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ESCOLA SUPERIOR DE BIOTECNOLOGIA
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QUALITY ASSESSMENT AND ANTI-LISTERIAL EFFICACY OF COLD PLASMA TREATMENT ON FRESH LETTUCE

by
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by
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Abstract

This work evaluates the potential of cold plasma technology as a decontamination method for fresh lettuce. Specifically, it investigates the effectiveness of cold plasma in reducing *Listeria innocua* (a non-pathogenic surrogate for *L. monocytogenes*) inoculated onto lettuce leaves, while simultaneously assessing its impact on quality attributes, including color (total color difference in relation to fresh samples, chroma, and browning index), pH, chlorophylls (*a, b*, and total), and total phenolic content. A conventional hot water treatment (blanching at 80 °C) was used as reference. By examining both microbial inactivation efficiency and preservation of physicochemical properties, the study aims to determine whether cold plasma can serve as a reliable approach for ensuring lettuce decontamination without compromising quality.

Cold plasma treatments (gliding arc discharge) with exposure times of 5, 10, 15, 20, 25, and 30 s were compared with thermal treatments of equal duration. pH values remained relatively stable (5.6–6.0), though thermal samples were more uniform, while plasma samples showed slightly greater variability. Color analysis showed treatment-dependent effects: plasma preserved color and vividness more effectively (higher chroma values), while thermal treatment better limited browning.

Chlorophyll *a* remained relatively stable under both treatments, with no significant differences across most time points. Chlorophyll *b* was more sensitive: at 5 and 30 s, plasma-treated samples retained significantly higher levels than thermal, suggesting superior preservation of pigments. Total phenolic content also differed markedly between treatments. Plasma-treated lettuce maintained or slightly increased phenolic levels over time (≈14–15 mg GAE/g FW), while thermal treatment caused significant reductions from 5 s onward, with final values more than two-fold lower than plasma.

Regarding microbial inactivation, thermal treatment completely eliminated *L. innocua* within 5 s (~7-log reduction), while cold plasma produced a slower decline, achieving only ~2-log reduction after 30 s. Despite its lower inactivation efficiency, cold plasma better preserved color, chlorophyll *b*, total chlorophyll, and phenolic content compared to thermal processing. Therefore, while thermal treatment ensures decontamination, cold plasma represents a promising non-thermal preservation strategy for lettuce with low microbial loads, provided its antimicrobial efficacy can be further optimized without compromising quality and freshness.

Keywords: Non-thermal treatments. Blanching. Vegetables. Decontamination. *L. innocua*.

Resumo

Este estudo avalia o potencial da tecnologia de plasma frio como método de descontaminação para alface fresca. É investigada a eficácia do plasma na redução de *Listeria innocua* (substituto não patogénico de *L. monocytogenes*) inoculada em folhas de alface, avaliando-se o impacto em características de qualidade, incluindo cor (diferença total de cor, croma e índice de escurecimento), pH, clorofilas (*a*, *b* e total) e teor fenólico total. Um tratamento convencional com água quente (branqueamento a 80 °C) foi utilizado como referência. Ao examinar tanto a eficiência da inativação microbiana quanto a preservação das propriedades físico-químicas, este estudo pretende determinar se o plasma frio pode descontaminar alface sem comprometer a sua qualidade.

Tratamentos com plasma frio (descarga de arco deslizante), com tempos de exposição de 5, 10, 15, 20, 25 e 30 s, foram comparados com tratamentos térmicos de igual duração. Os valores de pH mantiveram-se estáveis (5,6–6,0), embora as amostras submetidas a tratamento térmico tenham apresentado maior uniformidade, enquanto as amostras tratadas com plasma revelaram uma variabilidade superior. A análise da cor revelou efeitos dependentes do tratamento: o plasma preservou melhor a cor e vivacidade (croma mais elevado), enquanto o tratamento térmico limitou o escurecimento.

A clorofila *a* manteve-se relativamente estável com ambos os tratamentos, sem diferenças significativas ao longo do tempo. A clorofila *b* foi mais sensível: aos 5 e 30 s, as amostras com plasma mantiveram níveis significativamente mais elevados, sugerindo melhor preservação dos pigmentos. O teor fenólico total também diferiu entre tratamentos. Alface submetida a plasma manteve ou aumentou ligeiramente os níveis fenólicos (≈14–15 mg GAE/g FW), enquanto o tratamento térmico causou reduções significativas desde 5 s, com valores finais mais de duas vezes inferiores aos do plasma.

Relativamente à inativação microbiana, o tratamento térmico eliminou completamente a *L. innocua* em 5 s (~7-log de redução), enquanto o plasma frio provocou um declínio mais lento, alcançando apenas ~2-log de redução após 30 s. Apesar da sua menor eficiência de inativação, o plasma frio preservou melhor a cor, a clorofila *b,* a clorofila total e o teor de compostos fenólicos em comparação com o processamento térmico. Assim, enquanto o tratamento térmico assegura a descontaminação, o plasma frio representa uma estratégia de conservação não térmica promissora para alface com baixas cargas microbianas, desde que a sua eficácia antimicrobiana possa ser otimizada sem comprometer a qualidade e a frescura.

Palavras-chave: Tratamentos não térmicos. Branqueamento. Vegetais. Descontaminação. *L. innocua.*

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Ekin Ersoylu

Table of Contents

List of Figures

List of Tables

List of Abbreviations

APPJ – atmospheric pressure plasma jet

BI – browning index

CAP – cold atmospheric plasma

CFU – colony forming unit

CIE – Commission Internationale de l'Éclairage

DBD – dielectric barrier discharge

DNA – deoxyribonucleic acid

FW – fresh weight

GAE – gallic acid equivalent

IQR – interquartile range

PAW – plasma-activated water

ref – reference value

ROS – reactive oxygen species

RNS – reactive nitrogen species

TCD – total color difference

TPC – total phenolic content

TSAYE – tryptic soy agar with yeast extract

TSBYE – tryptic soy broth with yeast extract

UV – ultraviolet radiation

1. Introduction

Fresh produce, such as vegetables, plays a crucial role in human nutrition because of their high content of vitamins, minerals, and bioactive compounds. However, the perishable nature of these products makes them highly susceptible to spoilage and microbial contamination, which is a major contributor to food waste. Factors such as high moisture levels, elevated temperatures, exposure to air, and improper storage conditions accelerate deterioration. Over time, spoilage becomes evident through discoloration, tissue breakdown, loss of texture, exudation, and the development of off-flavors (Ponce et al., 2002).

Various mild processing techniques can be applied to extend the shelf life of vegetables and ensure their safety while maintaining a fresh appearance, which is critical for consumer acceptance. Preserving freshness is particularly important for vegetables consumed raw, where visual quality, crisp texture, and natural flavor play a decisive role in marketability and consumer preference. Washing vegetables in decontamination solutions (acidic agents, hypochlorite, or peroxide) is a widely used approach (Ramos et al., 2013). In recent years, however, several mild, non-thermal technologies, including modified atmosphere packaging, pulsed light, ultraviolet (UV-C) treatment, cold plasma, high-pressure processing, and ozone treatment, have also been investigated as pre-treatment strategies for fresh-cut produce (Ramos et al., 2013; Alexandre et al., 2016). These approaches, by avoiding heat, aim to inactivate microorganisms and delay spoilage while minimizing undesirable changes in sensory attributes and nutritional value. Among them, cold plasma has emerged as a particularly promising technology, designed to preserve the natural qualities of fresh produce, reduce economic losses, and ensure food safety (Lee at al., 2006; Lee et al., 2015).

1.1 Plasma and its Properties

Plasma, conventionally regarded as the fourth state of matter, is a partially or fully ionized gas distinct from solids, liquids, and gases (Rahman et al., 2022). Its generation follows the progressive addition of energy to matter (Figure 1): solids melt into liquids, liquids vaporize into gases, and further energy input to gases leads to ionization, producing plasma (Rabinovich et al., 2022).

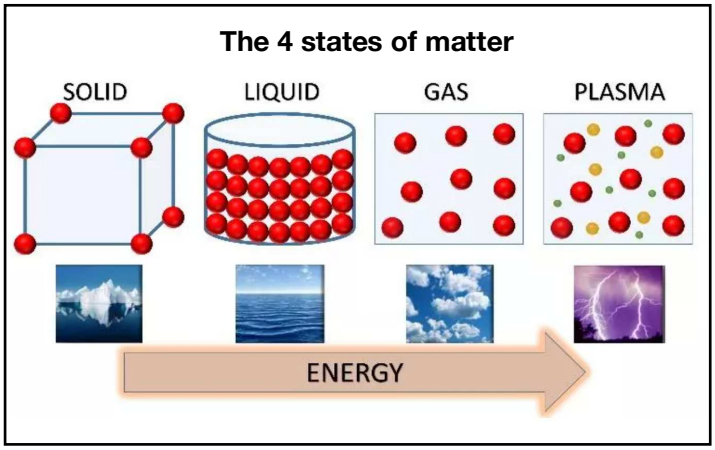

Figure 1. The solid, liquid, gas, and plasma states of matter (adapted from science.lu, 2020).

Plasma exhibits electrical conductivity and responsiveness to electromagnetic fields and is composed of particles in continuous interaction, including photons, electrons, ions, atoms, free radicals, and molecules in both excited and non-excited states (Critzer et al., 2007).

1.2 Classification of Plasma

Plasma can be broadly classified into high-temperature plasma and low-temperature plasma (Tolouie et al 2018), depending on the thermal equilibrium between electrons and heavier gas particles (Li et al., 1996).

High-temperature plasma

High-temperature plasma, with gas temperatures exceeding 10^6 K, dominates the universe and is found in stellar environments, nuclear blasts, and controlled fusion reactions (Li et al., 1996). Examples on Earth include lamp discharges, electric arcs, and other high-power discharges.

Low-temperature plasma

Low-temperature plasma, also known as cold plasma or non-thermal plasma, is a type of plasma characterized by low bulk gas temperatures, typically ranging from 30 °C to 60 °C and is commonly generated through glow discharges at low pressures (Li et al., 1996). Unlike thermal plasma systems, this plasma can operate near atmospheric pressure under non-equilibrium conditions, where electrons attain high energies and generate reactive species while the overall

gas temperature remains close to ambient (Bangar et al., 2022; Tolouie et al., 2018; Xu et al., 2017).

As a dry, non-thermal process, cold plasma is produced using electricity and a carrier gas such as air, nitrogen, argon, or oxygen (Deng et al., 2008; Laroussi, 1996; Perni et al., 2008). Its low-temperature, non-equilibrium nature enables effective microbial inactivation without causing thermal damage, preserving the sensory and nutritional quality of treated food products (Sasikumar et al., 2025).

