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Effect of Atmospheric Cold Plasma Treatment on Fruit Juice Quality

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ABSTRACT

Background:

Atmospheric cold plasma (ACP) is a novel non-thermal processing method, In the past decade, research has demonstrated that it is effective in inactivating microorganisms such as *Salmonella enterica subsp. enterica and E. coli O157:H7*, yeasts, and molds in food products. Moreover, it does not require any chemical reagents. The only cost of this treatment is natural gas and electricity. In this way, ACP is also a cost-efficient and environmentally friendly technology. Compared to thermal processing, non-thermal processing can result in greater nutritional value and quality attributes, such as color, pH, and texture. Therefore, it is important to determine the impact of ACP on quality attributes in determining the viability of ACP as alternative to thermal processing treatments.

Scope and approach:

This review paper discusses how ACP affects the sensory quality, nutritional quality, and microbial quality of fruit juice and summarizes the possible mechanisms involved ACP processing of juices.

Key findings and conclusions:

ACP treatment does not visually change the color of the fruit juices such as apple juice, orange juice compared to the untreated fruit juices. The decrease in pH of the juices treated with ACP is not significant. Ascorbic acid content and antioxidant capacity of ACP treated juices are lower than untreated juices but higher than thermally processed treated juices. However, the results on the effect of ACP on the anthocyanin content of juices is inconclusive. ACP treatment results in the reduction of microbial load in fruit juices. The extent of bacterial inactivation depends on the clarity, pH of the juice, depth of the treated sample, and source of cold plasma. In comparison to thermal processing, ACP treatment of fruit juices can produce a safe product by achieving the needed reduction in microbial load, with improved nutritional and sensory quality.

Key words: ACP treatment, cold plasma, sensory quality, nutritional quality, microbial quality

INTRODUCTION

In in past ten years, atmospheric cold plasma (ACP) has proven it can inactivate microorganisms in a variety of food products such as flour, beverages, and meat products. Because ACP is a non-thermal processing treatment method, compare to traditional thermal food processing methods, ACP can minimize the negative adverse effects of extreme heat on food products such as color degradation, texture change, and nutrient loss. Moreover, ACP generates plasma at or near atmospheric pressure and does not require any chemical addition. In this way ACP also is a cost efficient and environmentally friendly technology (Pankaj et al., 2018).

Chemistry of cold plasma

Plasma is the 4th state of matter which is composed of ionized gas consisting of ions, electrons, and uncharged particles (Hoffmann, et al., 2013). According to the thermal equilibrium, there are two kinds of plasma, thermal and low temperature plasma. Low temperature plasma can be divided into quasi-equilibrium plasma and non-thermal plasma (cold plasma, CP). The electrons, neutral species and ions of CP are in thermal non-equilibrium, which means the temperature of the species in the plasma are not same (Misra et al., 2016). Most of energy of CP is stored in the electrons. The temperature of free electrons in CP can reach to about 10000°C (Kljusuric et al., 2016) and the temperature of the ions and neutral particles in CP is approximately room temperature. The density of free electrons in CP is very low, with less than 1% or about one-hundred-million free electrons per cubic centimeter (Misra et al., 2016). Thus, the temperature of CP is relatively close to 40°C, the temperature of more abundant neutral atoms (Hoffmann, et al., 2013). As a result, the ACP is defined as a non-thermal processing method. This review will focus on CP because the temperature of the other nonthermal plasma (quasi-equilibrium plasma) usually ranges from 100°C to 150°C (Misra et al., 2016). The low temperature treatment can minimize the adverse effects of thermal treatments.

All gases, such as air, nitrogen, oxygen, or argon, can be used by ACP to generate cold plasma. Table 1. summarize the reactive species generated in CP from different gas source of the ACP, device systems, and parameters.

Table 1. Reactive species generated in CP.

