circulation system, in order to insulate the electrodes, as well as to cool down the plasma generation system, by circulating the oil through a heat exchanger, in order to dissipate the heat from the oil. Before turning on the plasma generation system,

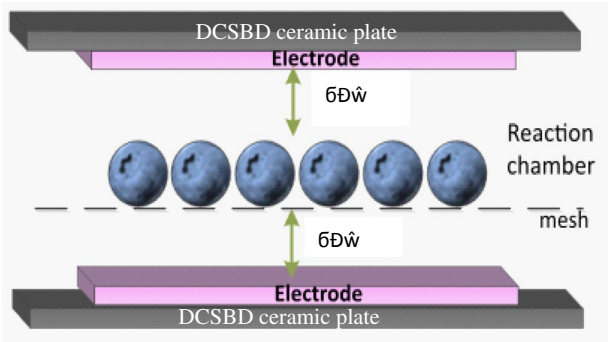

FIGURE 1 Graphical representation of the diffuse coplanar surface barrier discharge (DCSBD) plasma unit

75 g of blueberries was placed on the net and the plasma chamber was sealed by tightening the screws of the sealing mechanism of the chamber. Then the chamber was rinsed with nitrogen (N) for 2 min using a gas flow system connected to the plasma chamber. The duration of the plasma treatment was set to 5 and 10 min. An untreated sample (0 min) was used as the control. After treatment, the chamber was unsealed and the treated samples were transferred to polyethylene terephthalate trays with perforated lids (8 perforations of 0.8 mm). Each plasma treatment was carried out in triplicate, summing up to 45 packages. The packaged treated blueberries were kept at 7°C up to 10 days. At specific time intervals of 0, 1, 4, 7, and 10 days samples were taken from the cold room in order to be analyzed.

2.3 | Microbial assay

For the microbiological analysis of the blueberries, 6 g of each sample were diluted 1:10 with buffered peptone water. Then, the mixture was homogenized for 2 min at speed 4, using a bag mixer (BagMixer 400 CC, Interscience, France). A number of 10-fold serial dilutions of the sample homogenate were made in duplicate, using a peptone salt solution and 100 µl of each dilution was spread on top of sterile petri dishes, containing the appropriate substrate for microbial growth. In order to determine the aerobic mesophilic total viable count of the samples, Plate Count Agar (PCA, Carl Roth GmbH & Co. KG, Germany) was used as growth medium and the petri dishes were incubated at 30°C for 72 h. For enumeration of yeasts and molds, Rose Bengal Chloramphenicol Agar (RBC, Carl Roth GmbH & Co. KG, Germany) was used as growth medium and the petri dishes were incubated at 25°C for 5 days. The microbial load was expressed as log colony forming units per gram of sample (log CFU/g) (n = 6).

2.4 | Physical quality parameters

2.4.1 | Color

Surface color of the berries was measured using a digital chromameter (CM-2600d, Konica, Minolta Sensing Inc., Tokyo, Japan). Evaluation was performed on the basis of Commission Internationale del l'éclairage (CIE) color system (L^*—lightness, a^*—redness, and b^*—yellowness). Spectral reflectance measurement was taken at one point on the surface of each blueberry. The instrument was calibrated against a white and a black background before each measurement. All measurements are expressed as mean ± standard deviation (n = 63).

2.4.2 | Modulus of elasticity

The elasticity of the blueberries was determined by non-destructive compression test using a texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, UK) equipped with a spherical probe (d = 6.35 mm). The resistance to the maximum force was determined. Average diameter of berry fruit at the equatorial position was measured using Vernier calipers and the berry was subjected to compression test using texture analyzer. Maximum force (F) and the deformation (D) were recorded. The quasi-static elastic modulus E can be calculated using Equation (1):

$$E = \frac{0.531F(1-\mu^2)}{D^{3/2}}\left[\frac{2}{R}+\frac{4}{d}\right]^{1/2} \quad (1)$$

where μ is Poisson's ratio (assumed as 0.49), F is the maximum force (N), D is the deformation at maximum force, and R is the fruit radius. All measurements were expressed as mean ± standard deviation (n = 63).

