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Cold plasma technology: Innovative non-thermal preservation of fish and seafood products

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Abstract
Cold plasma technology has emerged as a promising non-thermal method for the preservation of fish and seafood products, addressing the growing demand for minimally processed yet microbiologically safe foods. This study explores the principles, mechanisms, and applications of cold plasma in extending shelf life and enhancing the safety of aquatic products without compromising their sensory and nutritional attributes. The research focuses on evaluating the efficacy of cold plasma treatments in microbial inactivation, particularly targeting spoilage organisms and common pathogens such as *Listeria monocytogenes*, *Salmonella spp.*, and *Escherichia coli*. Various plasma generation methods, including dielectric barrier discharge (DBD) and plasma jets, are discussed in the context of their effectiveness and suitability for seafood processing. Experimental results demonstrate that short-duration plasma exposure significantly reduces microbial loads on fish fillets, shellfish, and crustaceans, while maintaining product quality, texture, and color. Additionally, cold plasma has been shown to reduce biogenic amine formation and lipid oxidation, contributing further to seafood preservation. The study also examines the challenges in scaling up this technology for industrial applications, including equipment design, treatment uniformity, and regulatory acceptance. Consumer perception and environmental impacts are briefly reviewed, highlighting the need for further education and sustainability assessment. The findings underscore cold plasma's potential as a viable alternative to traditional preservation methods, offering a clean-label solution that aligns with modern food safety and quality standards. Continued research and development are recommended to optimize process parameters and fully integrate cold plasma into commercial seafood preservation systems.

Keywords: Cold plasma, non-thermal preservation, seafood safety, microbial inactivation, shelf life extension, dielectric barrier discharge, food quality, fish processing

Introduction

The Xinjiang Uyghur Autonomous Region, often portrayed through the prism of "re-education camps," "cultural assimilation," and "religious repression," has become a focal point in international discourse. Dominated largely by In recent decades, the global demand for fresh, safe, and high-quality seafood products has increased significantly, driven by growing health consciousness, urbanization, and evolving dietary patterns that favor protein-rich, low-fat food sources. Fish and seafood are highly valued for their nutritional benefits, particularly their content of high-quality proteins, omega-3 polyunsaturated fatty acids, essential amino acids, vitamins, and minerals. However, these products are also among the most perishable of all food commodities due to their high-water activity, neutral pH, and abundance of free amino acids and unsaturated lipids, which make them an ideal substrate for microbial growth and biochemical spoilage. As a result, ensuring the safety, freshness, and extended shelf life of seafood remains a critical challenge for the food industry, regulators, and researchers alike.

Traditional methods for preserving fish and seafood—such as refrigeration, freezing, salting, smoking, vacuum packaging, and chemical preservatives—have been widely used for decades. While effective to varying degrees, many of these approaches have limitations. For instance, cold storage alone may slow but not eliminate microbial activity. Freezing can alter texture and cause cellular damage. Chemical preservatives may raise health concerns and are increasingly rejected by consumers seeking clean-label products. Furthermore, thermal processing methods, although highly effective for microbial inactivation, often lead to undesirable changes in sensory attributes such as texture, flavor, and color, as well as degradation of thermolabile nutrients. These issues underscore the urgent need for innovative preservation technologies that can ensure microbial safety and shelf-life extension without compromising the sensory and nutritional integrity of seafood.

Non-thermal food preservation methods have attracted considerable attention in recent years as viable alternatives or complement to traditional processing. These include high-pressure processing (HPP), pulsed electric fields (PEF), ultraviolet (UV) light, and more recently, cold plasma (CP) technology. Among these, cold plasma has emerged as a particularly promising technique due to its efficacy in microbial inactivation, low energy consumption, and minimal impact on food quality. Cold plasma, also referred to as non-thermal or non-equilibrium plasma, is a partially ionized gas composed of reactive species such as electrons, ions, UV photons, free radicals, and neutral particles. When applied to food surfaces, these reactive agents interact with microbial cells, leading to cell membrane disruption, oxidative damage, and ultimately microbial death—all without the need for high temperatures. Cold plasma can be generated through several mechanisms, including dielectric barrier discharge (DBD), plasma jets, corona discharge, and gliding arc discharges. Among these, DBD systems are most commonly applied in food processing due to their relatively simple design and scalability. The treatment can be performed in two modes: direct (in-package or open-air treatment of food surfaces)

and indirect (plasma-activated water or gas used for rinsing). These modes allow flexibility in designing food-grade preservation systems suitable for different types of seafood, including finfish, mollusks, and crustaceans.

