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Cold Atmospheric plasma treatments trigger changes in sun-dried tomatoes mycobiota by modifying the spore surface structure and hydrophobicity

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1 **Cold Atmospheric plasma treatment trigger changes in sun-dried tomatoes**
2 **mycobiota by modifying the spore surface structure and hydrophobicity**
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4

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47 **Abstract**

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49 The contamination of sun-dried tomatoes during processing can have a decisive impact on the
50 quality of the finished product. In this study we investigated how Cold Atmospheric Plasma (CAP)
51 under high surface power density (SPD) values can reduce fungal contamination in sun-dried
52 tomatoes. In the application of this innovative processing method, the established “regime” for air
53 plasma chemistry was the transition regime or NO_x regime. First, we isolated and identified the
54 mycobiota present on the tomatoes surfaces by mean of the analysis of the ITS region. The analysis
55 revealed 32 different species, with *A. niger*, *A. tubingensis*, *A. chevalieri*, *A. flavus*, and *A.*
56 *alternata* being the most abundant. Then, to reduce the fungal population, CAP-NO_x was applied
57 for 5, 10, 20 and 30 min on the surface of dried tomatoes. After incubation for 10 days, we observed
58 that the antifungal effect was species and dose-dependent. *In vitro* investigation on the most
59 abundant species revealed that *A. chevalieri* PSJ144 was the most sensitive species (almost 90%)
60 immediately after 5 min of CAP treatment. With the increase of the exposure time up to 30 min, a
61 strong reduction ($p \leq 0.05$) of spore germination of *A. alternata* PSJ77 and *A. tubingensis* PSJ100
62 was observed (98 and 92%, respectively). However, spores of *A. niger* PSJ38 and *A. flavus* PSJ30
63 showed the highest resistance to the treatment.

64 Moreover, the reparameterized Weibull function allowed to obtain useful information about
65 germination kinetics as a function of time of CAP-NO_x treatment, revealing that the resistance of
66 the spores was: *A. chevalieri* < *A. alternata* < *A. tubingensis* < *A. flavus* < *A. niger*. *In situ* analyses
67 confirmed a significant effect on natural fungal contamination by CAP-NO_x treatment (76.5 % of
68 reduction), likely due to cell membrane rupture and cell death caused by plasma radicals. In
69 addition, Pearson correlation analysis showed that spore resistance was highly correlated ($p=0.98$)
70 with their hydrophobicity. In a nutshell, our results clearly indicate that CAP-NO_x treatment is an
71 effective technique to reduce fungal contamination in sun-dried tomatoes. Among non-thermal
72 processing methods, CAP shows promising perspectives of application in the tomato industry, to
73 mitigate the effects of energy price rises.

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80 **Introduction**

81

82 Daily consumption of fruits and vegetables, in sufficient quantity and quality, helps prevent
83 diseases such as cardiovascular diseases, diabetes and cancer, as well as deficiencies of
84 micronutrients and essential vitamins. The World Health Organization (WHO) ranks inadequate
85 consumption of fruits and vegetables sixth among the 20 risk factors for human mortality.
86 Although these products are known to contain a natural non-pathogenic epiphytic microbiota, they
87 can be contaminated with microorganisms that can be pathogens from human and animals, and
88 can grow during harvesting, transportation, processing, and handling.

89 Tomato (*Lycopersicon esculentum* L. var. Excell and Aranca) is a highly perishable and fragile
90 vegetable, which is highly susceptible to contamination by microorganisms and mechanical
91 damage during transportation, processing, and storage (Hegazy, 2017). They are not only
92 consumed as fresh produce, but also processed into a variety of products, such as pulp, ketchup,
93 sauces, paste, juices, and dried tomatoes (Sanzani et al., 2019). In this context, several strategies
94 are used to produce dried tomatoes. Sun-drying, which is the oldest among all the drying
95 techniques, is still one of the most commonly used methods to produce dried tomatoes. Nowadays,
96 sundried tomato is an important ingredient in the food and catering industry, but the quality of this
97 product is not always constant (Sohail et al., 2011). During production, the fruit is sliced to increase
98 the surface-area to volume ratio for the loss of moisture, and the pieces are then dried in open
99 spaces under the sun, where they can come into contact with microorganisms, dirt, soil and insects,
100 leading to possible microbial contamination of the product (Canakapalli et al., 2022). Tomato
101 slices are left in full sun for 4–8 days until they have lost most of their moisture content up to 10–
102 15% (Oberoi et al., 2007). In Italy, dried tomatoes are first cured with sodium chloride for 7 days,
103 and then stored at room temperature without humidity control, reaching a shelf-life of around 12
104 months at room temperature. Drying is a critical step in processing, which can lead to fungal
105 contamination from the environment, which can affect the quality of the product (Kakde & Kakde,
106 2012; Sanzani et al., 2019). In addition, rehydration of dried fruit under unsuitable storage
107 conditions may reactivate the fungal growth with subsequent mycotoxin formation (Karaca et al.,
108 2010). Some studies have reported the presence of fungal spores in dried tomatoes, which belonged
109 to the following species: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Mucor*

110 *spp.*, *Penicillium brevicopactum*, *Penicillium chrysogenum*, *Fusarium culmorum*, *Aspergillus*
111 *rugulovalvus* formerly *Aspergillus rugulosus* (syn *Emericella rugulosa* var. *lazuline*), *A. niger*, *A.*
112 *amstelodami*, *A. tubingensis*, *A. cristatus*, *R. oryzae*, *Cladosporium cladosporioides*, *Corynascus*
113 *sepedonium*, and *Alternaria sp.* (Molina-Hernandez et al., 2022; Sanzani et al., 2019; Suleiman et
114 al., 2017). Among these species, *Aspergillus ssp.*, *Alternaria sp.* and *Penicillium sp.* deserve
115 special attention since several species of these genera can produce mycotoxins. The incidence of
116 these toxic fungal metabolites in dried tomatoes results in economic losses for growers as
117 contaminated export products are rejected (Abdallah et al., 2020; Heperkan et al., 2012).
118 Therefore, the industry today uses strategies aimed to reduce the growth of these molds, which are
119 generally based on the use of chemicals. However, the increasing demand for foods with high
120 quality, with less or no additives, promotes the development of technological alternatives for
121 fungal control. In particular, due to the recent rise of energy prices, non-thermal processing
122 methods are appealing options for food manufacturers.

123 Over the last decades, various non-thermal technologies have been proposed for surface
124 decontamination of dried fruits, including gaseous ozone and ozonated water (Zorlugenç et al.,
125 2008), ultraviolet (UV-C) alone and in combination with clove essential oils (Gündüz & Korkmaz,
126 2019), pulsed light (PL) (Aguiló-Aguayo et al., 2013), ultrasound (Görgüç et al., 2021), gamma
127 (γ) irradiation (Hamanaka & Chandel, 2009), electron beam irradiation (Mousavi Khaneghah et
128 al., 2020), microwave (Popelářová et al., 2021), and cold atmospheric plasma (CAP) (Lee et al.,
129 2015; Molina-Hernandez et al., 2022). The latter technology involves the use of a mixture of
130 ionized gas consisting of charged particles, electric fields, ultraviolet (UV) photons, and reactive
131 species, which can exert a strong oxidative power (Laurita et al., 2021). The chemistry that governs
132 the atmosphere inside a plasma reactor depends on the surface power density (SPD), which is the
133 ratio between the power absorbed by the plasma and the surface area of the electrode. According
134 to Simoncelli et al. (2019), two different regimes can be observed below and above a SPD
135 threshold of 0.1 W/cm²: 1) small SPD values, where the chemistry is dominated by ozone
136 formation reactions (O₃ -regime); 2) high SPD values, where the formation of NO, N, NO₂, NO₃,
137 N₂O₅, O, O₃ dominates, in a condition that is known as transition regime or NO_x regime. Few
138 publications have reported the potential antibacterial activity through the generation of Reactive
139 Nitrogen Species (Wang et al. 2022) by plasma treatment (Shaw et al. 2018). Some researchers
140 have been proposed that NO and the production of intracellular derivatives, such as peroxinitrite

141 and carbonate radicals, can act as effective antimicrobial agents, causing biological effects (DNA
142 damage, binding to iron centers, oxidation of thiols, cysteine, etc.) that induce permanent damage
143 in microorganisms (Hao et al. 2014).

144 Recently, we have shown that CAP-O₃ treatments affect the fungal community structure in
145 sundried tomatoes, as fewer fungi were isolated, and their diversity decreased with prolonged CAP
146 treatments (Molina-Hernandez et al., 2022). To the best of our knowledge, there are no studies in
147 the scientific literature on the application of CAP under NO_x regime to decontaminate dried fruits.
148 Thus, the aim of this study was to evaluate the effects of CAP at NO_x regime on the fungal
149 population on the surface of sundried tomatoes collected in different regions of Italy. First the
150 predominant mycobiota in sundried tomatoes was identified by molecular methods before and after
151 treatment. Next, spore inactivation of the most abundant fungal species, isolated from dried tomato
152 samples, was investigated.

153 **2. Materials and methods**

154

155 *2.1. Samples*

156 Twenty-three batches of sundried tomatoes (2 kg) were randomly collected from retailers across
157 Abruzzo, Puglia, and Umbria regions in Italy during March 2021. All samples were of commercial
158 quality; samples were selected to avoid visible damage or macroscopic contamination by
159 filamentous fungi. To avoid fungal development without affecting the viability of spores present
160 in sundried tomato, samples were stored in vacuum packed at 20°C until analysis.

161

162 *2.2 Natural mycobiota in sundried tomatoes*

163 *2.2.1. Fungal isolation*

164 For each batch, ten sundried tomato fruits were randomly selected for each treatment and for the
165 untreated control. For the control and after the treatments, each fruit sample was cut into squares
166 of approximately 1.5 x 1.5 cm, and subsequently the different sub-samples were aseptically placed
167 on different culture media: Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Nutrient agar
168 (WL), and Dichloran Glycerol agar base (DG18) for xerophilic filamentous fungi.
169 Chloramphenicol (0.05 g L⁻¹), purchased from Liofilchem (Liofilchem, Roseto degli Abruzzi-
170 Italy), was added to all culture media with to prevent bacterial growth. All Petri dishes were

171 incubated for 7 to 10 days at 25°C with 12 h of light and 12 h of darkness in a humid chamber.
 172 The plates were then examined to measure the percentage of colonized sub-samples, and fungal
 173 diversity (morphotypes). The different colonies were tentatively identified according to their
 174 morphology (Samson, Hoekstra and Van Oorshot, 1984) and grouped by their morphological
 175 appearance. Pure cultures were obtained from hyphal tip transfer to PDA media and stored at 5°C.
 176 The percentage of frequency of each morphotype (MF) for each treatment was calculated
 177 according to:

$$178 \quad MF (\%) = (\sum \text{isolates of the morphotype}) / (\sum \text{all fungal isolates}) \times 100$$

179 Finally, to increase the rate of fungal growth, the plates were incubated at 30°C.

180

181

182 2.2.2. Phenotypical and molecular identification of filamentous fungi

183 To identify the isolates based on the morphological and growth characteristics, single colonies
 184 were purified in malt extract agar (MEA) (Liofilchem, Roseto degli Abruzzi-Italy), and
 185 subsequently isolated filamentous fungi were identified based on the morphological characteristics
 186 under a light microscope according to Munitz et al. (2013). Then, the fungal isolates were
 187 tentatively assigned to different genera based on the size and shape of the spores and mycelia. To
 188 confirm the identity of the fungi, molecular identification was then carried out according to the
 189 method reported by Delgado-Ospina et al. 2021. The PCR assay was performed using the primers
 190 listed in Table 1. The ITS region was amplified with the primer pair ITS1-ITS4, ITS1-ITS2.
 191 Additional loci (β -tubulin, Calmodulin) were used to identify *Aspergillus* species. All primers
 192 used were purchased from Sigma Aldrich (Saint Louis, Missouri, USA).