1.3 Production of Plasma

Plasma can be generated through high temperatures, strong electrical or magnetic fields, or intense electrical discharges. When high-voltage energy interacts with gas molecules, free electrons are released and collide with atoms and molecules, producing ions, radicals, photons, and other reactive species (Dinç, 2025; Tusek et al., 2001; Niemira, 2012a). The dominant mechanism is electron impact ionization, in which the kinetic energy of colliding electrons exceeds the ionization potential of atoms or molecules. Additional ionization pathways include heavy-particle collisions and photoionization by high-energy photons (Sasikumar et al., 2025). Recombination processes, where electrons rejoin ions, release energy in the form of heat, light, or chemical energy, thereby sustaining the plasma state (Boulos et al., 2023).

Plasma can be further categorized by generator type (radio frequency, low frequency, microwave), discharge type (e.g., Gliding Arc, Corona Discharge, Dielectric Barrier Discharge), or operating pressure (low vs. atmospheric) (Dinç, 2025).

Figure 2 illustrates a gliding arc cold plasma discharge, similar to the system employed in this study (Birania et al., 2022).

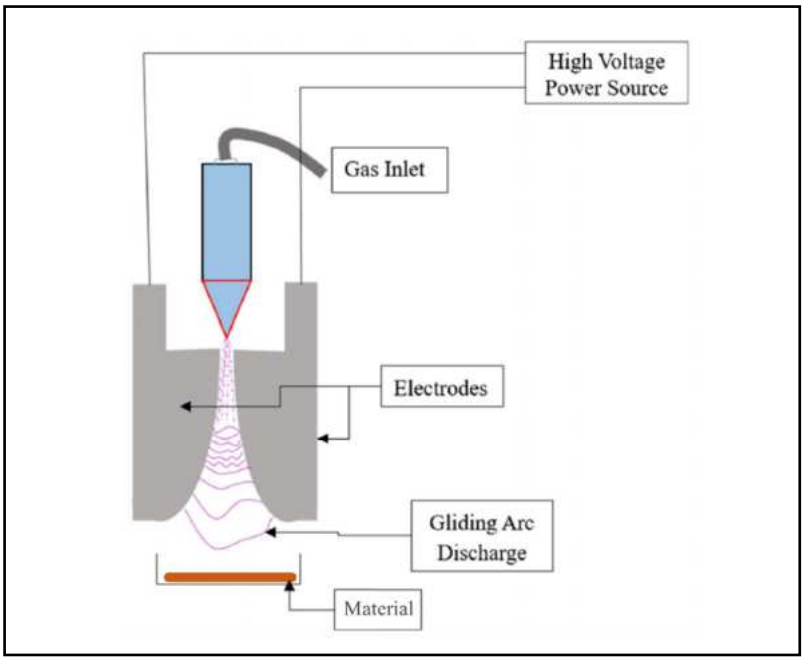

Figure 2. Gliding arc cold plasma discharge (Birania et al., 2022).

1.4 The Effect of Plasma on Microorganisms

The antimicrobial efficacy of plasma arises from a combination of synergistic mechanisms, including the generation of UV radiation, ozone, charged particles, reactive oxygen species (ROS), and reactive nitrogen species (RNS) (Laroussi & Leipold, 2003). These agents cause structural and functional damage to microbial cells, targeting membranes, proteins, and DNA (Fernández et al., 2013).

Several inactivation mechanisms have been proposed, including physicochemical interactions of plasma-derived species, ROS and RNS activity, and cell membrane permeabilization (Hou et al., 2008; Bermúdez-Aguirre & Corradini, 2012; Niemira, 2012a). Among these, hydroxyl (OH) radicals are particularly potent: they initiate oxidative reactions that disrupt microbial membranes, oxidize amino acid residues and unsaturated fatty acids, form peroxide radicals, and even cleave peptide bonds leading to severe damage of cellular structures and ultimately cell death (Surowsky et al., 2014).

Overall, the antimicrobial activity of plasma is mainly attributed to ROS and RNS, including O, O_2, O_3, NO, and NO_2. Hydroxyl radicals are considered the most effective in microbial

inactivation. Importantly, these reactive species dissipate within milliseconds, leaving no harmful residues and posing no risks to human health (Gürol et al., 2012).

The effectiveness of cold plasma depends strongly on treatment parameters such as the feed gas composition, the configuration of the plasma generation system, and the characteristics of the treated commodity. Food matrix properties, such as water activity, texture, and protein or fat content, also modulate microbial inactivation efficiency and influence product quality (Niemira, 2012a).

1.5 Plasma Applications

Plasma technology has a wide range of applications across materials science and food technology. In materials science, plasma is employed for surface treatments, thin-film deposition, ion implantation, and coating applications, enhancing the properties of composites, metals, ceramics, and functional materials such as diamond or superconductive oxides (Chen et al., 1993a, 1993b; Klemberg-Sapieha et al., 1993; Liston, 1993; Mittal et al., 1994; Clyne & Roberts, 1995; Mamalis et al., 1995; Zhang et al., 1995).

Cold plasma can rapidly decontaminate surfaces, including polymers used in packaging, without compromising structural integrity, and can form thin, homogeneous coatings without leaving chemical residues (Yasuda, 1984; Niemira, 2012a; Pankaj et al., 2014). Compared to conventional chemical or thermal treatments, it avoids extensive preparation, reduces chemical waste, and lowers energy consumption (Yangılar & Oğuzhan, 2013).

Antimicrobial potential in fresh produce

Within the food domain, cold plasma is increasingly recognized as a sustainable and environmentally friendly alternative to conventional sanitization methods.Operating at bulk gas temperatures of 30–60 °C, it effectively inactivates human pathogens on fresh produce, meats, dairy products, ready-to-eat foods, and packaging while preserving sensory, physical, and nutritional quality without significant heat generation (Misra et al., 2011; Niemira, 2012a; Fernández et al., 2013; Zhang et al., 2013; Dey et al., 2016; Bourke et al., 2017; Saremnezhad et al., 2021).

As previously presented, the antimicrobial efficacy of cold plasma is largely attributed to the synergistic action of reactive oxygen and nitrogen species, UV photons, and charged particles, which collectively induce oxidative damage to key cellular structures.

Cold plasma has been shown to reduce *L. monocytogenes*, *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungal contaminants such as *Aspergillus* and *Penicillium*, demonstrating broad-spectrum activity (Bermúdez-Aguirre & Corradini, 2012; Niemira, 2012b; Fernández et al., 2013; Surowsky et al., 2014; Hertwig et al., 2015; Hertrich et al., 2017). Efficacy depends on factors such as plasma type, exposure time, feed gas composition, and target surface characteristics.

Leafy fresh vegetables are highly perishable and prone to microbial contamination, making them a critical focus for decontamination strategies. Lettuce, in particular, serves as an ideal model for evaluating cold plasma treatments due to its widespread consumption, raw consumption, short shelf life, and nutritional importance (Singh et al., 2024). Different lettuce types vary in nutrient content: cutting types (also called loose-leaf or leaf lettuce) are rich in vitamin A and β-carotene, cos types (also called Romaine lettuce) have higher vitamin C and folate, and all types provide vitamin K and moderate levels of iron (Still, 2007; Singh et al., 2024). Its susceptibility to microbial contamination and consumption in minimally processed forms make it particularly relevant for non-thermal surface decontamination studies.

Several studies have confirmed the effectiveness of cold plasma for lettuce decontamination. Niemira (2012a) reported that cold plasma treatment could achieve a 3–4 log reduction of *L. monocytogenes* on fresh produce with minimal impact on product quality. Similarly, Ziuzina et al. (2014) demonstrated effective inactivation of *Listeria* on lettuce; however, achieving substantial reductions of bacteria attached to lettuce surfaces required extended treatment times of up to 300 seconds. Specifically, reductions of 2.4, 2.3, and 3.3 $\log_{10}$ CFU/sample were observed for *Salmonella*, *L. monocytogenes*, and *E. coli*, respectively. The study further explored the effects of plasma afterglow on these pathogens in various forms, including planktonic cultures, biofilms formed on lettuce surfaces, and bacteria internalized within lettuce tissue, highlighting the challenges of inactivating bacteria in complex matrices.

For *S. Typhimurium* on lettuce, a 15-minute treatment was required to achieve a 2.72 $\log_{10}$ reduction in viability (Fernández et al., 2013). The differing efficiency of cold plasma across various types of fresh produce is likely attributable to differences in surface characteristics, which can influence microbial attachment and exposure to reactive plasma species.

Hertrich et al. (2017) reported a modest reduction of 0.35 log CFU/g in *Salmonella* levels on lettuce when it was prepackaged in mixed salads containing tomato.

Table 1 summarizes recent research on cold plasma applications to lettuce and other leafy greens, highlighting key achievements, including reductions in pathogenic and spoilage microorganisms. While treatment efficacy varies depending on plasma type, exposure time, and gas composition, these studies collectively demonstrate the potential of cold plasma as a non-thermal decontamination strategy for fresh lettuce.

Table 1. Recent advances in cold plasma treatments for microbial reduction in lettuce

Reference	**Product**	**Plasma type & key conditions**	**Microbiological impact**
Özdemir et al. (2023)	Fresh green leafy vegetables (lettuce, spinach, arugula, etc.)	Various: DBD, APPJ, corona, plasma jets — multiple gases and parameters reviewed	Reports multiple studies with >2 log reductions for some conditions; efficacy strongly parameter-dependent
Supakitthanakorn et al. (2023)	Lettuce (disease control)	Atmospheric-pressure DBD plasma	Reported inhibitory effect on fungal pathogen (*Athenia rolfsii*) causing southern blight in lettuce
Han et al. (2024)	Romaine lettuce	Pilot-scale Plasma-Activated Water (PAW) treatment	~ 1.3 to 2.5 log CFU/g reduction in *E. coli*, *S. Typhimurium*, *L. monocytogenes*, and indigenous mesophilic aerobic bacteria
Laika et al. (2024)	Fresh-cut iceberg lettuce	Surface DBD CAP; reported: 6 kV, 23 kHz; exposure times 15–60 min	Slight reductions in mesophilic/psychrotrophic counts (small inactivation for short exposures)
Liu et al. (2024)	Tomato, cucumber, lettuce (fresh produce)	Indirect cold plasma combined with Modified Atmosphere Packaging	Reduced microbial load and slowed quality deterioration (lower respiration, delayed browning)

Treatment conditions vary widely across studies, and there is no standardized protocol for lettuce, making it difficult to compare results or implement the technology consistently. Addressing these gaps is essential for developing scalable, reliable cold plasma interventions for fresh produce.

Impact on quality and shelf life

Beyond its antimicrobial potential, cold plasma can extend the shelf life of fresh vegetables during refrigerated storage. By reducing initial microbial loads, plasma treatment delays spoilage and helps maintain quality attributes such as texture, color, and bioactive compounds. The studies summarized in Table 2 demonstrate that cold plasma treatments can have varying effects on lettuce quality, depending largely on the type of plasma, gas composition, power, and treatment duration. Most low-pressure plasma applications, such as those reported by Grzegorzewski et al. (2010) and Schnabel et al. (2015), caused minimal alterations to phenolic content, flavonoid stability, or sensory attributes, particularly under very short exposure times (7–40 s). This suggests that brief low-pressure plasma treatments are generally safe for preserving lettuce quality, although some subtle biochemical changes, such as faster flavonoid degradation, may occur under certain conditions.

