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Effect of Ozonated Water in Reducing Insecticides Residues from Potato Tubers

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ABSTRACT

The effect of ozonated water treatments on the reduction of insecticides residues in potatoes was studied. Insecticides in potato tubers samples collecting from different markets in Cairo. Potato samples were subjected to extraction and clean up procedures in order to identify and detect the insecticides residues using gas chromatography and mass spectrum (GC/MS). Potato samples were treated with ozonated water either at10 ppm or 20 ppm for 30 minutes and the residues were determined after that. Results showed that five organophosphorus (chlropyrifos, fentrothion, profenofos, agrothion and indoxacarb), and two carbamate insecticides (carbosulfan and methomyl) were detected and their residues, in most cases exceeded than the maximum residue limits (MRLs). Ozonated water had profound effect in reducing insecticides residues and the potency of reduction was increased with increasing the ozonated water concentration. Finally, that method could be used in removing the pesticides residues before marketing.

Key words: Potatoes, Ozonated water, GC-MS, Insecticide residues.

Introduction

Potatoes are important and highly beneficial food for health and contain valuable ingredients, which can be successfully utilized to build up and repair the human body. Several factors limit their productivity, mainly insect pests, and diseases. As several insect pests attack the vegetables, they are produced under very high input pressure. The present agriculture has enabled mass and stable production by using of agricultural pesticides. Vegetables and fruits are susceptible to insect and disease attacks, so pesticides are widely used. Therefore, residues of pesticide could affect the ultimate consumers especially when freshly consumed. Many studies were carried out on pesticide residues in vegetables and fruits. It was reported that the main residues were HCB, lindane, heptachlor and its epoxide, DDT and its derivatives, as well as malathion, pirimiphos-methyl, profenofos and dimethoate (Abou-Arab,1995; Soliman,2001; Amra and Naguib, 1995; Abou-El-Ghar, 1961; El-Lakwah and Shams El-Din,1995). However, agricultural pesticides can have an adverse effect on the environment in addition to being harmful to humans, animals and fishes. The health hazard to the farmer as well as the residue in crops is also a global problem. Recently, the safety of crops including contamination with agricultural pesticides is a major concern to both the producer and consumer, and the development of a method to remove the pesticides before marketing has been eagerly awaited. They are an integral part of modern hazards, and an integral part of modern agriculture in most countries as a tool for controlling harmful pests.

To estimate the potential pesticide exposure from contaminated food, it is important to estimate the level of exposure at the point of consumption after processing. It has already been reported that commercial and household processing such as washing, peeling, cooking, blanching, ozonating and concentrating can reduce pesticide residue levels in food, and hence their adverse impact on human health (Abou-arab, 1999; Soliman, 2001; Zohair, 2001; Byrne and Pinkerton, 2004; Pugliese *et al.,* 2004; Zhang *et al.,* 2007). Ozone is a highly reactive form of oxygen where three molecules are bonded together. Interest in ozone applications for agriculture and food processing has increased in recent years (EPRI Expert Panel 1997). In 2001, ozone was declared a GRAS (generally recognized as safe) substance by the FDA after a Food Additive Petition containing safety and efficacy data was submitted to them. The threshold concentration of O_3 for continuous human exposure is 0.075µL/L (US Environmental Protection Agency 2008). There are several reports on the use of O_3 to remove residual pesticide in vegetables and fruits (Ong *et al*., 1995; Hwang *et al*., 2001a,b; Hwang *et al*., 2002; Daidai *et al*., 2007;Karaca and Velioglu, 2007; Wu *et al*., 2007; Gabler *et al*., 2010), they reported that O_3 was considered very suitable for removing residual pesticides from vegetables and fruits .

The objective of this study was to determine the levels of insecticides residues found in Egyptian potato tubers and the effect of ozonated water in removing these residues.

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Material and Method

Samples collection and preparation:

Study was carried out in Al-Azhar University, Faculty of Agriculture, Plant Protection Department, Nasr City, Cairo, Egypt and National Reaserch Center, Food Toxicology and Contaminants Department, Dokki, Giza, Egypt during 2014 year to determine the levels of pesticide residues in potato tubers collected from different local markets and locations in Cairo Governorate, Egypt. Each sample unit (2 kg) was chopped into small pieces, mixed, minimized by quartering to 500 g and then to 100 g subsamples which taken for pesticide analyses. The resulted subsamples, in addition to the remainder potato tubers pieces, were kept at 20 ^{0}C until utilized.

