Quality Assurance and Safety of Crops & Foods, 2025; 17(1): 163–189

Application of cold plasma, a novel nonthermal method on fruit juices: A review on quality and safety

Gurveer Kaur*, Sandhya, Maninder Kaur, Vimal Challana*, Sumandeep Kaur

Department of Processing and Food Engineering, College of Agricultural Engineering, Punjab Agricultural University, Ludhiana, Punjab, India

***Corresponding Authors:** Gurveer Kaur and Vimal Challana, Department of Processing and Food Engineering, College of Agricultural Engineering, Punjab Agricultural University, Ludhiana, Punjab, India. Emails: kaurgurveer91@pau.edu; vimalchallana@pau.edu

Received: 10 September 2024; Accepted: 26 December 2024; Published: 6 February 2025

REVIEW ARTICLE

Abstract

Growing consumer demand for minimally processed foods with high quality and safety standards has gained interest in nonthermal decontamination methods for fruit juices. Cold plasma (CP) has emerged as a promising technology in the food industry, offering numerous advantages, such as safety, versatility, environmental friendliness and efficiency. This review paper provides a comprehensive examination of recent advances in CP application for fruit juice processing, focusing on microbial inactivation and its effect on bioactive components along with the interaction with active plasma species. Additionally, the paper also analyzes variations in operating parameters of CP that influence the quality of fruit juices. The data from the existing studies indicated that CP treatment resulted in microbial inactivation ranging from 0.15 to 7.4-log cycles. Additionally, enzymatic activity decreased by up to 54%, antioxidant activity improved by up to 261%, and anthocyanin levels increased by 35%, compared to the control samples. The CP shows promising results in retaining the juice's physicochemical properties with minimal degradation. However, the scaling up of CP technology commercially is still a challenge. Further experimental studies are required in fruit juice processing for industrial applications to get a better idea for understanding the interactions between plasma active species and juice components.

Keywords: cold-plasma; fruit-juices; quality; microbe; active species; degradation

Introduction

Food preservation has been an important practice for centuries, with conventional methods, such as sun drying, smoking, fermentation, salting and roasting, which have been used to extend the shelf-life and maintain the quality of food products (Nwabor *et al.*, 2022). With scientific advancements, these traditional techniques have been replaced by modern thermal preservation methods. These methods have been widely utilized to overcome spoilage caused by microbes. However, these methods have a number of drawbacks, which include color alteration, and loss of texture, flavor and nutritional content, all of which lower the quality of the product. These drawbacks are quite alarming in the case of fruit juices, where maintaining the nutritional value and sensory attributes are essential. Fruit juices, as defined by the Codex Alimentarius Commission (CAC, 2005) as 'unfermented but fermentable liquid obtained from the edible part of the sound, appropriately mature and fresh fruit or fruit maintained in sound condition by suitable means'. Fruit juices are very sensitive to this processing. Juices are cherished for their prompt availability, high nutritional content and desirable sensory attributes, which include attractive color, pleasant aroma and rich taste. The juice contains water, sugars (sucrose, glucose and fructose), acids, vitamins, minerals and organic compounds that are easily affected by processing.

Growing consumer demand for minimally processed foods that retain their natural attributes in response to the research in food preservation has deviated toward nonthermal preservation techniques. Unlike conventional thermal processing methods, which degrade the nutritional and sensory attributes of food, nonthermal techniques offer a promising solution by maintaining food safety while retaining these qualities. Techniques such as cold plasma (CP), ultraviolet, ultrasonication, thermosonication, high-pressure processing and pulsed electric fields have been explored for their potential applications and advantages. Among these innovative techniques, CP is considered as an advantageous nonthermal food-processing technique for juice, compared to other nonthermal treatments. Irradiation leads to alterations in aroma, color, flavor and decrement in nutritional properties (Khalili *et al.*, 2017). Although fermentation is a natural process, it is a time-consuming process and offers inconsistent product quality.

Additionally, pulsed electric field has challenges with higher costs, presence of already existing bubbles that can lead to nonuniform treatment, corrosion and fouling of electrodes and migration of electrode material into food (Pataro and Ferrari, 2020; Taha *et al.*, 2022). Ozone treatment poses risks of oxidative damage, which can result in the degradation of flavor, color and nutrient. High-pressure processing is considered one of the most effective treatments for most foods, but its higher installation costs and potential for texture alteration limit its usage. Ultraviolet (UV) treatment is also limited by its inability to penetrate viscous products, as color, heterogeneity, solid contents and viscosity significantly reduce its efficacy in diluting the effectiveness of microbial inactivation (Guerrero-Beltrn and Barbosa-Cnovas, 2004). CP is considered an effective, noninvasive, sustainable and chemical-free method for food preservation for maintaining food safety and quality (Chen *et al.*, 2019).

Cold plasma is considered a fourth state of matter, which consists of an ionized gas containing atoms, ions, radicals and electrons. CP has shown a number of potential applications across different food sectors, which includes fruits and fruit juices (Porto *et al.*, 2023; Sarangapani *et al.*, 2017; Tappi *et al.*, 2016, 2019; Ukuku *et al.*, 2019; Zhao *et al.*, 2023), meat products (Abdel-Naeem *et al.*, 2022; Akhtar *et al.*, 2022), nuts (Makari *et al.*, 2021; Medvecká *et al.*, 2024) and baked goods (Mahanta *et al.*, 2024; Palabiyik *et al.*, 2023). The effectiveness of CP treatment for microbial inactivation, with minimal effect on the physical and chemical properties of foods, has made it a powerful alternative to traditional thermal methods.

This review provides a detailed overview of CP technology as applied to preservation and for maintaining the quality of fruit juices. Its efficacy in microbial load reduction and impact on nutritional and sensory parameters makes it a promising, consumer-friendly alternative that meets consumer preferences for minimally processed and fresh-like natural products. As demand for functional and fortified beverages continues to rise, the application of CP plays a crucial role in preserving bioactive components, such as vitamins, antioxidants and anthocyanins, thereby meeting the increasing consumer demand for health-enhancing beverages.

Specifically, the review paper highlights the impact of CP's operating parameters, such as voltage, treatment time (TT), power, discharge gap, gas type and its flow rate, frequency and current product variables such as effect of sample size and composition on sensory attributes, nutritional retention, enzyme activity and microbial load. From the existing literature on CP applications in fruit juice processing, we highlight CP's potential as a sustainable and effective alternative to traditional thermal processing methods. The review aims to bridge existing gaps in the current research and highlights future opportunities for research and commercialization in the field of nonthermal fruit juice preservation.

Cold plasma technology

In nature, matter exists in three states: solid, liquid and gaseous. The phase transformation occurs from solid to liquid, and liquid to gas, with an increase in the levels of energy (Figure 1). If energy increases beyond a certain level, the gas molecules are converted into ionized molecules, resulting in plasma state.

Owing to the excessive energy supplied to molecules, the electrons are freed from the bonds with a nucleus, resulting in the separation of electrons and positive ions. In this process, the electrons collide with neutral atoms, and are ionized to generate free radicals, positive ions, electrons and neutral particles, collectively known as plasma-activated species. These ions and particles constitute plasma (Rao *et al.*, 2023). Figure 1 presents the classification of plasma and generation of plasma-activated species by applying a strong electric field to gas.

The plasma is categorized into thermal and nonthermal plasma. Thermal plasma is generated when a gas is ionized by a high-temperature energy source (which can exceed 20,000 K), which can be used for coating, welding and treating hazardous waste. Nonthermal plasma

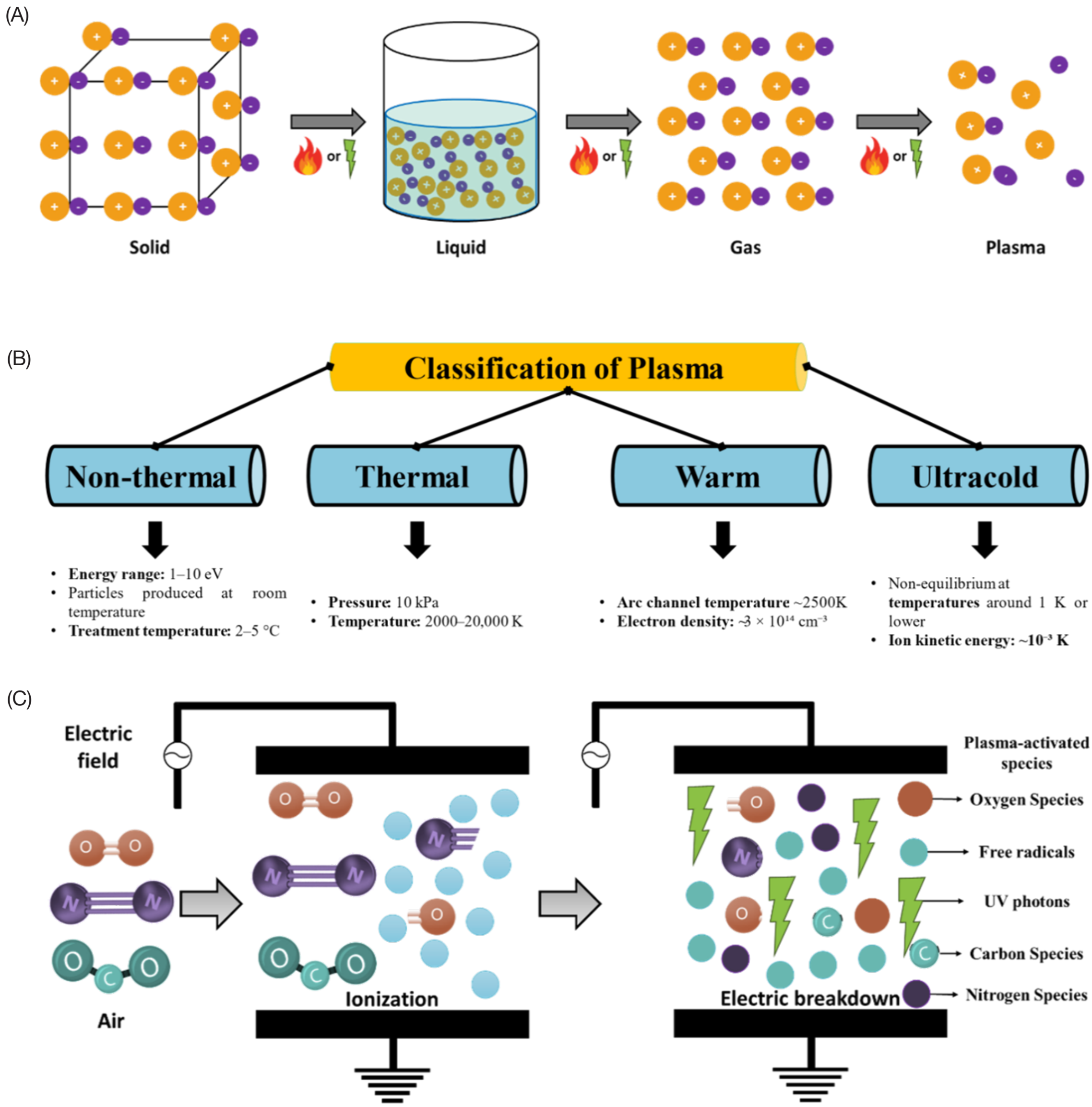

Figure 1. **(A) Different phases of matter, (B) classification of plasma and (C) generation of CP treatment by a strong electric field and generated species.**

is generated at a temperature that is near to room temperature. The temperature of an electron can control ionization and chemical processes. The plasma remains in the metastable state with almost net zero charge. The nonthermal plasma can be used for food product applications. Electrical, radio-frequency, and microwave power sources generate nonthermal plasma by inducing a high potential difference (Fernandes and Rodrigues, 2021). CP is generated by using gases such as helium, argon, air, nitrogen, and their mixtures. Most of the studies related to fruit juices have used air and argon in combination with O_2 and nitrogen. Moreover, CP is generated by getting power from alternative current, direct current, radio-frequency, or microwaves (Figure 2; El-Sheekh *et al.*, 2023). Various plasma-generation sources with principle and applications is summarized in Table 1.