193

194 Table 1. Primers used for PCR assay.

195

Gene name	Gene	Length bp	Primer	Sequences (5' → 3')	Reference
Internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S ribosomal DNA (rDNA) region	ITS (1-4)	420-825	ITS1 (F)	5'TCCGTAGGTGAACCTGCCGG3'	(Glass & Donaldson, 1995)
			ITS4 (R)	5'TCCTCCGCTTATTGATATGC3'	
	ITS (1-2)	565-613	ITS1 (F)	5' GGAAGTAAAGTCGTAACAAGG 3'	
			ITS2 (R)	5' TTGGTCCGTGTTTCAAGACG 3'	

β-tubulin	ben A	1125	β -tub 2a (F)	5'GGTAACCAAATCGGTGCTTTC 3'	(Makhlouf et al., 2019)
			β -tub 2b (R)	5'ACCCTCAGTGTAGTGACCCTTGGC 3'	
Calmodulin	cmdA	543	Cmd5 (F)	5'-CCGAGTACAAGGAGGCCTTC-3'	
			Cmd6 (R)	5'-CCGATAGAGGTCATAACGTGG-3'	

196 Abbreviation: F: Forward, R: Reverse

197

198

199 2.3. Plasma Treatments

200 A detailed description of the plasma system used for the treatments has already been given in a
 201 previous work (Molina-Hernandez et al., 2022), but the most relevant details are reported here for
 202 the sake of completeness. Cold Atmospheric Plasma (CAP) was generated by a Surface Dielectric
 203 Barrier Discharge (SDBD) placed at the top of a closed chamber, defining a confined atmosphere.
 204 A high voltage generator produced a sinusoidal waveform with a peak voltage of 6 kV and a
 205 repetition frequency of 23 kHz; the power density absorbed by the plasma source was of
 206 425.35 ± 25.79 W, resulting in a surface power density of $2,6$ W/cm². Treatments were carried out
 207 at room temperature (26 ± 1 °C). Thirty sundried tomato pieces were placed side and successively
 208 treated as described in paragraph 2.2.1. Afterward, the plate with the samples was subjected to
 209 CAP treatment at 20 cm perpendicularly from the SDBD.

210

211

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214

215 2.4. Optical Absorption Spectroscopy (OAS) measurements

216 OAS measurements were performed as described by Simoncelli et al. (2019). More specifically,
 217 OAS measurements rely on the Lambert-Beer law, which correlates the amount of light absorbed
 218 by a certain species to the absolute concentration of such species:

$$219 \quad n_k = -\frac{1}{L\sigma_k} \ln \frac{I}{I_0}$$

220

221 where n is the concentration of the k -th species, L is the optical path (25 cm), I_0 is the intensity of
 222 incident light, and I is the residual light intensity after the absorption.

223 The wavelengths selected for the absorption measurements performed in this study and the
224 absorption cross-sections of the absorbing species of interest, O₃ and NO₂, are listed in Table 2.
225 These wavelengths were defined, in accordance with Moiseev et al. (2014), to maximize the
226 absorption of the molecules while minimizing the contribution, and thus the interference from
227 other absorbing molecules.

228

229 **Table 2.** Absorption cross-sections in cm² of the species of interest at each selected wavelength.

230

Selected wavelength	O₃ cross-section	NO₂ cross-section
253±1.2 nm	(1,12±0.02) E-17	(1.1±0.3) E-20
400±1.2 nm	(1,12±0.08) E-23	(6.4±0.2) E-19

231

232 The setup conditions used to perform the OAS were the same described by Simoncelli et al. (2019).
233 Two LEDs were used as the light source, one with maximum emission at 255 nm and the other
234 with maximum emission at 400 nm. The light beam was focused using optical fibers and fused
235 silica lens to obtain a parallel beam passing inside the plasma chamber, at a distance of 20 cm from
236 the SDBD. The same distance was maintained between the SDBD and the samples during
237 treatments. The beam was then collected in a 500 mm spectrometer (Acton SP2500i, Princeton
238 Instruments) and spectrally resolved in the UV, VIS and near infrared (NIR) regions. OAS
239 acquisitions were performed using a grating with a resolution of 150 mm⁻¹ and setting a width of
240 10 µm for the inlet slit of the spectrometer. A photomultiplier tube (PMT-Princeton Instruments
241 PD439) connected to a fast oscilloscope (Tektronix MSO46) was used as detector to allow fast
242 acquisitions (time resolution of 40 ms). The PMT amplification factor was kept constant for all
243 acquisitions. Prior to every measurement, the plasma chamber was opened and flushed with air for
244 5 minutes to ensure identical initial conditions. Each measurement was repeated 3 times.

245

246 *2.5. Effect of CAP on natural contaminated sundried tomatoes*

247 To evaluate fungal inhibition, the most contaminated batches were subjected to decontamination.
248 For each batch, ten different tomatoes were subjected to the CAP-NO_x treatment (5, 10, 20, 30,
249 min) and successively treated as described in paragraph 2.2.1.

250

251 2.6. *Effect of CAP on spore germination*

252 For the most frequent species found in the control samples of sundried tomatoes, the *in vitro*
253 resistance/sensibility to CAP-NO_x exposure time was determined. For this purpose, the spores of
254 *A. niger* PSJ38, *A. tubingensis* PSJ100, *A. flavus* PSJ30, *Aspergillus chevalieri* PSJ144 and
255 *Alternaria alternata* PSJ77 were collected according to the method described by Molina-
256 Hernandez et al. (2022), standardized at a wavelength of 620 nm to obtain an optical density (OD)
257 of 0.1 AU, which corresponds to 1.0×10^5 spores/mL. The different spore suspensions were placed
258 on petri dishes and treated with CAP for 5, 10, 20, 30, 40 and 50 min, respectively. After the
259 treatment, an aliquot of 20 μ L of the spore suspension was inoculated into glass slides with a thin
260 layer of MEA and incubated at 30 °C for 16 h. Untreated spores were considered as controls. Then,
261 spore germination was observed with a light microscope. Spores were considered germinated when
262 their germ tube was longer than that of the same spore (Peralta-Ruiz et al., 2020). All the
263 experiments were performed in triplicate, and a total of 200 spores were counted for each sample.

264

265 2.7. *Assay of cell surface hydrophobicity*

266 The hydrophobicity index of the outermost surface of the spores was determined by microbial
267 adhesion to hydrocarbons, according to the methodology reported by Wang et al. (2017). The spore
268 suspension solutions were washed twice and then resuspended in PBS to an OD₆₀₀=1. The
269 absorbance of the spore suspension was measured and defined as A₁. Subsequently, one milliliter
270 of n-butyl alcohol was then added to 1 ml of the cell suspension in a 15-ml falcon tube. After
271 vortexing for 30 s and 3 minutes of incubation, separation occurred. The absorbance of the lower
272 aqueous phase was defined as A₂. Hydrophobicity is then expressed as percentage calculated using
273 the following equation:

274

$$275 \text{Hydrophobicity (\%)} = (A_1 - A_2) / A_1.$$

276

277 2.7. *Spore viability*

278 To analyze spore viability immediately after CAP treatment, the spores most frequently found in
279 sundried tomatoes were stained with a mixture of CFDA (carboxyfluorescein diacetate) and
280 propidium iodide (PI) according to methodology reported by Molina-Hernandez et al. (2022).

281 While the green fluorescent dye CFDA is able to penetrate both intact and damaged cell
282 membranes, the red fluorescent dye PI can only penetrate cells with a significant membrane
283 damage (Molina-Hernandez et al., 2021). 10^4 spore suspension in PBS was treated with CAP-NO_x
284 for 5, 10, 20, 30 and 40 min, then stained with both dyes. A Nikon A1R confocal imaging system
285 (Nikon Corp., Tokyo, Japan) was used to observe spore viability.

286

287 2.8. Texture analysis of the sundried tomatoes

288 Firmness (F), skin strength (SS) and elasticity (E) of control and treated tomato samples were
289 evaluated using a texture analyzer (TA.HDi 500; Stable Micro Systems, Godalming, UK)
290 according to the method described by Serhat Turgut et al. (2018). Briefly, a Perspex blade (A/LKB)
291 was used for F measurements of tomato samples at the speed of 2 mm/s. For each tomato, two
292 measurements were made. SS and E were determined using a 2-mm cylindrical stainless probe at
293 the speed of 1 mm/s. For each tomato, 3 points were punctured. For each sample, three tomato
294 slices were measured. F and SS were expressed in g. E was expressed in mm.

295

296 Statistical analyses

297 For the *in vitro* studies, to evaluate both the CAP effect on fungal spore germination and the
298 reduction in fungal mycelial growth in response to the treatments, data from each sampling point
299 were shown as the mean \pm SD and statistically analyzed by ANOVA, followed by individual
300 comparisons using Duncan's Multiple Range Test, at $p \leq 0.05$ and using Pearson correlation as a
301 measure of linear association between treatments.

302 To describe the kinetics of spore inactivation due to time of CAP-NO_x treatment, the
303 reparametrized Weibull model proposed by De Flaviis & Sacchetti (2022), in the form of a survival
304 function, was fitted. The model was reformulated as follows:

$$F(t) = N_0 \left\{ e^{\left[-\left(\frac{t}{\beta}\right)^{\frac{e\beta\mu\beta}{N_0}} \right]} \right\} \quad (1)$$

305 The Weibull model, extensively used in many applications (e.g., analysis of non-linear survival
306 curves), was chosen because it can fit different shapes of decay (e.g., sigmoidal curves, long-tailed
307 curves, first order decay) and is a very simple and flexible model (van Boekel, 2008). Moreover,

308 the reparametrized Weibull model utilized in this study allows to obtain three meaningful
 309 parameters for the interpretation of the kinetics of spore inactivation: i) the initial percentage of
 310 germination (N_0); ii) the failure time (β), corresponding to the CAP-NOx treatment time when
 311 63.2% of the kinetics are reached; iii) the reduction rate at the failure time (μ_β). The least squares
 312 criterion and the “Levenberg–Marquadt” method were used to fit the models and estimate the
 313 parameters N_0 , β and μ_β . The goodness of fit of the models was evaluated considering the R^2 , the
 314 coefficient of variation of the root means square error (CV(RMSE)) and the Akaike’s Information
 315 Criterion (AIC). Moreover, the maximum reduction rate (μ_{max}) and the lag phase (λ),
 316 corresponding to the so-called “shoulder effect” were calculated following the equations reported
 317 by De Flaviis & Sacchetti (2022):

$$\mu_{max} = \mu_\beta e \left(\frac{D}{e}\right)^D \quad (2)$$

$$\lambda = \beta[D^{-D} - (D^{-D} - D^{1-D})(e^D)] \quad (3)$$

318 Where D is:

$$D = \frac{\mu_\beta e \beta - N_0}{\mu_\beta e \beta} \quad (4)$$

319 Statistical differences of estimated and calculated parameters among fungal spores were analyzed
 320 by one-way ANOVA and Least Significant Difference (LSD) post-hoc test. Non-linear regressions
 321 were performed using Wolfram Mathematica software (Wolfram Research, Inc.).

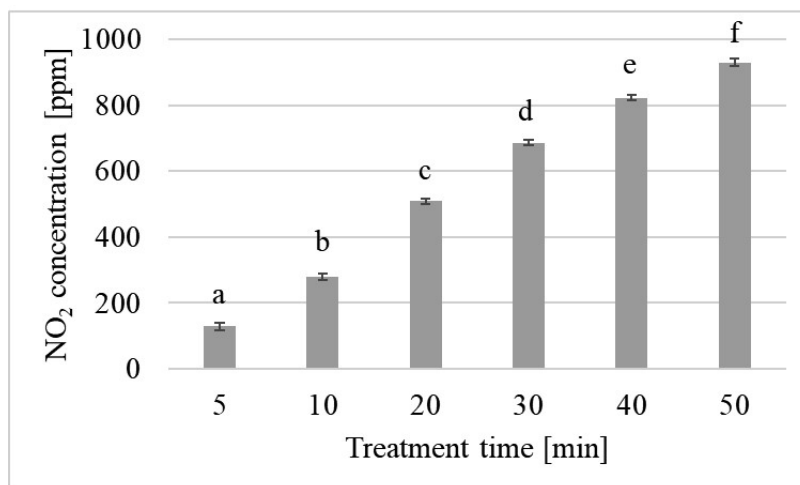
322

323 3. Results

324 3.1 Cold Atmospheric Plasma (CAP)

325 The temporal evolution of O_3 and NO_2 was monitored using OAS. Coherently with the high SPD
 326 involved in the treatments, no O_3 was detected, whereas a significant NO_2 concentration was
 327 measured and was observed to increase with time, as shown in Figure 1.

328



329
 330 **Figure 1.** Values of NO₂ after different treatment times. The values are the mean of three repetitions. In
 331 each panel, data are mean ± SD, and statistical significance is specified with letters (*p ≤ 0.05 as determined
 332 by paired Student t-test).

333

334 3.2 Fungal contamination in sundried tomatoes

335 In this study, sundried tomatoes samples were tested for the presence and levels of filamentous
 336 fungi contaminants. The analysis revealed that all the tomatoes samples were contaminated with
 337 spores that germinated and produced proliferous mycelia, although with high variability in the
 338 fungal incidence.

