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Organochlorine pesticide residues inraw and cooked fish fillets

Abdelrahman Said Talab*, Hala Elshahat Ghannam

National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

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Key words: Organochlorine pesticides, Microwave, Halogen.

Abstract

The effects of microwave and halogen cooking on organochlorine pesticides residues of five commercial fish species fillets (Mullet, Nile tilapia, Blue tilapia, Mango tilapia and Bayad catfish) were assessed. Results showed that dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane(HCH) were the predominant contaminants in fish fillets. Besides, concentrations of endrin, endosulfan I, endosulfan II, ensulfan sulfate, endrin aldehyde, endrinketon and methoxychlor compound were below detectable limits in raw and cooked fish fillets. The high levels of HCHs were found in raw fillets of Bayad catfish, Mango tilapia, Blue tilapia, Mullet and Nile tilapia as (321, 225, 159, 143 and111 ng/g ww, respectively).Concerning the effect of thermal processing methods microwave and halogen caused significant ($p<0.05$) losses in the concentrations of most examined pesticides. The reduction rates in total organochlorine pesticides residues in microwave and halogen cooked samples were 71.89 and 79.80%, respectively. The highest losses were occurred in halogen cooked samples as follows: Bayad catfish (79.80%), followed by Mango tilapia (73.97%), Mullet (71.95%), Nile tilapia (68.40%), Blue tilapia (64.43%). Halogen cooking method was more effective than microwave in reducing the content of persistent organochlorine pesticides and it can be used to enhance the nutritional value of fish products and promote good health.

***Corresponding Author:** Abdelrahman Said Talab ✉ Abdelrahman_saidh@yahoo.com

Introduction

Organochlorine pesticides (OCPs) are a common name of a group of pesticides consisting of benzene and chlorine and it can pose harmful effects to human health and ecosystems, because they are lipophilic compounds and have low chemical and biological degradation rates (Barakat *et al*., 2002;Pandit *et al*., 2005). Food safety is an area of growing worldwide concern on account of its direct bearing on human health. Generally, risk assessments due to chemical residues are based on residues levels in uncooked food, even though a large proportion of food consumed is either cooked or processed before consumption. To properly assess the risks of pesticide residues to consumer, it is necessary to consider the effects of cooking on the residues (Holden *et al*., 2001). Many studies have shown significant reductions in pesticide residues during household or industrial food processing (Ikeura*et al*., 2013; Mujawar *et al*., 2014; Zhao *et al*., 2014; Huan *et al*., 2015). However, the mechanisms involved in the transfer and or degradation of contaminants during cooking process are not clear (Zabik and Zabik,1999). Salama *et al*., (1998) found that, the most effective cooking methods for removing polychlorinated biphenyls (PCBs) from bluefish included smoking and microwave baking.

The percentages of PCBs lost were 65% and 60%, respectively. Also, Salem and Aid (2011)found that cooking processes of fish tissues reduced13.6-100% polychlorinated biphenyls (PCBs) concentration. Microwave oven and halogen cooker is widespread in European and Arabian households, where working women spend less time preparing meals, as well as ready-to-eat foods now available on everywhere. Therefore, the goal of this study is to investigate the effects of microwave and halogen cooking methods on organochlorine pesticides levels in the fillets of five economic fish species; Nile tilapia, Blue tilapia, Mango tilapia, Mullet and Bayad catfish, to guide consumers in cooking techniques which can reduce dietary OCPs exposure from important fish species consumption and to provide toxicologists with more accurate OCPs exposure estimates.

Materials and methods

Fish samples collection

A total of 50 fresh fish samples from five important fish species namely:Nile tilapia (*Oreochromisniloticus*), Blue tilapia (*Oreochromisaureus*), Mango tilapia (*Sarotherodongalilaeus*), Mullet (*Mugilcephalus*) and Bayad catfish (*Bagrusbajad*) of approximately similar size (10 of each), to minimize the effect of body weight were collected from local market at El-Kanater El-Khairia city, Egypt. The average weights and lengths of Nile tilapia, Blue tilapia, Mango tilapia, Mullet and Bayad catfish recorded (500.75±7.28 g and 33.45±1.35 cm), (300.60±13.05 g and 27.50±1.68 cm), (250±11.54 g and 23.10±0.85cm), (870.50±14.77 g and 45.75± 1.86 cm) and (937.20±25.14 g and 48.53±2.20), respectively.

