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Impact of cold plasma on microbial load and quality properties of maize flour

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ABSTRACT

The control of microbial contamination in flour represents a significant challenge for food safety. Traditional thermal methods often compromise product safety and quality as they cause undesirable changes. In addition, many existing technologies suffer from limitations such as uneven treatment, safety issues, and mainly superficial effects. To overcome these problems, a prototype system integrating atmospheric dielectric barrier discharges (DBD) cold plasma with mechanical fluidization was developed and applied to naturally contaminated maize flour for 5, 10, 20 and 30 min. Bacterial and fungal inactivation, physico-chemical properties, particle size distribution and microstructural properties were evaluated. A gradual reduction in mesophilic aerobic bacteria (MAB) was observed, with complete inactivation achieved after 30 min; however, bacterial regrowth was detected after 16 days of storage. Filamentous fungi were completely inactivated after 10 min and although partial recovery occurred during storage, fungal loads remained below those of the control. Fungal diversity was also altered, with Aspergillus flavus being more sensitive than Aspergillus oryzae. A notable reduction in water content and activity was observed for longer treatments. Modification of particle size and morphology likely occurred in the process with plasma associated to mechanical fluidization of flour. Overall, the effectiveness of ACP was influenced by treatment duration and fungal species, highlighting both its potential and limitations in flour decontamination, without detrimental effects on the selected physical quality attributes of the product.

1. Introduction

Cereals, such as rice (*Oryza sativa*), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.), are of paramount importance for human nutrition and are grown in many areas of the world (Bartholomew, Bradshaw, Jurick, & Fonseca, 2021). However, cereal grains are susceptible to contamination during ripening, harvesting, processing and storage. Grain-derived flour is often contaminated because it originates directly from the grain, and the flour is an inherent component of the grain mass. Consequently, microbial infection by various microorganisms can occur, which ultimately deteriorate the quality of the product (Deligeorgakis et al., 2023), leading to its spoilage, causing economic decline, shortening shelf life, and increasing wastage (Sami, Abedi, Mohammadi, & Mirlohi, 2020). Whole flour and products made from it are at higher risk of fungal and bacterial contamination, for example by the genera *Aspergillus*, *Penicillium* and *Fusarium* spp. (Cardoso et al., 2019; Sacco et al., 2020). This species produces mycotoxins that are carcinogenic, mutagenic, and genotoxic to humans and are highly resistant to processing and treatment technologies (Elaridi, Yamani, Al Matari, Dakroub, & Attieh, 2019; García-Ramón et al., 2025; Pankaj, Shi, & Keener, 2018).

The stability of flour largely depends on its water activity (a_w) and moisture content. According to the Food and Agriculture Organization (FAO), low-moisture or low-a_w foods are those with an $a_w \leq 0.70$ (Blessington, Theofel, & Harris, 2013; Rifna, Singh, Chakraborty, & Dwivedi, 2019). At such levels, microbial cell division is physiologically inhibited (Young et al., 2015) and foodborne pathogens and spoilage fungi can survive for extended periods in flour (Cardoso et al., 2019).

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Under unfavorable conditions, microorganisms remain inactive and do not present a potential hazard (Plavšić et al., 2017). However, once moisture levels increase above 12–14%, microorganisms can multiply rapidly, causing mildew development, flour agglomeration, and deterioration of quality and safety, which shortens product shelf life (Li et al., 2013). In addition rehydration of powder ingredients can enable microorganisms or spores to repair sublethal damage and resume growth, rendering the final food unsafe (Wang et al., 2015), as well as and being a potential health hazard (Eglezos, 2010).

Various strategies have been studied to inhibit microbial growth in food flours (Magallanes López & Simsek, 2021). Fumigation with ethylene oxide has been widely applied to reduce microbial loads (Sánchez-Maldonado, Lee, & Farber, 2018). However, the use of ethylene oxide has been forbidden in the food industry by the European Union due to the possible toxic residues and health hazards to workers (Sánchez-Maldonado et al., 2018). In addition, pulsed light (Du, Prasad, Gänzle, & Roopesh, 2020; Subedi et al., 2020), radio frequency heating and pasteurization (Fan, Zhao, Wang, Cao, & Jiang, 2017; Liu et al., 2018; Villa-Rojas, Zhu, Marks, & Tang, 2017), ozone (Li et al., 2013; Tiwari et al., 2010; Zhuang et al., 2020), vacuum steam treatment (Snelling, Malekmohammadi, Bergholz, Ohm, & Simsek, 2020), superheated steam (Huang, Guo, Manzoor, Chen, & Xu, 2021), gamma irradiation (Bhat et al., 2024; Mukisa et al., 2012), and microwave heating treatment (Cao, Zhang, Mujumdar, & Wang, 2019; Dababneh, 2013) have been applied for flour/powder decontamination. Even if the aforementioned technologies have reduced the microbial load, still these techniques have drawback such as relatively low penetrability limits, product color modifications and degradation (darker with a burnt characteristic), low efficacy in reducing microbial levels, non-uniform heating and treatment, and potential shadowing effect (Deng, Li, & Xiao, 2024). Therefore, the restrictions of traditional and existing decontamination methods have encouraged the development of other methods.

