

Quantitative Determination of Organophosphorus Compounds in Hard Cheese Using Gas Chromatography Coupled With Ion Trap-Tandam Spectrometry

Al-Julaifi M.Z.¹, Bazalou M.S.², Ibrahim .A.A.M³ and

Sanya T. El Ghamry⁴

1-Professor of Science, Molecular Biology and Biotechnology and The General Director of Veterinary Laboratories Department at Ministry of Agriculture, Riyadh, Kingdom of Saudi Arabia.

2- Senior Researcher of Biochemistry at Damietta Sea' Port Lab, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt.

3- Toxicology specialist - Veterinary Laboratories Department at Ministry of Agriculture, Riyadh, Kingdom of Saudi Arabia.

4- Researcher of Food Hygiene at Mansura Vet. Lab Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt.

Abstract

Organophosphorus pesticides have been used extensively especially in tropical countries to control of agricultural pests. However, much of the food we eat contain pesticide residues. Analyses of organophosphorus compounds (OP) residues are carried out worldwide because of their potential health hazardous effect on humans. In this study, hard cheese was monitored for (OP) using ion trap GCMS/MS. Fourteen Organophosphorus pesticide residues were examined (methamidophos, trichlorofon, demeton-s-methyl, diazinon, paraxon methyl, phosphamidon, chlorpyrifos methyl malaxon, fenitrothion, malathion, methidathion, bromophos-ethyl, ethion and azinophosmethyl) to determine the degree of contamination with these danger contaminants. The study included 50 samples of ripened hard cheese which were collected from different factories production batches. From the total ion chromatogram it was found that methamidophos was the first eluted compound (at 5.931 minutes) while azinophosmethyl was the last one (29.563). The linearity (r^2) of each compound standard curve was not less than 0.999 except for trichlorofon, demeton-s-methyl that were (0.868 and 0.818) respectively.

It was found that 4 (8%) samples were contaminated with diazinon while 2 (4%) samples only were contaminated with malathion with a total percentage of 12%.The concentrations of diazinon in the four samples were 0.030, 0.046, 0.052 and 0.021 mg/kg on whole product basis where these concentrations were 0.055, 0.90, 0.094 and 0.043 mg/kg on whole fat basis respectively. Meanwhile, the concentrations of malathion in the two samples were 0.025 and 0.073 mg/kg on whole product basis while they were 0.045 and 0.130 mg/kg on whole fat basis respectively.

Introduction

Milk production is strongly linked to the environment. The latter depends largely on human activities. Uncontrolled processes interfering with the food delivering ecological system or with its natural compounds lead to residues in milk, which are not very easy to be controlled. Most of the foods produced for human consumption grow using pesticides. Chemical control of weeds, insects, fungi and rodents has enabled agricultural productivity and intensity to increase. However, these economic benefits are not without their risks to human and environmental health.

The occurrence of chemical residues in the milk of lactating cows is a matter of public health concern, since dairy products are widely consumed by infants, children and many adults throughout the world. In view of this, many countries have enacted regulations that limit the level of chemical residues in milk and dairy products. Milk chemical safety is important for public health. Governments need to assessment of chemical risks in people who consume contaminated milk to achieve risk communication and risk management. Total quality management and the Hazard Analysis Critical Control Points (HACCP) approach has an important role for preventing and controlling of chemical contaminants in milk and dairy products. **(Khaniki, 2007)** .

Organophosphates are highly lipid-soluble agents and are well absorbed from the skin, oral mucous membranes, conjunctiva and gastrointestinal and respiratory apparatus. The onset, severity and duration of poisoning is depended to the dose, route of exposure, physicochemical properties of the OPs (e.g. lipid solubility), rate of metabolism (whether transformation in the liver is required before the compound becomes toxic) and whether the organophosphorylated cholinesterase ages rapidly(**Karalliedde et al.,2003**). Organophosphorus compounds are potent neurotoxin compounds that are extensively used in agriculture (agronomy and animal science), industry and medicine (**Abou-Donia, 2003**).

The mechanism of action of organophosphates (OP)/nerve agents is based on acetylcholinesterase (AChE) inhibition and subsequent accumulation of neuromediator acetylcholine at the cholinergic synapses, either peripheral or central, causing cholinergic hyper stimulation and development of symptoms of poisoning, followed by metabolic disbalance and, without effective prophylaxis/treatment leading to death(**Worek et al.,2005**) .

Generally, in the last few decades there is exponential increase in use of organophosphorus (OP) compounds as pesticides and insecticides leading to adverse

effect on human population and live stock (**Schipper et al. , 2008 and Gahlaut et.al.,2012**).

MATERIALS AND METHODS

(I)-MATERIALS

(I)-1-CHEMICALS

(I)1-a-REAGENT:

- (a) Anhydrous magnesium sulfate (MgSO₄), powder form; purity > 99.9%.
- (b) Acetonitrile (MeCN), quality of sufficient purity that is free of interfering compounds.
- (c) Acetic acid (HOAc), Glacial; quality of sufficient purity that is free of interfering compounds.
- (d) 1% HOAc in MeCN, prepared on a v/v basis (10 ml glacial HOAc in a 1 liter MeCN solution).
- (e) Primary secondary amine (PSA) sorbent, 40 mm particle size (Varian Part No. 12213024).
- (f) C18 sorbent, 40 µm particle size.