2.5 | Chemical quality parameters

2.5.1 | Total soluble solids, titratable acidity and ascorbic acid

Juice of the blueberries was extracted by mechanical pressing and then analyzed for total soluble solids (TSS), titratable acidity (TA), and ascorbic acid. TSS was measured using a handheld refractometer (DR301-95, Krüss Optronic, Hamburg, Germany) and expressed as °Bx. The ascorbic acid content was determined using a handheld reflectometer (RQflex 10, Merck, Darmstadt, Germany) and expressed as mg/L. For measurement of TA, an automated T50M Titrator with Rondo 20 sample changer (Mettler Toledo, Gießen, Germany) was used. Potentiometric titration with 0.1 mol/L NaOH to endpoint of pH 8.2 was performed and expressed as g/100 ml of citric acid based on fresh blueberry juice. All measurements are presented as mean ± standard deviation (n = 9).

2.5.2 | Anthocyanins

Procedure for anthocyanin determination was performed according to Caleb et al. (2016) with slight modifications. Blueberries (1.0–1.2 g) were put in a 50-ml flask containing 10 ml of acetone solvent

mixture (0.1% butylated hydroxytoluene (BHT)/ethanol (1.5:1, v/v) and dispersed using an Ultra Turrax (Ultra-Turrax, Janke& Kunkel, Staufen, Germany). The mixture was shaken for 30 min in an overhead shaker and then centrifuged using MiniSpin (Eppendorf AG, Germany) for 3 min at 21,733 g at room temperature. The mixture was filtered through a G4 frit (10–16 µm pores) (Waters GmbH, Eschborn, Germany). Next, 2.5 ml of the supernatant was transferred into a separating funnel and 7.5 ml of the above-mentioned solvent was added. Then, 10 ml of a mixture of hexane and double distilled water (1:8) (v/v) was added. The lower phase in the separating funnel was transferred to a 20-ml volumetric flask and 0.2 ml of concentrated HCl was added to it. Water was further added to make up for the rest of the volume in the flask. The mixture was then filtered and transferred to quartz cuvettes. The absorption was measured in the wavelength range from 350 to 800 nm using a spectrophotometer (Specord 210 plus, Analytik Jena AG, Germany) and the total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents per 100 g of fresh sample.

2.6 | Statistical analysis

Analysis of variance (ANOVA) was carried out using R software (version 3.6.1) to analyze the significant differences due to plasma treatment time and storage days of blueberries. Tukey´s test was used to confirm statistically significant differences and results with $p \leq .05$ were considered to be significant.

3 | RESULTS AND DISCUSSION

3.1 | Microbial analysis

This is the first study that evaluates the reduction in native microflora in tandem with the physical and chemical properties of blueberries treated by N-generated CAPP and stored for 10 days at 7°C. Results showed that the longer the treatment time with N-generated CAPP, the higher was the reduction in the population of native microflora on fresh blueberries (Figure 2). For aerobic mesophilic total viable counts, the treatment time resulted in a significant difference ($p < .05$) between untreated and 10-min treated sample within the same day for all sampling days (days 0, 1, 4, 7, and 10). During storage, within the same treatment groups, no significant differences were observed, except for 10 min. On the other hand, no significant differences ($p > .05$) on the counts of yeasts and molds were obtained between untreated and 5-min treated samples directly after plasma treatment (i.e., on day 0). This shows that for an immediate significant reduction ($p < .05$) of yeasts and molds, a treatment longer than 5 min should be applied. However, as storage time increases the difference between untreated, 5- and 10-min treated berries becomes more distinguished, as significant differences ($p < .05$) between untreated and 5- and 10-min treated samples were observed within the same day on all subsequent days after treatment (i.e., on days 1, 4, 7, and 10). For day 10, a reduction of 0.68 log CFU/g for 5-min and 1.25 log CFU/g for 10-min treatment was obtained. Comparing this to the 10-days reduction in aerobic mesophilic total viable counts, which was 0.37 log CFU/g for 5-min treatment and 1.14 log CFU/g for 10-min treatment, it is clear that N-generated CAPP treatment reduced the total mesophilic aerobic counts to a higher extent than the yeasts and molds throughout the storage period of 10 days. Similar results were found by Lacombe et al. (2015), in which air-generated CAPP (i.e., air plasma jet, 47 kHz and power consumption of 549 W) treatment significantly ($p < .05$) reduced the total aerobic count, whereas for yeasts and molds the reduction was not significant for blueberries, for most of the treatment times analyzed (i.e., 15, 30, 45, 60, 90, and 120 s) and throughout a storage period of 7 days. Similarly, in the study carried out by Dong and Yang (2019), the microbial reduction was much lower for fungi (0.58 $\log_{10}$ CFU/g $_{FW}$) than for total bacteria counts (2.01 $\log_{10}$ CFU/g $_{FW}$) of blueberries treated by air-generated CAPP (i.e., Dielectric barrier discharge [DBD] plasma discharge system, 3-mm discharge gap, 36 V, and current of 1.8A). The reason for that has been associated with the thickness and complexity of fungi cell walls, as compared to bacteria cell walls (Dong & Yang, 2019; Lee, Paek, Ju, & Lee, 2006). In our study, even though the microbial reduction between untreated and 10-min treated samples was not very high, it was still significant ($p < .05$).

