Assessment of Polychlorinated Biphenyl and Organochlorine Pesticide Residues in Some Cheese Types in the Egyptian Markets

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Abstract

A total of 75 samples of Roumy, Dumyati and Processed cheese (25 of each) were randomly collected during summer 2012 from different Egyptian markets to determine 10 polychlorinated biphenyl (PCBs) and 12 organochlorine pesticides.The obtained results indicate the absence of PCBs in all the examined samples. Meanwhile, αBHC, βBHC, heptachlor, heptachlor epoxide, γ chlordane, γ endosulfan, endrin, pp- DDE, and pp- DDD were detected in the mean levels ranged from 0.022 ±0.016 to 1.29 ±0.701 ppb of ppDDE and βBHC respectively. These mentioned 2 levels were recorded in Roumy cheese samples. The obtained results showed that the Roumy cheese contained slightly higher levels of the most detected organochlorine compounds than those the other two examined cheese types. On the other hand, heptachlor residues exceeded the permissible limit in one sample (4%) of both Roumy and Dumyati cheese. Meanwhile, γ chlordane exceeded the permissible limit in 2 samples (8%) of the processed cheese. Also, γ endosulfan residues were above the permissible limits in one sample (4%) out of Dumyati cheese. The levels of DDT derivatives were below its permissible limit in all the examined samples. Meanwhile, the permissible limits of αBHC, βBHC, heptachlor epoxide and endrin are not available. The organochlorine pesticide residues were detected in relatively low levels in the examined samples, although little samples recorded organochlorine levels exceeded the permissible limits. The continuous monitoring of organochlorine compounds in the milk products and the others greasy foods is recommended.

Key words: Organochlorine, Organochlorine Pesticide, Roumy, Dumyati, Processed cheese, γ endosulfan

Introduction

Polychlorinated biphenyls (PCBs) are toxic industrial chemical pollutants, because of their fat solubility, widespread and uncontrolled industrial applications, PCBs have become a persistent and ubiquitous contaminant in the environment **(USFDA, 2006).** PCBs are divided into 209 congeners supplied by the commission of the European community according to their chemical structure. Some PCBs congeners elicit a divers spectrum of toxic and biochemical response including body weigh loss, immunotoxicity **(Sormo *et al,* 2009)** and induction of gene expression **(El Nemr *et al,* 2003)**.

Organochlorine pesticides are man-made organic chemicals, developed in 1940s for use mainly as insecticides in 2nd world war, as PCBs, these compounds are fat soluble, long persistent in the environment and passes through the food chain **(Casarett and Doull, 2001)**. Therefore, these compounds were prohibited in many countries. In spite of, the organochlorine pesticides were prohibited in Egypt

since 1980s **(Dogheim *et al,* 1996),** the residues of these pesticides may found in the human food in Egypt due to previous applications or illegal use of these compounds **(Abbassy *et al,* 2003).** Organochlorine pesticides cause serious toxic symptoms by continuous exposure including developmental abnormalities and growth suppression, disruption of the endocrine system, impairment of immune function, and cancer promotion **(El Nemr *et al,* 2003)**.

Cheese is nutritious food made mostly from the milk of cows but also other mammals, including sheep, goats, buffalo, and camels. Around 4000 years ago people have started to breed animals and process their milk. That's when the cheese was born. The fat content of the most cheese types is higher than those in the milk **(Cheese breakdown, 2012)**. Therefore, the fat soluble pollutants residues in the cheese must be considered.

The present study was aimed for detection and determination polychlorinated biphenyl (PCBs) and organochlorine pesticide residues in Roumy, Dumyati and Processed cheese samples from the Egyptian markets and comparing their levels with the permissible limits.