Fish samples were cleaned and washed well with tap water and stored in icebox for transportation to the Fish Processing Technology laboratory, National Institute of Oceanography and Fisheries (NIOF), El-Kanater El-Khairia city. These fish species were selected on the basis of their importance to local human fish consumption in the Delta of River Nile region.

Fish samples preparation

All fish samples were washed with tap water, manually dissected, skinned, filleted and rinsed with tap water, then distilled water. Each speciefish fillets were divided into three groups, the first was kept raw, the second group was cooked by microwave and the third group was cooked by halogen cooker.

Fish fillets preparation

Fish fillets were divided into three groups; one was taken as control and other swereimmersed in brine solution (10% sodium chloride) for 3-5 min under ambient temperature, washed with tap water and then drained. Salted fish fillets were coated with batter solution [94% wheat flour, 2% egg yolk, 2%

skimmed milk, 1.8% salt and 0.2% cumin and water (1:3w/w) were good mixed with using electrical mixer for 2 min]. Coated fish fillets were rubbed with breadcrumb and left for 3-4 min till cooking process as described method by (Abdou *et al*., 2012).

Fish fillets cooking

Microwave cooking was done using standard household microwave oven (Samsung, 2450 MHz) for 3-5 minutes for each side. Halogen cooking was performed using halogen oven (LENTEL, KYR-912A, 1300watt) at 180°C for 3-5 min for each side.

Cooking times were varied to compensate for differences in fish species, steak size and to achieve similar degrees of doneness in all steaks. The cooking endpoint was based on the visual appearance of the tissue at the center of the steaks changing, from translucent to white. Cooked fish fillets were cooled under ambient temperature and packed in polyethylene bags till analysis.

Analytical methods

Standards and reagents

The pesticides standards were purchased from Supelco (Bellefonte, PA, USA). All other solvents, reagent and chemicals were HPLC grade.

Extraction of pesticides residues from fish fillets

Extraction of pesticides residues from fish raw, microwave and halogen cooked fish fillets were performed according to UNEP/IOC/ IAE (1989) and IOC (1993) as follows: Ten grams of macerated fish samples were homogenized with 30 g anhydrous sodium sulfate, till a fine homogenate was obtained. The mixture was transferred to a pre-cleaned extraction thimble and the dehydrated tissue was extracted with 200 ml of 50% methylene chloride in n-hexane for 8 hours in a Soxhlet apparatus cycling 5-6 times per hour. The extracted solvents were concentrated with rotary evaporator to about 1 ml.

Clean up of fish sample extracts

Fish samples extracts werecleaned and fractionating using 20 g of 0.5%deactivated florisil topped with 1 g anhydrous sodium sulfate then column was wet using 30 ml n-hexane then elution of sample was done with 200 ml of the following mixture dichloromethane : n-hexane: acetonitrile (50: 48.5: 1.5) (Mills *et al*., 1972). After that, fraction was transferred to rotary vacuum evaporator adjusted at 35°C and evaporated until the volume reached 2-3 ml. The final extract was transferred quantitatively by rinsing with aliquot of the organic solvent into a concentrator tube and evaporated to dryness. The residue was dissolved in 2 ml of n-hexane and transferred into autosampler vial for GC-ECD.

Preparation of blank solution

The same volume of solvents and chemicals, which used in extraction of chlorinated pesticides from fish samples were subjected to the same procedures and used as the blank.

