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Effect of Ras cheese manufacturing on the stability of DDT and its metabolites

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Residue levels of DDT were analyzed in 25 samples of liquid milk as well as 25 samples of Ras cheese collected from different regions in Great Cairo Governorates. Levels of DDT and its metabolites in liquid milk samples were higher than those in Ras cheese. The detected levels of pesticides in milk and cheese were lower than the maximum acceptable limits (MAL_s) recommended by FAO/WHO (1993). The reduction levels of total DDT in Ras cheese made from contaminated milk with different levels of DDT (0.1, 1.0 and 10.0 mg/kg fat) were 40.6, 33.9 and 25.5%, respectively, at the end of storage period. The isolated Ras cheese microorganisms reduced the total DDT residues by 10.8, 11.8 and 4.8% for Streptococci, lactobacilli and yeasts, respectively, at the end of incubation period.
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INTRODUCTION

Pesticides have played an important role in the dramatic increases in agricultural productivity which have been achieved in the developed world over the last few decades. Production and use of organochlorine pesticides has been declining in recent years. This suggests that residue problems will decrease but the persistence of these materials in the environment means that some residues will be encountered for many years (Fries *et al.*, 1969).

Organochlorine pesticides have not been intended for direct use on animals or feeds. This means that any milk contamination by these compounds results from either indirect contamination from environmental sources or improper use of the pesticides (Fries *et al.*, 1969). Several investigators have attempted to reduce the organochlorine pesticide contents of dairy products by certain processing techniques, chemical treatments of milk, or by administering drugs. The adventitious removal of residues by processing is influenced by the type of food, location of pesticide and especially by the processing operation (Downey, 1972; Al-Alfy, 1981; Abou-Donia *et al.*, 1985; Anb, 1987; Abou-Arab, 1991).

Chlorinated hydrocarbon pesticides have been considered very resistant to physical and microbial degradation. Recent interest has focused on the mechanisms by which microorganisms degrade these materials to less harmful products. Several investigators (Barker *et al.*, 1965; Chacko *et al.*, 1966; Mendel & Walton, 1966; Wedemeyer, 1966, 1968; Langlois *et al.*, 1970; Rachev *et al.*, 1974) have reported the reductive dechlorination of DDT to DDD by microorganisms. Although the DDT–DDD conversion has been reported in a number of systems, the exact mechanism of the reaction is still obscure. More recent data by Kim (1970) and Abou-Arab (1991) indicate that gram-positive lactic cultures were unable to cause any measurable degradation of aldrin, DDT or γ-BHC.

Ras cheese is considered as one of the popular hard cheese types which is highly regarded by Egyptian consumers. It however requires a long ripening period ranging from 4 to 6 months. With this in view, the present study was conducted to explore the incidence of the highly persistent pesticides (DDT and its metabolites) that may reach liquid raw milk and dairy products. The effects of manufacturing process and storage period of Ras cheese as well as the effect of isolated microorganisms from Ras cheese on the degradation of DDT and its metabolite residues are examined.

MATERIALS AND METHODS

Materials

Twenty five samples of each bulk milk and Egyptian Ras cheese were collected randomly from small dairy herds and different regions in great Cairo governorates (Cairo, Giza and Kalubia). All samples were collected during the period of January to July of 1995 to determine DDT and its metabolites.

Ras cheese making

Ras cheese was manufactured by the conventional method described by Abd-El-Tawab (1963) from contaminated buffalo's milk with different three levels of DDT (0.1, 1.0 and 10.0 ppm based on milk fat basis). A common starter culture was used. Pure strains of *Streptococcus thermophilus* (EMC 1225) and *Lactobacillus bulgaricus* (EMC 1322) were obtained from Egypt Microbial Collection at Microbiological Resource Centre (MIRCN), Faculty of Agriculture, Ain-Shams University. Animal rennet powder obtained from CH-Hansen Laboratory, Denmark, was used as coagulant. The cheese moulds were coated with paraffin wax and stored for ripening at 12°C. Samples were taken for analysis during the different steps of manufacturing and storage period for 6 months.

