Savi GD, Bortolloto T, Runtzel C, Scussel VM (2016) Effect of ozone gas on degradation of organophosphate and pyrethroid pesticide residues inwhole wheat (*Triticum aestivum*) grains effect of during storage. Pp. 220–224. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effect of ozone gas on degradation of organophosphate and pyrethroid pesticide residues in whole wheat (*Triticum aestivum*) grains during storage

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ABSTRACT

An experiment was conducted to study the effect of Ozone gas on degradation of organophosphate and pyrethroid residues in whole wheat (*Triticum aestivum* L.) grains during storage. Ozone (O_3) concentration was applied at 60 µmol/mol and times of exposure were 120 and 180 min. Humidity (moisture content – m.c. and water activity - a_w) was determined. Storage silos and wheat grains (before and after compounds of both pesticides groups spiking) were prepared and pesticides after O_3 gas treatment were analyzed. Fenitrothion content decreased (54%) following O_3 treatment after 180 min of exposure (at m.c. of 20% and a_w of 0.9). Similarly, after the same O_3 treatment with exposure times of 120 and 180 min, the deltamethrin residues significantly reduced to 83 and 85% in the wheat grains at lower m.c. and a_w (12% and 0.6). On the other hand, the grains with higher humidity (m.c. 20% and a_w 0.9) revealed better results, corresponding to reductions of 87 and 90%. The residues of bifenthrin also decreased after 180 min of exposure with 47% reduction. In the same experimental conditions, the pirimiphos-methyl residues, exhibited 77% reduction. After O_3 gas treatment, by-products of pirimiphos-methyl containing different molecular mass were identified by LC-MS. The O_3 gas was effective in degrading the pesticides and is a strong oxidizer that has shown potential in grain storage (as GRAS) in order to ensure food quality and safety.

Key words: Organophosphate, Ozone, Pesticides residues, Pyrethroid, Wheat

Pesticides are used worldwide in agriculture and urban environments in preventing or destroying biological contaminants. They are commonly applied to grains in the storage units, as liquid formulations, on the conveyor carriers prior being stored in silos to prevent insect infestation (Embrapa, 2015). However, they are persistent in the grains and their products as residue, have been linked to adverse health effects in living organisms (Lozowicka et al., 2014). Organophosphate (fenitrothion and pirimiphos-methyl) and pyrethroid (bifenthrin and deltamethrin) pesticides are some examples of pesticides widely used on wheat (*Triticum aestivum* L.) grains in the storage units. Therefore, to ensure food safety, maximum residue limits (MRLs) and storage security intervals have been set for the control of pesticide residues, since agricultural crops cannot be sold if they contain pesticides exceeding the residual limit (Brazil, 2009).

For wheat grains, the MRL under Brazilian regulations is 1.0 mg/kg for fenitrothion and deltamethrin. The storage-interval periods are about 30 and 120 days for deltamethrin and fenitrothion respectively. In addition, the MRL is 0.6 and 10.0 mg/kg for bifenthrin and pirimiphos-methyl respectively. The storage security-interval periods are approximately 30 days for both pesticides (Brazil, 2009).

Since insecticidal residues are persistent in the grains, it is necessary to develop methods to remove pesticides from grains. Application of O_3 gas is considered to be a potential chemical method for

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removing residual pesticides for food industry because it decomposes to molecular oxygen without leaving residues, and it is considered safe by the US Food and Drug Administration (FDA, 1982).The O_3 is a powerful antimicrobial agent owing to its potential oxidizing capacity and used used as disinfectant for microorganisms and viruses, odour and taste removal, and decomposition of organic matter (Khadre et al., 2001). Ozonation has been widely investigated for the removal of pesticides and extensively implemented on stored grains to control the development of insects, fungi and toxins (Ikeura et al., 2013, Mcdonough et al., 2011, Scussel et al., 2011, Savi et al., 2014).

The aim of this work was to study the O_3 application gas as a possibility to remove pesticide residues (widely used on wheat grains) in the storage units, as well as to evaluate their degradation by-products.

MATERIALS AND METHODS

Fenitrothion, deltamethrin, bifenthrin and pirimiphos-methyl were obtained from Sigma Aldrich Chemicals (St. Louis, MO, USA); acetonitrile and methanol with LC grade, dichloromethane and phosphoric acid were purchased from Vetec (Duque de Caxias, RJ, Brazil). The Milli-Q water was obtained from Synergy (Millipore, USA) and the solid phase extraction (SPE) columns Strata-X (200 mg/6 ml) from Phenomenex (Madrid, Avenue, Torrance, USA).

