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Comparison of the Effects of Cold Argon Plasma, Air, and Plasma-activated Water on the Shelf Life of Oyster mushrooms

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ABSTRACT

This study investigates the effects of three non-thermal technologies-cold argon plasma (CArP), plasma-activated air (CAP), and plasma-activated water (PAW) on the shelf life and quality of oyster mushrooms. The results demonstrate that all three methods significantly reduce microbial load and improve physicochemical properties, including moisture retention, texture, and acidity. PAW emerged as the most effective treatment, with a 99% reduction in microbial load and superior preservation of moisture and texture over a 14-day storage period. The reactive oxygen and nitrogen species (RONS) generated during PAW treatment were key to its antimicrobial efficacy and structural preservation. While the study highlights the potential of cold plasma as a sustainable alternative to conventional preservation methods, further research is needed to evaluate its long-term effects on nutritional value, flavor, and texture, as well as its comparative effectiveness against traditional techniques. These findings contribute to the growing body of research on non-thermal food preservation and underscore the need for extended observation periods to assess commercial viability.

Keywords: Cold plasma, Oyster mushrooms, Shelf life, Non-thermal preservation

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INTRODUCTION

In addition to their culinary versatility, mushrooms- especially oyster mushrooms [*Pleurotus ostreatus* (Jacq.) P. Kumm.] -are known throughout the world for their nutritional and practical qualities. In addition to proteins, dietary fiber, and Ascorbic Acid (vitamins C), Thiamine (B1), Niacin (B3), folic acid (B9), and Cobalamin (B12), they are also a great source of minerals like potassium (K), phosphorus (P), selenium (Se), and zinc (Zn). These mushrooms' nutraceutical and therapeutic value is further enhanced by the presence of bioactive substances such as polysaccharides, phenolics, terpenoids, and essential fatty acids. Oyster mushrooms are positioned as a promising ingredient in the creation of food products with added value and functionality because of these qualities [1, 2]. Because of their remarkable ability to decompose lignocellulosic agricultural waste and transform it into nutrient-dense food, oyster mushroom cultivation has attracted a lot of attention. In addition to lowering environmental pollution, this sustainable strategy improves food security. Oyster mushrooms are particularly well-suited for developing countries because they thrive in a range of climates and require minimal technological advancement. Their contribution to the global mushroom production of approximately 27% underscores their economic and ecological importance [3]. Apart from their benefits, oyster mushrooms are highly perishable; postharvest losses are attributed to their high moisture content, rapid microbial growth, and declining quality. To address these problems, conventional preservation techniques like gamma irradiation, cold storage, MAP, and edible coatings have been used. Nevertheless, these approaches frequently have drawbacks, such as exorbitant expenses, environmental issues, and detrimental impacts on nutritional and sensory qualities. Because of this, scientists are paying more attention to cutting-edge non-thermal preservation techniques [4, 5, 6]. Promising non-thermal technologies that provide effective microbial inactivation while maintaining the nutritional value and sensory appeal of fresh produce are cold plasma (CP) and PAW. RONS produced by CP efficiently lower microbial loads without the use of chemical preservatives or high temperatures. In a similar vein, PAW enhanced with RONS has proven effective in controlling microorganisms and has the potential to be a sustainable substitute for traditional chemical treatments. Environmental sustainability and consumer demand for natural, minimally processed foods are two important issues that both technologies address [7, 8]. It has been demonstrated that CP and PAW treatments affect the physicochemical characteristics of food products in addition to microbial inactivation. For instance, research has shown that treated produce has notable changes in texture, pH, acidity, and modulus of elasticity. Because these technologies preserve the structural and sensory integrity of perishable products, such as oyster mushrooms, while also extending their shelf life, they are suitable for their preservation [9, 10]. Furthermore, incorporating these technologies into existing preservation systems could increase the overall efficacy of supply chains. For instance, combining CP or PAW with other methods, like MAP or edible coatings, could result in hybrid preservation strategies that maximize microbial safety and quality retention while minimizing environmental impacts [6, 11, 12]. In light of these developments, the current study intends to thoroughly assess how oyster mushrooms are affected by cold argon plasma, air plasma, and plasma-activated water. Microbial load reduction, pH and acidity variations, textural characteristics like modulus of

elasticity and modulus of pressure, and overall shelf life extension are among the crucial parameters that are the focus of the investigation. Through the integration of findings from recent research, this study aims to address both scientific and practical challenges in the field and offer a comprehensive understanding of the potential of non-thermal plasma technologies in mushroom preservation.

MATERIALS AND METHODS

This study was conducted using PAW and CAP treatments on the oyster mushrooms as the experimental model. All experiments were performed according to ethical guidelines for scientific research. No human subjects or animals were involved in the study. The treatments applied, including CAP and PAW, were carried out to investigate their effects on the quality and structure of the mushrooms without causing harm to the specimens. Control groups were included in the experimental design to ensure the reliability and validity of the results. The methodology followed standard scientific practices to ensure transparency, reproducibility, and ethical compliance throughout the experiment. In this study, a control group was included to compare the effects of PAW and CAP treatments on oyster mushrooms. The control group consisted of untreated mushrooms, which were subjected to the same conditions as the treated samples (e.g., storage and handling) but without exposure to PAW or CAP treatments. This group served as a baseline to evaluate the impact of the treatments on microbial load, color changes, and other quality parameters. To guarantee consistency and dependability in the comparison, the control group was managed and examined using the same procedures as the experimental groups.

Design and Construction of Plasma-Activated Water Production Device

The device used for PAW production consists of a mass transfer absorption column with a height of 120 cm, designed to enhance plasma absorption in water. To facilitate interaction between water and plasma, trays were installed at 20 cm intervals within the column. The system includes a city water inlet positioned at the top of the column and a gas inlet connected to a compressor. The compressor, with a capacity of 1000 liters per minute, provides a steady flow of compressed air, while the water inlet ensures a continuous flow rate of 1.1 cubic meters per minute. To produce plasma-activated water, the water is treated for 20 minutes within the reactor. During this process, the generated volume of activated air is introduced into the absorption column. The untreated water, initially collected from a well at the Research Institute of Chemistry and Chemical Engineering of Iran, has an initial pH of 7.1 and was analyzed using the ICP method for detailed assessment. The reactor is equipped with two electrodes, one of which is coated with a dielectric layer to strengthen the electric field necessary for plasma generation. The voltage applied to the device ranges from 20 to 30 kV, creating a robust electric field, and the operating frequency ranges from 20 to 50 kHz, with a power output of 80 watts. Air is used as the carrier gas, entering the absorption column at a flow rate between 2 and 5 liters per minute, and the water treatment process occurs under atmospheric pressure.