Effect of Cold Plasma on Quality Parameters of Fruit Juices

Cold Plasma is applied to fruit juices at different stages of processing. It can be employed immediately after the juice is expressed from the fruit for microbial inactivation. Alternatively, CP treatment can be applied after concentrating the juice to the desired consistency. It can also be combined with other treatments, either before or after CP application, to enhance its effects. A general flow sheet of fruit juice processing treated with the CP is given in Figure 3.

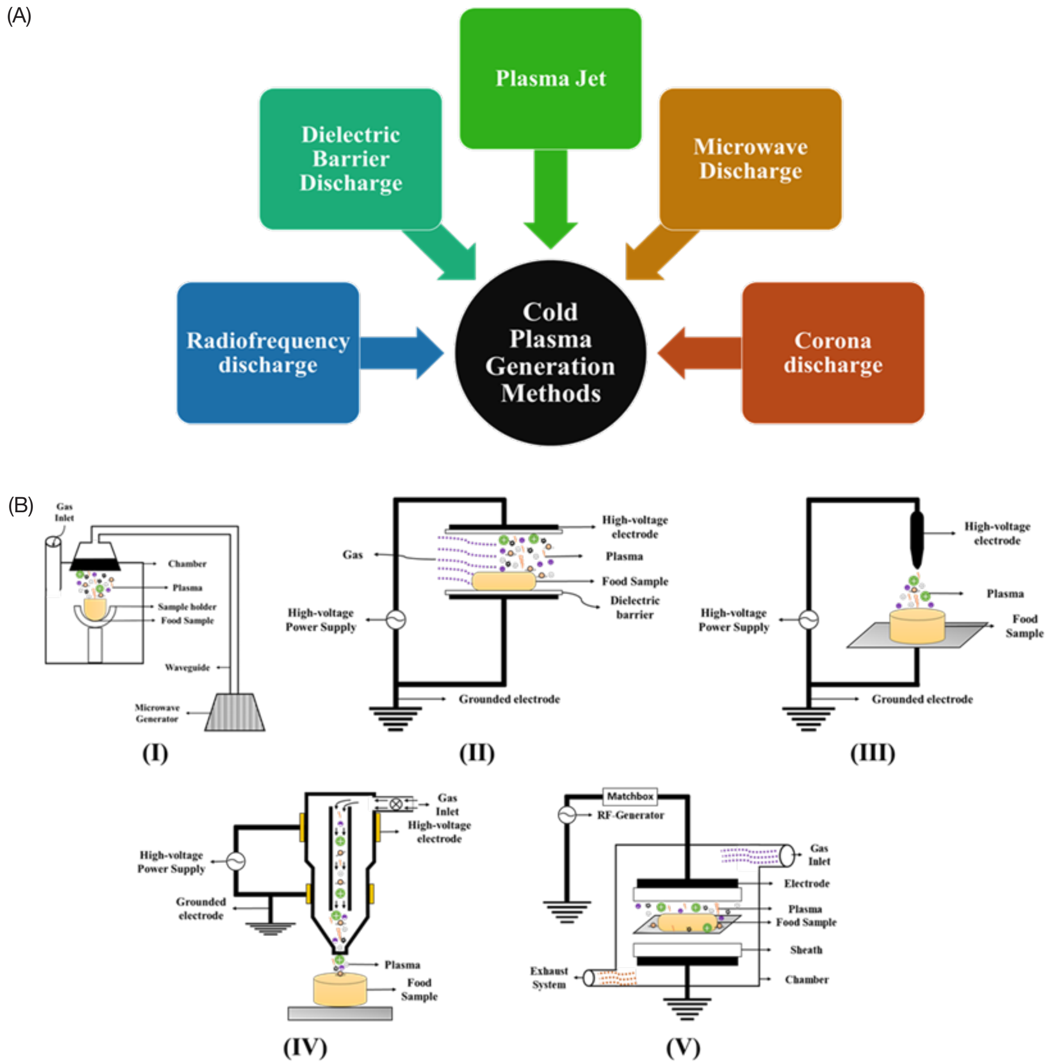

Figure 2. **(A) List of CP-generation sources, and (B) CP-generation equipment: (I) microwave discharge, (II) dielectric barrier discharge, (III) corona discharge, (IV) plasma jet, and (V) radiofrequency discharge.**

Numerous experiments were conducted to evaluate the efficacy of CP in improving various characteristics of fruit juices. The experiments focused on examining the impact of CP on a range of quality parameters such as nutritional content, sensory attributes, and microbial inactivation. To provide a clear understanding of the method used in the literature, Table 2 summarizes the techniques used for the determination of each parameter. Impact of CP treatment on quality parameters of different juices is presented briefly in Table 3.

Sensory attributes

The sensory attributes of food products are of utmost importance for any individual. Quality of a food product is evaluated through sensory analysis based on various factors, such as color, aroma, taste, consistency, mouth feel, and aftertaste. Proper sensory analysis is important as it directly affects consumer satisfaction, demand and market success. A proper sensory evaluation makes sure that products meet consumer expectations, enhancing their likelihood of acceptance in the market. Sensory evaluation is considered an indicator on which the industry can rely for the appeal of food products and potential market performance.

Chutia *et al.* (2020) reported that when the tender coconut juice was mixed with 1% orange juice, and the blended beverage was exposed to CP for 1.75 min at a voltage of 18.00 kV, it helped to mask chemical odor. The beverage was acceptable based on sensory characteristics. The masking of chemical odor could be attributed to the degradation of volatile compounds that cause

Table 1. Various plasma-generation sources, their working principle, advantages and applications.

Type	Configuration	Principle	Advantages	Applications	Applications for decontamination of fruit juices
Dielectric barrier discharge	• Electrode holder cap • Live electrode • Ground electrode • Dielectric barrier material • Airflow controller (Rathore and Nema, 2022)	Plasma is generated between two electrodes when a high-voltage current is supplied to gases (Garg and Maheshwari, 2023)	• Simple to use • Various gases can be used • Lower gas flow rate (GFR) • Consistent while discharging ignition • Flexible (for using different electrode geometries) (Lokesh, 2024)	• To enhance the shelf life of food • To treat wastewater • To reduce food spoilage (Garg and Maheshwari, 2023)	• Orange juice (Rodrigues and Fernandes, 2023) • Apple juice (Farias *et al.*, 2021) • Cammu-cammu juice (de Castro *et al.*, 2020 • Chokeberry juice Gan *et al.*, 2021
Corona discharge	• Live electrode • Ground electrode • Airflow controller • High-voltage power source • Stainless grid (Belhora *et al.*, 2013)	The plasma is generated by supplying high voltage on the sharp edges of the electrode made from metals. The power can be supplied in the form of AC, DC, or radiofrequency (El-Sheekh *et al.*, 2023)	• The reactive region remains at a low temperature (El-Sheekh *et al.*, 2023) • Easy to establish (Okubo and Kuwahara, 2020) • Lower investment and operating cost • Low energy yield • High removal efficiency (Unnisa and Hassanpour, 2017)	• Surface treatments • Electro-precipitation • Medical instruments' sterilization • Microbial decontamination (Lokesh, 2024)	• ------
Plasma jet	• Electrode holder cap • Live electrode • Ground electrode • Dielectric material • Airflow controller • Collimators • Optical emission spectroscopy (Tschang *et al.*, 2020)	The sinusoidal voltage excites discharge in the plasma jet, which releases electrons that collide with the molecules of the gas and produce various reactive species such as free radicals, excited atoms, photons, etc. (Jiang *et al.*, 2022; Shrestha *et al.*, 2016)	• Readily available • Simple to use • Economical (Lokesh, 2024)	• Sterilization • Algal biosynthesis enhancement and growth (El-Sheekh *et al.*, 2023) • Polymer etching • Decontamination of food • Water treatment • Biological tissue treatment (Viegas *et al.*, 2022)	• Chokeberry juice (Gan *et al.*, 2021) • Sour cherry juice (Hosseini *et al.*, 2021) • Pomegranate juice (Herceg *et al.*, 2016) • Blueberry juice (Hou *et al.*, 2019)
Microwave discharge	• Magnetron • Directional coupler • Wave guide • Dielectric material • Microwave plasma source (Hu *et al.*, 2023)	The interaction that occurs between microwave radiations and molecules of gas, which absorb energy from the waves, results in the ionization of molecules and the formation of plasma (El-Sheekh *et al.*, 2023)	• Electrode-less system (El-Sheekh *et al.*, 2023) • High plasma density • Energy efficient • Scalability (Tiwari *et al.*, 2020)	• Sterilization • Pasteurization • Thermal processing, like drying (Garg and Maheshwari, 2023) • Gasification of biomass • Treatment of waste gases (Tiwari *et al.*, 2020)	• Coconut liquid endosperm (Gabriel *et al.*, 2016)

(*continues*)

Table 1. Continued.

