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In package atmospheric cold plasma outperforms direct exposure for microbial decontamination of bread

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Abstract
Bread is a high water activity staple that is recontaminated by airborne bacterial and fungal propagules as soon as it leaves the oven, and its short mould free shelf life is conventionally managed with chemical preservatives that increasingly conflict with clean label expectations. This study evaluated a pins to parallel plate high voltage atmospheric cold plasma (CAP) reactor as a non thermal, residue free surface decontamination step for freshly baked bread. Bread cubes (5 × 5 × 5 cm) were exposed to an atmospheric glow discharge struck across a 40-45 mm inter electrode gap at 25 kV for 30, 45 and 60 s, either directly (T_1-T_3) or after sealing in the retail package (A_1-A_3), and compared against an untreated control. Moisture content, total plate count (TPC), yeast and mould count and 9 point hedonic sensory scores were determined. TPC fell from 1.7×10^5 cfu/g in the control to 0.5×10^5 cfu/g (0.53 log; −71%) after 60 s of direct exposure and to 0.3×10^5 cfu/g (0.75 log; −82%) after 60 s of in package exposure. Yeast and mould became undetectable after 60 s of direct exposure but after only 45 s of in package exposure. Moisture content was consistently and highly significantly ($P \leq 0.01$) higher in the in package samples (13.05-13.42%) than in directly exposed samples (12.05-12.13%) at every exposure time; within the in package series moisture declined significantly with treatment time ($F = 13.392$, $P \leq 0.01$), whereas the directly exposed series showed no significant time effect ($F = 1.311$, NS). Sensory scores declined progressively with direct exposure beyond 30 s but were retained in the in package series, which peaked at 45 s. Confining the discharge within the package therefore delivered a greater microbial reduction at a lower moisture and sensory cost than open air exposure, and 45-60 s at 25 kV in package is identified as the most favourable operating window for pins to parallel plate CAP treatment of bread.

Keywords: Atmospheric cold plasma, pins to parallel plate, in package treatment, brea, microbial decontamination and shelf life extension

1. Introduction
Bread is inexpensive, ubiquitous and nutritionally important, yet among the most perishable products on the retail shelf. Baking is effectively a pasteurisation, core temperatures of 95-98 °C destroying vegetative cells and most fungal propagules, so a loaf leaving the oven is essentially sterile. During cooling, slicing and wrapping the loaf collects airborne bacteria and fungal spores, which settle on a substrate offering a water activity of 0.94-0.97, a near neutral crumb pH of 5-6 and abundant fermentable sugars. Mould free shelf life is consequently measured in days, and the genera responsible (Penicillium, Aspergillus, Cladosporium, Rhizopus and Neurospora) are those whose conidia are hardest to exclude from a bakery (Axel *et al.,* 2017; Pacher *et al.,* 2022) [3, 11].
This risk has been managed for decades with weak acid preservatives, principally calcium propionate and sorbates, plus modified atmosphere packaging. These work but are out of step with the market: consumers treat an E number as a reason not to buy, several jurisdictions have tightened permitted levels, and dosing high enough to control mould can flatten flavour. Physical, residue free interventions after baking are the obvious way to close the resulting gap (Debonne *et al.,* 2024) [5].
Cold atmospheric plasma (CAP) is among the most promising of these. A strong electric field applied to air at atmospheric pressure drives electrons far above the bulk gas temperature, and their collisions with N_2 and O_2 create a chemically rich but thermally cold

environment. The resulting reactive oxygen and nitrogen species (RONS), with charged particles and UV photons, attack microbial cells on several fronts at once: membrane lipid peroxidation, protein oxidation and strand breakage in nucleic acids (Misra *et al.,* 2011; Thirumdas *et al.,* 2015) [10, 15]. The gas stays near ambient temperature, so the product is not cooked, and the species decay within minutes, leaving no residue. CAP is now a mainstream non thermal option (Bourke *et al.,* 2018; Gavahian & Mousavi Khaneghah, 2020) [4, 6].

Not all reactors suit bread, which presents a large, rough surface and is soft enough that moisture drawn from the crumb reads immediately as staling. Dielectric barrier discharge needs a dielectric layer and closely spaced electrodes; plasma jets cover only a few square centimetres; glow discharge needs vacuum hardware. The pins to parallel plate geometry is a useful compromise: field enhancement at each pin tip initiates ionisation at modest voltage while the counter plate spreads the discharge into a diffuse glow, giving a large footprint and high RONS flux with negligible heating. It has proved effective on cereal substrates without damaging the matrix (Potkule *et al.,* 2025; Ramkumar *et al.,* 2024) [12, 13].