Corona plasma treatments, as reported by Jahid et al. (2015) and Cui et al. (2018), also largely maintained color, texture, and sensory properties. Notably, Cui et al. (2018) observed positive effects on sensory evaluation, suggesting that under carefully controlled conditions, plasma may even enhance perceived quality. However, Song et al. (2015) highlighted that longer treatment times (10 min) or higher power levels can induce lightness changes due to enzymatic browning and oxidation of phenolic compounds, indicating that extended exposure can start to compromise quality despite leaving some parameters unchanged, such as ascorbic acid or antioxidant activity.

Overall, these findings prove the sensitivity of lettuce quality to both treatment duration and plasma operational parameters. Shorter exposures, particularly with low-pressure plasma, appear to preserve most quality attributes, while longer treatments or higher energy inputs increase the risk of biochemical and visual changes. The diversity of experimental conditions, ranging from very brief low-pressure plasma to extended high-power treatments, makes direct comparisons difficult and highlights the need for standardized protocols to optimize microbial inactivation while minimizing quality losses.

Cold plasma can be safely applied to lettuce if treatment parameters are carefully controlled, but further research is needed to establish clear guidelines on duration, power, and gas composition, particularly for treatments beyond the laboratory scale.

Table 2. Effect of cold plasma on quality attributes of lettuce

Reference	Quality assays	Plasma type & key conditions	Impact
Grzegorzewski et al. (2010)	Phenolic profile	Low-pressure plasma Gas: argon Time: 40 s	Insignificant decrease in polyphenolic or phenolic content Faster degradation of flavonoids
Jahid et al. (2015)	Color, texture, sensory evaluation	Corona plasma Gas: air Time: 50 s	Insignificant effects on the quality parameters analyzed
Schnabel et al. (2015)	Texture, appearance, odor	Low-pressure plasma Gas: air Time: 7 s	Insignificant effects on the quality parameters analyzed
Song et al. (2015)	Ascorbic acid, antioxidant activity, sensory evaluation	Low-pressure plasma Gas: N_2, N_2 + O_2, He Power: 400–900 W Time: 10 min	Lightness because of enzymatic browning and phenolic compound oxidation Insignificant change in color, ascorbic acid, antioxidant activities, physicochemical properties
Cui et al. (2018)	Surface color, texture, sensory evaluation	Corona plasma Gas: N_2 Power: 400–600 W Time: 2–30 min	Positive impact in sensory evaluation Insignificant change in other parameters
Laika et al. (2024)	Ascorbic acid, chlorophylls, total phenolic content, antioxidant activity, enzymes	Dielectric barrier discharge Time: 15, 30, 60 min	Reduction in all parameters analyzed

1.6 Pathogenic *Listeria* and Surrogates in Food Safety

The presence of pathogenic bacteria in food can cause serious foodborne illnesses, which are generally classified into food infections and food intoxications. Food infections occur when low numbers of microorganisms, such as *L. monocytogenes*, are ingested and colonize the intestine. This colonization can disrupt the normal intestinal flora, leading to gastrointestinal symptoms, including stomach ache and diarrhea, typically appearing 8–16 hours after consumption and lasting several days. A hallmark of food infections is fever, and while symptoms generally

resolve within a few days to a week, sensitive populations defined as YOPI (young, old, pregnant, or immunodeficient) may require hospitalization.

Certain pathogens, including *L. monocytogenes*, are invasive: they can cross the intestinal barrier, enter the bloodstream, and replicate within phagocytes (white blood cells), partially evading the immune system. This ability enables systemic dissemination and increases the severity of the infection. Listeriosis carries a high mortality rate, estimated at 15–30% (Devlieghere et al., 2013).

The genus *Listeria*, part of the phylum Firmicutes, comprises Gram-positive, facultatively anaerobic bacteria found ubiquitously in soil, water, and plant material (Linke et al., 2014; Vivant et al., 2013). Six species are recognized: *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, and *L. grayi*, with *L. monocytogenes* being the main human pathogen. Monitoring *Listeria* species in food production environments is often used as an indicator for the potential presence of *L. monocytogenes* (Devlieghere et al., 2013).

L. innocua, originally considered non-pathogenic and non-hemolytic (Orsi &Wiedmann, 2016), is psychrotrophic, growing at 1–45 °C (optimal 30–37 °C) and tolerating a pH range of 4.5–9.2 (Norrung, 2000; Ryser, 2011). Due to its close genetic and physiological similarity to *L. monocytogenes*, *L. innocua* is widely used as a surrogate organism in research. It allows the study of *Listeria* behavior and the evaluation of control strategies in food processing environments without posing health risks to researchers, as both species exhibit comparable growth under varying conditions of temperature, pH, water activity, and nutrient availability.

1.7 Thesis Aim

This study aims to evaluate cold plasma technology as a non-thermal decontamination method for fresh lettuce. Specifically, it investigates the effectiveness of cold plasma (Gliding Arc discharge type) in reducing *L. innocua*, the non-pathogenic surrogate for *L. monocytogenes*, on lettuce surfaces, while simultaneously assessing its impact on quality attributes, including color, pH, chlorophyll content, and total phenolic content.

Lettuce was selected as a case study vegetable due to its short shelf life, high sensitivity to temperature and storage conditions, and status as a widely consumed perishable product. These characteristics make it an ideal model for examining critical factors related to microbial safety and quality retention. Although some studies have investigated the use of cold plasma on lettuce, there remains a lack of comprehensive information on optimal treatment conditions,

pilot-scale applications, and standardized protocols, leaving significant opportunities and a clear need for further exploration.

Cold plasma treatments were applied for varying exposure times. For comparison, a conventional hot water treatment (blanching at 80 °C) was also included. Although thermal treatment is not typically used for fresh lettuce due to its adverse effects on texture, color, and bioactive compounds, it was incorporated as a reference to illustrate the effects of extreme thermal stress and provide a benchmark for microbial inactivation.

By evaluating both microbial reduction efficiency and preservation of quality properties, this research seeks to determine whether cold plasma can serve as a reliable strategy for ensuring microbial decontamination of lettuce without compromising quality characteristics and particular bioactive compounds.

2. Materials and Methods

2.1 Lettuce Samples

Lettuce (*Lactuca sativa* L.) used in the present study was obtained from a local supermarket. The leaves were separated, and any visible dirt or soil was carefully removed using a sterile paper towel. Prior to plasma treatment, each lettuce leaf was marked with a 7 × 7 cm square using paper tape to clearly identify the treatment area (Figure 3).

Figure 3. Example of lettuce sample preparation.

2.2 Treatments Applied

Samples were exposed to two different processes for various durations: cold plasma and thermal treatment (blanching) at 80 °C. The thermal treatment, applied for the same periods as plasma, served as a reference for comparison with a severe thermal effect.

Cold plasma

Samples (Figure 3) were exposed to cold plasma treatments for 5, 10, 15, 20, 25, and 30 seconds, while one untreated sample served as the control.

Plasma was generated using a gliding arc discharge device (Blown-arc™ Series, Enercon, USA) operating with compressed (70–90 psi), clean, dry, and oil-free air (Figure 4). The system consists of two flat metallic electrodes connected to a high-voltage power supply, with air injected at a constant flow rate through the discharge gap to create a plasma arc and generate reactive species carried downstream by the gas flow. During treatment, the plasma nozzle (6.5 cm) was positioned 4 cm above the lettuce surface and manually moved to ensure full exposure

of each sample. The lettuce surface temperature was monitored using an infrared thermometer (Fluke 62 max, Fluke Corporation, Everett, WA, USA).
The treatments were performed in triplicate.

Figure 4. Cold plasma system setup.

Thermal treatment

Lettuce leaves were treated individually, with each leaf representing a single sample and a single treatment time, in 500 mL of tap water heated to 80 °C using a laboratory pot and hot plate heater (Thermo Scientific™ SAFE-T™ HP6 Explosion-Proof Hotplate, Thermo Fisher Scientific, Waltham, MA, USA). Treatments were applied for 5, 10, 15, 20, 25, and 30 seconds, with one untreated sample serving as the control. The temperature of the water bath was monitored using a simple laboratory thermometer. The lettuce surface temperature was monitored using an infrared thermometer (Fluke 62 max, Fluke Corporation, Everett, WA, USA). After treatment, each leaf was immediately transferred to ice for rapid cooling before further analysis. All treatments were performed in triplicate.

2.3 Quality Analyses

Analyses were conducted on samples prior to and following plasma and thermal treatments.

pH

Lettuce leaf samples were homogenized using a pestle and then transferred to a 10 mL beaker. pH was measured with a pH meter (SensION+ PH31, Hach-Lange, Germany) using a pointed electrode fully submerged in the sample and rinsed with deionized water between measurements. The pH meter was calibrated daily with standard solutions of pH 4.01, 7.00, and

10.01, according to the manufacturer's instructions. Measurements were performed in duplicate for each of three independent samples.

Color

Color analysis of the lettuce samples was performed using a colorimeter (Minolta CR-400, Konica Minolta, Osaka, Japan), calibrated with a white standard tile before each measurement. CIE L* (lightness), a* (red-green), and b* (yellow-blue) values were recorded. L* represents lightness, ranging from 0 (black) to 100 (white). The a* coordinate indicates red when positive and green when negative, while the b* coordinate indicates yellow when positive and blue when negative. From these values, the following color parameters were calculated:

- Chroma (eq. 1), or saturation index, which measures the intensity or purity of a color; higher values indicate more vivid colors, while lower values indicate duller colors;
- Total color difference (TCD, eq. 2), reflecting overall color change in relation to one reference sample;
- Browning index (BI, eq. 3), which quantifies the degree of browning, often caused by processing, storage, or enzymatic reactions; higher BI indicates more browning, while lower BI indicates less.

These parameters were used to assess color changes induced by cold plasma and blanching treatments (Alibas, 2009; Ihns et al., 2011):

$$Chroma = \sqrt{a^{*2} + b^{*2}} \tag{1}$$

$$TCD = \sqrt{\left(L^* - L^*_{ref}\right)^2 + \left(a - a^*_{ref}\right)^2 + \left(b^* - b^*_{ref}\right)^2} \tag{2}$$

where the index *ref* indicates fresh/untreated sample.

$$BI = 100 \times \frac{(x-0.31)}{0.17} \tag{3}$$

in which

$$x = \frac{a^* + 1.750\ L^*}{5.645\ L^* + a^* - 3.012\ b^*} \tag{4}$$

Readings were taken from 10 different areas per sample, and measurements were performed in triplicate.

Chlorophyll content

Each lettuce sample (3.00 g) were homogenized using a mortar and pestle. The homogenized samples were immersed in 100% methanol (15 mL) for 15 minutes to facilitate extraction, after which they were filtered through filter paper into volumetric flasks. During the extraction, samples were wrapped in aluminum foil to prevent light exposure.