Device	Gas source	Parameters	Reactive species	Reference
Atmospheric pressure plasma jet (APPJ)	Air	25 kHz, 3000L/h, 650W 0-120s	atomic oxygen (O) ozone (O_3) hydroxyl radicals ($OH^{\cdot}$) nitrite (NO_2), nitrate (NO_3),	Akishev et al.,2018 Dasan, 2018

			hydrogen peroxide ($H_2O_{2,}$) nitrogen dioxide (NO_2)	
APPJ	Argon（purity 99.99%) with oxygen (concentration 0.5%-1%)	1000Hz, 11kV,1.0L/min 11kV, 2-6 min	argon ions (Ar^+) atomic oxygen (O) ozone (O_3) metastable oxygen hydroxyl radicals ($OH^·$) nitric oxide (NO)	Hou et al., 2019
Dielectric barrier discharge (DBD)	MA65(65%O_2 + 30%N_2 +5%CO_2)	60Hz, 90kV, 30-120s	nitric oxide (NO) nitrogen dioxide (NO_2) nitrate ion (NO_3^-) atomic oxygen (O) ozone(O_3) singlet oxygen hydroxyl radical ($OH^·$)	Xu et al., 2017

The reactive gas species, such as ozone and nitrogen oxides, generated in the plasma during the ACP treatment can be characterized by optical emission spectroscopy (OES) and composition and reactive species in the gas after the ACP treatment by optical absorption spectroscopy (OAS). OES uses a HR2000+ spectrometer and optical fibers to detect the reactive species in the gas. The peaks of reactive species can be identified by comparison with the NIST Atomic Spectra Database. (Xu et al., 2017). OAS detect the composition of gas after the treatment by UV-visible HR2000+ spectrometer with optical probes and the UV-visible deuterium-hydrogen lamp as the light source. Both optical probes and UV-visible lamp are inserted into the sealed packaged sample after the ACP treatment. The concentration of the reactive species can be calculated by Beer–Lambert law (Moiseeve et al., 2014).

Device systems of cold plasma

DBD and APPJ are two ACP device systems commonly used in the food processing. The DBD device consists of two metal electrodes with at least one of the electrodes with dielectric barrier. The dielectric barrier can limit the discharge current and avoid arc transition. The step-up transformer provides the voltage for the electrodes. APPJ consists of two concentric electrodes, of which one electrode connects with a radio frequency generator, to generate a radio frequency power at 13.56 MHz (Lu, 2014). The ionized gas flows through the nozzle in the device to contact the sample. The APPJ can produce stable discharge and avoids the arc transition due to the low power implanted to the device (Tendero et al., 2006).

LIMITATIONS OF THERMAL PROCESSING OF FRUIT JUICE

Most consumers determine high-quality juice products by whether it has natural color, flavor, and high nutritional value, such vitamin C and polyphenols. Thermal processing is widely used in the processing of fruit and vegetable juice products to achieve a 5-log reduction of the pertinent pathogen (Food and Drug Administration, 2001). Many quality attributes are degraded during thermal processing of juices due to the high processing temperature needed to produce a safe product. For example, thermal treatment can degrade several nutrients such as vitamin C and carotenoids. The color of the juice can also be changed by heating due to the Maillard reaction and the degradation of the natural pigments, such as chlorophylls, and carotenoids. (Awuah, et al., 2007). Therefore, ACP, as a non-thermal processing method, may improve these quality attributes and increase the nutritional value of the juices.

Table 2. shows the summary of the effect of ACP on the quality attributes of fruit juices. Different conditions and device systems of ACP treatment affect the quality attributes of different juice products in different ways. For example, the anthocyanin content of pomegranate juice is increased after ACP treatment. However, anthocyanin content in the blueberry juice is decreased. The following sections will discuss how ACP treatment affects the quality attributes

of fruit juices in more detail and summarize the possible mechanism for the affect of ACP on the quality attributes.

Table2. Summary of the effect of ACP processing on quality of juice products.