Determination of organochlorine pesticide residues

Organochlorine pesticide residues were measured using Gas Chromatography (an Agitech 7890A series instrument) supplied with an Electron Capture Detector. The gas chromatograph condition: DB-17 capillary column (30m length x 0.32 mm internal diameter (i.d.) x 0.25 um film thickness). Operating temperatures: column temperature was programmed: initial oven temperature, 160 °C for 2 min., raised at 3° C/ min to 220 ° C, then raised 15 °C to 270 °C and then held at 240 °C for 15 min. Injector temperature was 280 °C and detector temperature 320 °C with nitrogen carrier gas flow at 4 m1/min. All compounds were identified by their retention times compared to known standards. Peak areas were used as the basis for quantification.

Standard solutions of OCPs including: α-HCH, β-HCH, γ-HCH, δ-HCH, Endrin Keton, Endrin, Endrinaldhyde, Dieldrin, Aldrin, Alfa chlorodan, Gamma chlorodan, Heptachlor, Heptachlor epoxid, Methoxychlor, Endosulfan I, Endosulfan II, Endosulfan sulfate, 4,4'- DDD, 4,4'-DDE, 4,4'-DDT were purchased from Supelco (Bellefonte, PA, USA)

as mixed solution of 1000 µg/mL each in cyclohexane and toluene (50:50). A series of calibration standards were prepared by dilution from the mixed stock standard solution to produce final concentrations of (0.1, 0.2, 0.5, 1.0, and 2.0) µg/mL in hexane. Working standard solutions were stored at 4 °C and used for no longer than 3 months. All solvents used for sample processing and analyses (acetonitrile, dichloromethane, ethyl acetate, hexane and methanol) were HPLC grade. Each of the standards was run thrice in the gas chromatograph to check whether the retention time was reproducible. After measuring the retention times of each standard, a mixture of standards was analyzed to verify whether all the retention times remained the same. In order to check the reliability of the experimental results, samples were spiked with 10 µg/g of the mixed standards and the resultant peak areas were compared with the calculated values of the analytes. The concentration in samples was expressed in ng/g wet weight.

Results and discussion

Organochlorine pesticides residuesconcentrations in raw fish fillets

HCHs concentrations in studied raw fish fillets are shown in Table (1). γ-HCH compound is the highest concentration in raw fish fillets than other compounds. The ranges of HCHs concentrations (ng/g ww) in raw fish fillets were 14-75 α-HCH, 28-67 β-HCH, 470-97 γ-HCH, 150-82and δ-HCH,. In addition, the high levels of HCHs were found in rawBayad catfish followed by Mango tilapia, Mullet, blue tilapia and Nile tilapia fishfillets. However, γ-HCH compound was higher level (97 ng/gww) in raw Bayad catfish than that in Nile tilapiafillets (47 ng/gww).

Table 1. HCHs concentration (ng/g wet wt.) in raw, microwave and halogen cooked fish fillets.

Fish species	trails	α-HCH	β-HCH	γ-HCH	δ-HCH	Σ HCHs
Mullet	R	45	28	55	15	143
	M	17	13	19	13	62
	H	14	12	13	11	50
Nile tilapia	R	14	29	47	21	111
	M	12	BDL	15	BDL	27
	H	11	BDL	12	BDL	23
Blue tilapia	R	25	37	69	28	159
	M	14	11	18	12	55
	H	16	BDL	14	11	41
Mango tilapia	R	46	41	83	55	225
	M	13	15	15	14	57
	H	11	13	12	12	48
Bayad catfish	R	75	67	97	82	321
	M	15	15	18	27	75
	H	13	12	13	13	51

R: raw fish fillets; M: microwave cooked fish fillets; H: halogen cooked fish fillets; BDL: below detectable limits.

The results are in accordance with those findings by Said and Hamed (2005)who reported that high percentage of HCHs is due to municipal discharges. Also, Ghannam *et al.*, (2014) found that, *B. bajad* showed higher total pesticides followed by *S. galilaeus*, *M. cephalus*, *O. aureus* and *O. niloticus*. Kelly *et al.*, (2007) demonstrated that the less lipophilic pesticides HCHs, HCB and HBCDs, showed smaller variation in concentrations among fish species of different trophic levels and feeding habits,

owing to their faster rates of attaining equilibrium water concentrations. Furthermore, fish are known to bio-accumulate persistent organic pollutants (POPs) through diet and environmental exposure. A relationship between the concentration of POPs in human serum and high levels of fish consumption has been established for critical exposure groups, such as fishermen. Contaminants levels can vary significantly within the same fish species depending on the age, the fat content and the area where the fish was caught (Bocio *et al.*, 2003; Sidhu, 2003; Stefanelli *et al.*, 2004;Pandelova *et al.*, 2008).

