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Organochlorine Pesticide Residues in Butter and Kaymak in Afyonkarahisar, Turkey

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Abstract: Kaymak and butter are traditionally produced from cream of cow or buffalo milk and consumed as Turkish dairy products. About 16 Organochlorine Pesticide (OCP) residues in kaymak and butter marketed in Afyonkarahisar province of Turkey were analyzed by gas chromatography technique. The results showed that kaymak and butter samples were contaminated with 13 and 15 OCPs, respectively. The amount of total OCPs was determined as 672.46 ng g^{-1} in kaymak and 308.95 ng g^{-1} in butter samples. The gas chromatography results indicated that three OCPs [(Beta-HCH (90.01 ng g^{-1}), Aldrin (528.04 ng g^{-1}) and Endrin (7.31 ng g^{-1})] in kaymak and five OCPs [(Beta-HCH (214.18 ng g^{-1}), Heptachlor (10.38 ng g^{-1}), Aldrin (12.34 ng g^{-1}), Dieldrin (12.69 ng g^{-1}) and Endosulfan Sulfate (8.08 ng g^{-1})] in butter were found to exceed the accepted level set by the EU Codex. Overall, OCP residues of kaymak and butter could be a potential risk for public health due to their extensive consumption.

Key words: Organochlorine pesticide, butter, kaymak, cow milk, dairy products, Turkey

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 Dichlorodiphenyltrichloroethanes (DDTs), chlordanes, dieldrin, Aldrin, Heptachlor and Hexachlorocyclohexanes (HCHs) were limited or banned chemicals by Global Stockholm Convention (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). 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 cthese 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 (Pandit *et al*., 2002). 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). Kaymak is a product of buffalo and cattle milk and obtained by concentrating the cream conventionally. It is especially, produced in the districts of Afyonkarahisar, Edirne, Kocaeli, Istanbul, Bursa, Ankara and Izmir provinces of Turkey. Since, Buffalo kaymak has an enormous amount of fat and white color, it is preferred by the consumers (Akalin *et al*., 2006).

Both kaymak and butter are consumed widely by Turkish people due to their tastes.A number of researchers reported the residues of OCPs in milk and milk products from different parts of the world including Turkey (Kumar and Nath, 1996; Yentur *et al*., 2001; Waliszewski *et al*., 2003; Battu *et al*., 2004; Kumar*et al*., 2005; Nizamlioglu *et al*., 2005; Rajashekar *et al*., 2007; Jafari *et al*., 2008; Salem *et al*., 2009). Although, there are a few number of reports on milk's OCPs residue from Turkey, there has been no report on OCP residues of Buffalo kaymak's yet. The present study was designed to determine the OCP residues in both butter and kaymak obtained from buffalo milk. To the best of the knowledge it will be the first report on kaymak's contamination by OCPs.

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MATERIALS AND METHODS

Collection of samples: This study was based on 40 butter and kaymak samples obtained from different producers in June of 2009. Kaymak samples were collected from 5 producers and butter samples were obtained from 20 different producers. Samples were kept in cold ice during their transportation to the laboratory where they were kept at 4°C until analysis. Samples were subjected to analysis within 24 h from their arrival.

Chemicals and reagents: Acetone, acetonitrile, anhydrous sodium sulfate, dichloromethane, diethyl ether, n-hexane and petroleum ether of pesticide residue grade were purchased from Sigma-aldrich and Merck. Analytical standards with >99% purity were obtained from Dr. Ehrenstorfer, Germany. Florisil, 60/100 mesh obtained from Merck was activated at 550°C for 12 h and kept at room temperature.

Sample extraction: Three grams of fat was dissolved into 40 mL petroleum ether. This was partitioned three times into acetonitrile saturated with petroleum ether (3×30 mL). The acetonitrile fraction after dilution with saline (600 mL) was again partitioned 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 >5 mL to be used for Florisil clean up (Kodba and Voncina, 2007; Salem *et al*., 2009). Cleaning up of the extracted samples, to remove the residual fat was performed by transferring the extract into a glass chromatographic column (12 mm i.d.) containing 15 g activated Florisil (60/100 mesh) topped with 15 mm layer of anhydrous sodium sulfate.

