INVESTIGATING ATMOSPHERIC COLD PLASMA FOR IMPROVING JUICE SAFETY AND QUALITY

by

EMINE OZEN

(Under the Direction of Rakesh K. Singh)

ABSTRACT

Juices are important in providing essential nutrients and refreshment to consumers. To ensure consumer safety, the Food and Drug Administration (FDA) requires processors to achieve at least 5-log CFU/ml reduction in the population of selected pathogen of concern in finished pasteurized products. Thermal pasteurization is a widely used method; however, it often compromises the sensory, nutritional, and functional properties of juices. In response to this challenge, the atmospheric cold plasma (ACP) method has gained attention as a promising alternative. This research explored the application of ACP treatment as a non-thermal technique to enhance the safety and quality of apple and cantaloupe juices. This novel approach seeks to address the limitations of traditional thermal pasteurization and minimize detrimental effects on juice quality. The study focuses on investigating the efficacy of ACP in reducing pathogenic bacteria while preserving the sensory attributes and nutritional value of juices. ACP achieved 5-log CFU/ml reduction for both acid-adapted and non-adapted *E. coli* K12 within 120 s, and *E. coli* O157:H7 within 90 s in apple juice. ACP protected bioactive compounds in apple and cantaloupe juices. ACP increased the presence of certain aroma-contributing volatiles, such as 1-hexanol, 1-butanol, 2-methyl acetate in apple juice, and hexanal, heptanal, nonanal, decanal, 1-heptanol, and

1-nonanol in cantaloupe juice. However, ACP also decreased the levels of some volatiles, including nonanoic acid, methyl nonanoate, and methyl decanoate, among others.

INDEX WORDS: Atmospheric cold plasma, Apple Juice, Cantaloupe Juice, Quality, Safety, Volatile compounds, Nonthermal processing

INVESTIGATING ATMOSPHERIC COLD PLASMA FOR IMPROVING JUICE SAFETY AND QUALITY

by

EMINE OZEN

B.S., Pamukkale University, Turkiye, 2013

M.S., The University of Georgia, 2018

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2023

INVESTIGATING ATMOSPHERIC COLD PLASMA FOR IMPROVING JUICE SAFETY AND QUALITY

By

EMINE OZEN

Major Professor: Rakesh K. Singh
Committee: Abhinav Mishra
Ron Walcott
Govindaraj Dev Kumar

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2023

DEDICATION

I dedicate this work to my lovely family—my mother, Sukruye Ozen, my father, Hakim Ozen, and my brother, Irfan Ozen. Your constant love, guidance, and belief in me have been the driving force behind my accomplishments.

Thank you for always being there for me.

ACKNOWLEDGEMENTS

Firstly, I would like to express my tremendous appreciation to Dr. Rakesh Singh for his constant support and for being an exceptional mentor when I needed it the most. His guidance and encouragement were pivotal in helping me overcome obstacles and find my way back on track. I would like to extend my heartfelt appreciation to the esteemed members of my committee, Dr. Abhinav Mishra, Dr. Govindaraj Dev Kumar, Dr. Ron Walcott and Dr. Koushik Adhikari for their invaluable guidance, expertise, and support throughout my academic journey. Your commitment to excellence and your willingness to dedicate your time to reviewing my work and providing constructive feedback have been instrumental in shaping the success of my research. I am grateful for the thoughtful discussions, insightful suggestions, and critical insights that each of you shared during committee meetings and throughout the development of my dissertation. I would also like to thank the staff members of our Department of Food Science & Technology, Ms. Karen Simmons, Ms. Lisa Cash, Mr. Carl Ruiz and lab mates for their kind help and support that helped me navigate my PhD with ease. I would also like to thank to Dr. John P. Shields (Electron Microscopy, University of Georgia) for SEM imaging, Gwen Hirsch (Department of Food Science and Technology, University of Georgia) for helping with the BSL-2 laboratories, Dr. Ming-Hsun Shen (Department of Food Science and Technology, University of Georgia) for building the light source for optical absorption spectroscopy, Nikita Shah (Biological Sciences, University of Georgia) for her help, Lida Rahimi Araghi (Food Science and Technology Department, University of Georgia) for helping cantaloupe juice production.

It is my sincere pleasure to express my deep gratitude to the Turkish government for granting me a scholarship and for providing me with the incredible opportunity to study in the United States.

I also thank to my friends for their presence and camaraderie have made my time here even more meaningful, and I am grateful for the connections and friendships that have been formed.

Further and further, my parents for their constant dedication in raising me and their immense love and support for our family. I am deeply appreciative of all the experiences and opportunities that have shaped me into the person I am today.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Consumption of fruits play an important role in maintaining a well-balanced and healthy lifestyle, due to their remarkable amount of essential nutrients, vitamins, dietary fiber, and bioactive compounds etc. (Hertog et al., 1993; Kongkachuichai, Charoensiri, Yakoh, Kringkasemsee, & Insung, 2015; Liu, 2003). Not only do they taste good, but they also offer a multitude of health benefits, including improved digestion, strengthened immune system, and reduced risk of chronic diseases (Serrano, Goñi, & Saura-Calixto, 2007). However, in today's fast-paced society, many consumers find it convenient to drink fruit juices to enjoy their benefits, as it provides an easy way to consume them. While fruit juices offer convenience, ensuring their safety and quality is an important concern. To ensure the safety of the fruit juices, the Food and Drug Administration (FDA) mandates that processors achieve a minimum 5-log (CFU/ml) reduction in resistant microorganisms in pasteurized juice products (US FDA, 2001). Traditional thermal pasteurization, although effective in eliminating harmful microorganisms, can lead to undesirable changes in taste, texture, and nutritional composition (Gonzalez and Barrett, 2010). To preserve the benefits of fruits and maintain the highest quality, non-thermal pasteurization methods have emerged as a promising alternative. Innovative techniques such as high-pressure processing (Marszałek et al., 2017) or pulsed electric field technology (Buckow et al., 2013) have been implemented to maintain the natural flavors, vibrant colors, and essential nutrients of the juice,

while simultaneously ensuring its safety and longevity. Among these emerging technologies, non-thermal atmospheric plasma has garnered significant attention.

Plasma, which is the fourth state of matter, is an ionized gas comprised of electrons, ions, and neutral species-molecules with a near-zero net electrical charge (Bárdos & Baránková, 2010; Misra et al., 2011; Pankaj et al., 2014; Segat et al., 2016). Plasma can be categorized as atmospheric or low-pressure, depending on the pressure conditions. Atmospheric cold plasma (ACP), used in this research is generated under atmospheric conditions, preventing the need for expensive reaction chambers and pressure maintenance systems (Pankaj et al., 2018). Various technologies are employed to generate atmospheric pressure plasma for food processing applications, including microwave (MW), corona discharge (CD), direct barrier discharge (DBD), glow discharge (GDP), resistive barrier discharge (RBD), and atmospheric pressure plasma jet (APPJ) (Leins et al., 2014; Chang et al., 1991; Fridman, 2003; Montie et al., 2000; Ehlbeck et al., 2011). ACP offers the advantage of pathogen decontamination without elevating temperatures or introducing chemical additives. Although this technique has been employed successfully in decontaminating orange juice, white grape juice, apple juice, blueberry juice, sour cherry nectar and tomato juice, the application of this technique to juices lacks extensive research and widespread implementation, due to the processing variations, such as different plasma generation methods, different gases used in the system, different processing times, as well as variations in juice types and amounts (Ozen & Singh, 2020).

The aim of this research was to determine optimal time and gas compositions required to achieve safe and high-quality cloudy apple juice (also called apple cider) by exploring the inactivation mechanisms of selected pathogenic, non-pathogenic, and acid-adapted bacteria through inactivation modeling. Additionally, the study seeks to assess the quality of the cloudy

apple juice and cantaloupe juice by comparing them to both fresh and heat-pasteurized juices after and before storage. This research is among the first to analyze the effect of ACP on the volatile compound of apple and cantaloupe juices. Furthermore, the findings of this study will contribute value to cold plasma applications to juice processing, as a large quantity of juice was utilized, facilitating future scale-up of the system for industrial applications.

The central hypothesis of this research was that ACP processing with optimized time and gas composition can ensure the safety of juices while preserving their quality attributes.

The hypothesis was formulated based on the literature and our preliminary findings that demonstrated that (a) the type of the processing gas to generate ACP and the plasma exposure time are important factors in microbial inactivation, (b) ACP processing can protect the quality of the juices and (c) ACP can improve some juice aroma compounds. With the completion of this work, we were able to understand the effect of feed gas type and ACP treatment time on juice safety, physicochemical properties and volatile compounds of apple and cantaloupe juices.

There were three specific objectives in this project:

1. To investigate the effects of the feed gas used in ACP processing on the inactivation of *E. coli* K12, acid-adapted *E. coli* K12, and *E. coli* O157:H7 in apple cider and understand the inactivation mechanism.
2. To examine the effects of different gasses with different processing times and three weeks of storage on the quality of cloudy apple juice.
3. To determine the effects of ACP processing on apple and cantaloupe juice quality properties including volatile compounds.

References

Bárdos, L., & Baránková, H. (2010). Cold atmospheric plasma: Sources, processes, and applications. *Thin Solid Films*, *518*(23), 6705–6713. https://doi.org/10.1016/j.tsf.2010.07.044

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange juice: a review on microbial, enzymatic, nutritional, and sensory quality and stability. *Comprehensive Reviews in Food Science and Food Safety*, *12*(5), 455–467. https://doi.org/10.1111/1541-4337.12026

Chang, J. S., Lawless, P. A., & Yamamoto, T. (1991). Corona discharge processes. *IEEE Transactions on Plasma Science*, *19*(6), 1152–1166. https://doi.org/10.1109/27.125038

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, T., Brandenburg, R., Von Dem Hagen, T., & Weltmann, K. D. (2011). Low temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, *44*(1). https://doi.org/10.1088/0022-3727/44/1/013002

FDA. (2001). Hazard analysis and critical control points (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. *Federal Register*, *66*, 6137–6202.

Fridman. (2003). Dielectric-barrier discharges: their history, discharge physics, and industrial applications. *Plasma Chemistry and Plasma Processing*, *23*(1), 1–46. https://doi.org/10.1023/A:1022470901385

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. *Journal of Food Science*, *75*(7), R121–R130. https://doi.org/10.1111/j.1750-3841.2010.01763.x

Hertog, M. G. L., Feskens, E. J. M., Kromhout, D., Hertog, M. G. L., Hollman, P. C. H., Hertog, M. G. L., & Katan, M. B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, *342*(8878), 1007–1011. https://doi.org/10.1016/0140-6736(93)92876-U

Kongkachuichai, R., Charoensiri, R., Yakoh, K., Kringkasemsee, A., & Insung, P. (2015). Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. *Food Chemistry*, *173*, 838–846. https://doi.org/10.1016/j.foodchem.2014.10.123

Leins, M., Kopecki, J., Gaiser, S., Schulz, A., Walker, M., Schumacher, U., Stroth, U., & Hirth, T. (2014). Microwave plasmas at atmospheric pressure. *Contributions to Plasma Physics*, *54*(1), 14–26. https://doi.org/10.1002/ctpp.201300033

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, 78(3), 517S-520S.

Marszałek, K., Woźniak, Ł., Kruszewski, B., & Skąpska, S. (2017). The effect of high-pressure techniques on the stability of anthocyanins in fruit and vegetables. *International Journal of Molecular Sciences*, *18*(2), 277. https://doi.org/10.1093/ajcn/78.3.517S

Misra, N. N., Tiwari, B. K., Raghavarao, K. S. M. S., & Cullen, P. J. (2011). Nonthermal plasma inactivation of food-borne pathogens. *Food Engineering Reviews*, *3*(3–4), 159–170. https://doi.org/10.1007/s12393-011-9041-9

Montie, T. C., Kelly-Wintenberg, K., & Reece Roth, J. (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*. https://doi.org/10.1109/27.842860

Ozen, E., & Singh, R. K. (2020). Atmospheric cold plasma treatment of fruit juices: A review. *Trends in Food Science & Technology*, *103*, 144–151. https://doi.org/10.1016/j.tifs.2020.07.020

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'donnell, C. P., Bourke, P., Keener, K. M., & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*(1), 5–17. https://doi.org/10.1016/j.tifs.2013.10.009

Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of cold plasma on food quality: A review. *Foods*, *7*(1), 4. https://doi.org/10.3390/foods7010004

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2016). Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. *Food and Bioproducts Processing*, *98*, 181–188. https://doi.org/10.1016/j.fbp.2016.01.010

Serrano, J., Goñi, I., & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International*, 40(1), 15–21. https://doi.org/10.1016/j.foodres.2006.07.010

CHAPTER 2

ATMOSPHERIC COLD PLASMA TREATMENT OF FRUIT JUICES: A REVIEW

[1]Ozen, E., and Singh, R.K. 2020. *Trends in Food Science & Technology*. 103: 144-151. Reprinted here with permission of the publisher.

Abstract

Conventional thermal processing technologies for pasteurization and sterilization of fruit juices have been used to ensure microbiological safety and enzyme inactivation. However, they could cause quality changes that may lower consumer acceptance. Therefore, an increasing effort has been made in applying novel non-thermal processing technologies to preserve the sensory, nutritional, and functional properties of juices while providing safe products. Atmospheric cold plasma (ACP) is one of such novel non-thermal processing technologies. This review presents an overview of ACP processing applications for fruit juices. Specific areas of research are focused on microbial inactivation and the effect of ACP on nutritional and physicochemical properties of juices. While some general trends can be observed for all juices, the effect of ACP differs based on treatment parameters and juice properties such as, the type of fruit used to make the juice. Thus, an understanding of ACP applications is important for optimization of processing parameters to produce high quality and safe juices.

Introduction

The global consumption of fruit juices has an increasing trend, especially "refrigerated single strength and not from concentrate segments" (Jordão, 2018). Consumers are willing to pay higher prices for premium fruit juices including cold press than thermally processed ones, because of their fresh flavors and perceived health benefits (Bardzik, Ishdorj, & Jang, 2016; Jordão, 2018). Moreover, studies have shown that fruit consumption has a positive impact on health and wellbeing of humans (Duthie, Duthie, & Kyle, 2000; Netzel et al., 2007), due to bioactive compounds like carotenoids, polyphenols, phenolics, anthocyanins (Hertog, Feskens, Kromhout, Hollman, & Katan, 1993; Kongkachuichai, Charoensiri, Yakoh, Kringkasemsee, & Insung, 2015) and phytochemicals (Liu, 2003). Therefore, it been has reported that drinking fruit juices can prevent chronic diseases like cancer and cardiovascular diseases (Ruxton, Gardner, & Walker, 2006).

Conventional thermal processing is the most widely used technology for preservation of juices. Thermal processing has drawbacks of overprocessing due to heat transfer limitations which lead to lower sensory, nutritional and functional properties (Gonzalez & Barrett, 2010). A review of thermal processing technologies, including alternative thermal treatments such as microwave heating and ohmic heating for fruit and vegetable juices has presented information about the quality attributes affected by various techniques. Thermal treatment causes several physical and chemical changes, impairs sensory properties and decreases the bioavailability of some nutrients (Petruzzi et al., 2017). While thermal processing changes the organoleptic properties of juices in addition to other quality changes, consumers are more interested in minimally processed products. Therefore, high quality juices can be achieved by non-thermal processing technologies.

Even though non-thermal processing can produce high quality and nutritious juices, the Food and Drug Administration (FDA) requires processors to achieve at least 5 log reduction in the

population of pertinent pathogens in finished pasteurized products. Pertinent pathogens refer to the most resistant microorganisms and those that cause public health concerns. Pertinent pathogens include *Salmonella* or *Escherichia coli* O157: H7 depending on the treatment type and juice (US FDA, 2018). The FDA rule has accelerated research to find a novel and safe non-thermal process to ensure juice quality and safety. Therefore, several non-thermal technologies have been developed like pulsed electric fields (Buckow, Ng, & Toepfl, 2013), high pressure processing (Marszałek, Woźniak, Kruszewski, & Skapska, 2017), ultrasound processing (Adekunte, Tiwari, Scannell, Cullen, & O'Donnell, 2010), radiation processing (Tremarin, Brandão, & Silva, 2017), and membrane processing (Gurak, Cabral, Rocha-Leão, Matta, & Freitas, 2010) to meet the food safety standards by protecting the physical, nutritional and organoleptic characteristics of juices. Atmospheric cold plasma (ACP) is one of the novel methods with great potential for application in pasteurization of food products with high efficiency (Li & Farid, 2016; Pan, Cheng, & Sun, 2019; Sharma & Singh, 2020). Pankaj and Keener (2018) reviewed both cold plasma applications for fruit juices. The number of research publications have doubled in just a few years since the publication of Pankaj and Keener (2018). This review expanded the scientific knowledge on this topic.

The objective of this review is to present a brief description of ACP technology, including its fundamentals and process parameters in juice applications. The microbial decontamination effectiveness and quality changes are also discussed.

Fundamentals of Non-Thermal Plasma

"Plasma" is the fourth state of matter. It is a neutral ionized gas composed of ions, free radicals, excited and non-excited atoms, and molecules having roughly zero net electrical charge (Bárdos & Baránková, 2010; Misra, Tiwari, Raghavarao, & Cullen, 2011; Pankaj et al., 2014;

Segat, Misra, Cullen, & Innocente, 2016). It was first discovered by Crookes (1879) and after almost 50 years, Tonks and Langmuir (1929) developed plasma generating devices.

Plasma can be classified in many ways based on density, ionization degree, thermodynamic equilibrium, etc. Furthermore, plasma can be divided into thermal, non-thermal, and local thermal equilibriums on the basis of thermal conditions. All species (electrons, ions, neutral species) exist in thermodynamic equilibrium in thermal plasma. However, for non-thermal equilibrium plasmas, the temperature of plasma species is not in the same range. In contrast to thermal plasma, electron temperature (T_e) of non-thermal equilibrium plasma is higher than the gas temperature (T_g) consisting of heavy particles: molecules, atoms, and ions ($T_e >> T_g$) (Kogelschatz, Askihev, & Napartovich, 2005). The temperature of electrons, and positive and neutral ions are in the same range in localized areas in the local equilibrium plasma (Bogaerts, Neyts, Gijbels, & Van der Mullen, 2002; Misra, Pankaj, Segat, & Ishikawa, 2016), but the ion temperature in local equilibrium plasma is much higher than the ion temperature in non-thermal plasmas while the electron temperature is much lower (Liu & Lu, 2010). Non-thermal equilibrium plasmas will be referred to as cold plasma in this review.

Plasma can also be classified as atmospheric or low-pressure plasmas depending on the pressure conditions. Atmospheric plasma is generated at standard atmospheric conditions thereby eliminating the need for a costly reaction chamber and pumps to maintain pressure (Pankaj, Wan, & Keener 2018). The food industry is interested in atmospheric pressure plasma equipment design and process conditions because of the convenience of the plasma environment, which does not require high temperatures and pressure adjustments (Ekezie, Sun, & Cheng, 2017; Misra, Pankaj, Frias, Keener, & Cullen, 2015; Misra et al., 2011).

Different atmospheric pressure plasma generation technologies for food processing applications are used. These technologies are microwave (MW) (Leins et al., 2014), corona discharge (CD) (Chang, Lawless, & Yamamoto, 1991), direct barrier discharge (DBD) (Kogelschatz, 2003), glow discharge (GDP) (Montie, Kelly-Wintenberg, & Reece Roth, 2000), resistive barrier discharge (RBD) and atmospheric pressure plasma jet (APPJ) (Ehlbeck et al., 2011). All these plasma types initiate and sustain electron collision under their specified conditions like radio frequency, gas discharge, photo ionization, etc. to generate plasma (Kogelschatz et al., 2005; Lieberman & Lichtenberg, 2005).

Among all the plasma sources, APPJ and DBD plasmas have the most widely explored configurations for juice decontamination and quality research and are easy to adopt and simple in construction (Figure 1). Also, they are more commercially available and easy to use at atmospheric conditions. DBD plasma includes two electrodes, at least one covered with a selected dielectric barrier material. This barrier creates many micro-discharges by avoiding arc transition from the processing environment. Therefore, DBD plasma provides uniformity and stability in the decontamination of the samples by acting in a large area (Cullen et al., 2014;

Tendero, Tixier, Tristant, Desmaison, & Leprince, 2006; Yong et al., 2015). APPJ involves of two concentric electrodes inside a nozzle through which the carrier gas passes. The inner electrode applies a high voltage (100-250 V) at high frequency (13.56 MHz) to ionize the carrier gases like helium, oxygen, etc., or gas mixtures (Jeong et al., 1998). These gases also blow the active region of the jet plasma and help to push the stream consisting of active particles out of the electrodes (Scholtz, Pazlarova, Souskova, Khun, & Julak, 2015).

Applications of Atmospheric Cold Plasma to Fruit Juices --- effect of cold plasma on safety and quality on fruit juices

Microbiological inactivation by cold plasma

Understanding the mechanism of microbial inactivation by cold plasma is critical for applying this novel technology to food products. There are several studies that have successfully shown the mechanism of microbial inactivation by cold plasma treatments in recent years (Liao et al., 2017; Patil, Bourke & Cullen, 2016; Bermúdez-Aguirre, 2020). Although several studies have successfully demonstrated microbial inactivation of cold plasma, these findings are still inconsistent because of the complex plasma and microbial systems (Liao et al., 2017). Cold plasma inactivation of microorganisms can be biological or physical. In biological inactivation; the bacteria cell can be damaged by UV radiation, lipid peroxidation, protein modulation and induced apoptosis.

It is well known that UV radiation between 200 – 300 nm can induce the dimerization of thymine bases in bacerial DNA, which blocks the replication ability of the cell (Laroussi & Member, 2002). Cold plasma systems emit UV light increasing the inactivation efficacy of the process in addition to effects of reactive species, charged particles, and oxidative species. But, the role of UV radiation is controversial. Although, some researches report that UV radiation created by cold plasma treatment plays an important role in inactiving microorganisms and spores (Boudam et al., 2006; Muranyi, Wunderlich, & Langowski, 2010), others agree that decontamination effect of the plasma is not significantly affected by UV radiation and is mainly controlled by reactive species (Laroussi & Leipold, 2004; Deng, Shi, & Kong, 2006; Lu et al., 2008; Patil et al., 2014).

Oxidation causes damage to either cell lipids or proteins. The membrane lipids are the most vulnerable cell compounds to the plasma components, especially to the reactive oxygen species (ROS) because of their location near the cell membrane and their polyunsaturated fatty acid composition which are very sensitive to oxidation (Montie et al., 2000; Laroussi & Leipold, 2004; Liao et al., 2017). The cold plasma generated reactive species cause oxidative stress when they reach the cell membrane of the bacteria, then produce short-chained fatty acids by the peroxidation of lipids. Therefore, the structure of the membrane lipids is altered, and the cell membrane becomes more fluid (Farr & Kogoma, 1991), and further, the cell loses the ability to transportation in and out of the cell (Laroussi & Leipold, 2004). Yost & Joshi (2015) reported that severe cytotoxic damage occurs by following lipid peroxidation. They could interact with various macromolecules inside of the cell, like oxidizing lipids and damaging proteins,further DNA.

The cold plasma damages proteins and cytoplasmic enzymes during the treatment; Reactive species oxidize side chains of amino acids, break peptide bonds, lead to crosslinks within proteins and increase aggregation (López et al., 2019). Nonetheless, Dobrynin et al. (2009) proposed that plasma alters the 3D structure of proteins by cleaving the peptide bonds and directly decimates the 2D structure (Surowsky, Fischer, Schlueter, & Knorr, 2013; Zhong et al., 2007). However, the effect of the cold plasma on proteins and enzymes is still unknown (Liao et al., 2017; López et al., 2019)

The effect of ROS on intracellular oxidative stress and programmed cell death (PCD) of bacteria, like mammalian cells, has been discovered in recent years (Rice & Bayles, 2008; Bayles, 2014). The studies have proposed that intracellular ROS generated from non-thermal plasma induces apoptosis of bacteria, which is associated with ordered cellular disassembly in the living cells (Li, Zhang, & Huang, 2015; Lunov et al., 2016). However, there is not enough research to

prove this claim. Further research is needed to analyze the microbial decontamination of cold plasma by PCD and oxidative stress.

Microorganisms are also decontaminated by electrostatic disruption and electroporation. Electrostatic distruption is the most studied physical mechanism caused by the accumulation of the charged particles (Laroussi & Member, 2002). These particles create an electrostatic force by charge accumulation on the outer cell membrane surface, resulting in the cell membrane rupture (Mendis, Rosenberg, & Azam, 2000).

Cold plasma acts similarly to the pulse electric field (Misra et al., 2011). When the cells are subjected to the pulsed electric fields, instability in the cell membrane begins, and the electric field causes membrane perforations (Pothakamury, Monsalve-Gonzàlez, Barbosa-Cánovas, & Swanson, 1995). It is claimed that the electric pulses from cold plasma can lead to electroporation in the cell membrane (Misra et al., 2011).

The decontamination efficacy of cold plasma has been found to be affected by different factors that can be categorized as processing parameters, environmental elements, and microbial environment (Misra et al., 2011). The lethal effect of the cold plasma depends on various processing parameters like input power (Deng et al., 2007; Liao et al., 2017), treatment time (Critzer, Kelly-Wintenberg, South, & Golden, 2007), gas type (Eto, Ono, Ogino, & Nagatsu, 2008; Lee et al., 2012; Mogul et al., 2003), flow rate (Zhang, Ma, Qiu, Tang, & Du, 2017) and direct applications (Lu et al., 2008). Environmental factors such as pH (Mañas & Pagán, 2005), food Matrix (Bermúdez-Aguirre, Wemlinger, Pedrow, Barbosa-Cánovas, & Garcia-Perez, 2013; Butscher, Zimmermann, Schuppler, & Rudolf von Rohr, 2016; Fernández, Noriega, & Thompson, 2013) and relative humidity (Guo, Huang, & Wang, 2015) also play an essential role in the sterilization efficacy of the cold plasma. Additionally, the number of microorganisms (Azharonok

et al., 2009) and type of microorganism (Hertwig, Reineke, Ehlbeck, Knorr, & Schlüter, 2015; Montie et al., 2000; Stoffels, Sakiyama, & Graves, 2008) have significant influences on the bactericidal effect of cold plasma.

Table 2.1 shows published studies focused on the application of ACP on fruit juices, including the processing parameters to decontaminate orange juice (Dasan & Boyaci, 2018; Shi et al., 2011; Xu, Garner, Tao, & Keener, 2017), white grape juice (Pankaj, Wan, Colonna, & Keener, 2017), apple juice (Dasan & Boyaci, 2018; Liao et al., 2018; Montenegro, Ruan, Ma, & Chen, 2002; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014; Xiang et al., 2018), blueberry juice (Hou et al., 2019), sour cherry nectar (Dasan & Boyaci, 2018) and tomato juice (Dasan & Boyaci, 2018; Starek et al.,2019). All studies confirmed that ACP can inactivate microorganisms in the juices. But some of the processing conditions like power, current to calculate power by frequency and voltage, flow rate of the processing gas, sample amount, etc., are not indicated precisely. Additionally, more research is needed to understand the effect of food medium on the microbial inactivation. For instance, Gabriel and others (2016) used a microwave atmospheric cold plasma jet with 450 W and 650 W input power to inactivate *Staphylococcus sp.*, *Salmonella enterica, Listeria monocytogenes, Escherichia coli* O157: H7 in young coconut liquid endosperm. Gram-positive *Staphylococcus sp*. exhibited the least resistance compared to gram-negative bacteria, while gram-negative bacteria were more sensitive to ACP than gram-positive species. They concluded that the food medium could affect the susceptibility of microorganisms to ACP.

Understanding microbial inactivation kinetics due to ACP will help the development of predictive microbial inactivation modelling to validate the processing. To date, different kinetic models like Weibull distribution (Liao et al., 2018; Surowsky et al., 2014) and biphasic inactivation

kinetics (Dasan & Boyaci, 2018) have been proposed to describe the inactivation of microorganisms by ACP in juice.

The Weibull model has been successfully used for modelling inactivation of *E. coli* O157:H7 and *Citrobacter freundii* in apple juice by ACP. The shape factor (β) of the model in both studies was larger than 1 indicating that the remaining microbial population was more damaged and susceptible to ACP (Liao et al., 2018; Surowsky et al., 2014). On the other hand, Dasan and Boyaci (2018) observed, in the case of *E. coli*, inoculated in different types of juices, that ACP was lower at the beginning and increased with time. It was concluded that plasma active species accumulated on the surface and absorbed inside the samples during processing. The absorption of the species increased the inactivation rate and created two or three linear segments rather than a linear line in the inactivation curve. Therefore, a biphasic inactivation curve was suggested to present inactivation of microorganisms by ACP. When these studies are compared, a smaller amount of sample (< 4 ml) was used for the Weibull model than a larger amount (11 ml) used in the biphasic model. A larger sample volume might lead to change in the curve pattern. These results reveal that more studies are needed to establish appropriate ACP conditions for the fruit juices.

Effects of atmospheric cold plasma on quality parameters of fruit juices

Consumer acceptance is directly influenced by the quality of foods, which comprises factors like mouthfeel, color, and more, highlighting their sensory impact . There are few studies in the literature that evaluate the effect of ACP processing on the quality of fruit juices. Table 2.2 shows the published studies including experimental conditions, assays and the results of the research in detail.

Food product color is an important attribute in consumer preferences. While color is an indicator of nutritional and organoleptic quality changes of foods during processing treatment, it is used as a quality control parameter by the industry and by researchers. Because pigments (natural or synthetic) and chemical reactions (enzymatic or non-enzymatic) create the main color appearance of the food products, any change in the food color of foods gives information about the effect of processing (Barba, Esteve, & Frígola, 2012).

A varying effect of cold plasma treatment on the color of juices has been reported depending on the processing conditions and the type of the juices (Table 2.2). Increasing cold plasma processing time resulted in color changes like ascorbic acid degregration in fruit juices (Pankaj et al., 2017). Bursać Kovačević and others (2016) stated that the gas flow had the greatest effect on color change when cloudy pomegranate juice was treated with ACP. Increasing gas flow raised total anthocyanin content in the pomegranate juice, and thus the color changed. Overall, color measurements indicate that plasma treatment did not compromise the color of white grape, apple, orange and pomegranate juice (Almeida et al., 2015; Bursać Kovačević et al., 2016; Liao et al., 2018; Pankaj et al., 2017).

The quality of processed foods is closely attributed by pH and acidity. Any drastic change could lead to undesirable impact on consumer acceptability and product shelf-life. The acidity of the juice could be affected by the solubilization of hydroxyl radicals generated during the cold plasma discharge (Pankaj et al., 2017) and change in pH might be due to the formation of nitric acid (HNO_3) and nitrous acid (HNO_2) as well as hydrogen peroxide (H_2O_2) (Helmke et al., 2011; Liu et al., 2010; Oehmigen et al., 2010). Several studies reported that cold plasma treatment changed the pH and/or acidity of orange, white grape and apple juices (Liao et al., 2018; Pankaj et al., 2017; Shi et al., 2011; Xu et al., 2017). Liao and others (2018) stated that the exposure time

had a direct effect on pH and titratable acidity (TA) of apple juice. These results indicate that changes in pH and TA might vary depending on the processing time and the type of juice, since the juices have buffering capacity. Therefore, juices consisting of ascorbic acid, malic acid and citric acid have ability to resist changes in pH (Xu et al., 2017).

Juices mainly consist of carbohydrate as soluble solid. It is difficult for plasma generated active species to reach these macromolecules in their short lifetime (Liao et al., 2018). Therefore, ACP does not show an effect on soluble sugar content (^{o}Brix) of juices (Liao et al., 2018; Shi et al., 2011). Some researchers reported the effect of cold plasma on turbidity (Shi et al., 2011), electrical conductivity (Pankaj et al., 2017) and volatile compounds (Alves Filho et al., 2019; Ma & Lan, 2015) of the orange, white grape and tomato juices (Table 2.2).

There are few reports concerning the loss of bioactive compounds and antioxidant capacities in fruit juice after cold plasma treatment. The effect of cold plasma on the stability of vitamin C is the only vitamin studied by researchers related to juice processing. Vitamin C is a sensitive bioactive compound to different processing conditions (the alkaline environment, heat, oxygen, and UV); hence, it is generally used as a quality indicator, which is a valid criterion for other organoleptic or nutritional components (Barba, Esteve, & Frígola, 2012). Cold plasma treatment had no significant effect on ascorbic acid content of orange juice and tomato juice (Shi et al., 2011; Starek et al., 2019). However, Xu et al., (2017) observed a decrease in vitamin C content of orange juice with increased cold plasma treatment time. This have been a result of oxidation reactions initiated by plasma generated ROS and reactive nitrogen species (RNS) (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008).