Quantitative chlorophyll determination was performed by measuring absorbance at 665 nm ($A_{665.2}$) and 652 nm ($A_{652.4}$) using a spectrophotometer (GENESYS 30, Thermo Fisher Scientific, Waltham, MA, USA) with a 1.00 cm pathlength cuvette. Small nm offsets (e.g., 665 instead of 665.2 and 652 instead of 652.4) are routine. Chlorophyll *a* and *b* contents were calculated in µg/mL using equations 5 and 6 described by Lichtenthaler (1987):

$$Chlorophyll\ a\ =\ 16.72A_{665.2} - 9.16\ A_{652.4} \quad (5)$$

$$Chlorophyll\ b\ =\ 34.09A_{652.4} - 15.28A_{665.2} \quad (6)$$

The total chlorophyll content of the lettuce samples was obtained as the sum of chlorophylls *a* and *b*. Chlorophyll content was expressed as milligrams per 100 grams of lettuce fresh weight (mg/100 g FW).

All analyses were performed in triplicate, with a minimum of six measurements per replicate.

Total phenolic content

The total phenolic content (TPC) of lettuce samples was determined following the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965), using the same extract prepared for chlorophyll analysis. All procedures were performed in triplicate.

A calibration curve was constructed using gallic acid (3,4,5-trihydroxybenzoic acid, ≥ 98%, Alfa Aesar, Thermo Fisher GmbH, Germany). Stock solutions of 1 mg/mL gallic acid were prepared in distilled water, and appropriate dilutions were made to obtain the standard concentrations for the calibration curve.

For each sample or standard, 50 µL was mixed with 50 µL of Folin-Ciocalteu phenol reagent (Supelco, Merck KGaA, Darmstadt, Germany), 1 mL of 75 g/L Na_2CO_3 solution (Scharlau, Barcelona, Spain), and 1.4 mL of distilled water. The mixture was incubated for 1 hour in the dark at room temperature to allow chromophore development.

After incubation, the absorbance was measured at 750 nm using a visible spectrophotometer (GENESYS 30, Thermo Fisher Scientific, Waltham, MA, USA). Total phenolic content was calculated by interpolation from the gallic acid calibration curve (Oliveira et al., 2015) and expressed as milligrams of gallic acid equivalent per grams of lettuce fresh weight (mg GAE/g FW).

2.4 Microbiological Assays

Bacterial cultures

L. innocua (2030c) was used as the model organism in this study. Stock cultures were maintained at –80 °C in TSAYE (Biokar Diagnostics, Beauvais, France) with 30% glycerol (Merck, Darmstadt, Germany). For routine use, pure colonies were aseptically transferred into sterile 15 mL Falcon tubes containing TSBYE (Biokar Diagnostics, Beauvais, France) and incubated at 30 °C for 24 hours. Subsequently, 0.5 mL of this primary culture was transferred into 5 mL of fresh TSBYE and incubated for an additional 16 hours to obtain actively growing cultures suitable for experimental use.

Inoculation of *L. innocua* onto lettuce

Before inoculation, lettuce leaves were subjected to a decontamination treatment, in order to reduce the initial microbial load and possible contaminations. A decontamination solution was prepared by mixing 3000 mL of deionized water with 150 mL of food-grade vinegar (20 % v/v). Lettuce slices were immersed in this solution for 15 minutes prior to experimental treatments. After rinsing, the lettuce slices were allowed to air dry for 15 minutes. For artificial contamination, the *L. innocua* stock culture, prepared and incubated for 16 hours as described in the previous section, was used at a concentration of approximately 1.4×10^9 CFU/mL.

A sterile cotton swab was employed to evenly spread the culture over the surface of the lettuce slices, which were then allowed to sit for 15 minutes to facilitate microbial attachment. The inoculum was spread in a 7 x 7 cm square area. Following inoculation, cold plasma treatments were applied to the contaminated lettuce slices for the specified exposure times, while control samples were left untreated. For the blanching treatment, contaminated lettuce samples were placed in sterile stomacher bags containing 50 mL of pre-sterilized water. The bags were submerged in water maintained at 80 °C for the defined exposure times, after which they were

immediately transferred to an ice water bath to halt further heat effects. For both treatments, the time points used corresponded to those described in Section 2.2.

Sample collection and *L. innocua* enumeration

Immediately after treatment, samples treated with cold plasma were collected from the lettuce surface using sterile cotton swabs. Each swab was transferred into 1 mL of sterile saline solution for subsequent microbial analysis. The suspension was thoroughly mixed to release the bacteria from the swab. In the case of thermally treated samples, after cooling down, the samples were homogenized, and 1 mL was collected to a 1.5 mL tube for further dilution. All collected samples were serially diluted in sterile NaCl solution as required for accurate counting. Each dilution was homogenized using a vortex mixer to ensure even distribution of bacterial cells.

L. innocua enumeration was carried out using PALCAM Agar (Biokar Diagnostics, Beauvais, France), a selective and differential medium for *Listeria* spp. PALCAM Agar contains selective agents (including lithium chloride, acriflavine, polymyxin B, and ceftazidime), which inhibit the growth of most Gram-negative bacteria and many Gram-positive organisms, thereby reducing background microflora. Its differential properties rely on esculin hydrolysis: *Listeria* spp. hydrolyze esculin to esculetin, which reacts with ferric ammonium citrate to form a dark precipitate, resulting in green-grey colonies with beige halos. Other common contaminants, such as enterococci and staphylococci, ferment mannitol to produce yellow colonies on the cherry-red medium. Presumptive *Listeria* colonies were identified based on these characteristics, with confirmatory biochemical tests applied when necessary (A.B.E., 2024).

From each serial dilution, 100 µL was aseptically transferred onto Petri dishes containing PALCAM Agar and spread evenly using a sterile metal Drigalski spatula. Each sample was plated in triplicate. For the culture enumeration control, both PALCAM and TSAYE agar were used, with 10 µL of culture applied using the droplet plating method. For the calculations, only the values obtained for PALCAM medium were applied.

Plates were incubated at 30 °C for 48-72 hours. After incubation, colonies exhibiting the typical gray-green appearance with depressed centers and black-to-brown halos were counted as presumptive *L. innocua* colonies. Colony counts were used to calculate microbial loads considering the respective dilutions and are expressed as CFU/mL for each treatment condition.

2.5 Data Analyses

Experimental data were compared for each exposure time across the two different treatments. Statistical analysis was performed using the independent-samples Student's t-test to assess differences between groups.

To evaluate the effect of treatment time on *L. innocua* survival under plasma treatment, a one-way ANOVA was performed after verifying the assumptions of normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). Duncan's multiple range test was applied for post hoc comparisons. A 5% significance level was used in all analyses.

Results were presented as mean ± standard deviation. All analyses were conducted using IBM® SPSS® Statistics (version 28, IBM Corp., Armonk, NY, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3. Results and Discussion

Box plots were employed to illustrate the distribution of the experimental data, making it easier to compare results across treatments and over time. In these plots, the box indicates the interquartile range (IQR = $Q_3 - Q_1$), the difference between quartile 3 (Q_3) and quartile 1 (Q_1), which contains 50% of the observations. The median, representing the central tendency, is shown as a horizontal line within the box. The whiskers extend to the smallest and largest values that are not considered outliers. Outliers are displayed separately, with circles representing moderate outliers (values more than 1.5 × IQR beyond Q_1 or Q_3) and stars marking extreme outliers (values more than 3 × IQR beyond Q_1 or Q_3).

The temperature of the samples was continuously monitored during both plasma and thermal treatments (Table 3). During plasma treatment, the temperature increased from 32.5 ± 5.2 °C at 5 seconds to values ranging between 37.9 ± 2.3 °C and 45.3 ± 4.5 °C at longer exposure times. These values confirm the treatment as cold plasma.

In contrast, thermal treatment samples exhibited a more rapid temperature rise, reaching 53.4 ± 13.4 °C at 5 seconds and stabilizing within the range 60–67 °C for longer durations. These trends clearly illustrate the distinct thermal profiles of plasma versus conventional thermal treatments.

Table 3. Surface temperature of lettuce samples over time during plasma and thermal treatments

Time (s)	Surface temperature (°C)	
	Plasma	**Thermal**
5	32.5 ± 5.2	53.4 ± 13.4
10	37.9 ± 2.3	62.5 ± 3.3
15	40.6 ± 3.0	60.3 ± 3.2
20	45.3 ± 4.5	60.6 ± 10.1
25	44.2 ± 9.2	60.8 ± 9.8
30	41.0 ± 4.6	67.1 ± 6.8

Values are mean ± standard deviation

3.1 Quality Characteristics

pH

The effects of plasma and thermal treatments on lettuce pH across different exposure times are shown in Figure 5 and Table 4. At the initial measurement (0 s), both samples exhibited similar pH values (5.64 ± 0.26 for plasma and 5.73 ± 0.20 for thermal). Over the 30 s exposure period, the pH remained relatively stable, with only minor fluctuations observed for both treatments. The measured pH values were within the 5.5–6.5 range considered optimal for lettuce in hydroponic systems (Jia et al., 2025).

The box plots further highlight that plasma treatment produced a wider range of variability compared to thermal treatment, as reflected by larger interquartile ranges and the presence of moderate outliers. This suggests that plasma treatment may induce more heterogeneous effects on the lettuce matrix, possibly due to localized physicochemical changes triggered by reactive species. On the other hand, thermal treatment appeared to maintain a more uniform impact, with smaller dispersion around the median.

Overall, the findings indicate that both plasma and thermal treatments maintain lettuce pH within a moderately acidic range (5.6–6.0), with plasma showing slightly higher variability over time compared to the more stable thermal process.

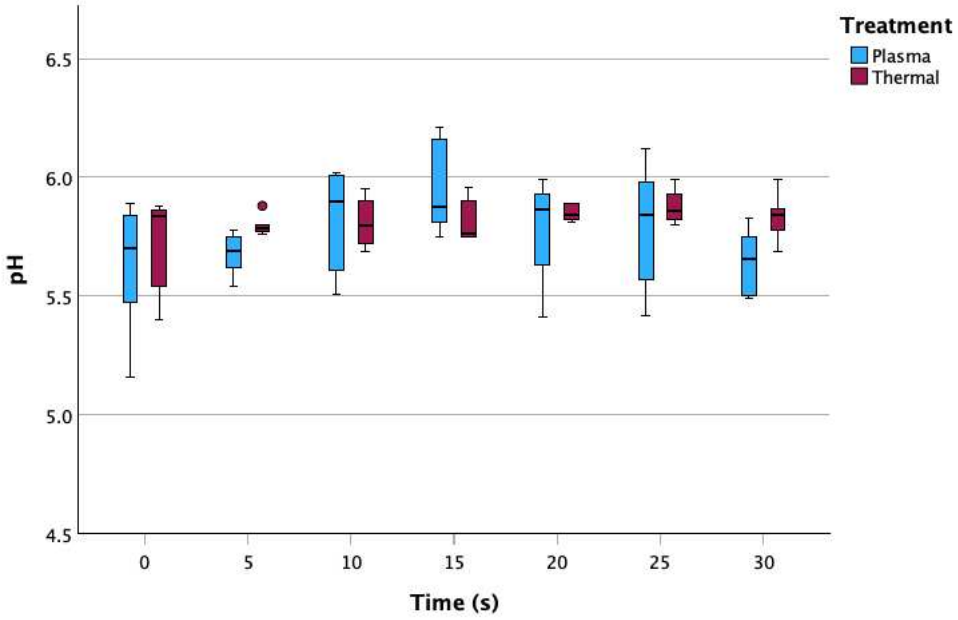

Figure 5. Boxplot of plasma and thermal treatment effects on lettuce pH over exposure time.

