

EFFECT OF ACIDIC NATURAL ANTIOXIDANT ON REMOVAL PESTICIDES FROM SOME CONTAMINATED CEREALS

Salim A.* and Azza Zohair**

* Food Technology and Dairy Dairy Department, National Research Center, Dokki, Cairo, Egypt.

**Faculty of Specific Education, Home Economic Department, Minufiya University. Ashmoun, Minofiya, Egypt.

ABSTRACT

The efficiencies of natural antioxidant solutions [Thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*,L.) and rosemary (*Rosmarinus officinalis*)] as well as tap water in elimination of organochlorine and organophosphorus pesticides from naturally contaminated cereal (wheat and corn) were examined. The results revealed the efficiency role of natural antioxidant solutions in elimination of pesticides under investigation. Washing by only tap water was less effective than the other solutions. The results also indicate that rosemary is the most effective in the elimination of pesticides followed by ginger and thyme solutions. The results also show that milling of corn and wheat to flour after soaking in ginger, rosemary and thyme solutions and drying eliminated pesticides residues completely.

Keywords: Pesticides, Cereals, Natural antioxidants, Wheat, Corn.

INTRODUCTION

Pesticide, despite of undoubtful economical benefits due to their use in agriculture can exert adverse effects on the nutritional quality of the crops. Some of pesticides can influence the biochemical processes in plants inhibiting the synthesis of protein, sugars and some vitamins (Berger and Cwiek, 1989). However, not only nutritive values of plants or animal food products are affected by pesticide use, but also the health risk of pesticides to humans is worsened by the fact that many of these substances have been shown to be carcinogenic and mutagenic (Murphy, 1986, Renwick *et al.*, 2003).

Organophosphorus pesticides have a higher acute toxicity than organochlorines, but they have the advantage of being rapidly degraded in the Environment. Organochlorine compounds have been banned but their residues still appear as polutants in food as well as the environment (Rea,1996 ;and Zohair2001).

Pesticide residues were detected in 75 (26%) of the 294 samples of cereal and cereal products analyzed in the EU surveys. Samples were analyzed for up to 40 pesticide compounds. Maximum Recommend Levels (MRLs) were exceeded in 3 (4%) of the cereal grain samples analyzed. In the 1998, cereals program showed no MRLs were exceeded but 150 (61%) of the 244 samples of cereal and cereal products tested contained pesticide residues (Annual Report,1998).

In Egypt, cereals are the main contribiours to dietary energy supply (DES), as they supply from 61.6% to 79.5% of the (DES). Cereals are mainly wheat, which are the main staple, rice and corn. Therefore this manuscript

aimed to study the effect of some acidic natural antioxidants on removing pesticides contamination from corn and wheat grains.

MATERIALS AND METHODS

Samples:

Twenty kilograms of both white corn and wheat grains were collected from the local market. A representative sample of about 2 kg was examined for pesticides, and washing treatment was carried out on corn and wheat samples previously naturally contaminated with pesticides.

Standards

Pesticide standards of dieldrin; lindane; aldrin, hexachlorobenzene (HCB), 2,2-dichloroethylene (*O,P*-DDE); 1,1-dichloro-2,2-bis (*P*-chlorophenyl) ethylene (*P,P*-DDE); 1-(*O*-chlorophenyl)-1-(*P*-chlorophenyle)-2,2-dichloroethane (*O,P*-DDD); 1,1-dichloro-2,2-bis (*P*-chlorophenyl)ethane (*P,P*-DDD); 1-(*O*-chlorophenyl)-1-(*P*-chlorophenyle) - 2,2,2-trichloroethane (*O,P*-DDT); 1,1,1-tri-chloro-2,2-bis chlorophenyl ethane (*P,P*-DDT), pirimiphos-methyl, malathion and, chlorpyrifos-methyl were purchased from Chem Service Inc. (WestChester, PA, USA).

Chemicals and reagents:

All used chemicals were obtained from E.Merck company (Germany). Ginger (*Zingiber officinale*), Thyme (*Thymus vulgaris*) and Rosemary (*Rosmarinus officinalis*) as natural antioxidant were obtained from Medicine plant and agricultural seed, Haraz Company. The plant solutions were prepared by immersing in hot water (50°C) at concentrations 5 and 10%.