3.2 | Physical quality parameters

It is well known that both texture and color are the most important physical quality parameters for consumers, as they affect their acceptability and willingness to buy and consume the product. Therefore, it is paramount to evaluate to what extent CAPP treatment affects these parameters. For texture, the effect of CAPP on blueberries was determined by means of the modulus of elasticity and was measured on day 0 (directly after treatment) and intermediate days (i.e., days 1, 4, and 7) until day 10 (Figure 3). The modulus of elasticity of nitrogen CAPP-treated blueberries did not change significantly ($p > .05$) between the untreated and 5-min treated samples at all days except day 4. However, between untreated and 10-min treated samples significant differences ($p < .05$) were detected within the same day for days 0, 4, and 7. This indicated that blueberries treated for 10 min tended to be slightly softer than the untreated samples. Therefore, in order to avoid significant changes in blueberry texture the treatment time in the DCSBD chamber should be kept below 10 min, when N is used as a working gas. Moreover, visually no display of physical damage was detected in the blueberries immediately after the 5- and 10-min treatments.

In the study conducted by Lacombe et al. (2015) the mean force (N) needed to compress the blueberry surface by 1 mm was higher in the untreated samples as compared to air CAPP (i.e., air plasma jet, 47 kHz and power consumption of 549 W)-treated berries, for all treatment times tested (i.e., 0, 15, 30, 45, 60, 90, and 120 s). This texture deterioration was also associated with collisions between the berries and the treatment container and due to temperature

