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Field and storage fungi inactivation and mycotoxins degradation by ozone gas in grains and nuts

V M SCUSSEL[1*], B N E GIORDANO[1], M BEBER[1], G D SAVI[1], H KREIBICH[1], D CHRIST[2]

[1]Laboratory of Mycotoxicology and Food Contaminants, Food Science and Technology Department, Center of Agricultural Sciences, Federal University of Santa Catarina, P.O. Box 476, Florianopolis – SC, CEP 88034-001, Brazil

ABSTRACT

Grains and nuts controlling and decontamination strategies regarding fungi (field: *Fusarium*; storage: *Aspergillus*/*Penicillium*) and mycotoxins (*field*: deoxynivalenol, fumonisins, zearalenone and *storage*: aflatoxins, Ochratoxin A, citrinin) using ozone (O_3) gas during storage were evaluated. Samples were treated with O_3 at different concentrations and exposure times keeping one as Control Group (C: no O_3). Fungi O_3 susceptibility was evaluated by colony counting and mycotoxins determined by liquid chromatography with different detectors, accordingly. The paddy rice (*Oryza sativa* L.), naturally fungi contaminated exhibited their growth reduction (O_3 treated 40 ppm; 30 min) to 99.9%. Similarly, O_3 gas decontamination behaviour was observed in stored wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), including toxin in wheat. The O_3 gas successfully inactivated fungi (*A. flavus* and *A. parasiticus*) spores of whole Brazil nut day one after application. Aflatoxins were not detected in any of the gas treated nut samples after the application (method LOQ: 1.34 µg/kg). The same occurred to cocoa beans. O_3 gas is internationally recognized safe and does not leave residues in food. It could be a promising method for fungi inactivation and toxin degradation (either solely or in combination with other decontamination methods) in storage units and industries to prevent food security and safety problems.

Key words: Cocoa, Fungi, Grains, Mycotoxins, Nuts, Ozone gas, Security, Safety

Food producing areas, worldwide, are mainly concentrated in regions where excess rainfall and high temperatures occur which induce biological contamination (insects, fungi and mycotoxins). To control these contaminants, pesticide application can be performed both in the field and storage grains.

However, if applied inappropriately, it can also turn as a contamination problem (pesticide residues persistence). Moreover, some contaminants are resistant to milling and heating processes and may remain in the food products, thus can enter in the food chain.

The increasing concern on environmental safety and human health, has stimulated the development and/or improvement of non-aggressive food decontaminantion atmosphere in order to avoid and/or minimize their application impact (Armor, 1999; Giordano et al., 2012; Savi and Scussel, 2014; Savi et al., 2014a; 2014b, 2015).

An oxidant, acceptable from the environmental/health point of view must have the following characteristics: (*a*) to react specifically with the living organism/compound to be destroyed/degraded; (*b*) not form toxic by-products (with toxicity equal to or higher than the target contaminant) and (*c*) be easy to obtain (Christ et al., 2016).

The green method that has been shown its decontamination efficiency to post-harvest high (fruits/vegetables) and low (grain/nuts/pulses) humidity food, without leaving residue is ozone (O_3), both as gas and in the liquid form (Sarig et al., 1996; Kells et al., 2001; Sharma et al., 2002, 2003, 2004; Di Renzo et al., 2005; Bataller et al., 2002, 2012).

The aim of this study was to evaluate grain and nuts controlling and decontamination strategies with respect to fungi (field: *Fusarium*; storage: *Aspergillus*/*Penicilium*) and some mycotoxins (*field*: deoxynivalenol - DON, fumonisins - FBs, zearalenone

[2]Storage and Prototypes Drying Laboratory, Technological and Exact Sciences Center, Western Parana State University, Jardim Universitario, Cascavel, PR, Brazil.

