COLD PLASMA TECHNOLOGY FOR MICROBIAL DECONTAMINATION OF SPICES: ANALYSIS, EFFICACY AND DESIGN

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Degree of Doctor of Philosophy

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Thesis/Dissertation submitted in partial fulfillment of the requirements for the degree

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DECLARATION

I declare that this is my own work, and this thesis/dissertation does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any other University or Institute of higher learning, and to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where the acknowledgement is made in the text. I retain the right to use this content in whole or part in future works (such as articles or books).

Signature: Date: 30th April 2025

The above candidate has carried out research for the PhD/~~MPhil/Masters~~ thesis/dissertation under my supervision. I confirm that the declaration made above by the student is true and correct.

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Signature of the Supervisor: Date: 30th April 2025

DEDICATION

"To my parents, my husband, and my two sons, for their unwavering sacrifice."

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- FP 125 Surface Sterilization of spices using non-thermal technologies
- TG 19/170 Spray drying of selected fruits, vegetable juices and yam pulps and innovative spray-dried powder-based products and development
- TG 21/222 Development of novel juice extraction technique for high-quality extracts from underutilized agro-based commodities, extract-based fermented/powder products

ABSTRACT

Spices and Foods are Inseparable. Sri Lanka is renowned for its spices, with the spice trade valued at approximately USD 923,000. Spices hold universal appeal in food and beverages. However, in recent decades, food safety outbreaks caused by contaminated spices have become increasingly common. Both thermal and non-thermal technologies have been applied to reduce microbial loads, with varying levels of effectiveness. This study aimed to investigate how microbial contaminants in spice seeds or particulates can be effectively eliminated while preserving the essential qualities of the spices in real-world conditions. The specific objectives were to identify the limitations of existing cold plasma systems in spice decontamination, design a spice-specific cold plasma sterilization system, and evaluate safety and quality changes in spices after treatment with cold plasma technology. The research began by assessing microbial contamination levels in commercial spice samples and analyzing spice processing from a farm-to-plate life cycle perspective. Spices showed significant contamination by aerobic bacteria and fungi. Additionally, a novel microflora associated with black pepper seeds and red chili powder was identified, with many species reported for the first time. Considering the microbial contamination levels and homogeneity of the samples, black pepper seeds, black pepper powder and red chili powder were selected for further investigations of the research. The study evaluated cold plasma technology, a novel decontamination method gaining global attention, through in-depth process analysis. The performance of existing cold plasma technologies was tested using two systems: Gliding Arc Plasma Discharge (GAPD) at 15 kV and 50 Hz reduced the Aerobic Plate Count (APC) and Yeast and Mould (Y&M) by 73% and 93% after 15 minutes, respectively. There were minor losses of volatile oils and some piperine degradation in black pepper seeds. The process led to a temperature rise of 65.2°C. Low-Pressure Cold Plasma (LPCP) at 13.56 MHz and 0.3 mbar, operating at 250 W for 20 minutes, reduced APC and Y&M by 90% and 99%, respectively, with a temperature increase of up to 41°C. When using nitrogen and air, LPCP reduced APC by 96% and 81%, respectively, and eliminated Y&M within 10 minutes in black pepper powder. However, a 60% loss of volatile oils was also observed. For red chili powder, LPCP reduced APC by 90% and Y&M by 99% in 9 minutes, but the treatment caused significant colour loss. Key drawbacks of the tested cold plasma systems included high temperatures, limited sample capacity, inconsistent microbial inactivation, and considerable quality degradation, especially in powdered spices. To address these challenges, a Rotary Dielectric Barrier Discharge (RDBD) system powered by radio frequency was developed. This system uses a radio frequency unit to convert gas inside a glass reactor into glow-like plasma, enabling uniform spice treatment near atmospheric pressure. The reactor chamber rotates at 10 rpm, increasing spice exposure to reactive plasma species. Perforated electrodes prevent arc discharges, while the air gap between the electrodes and the glass wall acts as a dielectric barrier, ensuring a uniform glow discharge for consistent treatment. Trials were conducted using black pepper seeds, both naturally contaminated and inoculated

with Bacillus cereus (ATCC 11778), comparing the performance of RDBD with LPCP. The decimal reduction times (D-values) for black pepper seeds were 4.35 minutes (GAPD), 6.41 minutes (LPCP), and 4.76 minutes (RDBD). RDBD demonstrated the least negative impact on quality attributes. Plasma characteristics for RDBD and LPCP were simulated using COMSOL Multiphysics. The time-averaged maximum electron temperature and electron density for RDBD were 1.2 eV and 1.4×10^{17} m^{-3}, respectively, while the center of the LPCP chamber reached over 2.67 eV and 10^{14} m^{-3}. Higher electron temperatures (~10 eV) can etch spice surfaces, causing physicochemical changes. Thus, RDBD's lower electron temperature (1.2 eV) ensures minimal surface damage during decontamination. Meanwhile, its high electron density enhances interaction with the spice surface, increasing microbial inactivation. Both LPCP and RDBD systems reached electric potentials capable of rupturing bacterial cell walls (26–110 V), supporting their effectiveness in microbial reduction.