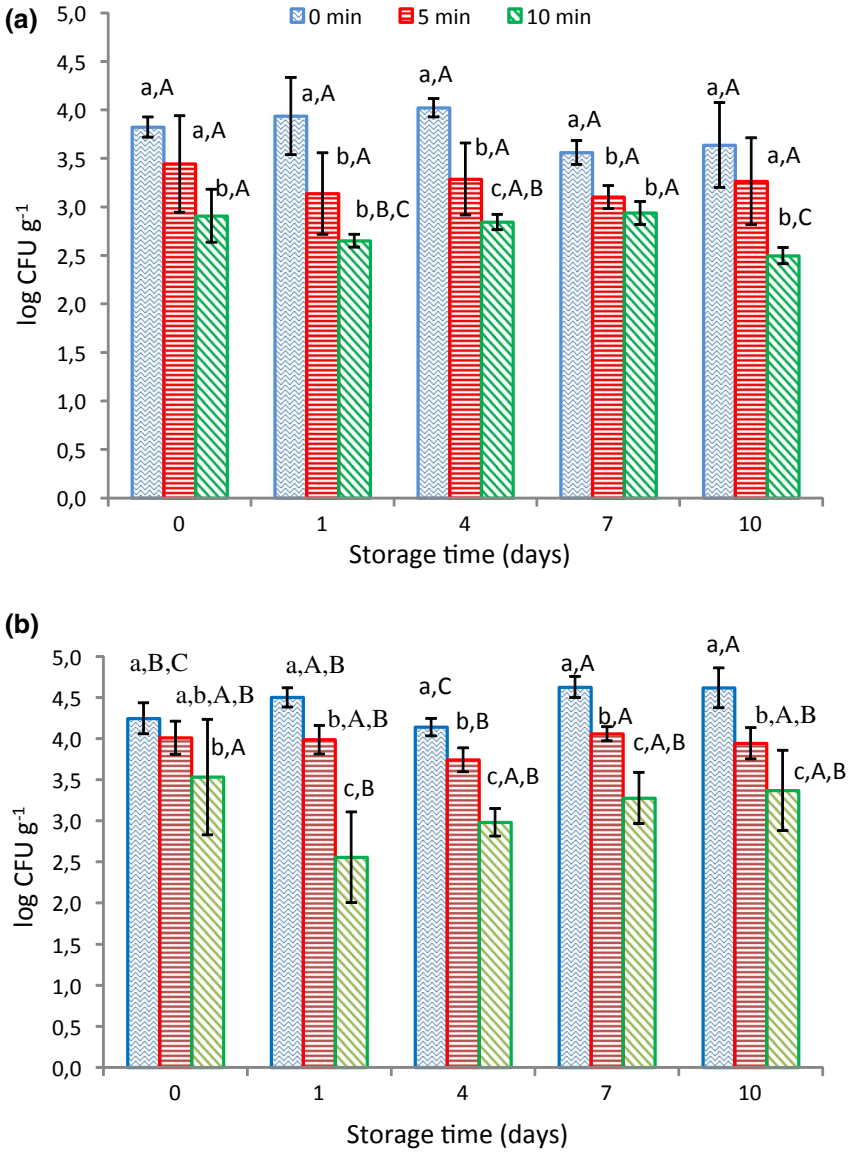

FIGURE 2 Impact of CAPP treatment on (a) the aerobic mesophilic total viable count and (b) yeasts and molds. Error bars represent standard deviation (*SD*) of mean values ($n = 6$). Different upper case letter within same treatment time and different lower case letter within the same storage day are significantly different based on Tukey (post hoc test) test at $p \leq .05$

rise during the treatment. Similarly, in the study carried out by Sarangapani et al. (2017) with air CAPP (i.e., in-package DBD, 60 and 80 kV)-treated blueberries, no significant changes were detected in the firmness between the control and sample treated for 1 min at 60 kV. For all other treatments, namely 5 min at 60 kV, 1 min at 80 kV, and 5 min at 80 kV significant changes were detected ($p < .05$). This might be an indication that the use of nitrogen as a working gas for CAPP treatment of blueberries does not have an impact on the texture as compared to the use of air. Nonetheless, it is hard to make a straightforward comparison, as there were other process parameters, apart from working gas, which differed between the studies (i.e., treatment time, power, and plasma source). Other studies with different fruits have also reported decrease in firmness and elasticity due to CAPP treatment (Misra, Pankaj, et al., 2014). This decrease has been associated with damage of outer cells as a result of internal structure degradation, with a subsequent softening of the produce surface (Sarangapani et al., 2017). Another well-known factor previously reported to affect texture of fresh produce throughout storage

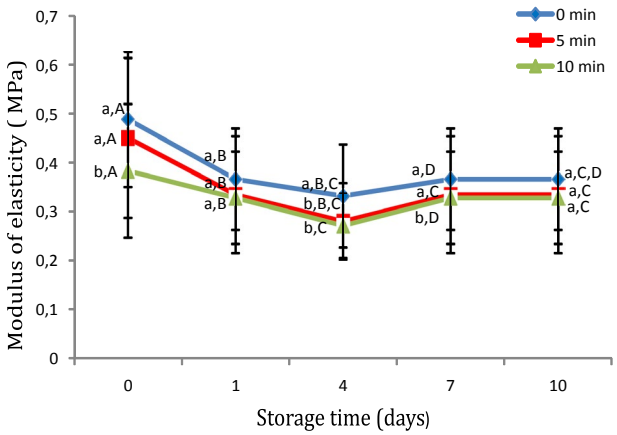

FIGURE 3 Impact of CAPP treatment on modulus of elasticity of blueberries. Error bars represent standard deviation (*SD*) of mean values ($n = 63$). Different upper case letter within a treatment and different lower case letter within the same storage day are significantly different based on Tukey (post hoc test) test at $p \leq .05$

TABLE 1 Changes in color parameters of control and N-generated CAPP-treated blueberries during storage

