Innovative Food Science and Emerging Technologies 58 (2019) 102230

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Effect of plasma bubbling on free radical production and its subsequent effect on the microbial and physicochemical properties of Coconut Neera

S. Aparajhitha, R. Mahendran*

Department of Food Packaging and System Development, Indian Institute of Food Processing Technology, Ministry of Food Processing Industries, Govt of India, Thanjavur, India

ARTICLE INFO

Keywords:
Atmospheric pressure cold plasma
Electron paramagnetic resonance
Microbial inactivation
Coconut neera

ABSTRACT

In this study, the effect of atmospheric pressure cold plasma on the physicochemical and antimicrobial properties of Coconut Neera – a natural sugar-rich health drink was evaluated. The drink was bubbled with plasma flowing at 5, 7 and 10 lph into 300 mL samples for 5 and 10 min at room temperatures of ~30 °C. The plasma input voltage was set at 150, 200 V and after treatment, pH, color and TSS of Neera were determined. The presence of OH radical was confirmed by EPR spectroscopy and qualitatively elucidated for every treatment combination to ascertain their role along with other free radicals in microbial inactivation (~80% reduction in CFU/mL). There was a considerable increase in pH (an increase of ~1.04) while the negligible change in color and TSS values. The nutritional profile, however, remained unchanged except for a slight decrease in vitamin C content (~4.7 mg/100 mL). Analogue thermal death time was calculated for the two given plasma treatments (30.51 and 13.8 min for 150 and 200 V respectively). The results from this study propose, plasma bubbling as a method for bulk sterilization for liquid foods that can be easily scaled up for industrial applications.

Industrial relevance: This study demonstrated the potential application of plasma bubbling for free radical production and its effect on the inactivation of microbes while retaining the nutritional properties of liquid foods (Coconut Neera in this study). This research gives an insight into better interaction of plasma species while bubbling through liquids food systems. The plasma bubbling described in this work is relevant to the liquid food processing and basis for industrial implementation.

1. Introduction

Atmospheric Pressure Cold Plasma is a novel technique that has recently been the subject of research due to its immense potential in various fields like pharmaceuticals (Laroussi & Lu, 2005; Wu, Wand, Huang, Lu, & Pan, 2011) biomedical (Helmke & Gerling, 2018) and in foods (Bourke, Ziuzina, Boehm, Cullen, & Keener, 2018; Coutinho et al., 2018). Plasmas are classified as high-temperature plasma and cold plasma with reference to its gas temperature. Various reactive plasma species are produced depending on the gas used and is increasingly being applied for surface sterilization of solid materials. One of its most striking features is that the process requires no heat or high temperature and has high efficacy in microbial inactivation thus making it idyllic for treatment of food samples. There are a few studies that have examined the use of cold plasma in liquid foods (Gurol, Ekinci, Aslan, & Korachi, 2012; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014). Other studies have explored the potential of cold plasma technology to increase the polyphenol and color stability of sour cherry juice (Garofulić et al., 2015), pomegranate juice (Herceg et al., 2016), chokeberry juice (Kovačević et al., 2016) and blueberry juice (Hou et al., 2019). Zhang et al. (2011) studied the effect of cold atmospheric plasma on microbial inactivation of orange juice while Almeida et al. (2015) and Fernandes, Santos, and Rodrigues (2019) explored its effect on the quality characteristics of orange juice and acerola juice respectively. Recent researches studying the effect of cold plasma on the physicochemical and thermal properties, bioactive compounds and microstructure of chocolate milk drink (Coutinho, Silveira, Fernandes, et al., 2019; Coutinho, Silveira, Pimentel, et al., 2019); physical and thermal properties of whey-based beverages (Silveira et al., 2019); curing efficacy on ground ham (Lee et al., 2018) and surface decontamination efficacy on RTE ham (Yadav et al., 2019), cheese and agar medium (Wan, Pankaj, Mosher, & Keener, 2019) add more emphasis on the flexibility in use and efficiency of cold plasma treatments in replacing conventional treatment methods to improve food quality and safety.

In the present study, Cold plasma gas has been bubbled through Coconut Neera at different input voltages and flow rates to study the

* Corresponding author.
E-mail address: mahendran@iifpt.edu.in (R. Mahendran).

https://doi.org/10.1016/j.ifset.2019.102230
Received 19 May 2019; Received in revised form 31 August 2019; Accepted 6 September 2019
Available online 10 September 2019

changes in microbial and physicochemical properties. Neera, an oyster white-colored liquid is obtained by tapping the immature, unopened coconut inflorescence (Kapilan, Kailayalingam, et al., 2015). It is a sweet, low Glycaemic Index drink (GI–35) with a neutral pH and high nutritional value (Kadere, 2012). Due to its richness in sugars, Neera readily undergoes fermentation to toddy with an alcohol content of 5–8%. Conversion of Neera into toddy is promoted by various microbial species like lactic acid bacteria followed by yeasts within few hours of its harvest. Existing methods of preservation in coconut Neera like pasteurization, centrifuging and deodorizing, ultrafiltration and addition of chemical preservatives have various shortcomings. Treatments like pasteurization, despite being successful in the elimination of microbes, can drastically affect the color and taste of the end product (Baliga & Ivy, 1961; Rupasinghe & Yu, 2012; Timmermans et al., 2011). Although Baliga and Ivy (1961) offer some insight into how different temperature-time combinations affect microbial count and nutritional aspects of Neera, there is no definitive study on how different properties of Neera get affected during pasteurization. Addition of chemical preservatives method has possible adverse side effects and a change in palatability leading to a decrease in consumer acceptance. Non-thermal processing like cold plasma is a promising technology that serves as an alternative tool to inactivate microbes at room temperatures and reducing detrimental effects on color, nutrition and other properties of food (Dasan & Boyaci, 2018).

During plasma bubbling, a comparatively large-sized stable and transient bubble is formed which causes the water (~80%) in Coconut Neera to dissociate into hydroxyl radicals and atoms like hydrogen. These reactive species mainly occur inside the bubble and are less concentrated in the gas-liquid interphase, similar to the distribution of radicals in an ultrasound field (Margulis, 1976). Plasma bubbling aids in better distribution of the reactive species thus resulting in a uniform dispersion of the antimicrobial effect. To evaluate the presence of free radicals during cold plasma treatment, Electron Paramagnetic Resonance (EPR)/Electron Spin Resonance (ESR) technique was used. It is a selective analytical tool to detect and quantify excited species having unpaired electrons (commonly called free radicals) (Wu et al., 2012).

Other detection tools such as Optical Emission Spectroscopy (OES) or Mass/energy analyzers (HPR 60) have considerable limitations due to the presence of microbes and the physical state of the Coconut Neera sample that lead to reduced signal-to-noise ratio and other complications (Malović, Puač, Lazović, & Petrović, 2010; Wu et al., 2012). Due to the extremely short lifespan of these free radicals, the addition of certain chemicals called Spin traps is necessary to stabilize the generated radicals to be able to detect them in solution. Takamatsu et al. (2014) and Tani, Ono, Fukui, Ikawa, and Kitano (2012) have reported the use of several spin traps in liquids treated with plasma. Hence, this paper aims to study the effect of plasma bubbling on the production of free radicals and its subsequent effect on the properties of Neera.

2. Materials and methods

2.1. Plasma setup

An indigenously developed cold plasma system (Fig. 1) was used with variable flow rates of 5,7 and 10 liters per hour (lph) using atmospheric air as the feed gas. Plasma was generated at 150 and 200 V with a primary input current of 0.2 and 0.24 A, respectively and was bubbled through 300 mL of fresh Neera for 5 and 10 min. The images of bubbles produced were captured (GigE vision area scan camera, C1600, Genie color series, DALSA), and their size was determined using ImageJ software (Fiji-ImageJ 1.42d, National Institutes of Health, USA). For all treatments in this study, three samples of Neera were taken for the three different flow rates 10, 7 and 5 lph and further, samples were taken from these stock Neera samples for treatments. Hence, the initial values in all experiments have been color-coded in accordance with the flow rates for better understanding and comprehension.