2. Materials and Methods.

2.1 Collection of samples.

A total of 75 samples of Roumy, Dumyati and Processed cheese (25 of each) were randomly collected during summer 2012 from different Egyptian markets to determine 10 polychlorinated biphenyl "PCBs" [PCB 28(2,4,4'), PCB44 (2,2',3,5'), PCB70 (2,3' ،(٥،'٤،PCB101 (2,2',4,5,5'), PCB138 (2,2',3,4,4',5), PCB152 (2,2',3,5,6,6'), PCB153 (2,2',4,4',5,5'), PCB180 (2,2',3,4,4',5,5'), PCB192 (2,3,3',4,5,5',6) and PCB194 (2,2',3,3',4,4',5,5')] and 12 organochlorine pesticides [αBHC, βBHC, γBHC, heptachlor, heptachlor epoxide, aldrin, γ chlordane, γ endosulfan, endrin, pp- DDE, pp- DDD and pp- DDT]. The examined samples (250 gm each) were collected in clean polyethylene bags. The samples were identified and transferred to the laboratory in ice box.

2.2 Preparation of samples:

Twenty five gm of each sample was thoroughly mashed with 50 ml of distilled water in a clean dry blender, then 10 ml from each prepared was measured into small glass containers with screw Teflon capped stoppers and kept deeply frozen at -20°C for analysis **(Suzuki *et al.,* 1979)**.

2.3 Analysis:

2.3.1 Extraction procedure:

Ten ml of prepared samples were placed into a 50ml centrifuge tube, and 20ml n-hexane, 5ml acetonitrile and 1ml ethanol were added and mixed by vigorous shaking for 1 minute, followed by centrifugation for 2 minutes at 2000 rpm. The hexane phase was then filtered through anhydrous sodium sulphate. The sample was extracted with 2 additional 20 ml portions of n-hexane (***Suzuki et al.,* 1979)**.

2.3.2-Clean up of samples

Sample extracts applied to chromatography column in 2-3 ml of hexane were eluted successively with hexane florisil (60/100 mesh) was activated at 250°C for 12-15 hours, placed in a desiccator until cool, deactivated with 0.5% H_2O, stored in a sealed container in a desiccator overnight, and then used within 72h. Columns were rinsed with 100 ml hexane collecting eluant in beaker. Stop the flow before the top of solvent reaches the top of sodium sulfate. Discard the eluant into the waste container. Samples extracts were applied to the column in 2-3 hexane, elute the column with 60 ml hexane, to elute the polychlorinated biphenyls (PCBs) and pp-DDE collecting eluant in the 100 ml flask as fraction 1 (F1). Next, add 35 ml of 30% dichloromethane in hexane, next add 45 ml of 50% dichloromethane in hexane to column and elute it to dryness, to elute all organochlorine in the samples. Reduce each fraction to 0.5ml ***(Khaled et al,* 2004).**

2.3.3-Preparation of blank solution

The same volumes of solvents and sodium sulfate anhydrous which used in the extraction of the cheese samples were subjected to the same extraction, partitioning and clean up procedures as the examined samples to detect any possible traces of the studies PCBs or pesti-

cides in the solvents or distilled water.

2.3.4-Gas chromatographic analysis

Chromatographic analysis were carried out at pesticide residue and environmental pollution department central agriculture pesticide laboratory. Hewlett Packard GC Model 6890 equipped with Ni^{63} – electron capture detector. GC conditions: HP- 5MS capillary column (30m length X 0.32mm internal diameter (i.d.), X 0.25μm film thickness), carrier gas: N_2 at a flow rate of 4 ml/min; injector and detector temperatures were 280°C and 300°C respectively. The initial column temperature was initial oven temperature, 180°C for 2 min, raised at 3 °C/min. and then held at 220°C for 1 min., then raised at 9°C /min. to 280°C and then held to 2 minutes, until a total time of 30 minutes had elapsed, DB-17 (J & scientific) capillary column (30m length X 0.32mm initial diameter (i.d.) X25 μm film thickness). Operating temperature were: column temperature was programmed 160°C to 230°C at a rate of 3°C / min. to 260°C at a rate 10°C then hold 10 minutes. Injector temperature were 300°C and detector temperature were 300°C with nitrogen carrier gas flow at 4 ml./ min was used to confirm the detected pesticides.

