Understanding the mechanisms of action of atmospheric cold plasma towards the mitigation of the stress induced in molds: the case of *Aspergillus chevalieri*

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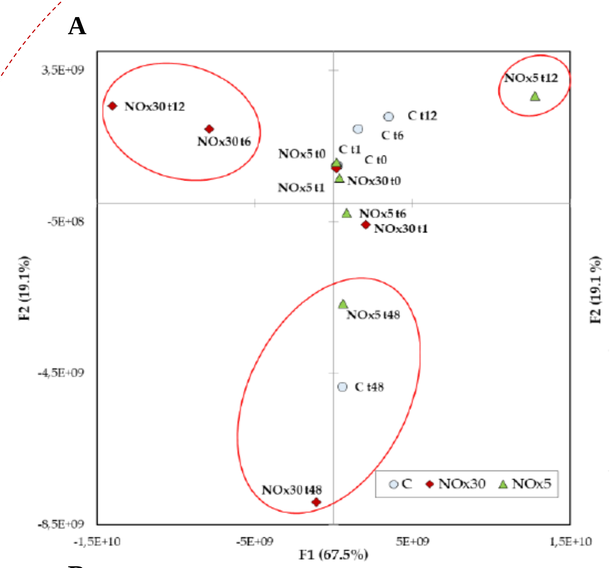
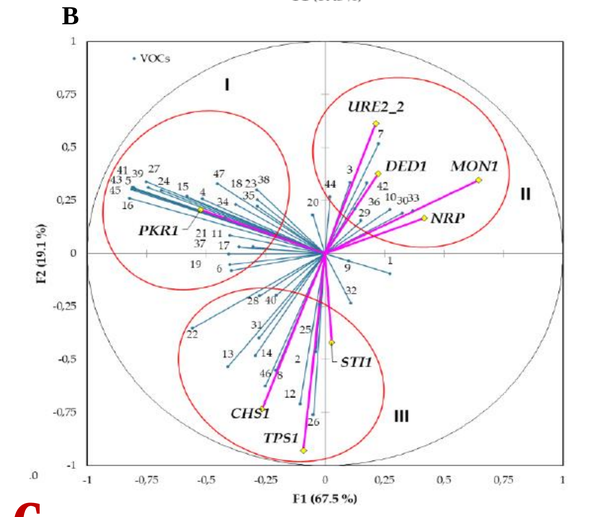
The application of cold atmospheric plasma (CAP) technology to avoid microbial growth in foods is considered a promising innovative technology for microbial inactivation on food surfaces. Utilization of CAP technology, a nonthermal technique, is encouraged because of its efficiency in maintaining natural aroma and flavor and product shelf-life.

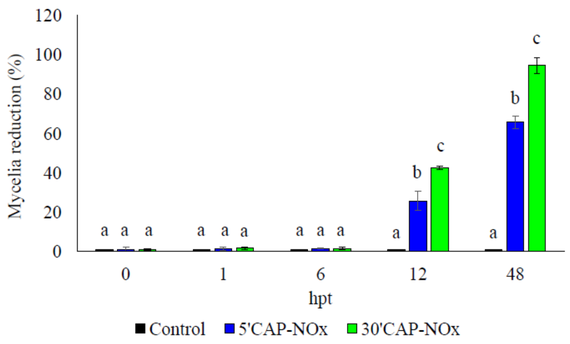
In this study the ability of non-thermal cold atmospheric plasma (CAP) at high power density (NOx) to affect biological process inducing the stress responses of *Aspergillus chevalieri*, a xerophilic/xerotolerant fungi affecting dried food products, were analysed.