Color attribute	Treatment time	Storage time				
		Day 0	Day 1	Day 4	Day 7	Day 10
L	0 min	$29.97 \pm 3.21^{a,A}$	$30.65 \pm 3.70^{a,A}$	$30.09 \pm 3.27^{a,A}$	$30.82 \pm 3.27^{a,A}$	$31.26 \pm 3.37^{a,A}$
	5 min	$28.82 \pm 4.09^{a,C}$	$28.06 \pm 3.72^{b,C}$	$29.22 \pm 3.03^{ab,BC}$	$31.12 \pm 2.91^{a,A}$	$30.81 \pm 3.74^{a,AB}$
	10 min	$28.97 \pm 3.16^{a,B}$	$28.24 \pm 3.22^{b,B}$	$28.65 \pm 3.19^{b,B}$	$31.29 \pm 2.99^{a,A}$	$31.60 \pm 3.12^{a,A}$
*a**	0 min	$-0.35 \pm 0.15^{a,A}$	$-0.27 \pm 0.51^{a,A}$	$-0.28 \pm 0.19^{b,A}$	$-0.31 \pm 0.17^{a,A}$	$-0.30 \pm 0.29^{a,A}$
	5 min	$-0.18 \pm 0.76^{a,A}$	$-0.20 \pm 0.23^{a,A}$	$-0.28 \pm 0.15^{ab,A}$	$-0.33 \pm 0.21^{a,A}$	$-0.30 \pm 0.18^{a,A}$
	10 min	$-0.27 \pm 0.16^{a,AB}$	$-0.26 \pm 0.14^{a,AB}$	$-0.17 \pm 0.40^{a,A}$	$-0.33 \pm 0.29^{a,B}$	$-0.35 \pm 0.17^{a,B}$
*b**	0 min	$-3.61 \pm 1.12^{a,A}$	$-3.79 \pm 2.45^{b,A}$	$-3.65 \pm 1.22^{a,A}$	$-4.28 \pm 1.23^{a,A}$	$-4.09 \pm 1.34^{a,A}$
	5 min	$-3.10 \pm 2.12^{a,AB}$	$-2.93 \pm 1.00^{a,A}$	$-3.38 \pm 1.15^{a,AB}$	$-4.39 \pm 1.14^{a,C}$	$-3.75 \pm 1.24^{a,BC}$
	10 min	$-2.97 \pm 1.21^{a,A}$	$-3.03 \pm 1.15^{a,A}$	$-3.25 \pm 0.99^{a,A}$	$-4.56 \pm 1.21^{a,B}$	$-4.18 \pm 1.22^{a,B}$

Note: Mean values ($n = 63$) ± standard deviation. Different uppercase letters within the same row and different lowercase letters within the same column for the same attribute are significantly different based on Tukey (post hoc test) test at $p \leq .05$.

is the transpiration process. The water vapor pressure differences between the product and the surrounding environment leads to water loss (Bovi, Caleb, Linke, Rauh, & Mahajan, 2016). This loss of water in turn has a direct effect on the produce texture leading to the decrease in the elastic modulus during storage in all samples.

In blueberries, the color has been described as a complex parameter dependent on the anthocyanin content and on the quantity and structure of wax on the berry surface (Albrigo, Lyrene, & Feeman, 1980; Kushman & Ballinger, 1975; Saftner, Polashock, Ehlenfeldt, & Vinyard, 2008; Silva, Marroquin, Matta, Garner, & Stojanovic, 2005). The *L*, *a**, and *b** color parameters were measured in the blueberries before and after treatment and during the storage (Table 1). The average values of *L*, *a**, and *b** for blueberries before treatment were observed as 29.97 ± 3.21, −0.35 ± 0.15, and −3.61 ± 1.12, respectively. During storage, within the same treatment no significant ($p > .05$) changes in *L*, *a**, *b** in untreated samples and in *a** in 5-min treated samples were observed. However, within other treatments, namely 5-min treatment (for *b** and *L*) and 10-min treatment (for *L*, *a**, *b**), a slight change was observed after day 4. However, in spite of these slight differences along storage days within the same treatment time, there was no significant difference ($p > .05$) between untreated and treated groups within the same day for sampling days (days 0, 7, and 10) in all three color parameters, namely, *L*, *a**, and *b**. This implies that the treatment time showed minimal abnormal color changes.

