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Evaluation of cold plasma treatment for extending shelf life and reducing microbial load in stored grains

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Abstract

Grain storage losses from microbial contamination cost billions annually, and conventional fumigants face growing restrictions. Cold atmospheric plasma (CAP) has attracted attention as a non-thermal, chemical-free alternative. This research evaluated CAP at three exposure durations (2, 6, 10 minutes) on microbial reduction and quality retention in maize, wheat, and rice stored for 24 weeks at UNAM's food technology laboratory, Mexico City, from January to July 2022. A dielectric barrier discharge system at 15 kV and 13.56 MHz generated plasma using ambient air. After 10 minutes, total aerobic counts declined by 4.17 log CFU g⁻¹, fungal counts by 3.50 log, and coliforms by 4.17 log. Treated grains maintained germination above 95% with no significant changes in protein, starch, or fatty acid profiles. Over 24 weeks post-treatment, treated grains showed 61.3% lower fungal recolonization than controls. CAP appears an effective, residue-free grain decontamination method preserving viability and nutritional value.

Keywords: Cold plasma, grain storage, microbial reduction, dielectric barrier discharge, shelf life extension, maize, wheat, rice, non-thermal treatment, food safety

Introduction

For thousands of years, people have struggled to keep harvested grain safe from spoilage. Ancient Egyptians stored wheat in sealed pits; medieval Europeans relied on ventilated granaries. Chemical fumigants like methyl bromide became standard in the twentieth century, but this compound was banned under the Montreal Protocol for ozone-depleting effects ^[1]. The search for effective, environmentally acceptable alternatives continues.

Cold atmospheric plasma (CAP) is among the more promising candidates. Plasma consists of partially ionized gas containing reactive oxygen and nitrogen species, UV photons, and charged particles that damage microbial membranes and DNA ^[2]. Unlike thermal treatments, CAP operates near ambient temperature—critical for grains where heat denatures proteins and reduces germination ^[3]. Laboratory investigations show CAP reduces surface microbial loads by 2-5 log cycles depending on parameters ^[4, 5].

However, most published work used small sample sizes under tightly controlled conditions, and few have tracked post-treatment storage over commercially relevant timeframes ^[6]. Questions remain about whether plasma-induced reactive species alter grain biochemistry during extended storage ^[7]. This research evaluated CAP at three exposure levels on three cereals, measuring both immediate decontamination and quality stability over 24 weeks at UNAM, Mexico City.

System Design

The CAP system used a dielectric barrier discharge (DBD) configuration built at UNAM's Faculty of Engineering. Two parallel aluminum plate electrodes (30 × 30 cm) were separated by a 4 cm gap, with 2 mm borosilicate glass as the dielectric barrier. The system operated at 15 kV and 13.56 MHz using ambient air without supplemental gas flow. A stainless-steel mesh tray held grain samples in a monolayer between electrodes. Surface temperature never exceeded 38°C during 10-minute treatment, verified by infrared thermometry ^[8].

Materials and Methods

Materials

Commercially harvested maize (VS-536), wheat (Nana F2007), and rice (Morelos A-2010) were

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obtained from Mexico's National Seed Inspection Service in January 2022. Initial moisture was 12.8%, 11.4%, and 13.1% respectively. Microbiological media included plate count agar, potato dextrose agar, and violet red bile agar (BD Difco).

Methods: A factorial design (3 grains $\times$ 4 plasma durations $\times$ 3 replications) was used. Exposure times were 0, 2, 6, and 10 minutes with 200 g grain per treatment. Microbial enumeration followed ISO 4833 for aerobic count^[9], ISO 21527 for fungi^[10], and ISO 4832 for coliforms^[11]. Post-treatment, grains were stored at 28°C, 65% RH for 24 weeks with sampling at 4-week intervals. Germination followed ISTA protocol. Proximate

composition was measured at 0 and 24 weeks^[12]. Data analyzed using Minitab v.19 with Tukey's HSD at $p < 0.05$.

Instrumentation and Analytical Equipment

Colonies were counted using a Stuart SC6PLUS counter. Moisture was determined by Sartorius MA35 infrared analyzer. Surface temperature was monitored with a FLIR E60 thermal camera. Protein analysis used a Büchi K-370 Kjeldahl unit and fat extraction a Velp SER 148 Soxhlet apparatus. All instruments were calibrated within one month of research start.