Determination of pesticides was carried out by the high-performance liquid chromatography (HPLC) Shimadzu (Kyoto, Japan), equipped with an isocratic pump (LC-20AT), column oven (CTO-20A), prominence communication bus module (CBM-20A), degasser (DGU-20A), autosampler (SIL-20A) and a detector diode array (DAD) (SPD-M 20A). Chromatographic separations were performed on a C18 reversed-phase column (250 × 4.6 mm, 4µ), Synergi Fusion-RP 80A (Phenomenex). The LC-MS experiments were carried out in a micrOTOF II mass spectrometer (Bruker Daltonics, Massachusetts, EUA) equipped with an ESI source and interfaced to a Prominence UFLC system (Shimadzu). MS data were collected in positive ion mode.

Artificial contamination of samples before ozone treatment

About 50 kg wheat grains were collected from vertical silos (stored for three months) in 2014 post-harvest, from Brazilian Agricultural Research Corporation (Embrapa Wheat). Samples were received after cleaning and drying (up to a maximum of 60ºC) steps in the storage unit, packed in a polyethylene bag and stored at 4ºC.The samples were artificially contaminated with a solution (100 µl) of each pesticides (1,000 mg/l) and spiked on wheat grains (50 g), separately and in triplicates. Then, it was stirred for 2 min and left to stand for 30 min.

In order to know the moisture content (m.c.), the wheat grains were subjected to drying in an oven (105 ± 5°C) up to constant weight through gravimetric method (AOAC, 2005). Besides, the water activity (aw) was performed in Aqua-Lab 4 TE equipment.

Ozone gas treatment in storage silos

The silos were made with vinyl polychloride tubes containing only two apertures: one for the input of O_3 gas and the other for the output. The artificial contaminated grains were packaged in the top part of the silos, on the polyamide screen surface. The bottom part of the silos was filled with 350 g wheat grains. The O_3 gas generated was applied into the pilots silos at 60 µmol/mol concentration up to 180 min, keeping one silo as Control (Group C:no O_3 gas). The O_3 gas generator system followed the procedures detailed by Giordano et al. (2012) and Savi et al. (2014) with minor modifications. The compressed air pump was connected to an air impurities remover to filter the room air. Afterwards, the air filtered was conducted to the adjusted flow meter for 1 l/min. The O_3 generator used (5 a 60 µmol/mol) was the corona discharge process. The control chamber (without O_3 gas) was ventilated with 'room air' in the same flow. The O_3 gas concentration was measured by the iodinemetric test, according to APHA (1999).

Pesticides analysis after ozone treatment

Whole samples of wheat grains were analyzed using SPE Strata-X column for clean-up step and LC/DAD for detection, as per D'Archivio et al. (2007), with some modifications. Briefly, each artificially contaminated wheat sample was ground with LC-grade acetonitrile (60 ml). The mixture was stirred for 1 min, followed by filtration and cleaning by SPE Strata-X column (previously conditioned with dichloromethane, acetonitrile and milli-Q water). The column was washed with LC-grade water:methanol (95:5, v/v) and the pesticides were slowly eluted with 1 ml 100% LC-grade acetonitrile and 1 ml 100% LC-grade methanol. The evaluate was evaporated to dryness using a heating block at 40ºC with gentle nitrogen stream and the dry residue was then redissolved with 500 µl mobile phase (acetonitrile : acidified water 0.1% H_3PO_4, 50:50, v/v for fenitrothion; acetonitrile : water, 92:8, v/v for deltamethrin; and acetonitrile:water, 85:15, v/v for bifenthrin and pirimiphos-methyl). The extract (50 µl) was

Table 1 Effect of gas ozone in the fenitrothion residue degradation in wheat grains with moisture content of 12% and 20% and a_w of 0.6 and 0.9

Wheat grains humidity	Ozone gas			
	Time of exposure	Control (without O_3)	Treatment (60 µmol/mol)	Reduction (%)
		Fenitrothion (mg/kg)		
m.c.: 12%	120	3.9 ± 0.1	4.0 ± 0.07	0
a_w: 0.6	180	4.6 ± 0.1	4.0 ± 0.8	0
m.c.: 20%	120	3.0 ± 0.3	2.6 ± 0.2	0
a_w: 0.9	180	3.3 ± 0.2	1.6 ± 1.2*	54

* Statistically significant when compared to Control group ($P < 0.05$) by Bonferroni post-test

Table 2 Effect of gas ozone in the deltamethrin residue degradation in wheat grains with moisture content of 12% and 20% and a_w of 0.6 and 0.9

Wheat grains humidity	Ozone gas			
	Time of exposure	Control (without O_3)	Treatment (60 µmol/mol)	Reduction (%)
		Deltamethrin (mg/kg)		
m.c.: 12%	120	8.4 ± 1.9	1.4 ± 0.3*	83
a_w: 0.6	180	9.8 ± 1.0	1.5 ± 0.4*	85
m.c.: 20%	120	9.3 ± 1.4	1.2 ± 0.3*	87
a_w: 0.9	180	10.7 ± 0.3	1.1 ± 0.1*	90