Pesticide analysis

DDT and its metabolites were extracted from different samples using diethyl ether, petroleum ether and acetonitrile. The extracts were purified using column chromatography on florisil (60/100 mesh) and eluting with a solvent mixture of petroleum ether and diethyl ether 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 series II equipped with Ni^{63} electron capture detector and Integrator 3395, fitted with HP-1 capillary column (methyl silicon gum), 30 m×0.25 mm, 0.2 μm film thickness. The column oven temperature was programmed from 80 to 160°C at a rate of 3°C/min, held for 2 min, then increased to 220°C at a rate of 5°C/min and held for 20 min. Injection and detector temperatures were 220 and 300°C, respectively. Pesticide standards of 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (PP′-DDT): 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane (OP′-DDT); 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (PP′-DDE); 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chiorophenyl) ethane (OP′-DDE); 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane (OP′-DDD) were provided by environmental protection agency (EPA).

Microbiological analysis

Streptococci, Lactobacilli and yeast were isolated from Ras cheese using the MRS medium (Man *et al.*, 1960) for lactobacilli, Elliker medium (Elliker *et al.*, 1956) for streptococci and Malt agar (Marth, 1978) for yeast. One percent of each isolated organism inoculated in DDT-contaminated medium (about 1.00 ppm). The inoculate media were incubated at 30°C, 37°C and 25°C for streptococci, lactobacilli and yeast, respectively. Samples were taken daily up to 10 days for analysis of DDT and its metabolites.

RESULTS AND DISCUSSION

Monitoring of DDT and its metabolites in raw milk and Ras cheese

Residue levels of DDT and its metabolites in 25 samples of raw milk as well as 25 samples of Ras cheese collected from different governorates in great Cairo (Cairo, Giza and Kalubia) are shown in Table 1. 52% of raw milk samples were found to contain DDT. However, other metabolites were also detected. DDE showed higher levels than DDT. This result is not surprising as DDE is more stable and more lipid-soluble than DDD or DDT (Fries, 1972; Snelson & Tuinstra, 1979). Moreover, significant concentrations of DDD occur in milk when DDT is present in the cow's diet (Guenzi & Beard, 1967). A number of anaerobic, microbial systems convert DDT to DDD. On the other hand, Mendel & Walton (1966) reported that gastrointestinal microorganisms are the major source of this conversion in the rat. The levels of DDT in the raw milk samples in this investigation were lower than the levels recorded by Frank *et al.* (1970), Khandekar *et al.* (1981), Kalra *et al.* (1983) and Yokomizo *et al.* (1984). On the other hand, the obtained results are in a good agreement with Abdrabo *et al.* (1989) and Abou-Arab (1991). However the level of DDT in this study was higher than those obtained by Frank *et al.* (1985).

The frequency distributions of the DDT and its metabolites in Ras cheese samples were P.P-DDD

Table 1. Frequency distribution of DDT and its metabolites in raw milk and Ras cheese

DDT and its metabolites	Frequency of positive samples (%)		Mean levels of positive samples (mg/kg fat)	
	Raw milk	Ras cheese	Raw milk	Ras cheese
P.P-DDT	8	ND	0.056	—
O.P-DDT	4	ND	0.003	—
P.P-DDD	36	16	0.017	0.009
O.P-DDD	32	12	0.070	0.006
P.P-DDE	12	4	0.064	0.004
O.P-DDE	24	4	0.084	0.005
Total DDT and its metabolites	52	32	0.100	0.008

ND: not detectable.

(16%), O.P-DDD (12%), P.P-DDE (4%), O.P.-DDE (4%) and total DDT (32%) with different levels (Table 1). No O.P-DDT and P.P-DDT could be detected. This may be attributed to the rapid degradation rates of DDT to its metabolites. Several investigators (Barker *et al.*, 1965, 1966) have reported the reductive dechlorination of DDT to DDD by microorganisms. Moreover, DDT and its metabolites are fat-soluble and their residues associated with lipoprotein material (Li *et al.*, 1970) and phospholipids (Hugunin & Bradley, 1971). Comparing the results obtained with Ras cheese in the current study with some types of Italian cheese, data indicated that DDT (and its metabolites) was found to be lower than those reported by Beniforti (1971), Grasso *et al.* (1972) and Corvi *et al.* (1976). Regarding other types of cheese such as Edam, Tilsit, Rokpol and Romadure, data revealed that the level of total DDT in Ras cheese was lower than those detected in these other types (Smoczynski, 1973; Smoczynski & Jaworski, 1974; Yokomizo *et al.*, 1984).

The detected levels of DDT and its metabolites in Ras cheese were lower than those detected in the raw milk. DDT and its metabolites must be concentrated in curd cheese. Thus, the reduction levels in cheese may attributed to the effect of different steps during manufacturing as well as the effect of the microorganisms during storage and ripening.