2.2. Electron spin resonance/electron paramagnetic resonance

ESR apparatus (Bruker Biospin, Germany; EMX Plus Source) was used for trapping OH· at room temperature with the following conditions; microwave frequency: 9.78 GHz; sweep width: 200 G; sweep time: 30.72 s; modulation frequency: 100 kHz; center field: 3480 G; the number of scans: 40; power level of 10 mW. Since OH· is one of the main reactive species produced due to the bubbling of plasma in Neera, its analysis was undertaken using DMPO (5,5-dimethyl-1-pyrroline N-oxide) (Sigma-Aldrich Co. Ltd., USA) by dissolving 50 mM into Neera before treatment. In this case, OH· radical has been selected for EPR analysis as it is one of the most important free radicals with high antimicrobial potency generated in the aqueous medium responsible for subsequent production of other radicals through chain reactions. DMPO was used as a spin trapping agent since it has a much higher reaction rate with OH· radicals and forms a more stable adduct with OH·. DMPO-OH· spectrum (Tresp, Hammer, Winter, Weltmann, & Reuter, 2013) is usually a typical four line signal with spin Hamiltonian parameters $a_N = a_H = 14.9$ mT (15 G) and g-value of 2.009. The principle of ESR is given by the following reaction (Kleschyov, Wenzel, & Munzel, 2007).

$$R* + ST \rightarrow R - SA*$$

where R* indicates the free radical intermediate, ST indicates the spin trap used and R-SA* represents the radical spin trap adduct formed. The intensity of the EPR signal is usually proportional to and is an indicator of the amount of spin adduct formed.

2.3. Microbiological analysis

The total bacterial count of fresh and treated Neera were determined using Plate Count Agar (PCA). Treated and untreated Neera samples of 1 mL were serially diluted to 10^{-5} and 10^{-6} concentrations and were then spread-plated onto petri dishes containing PCA agar according to AOAC *Official Methods of Analysis*, *sec* (940.37B) (Chemists & Horwitz, 1990). The plates were incubated at 38 °C for 24 h. Each microbial analysis was done in triplicate, and the results are represented as CFU/mL (Tran & Farid, 2004).

2.4. Physico-chemical properties

2.4.1. TSS, Color, and pH

A hand-held refractometer (Erma, Japan, 0-80^{O}Brix) was used to measure the total dissolved/suspended solids in Neera. The scale of representation is Brix which can be defined as equal to the percent sugar and other dissolved solids in a solution (Cavalcanti, Fernandes, Barbosa, & Vieira, 2008). Hunter-Lab Color Flex EZ, 45/0^{O} Color Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, Virginia, USA) was used for the measurement of fresh and treated Neera. This value is measured in terms of L*a*b* color space (Kathiravan, Nadanasabapathi, & Kumar, 2014). The color difference was calculated using the following equation

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$

where L*, a* & b* represent color values of the initial sample while L, a & b represent color values of plasma treated samples. The pH of fresh and treated samples was determined using the Laqua PH1100 pH meter (Horiba Scientific, Singapore) at ambient temperature (Alves, Nesterenko, Paull, Haddad, & Macka, 2018).

2.5. Nutritional analysis

Samples of 5 mL were analyzed by the Anthrone reagent method (McCready, Guggolz, Silviera, & Owens, 1950) for the evaluation of carbohydrate. Analysis of Vitamin C was done by 2,6-dichlorophenol titration using AOAC Method (Horwitz, 2000 Official Method 967.21,

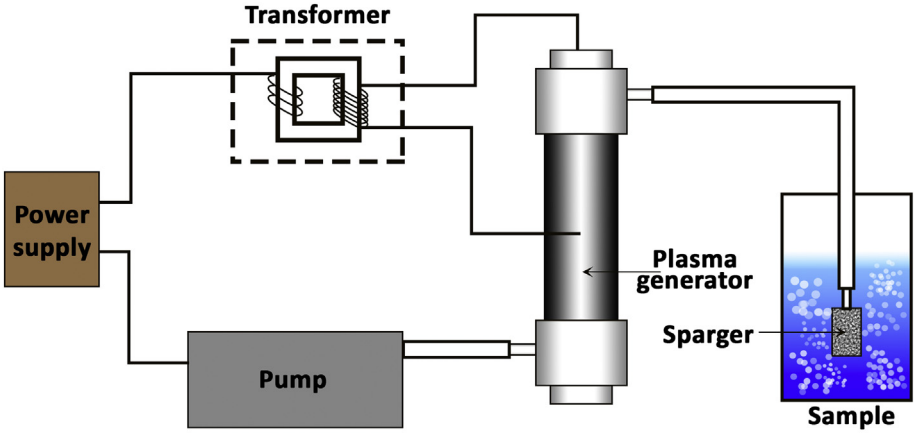

Fig. 1. Experimental plasma bubbling setup.

45.1.14). Moisture, total ash and protein content of Neera were determined using AOAC Methods (Horwitz, 2000 Official Method 925.45 Moisture in Sugars, 900.02, 991.20, respectively).

2.6. Thermal death time value

Thermal death time for conventional thermal treatments is the time required to reduce the original microbial population by 1 log or by 90% in a food (Zahoranová et al., 2016). It is commonly used as an indicator to ascertain the resistance of a given microorganism to heat treatment under specific treatment conditions. The analogue thermal death time (D value) was calculated to find the extent to which the given plasma treatment was effective in microbial reduction. D value was calculated using the formula

$$D = \log \frac{N_0}{N}$$

where N_0 and N are the initial and final microbial count respectively.

3. Results and discussion

3.1. Bubbling of plasma

It was observed that as the flow rate of plasma into Coconut Neera increased, the number of microbubble formed were lesser but the rate of a coalition of bubbles was more. The process of coalescence of two or more microbubble into a macro bubble has been described previously by Duineveld (1996). The increasing pressure inside these bubbles causes their surface to deform and thin. When the thickness of this surface goes below 0.1 μm, these bubbles coalesce into a bigger bubble. The effect of flow rate on bubble diameter shows a linearly increasing trend as shown by Fig. 2. Thus, at higher flow rates, the number of macro bubbles were higher resulting in more significant cavitation pressures.

As the surface area of the bubble becomes more extensive, there is usually an increase in the reactive species accumulated inside the bubble. When these bubbles reach their threshold size, they burst, and this phenomenon is usually called hydrodynamic cavitation. The bubbles are typically characterized based on their high pressure and temperature which contribute to the production of hydroxyl radicals in an aqueous medium. Such reactions are common at the bubble-liquid interface. It was also reported that the concentration of the generated free radicals like OH·, H· were the highest at the bubble center in ultrasound treatment (Riesz, Berdahl, & Christman, 1985). These larger sized bubbles accumulate several free radicals inside them, and when they burst with such high pressures, their antimicrobial effect is amplified when compared to lower flow rates.

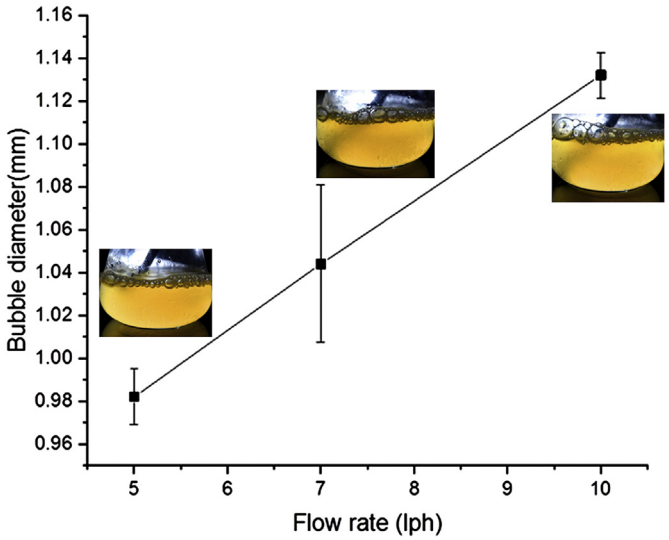

Fig. 2. Bubble diameter as a function of plasma flow rate.

3.2. Plasma species analysis by EPR

The extent of the effect of free radicals such as OH· varies with the number of such species produced. EPR is one of the techniques currently used for free radicals quantification that produces a spectrum of first derivative curves. The relative position of these peaks serves as a unique fingerprint of the reactive species produced while the height of each peak is indicative of their concentration. From the first derivative curves obtained (Fig. 3.), the center peak at g = 2.009 was analyzed. A similar analysis of DMPO-OH· peaks were conducted in a study by Wu et al. (2012) to demonstrate the role of OH· radicals in bacterial inactivation. The intensity of the center peak for each flow rate and treatment time at different input voltages are given in Table 1.

