Exploring Potential of a Remote Plasma Electrolysis System (RPES) for Fruit Surface Sterilization

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Abstract. This study investigates the potential of remote plasma electrolysis systems (RPES) for eliminating microorganisms on fruit surfaces. A plasma electrolysis reactor generated long-lived reactive oxygen and nitrogen species (RONS), including hydrogen peroxide (H_2O_2), nitrite (NO_2^-), nitrate (NO_3^-), nitrous acid (HNO_2), and nitric acid (HNO_3), which were introduced into a treatment chamber through airflow. The spatial distribution of these reactive species was visualized using a KI-starch agar gel reagent. The antimicrobial efficacy of RPES was tested in vitro against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*). Following optimization, the system was applied to sterilize 1.0 kg samples of rambutans and grapes, with a treatment time of 10 minutes. After treatment, aerobic bacteria on rambutan surfaces decreased by 98.3%, and yeasts and molds level dropped by 50.1%. On grape surfaces, RPES completely eliminated aerobic bacteria, yeasts, and molds. These findings demonstrate RPES's effectiveness in inactivating surface pathogens on fruits, highlighting its potential for broader applications in fruit sterilization.

1 Introduction

Postharvest technologies play a crucial role in maintaining a steady global supply of fresh fruits, supporting both local and large-scale production. Recent advancements in these technologies have emphasized sustainability, human safety, and long-term ecological impact. Among these innovations, remote cold atmospheric pressure plasma (RCAPP) has emerged as a promising tool for surface modification, including the sterilization of fresh fruit surfaces [1–3].

Unlike direct or indirect plasma treatments, RCAPP's chemical treatment method is highly adaptable, making it suitable for treating fruits of various shapes, sizes, and surface textures without causing electrical, thermal, or ion damage [1,4]. This flexibility also means that RCAPP is less affected by the type of plasma discharge (such as filamentary, glow, spark, or arc discharges) and the shape or size of the electrodes. Instead, the effectiveness of the treatment depends on the final concentration and chemical composition of the generated radicals.

RCAPP generates gases like ozone and nitric oxide, which are effective for surface treatment of fresh produce [5,6]. Ozone, commonly produced in corona discharge plasma reactors, disinfects fruit surfaces due to its strong oxidizing and antimicrobial properties, effectively targeting a broad range of microorganisms [7–9]. The U.S. Food and Drug Administration (FDA) has approved ozone for use in agricultural processing [10]. Nitric oxide treatment offers additional benefits, such as reducing respiration, minimizing water loss, preventing browning, and reducing postharvest diseases [5].

In previous experiments, a plasma electrolysis reactor was used to treat community wastewater, promoting duckweed growth [11]. The reactor produced long-lived reactive oxygen and nitrogen species (RONS), including hydrogen peroxide (H_2O_2), nitrite (NO_2^-), nitrate (NO_3^-), nitrous acid vapor (HNO_2), and nitric acid vapor (HNO_3), all of which play key roles in microorganism elimination [12]. Given the limited research on remote plasma systems generating radicals beyond ozone and nitric oxide, this study aims to investigate the potential of a remote plasma electrolysis system (RPES) to reduce microbial load on fresh fruits.

This study includes measuring the distribution of RONS using the KI gel reagent method, along with the concentrations of key radicals such as nitrite, nitrate, and hydrogen peroxide within the RPES treatment chamber. It will assess the bactericidal effectiveness of RPES against bacteria cultured on agar plates, as well as the system's efficiency in eliminating bacteria, fungi, and yeasts on the surfaces of grapes and rambutans treated with RPES.

2 Materials and Methods

Remote plasma electrolysis system

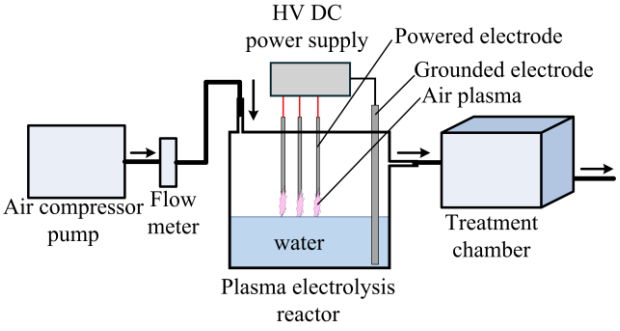

Fig. 1. Schematic diagram of the experimental setup for fruit surface sterilization by the remote plasma electrolysis system.