(I)-1-b-Organophosphorus compounds standards:

- (a) Pesticide standards: High purity reference pesticide standards mixture containing 14 pesticides namely (Pesticide Mix 174) was purchased from Dr. Ehrenstrofer Company for (reference materials for residue analysis-lot number 10919EA). The pesticides included in the mixture were methamidophos, trichlorofon, demeton-s-methyl, diazinon, paraxon methyl, phosphamidon, chlorpyrifos methyl malaxon, fenitrothion, malathion, methidathion, bromophos-ethyl, ethion and azinophosmethyl
- (b) A stock standard solution was prepared at highly concentration (2000 ng/mL) in ethyl acetate and stored in dark vials in the Freezer.

(I)-1-c- Triphenyl phosphate (TPP): A 15µg/ml of TPP solution in 1:1(acetonitrile : water) containing 0.1 formic acid was prepared as internal standard spiking solution (IS) where 100 µl was added to each sample at the time of analysis (**Zhao et al.,2012**)

(1)-2-SAMPLES

Fifty samples of ripened Romano hard cheese were collected from different production batches. At every time of collection each sample was collected aseptically in a

polyethylene bag and transported to the laboratory in ice box and kept frozen at -20 until the time of analysis that didn't exceed three days.

(II)-METHODS

(II) -1- SAMPLE PREPARATIO:

(a) Fifty grams of each sample were cut into small cups and the homogenized in a high speed laboratory homogenizer for 25 second.

(b) Fifty grams of the homogenized sample were placed in a FALCON centrifuge tube and subjected to the extraction process.

(II) -2- : EXTRACTION AND CLEAN UP

The QuEChERS method for pesticide residue analysis according to **Lehotay et.al.,(2005)** as recommended by **AOAC Official Method2007.01**, was conducted.The principle of the method has the following features

- (a)- The QuEChERS method uses a single-step buffered acetonitrile (MeCN) extraction salting out liquid–liquid partitioning from the water in the sample with MgSO₄.
- (b)- Dispersive-solid-phase extraction (dispersive-SPE) cleanup is the second step to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and MgSO₄.
- (c)- Then the extracts are analyzed by mass spectrometry (MS) techniques after a gas chromatographic analytical separation.

In brif :

- (a) Fifteen gm of homogenized sample with 15 ml acetonitrile (MeCN) containing 1% acetic acid were placed in a 50 ml wellcapped falcon centrifuge tube and shaken vigorously.
- (b) Six gm of anhydrous magnesium sulphate and 1.5 gm sodium acetate were added to the tube and shaken vigorously and centrifuged at 4500rpm.
- (c) one ml of the buffered MeCN extract underwent a cleanup step (in a technique known as dispersive solid –phase extraction) in another 2ml centrifuge tube containing 50mg each ofC18 and primary secondary amine sorbents plus 150mg MgSO₄ and centrifuged at 4500rpm.
- (d) Two hankered μ l of the clear extract was subjected to gas chromatography analysis and could be diluted if necessary. QuEChERS procedure flow chart was as flow.

QuEChERS Procedure For Extraction And Clean Up

- Fifteen g of homogenized sample were accurately weighed (± 0.05 g) in 50 mL centrifuge tube.
- samples were Spiked with 100 μ L IS solution and vortex for 1 min.
- Fifteen mL of 1% acetic acid in MeCN were added, then the centrifuge tube was shaken vigorously for 1 min.
- Bond Elut QuEChERS AOAC salt packet (6 g of anhydrous magnesium sulfate and 1.5 g sodium acetate) was added to the tubes that were capped and shaken vigorously for 1 min.
- The tube was centrifuged at 4500 rpm for 5 min.
- one mL of upper ACN layer was Transferred to Bond Elut AOAC (50 mg each of C18 and primary secondary amine sorbents plus 150 mg MgSO₄) dispersive SPE 2 mL tube.
- The tube was vortexed for 1 min then centrifuged at 4500 rpm for 5 min.
- Two hundred μ L extract were Transferred to autosampler vial, could be diluted with 800 μ L appropriate solution if necessary.
- Samples at this point were ready for GC/MS/MS injection at 2 μ L injection volume at splitless mode. (Shimadzu scientific instruments 2013) .

INSTRUMENTATION

The analysis of the selected pesticides was carried out by an integrated system of gas chromatography, equipped with automatic injection system and coupled to a mass spectrometric system with ion trap analyzer. Varian CP-3800 GC, Saturn-2200 mass spectrometer with auto injector CP-8410 was used for analysis. The mass spectrometer was autotuned using perfluorotributylamine (PFTBA). The separation of pesticide was done in a 30 meter length, 0.25 mm internal diameter and 0.25 μ m film thickness Varian VF-5MS column Cat No cp8944. The injector was used at constant temperature 280°C.