Recent studies have demonstrated the efficacy of cold plasma in reducing populations of pathogenic and spoilage microorganisms commonly associated with seafood, such as *Listeria monocytogenes*, *Salmonella spp.*, *Escherichia coli*, *Vibrio spp.*, and *Pseudomonas spp.* In addition to microbial decontamination, cold plasma has also been shown to inhibit enzymatic activity and oxidative reactions responsible for off-flavor development, color changes, and texture degradation. For instance, cold plasma treatment can reduce the formation of biogenic amines—such as histamine, putrescine, and cadaverine—that result from microbial decarboxylation of amino acids and are often used as spoilage indicators in seafood.

One of the most significant advantages of cold plasma technology is its ability to achieve microbial safety without raising the temperature of the food product. This allows for the preservation of thermally sensitive nutrients, such as long-chain omega-3 fatty acids (EPA and DHA), and minimizes sensory changes. Moreover, cold plasma treatments are typically conducted in short durations—from seconds to a few minutes—making the process time-efficient and energy-efficient. The use of air or inert gases (e.g., argon, helium) for plasma generation also reduces the need for chemical additives, aligning with clean-label and eco-friendly processing trends.

However, despite its considerable promise, the implementation of cold plasma technology in the seafood industry faces several challenges. One major concern is the standardization and optimization of process parameters, including voltage, treatment time, gas composition, and distance between electrodes and food surface. These variables significantly influence the generation and reactivity of plasma species, and therefore, the effectiveness and safety of the treatment. Furthermore, concerns regarding potential formation of toxic by-products (e.g., ozone or nitrogen oxides) and their residual presence on food products must be thoroughly investigated and mitigated through proper process design and risk assessment.

Scalability and industrial integration also present practical challenges. While several pilot-scale and lab-scale systems have shown positive results, translating these findings into continuous, large-scale operations requires advances in equipment engineering, automation, and packaging compatibility. In-package plasma treatment, for example, must consider the interaction between plasma and packaging materials, ensuring that neither the food nor the package is adversely affected. Additionally, regulatory frameworks concerning the use of cold plasma in food processing are still evolving in many countries. While the technology is generally recognized as safe when properly applied, formal approval and guidance from food safety authorities are necessary to build industry and consumer confidence.

From a consumer standpoint, there is growing interest in technologies that offer minimally processed, fresh-tasting, and preservative-free food products. Cold plasma aligns well with these preferences. Nevertheless, consumer education is essential to bridge the knowledge gap about the safety and benefits of plasma-treated foods, particularly since the term "plasma" may evoke concerns if not clearly explained. Transparent labeling, public engagement, and scientific communication will be crucial to facilitate market acceptance.

Environmental and sustainability considerations further support the adoption of cold plasma. The process requires relatively low energy input compared to thermal methods and does not produce significant liquid or solid waste. When implemented in conjunction with renewable energy sources and recyclable packaging materials, cold plasma has the potential to contribute to more sustainable seafood supply chains.

Given the critical importance of seafood in global nutrition and the growing pressure on food systems to improve safety, reduce waste, and enhance sustainability, cold plasma technology represents a forward-looking solution that warrants deeper investigation and development. This paper aims to comprehensively review the principles, mechanisms, and applications of cold plasma in the context of fish and seafood preservation. It will examine current research on microbial inactivation, effects on quality attributes, potential risks, and challenges in scaling up. By analyzing the current state of knowledge and identifying research gaps, the study seeks to contribute to the ongoing efforts toward the industrial adoption of cold plasma as an innovative, non-thermal preservation technology for seafood products.

Methods

Study Design Overview

This study was designed to evaluate the effectiveness of cold plasma (CP) as a non-thermal preservation technique for fish and seafood products, focusing on microbial inactivation, physicochemical properties, and overall shelf-life extension. A comparative approach was employed, contrasting cold plasma-treated samples with untreated controls stored under identical conditions. The study incorporated both laboratory-scale and semi-industrial setups to evaluate the practicality of scaling up the process. Both qualitative and quantitative analyses were conducted to assess the microbial, nutritional, and sensory attributes post-treatment.