Table 2. DDTs concentrations (ng/g wet wt.) in raw, microwave and halogen cooked fish fillets.

Fish species	trails	p,p'-DDE	p,p'-DDD	p,p'-DDT	Σ DDTs
Mullet	R	20	56	31	107
	M	BDL	12	15	27
	H	BDL	11	13	24
Nile tilapia	R	20	33	13	66
	M	12	17	11	40
	H	11	12	10	33
Blue tilapia	R	20	40	19	79
	M	16	16	12	44
	H	11	12	12	35
Mango tilapia	R	30	47	29	106
	M	15	14	13	42
	H	11	12	12	35
Bayad catfish	R	30	68	51	149
	M	13	16	13	42
	H	11	13	11	35

R: raw fish fillets; M: microwave cooked fish fillets; H: halogen cooked fish fillets; BDL: below detectable limits.

Table (2) exhibits the DDTs concentrations in studied raw fish fillets. Concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDT compounds were ranged 20-30, 330-68 and 13-51 (ng/g ww) in raw fish fillets, respectively. Levels of DDTs compounds in raw pelagic fish were similar in demersal fish fillets (fig.1). However, ΣDDTs residues in demersal fish filletsBayad catfishwere higher levels than pelagic ones Nile tilapia.

The results are in agreement with those reported by Zabik *et al.*, (1995)who reported DDE was the major constituent in the DDT complex and Yehouenou *et al.*,(2006)who revealed that DDT, its metabolites and isomers were the most frequently identified pesticides in fish flesh. On the other side, our results are lower levels than those finding by Svobodová *et al.*, (2003)in cultured fish species (7.9-86.5 ng/g) and also, Storelli *et al.*, (2007) whofound that the mean values of p,p'-DDE and p,p'-DDT in the muscle tissue of eels were 19.2 and 3.0 ng/g (ww), respectively. Also, Takahashi *et al.*, (2010)showed that the concentrations of DDTs and PCBs in twelve species of deep-sea fishes were 23,000 and 12,400ng/g lipid wt., respectively) as well as Abdallah and Morsy (2013)showed that concentrations of DDTs in fish tissues ranged from 0.638-3.390 ng/g for all studied fish species.

Levels of aldrin, dieldrin, heptachlor, and heptachlor epoxide compounds in studied raw fish fillets are shown in Table 3 and figure 1. Results showed that the aldrin, dieldrin, heptachlor, and heptachlor epoxide concentrations ranged 20-90, 30-55, 15-27 and 14-43 (ng/g ww) in studied raw fish fillets, respectively. ΣCyclodienescompounds were higher levels (149ng/gww) in Nile tilapia fish fillets than (105ng/gww) in Blue tilapia. Our results also are agreed with those reported by Abdallah and Morsy **(**2013)who demonstrated that aldrin is readily metabolized to dieldrin by humans, plants and animals.

Table 3. Cyclodienes concentrations (ng/g wet wt.) in raw, microwave and halogen cooked fish fillets.

Fish species	trails	Aldrin	Dialdrin	Heptachlor	Heptachlor Epoxide	ΣCyclodienes
Mullet	R	20	53	19	43	135
	M	13	18	19	BDL	50
	H	12	12	10	BDL	34
Nile tilapia	R	90	30	15	14	149
	M	15	17	18	18	68
	H	10	11	13	13	47
Blue tilapia	R	21	42	22	20	105
	M	12	12	16	18	58
	H	11	10	12	13	46
Mango tilapia	R	20	37	27	23	107
	M	14	BDL	15	11	40
	H	10	BDL	11	10	31
Bayad catfish	R	20	55	19	30	124
	M	13	18	19	BDL	50
	H	12	12	10	BDL	34

R: raw fish fillets; M: microwave cooked fish fillets; H: halogen cooked fish fillets; BDL: below detectable limits.

