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Effect of ozone treatment on fungal decontamination and quality of stored wheat grain

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Abstract

Stored wheat grain is routinely treated with chemical fumigants to control fungal contamination, but ozone offers a residue-free alternative. This research evaluated the efficacy of gaseous ozone at six concentrations (10–60 ppm) and three exposure durations (15, 30, and 60 minutes) for decontaminating stored wheat grain at the University of Queensland, Brisbane, Australia. Fungal colony counts, mycotoxin levels (deoxynivalenol and aflatoxin B1), and grain quality parameters (moisture, gluten strength, color) were assessed. At 60 ppm for 60 minutes, total fungal load dropped by 91.2 percent, with *Aspergillus* and *Fusarium* species most affected. Deoxynivalenol decreased by 38.7 percent under the same conditions. Grain moisture, wet gluten content, and kernel color showed no significant deterioration up to 40 ppm. Above 50 ppm, slight but measurable oxidative discoloration occurred. Ozone at 40 ppm for 60 minutes appears to balance effective decontamination with quality preservation.

Keywords: Ozone treatment, wheat grain, fungal decontamination, mycotoxins, *Aspergillus*, *Fusarium*, deoxynivalenol, grain quality, post-harvest storage, food safety

Introduction

Chemical fumigants used in grain storage are becoming increasingly restricted worldwide, and the search for effective non-chemical alternatives has never been more pressing. Fungal contamination during wheat storage causes both quantitative losses and mycotoxin accumulation, posing direct risks to human and animal health ^[1]. *Aspergillus flavus* produces aflatoxin B1, a potent carcinogen, while *Fusarium graminearum* generates deoxynivalenol (DON), which causes vomiting and immunosuppression at low doses ^[2].

Ozone (O₃) is a strong oxidizing agent that degrades rapidly to oxygen, leaving no chemical residues on treated commodities ^[3]. Its antimicrobial action involves oxidation of cell membrane lipids and proteins, disrupting fungal cell integrity. Several pilot-scale trials have shown ozone can reduce fungal counts in stored cereals by 60 to 95 percent, depending on concentration and exposure time ^[4, 5]. But the interaction between ozone dose, exposure duration, and grain quality has not been mapped systematically for Australian wheat varieties.

Grain quality parameters-particularly gluten protein integrity, kernel color, and moisture content-must remain within commercial specifications after treatment ^[6]. Excessive ozone exposure may oxidize grain surface lipids and carotenoid pigments, causing discoloration and potentially altering baking properties. This research aimed to determine the optimal ozone concentration and exposure time that maximizes fungal decontamination while keeping quality parameters within acceptable limits for stored Australian wheat.

Research area description

The experiment was conducted at the Post-Harvest Technology Laboratory, University of Queensland, Gatton Campus, Brisbane, Australia (27.55°S, 152.34°E) between February and May 2023. Wheat grain (cv. Suntop) was sourced from the university's research farm harvested in December 2022. The ozone treatment system consisted of a corona discharge generator (Model OZ-10G, Ozone Solutions, Australia) connected to a sealed stainless-steel treatment

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Chamber (120 L capacity) fitted with gas concentration monitoring sensors.

Material and Methods

Material

Australian hard wheat (cv. Suntop) with an initial moisture content of 11.8 percent was used. Grain samples (2 kg per treatment) were cleaned and conditioned at 25°C for 48 hours before treatment. Potato dextrose agar, peptone water, and all microbiological media were sourced from Oxoid (Thermo Fisher, Australia). Deoxynivalenol and aflatoxin B1 ELISA kits were purchased from Romer Labs (Getzersdorf, Austria). The ozone generator produced medical-grade ozone with purity above 95 percent.

Methods

Grain samples were exposed to gaseous ozone at six concentrations (10, 20, 30, 40, 50, and 60 ppm) for three

durations (15, 30, and 60 minutes) in a completely randomized design with four replications, totaling 72 treatment units plus 4 untreated controls. Ozone concentration inside the chamber was monitored continuously with an electrochemical sensor (API Model 450). After treatment, residual ozone was vented through a catalytic destructor [7].