In this investigation, the concentrations of DDT and its metabolites in both milk and Ras cheese were found at levels lower than the maximum acceptable limits (MALs) recorded by FAO/WHO (1993).

Effect of manufacture and storage of Ras cheese on DDT and its metabolite levels

The distribution of DDT and its metabolites in Ras cheese made from contaminated milk with three different levels of DDT are shown in Tables 2 and 3. Pasteurization at 72°C caused a reduction in total DDT by 2.97, 3.92 and 2.46% for contaminated milk with 0.1, 1.0 and 10.0 mg/kg fat DDT, respectively. This finding is in harmony with results obtained by Abou-Arab (1991) and Mann *et al.* (1950) who reported that pasteurization had little effect on the DDT levels in milk. On the other hand, Rachev *et al.* (1974) found that heat treatments on 93°C or 100°C for a few seconds had little effect on the P.P-DDT and its metabolites, P.P-DDE and P.P-DDD. However, Todorov *et al.* (1974) found 2% losses of DDT as effected by pasteurization did not affect greatly the reduction/elimination of DDT in milk.

Whey from cheese after pressing contained small amounts of DDT ranging from 0.002 to 0.3 mg/kg fat. This may be attributed to the absorption of DDT by the coagulated milk protein or its association with that portion of milk fat normally found in whey (Montoure & Muldoon, 1968). However, the remaining levels of total DDT after pressing were 91.1, 86.4 and 92.2% in cheese made from contaminated milk by 0.1, 1.0 and 10.0 mg/kg fat DDT, respectively. These results indicated that DDT is very resistant to processing techniques. DDT and its metabolites (as most of the chlorinated hydrocarbon pesticides) are lipid-soluble, thus they have been more frequently found in fatty portions of foods. So residue levels of DDT were much higher in cream, butter and cheese than skim milk and whey (Mann *et al.*, 1950; Beroza & Bowman, 1966).

Storage (ripening) periods for 6 months caused 40.6, 33.8 and 25.5% reduction in total DDT at the end of storage for cheese made from contaminated milk by 0.1, 1.0 and 10.0 mg/kg fat, respectively. This may be due to the effect of ripening microorganisms during storage. The reduction levels of total DDT during the storage period were 24.8, 23.4 and 19.2%, based on the levels of DDT in cheese made from contaminated milk, just after pressing. These results agree with those obtained by Montoure and Muldoon (1968) and Gertig and Moruszewska (1973).

Table 2. Effect of manufacturing process and storage of Ras cheese on DDT and its metabolites (mg/kg)

Phase	Cheese from contaminated milk by											
	0.1 mg/kg fat				1.0 mg/kg fat				10.0 mg/kg fat			
	DDT	DDD	DDE	Total	DDT	DDD	DDE	Total	DDT	DDD	DDE	Total
Raw milk (contaminated)	0.087	0.008	0.006	0.101	0.910	0.040	0.070	1.020	9.540	0.520	0.090	10.150
Pasteurized milk	0.082	ND	0.016	0.098	0.900	0.020	0.060	0.980	9.510	0.210	0.180	9.900
Curd	0.080	ND	0.014	0.094	0.900	0.011	0.050	0.961	9.500	ND	0.160	9.660
Whey at dipping	—	—	—	0.004	—	—	0.019	—	—	—	0.240	—
Whey from pressing	—	—	—	0.002	—	—	—	0.080	—	—	—	0.300
Cheese after pressing	0.080	0.006	0.006	0.092	0.820	0.011	0.050	0.881	9.000	0.2000	0.160	9.360
Storage period (month)												
1	0.076	0.006	0.006	0.088	0.800	ND	0.020	0.820	8.540	0.400	0.400	9.340
2	0.080	ND	ND	0.080	0.800	ND	ND	0.800	8.000	0.210	0.720	8.930
3	0.070	ND	0.008	0.078	0.770	0.010	0.010	0.790	7.900	0.212	0.641	8.753
4	0.060	0.004	0.006	0.070	0.700	0.020	0.016	0.736	7.500	0.020	0.751	8.271
5	0.040	0.009	0.015	0.064	0.600	ND	0.120	0.720	7.000	0.111	0.731	7.842
6	0.029	0.012	0.019	0.060	0.550	0.014	0.111	0.675	6.500	0.240	0.820	7.560

ND: not detectable.