[*]Corresponding author e-mail: *vildescussel_2000@yahoo.co.in*

Table 1 Fungi inhibition by ozone gas in naturally contaminated and inoculated grains and nuts

Food	O_3 treatment			Storage (days)	Fungi				
	Concentration (ppm)	Time (min)	Flow (l/min)		TFC (CFU/g) Initial	TFC (CFU/g) After O_3	Inhibition (%)	Genera and species (isolated/identified/ studied)	Culture media
Maize	20/40/60	30/105/180	1.0	30	10×10^2	NG	100	NP	PDA
Wheat (whole)	40/60	30/60/120/180	1.0	NA	48×10	ND	100[p]	*F. graminearum*	PDA
	40/60	30/60/120/180	1.0	NA	44×10	5.35×10^1	87.8	*A. flavus; A. parasiticus; P. citrinum; F. vrticilioides; A. flavus; P. citrinum*	PDA
Rice (paddy)	10/20/40	30.0	1.0	NA	3×10^5	1.4×10^2	99.9	*Aspergillus*; *Penicillium; Acremonium; Alternaria*	PDA
Brazil nuts (in-shell)	10	90.0	NI	1/30/60	1.83×10^4	NG	100	*A. flavus*; *A. parasiticus*	PDA
	10/14/31.5	180/300	NI	180	4.83 log	NG	100	*A. flavus*; *A. parasiticus*	MEA
Cocoa (post-ferment)	20/40/60	30/105/180	NI	30	5.6×10^2	NG	100	*A. flavus*	PDA

NP, not performed; NI, not informed; NG, no growth; PDA, potato dextrose agar; MEA, malt extract agar;TFC, total fungi count.

– ZON and *storage*: aflatoxins - AFLs, ochratoxin A - OTA, citrinin - CTR) using ozone (O_3) gas during storage.

MATERIALS AND METHODS

Decontamination trials were performed in pilot silos loaded with respective grains (paddy rice, whole wheat, maize) and nuts (in-shell Brazil nuts and post-fermentation drycocoa).

They were divided into two main Groups: O_3 treated and control (no O_3), which were timing exposed to gas treatments at different concentrations (Tables 1 and 2).

Fungi susceptibility to O_3 was evaluated by colony counting (total spores load on grains/nuts) according to APHA (1999) and mycotoxins (DON, FBs, ZON and *storage*: AFLs, OTA, CTR) determined by liquid chromatography with different detectors, accordingly (Scussel et al., 2011; Giordano et al., 2013; Savi et al., 2014a, b). Fungi microscopic degradation (spores germination inhibition) and gas effect on grain (germination and lipid oxidation) were also investigated (Savi and Scussel, 2014; Savi et al., 2015).

RESULTS AND DISCUSSION

Tables 1 and 2 show the O_3 treatment conditions, storage days and its effect on fungi and toxins, respectively. While the naturallyfungi contaminated

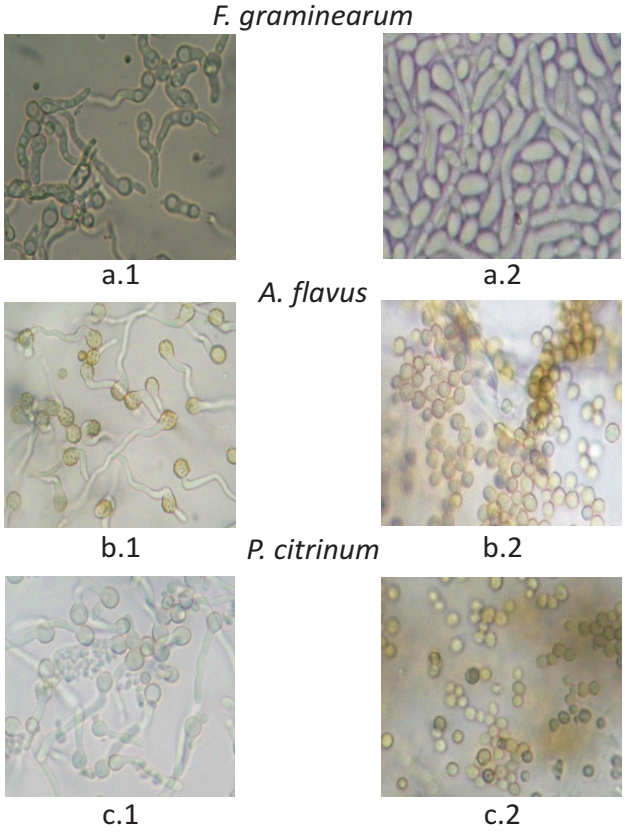

Fig 1. Effects of ozone gas (60 µmol/mol, 120 min exposure) on fungi conidia germination. [Conidia germination of groups control (a.1, b.1 and c.1) and treated (a.2, b.2 and c.2)]