A. chevalieri PSJ144 strain used in these studies, has been previously isolated from sundried tomatoes

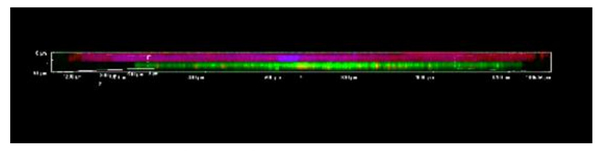




6. Gene expression associated to VOC profile. Mycelia modulated the metabolic and genetic responses on the basis of different CAP-NOx treatment. After 48 hpt the mycelia-response tends to merge independent of the CAP-NOx treatment (**A**). A clear variable separation into three main clusters was detected (**B**).



1. Strong antifungal activity exhibited by CAP-NOx against *A. chevalieri* mycelia inactivation was more pronounced after treatment lasting 30 min as opposed to 5 min.



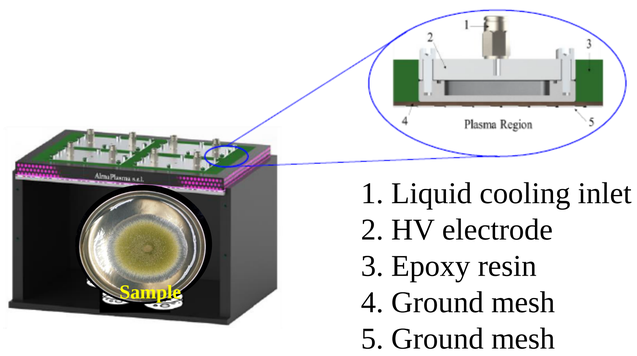
Confocal fluorescence image

2. Dead surface mycelium layers exposed to the treatments, evidenced by propidium iodide (PI) dye, (red fluorescence) and alive at the bottom (green fluorescence, carboxyfluorescein diacetate -CFDA)

Treatments:

✓5 min (5'CAP-NOx)

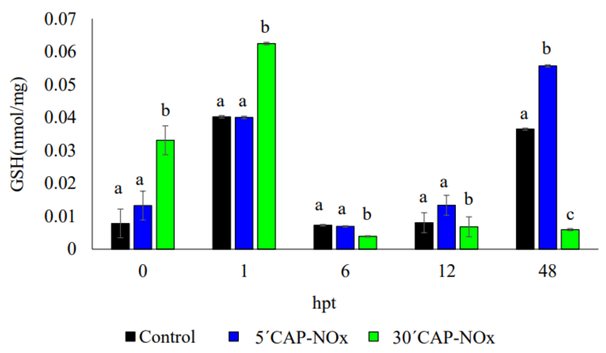
✓30 min (30'CAP-



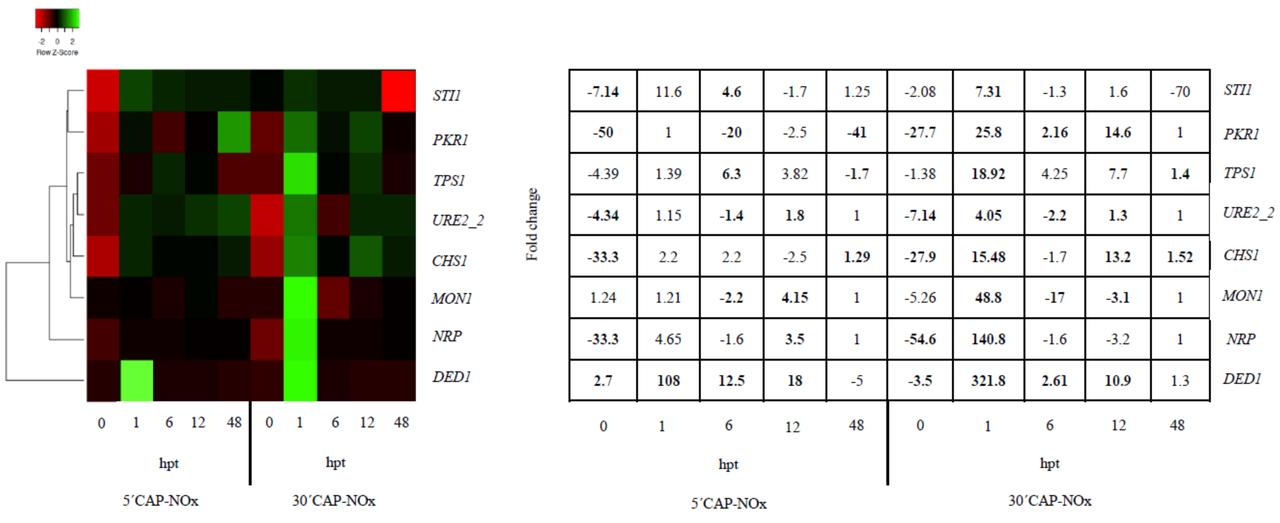
1. Liquid cooling inlet
2. HV electrode
3. Epoxy resin
4. Ground mesh
5. Ground mesh