Table 4. Plasma and thermal treatment effects on lettuce pH over exposure time

Time (s)	pH	
	Plasma	**Thermal**
0	5.64 ± 0.26	5.73 ± 0.20
5	5.68 ± 0.09	5.80 ± 0.04
10	5.82 ± 0.21	5.81 ± 0.10
15	5.95 ± 0.19	5.81 ± 0.09
20	5.78 ± 0.22	5.85 ± 0.03
25	5.80 ± 0.26	5.88 ± 0.07
30	5.65 ± 0.14	5.84 ± 0.10

Values are mean ± standard deviation

Color

Total color difference

Total color difference was calculated relative to fresh untreated lettuce, which served as the reference. Typical color appearances of treated and untreated samples are shown in Figure 6 (plasma treatment) and Figure 7 (thermal treatment).

Both plasma and thermal treatments produced noticeable deviations from the fresh control (Figure 8; Table 5). Across most exposure times, plasma resulted in significantly lower TCD values than thermal treatment ($p > 0.05$), indicating superior color preservation during short exposures. For example, at 5 seconds plasma-treated samples showed a TCD of 8.43 ± 4.55, compared with 13.62 ± 6.07 under thermal treatment. A similar trend was observed at 10, 15, 20, and 30 seconds, confirming that plasma limited color alterations more effectively than heat. The only exception occurred at 25 seconds, when both treatments displayed comparable TCD values. Overall, thermal treatment consistently produced greater deviations from the fresh reference, highlighting the susceptibility of lettuce pigments to heat-induced degradation. Plasma treatment, although not completely preventing color change, reduced these deviations and better maintained the visual quality of lettuce.

Figure 6. Representative color appearance of lettuce subjected to plasma treatment at different exposure times.

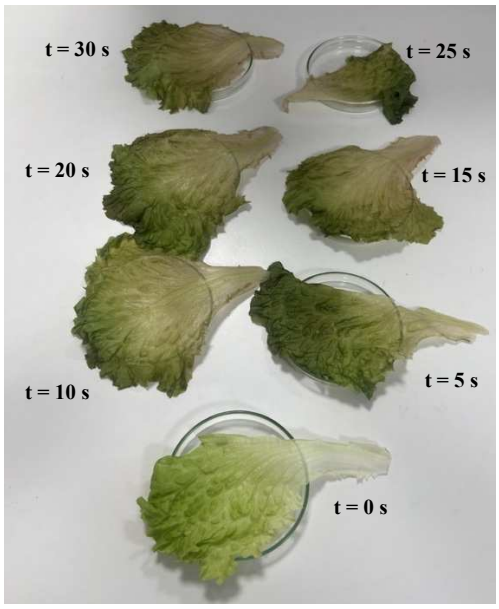

Figure 7. Representative color appearance of lettuce subjected to thermal treatment at different exposure times.

Smeu et al. (2012) observed that cold plasma treatment caused noticeable initial color differences in fresh-cut lettuce, primarily reflected in a* values, but overall preserved quality during storage. In contrast, Jahid et al. (2015) and Cui et al. (2018) reported insignificant effects of cold plasma on color changes in treated lettuce.

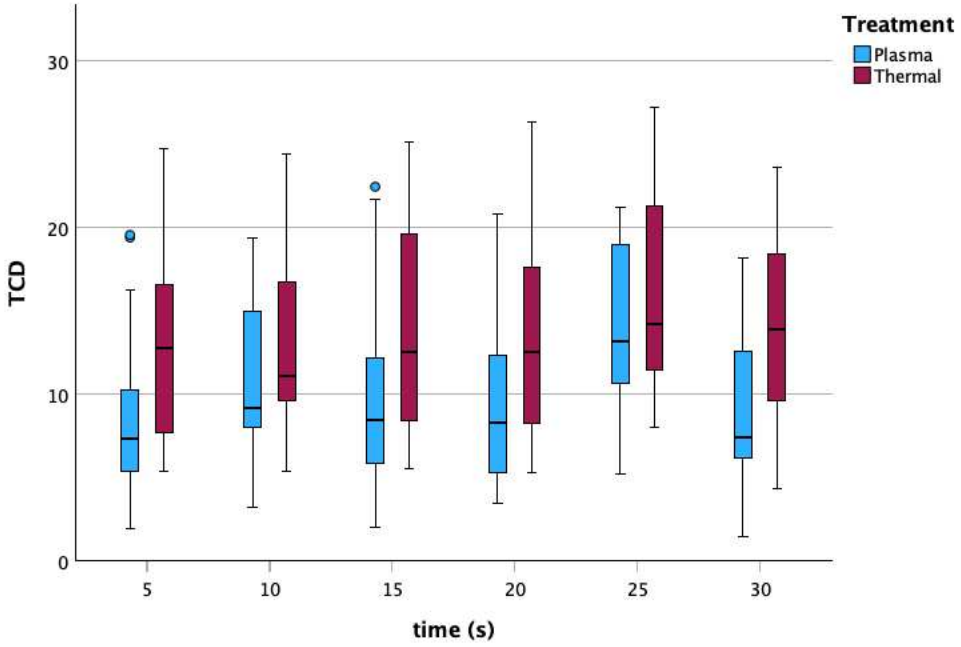

Figure 8. Boxplot of plasma and thermal treatment effects on lettuce TCD over exposure time.

Table 5. Plasma and thermal treatment effects on lettuce TCD over exposure time

Time (s)	TCD	
	Plasma	**Thermal**
5	8.43 ± 4.55 [a]	13.62 ± 6.07 [b]
10	10.90 ± 4.20 [a]	13.28 ± 5.53 [b]
15	10.07 ± 5.75 [a]	13.70 ± 6.15 [b]
20	9.50 ± 4.69 [a]	12.95 ± 5.64 [b]
25	13.91 ± 4.94 [a]	15.50 ± 5.35 [a]
30	9.05 ± 4.66 [a]	14.08 ± 5.32 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

Browning index

The browning index of lettuce samples subjected to plasma and thermal treatments is shown in Figure 9 and Table 6. At the initial time (0 s), no significant differences were observed, with BI values of 50.46 ± 12.73 (plasma) and 47.34 ± 15.83 (thermal), indicating comparable starting points. Over the course of treatment, BI values varied between 56 and 72, reflecting moderate

browning in both groups. Plasma-treated samples generally exhibited higher BI values compared to thermal treatment, though differences were not always statistically significant. Significant differences ($p < 0.05$) were found at 15, 25, and 30 s, where thermal treatment consistently resulted in lower BI values (55.57, 56.72, and 59.77, respectively) compared to plasma (67.52, 72.47, and 66.31, respectively). This indicates that thermal treatment was more effective in limiting browning at prolonged exposure times.

The boxplot further illustrates this trend: plasma-treated samples showed higher median BI values. This suggests that plasma treatment induced more variable color changes across lettuce tissues, possibly due to uneven exposure to reactive species generated by the plasma.

Mechanistically, the higher BI values observed under plasma may be associated with the oxidative effects of reactive oxygen and nitrogen species, which can accelerate pigment degradation and promote enzymatic or non-enzymatic browning reactions. By contrast, short-term thermal treatment may partially inactivate polyphenol oxidase and other browning-related enzymes, thereby limiting browning development.

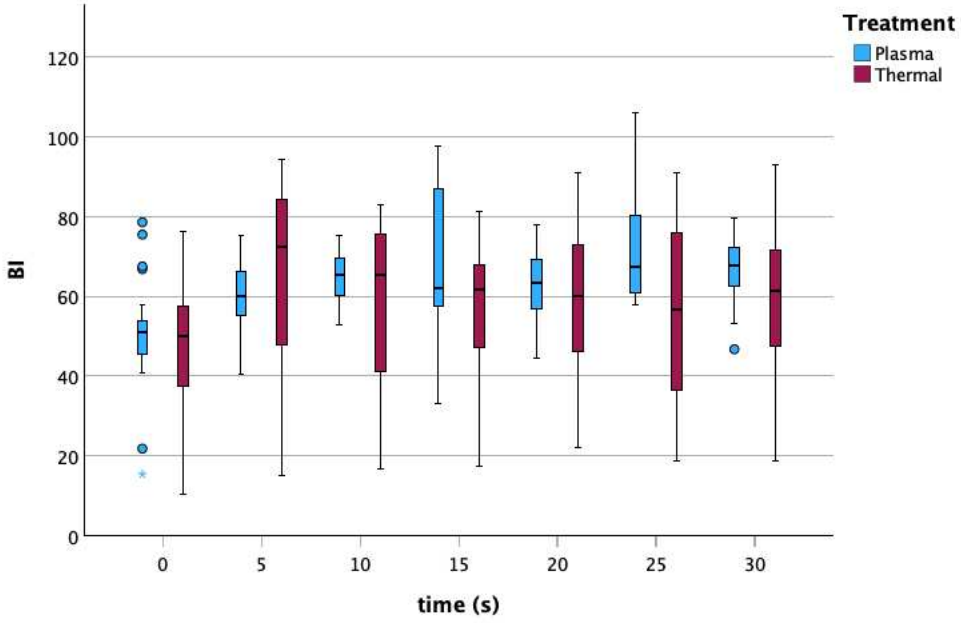

Figure 9. Boxplot of plasma and thermal treatment effects on lettuce browning index over exposure time.

Table 6. Plasma and thermal treatment effects on lettuce browning index over exposure time

Time (s)	BI	
	Plasma	Thermal
0	50.46 ± 12.73 [a]	47.34 ± 15.83 [a]
5	60.46 ± 8.56 [a]	65.95 ± 21.98 [a]
10	64.84 ± 5.94 [a]	58.84 ± 20.74 [a]
15	67.52 ± 16.58 [a]	55.57 ± 17.82 [b]
20	62.80 ± 7.72 [a]	57.86 ± 20.32 [a]
25	72.47 ± 13.40 [a]	56.72 ± 21.99 [b]
30	66.31 ± 7.80 [a]	59.77 ± 17.48 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

From a practical standpoint, both treatments maintained the browning index within a moderate range, unlikely to cause severe visual deterioration. Although BI indicated only subtle changes in plasma-treated samples, no visible browning was observed (Figure 6), suggesting that the instrumental differences were below the threshold of human perception. In contrast, thermally treated samples appeared visibly more browned (Figure 7). Nevertheless, the relatively lower BI values under thermal treatment suggest a slight advantage in preserving lettuce color stability compared to plasma, particularly at longer treatment durations.