The organochlorine and PCBs residue components were identified by comparing their retention times with those of the standards quantified by extrapolation of corresponding sample peak areas with those from standard curves prepared for each pesticide standard. Small variations in retention times and response factors of each compound during the experiments were corrected for by obtaining fresh chromatograms of the standard mixture after every nine injections. Standard solutions of concentrations ranging from 0.01 to 0.04 ppm were prepared for each pesticide standard and 1μl was injected into the GC. Peak areas of standard solutions were plotted against their concentrations. A line of best fit was drawn through the point and the limits of detection were taken at 5 times the detector noise level.

2.3.5- Determination of percentage rate of recovery

The reliability of analytical method was examined by fortifying the tested samples with known quantities of tested pesticides following the same procedures of extraction, partitioning, clean up and analysis. The percentage rate of recovery of organochlorine pesticide ranged from 69.90% to 95.85% for p-p DDT and γ chlordane respectively; while, in the PCBs the percentage rate of recovery varied from 85.17% to 97.95% for PCB28 and PCB138 respectively.

2.4 Statistical analysis

Statistical analysis of data was conducted using "Statistic for animal and veterinary science" ***(Petric and Watson,* 1999).**

3. Results and Discussion.

The polychlorinated biphenyl (PCBs) residues were not detected in all the examined cheese samples in the present investigation.

Table 1. showed the organochlorine pesticide concentrations in the different examined cheese types. The obtained levels of αBHC and βBHC in the examined cheese samples were lower than those detected by ***Bentabol and Jadral* (1995),** which recorded 46 and 46.5 ppb of αBHC and βBHC residues in the cheese samples respectively in Spain. Moreover, ***Nida et al.,* (2009)**, detected 15 and 30 ppb of αBHC and βBHC respectively in the examined cheese samples in Jordan. Heptachlore and heptachlore epoxide residues in the present investigation were lower than those estimated by ***Bentabol and Jadral* (1995)**. On contrarily, these mentioned organochlorine compounds were not detected in cheese samples examined by ***Nida et al.,* (2009)**. Moreover, ***Darko* (2003)** estimated higher levels of γ endosulfan residues than our figures in cheese samples in Ghana (2.7- 4.25 ppb). Also, estimated γ chlordane residues by ***Bentabol and Jadral* (1995)** in cheese samples (50.2 ppb) were obviously higher than our obtained levels. Furthermore, estimated endrin residues in cheese samples (27 ppb) by ***Nida et al.,* (2009)** clearly higher than those estimated in the present study. Con-

cerning DDT and its derivatives, as the other estimated organochlorine compounds, the residues of DDT derivatives in the current investigation recorded lower levels than those in the available previous studies. ***Suarez et al.,* (1997)** estimated 858- 904 ppb of DDT in cheese samples in Spain. ***Darko* (2003)** detected pp DDE in levels 31.5- 149 ppb in cheese samples in Ghana. Also, ***Nida et al.,* (2009)** recorded 64 ppb of pp DDE residues in cheese samples in Jordan. On contrarily; in the previous Egyptian study, pp DDE residues were not detected in the examined Karesh cheese samples ***(Neven and Maha,* 2007)**. On the other hand, γBHC, aldrin, and pp- DDT were not detected in all the examined cheese samples. The achieved results revealed relatively law organochlorine levels in the examined cheese samples comparing with the results of the available previous studies.

Table 1. Concentrations (ppb) of organochlorine pesticide residues in the examined cheese type samples (n = 25 for each type).