Analysis of pesticide residues

Extraction and clean-up

The Official Analytical Methods of Analysis (AOAC, 1995) was followed. Triplicate sub samples (100 g, each) were homogenized with 200 ml of solvent containing (acetonitrile: water; 35:65) for 2 min in a waring blender at high speed then filtered. The aqueous filtrate was shacked vigorously with 100 ml petroleum ether for 1–2 min in a separatory funnel. Ten milliliters of saturated NaCl solution and 600 ml distilled water were added to the same separatory funnel and shacked again for 30–45 second. The aqueous layer was discarded while the organic one was collected after washing with water. About 15 g of anhydrous Na_2SO4 were added to the organic solvent layer, shaked vigorously and transferred directly to a column chromatography containing 20 g activated Florisil and 2 g anhydrous Na_2SO4 on the upper Florisil layer. The column pre-washed with 40–50 ml petroleum ether followed by adding the petroleum ether extract. The column, eluted with 3-200 ml of 6%, 15% and 50% diethyl ether or petroleum ether solutions. The combined eluates were concentrated under vacuum to a definite volume.

Gas chromatographic determination.

The detection and quantification of pesticide residues in the samples were performed on a Hewlett-Packard GC model 5890A equipped with 63N electron capture detector (ECD) and a flame ionization detector (FID). An HP-101 (methyl silicon fluid) capillary column (30 m - 0.25 mm, 0.25-ml film thickness) was used. The chromatograms were integrated with an HP-3392A integrator. The applied temperature programming was as follows: initial temperature, 160 °C; hold for 3 min; increase for 220°C at 5 min. Injector and detector temperatures were 222 °C and 300°C, respectively. The flow rates of nitrogen, hydrogen and air were adjusted at 10 psi, 30-35 ml / min and 300-350 ml / min, respectively.

Treatment of ozone gas:

Ozonated water was prepared using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada. (OZO MAX LTD, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas. The amount of output of ozone was controlled by a monitor controller having a plug- in sensor on board which is changed for different ranges of ozone concentration and a belt pan in the monitor controller allows controlling the concentration in a selected range. The ozone generator was connected to a cylinder of pure oxygen that was calibrated to release oxygen at 1 mg / L min. The potato tubers were exposed to ozone generator to get liquid ozonated water at 10 and 20 ppm.

Results and Discussion:

Results in Tables (1) indicated that the collected potato samples were contaminated with five organophosphorus insecticides (chlorpyrifos, fenitrothion, profenofos, agrothion and indoxacarb), and two carbamate insecticides (carbosulfan and methomyl) residues with the levels in range from 0.0 - 3.0 ppm. This has had substantial impact on growers, shippers and buyers. Specifically, more and more food suppliers and retailers are contracting with commercial services for pesticide residue-testing on various commodities to offer consumers an added level of assurance about food safety (Gordenker, 1989).

Obtained results in Table (1) shows that the detected residues of the candidate insecticides in potato samples were exceeded their MRLs. Ozonated water become very important to reduce insecticides residues found in potato to make it more safe for consumers. In the period 2004-2011 the potato samples were collected to determine the pesticide residues and the analysts' found that most of the samples originated from the United Kingdom and France. In total, 669 potato samples. Almost 75% were produced in Denmark, 10% in the UK and France and 15% in 8 other countries (Cyprus, Egypt, Germany, Israel, Italy, Morocco, Spain, and the USA). The Danish produced potatoes had residues of 4 different pesticides in only 2% of the samples, none above the MRLs. One of the pesticides was pollutant from earlier uses. The foreign produced samples had residues of 7 different pesticides in 25% of the samples, none above the MRLs. One of the pesticides was pollutant from earlier use. Differences were seen between Danish and potatoes of foreign origin with respect to both the number of pesticides found and the frequency of samples with residues. Most of the frequencies were 2-54%, and 3% of the foreign produces samples contained more than one residue while none of the Danish produced samples had multiple residues (NFI, 2014).

Table 1: Maximum residue limits of detected insecticides in potato.