Atmospheric cold plasma (ACP) can be assessed as an alternative tool for decontamination technology from flour samples. ACP is created by a strong electric field that accelerates free electrons, which in turn dissociate, excite, or ionize gaseous molecules (Dasan et al., 2017; Edelblute, Malik, & Heller, 2015). As a result, it contains a vast array of reactive particles, including electrons, positive and negative ions, free radicals, gas atoms, and molecules in both ground and excited states, as well as quanta of electromagnetic energy, photons, and visible light (Fridman et al., 2008). It is considered non-thermal as characterized by temperature close to the ambient one. ACP was found to successfully deactivate a varied range of microorganisms including fungi, spores and viruses (Desai et al., 2024; Fayaz et al., 2025; Katsigiannis, Bayliss, Walsh, & Safety, 2022; Wei et al., 2026) in variety of food products. However, its application for the decontamination of flour remains largely unexplored, despite the growing interest and relevance to the food industry. To address this gap, further engineering solutions and advanced technologies are required to overcome current limitations, particularly the issues of limited penetration, adverse effect on product, and non-uniform treatment.

In the existing literature, ACP has mainly been applied to thin product layers under static conditions, without active mixing or particle movement. However, ACP is a surface-driven technology with limited penetration depth, as reactive species primarily interact with exposed surfaces. As a result, in particulate matrices such as flours, uneven treatment and shadowing effects are likely, leading to non-uniform particle exposure and potentially inconsistent decontamination efficacy (Gebremical et al., 2025). Moreover, when particles are aggregated, inner fractions may be shielded from plasma exposure due to the poor penetration of reactive species (Zhao et al., 2024; Zhu et al., 2025).

To solve the aforementioned limitation, improved system designs capable of enhancing homogeneity through continuous mixing, while ensuring efficient microbial inactivation and preservation of flour quality attributes are essential. In this context, a novel atmospheric cold plasma system with integrated mechanical fluidization (Gebremical et al., 2025) was used for the treatment of naturally contaminated maize flour to investigate the effect on microbial and fungal decontamination and on some quality parameters. The extent of microbial recovery during a 16-day storage period after treatment was also evaluated.

2. Materials and methods

2.1. Materials

Maize flour sample were purchased from was purchased from a producer located in Valtellina (Italy), and storage at 4 °C.

2.2. Cold atmospheric plasma (ACP) treatment of flour

The general scheme of the experiment in this study is shown in Fig. 1. The ACP source consists of a Dielectric Barrier Discharge (DBD) source powered by a pseudo-sinusoidal high voltage generator with an amplitude of up to 15 kV peak-to-peak, as detailed in our previous work (Gebremical et al., 2025).

The discharge frequency was approximately 33 kHz. Each discharge cycle delivered ~270 J, corresponding to an operating power of about 9 W. The treatment chamber had a volume of 3270 cm^3. The fan was powered by a regulated power supply (EA-PS2042–20) set to provide a constant output of 8.1 V and 3 A. The fan operated at ~7500 rpm with a blade length of 65 mm, generating an air velocity of 2.0–3.6 m/s depending on the distance from the fan (from the bottom to the top of the chamber). These operating conditions were selected to maintain the flour predominantly in a fluidized state throughout the treatment.

During the experiment, the temperature inside the treatment chamber was measured with a thermocouple. The treatments were carried out in triplicate for 5, 10, 20 and 30 min. The untreated sample was used as a control.

2.3. Microbial analysis

Microbial analysis was carried out at two time points: immediately after treatment (t_0) and after 16 days (t16) post-treatment, the samples were stored in 30 °C to assess decontamination and the possible microorganism's recovery over time. Therefore, we also performed molecular identification to determine which microorganisms were affected.

2.3.1. Determination of mesophilic aerobic bacteria (MAB)

MAB counts were determined using the method outlined in Chapter 3 of the FDA's Bacteriological Analytical Manual (BAM).

2.3.2. Natural mycobiota in flour

2.3.2.1. Fungal isolation and effect on natural mycobiota associated to flour after ACP. Fungal isolation was performed according to the method described by (Molina-Hernandez et al., 2023) with some modifications. For each sample, random aliquots of 10 g of flour were aseptically placed in sterile stomacher bags containing 90 ml of sterile physiological solution (0.9% *w*/*v*) and then homogenised sequentially in a Stomacher-400 Circulator (Seward, West Sussex, UK). Tenfold dilutions of the homogenate were prepared and 100 μl were inoculated onto potato dextrose agar (PDA) and Malt Extract Agar (MEA) for filamentous fungi and yeasts and DG18 for xerophilic filamentous fungi and incubated at 30 °C for 48 h.

The percentage of frequency of each morphotype (MF) for each sample was calculated according to:

$$MF(\%) = \left(\sum \text{isolates of the morphotype}\right) \Big/ \left(\sum \text{all fungal isolates}\right) \times 100$$