The injection volume was 1 μ L in splitless mode. The ion trap and transferline temperatures were set to 210 $^{\circ}$ C and 250 $^{\circ}$ C, respectively (**Dorđević et al.2013**). The mass spectrometer was used in SIM mode under electron impact at 70 eV and scan time 1 second. The computer that controlled the system also held a GS-MS library (NIST® library) for the confirmation of spectral analysis. The mass spectrometer was calibrated weekly with perfluoro-tributylamine. Helium (99.999%) at a flow-rate of 1 ml min⁻¹ and 9.6 psi pressure was used as carrier and collision gas. The initial oven temperature was 50 $^{\circ}$ C and ended at 280 $^{\circ}$ C. The total run time of chromatographic analysis was 41.6 minutes with 3 minutes post run.

The principle of analysis is that the ion trap mass spectrometer uses a scanning method where a precursor ion is selected from the analyte spectrum. This ion is isolated in the ion trap and all other ions are expelled. Then sufficient energy is applied to the single ion for fragmentation into a unique spectrum of its product ions, generating response in the low picogram levels with a signal to noise that enables high sensitivity. This type of detection is called tandem MS/MS. The adverse effects of the sample matrix are minimized while generating confirmational data for quantitation. The ion trap GC/MS was used to develop a quantitative method for the analysis of the selected organophosphorus mixture used in this study(**Rhee et al. 1995**)

RECOVERY STUDIES:

Percentage recovery of each organophosphorus was performed according to **Ogah and Coker (2012)** and it should be noted that these recovery experiments were carried out using blank samples that were tested previously and the specified pesticides were not detected .Six levels of mixed pesticide standard solutions were prepared and used for spiking the blank samples. Each standard solution) 1.0ml) was added to 15.0 g of ground sample to give fortification levels of 31.25 ,62.5 ,125 ,250 ,500 and 1000 ppb respectively. Each spiked sample was allowed to stand for six hours and then extracted, cleaned up and analyzed like the test samples. The standard solutions were also run on GC under the same conditions as the spiked samples. Internal standard (triphenylphosphate)was included in both extract and standard solutions. Peak area ratios of pesticides to those of internal standard were calculated for both standard solutions and spiked samples. The percent recovery of each pesticide was then calculated as follows:

$$\frac{\text{Peak Area ratio of pesticide in spiked sample}}{\text{Peak Area ratio of pesticide in standard solution}} \times 100$$

DETERMINATION OF FAT PERCENT IN CHEESE SAMPLES:

Fat percent of cheese samples were determined using the gravimetric method in accordance with **Chemical Laboratory Guide (CLG-FAT.03,2009)** of the United States Department of Agriculture-Food Safety and Inspection Service, Office of Public Health Science.

The instrument used for fat extraction was fully automated Soxhlet unit while the extraction was petroleum ether.

CALCULATIONS:

$$\text{Fat content, percent} = \frac{100(B - C)}{A}$$

Where A = Sample weight

B = Weight of cup after extraction

C = Weight of cup prior to extraction

N.B.

Expression of Fat % in hard cheese samples as percentage of fat in dry matter (FDM) according to Codex standard 283-1978

Results and discussion

Results are showed in tables 1-6 and Fig 1and 2

Discussion

Organophosphorus (OP) compounds have found wide applications as pesticides and insecticides in agriculture and as chemical warfare agents in military practice. Worldwide, OP compounds account for over 38% of the total pesticides used (**Singh.2009**). Commonly used organophosphates includes parathion, , methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos and azinphos methyl and malathion.They are widely used in agriculture, residential landscaping, public recreation areas and in public health pest control programs such as mosquito eradication (**EPA.2008**)

The use of OPs has a number of health advantages, including control of insect vector borne diseases and increasing the food and agriculture productions. However, the use of these toxic compounds on man, animals or in his immediate environment has caused potential hazards that seriously are painful for public health. Therefore there is an increasing awareness and concern on the part of food and health officials and of the dairy industry about the presence OPs in milk and other milk products because in dairy cattle farms, milk may serve as a vector for the transmission of substances of extrinsic origin which can be potentially toxic to the consumer (**Kazemi et al., 2012**).

Table (1) showed the name of each pesticide in the total ion chromatogram (figure1) and the specific parameters of each pesticide that were used to analyze the presence of the chosen organophosphorus compounds in hard cheese. Meanwhile the spectral data of the 14 pesticide which matched the fragmentation of individual pesticide in the NIST® spectral library software that was included with the instrument operating softwares were shown by figure (2).

Table (2) revealed that the response of each organophosphorus pesticide was in a good linearity with its concentrations; the linearity correlations were not less than 0.99 for all the examined organophosphorus pesticide except for trichlorofon and demeton-s-methyl.

Due to the complex nature of the matrix of samples related to animal origin and the presence of fat, selection of the appropriate technique for preconcentration and purification of the analytes, and its optimization is the most laborious work, but also it is very important aspect of the analysis. Although there is a huge number of different cleanup methods that were used in the determination of pesticides in milk, there is no universal technique, which would be entirely better than the others in terms of all analytical scopes. Meanwhile among the proposed cleanup methods MSPD (matrix solid phase dispersion) and DSPE (dispersive solid phase extraction) were stated as the most promising to the pesticides analysis in milk, but some drawbacks of these methods were recorded (**Kiljanek et al., 2013**). **Lehotay et al. (2005)** established that there is dependence between recovery of pesticides versus fat content of the sample in both MSPD and DSPE cleanup methods.