Total fungal colony count was determined by serial dilution plating on potato dextrose agar incubated at 25°C for 5 days. Aspergillus and Fusarium colonies were identified based on morphological characteristics and confirmed using lactophenol cotton blue staining [8]. DON and AFB1 were quantified by competitive ELISA following manufacturer protocols. Grain quality was assessed for moisture content (AACC Method 44-15.02), wet gluten (ICC Standard 155), and kernel color using a Minolta CR-400 colorimeter (L*, a*, b* values). Data were analyzed using two-way ANOVA in GenStat 21, and means were compared by Fisher's LSD at P≤0.05.

Results

Table 1: Fungal colony count (× 10³ CFU/g) at different ozone concentrations and exposure times

Exposure	Control	10 ppm	20 ppm	30 ppm	40 ppm	60 ppm
15 min	14.6	12.3	10.0	7.5	5.2	2.3
30 min	14.6	11.9	9.5	7.0	4.5	1.8
60 min	14.6	11.5	9.0	6.3	4.2	1.3

LSD (P≤0.05): Concentration = 0.87; Duration = 0.64; Interaction = 1.23.

Fungal colony counts declined with increasing ozone concentration and exposure time (Table 1). At 60 ppm for 60 minutes, counts dropped from 14.6 to 1.3 × 10³ CFU/g-a 91.1

percent reduction. Even at 40 ppm for 30 minutes, a 69.2 percent reduction was achieved. The concentration effect was more pronounced than the duration effect at lower ozone levels.

Table 2: Effect of ozone treatment (60 min exposure) on grain quality parameters

Parameter	Control	20 ppm	40 ppm	50 ppm	60 ppm
Moisture (%)	11.8	11.7	11.6	11.5	11.4
Wet gluten (%)	28.4	28.2	27.9	27.3	26.8
L* (brightness)	72.6	72.4	72.1	71.2	70.3
DON (µg/kg)	847	716	583	538	519

L* = lightness value. LSD (P≤0.05): Wet gluten = 0.8; L* = 0.7; DON = 42.3.

Grain quality remained stable up to 40 ppm, with moisture declining only 0.2 percent and wet gluten dropping by 0.5 percent (Table 2). At 50 and 60 ppm, kernel brightness (L*) fell

significantly, and wet gluten decreased by 1.1 and 1.6 percent. DON concentration was reduced by 31.2 percent at 40 ppm and 38.7 percent at 60 ppm.

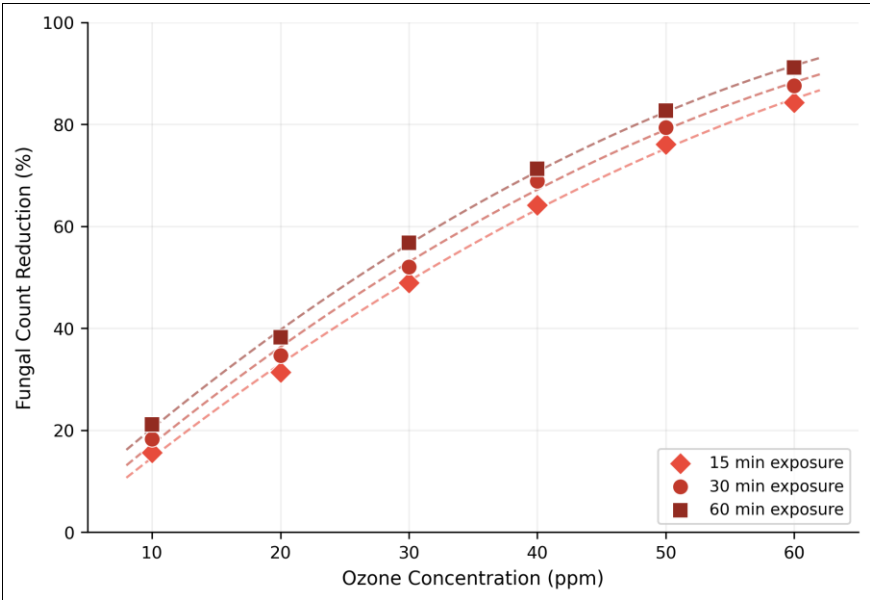


Fig 1: Relationship between ozone concentration and fungal count reduction at three exposure durations