To identify the isolates based on morphological and growth

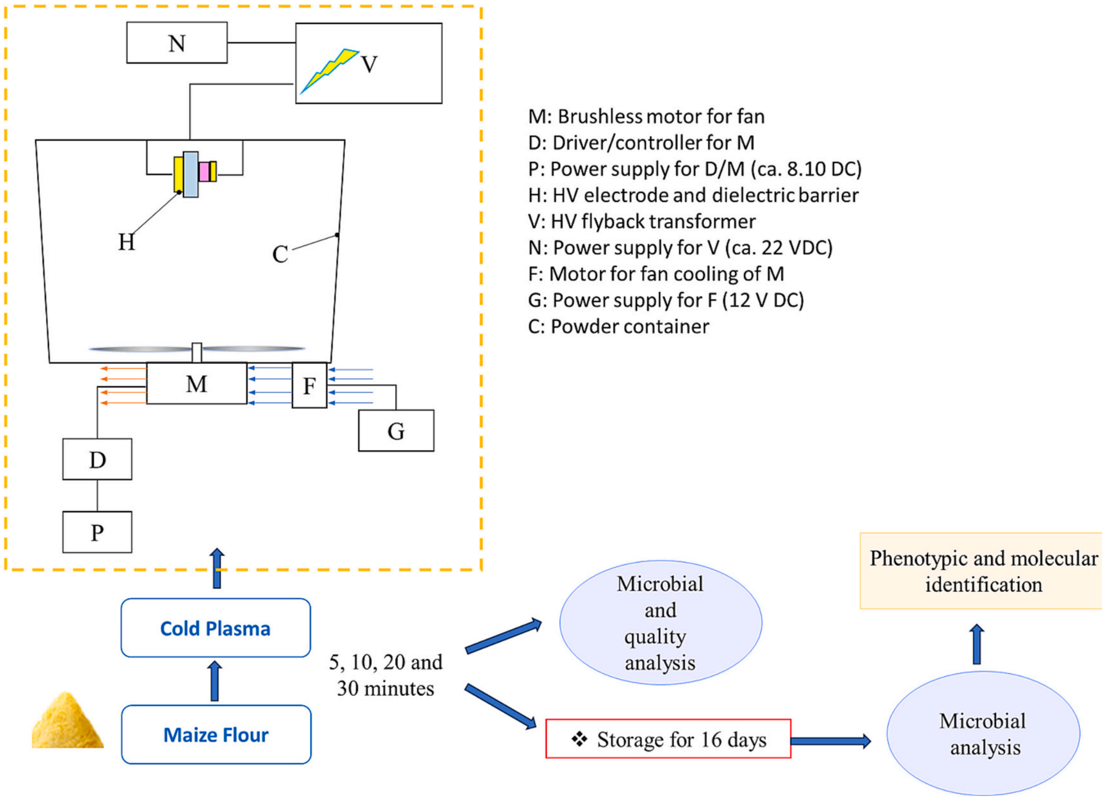

Fig. 1. Schematic diagram of the experiment.

characteristics, individual colonies were purified in malt extract agar (MEA) (Liofilchem, Roseto degli Abruzzi, Italy) and then isolated filamentous fungi were identified based on morphological characteristics under a light microscope according to (Munitz et al., 2013). The fungal isolates were then tentatively categorised into different genera based on the size and shape of the spores and mycelia.

To determine the effect of ACP on the mycobiota naturally associated to maize flours, treated and untreated samples were analyzed immediately after treatment (t_0) and after 16 days (t16) of storage (30 °C) according to the methodology described by (Molina-Hernandez et al., 2022).

2.3.2.2. Phenotypical and molecular identification of filamentous fungi. To determine the effect of ACP on the fungal species in untreated and treated maize flour samples, a random number of each colony species was taken from the different Petri dishes and identified based on morphological and growth characteristics. Colonies were inoculated into two different culture media, malt extract agar (MEA) and Czapeck yeast extract agar (Liofilchem, Roseto degli Abruzzi, Italy). Molecular identification was then performed according to the method described by (Delgado-Ospina et al., 2022). A PCR test was performed with the primers listed in Table 1. All genes were amplified with universal primers obtained from Sigma Aldrich (Saint Louis, MO, USA).

2.4. Quality analysis of maize flours

2.4.1. Particle size distribution

The device Mastersizer 3000 (Mastersizer 3000, Malvern Instruments Ltd., Malvern, UK) was used to analyze the flour particle size distribution according to (Branton & Jana, 2017; Coutinho et al., 2019). The following parameters were considered: D [4,3] (volume mean - μm), referring to the diameter of the sphere having the same volume of particles of the system; D [3,2] (surface weighed mean diameter- μm), which indicates the mean diameter of particles proportional to the ratio of the surface area to total volume; D_{10} (μm), D_{50} (μm), and D_{90} (μm) that represent particle diameter corresponding to 10%, 50%, and 90% of the cumulative distribution. Wet (Hydro EV) dispersion method was used for the analysis.

2.4.2. Physico-chemical properties

The values for lightness (L^*), redness or greenness (a^*), yellowness or blueness (b*) of maize flour samples before and after treatments for all treatments were recorded using a Spectro photo colorimeter (Color Flex, 40/0, Hunter Lab, Reston, VA, USA) was used. The instrument was calibrated before using white and black standard references and set with the D65 illuminant.

The a_w of the maize flour sample was measured using a water activity meter (Waterlab, Steroglass, Italy) at 25 °C. The moisture content was measured by drying using a convection oven (UF1060, Memmert,

Table 1
Primers used for PCR assay.

Gene target	Length bp	Primer	Sequences (5′-3′)	Reference
Internal transcribed spacer (*ITS*)	420–825	ITS1 (F) ITS4 (R)	5TCCGTAGGTGAACCTGCGG3′ 5TCCTCCGCTTATTGATATGC3′	(Glass & Donaldson, 1995)
β-tubulin (*BenA*)	1125	β-tub 2a (F) β-tub 2b (R)	5GGTAACCAAATCGGTGCTTTC 3′ 5ACCCTCAGTGTAGTGACCCTTGGC 3′	(Makhlouf et al., 2019)

Where: F: Forward, R: Reverse. BenA means B-tubulin and CaM mean calmodulin.

Germany) at 105 °C for 8 h. The change in moisture content of the sample after ACP treatment was calculated from the initial and final weights of the sample. The temperature of samples during ACP treatments was measured using a thermocouple.

2.5. Scanning electron microscopy (SEM)

Ultrastructural analysis of samples was performed with an Environmental Scanning Electron Microscope Quanta™ 250FEG ESEM (FEI, Hillsboro, OR, USA). The samples, after fixing to a stub with carbon double-sided tape, were directly analyzed in low vacuum mode (pressure chamber at 70 Pa) with a beam accelerating voltage of 7 kV. Magnification ranges of ESEM micrographs (≥10 micrographs acquired) were 1000–4000 × .