A second decision matters as much as geometry: open or in package exposure. In package treatment strikes the discharge in the pack headspace, so RONS are retained against the food and keep reacting during storage, evaporated water cannot escape, and recontamination during wrapping is eliminated (Alaguthevar *et al.,* 2024) [1]. Published work applying CAP to finished bread remains sparse. The most relevant is Starek-Wójcicka *et al.* (2022) [14], who treated gluten free and wheat-rye loaves with a nitrogen gliding arc discharge and reported complete suppression of mesophilic bacteria and fungi alongside moisture loss and increased crumb hardness. It established the principle but used a geometry, feed gas and duration (2-10 min) far removed from a bakery line, and did not compare open air with in package exposure. The present study treats freshly baked bread at 25 kV for 30, 45 and 60 s, directly and in package, to optimise the exposure protocol, quantify the microbial response and assess the quality and shelf life consequences.

2. Materials and Methods

2.1 Raw material, chemicals and glassware

Freshly baked white pan bread, produced without added preservatives or hydrocolloid improvers, was purchased from a local bakery in Alamathi, Chennai, and transported to the laboratory in sealed low density polyethylene bags within 2 h of baking.

2.2 Pins to parallel plate atmospheric cold plasma system

A pre developed pins to parallel plate high voltage plasma generation (HVPG) system was used (Fig. 1). The reactor consisted of an array of pointed stainless steel pin electrodes mounted above a flat grounded parallel plate electrode inside an acrylic treatment chamber. A DC regulated power supply delivered 12 V DC to a plasma generator module, which stepped the input up to a discharge potential of 25 kV across the pin-plate gap. The inter electrode gap was maintained at 40-45 mm, and an atmospheric pressure glow discharge was struck in ambient air within this gap. Sample temperature was monitored during treatment and did not exceed ambient by more than a few degrees, confirming the non thermal character of the process. The pin-plate arrangement was selected because field enhancement at the pin tips initiates ionisation at comparatively low applied power while the counter plate distributes the discharge into a diffuse glow over the full sample footprint, an arrangement previously validated for cereal and dried fruit substrates (Ramkumar *et al.,* 2024) [13].

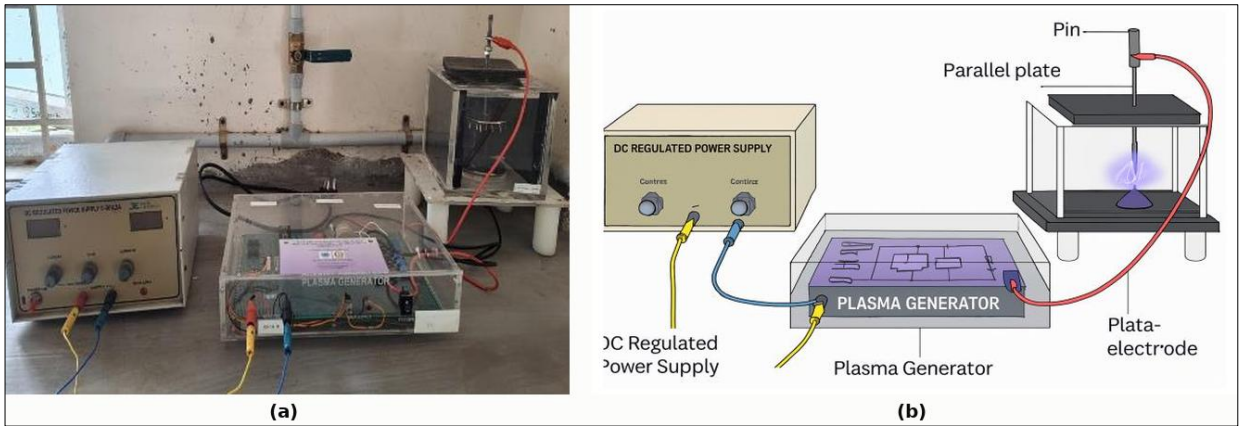

Fig 1: Pins to parallel plate atmospheric cold plasma system: (a) experimental set up; (b) schematic representation showing the DC regulated power supply, plasma generator, pin electrode and parallel plate electrode.

2.3 Ancillary equipment

Moisture content was determined in a hot air oven (Hasthas Scientific Instruments India Pvt. Ltd., Chennai). Microbiological work was performed in a laminar air flow cabinet, and inoculated plates were held in a laboratory incubator.

2.4 Sample preparation and experimental design

Bread was cut into cubes of 5 × 5 × 5 cm so that every sample presented an identical surface area and geometry to the discharge, ensuring homogeneous treatment. Samples were assigned to seven groups. An untreated control received no plasma exposure. Three groups (T_1, T_2, T_3) were placed uncovered on the grounded plate and exposed directly to the discharge for 30, 45 and 60 s respectively. Three further groups (A_1, A_2, A_3) were first sealed in their packaging and then exposed to the discharge for the same three durations, so that the plasma was struck in the confined package headspace. All treatments were performed at 25 kV. Treated and control samples were subsequently

stored under identical ambient conditions and drawn for analysis. Every determination was performed in ten replicates. The overall experimental scheme is summarised in Fig. 2.

