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Original Research Article

Risk assessment of some residues in milk and some dairy products

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ABSTRACT

Milk and dairy products are an important exposure route for organochlorine pesticides (OCPs) to humans. For this reason milk, kareish cheese and yoghurt analyzed for the presence of fourteen organochlorine pesticides namely Alpha HCH, Gamma HCH, Delta HCH, Aldrin, Gamma Chlordane, Endosulfan, Dieldrin, Endrin, , p,p'-DDE, p,p'-DDD, p,p'-DDT and methoxychlor using gas chromatography with electron capture detector (GC-ECD. A total of 90 random samples of raw cow's milk samples and dairy products (30 of each) were collected from 4 districts in Fayoum Governorate (Fayoum, Etssa, senoris and Tamia) during a period from September 2017 to January 2018. The results indicated that 6.60%(6/90) , 5.50%(5/90), 25.55%(23/90), 13.33%(12/9), 11.11%(10/90), 1.11%(1/90), 18.88%(17/90), 8.88%(8/90), 15.55%(14/90), 17.77%(16/90) and 22.22%(20/90) of the examined samples were contaminated with Alpha HCH, Gamma HCH, Delta HCH, Aldrin, Gamma Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDE, p,p'-DDD and methoxychlor,respectively. None of the examined samples revealed the presence of Dichlorodiphenyltrichloroethane (p,p'-DDT), Heptachlor and Heptachlor epoxide.

Generally the concentrations in the examined samples followed the order of Delta HCH > methoxychlor > Gamma HCH > p,p'-DDD > Dieldrin > Aldrin > Gamma Chlordane > Endosulfan > p,p'-DDE > Endrin > Alpha HCH.

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1. Introduction

Milk and dairy products play a significant role in human diet, regardless the consumers' age. It considered as a complete food because it is a good and cheap source of protein, fat and contains many valuable nutrients. Pesticides were used in agriculture and public health programs for combating pests and vector-borne disease. Uncontrolled and random application of these pesticides may led to significant contamination of food commodities **Ismail and Elkassas, (2016).**Milk-producing animals may be exposed to pollutants via contaminated feed, water, or air **Deti *et al*, (2014).**Foods, particularly animal-based foods, are the most important source of human exposure for many persistent organic compounds including OCPs. **Alcock *et al*, (2000**). Organochlorine pesticides due to their lipophilic properties are initially stored in fat-rich tissues and translocated and excreted in milk. Therefore, the consumption of dairy products together with other contaminated food may expose consumers to unexpected levels of organochlorine pesticides(OCPs). Some of OCPs have been considered as ‘‘endocrine-disrupting chemicals" **Colborn *et al*, (1993) and Kalpana, (1999)** and carcinogenic substances **Surendranath *et al*, (1998)** OCPs accumulate in fat-rich food products including dairy products, such as kareish cheese and raw milk **Salem *et al*, (2009)**.

The presence of organochlorine pesticides in raw milk and dairy products has been reported in different countries and regions.Therefor, this study quantify the concentrations of organochlorine pesticides residues in raw milk and dairy products in El Fayoum Governorate, Egypt.

2. Materials and methods

2.1. Sampling:

A total of 90 random samples of raw cow 's milk, kareish cheese and yoghurt (30 0f eash) were collected from 4 districts in fayoum Governorate (Fayoum, Etssa, Senoris and Tamia) during a period from september 2017 to january 2018.

The raw cow's milk and yoghurt were collected from super markets while kareish cheese were collected from public markets in villages .All milk samples examined for heat treatment by Storch's test (Lampert, L.M.1975)

All samples were labeled to identify the source, site and date of sampling, and transferred to central agricultural pesticides laboratory,Doki,Giza in boxes containing ice bags where they kept at -20 ^{o}C till further analysis.

2.2. Chemicals and reagents

Acetone, n-Hexane, Dichloromethane, Acetonitrile, Chloroform and Methanol of pesticides residue (PR) grade were purchased from Alliance Bio, USA.Petroleum ether, diethyl ether and anhydrous sodium sulfate, of PR grade were purchased from Merck (Germany). Analytical standards of Alpha HCH, Gamma HCH, Delta HCH, Heptachlor, Heptachlor epoxide, Aldrin, γ-Chlordane, Endosulfan, Dieldrin, Endrin, p,p'-DDE, p,p'-DDD and p,p'-DDT, methoxychlor were obtained from Dr. Ehrenstorfer, Augsburg in Germany, with purities larger than 98.5%,as illustrated in Fig(1).Florisil, 60-100 mesh, PR grade was purchased from (Sigma, USA), It was activated in an oven. at 130ºC for 24 hours column, before using in a column , it was cooled to room temperature in a desicator.