This column was rinsed with 100 mL petroleum ether and then the extracted sample was applied to 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 redissolved in hexane to a volume of 5 mL (Alawi *et al*., 1992; Salem *et al*., 2009). Finally, an aliquot of each extract was transferred to 2 mL injection vials to be ready for the analysis. The OCP residues were determined by using a Hewlett-Packard Gas Chromatograph (GC-HP 7890 A) equipped with a 63 Ni electron capture detector (Micro ECD) using a silica capillary column (HP-5 MS: 30 m, 0.25 mm i.d. with 0.25 μm film thickness). The carrier gas was helium at a flow rate of 24 psi min^{-1} through column and 60 mL min^{-1} make up. The gas chromatography oven temperature was initiated at 80°C for 1 min, raised to 175°C (at a rate of 30°C min^{-1}) then raised to 290°C (at a rate of 10°C min^{-1}) and held for 2 min. Injection port temperature and detector temperature were maintained at 250 and 320°C, respectively. The sample volume injected was 1 μL.

After creating the standard calibration curves, OCP residues were quantitatively determined by comparison of their retention time and peak heights/areas with of the standard solutions' values run under the same operating conditions. The concentrations of various residues in each sample were reported as ng g^{-1} on a fat basis.

Recovery study was also performed with pesticide standards. After extraction and solvent evaporation, the samples were analyzed according to the method mentioned above and the recovery values were calculated from calibration curves of standards of the OCP. Detection limits of the method were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC. Blank analyzes were also performed to check interference from the sample. Samples were analyzed in duplicate and represent the arithmetic mean.

Statistical analysis: The statistical evaluation of the results was performed using the Statistical Analysis System (SAS Institute, 1990). Data collected for OCPs residues of mils were analyzed by one-way analysis of variance in order to test for significant differences between treatments. The level of significance was set at the α value of 0.05.

RESULTS AND DISCUSSION

The detection limits, the average recoveries with their Standard Deviations (SDs) of OCPs are shown in Table 1. The average recoveries of OCPs in butter and kaymak were from 72.38-97.33% indicating satisfactory reproducibility of the method. The average OCP levels,

Table 1: Recovery of OCPs in butter and kaymak

Pesticides	Detection limit (ng g^{-1})	Recovery rate (%)±SD
α-HCH	0.10	92.39±1.22
Hexachlorobenzene (HCB)	0.15	89.13±0.60
β-HCH	0.10	96.53±1.66
γ-HCH	0.15	90.92±2.16
δ-HCH	0.20	84.15±1.55
Heptachlor	0.14	87.08±6.88
Aldrin	0.10	84.58±0.35
Heptachlor-endo-epoxide (trans isomer)	0.12	77.17±1.94
α-endosulfan	0.18	72.38±0.75
Dieldrin	0.10	75.11±3.10
4,4'-DDE	0.20	95.53±2.43
Endrin	0.16	94.74±2.85
β-endosulfan	0.30	77.87±2.25
4,4'-DDD	0.10	75.40±3.25
Endosulfan-sulfate	0.25	85.57±1.63
4,4'-DDT	0.10	97.33±0.74

Table 2: OCPs residue in butter and kaymak(ng g^{-1})

	Butter			Kaymak		
Pesticides	Frequency	Mean	Ranges	Frequency	Mean	Ranges
α-HCH	10	1.66	0.17-5.67	25	1.18	0.18- 3.24
Hexachlorobenzene (HCB)	52.5	7.74	0.15-27.17	37	2.01	0.15-4.20
β-HCH	57.5	214.18	40.71-597.64	82.5	90.01	32.31-204.71
γ-HCH	47.5	1.91	0.62-5.74	67.5	2.94	0.42-8.96
δ-HCH	7.5	2.47	0.29-4.55	20	3.63	0.80-8.49
Heptachlor	35	10.38	8.49-18.14	-	nd	nd
Aldrin	10	12.34	1.37-43.60	37.5	528.04	1.20-2245.69
Heptachlor-endo-epoxide (trans isomer)	-	nd	nd	-	nd	nd
α-endosulfan	5	2.98	2.22-3.74	22.5	1.46	0.18-3.77
Dieldrin	5	12.69	0.15-18.09	27.5	1.99	0.15-6.89
4.4'-DDE	5	4.65	0.68-8.61	17.5	7.36	1.34-23.46
Endrin	-	nd	nd	12.5	7.31	4.95-9.70
β-endosulfan	7.5	1.28	0.62-1.89	-	nd	nd
4.4'-DDD	7.5	3.27	0.48-7.06	57.5	3.59	0.10-54.27
Endosulfan-sulfate	57.5	8.08	0.64-23.64	52.5	3.83	1.94-8.11
4.4'-DDT	80	25.32	13.57-64.79	45	19.11	13.76-35.57

obtained from both kaymak and butter of Afyonkarahisar, after analyzing by GC are shown in Table 2. The results showed that kaymak samples were contaminated with 13 different OCPs and butter samples were contaminated with 15 different types of OCPs (Table 2). The total OCP levels were 672.46 and 308.95 ng g^{-1} in kaymak and butter, respectively (Table 2).