Other studies on the effect of air-generated CAPP on blueberries (Lacombe et al.on the surface of products, consequently exerting a , 2015; Sarangapani et al., 2017) reported a slight decrease in L (lightness) values (indicating development of a darker color) and an increase in *a** and *b** values (indicating shift in perceived color toward red and blue), after plasma treatment. The darkening of the color is attributed to loss of moisture and melting of the surface wax in berries, due to plasma treatment. However, the color changes reported in one of the above-mentioned studies on blueberry (Sarangapani et al., 2017) were not significant. Some studies on other fresh produce such as green apples, cucumbers, and carrots, also reported darkening of color after plasma treatment (Baier, 2015); in slices of cucumber, pears, and carrots the color change was reported to be minimal, with acceptable difference (Wang et al., 2012). Contrastingly, in other studies the changes in color parameters post-plasma treatment were reported to be insignificant, for example, in strawberries (Misra, Patil, et al., 2014), cherry tomatoes (Misra, Keener, Bourke, Mosnier, & Cullen, 2014), and tomatoes (Bermúdez-Aguirre, Wemlinger, Pedrow, Barbosa-Cánovas, & Garcia-Perez, 2013), which also corroborates with the results of the present study. Different treatment conditions could be responsible for the varied effect on loss of moisture and melting of wax on the surface of products, consequently exerting a variable effect on the color parameters.

3.3 | Chemical quality parameters

3.3.1 | Total soluble solids, titratable acidity, and ascorbic acid

In the present study, the total soluble solid content (TSS) of the untreated sample was measured to be 12.83 ± 0.43 °Bx on day 0 and was not significantly affected by the CAPP treatment or during the subsequent storage (Table 2). The titratable acidity (TA) expressed as citric acid equivalent (g/L) showed significant changes only within the untreated and 5-min treated groups on day 4 compared to previous storage days. Overall, no significant differences ($p > .05$) between untreated and treated samples were observed within the same day for all sampling days (days 0, 1, 4, 7, and 10) for both TSS and TA. Comparatively, Sarangapani et al. (2017) reported a significant increase in the TSS content of blueberries post-CAPP treatment, whereas TA did not significantly change ($p > .05$). In a 20-days storage study of blueberries, reported by Dong and Yang (2019), sugar content increased up to day 4 and then decreased.

Ascorbic acid, also known as vitamin C, is a water-soluble vitamin with a high antioxidant capacity and is present in significant amounts in fresh berries including blueberries (Skrovankova, Sumczynski, Mlcek,

TABLE 2 Changes in chemical quality parameters of control (0 min) and N-generated CAPP-treated blueberries (5 and 10 min) during 10-days storage

Color attribute	Treatment time	Storage time				
		Day 0	Day 1	Day 4	Day 7	Day 10
TSS (°Bx)	0 min	$12.83 \pm 0.43^{a,A}$	$13.10 \pm 0.68^{a,A}$	$13.17 \pm 0.22^{a,A}$	$13.12 \pm 0.68^{a,A}$	$13.24 \pm 0.68^{a,A}$
	5 min	$12.87 \pm 0.72^{a,A}$	$12.70 \pm 0.67^{a,A}$	$13.00 \pm 0.66^{a,A}$	$12.80 \pm 0.51^{a,A}$	$12.99 \pm 0.67^{a,A}$
	10 min	$12.83 \pm 0.64^{a,A}$	$12.89 \pm 0.63^{a,A}$	$12.76 \pm 0.49^{a,A}$	$13.00 \pm 0.59^{a,A}$	$13.14 \pm 0.34^{a,A}$
Ascorbic acid (mg/L)	0 min	$120.44 \pm 11.99^{a,B}$	$101.78 \pm 5.12^{a,C}$	$143.56 \pm 11.30^{a,A}$	$142.22 \pm 6.67^{a,A}$	$120.44 \pm 19.05^{a,B}$
	5 min	$114.44 \pm 8.47^{a,BC}$	$104.25 \pm 8.7^{a,C}$	$137.11 \pm 11.19^{a,A}$	$130.00 \pm 7.81^{b,A}$	$126.67 \pm 9.49^{a,AB}$
	10 min	$115.78 \pm 6.96^{a,C}$	$93.11 \pm 18.57^{a,D}$	$138.44 \pm 6.77^{a,A}$	$131.11 \pm 6.79^{b,AB}$	$122.44 \pm 10.71^{a,BC}$
Anthocyanins (mg/100 g FM)	0 min	$107.42 \pm 15.07^{a,A}$	$133.09 \pm 55.56^{a,A}$	$98.45 \pm 28.28^{a,A}$	$121.63 \pm 31.09^{a,A}$	$108.48 \pm 64.18^{a,A}$
	5 min	$109.42 \pm 22.57^{a,A}$	$89.71 \pm 18.89^{a,A}$	$131.91 \pm 99.29^{a,A}$	$109.17 \pm 63.70^{a,A}$	$127.33 \pm 40.26^{a,A}$
	10 min	$94.40 \pm 15.89^{a,A}$	$145.24 \pm 83.11^{a,A}$	$119.16 \pm 33.75^{a,A}$	$119.23 \pm 39.83^{a,A}$	$101.87 \pm 37.31^{a,A}$
Citric acid equi. (g/L)	0 min	$13.61 \pm 1.79^{a,B}$	$14.50 \pm 2.58^{a,B}$	$17.76 \pm 1.73^{a,A}$	$12.81 \pm 1.13^{a,B}$	$12.96 \pm 1.46^{a,B}$
	5 min	$12.86 \pm 1.41^{a,B}$	$13.14 \pm 1.51^{a,B}$	$16.77 \pm 1.67^{a,A}$	$12.28 \pm 1.10^{a,B}$	$13.06 \pm 1.18^{a,B}$
	10 min	$13.31 \pm 1.65^{a,B}$	$14.15 \pm 2.03^{a,B}$	$16.61 \pm 1.72^{a,A}$	$12.12 \pm 0.78^{a,B}$	$12.34 \pm 1.01^{a,B}$