The detection limits of pesticides were estimated as the lowest concentration that could be determined by the instrument under the performed analysis condition. The detection limits were in the range of 5–30ppb. Table (3) showed the recovery rate of the 14 pesticide at different concentrations. The recovery experiments were performed by blank sample spiked at different fortification levels. The recoveries were ranged from 78.76-129.76%.

Table (4) contained the maximum residues limit (MRL) of the selected organophosphorus compounds in milk (as a guide for dairy products) according to different authorities. In this instance CAC (2013) reported that: for a "milk product"

with a fat content less than 2%, the MRL applied should be half those specified for milk, but the MRL for "milk products" with a fat content of 2% or more should be 25 times the maximum residue limit specified for milk, expressed on a fat basis.

Table (5) represented the fat content in cheese samples expressed as percentage (%) and it was observed that fat percent exceed the figure 2 at which the maximum residue limit had to be calculated as 25 times the permitted maximum residue limit in milk for those compounds that were considered as fat soluble.

Table (6) expressed the number of positive samples, percentage of incidence and concentrations of organophosphorus pesticide in hard cheese samples based on whole product basis and on fat basis for the fat soluble compound (diazinon) and it was found that 4 (8%) samples were contaminated with diazinon while 2 (4%) samples only were contaminated with malathion with a total percentage of 12%.

The concentrations of diazinon in the four samples were 0.030 , 0.046 , 0.052 and 0.021 mg/kg on whole product basis where these concentrations were 0.055 , 0.90 , 0.094 and 0.043 mg/kg on whole fat basis respectively.

Meanwhile , The concentrations of malathion in the two samples were 0.025 and 0.073 mg/kg on whole product basis while they were 0.045 and 0.130 mg/kg on whole fat basis respectively.

The codex of (FAO/WHO, 2013) regulations for pesticide residues in food was taken as a reference to compare our results as the Egyptian MRLs were mainly those of the codex limits.

According to the available MRLs, all positive samples exceeded the established MRLs of diazinon and malathion on whole product basis (or as specified for milk) but did not exceed the limits when considering the factor of 25 specified for dairy products that contain fat content equal or more than 2%.

In Europe and by the force of the **Regulation (EC) No. 396/2005** a general principle is that: for any substance not covered by the Annexes of Regulation No. 396/2005 that falls under the definition of "pesticides residues" a default MRL of 0.01 mg/kg will applied . This means that this general limit is applicable 'by default in all cases where an MRL has not been specifically set for a product or product type.

From another point of view the World Health Organization reported that, every year there are three million pesticide poisonings, mostly OP-related, and 200,000 deaths worldwide that are attributed either as self-poisoning or occupational exposure [**Bird et.al.,2008**]. Besides human exposure, there is also concern that these pesticides could leak into ground and municipal water supplies and pollute surrounding environment. Reports in the literature have expressed concern over exposure to non target organisms

such as birds and fish, as well as the potential for human exposure from sources such as fresh fruits and vegetables and processed foods.

These toxins may originate in cow's milk from the ingestion of plants known to contain toxic substances or feeds contaminated with residues of pesticides (**Kazemi et al., 2012**) and grass or corn silage, and veterinary use against parasites and insects by application of pesticides directly on the dairy cattle, in barn or in dairy plants. (**Kiljanek et al., 2013**).

The contamination of milk with organophosphorus pesticide residues could originate through the feeding of dairy cattle on feeds contaminated with these pesticides and/or due to the exposing of the animals to direct atmosphere and to oral intake of organophosphorus pesticides (**IDF, 1979**) and **Kiljanek et al. (2013)** who stated that The main causes of (OP) residues in milk are carried over processes from contaminated feed, grass or corn silage, and veterinary use against parasites and insects by application of pesticides directly on the dairy cattle, in barn or in dairy plants.

In this issue, results of **Nasr et al., (2007)** at Gharbia Governorate, Egypt showed that no contamination with investigated pesticide residues found in all analyzed samples, excepted one sample in autumn season contaminated by malathion with a concentration of (0.018 mg kg^{-1}) and representing (0.02%) of all samples. Also other Egyptian authors at some other governorates showed that milk contamination with organophosphorus pesticide had a concentration range of $0.08 - 0.87 \text{ mg kg}^{-1}$ where diazinon and malathion were the most prevalent organophosphorus pesticides present (**Abd Rabo et al. 1989, Abd Alla et al. 1991. Dabiza et al. 1999 and Ahmed and Saleh .(2002). Abou Donia et al.(2010)** found no (OP) in buffalo nor cow milk dairy farms in Egypt, while searching for malathion, pirimiphos methyl and dimethoate residues.

Conclusion and Recommendation

The use of gas chromatography analysis coupled with ion trap and tandem mass spectrometry technology for the detection of organophosphorus pesticides residues in hard cheese gave satisfactory reliable results due to the high sensitivity and selectivity of the instrument. Meanwhile the extraction and clean-up protocol using QuECHERS method resulted in acceptable recoveries for a majority of the pesticides studied.