Radiofrequency discharge	• RF-generator • Matching unit • Live electrode • Ground electrode • Airflow controller • (Puligundla and Mok, 2020; Sahoo *et al.*, 2022)	Plasma is generated when a gas is placed inside the chamber subjected to high-frequency electromagnetic field generated by an RF generator (Jiang *et al.*, 2022)	• Low-temperature processing • Uniform distribution of plasma • Green technology • Scalable • Cost-effective (Emily, 2024)	• Surface treatment • Sterilization • Wastewater treatment (Emily, 2024)	• ------

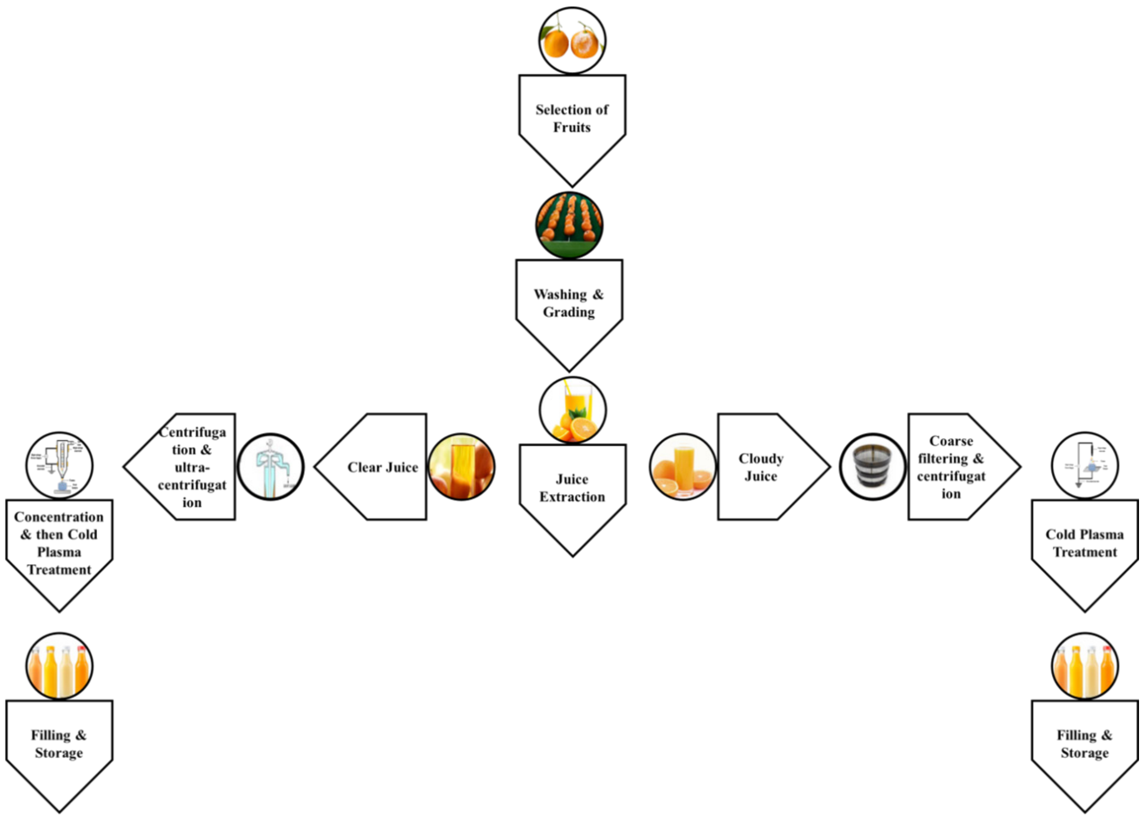

Figure 3. Flow diagram of fruit juice processing using CP treatment for microbial decontamination.

off-flavor through oxidation. Additionally, the enhanced citrus aroma of orange juice and microbial load reduction contribute to the enhanced flavor profile of the final juice. Porto *et al.* (2023) also observed a decrease in undesirable aromas in pineapple juice after its treatment with a dielectric barrier discharge-based CP system operated at 50 Hz for less than 10 min at 20 kV. The decrease in unwanted aroma enhances sensory appeal for consumers.

Color

It is a common saying that we eat first with our eyes. Color is the main component from where consumers make a perception about the freshness of the product, thus affecting the decision to purchase that product. After CP treatment, change in color is quite common. The color value is obtained as L^*, a^* and b^* using the colorimeter. The total color change (ΔE) is calculated with the help of these three values, and it is worth noting that in most cases, ΔE is required to be minimum. Color is also recorded in the form of chroma value (indicating intensity) and hue angle (indicating level of browning) (Bhatkar *et al.*, 2021).

Kovačević *et al.* (2016b) observed that L^*, a^* and b^* increased with increase in gas flow in the case of pomegranate juice. A similar trend was also reported for b^* in the case of camu-camu juice (Castro *et al.*, 2020), orange,

Table 2. Methodology followed for analyzing various parameters of fruit juices.

Parameters	Methods	References
Total soluble solids (TSS)	Refractometer	Liao *et al.*, 2018; Zhao *et al.*, 2023
Titratable acidity	Acid-base neutralization method	Liao *et al.*, 2018; Pankaj *et al.*, 2017
pH	Digital pH meter	Hosseini *et al.*, 2021; Liao *et al.*, 2018; Pankaj *et al.*, 2017; Zhao *et al.*, 2023
Color	Colorimeter	Fernandes *et al.*, 2019; Hosseini *et al.*, 2021; Hou *et al.*, 2019; Liao *et al.*, 2018; Pankaj *et al.*, 2017; Wang *et al.*, 2020; Zhao *et al.*, 2023
Sensory attributes	Fuzzy logic	Chutia *et al.*, 2020)
Total phenolic content	Folin–Ciocalteu method	Fernandes *et al.*, 2019; Hosseini *et al.*, 2021, 2020; Hou *et al.*, 2019; Liao *et al.*, 2018; Pankaj *et al.*, 2017; Rodríguez *et al.*, 2017; Zhao *et al.*, 2023
Anthocyanin	pH-differential method	Gan *et al.*, 2021; Hosseini *et al.*, 2020; Hou *et al.*, 2019
Vitamin C	Spectrophotometric method	Rodríguez *et al.*, 2017
	2,6-dichlorophenolindophenol (DCPIP)	Hou *et al.*, 2019
	Redox titration using iodine	Hosseini *et al.*, 2020
Antioxidant activity	2,2-diphenyl-1-picrylhydrazyl (DPPH)	Chutia *et al.*, 2020; Dantas *et al.*, 2021; Gan *et al.*, 2021; Hou *et al.*, 2019; Liao *et al.*, 2018; Pankaj *et al.*, 2017; Rodríguez *et al.*, 2017
	ABTS method (2,2- azino-bis-3-ethylbenzothiazoline-6- sulphonic)	Almeida *et al.*, 2015; Hou *et al.*, 2019; Rodríguez *et al.*, 2017; Zhao *et al.*, 2023
	Fluorescence recovery after photobleaching (FRAP) assay	Hou *et al.*, 2019; Rodríguez *et al.*, 2017
	Oxygen radical absorbance (ORAC) assay	Dantas *et al.*, 2021
Pectin methylesterase	Titrimetry method	Andreou *et al.*, 2023
Peroxidase	Peroxidase assay by spectrophotometric analysis	Chutia *et al.*, 2019; Dantas *et al.*, 2021; de Castro *et al.*, 2020; Pipliya *et al.*, 2022)
Polyphenol oxidase	Polyphenol assay by spectrophotometric analysis	Chutia *et al.*, 2019; Dantas *et al.*, 2021; de Castro *et al.*, 2020; Illera *et al.*, 2019; Pipliya *et al.*, 2022)

tomato, apple, and sour cherry juice (Dasan and Boyaci, 2018). Decrease in the b^* value could be attributed to the degradation of bioactive components, which produce a yellow-brown color (Castro *et al.*, 2020).

Dasan and Boyaci (2018) reported that in case of apple and sour cherry juice, ΔE value increased with increase in CP exposure time. This trend was in line with the findings of Pankaj *et al.* (2017) and Liao *et al.* (2018) for grape juice and apple juice, respectively. Increase in ΔE could be due to the polymerization of phenolic compounds, which are naturally present in fruit juices. The phenols are susceptible to structural changes under CP treatment. The active species generated during CP promotes oxidation and cross-linking of phenols, which leads to polymerization, resulting in a darker color. A longer treatment resulted in amplification and caused a more change in ΔE.

In contrast, Gan *et al.* (2021) reported that CP-treated juice afforded a lesser value of ΔE, compared to thermal-treated chokeberry juice, which could be ascribed to the degradation of heat-sensitive pigments during thermal treatment. Thermal processing results in the break down of pigments, resulting in more color loss. On the other hand, CP treatment does not involve high temperatures and preserves pigments, resulting in lower ΔE. Fernandes *et al.* (2019) reported that glow discharge plasma treatment improved the color of acerola juice, which could be ascribed to an increase in the concentration of free carotenoids. CP treatment breaks down the cell wall structure and facilitates the release of carotenoids and other pigments, resulting in the improved color of the juice.

pH and titratable acidity

The total acid concentration is measured in the form of titratable acidity. The most common organic acids present in the foods that we consume are lactic acid, citric acid, tartaric acid, malic acid, and acetic acid, and carbonic acid and phosphoric acid are the examples of inorganic acids present in foods. The presence of organic acids influences the flavor, color, and microbial stability of foods. Titrable acidity combined with sugar is used as a maturity indicator of fruits. Titratable acid is determined by the neutralization reaction (Tyl and Sadler, 2017). pH is a measure of the activity of hydronium ions (H_3O^+)

Table 3. Effect of CP processing on fruit juices.

Matrix	Plasma source	Gas	Operating conditions	Impact on physical properties	References
Pomegranate juice	Cold atmospheric plasma jet	Argon	TT: 3, 5, and 7 min SS: 3, 4, and 5 cm^3 GFR: 0.75, 1, 1.25 dm^3/min v: 25 kHz D: 1.50 cm V: 2.5 kV	• Anthocyanin content increases (21–35%) • TT and SS do not impact ΔE	Kovačevič *et al.*, 2016b
White grape juice	High-voltage atmospheric CP	Dry air	TT: 1,2,3, and 4 min V: 80 kV	• ΔE was very low • Change in pH, acidity, and electrical conductivity is not significant • Increase in total flavonols after treatment • TPC, antioxidant capacity, and flavonoids decrease with an increase in treatment time	Pankaj *et al.*, 2017
Siriguela juice	Glow discharge plasma generator	N_2	v: 50 kHz GFR: 10–30 mL/min TT: 5–15 min SS: 80 mL	• AA, TPC, pigments, and vitamin B increase after the treatment	Paixão *et al.*, 2019
Blueberry juice	CP jet	Argon and oxygen	GFR: 1.0 L/min V: 11 kV v: 1 kHz D: 2.0 cm T: 2, 4, and 6 min O_2 conc.: 0, 0.5, and 1%	• Anthocyanin, vitamin C, and antioxidant activity decrease with an increase in treatment time, but TPC increases • TPC and AA increase with increase in O_2 conc.	Hou *et al.*, 2019
Camu-camu juice	Glow plasma system	Synthetic air	υ: 80 kHz SS: 40 mL GFR: 10, 20, and 30 mL/min TT: 10, 20, and 30 min	• Increase in ascorbic acid bioavailability • ΔE increases with an increase in GFR • Total anthocyanins and ascorbic acid concentration increases after treatment	Castro *et al.*, 2020
Araça-boi juice	Glow discharge plasma	Air	GFR: 10, 20, and 30 mL/min TT: 10, 20, and 30 min SS: 30 mL	• Increase in conc. of sucrose • Conc. of malic acid, amino acids, fructose, and glucose decreases after treatment	Farias *et al.*, 2023
Pomegranate juice	Cold atmospheric plasma jet	Argon	T: 3, 5, and 7 min SS: 3, 4, and 5 cm^3 GFR: 0.75, 1, and 1.25 dm^3/min υ: 25 kHz D: 1.50 cm V: 2.5 kV	• TPC increases after CP treatment	Herceg *et al.*, 2016

(*continues*)

Table 3. Continued.