Phenolic compounds are beneficial components commonly found in fruits and vegetables. They could be the primary determinant of antioxidant potentials of foods hence they have been

associated with the health benefits (Heim, Tagliaferro, & Bobilya, 2002; Hertog et al., 1993; Parr & Bolwell, 2000). The phenolic compounds in cold plasma treated white grape, pomegranate, chokeberry, and sour cherry juices are shown in Table 2.2. Overall, some studies reported a decrease in the phenolic content of juices after cold plasma treatment (Almeida et al., 2015; Liao et al., 2018; Pankaj et al., 2017). On the other hand, other researchers found an increase in the phenolic content of cold plasma treated juices (Bursać Kovačevic et al., 2016; Garofulić et al., 2015; Herceg et al., 2016). The decrease in the phenolic compounds can be explained by their susceptibility to ozone attack, which is created by cold plasma. Ozone has been reported to be efficient in degrading the aromatic ring of phenolic compounds (Pérez, Torrades, Domènech, & Peral, 2002; Stalter, Magdeburg, Wagner, & Oehlmann, 2011). The increase in phenolic components might be contributed by plant cell wall disruptions. The distrupted cells release phenolic compounds out of the plant cell and this leads to an increase in their concentration in the media (Landbo & Meyer, 2001). Interestingly, most research papers show an increase in phenolic compounds and support the cell wall breakdown by plasma when used to treat cloudy juices. On the other hand, Dasan and Boyaci (2018) stated that the total phenolic content (TPC) of cold plasma treated juices was not affected by the turbidity of the juices. TPC was mainly changed with time. It decreased due to degradation in the aromatic rings of phenolic components in the beginning of the process then increased due to cell wall disruptions. Hence TPC might depend on the processing time for cloudy juices.

The antioxidant capacity of juice is another important quality parameter to be measured after the treatment. Plant-based foods provide dietary antioxidants like vitamin E, flavonoids, vitamin A and other phenolic compounds. Thus, the antioxidant capacity gives considerable information about oxidation resistance and antioxidant contribution, effective in preventing

diseases like cancer (Serrano, Goñi, & Saura-Calixto, 2007). The antioxidant capacity of white grape, apple, and orange juice was evaluated (Almeida et al., 2015; Liao et al., 2018; Pankaj et al., 2017) and while the antioxidant capacity of white grape juice increased with cold plasma treatment, the antioxidant capacity decreased in treated apple and orange juice. Pankaj et al. (2017) stated that the increase in the antioxidant capacity in the white grape juice might be due to an increase in the phenolic content. However, the effect of ACP treatment on phenolic compounds and antioxidant capacity of juice remains unclear.

Advantages and Limitations

Cold plasma treatment of fruit juices is a promising technology in that it is rapid and the increased temperature is acceptable (López et al., 2019). However, there are few studies reporting the effects of cold plasma on the fruit juices (Table 2.2). Even though cold plasma treatment has a good bacterial inactivation rate, only small amount of sample was used in the reported research. More research is needed using large sample to predict industrially important process parameters, like processing time and gas flow rates for the treatment of juices. Additionally, the reported experimental methodologies are not adequate to validate and independently verify the processing conditions. Therefore, further research is needed to be consistent with previous studies, which have been done on cold plasma juice treatment.

The use of air in cold plasma treatment is more cost-effective than other types of gasses (Niemira, 2012). Air as a processing gas was used in most of the juice studies, because it is a cheap and effective way to inactivate microorganisms while protecting product quality. For a full evaluation of the effect of cold plasma in juice, more food quality and safety research should be undertaken to provide more information about microbial inactivation, nutritional changes, color, etc. Moreover, while researchers claim this technology is safe for food processing, more research

is needed to prove that cold plasma treated food products do not have any toxic residues and other chemical changes.

Proposed Work

Atmospheric cold plasma (ACP) as a novel non-thermal processing technology application to fruit juices is reviewed in this paper. ACP applications have a potential to satisfy consumer demands when the juice industry adopts this novel technology. ACP is being used in many areas, but the reported research reveals that more studies are needed in the area of fruit juice processing. Furthermore, there is not enough standardization in processing conditions of the juices to make a comparison among various studies. Consequently, as uncertainty comes from the literature, the operating conditions should be reported in detail. More studies need to report details of processing conditions so the technology can be adapted for large-scale processes.

References

Adekunte, A., Tiwari, B. K., Scannell, A., Cullen, P. J., & O'Donnell, C. (2010). Modelling of yeast inactivation in sonicated tomato juice. *International Journal of Food Microbiology*, *137*(1), 116-120. https://doi.org/10.1016/j.ijfoodmicro.2009.10.006

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 Filho, E. G., Rodrigues, T. H. S., Fernandes, F. A. N., de Brito, E. S., Cullen, P. J., Frias, J. M., ... & Rodrigues, S. (2019). An untargeted chemometric evaluation of plasma and ozone processing effect on volatile compounds in orange juice. *Innovative Food Science & Emerging Technologies*, 53, 63-69. https://doi.org/10.1016/j.ifset.2017.10.001

Bogaerts, A., Neyts, E., Gijbels, R., & Van der Mullen, J. (2002). Gas discharge plasmas and their applications. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 57(4), 609-658. https://doi.org/10.1016/S0584-8547(01)00406-2

Barba, F. J., Esteve, M. J., & Frígola, A. (2012). High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: A review. *Comprehensive Reviews in Food Science and Food Safety*, *11*(3), 307–322. https://doi.org/10.1111/j.1541-4337.2012.00185.x

Bardzik, A., Ishdorj, A., & Jang, J. (2016). Super-premium fruit and vegetable beverages: A retail sales analysis and demand estimation. *Agricultural and Resource Economics Review*, 45(3),

563-580. https://doi.org/10.1017/age.2016.24

Bárdos, L., & Baránková, H. (2010). Cold atmospheric plasma: Sources, processes, and applications. *Thin Solid Films*, *518*(23), 6705–6713. https://doi.org/10.1016/j.tsf.2010.07.044

Bermúdez-Aguirre, D. (2020). Advances in the inactivation of microorganisms and viruses in food and model systems using cold plasma. *In Advances in Cold Plasma Applications for Food Safety and Preservation* (pp. 49-91). Academic Press. doi.org/10.1016/B978-0-12-814921-8.00002-5

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange juice: A review on microbial, enzymatic, nutritional, and sensory quality and stability. *Comprehensive Reviews in Food Science and Food Safety*, 12(5), 455-467. https://doi.org/10.1111/1541-4337.12026

Bursać Kovačević, D., Gajdoš Kljusurić, J., 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

Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, *190*, 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099

Chang, J. S., Lawless, P. A., & Yamamoto, T. (1991). Corona discharge processes. *IEEE Transactions on Plasma Science*, *19*(6), 1152–1166. https://doi.org/10.1109/27.125038

Crookes, W. (1879). On the illumination of lines of molecular pressure, and the trajectory of molecules. *American Journal of Science*. https://doi.org/10.2475/ajs.s3-17.99.218

Cullen, P. J., Misra, N. N., Han, L., Bourke, P., Keener, K., O'Donnell, C., … Milosavljević, V.

(2014). Inducing a dielectric barrier discharge plasma within a package. *IEEE Transactions on Plasma Science*, *42*(10), 2368–2369. https://doi.org/10.1109/TPS.2014.2321568

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

Duthie, G. G., Duthie, S. J., & Kyle, J. A. M. (2000). Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. *Nutrition Research Reviews*, *13*(1), 79–106. https://doi.org/10.1079/095442200108729016

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Von Woedtke, T., Brandenburg, R., … Weltmann, K. D. (2011). Low temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, *44*(1). https://doi.org/10.1088/0022-3727/44/1/013002

Ekezie, F. G. C., Sun, D. W., & Cheng, J. H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, 69, 46-58. https://doi.org/10.1016/j.tifs.2017.08.007

Garofulić, I. E., Režek Jambrak, A., 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

US Food and Drug Administration (US FDA). (2018). *Guidance for Industry: Questions and Answers on Juice HACCP Regulation.* www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-questions-and-answers-juice-haccp-regulation

Gabriel, A. A., Aba, R. P. M., Tayamora, D. J. L., Colambo, J. C. R., Siringan, M. A. T., Rosario, L. M. D., … Ramos, H. J. (2016). Reference organism selection for microwave atmospheric pressure plasma jet treatment of young coconut liquid endosperm. *Food Control*, *69*, 74–82. https://doi.org/10.1016/j.foodcont.2016.04.034

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. *Journal of Food Science*, 75(7), R121-R130. https://doi.org/10.1111/j.1750-3841.2010.01763.x

Gurak, P. D., Cabral, L. M. C., Rocha-Leão, M. H. M., Matta, V. M., & Freitas, S. P. (2010). Quality evaluation of grape juice concentrated by reverse osmosis. *Journal of Food Engineering*, 96(3), 421-426. https://doi.org/10.1016/j.jfoodeng.2009.08.024

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, 13(10), 572-584. https://doi.org/10.1016/S0955-2863(02)00208-5

Helmke, A., Hoffmeister, D., Berge, F., Emmert, S., Laspe, P., Mertens, N., … Weltmann, K. D. (2011). Physical and microbiological characterisation of *Staphylococcus Epidermidis* inactivation by dielectric barrier discharge plasma. *Plasma Processes and Polymers*, *8*(4), 278–286. https://doi.org/10.1002/ppap.201000168

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

Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P. C. H., & Katan, M. B. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The lancet,342(8878), 1007-1011. doi.org/10.1016/0140-6736(93)92876-U

Hertwig, C., Reineke, K., Ehlbeck, J., Knorr, D., & Schlüter, O. (2015). Decontamination of whole black pepper using different cold atmospheric pressure plasma applications. *Food Control*, 55, 221–229. https://doi.org/10.1016/j.foodcont.2015.03.003

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*, *290*, 79–86. https://doi.org/10.1016/j.foodchem.2019.03.123

Jeong, J. Y. J. Y., Babayan, S. E., Park, J., Selwyn, G. S. G. S. G. S., Hicks, R. F. R. F., Schutze, A., & Sch, A. (1998). The atmospheric-pressure plasma jet: a review and comparison to other plasma sources. *IEEE Transactions on Plasma Science*, *26*(6), 1685–1694. https://doi.org/10.1109/27.747887

Jordão, A. M. (2018). Phenolic compounds in fruit beverages. *Beverages*, 4(2). https://doi.org/10.3390/beverages4020035

Kogelschatz, U. (2003) Dielectric-barrier discharges: Their history, discharge physics, and industrial applications. *Plasma Chemistry and Plasma Processing*, 23(1), 1–46. https://doi.org/10.1023/A:1022470901385

Kogelschatz, U., Akishev, Y. S., Becker, K. H., Kunhardt, E. E., Kogoma, M., Kuo, S., ... & Schoenbach, K. H. (2005). DC and low frequency air plasma sources. *In Non-Equilibrium Air Plasmas at Atmospheric Pressure* (pp. 276-361).

Kogelschatz, U., Askihev, Y. S., & Napartovich, A. P. (2005). History of non-equilibrium air discharges. In K. H. Becker, U. Kogelschatz, K. H. Schoenbach, & R. J. Barker (Eds.), *Non-equilibrium air plasmas at atmospheric pressure* (pp. 17–25). London: Institute of Physics.

Kongkachuichai, R., Charoensiri, R., Yakoh, K., Kringkasemsee, A., & Insung, P. (2015). Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. *Food*

Chemistry, *173*, 838–846. https://doi.org/10.1016/j.foodchem.2014.10.123

Landbo, A. K., & Meyer, A. S. (2001). Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *Journal of Agricultural and Food Chemistry*, *49*(7), 3169–3177. https://doi.org/10.1021/jf001443p

Leins, M., Kopecki, J., Gaiser, S., Schulz, A., Walker, M., Schumacher, U., … Hirth, T. (2014). Microwave plasmas at atmospheric pressure. *Contributions to Plasma Physics*, *54*(1), 14–26. https://doi.org/10.1002/ctpp.201300033

Li, X., & Farid, M. (2016). A review on recent development in non-conventional food sterilization technologies. *Journal of Food Engineering*, *182*, 33–45. https://doi.org/10.1016/j.jfoodeng.2016.02.026

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 *Escherichia coli* inactivation in apple juice. *Journal of Food Science*, *83*(2), 401–408. https://doi.org/10.1111/1750-3841.14045

Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, *75*, 83–91. https://doi.org/10.1016/j.foodcont.2016.12.021

Lieberman, M. A., & Lichtenberg, A. J. (2005). *Principles of plasma discharges and materials processing*. John Wiley & Sons.

Liu, F., Sun, P., Bai, N., Tian, Y., Zhou, H., Wei, S., … Fang, J. (2010). Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. *Plasma Processes and Polymers*, *7*(3–4), 231–236. https://doi.org/10.1002/ppap.200900070

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic

combinations of phytochemicals. *The American journal of clinical nutrition*, 78(3), 517S-520S. https://doi.org/10.1093/ajcn/78.3.517S

Liu, D.W., & Lu, X.P. (2010). Introduction. In: Chu, P.K., Lu, X.P. (Eds.), *Low Temperature Plasma Technology: Methods and Applications*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, p. 3.

López, M., Calvo, T., Prieto, M., Múgica-Vidal, R., Muro-Fraguas, I., Alba-Elías, F., & Alvarez Ordóñez, A. (2019). A review on non-thermal atmospheric plasma for food preservation: Mode of action, determinants of effectiveness and applications. *Frontiers in microbiology*, 10, 622. https://doi.org/10.3389/fmicb.2019.00622

Ma, T. J., & Lan, W. S. (2015). Effects of non-thermal plasma sterilization on volatile components of tomato juice. *International Journal of Environmental Science and Technology*, *12*(12), 3767–3772. https://doi.org/10.1007/s13762-015-0796-z

Marszałek, K., Woźniak, Ł., Kruszewski, B., & Skapska, S. (2017). The effect of high pressure techniques on the stability of anthocyanins in fruit and vegetables. *International Journal of Molecular Sciences*, *18*(2). https://doi.org/10.3390/ijms18020277

Misra, N. N., Pankaj, S. K., Frias, J. M., Keener, K. M., & Cullen, P. J. (2015). The effects of nonthermal plasma on chemical quality of strawberries. *Postharvest Biology and Technology*, *110*, 197–202. https://doi.org/10.1016/j.postharvbio.2015.08.023

Misra, N. N., Pankaj, S. K., Segat, A., & Ishikawa, K. (2016). Cold plasma interactions with enzymes in foods and model systems. *Trends in Food Science and Technology*, *55*, 39–47. https://doi.org/10.1016/j.tifs.2016.07.001

Misra, N. N., Tiwari, B. K., Raghavarao, K. S. M. S., & Cullen, P. J. (2011). Nonthermal Plasma Inactivation of Food-Borne Pathogens. *Food Engineering Reviews*, *3*(3–4), 159–170.

https://doi.org/10.1007/s12393-011-9041-9

Montenegro, J., Ruan, R., Ma, H., & Chen, P. (2002). Inactivation of *E. coli* O157: H7 using a pulsed nonthermal plasma system. *Journal of food science*, 67(2), 646-648. doi.org/10.1111/j.1365-2621.2002.tb10653.x

Montie, T. C., Kelly-Wintenberg, K., & Reece Roth, J. (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*. https://doi.org/10.1109/27.842860

Netzel, M., Netzel, G., Kammerer, D. R., Schieber, A., Carle, R., Simons, L., … Konczak, I. (2007). Cancer cell antiproliferation activity and metabolism of black carrot anthocyanins. *Innovative Food Science and Emerging Technologies*. https://doi.org/10.1016/j.ifset.2007.03.011

Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, *3*(1), 125–142. https://doi.org/10.1146/annurev-food-022811-101132

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K. D., & Von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, *7*(3–4), 250–257. https://doi.org/10.1002/ppap.200900077

Pan, Y., Cheng, J. H., & Sun, D. W. (2019). Cold plasma-mediated treatments for shelf life extension of fresh produce: a review of recent research developments. *Comprehensive Reviews in Food science and Food safety*, 18(5), 1312-1326. https://doi.org/10.1111/1541-4337.12474s

Pankaj, S. K., & Keener, K. M. (2018). Cold plasma processing of fruit juices. *In Fruit juices (pp. 529-537)*. Academic Press. doi.org/10.1016/B978-0-12-802230-6.00026-6

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., O'Neill, L., Jiménez, A., Bourke, P., & Cullen, P. J. (2014). Characterization of polylactic acid films for food packaging as affected by dielectric barrier discharge atmospheric plasma. *Innovative Food Science and Emerging Technologies*, *21*, 107–113. https://doi.org/10.1016/j.ifset.2013.10.007

Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of cold plasma on food quality: A review. *Foods*, 7(1), 4. https://doi.org/10.3390/foods7010004

Pankaj, S. K., Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture*, *97*(12), 4016–4021. https://doi.org/10.1002/jsfa.8268

Parr, A. J., & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, *80*(7), 985–1012. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<985::AID-JSFA572>3.0.CO;2-7

Patil, S., Bourke, P., & Cullen, P. J. (2016). Principles of nonthermal plasma decontamination. *In Cold plasma in food and agriculture* (pp. 143-177). Academic Press. https://doi.org/10.1016/B978-0-12-801365-6.00006-8

Pérez, M., Torrades, F., Domènech, X., & Peral, J. (2002). Treatment of bleaching Kraft mill effluents and polychlorinated phenolic compounds with ozonation. *Journal of Chemical Technology and Biotechnology*. https://doi.org/10.1002/jctb.651

Petruzzi, L., Campaniello, D., Speranza, B., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Thermal treatments for fruit and vegetable juices and beverages: A literature overview. *Comprehensive Reviews in Food Science and Food Safety*, *16*(4), 668–691. https://doi.org/10.1111/1541-4337.12270

Ruxton, C. H., Gardner, E. J., & Walker, D. (2006). Can pure fruit and vegetable juices protect against cancer and cardiovascular disease too? A review of the evidence. *International journal of food sciences and nutrition*, 57(3-4), 249-272. https://doi.org/10.1080/09637480600858134

Scholtz, V., Pazlarova, J., Souskova, H., Khun, J., & Julak, J. (2015). Nonthermal plasma - A tool for decontamination and disinfection. *Biotechnology Advances*, *33*(6), 1108–1119. https://doi.org/10.1016/j.biotechadv.2015.01.002

Segat, A., Misra, N. N., Cullen, P. J., & Innocente, N. (2016). Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. *Food and Bioproducts Processing*, *98*, 181–188. https://doi.org/10.1016/j.fbp.2016.01.010

Serrano, J., Goñi, I., & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International*, *40*(1), 15–21. https://doi.org/10.1016/j.foodres.2006.07.010

Sharma, S., & Singh, R. K. (2020). Cold plasma treatment of dairy proteins in relation to functionality enhancement. *Trends in Food Science & Technology*, 120, 30-36. https://doi.org/10.1016/j.tifs.2020.05.013

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., & Shao, X. J. (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

Stalter, D., Magdeburg, A., Wagner, M., & Oehlmann, J. (2011). Ozonation and activated carbon treatment of sewage effluents: Removal of endocrine activity and cytotoxicity. *Water Research*. https://doi.org/10.1016/j.watres.2010.10.008

Starek, A., Pawłat, J., Chudzik, B., Kwiatkowski, M., Terebun, P., Sagan, A., & Andrejko, D. (2019). Evaluation of selected microbial and physicochemical parameters of fresh tomato juice after cold atmospheric pressure plasma treatment during refrigerated storage. *Scientific Reports*, 9(1), 1-11. https://doi.org/10.1038/s41598-019-44946-1

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

Tendero, C., Tixier, C., Tristant, P., Desmaison, J., & Leprince, P. (2006). Atmospheric pressure plasmas: A review. *Spectrochimica Acta - Part B Atomic Spectroscopy*, *61*(1), 2–30. https://doi.org/10.1016/j.sab.2005.10.003

Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. (2008). Kinetics of freshly squeezed orange juice quality changes during ozone processing. *Journal of Agricultural and Food Chemistry*, *56*(15), 6416–6422. https://doi.org/10.1021/jf800515e

Tonks, L., & Langmuir, I. (1929). Oscillations in ionized gases. *Physical Review*. https://doi.org/10.1103/PhysRev.33.195

Tremarin, A., Brandão, T. R. S., & Silva, C. L. M. (2017). Inactivation kinetics of *Alicyclobacillus acidoterrestris* in apple juice submitted to ultraviolet radiation. *Food Control*. https://doi.org/10.1016/j.foodcont.2016.07.008

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

Yong, H. I., Kim, H. J., Park, S., Alahakoon, A. U., Kim, K., Choe, W., & Jo, C. (2015). Evaluation of pathogen inactivation on sliced cheese induced by encapsulated atmospheric pressure dielectric barrier discharge plasma. *Food Microbiology*, *46*, 46–50. https://doi.org/10.1016/j.fm.2014.07.010

Tables

Table 2.1 Microbial inactivation by atmospheric cold plasma (ACP) in fruit juices.

Product	Target Microorganism	Provided Processing Conditions	Time	Max log reduction	References
		Dielectric Barrier Discharge (DBD)			
Apple juice	*Escherichia coli* O157:H7	Power: 50 W Gas: air Treatment amount: 3 ml	30 s	4.3 log CFU/ml	(Liao et al.,2017)
Apple Juice	*Zygosaccharomyces rouxii*	Power: 90 W Gas: air Sample amount: 3 ml	140 s	5 log CFU/ml	(Xiang et al., 2018)
Apple Juice	*Escherichia coli* O157:H7	Voltage: 9 kV Frequency: 100 Hz Sample amount: 0.8 ml	40 s	> 5 log CFU/ml	(Montenegro et al., 2002)
Orange juice	*Staphylococcus aureus* *Escherichia Coli* *Candida Albicans*	Power: 1.14 W/cm^2 Gas: air Sample amount: 50 µl	12 s 8 s 25 s	> 5 log CFU/ml	(Shi et al., 2011)
Orange Juice	*Salmonella enterica*	Voltage: 90 kV Frequency: 60 Hz Gas: MA65 Sample amount: 50 ml Sample condition: 24 h storage and packaged	120 s	4.7 log CFU/ml	(Xu et al., 2017)
White grape juice	*Saccharomyces cerevisiae*	Voltage: 80 kV Frequency: 60 Hz Gas: air Sample condition: packaged treatment	4 min	7.4 log CFU/ml	(Pankaj et al., 2017)

Table 2.1 Microbial inactivation by atmospheric cold plasma (ACP) in fruit juices (Cont.).

Product	Target Microorganism	Provided Processing Conditions	Time	Max log reduction	References
Atmospheric Jet Plasma (AJP)					
Apple juice Orange juice tomato Juice Sour Cherry Nectar	*Escherichia Coli*	Power: 650 W Gas: air Treatment amount: 11 ml Gas flow: 50 l/min	120 s	4 log CFU/ml 1.6 log CFU/ml 1.4 log CFU/ml 3.3 log CFU/ml	(Dasan & Boyaci, 2018)
Blueberry juice	*Bacillus sp.*	Frequency: 1000 Hz Voltage: 11 kV Gas: argon 1% oxygen Gas flow: 1 l/min	6 min	7.2 log CFU/ml	(Hou et al., 2019)
Gliding Discharge Plasma (GDP)					
Fresh tomato juice	Total aerobic mesophilic bacteria Total yeasts Total molds	Power: 40 W Voltage: 3.8 kV Frequency: 50 Hz Gas: Nitrogen Sample amount: 5 mL Gas flow: 440 l/min	5 min	> 5 log CFU/ml 4.9 log CFU/ml 3.5 log CFU/ml	(Starek et al.,2019)
Pasteurized tomato juice	*Candida albicans* *Saccharomyces cerevisiae*	Power: 40 W Voltage: 3.8 kV Frequency: 50 Hz Gas: Nitrogen Sample amount: 5 mL Gas flow: 440 l/min	1 min 5 min	3.7 log CFU/ml 3.5 log CFU/ml	(Starek et al.,2019)

Table 2.2 Effect of atmospheric cold plasma (ACP) on quality parameters of fruit juices.

Product	Quality Assays	Reported Processing Conditions	Results	References
Dielectric Barrier Discharge (DBD)				
Orange juice	pH, °Brix, total acid, turbidity, vitamin C and shelf life	Voltage: 20 kV Frequency: 60 kHz Gas: air Sample amount: 50 µl Control treatment: raw juice Time: 3-20 s	-Extension in the shelf life of the product -Insignificant change in other parameters	(Shi et al., 2011)
White grape juice	pH, color, acidity, total phenolics, antioxidant capacity, total flavonoids and electrical conductivity	Voltage: 80 kV Frequency: 60 Hz Gas: air Packaged treatment Control treatment: raw juice Time: 1-4 min	-No effect on pH -Very low browning in color -A significant increase in total flavonoids -A decrease in other parameters	(Pankaj et al., 2017)
Apple Juice	pH, color, °Brix, acidity, total phenolics and total antioxidant capacity	Power: 30, 40 and 50 W Frequency: 10 kHz Gas: air Treatment amount: 3 ml Control treatment: raw juice Time: 0-40s	-Insignificance difference in °Brix -A reduction in pH and increase in acidity - A decrease in L^* value, increase in $a^*$and b^* values -Insignificant change in total phenolic and antioxidant capacity	(Liao et al., 2018)
Orange Juice	Volatile components	Voltage: 70 kV Frequency: 50 Hz Gas: Air Treatment amount: 20 ml Control treatment: raw juice Time: 20-40 s	-A decrease in the stability of volatile compounds	(Alves Filho et al., 2019)
Prebiotic orange juice	pH, color, total phenolic content, total antioxidant activity and oligosaccharides	Voltage: 70 kV Frequency: 50 Hz Gas: Air Sample amount: 20 ml Treatment type: direct/indirect Control treatment: raw juice Time: 15-60 s	- A decrease in pH -Lighter and vivid color - A decrease in the phenolic content after 60s in indirect treatment -A significant reduction in antioxidant capacity of directly treated samples -Insignificant difference in oligosaccharides	(Almeida et al., 2015)

Table 2.2 Effect of atmospheric cold plasma (ACP) on quality parameters of fruit juices (Cont.).

Product	Quality Assays	Reported Processing Conditions	Results	References
Apple juice	pH, color, °Brix, TA, TPC, reducing sugars	Power: 90 W Gas: air Sample amount: 3 ml Control treatment: raw juice Time: 0-140 s	-A decrease in pH -An increase in TA and color difference -No effect on °Brix, reducing sugars and TPC	(Xiang et al., 2018)
Orange Juice	pH, color, °Brix, vitamin C, pectin methylesterase activity (PME)	Voltage: 90 kV Frequency: 60 Hz Gas: air and MA65 Sample amount: 25-50 ml Control treatment: pasteurized juice Sample condition: 24 h storage Time: 30-120 s	-Insignificant change in pH and °Brix -A Reduction in vitamin C concentration and PME activity -Slightly noticeable color difference	(Xu et al., 2017)
Atmospheric Jet Plasma (AJP)				
Pomegranate Juice	Phenolic compounds, total polyphenol content (TPC)	Power: 4 W Frequency: 25 kHz Gas: argon Sample amount: 3, 4 and 5 ml Control treatment: pasteurized and raw juice Gas flow: 0.75, 1, 1.25 L/min Time: 3-7 min	-An increase in TPC - An increase in ellagic acid, chlorogenic acid, ferulic acid, catchin and punicalagin 1 amounts -A decrease in protocatechuic acid, caffeic acid and punicalagin 2 amounts	(Herceg et al., 2016)
Chokeberry juice	The stability of polyphenols	Power: 4 W Frequency: 25 kHz Gas: argon Sample amount: 3, 5 and 7 ml Control treatment: pasteurized juice Gas flow: 0.75 L/min Time:3-5 min	-Insignificant change in hydrocinnamic acids -A decrease in flavanols and anthocyanins	(Bursać Kovacevic et al., 2016)

Table 2.2 Effect of atmospheric cold plasma (ACP) on quality parameters of fruit juices (Cont.).

Product	Quality Assays	Reported Processing Conditions	Results	References
Apple Juice Orange juice Tomato Juice Sour Cherry Nectar	pH, color, acidity and total phenolics	Power: 650 W Gas: air Treatment amount: 11 ml Control treatment: raw juice Gas flow: 50 L/min Time: 30-120 s	-No significant effect on color, except for apple juice - An increase in the total phenolics -No effect on pH	(Dasan and Boyaci, 2017)
Sour cherry Marasca juice	Anthocyanin and phenolic acid contents	Power: 4 W Gas: argon Sample amount: 2, 3 and 4 ml Control treatment: pasteurized and raw juice Gas flow: 0.75-1.25 L/min Time: 3-5 min	-An increase in anthocyanins concentration and phenolic acid content	(Garofulic et al., 2015)
Pomegranate Juice	Color and anthocyanins	Power: 4 W Gas: argon Sample amount: 3, 4 and 5 ml Control treatment: raw juice Gas flow: 1.5 L/min Time: 3-7 min	-An increase in individual and total anthocyanin content -A significant change in the color difference	(Bursać Kovacevic et al., 2016)
Blueberry juice	Color, TPC, antioxidant activity, anthocyanin content and vitamin C	Voltage: 11 kV Frequency: 1000 Hz Gas: argon with 0, 0,5 and 1% oxygen Control treatment: pasteurized juice Gas flow: 1 L/min Time: 2-6 min	-A decrease in anthocyanin content, vitamin C and total color difference -An increase in TPC	(Hou et al., 2019)

Table 2.2 Effect of atmospheric cold plasma (ACP) on quality parameters of fruit juices (Cont.).

Product	Quality Assays	Reported Processing Conditions	Results	References
Spark Discharge Plasma (SDP)				
Cloudy apple juice	pH, color, PPO activity, non-enzymatic browning, particle size distribution (PSD), TPC, Antioxidant capacity	Voltage: 8-10.9 kV Frequency: 20-65 kHz Gas: air Sample amount: 10 ml Control treatment: raw juice Time: 1-5 min Storage: 7-28 days	-No PPO activity after 7 days storage -An increase in lightness -A significant increase in non-enzymatic browning, TPC and antioxidant capacity, and decrease after storage -A decrease in PSD after 4 min treatment -A significant decrease in pH and maintained by storage	(Illera et al., 2019)
Liquid Plasma Sterilization Tube				
Tomato Juice	Volatile components	Voltage:230V Frequency: 50 Hz Gas: air Treatment amount: 20 ml Control treatment: raw juice Sample condition: packaged treatment and 24 h storage Time: 5 min	-No significant effect on the smell of tomato juice	(Ma & Lan, 2015)
Gliding Discharge Plasma (GDP)				
Tomato juice	Dry matter pH Lycopene Ascorbic acid content	Power: 40 W Voltage: 3.8 kV Frequency: 50 Hz Gas: Nitrogen Sample amount: 5 mL Control treatment: raw juice Gas flow: 440 L/min Time: 5 min	-No significant change in dry matter, lycopene and ascorbic acid content -Significant increase in pH	(Starek et al.,2019)

CHAPTER 3

INACTIVATION OF *ESCHERICHIA COLI* IN APPLE CIDER USING ATMOSPHERIC COLD PLASMA[1]

[1]Ozen, E., Kumar, G. D., Mishra, A., & Singh, R. K. 2022. *International Journal of Food Microbiology*. 382, 109913. Reprinted here with permission of the publisher.