Table 3. Reduction (%)* of total DDT during the manufacturing and storage of Ras cheese

Phase	Cheese from contaminated milk by		
	0.1 mg/kg fat	1.0 mg/kg fat	10.0 mg/kg fat
Raw milk			
Pasteurized milk	2.97	3.92	2.46
Curd	6.93	5.78	4.83
Whey at dipping	—	—	—
Whey from pressing	—	—	—
Cheese after pressing	8.91	13.6	7.78
Storage period (month)			
1	12.9	19.6	7.98
2	20.8	21.6	12.0
3	22.8	22.6	13.8
4	30.7	27.8	18.5
5	36.6	29.4	22.7
6	40.6	33.8	25.5

*Based on raw contaminated milk.

Table 4. Degradation of DDT and its metabolites as effected by isolated microorganisms during the incubation period

Incubation period (Days)	DDT and its metabolites (mg/l medium)											
	Streptococci				Lactobacilli				Yeasts			
	DDT	DDD	DDE	Total	DDT	DDD	DDE	Total	DDT	DDD	DDE	Total
Zero time	1.08	0.46	0.12	1.66	1.10	0.36	0.32	1.78	1.04	0.59	0.26	1.89
1	1.00	0.72	0.19	1.91	1.10	0.30	0.40	1.80	1.09	0.55	0.30	1.94
2	0.94	0.60	0.29	1.83	1.06	0.40	0.56	2.02	1.05	0.50	0.30	1.85
3	0.88	0.53	0.40	1.81	1.00	0.50	0.55	2.05	1.02	0.50	0.30	1.82
4	0.80	0.54	0.33	1.67	1.00	0.41	0.50	1.91	0.95	0.52	0.33	1.80
5	0.80	0.40	0.30	1.50	1.09	0.34	0.48	1.91	0.95	0.50	0.33	1.78
6	0.71	0.59	0.62	1.92	0.95	0.41	0.39	1.75	0.90	0.51	0.35	1.76
7	0.60	0.40	0.60	1.60	0.90	0.51	0.30	1.71	0.90	0.50	0.35	1.75
8	0.60	0.34	0.50	1.44	0.75	0.68	0.28	1.71	0.91	0.45	0.40	1.76
9	0.50	0.54	0.55	1.59	0.70	0.61	0.30	1.71	0.90	0.55	0.39	1.84
10	0.50	0.48	0.50	1.48	0.66	0.58	0.33	1.57	0.90	0.50	0.40	1.80

Effect of Ras cheese microorganisms on DDT and its metabolites

The isolated microorganisms from Ras cheese were incubated in DDT-containing media (1.0 ppm for each medium at 30°C (streptococci), 37°C (lactobacilli) and 25°C (yeasts) for 10 days. The concentrations of DDT added to media (1.0 ppm) represent the maximum permissible levels for milk and dairy products recorded by FAO/WHO (1993). On the other hand, this level did not affect the growth of microorganisms during the incubation periods. Table 4 indicates that the reduction in total DDT was 10.8, 11.8 and 4.8% at the end of incubation period as effected by streptococci, lactobacilli and yeast, respectively. However the levels of DDD and DDE were increased during incubation. This may be due to the degradation of DDT into DDD and DDE. Moreover, the maximum reduction in total DDT was observed after 8 days (13.3%), 10 days (11.8%) and 7 days (7.4%) for streptococci, lactobacilli and yeasts, respectively. Similar findings were observed by Ledford and Chem (1969). Kallman and Andrews (1963) and Wedemeyer (1967) reported that the conversion of DDT to DDD and DDE by yeast was not produced by organisms, but the reduction in the amounts was due to fermentation of lactose which resulted in lowering pH of the medium.

From the results, it is noticeable that the collected milk samples had higher levels of DDT and its metabolites than Ras cheese samples. On the other hand, the manufacturing process of Ras cheese removed only 7.78–13.63% DDT from the contaminated milk. Moreover, the reduction of DDT in Ras cheese may be attributed to the microorganisms in ripening cheese as well as the absorption of pesticide residues and interferences with the cellular metabolism of microorganisms (Hantke & Bradley, 1972; Chacko & Lackwood, 1967; Kim & Harmon, 1970).

Thus, it could be concluded that the consumption of Ras cheese is safer than the liquid contaminated milk.

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