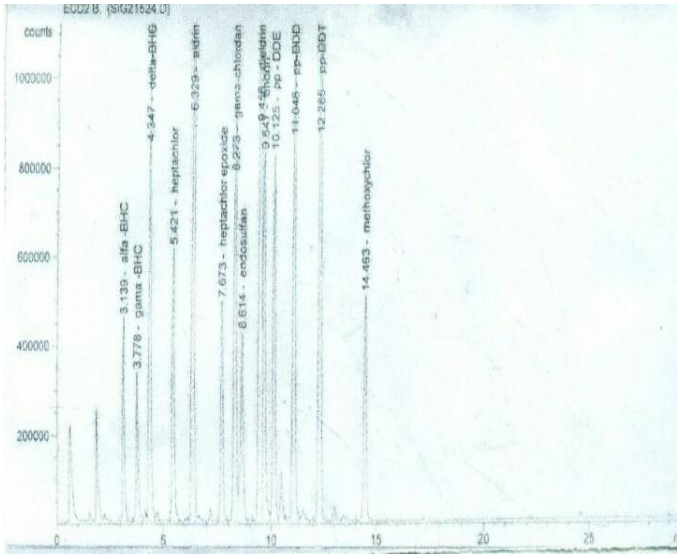

Fig.(1): GC-ECD Chromatogram of the Standards Mixture of organochlorine insecticides solution.

2.3. Sample preparation,Extraction and clean up:

Procedures for extraction of pollutants and lipids from raw milk ,kareish cheese and yoghurt described by (the Association of Official Analytical Chemists (AOAC, 1995) .Three grams of fat was dissolved into 40 ml petroleum ether, then partitioned three times into acetonitrile saturated with petroleum ether (3× 30 ml). Dilute acetonitrile fraction with saline (600 ml), partitioned it into petroleum ether (3× 100 ml). This was dried over anhydrous sodium sulfate, and concentrated at 30 C on a rotary vacuum evaporator to a volume less than 5 ml to be used for Florisil clean up (Kodba and Voncina, 2007).

Clean up of the extracted samples, to remove the residual fat, was performed by transferring the extract into a glass chromatographic column (25 mm i.d.) containing 25 g activated Florisil (60/100 mesh) topped with 1-cm layer of anhydrous sodium sulphate. The prepared column was rinsed with 100 ml petroleum ether, and then the extracted sample was transferred onto the column. The column was eluted with 300 ml eluent (20% dichloromethane + 80% petroleum ether). The collected eluate was concentrated to dryness on a rotary vacuum evaporator and dissolved in hexane to a volume of 5 ml (Alawi et al., 1992).Each extract was transferred to 2-ml injection vials to be ready for the analysis with the electron capture gas chromatography.

2.4. Determination of organochlorine pesticide residual concentrations:

The OCP residues were determined by analysis of samples using a gas liquid chromatograph (Hewlett-Packard Model 6890) equipped with electron capture detector Ni63, a split/splitless injection inlet, capillary column capability and a 7683A autosampler. Nitrogen was used as a carrier at flow rate of 3 ml /min. The chromatograph oven temperature was programmed from an initial temperature 160 (2 min hold) to 240 ºC at a rate of 5º C /min and was maintained at 240ºC for 20 min. Injector and detector temperatures were maintained at 260 and 320 ºC, respectively. The sample volume injected was 1µl.

2.5. Recovery studies

The method used for OCPs determination in raw milk and dairy products was was accredited by the United Kingdom Accreditation Service (UKAS) in 2003, as part of the ISO/IEC 17025 accreditation process of the laboratory (Salem et al., 2009).

Recovery study was performed on dairy products spiked with pesticide standards after extraction and solvent evaporation. The recovery values were calculated from peak area of the chromatograms obtained with standards of the OCPs. Detection limits is the lowest concentrations of the residues in each of the matrices measured at the GC. Blank analyzes

were performed to check interference from the sample. Samples were analyzed in duplicate and represent the arithmetic mean. The detection limits, the average recoveries with their standard deviations (SDs) of OCPs are shown in Table (1). The average recoveries of OCPs in raw milk and raw milk products were from 73.41% to 88.18 % which indicates that the reproducibility of the method was satisfactory.