* Statistically significant when compared to Control group ($P < 0.05$) by Bonferroni post-test

injected onto the LC/DAD System set at wavelengths of 270, 233, 190 and 275 nm for fenitrothion, deltamethrin, bifenthrin and pirimiphos-methyl. The mobile phase was delivered at a flow constant rate of 0.8, 1.0 and 1.4 ml/min for pirimiphos-methyl, fenitrothion and deltamethrin and bifenthrin respectively. The dry residue of the samples containing pirimiphos-methyl also was redissolved with 500 µl mobile phase LC-MS grade and inject into the LC/MS MicrOTOF system.

Data were statistically analysed using analysis of variance followed by Bonferroni post-test. The data were expressed as mean ± standard deviation and the values of $P<0.05$ were considered statistically significant.

Table 3 Effect of gas ozone in the bifenthrin residue degradation in wheat grains with moisture content of 12% and 20% and a_w of 0.6 and 0.9

Wheat grains humidity	Ozone gas			
	Time of exposure	Control (without O_3)	Treatment (60 µmol/mol)	Reduction (%)
		Bifenthrin (mg/kg)		
m.c.: 12%	120	1.6 ± 0.1	1.4 ± 0.1*	0
a_w: 0.6	180	2.0 ± 0.1	1.6 ± 0.4*	0
m.c.: 20%	120	1.5 ± 0.3	1.3 ± 0.4*	0
a_w: 0.9	180	1.9 ± 0.2	1.0 ± 0.3*	47

* Statistically significant when compared to Control group ($P < 0.05$) by Bonferroni post-test.

Table 4 Effect of gas ozone in the pirimiphos-methyl residue degradation in wheat grains with moisture content of 12% and 20% and aw of 0.6 and 0.9

Wheat grains humidity	Ozone gas			
	Time of exposure	Control (without O_3)	Treatment (60 µmol/mol)	Reduction (%)
		Pirimiphos-methyl (mg/kg)		
m.c.: 12%	120	8.6 ± 1.9	3.9 ± 1.0*	55
a_w: 0.6	180	9.3 ± 1.6	3.7 ± 0.6*	60
m.c.: 20%	120	9.0 ± 1.3	3.3 ± 0.8*	63
a_w: 0.9	180	11.7 ± 0.6	2.7 ± 0.9*	77

* Statistically significant when compared to Control group ($P < 0.05$) by Bonferroni post-test

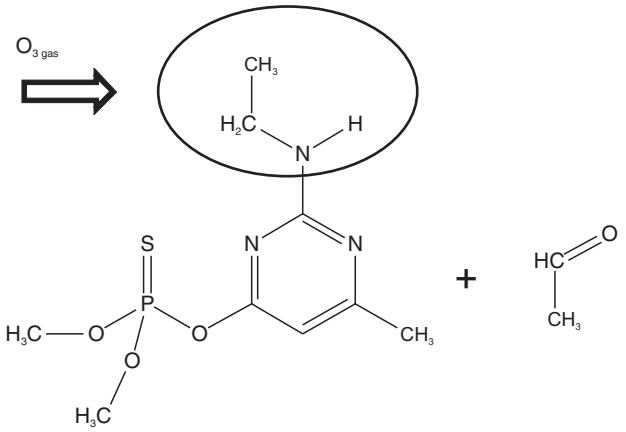

Fig. 1. By-product (278.1 *m/z*) formed after degradation of pirimiphos-methyl by ozone gas treatment.

RESULTS AND DISCUSSION

The LC/DAD method for pesticides chromatographic separation and the validation parameters (linearity, limit of detection - LOD, limit of quantification - LOQ, reproducibility, repeatability and recovery) were quite adequate. Under the chromatographic conditions the retention time (Rt) was 14 ± 0.5 min, 6.0 ± 0.5 min, 9.0 ± 0.5 min and 4.2 ± 0.5 min for fenitrothion, deltamethrin, bifenthrin and pirimiphos-methyl respectively. Linearity was confirmed using the calibration curve for each pesticide concentration, which was linear at 0.08–1 mg/l for fenitrothion and deltamethrin and 0.1–10 mg/l for bifenthrin and pirimiphos-methyl, with a correlation factor equal to 0.991, 0.992, 0.996 and 0.998 respectively. The LOD and LOQ were 0.01 and 0.2, 1.2 and 1.4, 0.3 and 0.6 and 0.8 and 2.1 mg/kg for the 4 pesticides respectively. The mean recovery of the extraction method for the concentrations of 100 and 1,000 mg/l were 80 and 93, 82 and 85, 85 and 91, 88 and 98% respectively.