2.6. Statistical analysis

All measurements were performed in triplicate. Results were analyzed using SPSS software (version 20.0, IBM, Armonk, USA). Analysis of variance (ANOVA) was performed, and Duncan post hoc multiple comparison tests was applied to identify the statistically significant differences between the means at $p < 0.05$.

3. Results and discusion

3.1. Effect of ACP on the Total Mesophilic Aerobic Bacteria and Filamentous Fungi

The number of natural mesophilic aerobic bacteria (MAB) and filamentous fungi in maize flour samples after 5, 10, 20 and 30 min of ACP treatment compared to the untreated samples (control) is shown in Table 2. MAB were detected in the untreated samples at low levels (3.3 ± 0.1 log CFU/g), whereas lower values were observed for filamentous fungi (2.0 ± 0.1 log CFU/g). Choi et al. (2012) investigated the microbiological contamination of rice flour and found a high level (4.9 log CFU/g). In the same trend, Cardoso et al. (2019) and Eglezos (2010) reported a high level of mesophilic bacteria in refined wheat flour (4.33–4.20 log CFU/g and 4.2 log CFU/g, respectively). In agreement with Cardoso et al. (2019), the authors also reported a slightly lower level of filamentous fungi (2.49 log CFU/g).

In general, a time-dependent effect was observed for ACP with similar results reported by (Zhao, Sheng, et al., 2024), with a notable contrast between its effects on bacteria and moulds. For bacterial load, a 10-min treatment was needed for a significant decrease (1 log CFU/g), and 30 min for complete inactivation ($p < 0.05$). Moulds, however, were reduced after a much shorter 5-min exposure. This delayed bacterial inactivation implies that brief ACP treatments may inflict only sublethal damage, a conclusion supported by (Eced-Rodríguez et al., 2024). In our previous work, we conducted a detailed characterization of the optical emission spectroscopy, identifying the main reactive species produced (Gebremical et al., 2025). Therefore, the observed effect might be due to the abundance of plasma reactive species in ACP, which could have long-term antimicrobial effects against microorganisms. On the contrary, Bahrami et al. (2016), reported that 1–2 min of ACP treatment to wheat flour generating at voltage at 15–20 kV, did not induce a significant reduction on the MAB, and moulds counts.

Table 2
Total mesophilic aerobic bacteria and filamentous fungi count naturally present in maize flours samples immediately after treatment (t0) and sixteen (t16) days of incubation after ACP treatment.

Samples	t_0	t_{16}	t_0	t_{16}
	M. Bacteria (Log CFU/g)		F. Fungi (Log CFU/g)	
Control	3.3 ± 0.1^a	3.7 ± 0.4^a	2.0 ± 0.1	4.6 ± 1.1^a
5	3.3 ± 0.1^a	4.9 ± 1.6^a	N.D.	5.5 ± 0.1^a
10	2.3 ± 0.02^b	4.4 ± 1.1^a	N.D.	3.7 ± 0.1^b
20	2.7 ± 0.02^c	4.8 ± 0.4^b	N.D.	2.0 ± 0.1^c
30	N. D	4.6 ± 0.1^b	N.D.	2.0 ± 0.1^c

At day 16, bacterial counts in treated samples showed evidence of recovery. Mean values in the 5 min (4.93 ± 1.60 log CFU/g) and 10 min (4.39 ± 1.11 log CFU/g) groups were higher than the control (3.65 ± 0.35 log CFU/g), although these differences were not statistically significant ($p > 0.05$) (Table 1). By contrast, the 20 min and 30 min treatments maintained significantly lower counts than the control at this endpoint ($p < 0.05$).

Despite the initial treatment efficacy, a resurgence was observed by day 16, with bacterial loads increasing to approximately 4.5 log CFU/g. This pattern suggests that a fraction of cells survived the treatment and/or repaired sublethal injury, enabling regrowth over time. One possible explanation is "competitive release", whereby the initial reduction in the microbial load creates a niche with reduced competition and increased nutrient availability, favouring proliferation of surviving cells. Future work should include more frequent sampling to better resolve the timing and dynamics of post-treatment recovery. On the contrary, mould counts demonstrated a treatment-time-dependent persistence, from 5.5 log CFU/g in samples treated for 5 min to 2.0 log CFU/g in those treated for 20 and 30 min. This resurgence indicates that a portion of the microbial population either withstood the treatment or rapidly repaired the sublethal damage. These results contrast with those of (Yang et al., 2024), who reported a sustained reduction of *Fusarium moniliforme* in yam flour, with log reductions between 1.50 and 3.73 log10 CFU/g observed nine days post-ACP treatment.

Several studies have been conducted investigating atmospheric cold plasma as an alternative for post-harvest decontamination of cereal material (Zhao, Sheng, et al., 2024). However, there is little information on its potential inactivation on flours (Deng et al., 2024). In this context, the effectiveness of ACP treatment depends on the equipment system and treatment duration. Microbial reduction usually increases with power and exposure time (Deng et al., 2024) and depends on the initial load of microorganisms (Pignata et al., 2014).

For example, Fernández-Felipe, Valdez-Narváez, Martinez, and Rodrigo (2024) investigated the use of low-pressure plasma (0.35 mbar) with oxygen and air to inactivate *Bacillus cereus* in a soybean powder matrix using O_2 and synthetic air as ionizing gasses and achieved a 3.71-log reduction in bacterial counts at 300 W and 30 min of treatment. Wang et al. (2024) have performed studies in other matrices with low water activity and achieved a 5-log reduction of vegetative *B. cereus* cells with a 10 min cold atmospheric plasma jet treatment, but the inactivation depended on the process gas used (air or nitrogen). In the present experiment, complete inactivation of MAB in maize flour was achieved with a 30 min treatment.