Times:

0, 1, 6, 12, and 48 h post treatments (hpt)



3. The cellular response to stress induced by CAP-NOx was confirmed by mycelial glutathione accumulation



-7.14	11.6	4.6	-1.7	1.25	-2.08	7.31	-1.3	1.6	-70	STI1
-50	1	-20	-2.5	-41	-27.7	25.8	2.16	14.6	1	PKR1
-4.39	1.39	6.3	3.82	-1.7	-1.38	18.92	4.25	7.7	1.4	TPS1
-4.34	1.15	-1.4	1.8	1	-7.14	4.05	-2.2	1.3	1	URE2_2
-33.3	2.2	2.2	-2.5	1.29	-27.9	15.48	-1.7	13.2	1.52	CHS1
1.24	1.21	-2.2	4.15	1	-5.26	48.8	-17	-3.1	1	MON1
-33.3	4.65	-1.6	3.5	1	-54.6	140.8	-1.6	-3.2	1	NRP
2.7	108	12.5	18	-5	-3.5	321.8	2.61	10.9	1.3	DED1

Fold change

0 1 6 12 48 0 1 6 12 48

hpt

5´CAP-NOx 30´CAP-NOx

5. CAP-NOx induced early downregulation of genes involved in fungal stress response.

DED1= DEAD-box ATP-dependent RNA helicase; NRP= Nonribosomal Peptide Synthetase; URE2_2 = Glutathione S-transferase, nitrogen catabolite repression regulator; PKR1 = SMK killer toxin resistance protein; MON1 = Vacuolar fusion protein; SII1 = Hsp90 cochaperone; CHS1 = Chitin synthase I; TPS1 = Trehalose-6-P synthase/phosphatase complex (TPS1).

