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THE EFFECT OF HEAT TREATMENT AND BACTERIAL FERMENTATION ON SOME ORGANOCHLORINE PESTICIDE RESIDUES IN RAW MILK

(With 4 Tables)

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تأثير المعاملات الحرارية والتخمر البكتيرى على متبقيات بعض المبيدات الكلورينية العضوية فى اللبن الخام

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تم جمع ٤٠ عينة عشوائية من اللبن الخام المباع بمدينة الاسكندرية وذلك لتقدير مدى تواجد وكمية متبقيات بعض المركبات الكلورينية العضوية بها وذلك باستخدام جهاز الكروماتوجرافى الغازى السائل وتشمل اللندان ود.د.ت ود.د.د. ود.د. اى والالدرين والديلدرين. وقد أثبتت الدراسة ان متبقيات مبيدات كل من اللندان ود.د.ت ود.د.د ود. د. اى هى أكثر تواجدا فى عينات اللبن الخام المباع بالأسواق والتى تم فحصها وقد وجدت بمستويات متفاوتة بينما كان مبيد الديلدرين متواجد فى عدد قليل من العينات ومن ناحية اخرى اظهرت الدراسة ان متبقيات مبيد الالدرين لم يمكن الكشف عنه فى جميع عينات اللبن الخام. كما تمت مقارنة مستويات متبقيات المبيدات الحشرية المكتشفة بالحدود القصوى المسموح بتواجدها والمحدده من قبل منظمة الصحة العالمية ومنظمة الاغذية والزراعة الدولية (FAO/WHO) حيث وجد أن مبيدات اللندان ود.د.ت ود.د.أى قد زاد عن الحد المسموح به فى ٩٧.١ % ، ٢.٧ % ، ٢.٨ % على التوالى مما يشكل خطورة على صحة الانسان المستهلك لهذا اللبن. وقد وجد أن لكل من البسترة والغليان تأثير معنوى فى تقليل قيمة متبقيات المبيدات فى اللبن وقد أدت عملية البسترة الى اختزال كل من د.د.ت ود.د. أى بنسبة ٢٩.٧٩ % ، ٢١.١١ % بينما أدت عملية الغليان الى اختزال هذه المركبات الى ٧١.٢٨ % ، ٧٠.٣٥ % على الترتيب وعلى العكس لوحظ ثبات كل من الديلدرين واللندان عند معاملة اللبن حراريا بالبسترة والغليان. بينما وجدت زيادة فى نسبة د.د.د. ومن ناحية اخرى وجد أن تصنيع اللبن الى زبادى له تأثير معنوى فى تكسير متبقيات المبيدات فى اللبن واختزال كل من د.د.ت. واللندان والديلدرين بالنسب الآتية

٦٥.٨٣ % ، ٥٦.١ %، ٨.٢ % على التوالى. ونوصى بوجوب اتخاذ هذه البيانات والنتائج كقاعدة للرقابة المستقبلية على متبقيات المركبات الكلورونية فى اللبن المنتج بمحافظات مصر المختلفة. كذلك التعامل معها من حيث اهميتها وتأثيرها على الصحة العامة للمستهلك وكيفية الإقلال أو التخلص من هذه الملوثات بمعالجة اللبن حراريا وميكروبيولوجيا (التخمر).

SUMMARY

A total of 40 random market raw milk samples were collected for detection of organochlorine pesticide residues using gas liquid chromatography. The obtained results from this study revealed that pesticide residues which most often found in examined milk samples were γ-HCH "lindane", P-P ′-DDT, P-P ′-DDD and P-P ′-DDE. While residues of dieldrin could be detected in a few number of samples, on the other hand residues of aldrin could not be detected in all examined samples.The detected levels of organochlorine pesticide residues were compared with the maximum residues limits of FAO / WHO. Increasing percentages of lindane, P-P ′-DDT and P-P ′-DDE to 97.1%, 2.7% and 2.8%, respectively, may act as health hazard for man consuming such milk. It has been found that both pasteurization and boiling of milk were effective in minimizing the pesticide residues. The reduction percentages of P-P ′-DDT and P-P ′-DDE residues were 29.79 and 21.11 % in response to pasteurization. While, values of 71.28 and 70.35 % were resulted by boiling. In contrast, residues of P-P ′-DDD were increased, while lindane and dieldrin were relatively constant under used temperatures. On the other hand,the processing of milk into yoghurt has a significant role in degradation of the insecticide residues in milk. The reduction percentages for each DDT and lindane residues were 65.83 and 56.1 % resp., while it was 8.2 % for dieldrin residues. This data can be used as guidline and a base line for future monitoring of organochlorine residues in milk in the Governorates to deal with public health aspects and to minimize the organochlorine pesticides in milk by heat and microbial treatments (fermentation).