Sample (size)	Plasma	Quality attribute	Reference
Apple juice (*Malus* spp.) (10 mL)	APPJ, 25 kHz, Air, 3000 L/h,650W, 0-120s Direct treatment	• No significant change in pH • Total color change • Phenolic contents increased with treatment longer than 90s	Dasan & Boyaci, 2018
Orange juice (*Citrus* spp.) (10 mL)	APPJ, direct treatment, 25 kHz, Air, 3000 L/h, 650W 0-120s Direct treatment	• No significant change in pH • No significant change in color. • Phenolic contents increased with treatment longer than 90s.	Dasan & Boyaci, 2018
Tomato juice (*Solanum lycopersicum* L.) (10 mL)	APPJ, direct treatment, 25 kHz, Air, 3000 L/h,640W 0-120s Direct treatment	• No significant change in pH • No significant change in color • Phenolic contents increased when the treatment longer than 90s	Dasan & Boyaci, 2018
Sour cherry nectar (*Prunus cerasus* L.) (10 mL)	APPJ, direct treatment, 25 kHz, Air, 3000 L/h,640W 0-120s Direct treatment	• No significant change in pH • No significant change in color • Phenolic contents increased the treatment longer than 90s	Dasan & Boyaci, 2018
White grape	DBD, direct	• No significant change in pH	Pankaj et al.,

juice (*Vitis* spp.) (fully covered the 15 cm diameter petri plate)	trématent, air, 80kV, 60 Hz, 1–4 min Direct treatment	• Juice color became more vivid • Total phenolic content flavonoid contents, DPPH free radicals scavenging, and antioxidant capacity are reduced, and total flavonols content increased.	2017
Pomegranate juice (*Punica granatum* L.) (1.5 mL)	APPJ, direct treatment, 25 kHz, argon, 2.5kV, 1.5dm^3/min, 0-7min Direct treatment	• Total anthocyanin content increased by 21–35% • No significant change in color	Putnik et al., 2016
Blueberry juice (*Vaccinium corymbosum* L.) (0.1–0.5 mL)	APPJ, 1000Hz, argon and oxygen, 11kV,1.0L/min, 11kV, 2-6 min Direct treatment	• Content of anthocyanin, Vitamin C, antioxidant activity decreases with increase of treatment time and concentration of oxygen change the content of anthocyanin at same treatment time. • Total phenolics content increase with the treatment time and concentration of oxygen increase • No significant change in color	Hou et al., 2019
Chokeberry juice (*Arnoia melanocarpa* (Michx.) Elliot) (1.5 mL)	APPJ, 25 kHz, argon, 0.75 dm^3 /min, 3-5 min Direct trématent	• Content of hydroxycinnamic acids increased • Content of anthocyanins decreased	Kljusuric et al., 2016
Coconut water (*Cocos nucifera* L.)	DBD, 60Hz, air, 90kV, 0.6L/min, 30s-120s	• Ascorbic acid content decrease • No significant change in color	Kumar et al., 2019

(25 mL)	Direct treatment		
Orange juice (*Citrus* spp.) (25 mL, 50 mL)	DBD, 60Hz, MA65, dry air, 90kV, 30-120s Direct treatment	• No significant change in pH • No significant change in color • Ascorbic acid content decrease	Xu et al., 2017

SENSORY QUALITY

Color

Color is one of quality attributes that influence whether consumers determine if a product has high quality or not. Therefore, color is one of the significant quality attributes of food products. The natural or artificial pigments and chemical reactions of food determine the color of the food. The effect of ACP on the color of the fruit juice products depends on the different conditions of ACP treatment (Pankaj et al., 2018). After apple juice, orange juice and tomato juice were treated with dry air ACP treatment for 120 s, only the apple juice had a noticeable visual color change, and there was no significant change in color for other two juices (Dasan, 2018). Blueberry juice treated with argon and argon with 0%, 0.5%, 1% oxygen cold plasma separately for 2 min, 5 min, 6 min to test its total color difference. A total color difference between 1.5 and 3 reflects visually noticeable color change. The color of blueberry juice had noticeable visual color change when it was treated with 0.5% oxygen in argon for 6 min (4.27 ± 0.26) and treated with 1% oxygen in argon for 6 min (4.58 ± 0.17). The color change is due to the degradation of the natural pigments, such as anthocyanin and chlorophyll, in the juice during processing (Pankaj et al., 2018).