339 A total of 220 filamentous fungi were isolated, and 78, representative of all the sundried tomatoes
 340 samples, were identified morphologically at a genus level and successively identified at a species
 341 level by PCR. The isolates belonged to 20 genera and represented a total of 32 different species
 342 (Table S1). Among the fungal species recovered, *Psathyrella candolleana* and *Trametes elegans*
 343 belonged to Basidiomycetes and the other species belonged to Ascomycetes. The relative
 344 abundance of the species identified showed that the genus *Aspergillus* supported 47% (94 isolates)
 345 of the cultivatable mycobiota, followed by *Alternaria* (6.8%), *Stemphylium* (6.41%), *Chaetomium*
 346 (8.1 %), *Arthrinium* (4.1%), and *Penicillium* (3.6 %). Other fungal genera such as *Rhizopus*,
 347 *Eutypella*, *Chrysonilia*, *Byssochlamis*, *Psathyrella*, *Trimmatothelopsis*, *Canariomyces*,
 348 *Corynoascus*, *Trametes*, *Eutypella*, *Amesia*, *Aporospora*, *Ovatospora*, *Cladosporium*,
 349 *Gymnascella* were also present at lower levels ranging from 1.8 % to 2.7 %.

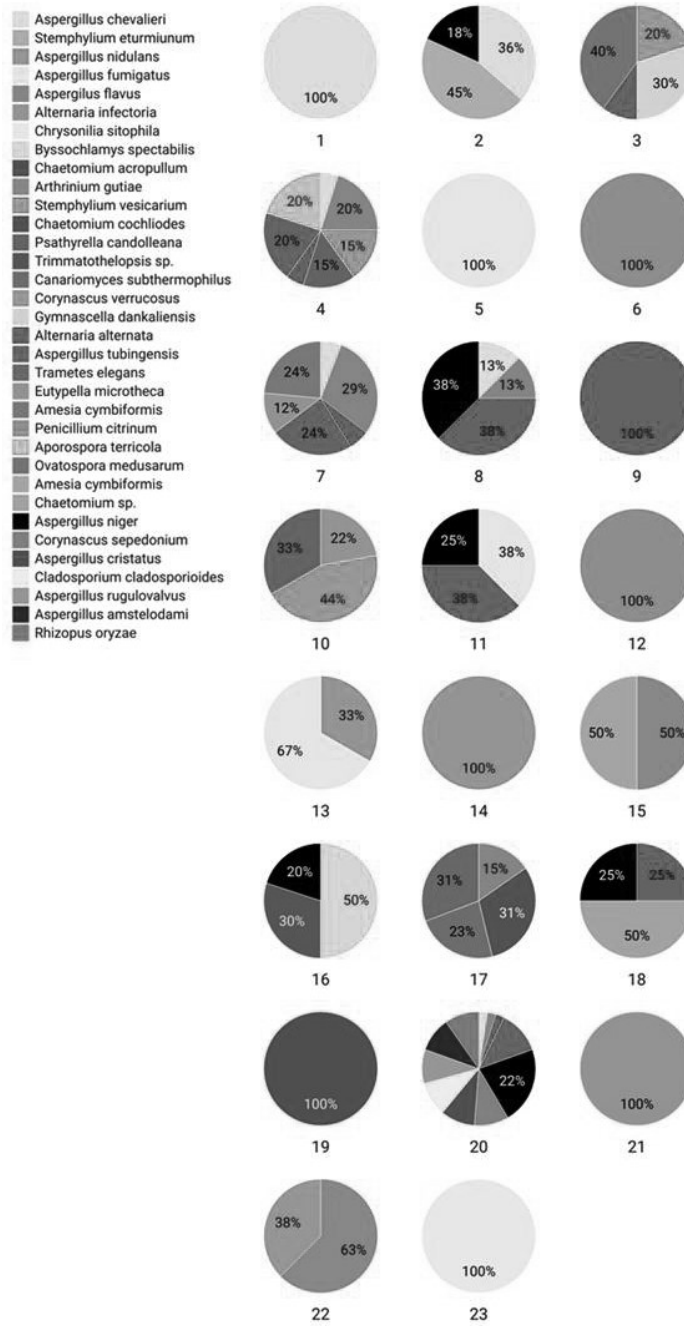
350 As shown in Figure 2, the most frequent and abundant species in sundried tomatoes were
 351 *Aspergillus niger* and *Aspergillus tubingensis*, which were present in 6 and 4 out of 24 samples,

352 representing respectively 9.1 % and 7.3 % of all isolated strains, followed by *Aspergillus chevalieri*
353 (5.9 %), *Aspergillus flavus* (5.9%), *Alternaria alternata* (5.9 %), and *Aspergillus fumigatus* (4.5%).
354 The other species were isolated less frequently, and their abundance was very low. It should be
355 highlighted that some batches showed very low fungal incidence, consisting of single species and
356 in particular of *A. chevalieri*, *A. fumigatus*, *A. alternata*, *P. citrinum*, *A. nidulans*, *A. cristatus*,
357 *Eutypella microtheca*, and *Crysonilia sitophyla* (batches 1, 5, 6, 9, 12, 13, 14, 19, 21, and 23,
358 respectively). The batches with low fungal incidence were probably added with high salt
359 concentration during production.

360

361 3.3. Effect of CAP-NOx treatments on the spores naturally present in sundried tomatoes

362 Different exposure times to CAP-NOx were tested to achieve the highest fungal inactivation on
363 the tomatoes surface. In detail, for the most contaminated batches (4, 7, 8 and 20), ten randomly
364 selected tomato samples were exposed to CAP-NOx for 5, 10, 20 and 30 min. Our results showed
365 a higher fungal inactivation by CAP treatment on the smooth surface of tomatoes than on the inner
366 face (data not shown), probably due to the lower accessibility of the CAP-NOx species to spores
367 adhering to the rough surfaces of the sundried tomatoes. Some of the fungal species isolated from
368 the control samples were rapidly inactivated and reached undetectable levels after only 10 min of
369 CAP-NOx. For example, in batch 7, we isolated *A. flavus*, *A. chevalieri*, *A. alternata*, *A. niger*, *A.*
370 *tubingensis*, *Eutypella microtheca*, *Amesia cymbiformis*, but after 5 min of treatment *Eutypella*
371 *microtheca*, *Amesia cymbiformis*, *A. chevalieri* and *A. tubingensis* were no longer found. With
372 the increase of exposure time to 10 min, we isolated only *A. alternata*, *A. flavus* and *A. niger* and
373 after 30 minutes of treatment, *A. niger* was the only isolated species under artificial environmental
374 conditions (95 % HR and 26°C) with a lower incidence than in the control samples. Thus, 30 mins
375 of exposure to CAP-NOx was able to reduce fungal contamination by 76.5 %.



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Figure 2. Frequency of the filamentous fungi isolated on the surface of sundried tomatoes belonging to different batches. Created with Datawrapper.

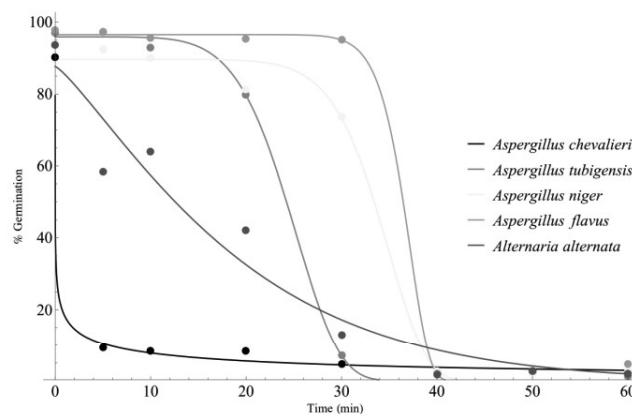
384 3.4. Effect of CAP-NOx time exposure on spore germination

385 To investigate the inactivation dynamics of CAP-NOx on spore germination *in vitro*, we
386 considered the spores of selected strains of the species most commonly isolated in the tomatoes
387 batches such as *A. alternata* PSJ77 *A. chevalieri* PSJ144, *A. tubingensis* PSJ100, *A. flavus* PSJ30,
388 and *A. niger* PSJ38. In general, the CAP-NOx treatments significantly reduced spore germination
389 but with different exposure times. In fact, spores of *A. chevalieri* PSJ144 showed a greater
390 reduction (almost 90%) immediately after 5 minutes of CAP treatment. Increasing the exposure
391 time up to 30 minutes, a strong reduction ($p \leq 0.05$) of spore germination was observed in *A.*
392 *alternata* PSJ77 and *A. tubingensis* PSJ100 (98 and 92%, respectively). However, spores of *A.*
393 *niger* PSJ38 and *A. flavus* PSJ30 showed the lowest percentage of non-germinated spores in the
394 same treatment. For these two species, the exposure time was extended to 50 min, and the
395 inhibition of spore germination of *A. niger* PSJ38 and *A. flavus* PSJ30 was achieved at 98 and
396 95%, respectively, after 40 minutes of CAP-NOx treatment, thus indicating a major resistance of
397 these two species to CAP-NOx treatments.

398 To study the spore inactivation dynamics, the reparameterized Weibull function was modeled to
399 provide useful species-specific information on germination kinetics as a function of time of CAP-
400 NOx treatment. The regressions are shown in Figure 3 and individually plots in figure S1, while
401 their parameters and goodness-of-fit indexes were listed in Table 3. The parameter β proved to be
402 an interesting indicator of spore resistance as it indicates the time required to reduce the initial
403 value (N_0) to the 63.2%. In this regard, *A. flavus* and *A. niger* showed the highest β time, followed
404 by *A. tubingensis* and *A. alternata*. *A. chevalieri* was the most sensitive spore, since β was reached
405 after only 0.1 minutes of treatment. Generally, μ_β and μ_{max} confirmed this trend, showing the
406 highest reduction rates in *A. chevalieri* that resulted the spore inactivated faster by the treatment.
407 It is noteworthy that although *A. alternata* showed a relatively low failure time (β), it was also
408 characterized by the lowest reduction rates. Concerning the goodness of fit, the models fitted
409 considerably well as shown by the CV(RMSD) in Table 2, except for *A. alternata* that fitted
410 slightly worse.

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413
 414 **Figure 3.** Kinetics of germination reduction in five different fungal spores, as a function of time of
 415 treatment, fitted by the Weibull reparametrized model. Dots indicate real data as means of three replications.
 416 The regression parameters were listed in Table 3.

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 418
 419 **Table 3.** Estimated and calculated parameters from germination kinetics. Different letters in the same
 420 column indicate significant differences ($p < 0.05$) according to LSD post-hoc test. Goodness of fit indexes
 421 were reported as mean \pm standard deviation.

	Estimated parameters ¹			Calculated parameters ²		Goodness of fit		
	N_0 (%)	μ_β (%/min)	β (min)	μ_{\max} (%/min)	λ (min)	R^2	CV(RMSD)	AIC
<i>Aspergillus flavus</i>	96.5 ^a	17.8 ^{ab}	37.2 ^a	17.9 ^a	33.8 ^a	0.999 \pm 0.000	3.38 \pm 0.15	43.09 \pm 0.82
<i>Aspergillus niger</i>	89.6 ^b	9.2 ^{ab}	35.3 ^b	9.2 ^b	29.2 ^b	0.997 \pm 0.000	6.55 \pm 0.33	51.91 \pm 0.54
<i>Aspergillus tubigenensis</i>	95.9 ^a	8.9 ^{ab}	25.9 ^c	9.0 ^b	19.1 ^c	0.999 \pm 0.000	3.74 \pm 0.40	40.59 \pm 1.75
<i>Alternaria alternata</i>	87.5 ^b	2.0 ^b	20.2 ^d	3.3 ^c	0.7 ^d	0.975 \pm 0.003	21.91 \pm 1.25	63.88 \pm 0.39
<i>Aspergillus chevalieri</i>	90.3 ^b	152.1 ^a	0.1 ^e	$\rightarrow\infty^3$	0 ³	0.998 \pm 0.000	8.65 \pm 1.01	36.59 \pm 1.84