The concentration of free radicals in the food sample was considerably affected by the volume of plasma bubbled inside. A similar trend of the increase was observed when the time of exposure as well as the plasma input voltage was increased. This increase can be seen in terms of an increase in the height of the peaks obtained from the EPR graph. The central peak was taken into consideration owing to its higher signal-to-noise ratio. This change in peak heights can be qualitatively used to determine the number of free radicals like OH· produced. Quantitative estimation of OH· radicals produced can also be done roughly using TEMPO spectrum as a reference standard, and the double integration of the ESR spectra obtained can be plotted against $TEMPO_{ref}$ concentrations (Wu et al., 2012).

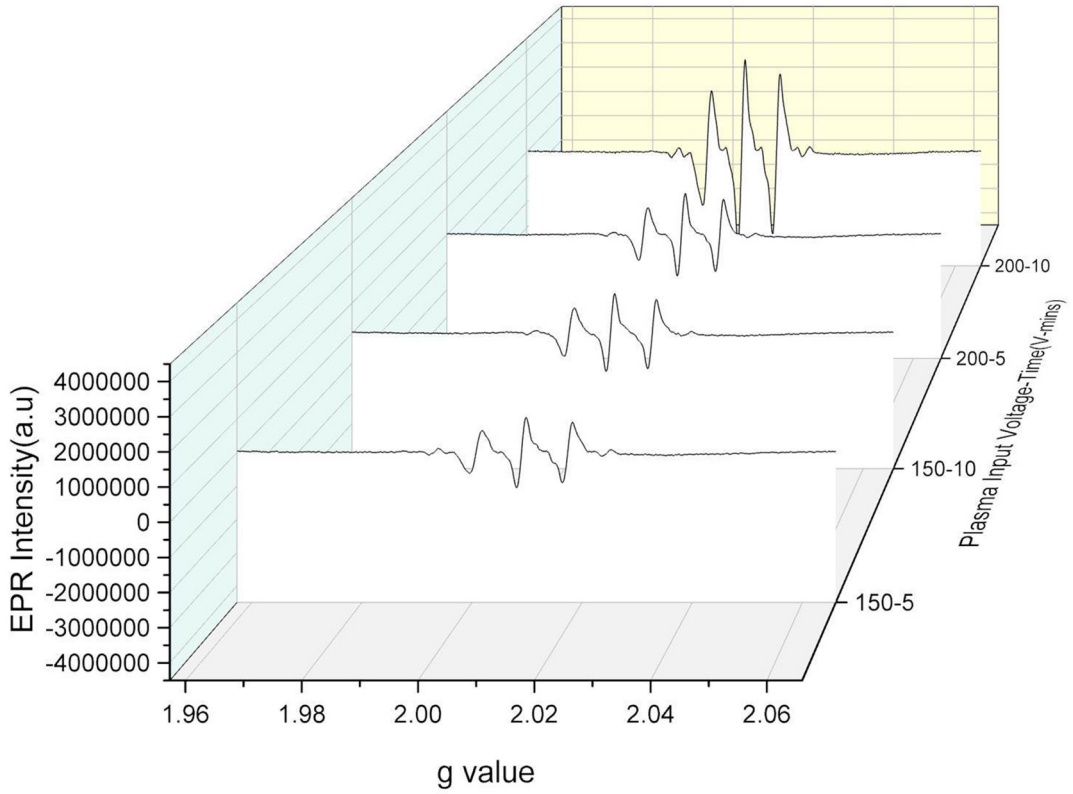

Fig. 3. EPR curves for the flow rate of 10 lph.

Table 1
EPR curve intensities at different treatment combinations.

Plasma flow rate (lpm)	EPR intensity (a.u)			
	150 V – 5 min	150 V – 10 min	200 V – 5 min	200 V – 10 min
5	245,220	278,510	423,850	517,900
7	677,900	747,120	800,950	859,620
10	1,050,700	1,326,200	1,505,000	3,675,400

3.3. Analysis of Physico-chemical properties

3.3.1. pH

It was found that the pH of the sample elevated as the rate and time of plasma bubbling increased (Fig. 4.). This rise in pH may be due to the production of OH· as a result of more splitting of water molecules in Neera as highly reactive radicals like OH·, HO_2 abstract a proton from water thus causing a chain reaction leading to the production of more free radicals. The reactions that take place can be given as follows (Kossyi, Kostinsky, Matveyev, & Silakov, 1992; von Woedtke et al., 2012)

$$O_2 + O_2 \rightarrow O_3 + O^{\cdot}$$

$$O_3 \rightarrow O_2 + O^{\cdot} + e$$

$$H_2O + O^{\cdot} \rightarrow 2OH^{\cdot}$$

Since Neera is primarily composed of sucrose and other sugars, these carbohydrates act as OH· scavengers. The hydroxyl radical abstracts a proton from the sucrose molecule resulting in a sugar-free radical lacking a proton (Peshev, Vergauwen, Moglia, Hideg, & den Ende, 2013). This reaction may be the reason behind the increase in pH unlike other studies involving cold plasma which have commonly reported a decrease in pH (Dasan & Boyaci, 2018; Ikawa, Kitano, & Hamaguchi, 2010; Kim et al., 2015; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013). The reaction between a sugar molecule and a hydroxyl radical was previously suggested by Morelli, Russo-Volpe, Bruno, and Lo Scalzo (2003).

$$(H - C - OH)_n + OH^{\cdot} \rightarrow (\cdot C - OH)_n + H_2O$$

From their study, it was found that disaccharides like sucrose and maltose had significant anti-hydroxyl radical activity and was found to be equal to the activity of the common free radical scavenger, chlorogenic acid. The study also states that the free radical intermediate that is formed after reaction with OH· radicals were more stable than the intermediates formed from other sugars analyzed.

3.3.2. Color value (ΔE value)

Color of Neera is highly influenced by phenolic compounds of which flavonoids constitute two-thirds. These phenolic compounds are believed to contribute to the color and antioxidant properties as suggested for Kalparasa (alternative name for fresh, unfermented Neera) and fresh-cut pitaya fruit (Hebbar, Arivalagan, Manikantan, Mathew, & Chowdappa, 2015; Li et al., 2019). When Neera was subjected to cold plasma treatment, ΔE value was found to be higher when plasma input voltage and time of exposure were increased with inflated flow rates (Fig. 5.).

This can be postulated as due to oxidation of phenolic compounds and flavonoids naturally present in Neera by the reactive species during plasma treatment (Xiang et al., 2018). This oxidation is partly suppressed due to the natural antioxidants present like Ascorbic acid and flavonoids, but when the concentration of free radicals exceeds the concentration of antioxidants, natural color of Neera slightly shifts towards pale white with the maximum color difference of 3.86 found for 10 lph – 200 V – 10 mins treatment. The results obtained are also in accordance with the EPR values (Fig. 3) which indicate maximum OH· radical production thus indicating possible oxidation of the phenolic compounds presents leading to a loss in color. The color difference between control and the plasma treated samples were slightly