Table (4) exhibits the concentrations of endrin, endosulfan I, endosulfan II, ensulfan sulfate, endrin aldehyde, endrinketon and methoxychlor compounds in raw and cooked fish fillets. All data showed that their residues were below detectable limits (<0.001 ng/g ww) in raw fish fillets. Our results are in agreement with those reported bySanterre, *et al.*, (2000)who found that the OCPs, organophosphate and parathyroid compounds were approximately 45% of catfish, 72% of trout and 92% of crayfish did not detectable residues.

Table 4. Compounds concentrations (ng/g wet wt.) in the studied raw and cooked fish fillets.

Fish species	trails	Endrin	Endosulfan I	Endosulfan II	Ensufan sulfate	Endrinaldhyde	Endrinketon	methoxychlor	Σ compounds
Mullet	R	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	M	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	H	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Nile tilapia	R	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	M	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	H	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Blue tilapia	R	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	M	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	H	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mango tilapia	R	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	M	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	H	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Bayad catfish	R	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	M	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	H	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

R: raw fish fillets; M: microwave cooked fish fillets; H: halogen cooked fish fillets; BDL: below detectable limits.

Effect of cooking methods on organochlorine pesticides residues

With regard to the effect of cooking methods on the HCHs levels, a high reduction in HCHs levels was found in halogen-cooked fish fillets compared to microwave-cooked fish fillets as exhibited in table 1 and fig.2 and 3.The levels of α-HCH, β-HCH, γ-HCHand δ-HCH compounds in microwave-cooked fish filletsreduced to reach12-17, 11-15, 15-19 and 12-27 ng/g (ww), respectively. The corresponding levels

in halogen-cooked fish filletswere 11-16, 12-13, 12-14 and 11-13ng/g (ww), respectively. Previous studies have been showed that OCPs residues in fish tissues showed an obvious variation in their loss and behavior among skinning, cooking and processing methods depending upon the nature and solubility of pesticide itself, fish species, preparation treatments, the method of cooking or processing and exposure level (Trotter *et al.*, 1989; Zabik*et al.*, 1995). In addition, fish are consumed mainly in a processed form, therefore the assessment of culinary treatment effects on toxic residues in fish products seems to be an important tissue, considering consumers safety (Witczak, 2009).

Table 5. Total OCPs concentrations (ng/g wet wt.) in raw, microwave and halogen cooked fish fillets.

Fish species	trails	Σ HCHs	Σ DDTs	ΣCyclodienes	Total OCPs	RE %
Mullet	R	143	107	135	385	-
	M	62	27	50	139	63.90
	H	50	24	34	108	71.95
Nile tilapia	R	111	66	149	326	-
	M	27	40	68	135	58.59
	H	23	33	47	103	68.40
Blue tilapia	R	159	79	105	343	-
	M	55	44	58	157	54.23
	H	41	35	46	122	64.43
Mango tilapia	R	225	106	107	438	-
	M	57	42	40	139	68.26
	H	48	35	31	114	73.97
Bayad catfish	R	321	149	124	594	-
	M	75	42	50	167	71.89
	H	51	35	34	120	79.80