Abstract

Atmospheric cold plasma (ACP) is a promising non-thermal technology that has the potential to inactivate microorganisms in foods. In this work, the inactivation of *E. coli* K12, acid-adapted *E. coli* K12, and *E. coli* O157:H7 in apple cider by ACP was investigated using as feed gases simulated air (SA) (80% N2 + 20% O2) and a mixture of 90% N2 + 10% O2 with various processing times (0 to 180 s). We obtained reduced populations of both acid-adapted and non-adapted *E. coli* K12 by 5-log CFU/ml within 120 s, and *E. coli* O157:H7 within 90 s. Additionally, no significant changes in the °Brix, pH, temperature, or titratable acidity (TA) of apple cider were observed after exposure to ACP. However, processing times longer than 120 s resulted in significant changes in the pH values. The highest concentration of ozone and hydrogen peroxide reached 0.22 ± 0.1 mg/L for CG in 180 s and 0.07 ± 0.01 mg/L for SA in 150 s, respectively. Both acid-adapted and non-acid adapted *E. coli* K12 was found to be more resistant to ACP exposure than *E. coli* O157:H7 after the 90 s, so it could serve as a surrogate for *E. coli* O157:H7. When we compared the effect of the gas type on bacterial inactivation on non-selective media, there were no significant differences between gas types, while selective media demonstrated significant differences. In optical absorption spectroscopy measurements of plasma species, primarily ozone peaks were observed. Furthermore, the optical absorption spectroscopy also revealed that the inactivation of the bacteria could be attributed to some plasma species with wavelengths between 190 and 308 nm. The findings provided a perspective on the use of ACP as a method for decontaminating fruit juices as a non-thermal processing.

Introduction

Escherichia coli is a rod-shaped, facultative anaerobe, Gram-negative bacterium. *E. coli* cells colonize the intestinal tracts of both humans and animals. Nevertheless, some strains of *E. coli* have evolved into pathogens as a result of acquiring virulence factors (Lim et al., 2010). One strain, *E. coli* O157:H7, has been associated with several outbreaks as a result of consumption of unpasteurized apple cider (CDC, 2005; Cody et al., 1999; Hilborn et al., 2000). The bacterium generally affects children, the elderly, and people with compromised immune systems, causing mild diarrhea to hemolytic-uremic syndrome (Cody et al., 1999; Hilborn et al., 2000). *E. coli* O157:H7 adheres to the mucosal surfaces of the gut and produces Shiga-like toxins acting both locally and systemically. Toxins contribute to colonic injury. When the colon is damaged, the toxins allow lipopolysaccharides and other inflammatory mediators to enter the circulation. The process leads to the hemolytic-uremic syndrome. Those with bloody diarrhea are more likely to develop the illness than those without bloody diarrhea (Boyce, Swerdlow and Griffin, 1995). The most severe outbreak of *E. coli* O157:H7 linked to contaminated apple cider occurred in 1996 in the United States. More than 70 people were sickened, and one child died (Cody et al., 1999). *E. coli* O157:H7 can survive at low pH conditions, as well as in the presence of preservatives or at low temperatures such as 4°C (Miller and Kaspar, 1994). As a result of increasing outbreaks associated with acidic juices, the Food and Drug Administration (FDA) changed the labeling laws requiring a warning on the package describing the likelihood of pathogen exposure if the juice is not pasteurized or does not undergo a 5-log CFU/ml reduction in pertinent microorganisms (FDA, 2001). A "pertinent" microorganism is defined as one that is most resistant in a particular juice. *E. coli* O157:H7 is considered as pertinent organism for apple juice and cider (Valappil, 2010). Therefore, the thermal pasteurization method is traditionally employed and still perceived as a

proven method for reducing pathogenic organisms by 5-log CFU/ml. However, many cider and juice processors, especially small producers, do not use pasteurization due to the reduction of enzymes, color, and flavor (Choi and Nielsen, 2005). The practice has resulted in safety concerns about apple cider and increased the need for non-thermal pasteurization. Thus, the design of non-thermal processing systems has drawn the attention of numerous food producers in recent years.

Atmospheric cold plasma (ACP) is one of the non-thermal technologies that can decontaminate food of pathogens without raising the temperature or using chemical additives. This can eliminate a variety of microorganisms at low temperatures within a short period of time (Ambrico et al., 2020; Bourke et al., 2018; Misra et al., 2011). Studies on antimicrobial efficacy of plasma have been conducted by several scholars. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) like atomic oxygen/nitrogen, free radicals, ions, ozone, etc. (Ambrico et al., 2020; Chen et al., 2019) and minor amounts of ultraviolet light (UV) are generated by plasma depending on the gas type and leads to microbial reduction in foods (Laroussi and Leipold, 2004). ROS and RNS are responsible for damaging the cell wall and DNA of the microorganisms (Korachi and Aslan, 2011; Yusupov et al., 2013). So far, some studies have utilized ACP to eliminate bacteria from fruit juices (Ozen and Singh, 2020). Yet, even fewer studies examined the decontamination of apple cider and juice with ACP (Dasan and Boyaci, 2018; Liao et al., 2018; Montenegro et al., 2002; Xiang et al., 2018). The effect of cold plasma processing on apple juice quality has also been studied by some researchers (Dasan and Boyaci, 2018; Illera et al., 2019; Liao et al., 2018; Montenegro et al., 2002; Xiang et al., 2018). There are differences in the results of these studies since different amounts of juice and cold plasma configurations like dielectric barrier discharge plasma, plasma jets, spark discharge plasma were used.

Therefore, our aims for this study were to (i) investigate the effects of the feed gas used in

cold atmospheric plasma (ACP) processing on the inactivation of *E. coli* K12, acid-adapted *E. coli* K12, and *E. coli* O157:H7 in apple cider, (ii) understand the inactivation mechanism ACP, (iii) compare *E. coli* K12 to *E. coli* O157:H7 for surrogate selection, and (iv) analyze the effects of processing on basic juice quality characteristics.

Materials and Methods

Bacterial strains and inoculum preparation

Inoculum preparation: Acid-adapted and non-adapted *E. coli* K12 as well as ampicillin-resistant *E. coli* O157:H7 (H1730), a lettuce outbreak isolated strain, were used in this study. The bacterial strains were obtained from the Food Microbiology Culture Strain Bank in the Department of Food Science and Technology at the University of Georgia. *E. coli* K12 was stored at -70 ℃. A loop of frozen culture was inoculated in tryptic soy broth (TSB) (Difco; Becton Dickinson Co., Sparks, MD) and incubated overnight at 37 ℃. A loop of bacteria from inoculated TSB was transferred into fresh TSB for second overnight incubation. The culture was used to prepare working cultures of both acid-adapted and non-acid-adapted *E. coli* K12. The bacteria were transferred daily from inoculated TSB to fresh ones in order to maintain their viability. Each independent non-acid adapted processing of *E. coli* K12 inactivation included inoculating 45 ml of TSB with 0.5 ml of previously inoculated TSB and incubating for 12-18 hours at 37 ℃. Cultures grown in a stationary phase from TSB were centrifuged at 1800 x g for 10 min, and the pellets were washed twice with sterilized 0.1% peptone water (PW) (Bacto; Becton Dickinson Co., Sparks, MD). To achieve an adequate number of bacteria per 100 ml of apple cider, the pellets were resuspended in 1 ml 0.1% PW.

E. coli O157:H7 (H1730) was stored in Tryptic Soy Agar (TSA) (Difco; Becton Dickinson Co., Sparks, MD) with 100 g /ml ampicillin (AMP) (Research Products International Corp., Mt.

Prospect, IL) at 4℃. The *E. coli* O157: H7 strain was resistant to ampicillin and streptomycin. Ampicillin resistance was obtained by transformation with a plasmid and the streptomycin resistance was obtained through incremental exposure (Hayman et al., 2022). Prior to each treatment, a loop of isolated colonies was streaked onto TSA plates with AMP and incubated at 37 ℃ for 12-18 h. Every plate was washed with 0.1% PW. Afterward, the cells were harvested by centrifugation in 1800 × *g* for 10 min and washed twice with 0.1% PW. The final pellet was resuspended in 1 ml of 0.1% PW for each plate.

Acid Adaptation: Acid-adapted *E. coli* K12 was grown in TSB adjusted to a pH value of 5 by adding lactic acid (Sigma Aldrich, St. Louis, MO). For each independent acid-adapted *E. coli* K12 inactivation experiment, 100 ml TSB (pH = 5) tubes were inoculated with cells from 1 ml of previously inoculated TSB (pH = 5) and incubated at 37 ℃ for 12-18 h. The stationary phase cultures from TSB (pH=5) were centrifuged at 1800 × *g* for 10 min, and the pellets were washed twice with 0.1% PW. As in non-acid adapted *E. coli* K12, the pellets were resuspended in 1 ml 0.1% peptone water to achieve the desired number of bacteria.

Apple cider

The cloudy apple juice was purchased from a local store (Trader Joe's, Monrovia, CA) and stored at 4℃ until use. The temperature of the juice was adjusted to room temperature prior to treatment. The juice had 12°Brix soluble solids, 3.54 pH, and 0.39% total acidity expressed as malic acid. The initial bacterial count was determined with TSA (Difco; Becton Dickinson Co., Sparks, MD). Since the samples were pasteurized, no bacteria were detected. Before ACP processing, 99 ml of juice were inoculated with a 1 ml suspension of washed bacteria suspended in PW.

Atmospheric cold plasma processing conditions

The ACP device used in this study is illustrated in Sharma and Singh (2022). The plasma system was equipped with a 1 kV-16A power generator (FG5001), plasma jet (CD50), and high voltage transformer (HTR11). The transformer converts the 1 kV output voltage to a 20 kV ignition voltage and a 2 kV arc drop voltage (15-25 Hz). The plasma jets used air and nitrogen with oxygen admixture to generate plasma by applying an electric field to the electrodes and ejecting from a nozzle head at a frequency of 15 - 25 kHz. Process gases pulled particles away from the ionizing area, forming a flow of active plasma species in the form of a small jet. Plasma generated at atmospheric pressure was passed directly into the apple cider samples via a PTFE (polytetrafluoroethylene) tube (3 mm inner diameter, 5 mm outer diameter, 12.5 cm length) attached to the nozzle outlet. The plasma injection created an unstable cavity in the cider. That resulted in bubble formation inside the sample and provided mixing. As feed gases for the plasma, we used (i) 80% nitrogen with 20% oxygen as simulated air (SA) and (ii) 90% nitrogen with 10% oxygen as a combined gas (CG). Under the plasma nozzle, a 250-ml beaker filled with 100-ml apple cider was positioned at 12.5 cm from the outlet of the plasma nozzle. Plasma carrier tubes were placed 3 cm into apple cider in order to ensure direct contact with plasma species and for mixing. The inactivation of acid-adapted and non-acid-adapted *E. coli* K12 and *E. coli* O157:H7 was conducted by exposing plasma generated by SA and CG for exposure times ranging from 0 to 150 s and 0 to 120 s, respectively. The flow rate of the feed gas was 5 L/min. Before and after ACP processing, the sample temperatures were recorded with a thermocouple (Fluke 52II, FLUKE, Everett, WA).

Bacterial enumeration

In order to count the bacteria, present in each treated apple juice sample, one ml from each treated sample was ten-fold serially diluted in 9 ml of 0.1% PW. Trypticase Soy Agar with 0.6% Yeast Extract (TSAYE) (Difco; Becton Dickinson Co., Sparks, MD) was used as a non-selective medium to enumerate all bacteria. MacConkey (MAC) agar (Difco; Becton Dickinson Co., Sparks, MD) was used as a selective media for enumeration of both acid-adapted and non-acid adapted *E. coli* K12, and SMAC (Difco; Becton Dickinson Co., Sparks, MD) agar was used for enumeration of *E. coli* O157:H7. Each medium was plated with 0.1 ml of sample or diluent. The number of colony-forming units (CFU) was determined by counting the colonies on the plates after 24 h incubation at 37 ℃.

When no colonies were detected (below the limit of detection, 1-log CFU/ml) after ACP treatment, the samples were incubated at 37℃ overnight and then plated on trypticase soy agar with 0.6% yeast extract (TSAYE) plates for bacterial detection. Only positive samples (when detected) were reported.

Microbial Modeling

The USDA Agricultural Research Service created the Integrated Pathogen Modeling Program (IPMP) 2013 to analyze data and make progress in the field of predictive microbiology. This study utilized the IPMP 2013 for analyzing survival models for ACP treated *E. coli* O157:H7 and K12 strains. Survival models included in IPMP 2013 are the linear model, reparametrized Gompertz model, Weibull model, and Buchanan two/three-phase linear survival models (Buchanan and Golden, 1995; Huang, 2009; Mafart et al., 2001; Peleg, 1999).

Ozone and hydrogen peroxide measurement

The ozone concentrations of ACP treated samples were measured immediately following the processing by using a commercially available ozone test kit (range 0 to 5 ppm: CHEMetrics, Vacu-vials, Ozone I-2019, Calverton, VA, U.S.A.). Hydrogen peroxide (H_2O_2) concentrations in the samples were determined using the H_2O_2 Fluorometric Hydrogen Peroxide Assay Kit (Sigma-Aldrich, MAK165- 1KT; St. Louis, MO). Fluorescence measurements were performed with a microplate reader (Spectra MAX, Gemini EM, Molecular Device) at 540/590 nm. In order to determine the concentration of H_2O_2 in the samples, the fluorescence values of the samples were compared with the standards.

pH, °Brix, TA, and temperature measurement

The °Brix of ACP-treated samples was measured at 25 ± 0.5℃ by a refractometer (Aichose refractometer, Xindacheng, Shandong, China). Titratable acidity (TA) was measured according to Wibowo et al. (2015) with the following modifications. A sample of untreated and treated apple cider (10 mL) was diluted with 90 ml of distilled water and placed in a 250 ml beaker. Afterwards, the mixture was titrated with 0.1 M of NaOH until the sample reached the endpoint (pH 8.2). The results were expressed as grams of malic acid per 100 g of apple cider and calculated using the equation (1).

$$Acidity\ (\%)\ =\ (V_b\ x\ C_{\ b} x\ f\ x100)/V_a \qquad (1)$$

The V_b is consumed NaOH volume (ml), C_b is NaOH concentration, V_a is apple cider volume (ml), and f is the acidic factor for malic acid. The pH of the ACP-treated apple cider was measured by a pH meter (1120x; Mettler-Toledo, Schwerzenbach, Switzerland) at 24.5 ± 1.1℃. To calibrate the pH meter, standard buffer solutions of pH 7.0 and pH 4.0 (Mettler-Toledo, Schwerzenbach, Switzerland) were used. A digital thermocouple was used to measure the temperature of the

samples before and after processing.

Scanning electron microscopy (SEM)

SEM images taken before and after ACP processing provide visual evidence of the morphological characteristics of the treated bacteria. The processing was carried out by utilizing simulated air (SA; 80% nitrogen and 20% oxygen) for 120 s. Samples with and without processing were centrifuged for 10 min at 1800 × *g*, and the pellet was collected. Precipitates were washed with 0.1% PW to ensure all possible protein base residues were removed rather than bacteria. The washed samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer for at least 2 h. The samples were then washed three times with 0.2 M cacodylate-HCl (pH =7.24). Coverslips were prepared for each sample by cleaning and coating with 0.1% poly-L-lysine. A few drops of each sample were added to the coverslips and incubated overnight for adhesion of the samples to the coverslip surface. The coverslips with samples were washed with 0.1 M cacodylate-HCl, then placed in a solution of 4% Osmium tetroxide (OsO_4)/ 0.1 M sodium cacodylate-HCl. The samples were gently washed after 1 h and washed once with 0.1 M cacodylate-HCl for 10 min. Then, the samples were washed for 10 min twice in Deionized (DI) water. Dehydration steps were carried out with 25%, 30%, 50%, 60%, 75%, 95%, and 100% ethanol series, and then the samples were immersed in hexamethyldisilazane (HMDS) and 100% ethanol solution in a 1:1 ratio for 30 min. The samples were then immersed in 100% HMDS for 30 min. Samples were removed from 100% HMDS and allowed to air dry overnight under the fume hood. Samples on coverslips were mounted on aluminum SEM stubs and gold-coated with a sputter coater (SPI Sputter Coater, SPI Supplies, PA, USA) to approximately 15 nm thickness, and imaged in scanning electron microscopy (Thermo Fisher Sci Teneo FE-SEM, Hillsboro, OR).

Optical absorption spectroscopy (OAS)

Optical absorption spectroscopy (OAS) was performed to characterize the gas composition of post-discharge (Moiseev et al. 2014). The absorption of plasma species was measured by a spectrometer with a self-assembled light source (Figure 1). A quartz xenon flash tube (L-4040Q, 4 mm x 40 mm, XenonFlashTubes, Haifa, Israel) was used to emit a continuous spectrum from 185 to 1100 nm. The lamp was powered by a 0.47μF capacitor (12v-PWR-STR-OD, XenonFlashTubes, Haifa, Israel). In order to trigger the flash, a microcontroller (Nano V3.0, Arduino LLC, MA, USA) was used to generate a 5 V pulse signal. Black construction paper was used to make an aperture (2 mm × 8 mm) for limiting flash flux entering the sample room. A transparent and colorless plastic jar of 80 mm in diameter and 100 mm in height was used as a sample room that had two holes allowing plasma in and out. The flash was collected by a UV/VIS collimating lens (LENSQ-COL, StellarNet Inc., FL, USA). The spectrum was measured by a Black Comet UV-VIS Spectrometer (StellarNet Inc., Tampa, USA) equipped with a F400 UV-VIS-SR fiber optic. The integration time was 5 ms. The measurements were taken at the ACP nozzle outlet inserted into one of the jar holes. Spectra of environment, SA and CG were measured ten times. A comprehensive spectral analysis software package (SpectraWiz, version 5.33) included with the spectrometer was used to analyze data.

The absorption curve was derived using the following equation (2):

$$A = log10\ (I_0 / I) \quad (2)$$

where A is the absorbance, I_0 and I are intensity of the environment and plasma at the given wavelength (photon counts) which was from 190 to 840 nm.

Statistical analysis

A one-way analysis of variance (ANOVA) and Student's t-test (paired) were used to evaluate the significance of the differences between the means of all treatments. Differences between the means at $p \leq 0.05$ were considered significant. All experiments were carried out independently and in triplicate. All statistical analyses were performed using open-source R software (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

Effect of plasma processing on inactivation of E. coli

The effect of ACP treatment on two non-pathogenic bacteria (*E. coli* K12 and acid-adapted *E. coli* K12) and a pathogenic bacterium (*E. coli* O157:H7) in apple cider under different processing times and gas compositions was investigated (Figures 3.2a, 3.2b, and 3.2c, respectively). The initial count of non-acid adapted *E. coli* K12 was 7.8±0.2 and 7.2 ±0.4 when TSAYE and MAC were used enumeration media, respectively. After the ACP treatment, A 5-log reduction of non-acid adapted *E. coli* K12 was achieved in 120 s for SA but it took 180 s for CG (Figure 3.2a). Meanwhile, acid-adapted *E. coli* K12 was less resistant to processing conditions than non-acid-adapted *E. coli* K12 (Figure 3.2b). The initial count of acid adapted *E. coli* K12 was 7.7±0.1 and 7.3±0.6 in TSAYE and MAC, respectively. After the processing, acid-adapted *E. coli* K12 exhibited a reduction of more than 5-log CFU/ml in only 120 seconds regardless of media or gas composition. Liao et al (2018b) demonstrated the effect of acid stress on the resistance of *E. coli* to ACP exposure. The researchers reported that long-term acid stress (24 h) reduced the resilience of *E. coli* cells to ACP processing, as the stres damaged both membranes and DNA, which is the same as the target of ACP processing.

Figures 3.2a and 3.2b show that the survival level of *E. coli* K12 slowly declined with

increasing ACP treatment times until reaching a reduction of 5-log populations. In contrast, *E. coli* O157:H7 numbers were 8.3±0.2 in TSAYE and 8.1±0.1 in SMAC, respectively, and bacteria numbers were reduced after ACP treatment. (Figure 3.2c). The SA and CG plasmas resulted in less than 1-log and 2-log reductions, respectively, after 60 s. Following this, there was no *E. coli* O157:H7 detected when the processing times reached to 90s. ACP processing was conducted by some researchers to inactivate *E. coli* in apple juice by using air as a feed gas. Xiang and others (2018) achieved a 4.3 log reduction of *E. coli* in 30 s for 3 ml apple juice, while Montenegro and others (2002) reached a 5-log reduction of *E. coli* in 40 s when they used 0.8 ml juice. Also, Dasan and Boyaci (2018) reduced *E. coli* by 4 logs in 120 seconds, using 11 ml apple juice. More than 5-log reduction was achieved in this study in 120 s for *E. coli* K12 and in 90 s for *E. coli* O157:H7 for 100 ml apple cider when SA (simulated air) was utilized. Therefore, all ACP processing protocols (including flow rates, power requirements, etc.) should be reported in detail to give researchers a better understanding of this new technology.

The effectiveness of any inactivation process is typically evaluated in the laboratory by utilizing the most relevant pathogens as challenge organisms. Before the process can be used in a commercial setting, it must be validated using surrogate microorganisms. At present, no surrogate identification and validation has been reported for *E. coli* O157:H7 treated with ACP in juices. ACP inactivation of *E. coli* K12 was compared to that of *E. coli* O157:H7 to establish whether the non-pathogenic strain is a suitable surrogate for the pathogenic strain. The fact that the count of *E. coli* O157:H7 did not decrease much until 60 s of ACP treatment indicates that nonpathogenic *E. coli* cannot be used as a surrogate for *E. coli* O157:H7 with less than 60 s. Furthermore, the slightly lower levels of inactivation for non-pathogenic versus pathogenic *E. coli* after 90 s of treatment represent that non-pathogenic *E. coli* might serve as a surrogate for pathogenic *E. coli* in situations

where the processing time is longer than 90 s for both feed gases.

The TSAYE agar as non-selective media for all bacteria, MAC agar as selective media for *E. coli* K12, and SMAC agar as selective media for *E. coli* O157:H7 were used to enumerate the bacteria. Comparing TSAYE and MAC for *E. coli* K12 and SMAC for *E. coli* O157:H7 could be used to determine whether ACP causes cell injury. Moosekian and others (2014) utilized SMAC and TSAYE media in order to determine the sanitizing efficacy of X-ray irradiation against *E. coli* O157:H7 damaged by chemical sanitizers. Deog and others (2006) indicated that MAC agar as a selective medium for *E. coli* inhibited the recovery of injured or stressed *E. coli* cells compared to TSAYE. Therefore, the injured cell tried to be identified by comparing selective and non-selective media in this study.

The number of colonies observed in our study varied depending on the media used. However, the difference was due to different initial counts of bacteria in both media before ACP processing. Accordingly, the inactivated bacterial number was calculated by subtracting the number of bacteria at the beginning and end of each treatment. Then, the results were compared. Overall, there were no statistically significant differences between non-selective and selective media ($p>0.05$). In contrast, both SA with 90 s and CG with 60 s ACP exposure for inactivating acid-adapted *E. coli* K12 resulted in significant differences between selective and non-selective media. This observation may have been due to irregularities in the system like fluctuations in gas flow etc.

The effect of the gas type on the inactivation of bacteria was examined. While SA inactivated bacteria slightly more than CG, the difference was not statistically significant when TSAYE agar was used. Furthermore, the bacterial reduction by ACP produced from SA is significantly higher when enumeration is done on SMAC and MAC for non-acid-adapted *E. coli* and *E. coli* O157:H7, respectively. As reported by the researchers, oxygen gas generated cold plasma is highly effective

in killing bacteria (Hong et al., 2009; Hury et al., 1998; Laroussi and Leipold, 2004; Xu et al., 2017). With the use of an oxygen-containing gas mixture, ACP produces highly reactive chemical species such as ozone (O_3) and hydroxyl radicals (OH). Specifically, these species produce strong oxidizing chemicals like H_2O_2, OH radicals, and hydronium ions H_3O^+, especially when in a liquid or humid environment (Dobrynin et al., 2009; Magureanu, 2012). This might explain why SA causes more damage to bacteria than CG. It has been observed that acid-adapted bacteria are more susceptible to processing when the nitrogen gas ratio is increased in the gas composition.

The impact of storage time on *E. coli* inactivation was evaluated (Table 3.1). The experiments were conducted only for *E. coli* 0157:H7 for 60 s. The initial bacterial count was 8.36 ± 0.1 log CFU/ml for TSAYE and 8.15 ± 0.1 log CFU/ml for SMAC. Incorporating the post-processing into ACP treatment significantly improved the inactivation of bacteria, especially when the samples were stored at 25 ℃ for 24 h after the processing. Han et al. (2016) as well as Xu et al. (2017) found that the post-treatment increased the inactivation efficacy of ACP as bacteria contact to reactive species for extended reaction time. As ACP is a non-thermal technology, post-treatment storage may reduce the time required for the process and prevent the juice quality from changing. However, it is important to consider any potential negative consequences of storing juice at room temperature for 24 hours after undergoing ACP treatment.

The survival curves of acid adapted *E. coli* K12, non-acid adapted *E. coli* K12, and *E. coli* O157:H7 treated with ACP with two different feed gases and enumerated in two different media were fitted to survival models including linear model, re-parameterized Gompertz survival model, Weibull model, and Buchanan two/three-phase linear survival models in IPMP 2013 (Table 3.2). In order to determine which model gives the best fit when bacteria are treated with ACP, the root means square errors (RMSE) were compared, as these values evaluate the performance of models.

The RMSE of Buchanan's two-phase linear survival model was 0.154, which was lower for non-acid adapted *E. coli* K12 than that of others when SA and TSAYE media were used. The RMSE value of Buchanan's two-phase linear survival model was also the lowest when MAC was used to enumerate non-acid adapted *E. coli* K12 in the same conditions. As a result, this model would provide a more accurate prediction of the survival of non-acid adapted *E. coli* K12.

On the other hand, the RMSE values increased for all survival models when CG was used for both media for non-acid adapted *E. coli* K12. The RMSE values of Buchanan's two-phase linear survival model and modified Gompertz model for acid-adapted *E. coli* K12 were 0.304 and 0.345, respectively, and lower than other models when the bacteria were treated with SA and then enumerated with TSAYE. When CG was used, the RMSE values were found to be unsatisfactory for the same bacteria. The Weibull model RMSE was 0.217 for *E. coli* O157:H7 when the bacteria treated with ACP generated from CG, and TSAYE were used. The observations from Table 3.2 did not provide an overall answer to the question of which model is most suitable. Due to the fact that the results varied according to the type of bacteria, the enumeration media, and the plasma gas. These results were consistent with previous studies in which the kinetics of microbial inactivation depend on the gas used, the amount of sample treated, and the type of microorganisms that are to be destroyed (Dasan and Boyaci, 2018; Liao et al., 2018a; Segura-Ponce et al., 2018; Surowsky et al., 2014).

Evaluation effect of ACP on apple cider properties

Plasma-liquid systems, such as the apple cider-ACP interaction presented in this paper, are highly complex environments. There is very limited understanding of the system and associated physical and chemical processes. However, in an effort to further investigate the mechanism of *E. coli* K12, acid-adapted *E. coli* K12, and *E. coli* O157:H7 inactivation; the experiments determining

temperature, acidity, pH, °Brix, ozone concentration, and H_2O_2 concentration were conducted to evaluate the change of the properties of the apple cider during the ACP processing (Table 3.3).

The pH values for apple ciders treated with ACP decreased from 3.54 ± 0.01 to 3.48 ± 0.01, but the reduction was significant when the apple cider was treated with SA for 120 and 150 s ($p<0.05$). It has been suggested that pH decreases result from nitrous acid (HNO_2), hydrogen peroxide (H_2O_2), and nitric acid (HNO_3) formation in ACP-treated samples (Helmke et al., 2011; Liu et al., 2010; Oehmigen et al., 2010). In addition, DI water was treated with ACP under the same conditions as apple cider (Table 4). In contrast to apple cider, DI water exhibited a drastic and significant decrease in pH ($p<0.05$). This may be due to citric acid, ascorbic acid, and malic acid having buffering capacities to resist changes in pH of juices (Oehmigen et al., 2010; Xu et al., 2017). °Brix of the treated ciders was not significantly changed ($p > 0.05$) after the ACP processing of the ciders (data not shown). The plasma generated reactive species are unable to reach the macromolecules (like sugars) of the juices in their short lifetime (Liao et al., 2018a). Therefore, ACP does not have any effect on the °Brix of the ciders (Liao et al., 2018a; Shi et al., 2011). When apple cider was treated with ACP generated from SA or CG, no significant changes in TA were observed ($p>0.05$). The temperature of the samples was not significantly affected by the processing ($p>0.05$).

The ozone concentrations of all treated apple juices were below 0.2 ppm (data not shown), while the ozone level of the sample treated with CG for 180 s was 0.22 ± 0.1 ppm which was the highest result among the treated samples. ACP-treated DI waters were measured for ozone concentrations (Table 3.4) and compared with ACP-treated apple juices. The difference in the results was not significant ($p>0.05$). Broadwater and others (1973) reported that vegetative bacteria were extremely sensitive to residual ozone even at low concentrations. *E. coli* can be killed at a

level of toxicity of 0.19 ppm of ozone. In contrast, when organic matter is present in the bacterial environment, low levels of ozone are ineffective since organic matter interferes with the activity of ozone. Thus, ozone might be a contributing factor to the bactericidal effect of ACP, but the effect of ozone cannot ensure complete inactivation.

The amount of H_2O_2 in apple ciders treated with ACP ranged from 0.010 ± 0.003 to 0.070 ± 0.005 ppm (Table 3.3). The level of H_2O_2 in the treated samples was significantly increased following the treatment. The concentration of H_2O_2 in DI water after ACP processing was less than 0.1 ppm (Table 3.4). Liu et al and others (2010) used ACP to inactivate *Staphylococcus aureus* in water. The H_2O_2 concentration was found to be 80 ppm after processing with ACP for 15 minutes. Various amounts of H_2O_2 were mixed in water to inactivate *S. aureus*. Once the H_2O_2 concentration reached 400 ppm, the number of *S. aureus* decreased. Thus, the ACP-produced H_2O_2 does not directly contribute to the inactivation of the processing. In conclusion, ACP can effectively inactivate *E. coli* in apple cider with minimal changes in its properties, but further research is required to determine how these changes impact inactivation effectiveness. Moreover, this study used a larger volume of juice (100 ml) than previous studies, and this will enable the system to be scaled up to industrial applications (Ozen and Singh, 2020).

Scanning electron microscopy (SEM)

The SEM images of *E. coli* K12, acid-adapted *E. coli* K12, and *E. coli* O157:H7 with and without ACP treatment generated from SA at 120 s are shown in Figure 3.3. The cells had a smooth surface before processing. The processing resulted in the development of holes on the surfaces. Particularly, the shape of *E. coli* O157:H7 was changed by the processing compared to other bacteria. In this case, the punctured appearance of the treated bacteria may be attributed to the etching effect of ROS and RNS generated by oxygen and nitrogen feed gases produced plasma

(Park et al., 2003).

Optical absorption spectroscopy of ACP

Using optical absorption spectroscopy (OAS), the plasma generated from SA and CG was assessed from nozzle outlet of plasma (Figure 3.4). It was observed that the plasma absorbance spectrum ranged between 190 and 308 nm. In view of the fact that the main absorption band of ozone lies between 200 and 300 nm (Hartley band); the absorption spectrum indicates that ozone is present in the plasma. In addition, the characteristic absorbance peaks of NO and excited NO_2 can be observed at 190–225 nm and 230–280 nm, respectively (Moiseev et al., 2014). Nitrogen oxides may therefore be present in plasma. Both plasma spectra of SA and CG exhibit similar patterns, however, the absorbance of plasma by SA is higher than that of CG. As a result, the formation of O_3 and nitrogen oxides by SA might lead to a higher inactivation rate. This finding is similar to other studies in which microbial inactivation increased as O_2 concentration increased in feed gas (Sureshkumar et al., 2010; Xu et al., 2017). The results of the OAS spectra, however, do not allow us to adequately discuss the results for each compound generated by the ACP device, separately.

Conclusions

This study was conducted to examine the effectiveness of ACP plasma against acid-adapted *E. coli*, non-acid-adapted *E. coli*, and *E. coli* O157:H7 in apple cider. Results showed that ACP resulted in a 5-log reduction of *E. coli* within 90 s to 120 s, based on the bacteria strain. The apple cider did not exhibit significant changes in pH, temperature, or titratable acidity after exposure to ACP. Ozone and hydrogen peroxide were detected in the treated samples at low concentrations (< 0.5 ppm). Therefore, we do not need to be concerned about their effects as there is not much residue. To determine whether cells had been damaged or killed, selective and non-selective media

were compared. A significant difference between the media was not observed. The significance of the gas type, however, was dependent upon the selectivity of the media. Even though the difference in the inactivation effectiveness of the different gases was not significant in non-selective media, the reduction rate of SA in selective media was significantly higher than that of CG. As the nitrogen content of the feed gas increased, acid-adapted bacteria were found to be more susceptible to the plasma, although the results were not significant. One of the other novel aspects of this study was using a large volume of apple cider (100 ml) as opposed to previous studies. In summary, the results demonstrate that ACP plasma can inactivate *E. coli* in apple cider with minimal changes in the juice properties. As ACP has the potential of addressing safety concerns, future studies should evaluate consumer acceptance and process optimization for scale-up applications of ACP to the juice industry.