Araça-boi juice	Dielectric barrier discharge plasma	Air	υ: 50, 200, 400, 600, and 800 Hz TT: 10, 15, and 20 min V: 20 kV SS: 30 mL	• Increase in conc. of sucrose • Conc. of malic acid, amino acids, fructose, and glucose decreases after treatment, but at 200 Hz increase in amino acids conc.	Farias *et al.*, 2023
Chokeberry juice	CP jet	Argon	SS: 1–5 mL TT: 1–5 min D: 2.4 cm	• CP treatment gives better retention of anthocyanin and vitamin C, reducing sugar, color and AA in comparison to thermal treatment	Gan *et al.*, 2021
Apple, orange, tomato juices, and sour cherry nectar	CP jet	Air	υ: 25 kHz GFR: 3,000 L/h D: 3.5 cm TT: 30, 60, 90, and 120 s	• ΔE and TPC increase with increase in TT	Dasan and Boyaci, 2018
Cashew apple juice	Benchtop plasma system PE-100	N_2	GFR: 10, 30, and 50 mL/min TT: 5,10, and 15 min Vacuum conditions: 30 kPa SS: 10 mL υ: 80 kHz	• Increase in vitamin C, flavonoid and polyphenol and antioxidant activity • Overexposure has a detrimental impact on bioactive components	Rodríguez *et al.*, 2017
Apple juice	Dielectric barrier discharge	Air	SS: 3 mL PI: 30, 40, and 50 W TT: 0–40 s	• Increase in TT and PI, titratable acidity, ΔE, and TSS increase • AA and TPC decrease with an increase in TT and PI	Liao *et al.*, 2018
Cashew apple juice	Dielectric barrier discharge	-	V: 20 kV υ: 200 and 700 Hz SS: 20 mL TT: 15 min	• Malic acid, lactic acid, and vitamin C concentration increases • Vitamin C bio-accessibility increases	(Leite *et al.*, 2021)
Pineapple Juice	Dielectric barrier discharge	Air	V: 20 kV υ: 50, 500 and 1,000 Hz SS: 20 mL TT: 10 and 20 min	• Demethylation of esters • Methyl esters converted into ethyl esters • Thioesters become more stable • The intense sweet aroma (methyl hexanoate) is reduced by low υ • Longer treatment time decreases the juice's freshness • The improvement in fresh and fruit descriptors of juice by mid-range υ	(Porto *et al.*, 2023)
Apple juice	Dielectric barrier discharge	Air	V: 20 and 30 kV υ: 50, 500 and 1,000 Hz SS: 20 mL TT: 1-8 min	• Improvement in color, sensory attributes, TPC, TSS and AA • pH reduces after treatment	(Zhao *et al.*, 2023)
Custard apple juice milk beverage	Dielectric barrier discharge	Argon	V: 35 kV υ: 50 kHz SS: 50 mL TT: 2 and 3 min	• pH decreases, and an increase in acidity was reported after CP treatment	(KM *et al.*, 2023)

(*continues*)

Table 3. Continued.

Orange juice	Dielectric barrier discharge	-	V: 70 kV υ: 50 Hz SS: 20 mL TT: 15, 30, 45, and 60 s	• Decrease in pH and hue angle after the treatment • Increase in chroma and L^* value	Almeida *et al.*, 2015
Coconut water	Dielectric barrier discharge	-	V: 18–28 kV TT: 1–3 min	• AA and transmittance get reduced with an increase in voltage and treatment time, but free fatty acid increases	Chutia *et al.*, 2020
Apple juice	Dielectric barrier discharge plasma	Synthetic air	υ: 50, 200, 400, 600, and 900 Hz SS: 20 mL V: 20 kV TT: 15 min	• Decrease in sucrose, glucose, and fructose content • Decrease in sweetening power and the sugar–acid ratio • Malic acid concentration increases	Farias *et al.*, 2021
Apple juice	Glow discharge plasma	Synthetic air	GFR: 10, 20, and 30 mL/min V: 80 kV SS: 40 mL TT: 10, 20, and 30 min	• Increase in conc. of glucose, fructose, and malic acid • Reduction in sucrose content and sugar–acid ratio	Farias *et al.*, 2021
Orange juice	Dielectric barrier discharge	-	υ: 50, 200, 400, and 600 Hz SS: 20 mL V: 20 kV TT: 15 min	• Improves the aroma of pasteurized orange juice	Rodrigues and Fernandes, 2023
Guava, açai, caja, and sapota	Glow discharge plasma	N_2	GFR: 10 and 30 mL/min TT: 5–15 min υ: 50 kHz SS: 10 mL V: 80 kV	• CP enhances the antioxidant capacity of açai (up to 101%), guava (up to 116%), and sapota (up to 261%) juices while reducing it in caja by up to 32% • An increase in TPC was observed in sapota juice (up to 91%), açai (up to 9%), caja (up to 2%), and guava (up to 17%) juices • An increase in TT decreases the bioactive components conc.	Rodriguez *et al.*, 2022
Sour cherry juice	Dielectric barrier discharge	Argon and oxygen	GFR: 5 standard L/min D: 2 cm υ: 20 kHz TT: 1, 5, and 9 min Applied FI: 25, 37.5, and 50 kV/cm SD: 0.5, 1, and 1.5 cm O_2 in argon: 0%, 0.5%, and 1%	• TPC did not change • Total anthocyanin and vitamin C reduce after treatment	Hosseini *et al.*, 2020
Sour cherry juice	Dielectric barrier discharge	Argon and oxygen	V: 10, 15, and 20 kV υ: 10, 15, and 20 kHz GFR: 3, 5, and 7 L/min O_2%: 0, 0.5, and 1 SS: 5, 10, and 15 mL TT: 1, 5, and 9 min	• Plasma jet length is affected by voltage than GFR, power supply, and gas composition • pH, color, and TPC did not change with plasma treatment	Hosseini *et al.*, 2021

(*continues*)

Table 3. Continued.

Chokeberry juice	-	Argon	GFR: 0.75 dm^3/min υ: 25 kHz D: 1.5 cm SS: 3, 5, and 7 mL TT: 3 and 5 min	Plasma-treated juice shows • Lower stability of flavonols and anthocyanin • Improved stability of hydroxycinnamic acids	Kovačević *et al.*, 2016a
Kiwi juice	Dielectric barrier discharge	-	TT: 1–5 min V: 10–40 kV SS: 10–20 mL	CP treatment shows • Reduced color loss • Improves the flavor	Liu *et al.*, 2021
Orange and carrot juice blend (80:20)	Dielectric barrier discharge	Air	TT: 5, 15, and 30 s SS: 100 mL	• Browning reactions reduce, and ΔE increases with an increase in TT	Campelo *et al.*, 2020
Mango pulp	Dielectric barrier discharge	Air	V: 25 kV SS: 5 g TT: 0, 2, 4, 6, 8, and 10 min	With the increase in TT • Conc. of ascorbic acid, TPC increases up to 6 min then decreases, and conc. of AA decreases • L^* intensity increases	Abedelmaksoud *et al.*, 2022
Acerola juice	Glow discharge plasma	N_2	GFR: 10, 15, and 20 mL/min υ: 80 kHz SS: 40 mL TT: 5, 10, and 15 min	• Conc. of vitamin A and carotenoid content increases • TPC decreases with an increase in GFR • Improvement in the color of the juice	Fernandes *et al.*, 2019
Apple juice	Electrical discharge plasma	Air	GFR: 150 L/h V: 15, 18, and 21 kV υ: 50 Hz TT: 0, 10, 20, and 30 min	• L^*, b^*, and chroma values decrease, and hue angle, pH, and TSS increase with increase in TT	Wang *et al.*, 2020

V: voltage, TT: treatment time, SS: sample size, GFR: gas flow rate, υ: frequency, D: distance between sample and plasma nozzle tip, O_2: oxygen, N_2: nitrogen, PI: power intensity, FI: field intensity, SD: sample depth, ΔE: total color change, TPC: total phenolic content, TFC: total flavonoid content, AA: antioxidant activity, L^*: lightness, b^*: blue-yellow, CP: cold plasma, Conc.: concentration.

(Andrés-Bello *et al.*, 2013) and is determined by using pH meters, glass electrodes, ISFET pH sensors, and pH indicators (Karastogianni *et al.*, 2016). Among these, pH meters are the most commonly used measure. It is observed that in general pH and titratable acidity have an inverse relationship (Blacker *et al.*, 2011).

Pankaj *et al.* (2017) observed in the case of white grape juice that with increase in the treatment time of CP, the pH value decreased, which was due to increase in the concentration of hydronium ions, resulting in more acidic juice. Zhao *et al.* (2023) also described a similar trend for apple juice.

Several studies have confirmed that CP results in a lower pH, compared to untreated samples. This trend has been observed for apple juice (Zhao *et al.*, 2023), custard apple juice, milk beverages (KM *et al.*, 2023), and orange juice (Almeida *et al.*, 2015). Liao *et al.* (2018) also reported that pH value dropped by 0.1 units if the juice was treated for 40 s with dielectric barrier discharge plasma. The trend could be ascribed to the production of hydroxyl radicals and reactive nitrogen species and the formation of new chemicals such as nitrous acid, nitric acid and hydrogen peroxide. The acids dissociate in water and increase the concentration of H_3O^+ ions, which in turn lowers the pH. Additionally, CP treatment resulted in water loss, leading to more concentrated acid content and contributing to lower pH value.

On the other hand, Wang *et al.* (2020) observed a reverse trend in the case of apple juice, in which increase in CP treatment time increased the pH value. The trend could be attributed to the generation of less acidic content, or facilitating the neutralization of acid, which increased the pH of juice. Additionally, differences in juice matrix, such as the chemical composition and buffering capacity of juice, influences the interaction of active species (Warne *et al.*, 2021). A similar trend was reported in the case of Araça-boi juice (Farias *et al.*, 2023). However, in the case of sour cherry juice, CP treatment time showed no significant change in recorded pH (Hosseini *et al.*, 2021).

Farias *et al.* (2021) observed that the malic acid concentration of apple juice increased with an increase in the treatment time of glow discharge plasma. A similar trend was reported by Liao *et al.* (2018) and KM *et al.* (2023) in the case of apple juice and custard apple juice milk beverages, respectively, which could be due to to the generation of free radicals and ozone, facilitating the oxidation of aldehydes that produced acid. In addition to this, the formation of reactive nitrogen species resulted in the formation of nitrogenous acids that also contributed to an increase in the acidity of juice. Liao *et al.* (2018) also observed that with an increase in power intensity during CP treatment, titratable acidity was reduced . The trend was ascribed to the fact that higher frequency of CP could lead to the increased break down of acidic compounds, which would reduce juice acidity.