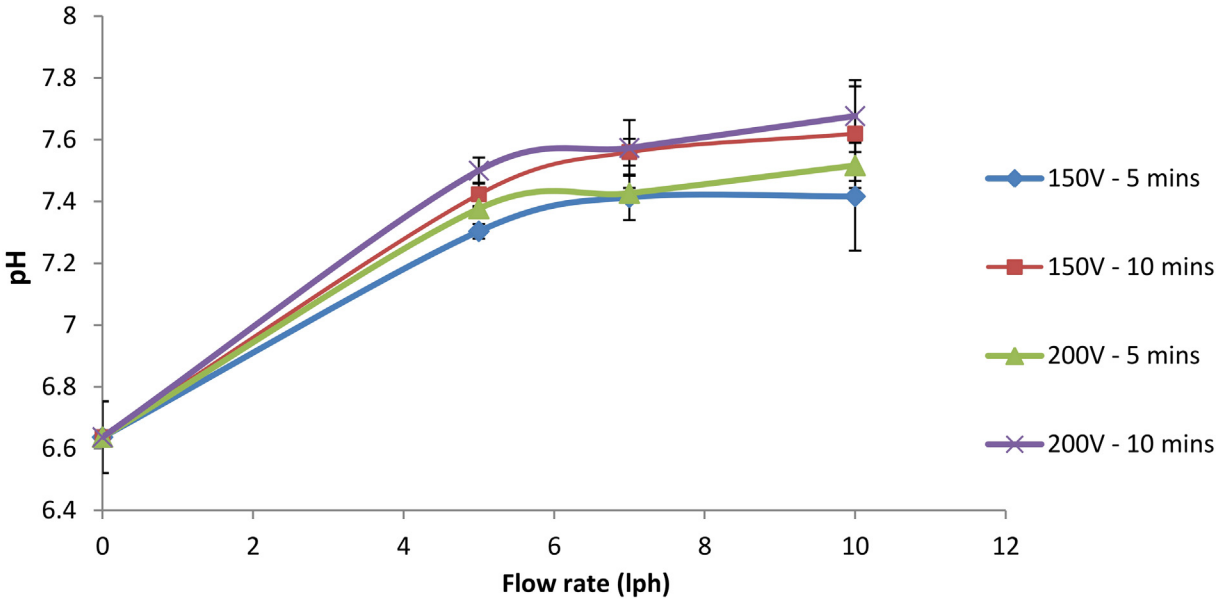

Fig. 4. pH variation with the plasma flow rate.

noticeable ($0.5 \leq \Delta E \leq 1.5$) to noticeable ($1.5 \leq \Delta E \leq 3.0$) for most samples (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006). Parameters like plasma input voltage (Sarangapani, O'Toole, Cullen, & Bourke, 2017) and treatment time (Almeida et al., 2015) along with an optimized volume of the carrier gas can considerably affect the color change in liquid foods. Extreme changes in color that affect the consumer preference of the product can be prevented by effectively optimizing various treatment parameters.

3.3.3. Total suspended solids (TSS)

It was found that cold plasma treatment did not have a considerable effect on TSS of Neera. The maximum increase of 0.6 ^{O}Brix was found at 10 lph - 200 V - 10 min treatment (Fig. 6). This increase can be postulated as due to splitting of water molecules. Liao et al. (2018) suggested a similar hypothesis for apple juice and Wang et al. (2012) for fresh fruit and vegetable slices. The findings from this study are following Zhang et al. (2011) who reported a negligible effect on ^{O}Brix for a DBD plasma treated orange juice.

3.4. Microbial analysis

The various mechanisms of plasma action on the microbial surface aid in antimicrobial effects (Kossyi et al., 1992). In cold plasma, the most common reactive species produced are O·, OH·, O_2 affects the cell membranes and walls of the microbes (Mounir Laroussi, Alexeff, & Kang, 2000). The reactive plasma species affect the microbial cells by oxidizing amino acids, unsaturated fatty acids of the membrane lipids and also by causing irreversible DNA damage. The presence of these radicals, especially OH· can deter the proper functioning of the membrane lipids thus leading to the microbial destruction. To estimate the time required to reduce a given microbial population, it is primordial first to study the complex microbiology of the medium. This helps in

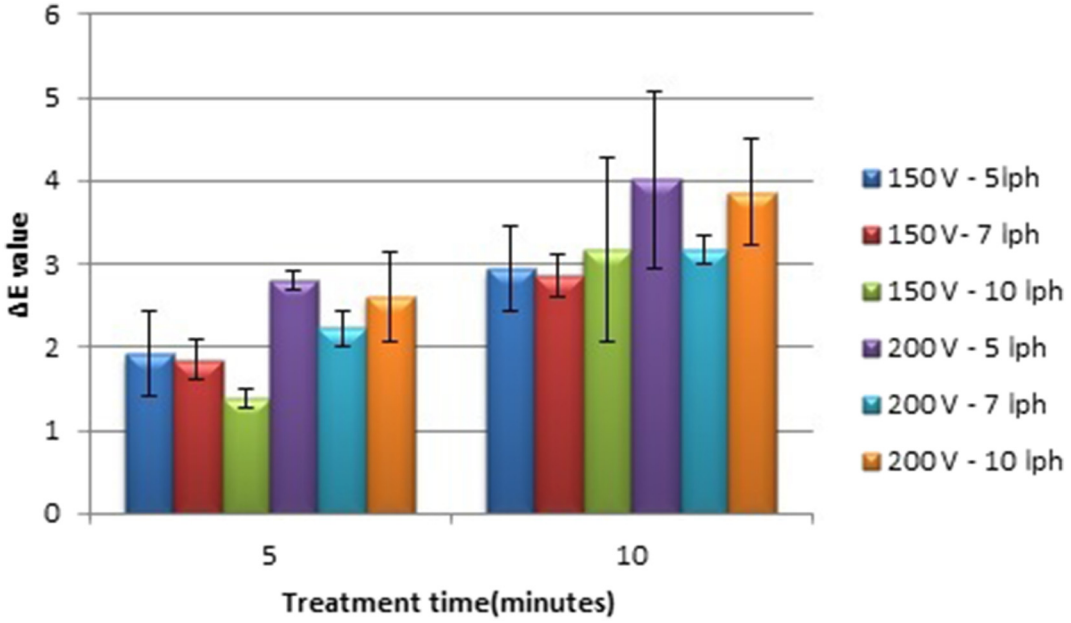

Fig. 5. ΔE value changes for different treatment times.

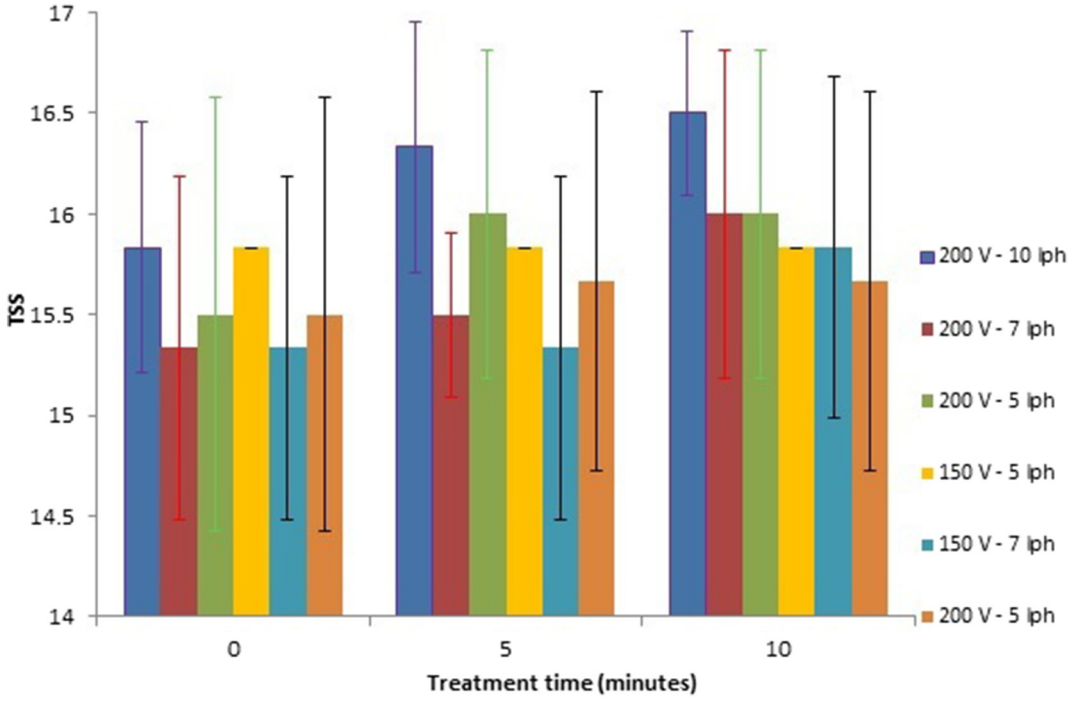

Fig. 6. Change in Total Soluble Solids with different exposure times.