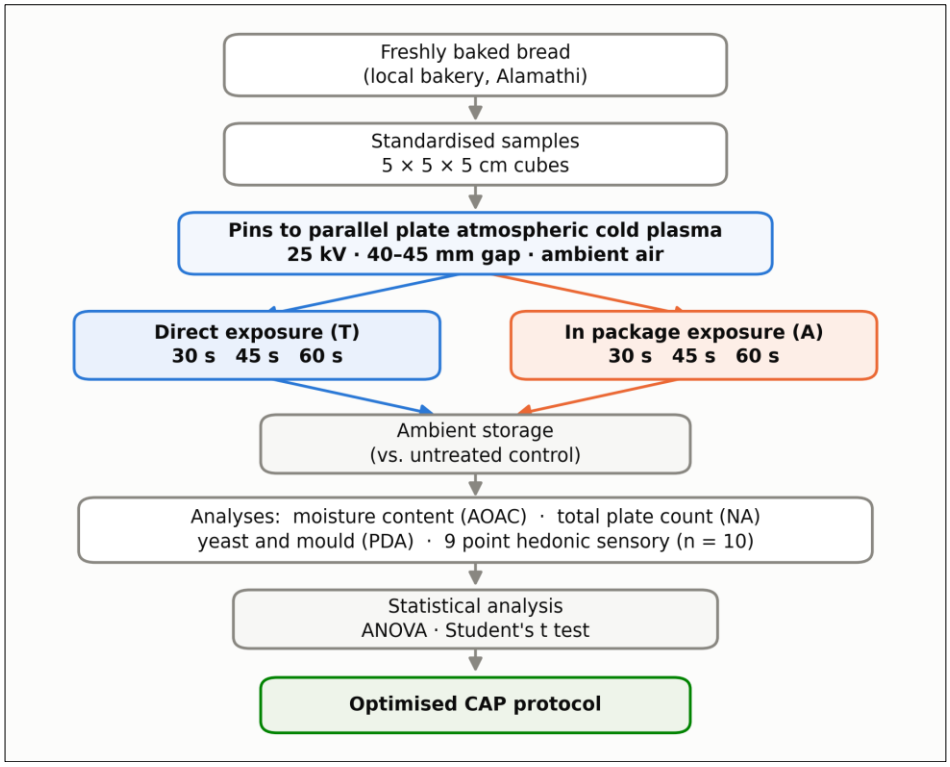

Fig 2: Overall flow of the experiment.

2.5 Determination of moisture content

Moisture content was determined by the hot air oven method following AOAC (2019) [2]. Four grams of sample were weighed into a pre dried, tared Petri dish and dried at 105±5 °C, cooled in a desiccator and reweighed; the heating and cooling cycle was repeated to constant weight. Moisture was calculated from the loss in weight on drying and expressed as a percentage:

$$\text{Moisture content (\%)} = [(W_2 - W_3) / W_2] \times 100$$

where W_2 is the weight of the sample with dish before drying and W_3 is the final weight of the sample after drying.

2.6 Microbiological analysis

Total plate count (TPC) and yeast and mould count were determined by the standard pour plate technique under aseptic conditions in a laminar air flow cabinet. Samples were homogenised in sterile diluent, serially diluted in decimal steps, and plated on Nutrient Agar for TPC and on Potato Dextrose Agar for yeast and mould. Plates were incubated at 25 °C and enumerated after 24 and 48 h. Counts were expressed as the mean number of colonies within each treatment group and reported as cfu/g. Where no colonies were recovered on any replicate plate the result is reported as not detected (ND). Log reduction was calculated as $\log_{10}(N_0) - \log_{10}(N)$, where N_0 is the control count and N the count after treatment. The enumeration and reporting scheme follows that used for plasma treated cereal and dried fruit substrates by Ramkumar *et al.* (2024) [13].

2.7 Sensory evaluation

Sensory evaluation was carried out by a panel of ten semi trained judges drawn from the academic staff and student body of the College of Food and Dairy Technology, Koduveli. Panellists assessed colour and appearance, flavour, body and texture, taste, and overall acceptability using a 9 point hedonic scale administered under standard sensory conditions, following the principles set out by Lawless and Heymann (2010) [9], where 9 = like extremely and 1 = dislike extremely. Samples were presented in randomised order under coded three-digit labels, and panellists rinsed with potable water between samples.

2.8 Statistical analysis

Data were tabulated as mean ± Standard error of ten replicates and subjected to analysis of variance to test the effect of exposure time within each exposure mode, and to Student's t test to compare direct and in package exposure at each exposure time. Treatment means were separated at the 5% and 1% levels; means bearing the same superscript letter within a column are on par. All analyses were performed in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., 2011) [7].