Total Soluble Solids

Total soluble solids content consists of all soluble constituents such as sugars, organic acids, phenolic and nitrogenous derivatives, and structural polysaccharides (Watrelot *et al.*, 2020). In strawberries, TSS consists of sugars, acids, trace amounts of vitamins, minerals, pigments, phenols, proteins, and fructans (Basak *et al.*, 2022). The TSS content is measured with a refractometer or other advanced nondestructive techniques, such as Raman spectroscopy, near-infrared spectroscopy, magnetic resonance imaging (MRI), and backscattering imaging (Li *et al.*, 2016).

It is observed that increase in the CP treatment exposure time results in higher TSS in apple juice (Wang *et al.*, 2020). Similar results were reported by Zhao *et al.* (2023) and Liao *et al.* (2018) for apple juice. The trend could be due to reduced moisture content with an increase in treatment time, which concentrates dissolved solids and another solutes present in the juice (Zhao *et al.*, 2023). As the duration of CP treatment increases, it leads to the evaporation of water present in the juice, resulting in increased concentration of soluble components. The effect could be further amplified by cellular structure disruption that allows the release of intracellular components, thus contributing to TSS. It was also reported by Liao *et al.* (2018) that higher power intesity of CP treatment resulted in higher Brix value (a measure of TSS), which could be due to reduced moisture content and increased cellular disruption.

It is worth noting that in the case of apple juice, with an increase in treatment time from 20 to 30 min, total sugar and sweetening power decreased if the juice was treated with glow discharge plasma as well as changes in the frequency of dielectric barrier discharge plasma (Farias *et al.*, 2021). The trend could be due to a longer plasma exposure, leading to the degradation or oxidation of sugars, which decreases juice's concentration and sweetening potential. Gan *et al.* (2021) discovered that the CP treatment was more effective in enhancing the TSS, compared to the thermal treatment in case of chokeberry juice. This could be due to the action of CP active species, which caused structural damage to cell membranes, resulting in the release of bound sugars present in the cells.

Total Phenolic Content (TPC)

Various operating parameters of CP treatment, such as the time of treatment, gas type, gas flow rate (GFR), voltage, and power, influence the phenolic content of juices. Dasan and Boyaci (2018) observed that the total phenolic content increases with increase in exposure time. The highest TPC was recorded at 120-s plasma treatment (among 30-, 60-, 90-s CP treatmet) for orange, apple, tomato juices, and sour cherry nectar with an increase of 9.52%, 14.43%, 14.81%, and 14.47%, respectively. The trend could be ascribed to the generation of plasma-activated species, which break down cell wall membranes and release the phenols bound with the cell wall, resulting in enhanced concentration of total phenols. Hou *et al.* (2019) also observed a similar trend in the case of blueberry juice, where the highest TPC was recorded at a treatment time of 6 min (compared to 2 and 4 min). The authors also reported an 11.7% higher retention of TPC in CP-treated blueberry juice, compared to thermally treated samples. The higher retention of TPC could be due to the nonthermal nature of CP, which minimizes the degradation of phenolic components by reducing heat and oxidative stresses.

Increase in TPC is time-dependent; with a further increase in time, the TPC might reduce. Abedelmaksoud *et al.* (2022) observed that TPC increased with an increase in time up to 6 min, after which it decreased. The maximum increase in TPC (approximately 1.28 mg GAE/100 mL) occurred at 4 min, while the maximum reduction (approximately 1.85 mg GAE/100 mL) was observed at 10 min. The decline in TPC value is due to the degradation of phenols by the action of reactive oxygen species (ROS), such as ozone, which damage the aromatic rings of phenols. Similar reduction in TPC at longer exposure to time were observed by Pankaj *et al.* (2017), Liao *et al.* (2018) and Almeida *et al.* (2015), who reported a decrease in TPC if the treatment time increased beyond certain limits.

However, in some cases, no significant effect was observed. Hosseini *et al.* (2021) did not observe any significant effect of CP treatment on phenols in the case of sour cherry juice.

Gas flow rate during CP also influences TPC, as these species are sensitive to atmospheric content. Paixão *et al.* (2019) analyzed the impact of GFR on the phenolic content of siriguela juice. It was reported that a higher GFR adversely affects phenolic concentration, which could be possible due to oxidative stress caused by an excess of reactive species. The author reported a 30% reduction of phenols when treated with N_2 with a GFR of 30 mL/min for a 10-min exposure, but TPC remained unaffected at a GFR of 10 mL/min, irrespective of exposure time. Fernandes *et al.* (2021) also observed that TPC content decreased with an increase in GFR and treatment time for acerola juice. In contrast, Rodríguez *et al.* (2017) recorded the opposite scenario and observed that an increase in GFR and treatment time led to an increase in TPC content. From these studies, it was concluded that the impact of GFR could vary depending on various factors, such as the type of gas used and specific experimental conditions.

Liao *et al.* (2018) further analyzed the effect of input power and treatment time of CP on the physicochemical properties of apple juice. It was concluded from the study that the power of CP treatment did not significantly affect the phenolic concentration for exposure up to 10 min, but beyond this time point, input power resulted in a sharp decrease in TPC. The study suggested that while low power may result in enhanced phenolic concentration, high power leads to degradation of phenolic compounds, which is similar to the effect observed with longer exposure time.

Overall, it was concluded that CP treatment enhanced the concentration of phenols up to a certain time point, beyond which further CP treatment led to reduction and degradation.

Anthocyanin Content

Anthocyanins are the pigments of red, blue or purple color found in plants. Anthocyanins are considered a part of the flavonoid family. Light, pH, temperature and structure affect the stability of anthocyanins. These pigments appear as red under acidic conditions, and blue color pigments exist under basic conditions. Cyanidin-3-glucoside is the major anthocyanin present in most plants. Anthocyanins serve the purpose of food colorants. Anthocyanin-rich fruits and flowers also have medicinal values and are used for curing different diseases (Khoo *et al.*, 2017).

Kovačević *et al.* (2016b) reported that the concentration of anthocyanins increased after CP treatment in case of pomegranate juice. It was also concluded that anthocyanin were affected by exposure time and volume of the sample, but GFR did not affect these pigments. Similarly, Castro *et al.* (2020) observed that CP treatment increased anthocyanin content, and Gan *et al.* (2021) also concluded that CP treatment was found to be promising in the case of retention of anthocyanins, compared to the thermal-treated chokeberry juice. Increase in anthocyanin concentration after CP treatment could be attributed to the break down of plant cell walls, which helps in the release and bioavailability of anthocyanins. In addition, reactive species also increase the formation of stable anthocyanin forms, which can contribute to more intense color in juices.

However, similar to the phenolic content, Hou *et al.* (2019) observed that with prolonged exposure to CP, anthocyanin content decreased in the case of blueberry juice. This decrease was due to the oxidative degradation of anthocyanins on prolonged exposure of the juice to reactive plasma-generated species. Hosseini *et al.* (2020) and Kovačević *et al.* (2016a) also reported similar findings in the case of sour cherry juice and chokeberry juice, respectively, when treated with CP. The optimization of CP operating parameters is essential to balance anthocyanin retention and degradation, because degradation is more pronounced during a longer exposure time.

Vitamin C Content

Vitamin C, which is also known as L-ascorbic acid, is popular for its health benefits. The primary sources of vitamin C are fruits and fruit juices. The concentration of vitamin C is quite high in citrus fruits.

Vitamin C in fruits is preserved largely with CP treatment as against thermal treatments. Gan *et al.* (2021) reported that CP treatment retained 30% more vitamin C than the conventional thermal treatment; however, the CP-treated juice degraded vitamin C by 28%, compared to the control sample. Similarly, Hosseini *et al.* (2020) observed a decrease in vitamin C levels with CP treatment in sour cherry juice.

The benefits of CP are not only limited to the better retention of vitamin C, as some authors observed an increment in the bioavailability of vitamin C with CP. Leite *et al.* (2021) also concluded that an increase in frequency also increased the bioaccessibility of vitamin C by approximately 5%. It is worth noting that the bioaccessibility of bioactive compounds is preferred over the concentration because it provides more health benefits.

Hou *et al.* (2019) observed that the longer duration of CP treatment resulted in lesser retention of vitamin C. It was also reported that the concentration of vitamin C decreased with increased oxygen levels. The degradation of vitamin C is linked to oxidation because of longer exposure of juices to reactive oxygen species.

Leite *et al.* (2021) reported that change in frequency did not affect vitamin C concentration in cashew apple juice. However, it was concluded that the frequency of CP treatment could change the content of vitamin C. This could be due to the reactive oxygen species generated during e CP treatment that induced modifications in chemical and physical aspects, thereby enhancing the release of vitamin C from suspended pulp.

In another study on cashew apple juice, Rodríguez *et al.* (2017) analyzed the impact of N_2 GFR and exposure time on vitamin C. It was observed at a GFR of 10 mL/min increased ascorbic acid by 10.4% and 10.8% after exposing the juice for 5 and 10 min to CP respectively. If the juice was treated for an increased duration, there was a reduction of 4.5% of vitamin C. A higher degradation was observed at higher GFR and longer exposure duration. This could be ascribed to increase in the generation of plasma reactive species from nitrogen gas such as nitric oxide. This increases the activity of dehydroascorbate reductase enzyme and maintains the cycle of ascorbate–glutathione, which helps in the formation of ascorbic acid from dehydroascorbic acid. Hence, at a lower GFR, the rate of regeneration of ascorbic acid is more than its decay, but at a higher GFR, prolonged exposure results in more plasma-generated species, thus decreasing ascorbic acid concentration. Hou *et al.* (2019) observed a 28.57% higher retention of vitamin C with a CP treatment of 11 kV, 1,000 Hz, and 0.5% oxygen for 2 min, compared to conventional thermal treatment. Higher retention with CP treatment could be due to milder conditions of CP processing, which minimize the degradation of bioactive components such as vitamin C. Thermal treatment involves higher temperatures that lead to significant losses of vitamin C whereas CP treatment is a nonthermal treatment that preserves the integrity of vitamin C by minimizing thermal degradation during processing.

Antioxidant Activity

Antioxidants are the compounds which are known for the neutralization of reactive oxygen species and free radicals. Antioxidants are of two types: natural and artificial. Antioxidants protect against many diseases such as heart disease, ageing, cancer, anemia and inflammation. Plums, oranges, lemons and blueberries have a high amount of antioxidants (Bhatkar *et al.*, 2021). Pankaj *et al.* (2017) reported a reduced antioxidant activity (AA) with an increase in treatment time and observed a 4.35% higher antioxidant activity in white grape juice with CP treatment at 80 kV for 1 min, compared to thermal treatment at 85°C for 43 s. A similar decrease in antioxidant activity was reported with an increase in treatment time in the case of blueberry juice (Hou *et al.*, 2019), apple juice (Liao *et al.*, 2018) and coconut water (Chutia *et al.*, 2020). Antioxidant activity depends on the concentration of vitamin C, anthocyanin (Wang *et al.*, 2020), lycopene, α-tocopherol (Rodriguez *et al.*, 2022) and TPC (Liao *et al.*, 2018; Pankaj *et al.*, 2017). The levels of these compounds directly influence antioxidant activity. Hence, oxidation of these components for a longer duration decreases antioxidant activity (Chutia *et al.*, 2020). Similarly, in the case of apple juice, when CP treatment operates at higher power, antioxidant activity was found to decrease (Liao *et al.*, 2018). The trend could be attributed to the oxidative degradation of antioxidant activity compounds during CP treatment. In some fruit juices, CP treatment increases antioxidant activity before decreasing with a longer treatment time. Abedelmaksoud *et al.* (2022) reported an initial increase in antioxidant activity, which decreased after 4 min of CP treatment in the case of mango pulp. This suggested that exposure for a shorter duration resulted in the release of some antioxidant components, but prolonged exposure resulted in degradation.