Kaymak specimens had much higher OCP residues than butter samples (Fig. 1). EU Food Codex regulates the maximum allowed OCP residues in foods as well as the milk products.

The analyses showed that β-HCH (90.01 ng g^{-1}), Aldrin (528.04 ng g^{-1}) and Endrin (7.31 ng g^{-1}) were over the acceptable levels in kaymak and B-HCH (214.18 ng g^{-1}), Heptachlor (10.38 ng g^{-1}), Aldrin (HHDN) (12.34 ng g^{-1}), Dieldrin (12.69 ng g^{-1}) and Endosulfan Sulfate (8.08 ng g^{-1}) in butter specimens (Table 1). The highest level of contaminants in kaymak and butter were Aldrin (528.04 ng g^{-1}) and β-HCH (214.18 ng g^{-1}) (Table 2), respectively. As OCPs tend to accumulate in both foods and environment, they cause a big problem for both ecosystem and human health.

Their usage began in 1945s in Turkey and they were banned in1983 (Barlas *et al*., 2006). However, some of them (Chlorpyrifos and Endosulfans) have still been reported to be used (Odabasi *et al*., 2008). Both butter and kaymak are traditional milk products and they should have 80/100 g milk fat and 60/100 g milk fat, respectively in accordance with Turkish Food Codex (Seckin *et al*., 2005).

Milk is an important way of exit of the pesticides taken into the animal body. The level of pesticides increases during the processing of dairy products such as cream and butter Therefore, determination of their residues in these food products has been continuously studied. Both producers and consumers are not aware of OCP's presence and contamination in the milk and milk

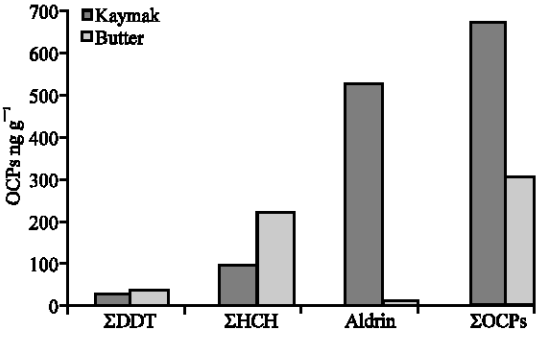

Fig. 1: OCPs residue in kaymak and butter (ng g^{-1})

products (John *et al*., 2001). OCPs are very stable and they tend to accumulate in fat rich tissues due to their lipophilic features (Snedeker, 2001). Since, there has been no literature on kaymak's contamination by OCPs, we were only forced to discuss butter.

John *et al.* (2001) reported the presence of Aldrin, α-HCH, β-HCH, γ-HCH, p,p'-DDD, p,p'-DDE, p,p'-DDT, Σ-DDT, Heptachlor, Heptachlor epoxide from buffalo milk and their values were 0.735, 0.198, 0.007, 0.080, 0.015, 0.515, 0.003, 0.533, 2.260, 4.944 and 8.757 mg L^{-1}, respectively.

When we compared the findings with this report, Aldrin, α-HCH, γ-HCH, p,p'-DDD, p,p'-DDE, Heptachlor, Heptachlor epoxide and total OCPs values were high, β-HCH, p,p'-DDT values were to be low (Table 2, Fig. 2). Waliszewski *et al.* (1996), investigated the OCPs in milk and butter and they found that HCB, α-HCH, β-HCH, γ-HCH, Heptachlor epoxide, p,p'-DDE, p,p'-DDT, Σ-DDT, α-endosulfan, β-endosulfan, endosulfansulfate average residue values were 0.016, 0.017, 0.078, 0.033, 0.035, 0.033, 0.031, 0.049, 0.015, 0.03 and 0.17 mg kg^{-1}, respectively. However, they did not report the presence of aldrin, heptachlor ve p,p'-DDD in their specimens. As in Table 2,