Sample Collection and Preparation

1. Seafood Selection

Three types of seafood products commonly consumed and known for high perishability were selected for this study:

- Atlantic salmon (*Salmo salar*) fillets
- Pacific white shrimp (*Litopenaeus vannamei*)
- Blue mussels (*Mytilus edulis*)

These samples were procured from certified local distributors under cold chain conditions and transported to the laboratory within 2 hours of harvest or thawing. All samples were visually inspected for freshness, and only intact, non-damaged products were used in the study.

2. Pre-Treatment Conditions

Samples were rinsed with sterile distilled water to remove surface contaminants and then divided into two groups

- **Treatment group:** Subjected to cold plasma treatment
- **Control group:** No treatment, stored under same conditions

Each group contained three replicates per seafood type, with each replicate consisting of a 100 g portion.

Cold Plasma Treatment System

1. Plasma Generation Equipment

Cold plasma was generated using a Dielectric Barrier Discharge (DBD) system, which consisted of two parallel aluminum electrodes, with one covered in a dielectric barrier (quartz). The system operated at

- **Voltage:** 20–40 kV (AC)
- **Frequency:** 20 kHz
- **Electrode gap:** 5 mm

The plasma chamber was a semi-closed environment allowing precise control of gas composition and humidity.

2. Working Gases

Two gas compositions were used to generate plasma

- Ambient air (natural mixture of N_2 and O_2)
- Argon (purity >99.99%) as an inert gas option

Gas flow rate was maintained at 1.5 L/min using mass flow controllers. Gas choice was randomized between batches to observe differences in reactive species generation and effect on samples.

3. Treatment Parameters

Each seafood sample was placed on a non-conductive, food-safe platform beneath the electrodes. Treatment durations were varied to study time-dependence

- 1 min, 3 min, and 5 min exposure durations
- Distance between electrodes and sample: 3 cm
- Temperature was monitored to confirm non-thermal conditions (below 40°C)

All plasma-treated samples were processed in a biosafety cabinet to ensure aseptic handling.

Microbiological Analysis

1. Sampling Time Points

Microbial analyses were conducted at four time points:

- Day 0 (immediately post-treatment)
- Day 3
- Day 7
- Day 10 (or until spoilage was evident)

Samples were stored at 4 ± 1°C between analyses to simulate cold-chain conditions.

2. Total Viable Count (TVC)

Samples (10 g) were aseptically homogenized in 90 mL of sterile peptone water using a stomacher. Serial dilutions were plated on Plate Count Agar (PCA) and incubated at 30°C for 48 hours.

3. Specific Pathogen Analysis

To assess food safety, selective agars were used

- ***Listeria monocytogenes*:** Oxford agar
- ***Salmonella spp.*:** XLD agar
- ***Escherichia coli*:** MacConkey agar

Plates were incubated under appropriate conditions for 24–48 hours, and colony-forming units (CFU/g) were recorded.

4. Spoilage Organisms

Spoilage-related bacteria (e.g., *Pseudomonas spp.*) were analyzed using Pseudomonas Agar Base with CFC supplement. Results were compared between control and treated samples over the storage period.

Physicochemical Analyses

To evaluate the preservation quality, several chemical and physical properties were analyzed.

1. pH Measurement

pH was measured using a calibrated pH meter. A 10 g portion of each sample was homogenized with 90 mL of distilled water, and pH was measured immediately.

2. Lipid Oxidation (TBARS Test)

Lipid peroxidation was assessed using the thiobarbituric acid reactive substances (TBARS) assay. Malondialdehyde (MDA) concentrations were expressed as mg MDA/kg sample, with values compared across treatment groups and over time.

3. Biogenic Amine Content

High-performance liquid chromatography (HPLC) was used to measure histamine, cadaverine, and putrescine levels. Samples were derivatized with dansyl chloride and analyzed under UV detection.

4. Color Analysis

A colorimeter was used to measure L* (lightness), a* (red/green), and b* (yellow/blue) values to determine any visible changes in appearance due to plasma treatment.

5. Texture Profile Analysis (TPA)

Texture was measured using a texture analyzer with a 50 mm cylindrical probe. Parameters such as hardness, cohesiveness, and springiness were recorded.

Sensory Evaluation

A trained sensory panel (n = 10) evaluated the seafood samples based on

- Appearance
- Odor
- Texture
- Overall acceptability

Sensory tests were conducted under controlled lighting and odor-neutral environments using a 9-point hedonic scale. Panelists were blinded to the treatment conditions. Evaluations were carried out on Day 0 and Day 5 post-treatment.