PAW Performance Optimization

A turbulent washing system measuring 150 cm in length, 20 cm in width, and 20 cm in height was designed to enhance PAW application. It included 5 cm paddles to generate enough turbulence. This system is intended to increase PAW's effectiveness in lowering microbial loads. Ten kilograms of oyster mushrooms were used in the test, which was conducted at a 30° C.

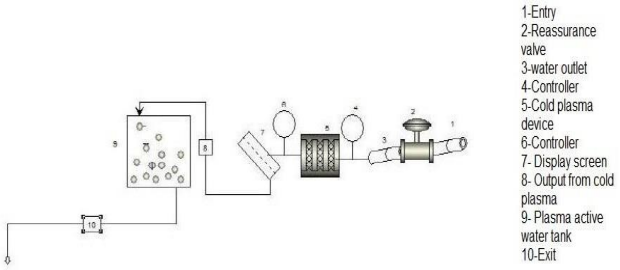

Fig. 1 Schematic diagram of PAW production system

Cold Air Plasma Device and cold Argon Plasma

The CAP device was powered by an energy source with a voltage range of 70 to 220 volts, supplied by a 1000-liter/minute compressor. The reactor consisted of two copper alloy electrodes, each 20 centimeters in length, spaced 1 centimeters apart. The gas was injected between the electrodes, generating DBD plasma. The applied electric field in the reactor facilitates the ionization of the gas, creating reactive species such as ions, electrons, and radicals that play a significant role in microbial inactivation and other applications. The CArP device was powered by an energy source with a voltage range of 70 to 220 volts. The reactor consisted of two copper alloy electrodes, each 20 centimeters in length, spaced 1 centimeter apart. The gas was injected between the electrodes, generating DBD plasma. The applied electric field in the reactor facilitates the ionization of the gas, creating reactive species such as ions, electrons, and radicals that play a significant role in microbial inactivation and other applications. The treatment time in the plasma device is crucial for optimizing the effect of the plasma treatment. Longer treatment times allow for more interaction between the plasma and the target surface, which can enhance the microbial inactivation process. However, prolonged exposure may also lead to undesirable effects on the treated material, such as degradation or damage; thus, it is important to balance treatment time with the desired outcome. For this study, plasma treatment durations of 0, 5, 10, and 20 minutes were selected based on previous studies and their effectiveness in microbial reduction.

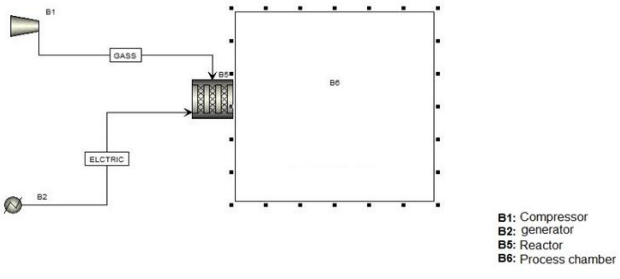

Fig. 2 Schematic diagram of CAP production system

Preparation of Samples and Microbiological Analysis

For the preparation of oyster mushroom samples, the mushrooms were carefully selected based on uniform size and similar characteristics to ensure consistency in the experiments. Each mushroom sample was cleaned to remove any external contaminants and soil particles. The average weight of each mushroom sample was approximately 20 grams. The samples were then cut into uniform pieces to ensure even exposure to both PAW and CAP. The samples were subjected to two distinct treatments: PAW and CAP. For the PAW, the mushrooms were immersed in

PAW for durations of 0, 5, 10, and 20 minutes. For the plasma-activated gas treatment, the mushrooms were exposed to plasma-activated air for the same duration. After treatment, the samples were immediately analyzed for microbial load to assess the effectiveness of the plasma treatment. For quality evaluation, three separate groups of oyster mushrooms, each with identical characteristics, were selected and treated accordingly. This ensured consistency across the trials, allowing for an accurate comparison of the effects of the treatments. To quantify microbial load, the enumeration of mesophilic aerobic bacteria, yeasts, and molds was carried out using the total count method. Appropriate culture media were selected based on the specific microbial type to ensure accurate growth. Following incubation, the number of colonies was counted, and the microbial load was calculated [13]. To assess the microbial load of samples, including aerobic mesophilic bacteria, yeast, and molds, the total count method was used. For counting aerobic mesophilic bacteria, sterile plate count agar culture medium and the pour plate culture method were used, and for counting molds and yeasts, sterile potato dextrose agar culture medium with 10% tartaric acid and the surface culture method were used [13]. Surface culture method. For counting molds and yeasts, a suspension of bacteria needs to be prepared for the surface culture method. In other words, a certain dilution of the desired bacteria must be prepared in a liquid medium, and then a certain amount of it is removed using a sampler and poured onto the surface of a sterile solid culture medium and transferred dry. Then, using a glass rod-shaped spreader with a diameter of about 3.5 mm and a length of 20 cm, and a right angle or material, it was spread evenly. In this method, for the cultivation of mold and yeast, 1000 microliters of each dilution were transferred to sterile plates with potato dextrose agar culture medium. Then, the cultured plates were placed in an incubator at 25 degrees Celsius for 2 days [14].

Search for Counting Aerobic Bacteria

To count total aerobic bacteria, it will be done according to standard number 5272. In the mixed culture method, a suspension of bacteria was first prepared. 100 microliters of the prepared suspension was poured into the bottom of a sterile plate and, 15-20 cc of culture medium was added to the plate and mixed thoroughly with Latin rotary movements. The plates cultured by the pour plate method and using the plate count agar culture medium were placed in the incubator at 3-5 degrees Celsius for 3 days. 2 observations were made from each dilution, and 3 replicates were cultured from each treatment. After incubation, the colony plates were counted. Finally, the microbial load was calculated [15].