Anthocyanin content

Anthocyanins are natural water-soluble pigments belonging to the flavonoid family. These pigments appear as blue, purple, red hues in fruits such as grape, blueberry and pomegranate. The color differences between anthocyanins are related to many factors such as the substitution pattern of the B-ring and glucosylation, pH, and temperature. Anthocyanins are not essential nutrients and currently there is no dietary reference in the United States. However, China recommends the daily intake level is 50 mg/d (Wallace & Giusti, 2015). The anthocyanin pigment is degraded by oxygen, light, metal ions, ascorbic acid, and enzymes. The anthocyanins not only function as pigments in foods, but also as antioxidants, and may help to prevent hypertension, liver disorders and diarrhea (Shipp & Abdel-Aal, 2010). In this way, food processors and consumers are very concerned about maintaining anthocyanin content in food products.

Pomegranate juice, chokeberry juice, and blueberry juice are considered good sources of anthocyanins. There is about 1480mg/100g and 365mg/100g anthocyanins in chokeberry and blueberry (Prior & Wu, 2006). The anthocyanin content of pomegranate juice ranged from 9-115 mg/L (Hasnaoui et al., 2011). The anthocyanin is not the essential nutrients, currently there is no dietary reference in the United States. China recommends the daily intake level is 50 mg/d (Wallace & Giusti, 2015). As this result, the performance of anthocyanins content after the ACP treatment is very important attribute for all these juices Cold plasma treatment has been shown to affect the anthocyanin content of these fruit juices (Kljusuric et al. 2016; Hou et al., 2019). The anthocyanin content of chokeberry juice treated by argon (purity 99.99%) plasma treatment for 3 and 5 minutes was 23% lower than untreated juice. It is proposed that the reactive atomic oxygen, metastable oxygen and hydroxyl radicals present in cold plasma cause oxidation of the anthocyanins. In this research, the anthocyanin content of the juice that underwent the thermal processing treatment (80 °C for 2 min) was higher than untreated juice (Kljusuric et al., 2016). Anthocyanins are relatively stable during heating at 70-80 °C, so the heat treatment did not cause degradation of the anthocyanins (Patras et al., 2010). The juice in this study was extracted by a cold press juicer, which resulted in a cloudy juice with many anthocyanins still in the cells. During heating, the cell membranes and cell wall are ruptured, contributing to a release of phenolic compounds and an increase in anthocyanin content after pasteurization (Khoddami, Wilkes, and Roberts 2013). However, the results of anthocyanin content in the case of pomegranate juice are totally different. Compared to untreated juice, the anthocyanin content in argon (purity 99.99%) CP treated juice increased by 21-35% (Putnik et al., 2016). The ROS in CP, such OH and O (3P) combine with Ar+ to contribute to damage of the epidermal tissue layers and allow the anthocyanins stored in the cell vacuoles to be released (Grzegorzewski et al. 2011).

Blueberry juice was treated with CP for 2, 4, 6 minutes with argon, argon with 0.5% oxygen, and argon with 1% oxygen as the plasma source. The anthocyanin content decreased as the treatment time increased (Hou et al., 2019) due to the oxidative degradation of the anthocyanins by the reactive atomic oxygen, metastable oxygen, and hydroxyl radicals in the plasma (Kljusuric et al., 2016). Furthermore, as the concentration of oxygen in the argon increased from 0%-0.5%, the anthocyanin content increased. However, as the concentration of oxygen in the argon increased from 0.5%-1.0%, the anthocyanin content decreased (Hou et al., 2019). The increase in anthocyanin content is attributed to the ROS in CP such as OH and O (3P) which can damage the epidermal tissue layers and allow more anthocyanins to be released (Grzegorzewski et al. 2011). However, at higher oxygen concentrations, there are more reactive oxygen species in the ROS which results in oxidation of the anthocyanins (Hou et al., 2019). Due to complex reaction, the mechanism about how CP affects anthocyanins still are not clear.