N	K.R.I.	Chemical Group	Compound	Cluster	Pathway	5 minutes NOx					30 minutes NOx				
						t0	t1	t6	t12	t48	t0	t1	t6	t12	t48
1	443.2	Alcohols	Ethanol	-	Gly	-0.68	-2.60	-6.92	-1.96	-9.94	-3.82	-6.05	-3.20	-4.26	-2.32
2	823.9		1-Butanol, 2,3-dimethyl-	III	E.P.	1.15	0.97	0.50	3.16	-1.24	-1.58	4.22	0.47	3.78	6.95
3	856		1-Octen-3-ol	II	F.A.O.	-13.84	-8.67	-6.62	-9.88	-1.46	-5.41	-5.45	-3.97	-2.99	-0.56
4	1039		2-Octen-1-ol, (Z)-	I	F.A.O.	-1.19	-0.33	-2.18	-0.66	-1.87	0.27	2.47	2.76	5.13	1.49
5	1051		2-Nonen-1-ol	I	F.A.O.	0.91	1.14	-2.41	-0.49	-1.33	1.10	6.24	0.48	1.40	6.05
6	1132		1-Nonanol	I	F.A.O.	-3.81	-3.89	-0.99	-0.76	-1.25	-3.87	-3.81	-0.18	-2.98	-1.01
7	1277		1-Octanol, 2-butyl-	II	F.A.O.	-0.22	-0.53	-0.77	-0.80	-2.12	0.06	2.06	0.10	1.71	6.52
8	1285		2-Undecen-4-ol	III	F.A.O.	1.40	0.16	0.38	2.15	1.73	0.40	4.32	0.21	1.66	3.90
9	1812		2-Decen-1-ol	-	F.A.O.	5.06	3.44	-1.65	-2.60	-1.76	6.74	6.30	-3.25	-3.72	-0.10
10	294	Alkanes	Propene	II	F.A.O.	-1.24	-3.59	-0.30	-1.07	-5.78	2.09	3.35	7.54	4.25	5.32
11	1218		Undecane, 3,7-dimethyl-	I	F.A.O.	1.03	2.44	0.39	4.51	2.65	5.04	2.74	0.57	7.44	13.55
12	1300		Tridecane	III	F.A.O.	0.66	2.50	3.09	4.71	2.66	2.37	3.36	6.43	8.81	13.84
13	1320		Dodecane, 2,7,10-trimethyl-	III	F.A.O.	-0.19	-0.62	-0.89	-1.73	-2.39	1.64	3.00	0.74	2.44	5.91
14	1200		Dodecane	III	F.A.O.	-0.04	-0.57	-5.32	-2.60	-7.79	3.12	1.12	0.04	2.13	1.48
15	1600		Hexadecane	I	F.A.O.	-1.42	-0.62	-3.40	-0.60	-1.24	-2.43	-2.28	7.41	10.82	8.26
16	2097		2-Butene, 1,4-diol-1,4-diphenyl	I	F.A.O.	-0.41	-1.16	-0.06	-1.16	-1.92	-0.47	-0.43	1.69	1.17	5.58
17	2243		Heptadecane, 9-hexyl-	I	F.A.O.	-6.42	-6.58	-0.91	-0.27	-0.34	-1.59	-5.67	4.90	5.06	6.68
18	1500	Aldehydes	Lilial	I	F.A.O.	5.87	2.68	3.60	1.29	2.36	5.89	2.64	2.25	3.02	6.08
19	1502		Tetradecanal	I	F.A.O.	-0.41	-1.60	-6.87	-4.30	-1.71	-0.40	-1.43	-3.24	-0.18	-0.63
20	927.2		Benzaldehyde	-	P.P.	-2.78	-4.88	-1.97	-2.00	-0.33	3.55	5.51	3.75	3.26	5.34
21	1386		Dodecanal	I	F.A.O.	-5.33	-5.97	-1.01	-0.53	-0.25	2.57	1.22	2.22	3.18	9.46
22	1795		Hexadecanal	III	F.A.O.	0.89	0.57	0.37	2.92	2.56	3.68	0.18	3.19	0.63	2.18
23	1909		4,8,12-Tetradecatrienal, 5,9,13-trimethyl-	I	F.A.O.	1.95	0.67	1.82	1.03	0.24	1.92	1.93	2.55	2.92	1.56
24	953	Ketones	4-Octanone	I	F.A.O.	0.39	0.14	3.94	3.90	0.80	8.40	2.04	0.32	4.30	7.58
25	1070		2 nonanone	III	F.A.O.	3.52	0.58	6.25	5.93	11.50	6.98	0.68	5.33	5.50	14.14
26	1427		5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	III	F.A.O.	0.53	3.66	3.19	3.36	1.10	3.80	3.79	5.34	6.32	3.45
27	1005	Esters	Heptanoic acid, methyl ester	I	Gly	0.27	3.12	1.04	1.45	3.49	6.04	0.41	1.65	5.87	4.45