References

Ambrico, P.F., Šimek, M., Rotolo, C., Morano, M., Minafra, A., Ambrico, M., Pollastro, S., Gerin, D., Faretra, F., De Miccolis Angelini, R.M., 2020. Surface dielectric barrier discharge plasma: a suitable measure against fungal plant pathogens. *Sci. Rep.* 10, 1–17. https://doi.org/10.1038/s41598-020-60461-0

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

Broadwater, W.T., Hoehn, R.C., King, P.H., 1973. Sensitivity of three selected bacterial species to ozone. *Appl. Microbiol*. 26, 391–393. https://doi.org/https://doi.org/10.1128/am.26.3.391-393.1973

Buchanan, R.L., Golden, M.H., 1995. Model for the non-thermal inactivation of *Listeria monocytogenes* in a reduced oxygen environment. *Food Microbiol.* 12, 203–212. https://doi.org/10.1016/S0740-0020(95)80099-9

CDC, 2005. No Title [WWW Document]. Outbreak Surveill. data. URL http://www.cdc.gov/foodborneoutbreaks/outbreakdata.htm

Chen, D., Peng, P., Zhou, N., Cheng, Y., Min, M., Ma, Y., Mao, Q., Chen, P., Chen, C., Ruan, R., 2019. Evaluation of *Cronobacter sakazakii* inactivation and physicochemical property changes of non-fat dry milk powder by cold atmospheric plasma. *Food Chem*. 290, 270–276. https://doi.org/10.1016/j.foodchem.2019.03.149

Choi, L.H., Nielsen, S.S., 2005. The effects of thermal and nonthermal processing methods on

apple cider quality and consumer acceptability. *J. Food Qual*. 28, 13–29. https://doi.org/https://doi.org/10.1111/j.1745-4557.2005.00002.x

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G., Vugia, D.J., 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann. Intern. Med.* 130, 202–209. https://doi.org/10.7326/0003-4819-130-3-199902020-00005

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 Bioprocess Technol*. 11, 334–343. https://doi.org/10.1007/s11947-017-2014-0

Deog, M.K., Delaquis, P., Toivonen, P., Bach, S., Stanich, K., Harris, L., 2006. Destruction of *Escherichia coli* O157:H7 by vanillic acid in unpasteurized juice from six apple cultivars. J. *Food Prot.* 69, 542–547. https://doi.org/10.4315/0362-028x-69.3.542

Dobrynin, D., Fridman, G., Friedman, G., Fridman, A., 2009. Physical and biological mechanisms of direct plasma interaction with living tissue. *New J. Phys.* 11. https://doi.org/10.1088/1367-2630/11/11/115020

FDA, 2001. Hazard analysis and critical control points (HACCP); procedures for the safe and sanitary processing and importing of juice; *final rule. Fed. Regist*. 6137–6202.

Han, L., Patil, S., Boehm, D., Milosavljević, V., Cullen, P.J., Bourke, P., 2016. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus. Appl. Environ. Microbiol.* 82, 450–458. https://doi.org/10.1128/AEM.02660-15

Hayman, K., Oguadinma, I. C., Mishra, A., & Kumar, G. D., 2022. Evaluation of the use of

ampicillin-and streptomycin-resistant *Shiga* toxin-producing *Escherichia coli* to reduce the burden of background microbiota during food safety studies. *Journal of Food Safety*, 42(1), e12950. https://doi.org/10.1111/jfs.12950

Helmke, A., Hoffmeister, D., Berge, F., Emmert, S., Laspe, P., Mertens, N., Vioel, W., Weltmann, K.D., 2011. Physical and microbiological characterisation of *Staphylococcus epidermidis* inactivation by dielectric barrier discharge plasma. *Plasma Process.* Polym. 8, 278–286. https://doi.org/10.1002/ppap.201000168

Hilborn, E.D., Mshar, P.A., Fiorentino, T.R., Dembek, Z.F., Barrett, T.J., Howard, R.T., Cartter, M.L., 2000. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. *Epidemiol. Infect*. 124, 31–36. https://doi.org/10.1017/S0950268899003258

Hong, Y.F., Kang, J.G., Lee, H.Y., Uhm, H.S., Moon, E., Park, Y.H., 2009. Sterilization effect of atmospheric plasma on *Escherichia coli* and *Bacillus subtilis* endospores. *Lett. Appl. Microbiol*. 48, 33–37. https://doi.org/10.1111/j.1472-765X.2008.02480.x

Huang, L., 2009. Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions. *J. Food Eng.* 90, 380–387. https://doi.org/10.1016/j.jfoodeng.2008.07.011

Hury, S., Vidal, D.R., Desor, F., Pelletier, J., Lagarde, T., 1998. A parametric study of the destruction efficiency of *Bacillus* spores in low pressure oxygen-based plasmas. *Lett. Appl. Microbiol.* 26, 417–421. https://doi.org/10.1046/j.1472-765X.1998.00365.x

Illera, A.E., Chaple, S., Sanz, M.T., Ng, S., Lu, P., Jones, J., Carey, E. and Bourke, P., 2019. Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. *Food chemistry:* X, 3, p.100049.

https://doi.org/10.1016/j.fochx.2019.100049

Korachi, M., Aslan, N., 2011. The effect of atmospheric pressure plasma corona discharge on pH, lipid content and DNA of bacterial cells. *Plasma Sci. Technol*. 13. https://doi.org/10.1088/1009-0630/13/1/20

Laroussi, M., Leipold, F., 2004. Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *Int. J. Mass Spectrom.* 233, 81–86. https://doi.org/10.1016/j.ijms.2003.11.016

Liao, X., Li, J., Muhammad, A.I., Suo, Y., Chen, S., Ye, X., Liu, D., Ding, T., 2018a. Application of a Dielectric Barrier Discharge Atmospheric Cold Plasma (Dbd-Acp) for *Eshcerichia Coli* Inactivation in Apple Juice. *J. Food Sci.* 83, 401–408. https://doi.org/10.1111/1750-3841.14045

Liao, X., Li, J., Suo, Y., Ahn, J., Liu, D., Chen, S., Hu, Y., Ye, X., Ding, T., 2018b. Effect of preliminary stresses on the resistance of *Escherichia coli* and *Staphylococcus aureus* toward non-thermal plasma (NTP) challenge. *Food Res. Int.* 105, 178–183. https://doi.org/10.1016/j.foodres.2017.11.010

Lim, J.Y., Yoon, J.W., Hovde, C.J., 2010. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *J. Microbiol. Biotechnol.* 20, 1–10. https://doi.org/10.4014/jmb.0908.08007

Liu, F., Sun, P., Bai, N., Tian, Y., Zhou, H., Wei, S., Zhou, Y., Zhang, J., Zhu, W., Becker, K., Fang, J., 2010. Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. *Plasma Process.* Polym. 7, 231–236. https://doi.org/10.1002/ppap.200900070

Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2001. On calculating sterility in thermal preservation methods : Application of the Weibull frequency distribution model. *Acta Hortic*.

566, 107–114. https://doi.org/10.17660/ActaHortic.2001.566.11

Magureanu, M., 2012. Pârvulescu, V. I., Magureanu, M., & Lukes, P. (Eds.). (2012). Plasma chemistry and catalysis in gases and liquids. John Wiley & Sons. Wiley-VCH, Weinheim, Germany.

Miller, L.G., Kaspar, C.W., 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *J. Food Prot*. 57, 460–464. https://doi.org/10.4315/0362-028X-57.6.460

Misra, N.N., Tiwari, B.K., Raghavarao, K.S.M.S., Cullen, P.J., 2011. Nonthermal Plasma Inactivation of Food-Borne Pathogens. *Food Eng. Rev.* 3, 159–170. https://doi.org/10.1007/s12393-011-9041-9

Moiseev, T., Misra, N.N., Patil, S., Cullen, P.J., Bourke, P., Keener, K.M., Mosnier, J.P., 2014. Post-discharge gas composition of a large-gap DBD in humid air by UV-Vis absorption spectroscopy. *Plasma Sources Sci. Technol.* 23. https://doi.org/10.1088/0963-0252/23/6/065033

Montenegro, J., Ruan, R., Ma, H., Chen, P., 2002. Inactivation of *E. coli* O157: H7 using a pulsed nonthermal plasma system. *J. Food Sci.* 67, 646–648. https://doi.org/10.1111/j.1365-2621.2002.tb10653.x

Moosekian, S.R., Jeong, S., Ryser, E.T., 2014. Inactivation of sanitizer-injured *Escherichia coli* O157: H7 on baby spinach using X-ray irradiation. *Food Control* 36, 243–247. https://doi.org/10.1016/j.foodcont.2013.08.024

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K.D., Von Woedtke, T., 2010. The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Process. Polym*. 7, 250–257. https://doi.org/10.1002/ppap.200900077

Ozen, E., Singh, R.K., 2020. Atmospheric cold plasma treatment of fruit juices: A review. *Trends*

Food Sci. Technol. 103, 144–151. https://doi.org/10.1016/j.tifs.2020.07.020

Pankaj, S.K., Wan, Z., Colonna, W., Keener, K.M., 2017. Effect of high voltage atmospheric cold plasma on white grape juice quality. *J. Sci. Food Agric*. 97, 4016–4021. https://doi.org/10.1002/jsfa.8268

Park, B.J., Lee, D.H., Park, J.C., Lee, I.S., Lee, K.Y., Hyun, S.O., Chun, M.S., Chung, K.H., 2003. Sterilization using a microwave-induced argon plasma system at atmospheric pressure. *Phys. Plasmas* 10, 4539–4544. https://doi.org/10.1063/1.1613655

Peleg, M., 1999. On calculating sterility in thermal and non-thermal preservation methods. *Food Res. Int.* 32, 271–278. https://doi.org/10.1016/S0963-9969(99)00081-2

Segura-Ponce, L.A., Reyes, J.E., Troncoso-Contreras, G., Valenzuela-Tapia, G., 2018. Effect of Low-pressure Cold Plasma (LPCP) on the Wettability and the Inactivation of *Escherichia coli* and *Listeria innocua* on Fresh-Cut Apple (Granny Smith) Skin. *Food Bioprocess Technol.* 11, 1075–1086. https://doi.org/10.1007/s11947-018-2079-4

Sharma, S., Singh, R.K., 2022. Effect of atmospheric pressure cold plasma treatment time and composition of feed gas on properties of skim milk. *Lwt* 154, 112747. https://doi.org/10.1016/j.lwt.2021.112747

Shi, X.M., Zhang, G.J., Wu, X.L., Li, Y.X., Ma, Y., Shao, X.J., 2011. Effect of low-temperature plasma on microorganism inactivation and quality of freshly squeezed orange juice. *IEEE Trans. Plasma Sci*. 39, 1591–1597. https://doi.org/10.1109/TPS.2011.2142012

Sureshkumar, A., Sankar, R., Mandal, M., Neogi, S., 2010. Effective bacterial inactivation using low temperature radio frequency plasma. *Int. J. Pharm.* 396, 17–22. https://doi.org/10.1016/j.ijpharm.2010.05.045

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. *Int. J. Food Microbiol*. 174, 63–71. https://doi.org/10.1016/j.ijfoodmicro.2013.12.031

Valappil, Z.A., 2010. Impact of thermal and non-thermal processing techniques on apple and grapefruit juice quality. *University of Florida.*

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 Chem*. 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 Bioprocess Technol.* 10, 1778–1791. https://doi.org/10.1007/s11947-017-1947-7

Yusupov, M., Bogaerts, A., Huygh, S., Snoeckx, R., Van Duin, A.C.T., Neyts, E.C., 2013. Plasma-induced destruction of bacterial cell wall components: A reactive molecular dynamics simulation. *J. Phys. Chem.* C 117, 5993–5998. https://doi.org/10.1021/jp3128516

Tables

Table 3. 1 The effect of post-treatment storage on the inactivation of *Escherichia coli* O157:H7 treated for 60 s.

Treatment (60 s)	Media for Microorganisms	
	TSAYE (log CFU/ml)	**SMAC (log CFU/ml)**
Combined Gas (CG) (90% nitrogen and 10% oxygen)		
CG60 (after treatment)	7.75 ± 0.02^{a}	3.54 ± 0.76^{a}
CG60 (1 h after treatment)	7.28 ± 0.13^{a}	3.42 ± 0.38^{b}
CG60 (24 h after treatment at 4°C)	5.88 ± 0.23^{b}	2.49 ± 0.02^{bc}
CG60 (24 h after treatment at 25°C)	3.04 ± 0.27^{c}	1.00 ± 0.00^{c}
Simulated Air (SA) (80% nitrogen and 20% oxygen)	**TSAYE**	**SMAC**
SA60 (after treatment)	5.04 ± 3.46^{a}	1.00 ± 0
SA60 (1 h after treatment)	6.92 ± 6.92^{a}	1.00 ± 0
SA60 (24 h after treatment at 4°C)	6.15 ± 0.05^{ab}	1.00 ± 0
SA60 (24 h after treatment at 25°C)	2.02 ± 0.35^{b}	1.00 ± 0

[a, b] and [c] mean in the same column and the same treatment followed by different superscript letters are significantly different ($P<0.05$).

TSAYE = Trypticase soy agar with 0.6% yeast extract

SMAC = Sorbitol MacConkey agar

Table 3. 2 Root means square error (RMSE) of each inactivation model for the treated bacteria with different gas composition and enumeration media.

	***E. coli* K12 without acid adaptation**				***E. coli* K12 with acid adaptation**				***E. coli O157:H7***			
	SA		CG		SA		CG		SA		CG	
Survival Models	TSAYE	MAC	TSAYE	MAC	TSAYE	MAC	TSAYE	MAC	TSAYE	SMAC	TSAYE	SMAC
Linear Model	0.663	0.436	0.917	1.073	0.721	0.832	1.365	0.725	1.942	2.076	1.626	1.661
Modified Gompers Model	0.205	0.234	0.597	1.184	0.345	0.613	0.518	0.660	NA	NA	NA	0.551
Weibull Model	0.162	0.386	0.679	1.224	0.625	0.955	1.115	0.831	1.712	1.816	0.217	1.514
Buchanan Two-Phase Linear Survival Model	0.154	0.231	0.609	1.188	0.304	0.947	0.938	0.834	1.530	1.605	0.373	1.981

NA: Not applicable

SA: Simulated air containing 80% nitrogen and 20% oxygen.

CG: Combined gas containing 90% nitrogen and 10% oxygen.

Table 3. 3 The effect of atmospheric cold plasma on the quality of apple cider (pH, TA, temperature and H_2O_2).

	Simulated air (80% Nitrogen + 20% Oxygen)				Combined gas (90% Nitrogen + 10% Oxygen)			
Treatments (s)	pH	TA (% Malic Acid)	Temperature (°C)	H_2O_2 (ppm)	pH	TA (% Malic Acid)	Temperature (°C)	H_2O_2 (ppm)
0*	3.54 ± 0.01^a	0.39 ± 0.00^a	25.2 ± 2.1^a	0.000 ± 0.003^a	3.54 ± 0.01^a	0.39 ± 0.00^a	25.2 ± 2.1^a	0.000 ± 0.003^a
30	3.58 ± 0.03^a	0.41 ± 0.05^a	24.2 ± 0.2^a	0.013 ± 0.002^a	3.55 ± 0.01^a	0.44 ± 0.05^a	25.0 ± 1.0^a	0.008 ± 0.003^a
60	3.54 ± 0.01^a	0.49 ± 0.11^a	25.2 ± 0.8^a	0.031 ± 0.010^a	3.54 ± 0.04^a	0.41 ± 0.06^a	24.6 ± 1.9^a	0.011 ± 0.018^a
90	3.52 ± 0.04^a	0.45 ± 0.03^a	24.0 ± 0.4^a	0.037 ± 0.016^a	3.52 ± 0.00^a	0.47 ± 0.05^a	24.4 ± 0.1^a	0.030 ± 0.001^b
120	3.48 ± 0.01^b	0.40 ± 0.03^a	25.5 ± 1.7^a	0.068 ± 0.010^a	3.53 ± 0.01^a	0.45 ± 0.03^a	24.2 ± 0.2^a	0.042 ± 0.003^b
150	3.52 ± 0.01^b	0.41 ± 0.03^a	25.0 ± 1.4^a	0.069 ± 0.005^a	3.52 ± 0.01^a	0.45 ± 0.03^a	23.5 ± 0.1^a	0.052 ± 0.002^b
180	-	-	-	-	3.52 ± 0.01^a	0.45 ± 0.02^a	22.8 ± 1.3^a	0.059 ± 0.008^b

^{a,b} means in the same column followed by different superscript letters are significantly different ($P<0.05$).

* is control (without processing).

Table 3. 4 The effect of atmospheric cold plasma on deionized water.

Treatments (s)	Air Treatment (80% Nitrogen + 20% Oxygen) pH	Ozone (ppm)	H_zO_2 (ppm)	Combined Gas Treatment (90% Nitrogen + 10% Oxygen) pH	Ozone (ppm)	H_zO_2 (ppm)
0	7.32 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	7.32 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
30	3.88 ± 0.01	0.25 ± 0.21	0.04 ± 0.00	3.90 ± 0.06	0.04± 0.04	0.00 ± 0.00
150	3.01 ± 0.04	0.23 ± 0.08	0.04 ± 0.01	-	-	-
180	-	-	-	3.01 ± 0.03	0.26 ± 0.05	0.01 ± 0.01

Figures

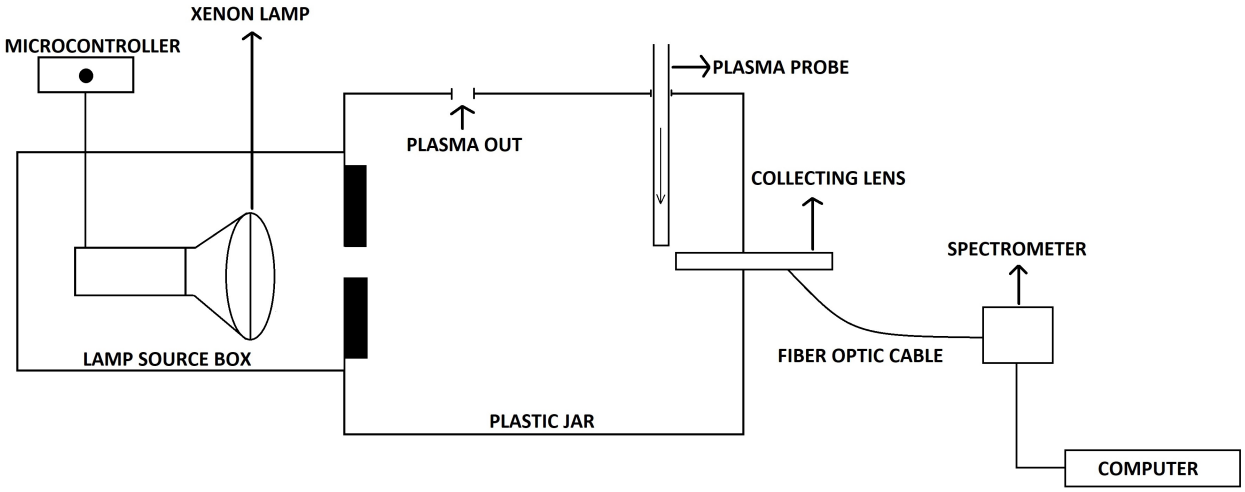

Figure 3. 1 Schematic diagram showing the setup for diagnostics of atmospheric cold plasma by optical absorption spectroscopy.

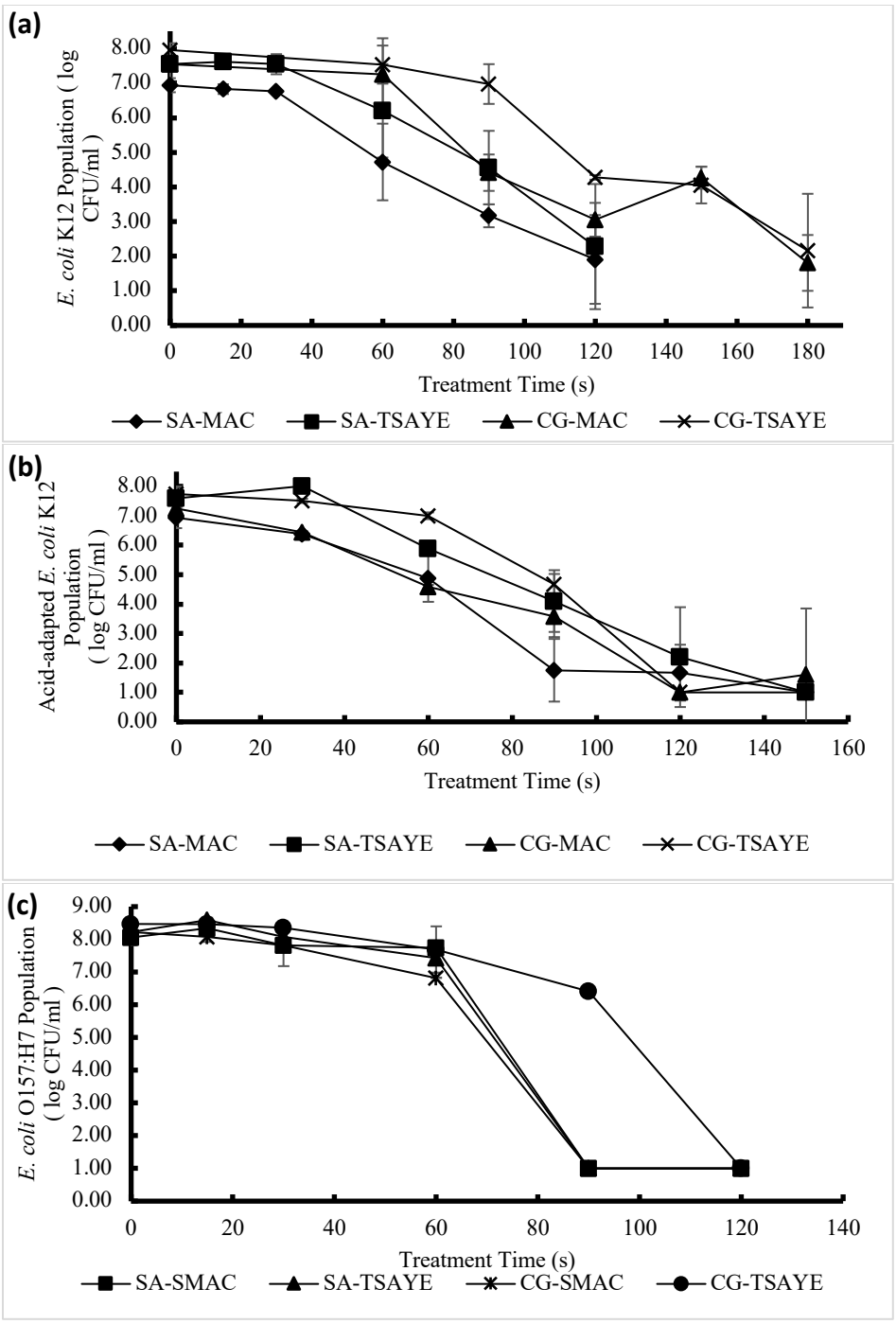

Figure 3. 2 The inactivation of (a) *E. coli* K12, (b) acid adapted *E. coli* K12 and (c) *E. coli* O157:H7 inoculated into apple cider exposed to simulated air (SA) or combined gas (CG) and enumerating the colonies on trypticase soy agar with 0.6% yeast extract (TSAYE) and MacConkey (MAC) mediums as a function of the exposure time.

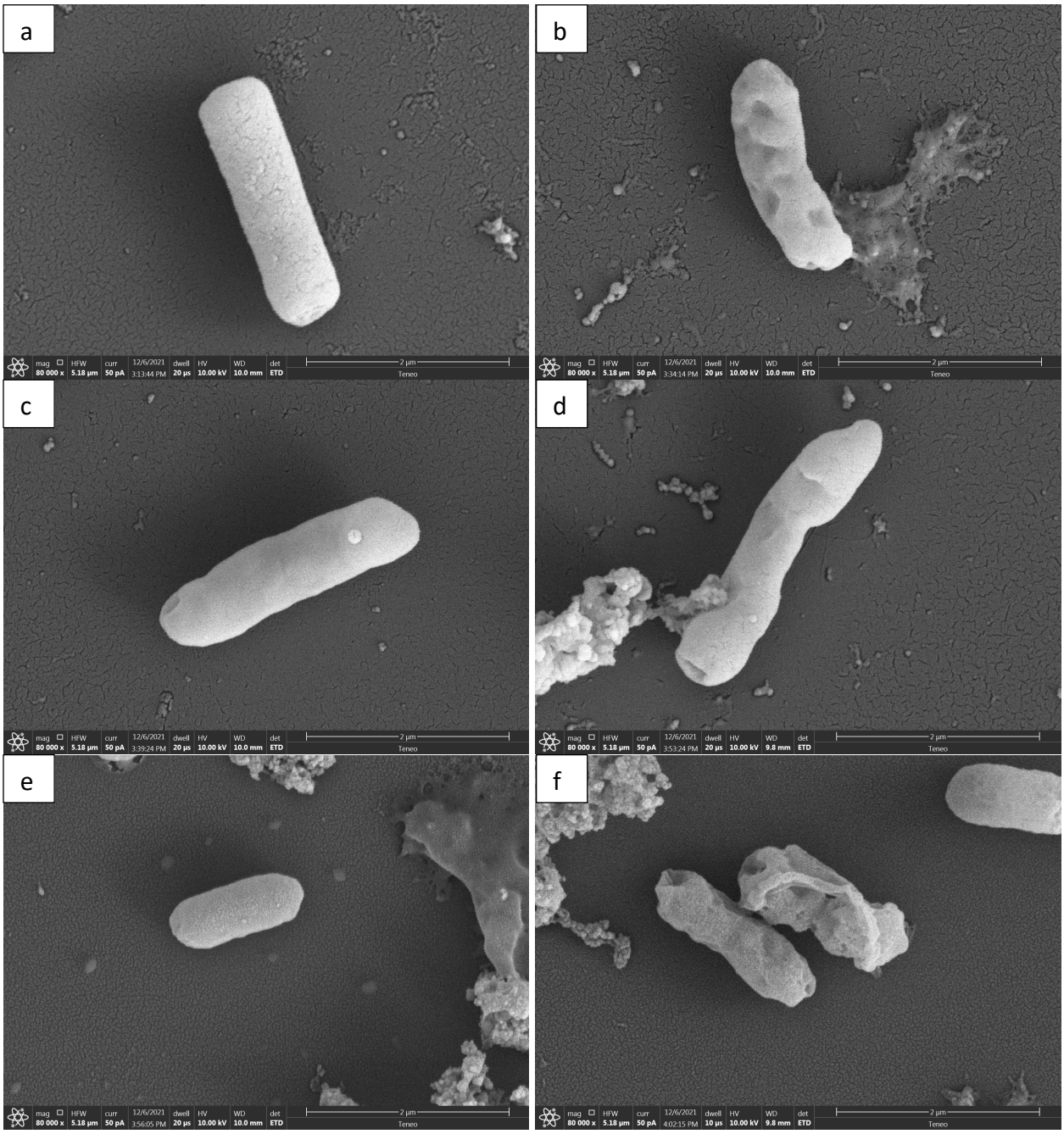

Figure 3. 3 SEM images (with 80,000 magnification) of *E. coli* K12 (a) before and (b) after treatment, acid-adapted *E. coli* K12 (c) before and (d) after treatment, and *E. coli* O157:H7 (e) before and (f) after treatment with simulated air generated ACP for 120 s.

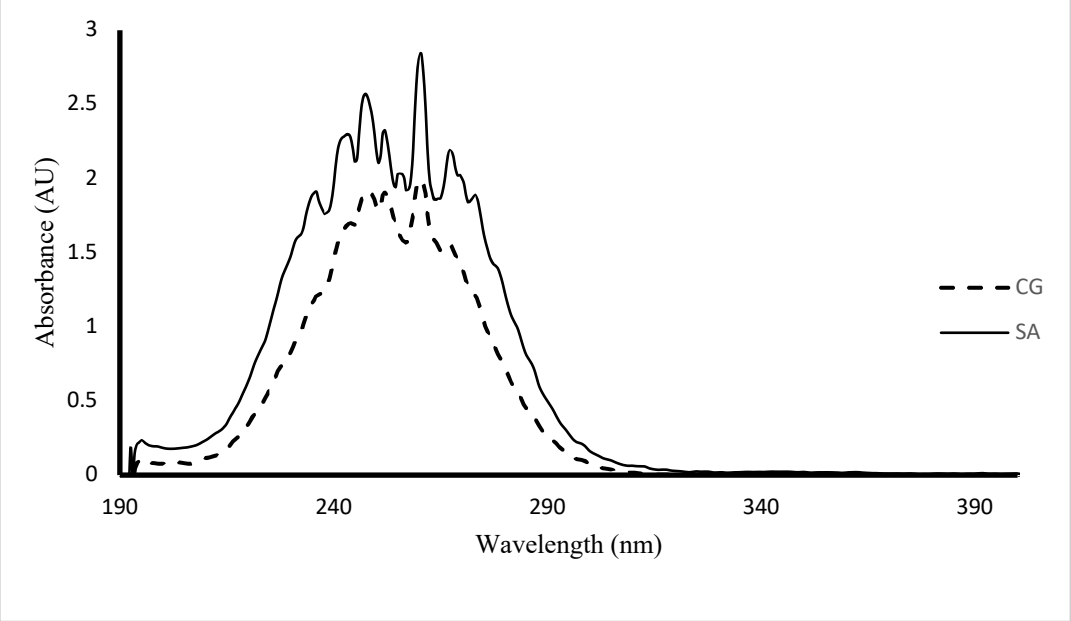

Figure 3. 4 Optical absorption spectroscopy of atmospheric cold plasma generated by simulated air (SA; 80% nitrogen and 20% oxygen) (___) and combined gas (CG; 90% nitrogen and 10% oxygen) (----).

CHAPTER 4

ATMOSPHERIC COLD PLASMA TREATMENT EFFECTS ON QUALITY OF CLOUDY APPLE JUICE DURING STORAGE[1]

[1]Ozen, E. To be submitted to *Food Research International.*

Abstract

The present study aimed to assess the impact of atmospheric cold plasma (ACP) treatment on the quality of cloudy apple juice. Specifically, the study investigated the effect of ACP processing using two different feed gases (simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen)) for varying durations (30, 60, 90, 120, and 150 s) and the effect of storage for three weeks at 4°C on the physicochemical characteristics (pH, color, viscosity, titratable acidity, soluble solids content, etc.) and bioactive compounds (total phenolic contents, antioxidant capacity, etc.) of cloudy apple juice (CAJ). Additionally, the study evaluated the effectiveness of ACP processing in reducing microbial survival, specifically total plate count, yeast and mold count, and *Alicyclobacillus acidoterrestris* spores, which are common spoilage agents in apple juice. Overall, the physicochemical characteristics of apple juice were unaffected by ACP treatment when compared to untreated and thermally pasteurized juices. The antioxidant activity and total phenolic content of ACP-treated juices were not significantly different from untreated and un-stored juices. In contrast, the antioxidant activity and total phenolic content of plasma-treated juices were significantly higher after storage. However, the ACP treatment was ineffective in reducing the survival of *Alicyclobacillus acidoterrestris* spores, and total yeast and mold count.

Introduction

Juices are an important source of essential nutrients and bioactive compounds needed for maintaining human health (Carrillo et al., 2014). Fruit juices generally meet consumer demands in terms of taste, and ease of consumption (Gomes et al., 2017). It has been reported that cloudy apple juice is more beneficial to health than clear apple juice, as it contains prebiotics and bioactive substances such as pectin, polyphenols, and ascorbic acid (Fonteles & Rodrigues, 2018; Oszmianski et al., 2007). However, the juices must be pasteurized to ensure their preservation until consumption.