Key words: *Raw milk, heat treatment, bacterial fermentation, organochlorine pesticide.*

INTRODUCTION

Organochlorine Compounds (OC) are dangerous pollutants due to their toxicity, stability and solubility in fat and long term biological half life (Serrano *et al.,* 2008). Organochlorine Pesticides (OCPs) like Dichlorodiphenyltrichloroethane's (DDTs), dieldrin, aldrin, and Hexachlorohexanes (γ-HCHs) were limited or banned chemicals by Global Stockholm Convention (Zhiwei *et al.,* 2007 and Wang *et al.,* 2008). The OCPs present in the environment contains agricultural and industrial chemicals (Snedeker, 2001). Appropriate and timely usage of pesticides provides a number of benefits for food production; otherwise they may become very harmful for human health. Their detection in the food and drinking water is an indication that they may enter food chain (Snedeker, 2001 and Kelly *et al.,* 2007). The highly lipophilic features of OCPs increases their likelihood of being found in fatty foods such as milk products (Darko and Acquaah, 2008). The toxicity of OCP residues shows their effects slowly in the chose of time.

The accumulated OCP residues in the fat tissue affects some vital organs such as heart, kidneys, liver and some glands e.g., thyroid & mammary glands and testicles (John *et al.,* 2001; Pandit *et al.,* 2002; Bogialli *et al.,* 2004). While their acute toxic effects could be easily diagnosed, the effects of long term exposures could not be identified as easy as the acute one. Therefore, it is necessary to monitor their residues in the food and the environment (Darko *et al.,* 2008). These pollutants are excreted into the milk by simple diffusion as milk is more acidic than plasma also considerable portion of milk is lipid which considered an important channel for excretion of fat soluble pesticides (Casarett and Doull, 1980; Battu *et al.,* 2004; Jafari *et al.,* 2008).

Up to date, no entirely safe pesticides have been developed, so the presence of significant amounts of OCP residues in milk is undesirable. As consequence these compounds have been reviewed by regulatory governmental agencies, including Food and Agriculture Organization for establishing an acceptable daily intake (ADI) and tolerance limits for pesticides (FAO/WHO, 2005). Therefore, it is plausible to throw light on the presence of OCP residues in raw market milk.

The effect of heat treatments on OCP residues occurring in raw milk have been reported by several workers (Leshchev *et al.,* 1972; Skibniewska and Smoczynski, 1985; Korolev, 1987; Abdrabo *et al.,* 1989; Fekry and Bahout, 1992; El-Hoshy, 1997; Zhang *et al.,* 2005). However, the influence of milk processing on the level of OCP residues was determined by Mendez *et al.* (1979); Serjeeva (1979); Sreenivasa *et al.* (1983); Abdel-Shaheed (1984); Ali *et al.* (1993); Rajashekar *et al.* (2007).

The worldwide use of pesticides and their hygienic importance make it urgent to know as much as possible a more information about this subject. Therefore, this work was carried out to study:

1- The presence of organochlorine pesticide residues in raw milk in Alexandria City.
2- The influence of pasteurization and boiling on the level of pesticide residues in raw milk.
3- Effect of yoghurt processing on the content of pesticide residues in milk.

MATERIALS and METHODS

*** Occurrence of OCP residues in raw milk:**

1 - Collection and preparation of milk samples:

Forty composite raw milk samples were collected in triplicate from street peddlers and dairy shops in Alexandria city, Egypt. The samples were collected in glass containers of one liter capacity and immediately transferred to the laboratory for analysis. If analysis delayed for a few days, 10ml milk was perfectly mixed in small glass container with screw capped stoppers and kept deeply frozen at -40ºC for OCP residues analysis.