pH

The pH of food products should be strictly controlled because pH is related to the taste and shelf life (Pankaj et al., 2018). The pH of some juices after CP treatment usually was slightly reduced as treatment time increased, but not significantly. The pH of white grape juice decreased from 3.38 to 3.30 with the ACP treatment for 1 to 4 minutes (Pankaj et al., 2017) and the pH of orange juice decreased from 3.86 to 3.80 with the atmospheric air cold plasma treatment for 0 to 120 seconds. However, pH change in white grape juice and orange juice are not significant. The reason that pHs of juices are slightly reduced is that the reactive nitrogen species, such as NO, in plasma induce formation of nitric acid and nitrous acid in the products (Xu et al., 2017). However, not all treated fruit juice samples have the same reported outcomes for example, the pH of tomato juice treated by dry air ACP for 120 s was slightly increased but also not significant (Dasan, 2018)

NUTRITIONAL QUALITY

Vitamin C

Vitamin C is a water-soluble essential nutrient with primary roles as an antioxidant against free radicals, and other roles in tissue growth and formation of neurotransmitters. L-ascorbic acid and L-dehydroascorbic acid are the active forms of vitamin C for humans. However, vitamin C is not synthesized by the human body. Diseases associated with deficiencies of vitamin C in the diet, include anemia, scurvy, and bleeding gums. The major dietary sources of vitamin C are citrus fruits, strawberries (*Fragaria ×ananassa* Duch.), and tomatoes (Iqbal, 2004). Vitamin C is degraded by many factors such as temperature, pH, metal catalysts, salt, light, and oxygen. (Lima et al., 1999). The ascorbic acid in the juice (liquid) is a strong reductant, it can be quickly oxidized to dehydroascorbic acid and this reaction is reversible. The oxidation can be catalyzed by many oxidative compounds such as molecular oxygen and heavy metal ions. Dehydroascorbic acid also can be further oxidized to 2,3-diketogulonic acid and other compounds such as threonic acid and oxalic acid especially in the alkaline solution resulting in a loss in vitamin C activity (Washko et al., 1992). Due to the labile nature of vitamin C, the vitamin C content of juices after processing is an important quality attribute.

The treatment of coconut water (Kumar et al., 2019) and blueberry juice (Hou et al., 2019) with atmospheric cold plasma, resulted in a decrease in l-ascorbic acid content (without the dehydroascorbic acid). The treatment of coconut water, by dry air atmospheric cold plasma for 30s to 120s at 90kV did not decrease the l-ascorbic acid content significantly. The reason may be because the initial ascorbic acid content was too low. However, when 400 ppm of ascorbic acid was added to the coconut juice and then treated with the same ACP conditions, the l-ascorbic content decreased 84.35% (Kumar et al., 2019).

Blueberry juice was treated by atmospheric cold plasma at 11kV for 2 min to 6 min. The source of plasma was argon with 0%, 0.5%, and 1% oxygen (purities all above 99.99%). The l-ascorbic acid content of blueberry juice also was measured after the juice was treated by thermal treatment at 85 °C for 15 min. The l-ascorbic in untreated juice is about 0.4 mg/100 ml in fresh blueberry juice. The l-ascorbic acid content after thermal treatment was 2.5µg/ml. At the same concentration of oxygen, l-ascorbic acid content decreased as the treatment time increased. When the treatment time was held constant, the l-ascorbic acid content decreased as the concentration of oxygen increased. Blueberry juices treated with 0% oxygen for 2 min to 6 min treatment time and with 0% to 1% of oxygen for 2 min treatment time and had a higher l-ascorbic acid content that thermally treated blueberry juice. These studies showed that cold plasma treatment decreased the l-ascorbic acid content of the juices but in some specific conditions of ACP the l-ascorbic acid content in fruit juice is higher than thermally treated juice. The ascorbic acid scavenges the free radicals formed during cold plasma treatment and is converted to dehydroascorbic acid, and dehydroascorbic acid also can be further oxidized to