Plasma Characterization

To better understand the plasma's reactive environment, in-situ plasma diagnostics were performed.

1. Optical Emission Spectroscopy (OES)

Emission spectra were collected during plasma discharge to identify key reactive species (e.g., $O\cdot$, $N\cdot$, $OH\cdot$, NO, O_3). This analysis helped correlate specific plasma species with microbial inactivation levels.

2. Gas Temperature Measurement

Optical methods and thermocouple readings confirmed that the treatment remained within non-thermal ranges (below 40°C), ensuring that microbial reduction was not due to heat.

Data Analysis

All experiments were conducted in triplicate (n = 3), and results are reported as mean ± standard deviation. Statistical analysis was performed using ANOVA (one-way and two-way) followed by Tukey's post hoc test to assess significant differences between treatments ($p < 0.05$ considered significant). Statistical software such as SPSS v25 and GraphPad Prism was used for data handling and visualization.

Experimental Controls and Validation

- **Negative control:** Samples without plasma exposure
- **Positive control:** Heat-treated samples (70°C for 1 minute) for reference microbial inactivation
- **Validation:** Replicate runs on different days to account for environmental or equipment variability
- **Repeatability:** Plasma equipment was calibrated before each use, and electrode cleaning protocols were maintained

Summary of Methodological Rationale

This comprehensive methodological framework was designed to capture both microbial safety and quality aspects of seafood following cold plasma treatment. The use of multiple seafood types ensured applicability across different biological matrices. Combining plasma diagnostics with microbiological and physicochemical analyses allowed for a holistic assessment of treatment efficacy. By including sensory evaluation and shelf-life tracking, the study aligns technological potential with consumer expectations, laying the groundwork for potential industrial application.

Content: Research Design, Subjects, Materials, and Procedures

Research Design

This study employed a mixed-model approach combining exploratory laboratory-scale experiments and semi-industrial pilot-line trials to evaluate the efficacy and mechanisms of cold plasma (CP) in preserving diverse seafood products. It was structured as a randomized block design with treatments and controls, enabling thorough comparison of outcomes across product types and treatment variations.

- **Objective:** To determine how cold plasma treatments—across variables like gas composition, exposure time, and power settings—affect microbial loads, product quality, and shelf stability in fish and seafood.

- **Experimental Groups:** Three seafood types (Atlantic salmon fillets, Pacific white shrimp, blue mussels) × three treatment modalities (no plasma—control; air plasma; argon plasma) × three exposure durations (1, 3, and 5 minutes). Each group comprised triplicate samples.

- **Time Points for Analysis:** Samples evaluated immediately post-treatment (Day 0), and stored at 4 ± 1 °C, were re-evaluated on Days 3, 7, and 10—or until overt spoilage was observed.

This randomized block design allowed for precise estimation of treatment effects and interactions, while the semi-industrial pilot component assessed practical scalability.

Subjects

1. Selection of Seafood Types

- **Atlantic salmon (*Salmo salar*):** Chosen for its high fat content and soft muscle texture, which are sensitive to oxidation and microbial spoilage.
- **Pacific white shrimp (*Litopenaeus vannamei*):** Representative of crustacean shellfish with high surface complexity and microbial affinity.
- **Blue mussels (*Mytilus edulis*):** Selected due to their bivalve shell structure and internal cavity prone to microbial colonization and enzymatic spoilage.

All seafood was sourced from reputable local suppliers certified for food safety and handled under strict cold chain protocols (4 °C) using insulated coolers and ice packs, reaching the lab within 2 hours of procurement.

2. Sample Preparation

Upon arrival, samples were assessed visually and olfactorily; only those with no signs of deterioration or off-odors were retained. Fillets and shrimp were rinsed under sterile, chilled deionized water. Mussels were rinsed and briefly depurated in aerated sterile seawater at 4 °C for 30 minutes to expel gut particulates.

Samples were then portioned into 100-gram replicates. Each portion was placed on sterile trays in a biosafety cabinet for aseptic handling and assigned to treatment groups using randomization software to eliminate bias.

Materials and Equipment

1. Cold Plasma Generation System

A custom-built Dielectric Barrier Discharge (DBD) apparatus was used, featuring

- Two flat aluminum electrodes (10 × 10 cm), one coated with a 3 mm quartz dielectric layer.
- Adjustable AC high-voltage input (20–40 kV) at a frequency of ~20 kHz.
- Electrode gap maintained at 5 mm, monitored via digital calipers.
- Temperature sensors embedded in sample trays to ensure non-thermal operation (≤40 °C).
- Gas-control manifold allowing regulated gas flows (1.5 L/min) of compressed sterile air or high-purity argon (≥99.99%).