2.4 Determination of pesticides

Pesticide residues were extracted from corn and wheat grains according to the methods of AOAC (1995) and Pesticide Analytical Manual (1991). Aliquots of 1-2 µl of extract were injected into a Hewlett-packard gas chromatography Model 5890 equipped with an Ni⁶³ electron capture detector (ECD), flame ionization detector (FID), and integrator 3392, fitted with HP-101 capillary column (cross-linked methyl silicon gum), 30 m X 0.25 mm X 0.25 µm film thickness. The oven temperature was programmed from 160°C to 220°C at the rate of 5°C/min, held for 20-min injection and detector temperatures were 220 and 300°C, respectively.

Treatment of Contaminated corn and wheat:

The contaminated corn and wheat samples were soaked for 10 min in (i) tap water; (ii) natural antioxidant ginger, thyme and rosemary solutions at concentrations of 5 and 10% (iii) corn and wheat grains were dried after soaking in different tested solutions. White corn grains were milled to 600 micron by using an Attenzione Mill, Type HZ50, and 220 Volts Italy. Wheat grains were milled using Brabender, OHG, Duisburg, Germany for obtaining the whole flour.

RESULTS AND DISCUSSION

Residue levels of pesticide in corn and wheat grains are shown in Table(1). Data indicate that tested corn and wheat samples contained

different types of pesticide. Organophosphorus compounds were present at higher levels than the organochlorine compounds indicating that organophosphorus compounds are widely used.

Table 1. Level of pesticide residues in corn and wheat grains.

Compound	^a Levels of pesticide (Mg/Kg ±SD)	
	Corn	Wheat
Organophosphorus		
Malathion	2.700±0.700	3.000±0.500
Pirimiphos-methyl	8.200±2.000	4.100±1.200
Chloropyrifos-methyl	5.300±1.800	6.000±2.000
Organochlorines		
Aldrin	0.130±0.030	0.100±0.040
Dieldrin	0.050±0.010	0.060±0.020
Lindane	0.230±0.100	0.220±0.100
HCB	0.070±0.020	0.090±0.020
O,P'-DDE	--	--
P,P'-DDE	0.102±0.030	0.113±0.027
O,P'-DDD	--	--
P,P'-DDD	0.097±0.022	0.075±0.020
O,P'-DDT	--	--
P,P'-DDT	0.068±0.020	0.081±0.030

^a Values given are mean of three replicates

^b Non detectable

It is not surprising that a pirimiphos-methyl residue is detected in corn and wheat since it is most widely used post harvest insecticide for cereal grains. In 1998 the Working Party extended its range of pesticides sought in cereal grains to include a wide range of herbicides and desiccants as well as the usual post-harvest pesticides. This explains the increased frequency of residues found in the grain survey with 97% of samples analysed containing detectable residues. The contamination by DDT indicated that this insecticide is probably still being applied despite the Ministry of Agriculture recommendation.

The effect of soaking in natural antioxidant solutions (5 and 10%) on pesticides residues in corn and wheat were shown in tables (2-5). The results indicate the efficient role of washing by acidic natural antioxidant solutions rosemary, thyme and ginger in elimination or reduction of organochlorine (aldrin, dieldrin, lindane, HCB and DDT) and organophosphorus (pirimiphos-methyl, malathion and chloropyrifos-methyl) pesticides from naturally contaminated corn and wheat. It was noticed that rosemary solution (10%) is the most effective solution in the elimination of pesticide residues.

It removed 100% of pesticides under investigation from contaminated corn and wheat grains on the other hand, ginger solution (10%) caused reduction of pesticides under study ranged from 80-100%. As well as thyme solution (10%) caused reduction ranged from 76-100%. Regarding to 5% solutions, data proved that reduction of pesticides ranged from (90.0 to 99.97%), (76.0 to 98.52%) and (72.0 to 94.47%) with rosemary, ginger and thyme respectively. Similar results were obtained by (Zohair, 2001) who reported that acidic detergent solutions are more effective in the elimination of the organochlorine pesticides.

Table (2): Removal of organochlorine residues in corn grains by soaking in acidic natural antioxidant solutions for 10 min.