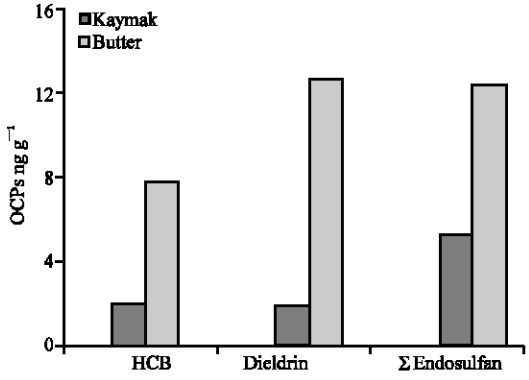

Fig. 2: OCPs residue in kaymak and butter (ng g^{-1})

β-HCH, aldrin, Heptachlor and p,p'-DDD values were lower than thes in their study. On the other hand, their HCB, α-HCH, γ-HCH, Heptachlor epoxide, p,p'-DDE, p,p-DDT, p,p'-DDD, Σ-DDT, α-endosulfan, β-endosulfan, endosulfansulfate levels were higher than the findings in butter specimens. Pandit *et al.* (2002) looked into the OCPs in all milk products including the butter and reported the presence of α-HCH, β-HCH, γ-HCH, DDE, DDT and total DDT average values as 0.003, 0.006, 0.013, 0.021, 0.071 mg kg^{-1}, respectively. The average values in the findings for α-HCH (1.66 ng g^{-1}), γ-HCH (1.91 ng g^{-1}), DDE (4.65 ng g^{-1}) and Σ DDT (33.4) were lower and β HCH (214.18 ng g^{-1}) and DDT (25.32 ng g^{-1}) levels were higher than that of Pandit *et al.* (2002)'s findings (Table 2).

Kumar *et al.* (2005) reported some OCPs with their average values from butter specimens as α-HCH: 0.024 mg kg^{-1}, β-HCH: 0.052 mg kg^{-1}, γ-HCH: 0.056 mg kg^{-1}, pp-DDT: 0.043 mg kg^{-1}, op-DDT: 0.02 mg kg^{-1}, pp-DDE: 0.057 mg kg^{-1}. The β-HCH values in both butter (214.18 ng g^{-1}) and kaymak (90.01 ng g^{-1}) were found to be higher that that of Kumar *et al.* (2005)'s values which they obtained from butter specimens (0.052 mg kg^{-1}) (Table 2).

Yentur *et al.* (2001), investigated the OCPs in butter and bulgur samples from Ankara and reported the presence of pp-DDT and pp-DDE in bulgur specimens. Salem *et al.* (2009) reported the detection of only pp-DDE and β-HCH with the average values of 0.009 mg $kg^{-1}$and 0.019 mg kg^{-1}in butter samples. They were unable to detect Aldrin, pp'-DDD, Dieldrin, α-Endosulfan, β-Endosulfan, Endrin, α-HCH, gamma-HCH, Heptachlor, Hexachlorobenzene in their study. Their DDE value (4.65 ng g^{-1}) was higher than thes and β-HCH level (214.18 ng g^{-1}) was lower than the findings. Although, cattles and buffalos belong to ruminants, their metabolisms are markedly different. Their exposures to OCPs may not be the same. Indeed, Salem *et al.* (2009) reported that the levels of OCP residues could differ due to differences in the distribution and clearances of OCPS. Additionally, Bentabol and Jodral, suggested that milk animals might be exposed to OCPs by their contaminated fodder, fresh or dried and/or the atmosphere. Since, the OCPs accumulate in fatty tissues they may be translocated and discharged by milk fat (John *et al.*, 2001). To this end, a number of studies have been carried out in different countries including Turkey. In these studies, OCP residues were detected in breast milk which can very risky for infants (Stuetza *et al.*, 2001; Yentur *et al.*, 2001; Nizamlioglu *et al.*, 2005; Zhiwei *et al.*, 2007; Alpay *et al.*, 2008). These findings suggest that OCPs can be transferred to food chain rather easily.

CONCLUSION

OCPs are toxic and cancerogenic compounds and could cause very serious health problems for both animals and human beings as well as for the environment (Safe, 1992). This study determined the presence of OCPs in both butter and kaymak. When we consider the risks of OCPs, their contamination in milk products could cause very high risks for both children who are depended on breast milk and public in general. Additionally, women after childbirth are given kaymak in order to increase their breast milk. This traditional fact may pose complications for both mothers and children's health. Briefly, some preventive measures should be taken to reduce the pesticidal contamination problems. To achive this, the milk or/and milk product producers should be educated and an organization should be established to monitor the residues of pesticides strictly in long term.

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