The level of oxygen also plays an important role in the retention of antioxidant activity in case of CP-treated juices. Hou *et al.* (2019) reported that blueberry juice exposed to a higher concentration of oxygen showed reduced antioxidant activity. The trend could be attributed to the formation of hydroxy radicals (•OH) generated by CP, which lowered free radical scavenging activity. Contrarily, some juices exhibited increased antioxidant activity with extended CP treatment. Paixão *et al.* (2019) reported a gas flow rate of 20 mL/min; antioxidant activity increased with an increase in treatment time. Antioxidant activity increased by 66% when exposed for 15 min whereas an increase of 64% and only 2.64% was recorded when the siriguela juice was treated for 10 and 5 min, respectively. Gan *et al.* (2021) reported that CP resulted in better retention of antioxidant activity, compared to thermal treatment in case of chokeberry juice. Zhao *et al.* (2023) reported similar findings in apple juice when CP operated at higher input voltage, which could be due to disruption of the cell wall that releases more bioactive components, thus increasing antioxidant activity.

Variability is observed in antioxidant activity responses across different juices. Rodriguez *et al.* (2022) reported that CP treatment enhanced the antioxidant activity of guava juice (up to 116%), sapota juice (up to 261%) and açai juice (up to 101%). However, in caja juice 32%

decrease in antioxidant activity was observed. The variation in antioxidant activity in similar operating conditions could be linked to the composition of antioxidants and the effect of CP on different bioactive components.

Overall, the effect of CP on antioxidant activity in fruit juices is dependent on various parameters such as treatment time, voltage, power, GFR, and juice composition. CP treatment can result in enhanced antioxidant activity in shorter term due to the release of bound antioxidant components because of structural changes. On the other hand, prolonged exposure to CP resulted in reduced antioxidant activity because of oxidative degradation. Hence, optimization of CP parameters is necessary for maximizing the retention of antioxidant activity during processing of fruit juices. It is worth noting that each juice type responds differently depending on its composition and oxidative effects.

Enzyme inactivation/browning

Enzymes are the molecules that originate from proteins. They play an important role in the food sector by inducing desirable or undesirable chemical reactions and are not involved as a reagent in the reactions. Enzymes consist of two main parts: apoenzyme (protein part) and cofactor or coenzyme (non-protein part). The enzymes are specific and can accelerate the speed of reactions. Enzymes can be used as indicators of pasteurization (lactoperoxidase), meat tenderization (papain, ficin and bromelain), and cheese-making (renin). Enzymes can also induce undesirable changes, such as browning (peroxide enzyme) and texture changes in fruits and vegetables (pectinase). Enzymes are affected by temperature, pH and water activity (Motta *et al.*, 2023). Various efforts have been made to inactivate or decrease the activity of undesirable enzymes such as peroxidase (POD), polyphenol oxidase (PPO), and pectin methyl esterase (PME) by varying the operating parameters of CP treatment. Changes in enzymes and their activity induced by CP treatment is summarized in Table 4.

Illera *et al.* (2019) reported that by increasing treatment time, the residual activity of PPO could be decreased. On increasing the treatment time from 4 to 5 min, a 42% reduction was observed in the activity of PPO. Abedelmaksoud *et al.* (2022) reported the reduced activity of POD, PME and PPO to the least value at maximum treatment time in the case of mango pulp.

de Castro *et al.* (2020) observed that in the case of camu-camu juice, as the excitation frequency of CP was increased, the enzyme activity of POD and PPO decreased up to a certain threshold (698 Hz). However, the enzymatic activity increased by further increasing the frequency (960 Hz). This could be ascribed to the generation of free radicals that act on N–H, C––N and C–H protein bonds and the change the secondary structure of the enzymes by reacting with free radicals, such as OH•, NO•, O^{-2}• and HOO•, through which enzymatic activity alternates. The increase in enzymatic activity could be the result of the release of intracellular enzymes on depolymerization of cells. Dantas *et al.* (2021) observed the opposite trend, concluding that lower frequency leads to higher inactivation of enzymes. The highest reduction in POD was about 60% when the açai pulp was treated at a frequency of 50 Hz for 5 and 15 min. PPO activity is reduced at a lower frequency range, but it increases with increase in the frequency. However, exposure for a prolonged duration reduces PPO activation. The increase is observed due to an increase in reactive oxygen species, which triggers the defense response of PPO.

Pipliya *et al.* (2022) observed that the efficacy of reducing the enzymatic treatment increased on increasing the applied voltage. When the frequency increased from 25 kV to 45 kV, the activity of POD reduced from 61.3% to 30.9%, and the PPO activity varied from 52% to 23.9% for a treatment of 0–10 min. Hence, it is concluded that POD is more resistant to CP than PPO. Chutia *et al.* (2019) also reported similar findings in the case of coconut water. The inactivation of POD and PPO is directly linked with CP treatment's voltage and time. The increase in voltage as well as treatment time reduces the enzymatic activity.

Paixão *et al.* (2019) observed in the case of siriguela juice that CP decreases the PPO activity by 20% when treated with N_2 gas at GFR of 20 mL/min for 15 min, but an increase in the POD activity was increased in some of the processing conditions.

Xu *et al.* (2017) analyzed the impact of two different gases and treatment periods (30 s, 60 s and 120s), which were dry air and modified atmospheric gas (which contains 65% O_2, 30% CO_2 and 5% N_2), on the PME activity. It was observed that the PME activity reduced with the increase in treatment time, and MA65 gas was more effective in reducing the enzyme activity than air. The CP generated reactive oxygen species, which affected the structural integrity of enzymes, resulting in the loss of enzyme activity or functionality. The MA65 gas was more effective than air because it had a higher concentration of O_3 than in air, and this higher concentration of O_3 generated in MA65 gas indicated higher oxidative capacity. Andreou *et al.* (2023) reported that higher inactivation of PME in orange juice was achieved by applying high voltage with lower gas (helium) flow. The PME activity was reduced by 44 units when the treatment voltage increased from 4.0 kV to 7.0 kV. When the gas flow

Table 4. Changes in enzymes and their activity induced by CP treatment

Matrix	Plasma source	Gas	Operating conditions	Enzyme	Key findings	References
Apple juice	Spark and glow discharge	Argon	D: 5 mm SS: 10 mL SD: 4.2 mm V: 7.9375–10.875 kV TT: 1–5 min	PPO	Increasing TT from 4–5-min, residual activity decreases by 42%	Illera *et al.*, 2019
Camu-camu juice	Dielectric barrier atmospheric CP	-	SS: 40 mL TT: 15 min V: 24 kV υ: 200, 420, 583, 698, and 960 Hz D: 15 mm	PPO and POD	Enzyme activity decreases with an increase in frequency	de Castro *et al.*, 2020
Siriguela juice	Glow discharge plasma	N_2	SS: 80 mL TT: 5–15 min GFR: 10–30 mL/min	PPO and POD	Minimum 78.54% and 92.44% RA was observed in PPO and PO	Paixão *et al.*, 2019
Açai pulp	Dielectric barrier discharge (atmospheric CP)	-	SS: 30 mL V: 20 kV υ: 50, 500, and 750 Hz TT: 5, 10, and 15 min	PPO and POD	Low υ values are affective in inactivating POD (42.3%) and PPO (82.4%)	Dantas *et al.*, 2021
Pineapple juice	Dielectric barrier discharge	Air	SS: 30 mL V: 25, 35, and 45 kV TT: 1–10 min P: 90, 120, and 220 W D: 8 mm	PPO and POD	POD was more resistant than PPO	Pipliya *et al.*, 2022
Coconut water	Dielectric barrier discharge plasma	Air	V: 18, 23, and 28 kV υ: 50 Hz SS: 15 mL TT: 1–5 min	PPO and POD	Inactivation of POD and PPO increases with an increase in treatment time and voltage	Chutia *et al.*, 2019
Orange juice	High-voltage atmospheric CP treatment	Air and MA65 (65% O_2 + 30% N_2 + 5% CO_2)	EG: 4.44 cm SS: 25 and 50 mL TT: 30, 60, and 120 s	PME	PME activity decreases with an increase in TT, and MA65 gas is more effective than air	Xu *et al.*, 2017
Orange juice	Dielectric barrier discharge	Helium	SS: 10 mL D: 4.3 mm υ: 85 kHz TT : 2–30 min V: 4.0–7.0 kV GFR: 0.5–2.0 Standard L/min	PME	Effectively inactivation of PME	Andreou *et al.*, 2023
Mango pulp	Dielectric barrier discharge	Air	SS: 5 g V: 25 kV TT: 0, 2, 4, 6, 8, and 10 min	PPO, POD, and PME	The residual enzyme activity decreases with an increase in TT	Abedelmaksoud *et al.*, 2022

V: voltage, TT: treatment time, SS: sample size, GFR: gas flow rate, υ: frequency, EG: electrode gap, D: distance between sample and plasma nozzle tip, SD: sample depth, PME: pectin methyl esterase, POD: peroxidase, PPO: polyphenol oxidase, CP: cold plasma, Conc.: concentration, O_2: oxygen, N_2: nitrogen, CO_2: carbon dioxide.

decreased from 2.0 to 0.5 standards L/min, nearly 50% more reduction in the PME activity was observed, which could be ascribed to the production of higher levels of NO^{-}_{2} and NO^{-}_{3}, which led to the formation of HNO_2 and HNO_3.

Effect on Microorganism

The growth of microorganisms in food causes various changes in the attributes of food, which could be due to their metabolic activity. The changes that are induced by microbes are both desirable, such as in the case of fermentation, and undesirable, which can lead to food poisoning and spoilage. The growth of microbes is dependent on various factors, such as intrinsic factors (nutrient content, pH, water activity etc.), extrinsic factors (humidity, temperature of storage etc.), processing factors, and factors affecting each other. Various thermal and nonthermal treatments were induced to keep check the microbial load. CP is one of the promising nonthermal treatments that is effective in inactivating microorganisms without impacting the quality parameters of foods. The effectiveness of CP treatment for microbial inactivation depends on operating factors as well as food factors listed in Figure 4B. The operating parameters of CP were changed to observe impact on microbial load.