The fenitrothion content decreased significantly by the O_3 treatment (60 µmol/mol) only after 180 min of exposure (54% of reduction), with m.c. of 20% and a_w of 0.9 (Table 1). However, the same O_3 treatment with a exposure time of 120 and 180 min, deltamethrin residues significantly reduced to 1.4 ± 0.3 and 1.5 ± 0.4 mg/kg (Control: 8.4 ± 1.9 and 9.8 ± 1.0 mg/kg), indicating a reduction of 83 and 85% in the wheat grains that exhibited lower m.c. and a_w (12% and 0.6). On the other hand, the grains with higher m.c. and a_w (20% and 0.9) revealed reduction in pesticide to 1.2 ± 0.3 and 1.1 ± 0.1 mg/kg with 120 and 180 min of O_3 exposure (Control: 9.3 ± 1.4 and 10.7 ± 0.3 mg/kg), indicating reduction of 87 and 90% (Table 2).

The residues of bifenthrin decreased (47%) after 180 min of exposure with 20% m.c. and 0.9 a_w (Table 3). On the other hand, in the same experimental conditions, the pirimiphos-methyl residues showed at 77% reduction (Table 4).

With the exposure times of 120 and 180 min, the pirimiphos-methyl residues significantly reduced to 3.9 ± 1.0 mg/kg and 3.7 ± 0.6 mg/kg (Control: 8.6 ± 1.9 and 9.3 ± 1.6 mg/kg), indicating reduction of 55 and 60% in the wheat grains that presented lower m.c. and a_w (12% and 0.6). On the other hand, the grains with higher m.c. and a_w (20% and 0.9) exhibited a decrease in pesticide contents to 3.3 ± 0.8 and 2.7 ± 0.9 mg/kg with 120 and 180 min of O_3 exposure (Control: 9.0 ± 1.3 and 11.7 ± 0.6 mg/kg), reduction being 63 and 77%.

The by-products of pirimiphos-methyl were identified through different regions of chromatogram by the LC-MS. It was possible to observe peaks at 278.1 and 306.1 *m/z*. The 306.1 *m/z* should correspond to the $[M+H]^+$ form of the remaining pirimiphos-methyl content, while the 278.1 *m/z* correspond to its by-products (Fig 1). The reaction of this pesticide with O_3 gas is mainly related to H_{20} abstraction leading to an N-dealkylated product, which MW=277.1 *m/z* and an acetaldehyde molecule (Yang et al., 2015).

According to results, it was possible to observed that the presence of higher humidity in the grains enhances the O_3 degradation effect, increasing its efficiency even under shorter exposure times (Ikeura et al., 2013). The humidity presence is important for O_3 reactivity with grain because the water solubilizes O_3 and increases the contact between gas and contaminated grains with pesticide residues.

Freitas et al. (2014) also reported that O_3 was effective in removing residues of bifenthrin and pirimiphos-methyl. The highest efficiency was for pirimiphos-methyl with degradation rate of 88%. Other also reported potential of O_3 in pesticide residue degradation in grapes, citrus fruits and vegetables (Gabler et al., 2010; Kusvuran et al., 2012; Ikeura et al., 2013).

The concentration and exposition time of the O_3 gas used in this study (60 µmol/mol, 120–180 min) did not show significant changes of physical and chemical characteristics of wheat grain, such as the carboxyl and carbonyl content, X-ray diffraction, lipid peroxidation, protein analysis, microstructural analysis and finally seed germination (Savi et al., 2014). In addition, Trombete et al. (2016) demonstrated that ozonation, when applied under the conditions of 10 to 60 mg/l, from 2 to 5 h of exposure, also did not cause any negative impact on the wheat quality, taking into account parameters of alveography, as well as

farinography, gluten content, chemical composition, mineral and sensory profiles.

CONCLUSION

The O_3 gas treatment can reduce the pesticides levels in the grains at efficient concentrations for degradation, with no grain-quality alteration. As O_3 gas is internationally generally recognized as safe (GRAS) and does not leave residues in food, could be a promising method of decontamination in industries and storage units during the wheat grain storage, in order to avoid contamination and ensure food security to the consumer.

ACKNOWLEDGMENT

We are grateful the Brazilian Agricultural Research Corporation (EMBRAPA Wheat) of Passo Fundo, RS, Brazil for providing the wheat grain samples and the Juriti Cooperative Industry, from Massaranduba city, SC, Brazil, for supporting part of this research. Finally, we also thank CAPES (Coordination for the Improvement of Higher Education Personnel) and CNPq (National Council of Technological and Scientific Development) for financial support.

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