Concerning the DDTs residues, a high reduction in DDTs levels was found in halogen-cooked fish fillets compared to microwave-cooked fish fillets. Concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDT compounds decreased to record <12-16, 12-17 and 11-15ng/g (ww) in microwave-cooked fish fillets, respectively. The corresponding levels in halogen-cooked fish filletswere <0.001-11, 11-13 and 10-13ng/g (ww), respectively as shown in table (2) and figures(2 & 3).In addition, a high reduction in levels of aldrin, dieldrin, heptachlor, and heptachlor epoxide was found in halogen-cooked fish fillets compared to microwave-cooked fish fillets. Also, concentrations of aldrin, dieldrin, heptachlor, and heptachlor epoxide compounds decreased to range <12-15, <0.001-18, 15-19 and <0.001-18ng/g (ww) in microwave-cooked fish fillets, respectively. The corresponding levels in halogen-cooked fish filletswere 10-12, <0.001-12, 10-13 and <0.001-13 ng/g (ww), respectively as presented in table (3) and fig.(2 & 3).These results are in accordance with those findings by Zabik *et al.*,(1979)who showed that cooking fillets by microwave reduced DDT compounds by an average of 54% and dieldrin by an average of 47%. In general, from table (5) total OCPs levels were ranged 326-594ng/g (ww) in raw studied fish fillets and reduced to 135-167 and 103-122ng/g in both microwave and halogen-cooked fish fillets, respectively. So, the mean losses in total OCP residues in microwave and halogen cooked samples were 71.89 and 79.80%, respectively. The highest losses was occurred in halogen cooked samples as follows: Bayad catfish (79.80%), followed by Mango tilapia (73.97%), Mullet (71.95%), Nile tilapia (68.40%), Blue tilapia (64.43%).High reduction rats in OCPs during cooking are due to chemical degradation and physical removal with the moisture or, primarily, the lipid phase (Cin and Kroger 1982). Also, Lee and Lee (1985) found that the residues of pesticide were gradually decreased with the increase of treatment temperature, when heated in dry oven, showed noticeable changes in BHC and endosulfan at 60°C or

80°C. Aldrin showed 78.8% loss at 100°C. Also, the reduction rates in β-BHC, Heptachlor, DDT complex, HCH, total PCBs, endrin residues in fried fish samples were 26.30, 37.84, 29.96, 38.81, 46.19 and 24.09%, respectively. Besides, losses (%) in OC residues in grilled and microwave-cooked products were localized between the results of fried and boiled products. Also, the loss rates in OC levels were more in grilled than microwave-cooked products. However, lindane, heptachlor epoxide, and chlordane residues were not detected (Bayen *et al.*, 2005).Witczak (2009)reported that heat treatment reduced the content of persistent OCPs not only in fish meat but also in other foodstuffs. Salem and Eid (2011)reported that cooking had potential significant decrease PCBs concentration in fish tissues. The decrease was observed to be ranged from 13.6 to 100%.

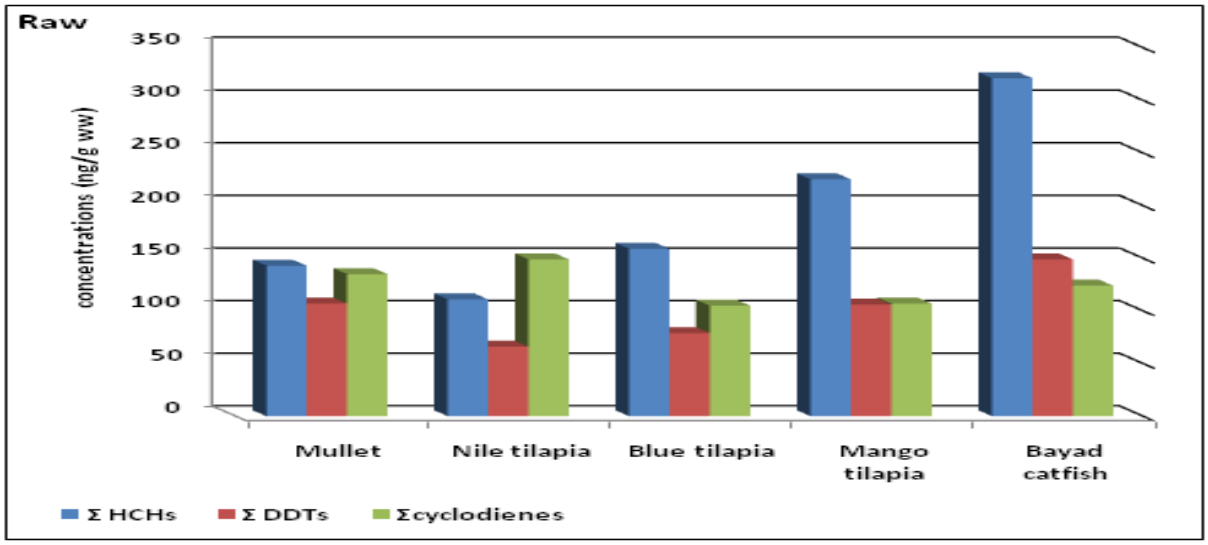

Fig. 1. Organochlorine pesticides concentrations (ng/g ww) in raw fish fillets.