Microbial Count

The microbial count was determined by multiplying the inoculated culture volume by the dilution factor and the number of colonies counted. Standard methods for preparing physiological serum and sterile culture media, including autoclaving, were used. These procedures were carried out to investigate the effects of CAP and PAW on the shelf life of oyster mushrooms. The equation for calculating microbial count in log CFU/ml is as follows: the number of colonies is multiplied by the dilution factor and divided by the volume of the inoculated sample [13].

$$Microbial\ count\ (Log\ CFU/ml) = \frac{Number\ of\ Colonies \times Dilution\ Factor}{Volum\ Plated\ (ml)}$$

To prepare physiological serum, 5.8 g of sodium chloride salt was added to 1000 cc of distilled water, and after dissolving, it was placed in an autoclave to sterilize. In preparing the culture media of Plate Count Agar and Potato Dextrose Agar 2, 22.5 g and 39 g of Plate Count Agar and Dextrose Agar powders were added to 1000 cc of distilled water, respectively. The culture media and the required equipment were also sterilized at 121 degrees Celsius and 15 pounds of pressure for 15 minutes using an autoclave. The physiological serum and sterilized culture media were stored in a sterile refrigerator until use. 100 cc of sterile potato dextrose agar culture medium was mixed with 1 cc of 10% tartaric acid sterilized with a 45-micron filter to create an acidic environment that would support the growth of mold and yeast while also inhibiting the growth of other microorganisms [13].

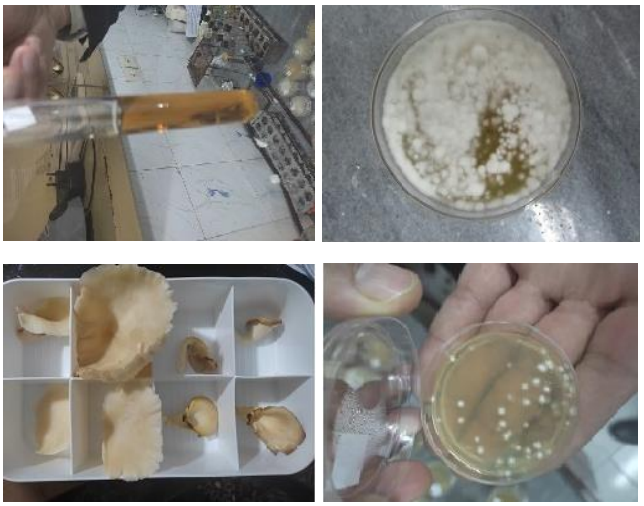

Fig. 3 a visual representation of the methodology used in this study, showcasing sample handling and microbial analysis

Microbial Inoculation

To evaluate the efficacy of PAW and CAP treatments, oyster mushrooms were inoculated with a suspension of Pseudomonas syringae pv. syringae at a concentration of 10 CFU/ml. After inoculation, the mushrooms were tested for the presence of bacteria to confirm successful inoculation. The treated and untreated samples were subsequently analyzed to determine the reduction in microbial load [16, 17].

Temperature Measurement

The surface temperature of the mushrooms was measured using a FLIR E8 thermal imaging camera. Thermal images were captured at regular intervals during the storage period from the mushroom caps. The samples were stored under controlled conditions with constant temperature and humidity for a period of 14 days. Temperature measurements were taken at the beginning and after 14 days of storage. The data obtained from the thermal imaging camera were processed, and the average surface temperature for each sample group was calculated. Additionally, temperature differences between the control and samples treated with PAW and CAP were evaluated. These measurements aimed to assess temperature variations and the effectiveness of the different treatments in maintaining the thermal stability of the product [18].

Experimental Design and Treatments

The experimental design included three groups: untreated control samples, CAP-treated samples, and PAW-treated samples. The details of the CAP and PAW treatments are described in Sections 2-2 and 2-3, respectively. After the treatments, the mushrooms were stored for up to 14 days, with measurements of color parameters (weight, pH, Emod permeability). The experimental design included three groups: untreated control samples, CAP-treated samples, and PAW-treated samples. The details of the CAP

and PAW treatments are described in Sections 2-2 and 2-3, respectively. After the treatments, the mushrooms were stored for up to 14 days, with measurements of color parameters (weight, pH, Emod permeability) taken at 0, 3, 7, and 14 days of storage to monitor changes over time [19, 20].

Statistical Method and Data Analysis

Data analysis was conducted using a completely randomized design. To assess statistical significance, a two-way ANOVA was applied, with a significance level of $p< 0.01$ or $p> 0.01$. Post-hoc mean comparisons were performed using Duncan's multiple range test. Additionally, various parameters, including wavelength calculations, energy levels, homogeneity, transition probabilities for emission lines, plasma jet temperature, gas concentration, and microbial viability, were also calculated to further analyze the data [21].

RESULTS AND DISCUSSION

The Elements of Air and Argon before and after CP Treatment, as well as the Elements of Water after CP Treatment and the Composition of Water Elements after CP Treatment

Given that cold plasma activates active species in air (air is made up of CO_2, Ar, N, and O), which leads to the creation of species, cold plasma treatment is applied to air, argon, and water in order to produce CAP, PAW, and CArP. In nitrogen, it generates, like nitrogen ion (N^+), dinitrogen ion (N_2^+), nitric oxide (NO), nitronium ion (NO_2^+), excited nitric oxide (NO*), excited oxygen (O*), dioxygen ion (O_2^+), ozone (O_3), and oxygen ion (O^+) It is activated from these elements, including carbon monoxide ion (CO^+), carbon dioxide ion (CO_2^+), excited carbon monoxide (CO), argon ion (Ar^+), and excited argon (Ar*) Cold plasma, a non-thermal technique, has emerged as a promising alternative to chemical and thermal food treatments due to its short treatment duration and use of moderate temperatures. It is a partially ionized gas formed by providing energy to a neutral gas through radio frequencies, electric fields, or microwaves, leading to ionization, excitation, and the formation of RONS. Unlike hot plasma, cold plasma operates with elevated electron temperatures (1–10 eV) while maintaining the translational energy of heavier particles near room temperature. This non-local thermodynamic equilibrium makes cold plasma energy-efficient compared to hot plasma. Its applications include shelf-life extension, food decontamination, seed germination, and food component modification, such as starches and proteins [22], and in another study, his study investigates the improvement of the physicochemical properties of coconut globulin (CG) through covalent cross-linking with tannic acid (TA) using CAP. CAP treatment shifted the interaction between CG and TA from non-covalent to covalent in a voltage-dependent manner, resulting in structural modifications of CG. The treatment enhanced the spherical structure of CG, reducing particle size from 474 to 384 nm. This size reduction was further amplified by the exposure of charged groups induced by CAP treatment. As a result, the solubility, surface hydrophobicity, and viscosity of CAP-treated CG-TA increased, leading to an elevated denaturation temperature and improved physical stability. These findings suggest a viable approach to enhancing the suboptimal physicochemical properties of plant proteins [23]. The following is a description of the chemical reaction between argon and air:

Formula (1) Plant ionization of cold plasma treatment on pastry $O_2 + e^- \rightarrow O_2^+ + 2e^-$

Formula (2) Molecular fission caused by plasma treatment on baking $O_2 + e^- \rightarrow O + O + e^-$

Formula (3) Ozone production in cold plasma treatment on flavor $O + O_2 \rightarrow O3$

Formula (4) Unit analysis due to cold plasma treatment $O_2^+ + e^- \rightarrow O + O$

Formula (5) Nitrogen ionization due to cold plasma treatment on nitrogen $N_2 + e^- \rightarrow N^{2}_{+} + 2e^-$

Formula (6) Ionized nitric oxide production process due to cold plasma treatment $N_2+ O_2 \rightarrow NO+ + NO$

Formula (7) Nitric formation process due to cold plasma treatment $N_2 + O \rightarrow NO + N$

Formula (8): Argon ionization due to cold plasma treatment $Ar + e^- \rightarrow Ar + 2e^-$

Formula (9) Excited argon production Due to cold plasma treatment $Ar+ e^- \rightarrow Ar^*$

Formula (10) Carbon dioxide ionization due to cold plasma treatment: $CO_2 + e^- \rightarrow CO_2+ + 2e^-$

Formula (11) Molecular fission of carbon dioxide $CO_2 + e- \rightarrow CO + O + e-$

Formula (12) Re-formation of carbon dioxide $CO + O \rightarrow CO_2$

Following the creation of CP, CAP was added to the water to create PAW, and the water's ability to function effectively with cold plasma present was confirmed. Untreated well water from the Iranian Institute of Chemistry and Chemical Engineering was prepared and treated to confirm the plasma's efficacy on the water. Table 1 shows the levels of P (0.16), Sr (0.51), Zn (0.30), and B (0.24) in the water prior to cold plasma treatment. The following elements were present in the water after it was treated with cold plasma: P (0.15), Sr (0.43), Zn (13), and B (0.18). The water is viable after ten minutes of cold plasma treatment, demonstrating the treatment's effectiveness and making the water's plasma transformation evident. Following the discovery that the aforementioned water possesses plasma properties, additional reactions in the water's structure, such as the production of Nitric acid (HNO_3) and the hydroxide ion (OH^-), as well as the simultaneous production of carbonic acid (H_2CO_3) and formic acid (HCOOH), and gases like ozone (O_3) and nitrite ion (NO^-) could be the cause The study investigates how to improve the effectiveness of PAW for chemical and biological decontamination by combining a liquid stirrer with a plasma-jet-activated gas bubble sparger. In the study in the PAW, through rotation, the stirrer in the study generates negative pressure, which enhances gas-liquid mass transfer and the dispersion of reactive species in plasma. This synergy increases the concentration of aqueous reactive oxygen and nitrogen species and increases the oxidative capacity of PAW. As a result, the kinetic rate constants and energy yield in the breakdown of methyl orange are enhanced. This technique offers a workable alternative to breaking down difficult chemicals without the need for chemical additives. [24] Another study looked at the effects of various cold plasmas, including O, N, air, and Ar, on the temperature, pH, and electrical conductivity of tap water under atmospheric pressure for a variety of applications. The results showed that non-homogenized water increased both electrical conductivity and temperature, while homogenized water increased both conductivity and temperature. Plasma treatments with Ar, N, air, and O resulted in the acidification of the homogenized water. Since oxygen plasma had the greatest pH reduction, it was selected to alter the acidic result. After being treated with O plasma and filtered through Ar gas, the water became more basic. Because the same reactor can yield different results, this study demonstrates that

the plasma process is versatile for a variety of applications [25]. In order to enhance the transfer of active species from plasma discharge to water, this study presents an MicroBubble-enhanced Cold Activation Plasma (MB-CAP) system. In order to maximize this transfer under different liquid flow conditions, new Venturi-type microbubble generators were created. In tests using river water, buffered saline, and pure water, the system successfully eliminated pathogens, chemical pollutants, and antibiotic residues. Additionally, MB-CPA demonstrated cytotoxic effects on cancerous cells, indicating its potential for use in biomedical and water treatment applications. This technology offers a green and sustainable way to produce PAW on a large scale. [26]. These reactions are explained as follows:

Formula (13) Oxygen ionization $O_2^+ + 2e^- \rightarrow O_2 + e^-$

Formula (14) Oxygen radical production $H^+ + OH + O_2 \rightarrow H_2O + O_2^+$

Formula (15) Oxygen cleavage $O_2 + e^- \rightarrow O + O$

Formula (16) OH production in water $H_2O + O \rightarrow OH + OH$

Formula (17)Ozone production reaction $O_2 + O \rightarrow O_3$

Formula (18) Peroxide production reaction $O_3 + H_2O \rightarrow H_2O_2 + O_2$

Formula (19) Nitrogen ionization $N^+_2 + O_2 \rightarrow N_O^+ + NO$

Formula (20) Nitric acid production reaction $NO^+ + H_2O \rightarrow H_2NO + H^+$

Formula (21) Argon ionization $Ar^+ + e^- \rightarrow Ar^*$

Formula (22) Oxy-argon production reaction $Ar^- + H_2O \rightarrow Ar + OH + H^+$

Formula (23) Carbon dioxide ionization $CO_2^+ + e^- \rightarrow CO + O + e^-$

Formula (24) Carbonic acid production $CO + H_2O + O \rightarrow H_2CO_3$

Formula (25) Hydroxyl production reaction $CO + H2O \rightarrow HCOOH$

Table 1 Atomic information for untreated water and PAW

Element (a)	Mg/l	Element	Mg/L	Element	Mg/L	Element	Mg/L
Ag	<0.01	Al	<0.01	As	<0.01	B	0.24
Ba	<0.01	Bi	<0.01	Ca	51.3	Cd	<0.01
Co	<0.01	Cr	<0.01	Cu	<0.01	Fe	<0.01
Ga	<0.01	In	<0.01	K	8.39	Li	<0.01
Mg	9.02	Mn	<0.01	Mo	<0.01	Na	145.3
Ni	<0.01	Pb	<0.01	P	0.16	Sb	<0.01
Si	7.53	Sn	<0.01	Sr	0.51	Ti	<0.01
Tl	<0.01	V	<0.01	Zn	0.30	-	-