understanding the effect of the given treatment on the various microorganisms present. Previous studies have analyzed that the microbiology of palm as well as coconut Neera, indicates it is usually dominated by Gram-positive bacteria and yeasts (Atputharajah, Widanapathirana, & Samarajeewa, 1986; Nwachukwu, Ibekwe, Nwabueze, & Anyanwu, 2006; Ogbulie, Ogbulie, & Njoku, 2007). Montie, Kelly-Wintenberg, and Roth (2000) found that Gram-negative bacteria get severely damaged and fragmented on plasma treatment. This was further supported by Mendis, Rosenberg, and Azam (2000) who reported that due to the accumulation of charges on the surface, the cell membrane of microbes gets ruptured thus leading to their death. Laroussi, Richardson, and Dobbs (2001) studied the surface morphology of Gram-negative and Gram-positive bacterial species before and after plasma treatment. They proposed a possible explanation for the lack of morphological change despite a decrease in cell viability in the case of Gram-positive species. This is because some reactive plasma species diffuse through the tough outer membrane of Gram-positive bacteria and react with the biomolecules inside. This can either alter the integrity of the cell, eventually leading to its death or can delay/hinder its reproduction without killing the microorganism. They also found that exposure to plasma for short periods only altered their metabolic character but did not necessarily kill them. Montie et al. (2000) reported three other mechanisms of plasma action that lead to microbial death. These include oxidation of microbial proteins, oxidation of DNA due to the formation of base adducts through reaction with oxygen free radicals and peroxidation of membrane lipids by OH· radicals.

From the microbial analysis, it was observed that as plasma input voltage increased, the production of reactive species was amplified resulting in better efficacy in microbial reduction. The EPR analysis also confirmed that as exposure time and flow rate increased, the number of OH· radicals production was enhanced, thus contributing as an additional factor in inactivating microorganisms. Fig. 7. shows the effect of cold plasma on microbial population. A CFU/mL reduction of < 1 log (~80% reduction in CFU/mL values) was observed for the given plasma treatment. This is due to the fact that the flow rate used for inactivation of microorganisms is very less (≤ 10 lph) unlike other cold plasma studies that have employed higher flow rates (Sun et al., 2012). It can be interpreted that as voltage and exposure time were increased, the number of survivors decreased with higher flow rates. This effect can be attributed to the fact that there is a higher input of free radicals from the plasma source as well as their subsequent formation in the liquid food containing bacteria. Although Adubofuor, Amoah, and Osei-Bonsu (2016) have reported a reduction in CFU/mL of up to 93%, they also found that it decreased the nutritional and sensorial aspect of the coconut water samples. This drawback can be eliminated with the proposed cold plasma bubbling method. The extent of microbial survival after cold plasma treatment can be decreased by scaling up flow rate or plasma input voltage resulting in a lesser D value.

3.5. Nutritional analysis

The maximum efficacy of microbial reduction as stated before was obtained for 10 lph-200 V-10 min treatment. Hence to ascertain that there is no loss of nutrients, fresh and treated Neera was analyzed (Table 2.). It was observed that there was a very negligible loss. A slight decrease in Vitamin C content was observed which may be due to the high concentration of the reactive oxygen and nitrogen species generated by cold plasma treatment. A similar decrease was observed by Xu, Garner, Tao, and Keener (2017). Thus, cold plasma treatment is a viable technology that can be used without adversely affecting its nutritional profile.

3.6. Thermal death time value

The analogue of D value found in the present study decreased as the flow rate, and plasma input voltage increased. The least D value of 13.8 min was found for 10 lph-200 V treatment while the maximum D value of 30.51 min was obtained for 5 lph-150 V treatment for an average initial load of 6.32 log CFU/mL (Fig. 8.). The higher D values obtained is due to the combination of lower flow rates-input voltages. By increasing the flow rate as well as input voltage, the efficiency can be increased while bringing down the treatment time. This marked difference in the D values clearly explains the effect of flow rate and plasma voltage on microbial reduction. Similar studies have been conducted by researchers on the effect of various process parameters on

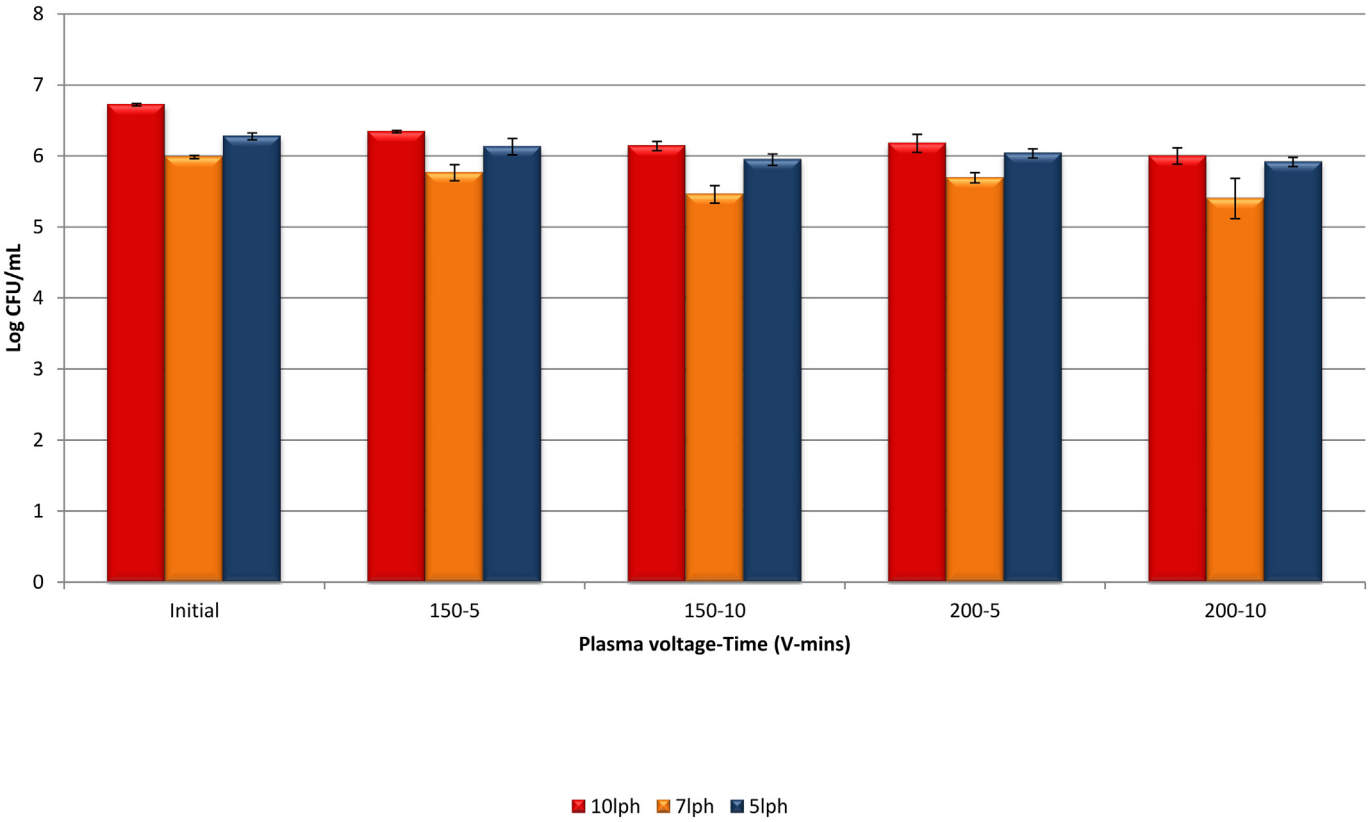

Fig. 7. Microbial reduction (in log CFU/mL) as a function of plasma input voltage and exposure time.

Table 2
Nutritional composition for fresh and plasma treated Neera.