Chroma

The chroma values of lettuce samples subjected to plasma and thermal treatments are shown in Figure 10 and Table 7. At time zero, no significant differences were observed between samples, which exhibited chroma values around 38, indicating comparable initial color intensity. As exposure time increased, a clear trend emerged: plasma-treated samples consistently maintained higher chroma values than thermally treated samples. Significant differences ($p < 0.05$) were observed from 10 s onward, where plasma treatment preserved color vividness, while thermal treatment resulted in a reduction of chroma. By 30 s, the difference persisted, with plasma-treated lettuce (39.93 ± 3.22) maintaining significantly higher chroma than the thermal group (33.91± 8.64).

The boxplots further illustrate these findings: plasma-treated samples displayed narrower distributions and consistently higher median values compared to thermal treatment. Thermal-

treated samples, in contrast, exhibited greater variability and a tendency toward lower chroma values across exposure times.

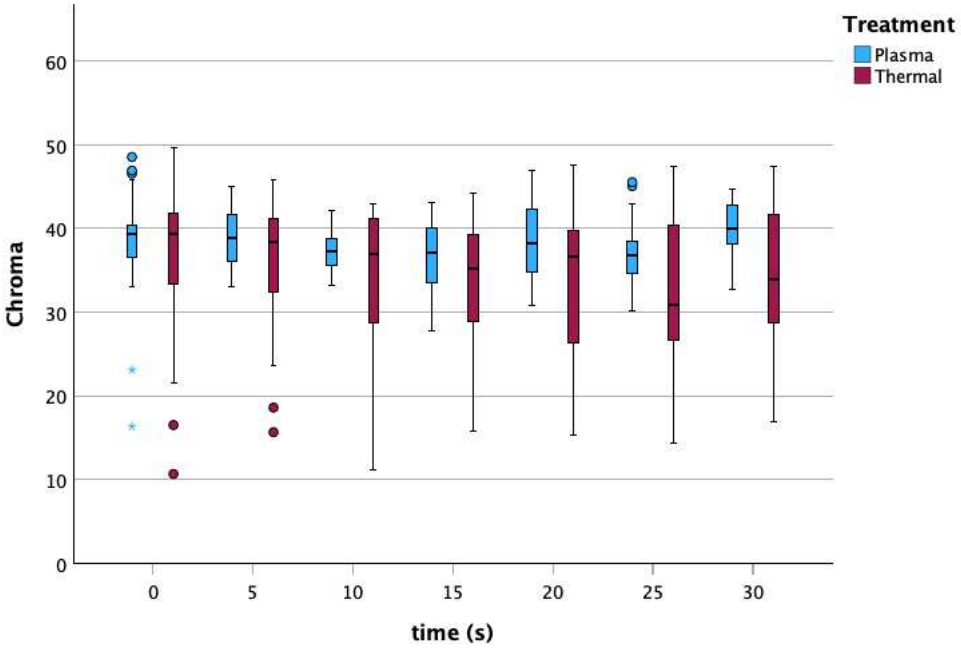

Figure 10. Boxplot of plasma and thermal treatment effects on lettuce chroma over exposure time.

Table 7. Plasma and thermal treatment effects on lettuce chroma over exposure time

Time (s)	Chroma	
	Plasma	**Thermal**
0	38.55 ± 6.44 [a]	37.40 ± 9.01 [a]
5	38.75 ± 3.44 [a]	36.10 ± 8.29 [a]
10	37.17 ± 2.27 [a]	33.35 ± 9.32 [b]
15	36.56 ± 4.62 [a]	32.71 ± 8.92 [b]
20	38.49 ± 4.56 [a]	33.24 ± 9.14 [b]
25	36.94 ± 3.71 [a]	32.12 ± 9.46 [b]
30	39.93 ± 3.22 [a]	33.91 ± 8.64 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

These results suggest that plasma treatment is more effective at preserving the saturation and purity of lettuce color, likely due to its non-thermal nature, which minimizes pigment degradation. In contrast, thermal treatment may accelerate pigment loss or degradation of chlorophylls and carotenoids, leading to a duller appearance with reduced color intensity.

Min et al. (2017) found no significant chroma changes in romaine lettuce treated with a dielectric barrier discharge plasma system, confirming that cold plasma can preserve color parameters in fresh produce. Similarly, Shah et al. (2019) observed that baby kale leaves exposed to cold plasma for up to 300 s maintained stable chroma and overall color quality, whereas excessive treatment led to a visible reduction in chroma. They further emphasized that, when applied within optimal limits, plasma treatment helps maintain the saturation and purity of leaf color during storage.

From a practical perspective, maintaining higher chroma values is desirable, as it reflects fresher, more vivid lettuce color, which is a key quality attribute influencing consumer acceptance. The ability of plasma to retain chroma over time reinforces its potential as a promising non-thermal technology for extending the visual quality of fresh-cut produce.

Chlorophyll content

The effects of plasma and thermal treatments on lettuce chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were evaluated over different exposure times.

Chlorophyll a

The results for chlorophyll *a* retention in lettuce after the treatments are presented in Figure 11 and Table 8. Overall, no significant differences were detected between plasma and thermal treatments across most time points ($p > 0.05$). Both treatments showed some variability, but the values generally fluctuated around the initial baseline (≈8–9 mg/100 g FW). These results indicate that chlorophyll *a* is relatively resistant to both plasma and thermal treatments within the tested time frame.

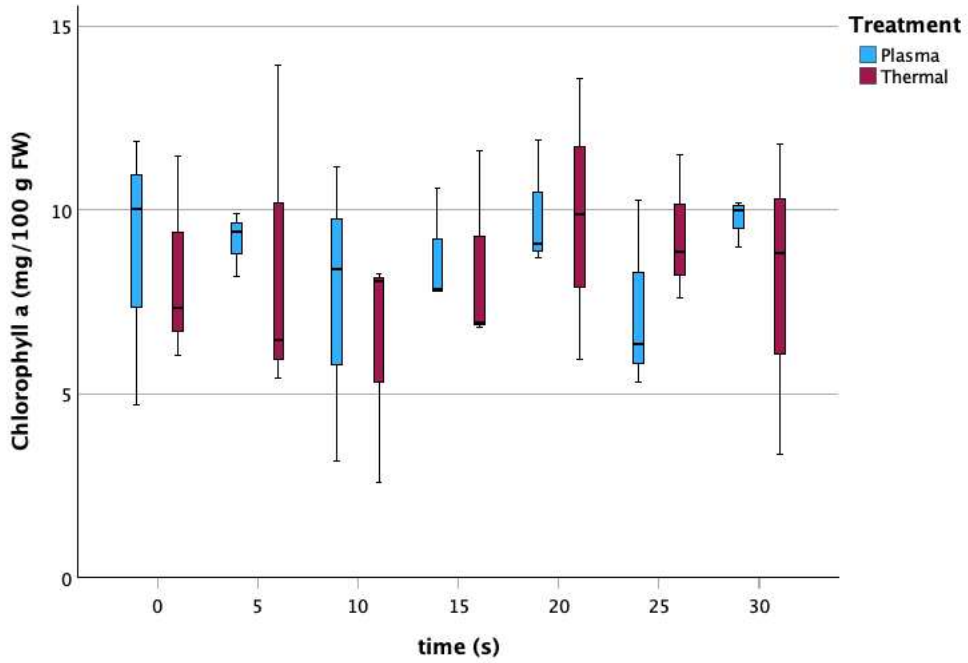

Figure 11. Boxplot of plasma and thermal treatment effects on lettuce chlorophyll *a* content over exposure time.

Table 8. Plasma and thermal treatment effects on lettuce chlorophyll *a* content over exposure time

Time (s)	Chlorophyll *a* (mg/100 g FW)	
	Plasma	Thermal
0	8.86 ± 3.72 [a]	8.28 ± 2.84 [a]
5	9.15 ± 0.88 [a]	8.61 ± 4.66 [a]
10	7.57 ± 4.06 [a]	6.29 ± 3.23 [a]
15	8.73 ± 1.61 [a]	8.46 ± 2.74 [a]
20	9.89 ± 1.76 [a]	9.79 ± 3.82 [a]
25	7.30 ± 2.61 [a]	9.32 ± 1.98 [a]
30	9.73 ± 0.65 [a]	7.98 ± 4.29 [a]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

Chlorophyll b

In contrast, chlorophyll *b* showed greater sensitivity to treatment conditions (Figure 12 and Table 9). At 5 s and 30 s, significant differences were observed, with plasma-treated samples maintaining higher chlorophyll *b* levels compared with thermal treatment ($p < 0.05$). For instance, at 5 s, plasma retained 3.98 ± 0.50 mg/100 g FW, while the thermal treatment reduced

59% chlorophyll *b* to 1.64 ± 1.26 mg/100 g FW. For 30 s a 27% reduction was observed. This suggests that plasma treatment may better preserve chlorophyll *b*, whereas thermal treatment can accelerate degradation. However, high variability at certain time points (e.g., 15 and 25 s) complicates a clear trend, possibly reflecting heterogeneity in leaf tissue response.

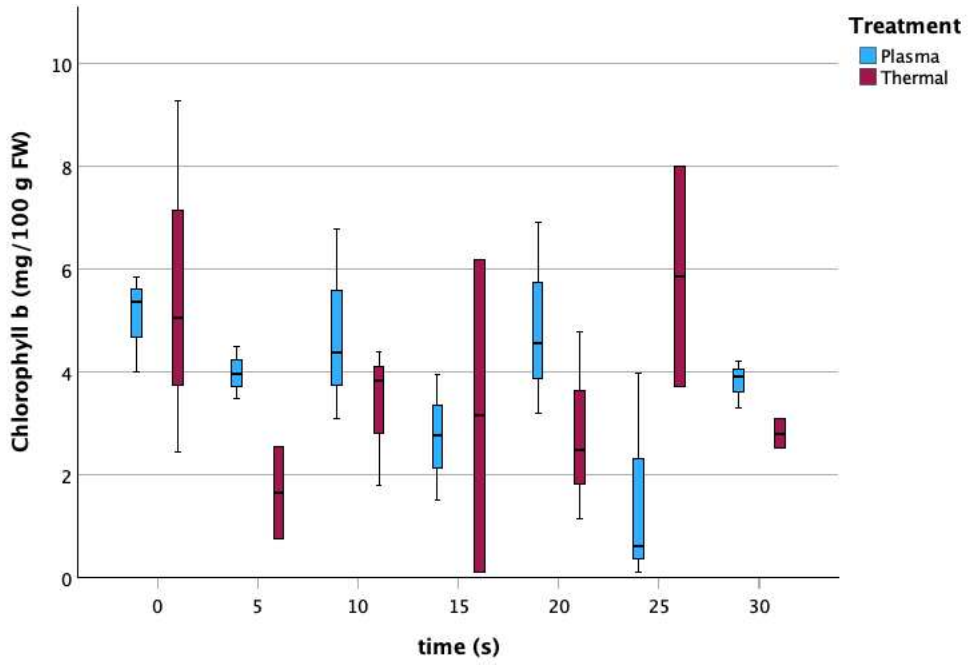

Figure 12. Boxplot of plasma and thermal treatment effects on lettuce chlorophyll *b* content over exposure time.