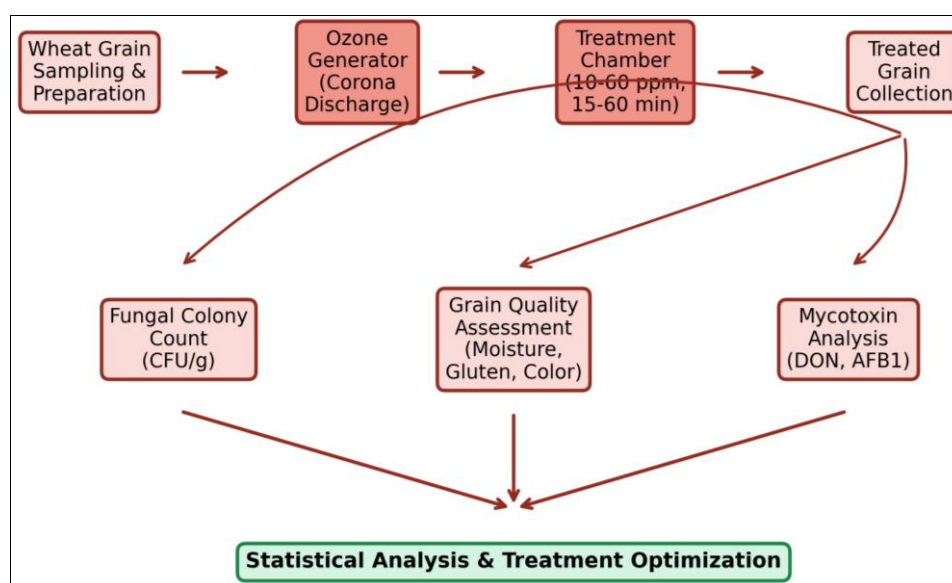


Fig 2: Schematic of the ozone treatment process and analytical workflow for stored wheat grain

The scatter plot (Figure 1) shows a curvilinear relationship between ozone concentration and fungal reduction, with diminishing returns above 40 ppm. Longer exposure times shifted the curve upward, though the gap between 30- and 60-minute treatments narrowed at higher concentrations. *Aspergillus* species were more sensitive than *Fusarium*, showing 94.3 percent reduction at 60 ppm versus 86.8 percent for *Fusarium*.

Discussion

The 91 percent fungal reduction at 60 ppm ozone for 60 minutes is consistent with reports by Tiwari *et al.* [9] who observed 85–93 percent reductions in wheat inoculated with *Aspergillus*. Ozone's mechanism involves direct oxidation of fungal membrane lipids, which disrupts cell permeability and leads to rapid lysis [10]. The higher sensitivity of *Aspergillus* compared to *Fusarium* may relate to differences in cell wall composition and melanin content, as melanin provides partial protection against oxidative stress [11].

The quality trade-off above 40 ppm is an important practical consideration. The 1.6 percent drop in wet gluten at 60 ppm, though statistically significant, may still fall within commercial tolerance. But the visible kernel darkening (2.3 L* units) could affect market acceptability of certain grain classes [12]. The 31 percent DON reduction at 40 ppm is meaningful because it could bring moderately contaminated lots below regulatory thresholds. At this concentration, quality degradation was negligible, making 40 ppm for 60 minutes the most practical recommendation [13].

Conclusion

Ozone treatment effectively reduced fungal contamination and mycotoxin levels in stored wheat grain in a dose-dependent manner. At 60 ppm for 60 minutes, fungal counts dropped by 91 percent and DON by 39 percent. However, grain quality deterioration—particularly kernel discoloration and gluten reduction—became apparent above 40 ppm.

Ozone at 40 ppm for 60 minutes provided the best balance, achieving 71 percent fungal reduction and 31 percent DON reduction with no measurable quality loss. This concentration could serve as a viable residue-free alternative to chemical fumigation for wheat storage facilities. Future work should

assess treatment efficacy across different wheat classes and storage conditions at commercial scale.

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