2,3-diketogulonic acid and other compounds resulting in a loss in Vitamin C activity (Hou et al., 2019).

The l-ascorbic acid in untreated juice is about 0.4 mg/100 ml in fresh blueberry juice (Hou et al., 2019) and there is 0 mg/100 ml in coconut water (United State Department of Agriculture, 2018). The daily recommended amounts of vitamin C for men is 90 mg and women is 75 mg. The vitamin C content in both of juice is less than 1% of daily recommended amounts. That means both blueberry juice and coconut water are not good sources of vitamin C and this nutritional quality attribute is not affected the nutrition values of these two juices a lot.

Antioxidant capacity

Due to the metabolism of the human body and external factors, free radicals are formed which can lead to body tissue damage, rheumatoid arthritis, and inflammatory bowel disease. Antioxidants play an important role in scavenging free radicals and preventing free radical formation. As people pay more attention to health, more and more people will consciously select food which contains high level antioxidants. Juice products contain many antioxidants such as Vitamin C, β-carotene, flavonoids, and other plant phenolics (Halliwell, 1996). Therefore, the antioxidant capacity of the juice after treatment is an essential quality attribute.

In two separate studies, the antioxidant capacity of white grape juice (Pankaj et al., 2017) and blueberry juice (Hou et al., 2019) decreased after cold plasma treatment. The cold plasma treatment with air generates ROS and ozone. These reactive species can degrade these compounds and reduce antioxidant capacity. The antioxidant activity of white grape juice treated by cold plasma with atmospheric air for 1 to 4 minutes was compared to juice thermally treated at 95°C for 7 min. The antioxidant capacity after cold plasma treatment decreased as the treatment time increased. However, antioxidant capacity of the juice treated with cold plasma for 1 min and 2 min was higher than the thermally treated juice. The major antioxidants of white grape juice are phenolic compounds and ascorbic acid (Pankaj et al., 2017).

The blueberry juice was treated by cold plasma with argon and different concentration of oxygen (0%, 0.5%, 1%) for 4 to 6 minutes. Increases in treatment time and oxygen concentration both caused reductions in the in the anthocyanins and ascorbic acid, main antioxidants in blueberry juice. The previous part discussed that the degradation of both these antioxidants are related to ROS in cold plasma and the concentration of oxygen (Hou et al., 2019). Losses in ascorbic acid content are attributed to ascorbic acid's role as a free radical scavenger as discussed in the previous section (Hou et al., 2019). The ozone can degrade the aromatic rings of phenolic compounds efficiently, and form hydroxylated and quinone compounds (Almeida et al., 2015), to degrade the phenolic compounds.

MICROBIAL QUALITY

ACP inactivates microorganisms by ionizing air to generate groups of reactive species, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), ultraviolet (UV) radiation, charged particles and electrons (Niemira, 2012). All those active species, especially ROS, play an important role in the inactivation of microorganisms. The study of cold plasma is still in the early stages, so there is no specific mechanism about how cold plasma inactivates microorganisms (Scholtz et al., 2015). However, it is believed that reactive species generated by CP target cell walls, cell membranes, nucleic acids, cytoplasm, proteins and enzymes, and lipids and fatty acids. For example, the reactive species target the cell walls and, cell membranes to damage the internal cellular components, DNA and RNA, and allow the cytoplasm to leak out. The reactive species also can cause protein denaturation and lipid peroxidation to inactive the microorganisms (Niemira, 2012). Moreover, ROS are effective to sterilize Bacillus *Subtilis* and deactivate Bacillus spores. Charged particles play an important role to damage the outer membranes of bacterial cells due to the charge accumulation on the outer surface of the cell membrane. The electrostatic force overcomes the tensile strength of the membrane and ruptures the membrane. Due to the thinner cell membrane of gram-negative bacteria , charged particles are more effective in inactivating gram-negative bacteria than gam-positive bacteria (Hoffmann, et al., 2013).