28	1019		Hexanoic acid, 3-ethyl-, methyl ester	III	Gly	0.94	3.68	4.93	1.00	0.72	4.38	2.01	3.68	0.24	7.95
29	1284		2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	II	Gly	-4.21	-3.79	0.29	1.10	2.48	-0.95	-0.89	-1.22	0.04	3.90
30	1389		Ethyl trans-4-decenoate	II	Gly	0.83	-2.05	-0.54	-1.75	0.72	-0.88	-0.90	1.03	0.05	5.99
31	1581		Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	III	Gly	-1.99	-3.09	1.85	2.01	3.62	1.24	2.00	0.17	1.14	6.75
32	1606		Tridecanoic acid, methyl ester	-	Gly	0.36	1.63	8.82	10.99	3.70	0.21	0.58	6.36	9.02	7.47
33	1607		Dodecyl acrylate	II	Gly	2.17	-0.04	-0.68	-0.86	-3.10	7.93	0.87	0.99	4.75	2.24
34	1814		Pentadecanoic acid, 14-methyl-, methyl ester	I	Gly	3.98	-1.91	-11.55	-3.14	-8.02	3.15	6.51	0.32	3.20	1.40
35	1819		1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	I	Gly	-1.12	-1.35	-0.95	-2.76	-3.92	-1.12	-1.19	-2.77	-1.95	-1.38
36	1926		Tetradecanoic acid, 1-methylethyl ester	II	Gly	0.74	0.39	0.20	1.15	1.68	0.17	6.23	2.04	4.53	4.81
37	2113		9-Octadecenoic acid (Z)-, methyl ester	I	Gly	2.10	3.52	7.12	6.52	6.02	-0.88	-8.20	6.97	7.69	6.03
38	2180		Hexadecanoic acid, 15-methyl-, methyl ester	I	Gly	-8.17	-0.56	-5.73	-2.00	-0.90	-4.43	2.39	4.63	9.05	6.03
39	2433		Oxalic acid, allyl hexadecyl ester	I	Gly	1.90	0.71	0.22	1.18	0.80	0.93	2.75	0.04	1.44	3.73
40	2934		Hexacosanoic acid, methyl ester	III	Gly	0.31	1.26	2.41	2.93	4.93	0.52	0.35	7.35	8.05	7.93
41	1380	Acids	Butanoic acid, 2-(aminooxy)-4-(methylthio)-	I	Gly	-6.98	-6.19	-5.16	-5.20	-2.49	-7.04	-5.91	-7.29	-6.43	-1.79
42	1670		Tridecanoic acid	II	Gly	-1.65	-3.09	-2.90	0.82	0.71	2.29	3.60	4.83	9.31	3.96
43	1942		Hexadecanoic acid	I	Gly	-2.14	-1.72	-1.82	-1.76	-2.56	-2.59	-0.60	-2.13	-1.49	-0.08
44	1407		Dodecanoic acid	II	Gly	-7.58	-0.62	-3.22	-0.96	-3.35	-0.76	-4.17	2.78	5.94	0.55
45	3094	Steroids derivatives	Ethyl iso-allocholate	I	n.a.	-0.77	-0.79	-0.22	-2.50	-4.72	-1.61	-3.80	-0.07	3.37	6.97
46	1539	Phenols	Phenol, 2,4-bis(1,1-dimethylethyl)-	III	n.a.	0.42	1.80	0.18	2.01	1.75	2.81	0.71	1.99	2.61	3.00

4. The CAP-NOx treatment provoked marked modifications of the volatile organic compounds (VOCs) profiles and increased fungal ketones and alkenes emitted suggesting change on lipid metabolism.

REFERENCE: Molina-Hernandez, J. B., Landi, L., De Flaviis, R., Laika, J., Romanazzi, G., Chaves-Lopez, C. (2023). Understanding the mechanisms of action of atmospheric cold plasma towards the mitigation of the stress induced in molds: the case of *Aspergillus chevalieri*. *Innovative Food Science & Emerging Technologies, 90, 103492.* https://doi.org/10.1016/j.ifset.2023.103492

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