Table 9. Plasma and thermal treatment effects on lettuce chlorophyll *b* content over exposure time

Time (s)	Chlorophyll *b* (mg/100 g FW)	
	Plasma	Thermal
0	5.07 ± 0.96 [a]	5.59 ± 3.44 [a]
5	3.98 ± 0.50 [a]	1.64 ± 1.26 [b]
10	4.75 ± 1.87 [a]	3.33 ± 1.37 [a]
15	2.74 ± 1.22 [a]	3.15 ± 4.31 [a]
20	4.89 ± 1.88 [a]	2.80 ± 1.84 [a]
25	1.57 ± 2.11 [a]	5.86 ± 3.02 [a]
30	3.81 ± 0.47 [a]	2.80 ± 0.40 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

Plasma exposures appear to better preserve chlorophyll *b* compared to thermal treatment, which is consistent with the observations of Laika et al. (2024). They reported that cold plasma treatment of iceberg lettuce significantly reduced chlorophyll *a* and *b* contents after extended exposures (15–60 min), while shorter treatments sometimes maintained levels comparable to the control, likely due to increased pigment extractability.

Total Chlorophylls

Patterns in total chlorophyll content (Figure 13 and Table 10) largely mirrored those observed for chlorophyll *b*. Significant differences emerged at 5 s and 30 s, with plasma-treated lettuce consistently retaining higher pigment levels than thermally treated samples. At 5 s, thermal treatment resulted in a marked 42% decrease (7.58 ± 2.00 mg/100 g FW) compared with plasma (13.13 ± 1.37 mg/100 g FW). These findings reinforce the suggestion that thermal exposure may promote early degradation of pigments, particularly chlorophyll *b*, leading to an overall reduction in total chlorophyll. Nevertheless, at later times (e.g., 25 s), thermal-treated samples presented unexpectedly higher total chlorophyll values (16.02 ± 4.89 mg/100 g FW) compared to plasma (8.87 ± 4.71 mg/100 g FW). This anomalous increase could be due to experimental variability or differences in extraction efficiency and should be interpreted with caution.

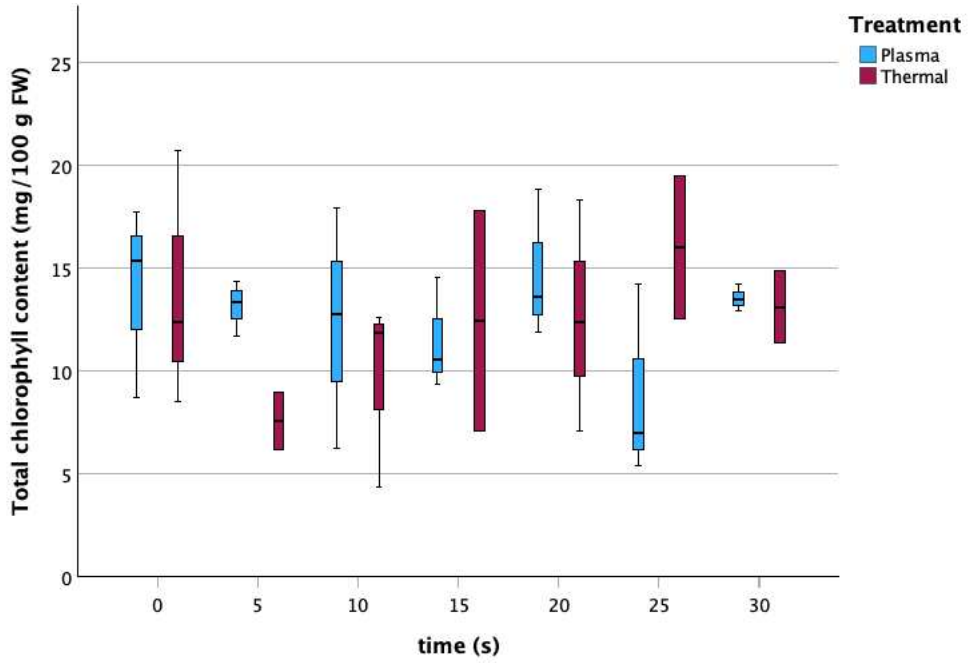

Figure 13. Boxplot of plasma and thermal treatment effects on lettuce total chlorophyll content over exposure time.

Table 10. Plasma and thermal treatment effects on lettuce total chlorophyll content over exposure time

Time (s)	Total chlorophyll (mg/100 g FW)	
	Plasma	**Thermal**
0	13.93 ± 4.68 [a]	13.87 ± 6.26 [a]
5	13.13 ± 1.37 [a]	7.58 ± 2.00 [b]
10	12.32 ± 5.85 [a]	9.62 ± 4.58 [a]
15	11.47 ± 2.73 [a]	12.44 ± 7.61 [a]
20	14.77 ± 3.61 [a]	12.59 ± 5.64 [a]
25	8.87 ± 4.71 [a]	16.02 ± 4.89 [a]
30	13.53 ± 0.66 [a]	13.11 ± 2.50 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

The results suggest that plasma treatment is at least as effective as thermal treatment in maintaining chlorophyll content, with some evidence of superior preservation of chlorophyll *b* and total chlorophyll. The absence of consistent and significant degradation trends across the time series indicates that both treatments, under the tested conditions, do not drastically compromise pigment stability. However, the relatively large standard deviations observed in several cases highlight variability in the data, which may stem from biological differences in lettuce samples or extraction procedures.

These findings are relevant from both nutritional and quality perspectives, as chlorophylls contribute to the visual appeal and potential health-promoting properties of leafy vegetables. Plasma treatment, by better preserving chlorophyll, could represent a promising alternative to thermal processing, particularly in applications where color retention is critical. These findings are consistent with the review of Amorim et al. (2023), who reported that cold plasma generally causes only slight reductions in chlorophyll content, mainly affecting the lateral side chains of the molecule rather than the porphyrin ring, which explains why major pigment loss is rarely observed.

Total phenolic content

The results of the total phenolic content of lettuce subjected to plasma and thermal treatments are in Figure 14 and Table 11.

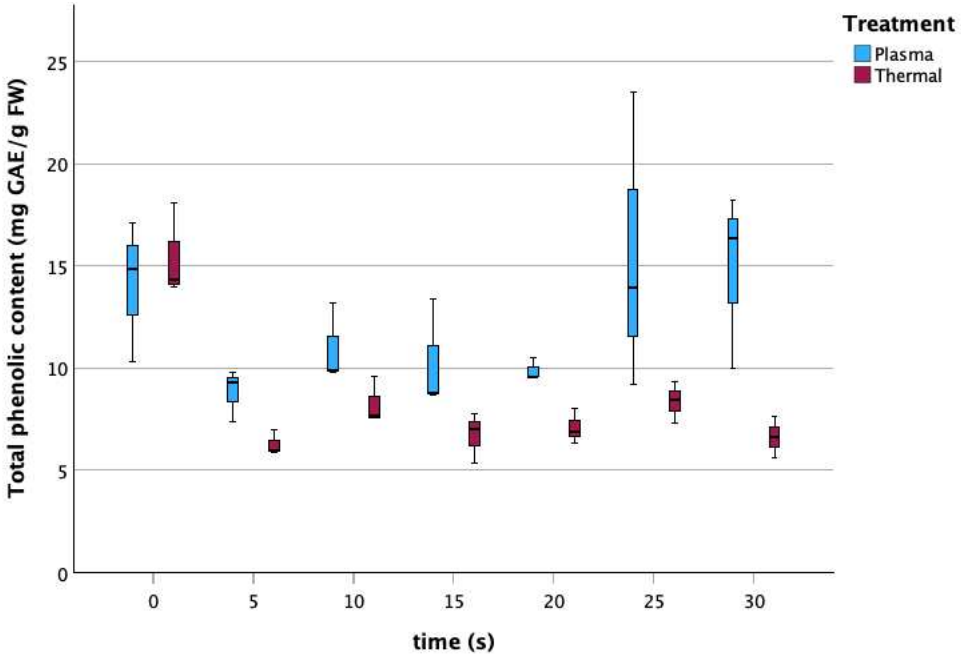

Figure 14. Boxplot of plasma and thermal treatment effects on lettuce total phenolic content over exposure time.

Table 11. Plasma and thermal treatment effects on lettuce total phenolic content over exposure time

Time (s)	Total phenolic content (mg GAE/g FW)	
	Plasma	Thermal
0	14.10 ± 3.45 [a]	15.46 ± 2.31 [a]
5	8.82 ± 1.28 [a]	6.29 ± 0.62 [b]
10	10.95 ± 1.95 [a]	8.26 ± 1.15 [b]
15	10.27 ± 2.70 [a]	6.69 ± 1.23 [b]
20	9.90 ± 0.53 [a]	7.09 ± 0.85 [b]
25	15.55 ± 7.26 [a]	8.36 ± 1.02 [b]
30	14.84 ± 4.32 [a]	6.63 ± 1.02 [b]

Values are mean ± standard deviation; for a given time values with different letters differ significantly ($p < 0.05$)

At the initial time point (0 s), no significant difference was observed between untreated control samples (14.10 ± 3.45 and 15.46 ± 2.31 mg GAE/g FW for plasma and thermal, respectively). However, as exposure progressed, plasma-treated samples consistently exhibited higher TPC compared to thermally treated samples, with significant differences at all time points ($p < 0.05$). Under plasma treatment, TPC values fluctuated but generally remained stable around the initial baseline. Peaks were observed at 25 s (15.55 ± 7.26 mg GAE/g FW) and 30 s (14.84 ± 4.32 mg GAE/g FW), which were comparable or slightly higher than the initial values. This suggests that plasma treatment did not induce degradation of phenolic compounds and may even have promoted their release from the plant tissue matrix, possibly through cell wall disruption and enhanced extractability.

In contrast, thermal treatment resulted in a significant reduction of TPC across nearly all time points compared to plasma. After only 5 s, phenolic content dropped 59% to 6.29 ± 0.62 mg GAE/g FW, and similar reductions persisted up to 30 s (6.63 ± 1.02 mg GAE/g FW). This decline is consistent with the known thermal sensitivity of many phenolic compounds, which can undergo oxidation, polymerization, or degradation when exposed to elevated temperatures. The contrasting effects observed highlight the potential advantage of plasma treatment over thermal processing in preserving or even enhancing phenolic content. While thermal treatment caused consistent losses, plasma maintained higher levels, with final values (30 s) more than double those of the thermally treated samples. This finding aligns with previous studies showing that non-thermal plasma can improve retention of bioactive compounds by avoiding heat-induced degradation, while also increasing extractability of bound phenolics.

From a nutritional perspective, phenolic compounds are key contributors to the antioxidant activity and health-promoting potential of lettuce. The preservation of TPC under plasma treatment is therefore relevant not only for maintaining functional quality but also for meeting consumer expectations regarding fresh-like nutritional properties. The results strongly suggest that plasma treatment could be a promising alternative to conventional thermal methods in fresh produce processing, particularly where the retention of phenolic compounds is critical.