The results in this study revealed that although some of the hard cheese samples were positive for some organophosphorus residues, the remainder of samples were clean from these toxins and those positive samples did not exceed the recommended maximum residues limits specified for dairy products with fat percent equal or more than 2%. Therefore the results of this study indicated that occurrence of organophosphorus compounds residues in hard cheese were slight, probably because of slight or moderate contamination of feedstuffs consumed by the milking cows. It could also be concluded that contamination of organophosphorus compounds residues in dairy

products chain did not appear to be a serious public health problem at the moment. However, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of organophosphorus pesticides contamination in dairy farms. Implementing a food control system, such as the Hazard Analysis and Critical Control Point (HACCP) system in the dairy products industries is of great importance in keeping dairy product away from many types of contaminants including organophosphorus compounds .

Egyptian Standards must conclude the permissible limits of all pesticide (not only the organophosphorus ones) that can reach the Egyptian consumer food by any way.

Fig (1) Total ion Chromatograph (TIC) of the 14standards mix- full scan at 2µg/ml

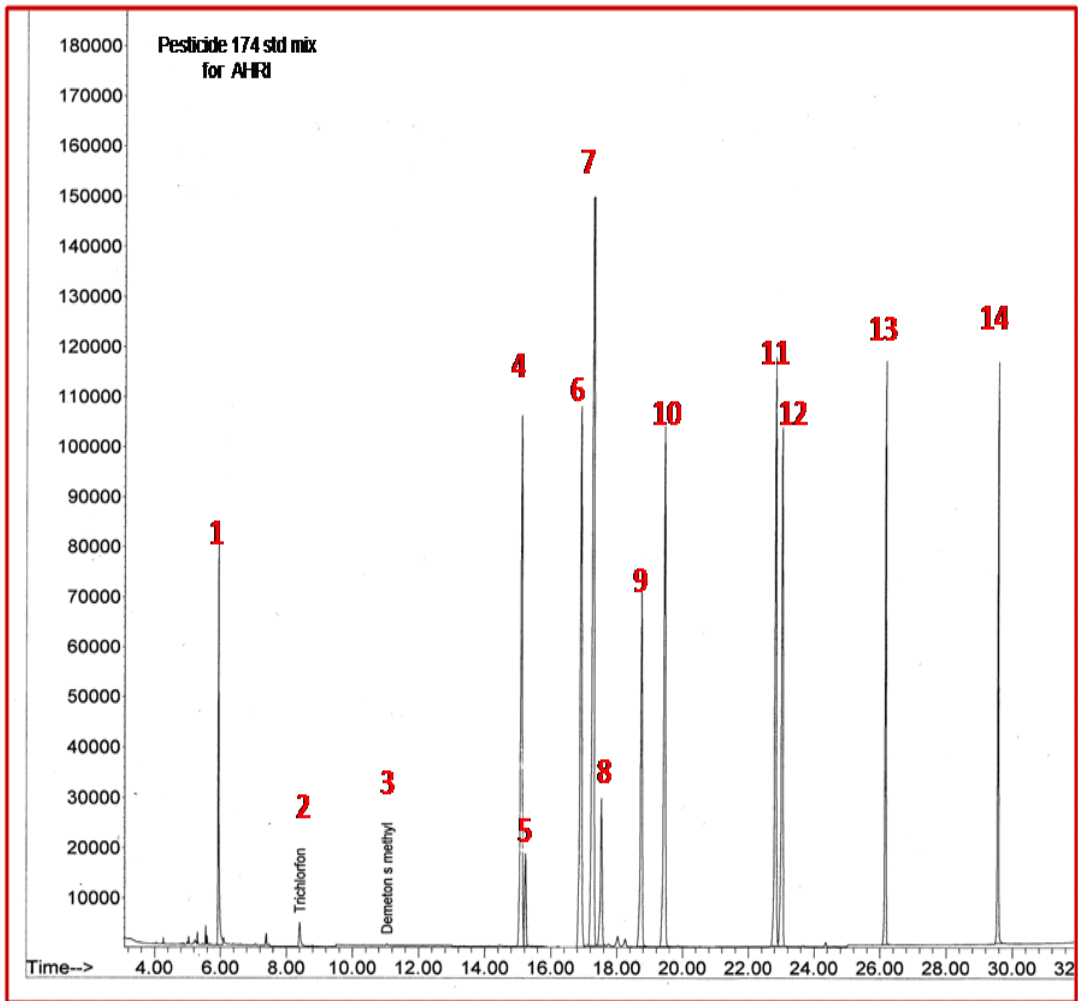


Table (1) Individual pesticide parameters, the serial numbers indicated the name of each pesticide in the total ion chromatogram

Serial	Compounds	Molecular weight	Quantifier ion	Qualifier Ions
1	Methamidophos	141	95	47-79
2	Trichlorofon	257	109	79-145
3	Demeton-s-methyl	230	88	60-142
4	Diazinon	304	179	137-152
5	Paraxon Methyl	247	109	79-96
6	Phosphamidon	299	127	72-264
7	Chlorpyrifos methyl	321	125	79-286
8	Malaxon	314	127	99-109
9	Fenitrothion	277	125	109-260
10	Malathion	330	125	93-173
11	Methidathion	302	145	85-125
12	Bromophos-ethyl	394	97	242-359
13	Ethion	384	231	97-153
14	Azinophosmethyl	317	160	77-132

Fig(2) Demonstrates the spectral data of the 14 pesticide which had matched the fragmentation of individual pesticide in the NIST® spectral library