Element (b)	Mg/l	Element	Mg/l	Element	Mg/l	Element	Mg/l
Ag	<0.01	Al	<0.01	As	<0.01	B	0.18
Ba	<0.01	Bi	<0.01	Ca	47.3	Cd	<0.01
Co	<0.01	Cr	<0.01	Cu	<0.01	Fe	<0.01
Ga	<0.01	In	<0.01	K	0.97	Li	<0.01
Mg	7.86	Mn	<0.01	Mo	<0.01	Na	131.1
Ni	<0.01	Pb	<0.01	P	0.15	Sb	<0.01
Si	5.86	Sn	<0.01	Sr	0.43	Ti	<0.01
Tl	<0.01	V	<0.01	Zn	0.13	-	-

Sample a: Water before CP Treatment
Sample b: Water after CP Treatment

Because of their interaction with the elements and compounds that are present, the active species OH, O_3, and hydrogen peroxide (H_2O_2), in the reactions produced by cold plasma, turn into more soluble or insoluble forms and either convert to gases or form complexes or sediments. The decrease in strontium levels caused by this factor may result in the formation of complexes and sediments. The oxidation-reduction process on the elements' surface can result in a change in their chemical formula when looking at another process that reduces elements. For instance, the presence of CP can oxidize zinc (ZnO) metal, turning it into ZnO. Lastly, hydration or the breakdown of compounds that can break down both organic and inorganic materials is another process that results in the reduction of elements in water. This article's explanations aimed to demonstrate the reactions that cold plasma produces in the structure of plasma-treated water, as well as its potential for reducing metals, elements, and toxins, as well as the presence of CP can influence the chemical contamination on the oyster mushroom surface in addition to lowering the microbial load in the study examines how the physicochemical characteristics of PAW generated with air, such as temperature, electrical conductivity (EC), contact angle, pH, H_2O_2, and NO_2^- levels, are affected by different exposure times (0–15 minutes). Additionally, PAW storage at 20°C for up to 20 days is examined. Reactive oxygen species (ROS) and Nitrogen species (RNS), specifically H_2O_2 and NO_2^-, are found in PAW, according to the results. These species are stable at 20°C for more than 20 days. Over time, plasma treatment lowers pH and increases water conductivity, offering important information for PAW's applications in a variety of fields [27]. For this reason, the following is a description of the chemical process that occurs in the water:

When phosphorus is exposed to HNO_3 or OH, it is likely to change into more soluble or insoluble compounds.

Formula (26) Reaction to produce insoluble Sr $Sr_2^+ + 2OH \rightarrow Sr_2(OH)^-$

Formula (27)reaction to produce ZnO or complexes with H_2O_2 $Zn_2^+ + O_3 + H_2O \rightarrow ZnO + H_2O_2$

Formula (28) Reaction to produce insoluble B_2O_3 in water $B + O_3 \rightarrow B_2O_3$

The Conductivity

The conductivity coefficient of the control water was 650 microsiemens, which decreased to 500 microsiemens after cold plasma treatment. This reduction may be due to the decrease in water salinity after cold plasma treatment, a phenomenon observed in other studies such as Bai et al., 2023, which reported similar findings regarding conductivity coefficient changes in grape water treated with cold plasma [28].

Microbiological Analysis

The total count dropped to zero when the microbial load of cold plasma was reduced by obtaining Psoudomonas syringae sub.sp.syringae, and we saw a dramatic decrease in the microbial load in 380 seconds when the mold and yeast were reduced. ($p< 0.0001$) This is because of the 13 reactions of cold plasma-activated water and the 12 reactions of air and argon gases that were shown in the CP. RONS, which has reduced the microbial load. Formula 1 illustrates how the cold plasma changes the O molecule into an active O species, which subsequently targets and eliminates the cell wall of the microbe. The complex plasma process continues by cutting the O molecule, which causes the vital protein and DNA structure of Psoudomonas syringae sub.sp.syringae to break down. At the same time, active nitrogen ions and ionized nitric oxide are produced, and nitric oxide causes the microorganism's structure to be destroyed, and it lowers the microbial load by producing carbon monoxide radicals and molecular fission of carbon monoxide. Then, in addition to lowering the microbial load, the likelihood of recontamination is significantly decreased by reproducing CO_2. Ionized and excited Ar is then used to denature the microorganism's protein and cell membrane and break down its structure. Every mechanism by which various gases activated by cold plasma reduce the microbial load was described in the written analysis. We will now explain this problem in light of the fact that water reduces the microbial load more quickly than argon plasma and air plasma methods. It is determined that these reactions are what led to the creation of chemically active compounds based on chemical formulas 1 through 13. CP in water generated hydroxyl radicals, O3, H_2O_2, HNO_3, H_2CO_3, and HCOOH. All of these substances aid in lowering the microbial load, rendering bacteria inactive, and enhancing water's antimicrobial qualities. The characteristics that come from these reactions turn water into a highly disinfecting environment. In the study on the spinach CP treatment successfully decreased the microbial load on spinach leaves in the study, showing great promise for extending shelf life and lowering post-harvest losses. This technique preserved quality characteristics like color and Total phenolic content (TPC) while achieving a noticeable reduction in the microbial count. The effectiveness of cold plasma in eliminating pesticide residue further demonstrated its benefits over more conventional methods such as hypochlorite wash [29].and in the another study on the Pseudomonas fluorescens and Pseudomonas putida By producing reactive species (RS) such as O_3, H_2O_2, and nitric oxide, cold atmospheric plasma (CAP) demonstrated potent antibacterial activity against Pseudomonas fluorescens and Pseudomonas putida. These RSs damaged cell membranes, deactivated enzymes (like malic dehydrogenase), and harmed bacterial DNA. CAP has the potential to be a novel preservation method for reducing microbial load and extending food shelf life, as evidenced by preservation tests that showed it significantly reduced microbial counts in red shrimp paste during refrigeration [30]. As illustrated in the figure. 3, it is evident that the effectiveness of three treatments, CArP and PAW, in reducing the Pseudomonas syringae bacterial load was investigated at various time points. The initial bacterial load of 5.925 in all samples at the start of the experiment (0 seconds) served as the baseline for comparison. The bacterial load in the air-treated sample decreased by 21.12% to 4.665 (Fig. 4) after 70 seconds of exposure. ($p <0.0001$) The bacterial load decreased less with argon exposure, reaching 5.441 (8.2% reduction.($p <0.0001$) Similarly, PAW-treated samples showed a bacterial load of 4.05 (Fig. 4), which was similar to argon (32% reduction). ($p< 0.0001$). At 180 seconds, more reductions were observed. The bacterial load decreased to 4.455 (a reduction of 24.8%) in argon-treated samples and to 3.88 (a reduction of 34.5%) in air-treated samples.($p< 0.0001$) In comparison to samples treated with air and argon, samples treated with PAW exhibited a more moderate 49% reduction in bacterial load, with a bacterial load of 3.0.($p< 0.0001$) The bacterial load in the air-treated samples decreased to 2.77 (a 53.3% reduction) after 280 seconds, whereas the samples exposed to argon showed a load of 2.975 (a 49.8% reduction ($p < 0.0001$). A 74% decrease, or 1.57, was the outcome of the PAW treatment.The most notable reduction in bacterial load attained by air treatment was observed at the end of the 380-second interval, when the bacterial load dropped to 0.95 (84% reduction). ($p< 0.0001$) Argon-treated samples had a bacterial load of 1.519 (a reduction of 74.3%), whereas PAW-treated samples had a load of 0.15 (a reduction of 99%). ($p< 0.0001$). These findings demonstrate that air treatment with cold plasma was the most effective method of reducing the bacterial load because oxygen has oxidative qualities. In the study on the effects of cleaning conditions, PAW was found to be less effective than argon, despite its well-known disinfecting qualities, in the experimental setup. The microbes that PAW efficiently eliminates from wounds include Staphylococcus aureus, Candida albicans, and polymicrobials. By modifying the gas flow rate and exposure duration, this technology has optimized the Decimal reduction time (D-value) for pathogens. PAW is advised for medical applications and wound healing since the results demonstrated that polymicrobials had the best microbial reduction. [31] and for further study on the effect of cold plasma treatment absorb. The microbial load of raw potato slices was considerably decreased after pretreatment with CAP over a 14-day storage period. Mesophilic, psychrophilic, and fungal microorganisms were significantly reduced after a 15-minute exposure to cold plasma. These findings show that CAP is a useful tool for enhancing the hygienic quality of potato products. [32] and researchers looked into how the combined effects of UV-C and Pulsed electric field (PEF) radiation affected the composition of crude protein extracts from fish gills and the reduction of microbial load. The findings demonstrated that longer duration and high-intensity PEF successfully decreased the microbial load. Additionally, the microbial load was further reduced by the combination of PEF and UV-C. The potential for enhancing food quality and microbiological safety is substantial with these non-thermal technologies [33]. In the study, the a Cold atmospheric pressure plasma jet (CAPJ) was successful in lowering the microbial load of bacteria that are resistant to drugs, like S. aureus and E. coli. After 180 seconds of exposure to CAPJ, E. Coli decreased by 5 logarithmic units, while S. aureus decreased by 3.4-4.6 logarithmic units. Surface modifications and gaseous species like RONS are involved in lowering the microbial load, according to the study. [34] Sterilizing the fungus Diutina catenulata with plasma that contained argon gas, air, and an argon-air mixture worked well. It has been established that plasma has great potential for food processing and safety [35].