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The remote plasma electrolysis system used in this study comprises an air compressor pump, an air flow meter, a plasma electrolysis reactor, and a treatment chamber as shown in Fig. 1. The details of the plasma reactor were described elsewhere [11]. Briefly, the air plasma was generated by a pin-to-liquid plane configuration with a liquid-grounded electrode. The electrical discharges were produced with 3 anodes and a 1.00-cm discharge gap. The pulse-average electrical power dissipated in a plasma discharge was 75.2 W, while the electric power consumed by the multi-channel DC power supply was 230.4 W. The system was enclosed in a container with gas exchange ports. The treatment chamber was a rectangular plastic container measuring 25 cm in width, 36 cm in length, and 27 cm in height, with a volume of 24,300 cm^3. It was equipped with ports for gas inlet and outlet. The air compressor pump pulled in ambient air, filtered out moisture and dust, and delivered the clean air to the plasma electrolysis reactor for plasma generation. The airflow rate entering the plasma reactor was regulated by an air flowmeter. RONS generated in the plasma electrolysis reactor were then carried through the pipeline into the treatment chamber. Unused residual RONS and by-products from chemical reactions between plasma-generated RONS and microorganisms, cells, or chemical residues on the fruit's surface were vented into the surrounding environment through an exhaust air pipe.

Visualization of the spatial distribution of reactive oxygen and nitrogen species (RONS) on the treated surface

In order to study the effect of air flow rate and treatment time on the final concentration of RONS within the treatment chamber, the potassium iodide (KI)-starch gel reagent method was used to visualize the spatial distribution of RONS on the treated surface [13]. To prepare the gel reagent, commercial agar powder was mixed with deionized water to create a 0.5% w/v concentration solution. Potassium iodide (KI) and starch were then dissolved in the agar solution, also at a concentration of 0.5% w/v. The solution was poured into a Petri dish and sterilized in an autoclave at 121 °C. The solution solidified into a gel at room temperature.

Measuring density of H_2O_2, NO_2^-, and NO_3^- in the treatment chamber

The concentrations of hydrogen peroxide, nitrite, and nitrate in the treatment chamber were determined by using a colorimetric method with two semi-quantitative test strips. 91319: Quantofix® Peroxide test strips (measuring range 0.5 – 25 mg/L H_2O_2, MACHEREY-NAGEL, Düren, Germany) were employed to measure hydrogen peroxide concentration, while 91313: Quantofix® Nitrate & Nitrite test strips (measuring range 10 – 500 mg/L NO_3^- and 1 – 80 mg/L NO_2^-, MACHEREY-NAGEL, Düren, Germany) were used to measure nitrite and nitrate concentration.

Bactericidal effects of RPES against pathogenic bacteria

To assess the efficacy of the RPES in inactivating hazards bacteria commonly found on fresh produce surfaces and cross-contamination from several points in the food production chain: methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli (E. coli)* grown on agar culture media dishes were treated within the treatment chamber. The dishes were placed in the center of the treatment chamber. These two bacterial species were chosen to represent gram-positive and gram-negative bacteria.

Microbiological analysis

Enumerations of aerobic bacteria, yeasts, and molds remaining on the treated fruit surface were performed following the FDA's Bacteriological Analytical Manual (BAM), Chapters 3 and 18, respectively. The results expressed in colonies forming units (CFU) per sample weight were calculated. The germicidal efficiency was determined as follows:

$$\text{Germicidal efficiency} = |N_t\text{-}N_0|/N_0 \times 100\% \quad (1)$$

where N_t and N_0 are the number of colony-forming units of the RPES-treated and untreated samples, respectively.