2. Consumables and Reagents

- **Microbiological media:** Plate Count Agar (PCA), Oxford agar, Xylose Lysine Deoxycholate (XLD) agar, MacConkey agar, Pseudomonas Agar Base with CFC supplement.
- **Chemical assays:** Thiobarbituric Acid Reactive Substances (TBARS) reagents; biogenic amines (histamine, cadaverine, putrescine) standards; dansyl chloride derivatization kit; calibration buffers for pH.
- **Instrumentation:** Analytical balance (±0.01 g), pH meter, texture analyzer with cylindrical probe, colorimeter (CIELAB), HPLC system with UV detector, optical emission spectrometer (OES), thermocouples, and temperature-tracking data loggers.

3. Sensory Evaluation Setup

- **Panel:** 10 trained evaluators, trained following ISO sensory methods, age 21–50, screened for normal odor and taste perception.

- **Environment:** Standardized sensory booth with neutral lighting and air filtration to minimize cross-sample bias.
- **Materials:** Individual evaluation booths, odorless disposable utensils and trays, palate cleansers.

Procedures

1. Cold Plasma Treatment

1. Ensure electrode surfaces are clean and free from residuals.
2. Power up the DBD system and calibrate voltage, frequency, and gas flow per treatment setup.
3. Place each sample on non-conductive, sterile trays located centrally beneath electrodes.
4. Expose the sample to plasma (air or argon) for the predetermined duration (1, 3, or 5 min).
5. Monitor sample surface temperature using thermocouples to confirm it remains under 40 °C.
6. Immediately transfer treated samples to sterile containers and store at 4 ± 1 °C until analysis.

Control samples are handled identically (placement, handling, packaging) but without plasma exposure.

2. Microbiological Assessment

At each time point

1. Homogenize a 10 g portion in 90 mL sterile peptone water using a stomacher.
2. Prepare serial dilutions and plate:

- PCA for total viable counts (30 °C, 48 hours)
- Oxford agar for *L. monocytogenes* (35 °C, 48 hours)
- XLD agar for *Salmonella* (37 °C, 24 hours)
- MacConkey agar for *E. coli* (37 °C, 24 hours) Pseudomonas Agar with CFC for spoilage organisms (25 °C, 48 hours)

3. Express microbial counts as CFU per gram of sample.

3. Physicochemical Analyses

- **pH:** Homogenize 10 g in 90 mL deionized water and measure using a calibrated pH meter.
- **Lipid Oxidation (TBARS):** React homogenate with TBA reagent; measure absorbance at 532 nm. Express as mg MDA/kg of tissue.
- **Biogenic Amines:** Derivatize homogenized extracts with dansyl chloride and analyze via HPLC, using standard calibration curves.
- **Color (CIELAB):** Record L*, a*, and b* coordinates at three separate points per sample.
- **Texture:** Perform double-compression (50%) profile using a texture analyzer; compute hardness, cohesiveness, springiness.

4. Sensory Evaluation

- Conduct blind, randomized panel sessions on Days 0 and 5.
- Provide each panelist with coded samples in identical portion sizes.
- Evaluate appearance, odor, texture, and overall acceptability on a 9-point hedonic scale.
- Use water and plain bread as palate cleansers between samples.

5. Plasma Characterization

- **Optical Emission Spectroscopy (OES):** During treatment, collect emission spectra to identify reactive species like atomic O, N_2, OH radicals, NO, and ozone.
- **Temperature Logging:** Monitor both sample and tray surfaces throughout treatment to ensure non-thermal conditions maintained.

6. Scaling-Up Trial

In a semi-industrial setting

- Process 5 kg batches in a conveyor-fed DBD module under identical electrical/gas parameters used in lab.
- Evaluate sample uniformity in treatment across conveyor width.
- Perform microbial and physicochemical assessments akin to lab trials to validate real-world applicability.

Data Collection and Statistical Analysis

- Data points collected in triplicate for each combination of sample type, treatment, and time.
- Compute means (± standard deviations) for each metric.
- Use one- and two-way ANOVA, followed by Tukey's post hoc test, to assess significance ($p < 0.05$).
- Statistical software: SPSS v25 and GraphPad Prism used for analysis and graphing.
- In sensory analyses, treat panelists as random effects to model inter-individual variability.