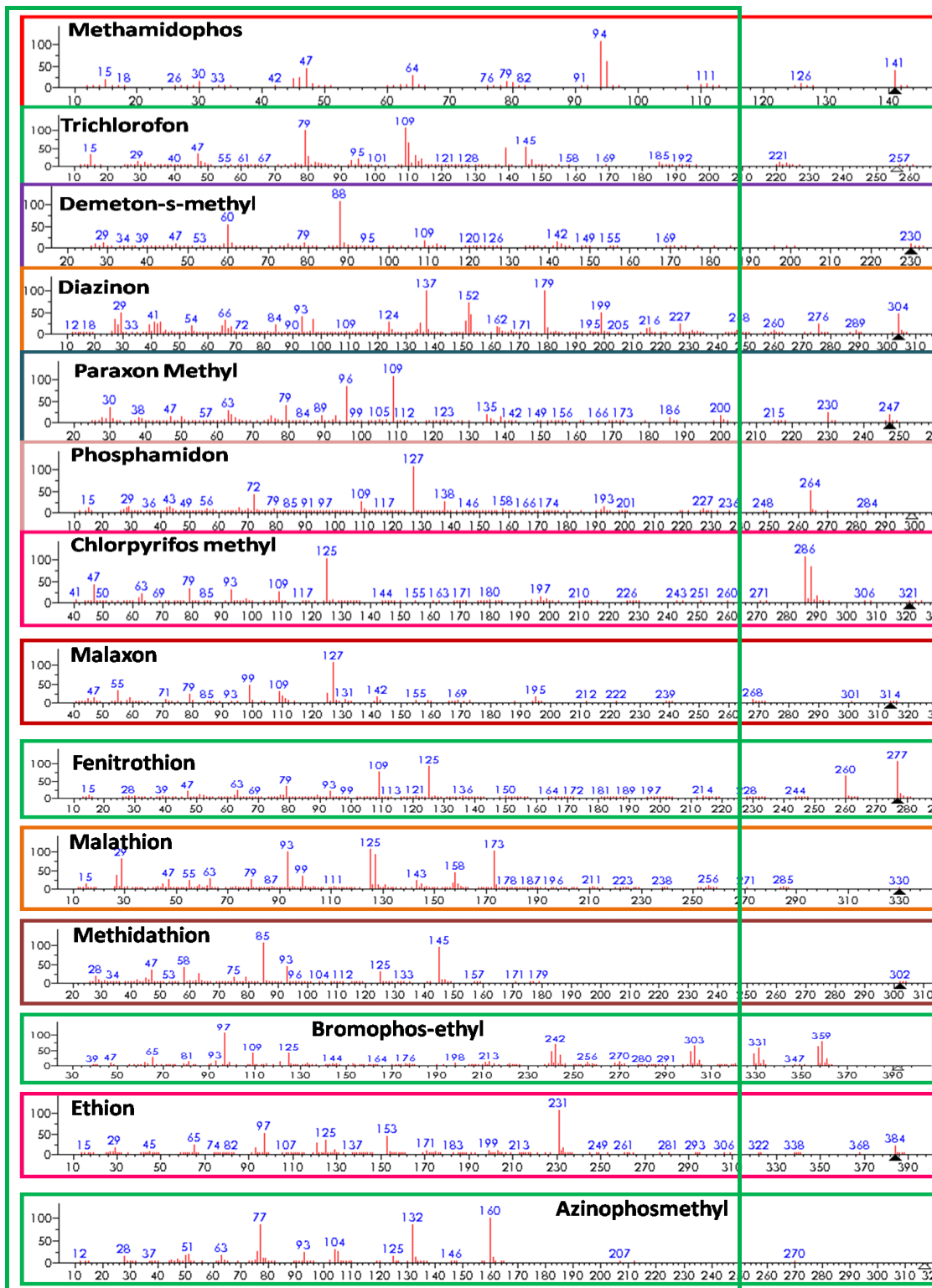


Table (2) : Retention time of the tested organophosphorus compounds and their specific standard curve linearity

Serial	Organophosphorus	Retention time (minute)	Linearity (r ²)
1	Methamidophos	5.931	0.999
2	Trichlorofon	8.400	0.868
3	Demeton-s-methyl	11.843	0.819
4	Diazinon	15.103	0.999
5	Paraxon Methyl	15.226	0.999
6	Phosphamidon	16.908	0.999
7	Chlorpyrifos methyl	17.272	0.999
8	Malaxon	17.530	0.999
9	Fenitrothion	18.740	0.999
10	Malathion	19.443	0.999
11	Methidathion	22.823	0.999
12	Bromophos-ethyl	23.022	0.999
13	Ethion	26.160	0.999
14	Azinophosmethyl	29.563	0.999

Table (3) Recovery rate of the 14 pesticide at different concentrations (ppb) in blank samples and their detection limits