3. Results and Discussion

This section presents and interprets, parameter by parameter, the response of bread to pins to parallel plate atmospheric cold plasma applied at 25 kV for 30, 45 and 60 s in direct and in package configurations.

3.1 Sensory quality and treatment optimisation

Sensory acceptability set the ceiling on treatment intensity, and the two modes differed sharply (Fig. 3). Directly exposed bread scored well at 30 s, all five attributes falling in the 6.5-7.0 band, but deteriorated steadily: by 60 s flavour, texture and taste had fallen to around 4.5 and overall acceptability to 5.0, the borderline of consumer

acceptance. Panellists described these samples as drier and firmer, with a less fresh aroma. In package samples retained acceptability across the whole range, peaking at 45 s (colour 8.0, body and texture 8.0, overall acceptability 7.5) and falling only to about 7.0 at 60 s. Colour was the most robust attribute in both series, consistent with the absence of thermal browning; flavour and texture the most sensitive.

Two processes degrade sensory quality under CAP, and packaging suppresses both. The first is dehydration of the crust and outer crumb: an open discharge sweeps evaporated water away and sustains a steep vapour pressure gradient, whereas a sealed package saturates its headspace within seconds and the gradient collapses. That the sensory data mirror the moisture data (Section 3.2) almost exactly is the signature of dehydration rather than chemical damage. The second is oxidative modification of surface lipids and flavour active volatiles by RONS. Starek-Wójcicka *et al.* (2022) [14] recorded the same pattern in CAP preserved bread, reporting hardness and springiness increases that intensified from 2 to 10 min and attributing them to plasma induced moisture removal. Ramkumar *et al.* (2024) [13], using the same pins to plate geometry on raisins, likewise found quality optimised at intermediate rather than maximal doses.

Two lines of evidence support reading this as a real process benefit rather than panel variability. Alaguthevar *et al.* (2024) [1] identify moisture and volatile retention in the sealed headspace as a principal advantage, noting that it permits longer exposure before quality penalties appear. Khan *et al.* (2023) [8] supply the matrix level explanation: non thermal plasma altered water activity and gluten cross linking in wheat flour while changing free moisture by less than 1%, showing that plasma acts on how water is bound rather than simply removing it. Since freshness in bread is perceived through crumb softness, and therefore water mobility, that distinction separates a 45 s in package treatment scoring 7.5 from a 60 s open air treatment scoring 5.0. On sensory grounds 45 s in package was optimal, 60 s acceptable, and direct exposure beyond 30 s not recommended.

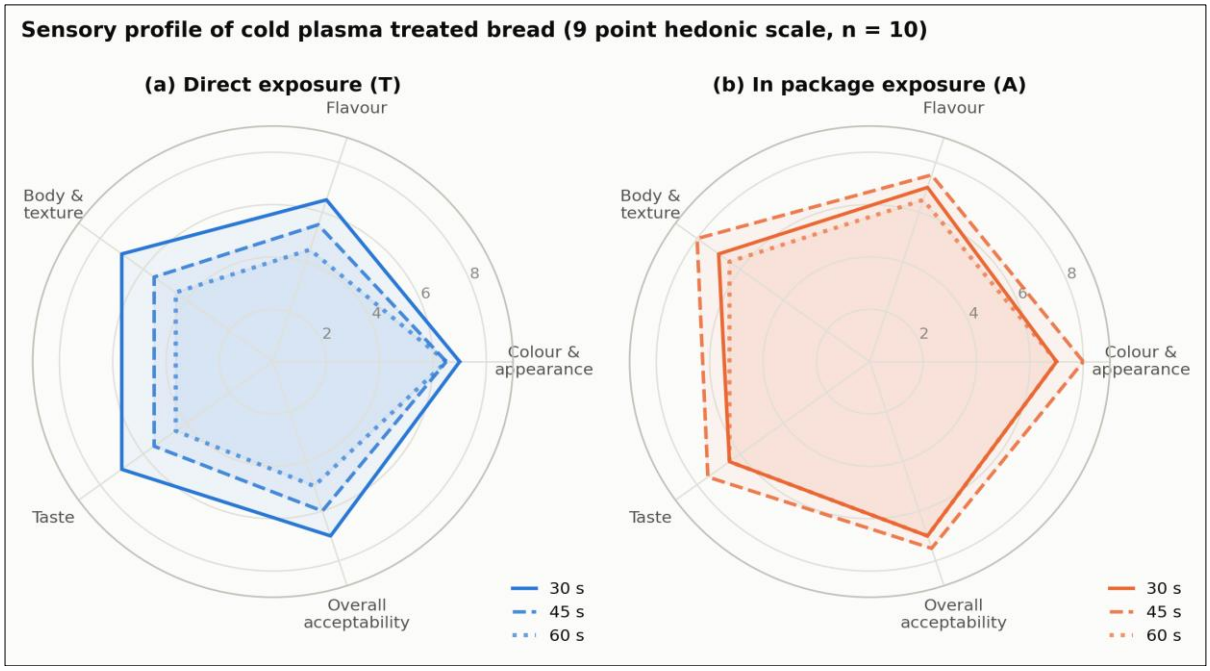

Fig 3: Sensory profile of cold plasma treated bread on a 9 point hedonic scale (n = 10 semi trained panellists): (a) direct exposure (T), (b) in package exposure (A). Values are panel means digitised from the original radar plots of the underlying study.