Quality Controls

- **Negative Control:** Untreated samples to account for baseline microbial and quality changes.
- **Positive Control:** Brief heat treatment (70 °C for 1 min) to benchmark maximum microbial kill.
- **Replication and Reproducibility:** Duplicate processing runs on different days; instrument calibration before each run; routine equipment cleaning; cross-check data entry.

Results

This study investigated the efficacy of cold plasma (CP) technology, using both air and argon as carrier gases, in preserving the microbiological and physicochemical quality of three seafood products—Atlantic salmon, Pacific white shrimp, and blue mussels—over refrigerated storage for ten days. The findings are presented below, categorized by key evaluation parameters: microbial reduction, physicochemical properties, sensory characteristics, and plasma characterization.

Microbial Inactivation

1. Total Viable Count (TVC)

Cold plasma treatment significantly reduced microbial load across all seafood samples. Initial TVC at Day 0 averaged 6.2 log CFU/g across all treatments. In control samples (no CP treatment), microbial counts rose steadily over storage, reaching 8.9 log CFU/g by Day 10, indicating progressive spoilage.

In contrast, samples treated with air plasma exhibited markedly slower microbial growth. On Day 3, TVC increased modestly to 6.3 log CFU/g, reaching only 7.2 log CFU/g by Day 10. Argon plasma-treated samples showed the most substantial inhibition, with counts only increasing from 6.2 log CFU/g on Day 0 to 6.7 log CFU/g on Day 10.

This indicates that CP treatment, especially with argon, significantly delays microbial growth without requiring thermal processing. A two-way ANOVA revealed that both treatment type and storage time had statistically significant effects on microbial load ($p < 0.001$), and their interaction was also significant ($p < 0.01$).

2. Pathogen Reduction

Selective enumeration of key foodborne pathogens revealed significant reductions following CP treatment. *Listeria monocytogenes*, *Salmonella spp.*, and *Escherichia coli* were initially detected at 3.0–3.5 log CFU/g on untreated samples. After a 3-minute cold plasma exposure:

- *Listeria monocytogenes* was reduced below detectable levels (<1 log CFU/g) in both shrimp and salmon treated with argon plasma.
- Air plasma reduced *Listeria* to 1.2–1.4 log CFU/g, still a >2 log reduction.
- *Salmonella spp.* and *E. coli* followed similar patterns, with >2.5 log reductions in argon-treated samples and approximately 2 log reductions with air plasma.

These reductions were sustained through storage, with re-growth suppressed in CP-treated samples compared to controls.

3. Spoilage Organisms

Spoilage bacteria (*Pseudomonas spp.* and *Shewanella spp.*) proliferated rapidly in control samples, exceeding 8 log CFU/g by Day 7. However, in CP-treated samples:

- Air plasma delayed spoilage organism growth until Day 7, with counts remaining under 6.5 log CFU/g by Day 10.
- Argon plasma extended this delay further; spoilage organisms stayed below 6 log CFU/g through Day 10.

These results demonstrate cold plasma's potential to prolong shelf life by effectively targeting both pathogenic and spoilage microorganisms.

Physicochemical Properties

1. pH Changes

Initial pH values of the seafood ranged from 6.3 to 6.5. Control samples showed significant increases over storage (up to pH 7.2 by Day 10), likely due to microbial metabolism producing basic compounds (e.g., ammonia). CP-treated samples exhibited more stable pH profiles

- Air plasma-treated samples increased modestly to pH 6.8.
- Argon plasma samples maintained near-original pH values (~6.5), suggesting reduced microbial and enzymatic activity.

2. Lipid Oxidation (TBARS)

The TBARS (thiobarbituric acid reactive substances) assay measured oxidative rancidity. Initial TBARS values were low (~0.3 mg MDA/kg). After 10 days:

- Control samples showed a significant increase (0.8–1.0 mg MDA/kg), especially in fatty fish like salmon.
- Air plasma-treated samples rose to 0.6–0.7 mg MDA/kg.
- Argon plasma-treated samples maintained the lowest values (0.4–0.5 mg MDA/kg), indicating better oxidative stability.

This suggests that CP, particularly with inert gases like argon, does not significantly promote lipid oxidation, preserving product quality.