The thermal (conventional) treatment of juice is a widely used method for preserving fruit juices by inactivating spoilage bacteria and enzymes (Gonzalez & Barrett, 2010). Even though thermal processing provides microbiological safety and long shelf life for juices, it alters their sensory and quality parameters, such as color, vitamins, phenolic content, etc. (Gonzalez & Barrett, 2010; Wang et al., 2012). It remains a challenge for the juice industry to produce juices that have fresh-like characteristics and an extended shelf life (Ramos et al., 2013). As a result of this challenge, non-thermal processing techniques are being developed like electric fields (Buckow et al., 2013), ultrasound processing (Adekunte et al., 2010), pulsed radiation processing (Tremarin et al., 2017), high pressure (Marszałek et al., 2017) and membrane processing (Gurak et al., 2010).

Atmospheric cold plasma (ACP) is a non-thermal processing technique that has attracted attention of researchers and food processors (Ozen & Singh, 2020; Pankaj et al., 2014). It consists of a neutral, ionized gas composed of electrons, free radicals, and atoms in excited or non-excited states at room temperature (Niemira, 2012). Depending on the gas type used, reactive plasma species such as singlet oxygen, OH radicals, and NO radicals may be generated, directly affecting microbial inactivation and food quality (Pankaj et al., 2018). It is important to note that the

effectiveness of generated reactive species is dependent on the plasma source, which is in turn dependent on the flow rate of the feed gas, the type of gas used, the voltage of the device, etc. (Pankaj et al., 2014; Ramos et al., 2013).

This study aims to evaluate the effect of ACP processing on the quality of cloudy apple juice (CAJ) from various perspectives, including color, antioxidant activity, and turbidity. A quality-based comparison of ACP processing of CAJ was conducted in comparison with non-processed and thermally processed CAJ. Additionally, the purpose of this work was to examine the effects of different gasses (simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen)) with different processing times and three weeks of storage on the quality of CAJ.

Material and Methods

Sample Preparation

Red Delicious apples were purchased from a local Georgia, USA market and stored overnight at an average temperature of 4°C. Then, the apples were individually brushed while being washed to remove dirt and wax. Washed apples were cut in half and crushed in a screw extractor (A200, Hobart Co. Troy, OH). Three layers of cheesecloth were used to filter crushed apples. To slow down the enzymatic browning of apple juice, 0.1% ascorbic acid was added. The juice was prepared in a cold room at 4 °C. Until further use, all juices were kept in a freezer at -18°C. A pasteurized clear apple juice was used (Great Value, Wal-Mart Stores, Inc. Bentonville, AR) to evaluate the inactivation of *Alicyclobacillus acidoterrestris* by ACP.

Atmospheric Cold Plasma (ACP) Treatments

The ACP device (CD50, Plasmatreat, Hayward, CA) used to process cloudy apple juices (CAJ) is illustrated and described in Sharma and Singh (2022). As part of the device, there is a 1 kV–16

A power generator (FG5001), a plasma jet (CD50), and a high-voltage transformer (HTR11). Using the transformer, the output voltage of 1 kV is converted into a 20 kV ignition voltage and a 2 kV arc drop voltage (15–25 Hz). During this research, oxygen and nitrogen mixtures were used as processing gases to pass through the electrodes and form a flow of plasma active species from a small jet. A PTFE (polytetrafluoroethylene) tubing (3 mm inside diameter, 5 mm outside diameter, 12.5 cm length) was attached to the nozzle outlet to deliver ACP into CAJ. As a result of the plasma passing through the CAJ, bubbles are generated inside the sample which agitates. Various gas mixtures were used in this study: (i) simulated air (SA) comprised of 80 % nitrogen and 20 % oxygen and (ii) combined air (CG) composed of 90 % nitrogen and 10 % oxygen. The processing times were 30, 60, 90, 120, and 150 s. A 1-L flask filled with 100 ml of CAJ was positioned at 28.5 cm from the plasma nozzle's outlet under the plasma nozzle. To ensure good mixing and direct contact with the plasma species, PTFE was placed into the sample at a depth of 0.7 cm. Processing times ranged from 0 to 150 s, and plasma flow rates were 5 l/min.

Inactivation of *Alicyclobacillus acidoterrestris* by ACP was carried out using 20 ml pasteurized clear apple juice. A 50 ml Falcon® centrifuge tube was filled with 20 ml of *A. acidoterrestris* inoculated juice and was positioned at 12.5 cm from the plasma nozzle's outlet under the plasma nozzle. Nozzles were placed inside the juice and were positioned so that they were 2.5 cm deep from the surface of the juice. The juices was treated for 3 min for both plasma gas mixture.

Pasteurization

For pasteurization, batch processing (non-flow) using a water bath (#SHWB10, Cole-Parmer Instrument Co., Chicago, USA) was conducted. For each sample, s 250 ml wide-mouth polypropylene bottle (Fisher Scientific, Pittsburgh, PA, USA) was filled with 200 ml of CAJ and placed in a water bath. A thermocouple wire was attached to each bottle cap, inserted into the CAJ,

and connected to a data logger (Fluke 52II, FLUKE, Everett, WA). Holding temperature and time were 90°C and 1 min, respectively. The samples were immediately cooled in an ice bath after the holding time.

Color, pH, °Brix, titratable acidity, and reducing sugar measurements

The CIELAB coordinates of the juice samples were measured with a colorimeter (model EZ 4500 L, Hunter Associates Laboratory Inc., Reston, VA). Before measurement, the colorimeter was calibrated with white and black tiles. For protection against the possible effects of environmental light, 35 ml of apple juice was placed in an opaque white foam and in a box. The L∗(lightness), a∗(greenness/redness), and b∗(blueness/yellowness) values of the juice samples were measured. Equations (1) and (2) were used to calculate the chroma (C*) and hue angle (h) in each juice sample.

$$C *= (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (1)$$

$$h = archtan\left(\frac{b^*}{a^*}\right) \quad (2)$$

The pH and soluble solid contents (SSC) of the samples were measured using a pH meter (Accumet AB150, Fisherbrand, OTT, Canada) and a refractometer (MA 884, Milwaukee Instruments, Inc., Rocky Mount, NC, USA). The titratable acidity (TA) of apple juices was measured following AOAC method no. 942.15, the final results expressed as grams of malic acid per 100 ml of juice.

To determine the reducing sugars, the 3,5-dinitro salicylic acid (DNS) method was used (Miller, 1959). Each sample was mixed with the same amount of DNS reagent in a tube and then placed in boiling water for 10 min. Immediately after the tubes were cooled in an ice bath, they were removed. The absorbance of samples at 540 nm was measured. The glucose solutions ranging

from 0.2 mg/ml to 1 mg/ml were prepared as standard solutions, and the sample results were expressed in mg glucose/ml of apple juice.

Turbidity, cloud stability, and particle size distribution (PSD) measurements

The turbidity or cloud value of CAJ was measured according to Bhat and Goh (2017). The absorbance of the samples was measured by a spectrometer (Model #1200, Cole-Parmer Instrument Co., Chicago, USA) at 660 nm, and turbidity was calculated by Eq. (3) and (4).

Cloud stability of CAJ was determined and reported as relative turbidity (%T), according to De Paepe et al. (2015). The absorbance of the samples at 660 nm was measured (T_0). The absorbance of the supernatant was also measured (Tc) after centrifugation at 4200 x g for 10 min. The cloud stability was then calculated according to Eq. (5):

$$Transmittance = 100\ x\ 10^{-Absorbance} \quad (3)$$

$$Turbidity(T) = 100 - Tranmittance \quad (4)$$

$$\%T = \frac{T_c}{T_0} x\ 100 \quad (5)$$

The Particle Size Distribution (PSD) of the samples was determined by a particle size analyzer (MAM 5004, Malvern Master sizer, Worcestershire, United Kingdom). The particles ranged between 0.1 to 1000 µm measured by laser light diffraction. The values of $D_{[4,3]}$ (µm), $D_{[3,2]}$ (µm), D10 (µm), D50 (µm), D90 (µm) and span were calculated by the software of the analyzer.

Total phenolic content (TPC) and antioxidant activity determination

Total phenolic content (TPC) was measured using the Folin-Ciocalteau method described by Singleton et al. (1999). Exactly 200 µl of juice samples were diluted with 1.8 ml of distilled water. A combination of 3 ml water, 200 µl Folin-Ciocalteau reagent (Sigma-Aldrich, St. Louis, MO, USA), and 200 µl diluted juice was mixed and waited for 10 min. Thereafter, 600 µl of sodium carbonate solution (80 µL, 20g/dl) was added. The mixture was incubated at 30°C for 2 h. The

absorbance of the samples at 762 nm was measured. To produce a standard curve, gallic acid solutions were used, and the results were expressed as milligrams of gallic acid equivalent per liter of apple juice (mg GAE/l).

Antioxidant capacity was determined by the DPPH assay described by Illera et al. (2019). The samples were mixed with the same amount of ethanol and centrifuged at 5000 x g for 5 min, then 3.5 ml of DPPH (0.2 mM) (Cayman Chemical Company, Ann Arbor, MI, USA) with 1 ml of the supernatant. The mixture was incubated at room temperature for 30 min. The sample absorbance at 517 nm was measured. A DPPH solution containing one ml of ethanol was used as a control. The antioxidant inhibition was calculated by eq (6);

$$The\ DPPH\ inhibition(\%) = \left(1 - \frac{A_S}{A_C}\right) x100 \quad (6)$$

A_s and A_c indicate the absorbance of the sample and control, respectively.

Microbial quality measurements

Plate count agar (PCA; Difco, BD, USA) and Dichloran Rose Bengal Chloramphenicol (DRBC; Difco, BD, USA) were used to enumerating the total plate count and total fungal count, respectively, using the 0.1 ml surface plating method. The PCA plates were kept in an incubator at 37°C for 24 h, while the DRBC plates were incubated at 25°C for five days. The results are presented as the number of colony-forming units (CFU)/ml.

To sporulate *Alicyclobacillus acidoterrestris* (ATCC 49025), orange serum agar (OSA) was adjusted to pH 3.7 with 10% tartaric acid, cultured for two days at 43 °C, and sub-cultured monthly at 4 °C. After streaking the growth cultures onto potato dextrose agar (PDA) adjusted to pH 5.6 with 10% tartaric acid, they were incubated at 43 °C for seven days. Then, spores were stained by the Schaeffer-Fulton method described by Schaeffer (1933), the sporulation was checked by microscopy (expected: at least 80% sporulation). Afterward, the sporulated cells were harvested

and centrifuged for 20 min at 3000x g. Pelleted cells were then inoculated into 20 ml of pasteurized apple juice. A final concentration of 10^5–10^6 spores/ml is achieved.

Statistical Analysis

All measurements taken three times. The data were analyzed by analysis of variance (ANOVA) with SPSS v.20 software (SPSS Inc., Chicago, IL, U.S.A.). Tukey test was used for comparison of sample, significant at $P<0.05$.

Results and Discussion

Effect of atmospheric cold plasma on physical attributes of cloudy apple juice

Table 4.1 shows the major physical characteristics of CAJ with thermal pasteurization, ACP processing, and no processing (TSS, TA, pH, and color). Acidity and pH are closely related to the quality of processed foods. Any drastic change in them could adversely affect consumer acceptability and shelf-life of the foods. Cold plasma discharge might affect juice acidity by solubilizing hydroxyl radicals (Pankaj et al., 2017), and pH might be affected by nitric and nitrous acids as well as hydrogen peroxide (H_2O_2) (Helmke et al., 2011; Liu et al., 2010; Oehmigen et al., 2010). It was found that there was no significant difference in pH between samples that were thermally processed, ACP-treated and samples that were not treated ($p>0.05$). Dasan and Boyaci (2018) and Tarabová et al. (2021) also found that ACP had no significant impact on apple juice pH. Since cold plasma processing generally causes pH changes in juices, it is also predicted that the juice composition would act as a buffer to preserve pH (Lukes et al., 2014; Machala et al., 2019). However, compared to juices without processing, all CAJ processed with ACP had significantly lower pH after three weeks of storage ($p<0.05$) (Table 2). In a comparison of thermally processed samples and ACP-treated samples after three weeks of storage, the pH of juices significantly decreased after 30 s for SA from 3.8 ± 0.0 to 3.66 ± 0.0 and 120 s for CG from

3.8 ± 0.0 to 3.65 ± 0.01. These findings agree with those of Liao et al. (2018) and Xiang et al. (2018) that pH of apple juice decreased after ACP processing. Changes in pH are associated with juice type, plasma type, juice amount, plasma active species production, etc. (Tarabová et al., 2021). No significant change in TA was found after treatment and after storage for three weeks (Table 4.2).

There was no significant difference in °Brix and viscosity between thermally processed, ACP processed, and non-processed samples ($p>0.05$) (Tables 4.1 and 4.2). Although carbohydrates accounted for the majority of soluble solids in juices, it is difficult for plasma-generated species to react with these macromolecules due to the short lifetimes of the species (Liao et al., 2018). Between just after the processing and after three weeks of storage, no significant differences in °Brix and viscosity were observed.

The color parameters for processed and non-processed juices without storage are given in Table 4.1 and those for three weeks stored samples in Table 4.2. Lightness (L) was not significantly different between non-processed samples and ACP-processed samples ($p>0.05$). L-values were not significantly affected by the gas composition or processing time. ACP-processed juices with SA for 120 and 150 s and CG for 120 s showed significantly lower L-values than those for thermally processed juices. After three weeks of storage for ACP treatment, the L-values ranged from 27.43 ± 1.39 to 32.03 ± 0.09 for SA and from 25.10 ± 0.23 to 32.06 ± 0.83 for CG (Table 4.2). Both gas compositions showed slightly increased L-values with increased treatment time, but the change was insignificant. When the treatment time reached 150 s for both gas mixtures, the CAJ color became significantly darker compared to un-processed samples. ACP-processed samples were all lighter than thermally processed samples. The results agree with those of Liao et al. (2018), who also found that increasing the plasma processing time decreased the lightness of apple juice. The

polymerization of phenolic content and the oxidation of pigment compounds by cold plasma species might impact color change (Kovačević et al., 2016; Wang et al., 2012).

Chroma (C*) describes how vibrant or dull a color is, depending on whether it is pure or gray. As C* values increase, the color becomes more vivid. Overall, ACP processing increased C*-values over time (Table 4.1). ACP-processed samples were not significantly different from raw and thermally pasteurized samples. After three weeks of storage, the C*-value of non-treated samples, 33.84 ± 3.95, was not significantly different from ACP-treated samples, but the samples treated with CG for 120 and 150 s were lower than non-treated samples (Table 4.2). The lower ACP treatment time showed significantly lower C*-values than pasteurized samples. The C*-values of ACP-treated CAJ were decreased by increasing processing time when the values were measured just after treatment. The finding agreed with Dasan and Boyaci (2018) and Wang et al. (2023) finding that apple juice saturation increased with plasma processing time.

There was no significant difference in hue values between the treatments when the measurement was completed just after treatment (Table 4.1). While the hue values of pasteurized juices were not significantly different from ACP-treated samples, pasteurized samples were significantly darker after three weeks of storage (Table 4.2).

ACP treatment did not result in any significant changes in reducing sugar in either stored or non-stored samples ($p > 0.05$) (Figure 4.1). The reducing sugar content of ACP-treated CAJ was not affected by the gas composition used to produce plasma or by processing time except for CG after 150 s with three weeks of storage. This increase may be explained by the ozone generated by the ACP breaking the glycosidic bonds in the polysaccharides and degrading them into monosaccharides (Ben'Ko et al., 2013; Umair et al., 2019).

Effect of atmospheric cold plasma on cloud value, cloud stability, and particle size distribution (PSD) of apple juice

Like other juices, cloud value, and stability are critical factors in CAJ appearance and mouthfeel. Figures 4.3 and Table 4.3 demonstrate how CAJ processing affects cloud value, cloud stability, and particle size distribution (PSD). Cloud values of ACP-treated samples with SA were significantly lower than those of pasteurized and non-treated apple juices without storage, but not when CG was used as a processing gas mixture (Figure 4.3). Overall, no changes were observed over the storage period, while the cloud value of CG for 150 s and pasteurized CAJ decreased significantly. As a result of comparing ACP-treated samples, neither the treatment time nor the gas composition impacted the cloud value. Cloud stability was not significantly different between all samples after three weeks of storage, and processing parameters were not significant on cloud stability, and the effects of processing parameters on cloud stability were not significant (Figure 4.3). Cloud stability generally did not change significantly over time, except for samples treated with SA for 150 s by ACP. Suspended pectin molecules mostly cause fruit juice cloudiness. Juice cloud loss is primarily caused by pectin de-esterification by pectinesterase (PE). A free carboxyl group adjacent to a methyl ester group is attacked by PE, leading to methanol and polygalacturonic acid forming. Therefore, cloud loss is not desirable by consumers as they associate it with quality degradation and spoilage (Baker & Cameron, 1999; Krop & Pilnik, 1974).

Overall, the particle volume mean diameter $D_{[4,3]}$ and the surface area mean diameter $D_{[3,2]}$ of ACP-treated samples were not different from the untreated sample ($D_{[4,3]}$ = 16.05 ± 1.97 μm; $D_{[3,2]}$ = 2.69 ± 0.07 μm) and the sample treated thermally ($D_{[4,3]}$ = 19.05 ± 2.33 μm; $D_{[3,2]}$= 2.37 ± 0.51 μm). There is no effect of the gas type used to produce plasma and processing time on $D_{[4,3]}$ and $D_{[3,2]}$ values, while they increased with increasing plasma time after three weeks of storage.

$D_{[4,3]}$ is significantly lowered when the plasma time for both gases was 30 s after the storage. Interestingly, there was a considerable increase in the particle volume mean diameter when the samples were treated with CG for 150 s and stored over three weeks.

The terms d(0.1), d(0.5), and d(0.9) indicate that 10%, 50%, and 90% of the total particles are smaller than the measured size, respectively. d(0.1), d(0.5), and d(0.9) of the samples were not significantly different from one another, except that ACP treatment with CG increased significantly ($p<0.05$).

The span value, the distribution width, of ACP-treated samples ranged from 5.56 ± 1.04 to 4.94 ± 0.27 for SA and from 6.73 ± 0.42 to 4.28 ± 0.46 for CG, which are not significant ($p<0.05$) different from non-processed and thermally processed samples. After three weeks of shelf life, the span of ACP samples with CG dropped significantly. The span value indicated the homogenization of the samples, and the samples with a lower span value demonstrated more cloud stability (Illera et al., 2019).

Effect of atmospheric cold plasma on antioxidant activity and phenolic content of apple juice

An antioxidant scavenging assay was conducted to compare the antioxidant capacities of processed and unprocessed apple juices (Figure 4.3). As a result of the ACP processing, antioxidant activity was slightly reduced. According to Dasan and Boyaci (2018), this overall decrease may be related to the fact that free radicals generated by ACP react with antioxidant compounds in apple juice and decrease their concentration. A significant increase in DPPH inhibition was observed in thermally pasteurized juices ($p<0.05$). The results were similar after three weeks of storage. Neither ACP-treated nor thermally pasteurized juices were affected by three weeks of storage, while raw juices lost significant antioxidant capacity. As a result of cold plasma processing, juice antioxidant capacity did not change significantly. Liao et al. (2018) and

Almeida et al. (2015) treated apple and prebiotic orange juices, respectively. It was found that plasma treatment of both juices reduced their antioxidant capacity slightly but not significantly. According to Wang et al. (2023), plasma treatment times higher than 2 min significantly decreased antioxidant activity. The results were similar to those of Pankaj et al. (2017) and Rodríguez et al. (2017), which used grape juice and cashew apple juice, respectively. Nevertheless, this study used a much greater amount of 100 ml juice than previous studies. Similarly, to most other quality parameters, the significance of the ACP processing in antioxidant capacity varies with juice amount, treatment time, etc.

The total phenolic content (TPC) of ACP-treated samples was decreased by increasing plasma time compared to non-processed and thermally processed samples (Figure 4.4). There was no significant influence of plasma gas type on phenolic content. Following three weeks of storage, phenolic content decreased after shorter plasma treatment times and increased after longer plasma treatment times. The phenolic content of plasma-treated samples was higher than that of non-processed samples and lower than that of thermally processed samples after the storage. The same trend was observed in changes in TPC with antioxidant capacity (Figure 4.3). It was stated that TPC is related to the antioxidant activity of foods (Muhammad et al., 2018).

The effect of atmospheric cold plasma on microbial quality

Pasteurized clear apple juices were inoculated with *Alicyclobacillus acidoterrestris* spores and then treated with ACP for 3 min with SA and CG feeding gas mixtures. Although ACP proved effective for killing microorganisms in fruit juices (Ozen & Singh, 2020), it proved less effective for killing spores. The results showed 1.06 ± 0.35 log CFU/ml reduction after 3 min processing with SA and 0.15 ±0.07 log CFU/ml reduction after 3 min processing with CG. Unlike vegetative cells, bacterial spores have hard and multilayered coats, making them more difficult to inactivate

by ACP (Tseng et al., 2012). Wang et al. (2022) inactivated *A. acidoterrestris* (vegetative cells) in 0.85% saline solution. The bacteria were killed up to 4.9 log CFU/ml in 2 min when 30 kV power was used. No studies have been conducted on the inactivation of *A. acidoterrestris* spores.

No bacteria were detected in samples even after three weeks of storage as the apple juice environment is unsuitable for bacterial growth (pH 3.8). The effects of ACP processing on yeast and mold (YMC) survival are presented in Figure 4.5. The non-processed apple juice contained 2.70 ± 0.14 log CFU/ml of yeast and molds, and ACP treatment had no significant effect on YMC. All samples except thermally pasteurized juices showed a significant increase in yeast and mold counts over three weeks. ACP was used in processing 5 ml tomato juice by Starek et al. (2019), which resulted in the inactivation of all total yeasts and molds after 5 min of processing. In this study, 100 ml samples and shorter processing times were used; thus, yeast and mold inactivation were expected to be lower or not at all, since yeast and mold have chitin-containing cell walls, making them more resistant than bacteria (Liao et al., 2017). Another possibility could be the disruption of yeast and mold biofilm from ACP treatments that could have released the yeast and mold cells. More research is needed to confirm this hypothesis.

Conclusions

The use of atmospheric cold plasma (ACP) treatment was effective for preserving the quality of cloudy apple juice (CAJ). The physicochemical characteristics of the juice, including pH, °Brix, viscosity, turbidity, and cloud stability, were not significantly affected by the plasma treatment. However, an increase in pH was observed after three weeks of storage due to the formation of plasma reactive species. The color of ACP-processed juices was generally between non-treated and thermally pasteurized juices. Furthermore, the juices' antioxidant capacity and phenolic content after three weeks of storage were higher than those of non-treated juices. Notably,

ACP processing did not affect the inactivation of *A. acidoterrestris* spores, yeast, and molds, as a high volume of juice (100 ml) was used in this study.

Our study contributes to the advancement of implementing cold plasma technology in the juice processing industry, given the large volume of juice (100 ml), different ACP gas compositions, and processing times evaluated. However, before scaling up this technology, it is crucial to assess its impact on non-acidic juices and evaluate its sensory impact.

References

Adekunte, A., Tiwari, B. K., Scannell, A., Cullen, P. J., & O'donnell, C. (2010). Modelling of yeast inactivation in sonicated tomato juice. *International Journal of Food Microbiology*, *137*(1), 116–120. https://doi.org/10.1016/j.ijfoodmicro.2009.10.006

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

AOAC. (2016). Official methods of analysis of AOAC international - 20th Edition, 2016. *20th Ed. Gaithersburg: AOAC.*

Baker, R. A., & Cameron, R. G. (1999). Clouds of citrus juices and juice drinks. *Food Technology*, *53*(1).

Ben'Ko, E. M., Manisova, O. R., & Lunin, V. V. (2013). Effect of ozonation on the reactivity of lignocellulose substrates in enzymatic hydrolyses to sugars. *Russian Journal of Physical Chemistry A*, *87*, 1108–1113. https://doi.org/10.1134/S0036024413070091

Bhat, R., & Goh, K. M. (2017). Sonication treatment convalesce the overall quality of hand-pressed strawberry juice. *Food Chemistry*, *215*, 470–476. https://doi.org/10.1016/j.foodchem.2016.07.160

Buckow, R., Ng, S., & Toepfl, S. (2013). Pulsed electric field processing of orange juice: A review on microbial, enzymatic, nutritional, and sensory quality and stability. *Comprehensive Reviews in Food Science and Food Safety*, *12*(5), 455–467. https://doi.org/10.1111/1541-4337.12026

Carrillo, E., Fiszman, S., Lähteenmäki, L., & Varela, P. (2014). Consumers' perception of symbols and health claims as health-related label messages. A cross-cultural study. *Food Research International*, *62*, 653–661. https://doi.org/10.1016/j.foodres.2014.04.028

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

De Paepe, D., Coudijzer, K., Noten, B., Valkenborg, D., Servaes, K., De Loose, M., Diels, L., Voorspoels, S., & Van Droogenbroeck, B. (2015). A comparative study between spiral-filter press and belt press implemented in a cloudy apple juice production process. *Food Chemistry*, *173*, 986–996. https://doi.org/10.1016/j.foodchem.2014.10.019

Fonteles, T. V., & Rodrigues, S. (2018). Prebiotic in fruit juice: Processing challenges, advances, and perspectives. *Current Opinion in Food Science*, *22*, 55–61. https://doi.org/10.1016/j.cofs.2018.02.001

Gomes, W. F., Tiwari, B. K., Rodriguez, Ó., de Brito, E. S., Fernandes, F. A. N., & Rodrigues, S. (2017). Effect of ultrasound followed by high pressure processing on prebiotic cranberry juice. *Food Chemistry*, *218*, 261–268. https://doi.org/10.1016/j.foodchem.2016.08.132

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. *Journal of Food Science*, *75*(7), R121–R130. https://doi.org/10.1111/j.1750-3841.2010.01763.x

Gurak, P. D., Cabral, L. M. C., Rocha-Leão, M. H. M., Matta, V. M., & Freitas, S. P. (2010). Quality evaluation of grape juice concentrated by reverse osmosis. *Journal of Food Engineering*. https://doi.org/10.1016/j.jfoodeng.2009.08.024

Helmke, A., Hoffmeister, D., Berge, F., Emmert, S., Laspe, P., Mertens, N., Vioel, W., & Weltmann, K. (2011). Physical and microbiological characterization of *Staphylococcus epidermidis* inactivation by dielectric barrier discharge plasma. *Plasma Processes and Polymers*, *8*(4), 278–286. https://doi.org/10.1002/ppap.201000168

Illera, A. E., Chaple, S., Sanz, M. T., Ng, S., Lu, P., Jones, J., Carey, E., & Bourke, P. (2019). Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. *Food Chemistry: X*, *3*, 100049. https://doi.org/10.1016/j.fochx.2019.100049

Kovačević, D. B., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Jambrak, A. R., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, *190*, 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099

Krop, J. J. P., & Pilnik, W. (1974). Effect of pectic acid and bivalent cations on cloud loss of citrus juice. *LWT Lebensmitt Wissensch Technol*, 7, 62-63.

Liao, X., Li, J., Muhammad, A. I., Suo, Y., Chen, S., Ye, X., Liu, D., & 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

Liao, X., Liu, D., Xiang, Q., Ahn, J., Chen, S., Ye, X., & Ding, T. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. *Food Control*, *75*, 83–91. https://doi.org/10.1016/j.foodcont.2016.12.021

Liu, F., Sun, P., Bai, N., Tian, Y., Zhou, H., Wei, S., Zhou, Y., Zhang, J., Zhu, W., & Becker, K. (2010). Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. *Plasma Processes and Polymers*, *7*(3-4), 231–236. https://doi.org/10.1002/ppap.200900070

Lukes, P., Dolezalova, E., Sisrova, I., & Clupek, M. (2014). Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H_2O_2 and HNO_2. *Plasma Sources Science and Technology*, *23*(1), 015019. https://10.1088/0963-0252/23/1/015019

Machala, Z., Tarabová, B., Sersenová, D., Janda, M., & Hensel, K. (2019). Chemical and antibacterial effects of plasma activated water: Correlation with gaseous and aqueous reactive oxygen and nitrogen species, plasma sources and air flow conditions. *Journal of Physics D: Applied Physics*, *52*(3). https://doi.org/10.1088/1361-6463/aae807

Marszałek, K., Woźniak, Ł., Kruszewski, B., & Skąpska, S. (2017). The effect of high pressure techniques on the stability of anthocyanins in fruit and vegetables. *International Journal of Molecular Sciences*, *18*(2), 277. https://doi.org/10.3390/ijms18020277

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428.

Muhammad, A. I., Liao, X., Cullen, P. J., Liu, D., Xiang, Q., Wang, J., Chen, S., Ye, X., & Ding, T. (2018). Effects of nonthermal plasma technology on functional food components. *Comprehensive Reviews in Food Science and Food Safety*, *17*(5), 1379–1394. https://doi.org/10.1111/1541-4337.12379

Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, *3*, 125–142. https://doi.org/10.1146/annurev-food-022811-101132

Oehmigen, K., Hähnel, M., Brandenburg, R., Wilke, C., Weltmann, K., & Von Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, *7*(3-4), 250–257.

Oszmianski, J., Wolniak, M., Wojdylo, A., & Wawer, I. (2007). Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *Journal of the Science of Food and Agriculture*, *87*(4). https://doi.org/10.1002/jsfa.2707

Ozen, E., & Singh, R. K. (2020). Atmospheric cold plasma treatment of fruit juices: A review. *Trends in Food Science & Technology*, *103*, 144–151. https://doi.org/10.1016/j.tifs.2020.07.020

Pankaj, S. K., Bueno-Ferrer, C., Misra, N. N., Milosavljević, V., O'donnell, C. P., Bourke, P., Keener, K. M., & Cullen, P. J. (2014). Applications of cold plasma technology in food packaging. *Trends in Food Science & Technology*, *35*(1), 5–17. https://doi.org/10.1016/j.tifs.2013.10.009

Pankaj, S. K., Wan, Z., Colonna, W., & Keener, K. M. (2017). Effect of high voltage atmospheric cold plasma on white grape juice quality. *Journal of the Science of Food and Agriculture*, *97*(12), 4016–4021. https://doi.org/10.1002/jsfa.8268

Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of cold plasma on food quality: A review. *Foods*, *7*(1), 4. https://doi.org/10.3390/foods7010004

Ramos, B., Miller, F. A., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2013). Fresh fruits and vegetables—an overview on applied methodologies to improve its quality and safety. *Innovative Food Science & Emerging Technologies*, *20*, 1–15. https://doi.org/10.1016/j.ifset.2013.07.002

Rodríguez, Ó., Gomes, W. F., Rodrigues, S., & Fernandes, F. A. N. (2017). Effect of indirect cold plasma treatment on cashew apple juice (*Anacardium occidentale L.*). *LWT- Food Science and Technology*, *84*, 457–463. https://doi.org/10.1016/j.lwt.2017.06.010

Schaeffer, A. B. & F. M. D. (1933). A simplified method of staining endospores. *Science*, *77*(1990), 194–194.

Sharma, S., & Singh, R. K. (2022). Effect of atmospheric pressure cold plasma treatment time and composition of feed gas on properties of skim milk. *LWT- Food Science and Technology*, *154*, 112747. https://doi.org/10.1016/j.lwt.2021.112747

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*, 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Starek, A., Pawłat, J., Chudzik, B., Kwiatkowski, M., Terebun, P., Sagan, A., & Andrejko, D. (2019). Evaluation of selected microbial and physicochemical parameters of fresh tomato juice after cold atmospheric pressure plasma treatment during refrigerated storage. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-44946-1

Tarabová, B., Tampieri, F., Maran, E., Marotta, E., Ostrihoňová, A., Krewing, M., & Machala, Z. (2021). Chemical and antimicrobial effects of air non-thermal plasma processing of fresh apple juice with focus on safety aspects. *Foods*, *10*(9), 2055. https://doi.org/10.3390/foods10092055

Tremarin, A., Brandão, T. R. S., & Silva, C. L. M. (2017). Inactivation kinetics of *Alicyclobacillus acidoterrestris* in apple juice submitted to ultraviolet radiation. *Food Control*, *73*, 18–23. https://doi.org/10.1016/j.foodcont.2016.07.008

Tseng, S., Abramzon, N., Jackson, J. O., & Lin, W.-J. (2012). Gas discharge plasmas are effective in inactivating *Bacillus* and *Clostridium* spores. *Applied Microbiology and Biotechnology*, *93*, 2563–2570. https://doi.org/10.1007/s00253-011-3661-0

Umair, M., Jabbar, S., Nasiru, M. M., Sultana, T., Senan, A. M., Awad, F. N., Hong, Z., & Zhang, J. (2019). Exploring the potential of high-voltage electric field cold plasma (HVCP) using a dielectric barrier discharge (DBD) as a plasma source on the quality parameters of carrot juice. *Antibiotics*, *8*(4). https://doi.org/10.3390/antibiotics8040235

Wang, L.-H., Chen, L., Zhao, S., Huang, Y., Zeng, X.-A., & Aadil, R. M. (2022). Inactivation efficacy and mechanisms of atmospheric cold plasma on *Alicyclobacillus acidoterrestris*: Insight into the influence of growth temperature on survival. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.1012901

Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., Zhu, W. D., Becker, K. H., & 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*, 1–7. https://doi.org/10.1140/epjd/e2012-30053-1

Wang, Z., Jia, H., Yang, J., Hu, Z., Wang, Z., Yue, T., & Yuan, Y. (2023). Inactivation of *Alicyclobacillus* contaminants in apple juice by dielectric barrier discharge plasma. *Food Control*, *146*, 109475. https://doi.org/10.1016/j.foodcont.2022.109475

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*(136), 201–207. https://doi.org/10.1016/j.foodchem.2018.