3.2 Moisture content

Moisture responded to both exposure mode and time, but far more strongly to the former (Table 1, Fig. 4). In package samples averaged 13.21% moisture against 12.10% for direct exposure, a gap of 1.1 percentage points, highly significant at every exposure time ($t = 15.714$, 19.756 and 18.786; all $P \leq 0.01$). The consistent t statistic shows the packaging effect is dose independent, established within 30 s and maintained thereafter.

The time response differed within each series. In package, moisture declined monotonically from 13.420±0.063% at 30 s to 13.169±0.052% at 45 s and 13.048±0.035% at 60 s, a significant decline ($F = 13.392$, $P \leq 0.01$) in which only the 30 s mean was distinct (b). Under direct exposure moisture was already low at 30 s (12.127±0.052%) and barely moved thereafter (12.131±0.002%; 12.054±0.039%), with no time effect ($F = 1.311$, NS). Open air exposure evidently strips the evaporable surface moisture within 30 s and then plateaus, whereas in the package the loss is slower and still progressing at 60 s. Extending in package treatment to 60 s costs only 0.37 percentage points, worth paying for the microbial gain in Section 3.3.

These findings agree with the two most comparable studies. Starek-Wójcicka *et al.* (2022) [14] reported progressive dehydration of gluten free and wheat-rye bread, the 10 min exposure producing greater loss than the 2 min: the same dose dependence seen here, on a longer time base suited to their lower power source. They also noted attenuated moisture change in loaves held in closed foil bags, the effect quantified in Table 1. Ramkumar *et al.* (2024) [13] similarly observed steady moisture decline in pin to plate treated raisins, concluding that controlled dehydration was partly beneficial, since it lowered water availability to spoilage organisms.

Table 1: Moisture content (%) of bread treated by pins to parallel plate atmospheric cold plasma at 25 kV

S. No.	Exposure time	In package (A)	Direct (T)	t value
1	30 s, 25 kV	13.420±0.063^{b}	12.127±0.052^{a}	15.714**
2	45 s, 25 kV	13.169±0.052^{a}	12.131±0.002^{a}	19.756**
3	60 s, 25 kV	13.048±0.035^{a}	12.054±0.039^{a}	18.786**
	F value	13.392**	1.311^{NS}	

Mean ± SE of ten replicates. ** = highly significant (P ≤ 0.01); NS = not significant. Means bearing the same superscript letter within a column are on par.

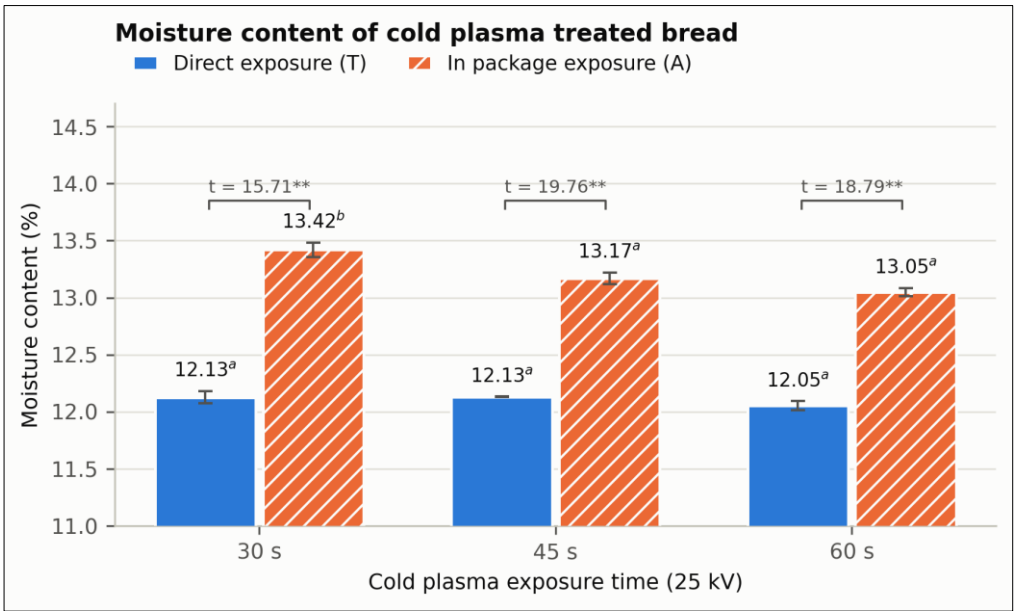

Fig 4: Moisture content of bread after direct and in package pins to parallel plate cold plasma treatment at 25 kV.