Treatment	Aldrin		Dieldrin		Lindane		HCB		P,P'-DDE		P,P'-DDD		P,P'-DDT	
	Mean	Red. %	Mean	Red. %	Mean	Red. %	Mean	Red. %	Mean	Red. %	Mean	Red. %	Mean	Red. %
Contaminated corn sample	0.13±0.03		0.05±0.01		0.23±0.1		0.07±0.02		0.102±0.03		0.097±0.022		0.068±0.02	
Ginger solution 5%	0.015±0.008	88	0.012±0.032	76	0.034±0.033	85	0.007±0.006	89	0.007±0.004	93	0.005±0.002	94.8	0.005±0.002	92.6
Ginger solution 10%	0.010±0.005	92	0.01±0.01	80	0.029±0.009	87	0.007±0.005	90	0.005±0.003	95	0.004±0.001	95.87	0.004±0.002	94.6
Thyme solution 5%	0.018±0.006	86	0.014±0.012	72	0.043±0.01	81	0.015±0.007	83	0.010±0.013	90	0.008±0.002	91.7	0.006±0.004	91
Thyme solution 10%	0.013±0.004	90	0.012±0.01	76	0.034±0.013	85	0.009±0.005	86	0.008±0.004	92	0.007±0.006	92.8	0.005±0.001	92.6
Rosemary solution 5%	0.004±0.001	97	0.005±0.002	90	0.018±0.007	92	0.004±0.001	94	0.004±0.002	96	0.005±0.001	94.8	0.004±0.002	94
Rosemary solution 10%	b ---	100	---	100	----	100	----	100	---	100	---	100	----	100
Tap water	0.112±0.04	13.8	0.046±0.02	8	0.2±0.15	13	0.063±0.02	10	0.098±0.04	2.9	0.09±0.03	3.5	0.065±0.04	4.4

^aMean = mg/Kg ±S.D. values given are mean of three replicates

^b Non detectable

Table (3): Removal of organophosphorus residues in corn grains by soaking in acidic natural antioxidant solutions for 10 min.

Treatment	Malathion		Pirimphos-methyl		Chlorpyrifos-methyl	
	Mean	Reduction%	Mean	Reduction%	Mean	Reduction%
Contaminated corn sample	2.7±0.7		8.2±2		5.3±1.8	
Ginger solution 5%	0.085±0.03	96.85	0.18±0.092	97.8	0.078±0.008	98.52
Ginger solution 10%	b ----	100	----	100	----	100
Thyme solution 5%	0.082±0.02	96.96	0.15±0.05	98.17	0.081±0.007	98.47
Thyme solution 10%	---	100	---	100	---	100
Rosemary solution 5%	0.073±0.02	99.97	0.13±0.03	98.91	0.067±0.006	98.73
Rosemary solution 10%	----	100	----	100	----	100
Tap water	2.3±06	14	7.4±0.17	9.6	4.6±1.8	12.8

^aMean = mg/Kg ±S.D. values given are mean of three replicates

^b Non detectable

Table (4): Removal of organochlorine residues in wheat grains by soaking in acidic natural antioxidant solutions for 10 min.

Treatment	Aldrin		Dieldrin		Lindane		HCB		P,P'-DDE		P,P'-DDD		P,P'-DDT	
	Mean	Reduction %	Mean	Reduction %	Mean	Reduction %	Mean	Reduction %	Mean	Reduction %	Mean	Reduction %	Mean	Reduction %
Contaminated corn sample	0.1±0.04		0.06±0.02		0.22±0.1		0.09±0.02		0.113±0.027		0.075±0.02		0.081±0.03	
Ginger solution 5%	0.011±0.002	89	0.008±0.006	86.6	0.006±0.002	97	0.007±0.003	92	0.01±0.005	91	0.006±0.003	92	0.004±0.002	95
Ginger solution 10%	0.007±0.004	93	0.007±0.005	88	^b -----	100	0.004±0.002	95.5	0.004±0.002	96	0.004±0.003	94.6	-----	100
Thyme solution 5%	0.012±0.003	88	0.009±0.006	85	0.015±0.008	93	0.005±0.003	94	0.009±0.003	92	0.007±0.002	90.6	0.007±0.003	91
Thyme solution 10%	0.010±0.009	90	0.007±0.007	88	0.004±0.003	98	0.004±0.002	95.5	0.006±0.003	94.7	0.005±0.002	93	0.005±0.002	93.8
Rosemary solution 5%	0.005±0.002	95	0.004±0.002	93	0.005±0.001	99	0.006±0.002	93	0.004±0.002	96	0.004±0.001	94.7	0.004±0.003	95
Rosemary solution 10%	-----	100	-----	100	-----	100	-----	100	-----	100	-----	100	-----	100
Tap water	0.086±0.06	14	0.055±0.02	7.8	0.19±0.12	13.6	0.081±0.01	10	0.109±0.03	3.5	0.072±0.013	4	0.077±0.03	4.9