Tables

Table 4. 1 The effect of atmospheric cold plasma generated from simulated air (SA; 80% nitrogen and 20% oxygen) or combined gas (CG; 90% nitrogen and 10% oxygen) for 30, 60, 90, 120 and 150 s on the quality of apple juice after processing (without shelf life).

TREATMENT	°BRIX	pH	VISCOSITY (10^{-3} Pa.s)	COLOR (L)	Color (Chroma)	Color (Hue)	TA (% Malic Acid)
SA30	12.80 ± 0.00^{a}	3.8 ± 0.0^{ab}	1.87 ± 0.01^{a}	41.67 ± 6.32^{ab}	49.85 ± 5.85^{ab}	71.00 ± 4.96^{a}	0.26 ± 0.04^{a}
SA60	12.80 ± 0.28^{a}	3.9 ± 0.0^{b}	1.98 ± 0.04^{a}	41.50 ± 5.38^{ab}	51.30 ± 4.92^{ab}	69.15 ± 3.96^{a}	0.22 ± 0.03^{a}
SA90	12.95 ± 0.35^{a}	3.9 ± 0.1^{ab}	1.99 ± 0.09^{a}	41.06 ± 5.91^{ab}	52.14 ± 3.95^{b}	68.96 ± 4.49^{a}	0.23 ± 0.00^{a}
SA120	12.80 ± 0.14^{a}	3.8 ± 0.0^{ab}	1.92 ± 0.06^{a}	24.32 ± 2.76^{a}	32.58 ± 3.35^{a}	60.29 ± 1.25^{a}	0.27 ± 0.02^{a}
SA150	13.25 ± 0.07^{a}	3.8 ± 0.1^{ab}	1.97 ± 0.17^{a}	23.82 ± 2.59^{a}	32.19 ± 4.08^{a}	59.54 ± 1.05^{a}	0.24 ± 0.04^{a}
CG30	12.85 ± 0.07^{a}	3.9 ± 0.1^{ab}	2.04 ± 0.01^{a}	36.72 ± 12.08^{ab}	44.95 ± 11.29^{ab}	67.39 ± 8.47^{a}	0.25 ± 0.02^{a}
CG60	12.65 ± 0.21^{a}	3.8 ± 0.0^{ab}	2.06 ± 0.06^{a}	29.52 ± 2.55^{ab}	39.86 ± 3.12^{ab}	62.27 ± 1.99^{a}	0.24 ± 0.02^{a}
CG90	12.75 ± 0.35^{a}	3.8 ± 0.0^{ab}	2.05 ± 0.01^{a}	25.82 ± 0.93^{ab}	35.66 ± 1.32^{ab}	59.63 ± 0.57^{a}	0.24 ± 0.02^{a}
CG120	12.15 ± 0.92^{a}	3.8 ± 0.0^{ab}	2.10 ± 0.33^{a}	23.90 ± 1.71^{a}	33.05 ± 1.81^{ab}	58.72 ± 0.22^{a}	0.23 ± 0.02^{a}
CG150	12.85 ± 0.21^{a}	3.7 ± 0.0^{a}	2.04 ± 0.11^{a}	31.07 ± 7.42^{ab}	39.04 ± 4.89^{ab}	64.97 ± 6.49^{a}	0.25 ± 0.01^{a}
Nontreated	12.50 ± 0.14^{a}	3.8 ± 0.0^{ab}	1.76 ± 0.11^{a}	40.83 ± 9.71^{ab}	47.78 ± 4.62^{ab}	70.96 ± 8.45^{a}	0.34 ± 0.09^{a}
Pasteurized	13.00 ± 0.14^{a}	3.8 ± 0.1^{ab}	1.94 ± 0.18^{a}	48.28 ± 1.21^{b}	42.72 ± 0.14^{ab}	74.06 ± 1.54^{a}	0.24 ± 0.02^{a}

^{a} and ^{b} mean in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Table 4. 2 The effect of atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) as feed gasses for 30, 60, 90, 120 and 150 s on the quality of apple juice after three weeks storage at 4°C.

TREATMENT	°BRIX	pH	VISCOSITY (10^{-3} Pa.s)	L - value	Chroma	Hue	TA (% Malic Acid)
SA30	11.80 ± 0.28^{a}	3.66 ± 0.04^{bc}	1.74 ± 0.02^{a}	27.43 ± 1.39^{bcd}	33.96 ± 1.79^{b}	63.07 ± 0.76^{ab}	0.26 ± 0.00^{a}
SA60	11.70 ± 0.28^{a}	3.61 ± 0.04^{c}	1.75 ± 0.06^{a}	28.42 ± 0.85^{bcd}	34.57 ± 0.91^{bd}	64.04 ± 0.06^{a}	0.25 ± 0.02^{a}
SA90	12.65 ± 0.49^{a}	3.63 ± 0.04^{c}	1.73 ± 0.06^{a}	29.81 ± 0.87^{bcd}	38.23 ± 0.65^{ab}	63.96 ± 0.40^{a}	0.26 ± 0.00^{a}
SA120	11.10 ± 1.13^{a}	3.62 ± 0.06^{c}	1.72 ± 0.07^{a}	31.17 ± 1.39^{bd}	38.34 ± 1.87^{ab}	64.76 ± 0.26^{a}	0.23 ± 0.02^{a}
SA150	11.70 ± 0.14^{a}	3.62 ± 0.05^{c}	1.69 ± 0.01^{a}	32.03 ± 0.09^{d}	39.17 ± 0.51^{ab}	64.37 ± 0.69^{a}	0.22 ± 0.07^{a}
CG30	13.15 ± 0.07^{a}	3.72 ± 0.01^{ac}	1.85 ± 0.02^{a}	25.10 ± 0.23^{c}	33.50 ± 0.37^{b}	60.73 ± 0.05^{b}	0.24 ± 0.03^{a}
CG60	12.60 ± 0.14^{a}	3.69 ± 0.00^{ac}	1.74 ± 0.05^{a}	27.90 ± 0.16^{bcd}	37.47 ± 0.84^{ab}	62.44 ± 0.13^{b}	0.23 ± 0.02^{a}
CG90	12.40 ± 1.13^{a}	3.67 ± 0.00^{bc}	1.80 ± 0.10^{a}	30.07 ± 0.25^{bcd}	39.63 ± 0.46^{ad}	64.35 ± 0.08^{a}	0.24 ± 0.02^{a}
CG120	12.60 ± 0.57^{a}	3.65 ± 0.01^{bc}	1.80 ± 0.12^{a}	31.39 ± 0.01^{d}	40.89 ± 0.28^{a}	65.03 ± 0.06^{a}	0.23 ± 0.00^{a}
CG150	10.70 ± 0.85^{a}	3.60 ± 0.01^{c}	1.72 ± 0.02^{a}	32.06 ± 0.83^{d}	42.25 ± 1.64^{ac}	65.43 ± 0.57^{a}	0.23 ± 0.02^{a}
Nontreated	11.60 ± 0.71^{a}	3.81 ± 0.01^{a}	1.70 ± 0.04^{a}	26.30 ± 3.56^{bc}	33.84 ± 3.95^{bd}	62.51 ± 2.23^{ab}	0.24 ± 0.02^{a}
Pasteurized	12.10 ± 0.14^{a}	3.76 ± 0.03^{ab}	1.81 ± 0.10^{a}	45.51 ± 0.27^{a}	40.56 ± 0.01^{a}	73.62 ± 0.11^{c}	0.26 ± 0.00^{a}

[a], [b], [c] and [d] mean in the same column followed by different superscript letters are significantly different ($P < 0.05$).

Table 4. 3 The effect of atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) as feed gasses for 30, 60, 90, 120 and 150 s processing times on the particle size distribution (PSD) of apple juice after the processing (W0) and after three weeks of storage at 4°C (W3). Values followed by different superscript letters in the same column and in the same storage time are significantly different ($p < 0.05$).

Storage	Treatment	D[4,3] (µm)	D[3,2] (µm)	D(0.1) (µm)	D(0.5) (µm)	D(0.9) (µm)	Span*
0 days	**SA30**	22.95 ± 3.36^{ab}	2.56 ± 0.17^{a}	1.11 ± 0.09^{a}	8.43 ± 0.67^{a}	48.32 ± 12.59^{a}	5.56 ± 1.04^{ab}
	SA150	23.25 ± 0.65^{ab}	2.37 ± 0.41^{a}	1.09 ± 0.21^{a}	8.81 ± 0.53^{a}	44.49 ± 0.42^{a}	4.94 ± 0.27^{ab}
	CG30	27.16 ± 2.00^{b}	2.39 ± 0.26^{a}	1.03 ± 0.12^{a}	8.28 ± 0.21^{a}	60.35 ± 7.11^{a}	6.73 ± 0.42^{b}
	CG150	18.65 ± 1.42^{ab}	2.41 ± 0.07^{a}	1.04 ± 0.06^{a}	8.20 ± 0.38^{a}	36.07 ± 2.09^{a}	4.28 ± 0.46^{a}
	Nontreated	16.05 ± 1.97^{ab}	2.69 ± 0.07^{a}	1.23 ± 0.01^{a}	7.91 ± 0.14^{a}	34.04 ± 2.04^{a}	4.14 ± 0.18^{ab}
	Pasteurized	19.05 ± 2.33^{a}	2.37 ± 0.51^{a}	0.87 ± 0.08^{a}	8.93 ± 0.88^{a}	41.85 ± 5.73^{a}	4.63 ± 0.11^{ab}
3 Weeks	**SA30**	8.15 ± 0.94^{A}	1.58 ± 0.45^{A}	0.57 ± 0.12^{A}	5.27 ± 0.13^{A}	20.57 ± 3.39^{A}	3.79 ± 0.58^{ABC}
	SA150	20.13 ± 2.73^{B}	2.91 ± 0.06^{AB}	0.99 ± 0.02^{A}	5.84 ± 0.16^{A}	34.01 ± 5.02^{A}	5.67 ± 1.01^{C}
	CG30	10.38 ± 1.79^{AB}	1.74 ± 0.48^{A}	0.62 ± 0.10^{A}	5.83 ± 0.08^{A}	24.25 ± 1.73^{A}	4.06 ± 0.23^{AC}
	CG150	53.30 ± 3.85^{C}	3.40 ± 0.81^{B}	1.71 ± 0.45^{B}	11.27 ± 0.37^{B}	197.44 ± 24.60^{B}	1.73 ± 0.16^{B}
	Nontreated	6.59 ± 0.86^{A}	1.89 ± 0.09^{A}	0.66 ± 0.02^{A}	4.65 ± 0.21^{A}	15.53 ± 3.11^{A}	3.19 ± 0.52^{AB}
	Pasteurized	12.60 ± 5.61^{AB}	1.55 ± 0.16^{A}	0.54 ± 0.04^{A}	5.86 ± 0.61^{A}	27.54 ± 7.50^{A}	4.57 ± 0.79^{AC}

*span has no units.

Figures

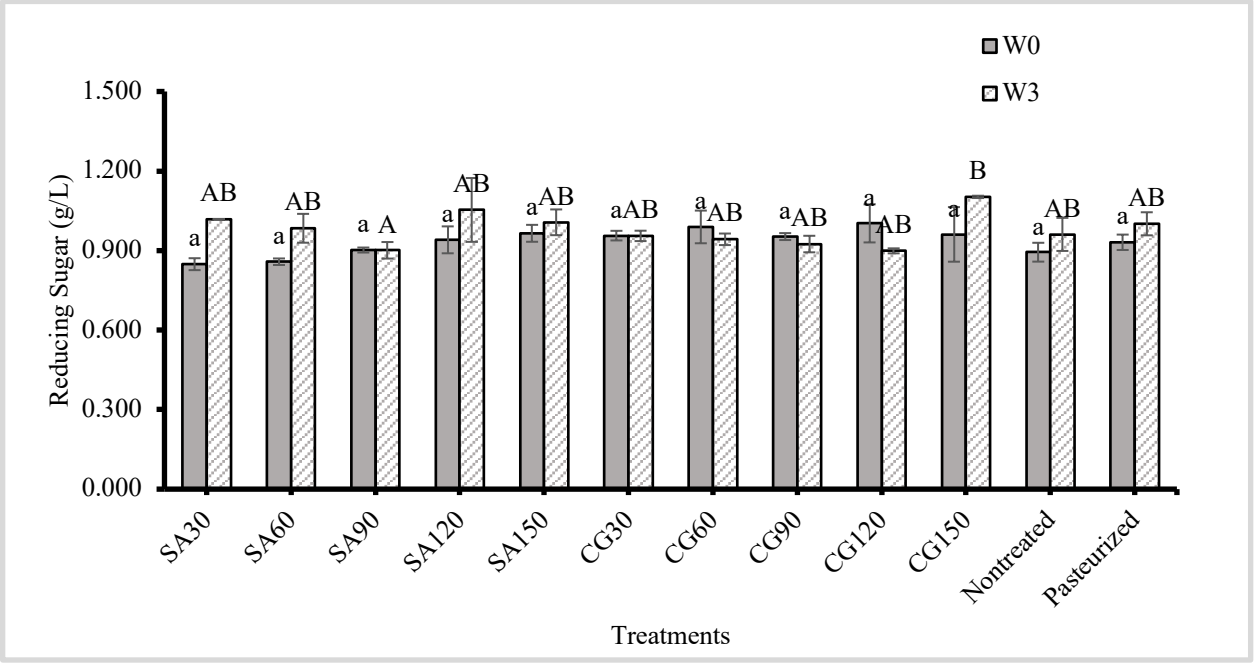

Figure 4. 1 The effect of atmospheric cold plasma generated from simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as feed gasses for 30, 60, 90, 120 and 150 s processing times on reducing sugar in apple juice after the processing (W0) and after three weeks of storage at 4°C (W3). Lowercase and uppercase letters show significant differences ($p < 0.05$) for the effect of the treatment for W0 and W3, respectively.

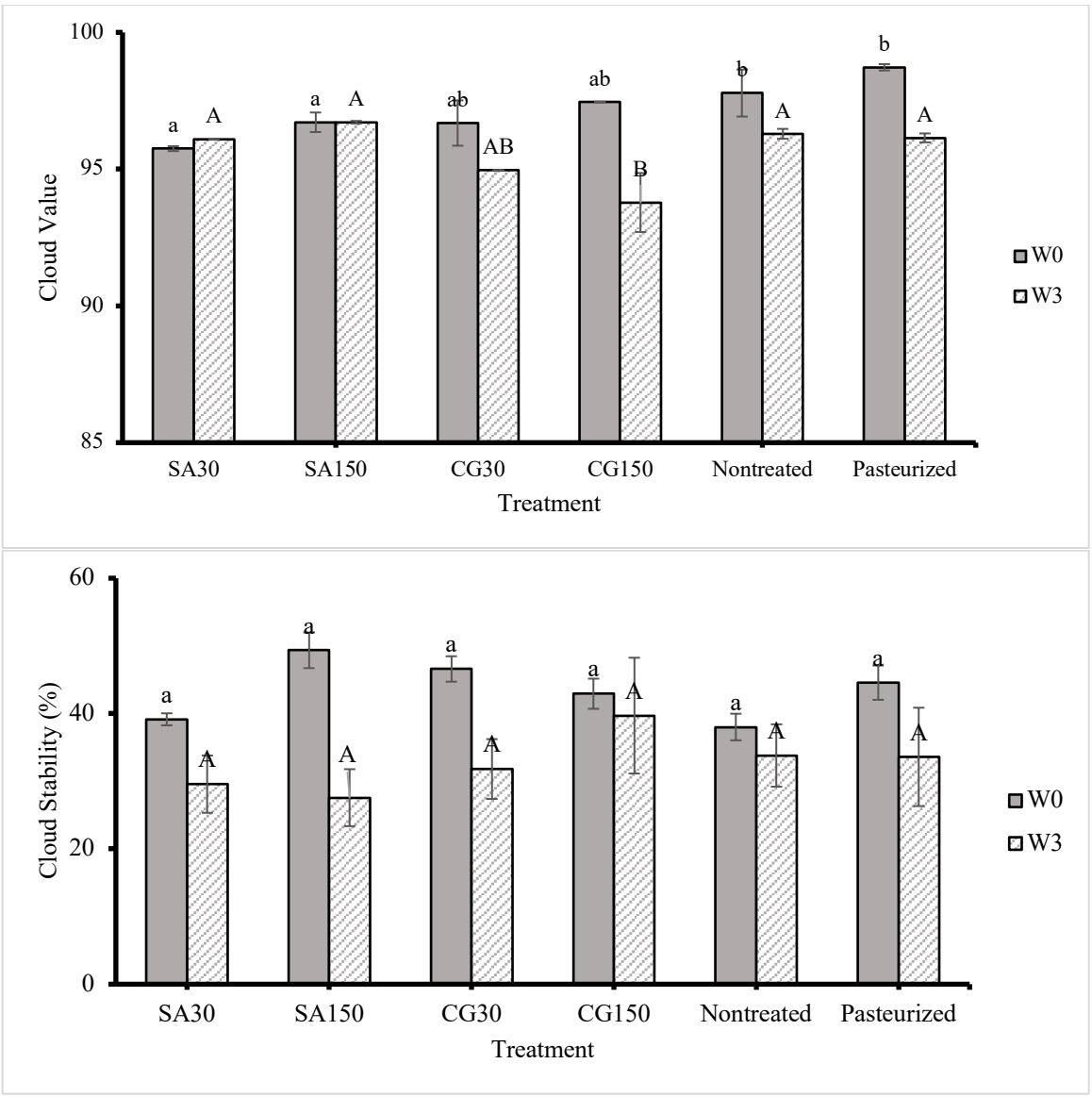

Figure 4. 2 The effect of atmospheric cold plasma generated by simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as working gasses for 30, 60, 90, 120 and 150 s processing times on cloud value and cloud stability of apple juice after the processing (W0) and after three weeks of storage at 4°C (W3). Values followed by different superscript letters are significantly different ($p < 0.05$).

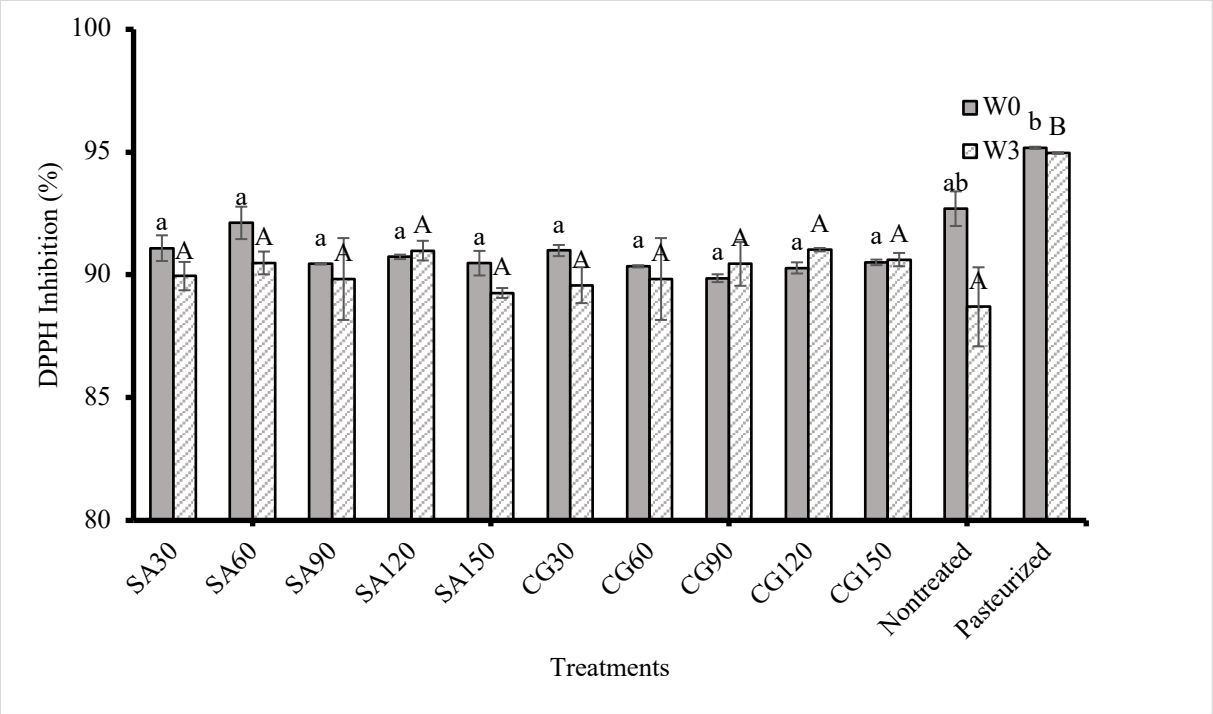

Figure 4. 3 The effect of atmospheric cold plasma with simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as working gasses for 30, 60, 90, 120 and 150 s processing times on antioxidant activity in apple juice after the processing (W0) and after three weeks of storage at 4°C (W3). Lowercase and uppercase letters show significant differences ($p < 0.05$) for the effect of the treatment for W0 and W3, respectively.

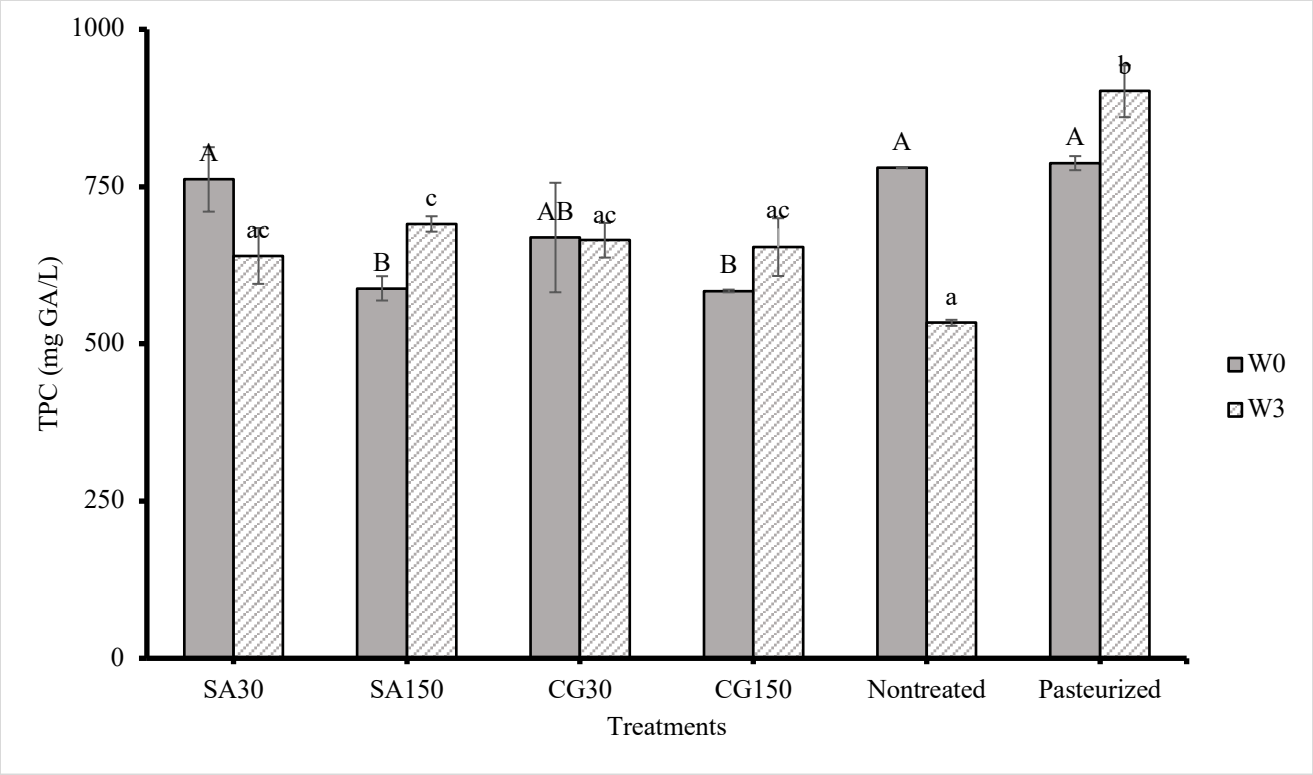

Figure 4. 4 The effect of atmospheric cold plasma with simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as working gasses for 30, 60, 90, 120 and 150 s processing times on total phenolic content (TPC) in apple juice after the processing (W0) and after three weeks of storage at 4°C (W3). Uppercase and lowercase letters show significant differences ($p < 0.05$) for the effect of the treatment for W0 and W3, respectively.

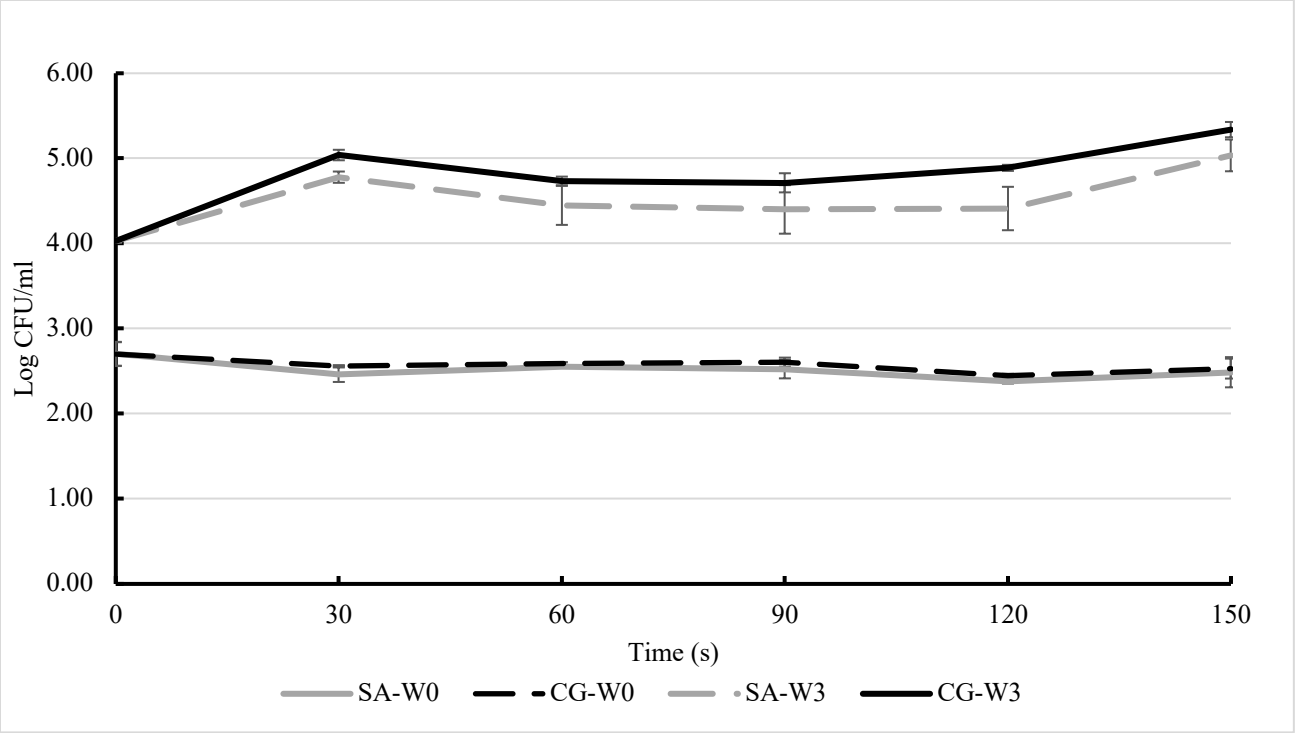

Figure 4. 5 The effect of atmospheric cold plasma generated by simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) as feed gasses for 30, 60, 90, 120 and 150 s on total yeast and mold counts of apple juice just after the processing (W0) and three weeks shelf life (W3) at 4°C.

CHAPTER 5

EFFECT OF ATMOSPHERIC COLD PLASMA ON THE PHYSICOCHEMICAL PROPERTIES AND VOLATILE COMPOUNDS OF APPLE AND CANTALOUPE JUICES[1]

[1]Ozen, E. To be submitted to *Food and Bioprocess Technology*

Abstract

Thermal pasteurization is widely used method for preservation of juices, but it alters the quality and the aroma of the juice. Atmospheric cold plasma (ACP) is a non-thermal processing technology with potential applications for juice processing. The purpose of this study was to assess how ACP processing affects the quality of cloudy apple juice (CAJ) and cantaloupe juice (CJ). Specifically, two different gasses were used in ACP treatment, which is simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) for 90 s. The effect of ACP treatment on the physicochemical characteristics, bioactive compounds, and volatile compounds were measured and compared with untreated and pasteurized juices. Overall, ACP did not significantly affect the juices' physicochemical characteristics. The color of CJ was similar to that of untreated CJ, whereas the color of CAJ was similar to thermally pasteurized CAJ. The antioxidant activity of CJ was significantly reduced from 65.8% to 33.4% when SA was used as a processing gas. Bioactive compounds in CAJ did not change with gas type and they were protected in CJ when ACP with CG was employed. ACP enhanced some juice flavors while destroying others as evaluated by change in volatile compounds.

Introduction

Since juices contain a variety of bioactive compounds such as pectin, polyphenols, and acids (Fonteles & Rodrigues, 2018; Oszmianski et al., 2007; Rodríguez et al., 2017), they satisfy consumer demands for health, taste, and ease of consumption. Juices, therefore, play a significant role in our diets. In order to preserve them, they must be pasteurized before consumption. The thermal pasteurization method is widely used to ensure the safety of juice. The downside of this technology is that it changes the nutritional and sensory properties of juices (Gonzalez & Barrett, 2010). Due to this, nonthermal pasteurization has become increasingly important. Atmospheric cold plasma is one of the nonthermal processes. In contrast to traditional thermal treatment methods that use high temperatures, atmospheric cold plasma processing uses temperatures close to ambient, thus reducing the risk of heat-related damage to the sensitive components of fruits and vegetables. In addition to preserving sensory properties, this non-thermal method also preserves bioactive compounds that contribute to fruit juice's health-promoting effects (Ozen & Singh, 2020).

A plasma is an energized mixture of ions, electrons, and neutral species that is often known as the fourth state of matter. Under atmospheric pressure or in vacuum conditions, an electric field is applied to a gas to create atmospheric or vacuum cold plasma (Bárdos & Baránková, 2010). Depending on the type of gas used and the environment where it was produced, reactive oxygen species (ROS), reactive nitrogen species (RNS), and small amounts of ultraviolet light (UV) were produced (Laroussi & Leipold, 2004).

In the USA, apples are one of the most important crops, and they are a good source of prebiotics and bioactive substances (Fonteles & Rodrigues, 2018; Oszmianski et al., 2007). In light of this, cloudy apple juice producers avoid pasteurization processes, such as hot-fill processes, because they do not want to adversely affect apple juice's bioactive compounds, quality, etc. (Choi & Nielsen, 2005). Similarly, to apple juice, cantaloupe juice contains functional substances. This fruit is suitable for juice production since it has a pleasant aroma and a high sugar content (Vaillant et al., 2005). Fruits such as this are highly perishable and are sensitive to thermal treatments (Fonteles et al., 2012; Hayashi, 1996).