Mehta and Yadav (2019) observed that with an increase in CP treatment, the microbial load reduced in the case of strawberry juice. Decrease in the total bacterial count was recorded more when the juice was exposed to 15 min of CP treatment than 10-min exposure. Similar results were obtained in apple juice (Liao *et al.*, 2018; Surowsky *et al.*, 2014; Wang *et al.*, 2020), sour cherry juice (Hosseini *et al.*, 2020), orange juice (Shi *et al.*, 2011; Xu *et al.*, 2017), pineapple juice (Sohbatzadeh *et al.*, 2021), white grape juice (Pankaj *et al.*, 2017), blueberry juice (Hou *et al.*, 2019), coconut water (Mahnot *et al.*, 2019) and chokeberry juice (Gan *et al.*, 2021). The microbial inactivation could be due to the generation of reactive species that affect microbes by causing DNA alterations, cell integrity loss, and lysis of cells. The charged species are the major contributors to microbiocidal effect. The reactive oxygen species can initiate the break down of DNA inside the cells (Xu *et al.*, 2017). The plasma-activated species can result in the rupturing of cell membranes by oxidizing the lipids and sugars present in cells. The activated species generated from nitrogen and oxygen results in the breakage of bonds of cell walls and peptidoglycan. The entire cell structure is damaged when active species break down the bonds between C-N and C-O. The generation of UV during CP treatment induces a bactericidal effect by forming thymine dimer when the microbe absorbs energy. It was reported that Gram-positive bacteria are more resistant to CP treatment than Gram-negative bacteria because of their thicker peptidoglycan layer (Rao *et al.*, 2023). Inactivation of different targeted microbes using CP treatment is shown in Table 5. The mechanism behind microbial inactivation is presented in Figure 4A.

The power and voltage supplied to CP treatment for generating active species also affect the inactivation of microbial load. The increase in voltage by 15 kV doubled the log reduction of aerobic mesophiles and more than double the log reduction recorded for yeast and mold in the case of sugarcane juice when treated for 2 min. Similar conclusions were drawn by various studies concerning apple juice (Ding *et al.*, 2023; Liao *et al.*, 2018). Liao *et al.* (2018) recorded that when the apple juice was treated with 30 W for 40 s, the microbial load (*Escherichia coli*) was decreased by 4.2 log units, and in another trial with higher power and shorter treatment time, that is, 50 W and 30 s, a greater log reduction of 4.34 log units was observed.

The sample size also affected the inactivation of microbes. Xu *et al.* (2017) observed an inverse relationship between microbial load reduction and the sample size. Large samples with smaller exposure areas require more time to achieve the desired microbial reduction. It was reported that when the volume of orange juice increased from 25 mL to 50 mL while maintaining the same exposure area, it resulted in a different inactivation time for *S. enterica*. A 5-log units reduction was achieved in 30 s for 25-mL orange juice, while it took 120 s for a 50-mL sample to achieve similar results. Doubling the exposure area and reducing the sample size facilitate the diffusion of plasma-activated species in the juice, and a 5-log units reduction of *S. enterica* was achieved within 60 s. Similar results were recorded in case of sour cherry juice (Hosseini *et al.*, 2020) and chokeberry juice (Gan *et al.*, 2021).

It is worth noting that the type of microbe and treatment provided to inactivate microbes also influence log reduction. Shi *et al.* (2011) reported that to achieve a 5-log units reduction using dielectric barrier discharge, *E. coli* needs the least time of 8 s, *S. aureus* requires 12 s, and the maximum time was recorded for *C. albicans*. Variation in the treatment period to achieve a similar result was the result of the difference that occurred in the composition of bacteria cell walls and cell matrix. *E. coli* is a Gram-negative bacteria, while *S. aureus* is a Gram-positive bacteria and *C. albicans* is a fungus. Gram-negative bacteria are more sensitive than Gram-positive bacteria because of the absence of an extra layer of peptidoglycan in their cell walls, which makes *S. aureus* resistant in comparison to *E. coli* (Rao *et al.*, 2023). The fungi are more resistant to treatments than bacteria because of their cell wall

(A)

(B)

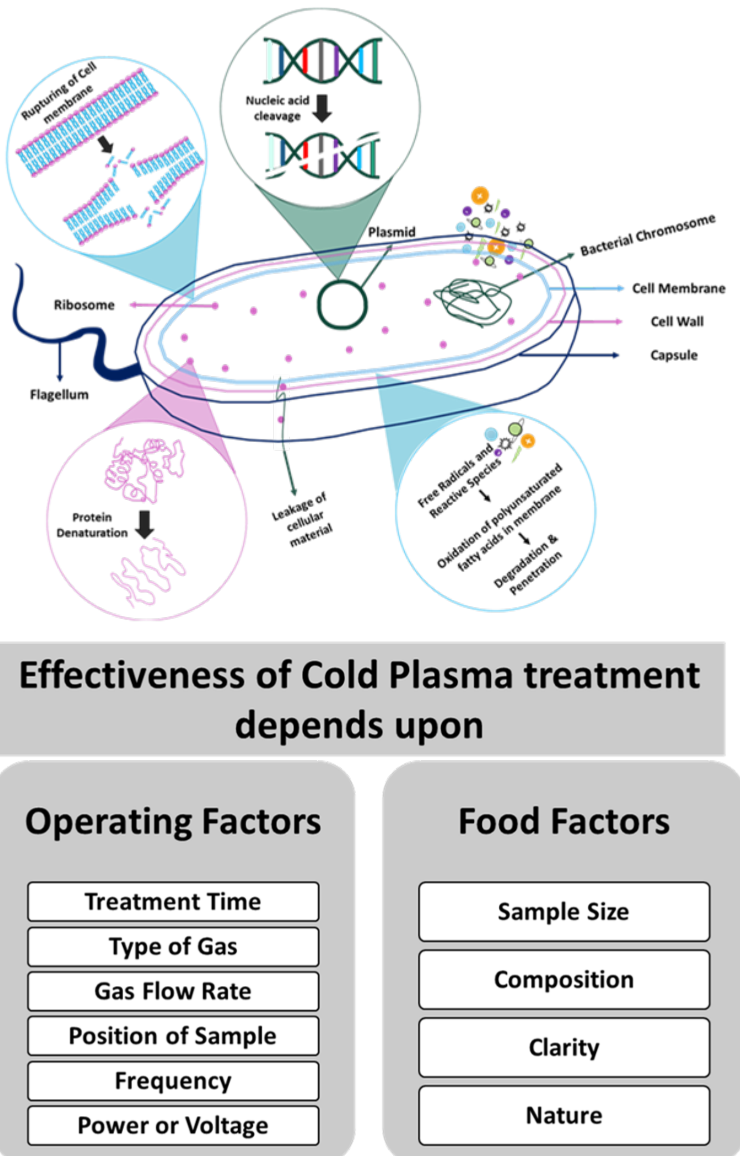

Figure 4. (A) Mechanism of inactivation of microbes during CP treatment, (B) factors affecting the effectiveness of CP treatment.

structure, which contains chitin, glucans and mannoproteins (non-filamentous glycoproteins localized in the outermost layer of yeast's cell wall), as well as their membrane composition and spore-forming ability to protect them against environmental stress. Additionally, fungi are generally larger and can produce antioxidants, which act as an extra layer of protection, compared to bacteria. Manzoor *et al.* (2020) recorded a similar result while inactivating aerobic mesophiles, and yeast and molds in sugarcane juice. It was found that the inactivation efficiency was lower for yeast and molds, compared to bacteria.

Xu *et al.* (2017) concluded that when the plasma treatment was afforded directly to orange juice, more reduction in *S. enterica* was observed than the indirect treatment; this could be the result of direct diffusion of plasma-activated species into the liquid or juice, which could further lead to the formation of microbicidal compounds. Additionally, highly reactive short-lived species, such as free radicals and charged particles, are added to the surface of juices because of direct treatment.

The gas used during the CP treatment plays an important role in determining antimicrobial efficacy and plasma chemistry. Different gases resulted in the production of various reactive species, which impact the efficacy of the treatment. The oxygen-rich environment resulted in higher microbial load reduction in comparison to inert

Table 5. Inactivation of microbes using CP.

Medium	Plasma source	Process gas	Treatment condition	Target microorganism	Key findings	References
Orange juice	High-voltage atmospheric CP treatment	Air and MA65 gas (O_2:N_2:CO_2 = 13:6:1)	D: 4.44 cm V: 90 kV TT: 30–120 s SS: 25–50 mL	*S. enterica serovar Typhimurium* (ATCC 14028)	• An increase in TT increases the inactivation rate • MA65 is more effective than air • Direct treatment is more effective • The smaller the sample size, more is the inactivation rate	Xu *et al.*, 2017
Apple juice	Dielectric barrier discharge	Air	SS: 3 mL TT: 0–40 s I: 30–50 W	*Escherichia coli*	• Input power is directly linked with microbial inactivation • An increase in TT increases microbial log reduction • Treatment of: 50 W and 30 s → 4.34 log reduction 30 W and 40 s → 4.2 log reduction	Liao *et al.*, 2018
Apple juice	Plasma jet	Argon and a mix of argon and O_2	υ: 1.1 MHz TT: 0–480 s V: 65 kV EG: 10 mm O_2: 0.025, 0.05, 0.075, and 0.1%	*Citrobacter freundi*	• Pure Argon gas doesn't influence microbial inactivation • Increase in conc. of O_2 and TT increases antimicrobial activity • Treatment of: 0.025% O_2 and 480 s→ 1.5 log reduction 0.1% O_2 and 480 s → 4.4 log reduction	Surowsky *et al.*, 2014
Sour cherry juice	Dielectric barrier discharge	Argon and O_2	TT: 1–9 min υ: 20 kHz SD: 0.5–1.5 cm V: 0–20 kV G: 20 mm GFR: 5 std. L/min O_2 in Ar: 0, 0.5, and 1%	*Escherichia coli*	The inactivation rate can be enhanced by: • Increasing the TT • Lowering the depth of the sample • Increasing O_2 concentration	Hosseini *et al.*, 2020
Orange juice	Dielectric barrier discharge	-	TT:12–25 s V: 30 kV υ: 60 kHz DG: 3 mm	• *Staphylococcus aureus* • *Escherichia coli* • *Candida albicans*	• An increase in TT results in an increase in the inactivation of microbial load • *E coli* is most sensitive to CP than *S. aureus* and *C. albicans*	Shi *et al.*, 2011
Strawberry juice	Dielectric barrier discharge	Air	TT: 10 and 15 min υ: 50 Hz V: 60 kV	Bacterial load	• Bacterial load reduction is more at 15 min of TT, compared to 10 min	Mehta and Yadav, 2019

(*continues*)

Table 5. Continued.