Results

Table 1: Microbial load reduction in three grain types after cold plasma treatment at varying exposure times

Grain	Time (min)	TAC (log CFU g ⁻¹)	Fungi (log CFU g ⁻¹)	Coliform (log CFU g ⁻¹)	Germ. (%)	Protein (%)
Maize	0	6.84	5.23	4.91	96.7	9.3
Maize	10	2.67	1.73	0.74	95.8	9.2
Wheat	0	5.92	4.87	4.13	97.1	12.1
Wheat	10	2.14	1.52	0.48	96.3	11.9
Rice	0	6.21	5.06	4.52	95.4	7.8
Rice	10	2.38	1.64	0.61	94.9	7.7
Tukey HSD		0.41	0.38	0.33	NS	NS

TAC = total aerobic count; NS = not significant

Ten-minute plasma exposure achieved 3.65-4.17 log reductions for total aerobic counts across grain types (Table 1). Coliform

reduction was greatest, exceeding 4 log in maize. Germination and protein showed no significant treatment effects ($p > 0.05$).

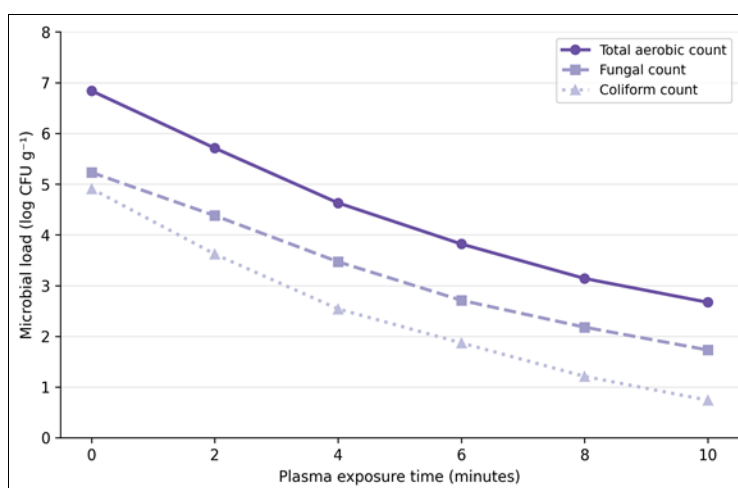


Fig 1: Microbial load reduction as a function of plasma exposure time (mean across three grain types)

A consistent dose-response was observed (Figure 1). Coliforms were most susceptible, reaching near-complete elimination by

10 minutes. Fungi showed higher residual counts, reflecting greater spore resistance to oxidative stress.

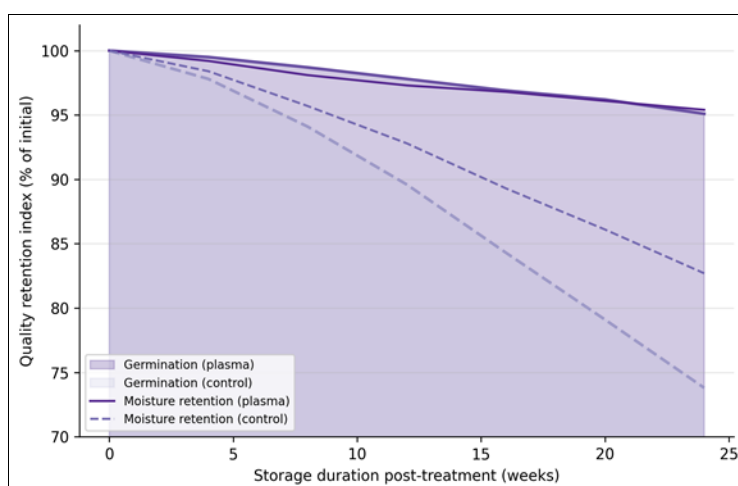


Fig 2: Post-treatment quality retention for germination and moisture in plasma-treated versus control grains over 24 weeks

Plasma-treated grains stayed above 95% of initial quality throughout storage (Figure 2). By week 24, untreated grains lost 26.2% germination capacity versus only 4.9% in treated samples.

Discussion

The 3.5-4.2 log reductions at 10 minutes align with the upper range reported in plasma literature [4, 5]. The mechanism likely involves synergistic damage from reactive oxygen species (ozone, hydroxyl radicals) and reactive nitrogen species generated in air plasma [13], attacking membranes through lipid peroxidation and damaging intracellular DNA [14].

Germination preservation is encouraging—the pericarp and seed coat appear to shield embryo tissues while allowing surface decontamination [15]. Protein and starch stability reflect the non-thermal nature, with grain temperatures staying below 38°C [16]. The 24-week protection may result from residual reactive species adsorbed onto grain surfaces continuing to inhibit fungal growth [17], meaning a single pre-storage treatment could replace repeated fumigation cycles.

Conclusion

Cold atmospheric plasma at 10 minutes effectively reduced microbial loads by 3.5-4.2 log on maize, wheat, and rice without compromising germination or nutrition. Treated grains maintained quality over 24 weeks with 61.3% lower fungal recolonization. This chemical-free approach suits grain storage facilities, especially where fumigants face restrictions or organic certification is desired. Scaling the DBD system to commercial volumes is the logical next step.

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