Pesticide	LOD	LOQ	RSD %	% Rec	RT
α-BHC	0.005	0.015	17	73.41	3.139
Ɣ-BHC	0.005	0.015	17	82.13	3.778
Δ-BHC	0.001	0.004	15	79.21	4.347
Heptachlor	0.01	0.03	15	75.11	5.421
h.epoxide	0.001	0.003	17	77.14	6.329
Aldrin	0.01	0.05	13	78.42	7.673
Ɣ-chlordan	0.001	0.004	14	88.13	8.273
endosulfan	0.01	0.04	13	73.44	8.614
dieldrin	0.001	0.003	14	81.22	9.456
endrin	0.001	0.003	15	74.41	9.647
pp DDE	0.002	0.005	14	84.13	10.125
pp DDD	0.002	0.005	12	85.14	11.048
pp DDT	0.002	0.005	16	88.13	12.285
methoxychlor	0.001	0.004	13	79.13	14.463

Table (1) : (LOD) limit of detection mg/kg , (LOQ) Limit of Quantitation mg/kg , (RSD) relative standard deviation %, (Rec) average recoveries % and (RT) retention time/ mint of organochlorine pesticides in raw cow 's milk, Kareish kareish cheese and yoghurt sampling GC-ECD (organochlorine validation parameters).

3. Results

3.1. Variations in organochlorine among the different dairy products

Amongst the dairy products examined (milk, kareish cheese and yoghurt) in the present study, the incidence of contamination of all OCPs detected in raw milk were higher than those detected in other products (Fig. 2). The order of contamination in the analyzed samples was, raw milk (93.33 %) heigher than kareish cheese (86.66 %) and yoghurt (86.66 %).

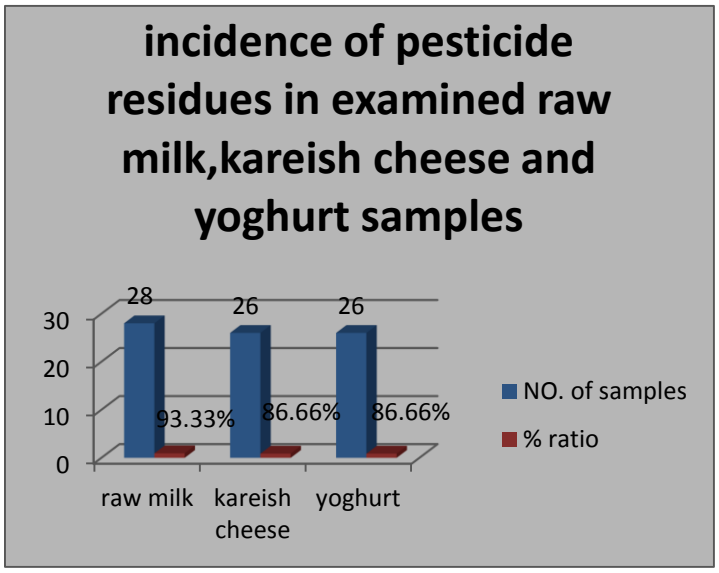

Fig. 2 Incidence of pesticide residues in examined raw milk, kareish cheese and yoghurt samples

3.2. Incidence of contamination with the different organochlorines

Generally, the incidence of contamination of the examined samples by the OCPs followed the order of Delta HCH > methoxychlor > Gamma HCH > p,p'-DDD > Dieldrin > Aldrin > Gamma Chlordane > Endosulfan > p,p'-DDE > Endrin > Alpha HCH.Fig (3)

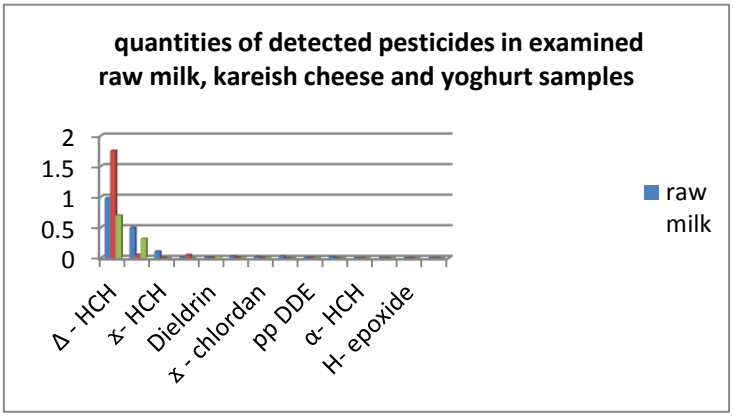

Fig (3) quantities of detected pesticides in examined raw milk, kareish cheese and yoghurt samples.