Error bars denote standard error (n = 10); superscript letters indicate means on par within an exposure mode; t values compare the two modes at each exposure time (** P ≤ 0.01). Two mechanistic studies justify this reading. Khan *et al.* (2023) [8] showed that non thermal plasma reduced the water activity of wheat flour below 0.7 while altering free moisture by less than 1%, linking this to disulphide cross linking within the glutenin fraction. The CAP associated firming reported here and by Starek-Wójcicka *et al.* (2022) [14] is therefore not solely evaporative: plasma also redistributes water between starch and protein phases and stiffens the gluten network. Alaguthevar *et al.* (2024) [1] add that a sealed headspace saturates with vapour almost immediately, largely eliminating the evaporative driving force that dominates open air treatment. Together these explain Table 1: a large, dose independent packaging effect on a small, dose dependent decline.

3.3 Total plate count

The untreated control carried 1.7 × 10^5 cfu/g, confirming substantial post bake recontamination despite the lethality of baking (Table 2, Fig. 5). Every treatment reduced this count, monotonically with exposure time in both modes. Direct exposure gave 1.2, 0.8 and 0.5 × 10^5 cfu/g at 30, 45 and 60 s (29.4%, 52.9% and 70.6%; 0.15, 0.33 and 0.53 $\log_{10}$). In package exposure was better at every duration: 0.9, 0.7 and 0.3 × 10^5 cfu/g (47.1%, 58.8% and 82.4%; 0.28, 0.39 and 0.75 log).

Two features of the dose-response deserve comment (Fig. 6). First, the in package advantage appears from the shortest exposure: 30 s in package (0.28 log) nearly doubled 30 s direct (0.15 log) and approached 45 s direct (0.33 log), so packaging buys intensity at no time cost. Second, the curves diverge: between 45 s and 60 s the direct series gained 0.20 log against 0.37 log in package. This is the signature of RONS accumulation: in the open chamber short lived species are swept away and lethality scales linearly with time, whereas in a sealed package ozone and nitrogen oxides accumulate and effective dose scales with the integral of a rising concentration.

Table 2: Microbiological quality of bread treated by pins to parallel plate atmospheric cold plasma at 25 kV

Microbial attribute	Control	T_1	T_2	T_3	A_1	A_2	A_3
Total plate count (×10^5 cfu/g)	1.7	1.2	0.8	0.5	0.9	0.7	0.3
$\log_{10}$ reduction (TPC)	n/a	0.15	0.33	0.53	0.28	0.39	0.75
Reduction (%)	n/a	29.4	52.9	70.6	47.1	58.8	82.4
Yeast and mould (×10^5 cfu/g)	0.8	0.5	0.4	ND	0.4	ND	ND

T = direct exposure, A = in package exposure; subscripts 1, 2 and 3 denote 30, 45 and 60 s at 25 kV. ND = not detected. Log reduction and percentage reduction are calculated against the untreated control.

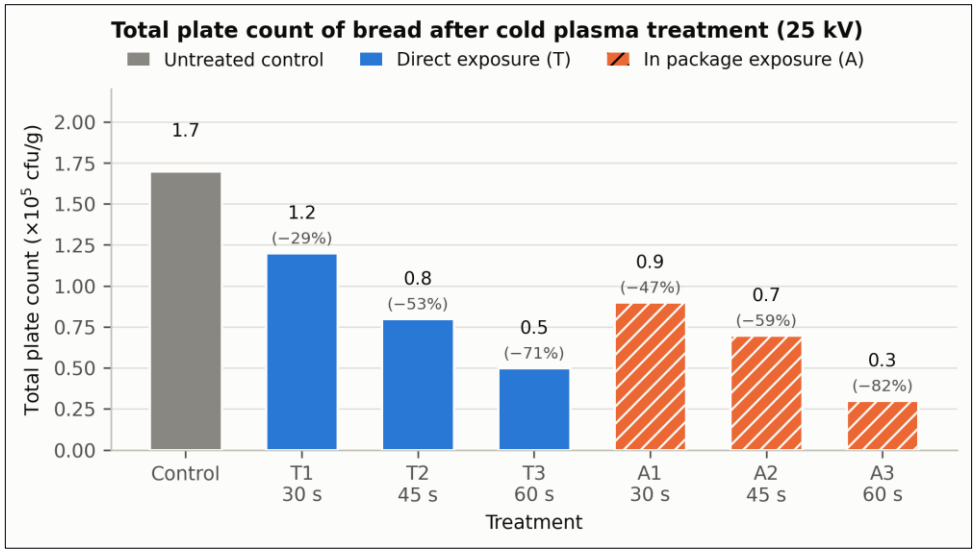

Fig 5: Total plate count of bread after direct and in package pins to parallel plate cold plasma treatment at 25 kV. Percentage reductions relative to the untreated control are shown within each bar