Keywords: microbial contamination, black pepper, red chili, Gliding Arc Plasma Discharge, Low-Pressure Cold Plasma, Rotary Dielectric Barrier Discharge Reactor

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LIST OF ABBREVIATIONS

Abbreviation	Description
AC	Alternating Current
APC	Aerobic Plate Count
ASTA	American Spice Trade Association
ATCC	American Type Culture Collection
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
CCP	Capacitively Coupled Plasma
CFU	Colony Forming Units
CPT	Cold Plasma Technology
DBD	Dielectric Barrier Discharge
DC	Direct Current
DCSBD	Diffuse Coplanar Surface Barrier Discharge
DGA	Dichloro Glycerol Agar
DNA	Deoxyribonucleic Acid (RNA)
DSC	Differential Scanning Calorimetry
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drugs Administration
GAPD	Gliding Arc Plasma Discharge
GAP	Good Agricultural Practices
GC – FID	Gas Chromatography Flame Ion Detection
GC – MS	Gas Chromatography Mass Spectroscopy
GMP	Good Manufacturing Practices
HPP	High Pressure Processing
ICP	Inductively Coupled Plasma
IR	InfraRed
LPCP	Low-Pressure Cold Plasma
MT	Metric Tons
NCBI	National Centre for Biotechnology Information

OES	Optical Emission Spectroscopy
PCA	Plate Count Agar
PCD	Programmed Cell Death
PDE	Partial Differential Equations
PEF	Pulsed Electric Field
PSD	Particle Size Distribution
RDBD	Rotary Dielectric Barrier Discharge
RF	Radio Frequency
RHS	Reactive Hydrogen Species
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
UV	Ultra Violet
WHO	World Health Organization
XRD	X Ray Diffractograms
Y&M	Yeasts and Moulds
1D	One Dimensional
2D	Two Dimensional

NOMENCLATURE

Γ	Incomplete gamma function
Γ_e	Electron flux
Γ_ε	Electron energy flux
h_V	Energy of the photons
E_o	Amplitude of the electromagnetic field
$E_{s,c}$	Critical surface energy
E_s	Surface energy
F_t	Tensile strength of the cell membrane
I_D	Discharge current
K_B	Boltzmann constant
N_n	Negative ions density
N_o	Initial microbial counts
N_p,	Positive ions density
N_t	Microbial counts after time t
P_{abs}	Power absorption
Q_{gen}	Generalized heat source (W/m^3)
R_e	Production rate of new electrons
R_t	Varying load resistance.
S_{en}	Energy loss or gain due to inelastic collisions ($V/m^3.s$)
T_e	Electron temperature
T_g	Gas temperature
T_o	Peak enthalpy
T_o	Onset temperature
T_v	Vibrational temperature
V_B	Breakdown voltage
V_s	Voltage at output of voltage source
e	Electron charge
k_1	Rate of microbial inactivation
m_e	Electron mass
n, n_e, N_e	Electron density

ε	Dielectric permittivity
ε_o	Vacuum permittivity
$\emptyset$	Mean electron energy (eV)
ω	Angular frequency of the electromagnetic field
A	Viable microorganisms (CFU/g) before treatment
Ar	Argon atom
B	Viable microorganisms (CFU/g) after treatment
e	Electron
Ɛ	Mean electron energy
h	Shell thickness
M	Moisture content of the sample (%)
r_1	Number of colonies counted in replicate 1 of 10^{-2}
R_1	Number of colonies counted in replicate 1 of 10^{-5}
r_2	Number of colonies counted in replicate 2 of 10^{-2}
R_2	Number of colonies counted in replicate 2 of 10^{-5}
r_3	Number of colonies counted in replicate 1 of 10^{-3}
R_3	Number of colonies counted in replicate 1 of 10^{-6}
r_4	Number of colonies counted in replicate 2 of 10^{-3}
R_4	Number of colonies counted in replicate 2 of 10^{-6}
V_o	Volume of oil (mL)
V_w	Volume of water (mL)
W	Weight of the sample (g)
ύ	Average velocity of electrons
D	Diffusivity (m^2/s)
E	Young modulus of the bacterium wall
E	Electric field (V/m)
N	Gas moles
P	Pressure
Q	Electrostatic potential of a bacterium
Q	External heat source (W/m^3)
R	Shell radius
R	Approximate radius of the microbe

T	Absolute temperature
d	Distance between electrodes
k	Electrical charge of bacterium
m, m_e	Mass of an electron
q	Unit charge (C)
r	Radius of the hemispherical shape of the microbe
r	Diameter of the atom
u	Mass averaged velocity field (m/s)
v	Electron neutral collision frequency
v	Volume
Δ	Thickness of the cell wall
λ	Mean free path of electrons
$\phi(V)$	Surface potential required to rupture cell wall

LIST OF APPENDICES