Table 2. Aflatoxins and others mycotoxins degradation in naturally contaminated and inoculated grains and nuts

Food		Toxins			O_3 treatment			AFLs					Method applied		
Type	Weight (kg)	AFLs initial (µg/kg)		Silo (L)	Conc (ppm)	Time (min)	Storage (days)	Degradation (µg/kg)					Inctivation (%)	Detection	LOD* and LOQ**
		Artificial	Natural	Bulk				AFB_1	AFB_2	AFG_1	AFG_2	AFL stotal			
Wheat (whole)	0.35	231.9 /265.8 239.9 / 199.4	NA	2	40/60	30 – 180	NA	12.51	41.06	47.96	37.81	42.90 (CTR)	94.6 / 84.5 80.0/ 81.0	LC/ FLD	0.26 & 3.1 / 0.002 & 0.02 / 0.28 & 1.41 / 0.005 & 0.03
Brazil nut (in-shell)	10	NA	10.6	0.26	10	90	1.0–60	NI	NI	NI	NI	<0.36	100	LC-MS^n	NI / 0.36
	2	NA	5.2	14.1	14/ 31.5	60 – 300	1.0–180	ND	ND	ND	ND	ND	100	LC/ FLD	NI & 0.5 / NI & 0.17 / NI & 0.5 / NI & 0.17 /NI & 1.34 AFG_{total}
Other Toxins															
Wheat (whole)	0.35	1065.10	DON	2	40/ 60	30 – 180	NA	NA	NA	NA	NA	NA	100	LC/ UV	67 / 119
	0.35	173.5	CTR	2	40/ 60	30 – 180	NA	12.51	41.06	47.96	37.81	42.90	75.3	LC/ FLD	0.2 & 1.2 CTR

AFLs, Aflatoxins; LOD, limit of detection; LOQ, limit of quantification (AFB_1; AFB_2; AFG_1; AFG_2 respectively); LC/FLD-MS-UV, liquid chromatography with fluorescence or mass or ultraviolet detectors; DON, deoxynivalenol; CTR, citrinin; NA, not applicable; &, and

paddy rice showed fungal growth reduction after O_3 application (40 ppm; 30 min) to 99.9% (from 3×10^5 to 1.4×10^2 CFU/g), the field (*Alternaria*) and storage (*Aspergillus, Penicillium*) fungi were still detected and growing in Group C.

Similarly, O_3 gas behaviour was observed for stored wheat and maize decontamination. Regarding wheat, the gas also showed strong effect on conidia (fungi spores) germination as seen in Fig1 for field and storage (*Fusarium graminearum, Aspegillus flavus, Penicillium citrinum*) and on different mycotoxins (DON, ZON, FBs and CTR) degradation.

The whole in-shell Brazil nuts at O_3 concentrations (10, 14, 31.5 ppm) and time (5 h exposition) was able to successfully destroy fungi to NG (no grow), including the *A. flavus* and *A. parasiticus* species, since day one after application. Independent of the O_3 concentrations applied, AFLs were not detected in any of the gas treated nut samples since day one of application up to the method LOQ of 1.34 µg/kg. The natural toxin contamination level was low (ca. 5 ppb). The same occurred to cocoa beans.

CONCLUSION

As O_3 gas is internationally recognized safe and does not leave residues in food, it could be a promising method for fungi and toxin inactivation and degradation (either solely or in combination with other decontamination methods)in storage units and industries to avoid problems of food security and safety.

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