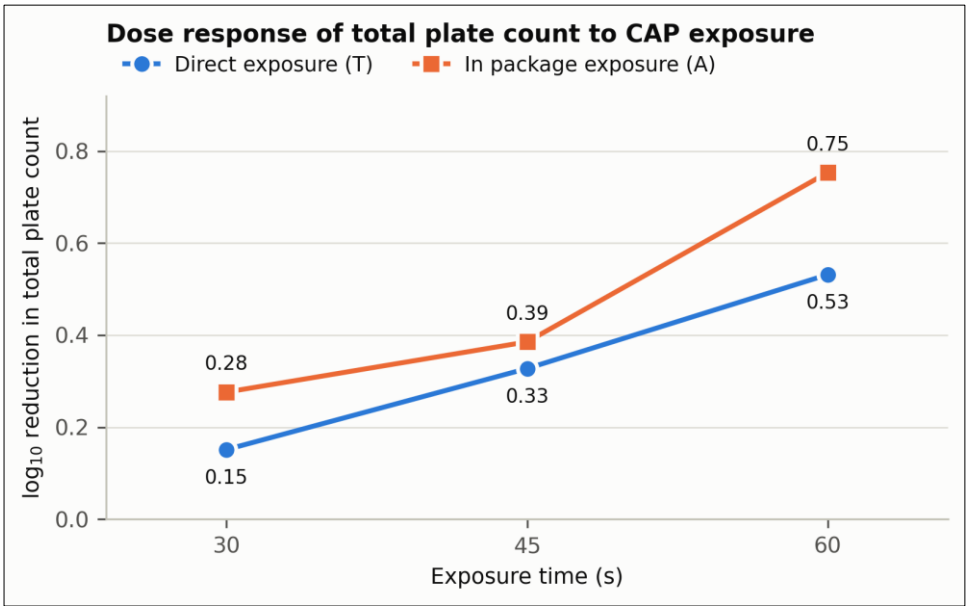

Fig 6: Dose-response of total plate count to cold plasma exposure time, expressed as $\log_{10}$ reduction relative to the untreated control. The divergence of the two curves beyond 45 s reflects accumulation of long lived reactive species in the sealed package headspace.

The reductions are modest beside Starek-Wójcicka *et al.* (2022) [14], who recovered no mesophilic bacteria or fungi after 10 min of nitrogen gliding arc exposure. The difference is dose, not mechanism: their treatment was ten to twenty times longer than the present 30-60 s. Read as a dose series the studies agree, both showing monotonic, non saturating reductions. Ramkumar *et al.* (2024) [13] reported a comparable effect for pin to plate plasma on raisins, mesophilic counts falling from 4.65 to 2.42 log cfu/g over 15 days, the advantage widening during storage.

Two established findings justify the pattern. Misra *et al.* (2011) [10] showed that plasma inactivation proceeds by simultaneous oxidative attack on the cell envelope, cytoplasmic proteins and nucleic acids, with dose dependent, often biphasic kinetics in which a resistant sub population forms the survivor tail. The shallow slope at 30-45 s and steeper decline at 60 s in package fit that description once accumulating RONS are allowed for. Alaguthevar *et al.* (2024) [1] add that in package plasma retains ozone and nitrogen oxides in the headspace, where they act after the discharge is switched off; this residual activity is why in package treatment outperforms open air at equal electrical dose. The 0.75 log reduction in 60 s in package, against 0.53 log in the open, measures that effect on bread.

3.4 Yeast and mould count

Yeasts and moulds, which determine when a loaf is rejected, responded far more sharply than the mesophilic population (Table 2, Fig. 7). Against a control of 0.8×10^5 cfu/g, direct exposure gave 0.5 and 0.4×10^5 cfu/g at 30 and 45 s (37.5% and 50%), eliminating detectable growth at 60 s. In package exposure reached that endpoint a step earlier: 0.4×10^5 cfu/g at 30 s and nothing detectable at 45 or 60 s. Complete suppression therefore required only 45 s in package, compatible with an in line bakery conveyor.

This differential susceptibility is the study's most useful finding, because bread spoilage is overwhelmingly fungal. Conidia lodge on the crust and germinate there, large and thin walled at the germ tube stage and, unlike bacteria

sheltered within crumb pores, fully exposed to a surface treatment. Axel *et al.* (2017) [3] argue that shelf life is set by time to visible mould rather than total viable count, so surface directed interventions deliver disproportionate benefit.