Organochlorine pesticides	Roumy cheese			Dumyati cheese			Processed cheese		
	Min.	Max.	Mean ±S.E.	Min.	Max.	Mean ±S.E	Min.	Max	Mean ±S.E
αBHC	N.D.	3.18	0.456 ±0.195	N.D.	1.5	0.09 ±0.066	N.D.	3.52	0.457 ±0.198
βBHC	N.D.	15.01	1.29 ±0.701	N.D.	8.39	0.925 ±0.462	N.D.	N.D.	N.D.
Heptachlor	N.D.	8.12	0.952 ±0.424	N.D.	6.87	0.502 ±0.349	N.D.	N.D.	N.D.
Heptachlor epoxide	N.D.	6.5	0.636 ±0.368	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
γ Endosulfan	N.D.	N.D.	N.D.	N.D.	11.31	0.936 ±0.547	N.D.	3.44	0.538 ±0.223
γ Chlordane	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	6.26	0.458 ±0.318
Endrin	N.D.	8.41	0.772 ±0.453	N.D.	5.55	0.45 ±0.267	N.D.	N.D.	N.D.
pp- DDE	N.D.	0.35	0.022 ±0.016	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
pp-DDD	N.D.	7.29	0.755 ±0.421	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. not detected (for detectable values)
LOD ranged between (0.01 → 0.04) Ppb

Table 2. Frequency distribution of organochlorine pesticide residues in the examined cheese type samples (n = 25 for each type).

Organo-chlorine Pesticide	Roumy cheese						Dumyati cheese						Processed cheese					
	N.D.		Within P.L.*		Over P.L.		N.D.		Within P.L.		Over P.L.		N.D.		Within P.L.		Over P.L.	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Hepta-chlor	20	80	4	16	1	4	23	92	1.0	4.0	1.0	4.0	25	100	0.0	0.0	0.0	0.0
γ Chlor-dane	25	100	0.0	0.0	0.0	0.0	25	100	0.0	0.0	0.0	0.0	23	92	0.0	0.0	2.0	8.0
γ En-dosulfan	25	100	0.0	0.0	0.0	0.0	22	88	2.0	8.0	1.0	4.0	19	76	6.0	24	0.0	0.0
Total DDT	20	80	5	20	0.0	0.0	25	100	0.0	0.0	0.0	0.0	25	100	0.0	0.0	0.0	0.0

*: P.L.: Permissible limits of Heptachlor, γ Chlordane, γ Endosulfan and DDT were 6, 2, 10 and 20 ppb in milks ***(Codex Alimintarius, 2010).*** The permissible limits of αBHC, βBHC, Heptachlor epoxide and Endrin are not available.
N.D. not detected (for detectable values)

Table 2. revealed that organochlorine pesticides residues could not be detected in the most of examined samples. Heptachlor residues exceeded the permissible limit in one sample (4%) of both Roumy and Dumyati cheese. Meanwhile, γchlordane exceeded the permissible limit in 2 samples (8%) of the processed cheese. Also, γ endosulfan residues were above the permissible limits in one sample (4%) out of Dumyati cheese. The levels of DDT derivatives were below its permissible limit in all the examined samples. Meanwhile, the permissible limits of αBHC, βBHC, Heptachlor epoxide and endrin are not available.

The obtained results showed that the Roumy cheese contained slightly higher levels of the most detected organochlorine compounds than the other two examined cheese types. This result explained by the lipophilic nature of the organochlorine compounds, the Roumy cheese had the higher fat contents than that in the other examined 2 cheese types. Moreover, the variations of lipid contents and sources of milk between the samples within the same cheese types, lead to fluctuations of the organochlorine levels between samples. On the other hand, the absence of PCBs residues in the examined samples indicates the cleanliness the ingredients of theses cheese samples from this industrial environmental pollutants.

From aforementioned results, its concluded that the organochlorine compounds were detected in relatively low levels in the examined samples, although little samples were recorded organochlorine compounds in levels exceeded the permissible limits. The very long persistent of organochlorine compounds in the environment ***(Gao et al.,* 2013)** may explained this result. The continuous monitoring of organochlorine compounds in the milk products and the others greasy foods is recommended.

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