2 - Detection of heat-treated milk:

Each sample of raw milk was subjected to Guaiac test (Schonberg, 1956) to prove that the milk samples were raw.

3 - Glass - ware:

All glasses were prepared for analysis according to steps described by Suzuki *et al.* (1979); Kodba and Voncina (2007); Seccia *et al.* (2008).

4 - Chemical and solvents:

- Analytical grade of anhydrous sodium sulphate was dried at 135ºC for several days prior to use.
- Florisil, PR grade (60-100 mesh) was prepared by extensive washing with water, drying at 100ºC and then overnight firing at 550ºC, the washed, fired Florisil was kept at 135 ºC till use.
- Acetonitrile, ethanol, N-hexane, benzene and ethyl acetate were distilled from glass wares subjected to general purity test.

5 - Gas liquid chromatography (GLC):

ATI UNICAM-610 series gas chromatography with split less injector, equipped with an electron capture detector (Ni^{63}), flame photometric detector and ATI UNICAM chromate jet integrator was used. The injector was connected to 30m x 0.22mm I.D. Silla capillary column containing DP-17 with 0.25 um film thickness. Nitrogen was used as a carrier gas, at flow rate of 2ml/min. The injector temperature was 260ºC and the detector temperature was 300ºC. Split less injector was performed at oven temperature of 250ºC. The OCP residues were quantitatively determined by comparison with standard solution injected under identical GLC conditions.

6 - Pesticides reference standards:

Generally, 0.01 gm was accurately weighed and quantitatively transferred to a 100 ml volumetric flask as a stock standard solution using n-hexane. An aliquot from each of these stock standard solutions was diluted to a proper concentration from which a 5µl. Injection gives a half full scale deflection peak. These working standard solution were used in determining the retention times of each of the investigated OCPs. All reference standards were kindly provided by the National Food Administration, Food Research dept., Uppsala, Sweden.

7 - Organochlorine pesticides under investigation:

Investigated pesticides includes different isomers of Hexachlorocyclohexane (γ-HCH "lindane"), DDT complex (P-P′-DDT, P-P′-DDD & P-P′-DDE), Aldrin and Dieldrin.

8 - Extraction and clean-up procedures:

The method used for extraction and clean-up procedures was described by A.O.A.C. (1990) and Salem *et al.* (2009).

9 - Recovery experiment:

Duplicate sub-samples from raw milk were subjected to all the previously mentioned steps of extraction, clean up and gas liquid chromatographic determination. To one of the duplicate sub-samples, an aliquot of the mixture contained the organochlorine pesticides standards were added. The amounts recovered from each pesticide was calculated and compared to the other duplicate to which no pesticides were spiked. The difference between the duplicates indicates the actual recovery of the pesticides.

Retention times and recovery percent of OCPs from fortified milk samples (Saad *et al.*, 2008).

Pesticides	Retention time	Fortified level	Recovery %

	(minutes)	(ppm)	
Γ-HCH "lindane"	5.21	0.2	84.30
P-P΄-DDT	11.69	5.0	91.80
P-P΄-DDD	10.05	5.0	100.14
P-P′-DDE	10.29	4.0	88.90
Aldrin	6.15	0.8	84.14
Dieldrin	8.79	1.6	98.90

**** Effect of heat treatments on the levels of OCP residues in milk:**

1 - Pasteurization:

Five positive milk samples were heated to 62.8 ºC for 1/2 hours then cooled immediately to 10 ºC (laboratory pasteurization).

2 - Boiling:

Five milk samples contained various types of present pesticide residues were heated with stirring to boiling point for five minutes then cooled.

***** Effect of yoghurt processing on the levels of OCP residues in milk:**

Five positive milk samples were heated to 85ºC in water bath for 20 minutes, cooled to 40:42 ºC, inoculated with 2% yoghurt starter culture (1:1). *Streptococcus thermophilous* and *Lactobacillus bulgaricus*, then incubated at 40 ºC for 3-4 hours until it was coagulated and then refrigerated over night. Yoghurt samples were then analyzed for pesticide residues.