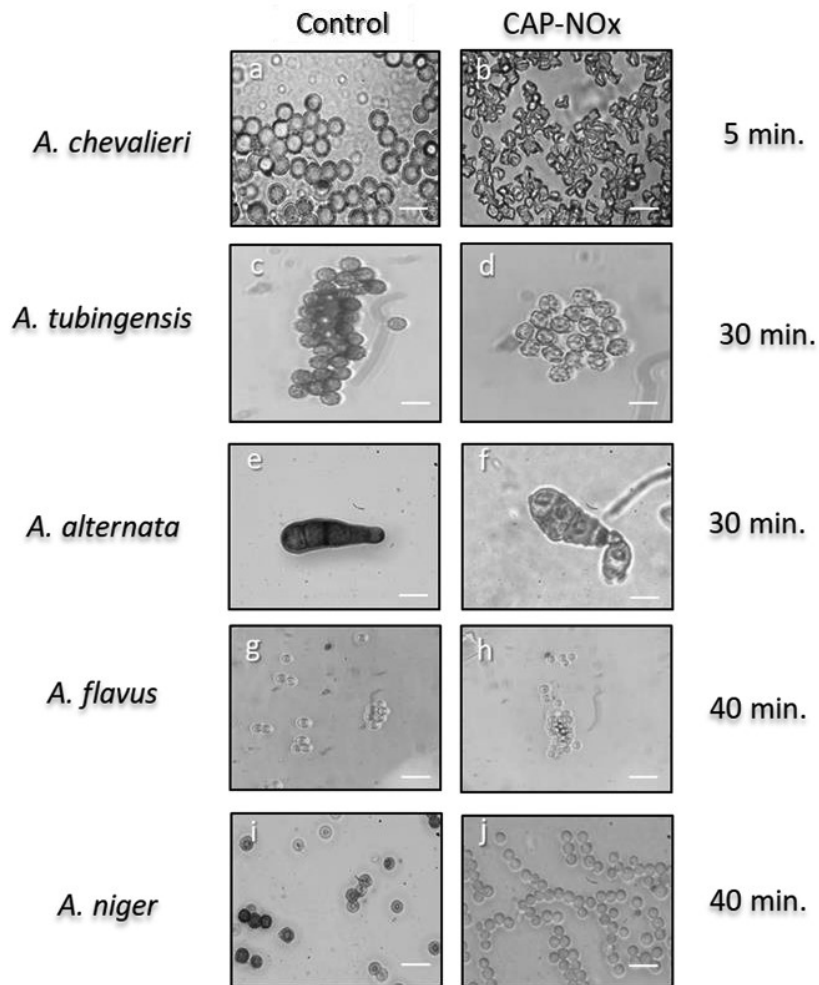
422 ¹ Computed by fitting Eq. 1.
 423 ² Computed by using Eq. 2 and 3.
 424 ³ Values theoretically assigned, as it is impossible to calculate these parameters when no inflection point is present.

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 427 **3.5. Changes in spore morphology**
 428 Microscopical analyses revealed differences in cell morphology as shown in Figure 4. In this case,
 429 a clear loss of spore integrity was observed after only 5 and 30 minutes of CAP-NOx for the spores
 430 of *A. chevalieri* PSJ144 and *A. tubigenensis* PSJ100, respectively. Intense bombardment with NOx
 431 radicals in *A. chevalieri* PSJ144 and *A. alternata* PSJ77 caused serious lesions on the spore surface,

432 where the spores appeared perforated. The loss of pigmentation of the spores of *A. niger* PSJ38
433 with increasing exposure time was very interesting, with the color changing from classical black
434 to pale yellow, while in the spores of *A. flavus* PSJ30 the color changed from yellow-green to
435 white. In addition, the spore surface appeared very smooth and homogeneous after CAP-NOx
436 treatment.

437 To determine if phenotypic changes were correlated with spore viability, fungal spores subjected
438 to CAP-NOx treatment were examined for viability, using CFDA and PI. Live spores stained green
439 and dead spores stained both red (Fig 5). Membrane permeability detection showed that almost all
440 the spores treated with CAP-NOx were stained red after 30 minutes or longer, suggesting that the
441 cell membrane was unable to maintain its function, which could lead to cell death. These
442 observations were reinforced by the lack of viability in extended CAP-NOx treatment.

443 As observed, the spores of *A. chevalieri* PSJ144 exhibited a cell membrane damage after 5 min of
444 treatment, which reduced their viability by 96.77%, while the spores of *A. tubingensis* PSJ100 and
445 *A. alternata* PSJ77 required more time (30 min) to reach similar values (94.59 and 97.22
446 respectively). In contrast, the spores of *A. niger* PSJ38 and *A. flavus* PSJ30 were strongly resistant,
447 as they required 40 mins treatment to show a loss of viability of 93.33 and 94.12%, respectively.



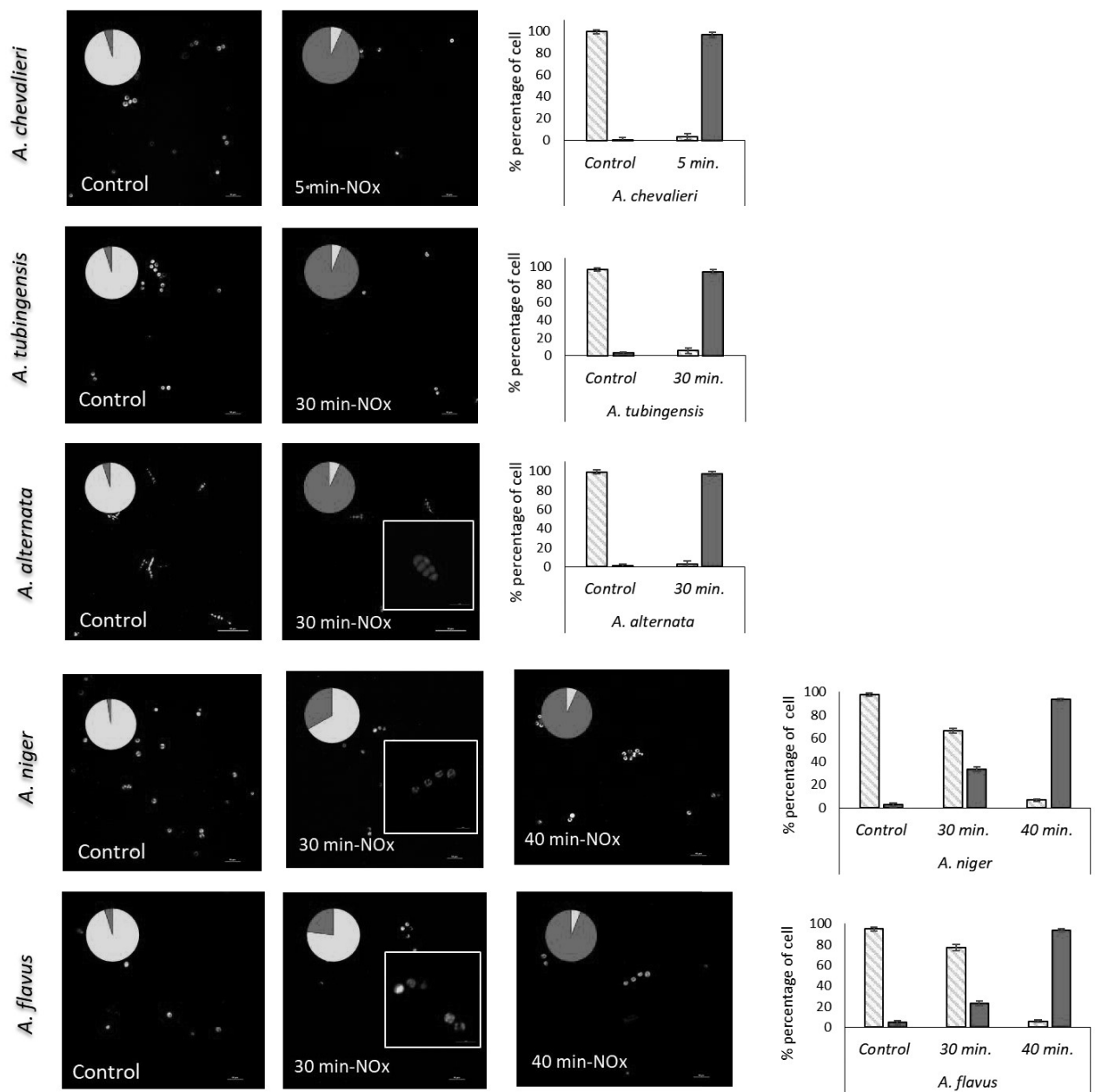
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451 **Figure 4.** Microscopic visualization of *A. chevalieri* PSJ144, *A. tubingensis* PSJ100, *A. alternata* PSJ77,

452 *A. flavus* PSJ30 and *A. niger* PSJ38 spores before and after treatment with CAP-NOx. Scale bars, 10 μ m.



453
 454 **Figure 5.** Confocal laser scanning microscopy analysis of cell viability in *A. chevalieri* PSJ144, *A.*
 455 *tubingensis* PSJ100, *A. alternata* PSJ77, *A. flavus* PSJ30, and *A. niger* PSJ 38 after treatment with CAP-
 456 NOx. Cells were stained with green fluorescence CFDA (carboxyfluorescein diacetate) and red propidium
 457 iodide (PI) dyes. Bars indicate the percentage of cell live (green) and death (red) spore. Image zoom of
 458 spores indicate a total loss of viability after treatment with CAP-NOx. Scale bar 10 um.

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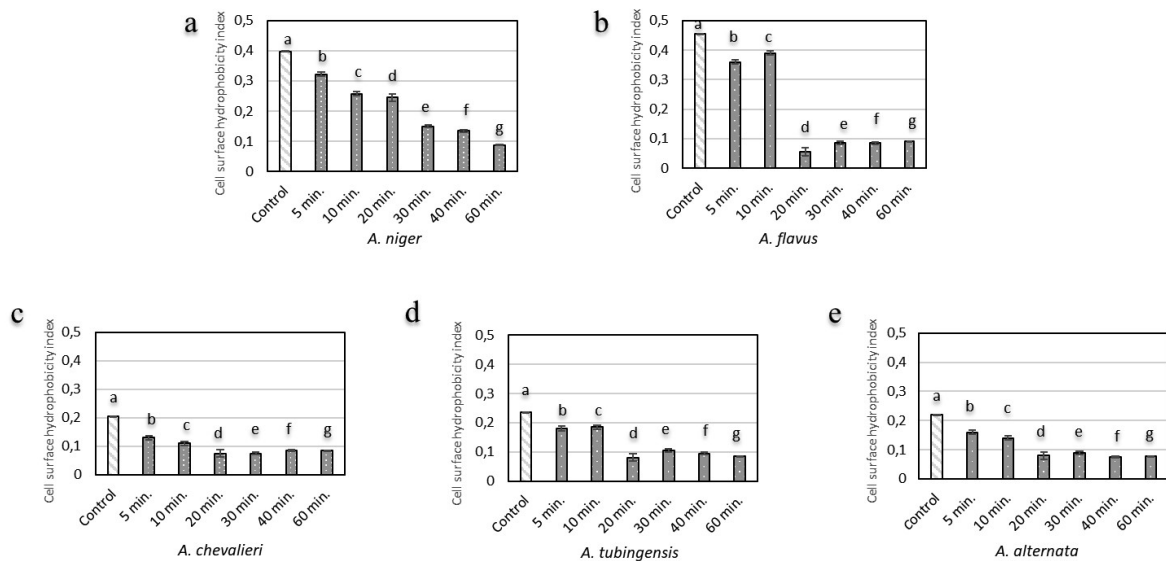
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463 3.6. Changes in spore hydrophobicity

464 Hojnik et al. (2019) suggested that the resistance of *A. flavus* to direct exposure to gaseous plasma
 465 is due to the surface properties of *Aspergillus* spp. spores, which have extremely hydrophobic
 466 properties. For this reason, we measured this property in the spores of the species studied here. As
 467 can be seen in Figure 6, the most resistant strains to CAP-NOx treatments, namely *A. niger* PSJ38
 468 and *A. flavus* PSJ30, had the highest hydrophobicity index compared to the most sensitive strains
 469 *A. chevalieri* PSJ144, *A. tubingensis* PSJ100, and *A. alternata* PSJ77. Statistical analysis using
 470 Pearson correlation showed a high correlation ($p=0.98$) between hydrophobicity and resistance to
 471 CAP-NOx. It should be emphasized that hydrophobicity was significantly reduced in all strains
 472 after only 5 min of exposure; however, in the most resistant strain (*A. niger* PSJ38), exposure to
 473 CAP-NOx promoted a gradual change from the hydrophobic to the hydrophilic state of the spore
 474 surface.

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 478 **Figure 6.** Analysis of the surface hydrophobicity of the *A. niger* PSJ38 (a); *A. flavus* PSJ30 (b); *A. chevalieri*
 479 PSJ144(c); *A. tubingensis* PSJ100(d), and *A. alternata* PSJ77(e) after treatment with CAP-NOx. Data were
 480 obtained from three independent experiments. Different letters represent significant differences among the
 481 sample ($p < 0.05$; Tukey HSD post-hoc test).