Note: Mean values ($n = 9$) ± standard deviation. Different uppercase letters within the same row and different lowercase letters within the same column for the same attribute are significantly different based on Tukey (post hoc test) test at $p \leq .05$.

Jurikova, & Sochor, 2015). In the present study, the N-generated CAPP treatment did not have a significant effect ($p > .05$) on ascorbic acid content of blueberries between untreated and treated samples within the same day for most sampling days (days 0, 1, 4, and 10). However, within each treatment (untreated, 5 min, and 10 min) storage days had a significant effect. Within each treatment the ascorbic acid content decreased from day 0 to day 1, increased from days 1 to 4 and decreased again from day 4 to day 10. The highest ascorbic acid contents were quantified on day 4, being 143.56, 137.11, and 138.44 mg/L for untreated, 5, and 10 min of plasma treatment, respectively. These variations in vitamin C during storage were also observed in air-generated CAPP treatment of blueberries stored for 20 days (Dong & Yang, 2019). Other studies of CAPP-treated blueberries have reported the ascorbic content only before and immediately after plasma treatment (Sarangapani et al., 2017).

3.3.2 | Anthocyanins

Anthocyanins are pigments responsible for blue, violet, purple, and red color in fruits (Lohachoompol, Srzednicki, & Craske, 2004). In the case of blueberries, anthocyanins are present in the vacuoles on the surface skin of the fruit (Sarangapani et al., 2017). In the present study, the anthocyanin content of blueberries in the untreated sample were found to be 107.42 ± 15.07 mg of cyanidin-3-glucoside equivalents per 100 g of fresh sample, which was not significantly affected by the CAPP treatment or during storage. As anthocyanin content is correlated with color, the results corroborated with the results of the color in this study, which also showed statistically insignificant changes between untreated and CAPP-treated groups.

Stability of anthocyanins is affected by many factors, including temperature and reactive oxygen species. Generally, CAPP treatment has been reported to have a detrimental effect on anthocyanin content. Studies on blueberries also showed reduced anthocyanin content after CAPP treatment, attributed to the elevated temperature at the point of application of plasma, as well as due to the generated reactive species (Lacombe et al., 2015; Sarangapani et al., 2017). Contrastingly, in a study on CAPP treatment of blueberries followed by storage, the anthocyanin content was found to be always higher than that of the control (Dong & Yang, 2019). It was speculated that CAPP treatment could have deformed/ruptured the surface exposed cells, resulting in better extractability of anthocyanin content than in the control. The inconsistency of the results could be attributed to the different treatment conditions.

4 | CONCLUSIONS

The present study supports N-generated CAPP as a disinfection technology, in order to reduce the microbial load in blueberries, without significantly impacting most chemical and physical quality parameters. In addition, it is evident from the study that CAPP treatment was more effective in inhibiting bacterial growth than fungal growth. During the subsequent storage, the quality parameters did not differ significantly in treated samples from the untreated sample within the same day. Thus, it can be concluded that N-generated CAPP can be an effective disinfection technique for blueberries, having minimal effects on the quality parameters.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest for this article.

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