****** Statistical analysis:**

The obtained data was analyzed statistically using F- test and T-test according to Snedecor and Cochran (1969).

RESULTS

The obtained data are recorded in Tables 1, 2, 3 & 4.

Table 1: Residues levels of organochlorine pesticide residues in raw milk samples (ppb).

Pesticides Residues	Incidence (Positive samples)	Levels (ppb)

	No./40	%	Min.	Max.	Mean ± S.E.
γ-HCH "lindane"	35	88	1	53	42 ± 0.013
P-P′-DDT	37	93	3.2	59	9 ± 0.023
P-P′-DDD	26	65	0.56	29	3.67 ±0.018
P-P′-DDE	36	90	2.6	66	20.2 ±0.009
Aldrin	0.0	0.0	0.0	0.0	0.00±0.000
Dieldrin	6	15	0.52	2.93	1.63 ±0.030

Table 2: Organochlorine pesticide residues of positive examined raw milk samples on basis of maximum residues limits (MRL*).

Pesticides residues	Maximum residues limits (MRL) (ppb)	Within MRL		Over MRL	
		No.	%	No.	%
γ-HCH "lindane"	10	1	2.9	34	97.1
P-P′-DDT	50	36	97.3	1	2.7
P-P′-DDD	50	26	100	0.0	0.0
P-P′-DDE	50	35	97.2	1	2.8
Aldrin	6	0.0	0.0	0.0	0.0
Dieldrin	6	6	100	0.0	0.0

The maximum residues limits of organochlorine pesticide residues in milk proposed by FAO / WHO (2005).

Table 3: Effect of common heat treatments on the levels of organochlorine pesticide residues in raw milk (ppb).

Type of treatments	Raw milk	Pasteurization			Boiling		
OCPs	Mean ± S.E.	Mean ± S.E.	Degradation %	Increasing %	Mean ± S.E.	Degrada-tion %	Increasing %
γ-HCH "lindane"	28±0.009	27±0.007***	1.00		0.00±0.000***		
P-P′-DDT	18.8±0.037	13.2±0.026***	29.79		5.4±0.011***	71.28	
P-P′-DDD	25±0.066	31.5±0.078***	-	26	41.2±0.0098***		64.8
P-P′-DDE	19.9±0.053	15.7±0.044***	21.11		5.9±0.009***	70.35	
Dieldrin	0.92±0.024	0.91±0.023 NS	1.10		0.00±0.000		

N.B. *** very significant change between means (at $p \leq 0.001$)
NS = Non significant change between means

Table 4: Effect of yoghurt processing on the levels of organochlorine pesticide residues in raw milk (ppb).

Type of OCPs	Raw milk (before heat TTT) Mean ± S.E.	Yoghurt		
		Mean ± S.E.	Loss	Degradation %
Total DDT	99.8 ± 0.109	34.1 ± 0.092***	65.7	65.83
Lindane	41 ± 0.013	18 ± 0.005***	23.0	56.1
Dieldrin	85 ± 0.028	78 ± 0.019***	7.0	8.2

N.B. *** very significant change between means (at $p \leq 0.001$)

DISCUSSION

1 - Occurrence of OCP residues in raw milk:

The residual properties and rate of the chemical which make them effective for long periods have been a cause of considerable importance in milk pollution. It is obvious clear from the results illustrated in Table 1 that γ-HCH (lindane) was present in 88% of the examined raw milk samples. The residues levels ranged from 1.0-53.0 ppb, with a mean level of 42 ± 0.013 ppb. Similar results were obtained by El-Hoshy (1997); Francois *et al.* (2002); Saad *et al.* (2008). More or less similar levels were reported by Abouzeid (1994); Cerkvenik *et al.* (2000); Pardio *et al.* (2003).

According to the maximum residues limits recorded by FAO / WHO (2005), the results presented in Table 2 revealed that 97.1% of the examined raw milk samples containing lindane beyond the maximum residues limits. The sources of food contamination by lindane were identified by Viccellio *et al.* (1998) used as pesticide, scabicide and pedialocides. It was the last OCPs to be banned from agriculture practice in France in 1988 and it has been banned in Canada, United States, China, Soviet Union and Australia in 1971, 1976, 1983, 1990 and 1994, respectively, (Li, 1999 and Francois *et al.,* 2002). Lindane still used extensively in a number of African countries, India and Brazil (Li, 1999), derived from many industries activities (Frank and Ripley, 1990) and high persistence of lindane in the environment (Uhnak *et al.,* 1986).