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485 3.7. Effect on the texture of the sundried tomatoes

486 Table 4 shows the texture parameters measured in sundried tomatoes after different exposure
487 times. After an exposure time of 5 min, no significant differences were observed in the parameters
488 considered. On the contrary, with increasing treatment times over 10 min, some changes were
489 observed. Compared with the untreated sample, firmness was higher after 10 min but lower after
490 20 and 30 min. Skin Strength decreased after 20 min, but increased after 30 min. Elasticity was
491 unchanged until the 20 min treatment, and then increased after 30 min.

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Table 4. Textural values in sundried tomatoes untreated and treated with CAP treatments.

Property	Control	5 min.	10 min.	20 min.	30 min.
Firmness	483.3±133.1 ^a	426.0±104.9 ^a	606.6±124.8 ^b	252.5±139.8 ^c	426.0±104.9 ^c
Skin Strength	1325.8±460.7 ^a	1307.3±202.3 ^a	1319.0±353.5 ^a	823.4±521.5 ^b	1697.5±161.4 ^c
Elasticity	5.7±2.7 ^a	6.5±1.0 ^a	6.5±1.6 ^a	6.1±2 ^a	7.6±1.8 ^b

497 ^{a,b,c} Means in the same row with different superscripts are significantly different (P 0.05).
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Discussion

501 Fungal decontamination is one of the biggest challenges for the food industry during production.
502 Some fungal species cause a wide range of diseases affecting numerous economically important
503 host plants, including tomatoes, cereals, potatoes, cabbage, broccoli, carrots, ornamentals, citrus
504 fruits, and apples (Kokaeva et al., 2018). Moreover, fungal contamination occurs not only at
505 harvest or post-harvest, but also during processing. In this context, tomatoes (*Solanum*
506 *Lycopersicum* L.) are processed into a variety of products such as dried tomatoes, where drying is
507 a critical processing step that can lead to contamination by fungi from the environment, which can
508 affect the quality of the product (Zansani et al., 2019). In fact, the importance of fungal
509 contamination in food products refers not only to the possible degradation activity, but also to the
510 ability of many of them to produce mycotoxins. In this study, we investigated the diversity and
511 occurrence of fungal species in sundried tomatoes from the market, and for the first time, the
512 efficacy of the CAP-NOx regime on the decontamination of fungal spores.

513 As our results from 24 different batches showed, the most frequently occurring fungal genus found
514 in sundried tomatoes was *Aspergillus* (47.4%). This result is in agreement with Kalyoncu et al.
515 (2005), who described this genus as one of the most important genera isolated in tomatoes and

516 tomato paste. The consequence of the presence of *Aspergillus* is the possible contamination with
517 mycotoxins, of which the most important are aflatoxins (B₁, B₂, G₁, G₂), ochratoxin A, and, at to
518 a lesser extent, fumonisins. In different studies, the presence of fungal species potentially
519 producing mycotoxins such as *A. flavus* (aflatoxin, sterigmatotistin) *A. fumigatus* (fumitremorgin),
520 *A. niger* and *A. tubingensis* (ochratoxin A) was detected in tomatoes, tomato paste, and sundried
521 tomatoes (Han et al., 2019; Kalyoncu et al., 2005; Kokaeva et al., 2018; Sanzani et al., 2019).
522 Other genera that showed high frequency in our batches were *Alternaria* (6.8%) and *Chaetomium*
523 (6.3%). The genus *Alternaria* (*A. alternata* and *A. infectoria*), which were present in a significant
524 number of samples (62%), are capable of producing a large number of toxins, including alternariol,
525 alternariol monomethylether, altenuene, tenoxin and tenuazonic acid. *A. alternata* is a saprophytic
526 pathogen of tomato, a necrotrophic latent fungus that causes black spots on the surface of ripening
527 tomatoes, resulting in frequent postharvest losses (Encinas-Basurto et al., 2017). On the other
528 hand, *Chaetomium* with the species *Chaetomium cochliodes* and *Chaetomium acropollum*, are
529 globally ubiquitous fungi found in soil and degraded cellulosic materials (Salo et al., 2020).
530 The presence of potentially mycotoxigenic fungi in sundried tomatoes makes it important to adopt
531 systems for disinfection and to minimize their impact on the quality of the treated product. Our
532 results demonstrate that CAP-NO_x regime can be a promising strategy to decontaminate the
533 surface of sundried tomatoes from fungal spores. In fact, the reactive species generated by CAP
534 under NO_x regime compromise to a great extent the integrity of cells (Misra et al., 2011). In our
535 study, the NO_x regime was achieved by applying a sufficiently high surface power density to the
536 plasma discharge. Indeed, NO_x production with cold atmospheric plasmas in air is known to be a
537 threshold process guided by the SPD by controlling the excitation and dissociation of N₂ molecules
538 (Simoncelli et al., 2019). Once these molecules are sufficiently excited, their reaction with O₂ and
539 O₃ molecules leads to the formation of a complex NO_x chemistry, including NO, N, NO₂, NO₃,
540 N₂O₅ (Hojnik et al., 2019; Molina-Hernandez et al., 2022).
541 Although the antifungal activity of CAP-O₃ has been demonstrated by several authors (Ambrico
542 et al., 2020; Kang et al., 2014; Molina-Hernandez et al., 2022; Panngom et al., 2014), studies on
543 the antifungal activity of CAP-NO_x are lacking. Our data support the hypothesis that exposure of
544 tomatoes to CAP-NO_x may be associated with the decontamination of fungal spores on the surface
545 of sundried tomatoes, which depends on the CAP-NO_x exposure time, and the characteristics of
546 the target fungi. The results of our *in-situ* study pointed out the efficacy of the treatment in terms

547 of spore inactivation in naturally contaminated tomatoes, which increased with increasing
548 exposure time to CAP-NO_x. In fact, we observed a reduction of fungal contamination of 76.5 %
549 after 30 mins of exposure to CAP-NO_x. In this regard, the spores of *A. flavus* PSJ30 and *A. niger*
550 PSJ38 were the most resistant after 30 min treatment, resulting in a high survival of these fungi
551 compared to other fungal species found in the tomatoes batches here analyzed. These results
552 indicate that the increased levels of reactive nitrogen species (RNS) following plasma treatment
553 likely played a role in spore inactivation.

554 The antifungal effect of NO has been previously reported (Weller et al., 2001). However, the
555 comparison of the efficacy of the CAP treatment with previous studies is very difficult because it
556 depends on the generator device, voltage, exposure time, initial microbial density, process gas,
557 working distance, and plasma exposure. Wang et al. (2022) reported 96.84% loss of cell viability
558 and membrane integrity of *Fusarium* spp. after 3-min of CAP using a dielectric barrier surface
559 micro-discharge (SMD) plasma generator. Jo et al. (2014) observed 92% fungal colony forming
560 units (CFU) inhibition of *Gibberella fujikuroi* on the rice seed surface, after 120 s of exposure.
561 Our results are in agreement with those obtained by Ambrico et al. (2020), who reported that the
562 inactivation of the fungal growth is species dependent. In fact, they observed different survival
563 rates of spores belonging from different fungal species (*Botrytis cinerea*, *Monilinia fructicola*,
564 *Aspergillus carbonarius* and *Alternaria alternata*) exposed to SDBD plasma. It is important to
565 highlight that inhibition of spore germination, reduction of viability and morphological alterations
566 of cell surface up to spore destruction of these species, demanded diverse treatment times.
567 Moreover, Pańka et al. (2022) found diverse efficacy of Cold plasma (DBD) treatments on
568 different fungal spores present in seeds, by using 400 W power and 15 s of exposure time. In this
569 case, the treatment was very effective against *Alternaria*, *Aspergillus*, *Colletotrichum*, *Fusarium*,
570 *Penicillium* genera, being *Aspergillus ochraceus* the most resistant species. For more intense direct
571 DBD systems, a notable study using an air direct DBD on agar plates achieved significant
572 reductions against fungi (1.7 and 1.0 log CFU reductions of *Aspergillus oryzae* and *Alternaria*
573 conidia, respectively, within 10 min) (Julák et al., 2018).

574 In our study, the specific effect of CAP-NO_x treatment on fungal spores was confirmed *in vitro*,
575 demonstrating that the spores of the species studied here were resistant to CAP-NO_x in the
576 following order: *A. chevalieri* PSJ144 < *A. alternata* PSJ77 < *A. tubingensis* PSJ100 < *A. flavus*
577 PSJ30 < *A. niger* PSJ38. Interestingly, although *A. alternata* showed a relatively low failure time

578 (β), it was also characterized by the lowest rates, suggesting that a small group of cell spores could
579 survive a longer treatment time. This may be because *Alternaria* spp. is the only multicellular
580 conidia studied in this work, characterized by a primary cell wall that is melanized and a secondary
581 wall that is not melanized (Ambrico et al., 2020), which may have resulted in a different response
582 to CAP-NO_x treatment.

583 Fungal spore survival in the harsh environment of plasma treatment generally depends on: 1) the
584 spore cell wall, which helps prevent radiation damage to DNA, 2) the type of gas used and the gas
585 flow rate, 3) the relative humidity, and 4) the substrate. Fungal spores are very dense and compact
586 structures surrounded by different layers. In particular, the outer layer of most asexual fungal
587 spores is composed of polysaccharides (chitin and combination of a-glucans and b-glucans), and
588 is surrounded by a rodlet layer with a complex structure composed of a phenolic compound
589 (melanin) and a hydrophobic protein (hydrophin) (El Enshasy, 2022). These compounds form a
590 well-structured monolayer in which a combination of neutral, hydrophilic and hydrophobic amino
591 acids are present and which exhibits a uniform hydrophobicity on the outside of the cell (Moonjely
592 et al., 2018; Wu et al., 2017). Ambrico et al. (2020) observed that RNS have a direct effect on
593 cells, and especially on the outermost layer, the polysaccharide-rich wall. Other authors suggested
594 that the radicals produced during CAP may oxidize the protein of the spore envelope, leading to a
595 loss of the envelope integrity and therefore making the spore more vulnerable to attack by the
596 radicals generated by plasma (Devi et al., 2017). In fact, bombardment with reactive plasma
597 species creates active sites on the surface of the protein, and the RNS introduced by plasma into
598 the protein structure can also act as quenchers (Bußler et al., 2015). In addition, the accumulation
599 of charged particles on the surface of spores and electrostatic forces can lead to rupture of the cell
600 membrane and subsequently cause cell death (Laroussi et al., 2003; Mendis et al., 2000). In a study
601 conducted by Hojnik et al. (2019) on spores of *A. flavus* treated with both direct gaseous plasma
602 treatment and indirect treatment with plasma activated aqueous broth (PAB), it was found that
603 direct treatment was more effective than PAB, and this result was attributed to the hydrophobic
604 surface properties of the spores, which make them much more resistant to RONS in the liquid
605 phase. Herein, we found that the survival of spores of the 5 different strains studied to CAP-NO_x
606 was highly dependent on spore hydrophobicity. In fact, the spores of *A. niger* PSJ38 and *A. flavus*
607 PSJ30, which turned out to be the most hydrophobic ones, were also more resistant to plasma
608 treatments than *A. chevalieri* PSJ144 spores, which were less hydrophobic. In this respect, spore

609 hydrophobicity might have contributed to the spore resistance, due to the lower interaction
610 between NOx radicals and the small secreted amphipathic proteins called hydrophobins, which
611 can self-assemble into a monolayer that exhibits a uniform hydrophobicity on the cell exterior
612 (Moonjely et al., 2018). In particular, the amino acids that form the hydrophobins of *A. chevalieri*
613 PSJ144 might be more polar and therefore more easily targeted by radical species than less polar
614 ones. In addition, it is important to underline that after each CAP-NOx treatment the spores lost
615 their hydrophobicity, which suggests a direct or an indirect effect of CAP on the protein
616 architecture of the cell wall, leading to a reduction in cell vitality. The degradation of cellular
617 proteins by CAP is poorly described in literature and should be investigated.