Pineapple juice	Dielectric barrier discharge and plasma jet	Ar	TT: 30, 90, 180, 300, and 420 s (only in DBD) DG: 2 cm V: 12 kV υ: 6.2 kHz SS: 10 mL GFR: 4 std. L/min D: 10 mm	*Enterococcus faecalis*	• A plasma jet is more effective in reducing bacterial load in comparison to a dielectric barrier discharge Almost complete sterilization is observed in jet plasma after 5 min of TT and 7 min for dielectric barrier discharge	Sohbatzadeh *et al.*, 2021
White grape juice	Dielectric barrier discharge	Air	V: 80 kV TT: 1–4 min	*Saccharomyces cerevisiae*	• An increase in TT results in an increase in the reduction of yeast 80 kV for 4-min → 7.4 log reduction	Pankaj *et al.*, 2017
Sugarcane juice	Dielectric barrier discharge	-	P: 0–220 V V: 30–45 kV F: 10 kHz TT: 2 min	• Aerobic mesophile • Yeast and mold	• Higher voltage leads to an increase in microbial reduction • Aerobic mesophiles 30 kV for 2 min → 1.8 log reduction 45 kV for 2 min → 3.6 log reduction • Yeast and mold 30 kV for 2 min → 0.15 log reduction 45 kV for 2 min → 0.50 log reduction	Manzoor *et al.*, 2020
Apple juice	Dielectric barrier discharge	Air	TT: 0–140 s V: 15–21 kV	*Zygosaccharomyces rouxii*	• Microbial load reduces sharply after 80 s of CP treatment • 90 W for 140 s → 5 log reduction	Wang *et al.*, 2020
Blueberry juice	Nonthermal plasma	O_2	V: 11 kV υ: 1,000 Hz TT: 2,4, and 6 min G: 2 cm O_2 conc.: 0, 0.5, and 1%	*Bacillus* sp.	• Increase in TT and conc. of O_2 bactericidal effect increases 1% O_2 conc. and 6 min TT → 7.2 log reduction	Hou *et al.*, 2019
Apple juice	Discharge reactor	Air	υ: 7.0 kHz V: 1.32, 2.20, 4.64, and 6.86 kV SS: 330 mL TT: 0–720 s GFR: 80, 130, and 180 mL/min	*A. acidoterrestris*	• Higher voltage, GFR, and longer TT have better bactericidal effect	Ding *et al.*, 2023
Coconut water	Dielectric barrier discharge	Air	TT: 30–120 s V: 90 kV DG: 15 mm D: 5 cm	*Salmonella enterica*	• 5-log reduction can be achieved with CP treatment at 90 kV for 120 s followed by 24 h refrigerated storage	Mahnot *et al.*, 2019

(*continues*)

Table 5. Continued.

Chokeberry juice	Dielectric barrier discharge	Argon	TT: 1–5 min SS: 1–5 mL V: 9 KV υ: 1,500 Hz GFR: 1.5 L/min D: 1.5 cm	• *Saccharomyces cerevisiae* • *Escherichia coli*	The bactericidal effect increases: • if the sample size is small • if TT is more	Gan *et al.*, 2021

V: voltage, TT: treatment time, SS: sample size, GFR: gas flow rate, υ: frequency, EG: electrode gap, D: distance between sample and plasma nozzle tip, CP: cold plasma, Conc.: concentration, O_2: oxygen, N_2: nitrogen, CO_2: carbon dioxide.

gases. Rowan *et al.* (2007) reported that *Bacillus cereus* exhibited the highest sensitivity to CP treatment in the presence of oxygen, followed by carbon dioxide, air and nitrogen.

Rød *et al.* (2012) also observed a limited antimicrobial effect of argon alone. The efficacy of the CP treatment improved when combined with oxygen. Surowsky *et al.* (2014) also reported that pure argon gas does not influence microbial inactivation. However, the bactericidal effect was observed if the argon gas was mixed with oxygen. On increasing the concentration of oxygen from 0.025% to 0.1% for 480 s, the bacterial log reduction increased by 2.9 units. The increase in concentration results in an increase in the concentration of atomic oxygen in plasma that forms hydrogen peroxide in juice and induces a bactericidal effect. Similar results were recorded in sour cherry juice (Hosseini *et al.*, 2020) and blueberry juice (Hou *et al.*, 2019).

Xu *et al.* (2017) reported that direct CP treatment of orange juice for 120 s, and stored for 24 h at 4°C resulted in higher *S. enterica* reduction in a 65% O_2, 30% N_2 and 5% CO_2 atmosphere (4.7-log units) than in air (2.9-log units). Ding *et al.* (2023) concluded that increasing GFR increases the inactivation number of microbes. Increase in GFR lowers the time required to achieve the desired inactivation result. The fastest sterilization was observed when the GFR was 180 mL/min for 240 s.

Spores are typically present in foods in their dormant stage. Spores are heat-resistant form of microorganisms that can endure harsh conditions and pose potential health risks. Therefore, proper food processing and storage are necessary to inhibit the growth and germination of spores. Zhao *et al.* (2023) found that CP alone achieved limited inactivation of *A. acidoterrestris* spores (2.5-log units) whereas combining CP with mild heat (85°C) enhanced spore reduction (4-log units). Similarly, Ding *et al.* (2024) demonstrated that 1 min of CP treatment was equivalent to 12 min of heat treatment at 95°C, reducing *A. acidoterrestris* spores by 0.4-log units in apple juice. Additionally, higher input power and GFR resulted in a higher inactivation rate. Wang *et al.* (2023) observed a nearly 3-log units reduction after 7 min of CP treatment at 30 kV. These studies underscored the potential of the CP treatment, especially when combined with other treatments.

Safety concerns related to the usage of cold plasma

Cold plasma is known for its effective microbial reduction in juices without exposing them to high temperatures. Certain safety issues inhibit its adoption in the food industry. CP generates free radicals, reactive oxygen species, electrons, protons, ozone, and nitrogen oxides, which interact with food surfaces and form byproducts that are harmful to human health. The Food and Drug Administration (FDA) has regulated ozone levels for CP-treated foods, but there are no guidelines for other reactive oxygen species (Sarangapani *et al.*, 2018). This lack of guidelines creates a regulatory gap that poses challenges for the safer application of CP in the food industry. Han *et al.* (2016) conducted a toxicological study on rodents, and suggested that CP is generally safe when applied under standard conditions. However, a main safety concern remains with the bacteria that can survive CP treatment in a viable but nonculturable (VBNC) state. These states could pose a serious health risk if recovered after the treatment. Hence, complete bacterial inactivation is necessary to mitigate public health risks and ensure food safety.

Limitations

Several limitations of the CP treatment restrict its application only to laboratory scale, with industry adoption still a huge challenge. The oxidative effect of reactive oxygen and nitrogen species is also a key issue. These reactive species degrade organic compounds such as vitamin C and also induce browning, and alter the color parameters (Wang *et al.*, 2020). Increase in the CP treatment time degrades certain quality traits such as phenols, flavonoids and antioxidants (Pankaj *et al.*, 2017). CP treatment is unable to fully inactivate the enzyme activity of pectin methyl esterase, peroxidase and polyphenol oxidase. In addition, plasma treatment may induce bacteria into a VBNC state, which poses a serious risk to food safety. The limited penetration of CP into tissue structures resulted in lower disinfection efficacy and prolonged treatment time for improving microbial inactivation; all this increases the operating cost and compromises the quality of juice. A limited number of studies exist on the impact of CP treatment and plasma-activated species on sensory attributes of fruit juices, such as aroma, consistency, appearance and taste. These limitations need to be addressed before the CP system is effectively scaled for industrial applications.

Future Directives

The CP treatment is a promising technology for laboratory-scale microbial inactivation in juices, but limited efforts are made for scaling CP to pilot or industrial use. The proposed aseptic system, illustrated in Figure 5, outlines a continuous CP decontamination system for the juice processing industry, which also integrates bottling and aseptic packaging operations. Optimization of CP parameters for various types of juices, such as high-acid, low-acid, clear, and cloudy juices. The optimization is essential to improve the efficacy of the treatment. Understanding the impact of plasma-activated species and microbes is essential, as the exact mechanism of inactivation is not fully clear yet and needs further experimentation.

Additionally, more research efforts are required to interact with active species with nutritional and bioactive compounds in juices, as well as the effects of CP treatment and plasma species on sensory attributes, such as aroma, taste, consistency and appearance. The combination of CP with other preservative methods could be explored to enhance microbial inactivation. The CP treatment also forms viable but nonculturable microbes, which could pose a risk, and hence need further experimental studies. Regulatory guidelines for the usage of CP in food processing need to be standardized to ensure safe and consistent applications.

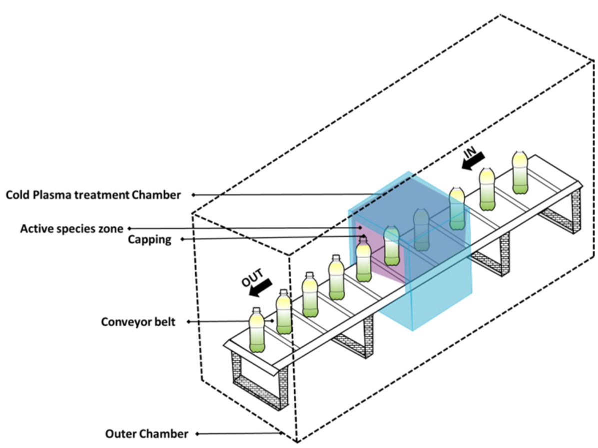

Figure 5. Conceptual design of a continuous CP decontamination system for industrial juice processing.

Conclusions

In recent years, the demand for novel nonthermal technologies that can preserve the nutritional, sensory and functional properties of fruit juices has increased significantly. CP has emerged as a promising nonthermal technology for juice decontamination with minimal quality and nutritional degradation. Despite promising laboratory-scale results, industrial adoption remains limited due to challenges in scaling up and optimizing CP parameters for various juice types.

Future research should focus on exploring the synergistic effects of CP with other preservation methods to enhance microbial inactivation and to extend shelf-life. Additionally, further efforts are needed to understand the interaction between plasma-activated species and bioactive components and their effect on sensory attributes and consumer health. Addressing these gaps through collaborative pilot-scale trials and industry collaboration is important for accelerating CP's commercialization and broader application in the juice industry.

Acknowledgements

The authors thanked the Department of Science and Technology, Punjab Agricultural University, for facilities.

Data Availability Statement

This study is a review paper; data were taken from previous studies and no new data were created.

Author Contributions

Conceptualization: Gurveer Kaur and Vimal Challana; methodology: Gurveer Kaur; validation: Gurveer Kaur, Vimal Challana and Sandhya; formal analysis: Gurveer Kaur; investigation: Gurveer Kaur, Sandhya and Vimal Challana; resources: Vimal Challana and Sumandeep Kaur; data curation: Vimal Challana, Gurveer Kaur and Sumandeep Kaur; writing of original draft: Vimal Challana, Gurveer Kaur and Sumandeep Kaur; review and editing of paper: Gurveer Kaur and Sandhya; visualization and supervision: Gurveer Kaur, Sandhya and

Maninder Kaur; project administration: Sandya, Gurveer Kaur, and Maninder Kaur.

Conflicts of Interest

The authors declared no conflict of interest.

Funding

None.

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