3 Results and Discussion

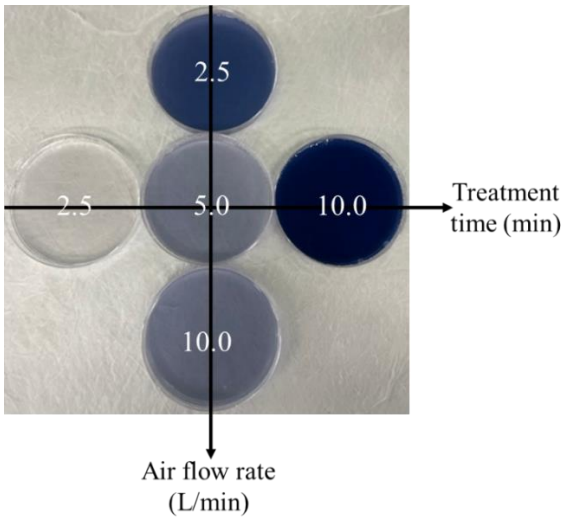
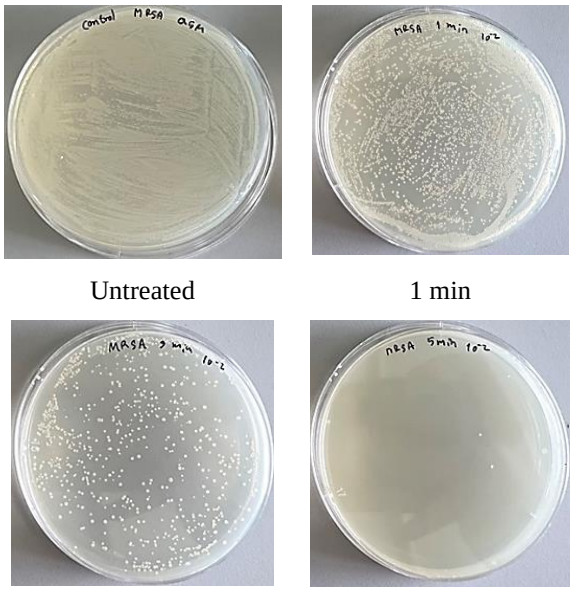


Fig. 2. Spatial distribution of RONS deposit on the KI–starch agar gel reagent after treatment by the RPES with various exposure times and air flow rates.

The concentration of reactive oxygen and nitrogen species (RONS) in the treatment chamber is a key factor influencing the effects of the remote plasma electrolysis system (RPES) on the surface being treated, particularly its sterilization effectiveness. The air flow rate for transporting reactive species from the plasma electrolysis reactor was optimized by measuring RONS

concentrations using KI-starch agar gel. The visual method relies on a traditional iodine-starch color reaction, which results in a blue color. RONS species that can be found in treatment chamber, such as OH, ONOOH, O_3, H_2O_2, HO_2, NO, NO_2, N_2O, and HNO_2, have a higher oxidation potential compared to I_2 [12,14–16] and are capable of oxidizing the gel reagent's iodide (I^-) into iodine (I_2). A reaction between I_2 and another I^- forms triiodide (I_3^-). The color turns blue when triiodide combines with starch. Since the most reactive species have a greater oxidation potential than I_2, the KI–starch gel method can display RONS distribution within a single assessment. Areas that darken indicate a higher concentration of reactive species deposited there [12]. As shown in Fig. 2, the optimal air flow rate for RPES was determined to be 2.5 L/min. The uniform color of the KI gel indicated a homogeneous distribution of RPES-produced reactive species on the treated surface. The figure also illustrates that longer plasma exposure increases the reaction rate of chemicals derived from RPES at the designated treatment site. The concentrations of H_2O_2, NO_2^-, and NO_3^- within the treatment chamber, where the air flow rate was 2.5 L/min and determined after 5 min of exposure time, were 50 mg/L, 80 mg/L, and 500 mg/L, respectively.

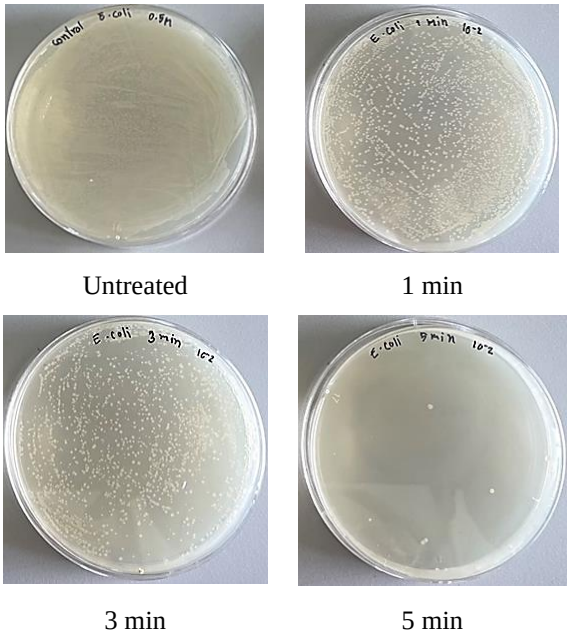


Untreated 1 min

3 min 5 min

Fig. 3. Effects of the remote plasma electrolysis system on MRSA inactivation as a function of treatment time. Bacteria were cultured in nutrient broth before being spread onto agar plates. They were then exposed to RPES-derived RONS within the treatment chamber at an airflow rate of 2.5 L/min.