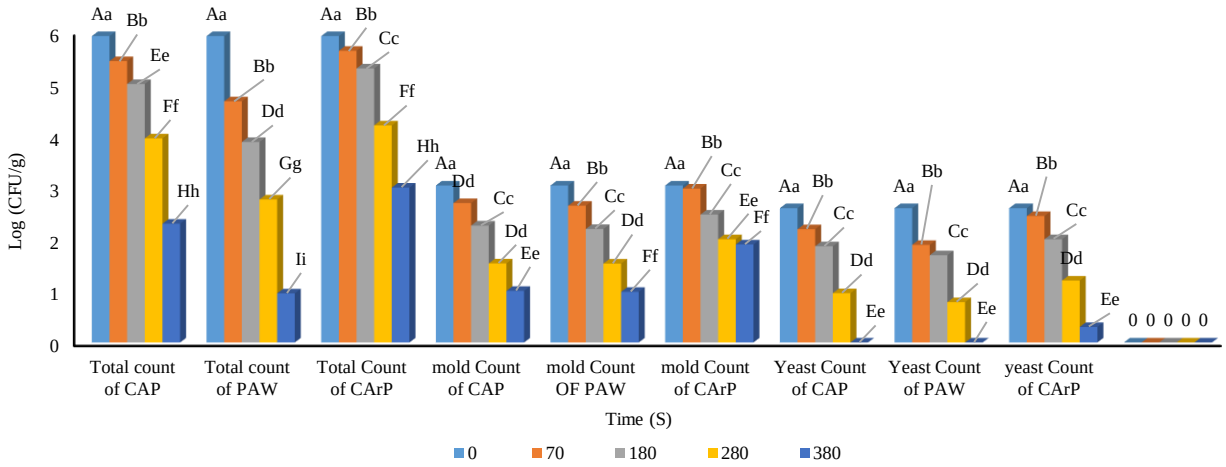

Fig. 4 Changes in the total number of micro organisms in oyster mushrooms treated with CAP and PAW during storage, as well as changes in the number of mold and yeast microorganisms

Table 2 ANOVA for the Total count of oyster mushrooms treated with PAW and CAP and CArP

Source	The sum of squares (SS)	df	Mean square (MS)	F	Sig
Treatment	5162.018	51	197.672	-	-
Time	860.878	1	850	32.071	0.0001
Treatmnt× time	4301.140	50	165.507		

Table 3 ANOVA for oyster mushroom mold count after PAW and CAP And CArP treatments

Source	Sum of squares (SS)	df	Mean square (MS)	F	Sig
Total	5162.018	51	199.122	-	-
Time	860.878	1	560	29.041	0.00
Residual	4301.140	50	166.202		01

Table 4 ANOVA for oyster mushrooms treated with PAW and CAP and CArP in terms of their yeast count

Source	The sum of squares (SS)	df	Mean square (MS)	F	Sig
Total	28.37	51	201.369	-	-
Time	17.54	1	17.54	30.22	0.0005
Residual	10.83	50	155.549		

Percentage of Weight Loss

Over the 14 days, the weight loss of oyster mushrooms after various treatments, CAP, CArP, PAW, and the control sample, is shown in the chart (Fig. 4). Among the significant discoveries are the following:

The Two-Chart Displays yellow PAW Bars

PAW shows the least amount of weight loss during the day 14. This implies that it is successful in maintaining the structural integrity of the oyster mushroom, most likely due to its antimicrobial and oxidative properties, which reduce water loss and microbially induced decay.