Pesticide	Recovery rate (%) at different concentrations						Detection limit (ppb)
	31.25ppb	62.5ppb	125ppb	250ppb	500ppb	1000ppb	
Methamidophos	78.76	93.55	102.37	96.96	100.06	100.3	10
Trichlorofon	ND	ND	23.5	27.3	27.9	35.2	50
Demeton-s-methyl	ND	ND	27.8	29.6	29.7	31.11	50
Diazinon	105.95	98.34	99.64	96.40	101.09	119	5
Paraxon Methyl	114.94	99.23	102.42	94.80	100.70	113.85	10
Phosphamidon	116.86	105.47	101.49	95.13	101.01	106.77	10
Chlorpyrifos methyl	110.85	97.73	99.86	97.10	100.44	120.53	4
Malaxon	124.86	117.82	94.98	95.96	101.04	118	10
Fenitrothion	129.76	103.58	97.02	92.15	101.95	120	15
Malathion	129.52	91.06	92.68	92.50	102.19	120.1	10
Methidathion	122.40	109.22	99.42	94.03	103.44	114.8	10
Bromophos-ethyl	120.64	99.86	97.25	95.70	101.14	114	10
Ethion	129.76	106.60	96.40	91.60	102.08	119	10
Azinophosmethyl	71.07	93.73	110.33	98.93	99.79	118	10

Table (4) : Maximum residues limit (MRL) of the selected organophosphorus compounds in milk (as guide for dairy products) according to different authorities

COMPOUND	(MRL(mg/kg		
	MILK (CAC) ^a	MILK (JAPANESE MRL) ^b	MILK ^c (86/363/EEC)
Methamidophos	0.02	0.02	0.01
Trichlorofon	NE	NE	(0.01)
Demeton-s-methyl	NE	NE	(0.01)
Diazinon	##and 0.5 #0.02	0.02	##and 0.25 #0.01
Paraxon Methyl	NE	NE	(0.01)
Phosphamidon	NE	NE	(0.01)
Chlorpyrifos methyl	0.01	NE	0.01
Malaxon	NE	NE	(0.01)
Fenitrothion	0.01	0.002	(0.01)
Malathion	##0.5	0.5	(0.01)
Methidathion	0.001	0.001	0.02
Bromophos-ethyl	NE	NE	(0.01)
Ethion	NE	in fat 0.5	(0.01)
Azinophosmethyl	NE	NE	0.01

a :Codex Alimentarius Commission Joint FAO/WHO b: The Japan Food Chemical Research Foundation((2006) :European Council Directive (1986): # on whole product basis:## .on fat basis .NE: Not Established (0.01): Proposed MRL of pesticides that don't have MRL until now as recommended by EEC/363/86.

Table (5): Percentage of fat in dry matter (FDM) in the examined hard cheese samples.

Number of samples	minimum	maximum	Mean fat %	Standard error
50	48.4	56	53.224	0.4

Table (6): Prevalence of the selected organophosphorus pesticide (OP) in the examined hard cheese samples. No 50

Serial	Organophosphorus	Number of positive samples	Percentage of positive samples	Concentration of (OP) on whole product basis (mg/kg)	Concentration of (OP) FDM (mg/kg)	
1	Methamidophos	ND				
2	Trichlorofon	ND				
3	Demeton-s-methyl	ND				
4	Diazinon	4	%8	0.030	*(%54.8)	0.055
				0.046	*(%50.9)	0.090
				0.052	*(%55.1)	0.094
				0.021	*(%48.1)	0.043
5	Paraxon Methyl	ND				
6	Phosphamidon	ND				
7	Chlorpyrifos methyl	ND				
8	Malaxon	ND				
9	Fenitrothion	ND				
10	Malathion	2	%4	0.025	*(%56.0)	0.045
				0.073	*(%56.0)	0.130
11	Methidathion	ND				
12	Bromophos-ethyl	ND				
13	Ethion	ND				
14	Azinophosmethyl	ND				
Total		6	%12	-	-	

* FDM = Fat in dry matter

ND = not detected

References

- Abd-Alla E.A.M., Sayed A.F. and Ahmed N.S (.1991) :**(Incidence of some organophosphorus pesticide residues in buffalo milk in Giza Governorate. Egypt. J . DairySci.19:243-248 .
- Abd-Rabo, F.H., A.E. Abou-Dawood, N.S. Ahmed and F.A.M. Hassan,**

(1989): Heat treatment change amounts of pesticide residues in buffalo`s milk. Egypt. J. Dairy Sci., 17: 349-357.

ABOU DONIA M. A., ABOU-ARAB A. A. K., ENB, A., EL-SENAIT, M. H. and ABD-RABOU N. S. (2010): Chemical composition of raw milk and the accumulation of pesticide residues em milk products. Global Veterinaria, Dubai, v. 4, n. 1, p. 6-14.

Abou-Donia M.B. (2003): Organophosphorus ester-induced chronic neurotoxicity. Arch. Environ. Health. 58(8):484-497.

Ahmed E.E.K., and Saleh S.K. (2002): Effect of milk treatment and manufacturing on pesticide residues. Proceedings of the 1st Conference of Central Agriculture Pesticide Laboratory, September 3-5, 2002, IEEE Xplore, pp: 27-34.

AOAC Official Method 2007.01(2007): Pesticide Residues in Foods by Acetonitrile. Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry First Action 2007. J. AOAC Int. 90, 485.

Bird S.B., Sutherland T.D., Gresham C., Oakeshott J., Scott C. and M. Eddleston M.(2008): OpdA, a Bacterial Organo-phosphorus Hydrolase, Prevents Lethality in Rats after Poisoning with Highly Toxic Organophosphorus Pesti-cides. Toxicology, Vol. 247, No. 2-3, pp. 88-92.