618 Another aspect to consider in spore resistance to CAP-NOx is the presence of melanin, a pigment
619 known to contribute to the rigidity of spore cell walls, protecting the cell from stressors such as
620 temperature, UV-radiation, and reactive oxygen species (Ott et al., 2021). In this respect, our data
621 support the observations of Ambrico et al. (2020), who found that the darkest-colored spores of *A.*
622 *carbonarius* and *A. Alternaria* showed higher resistance to the treatment compared to the lighter
623 pigmented and thinner walled spores of *B. cinerea* and *M. fructicola*. In fact, in our study the spores
624 of *A. niger* PSJ38 and *A. alternata* PSJ77 were more resistant to CAP-NOx than those of *A.*
625 *chevalieri* PSJ144, which showed a 98% inactivation after 5 min of plasma exposure. It should be
626 emphasized that melanin pigments quench reactive oxygen and nitrogen species, and therefore
627 spores depleted of this pigment are more susceptible to ROS and RNS (Ott et al., 2021). This may
628 be the case for *A. niger* PSJ38, which was depigmented after 40 min of treatment and showed an
629 increasing percentage of spore inactivation. Pal et al. (2014) also found that loss of melanin was
630 associated with increased susceptibility of *Aspergillus* spp. to damage by reactive oxygen species.
631 Therefore, we can hypothesize that proteins of the wall are one of the first targets for biologically
632 active agents generated by CAP-NOx, and that the efficacy of plasma in spore inactivation depends
633 not only on spore hydrophobicity, but also on the presence of melanin that could mitigate the
634 effects of CAP-NOx on spore survival.

635 Textural properties are important characteristics that determine the product quality, as they
636 strongly influence consumer's perception. The observed changes in the considered parameters
637 occurred after 10 min of treatment but did not show a clear trend with respect to treatment duration.
638 The texture of sun-dried tomatoes is very complex, and, to our knowledge, there are no reports on
639 the effects of CAP on the structural properties of these products, so it is very difficult to draw

640 conclusions. To understand whether the observed changes cause significant effects on dried tomato
641 quality, the texture should probably be evaluated by a panel test.
642 Compared with other methods for reducing fungal growth, CAP-NOx is a fast technology that can
643 decontaminate foods and does not leave toxic residues or post-processing exhaust gases. In
644 addition, it represents a non-thermal alternative for food decontamination, which can be considered
645 appealing in view of the recent rise of energy prices. Moreover, this method is certainly
646 sustainable, because it avoids the impact of chemicals on both the product and the environment.
647 Thus, CAP-NOx could be an attractive approach for producing high-quality tomato products with
648 an extended the shelf-life, with interesting potential effects on the market, considering the
649 possibility to enlarge the trade, differentiate the range of commercialized products, reduce the
650 waste and the cost of the discharge, with economic and social advantages. Furthermore, post-
651 harvest treatments with chemicals involve costs for controlling residues, with a direct impact on
652 the price of the finished product (Hernández-Torres et al., 2022).

653

654

655 **CONCLUSIONS**

656 This study is a first attempt to apply CAP-NOx for treating a dried fruit. The results presented in
657 this paper show for the first time the inhibitory activity of CAP-NOx species against the spores of
658 the most frequent species found in sundried tomatoes, and namely *A. alternata*, *A. chevalieri*, *A.*
659 *tubingensis*, *A. flavus* and *A. niger*. We demonstrated that CAP treatment can effectively reduce
660 the survival rate (76.5%) of fungi on the surface of sundried tomatoes. The Weibull
661 reparameterized model proposed by De Flaviis & Sacchetti (2022) gave useful information on the
662 species-specific inactivation kinetics after CAP-NOx treatment, which involves a differential
663 germination of the fungal spores associated with sundried tomatoes. Although four of the tested
664 *Aspergillus* species have a similar asexual morphological type, they perform differently under the
665 nitrosative stress induced by CAP-NOx. This behavior may be correlated with spore
666 hydrophobicity, which was major in the most resistant spores as *A. niger*. However, more research
667 needs to be done to better explain the vector of environmental adaptations in *A. niger* against CAP-
668 NOx. In conclusion, the results of this study open up new perspectives on the potential application
669 of CAP-NOx for fungal decontamination of dried foods.

670

671 **CRedit authorship contribution statement**

672

673 **Junior Bernardo Molina-Hernandez:** Conceptualization, Methodology, Software, Validation,
674 Formal analysis, Investigation, Writing – original draft, **Silvia Tappi:** Methodology, Formal
675 analysis, Writing – review & editing; **Matteo Gherardi:** Methodology, Formal analysis, Writing
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677 Investigation, Formal analysis, **Yeimmy Yolima Peralta-Ruiz:** Investigation, review & editing,
678 **Antonello Paparella:** Supervision, Writing – review & editing: **Clemencia Chaves-López:**
679 Conceptualization, Supervision, Writing – original draft, Project administration.

680

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682 The authors declare no conflict of interest

683

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- *A. niger*, *A. tubingensis*, were most abundant species in dried tomatoes.
- CAP reduced the fungal contamination on the dried tomatoes by 76.5 %.
- *A. niger* and *A. flavus* spores were more resistant ones to CAP.
- *A. chevalieri* spores were inactivated after 0.1 min of CAP-NO_x.
- Spore hydrophobicity was correlated with the spore resistant to CAP.

Declarations of interest

The authors of the present paper “Cold Atmospheric plasma treatment trigger changes in Sundried tomatoes mycobiota by inducing changes spore surface structure and hydrophobicity of *Aspergillus* species”, Junior Bernardo Molina-Hernandez, Silvia Tappi, Matteo Gherardi, Riccardo de Flaviis, Jessica Laika, Yeimmy Yolima Peralta-Ruiz, Antonello Paparella and Clemencia Chaves-López **don't have any conflict of interest**

CRedit authorship contribution statement

Junior Bernardo Molina-Hernandez: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, **Silvia Tappi:** Methodology, Formal analysis, Writing – review & editing; **Matteo Gherardi:** Methodology, Formal analysis, Writing –review & editing; **Riccardo de Flaviis:** Methodology, Data curation, **Jessica Laika:** Investigation, Formal analysis, **Yeimmy Yolima Peralta-Ruiz:** Investigation, review & editing, **Antonello Paparella:** Supervision, Writing – review & editing: **Clemencia Chaves-López:** Conceptualization, Supervision, Writing – original draft, Project administration.

Table 1. Primers used for PCR assay

Table 2. Absorption cross-sections in cm^2 of the species of interest at each selected wavelength.

Table 3. Estimated and calculated parameters from germination kinetics. Different letters in the same column indicate significant differences ($p < 0.05$) according to LSD post-hoc test. Goodness of fit indexes were reported as mean \pm standard deviation.

Table 4. Textural values in sundried tomatoes untreated and treated with CAP treatments.

Table S1. Identification of the fungi isolates from dry tomatoes, determined by amplifying Internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S ribosomal DNA (rDNA) region, β -tubulin and Calmodulin gene and nucleotide sequences.

Figure 1. Values of NO_2 after different treatment times. The values are the mean of three repetitions. In each panel, data are mean \pm SD, and statistical significance is specified with letters ($*p \leq 0.05$ as determined by paired Student t-test).

Figure 2. Frequency of the filamentous fungi isolated on the surface of sundried tomatoes belonging to different batches. Created with Datawrapper.

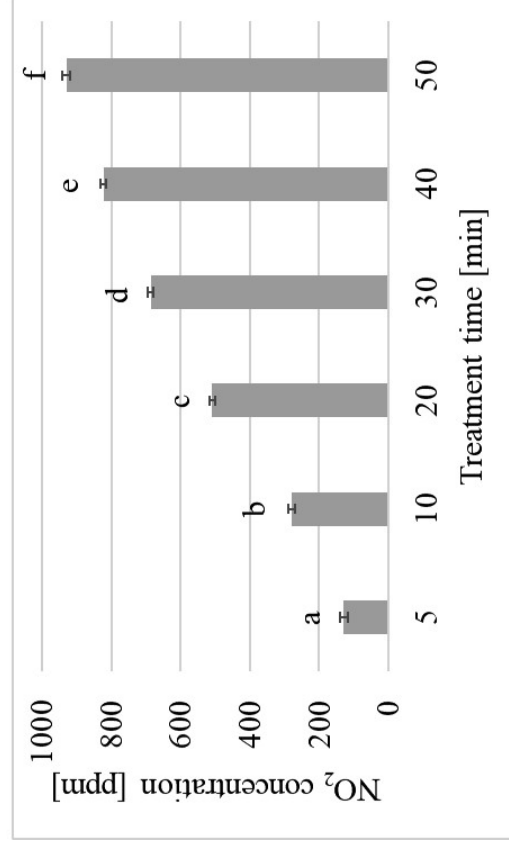
Figure 3. Kinetics of germination reduction in five different fungal spores, as a function of time of treatment, fitted by the Weibull reparametrized model. Dots indicate real data as means of three replications. The regression parameters were listed in Table 3.

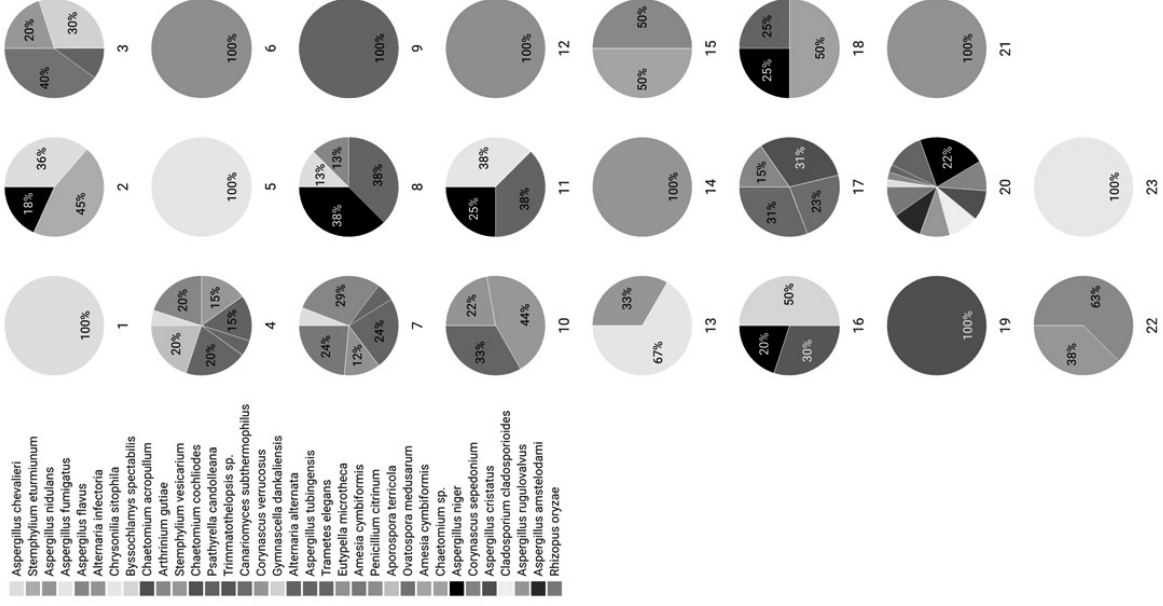
Figure 4. Microscopic visualization of *A. chevalieri* PSJ144, *A. tubingensis* PSJ100, *A. alternata* PSJ77, *A. flavus* PSJ30 and *A. niger* PSJ38 spores before and after treatment with CAP-NOx. Scale bars, 10 μm .