Mean and standard deviation of three repetitions in two different experiments. Different letters in the same line mean significant differences between the treatments ($p < 0.05$; Duncan post-hoc test). N.D.; non-detected.

As previously mentioned, comparing the efficacy of ACP treatment with previous studies is challenging, as it depends on several factors, including voltage, exposure time, initial microbial density, process gas, working distance and plasma exposure time (Gavahian, Peng, & Chu, 2019). In general, information on the sensitivity of fungal spores to plasma is limited and primarily focused on the decontamination of flours, which poses challenges due to sample mixing and uneven treatment.

A limitation of the present study is the long sampling interval between Day 0 and Day 16. Although this design was sufficient to document recovery by the end of the storage period, the absence of intermediate time points limits a detailed kinetic interpretation. In particular, it prevents accurate estimation of the lag phase duration and precludes identification of the onset of regrowth (e.g., whether recovery began on Day 10, 12, or 14).

This limitation highlights the need for higher temporal resolution in future work. To robustly model recovery kinetics and pinpoint the transition from lag to active growth, subsequent studies should include more frequent sampling during the critical recovery window, thereby providing a more complete description of the underlying temporal dynamics.

3.2. Effect of the ACP on the fungal species

To assess whether ACP treatments exerted species-dependent effects, filamentous fungi associated with treated and untreated maize flour samples were identified by PCR amplification and sequencing of the internal transcribed spacer (ITS) region and partial β-tubulin and calmodulin genes. As this work relied on culture-dependent methods, the identified taxa likely represent only a subset of the total mycobiome, i.e., the cultivable fraction present in the original samples. Obligate, fastidious, or otherwise non-cultivable fungi that could not grow under the incubation conditions used would not have been detected.

From 19 fungal isolates, a total of 4 different fungal morphotypes were found in the analyzed samples, that belonged to the species *Aspergillus flavus* Aspergillus *oryzae*, formerly *Aspergillus rugulosus, Aspergillus parvisclerotigenus* and *Davidella* sp. (non-pathogenic specie). In particular, sequencing of the ITS regions, the b-tubulin and calmodulin genes and their analysis have several specific advantages to identify the *Aspergillus* species at the genus and section level (Balajee, 2008). The species isolated here are of particular nutritional interest as they can grow rapidly over a wide range of temperature and a_w (minimum ~0.70–0.72 aw). *Aspergillus* in particular was frequently observed in wheat grain and its flours by different investigators (Minutillo, Ruano-Rosa, Abdelfattah, Schena, & Malacrinò, 2022). In addition, the presence of *Aspergillus* ssp. is of particular concern as several species of this genus are capable of producing mycotoxins. For example, *A. flavus* can produce a secondary metabolite called aflatoxin, which is a potent carcinogen. Among the more than 20 identified forms of aflatoxin, AFB1, AFB2, AFG1 and AFG2 are the most common, with AFB1 being the most hazardous (Wang et al., 2022). On the other hand, *Davidiella* sp. a non-pathogenic fungus that can play beneficial roles in agricultural systems by supporting crop health and inhibiting pathogens through competitive exclusion or the secretion of antifungal compounds was identify in untreated maize flour sample (Sharon et al., 2024).

As shown in Fig. 2, ACP treatments affected the fungal community structure in maize flour, as fewer fungi were isolated, and their diversity decreased with prolonged treatment with ACP. Although no significant differences in the number of colonies were observed after 5 min of treatment compared to the control samples, only *A. flavus* and *A. oryzae* were detected. After 10 min, while an overall decrease was observed, their ratio was maintained farily similar. In contrast, after longer exposures, *A. flavus* showed to be more sensitive compared to *A. oryzae*.

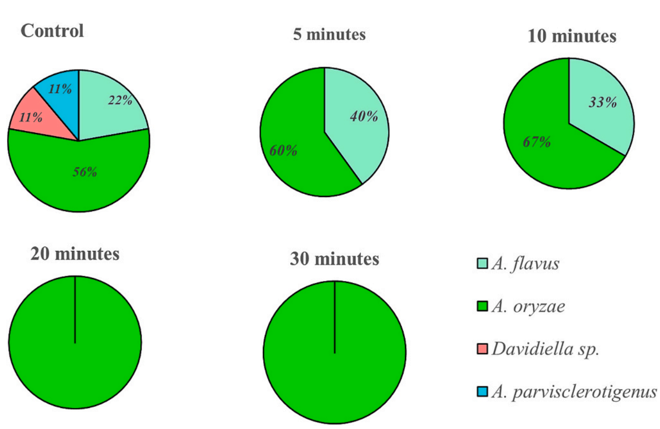

Fig. 2. Incidence of the fungal species on flour maize samples before and after the ACP treatments.

Indeed, only the latter was detected after 20 and 30 min of exposure. This effect may be attributed to ACP-generated reactive species causing sublethal damage to spores and thereby delaying their recovery (Zhao et al., 2025). I. As mentioned above, it is important to emphasize that the longer duration of the treatment means a longer exposure of the cells to plasma reactive species. Similar results were reported by (Zhao et al., 2025; Zhao, Sheng, et al., 2024). Thus, our results indicate that the applied treatment was efficient in the inactivation of spores of species that are frequently reported as contaminants and may produce important mycotoxins.