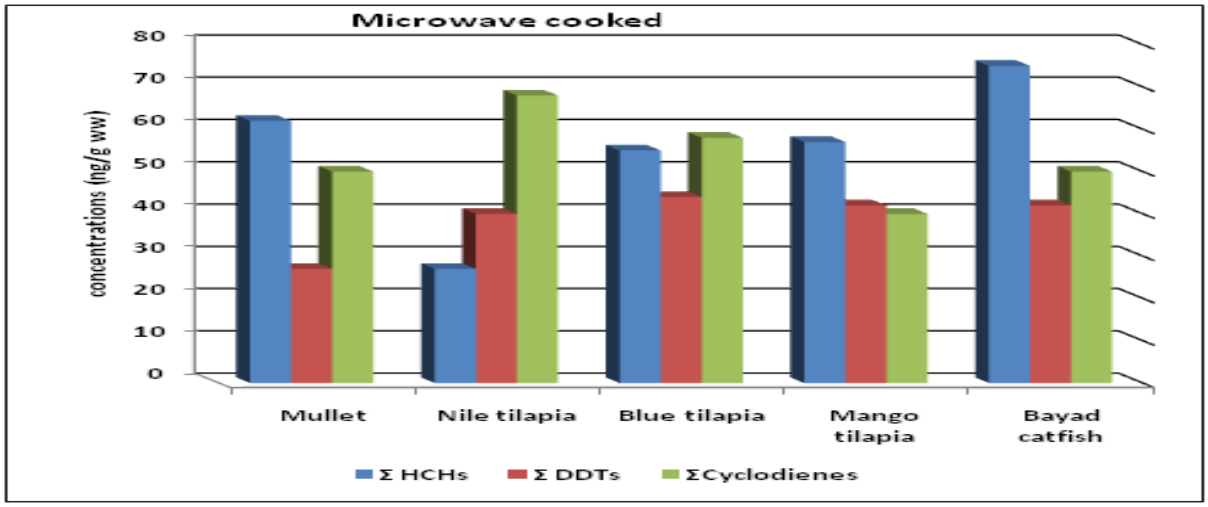

Fig. 2. Organochlorine pesticides concentrations (ng/g ww) in microwave-cooked fish fillets.

The loss of pesticide residues (Table 4) during microwave and halogen cooking may be due to some physicochemical processes, e.g. evaporation, co-distillation and thermal degradation which may vary with the chemical nature of the individual pesticides. During the process the water contained in the tissue could entrain pesticide molecules (co-distillation) while heat causes evaporation and degradation (Sharma *et al.*, 2005).