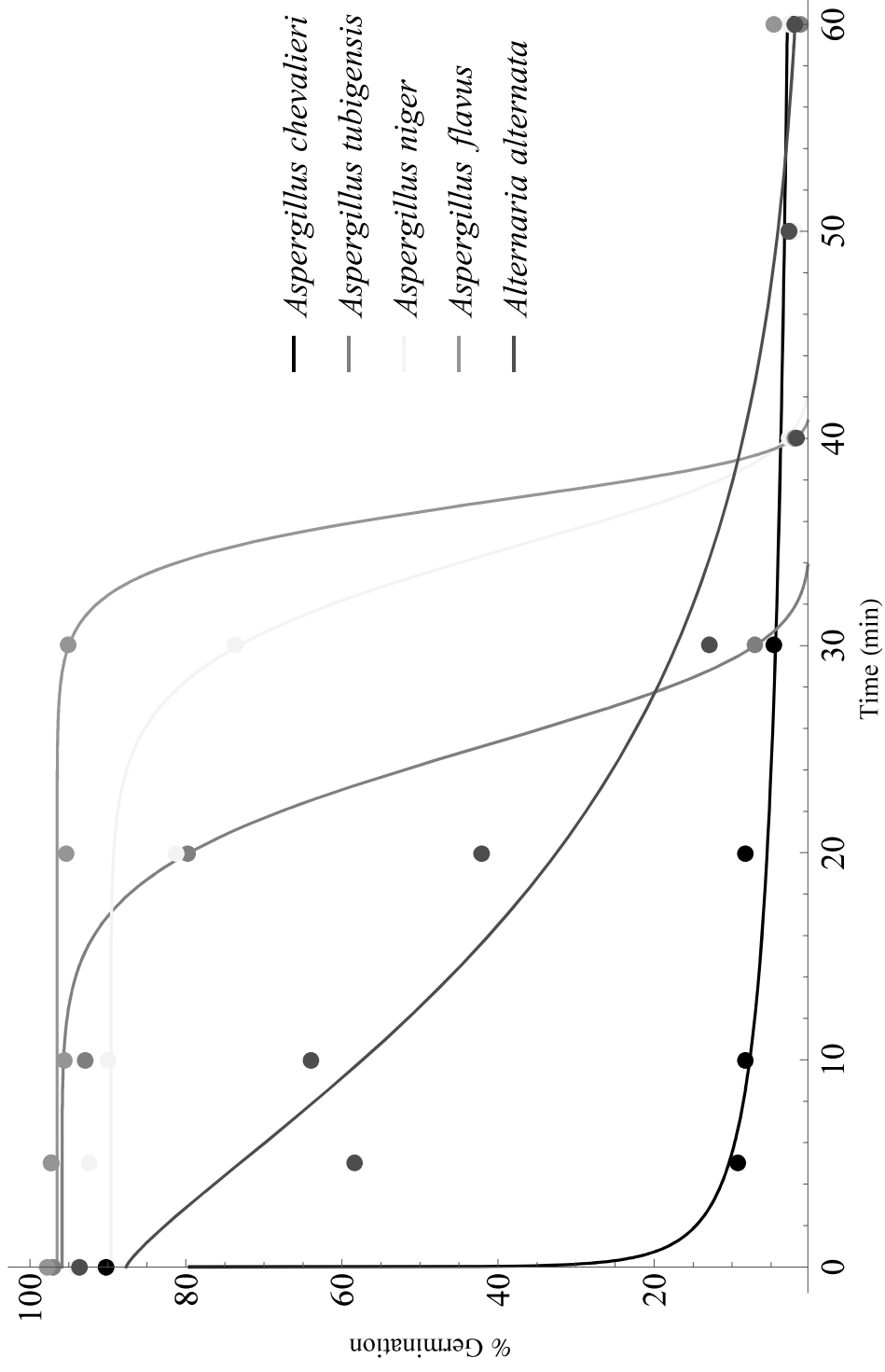
Figure 5. Confocal laser scanning microscopy analysis of cell viability in *A. chevalieri* PSJ144, *A. tubingensis* PSJ100, *A. alternata* PSJ77, *A. flavus* PSJ30, and *A. niger* PSJ 38 after treatment with CAP-NOx. Cells were stained with green fluorescence CFDA (carboxyfluorescein diacetate) and red propidium iodide (PI) dyes. Bars indicate the percentage of cell live (green) and death (red) spore. Image zoom of spores indicate a total loss of viability after treatment with CAP-NOx. Scale bar 10 μm .

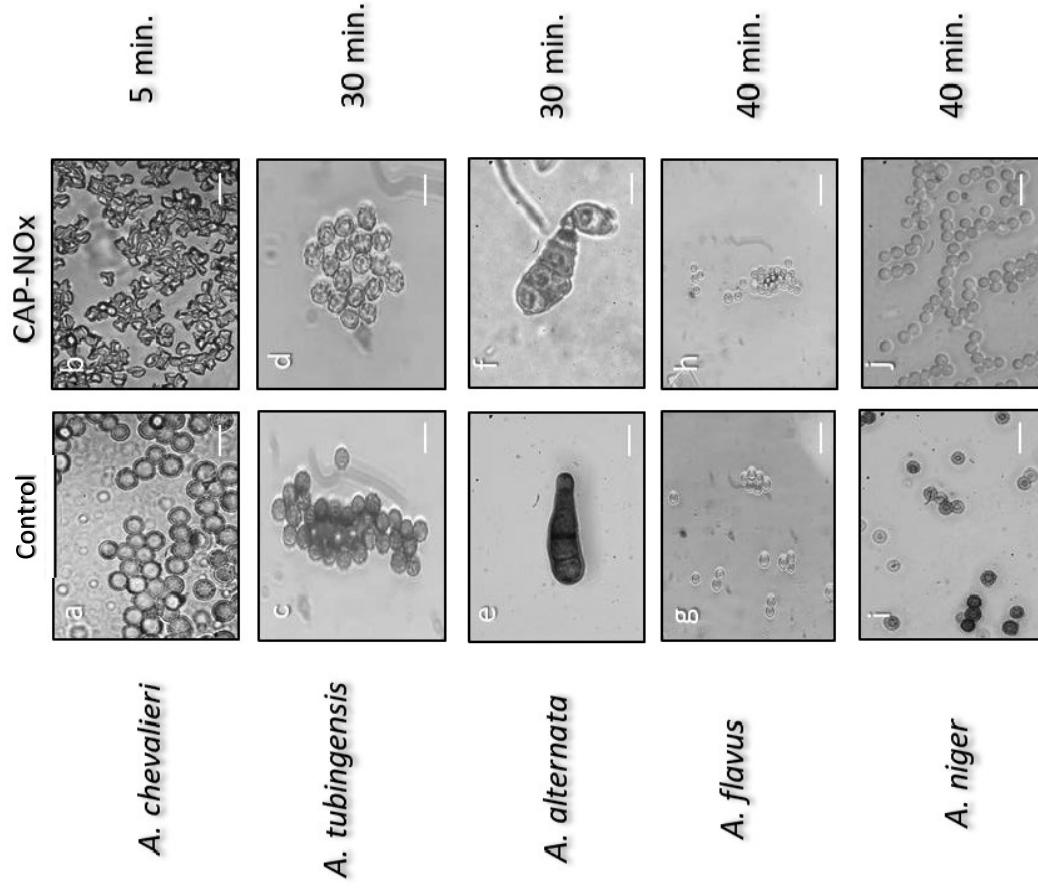
Figure 6. Analysis of the surface hydrophobicity of the *A. niger* PSJ38 (a); *A. flavus* PSJ30 (b); *A. chevalieri* PSJ144(c); *A. tubingensis* PSJ100(d), and *A. alternata* PSJ77(e) after treatment with CAP-NOx. Data were obtained from three independent experiments. Different letters represent significant differences among the sample ($p < 0.05$; Tukey HSD post-hoc test).

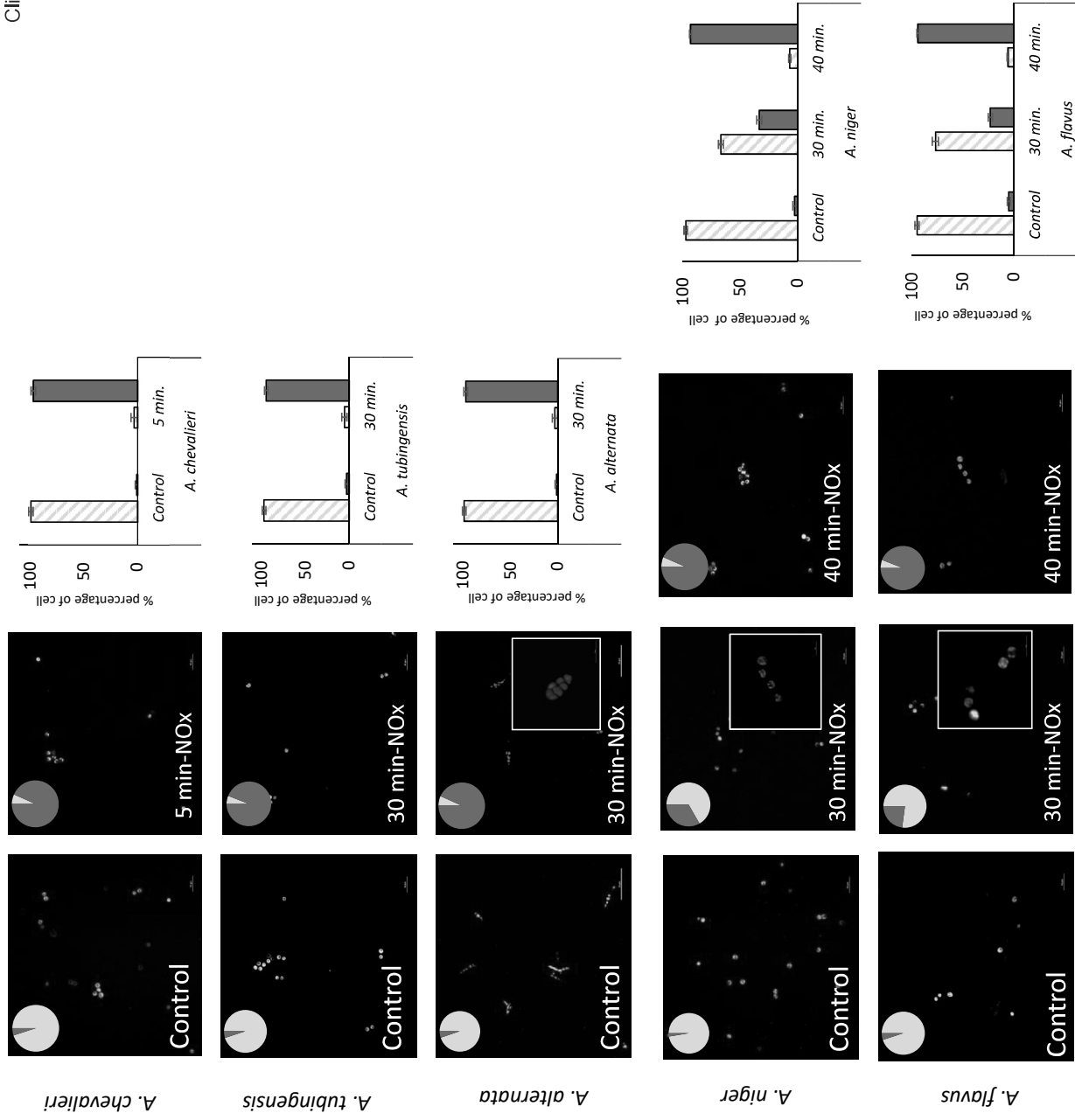
Figure S1. Individually plots of germination kinetics in five different fungal spores for three replicates fitted by the Weibull reparametrized model. Dots indicate real data. X axis and Y axis indicate time of treatment and percentage of germination respectively.











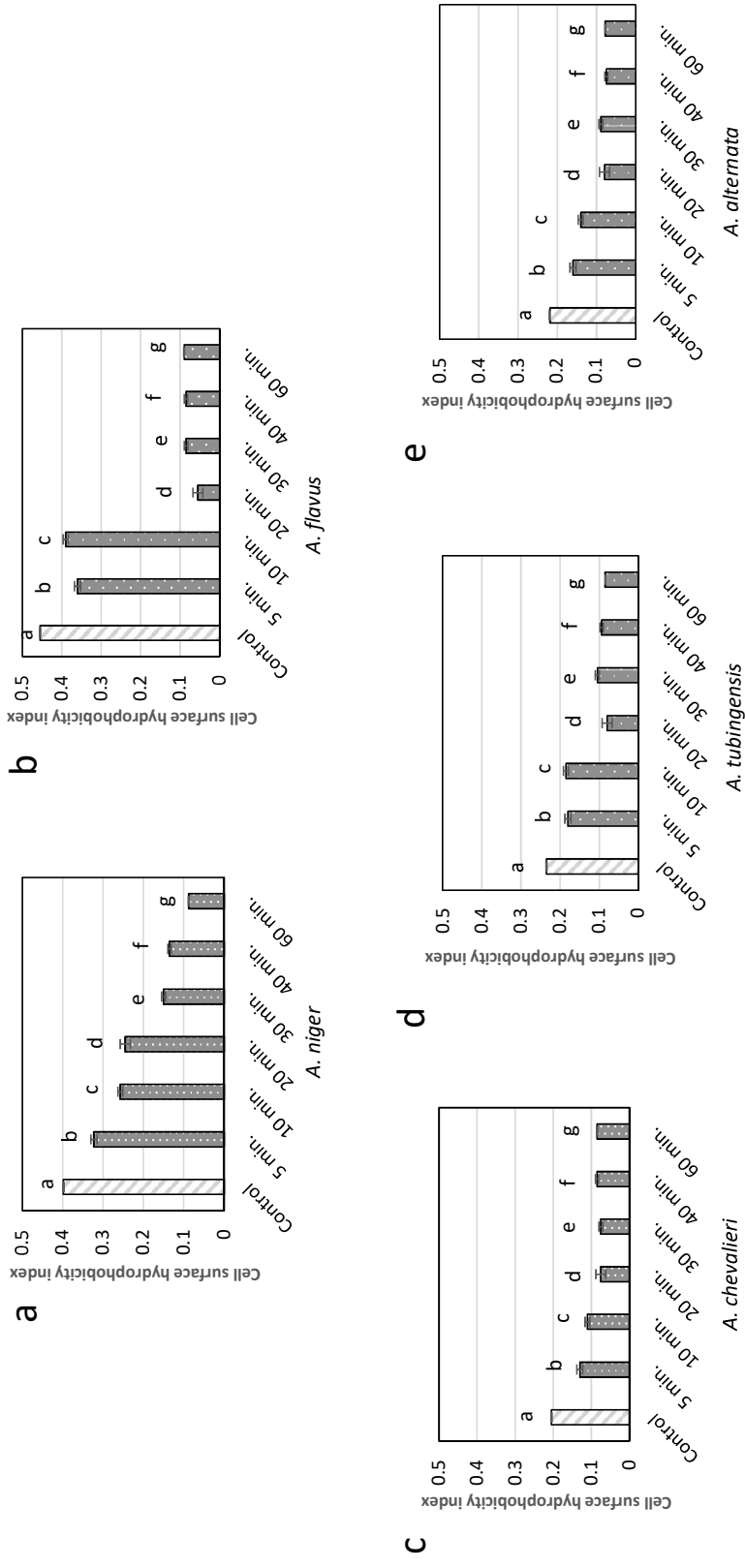


Table 1. Primers used for PCR assay.