3.3. Organochlorine pesticide residues in raw milk, kareish cheese and yoghurt

4. discussion:

4.1. HCHs (hexachlorocyclohexane isomers)

Among three different isomers of HCH (α-isomer, Ɣ-isomer and Δ -isomer), α- HCH presented in 1 (3.33%), 2 (6.66 %) and 3 (10%) of raw milk, kareish cheese and yoghurt examined samples with mean concentrations of 0.001, 0.0011 ± 0.0012 and 0.00013 ± 0.00006mg/kg, respectively.Ɣ-HCH presented in 2 (6.67 %) , 2 (6.67 %) and (3.33 %) samples of raw milk, kareish cheese and yoghurt at mean concentrations of 0.054 ± 0.06 ,0.0025 ± 0.001 and 0.0001mg/kg.

While Δ- HCH detected in 10(33.33%) , 6 (20 %) and 7(23.7 %) of raw milk, kareish cheese and yoghurt samples at mean concentrations of 0.098 ± 0.09 , 0.29 ± 0.3 and 0.099 ±0.1mg/kg . Concentrations of α- HCH and Ɣ-HCH were below values setted by (EU,2014) , while concentrations of Δ- HCH exceeded the MRLs in 7 raw milk, 5 kareish cheese and 3 yoghurt samples. **Salem *et al*, (2009)** detected α- HCH and Ɣ-HCH in cheese and yoghurt samples at mean concentrations of 0.027 ,0.44 mg/kg and 0.030 , 0.22 mg/kg while Ɣ-HCH could not be found at any milk samples.

4.2. Aldrin, Dieldrin and Endrin

Aldrin detected in 4 (13.33 %) of milk, kareish cheese and yoghurt at mean concentrations of 0.008 ± 0.015 ,0.0022 and 0.0011 mg/kg.These concentrations were below values setted by (FAO/WHO,EU) except one raw milk sample exceeded the MRLs. However, Aldrin, Dieldrin and Endrin have not been detected in any of the samples analysed by **(Salem et al., 2009)**.

4.3. Heptachlor and Heptachlor epoxide

Heptachlor and Heptachlor epoxide have not been detected in any of raw milk, kareish cheese and yoghurt samples in our study. However , Heptachlor was detected in concentrations exceeded (FAO/WHO) MRLs in two raw milk samples with a mean concentration of 0.026 mg/ kg ***Salem et al*, (2009)**.

4.4. DDTs (dichlorodiphenyltrichloroethane and its metabolites)

The pp -DDE was detected in raw milk, kareish cheese and yoghurt samples in this study at mean concentration of 0.0023 ± 0.003 , 0.001 ± 0.001 and 0.002 ± 0.001 mg/kg, While pp –DDD founded at mean concentration of 0.0022 ± 0.002 , 0.007 ± 0.1 and 0.0002 ± 0.0001 mg/kg.These concentrations were detected in levels lower than the MRLs. DDT was not detected at any detected samples. Avancini *et al*, (2013) detected p,p -DDE and p,p –DDD in bovine raw raw milk samples which is nearly similar to our result had values lower than the reference values. While higher level of p,p -DDE, p,p -DDD and p,p -DDT obtained by Ismail1 and Elkassas, (2016) above their respective MRLs recommended by FAO/WHO and European Union legislation. Lower pp -DDE concentration detected in kareish cheese analyzed in Maharashtra Pandit *et al*, (2002) and Mumbai, India Pandit and Sahu, (2002). In contrast, higher levels were detected in kareish cheese in Ghana Darko and Acquaah, (2008) and Spain (Suarez *et al*, 1998) and in yoghurt from Ghana Darko and Acquaah, (2008).

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