To investigate the effects of cold plasma processing on quality attributes, cloudy apple juice (CAJ) and cantaloupe juice (CJ) were used, as they have distinct flavors and promising nutritional characteristics. Some researchers have studied the effects of atmospheric cold plasma on CAJ quality (Dasan & Boyaci, 2018; Illera et al., 2019; Liao et al., 2018; Montenegro et al., 2002; Xiang et al., 2018), but no research has been conducted on cantaloupe juice. Furthermore, no research has been conducted on the effect of atmospheric cold plasma on volatile compounds of apple and cantaloupe juices. The purpose of this study was to examine the effects of atmospheric cold plasma processing on apple and cantaloupe juice quality properties and volatile compounds in comparison with untreated and thermally pasteurized juices.

Materials and Methods

Sample Preparation

Red Delicious apples were purchased from a local market in Georgia, USA, and were stored overnight at 4°C. Afterward, each apple was brushed and washed individually to eliminate dirt and wax. The washed apples were then halved and crushed using an extractor (A200, Hobart Co. Troy, OH). The crushed apples were filtered through three layers of cheesecloth. To prevent

the apple juice from undergoing enzymatic browning, 0.1% ascorbic acid was added. The juice was prepared in a cold room at 4°C and stored in a freezer at -40°C until further use.

Minerva cantaloupes were purchased from a local market in Georgia, USA, and stored overnight at 4°C. Each cantaloupe was washed, cut in half, seeds were removed, and fruits were sliced into 1-inch pieces. The pieces were dipped in boiling water until their center temperature reached 60 °C. By immediately immersing them in an ice bath, the pieces were brought to an internal temperature of 5 °C. After peeling the pieces, they were ground into a puree with a grinder (A200, Hobart Co. Troy, OH) and blended (Ninja Professional Blender BL621, SharkNinja Operating LLC, QC, Canada). A three-layer cheesecloth filter was used to filter blended juice, and the juice was then stored at -40 °C until needed.

Atmospheric Cold Plasma (ACP) Treatments

In this study, the ACP device (CD50, Plasmatreat, Hayward, CA) was utilized to treat cloudy apple juices (AJ) and cantaloupe juices (CJ) illustrated by described in (Sharma & Singh, 2022). The device consists of a power generator (FG5001) with a voltage of 1 kV and a current of 16 A, a plasma jet (CD50), and a high-voltage transformer (HTR11). The transformer converts the output voltage to a 20 kV ignition voltage and a 2 kV arc drop voltage (15-25 Hz). Oxygen and nitrogen mixtures were employed as processing gases, passing through the electrodes to generate a flow of plasma active species from a small jet. To deliver ACP into the juices, a PTFE tubing (3 mm inside diameter, 5 mm outside diameter, 12.5 cm length) was attached to the nozzle outlet. To achieve proper mixing and direct interaction with the plasma species, a 1-liter flask containing 100 ml of the juice was placed beneath the plasma nozzle, positioned at a distance of 28.5 cm from the nozzle's outlet. In order to facilitate this mixing, a PTFE tubing was inserted into the sample at a depth of 0.7 cm. Two gas mixtures were used: simulated air (SA) containing 80% nitrogen and

20% oxygen, and combined air (CG) consisting of 90% nitrogen and 10% oxygen. The processing time was 90 s and the plasma flow rates was 5 liters per minutes. After the plasma treatment, ACP processed, untreated and thermally pasteurized juices were stored at 4 °C for 2 weeks, then analyses were conducted.

Pasteurization

To carry out the pasteurization process, a batch processing method was employed using a water bath (model #SHWB10, Cole-Parmer Instrument Co., Chicago, USA). Each sample was placed in a 250 ml wide-mouth polypropylene bottle (Fisher Scientific, Pittsburgh, PA, USA), with 200 ml of AJ and CJ being added to each bottle. To monitor the temperature, a thermocouple wire was attached to the bottle cap, inserted into the juices, and connected to a data logger (Fluke 52II, FLUKE, Everett, WA). The holding temperature and time were set at 90°C and 1 min, respectively. Following the holding time, the samples were promptly cooled in an ice bath.

Color, pH, °Brix, titratable acidity, and reducing sugar

A colorimeter (EZ 4500 L, Hunter Associates Laboratory Inc., Reston, VA) was used to measure the CIELAB coordinates of the juice samples. Prior to the measurements, the colorimeter underwent calibration using white and black tiles. To minimize the impact of environmental light, 35 ml of apple juice was placed inside an opaque white foam container and enclosed within a box. The lightness (L∗), greenness/redness (a∗), and blueness/yellowness (b∗) values of the juice samples were then determined. The chroma (C*) and hue angle (h) in each juice sample were calculated using Equations (1) and (2).

$$C* = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (1)$$

$$h = archtan\left(\frac{b^*}{a^*}\right) \quad (2)$$

Apple and cantaloupe juices were tested for pH and SSC using pH meters (Accumet AB150, Fisherbrand, OTT, Canada) and refractometers (MA 884, Milwaukee Instruments, Inc., Rocky Mount, NC, USA). AOAC method no. 942.15, with the final results expressed in grams of malic acid for CAJ and citric acid for CJ per 100 ml of juice, was used to determine the titratable acidity of CAJ and CJ.

The 3,5-dinitro salicylic acid (DNS) method, as described by Miller (1959), was employed to determine the reducing sugars of the juice samples. In this method, each sample was mixed with an equal volume of DNS reagent in a tube, and the tubes subsequently inserted into boiling water for 10 min. After cooling the tubes in an ice bath, their absorbance at 540 nm was measured. Standard solutions of glucose ranging from 0.2 mg/ml to 1 mg/ml were prepared, and the results of the samples were expressed in mg of glucose per ml of the juices.

Turbidity, cloud stability, and particle size distribution (PSD)

The turbidity (cloud value) of CAJ and CJ was measured according to Bhat and Goh (2017). Sample absorbance was measured at 660 nm using a spectrometer (Model #1200, Cole-Parmer Instrument Co., Chicago, USA), and turbidity was calculated using Eq. (3) and (4).

$$Transmittance = 100\ x\ 10^{-Absorbance} \quad (3)$$

$$Turbidity(T) = 100 - Tranmittance \quad (4)$$

Cloud stability of CAJ and CJ was measured and reported as relative turbidity (%T), according to De Paepe et al. (2015). Samples were measured for their absorbance at 660 nm (T0). After centrifugation at 4200 x g for 10 min, the supernatant absorbance (Tc) was also measured. As a result, cloud stability was calculated using Eq. (5):

$$\%T = \frac{T_c}{T_0}\ x\ 100 \quad (5)$$

The Particle Size Distribution (PSD) of AJ and CJ was determined by a particle size analyzer (MAM 5004, Malvern Master sizer, Worcestershire, United Kingdom). The particles measured by laser light diffraction and D[4,3] (µm), D[3,2] (µm), D10 (µm), D50 (µm), D90 (µm), and span values were calculated by the device's software.

Total phenolic content (TPC) and antioxidant activity

By using the Folin-Ciocalteau method described by Singleton et al. (1999), the total phenolic content (TPC) was determined. The 200 µl juice samples were diluted with 1.8 ml of distilled water. The mixture of 3 ml water, 200 µl Folin-Ciocalteau reagent, and 200 µl diluted juice is mixed and incubated for 10 minutes at room temperature. Afterward, 600 µl of sodium carbonate solution (80 µL, 20 g/dl) was added and mixed. After 2 hours of incubation at 30°C, the absorbance of the samples at 762 nm was measured. To create a standard curve, gallic acid solutions were utilized, the findings were presented as milligrams of gallic acid equivalent per liter of CAJ and CJ (mg GAE/l).

The DPPH assay, as described by Illera et al. (2019), was used to measure the antioxidant capacity. The samples were combined with an equal volume of ethanol, followed by centrifugation at 5000 x g for 5 minutes. Subsequently, 3.5 ml of a DPPH solution (0.2 mM) (Cayman Chemical Company, Ann Arbor, MI, USA) was mixed with 1 ml of the resulting supernatant. The mixture was then incubated at 25 °C for 30 min. The sample's absorbance was measured at 517 nm. As a control, a DPPH solution containing 1 ml of ethanol was used. The inhibition of antioxidant activity was calculated using equation (6).

$$The\ DPPH\ inhibition(\%) = \left(1 - \frac{A_S}{A_C}\right) x100 \quad (6)$$

The absorbance of the sample and control are denoted by As and Ac, respectively.

Volatile compounds analysis by GC-MS

Solid-phase microextraction (SPME) and gas chromatography with mass spectrometry (GC-MS) were used to extract and analyze the volatile compounds of AJ and CJ. The method was used according to Mohammed et al. (2021) with some modifications. Before analysis, frozen juice samples were thawed at room temperature. Two ml of each sample was transferred into 20 ml vials and 20 µL of 0.005 mg/ml 1-3-dichlorobenzene (Sigma-Alrich, St. Louis, MO, USA) was added to the juices. The vials were sealed with an aluminum cover with Teflon septum and then placed into the tray of an autosampler (Model GC Sampler 80, Agilent Technologies, Santa Clara, CA, USA). Every sample was separately placed into the oven in the autosampler to promote absorption from the headspace at 50 ◦C for 15 min. It was agitated at 250 rpm with on and off steps of 5 s and 2 s, respectively. An SPME fiber coated with 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was automatically inserted into the headspace to absorb the volatiles at 50 °C for 15 min then the fiber moved to the injection port of gas chromatography (Model 7890A, Agilent Technologies, Santa Clara, CA, USA) – mass spectrometry (Model 5977A, Agilent Technologies, Santa Clara, CA, USA) (GC–MS) and desorbed the absorbed compounds into the port at 270 °C for 5 min in spitless mode. The GC was equipped with an HP-5MS column (30 m × 250 µm i.d. × 0.25 µm film thickness). The system was operated in splitless and electron impact ionization mode (70 eV) scanning a mass range (m/z) from 30 Da to 400 Da. Helium gas is used as carrier gas with a 1 ml/min flow rate. The column temperature program was as follows: the column temperature was maintained at 40 °C for 1.4 min, then the temperature increased 5 °C/min to 175 °C and held for 1 min, then increased 10 °C/min to 275 °C, held for 3 min with a total run time of 44 min.

The volatile compounds of the juices were identified by comparing their mass spectra with the NIST mass spectra library [National Institute of Standards and Technology (NIST) mass spectral library, Version 2.2, 2014]. The retention indices (RI) of the identified compounds were determined according to Van Den Dool & Kratz (1963) based on n-alkanes (C10-C26) by the equation (7). Then, the calculated RI was compared to RI found in the literature (NIST spectra library collection) to identify the volatiles. Additionally, the peak area of the internal standard (IS) was used to calculate the relative concentrations of the identified volatiles for semi-quantification.

$$RI_x = 100n + 100\left(\frac{t_{Rx}-t_{Rn}}{t_{Rn+1}-t_{Rn}}\right) \quad (7)$$

Where t_R is the retention times of n-alkanes (C10-C26) and t_{Rx} is the retention time of the identified volatiles from juices with carbon number between n and n+1 alkanes.

Statistical Analysis

All measurements taken three times. The data were analyzed by analysis of variance (ANOVA) with SPSS v.20 software (SPSS Inc., Chicago, IL, U.S.A.). Tukey test was used for comparison of sample, significant at $P<0.05$.

Results and Discussion

Impact on physical attributes

The sensory perception of foods is influenced by their physical characteristics. A change in these characteristics could negatively affect consumer acceptance. Therefore, minimal or no changes are expected when food is processed. Table 5.1 shows the main physical characteristics (TSS, TA, pH, and color) of apple and cantaloupe juices after thermal pasteurization, ACP treatment, and without any treatments. The pH of ACP-processed apple juices was 3.61 ± 0.02 and 3.63 ± 0.01 for SA and CG, and not significantly different from the control. Pasteurized apple juices had a significantly higher pH, 3.75 ± 0.00. Cantaloupe juice samples treated with ACP were

not significantly different from nontreated and pasteurized samples. TA did not significantly differ between unprocessed and pasteurized juices when treated with ACP. Reactive oxygen and nitrogen species produced by cold plasma have been reported to affect pH (Lukes et al., 2014b; Machala et al., 2018). A number of factors influence the changing intensity of pH in the samples, including the plasma source, the gas composition, the juice type, etc. (Ozen & Singh, 2020; Tarabová et al., 2021). According to the results of this study, the pH of CAJ was slightly decreased, whereas the pH of CJ was increased compared to untreated juices. There may be a slight difference between the buffering capacities of the juices due to their complex organic compositions (Xu et al., 2017).

The total soluble solid content (TSS) or Brix° representing sugars in the juices showed no significant change for both juices. The findings were in accordance with the observations of Liao et al. (2018) and Shi et al. (2011) who found that atmospheric cold plasma processing had no effect on the °Brix of apple and orange juices, respectively. Due to the macromolecular nature of carbohydrates, cold plasma species cannot interact with them (Liao et al., 2018b).

Color serves as an indicator of freshness and quality, which impacts consumers' purchasing decisions. The color parameters of CAJ and CJ with and without treatments is shown in Table 5.1. The lightness (L*) represents achromatic colors (white, grey, and black) ranging from 0 (black) to 100 (white). ACP-treated AJ had higher L-values than untreated CAJ and lower L-values than pasteurized CAJ. The L-values of ACP-treated CJ did not differ significantly from those of untreated or pasteurized juices (Table 5.1). Chroma (C*) indicates how vivid or saturated the color is. An increase in chroma represents a more vivid color. The C* values of the ACP-treated AJ samples were significantly higher than untreated samples, but not significantly different from pasteurized samples. While the C* values of ACP-treated CJ with SA showed similar results to untreated CJ, the values increased when CG was used as plasma gas. There was no significant

difference between pasteurized CJ and ACP with CG treated samples. Hue value is a characteristic of colors determined by their position in the color spectrum. The Hue value does not represent the brightness or intensity of a color. Specifically, it shows the color we are measuring directly. ACP-treated CAJ had similar hue values to untreated samples, but lower than pasteurized samples. In the case of CJ, similar results were observed**.** During plasma processing, pigment isomerization, and phenolic compound isomerization may have caused the change in juice color (Kovačević et al., 2016b; Wang et al., 2012). Furthermore, the free radicals cause oxidation reactions that change the color of the treated samples (Bhat, 2016). As previously discussed (Ozen & Singh, 2020), color change in juices or any samples is dependent on the nature of the sample and the parameters of the processing. While this study examined two juices with the same processing parameters, the difference was proven in the effect of ACP treatment on different color parameters of juices. Lastly, ACP treatment did not significantly change reducing sugars of both AJ and CJ samples compared to untreated juices ($p > 0.05$) (Figure 5.1).

Impact on cloud value, cloud stability, and particle size distribution (PSD)

The cloud value (turbidity), the cloud stability, and the particle size of fresh juice are all important parameters to consider. As a result, these parameters have a significant impact on the juice's quality and shelf life. Untreated CAJ and CJ had initial turbidities of 97.9 ± 0.1 and 95.5 ± 2.2, respectively (Figure 5.2). The turbidity of ACP-treated CAJ was not significantly different from that of pasteurized samples, whereas CG significantly reduced the turbidity of CAJ. The turbidity of CJ was lower after pasteurization, but it was no different after ACP processing. The cloud stability of CAJ was not affected by ACP treatment. CJ's cloud stability was lowered both by pasteurization and by ACP treatment (Figure 2). Since pectins, polyphenols, and proteins

contribute to the cloud appearance of juice (Zhao et al., 2008), the cold plasma reacts with these components, altering turbidity and cloud stability.

In Table 5.2, particle size distributions (PSD) of CAJ and CJ are presented by D[4,3], D[3,2], d0.1, d0.5, d0.9, and span values. D[4,3] and D[3,2] are the average particle sizes in the distribution based on the volume and surface area of the particles, respectively. A particle with a diameter less than 10%, 50%, and 90% of its distribution is labeled d0.1, d0.5, and d0.9, respectively. Span value is the measurement of the spread of PSD. A smaller span value indicates a smaller PSD and a larger span value indicates a wider PSD.

The D[4,3] values for untreated and pasteurized CAJ significantly decreased from 40.27 ± 0.52 μm to 14.67 ± 2.81 μm . ACP-processed CAJ had significantly lower D[4,3] values than untreated CAJ ($p<0.05$). D[4,3] values of CJ were not affected by ACP treatment. Despite all PSD parameters of ACP-treated CJ being higher than both untreated and pasteurized juices, the effect of ACP was insignificant ($p>0.05$). The PSD parameters of ACP-processed CAJ were between untreated and pasteurized juices. Overall, the PSD of ACP-processed CAJ showed a similar pattern with pasteurized CAJ.

Impact on antioxidant activity and phenolic content

The antioxidant capacity of foods is another crucial quality parameter to consider when a new processing method is introduced. In this manner, antioxidant capacity measurements after processing provide information about the antioxidant contribution of plant-based foods, which may help prevent some oxidative stress-related diseases like cancer (Serrano et al., 2007). Figures 5.3 illustrates the effects of ACP processing on antioxidant activity by inhibiting DPPH as well as phenol content in CAJ and CJ, respectively. The antioxidant activity of untreated CAJ was not significantly affected by both ACP and pasteurization while the antioxidant activity of CJ after

thermal pasteurization and ACP processing with CG increased significantly ($p<0.05$) compared to untreated CJ. Using SA as a processing gas significantly decreased the DPPH inhibition of CJ from 65.8% to 33.4%. Since cold plasma is an oxidative process, it adversely affects antioxidant compounds in food (Gavahian et al., 2018). Due to the presence of higher reactive oxygen species in SA than in CG, CJ's antioxidant capacity drastically decreases when ACP treatment is combined with SA. As a result, these species increase the oxidation rate of antioxidants (Fernandes et al., 2019). Almeida et al. (2015) and Liao et al. (2018) have reported that cold plasma processing caused an insignificant change in antioxidant capacity in orange and apple juices while Illera et al., 2019) reported that plasma treatment increased cloudy apple juice antioxidant activity.

While the total phenolic content (TPC) of ACP-treated CJ showed a similar trend to the antioxidant activity of CJ, both pasteurization and ACP treatment increased the TPC of CAJ. Almeida et al. (2015) found that plasma treatment increased TPC in orange juice, while Liao et al. (2018) and Xiang et al. (2018) found that cold plasma treatment did not affect TPC in apple juice. In addition, Herceg et al. (2016), Hou et al. (2019), and Illera et al. (2019) reported that the processing increased TPC in pomegranate, blubbery, and cloud apple juices, respectively. There is still work to be done to understand how cold plasma technology affects the antioxidant activity and TPC of foods.

Impact on volatile compounds

An important quality parameter of food is its flavor, which is determined by volatile aroma compounds. As food-volatile aroma compounds contain a variety of volatiles, they are complex mixtures. The volatile compounds of ACP processed, thermally pasteurized, and untreated CAJ and CJ are shown in Table 5.3 and Table 5.4.

ACP treatment (especially with CG) leads to the disappearance or formation of some compounds found in both untreated and pasteurized CAJ, as shown in Table 5.3. While non-treated CAJ had 74% of aldehydes and ketones, 13% of alcohols, and 13% esters; ACP-treated samples had 65% of aldehydes and ketones, 20% of alcohols, and 15% esters for SA and 11% of aldehydes and ketones, 48% of alcohols, and 41% esters for CG. There were 91% of aldehydes and ketones, 3% of alcohols, and 6% of esters present in pasteurized CAJ, but 79% of aldehydes and ketones were nonanal, which might be due to the breakdown of precursor molecules during thermal treatment (Li et al., 2009). It was easy to observe the overall change in volatile composition when the compounds were divided by their chemical groups. It is difficult to distinguish all compounds individually, therefore we will discuss those compounds which have shown drastic changes.

Hexanal imparts a green apple flavor to the apple, making it one of the main flavor contributors (Zheng et al., 2004). In untreated samples, approximately 5.85 x 1.43 μg/l of hexanal was found, and this amount decreased after plasma treatment. However, when SA was utilized as the processing gas, the change in hexanal content was not statistically significant. 1-Octen-3-one is formed by the oxidation of linoleic acids found in the fruits' membranes after they are damaged (Synos et al., 2015). It adds a metallic flavor to food (Hashizume et al., 2007). It was detected in untreated, pasteurized, and ACP (with SA) treated CAJ. In contrast to thermal pasteurization, ACP decreased the amount of this off-flavor compound, and it was not detected in the samples treated with CG. However, 3-Octanone, another off-taste component, was only detected in CAJ treated with ACP, though its amount was not significant. 1-hexanol gives a vegetal and herbaceous aroma to the juices (Deshou et al., 2019). The plasma treatment with SA significantly increased 1-hexanol levels. 1-Butanol, 2-methyl-, acetate provides a fruity flavor to the samples (Jirapong et al., 2015).

ACP treatment increased the content of 1-Butanol, 2-methyl-, acetate in AJ but the increase was not significant ($p>0.05$).

Untreated CJ had 33% aldehydes and ketones, 16% alcohols, and 23% esters; ACP-treated samples had 58% aldehydes and ketones, 33% alcohols, and 8% esters for SA; and had 41% aldehydes and ketones, 35% alcohols, and 19% esters for CG. In the untreated samples, no Hexanal, Heptanal, Nonanal, and Decanal were detected. However, in the ACP-treated samples, the concentrations of these compounds were as follows: SA treatment resulted in 10.60 ± 1.56 µg/L of Hexanal, 25.41 ± 6.40 µg/L of Heptanal, 22.74 ± 8.45 µg/L of Nonanal, and 4.95 ± 1.25 µg/L of Decanal, while CG treatment led to 4.71 ± 0.20 µg/L of Hexanal, 10.16 ± 1.66 µg/L of Heptanal, 25.29 ± 7.37 mg/L of Nonanal, and 4.33 ± 0.46 mg/L of Decanal. The flavor of these components gives green apple flavor from hexanal, pungency from heptanal, the orange-rose notes of nonanal, and the citrusy richness of decanal (Espino-Díaz et al., 2016; Fraternale et al., 2011; Zheng et al., 2004). After ACP treatment, 1-hexanol concentrations increased significantly from 3.09 ± 0.07 µg/L to 12.02 ± 2.53 µg/L for SA and 17.15 ± 0.85 µg/L for CG. While there was no 1-Heptanol and 1-Nonanol were detected in untreated and pasteurized juices, ACP-treated samples showed 1-Heptanol in the amount of 3.85 ± 0.22 µg/L for SA and 9.79 ± 1.65 µg/L for CG and 1-Nonanol in the amount of 7.94 ± 1.13µg/L for SA and 6.12 ± 0.54 µg/L for CG. 1-hexanol contributes a vegetal and herbaceous aroma to juices (Deshou et al., 2019). Additionally, 1-heptanol is associated with a green flavor, while 1-nonanol provides a watermelon flavor. These findings suggest that ACP treatment may have enhanced the overall flavor of the juices. After ACP treatment, there was an increase in the concentration of certain alcohol compounds; however, both 1-Octanol and Benzyl alcohol exhibited a decrease. These particular flavors are known to contribute to floral and fruity flavor profiles (Jensen et al., 2001; Martin et al., 2016). It has been

also found that nonanoic acid, methyl ester (methyl nonanoate), and decanoic acid, methyl ester (methyl decanoate), are present in ACP-treated CJ. These compounds provide a fruity-sweet flavor to fruits (Liu et al., 2018).

In cold plasma processing, reactive oxygen and nitrogen species contribute to the production or degradation of some aroma compounds. As un-saturated long-chain fatty acids are oxidized, they are converted into aldehydes, which are then reduced to alcohols by isomerization, and further esters are produced by esterification (Hu et al., 2018). Rodrigues and Fernandes (2023) treated orange juice with cold plasma and measured volatile compound changes. The research observed that by increasing the frequency of plasma treatment, the amount of some aldehydes and alcohols increased in plasma-treated samples. The plasma application oxidized the components. As of now, research on cold plasma's effect on food volatiles is limited, and further investigation is needed to evaluate and discuss its impact.

Conclusions

The need for safe juices has heightened the significance of pasteurization. However, the widely adopted method of thermal pasteurization has drawbacks that impact the quality of juices, making it unfavorable among consumers. Therefore, a non-thermal process ACP was employed in this study to preserve juice quality. Overall, the quality of ACP-processed juices did not show significant differences compared to untreated juices. However, some minor changes were observed in various parameters for different juice types. The bioactive compounds, such as antioxidant activity and phenolic content, of CAJ remained unaffected by ACP treatment, whereas CJ experienced a significant decrease in these compounds when CG was utilized as the plasma gas mixture. Additionally, ACP treatment helped preserve the color of CJ, while ACP-processed CAJ yielded similar results to pasteurized CAJ. Analysis of the volatile compounds in the juices

revealed that ACP increased the presence of certain aroma-contributing volatiles, such as 1-hexanol, 1-butanol, 2-methyl acetate in CAJ, and hexanal, heptanal, nonanal, decanal, 1-heptanol, and 1-nonanol in CJ. However, it also decreased the levels of some volatiles, including nonanoic acid, methyl nonanoate, and methyl decanoate, among others. Further research is needed to gain a more detailed understanding of the effects of ACP on volatiles.

References

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

AOAC. (2016). Official Methods of Analysis of AOAC International - 20th Edition, 2016. 20th Ed. Gaithersburg: AOAC.

Bárdos, L., & Baránková, H. (2010). Cold atmospheric plasma: Sources, processes, and applications. *Thin Solid Films*, 518(23), 6705–6713. https://doi.org/10.1016/j.tsf.2010.07.044

Bhat, R. (2016). Impact of ultraviolet radiation treatments on the quality of freshly prepared tomato (*Solanum lycopersicum*) juice. *Food Chemistry*, 213, 635–640. https://doi.org/10.1016/j.foodchem.2016.06.096

Bhat, R., & Goh, K. M. (2017). Sonication treatment convalesce the overall quality of hand-pressed strawberry juice. *Food Chemistry*, 215, 470–476. https://doi.org/10.1016/j.foodchem.2016.07.160

Choi, L. H., & Nielsen, S. S. (2005). The effects of thermal and nonthermal processing methods on apple cider quality and consumer acceptability. *Journal of Food Quality*, 28(1), 13–29. https://doi.org/10.1111/j.1745-4557.2005.00002.x

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, 334–343. https://doi.org/10.1007/s11947-017-2014-0

De Paepe, D., Coudijzer, K., Noten, B., Valkenborg, D., Servaes, K., De Loose, M., Diels, L., Voorspoels, S., & Van Droogenbroeck, B. (2015). A comparative study between spiral-filter press and belt press implemented in a cloudy apple juice production process. *Food Chemistry*, 173, 986–996. https://doi.org/10.1016/j.foodchem.2014.10.019

Deshou, M., liu, H., zhengfeng, L., yunwei, N., zuobing, X., fengmei, Z., & jiancai, Z. (2019). Characterization of aroma-active compounds in delicious apples juices by gas chromatography-mass spectrometry (GC-MS), gas chromatography–olfactometry (GC-O) and odor activity value (OAV). *BioRxiv*, 611061. https://doi.org/10.1101/611061

Espino-Díaz, M., Sepúlveda, D. R., González-Aguilar, G., & Olivas, G. I. (2016). Biochemistry of apple aroma: A review. *Food Technology and Biotechnology*, 54(4), 375–394. https://doi.org/10.17113/ftb.54.04.16.4248

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

Fonteles, T. V., Costa, M. G. M., de Jesus, A. L. T., de Miranda, M. R. A., Fernandes, F. A. N., & Rodrigues, S. (2012). Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters. *Food Research International*, 48(1), 41–48. https://doi.org/10.1016/j.foodres.2012.02.013

Fonteles, T. V., & Rodrigues, S. (2018). Prebiotic in fruit juice: processing challenges, advances, and perspectives. *Current Opinion in Food Science*, 22, 55–61. https://doi.org/10.1016/j.cofs.2018.02.001

Fraternale, D., Ricci, D., Flamini, G., & Giomaro, G. (2011). Volatiles profile of red apple from Marche Region (Italy). *Records of Natural Products*, 5(3), 202.

Gavahian, M., Chu, Y.-H., Khaneghah, A. M., Barba, F. J., & Misra, N. N. (2018). A critical analysis of the cold plasma induced lipid oxidation in foods. *Trends in Food Science & Technology*, 77, 32–41. https://doi.org/10.1016/j.tifs.2018.04.009

Gonzalez, M. E., & Barrett, D. M. (2010). Thermal, high pressure, and electric field processing effects on plant cell membrane integrity and relevance to fruit and vegetable quality. *Journal of Food Science*, 75(7), R121–R130. https://doi.org/10.1111/j.1750-3841.2010.01763.x

Hashizume, M., Gordon, M. H., & Mottram, D. S. (2007). Light-induced off-flavor development in cloudy apple juice. *Journal of Agricultural and Food Chemistry*, 55(22), 9177–9182. https://doi.org/10.1021/jf0715727

Hayashi, R. (1996). An overview of the use of high pressure in bioscience and biotechnology. *Progress in Biotechnology*, 13, 1–6. https://doi.org/10.1016/S0921-0423(06)80003-1

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

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*, 290, 79–86. https://doi.org/10.1016/j.foodchem.2019.03.123

Hu, C.-J., Li, D. A., Ma, Y.-X., Zhang, W., Lin, C., Zheng, X.-Q., Liang, Y.-R., & Lu, J.-L. (2018). Formation mechanism of the oolong tea characteristic aroma during bruising and withering treatment. *Food Chemistry*, 269, 202–211. https://doi.org/10.1016/j.foodchem.2018.07.016

Illera, A. E., Chaple, S., Sanz, M. T., Ng, S., Lu, P., Jones, J., Carey, E., & Bourke, P. (2019). Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters

of the juice during storage. *Food Chemistry*: X, 3, 100049. https://doi.org/10.1016/j.fochx.2019.100049

Jensen, K., Christensen, L. P., Hansen, M., Jørgensen, U., & Kaack, K. (2001). Olfactory and quantitative analysis of volatiles in elderberry (*Sambucus nigra L.*) juice processed from seven cultivars. *Journal of the Science of Food and Agriculture*, 81(2), 237–244. https://doi.org/10.1002/1097-0010(20010115)81:2<237::AID-JSFA809>3.0.CO;2-H

Jirapong, C., Wongs-Aree, C., Noichinda, S., Uthairatanakij, A., & Kanlayanarat, S. (2015). Assessment of volatile and non-volatile organic compounds in the liquid endosperm of young 'Nam Hom'coconut (*Cocos nucifera L.*) at two stages of maturity. *The Journal of Horticultural Science and Biotechnology*, 90(5), 477–482. https://doi.org/10.1080/14620316.2015.11668703

Kovačević, D. B., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Jambrak, A. R., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, 190, 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099

Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*, 233(1–3), 81–86. https://doi.org/10.1016/j.ijms.2003.11.016

Li, C., Hao, J., Zhong, H., Dang, M., & Xie, B. (2009). Aroma components at various stages of litchi juice processing. *Journal of the Science of Food and Agriculture*, 89(14), 2405–2414. https://doi.org/10.1002/jsfa.3736

Liao, X., Li, J., Muhammad, A. I., Suo, Y., Chen, S., Ye, X., Liu, D., & 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

Liu, Y., He, C., & Song, H. (2018). Comparison of SPME versus SAFE processes for the analysis of flavor compounds in watermelon juice. *Food Analytical Methods*, 11, 1677–1689. https://doi.org/10.1007/s12161-018-1153-x

Lukes, P., Dolezalova, E., Sisrova, I., & Clupek, M. (2014). Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H_2O_2 and HNO_2. *Plasma Sources Science and Technology*, 23(1), 015019. https://10.1088/0963-0252/23/1/015019

Machala, Z., Tarabová, B., Sersenová, D., Janda, M., & Hensel, K. (2018). Chemical and antibacterial effects of plasma activated water: Correlation with gaseous and aqueous reactive oxygen and nitrogen species, plasma sources and air flow conditions. *Journal of Physics D: Applied Physics*, 52(3), 034002. https://10.1088/1361-6463/aae807

Martin, V., Giorello, F., Fariña, L., Minteguiaga, M., Salzman, V., Boido, E., Aguilar, P. S., Gaggero, C., Dellacassa, E., & Mas, A. (2016). De novo synthesis of benzenoid compounds by the yeast Hanseniaspora vineae increases the flavor diversity of wines. *Journal of Agricultural and Food Chemistry*, 64(22), 4574–4583. https://doi.org/10.1021/acs.jafc.5b05442

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3), 426–428.