	Fresh	200/10
Vitamin C (mg/100 mL)	33.33333	28.57143
Protein [mg/100 mL]	437.8125	437.8125
Carbohydrate [g/100 mL]	2.486583	2.336315
Moisture (W.B)%	79.00162	81.5739
Ash [mg/100 mL]	243.368	233.59

the D value of microorganisms in solid and liquid foods. Fernandez, Shearer, Wilson, and Thompson (2012) observed that the D value for any microorganism depended on the initial load present in the liquid medium and came up with a linear equation. They claimed that D value showed a significant increase with the increase in initial microbial load. Basaran, Basaran-Akgul, and Oksuz (2008) compared the D values for two different plasmas at different treatment conditions on the surface load of hazelnuts. They found SF_6 plasma to be more effective than air gas plasma with D values of 1.1 and 4.2 mins respectively in the elimination of fungal species *Aspergillus* from both shelled and unshelled nuts. Jahid, Han, and Ha (2014) compared and studied the effect of CAP on planktonic cells and biofilms of *A. hydrophila* on lettuce. They observed that there was a 4D reduction within 6 s in the case of planktonic cells at any temperature while the D value increased with increase in temperature for lettuce with a biofilm on it. They also found that these values were in proportion to the initial load and incubation temperatures. This effect is because, despite inactivation of the top layer of microbes, they form a physical barrier to shield the lower layers of microorganisms from the effect of plasma, thus increasing their chances of survival. This effect is more with higher initial loads (Fernandez et al., 2012) and hence requires longer treatment times as well as higher flow rates and energy input for a sufficient microbial inactivation as observed in this study wherein microbial inactivation increased with higher flow rates as well as input voltages and treatment times.

4. Conclusion

The effect of cold plasma bubbling on microbial inactivation has been ascertained through electron paramagnetic resonance to detect OH· radicals in the solution. No significant changes ($p > 0.05$) in pH, TSS, and color due to plasma treatment were observed while there was a significant reduction in the microbial count due to plasma bubbling ($p < 0.05$). In industrial conditions involving sterilization of bulk quantities of liquid food, this plasma bubbling technique has an edge over thermal treatment methods owing to the better dissolution of plasma species for effective microbial inactivation without significant quality changes. As a future scope of research, similar to z value in thermal treatments, an analogue Z value for plasma bubbling treatments can be found for different flow rates. At present, thermal treatments dominate at the industrial level where commercial sterility is of prime importance but also result in huge losses in nutritional as well as sensorial appeal. The suggested cold plasma bubbling method can be easily used to achieve commercial sterility while also reducing losses in terms of energy as well as nutrition. The ease with which the process parameters can be controlled makes this a promising method capable of delivering safe and nutritious food products to the consumer.

Declaration of competing interest

Authors do not have any conflict of interest.

Acknowledgments

We acknowledge the Science and Engineering Research Board of Department of Science and Technology, Government of India for supporting this research work (SERB, EMR/2017/000247).

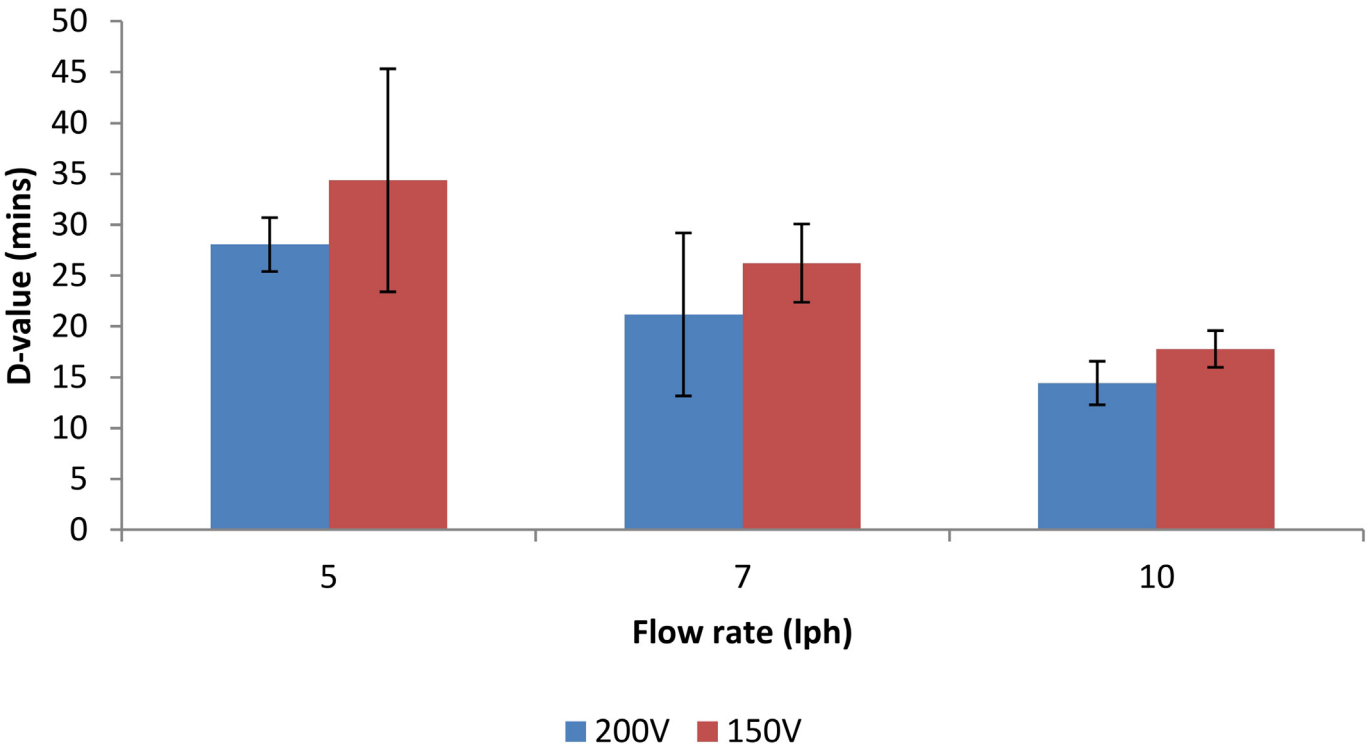

Fig. 8. D value as a function of the flow rate at a different plasma input voltage.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2019.102230.

References

Adubofuor, J., Amoah, I., & Osei-Bonsu, I. (2016). Sensory and physicochemical properties of pasteurized coconut water from two varieties of coconut. *Food Science and Quality Management, 54*(54), 3–12.

Almeida, F. D. L., Cavalcante, R. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N., & Rodrigues, S. (2015). Effects of atmospheric cold plasma and ozone on prebiotic orange juice. *Innovative Food Science and Emerging Technologies, 32*, 127–135. https://doi.org/10.1016/j.ifset.2015.09.001.

Alves, M. N., Nesterenko, P. N., Paull, B., Haddad, P. R., & Macka, M. (2018). Separation of superparamagnetic magnetite nanoparticles by capillary zone electrophoresis using non-complexing and complexing electrolyte anions and tetramethylammonium as dispersing additive. *Electrophoresis, 39*(12), 1429–1436. https://doi.org/10.1002/elps.201800095.

Atputharajah, J. D., Widanapathirana, S., & Samarajeewa, U. (1986). Microbiology and biochemistry of natural fermentation of coconut palm sap. *Food Microbiology, 3*(4), 273–280. https://doi.org/10.1016/0740-0020(86)90009-2.

Baliga, B. P., & Ivy, A. C. (1961). Beverage preservation, pasteurization of palm sap (Neera). *Journal of Agricultural and Food Chemistry, 9*(2), 149–151. https://doi.org/10.1021/jf60114a018.

Basaran, P., Basaran-Akgul, N., & Oksuz, L. (2008). Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment. *Food Microbiology, 25*(4), 626–632. https://doi.org/10.1016/j.fm.2007.12.005.

Bourke, P., Ziuzina, D., Boehm, D., Cullen, P. J., & Keener, K. (2018). The potential of cold plasma for safe and sustainable food production. *Trends in Biotechnology, 36*(6), 615–626. https://doi.org/10.1016/j.tibtech.2017.11.001.

Cavalcanti, A. L., Fernandes, L. V., Barbosa, A. S., & Vieira, F. F. (2008). pH, titratable acidity acidity and total soluble solid content of pediatric antitussive medicines. *Acta Stomatologica Croatica, 42*(2), 164–170.

Chemists, A. A., & Horwitz, W. (1990). *Official methods of analysis* (15th ed.). *Vol. I*, Arlington, VA: AOAC489.

Coutinho, N. M., Silveira, M. R., Fernandes, L. M., Moraes, J., Pimentel, T. C., Freitas, M. Q., et al. (2019). Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry, 278*, 276–283. https://doi.org/10.1016/j.foodchem.2018.11.061.