Orange Bars for CAP are Displayed

Moderate weight loss results from the CAP treatment. Although it helps delay weight loss, its performance is worse than that of the control because it is less reactive than PAW.

Blue Bars for CArP are Displayed

The weight reduction in samples treated with CArP is less than that of the control but greater than that of PAW. Perhaps because CArP can create an environment that is comparable to anaerobic conditions, the treatment slows down the degradation process even though it has inert properties.

Gray Bars are Used as the Control Sample

Over the 14 days, the control sample loses the most weight. This result demonstrates how microbial growth and natural decay impact untreated samples.

The best method for maintaining the weight of oyster mushrooms is plasma-activated water, which is followed by argon and air treatments. These results show how cutting-edge plasma-based treatments can increase fresh produce's shelf life. The optimization of plasma-activated water's use for a wider range of food preservation applications may be the main goal of future research projects. In actuality, cold plasma absorption was demonstrated in the study to lessen the weight loss of mandarins during storage without appreciably altering other quality parameters like pH, soluble solids, or CO_2 generation. CPT was identified in the study as a promising postharvest technology for enhancing fruit quality and storability [36]. In the study on the PAW, by delaying moisture loss and microbial growth on the fruit's surface, PAW treatment successfully decreased weight loss in goji berries during storage while preserving quality characteristics like texture and bioactive compounds [37]. Surface dielectric barrier discharge plasma treatment, particularly with H_2O_2 vapor and air for 180 seconds, effectively reduced weight loss in button mushrooms during a 21-day storage period at 4 °C by preserving stiffness, color, and moisture content [38].

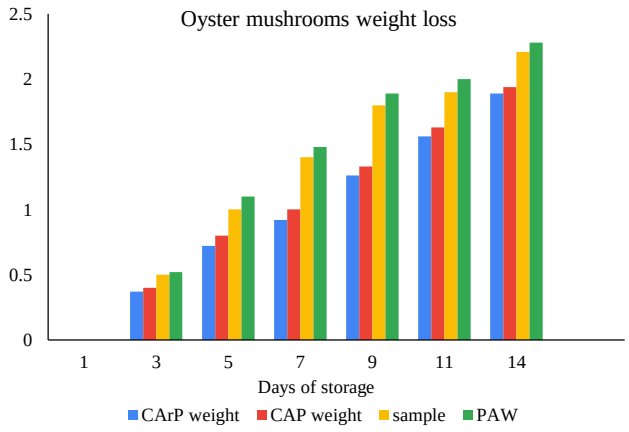

Fig. 5 Changes in weight in the oyster mushrooms treated with CAP and PAW and CArP during storage

Table 5 ANOVA for oyster mushrooms treated with PAW and CAP and CArP in terms of weight in the oyster mushrooms

Source	The sum of squares (SS)	df	Mean square (MS)	F	Sig
Total	28.37	51	211.309		
Time	17.54	1	15.64	54.22	0.0005
Residual	10.83	50	0.999		

The impact of Acidity (%)

The chart shows (Figure 6) a smaller drop in acidity, indicating that these processes have a milder effect on pH.

Compared to the other treatments, PAW has a higher acidity. This change is linked to the interaction of plasma-activated species with water, which results in the formation of weak acids like formic acid, HCOOH, and HCO_3 (formulas 24 and 25). Higher acidity levels result from this, as the chart makes evident. Overall, the chart's variations in acidity levels illustrate the unique impacts of the active species produced in each treatment on chemical reactions and sample pH variations. These differences are statistically significant ($p< 0.0001$), showing how well each treatment modifies the acidity of the samples. In the review on the impacts of cold plasma treatment on the foods, it emphasizes how the non-thermal process of cold plasma processing causes notable changes in food characteristics, such as adjustments to pH. When plasma reactive species interact with food ingredients, they can affect pH, bioactive compounds, and enzymes in both positive and negative ways. These modifications highlight cold plasma's potential as a cutting-edge technique to alter the acidity and quality of food. [39] in the other study demonstrated that plasma activation time has a significant impact on the pH value of PAW, underscoring the influence of PAW's physicochemical characteristics on its functionality. This is especially important for applications where acidic conditions are crucial, like microbial inactivation and quality preservation. [40].

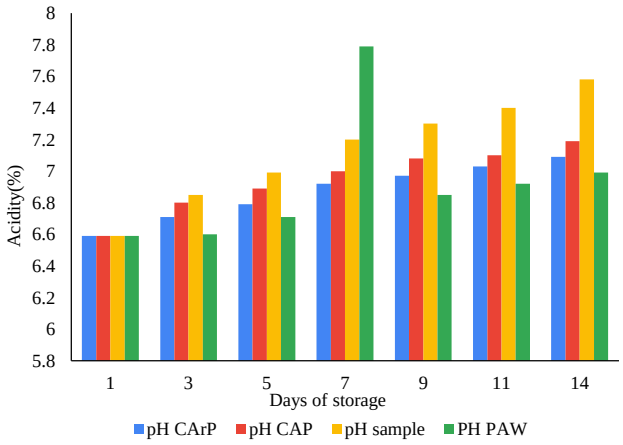

Fig. 6 Acidity variations in oyster mushrooms treated with CAP, PAW, and CArP over time

Table 6 ANOVA for oyster mushrooms treated with PAW and CAP and CArP in terms of Acidity

Source	The sum of squares (SS)	df	Mean square (MS)	F	Sig
Total	28.37	35	186.789	-	-
Time	17.54	1	20.54	62.22	0.0005
Residual	10.83	34	1.0158		

The ability of argon plasma to effectively maintain the structural integrity of mushrooms while still regulating microbial growth may have been hampered by its lower oxidative capacity, which generates fewer reactive species than CAP. In summary, the