Zhao et al. (2024) investigated the sporicidal activity of ACP using dielectric barrier discharge plasma (DBDP) on aflatoxin producers *A. flavus* spores and reported that after a 6 min 60 V treatment, the total number of viable spores decreased by 4.47 log10 CFU/ml and the *A. flavus* value was significantly lower after treatment ($p < 0.05$). In the same trends, (Esmaeili, Hosseinzadeh Samani, Nazari, Rostami, & Nemati, 2023) investigated the effect of ACP using a plasma jet, with exposure time of 5–10–15 min, and a supply voltage of10–15–20 kV for the reduction of *A. flavus*. Highest fungal reduction (5.14 log) was reached after a plasma exposure time of 15 min, with a voltage of 20 kV, and a gas mixture including 62.23% of argon, while the maximum aflatoxin reductions (B1, 83.70%; B2, 74.36%; G1 41.39% and G2, 50.74%;) were obtained after a plasma exposure time of 15 min, 54.26% argon in the gas mixture and a fixed dose of 20 ppb aflatoxin. In the present study, *A. flavus* spores were completely inactivated after 20 min of plasma generated at 33 kHz and 15 kV (peak-to-peak).

In this work, culture-dependent methods provided only a partial view of the microbial community and may have overlooked dominant or non-cultivable members. Moreover, the lack of high-throughput sequencing prevented evaluation of whether the cultured isolates were ecologically representative or merely opportunistic taxa favoured by the cultivation conditions. Future integration with amplicon-based profiling and/or metagenomics will be essential to capture unculturable fungi and to contextualise these results within the full community diversity.

3.3. Effect of ACP on particle size distribution

The particle size distribution of the maize flour samples is shown in Fig. 3. The particle size distribution (PSD) of the maize flour samples was influenced by the duration of the atmospheric cold plasma treatment and possibly by the friction/agglomeration. The untreated control exhibited a broad distribution with a dominant peak at 100–150 μm and a small shoulder in the lower range (~10–20 μm), reflecting the heterogeneous nature of the control flour particles. After a 5 min plasma treatment, no significant differences were observed. A longer treatment (20 min) resulted in a sharper and higher peak compared to the control, indicating a shift towards a narrower and more homogeneous PSD. In particular, the 30 min plasma treatment led to the most pronounced effect, with the distribution curve showing the highest and sharpest peak, which was slightly shifted towards larger particle sizes. This result shows that a longer plasma treatment affects the size and homogeneity of the particles. The changes in PSD might be also related to the changes in morphology as indicated in Fig. 4.

Table 3 presents the numerical data obtained from the analysis of the distribution, which allows for a better understanding of the observed phenomena. No significant differences were observed between the 5 min sample and the control; however, both D [3,2] (Surface-weighted mean diameter) and D [4,3] (volume mean) increased significantly after 10 min, with the sample treated for 30 min showing the highest values (100 μm and 179 μm, respectively). The control and 5 min treated samples had the lowest, not significantly different ($p > 0.05$), values. Additionally, the cumulative particle size distribution values D10, D49, and D90 μm showed corresponding changes, confirming the increase in particle size after treatment. Similar results were observed for wheat flour treated with atmospheric cold plasma (Zhang, He, Zhang, & Jiang, 2024). The increase in particle size with longer treatment times may be

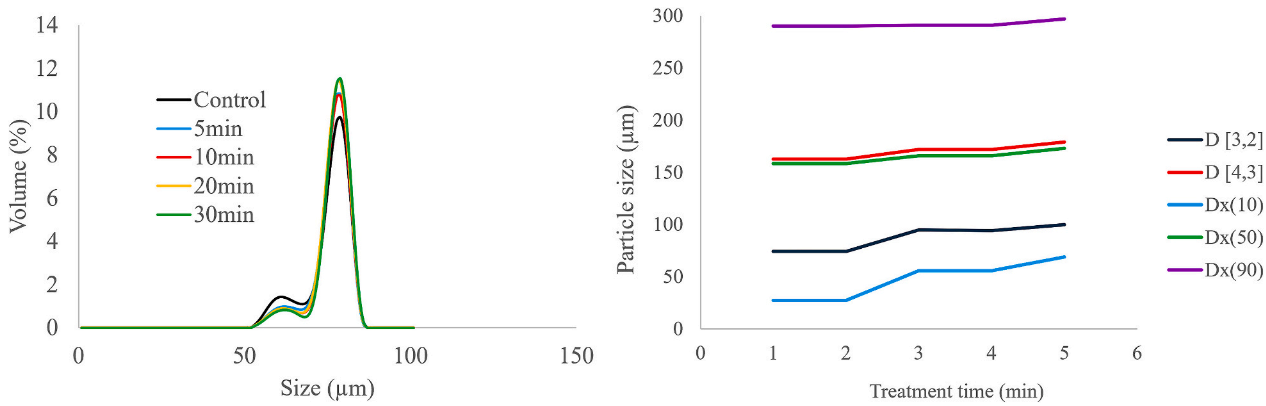

Fig. 3. Particle size distribution of maize fluidized maize flour treated with cold plasma.