P-P′-DDT is considered one of the most important pollutants in the environment, with a potential toxicity for all biological systems, as it accumulates in human tissues. The levels of p-p′-DDT concentrations were ranged from 3.2 to 59 ppb, with a mean level of 9 ± 0.023 ppb. 93% of examined market raw milk samples containing p-p′-DDT Table 1. Nearly similar findings were reported by Abouzeid (1994) and Saad *et al.* (2008), who recorded that the mean levels of p-p′-DDT were 8.902 and 9.000 ppb in their examined milk samples,respectively. On the other hand, high figures were recorded by several workers (Cerkvenik *et al.*, 2000; Francois *et al.,* 2002; Waliszewski *et al.,* 2003; Zhong *et al.,* 2003). Lower levels were obtained by Aman and Bluthgen (1997), they mentioned that p-p′-DDT was detected with a mean level of 7.67ppb.

DDT is considered one of the most important pollutants in the environment and distributed widely in different classes of animal feeds. The major sources of DDT in the environment arise from application of DDT for control of pests in the animal feeds, flies and ectoparasites. Although DDT was banned in the early 1970, it was still manufactured in the United States and exported at rate of one ton/day for developing and tropical countries in 1994 (Smith, 1995).

It is evident from the results illustrated in Table1 that p-p′-DDD residues were detected in 65% of the examined market raw milk samples. The levels ranged from 0.56 to 29 ppb, with a mean level of 3.67± 0.018 ppb. Nearly similar findings were obtained by Abouzeid (1994). On the contrary, Ali *et al.* (1993) and El-Hoshy (1997) recorded much higher levels of p-p′-DDD in the examined raw milk samples.

Inspection of Table1 revealed that p-p′-DDE residues were detected in 90% of samples. The values ranged from 2.6- 66 ppb, with a mean level of 20.2 ± 0.009 ppb. More or less similar levels were obtained by Awasthi and Ahuja (1995); Wong and Lee (1997); Storelli *et al.* (2001); Francois *et al.* (2002) and Bayoumi (2003). Level of DDT in milk is lower than its metabolites p-p′-DDE. These results proven that p-p′-DDT undergo several metabolic degradation to its metabolites as p-p′-DDE (IDF, 1991). This data might indicate DDT, aging DDT stored in food results in higher p-p′-DDE concentration as derivatives Fries *et al.* (1972); El-Marsafy *et al.* (1999); Eskenazi *et al.* (2009).

The results displayed in Table 2 revealed that 2.7%, 0% and 2.8% of market raw milk samples contained p-p′-DDT, p-p′-DDD and p-p′-DDE, respectively, beyond the maximum residues limits recorded by FAO / WHO (2005).

It is obvious clear from the results presented in Table1 that dieldrin was detected in 15% of samples. The concentrations various from 0.52 to 2.93 ppb, with a mean level of 1.63± 0.030 ppb. Similar results were obtained by Bluthgen *et al.* (1984); Abouzeid (1994). Considerable higher levels were recorded by El-Hoshy (1997); Wong and Lee (1997); Francois *et al.* (2002). On the other, Elafi *et al.* (1997) reported that dieldrin was not detected in any of analyzed milk samples in Libya.

Results presented in Table 2 indicated that all examined milk samples contained dieldrin within the permissible residues limits recorded by FAO / WHO (2005). Although dieldrin is used in Egyptian agriculture since 1970, it was detected at lower levels in any of analyzed raw milk samples according to FAO / WHO (2005). This may be due to that the dieldrin was destroyed by acid produced by soil bacteria (UNEP/IOC/IAEA, 1986).

The results shown in Table 1 revealed that aldrin could not be detected in all the examined raw milk samples. Similar results were recorded by Abouzeid (1994) and El-Hoshy (1997). The absence of aldrin in the examined raw milk samples could be attributed to its continuous degradation into dieldrin within the living tissues through the different metabolic process (Dogheim *et al.,* 1988).