Gene name	Gene	Length bp	Primer	Sequences (5' → 3')	Reference
Internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S ribosomal DNA (rDNA) region	ITS (1-4)	420-825	ITS1 (F)	5'TCCGTAGGTGAACCTGCCGG3'	(Glass & Donaldson, 1995)
			ITS4 (R)	5'TCCTCCGCTTATTGATATGC3'	
	ITS (1-2)	565-613	ITS1 (F)	5' GGAAGTAAAGTCGTAACAAGG 3'	
			ITS2 (R)	5' TTGGTCCGTGTTCAAGACG 3'	
β -tubulin	ben A	1125	β -tub 2a (F)	5'GGTAACCAAATCGGTGCTTTC 3'	(Makhlouf et al., 2019)
			β -tub 2b (R)	5'ACCCTCAGTGTAGTGACCCTTGGC 3'	
Calmodulin	cmdA	543	Cmd5 (F)	5'-CCGAGTACAAGGAGGCCTTC-3'	
			Cmd6 (R)	5'-CCGATAGAGGTCATAACGTGG-3'	

Abbreviation: F: Forward, R: Reverse

Table 2. Absorption cross-sections in cm^2 of the species of interest at each selected wavelength.

Selected wavelength	O₃ cross-section	NO₂ cross-section
253±1.2 nm	(1.12±0.02)E-17	(1.1±0.3)E-20
400±1.2 nm	(1.12±0.08)E-23	(6.4±0.2)E-19

Table 3. Estimated and calculated parameters from germination kinetics. Different letters in the same column indicate significant differences ($p < 0.05$) according to LSD post-hoc test. Goodness of fit indexes was reported as mean \pm standard deviation.

	Estimated parameters ¹			Calculated parameter ²		Goodness of fit		
	N_0 (%)	μ_β (%/min)	β (min)	μ_{\max} (%/min)	λ (min)	R^2	CV(RMSD)	AIC
<i>Aspergillus flavus</i>	96.5 ^a	17.8 ^{ab}	37.2 ^a	17.9 ^a	33.8 ^a	0.999 \pm 0.000	3.38 \pm 0.15	43.09 \pm 0.82
<i>Aspergillus niger</i>	89.6 ^b	9.2 ^{ab}	35.3 ^b	9.2 ^b	29.2 ^b	0.997 \pm 0.000	6.55 \pm 0.33	51.91 \pm 0.54
<i>Aspergillus tubigenensis</i>	95.9 ^a	8.9 ^{ab}	25.9 ^c	9.0 ^b	19.1 ^c	0.999 \pm 0.000	3.74 \pm 0.40	40.59 \pm 1.75
<i>Alternaria alternata</i>	87.5 ^b	2.0 ^b	20.2 ^d	3.3 ^c	0.7 ^d	0.975 \pm 0.003	21.91 \pm 1.25	63.88 \pm 0.39
<i>Aspergillus chevalieri</i>	90.3 ^b	152.1 ^a	0.1 ^c	$\rightarrow\infty^3$	0 ³	0.998 \pm 0.000	8.65 \pm 1.01	36.59 \pm 1.84

¹ Computed by fitting Eq. 1.

² Computed by using Eq. 2 and 3.

³ Values theoretically assigned, since it is impossible to calculate these parameters when no inflection point is present.

Table 4. Textural values in sundried tomatoes untreated and treated with CAP treatments.

Property	Control	5 min.	10 min.	20 min.	30 min.
Firmness	483.3±133.1 ^a	426.0±104.9 ^a	606.6±124.8 ^b	252.5±139.8 ^c	426.0±104.9 ^c
Skin Strenght	1325.8±460.7 ^a	1307.3±202.3 ^a	1319.0±353.5 ^a	823.4±521.5 ^b	1697.5±161.4 ^c
Elasticity	5.7±2.7 ^a	6.5±1.0 ^a	6.5±1.6 ^a	6.1±2 ^a	7.6±1.8 ^b

^{a,b,c} Means in the same row with different superscripts are significantly different (P 0.05).

Table S1. Identification of the fungi isolates from dry tomatoes, determined by amplifying Internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S ribosomal DNA (rDNA) region, β -tubulin and Calmodulin gene and nucleotide sequences.

Sample	Closed relative	Primer	Identity %	Accession number	
PSJ 77	<i>Alternaria alternata</i>	ITS1-ITS4	100	KF881759.1	
PSJ 65		ITS1-ITS4	100	KF881759.1	
PSJ 79		ITS1-ITS4	100	KF881759.1	
PSJ 70	<i>Alternaria infectoria</i>	ITS1-ITS4	100	MK226292.1	
PSJ 70-1		ITS1-ITS4	100	MK226292.1	
PSJ 74	<i>Amesia cymbiformis</i>	ITS1-ITS4	100	MH861721.1	
PSJ 72	<i>Aporospora terricola</i>	ITS1-ITS4	99	AF049088.1	
VJT-20	<i>Aspergillus amstelodami</i>	β t2a- β t2b	99,7	FR775356.2	
PSJ-76-1	<i>Aspergillus chevalieri</i>	ITS1-ITS4	100	MT316337.1	
PSJ 79		ITS1-ITS4	100	MT316337.1	
PSJ 132-1		ITS1-ITS4	100	MN174037.1	
PSJ 150		ITS1-ITS4	100	MN174037.1	
PSJ 131		ITS1-ITS4	100	MN174037.1	
PSJ 144		ITS1-ITS4	99	MT316339.1	
VJT-2		<i>Aspergillus cristatus</i>	ITS1-ITS4	99,32	KY828916.2
PSJ 30	<i>Aspergillus flavus</i>	ITS1-ITS2	99	JX501356.1	
PSJ 14		ITS1-ITS4	99	MT645322.1	
PSJ 106		ITS1-ITS4	100	MT558941.1	
PS154		ITS1-ITS4	100	MT292809.1	
PSJ 40-1		ITS1-ITS4	99	MF379664.1	
PSJ 9	<i>Aspergillus fumigatus</i>	ITS1-ITS4	100	MK841416.1	
PSJ 10		ITS1-ITS4	99	MT487775.1	
PSJ 50		ITS1-ITS4	100	OK067466.1	
PSJ 48		<i>Aspergillus nidulans</i>	ITS1-ITS4	100	MK397763.1
PSJ 105		ITS1-ITS4	99	MT316339.1	
VJT 7	<i>Aspergillus niger</i>	CMD5-CMD6	99,6	HQ632731.1	
VJT 14		β t2a- β t2b	99,7	MN907662.1	
VJT 12		ITS1-ITS4	99,1	MN493772.1	
VJT 28		ITS1-ITS4	99,1	LC577101.1	
VJT 15		β t2a- β t2b	99,7	JX545078.1	
VJT 18		ITS1-ITS4	99,1	MTI23512.1	
VJT 1		β t2a- β t2b	99,12	MT597823.1	
VJT 6		β t2a- β t2b	98,8	KY990205.1	
VJT 17		β t2a- β t2b	99,7	KU865178.1	
VJT 26		ITS1-ITS4	98	MK138359.1	
PSJ 38		β t2a- β t2b	99,1	KJ36066.1	
		CMD5-CMD6	99,1	MH447369.1	
VJT-5		<i>Aspergillus rugulovalvus</i>	β t2a- β t2b	100	AB248319.1
PSJ 100		<i>Aspergillus tubingensis</i>	CMD5-CMD6	100	MK166185.1
PSJ 109	CMD5-CMD6		100	KY612372.1	
VJT 10	CMD5-CMD6		98,9	KY612372.1	
VJT 30	CMD5-CMD6		98,9	KX231824.1	
VJT 8	ITS1-ITS2		100	KY612372.1	
VJT 16	ITS1-ITS4		100	MK166185.1	
PSJ 16	β t2a- β t2b		98,9	MK166185.1	
PSJ 12	ITS1-ITS4		100	MN634560.1	
PSJ 143	<i>Arthrinium gutiae</i>		ITS1-ITS4	100	MN634560.2
PSJ 13	<i>Byssochlamys spectabilis</i>		ITS1-ITS4	99	MW335157.1
PSJ 140	<i>Canariomyces subthermophilus</i>	ITS1-ITS4	98	MK926804.1	
PSJ 135	<i>Chaetomium cochliodes</i>	ITS1-ITS4	100	MH590621.1	
PSJ 40	<i>Chaetomium acropullum</i>	ITS1-ITS4	99	MH550490.1	
PSJ 40-2		ITS1-ITS4	99	KU571511.1	
PSJ 83		ITS1-ITS4	99	KU571511.1	
PSJ 119	<i>Corynascus verrucosus</i>	ITS1-ITS4	100	KY065360.1	
PSJ 15		ITS1-ITS4	98	GU192459.1	
PSJ 15-1	<i>Chrysonilia sitophila</i>	ITS1-ITS4	99,9	GU192459.1	
VJT-4	<i>Corynascus sepedonium</i>	ITS1-ITS4	99,43	MK919294.1	
VJT-3	<i>Cladosporium cladosporioides</i>	ITS1-ITS4	99,3	KY039309.1	

PSJ 55		<i>ITS1-ITS4</i>	99	<i>MF359643.1</i>
PSJ 96	<i>Eutypella microtheca</i>	<i>ITS1-ITS4</i>	100	<i>MH864886.1</i>
PSJ 98		<i>ITS1-ITS4</i>	99	<i>MH864886.1</i>
PSJ 151	<i>Gymnascella dankaliensis</i>	<i>ITS1-ITS4</i>	100	<i>AY304514.1</i>
PSJ 86		<i>ITS1-ITS4</i>	99	<i>MN418435.1</i>
PSJ 70	<i>Penicillium citrinum</i>	<i>ITS1-ITS4</i>	99	<i>MG575517.1</i>
PSJ 73		<i>ITS1-ITS4</i>	100	<i>LC514694.1</i>
PSJ 76	<i>Ovatospora medusarum</i>	<i>ITS1-ITS4</i>	100	<i>MH860651.1</i>
PSJ 110	<i>Psathyrella candolleana</i>	<i>ITS1-ITS4</i>	99	<i>MT424873.1</i>
VJT-22		<i>ITS1-ITS4</i>	99,7	<i>LC514326.1</i>
VJT-25	<i>Rhizopus oryzae</i>	<i>ITS1-ITS4</i>	99,7	<i>MT603963.1</i>
VJT 11		<i>ITS1-ITS4</i>	99,6	<i>LC514321.1</i>
PSJ 87-1	<i>Stemphylium eturmiunum</i>	<i>ITS1-ITS4</i>	100	<i>MW883450.1</i>
PSJ 3		<i>ITS1-ITS4</i>	100	<i>MG065802.1</i>
PSJ 51		<i>ITS1-ITS4</i>	100	<i>MG065802.1</i>
PSJ 102	<i>Stemphylium vesicarium</i>	<i>ITS1-ITS4</i>	100	<i>MW245000.1</i>
PSJ 81		<i>ITS1-ITS4</i>	100	<i>MN328401.1</i>
PSJ 58	<i>Trametes elegans</i>	<i>ITS1-ITS4</i>	100	<i>MT597442.1</i>
PSJ 137	<i>Trimmatothelopsis sp.</i>	<i>ITS1-ITS4</i>	95	<i>MK948457.1</i>