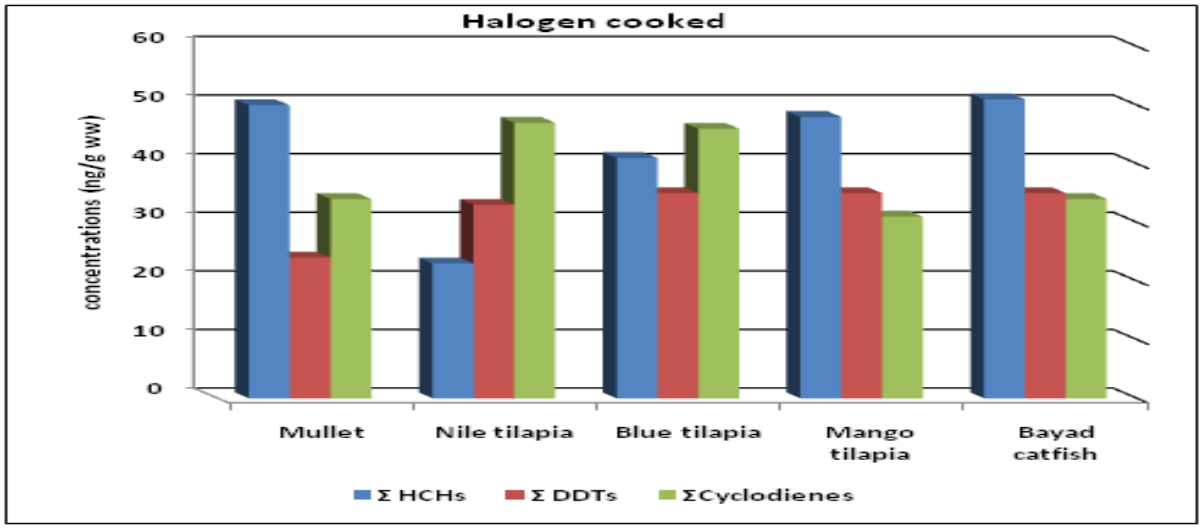

Fig. 3. Organochlorine pesticides concentrations (ng/g ww) in halogen-cooked fish fillets.

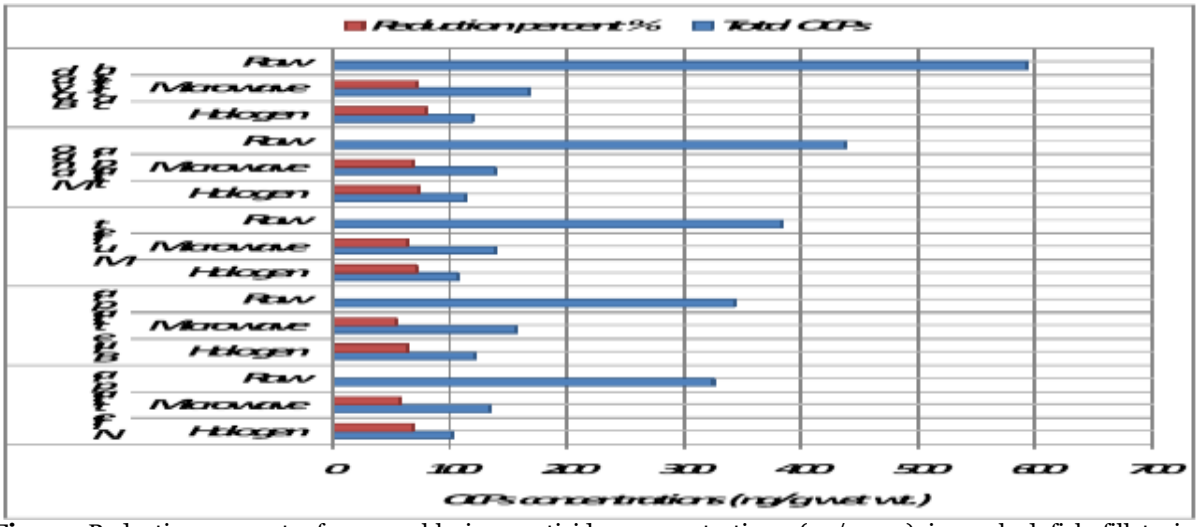

Fig. 4. Reduction percent of organochlorine pesticides concentrations (ng/g ww) in cooked fish fillets in comparison with raw samples.

Finally, the harmful effects of OCPs on human health depend on their maximum permissible levels (MPLs) and acceptable daily intake (ADI) that reported by (FAO/WHO, 1998). The levels of heptachlor, chlordane, total PCBs, aldrin and dieldrin residues were above than the MPLs (0.3, 0.1, 2.0, 0.2 and 0.2 ppm) as reported by EPA (1992).

Conclusion

Residues of OCPs varied among fish speciesand different cooking methods. Skinning, batter coating and thermal processes reduced OCPs residues in fish fillets. Most OCPs detected were below maximum permissible limits (MPLs). Continuous monitoring of cooked fish products for the presence of various pesticide residues and attempts should be made to define the source of contamination as a means to correct the situation.

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