2 - Effect of common heat treatments on the levels of natural OCP residues in milk:

Table 3 revealed that highly significant ($p \leq 0.001$) reduction percentages of each p-p′-DDT and p-p′-DDE residues as result of pasteurization were 29.79 and 21.11%, respectively. likewise, highly significant ($p \leq 0.001$), reduction of 71.28 & 70.35%, respectively, were resulted due to boiling. On the other hand, p-p′-DDD residues were highly significantly ($p \leq 0.001$), increased at percentages of 26% and 64.8% due to pasteurization and boiling, respectively. These results are in accordance with that mentioned by Abdrabo *et al.* (1989) and Amr *et al.* (1996). Moreover, the increasing level of p-p′-DDD may be attributed to decomposing of p-p′-DDT& p-p′-DDE during thermal treatment into p-p′-DDD. The conversion of p-p′-DDT by thermal treatment into p-p′-DDD has reported by many authors (Hiroko *et al.,* 1971; Dick *et al.,* 1978; Heeschen *et al.,* 1978). It is evident from the data illustrated in Table 3 that lindane and dieldrin residues were thermo stable against pasteurization and boiling and non significantly changed. Similar finding was determined by Abdrabo *et al.* (1989).

3 - Effect of yoghurt processing on the levels of natural OCP residues in milk:

It is evident from the data illustrated in Table 4 that processing of milk into yoghurt highly significantly ($p \leq 0.001$) reduced the content of OCP residues at percentages of 65.83%, 56.1% and 8.2% for total DDT, lindane and dieldrin, respectively. These results are nearly similar to Ali *et al.* (1993). The less reduction in dieldrin residues may be attributed to inability of lactic acid bacteria to destruct this compound.

Generally, the results obtained about the efficiency of lactic acid bacteria in the degradation of pesticides in milk are coincided with those reported by Serjeeva (1979) who indicated that polluted milk with pesticides should be preferably processed into yoghurt or white pickled cheese. However, a contradictory opinion was given by Mendez *et al.* (1979).

From the public health point of view, organochlorines have been related to an increase in the incidence of some kinds of tumors, such as leukemia and solid tumors. Reproductive effects, due to anti-androgenic and estrogenic action, the incidence of abortion and the frequency of prematurely, have also been observed. The accumulation of the organochlorines in the adipose tissues is positively correlated to the increase in aging and could be implicated in development of aging diseases in man who consume the milk and milk products, such as Parkinson′s disease who stated by Nunes-Monica and Tajara-Eloiza (1998); Bogialli *et al.* (2004); Cedergreen *et al.* (2008). DDT is suspected to being a human carcinogen, this suspicion is based on the fact that DDT has been showen to cause liver tumor in mice Edward (1993). Likewise, Helleday *et al.* (1996); Bulut *et al.* (2010), concluded that DDT has adverse effect on human health in terms of inducing genetic recombination which is known to provoke a number of diseases including Cancer. Furthermore, Langnecker *et al.* (1997); Soderlund *et al.* (2002), reported that high level exposure to selected organochlorines (including DDT) in man appeared to cause abnormal functioning of the liver, skin and nervous system. Ruehlnam *et al.* (1998); Sereda *et al.* (2009), found that DDT inhibits the L-type Ca channel in vascular smooth muscle cells and evoke rapid relaxation of coronary vasculature. Thus inhibition of calcium influx in vascular smooth muscle cells explaining the acute vasodilator action of DDT.

In conclusion, the results of this study revealed that the levels of organochlorine pesticide residues in the examined raw milk samples were high and over the maximum residues limits in most of the examined samples which may constitute a possible public health hazards. The predominant compounds in milk were lindane, DDT and DDE. Hence from hygienic health point of view, milk containing pesticide residues should be directed to industrial manufacturing as some of these compounds affected by boiling and pasteurization, as well as, by high acidity produced during processing of fermented milk such as yoghurt. These data can be used as guideline and a base line for future monitoring of organochlorine residues in milk in the Governorates should be done to deal with public health aspects and efforts should be directed to minimize the organochlorine pesticides contamination in milk.

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