^aMean = mg/Kg ±S.D. values given are mean of three replicates

^b Non detectable

Table (5): Removal of organophosphorus residues in wheat grains by soaking in acidic natural antioxidant solutions for 10 min.

Treatment	Malathion		Pirimphos-methyl		Chlorpyrifos-methyl	
	*Mean	Reduction	Mean	Reduction	Mean	Reduction
Contaminated corn sample	3± 0.5	%	4.1±1.2	%	6±2	%
Ginger solution 5%	0.096±0.05	96.8	0.082±0.035	98	0.09±0.04	98.5
Ginger solution 10%	b ---	100	----	100	----	100
Thyme solution 5%	0.15±0.012	95	0.10±0.003	97.5	0.18±0.04	97
Thyme solution 10%	----	100	b	100	N.D	100
Rosemary solution 5%	0.069±0.021	97.7	0.049±0.01	98.8	0.12±0.05	98
Rosemary solution 10%	----	100	----	100	----	100
Tap water	2.586±0.4	13.8	3.69±0.9	10	5.25±2	12.5

*Mean = mg/Kg ±S.D. values given are mean of three replicates

b Non detectable

The results clearly indicate that organophosphorus pesticides are more rapidly degraded than organochlorines. Ginger, rosemary and thyme (10%) solutions eliminated pirimphos-methyl, malathion and chlorpyrifos-methyl residues completely.

The data from tables demonstrate that there was a gradual increase in the percentage of reduction due to the increase concentration of different washing solutions, being more efficient than tap water. These results agreed with those obtained by Ismail *et al.*, (1993); Abou Arab *et al.*, (1998) and Zohair (2001).

Spices ginger, rosemary and thyme having an inhibitory effect against mold growth (Kunz,1994 and Hassan *et al.*, 2001). Marja *et al.*, 1999, reported that there were some herbs and medicinal plants with considerably strong antioxidant response (over 90% inhibition). Thyme showed the highest activity. Thymol and carvacrol are major aroma components of essential oil of thyme and both show high antioxidant and antimicrobial activity (Nakatani.,1997; Hirasa and Takemasa,1998).

Gingerol, Zingeron, Camphene and linalol are major component of essential oil of ginger. Ahmed *et al.*, (2000), proved that ginger has a highly protective effect against malathion induced oxidative damage, Rosemary also has antioxidant properties. It shows antimutagenic activity in bacteria (Minnunni *et al.*,1992) and anticarcinogenic properties in various animal systems (Ho *et al.*,1994 and Offord *et al.*,1995). The most active constituents are carnosol and carnosonic acid which account for 90% of antioxidant activity (Aruoma *et al.*,1992). Also rosemary showed antigenotoxicity against the hazards induced by the unconventional pesticides abametin and thuringiensin (Fahmi and Salama,1998).

The results in Tables 6&7 show the effect of milling of corn and wheat grains to flour after soaking in 5% ginger, rosemary and thyme solutions and drying on the elimination of pesticides. It was noticed that this process eliminated pesticide residues. Washing and cleaning, which are the initial steps in most processing procedures, frequently result in a reduction of residues, particularly those of nonsystemic pesticides. Many types of processing are accompanied by a significant lowering of residue levels for example, the milling of cereals to flour and the polishing of rice.(Annual Report 1999)

Table 6. Effect of milling of corn to flour on pesticide residues.