Mushroom Elasticity and Texture

Over the course of the 14-day storage period, there were notable variations in the modulus of elasticity (Emod) (Fig. 7 and 8), which measures the firmness and structural integrity of oyster mushrooms, between the treatments (CArP, CAP, PAW, and control) ($p< 0.0001$). Starting at 0.12 GPa on Day 1 and progressively dropping to 0.12 GPa by Day 14, the PAW-treated samples maintained the highest Emod values throughout the experiment, as the graph illustrates. This demonstrates how well PAW treatment preserved firmness, as evidenced by the 29.4% decrease in Emod over the storage period. RONS, such as OH radicals and H_2O_2, were present in PAW and probably helped to inhibit microbes while reducing structural deterioration.On the other hand, the Emod values of the untreated control samples were somewhat lower than those of PAW, ranging from 0.14 GPa on Day 1 to 0.12 GPa by Day 14. The Emod of the control samples decreased by 14.3%, mostly as a result of enzymatic activity and inherent moisture loss during storage. This implies that the untreated samples initially retained their structural firmness in the absence of microbial control, but by the end of storage, they were comparable to mushrooms treated with PAW. Samples treated with CAP showed intermediate Emod values, settling between 0.09 GPa and 0.06 GPa over the days 14. By Day 14, the CAP treatment, which produces RONS like NO, O_3, and OH, caused structural alterations and a 33.3% decrease in firmness when compared to untreated mushrooms. Despite CAP's efficient microbial inhibition, which extended storage quality, this reduction is suggestive of oxidative effects on the cellular structure. Samples treated with CArP had the lowest Emod values at all time points, ranging from 0.07 GPa to 0.05 GPa. By Day 14, the Emod of CARP-treated mushrooms was up to 58.8% lower than that of PAW-treated samples.

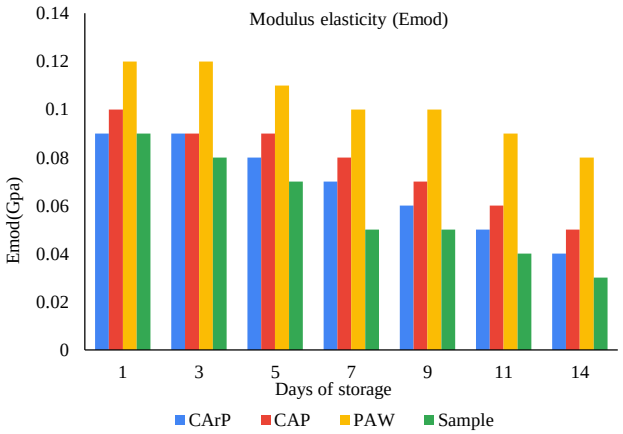

Fig 7 Elasticity variations in oyster mushrooms treated with CAP, PAW, and CArP over time

Table 7 ANOVA for oyster mushrooms treated with PAW and CAP and CArP in terms of 4 Elasticity

Source	The sum of Squares (SS)	df	Mean square (MS)	F	Sig
Total	28.37	35	191.369	-	-
Time	17.54	1	18.43	61.21	0.0005
Residual	10.83	34	0.518		

updated graph highlights how well the PAW treatment performs in maintaining the modulus of elasticity, with the control samples, CAP, and CArP treatments coming in second and third, respectively. These results demonstrate the dual function of plasma treatments in both structural preservation and microbial

control, with PAW being the most successful in preserving the oyster mushrooms' textural characteristics over time. In the study on the storage stability of mushrooms, by lowering the microbial load (16.5%), preserving protein and vitamin C content, and reducing browning (26.9%), cold plasma treatment greatly enhanced the postharvest quality of Agaricus bisporus. The best treatment parameters, which showed promise for extending shelf life, were 95 kV voltage, 130 Hz frequency, and 10 minutes processing time. [41] The author of another study on button mushroom postharvest quality finds that PAW soaking successfully decreased Agaricus bisporus microbial counts over seven days of storage at 20°C (0.5 log for fungi and 1.5 log for bacteria). Without appreciably altering color, pH, or antioxidant qualities, it maintained firmness, respiration rate, and electrical conductivity, delaying softening and making it a viable postharvest preservation technique. [54] It is evident from data from earlier investigations that the findings of the oyster mushroom study are consistent with those of earlier studies.

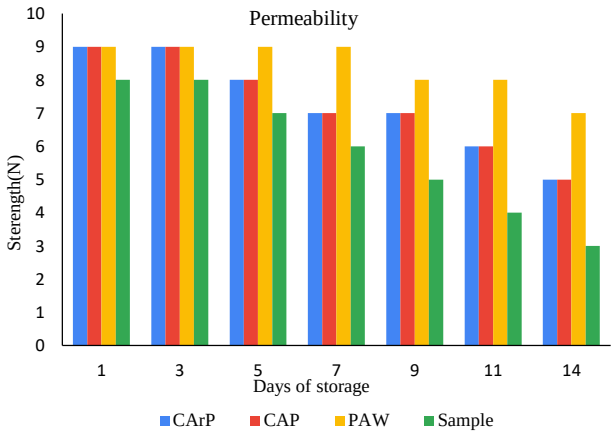

Fig. 8 Comparing the control sample, samples treated with CAP, CArP, PAW, and Sample to examine the permeability of oyster mushroom surfaces

Table 8 ANOVA for oyster mushrooms treated with PAW and CAP and CArP in terms of permeability

Source	The sum of squares (SS)	df	Mean square (MS)	F	Sig
Total	28.37	51	190.032	-	-
Time	17.54	1	18.04	56.12	0.0005
Residual	10.83	50	0.719		

CONCLUSION

This study examined how the shelf life of oyster mushrooms was affected by three innovative preservation techniques: cold plasma, CAP, and PAW. According to the findings, all three techniques successfully decreased the microbial load, preserved the product's chemical and physical characteristics, and considerably extended the mushrooms' shelf life. Nonetheless, the PAW technique proved to be the most successful in lowering the microbial load while preserving the mushrooms' general quality. Reactive RONS, which were produced as a result of the use of PAW, produced a disinfecting environment and successfully decreased the microbial load. Additionally, this technique improved the texture and preserved moisture in the mushrooms while having minimal effects on pH, which is in line with earlier research findings [8, 44]. Additionally, RONS's unique properties, like its ability to produce ozone and hydrogen peroxide, are responsible for this method's higher efficacy when compared to CAP and CArP [25, 45, 27]. Applying cold plasma technology to other fresh products, like fruits and button mushrooms, has also demonstrated that it can lower microbial load and extend shelf life while preserving the products' nutritional value and sensory appeal, according to related research [41, 42]. Because of its high efficiency and minimal environmental impact, PAW is presented as a sustainable and eco-friendly substitute for chemical and thermal methods [8, 30]. The study's overall findings demonstrate the great potential of cold plasma technology, especially PAW, in the food industry to reduce post-harvest waste, improve product quality, and meet consumer demand for minimally processed foods [4, 5]. Subsequent investigations may concentrate on refining this technology and expanding its uses for a greater variety of food items [43, 8].

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