Coutinho, N. M., Silveira, M. R., Pimentel, T. C., Freitas, M. Q., Moraes, J., Fernandes, L. M., et al. (2019). Chocolate milk drink processed by cold plasma technology: Physical characteristics, thermal behavior and microstructure. *LWT, 102*, 324–329. https://doi.org/10.1016/j.lwt.2018.12.055.

Coutinho, N. M., Silveira, M. R., Rocha, R. S., Moraes, J., Ferreira, M. V. S., Pimentel, T. C., ... Cruz, A. G. (2018). Cold plasma processing of milk and dairy products. *Trends in Food Science and Technology, 74*, 56–68. https://doi.org/10.1016/j.tifs.2018.02.008.

Cserhalmi, Z., Sass-Kiss, A., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies, 7*(1–2), 49–54. https://doi.org/10.1016/j.ifset.2005.07.001.

Dasan, B. G., & Boyaci, I. H. (2018). Effect of cold atmospheric plasma on inactivation of Escherichia coli and physicochemical properties of apple, orange, tomato juices, and sour cherry nectar. *Food and Bioprocess Technology, 11*(2), 334–343. https://doi.org/10.1007/s11947-017-2014-0.

Duineveld, P. C. (1996). *Bouncing and coalescence of two bubbles in water*.

Fernandes, F. A. N., Santos, V. O., & Rodrigues, S. (2019). Effects of glow plasma technology on some bioactive compounds of acerola juice. *Food Research International, 115*, 16–22. https://doi.org/10.1016/j.foodres.2018.07.042.

Fernandez, A., Shearer, N., Wilson, D. R., & Thompson, A. (2012). Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of Salmonella enterica serovar Typhimurium. *International Journal of Food Microbiology, 152*(3), 175–180. https://doi.org/10.1016/j.ijfoodmicro.2011.02.038.

Garofulić, I. E., Jambrak, A. R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., & Herceg, Z. (2015). The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (Prunus cerasus var. Marasca) juice. *LWT-Food Science and Technology, 62*(1), 894–900. https://doi.org/10.1016/j.lwt.2014.08.036.

Gurol, C., Ekinci, F. Y., Aslan, N., & Korachi, M. (2012). Low temperature plasma for decontamination of E. coli in milk. *International Journal of Food Microbiology, 157*(1), 1–5. https://doi.org/10.1016/j.ijfoodmicro.2012.02.016.

Hebbar, K. B., Arivalagan, M., Manikantan, M. R., Mathew, A. C., & Chowdappa, P. (2015). Kalparasa collection and value addition. *Technical Bulletin, 92*, 28.

Helmke, A., & Gerling, T. (2018). Comprehensive clinical plasma medicine. *Comprehensive clinical plasma medicine* (pp. 23–41). . https://doi.org/10.1007/978-3-319-67627-2.

Herceg, Z., Kovačević, D. B., Kljusurić, J. G., Jambrak, A. R., Zorić, Z., & Dragović-Uzelac, V. (2016). Gas phase plasma impact on phenolic compounds in pomegranate juice. *Food Chemistry, 190*, 665–672. https://doi.org/10.1016/j.foodchem.2015.05.135.

Horwitz, W. (2000). *Official methods of analysis of AOAC international* (17th ed.). Gaithersburg, MD: Association of Analytical Chemists International.

Hou, Y., Wang, R., Gan, Z., Shao, T., Zhang, X., He, M., & Sun, A. (2019). Effect of cold plasma on blueberry juice quality. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2019.03.123.

Ikawa, S., Kitano, K., & Hamaguchi, S. (2010). Effects of pH on bacterial inactivation in aqueous solutions due to low-temperature atmospheric pressure plasma application. *Plasma Processes and Polymers, 7*(1), 33–42. https://doi.org/10.1002/ppap.200900090.

Jahid, I. K., Han, N., & Ha, S.-D. (2014). Inactivation kinetics of cold oxygen plasma depend on incubation conditions of Aeromonas hydrophila biofilm on lettuce. *Food Research International, 55*, 181–189. https://doi.org/10.1016/j.foodres.2013.11.005.

Kadere, T. T. (2012). *Baseline survey, biochemical, microbial, and technological studies on "Mnazi"*.

Kapilan, R., Kailayalingam, R. S. M., et al. (2015). Optimization of the usage of commercial lime for the inhibition of fermentation of sweet sugary saps of Borassus