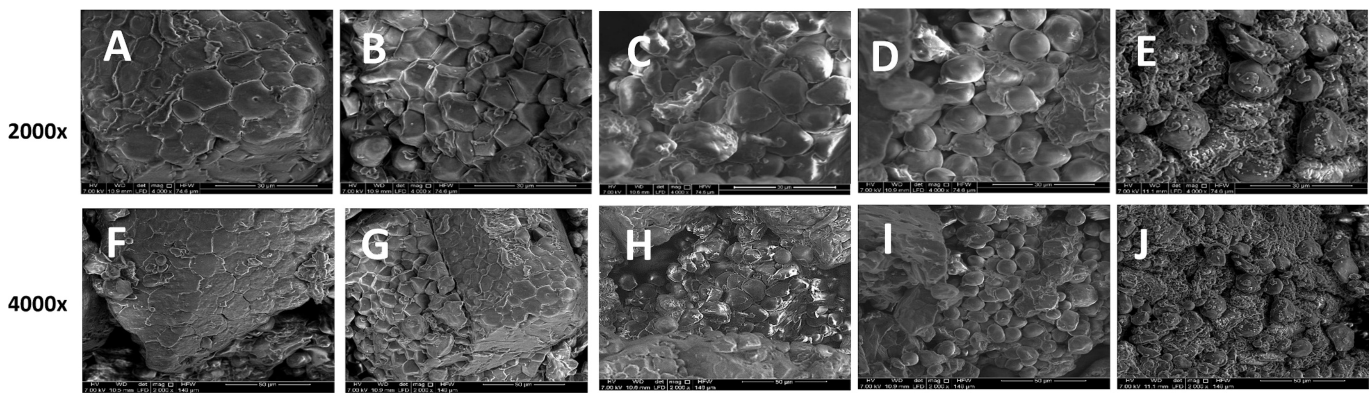

Fig. 4. SEM images of control and ACP treated maize flour; at 2000×: A) control, B) 5 min, C) 10 min, D) 20 min E) 30 min and 4000×: F) control, G) 5 min, H) 10 min, I) 20 min J) 30 min.

Table 3
Distribution of particle sizes of Maize flour.

Samples	Particle size distribution				
	D [3,2] (μm)	D [4,3] (μm)	D_{10}(μm)	D_{50}(μm)	D_{90}(μm)
Control	74.46 ± 0.92^{a}	162.75 ± 0.97^{a}	27.43 ± 0.56^{a}	158.50 ± 0.77^{a}	290.25 ± 1.60^{a}
5 min	74.48 ± 0.45^{a}	162.75 ± 0.82^{a}	27.43 ± 1.35^{a}	158.50 ± 1.22^{a}	290.25 ± 0.82^{a}
10 min	95.00 ± 0.91^{b}	172.00 ± 0.34^{b}	55.90 ± 0.21^{b}	166.00 ± 1.10^{b}	291.00 ± 0.57^{a}
20 min	94.20 ± 0.52^{b}	172.00 ± 0.77^{b}	55.90 ± 0.78^{b}	166.00 ± 0.31^{b}	291.00 ± 0.83^{a}
30 min	100.00 ± 0.99^{c}	179.00 ± 1.4^{c}	68.90 ± 0.92^{c}	173.00 ± 0.19^{c}	297.00 ± 0.69^{b}

Values are given as mean ± standard deviation. Different letters in the same column indicate significant ($p < 0.05$) differences between the means.

Table 4
Color, moisture and water activity of maize flour sample before and after ACP treatment.

Sample				Color		
	a_w	T (°C)	Moisture (db %)	*L**	*a**	*b**
Control	0.61 ± 0.02^{a}	29.50 ± 0.21^{c}	15.50 ± 0.02^{a}	92.03 ± 0.10^{b}	0.19 ± 0.06^{e}	12.67 ± 0.01^{a}
5 min	0.57 ± 0.02^{a}	31.50 ± 0.49^{b}	14.90 ± 0.07^{a}	92.11 ± 0.11^{b}	0.31 ± 0.01^{d}	12.64 ± 0.02^{a}
10 min	0.43 ± 0.01^{b}	32.60 ± 0.12^{ab}	11.30 ± 0.6^{b}	92.37 ± 0.21^{b}	0.45 ± 0.01^{c}	12.11 ± 0.01^{b}
20 min	0.39 ± 0.03^{c}	32.90 ± 0.31^{a}	10.00 ± 0.02^{c}	92.54 ± 0.13^{b}	0.51 ± 0.01^{b}	11.31 ± 0.03^{c}
30 min	0.36 ± 0.03^{d}	33.00 ± 0.09^{a}	10.00 ± 0.03^{c}	93.80 ± 0.43^{a}	0.73 ± 0.01^{a}	11.26 ± 0.03^{c}

Values are given as mean ± standard deviation. Different letters in the same column indicate significant ($p < 0.05$) differences between the means. Dry based (d.b.).

due to the fluidization of the flour combined with cold plasma, resulting in agglomeration, especially of small particles. When the particle size becomes smaller, the relative magnitude of the inter-particle forces increases. Agglomeration is a common phenomenon observed during the fluidization of such flours (Valverde & Castellanos, 2007), as strong interparticle forces cause individual particles to stick together and form agglomerates (Kaliyaperumal, Barghi, Briens, Rohani, & Zhu, 2011).

3.4. Effect of ACP on physico-chemical properties

Table 4 reports the physico-chemical properties measured in the maize flour samples at different plasma exposure times. ACP treatments resulted in an increase of the surface temperature of the maize flour from 29.5 (Control) to 33 °C (30 min). The surface temperature of the maize flour significantly increased within the first 5–10 min of the ACP treatment and later became stable when the plasma discharge energy supplied by the system and the sample attained equilibrium state.

Moisture and water activities were analyzed before and after ACP treatment, and the results are shown in Table 4. In the control, the moisture content was 15.5%. Significant changes ($p < 0.05$) were observed starting from the 10 min treatment. In the long duration treatments (30 min), the moisture content decreased from 15.5 to 10%

(30 min). The decrease in moisture content could be due to the utilization of moisture for the generation of plasma reactive active species and the transfer of moisture to the surface by etching and electron bombardment during the treatment. These results are consistent with the studies conducted with different flours (Sarangapani et al., 2016; Thirumdas et al., 2016; Zhang et al., 2024).