Laika et al. (2024) observed that prolonged cold atmospheric plasma treatments of iceberg lettuce (15-60 min) led to a significant decrease in total phenolic content, with losses up to 50% of initial value. The authors attributed this reduction to the oxidative reaction of reactive oxygen species and ozone on phenolic aromatic rings, leading to their degradation.

3.2 *L. innocua* survival

The survival of *L. innocua* (expressed as log N, where N represents colony-forming units per mL) was significantly affected by the applied treatments (Figure 15 and Table 12).

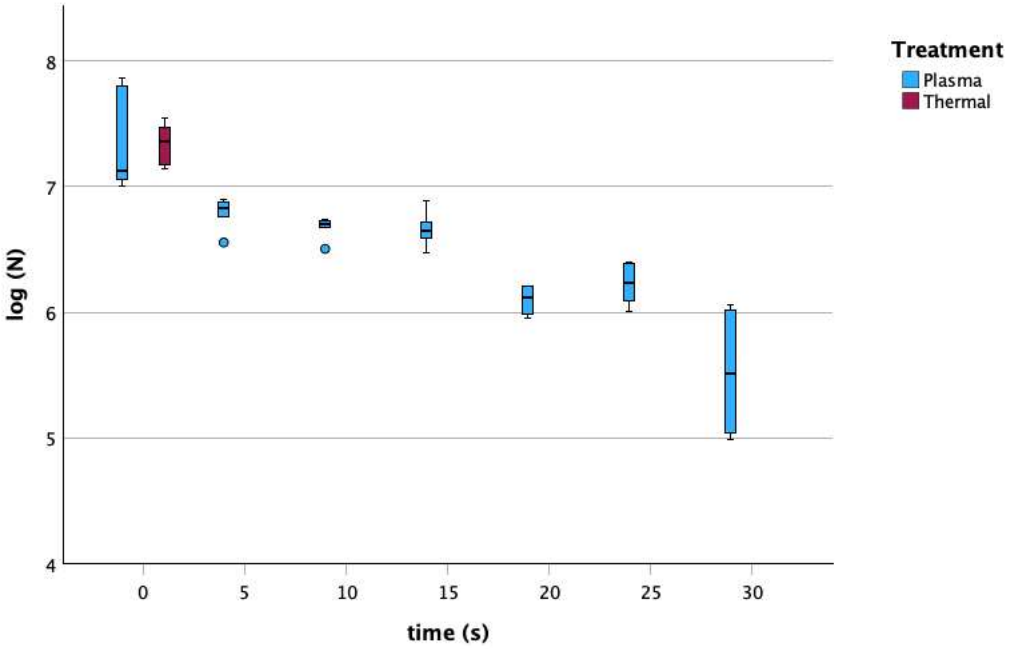

Figure 15. Boxplot of plasma and thermal treatment effects on lettuce *L.innocua* survival over exposure time.

Table 12. Plasma and thermal treatment effects on lettuce *L.innocua* survival over exposure time

Time (s)	log N	
	Plasma	**Thermal**
0	7.33 ± 0.40 [a]	7.34 ± 0.17 [a]
5	6.79 ± 0.13 [b]	Not detected
10	6.68 ± 0.09 [b]	Not detected
15	6.66 ± 0.14 [b]	Not detected
20	6.10 ± 0.12 [c]	Not detected
25	6.22 ± 0.17 [c]	Not detected
30	5.52 ± 0.54 [d]	Not detected

Values are mean ± standard deviation; for a given treatment, values with different letters differ significantly ($p < 0.05$)

In the case of thermal treatment, no viable *L. innocua* cells were detected at any of the evaluated time points, indicating complete inactivation. This result is consistent with the well-known sensitivity of *Listeria* species to heat and confirms that thermal processing is highly effective in eliminating this microorganism under the tested conditions.

In contrast, plasma treatment showed a more gradual reduction in *L. innocua* counts, as reflected in the log survival values. The initial population at time 0 was 7.33 ± 0.40 log CFU/mL. Over time, survival decreased progressively, with the lowest value recorded after 30 s of plasma exposure (5.52 ± 0.54 log CFU/mL). However, complete inactivation was not achieved, even at the longest exposure time tested. Özdemir et al. (2023) and Han et al. (2024) reported similar microbial reductions in plasma-treated lettuce, although the latter specifically focused on *L. monocytogenes* in plasma-activated water.

The statistical grouping indicates that survival levels at 20 s (6.10 ± 0.12 log CFU/mL) and 25 s (66.22 ± 0.17 log CFU/mL) did not differ significantly from each other, but both were significantly lower than earlier time points (5–15 s). After 30 s, *L. innocua* survival was further reduced, falling into a distinct group, suggesting a threshold effect where prolonged plasma exposure enhances microbial reduction.

Overall, plasma treatment significantly reduced *L. innocua* counts compared to the control, achieving approximately a 2-log cycle reduction after 30 s of exposure. However, it was less effective than thermal treatment, which ensured complete inactivation, corresponding to a 7-log cycle reduction within just 5 s. These results highlight plasma treatment as a promising non-thermal alternative for microbial control, although further optimization of exposure time and intensity is required to achieve safety levels comparable to those obtained by thermal processing.

4. Conclusions

This study demonstrates that cold plasma (gliding arc discharge) and thermal treatments affect the quality and microbial safety of fresh lettuce in distinct ways. Both treatments maintained pH within a moderately acidic range (5.6–6.0) over the 30 s exposure period. Thermal treatment showed slightly higher pH uniformity, whereas plasma-treated samples exhibited greater variability, likely due to localized physicochemical changes induced by reactive species.

Color analysis showed that plasma preserved lettuce visual quality more effectively than thermal treatment. Total color difference, calculated relative to fresh untreated lettuce, was significantly lower under plasma at most exposure times (e.g., 9.05 ± 4.66 vs. 14.08 ± 5.32 at 30 s). This indicates that plasma limited deviations from the fresh reference, whereas thermal treatment caused stronger alterations consistent with heat-induced pigment degradation. Plasma also maintained higher chroma values, reflecting better color vividness; at 30 s, chroma reached 39.93 ± 3.22 under plasma compared with 33.91 ± 8.64 under thermal treatment. In contrast, thermal processing more effectively limited browning, with a browning index of 59.77 ± 17.48 at 30 s versus 66.31 ± 7.80 under plasma, suggesting partial inactivation of browning-related enzymes during short-term heating.

Chlorophyll stability varied with pigment type. Chlorophyll *a* remained relatively stable under both treatments (≈7–10 mg/100 g FW), whereas chlorophyll *b* was more sensitive. At 5 s, plasma retained 3.98 ± 0.50 mg/100 g FW of chlorophyll *b* compared with only 1.64 ± 1.26 mg/100 g FW in thermal samples. Total chlorophyll mirrored this trend, with plasma maintaining higher pigment content at early times (13.13 ± 1.37 mg/100 g FW at 5 s vs. 7.58 ± 2.00 mg/100 g FW for thermal). These results indicate that cold plasma better preserves chlorophyll *b* and total chlorophyll, potentially supporting nutritional and visual quality.

Total phenolic content was markedly influenced by treatment. Plasma maintained or slightly increased phenolic levels (14.84 ± 4.32 mg GAE/g FW at 30 s), while thermal treatment caused rapid and sustained reductions (6.63 ± 1.02 mg GAE/g FW at 30 s), highlighting the sensitivity of phenolic compounds to heat. The ability of plasma to preserve total phenolic content suggests that non-thermal plasma may prevent heat-induced degradation and even enhance extractability of bioactive compounds. These results suggest that plasma avoids heat-induced phenolic degradation and may enhance extractability through tissue disruption.

Regarding microbial inactivation, thermal treatment achieved complete elimination of *L. innocua* within 5 s (≈7-log reduction), confirming the effectiveness of heat. In contrast, plasma reduced *L. innocua* counts progressively, from 7.33 ± 0.40 log CFU/mL (untreated sample) to 5.52 ± 0.54 log CFU/mL at 30 s, corresponding to approximately a 2-log reduction. While plasma significantly reduced microbial load, full inactivation was not achieved, indicating that optimization of exposure time and intensity is required to reach the safety levels obtained by thermal processing.

It should be emphasized that the purpose of including the thermal treatment in this study was not to recommend it as a practical approach for lettuce processing, but rather to provide a benchmark for comparing the impacts of thermal versus non-thermal treatments on quality and microbial reduction.

Cold plasma shows strong potential as a non-thermal preservation technology for lettuce. It effectively maintains color intensity, chlorophyll *b*, total chlorophyll, and phenolic content, while moderately reducing microbial counts. Thermal treatment ensures complete microbial inactivation and limits browning but at the cost of pigment and phenolic degradation. These findings suggest that cold plasma could serve as a complementary or alternative approach to conventional thermal treatments, particularly in applications where maintaining visual quality and bioactive compounds is critical. Further research is needed to optimize plasma parameters to balance microbial safety with quality preservation, making it a viable tool for fresh produce processing.

5. Future Work

Although this study has provided valuable insights into the effects of cold plasma on lettuce, several areas warrant further investigation. The findings of this thesis suggest multiple promising directions for future research:

- **Plasma Dose Optimization -** This study tested a relatively narrow range of plasma settings. Future work could explore a wider range of voltages, frequencies, and gas flow rates. Such optimization would help standardize results and facilitate comparisons across different studies;
- **Geometry Effects -** In this research, lettuce leaves were positioned at a fixed distance from the electrodes. Varying the distance could significantly alter treatment efficacy. For industrial-scale applications, conveyor belt systems could be evaluated to reduce uneven treatment across the leaf surface;
- **Alternative Gases -** Cold plasma treatment in this study used only air. Future investigations could explore other gases, such as nitrogen or argon, which may enhance microbial inactivation or reduce negative effects on product quality;
- **From Surrogate to Real Pathogens -** While *L. innocua* was used as a safe surrogate in this study, future work should test actual pathogens, including *L. monocytogenes*, *E. coli*, and *Salmonella*, to evaluate the real antimicrobial impact. Additionally, spoilage microorganisms could be studied to assess plasma effects on lettuce shelf life;
- **Shelf Life Trials -** This research measured microbial and quality parameters immediately after treatment. Future studies should monitor these parameters over several days or weeks to determine the actual shelf life of treated lettuce;
- **Sensory Analysis -** Consumer panels could be employed in future experiments to evaluate the effects of plasma on taste, smell, appearance, and texture, providing insights into potential impacts on eating quality;
- **Nutritional Compounds -** Additional quality parameters, such as vitamin C content and antioxidant capacity, could be measured to provide a more complete nutritional profile of plasma-treated lettuce;
- **Comparison with Other Technologies -** Future research could compare cold plasma with other non-thermal methods, such as UV-C, ozone, or pulsed light treatments, to determine whether plasma offers unique advantages in microbial inactivation or quality preservation.

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