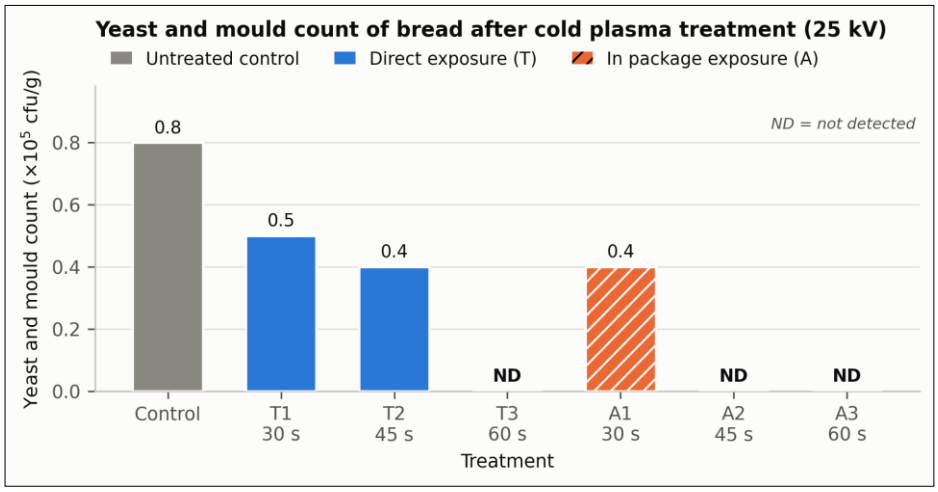

Fig 7: Yeast and mould count of bread after direct and in package pins to parallel plate cold plasma treatment at 25 kV. ND = not detected.

Both comparable studies agree. Starek-Wójcicka *et al.* (2022) [14] eliminated yeast and mould in gluten free and wheat-rye bread at 2 min while needing the full 10 min for mesophilic bacteria, the same ordering seen here, where fungi disappeared at 45 s in package with TPC only 0.39 log reduced. Ramkumar *et al.* (2024) [13] likewise found fungal counts on pin to plate treated raisins suppressed more rapidly and durably than bacterial counts. Misra *et al.* (2011) [10] explain why: fungal spores are vulnerable to RONS because their large surface area to volume ratio and thin, lipid rich outer layers make efficient peroxidation targets, and germinating conidia lose their dormant state resistance. Khan *et al.* (2023) [8] add a cereal specific justification, reporting 20-80% reductions in Enterobacteriaceae on plasma treated wheat flour with no change in starch properties: antimicrobial action on a cereal surface is achievable within a dose window that leaves the matrix intact.

3.5 Integrated process optimisation

The three parameters define a clear optimum. Direct exposure is dominated by cost: 1.1 percentage points more moisture removed, acceptability below 6.0 beyond 30 s, and 60 s needed to eliminate fungi. In package exposure inverts each trade off: 45 s is where yeast and mould first become undetectable and acceptability peaks, at a moisture cost of 0.25 points relative to 30 s; extending to 60 s raises the TPC reduction from 0.39 to 0.75 log for a further 0.12 points and a small sensory decrement. The recommended window is 45-60 s at 25 kV in package: 45 s where sensory quality is paramount, 60 s where the extra 0.36 log justifies a firmer crumb.

4. Conclusion

Pins to parallel plate atmospheric cold plasma at 25 kV is an effective, residue free decontamination step for freshly baked bread, and the configuration matters as much as the dose. In package exposure outperformed direct on every parameter: total plate count reduced by 0.75 log (82.4%) in 60 s against 0.53 log (70.6%); yeast and mould eliminated in 45 s against 60 s; 1.1 percentage points more moisture retained at every exposure time ($P \leq 0.01$); and sensory acceptability preserved across the range, peaking at 45 s where direct exposure reached its margin by 60 s. Sealing the package does two things at once. It saturates the headspace, collapsing the evaporative gradient behind plasma induced drying and the firming and flavour loss that follow, and it retains long lived reactive species at the product surface, where they act after the discharge is extinguished. The first protects quality, the second amplifies lethality. The most consequential finding for bread is the differential susceptibility of fungi. Consumers reject bread when mould becomes visible, not when total viable count crosses a threshold, so a 45 s in package treatment rendering yeast and mould undetectable addresses that failure mode while leaving the crumb soft. On this evidence 45-60 s at 25 kV in package is the recommended window, compatible with continuous bakery operation. Further work should convert these reductions into a quantified shelf life gain through extended storage trials tracking time to visible mould, and should separate evaporative drying from plasma induced changes in water binding using instrumental texture profile analysis and low field NMR. Beyond that, the process needs optimisation of voltage, electrode gap, pin density and headspace gas on improver containing formulations that may tolerate longer exposures, followed by pilot scale trials establishing throughput, energy use and cost per loaf.

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