flabellifer and Caryota urens. (pp. 2(12): 60-66). *2*(12), 60–66.
Kathiravan, T., Nadanasabapathi, S., & Kumar, R. (2014). Standardization of process condition in batch thermal pasteurization and its effect on antioxidant, pigment and microbial inactivation of Ready to Drink (RTD) beetroot (Beta vulgaris L.) juice. *International Food Research Journal, 21*(4), 1305.
Kim, H.-J., Yong, H. I., Park, S., Kim, K., Choe, W., & Jo, C. (2015). Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. *Food Control, 47*, 451–456. https://doi.org/10.1016/j.foodcont.2014.07.053.
Kleschyov, A. L., Wenzel, P., & Munzel, T. (2007). Electron paramagnetic resonance (EPR) spin trapping of biological nitric oxide. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 851*(1–2), 12–20. https://doi.org/10.1016/j.jchromb.2006.10.006.
Kossyi, I. A., Kostinsky, A. Y., Matveyev, A. A., & Silakov, V. P. (1992). Kinetic scheme of the non-equilibrium discharge in nitrogen-oxygen mixtures. *Plasma Sources Science and Technology, 1*(3), 207. https://doi.org/10.1088/0963-0252/1/3/011.
Kovačević, D. B., Kljusurić, J. G., Putnik, P., Vukušić, T., Herceg, Z., & Dragović-Uzelac, V. (2016). Stability of polyphenols in chokeberry juice treated with gas phase plasma. *Food Chemistry, 212*, 323–331. https://doi.org/10.1016/j.foodchem.2016.05.192.
Laroussi, M., Richardson, J. P., & Dobbs, F. C. (2001). Biochemical pathways in the interaction of non-equilibrium plasmas with bacteria. *Proc. Electromed 2001 (Portsmouth, VA, 2001). 33*.
Laroussi, M., Alexeff, I., & Kang, W. L. (2000). Biological decontamination by nonthermal plasmas. *IEEE Transactions on Plasma Science, 28*(1), 184–188. https://doi.org/10.1109/27.842899.
Laroussi, M., & Lu, X. (2005). Room-temperature atmospheric pressure plasma plume for biomedical applications. *Applied Physics Letters, 87*(11), 113902. https://doi.org/10.1063/1.2045549.
Lee, J., Jo, K., Lim, Y., Jeon, H. J., Choe, J. H., Jo, C., & Jung, S. (2018). The use of atmospheric pressure plasma as a curing process for canned ground ham. *Food Chemistry, 240*, 430–436. https://doi.org/10.1016/j.foodchem.2017.07.148.
Li, X., Li, M., Ji, N., Jin, P., Zhang, J., Zheng, Y., ... Li, F. (2019). Cold plasma treatment induces phenolic accumulation and enhances antioxidant activity in fresh-cut pitaya (Hylocereus undatus) fruit. *LWT, 115*, 108447. https://doi.org/10.1016/j.lwt.2019.108447.
Liao, X., Li, J., Muhammad, A. I., Suo, Y., Chen, S., Ye, X., ... Ding, T. (2018). Application of a dielectric barrier discharge atmospheric cold plasma (Dbd-Acp) for Eshcerichia coli inactivation in apple juice. *Journal of Food Science, 83*(2), 401–408. https://doi.org/10.1111/1750-3841.14045.
Malović, G., Puač, N., Lazović, S., & Petrović, Z. (2010). Mass analysis of an atmospheric pressure plasma needle discharge. *Plasma Sources Science and Technology, 19*(3), https://doi.org/10.1088/0963-0252/19/3/034014.
Margulis, M. (1976). A. Cavitation-diffusion model of the spatial distribution of radicals in an ultrasonic field. *Russian Journal of Physical Chemistry, 50*, 534–537.
McCready, R. M., Guggolz, J., Silviera, V., & Owens, H. S. (1950). Determination of starch and amylose in vegetables. *Analytical Chemistry, 22*(9), 1156–1158. https://doi.org/10.1021/ac60045a016.
Mendis, D. A., Rosenberg, M., & Azam, F. (2000). A note on the possible electrostatic disruption of bacteria. *IEEE Transactions on Plasma Science, 28*(4), 1304–1306. https://doi.org/10.1109/27.893321.
Montie, T. C., Kelly-Wintenberg, K., & Roth, J. R. (2000). An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials. *IEEE Transactions on Plasma Science, 28*(1), 41–50. https://doi.org/10.1109/27.842860.
Morelli, R., Russo-Volpe, S., Bruno, N., & Lo Scalzo, R. (2003). Fenton-dependent damage to carbohydrates: Free radical scavenging activity of some simple sugars. *Journal of Agricultural and Food Chemistry, 51*(25), 7418–7425. https://doi.org/10.1021/jf030172q.
Nwachukwu, I. N., Ibekwe, V. I., Nwabueze, R. N., & Anyanwu, B. N. (2006). Characterisation of palm wine yeast isolates for industrial utilisation. *African Journal of Biotechnology, 5*(19), https://doi.org/10.5897/AJB06.487.
Ogbulie, T. E., Ogbulie, J. N., & Njoku, H. O. (2007). Comparative study on the microbiology and shelf life stability of palm wine from Elaeis guineensis and Raphia hookeri obtained from Okigwe, Nigeria. *African Journal of Biotechnology, 6*(7), https://doi.org/10.5897/AJB2007.000-2110.
Peshev, D., Vergauwen, R., Moglia, A., Hideg, É., & den Ende, W. (2013). Towards understanding vacuolar antioxidant mechanisms: A role for fructans? *Journal of Experimental Botany, 64*(4), 1025–1038. https://doi.org/10.1093/jxb/ers377.
Riesz, P., Berdahl, D., & Christman, C. L. (1985). Free radical generation by ultrasound in aqueous and nonaqueous solutions. *Environmental Health Perspectives, 64*, 233–252. https://doi.org/10.1289/ehp.8564233.
Rupasinghe, H. P. V., & Yu, L. J. (2012). *Emerging preservation methods for fruit juices and beverages. FOOD ADDITIVE.* 65. https://doi.org/10.5772/32148.
Sarangapani, C., O'Toole, G., Cullen, P. J., & Bourke, P. (2017). Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science and Emerging Technologies, 44*, 235–241. https://doi.org/10.1016/j.ifset.2017.02.012.
Silveira, M. R., Coutinho, N. M., Rocha, R. S., Moraes, J., Esmerino, E. A., Pimentel, T. C., et al. (2019). Guava flavored whey-beverage processed by cold plasma: Physical characteristics, thermal behavior and microstructure. *Food Research International, 119*, 564–570. https://doi.org/10.1016/j.foodres.2018.10.033.
Sun, P., Wu, H., Bai, N., Zhou, H., Wang, R., Feng, H., ... Fang, J. (2012). Inactivation of Bacillus subtilis spores in water by a direct-current, cold atmospheric-pressure air plasma microjet. *Plasma Processes and Polymers, 9*(2), 157–164. https://doi.org/10.1002/ppap.201100041.
Surowsky, B., Fröhling, A., Gottschalk, N., Schlüter, O., & Knorr, D. (2014). Impact of cold plasma on Citrobacter freundii in apple juice: Inactivation kinetics and mechanisms. *International Journal of Food Microbiology, 174*, 63–71. https://doi.org/10.1016/j.ijfoodmicro.2013.12.031.
Takamatsu, T., Uehara, K., Sasaki, Y., Miyahara, H., Matsumura, Y., Iwasawa, A., ... Okino, A. (2014). Investigation of reactive species using various gas plasmas. *RSC Advances, 4*(75), 39901–39905. https://doi.org/10.1039/c4ra05936k.
Tani, A., Ono, Y., Fukui, S., Ikawa, S., & Kitano, K. (2012). Free radicals induced in aqueous solution by non-contact atmospheric-pressure cold plasma. *Applied Physics Letters, 100*(25), 2012–2015. https://doi.org/10.1063/1.4729889.
Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., Der Plancken, I. V., ... Matser, A. M. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innovative Food Science and Emerging Technologies, 12*(3), 235–243. https://doi.org/10.1016/j.ifset.2011.05.001.
Tran, M. T. T., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science & Emerging Technologies, 5*(4), 495–502. https://doi.org/10.1016/j.ifset.2004.08.002.
Tresp, H., Hammer, M. U., Winter, J., Weltmann, K. D., & Reuter, S. (2013). Quantitative detection of plasma-generated radicals in liquids by electron paramagnetic resonance spectroscopy. *Journal of Physics D: Applied Physics, 46*(43), https://doi.org/10.1088/0022-3727/46/43/435401.
von Woedtke, T., Oehmigen, K., Brandenburg, R., Hoder, T., Wilke, C., Hähnel, M., & Weltmann, K.-D. (2012). Plasma-liquid interactions: Chemistry and antimicrobial effects. *Plasma for bio-decontamination, medicine and food security* (pp. 67–78). . https://doi.org/10.1007/978-94-007-2852-3_6.
Wan, Z., Pankaj, S. K., Mosher, C., & Keener, K. M. (2019). Effect of high voltage atmospheric cold plasma on inactivation of Listeria innocua on Queso Fresco cheese, cheese model and tryptic soy agar. *LWT, 102*, 268–275. https://doi.org/10.1016/j.lwt.2018.11.096.
Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., ... Fang, J. (2012). Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: Inactivation and physiochemical properties evaluation. *The European Physical Journal D, 66*(10), 276. https://doi.org/10.1140/epjd/e2012-30053-1.
Wu, H., Sun, P., Feng, H., Zhou, H., Wang, R., Liang, Y., ... Fang, J. (2012). Reactive oxygen species in a non-thermal plasma microjet and water system: Generation, conversion, and contributions to bacteria inactivation—An analysis by electron spin resonance spectroscopy. *Plasma Processes and Polymers, 9*(4), 417–424. https://doi.org/10.1002/ppap.201100065.
Wu, S., Wand, Z., Huang, Q., Lu, X., & Pan, Y. (2011). Study on a room-temperature air plasma for biomedical application. *IEEE Transactions on Plasma Science, 39*(6), 1489–1495. https://doi.org/10.1109/TPS.2011.2132152.
Xiang, Q., Liu, X., Li, J., Liu, S., Zhang, H., & Bai, Y. (2018). Effects of dielectric barrier discharge plasma on the inactivation of Zygosaccharomyces rouxii and quality of apple juice. *Food Chemistry, 254*, 201–207. https://doi.org/10.1016/j.foodchem.2018.02.008.
Xu, L., Garner, A. L., Tao, B., & Keener, K. M. (2017). Microbial inactivation and quality changes in orange juice treated by high voltage atmospheric cold plasma. *Food and Bioprocess Technology, 10*(10), 1778–1791. https://doi.org/10.1007/s11947-017-1947-7.
Yadav, B., Spinelli, A. C., Govindan, B. N., Tsui, Y. Y., McMullen, L. M., & Roopesh, M. S. (2019). Cold plasma treatment of ready-to-eat ham: Influence of process conditions and storage on inactivation of Listeria innocua. *Food Research International, 123*, 276–285. https://doi.org/10.1016/j.foodres.2019.04.065.
Zahoranová, A., Henselová, M., Hudecová, D., Kalináková, B., Kováčik, D., Medvecká, V., & Černák, M. (2016). Effect of cold atmospheric pressure plasma on the wheat seedlings vigor and on the inactivation of microorganisms on the seeds surface. *Plasma Chemistry and Plasma Processing, 36*(2), 397–414. https://doi.org/10.1007/s11090-015-9684-z.
Zhang, G.-J., Shao, X.-J., Shi, X.-M., Wu, X.-L., Ma, Y., & Li, Y.-X. (2011). Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Transactions on Plasma Science, 39*(7), 1591–1597. https://doi.org/10.1109/tps.2011.2142012.
Ziuzina, D., Patil, S., Cullen, P. J., Keener, K. M., & Bourke, P. (2013). Atmospheric cold plasma inactivation of E scherichia coli in liquid media inside a sealed package. *Journal of Applied Microbiology, 114*(3), 778–787. https://doi.org/10.1016/j.fm.2014.02.007.