Compound	Ginger solution 5%			Thyme solution 5%			Rosemary solution 5%		
	Grains	Flour	Reduction	Grains	Flour	Reduction	Grains	Flour	Reduction
	^a Mean	^a Mean		Mean	Mean		Mean	Mean	
Organochlorines									
Aldrin	0.015±0.008	^b -----	100	0.018±0.006	----	100	0.004±0.001	-----	100
Dieldrin	0.012±0.032	-----	100	0.014±0.012	----	100	0.005±0.002	-----	100
Lindane	0.034±0.033	-----	100	0.043±0.01	-----	100	0.018±0.001	-----	100
HCB	0.007±0.006	-----	100	0.015±0.007	-----	100	0.004±0.004	-----	100
P,P'-DDE	0.007±0.004	-----	100	0.01±0.013	-----	100	0.004±0.003	-----	100
P,P'-DDD	0.005±0.002	-----	100	0.008±0.002	-----	100	0.005±0.001	-----	100
P,P'-DDT	0.005±0.002	-----	100	0.006±0.002	-----	100	0.004±0.002	-----	100
Organophosphorus									
Malathion	0.085±0.03	-----	100	0.073±0.02	-----	100	0.082±0.02	-----	100
Pirimphos-methyl	0.18±0.092	-----	100	0.13±0.03	-----	100	0.15±0.05	-----	100
Chlorpyrifos-methyl	0.078±0.008	-----	100	0.067±0.006	-----	100	0.081±0.007	-----	100

^aMean = mg/Kg ±S.D. values given are mean of three replicates

^b Non - detectable

Table 7. Effect of milling of wheat to flour on pesticide residues.

Compound	Ginger solution 5%			Thyme solution 5%			Rosemary solution 5%		
	Grains	Flour	Reduction	Grains	Flour	Reduction	Grains	Flour	Reduction
	^a Mean	^a Mean		Mean	Mean		Mean	Mean	
Organochlorines									
Aldrin	0.011±0.002	^b -----	100	0.012±0.003	-----	100	0.005±0.002	-----	100
Dieldrin	0.008±0.006	-----	100	0.009±0.006	-----	100	0.004±0.002	-----	100
Lindane	0.006±0.002	-----	100	0.015±0.008	-----	100	0.005±0.001	-----	100
HCB	0.007±0.003	-----	100	0.005±0.003	-----	100	0.0035±0.002	-----	100
P,P'-DDE	0.01±0.005	-----	100	0.009±0.003	-----	100	0.004±0.002	-----	100
P,P'-DDD	0.006±0.003	-----	100	0.007±0.002	-----	100	0.004±0.001	-----	100
P,P'-DDT	0.004±0.002	-----	100	0.007±0.003	-----	100	0.004±0.002	-----	100
Organophosphorus									
Malathion	0.096±0.05	-----	100	0.15±0.012	-----	100	0.069±0.021	-----	100
Pirimphos-methyl	0.082±0.035	-----	100	0.10±0.003	-----	100	0.049±0.01	-----	100
Chlorpyrifos-methyl	0.09±0.04	-----	100	0.18±0.04	-----	100	0.12±0.05	-----	100

^aMean = mg/Kg ±S.D. values given are mean of three replicates

^b Non detectable

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تأثير مضادات الأوكسدة الحامضية علي إزالة التلوث بالمبيدات من بعض أنواع الحبوب
أبوبكر سالم* و عزة زهير**
* المركز القومي للبحوث - الدقي - مصر
** كلية التربية النوعية - جامعة المنوفية

تهدف هذه الدراسة إلي دراسة تأثير بعض مضادات الأوكسدة الحامضية علي إزالة التلوث الطبيعي بالمبيدات الكلورونية والفسفورية من حبوب القمح والذرة. حيث تم نقع الحبوب الملوثة في محاليل ذات تركيزات مختلفة من الزعتر والزنجبيل وحصلان لمدة ١٠ دقائق ثم تم تجفيف الحبوب وطحنها. ولقد استخدم جهاز كروماتوجراف الغاز (GC) لتقدير مستوي المبيدات. ولقد أسفرت الدراسة عن فعالية المحاليل الثلاثة السابق الإشارة إليها (بتركيز ١٠%) في إزالة التلوث بالمبيدات الفسفورية حيث كانت نسبة الإزالة ١٠٠%. ولقد وجد أن حاصلان بتركيز ١٠% كان أكثر المحاليل فعالية لإزالة التلوث بالمبيدات الكلورونية وتلاه الزنجبيل ثم الزعتر. ولقد أدت عملية التجفيف والطحن إلي دقيق بعد النقع في محاليل الزعتر والزنجبيل وحصلان بتركيز ٥% إلي الإزالة الكلية للمبيدات الكلورونية.