Mohammed, J., Belisle, C. E., Wang, S., Itle, R. A., Adhikari, K., & Chavez, D. J. (2021). Volatile profile characterization of commercial peach (*Prunus persica*) cultivars grown in Georgia, USA. *Horticulturae*, 7(12), 516. https://doi.org/10.3390/horticulturae7120516

Montenegro, J., Ruan, R., Ma, H., & Chen, P. (2002). Inactivation of *E. coli* O157: H7 using a pulsed nonthermal plasma system. *Journal of Food Science*, 67(2), 646–648. https://doi.org/10.1111/j.1365-2621.2002.tb10653.x

Oszmianski, J., Wolniak, M., Wojdylo, A., & Wawer, I. (2007). Comparative study of polyphenolic content and antiradical activity of cloudy and clear apple juices. *Journal of the Science of Food and Agriculture*, 87(4), 573–579. https://doi.org/10.1002/jsfa.2707

Ozen, E., & Singh, R. K. (2020). Atmospheric cold plasma treatment of fruit juices: A review. *Trends in Food Science & Technology*, 103, 144–151. https://doi.org/10.1016/j.tifs.2020.07.020

Rodrigues, S., & Fernandes, F. A. N. (2023). Effect of dielectric barrier discharge plasma treatment in pasteurized orange juice: changes in volatile composition, aroma, and mitigation of off-flavors. *Food and Bioprocess Technology*, 16(4), 930–939. https://doi.org/10.1007/s11947-022-02976-0

Rodríguez, Ó., Gomes, W. F., Rodrigues, S., & Fernandes, F. A. N. (2017). Effect of indirect cold plasma treatment on cashew apple juice (*Anacardium occidentale L.*). *Lwt*, 84, 457–463. https://doi.org/10.1016/j.lwt.2017.06.010

Serrano, J., Goñi, I., & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International*, 40(1), 15–21. https://doi.org/10.1016/j.foodres.2006.07.010

Sharma, S., & Singh, R. K. (2022). Effect of atmospheric pressure cold plasma treatment time and composition of feed gas on properties of skim milk. *LWT – Food Science and Technology*, 154, 112747. https://doi.org/10.1016/j.lwt.2021.112747

Shi, X. M., Zhang, G. J., Wu, X. L., Li, Y. X., Ma, Y., & Shao, X. J. (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

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, Vol. 299, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Synos, K., Reynolds, A. G., & Bowen, A. J. (2015). Effect of yeast strain on aroma compounds in Cabernet franc icewines. *LWT-Food Science and Technology*, 64(1), 227–235. https://doi.org/10.1016/j.lwt.2015.05.044

Tarabová, B., Tampieri, F., Maran, E., Marotta, E., Ostrihoňová, A., Krewing, M., & Machala, Z. (2021). Chemical and antimicrobial effects of air non-thermal plasma processing of fresh apple juice with focus on safety aspects. *Foods*, 10(9), 2055. https://doi.org/10.3390/foods10092055

Vaillant, F., Cisse, M., Chaverri, M., Perez, A., Dornier, M., Viquez, F., & Dhuique-Mayer, C. (2005). Clarification and concentration of melon juice using membrane processes. *Innovative Food Science & Emerging Technologies*, 6(2), 213–220. https://doi.org/10.1016/j.ifset.2004.11.004

Van Den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, 11, 463-471.

Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., Zhu, W. D., Becker, K. H., & 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, 1–7. https://doi.org/10.1140/epjd/e2012-30053-1

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, 1778–1791. https://doi.org/10.1007/s11947-017-1947-7

Zhao, G.-Y., Zong, W., & An, G.-J. (2008). Effect of storage on cloud stability of cloudy apple juice. *Food Science and Technology International*, 14(1), 105–113. https://10.1177/1082013208090176

Zheng, C. H., Kim, T. H., Kim, K. H., Leem, Y. H., & Lee, H. J. (2004). Characterization of potent aroma compounds in *Chrysanthemum coronarium L.* (Garland) using aroma extract dilution analysis. *Flavour and Fragrance Journal*, 19(5), 401–405. https://doi.org/10.1002/ffj.1447

Tables

Table 5. 1 The effect of atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) as feed gasses for 90 s on the quality of apple and cantaloupe juices.

Juice Type	Treatment	°Brix	pH	Color (L)	Chroma	Hue	TA (% Malic Acid)
Apple Juice	**SA90**	11.20 ± 0.42^{a}	3.61 ± 0.02^{a}	30.31 ± 0.41^{ab}	39.29 ± 0.10^{a}	63.10 ± 0.74^{a}	0.27 ± 0.02^{a}
	CG90	11.70 ± 0.00^{a}	3.63 ± 0.01^{a}	29.64 ± 0.84^{ab}	40.24 ± 1.86^{a}	62.56 ± 0.59^{a}	0.24 ± 0.02^{a}
	Nontreated	10.25 ± 0.78^{a}	3.65 ± 0.01^{a}	24.24 ± 1.81^{a}	31.23 ± 2.20^{b}	61.42 ± 0.29^{a}	0.25 ± 0.03^{a}
	Pasteurized	10.65 ± 0.49^{a}	3.75 ± 0.00^{b}	49.19 ± 0.36^{b}	41.25 ± 0.35^{a}	75.01 ± 0.07^{b}	0.23 ± 0.00^{a}
Cantaloupe Juice	**SA90**	7.50 ± 0.28^{A}	4.98 ± 0.23^{AB}	48.39 ± 0.44^{A}	37.57 ± 1.47^{A}	59.02 ± 2.51^{A}	0.20 ± 0.11^{A}
	CG90	8.05 ± 0.07^{A}	4.97 ± 0.14^{AB}	46.66 ± 0.21^{A}	47.14 ± 1.52^{B}	64.38 ± 0.62^{AB}	0.15 ± 0.02^{A}
	Nontreated	7.90 ± 0.00^{A}	4.54 ± 0.22^{B}	46.88 ± 0.74^{A}	40.63 ± 0.74^{A}	59.89 ± 0.38^{A}	0.25 ± 0.05^{A}
	Pasteurized	7.90 ± 0.42^{A}	5.43 ± 0.05^{A}	47.37 ± 0.19^{A}	46.70 ± 0.38^{B}	66.25 ± 0.99^{B}	0.11 ± 0.01^{A}

Values followed by different superscript letters in the same column within each juice are significantly different ($p < 0.05$).

TA = Titratable acidity

Table 5. 2 The effect of atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) and combined gas (CG; 90% nitrogen and 10% oxygen) for 90 s on the particle size distribution (PSD) of apple and cantaloupe juices. Values followed by different superscript letters in the same column within each juice are significantly different ($p < 0.05$).

Juice Type	Treatment	D[4,3] (µm)	D[3,2] (µm)	D(0.1) (µm)	D(0.5) (µm)	D(0.9) (µm)	Span*
Apple Juice	**SA90**	25.79 ± 3.25^{a}	2.36 ± 0.41^{a}	0.96 ± 0.23^{ab}	11.13 ± 1.10^{a}	73.43 ± 11.08^{a}	6.60 ± 1.67^{a}
	CG90	22.87 ± 3.85^{a}	1.77 ± 0.02^{ab}	0.70 ± 0.04^{ac}	8.97 ± 1.61^{a}	64.78 ± 12.39^{a}	7.14 ± 0.10^{a}
	Nontreated	40.27 ± 0.52^{b}	2.69 ± 0.19^{a}	1.26 ± 0.08^{b}	22.10 ± 3.68^{b}	105.13 ± 5.25^{b}	4.79 ± 1.04^{a}
	Pasteurized	14.67 ± 2.81^{a}	1.18 ± 0.04^{b}	0.41 ± 0.01^{c}	5.57 ± 0.42^{a}	42.53 ± 9.40^{a}	7.53 ± 1.12^{a}
Cantaloupe Juice	**SA90**	219.82 ± 21.91^{A}	100.26 ± 8.27^{A}	49.69 ± 4.37^{A}	169.50 ± 21.47^{A}	470.81 ± 42.18^{A}	2.50 ± 0.10^{A}
	CG90	210.73 ± 39.46^{A}	102.70 ± 5.86^{A}	52.55 ± 0.46^{A}	169.61 ± 25.93^{A}	430.40 ± 113.50^{A}	2.20 ± 0.33^{A}
	Nontreated	199.17 ± 32.12^{A}	90.28 ± 0.54^{A}	47.49 ± 0.03^{A}	155.50 ± 18.99^{A}	419.66 ± 92.09^{A}	2.38 ± 0.30^{A}
	Pasteurized	133.23 ± 25.77^{A}	56.03 ± 1.22^{B}	26.74 ± 0.33^{B}	98.65 ± 6.98^{A}	202.27 ± 208.02^{A}	2.67 ± 0.63^{A}

*span has no units

Table 5. 3 Relative concentration (based on the internal standard concentration) of volatile compounds identified in apple juices (µg/L) by headspace solid-phase microextraction gas chromatography–mass spectroscopy (HS-SPME/GC–MS).

Category	Compound	RI (Exp)[a]	RI(Lit)[b]	Apple juices with/without treatments (µg/L)			
				Untreated	Pasteurized	SA90[c]	CG90[d]
Aldehydes And Ketones	Hexanal	804	800	5.85 ± 1.43	8.62 ± 0.87	2.25 ± 2.29	n.d.[e]
	2-Hexenal, (E)-	855	855	1.86 ± 0.19	n.d.[e]	n.d.[e]	n.d.[e]
	2-Heptenal, (Z)-	957	964	8.15 ± 1.48	13.18 ± 2.64	9.02 ± 4.21	n.d.[e]
	1-Octen-3-one	980	980	4.12 ± 0.50	13.98 ± 3.40	5.43 ± 3.05	n.d.[e]
	Benzeneacetaldehyde	1046	1044	0.52 ± 0.09	n.d.[e]	n.d.[e]	n.d.[e]
	2-Octenal, (E)-	1060	1064	2.80 ± 0.30	12.51 ± 1.50	3.60 ± 2.35	1.48 ± 1.24
	Decanal	1206	1204	0.99 ± 0.11	7.01 ± 0.46	1.94 ± 0.78	0.89 ± 0.60
	2-Undecenal	1363	1359	2.80 ± 1.13	n.d.[e]	3.51 ± 2.06	n.d.[e]
	3-Octanone	989	985	n.d.[e]	n.d.[e]	0.39 ± 0.04	0.61 ± 0.40
	Ethanone, 1-(4-ethylphenyl)-	1342	1281	n.d.[e]	n.d.[e]	0.66 ± 0.00	n.d.[e]
	Heptanal	904	902	n.d.[e]	1.64 ± 0.29	n.d.[e]	n.d.[e]
	Nonanal	1106	1108	n.d.[e]	78.55 ± 13.12	n.d.[e]	n.d.[e]
	4-Decanone	1133	1111	n.d.[e]	1.30 ± 0.02	n.d.[e]	n.d.[e]
	2-Decenal, (E)-	1261	1262	n.d.[e]	3.29 ± 0.72	n.d.[e]	n.d.[e]
	Undecanal	1306	1305	n.d.[e]	0.70 ± 0.05	n.d.[e]	n.d.[e]
Alcohols	1-Hexanol	874	870	4.79 ± 1.03	2.91 ± 0.82	8.04 ± 3.01	12.06 ± 0.76
	1-Nonanol	1173	1171	n.d.[e]	n.d.[e]	n.d.[e]	1.34 ± 0.41
	1-Octanol	1074	1076	n.d.[e]	1.22 ± 0.31	n.d.[e]	n.d.[e]
Esters	1-Butanol, 2-methyl-, acetate	883	883	4.12 ± 0.55	7.32 ± 1.08	6.17 ± 4.88	9.44 ± 0.11
	Acetic acid, hexyl ester	1017	1014	0.80 ± 0.13	0.58 ± 0.16	0.65 ± 0.04	0.58 ± 0.16
	2-Hexen-1-ol, acetate, (Z)-	1020	1005	n.d.[e]	1.14 ± 0.08	n.d.[e]	1.27 ± 0.24

[a]: RI(Exp), retention indices calculated by a series of hydrocarbons based on the HP-5MS column, except for 4-Decanone which is based on a DB-1 column.

[b]: RI(Lit), retention indices obtained from the NIST library.

[c]: Juices processed with atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) for 90 s.

[d]: Juices processed with atmospheric cold plasma with combined gas (CG; 90% nitrogen and 10% oxygen) for 90 s.

[e]: n.d. – Not detected.

Table 5. 4 Relative concentration (based on the internal standard concentration) of volatile compounds identified in cantaloupe juices (μg/L) by headspace solid-phase microextraction gas chromatography–mass spectroscopy (HS-SPME/GC–MS).

Category	Compound	RI (Exp)[a]	RI(Lit)[b]	Cantaloupe juices with/without treatments (μg/L)			
				Untreated	Pasteurized	SA90 [c]	CG90 [d]
Aldehydes And Ketones	Hexanal	804	800	10.60 ± 1.56	4.71 ± 0.20	n.d.[e]	n.d.[e]
	Heptanal	904	902	25.41 ± 6.40	10.16 ± 1.66	n.d.[e]	1.08 ± 0.16
	1-Octen-3-ol	982	981	1.92 ± 0.29	2.73 ± 0.03	3.55 ± 0.12	2.39 ± 0.39
	Nonanal	1105	1106	22.74 ± 8.45	25.29 ± 7.37	n.d.[e]	8.94 ± 2.98
	Decanal	1206	1204	4.95 ± 1.25	4.33 ± 0.46	n.d.[e]	n.d.[e]
	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	1451	1455	3.81 ± 0.92	n.d.[e]	3.51 ± 2.38	3.58 ± 1.51
	5-Hepten-2-one, 6-methyl-	990	985	n.d.[e]	n.d.[e]	n.d.[e]	0.31 ± 0.04
n.d.[e] Alcohols	1-Hexanol	874	870	12.02 ± 2.53	17.15 ± 0.85	3.09 ± 0.07	n.d.[e]
	1-Heptanol	974	974	3.85 ± 0.22	9.79 ± 1.65	n.d.[e]	n.d.[e]
	Eucalyptol	1032	1028	1.54 ± 0.11	1.70 ± 0.15	1.58 ± 0.10	1.75 ± 0.24
	Benzyl alcohol	1038	1037	1.56 ± 0.43	1.44 ± 0.09	6.16 ± 0.74	1.07 ± 0.02
	1-Octanol	1074	1076	4.49 ± 1.29	3.47 ± 0.05	n.d.[e]	n.d.[e]
	1-Nonanol	1173	1171	7.94 ± 1.13	6.12 ± 0.54	n.d.[e]	n.d.[e]
	1-Decanol	1272	1279	8.07 ± 0.49	n.d.[e]	n.d.[e]	n.d.[e]
	3-Hexen-1-ol, (Z)-	1010	1007	n.d.[e]	1.70 ± 0.07	4.20 ± 0.06	4.96 ± 0.13

[a]: RI(Exp), retention indices calculated by a series of hydrocarbons based on the HP-5MS column.

[b]: RI(Lit), retention indices obtained from the NIST library.

[c]: Juices processed with atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) for 90 s.

[d]: Juices processed with atmospheric cold plasma with combined gas (CG; 90% nitrogen and 10% oxygen) for 90 s.

[e]: n.d. – Not detected.

Table 5. 4 Relative concentration (based on the internal standard concentration) of volatile compounds identified in cantaloupe juices (µg/L) by headspace solid-phase microextraction gas chromatography–mass spectroscopy (HS-SPME/GC–MS) (Cont.).

Category	Compound	RI (Exp)[a]	RI(Lit)[b]	Cantaloupe juices with/without treatments (µg/L)			
				Untreated	Pasteurized	SA90 [c]	CG90 [d]
Esters	Acetic acid, hexyl ester	1017	1014	5.77 ± 1.95	6.65 ± 0.11	7.24 ±0.24	7.65 ± 0.64
	Heptanoic acid, methyl ester	1028	1025	0.40 ± 0.10	0.85 ± 0.37	2.16 ± 0.00	n.d.[e]
	Octanoic acid, methyl ester	1126	1126	n.d.[e]	1.20 ± 0.51	n.d.[e]	n.d.[e]
	Acetic acid, phenylmethyl ester	1166	1162	0.32 ± 0.05	0.78 ± 0.14	n.d.[e]	2.18 ± 0.45
	Acetic acid, octyl ester	1212	1211	0.74 ± 0.52	1.11 ± 0.18	1.16 ± 0.01	0.82 ± 0.42
	Nonanoic acid, methyl ester	1225	1225	0.71 ± 0.42	1.89 ± 1.22	n.d.[e]	n.d.[e]
	Decanoic acid, methyl ester	1323	1327	1.14 ± 0.31	1.78 ± 0.76	n.d.[e]	n.d.[e]
	1-Butanol, 2-methyl-, acetate	883	883	n.d.[e]	2.49 ± 0.16	4.76 ± 0.04	7.97 ± 0.81
	3-Hexen-1-ol, acetate, (Z)-	1010	1007	n.d.[e]	n.d.[e]	1.23 ± 0.05	n.d.[e]
	Heptanoic acid, ethyl ester	1028	1025	n.d.[e]	1.92 ± 1.29	n.d.[e]	n.d.[e]
	Octanoic acid, ethyl ester	1126	1126	n.d.[e]	1.20 ± 0.51	n.d.[e]	n.d.[e]
	Isobutyl acetate	784	781	n.d.[e]	n.d.[e]	5.06 ± 0.55	n.d.[e]
Carboxylic Acis	Nonanoic acid	1323	1327	n.d.[e]	6.23 ± 1.64	-	n.d.[e]
	Acetic acid	697	660	n.d.[e]	n.d.[e]	16.17 ± 5.51	n.d.[e]

[a]: RI(Exp), retention indices calculated by a series of hydrocarbons based on the HP-5MS column.

[b]: RI(Lit), retention indices obtained from the NIST library.

[c]: Juices processed with atmospheric cold plasma with simulated air (SA; 80% nitrogen and 20% oxygen) for 90 s.

[d]: Juices processed with atmospheric cold plasma with combined gas (CG; 90% nitrogen and 10% oxygen) for 90 s.

[e]: n.d. – Not detected.

Figures

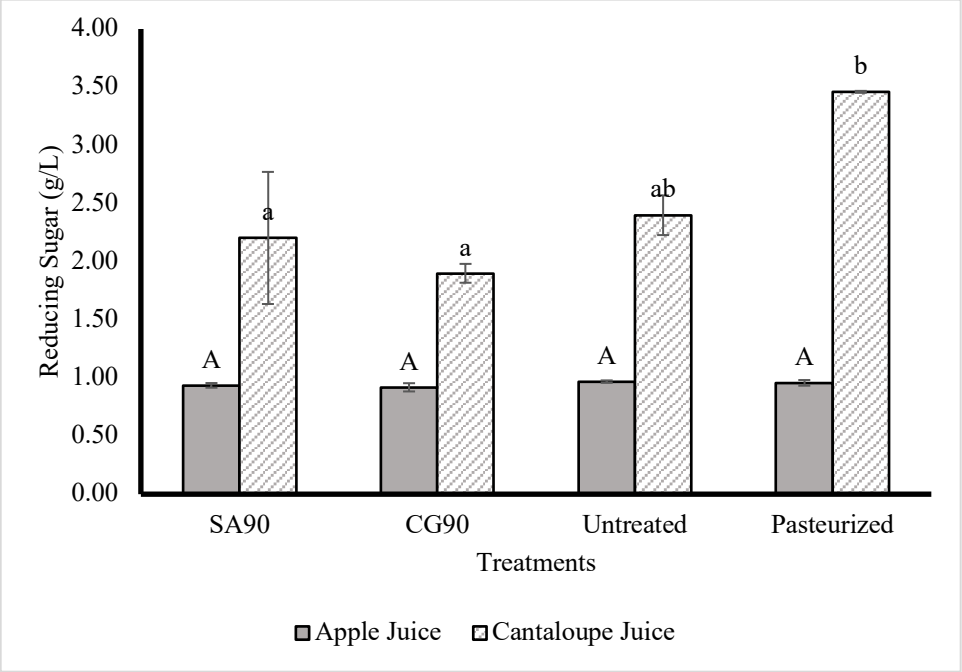

Figure 5. 1 The effect of atmospheric cold plasma with simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as working gasses for 90 s processing times on reducing sugar of apple and cantaloupe juices. Uppercase and lowercase letters show significant differences ($p < 0.05$) for the effect of the treatment for apple and cantaloupe juices, respectively.

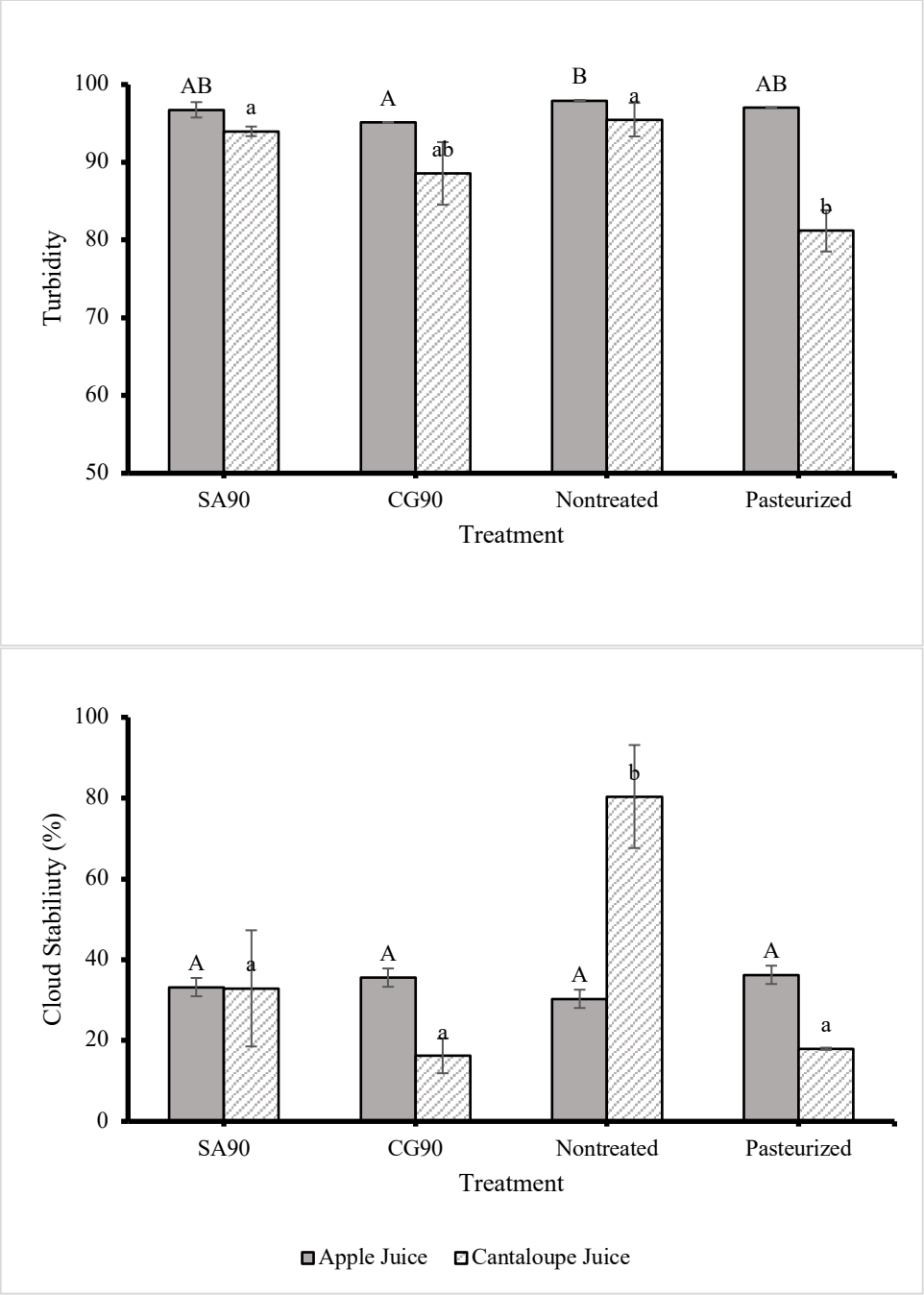

Figure 5. 2 The effect of atmospheric cold plasma with simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) for 90 s on turbidity and cloud stability of apple and cantaloupe juices. Uppercase and lowercase letters show significant differences ($p < 0.05$) for the effect of the treatment for apple and cantaloupe juices, respectively.

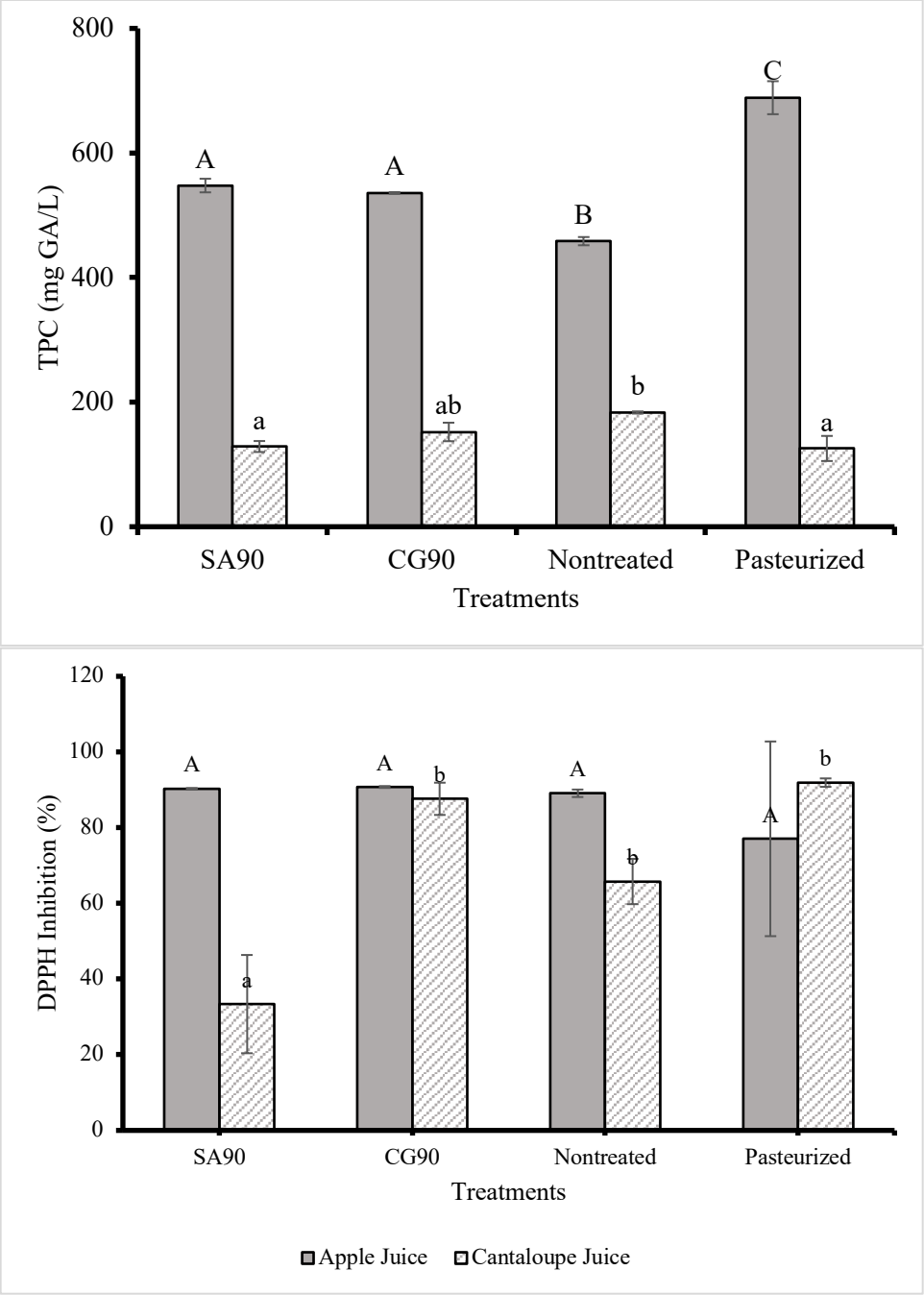

Figure 5. 3 The effect of atmospheric cold plasma with simulated air (SA) (80% nitrogen and 20% oxygen) and combined gas (CG) (90% nitrogen and 10% oxygen) as working gasses for 90 s processing times on total phenolic content and antioxidant activity of apple and cantaloupe juices. Uppercase and lowercase letters show significant differences ($p < 0.05$) for the effect of the treatment for apple and cantaloupe juices, respectively.

CHAPTER 6

CONCLUSIONS & FUTURE RECOMMENDATIONS

This study investigated the effects of atmospheric cold plasma (ACP) treatment on juice quality and safety, with a focus on microbial inactivation and preservation of volatile compounds relevant to sensory attributes. In Chapter 3, the effect of ACP on *E. coli* in apple juice was investigated. Chapter 4 examined the effect of plasma time, gas compositions, and storage on apple juice quality. Lastly, the quality and volatile compounds of apple and cantaloupe juices after ACP treatment were studied and reported in Chapter 5. The results demonstrated that atmospheric cold plasma treatment effectively reduced microbial populations, including pathogens, in juice samples while preserving most quality content. Moreover, volatile compounds increased and/or decreased for both apple and cantaloupe juices. These findings have significant implications for the juice industry as ACP treatment offers a promising non-thermal method for enhancing juice safety by eliminating harmful microorganisms without compromising quality value. Although ACP is a non-thermal and promising technology for juice processing, future studies are recommended with a long-term goal of commercializing ACP pasteurization as follows:

1) Reporting detailed ACP processing configurations: Given the diverse range of plasma devices available, providing comprehensive information by researchers on the specific parameters used during cold plasma treatment is crucial. This includes details such as the type of plasma source, discharge power, treatment duration, gas composition, and treatment distance. Clear documentation will facilitate reproducibility and comparison of results across studies.

2) Research on spore inactivation in cold plasma-treated juices: While studies have examined the inactivation of bacteria, yeast, and molds, there is a need to investigate the efficacy of atmospheric cold plasma in eliminating spores. Spores can be resilient to various preservation methods and pose a risk to juice quality.

3) Investigating the effect of cold plasma on yeast and molds during storage: Shelf life is a critical aspect of juice quality, and the growth of yeast and molds during storage can lead to spoilage. Further research is needed to assess the long-term effects of cold plasma treatment on yeast and mold growth and viability in juices. This will provide insights into the potential for atmospheric cold plasma to extend juice shelf life.

4) Exploring the effect of cold plasma on juice volatiles: Juice volatile compounds contribute significantly to their sensory attributes and overall quality. Investigating the impact of cold plasma treatment on juice volatile profiles is essential to assess any potential alterations in aroma, flavor, and overall sensory experience. This research provided valuable information on the suitability of ACP for preserving juice's volatile characteristics, but more research is needed.

5) Assessing the effect of cold plasma on juice sensory properties: Consumer acceptance and preference are crucial factors in the success of any food processing technology. Hence, it is important to conduct sensory evaluations to determine the effect of cold plasma treatment on juice sensory attributes. This includes assessing taste, color, texture, and overall consumer perception. Such investigations will help gauge the acceptability of cold plasma-treated juices in the market.

6) Further research on inactivation modeling: Developing accurate mathematical models to predict pathogen inactivation kinetics during ACP treatment is necessary. This will enable the optimization of processing parameters for different juice types and provide insights into the

efficacy and safety of the treatment. Additional research is needed to refine and validate existing models or develop new ones specific to ACP inactivation.

7) More research on pathogen surrogate selection: Identifying suitable surrogate microorganisms that mimic the behavior and resistance of pathogens is crucial for conducting research on cold plasma treatment. Further investigation is needed to determine and validate appropriate surrogate microorganisms that can effectively represent the behavior of various pathogens in juice. This will enhance the reliability and relevance of research outcomes.

8) Assessing scalability and cost-effectiveness: Research should address the scalability and cost-effectiveness of implementing ACP technology in juice processing facilities. This includes evaluating the feasibility of integrating ACP systems into existing processing lines, estimating operational costs, and conducting cost-benefit analyses. Such investigations will provide valuable information for industry stakeholders considering cold plasma treatment.

By addressing these future research areas, further advancements can be made in ACP applications for juices. This will contribute to enhanced microbial safety, extended shelf life, and improved sensory quality.