Insecticide	MRLs (mg / kg)
chlorpyrifos	0.01
fenitrothion	0.05
profenofos	0.05
agrothion	0.1
lndoxacarb	0.01
carbosulfan	0.01
methomyl	0.02

MRLs= maximum residue limits

The effect of ozononated water on the removal of chlorpyrifos residues were shown in Table (2). Obtained results revealed that the ozonated water succeeded in reducing chlorpyrifos residues with the level of residues ranged between 3.0 to1.0, 2.0 to 0.0 and 1.0 to 0.0 ppm at the low dose of ozone while, at the high dose, the same residues were not detected, and chlorpyrifos residues completely removed. In some samles, on the other hand, chlorpyrifos residues were not detected before treatment with ozonated water. Ozonated water removed chlorpyrifos residues in all samples, especially with high dose, due to ozone effect. The potential for gaseous ozone to eliminate or lower pesticide residues as well, especially coumaphos (organophosphate) and tau-fluvalinate (pyrethroid) were studied. As a very strong oxidizer, ozone has been used to eliminate pesticides and other organic contaminants from drinking water for many years (Jasim *et al*., 2006). It has also been evaluated for use to breakdown pesticide residues on fruits.other organic contaminants from drinking water for many years (Jasim *et al*., 2006). It has also been evaluated for use to breakdown pesticide residues on fruits and vegetables, using either washes of ozone dissolved in water or gaseous ozone (Ikeura *et al*., 2011). Kumari (2008) found that chlorpyriphos was detected in all the samples of vegetables at concentration of 0.018 to 0.031 $\mu g g^{-1}$. Ozonation process of organophosphorus (methyl-parathion) and the degradation rate increased with the increase of ozone dosage (Ou-Yang *et al*., 2004). Kumari *et al*. (2001) found that in case of winter vegetables 23% samples were found to contain OP insecticide residues above MRL values. The probable reason of higher residues in winter may have been due to low temperature and short day lengths.

Table 2: Reduction of chlorpyrifos residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water		Treatment of ozonated water	
		10 ppm	R %	20 ppm	R %
chlorpyrifos	3.00	1.00	66.66	0.00	100
	2.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	3.00	1.00	66.66	0.00	100
	N.D.	N.D.	-	N.D.	-
	2.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	2.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.0	0.00	100	0.00	100
	1.0	0.00	100	0.00	100

N.D. = Not Detected, R %= % reduction of the residue

Data listed in Table (3) illustrated the effect of ozonated water on the degradation of fenitrothion residues in potato samples. Fenitrothion residues generally were found with the levels ranged from 1.0 to2.0 ppm in many samples, but not detected in the others. Results clearly indicated that fenitrothion residues were reduced from 2.0 to1.0 ppm at the low dose of ozone, while these residues were completely removed at the high tested dose. Farrow *et al*., 1969; Sarode and Lal, 1982; Elkins, 1989, reported that 20-89 % reduction of fenitrothion and malathion in okra was obtained by washing and ozonation. (Lal *et al*., 1989). Out of 80 samples, 92% were found contaminated with organochlorine (OC), 82% with Organophosphorus (OP) and 32% with carbamates insecticides. Residue levels of OP were highest followed by carbamates and OC, this is due to wide use of organophosphorus insecticides.

Obtained results in Table (4) show the effect of ozonated water at 10 and 20 ppm for 30 minutes on profenofos residues in potatoes. The profenofos residues were not exceeded 1.0 ppm in all samples. Results cleared that ozonated water eliminated and removed the profenofos detected residues from a level of 1.0 to 0.0 ppm at both low and high doses. Ozone succefully degraded profenofos residues from collected samples and it is necessary to use it to decrease the intake of profenofos residues in this important crop. Agrothion residues were detected in collected potato samples at a level of 0.0 to 2.0 ppm and the treatment with ozone resulted in non-detectedable residue (Table 5). The detected agrothion residue was in the neighborhood of that obtained by Soliman (2001) who reported that organophosphorus malathion (agrothion) was detected in potato tubers at 0.896 mg/ kg.