CAC. Codex Alimentarius Commission Joint FAO/WHO (2013): CODEX MAXIMUM RESIDUE LIMITS FOR PESTICIDES: Codex Maximum Residue Limits/Extraneous Maximum Residue Limits for Milk and Milk Products. Codex Alimentarius Commission Joint FAO/WHO Food Standards Programme .FAO, 00100 Rome, Italy.

Chemical Laboratory Guide (2009) Determination of Fat .CLG-FAT.03.

United States Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science .

Codex stand 283-1978 : Codex General Stansard for cheese.

Dabiza N.M.A., El-Senaity M.H. and Zin-El-Dein M.M. (1999): Pesticide residues in some market dairy products and distribution of malathion in butter, ghee and Domiati chese during manufacture. Egypt. J. Dairy Sci., 27: 345-357.

Đorđević T., Šiler-Marinković S., Đurović-Pejčev1 R., Dimitrijević-Branković S. and Umiljendić J.G.(2013): Efficiencies of different methods for determination of organophosphate pesticide residues in fermented wheat substrate. Pestic. Phytomed. (Belgrade), 28(2), 133–140.

EEC 86/363 (1986): Council Directive of 24 July 1986 on the fixing of maximum levels for pesticide residues in and on foodstuffs of animal origin (86/363/EEC). (OJ L 221, 7.8.1986, p. 43).

EPA (Environmental Protection Agency), (2008): Malathion for Mosquito Control.[http://epa.gov/opp00001/health/mosquitoes/malathion for mosquitoes.html](http://epa.gov/opp00001/health/mosquitoes/malathion%20for%20mosquitoes.html).

FAO/WHO Codex Alimentarius Commission Joint FAO/WHO (2013): Pesticide residues in food and feed. Commodity details: ML 0106 Milks.Class:Primary food commodities of animal origin. Type: Mammalian product.Category:Milks.

Gahlaut A., Gothwal ., Chhillar .K.and Hooda V.(2012): Electrochemical Biosensors for Determination of Organophosphorus Compounds: Review. Open Journal of Applied Biosensor, 2012, 1, 1-8.

IDF,International Dairy Federation (1979): Chemical residues in milk and milk products. IDF. Document, pp: 113.

Karalliedde L., Edwards P.and Marrs T.C. (2003): Variables influencing the toxicity of organophosphates in humans. Food Chem. Toxicol. 41:1–13.

Kazemi M., Tahmasbi A.M., Valizadeh R., Naserian A.A., Soni A. and Moheghi M.M.(2012): Importance and toxicological effects of organophosphorus pesticides: A comprehensive review. Basic Research Journal of Agricultural Science and Review ISSN 2315-6880 Vol. 1(3) pp. 43-57.

Khaniki G. J. R. (2007): Chemical contaminants in milk and public health concerns: A review. Int. J. Dairy. Sci., 2: 104-115.

Kiljanek T., Niewiadowska A. and Semeniuk S.(2013): Determination of organophosphorus pesticide residues in milk. Bull Vet Inst Pulawy 57, 185-189.

Lehotay S.J ., Mastovská K. and Yun S.J.(2005): Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrixes. J AOAC Int; 88(2):630-638.

Nasr I.N. A., Sallam A.A. and Abd El-Khair A.A. (2007): Monitoring of Certain Pesticide Residues and Some Heavy Metals in Fresh Cow`s Milk at Gharbia Governorate, Egypt. Journal of Applied Sciences, 7: 3038-3044.

Ogah C.O. and Coker H.B.(2012): Quantification of Organophosphate and Carbamate Pesticide Residues in Maize. Journal of Applied Pharmaceutical Science Vol. 2 (9), pp. 093-097 .

Regulation (EC) No 396/2005 (2005): Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC(Text with EEA relevanc). OJ L 70, 16.3.2005, p. 1–16. Last reviewed 9/7/2013.

Rhee J.S., Park H.M. and Er y.w.(1995):Study on the residue analysis of organophosphorus and organochlorine pesticides with Ion Trap and Quadrupole Mass Spectrometer.Journal of the Korean Chemical Society.VOL.39,No.12.

Schipper P.N., Vissers M.J.and Van Der Linden A.M. (2008): Pesticides in ground water and drinking water wells: overview of the situation in the Netherlands. Water Sci. Technol. 57(8): 1277-1286.

SHIMADZU SCIENTIFIC INSTRUMENTS (2013): Analysis of Organophosphorus Pesticides in Baby Foods Using a Triple-Quadrupole GC/MS/MS System. Application News No. GCMS-1304

Singh B.K.(2009): Organophosphorus-degrading Bacteria: Eco- logy and Industrial Applications,” Nature Reviews Mi-crobiology, Vol. 7, pp. 156-164.

The Japan Food Chemical Research Foundation (2006): List of MRLs, according to individual agricultural chemicals.

Worek F, Koller M, Thiermann H, Szinics L. (2005): Diagnostic aspects of organophosphate poisoning. Toxicol. 214, 182-189.

Zhao L. and Stevens J.(2012) :(Analysis of pesticide residues using Agilent bond elut QuECHERS AOAC kits by GS/MS.Agilent technologies application note No 5990-4305EN