Yeasts

Yeasts are eukaryotic microorganisms that, they are usually acid tolerant, with the optimal pH range from pH 5.3 to 6.5 and an optimal temperature around 23°C (Hoffman et al., 2015; Gorret et al., 2001). Therefore, yeasts grow easily in unheated fruit juice products. Yeasts can change color, flavor and odors of food products and they can also cause human illness. The ability of atmospheric cold plasma treatments to inactivate yeast is important to fruit juice products. White grape juice was inoculated with ethanol red yeast at an initial concentration of 7.3 log CFU/ml. The juice samples were treated with dry air ACP treatment for 1 to 4 minutes at 90 kV. The yeasts in the white grape juice decreased as the treatment time increased. After 4 minutes, the concentration of yeast was reduced to 0 CFU/ml. The yeast inactivation is due to the reactive gas species generated by atmospheric cold plasma. These reactive gas species, through electroporation, lipid peroxidation, inactivation of enzymes and cleavage of DNA lead to cell death (Xu et al., 2017).

Bacteria

S. enterica and *E. coli* such as *Salmonella enterica* subsp. *enterica* and *E. coli* O157:H7 are common microorganisms that cause foodborne illness and can contaminate a variety of foods, such as eggs, vegetables, fruits, poultry, dairy products, and processed foods (Centers for Disease

Control and Prevention, 2020). The CDC estimates that there are about 1 million foodborne illnesses caused by *S. enterica* every year (Centers for Disease Control and Prevention, 2016). In 2019, there were 382 reported food outbreaks related to pathogenic *E. coli* (Centers for Disease Control and Prevention, 2019). There are several reasons that fruit juice may become contaminated *Salmonella* and *E. coli*. First, fruits may be sprayed with contaminated water and not cleaned thoroughly before juicing. Second, the fruits, or fruit juice may not be stored properly (temperature higher than 40°F) (United States Department of Agriculture, 2013). The thermal processing is the traditional treatment for juice products to achieve a 5-log reduction in bacterial counts, as required by the FDA (Food and Drug Administration, 2001). However, thermal processing decreases the overall quality of juice, such as flavor, color, and nutrition (Awuah et al., 2007). For example, orange juice with initial concentration 104CFU/mL total microbial counts, treated by thermal processing for 75°C for 10 seconds, after the treatment the total microbial counts is lower than detectable levels, but the thermal treatment resulted in an increase in cloudiness of the juice, as measured by a reduction in the light transmission from 23% to 1% (Parish, 1998) Non-thermal processing is an alternative to thermal processing to minimize losses in the nutritional and sensory quality of foods and still achieve a 5-log reduction in bacterial counts. Therefore, it is important to determine, the ability of ACP treatment to inactivate S. enterica and E. coli.