The effects of chemical treatment of the remote plasma electrolysis system on the decontamination of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*) grown on agar plate are shown in Fig. 3 and 4, respectively. RPES treatment for 5 min effectively eliminated nearly all *E. coli* and MRSA microorganisms. These results demonstrate that RPES is effective against various bacteria, including gram-positive, gram-negative, and antibiotic-resistant strains, with its bactericidal effect increasing over time.



Untreated 1 min

3 min 5 min

Fig. 4. Effects of the remote plasma electrolysis system on *E. coli* inactivation as a function of treatment time. Bacteria were cultured in nutrient broth before being spread onto agar plates. They were then exposed to RPES-derived RONS within the treatment chamber at an airflow rate of 2.5 L/min.

Table 1. Enumeration of microorganisms on rambutan and grape surfaces before and after RPES treatment.

Sample	Treatment time (min)	Total plate count (CFU/g)	Yeasts and molds (CFU/g)
rambutan	0	9,680	6,685
	10	165	3,335
grape	0	1,650	1,640
	10	0	0

To assess the antimicrobial effectiveness of RPES on fresh fruits, 1.0 kg samples of rambutans and grapes, sourced from a local market in Rayong, Thailand, were treated in an RPES chamber for 10 minutes at an airflow rate of 2.5 L/min. The fruits were placed on a stainless steel mesh tray, positioned at the center of the treatment chamber. After treatment, aerobic bacteria on rambutan surfaces decreased by 98.3%, while yeasts and molds level dropped by 50.1%, as shown in Table 1. On grape surfaces, RPES completely eliminated aerobic bacteria, yeasts, and molds. These findings indicate that RPES could be a viable tool for sterilizing fresh fruit surfaces.

RPES-generated reactive oxygen and nitrogen species (RONS) deactivate pathogens on fruit through several mechanisms. RONS attack microbial cell membranes, increasing permeability and causing leakage of intracellular contents, which ultimately leads to cell death. Within cells, RONS induce oxidative stress, resulting in lipid peroxidation, protein oxidation, and DNA damage, all of which disrupt essential cellular

functions. Additionally, plasma-generated species can break down biofilm extracellular matrices, making embedded cells more vulnerable to inactivation [17–20].

The effectiveness of microbial reduction on fruit surfaces, however, depends on surface characteristics like roughness or features such as pliable spines that could shield some microorganisms from RPES exposure. To improve RPES efficacy across differentfruit types, specific treatment protocols could be developed to match each fruit's unique characteristics. This might include optimizing treatment duration, adjusting the concentration of RONS, and refining other parameters to achieve effective decontamination while minimizing potential fruit damage. Further research is needed to investigate any toxicity or undesirable effects of RPES-treated fresh fruits to support the safe use of remote cold plasma technology in the fruit industry.

4 Conclusion

In summary, this study demonstrates that remote plasma treatment with the plasma electrolysis reactor has the potential to sterilize fresh fruit surfaces. Deposition on the treated surface of long-lived reactive species within the plasma chamber deriving from the plasma reactor was visualized using a KI–starch agar gel reagent. The visualization indicated the uniform spatial distribution of reaction species on the treated surface. Results from in vitro microbial inactivation studies using MRSA and *E. coli* demonstrated the efficacy of remote plasma electrolysis system (RPES) in eliminating microorganisms on 2D surfaces. The RPES treatment demonstrated significant antimicrobial effects on both rambutan and grape. While it completely eliminated microbial contamination on grape surfaces, the reduction on rambutan surfaces was slightly lower, possibly due to the fruit's surface morphologies.

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Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT [chat.openai.com], Google Gemini [gemini.google.com], and QuillBot [quillbot.com] in order to improve only language and readability. After using these tools/services, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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