3. Biogenic Amine Formation

Biogenic amines such as histamine, cadaverine, and putrescine were measured via HPLC. Control samples exhibited rapid accumulation, with histamine levels reaching 150 mg/kg in mussels by Day 10—surpassing safety thresholds in some regulatory jurisdictions.

CP-treated samples showed substantially lower biogenic amine levels

- Histamine in argon-treated mussels stayed below 50 mg/kg throughout.
- Cadaverine and putrescine were also significantly reduced in plasma-treated shrimp and salmon.

These reductions align with the suppressed microbial activity observed, since amine production is microbially driven.

4. Texture and Color

Texture profile analysis revealed that CP had minimal impact on textural properties. Hardness and cohesiveness in shrimp and salmon remained unchanged across plasma treatments. Mussels showed slightly increased firmness after 5-minute plasma exposure, but this was not perceived negatively in sensory testing.

Color measurements (L*, a*, b*) showed negligible changes in lightness or hue values across all treatments. This supports the non-destructive nature of CP and confirms its suitability for fresh seafood products, where appearance is critical for consumer acceptance.

Sensory Evaluation

Sensory assessments on Days 0 and 5 revealed important trends

- Control samples were rated poorly by Day 5, particularly for odor (mean 4.2/9) and texture (4.6/9).
- Air plasma-treated samples maintained acceptable ratings across all categories through Day 5.
- Argon plasma-treated samples scored highest overall (appearance: 8.1, odor: 7.9, texture: 8.0), with no detectable off-odors or discoloration.

Panelists could not distinguish CP-treated samples from fresh untreated controls on Day 0, indicating no sensory compromise from treatment. No panelist reported metallic taste or residual gas flavor, addressing a common consumer concern.

Plasma Characterization

Optical Emission Spectroscopy (OES) identified the presence of reactive species generated during CP treatment:

- Air plasma generated reactive oxygen species (ROS) such as $O\cdot$, $OH\cdot$, and ozone (O_3), along with nitrogen-based species like NO and NO_2.
- Argon plasma generated a more targeted spectrum with fewer ROS but higher concentrations of metastable argon species, which contributed to efficient microbial disruption with less oxidative stress.

These plasma signatures correlated with microbiological outcomes: air plasma being moderately effective but

inducing more oxidative side effects; argon plasma offering superior microbial inactivation with lower collateral degradation.

Shelf-Life Extension

Shelf-life estimation was based on microbiological thresholds (TVC < 7 log CFU/g and acceptable sensory scores). Control samples reached spoilage thresholds by Day 5–6. In contrast:

- Air plasma-treated samples remained acceptable until Day 8.
- Argon plasma-treated samples-maintained quality and safety until at least Day 10.

This indicates a potential 3–5 day extension in refrigerated shelf life using CP treatment, especially with inert gases.

Summary of Key Findings

- Cold plasma, particularly with argon gas, significantly reduces microbial loads in seafood without heat or chemical additives.
- Pathogens and spoilage organisms are effectively controlled, enhancing food safety and shelf life.
- Physicochemical properties such as pH, oxidation, texture, and color remain stable, confirming the non-destructive nature of CP.
- Sensory quality is preserved, with no off-flavors or undesirable visual changes.
- Plasma-generated reactive species differ by gas composition, influencing treatment efficacy and oxidative side effects.

Discussion

The present study comprehensively evaluated the potential of cold plasma (CP) technology as a non-thermal preservation method for fish and seafood products. The results demonstrate that CP, particularly when generated with argon gas, offers significant advantages in microbial inactivation, physicochemical stability, sensory preservation, and shelf-life extension.

Interpretation of Results

The observed reductions in total viable counts and targeted foodborne pathogens such as *Listeria monocytogenes*, *Salmonella spp.*, and *Escherichia coli* affirm cold plasma's potent antimicrobial efficacy. These findings align with prior research, such as Misra *et al*. (2019) [7], who demonstrated that dielectric barrier discharge plasma can achieve 3–5 log reductions in microbial populations on seafood surfaces without heat damage. The higher microbial reductions observed with argon plasma correspond with reports by Boehm *et al*. (2017) [1], indicating that inert gas plasmas generate reactive species that penetrate microbial cells more effectively while minimizing oxidative damage to food matrices.

The stable pH values and suppressed lipid oxidation in CP-treated samples are particularly noteworthy. Oxidative spoilage is a major quality concern in fatty fish, as documented by Falowo *et al*. (2014) [2]. Our results show that argon plasma induces less lipid peroxidation than air plasma, consistent with the findings of Siddiqui *et al*. (2020) [10], who reported that nitrogen or argon plasmas limit free radical formation compared to oxygen-containing plasmas. This reduces rancidity risks and helps maintain nutritional quality.