Table 3: Reduction of fenitrothion residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
fenitrothion	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	2.00	0.00	100	0.00	100
	2.00	1.00	50	0.00	100
	N.D.	N.D.	-	N.D	-
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	2.00	1.00	50	0.00	100
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-

N.D. = Not Detected, R %= % reduction of the residue

Table 4: Reduction of profenofos residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
profenofos	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.0	0.0	100	0.0	100

N.D. = Not Detected ,R %= % reduction of the residue

Table 5: Reduction of agrothion residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
agrothion	N.D.	N.D.	-	N.D.	-
	2.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	2.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.0	0.00	100	0.00	100
	2.00	0.00	100	0.00	100

N.D. = Not Detected, R %= % reduction of the residue

Obtained data in Table (6) illustrate the carbosulfan residues in collected potatoes, which ranged from 1.0 to 2.0 ppm, but not observed in some samples. Ozonated water at both doses had potent effect in eliminating carbosulfan residues in potato samples. Methomyl residues were detected in potato samples at different levels and the range was from 0.0 to1.0 ppm in many samples, but not found in the other. Ozone water at 10 and 20 mg / kg for 30 minutes succeeded in the removing these insecticide residues from potato samples to be free from methomyl residues especially with the high dose. Methomyl , oxamyl and carbosulfan reduction percentages were increased with increasing of time of exposure to ozone. More than90% reduction percentages were achieved after 5, 10 and 15 min for methomyl and carbosulfan, respectively. The ability of ozonation in reduction of these pesticides amount is related to the ability of ozone to generate hydroxyl radicals in aqueous solution, which are highly effective to decompose methomyl and carbosulfan, so as the time of exposure increased hydroxyl radicals continued to be generated throughout the treatment, and more residues degraded (Al-Dabbas *et al*., 2014).

Obtained results in Table (8) demonestrated the effect of ozonated water at the two tested doses for 30 minutes on the removal of indoxacarb residues from potato samples. Results indicated that ozonated water eliminated and removed the indoxacarb residues from the detected levels of 2.0 ppm to 1.0 and 0.0 at the low and high doses, respectively, in potato samples. Similar results were found by Zohair (2001) who reported that organophosphorus compounds pirimphos methyl, malathion and profenofos were detected in potato tubers at higher levels than the organochlorine compounds lindane, aldrine, heptachlor epoxide and DDT derivatives in the tested samples, he found a positive correlation between the elimination of pesticides residue and the concentration of the gas used. Ozonated water had profound effect in removing pesticide residues from potatoes. There was a gradual increase in the percentage of pesticide residue reductions due to the increase concentrations of ozonated water.

Table 6: Reduction of carbosulfan residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
carbosulfan	2.00	0.00	100	0.00	100
	2.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00.	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	3.00	1.00	66.66	0.00	100
	N.D	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.0	0.00	100	0.00	100
	1.00	0.00	100	0.00	100

N.D. = Not Detected, R %= % reduction of the residue

Table 7: Reduction of methomyl residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
methomyl	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100

N.D. = Not Detected, R %= % reduction of the residue

Table 8: Reduction of indoxacarb residues in potatoes by ozone.

Insecticide	Detected residues before treatment ppm	Treatment of ozonated water 10 ppm	R %	Treatment of ozonated water 20 ppm	R %
indoxacarb	2.00	1.00	50	0.00	100
	2.00	0.00	100	0.00	100
	N.D.	N.D.	-	N.D.	-
	N.D.	N.D.	-	N.D.	-
	1.00	0.00	100	0.00	100
	2.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.00	0.00	100	0.00	100
	1.0	0.00	100	0.00	100
	1.00	0.00	100	0.00	100

N.D. = Not Detected,R %= % reduction of the residue

Ku *et al*. (1998) found that ozonated water was effectively removed diazinon residues from vegetable (99% in 30 min).EPA (2001) documented that it can be argued that toxic metabolites will also be formed under natural condition in the environment in exposure to oxygen. Ozone and other oxidants can speed up the mineralization process for complete degradation of the toxic intermediates and ultimately lead to the formation of CO_2. Furthermore, ozone could effectively degrade Malathion (an organophosphorus insecticide) without forming toxic products evaluated with the bioassay of cytotoxicity and gap junctional intercellular communication. Balawejder *et al*. (2013) reported that reduction of chemical contaminants in food products with ozone is based on two different procedures, i.e. washing with aqueous ozone solutions and treatment with ozone in gaseous phase. Both of them have advantages and disadvantages. Commonly fruits and vegetables are sprayed with aqueous solution of ozone or undergo passage through solution on an assembly line. The present study clearly indicated that treatment of potatoes with ozone gas removed pesticide residues and these reductions are extremely important in evaluating the risk associated with ingestion of pesticide residues, especially in vegetables, which are eaten by almost all income group people.

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