The initial a_w value of the maize flour was 0.61. A significant decrease was observed with the increasing duration of ACP treatment. Specifically, the a_w value decreased to 0.43, 0.39, and 0.36 for 10, 20, and 30-min ACP treatments, respectively. A similar result was reported (Kaur & Annapure, 2023).

The L^*, a^* and b^* values of maize flour samples treated for different treatment times were compared in Table 4. Plasma contains various reactive species and can cause surface etching in the treated product, therefore changes in color values are to be expected (Joy et al., 2022).

L^* values remained similar up to 20 min of treatment, whereas a significant increase was observed only after 30 min. The a^* parameter showed a significant increase with longer treatment duration. Conversely, the b^* value decreased significantly from 10 min onward, suggesting a reduction in yellowness. The lowest b^* values were observed after 20 and 30 min of treatment. Similar increases in L^* value following atmospheric cold plasma treatment have been reported by (Chaple et al., 2020; Sarangapani et al., 2015; Zhang et al., 2024) in rice and wheat flours. These changes might be due to the reaction between plasma ROS and RNS and also changes in moisture content. The changes in a* and b^* values indicate that ACP treatment made the maize flour more red and less yellow. ACP is generated with rich RONS, and these reactive species could cause etching, fracture, and might result in pigments decomposition in maize flour.

3.5. Scanning Electron Microscopy (SEM) of ACP-treated maize flour

The surface structure of maize flour was examined by SEM to visualize the effects of different ACP treatment times, as shown in Fig. 4, revealing notable changes. The control sample (Fig. 4A) displayed granules with a well-defined, compact, and intact appearance, indicating that the maize flour granules and their surroundings retained their natural structure. After 5 min of ACP exposure (Fig. 4B), small surface pits and slight roughness appeared.

This might suggest the initial signs of interaction between plasma's reactive species and the outer layer of the flour particles but also the friction of the particle against the wall of the container and between the particles themselves that, as well as the impact, can contribute to modifying the surface. Therefore, what we are observing might be an etching due to a combination of plasma and mechanical effects.

When the treatment was increased to 10 min (Fig. 4C), the granules became more irregular and partially altered, and the surfaces appeared rougher, with some edges deformed. This may result from the combined effects of bombardment by ACP reactive species (Wani et al., 2015). With 20 min of treatment (Fig. 4D), the damage became more apparent: the granules lost their clear boundaries, began to collapse, and formed aggregates, possibly due to surface etching, microcracking, and fusion. Similar results were observed in protein, starch, and flour as treatment time increase (Ji et al., 2020; Yang et al., 2024). After 30 min (Fig. 4E), the particles appeared heavily eroded, compacted, and fused into irregular clusters. Such structural changes are typical of extended plasma exposure (Yang et al., 2024), where continuous bombardment by reactive species causes erosion, cracks, and holes on the surfaces of the granules, resulting in surface destruction.

Overall, the SEM images reveal clear time-dependent morphological changes on the maize flour surface. Short plasma treatments (up to 10 min) mainly produce surface activation without severely affecting the structure. However, longer exposures (20–30 min) lead to extensive degradation of the granules. Similar results have been reported for plasma-treated cereal and starch flours (Hosseinpour, Fazaeli, Hedayati, & Niakousari, 2025; Jaddu, Pradhan, Dwivedi, & Technologies, 2022; Sarkar et al., 2023; Zare, Mollakhalili-Meybodi, Fallahzadeh, & Arab, 2022).

4. Conclusion

Atmospheric cold plasma (ACP) treatment showed high efficacy in inactivating microorganisms in maize flour, with longer exposure times leading to significantly greater reductions in microbial load. Complete inactivation of filamentous fungi was achieved after 5 min of treatment, whereas mesophilic aerobic bacteria required 30 min. During 16 days of storage, bacterial populations in treated flour exhibited significant recovery, reaching levels comparable to, if not higher than, the untreated control. In contrast, although fungal populations also showed some recovery, they remained substantially lower than in the control samples.

The impact of ACP on the fungal community was species dependent. Physicochemical analyses indicated that prolonged plasma exposure reduced moisture content and water activity and altered particle size distribution and microstructural characteristics, while only minor color changes were detected. Overall, these results suggest that ACP—particularly when coupled with continuous mixing—represents a promising technology for fungal decontamination of flour. However, extended treatments may induce measurable changes in selected quality attributes.

These findings also support the view that resistance and susceptibility to ACP are intrinsic, species-specific traits that influence treatment efficacy across food matrices. Nevertheless, further studies are needed to better characterize the full extent of microbial decontamination and post-treatment recovery. Future research should specifically investigate how variability in flour composition relates to initial microbial loads and how this variability affects both ACP effectiveness and its impact on physicochemical properties and product safety. Finally, validation under standard supply-chain storage conditions will be required to confirm any shelf-life extension.

CRediT authorship contribution statement

Gebremedhin Gebremariam Gebremical: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Junior Bernardo Molina Hernandez:** Writing – review & editing, Methodology, Formal analysis. **Silvia Tappi:** Writing – review & editing, Supervision. **Luigi Ragni:** Writing – review & editing, Conceptualization. **Clemencia Chaves Lopez:** Writing – review & editing, Project administration. **Massimiliano Rinaldi:** Writing – review & editing, Supervision, Methodology. **Pietro Rocculi:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

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Data availability

Data will be made available on request.

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