Moreover, the significant reduction in biogenic amines in treated samples underscores the indirect microbial inhibition mechanism, since amines primarily arise from bacterial decarboxylation of amino acids (Suzzi & Gardini, 2003) [11]. The decrease in histamine and other amines enhances product safety, especially for species prone to scombroid poisoning like tuna and mackerel (Taylor, 1996) [13].

Sensory evaluation results, showing no negative impact on odor, texture, or appearance, reinforce CP's non-destructive nature. This is critical for consumer acceptance, where even minimal sensory alterations can deter purchase (Jayasinghe *et al*., 2020) [5]. The lack of metallic or off-flavors dispels common misconceptions about plasma treatments imparting undesirable tastes (Niemira & Sites, 2008) [9].

Comparison to Previous Studies

Several studies corroborate the antimicrobial effectiveness and quality preservation of cold plasma in seafood. For example, Ziuzina *et al*. (2015) reported similar microbial inactivation in oysters using cold plasma, with no compromise on organoleptic properties. Lee *et al*. (2018) [6] demonstrated shelf-life extension in shrimp treated with atmospheric plasma, consistent with our findings of up to five additional days of refrigerated storage.

However, some variability exists in reported results, largely dependent on plasma source, gas composition, exposure time, and seafood type (Niemira, 2012) [8]. This study's use of both air and argon gases provides comparative insights often lacking in literature, highlighting argon's superiority for seafood preservation.

Implications

The implications for the seafood industry are significant. Cold plasma offers a sustainable, chemical-free, and low-energy alternative to traditional preservation methods like freezing, irradiation, or chemical additives, which often alter product quality or consumer perception (Tappi *et al*., 2021) [12]. The ability to extend shelf life by several days can reduce food waste and economic losses along the supply chain (FAO, 2021).

Moreover, CP's efficacy against both spoilage organisms and pathogens addresses food safety concerns, potentially reducing reliance on antibiotics and preservatives, which aligns with increasing regulatory and consumer demands for clean-label products (Huang *et al*., 2021) [4].

Limitations and Future Research

Despite promising outcomes, several limitations warrant discussion. First, the study was limited to three seafood types; results may vary with other species, especially those with different surface properties or microbial flora. Second, the scalability of plasma systems remains challenging, as semi-industrial trials showed some variation in treatment uniformity. Further engineering refinements are necessary to ensure consistent exposure on larger processing lines.

Additionally, while sensory evaluation was conducted with trained panelists, consumer acceptability studies across broader demographics are needed to validate market readiness. Long-term storage effects beyond 10 days and the impact on nutritional compounds such as vitamins and omega-3 fatty acids should be investigated.

Lastly, mechanistic understanding of plasma interactions with complex seafood matrices remains incomplete. Advanced molecular and metabolomic analyses could elucidate subtle biochemical changes, aiding optimization of treatment parameters.

Conclusion

This study confirms cold plasma technology, especially argon-based dielectric barrier discharge plasma, as an effective non-thermal preservation method for fish and seafood. CP treatment substantially reduces microbial loads, including critical pathogens, thereby enhancing food safety. The process effectively delays spoilage organism growth, extending refrigerated shelf life by up to five days compared to untreated controls.

Importantly, cold plasma does not compromise key quality attributes such as pH, lipid stability, texture, color, or sensory acceptability. These results demonstrate CP's capacity to maintain freshness and sensory appeal without introducing heat damage or chemical residues. The use of argon as the working gas offers superior microbial inactivation with minimal oxidative side effects, highlighting the importance of plasma composition in treatment efficacy.

The findings have significant implications for the seafood processing industry by providing a clean-label, sustainable alternative to conventional preservation techniques. This can reduce food waste, enhance safety, and meet consumer demand for minimally processed seafood products.

Nonetheless, challenges remain in scaling technology to commercial processing lines and fully understanding the biochemical impacts on seafood matrices. Future research should focus on optimizing plasma system design, exploring effects on a wider range of seafood species, and conducting consumer acceptance studies. Integrating molecular analyses will further refine mechanistic insight.

In summary, cold plasma technology holds strong promise for revolutionizing seafood preservation, aligning with modern food safety and sustainability goals.

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