The initial concentration of *E. coli* ATCC 25922 was between 6.18 log CFU/ml and 6.50 log CFU/ml in apple, orange, tomato juices and sour cherry nectar. The juice samples were treated by high voltage atmospheric cold plasma with atmospheric air at 20 °C for 30, 60, 90, and 120 seconds. The survival colony counts decreased as treatment time increased. After 120 seconds, *E. coli* was reduced to 1.59 ± 0.17, 1.43 ± 0.22, 4.02 ± 0.03, and 3.34 ± 0.09 log CFU/mL in orange, tomato, apple juices, and sour cherry nectar, respectively. The high voltage atmospheric cold plasma was better able to inactivate *E. coli* in apple juice and sour nectar than orange and tomato juices. The differences in clarity and pH of the juice affect the effectiveness of the cold plasm treatment. Cloudy juices like orange juice have non-uniform turbid particles and tomato juice has a viscous structure. The solids and components in juices contribute to cloudiness of juice which can protect bacteria from the active species in the cold plasma. These active species are mainly responsible for inactivating microorganisms (Dasan and Boyaci, 2018). The pH of juice is also an important factor that influences the ability of ACP treatment to inactivate microorganisms. When the pH is lower than the 4.7, the ability to inactivate microorganisms is very strong but when the pH is higher than 4.7, bacterial inactivation is relatively weak or almost absent.

In the case of orange juice, *S. enterica* serovar Typhimurium ATCC 14028 with an initial level between 5 to 8 log CFU/ mL was inoculated to 25 ml and 50 ml sample juice, respectively and treated by MA65 (65%O2 + 30%N2 +5%CO2, <5% relative humidity) and dry air high voltage atmospheric cold plasma for 30 s to 120 s with refrigerated storage (4°C) for 24 h before analysis. The effect of sample depth on bacterial inactivation by cold plasma was determined. The population of S. enterica in both samples decreased as the treatment time increased. The orange juice sample with 50 ml (higher depth) treated by MA65 cold plasma for 120 s to result in a 5log reduction while only a 2.81 log reduction was observed for the juice samples treated by air cold plasma. The sample with 25 ml (lower depth) orange juice treated by MA65 cold plasma achieved a 5 log reduction in less than 30s. This research demonstrated that the bacterial inactivation ability of ACP treatment is not only dependent on the treatment time but also the source of cold plasma and the depth of the sample (Xu et al., 2017)

In another study, coconut water, with an initial Salmonella enterica serovar Typhimurium LT2, ATCC 14028 concentration of 4 log CFU/ml was treated by dry air high ACP for 30 s to 120 s followed by refrigerated storage for 24 hours (4 °C) before testing. The result show that there is 1.30±0.30 log reduction in coconut water after 120 s treatment time (Kumar et al., 2019). Ozone, nitrous gases, reactive oxygen species (ROS), such as hydroxyl radicals, and reactive nitrogen species (RNS), such as NO, in the plasms are antibacterial agents that inactivate the microorganisms (Kumar et al., 2019 ; Brandenburg et al., 2010). The compounds target the cell wall, cell membranes to damage the internal cellular components, DNA and RNA, and allow the cytoplasm to leak out (Niemira.2012). NO can also react with ROS to form peroxynitrite that can oxidize the cell membranes to inactivate microorganisms. Ozone is also proven to be an effective antimicrobial agent can attack many cellular constituents, such as enzymes, nucleic acids, and proteins (Khadre, et al., 2001).

CONCLUSIONS

ACP is a novel non-thermal processing method as an alternative to thermal processing. The advantage of ACP is that improved quality attributes, such as color, and pH. In different reported studies about ACP treatment on fruit juice show that most juice has no significant change in color. Compared to the juice samples treated thermally ACP retains more antioxidant capacity. The effect of ACP on the juice quality is related to treatment time, compositions of fruit, and the source of the plasma. The ACP treatment is effective in inactivating microorganisms in fruit juice. The effect of ACP treatment on the inactivation of microorganisms is not only related to treatment time, and source of plasma, but also depends on the depth and pH of the sample. For ACP treatments to be widely used in fruit juice production in the future, further studies should focus on how ACP affects the flavor compounds in juice and shelf life of the products. Several research articles showed that using different APC devices systems (DBD and APPJ) with different conditions of ACP treatment, such as different flow rate, voltage and source of CP to treat fruit juice, affect the quality attributes differently. There are already a few papers discuss how different source of CP to effect quality attributes of juice. Additional research is needed to determine how different device systems, flow rate, and voltage of ACP influence juice quality.

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