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MULTIRESIDUE ANALYSIS OF PESTICIDES IN BEEF AND MUTTON SAMPLES AND STUDY ON EFFECT OF COOKING ON RESIDUAL LEVELS OF ALDRIN AND DIELDRIN

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Abstract: A study was conducted to estimate certain organochlorine (DDT, HCH and cyclodiene compounds), organophosphorus (Methyl-Parathion, Malathion, Chlorpyrifos and Methyl-Chlropyrifos) and synthetic pyrethroid (Cypermethrin and Deltamethrin) residues in beef and mutton samples (40 each) collected from different regions of Andhra Pradesh by gas chromatography (GC). The contamination levels of Organochlorine (OC) pesticides detected in beef and mutton samples were 0.107 and 0.092 ppm for p,p'DDT- para para dichloro diphenyl trichlore ethane, 0.086 and 0.067 ppm of p,p'DDE- para para dichlorodiphenyl dichlore ethane, 0.077 and 0.058 ppm of p, p'DDD- para para dichloro diphenyl dichloroethylene, 0.074 and 0.039 ppm of α HCH (hexachloro cyclo hexane), 0.058 and 0.046 ppm of β HCH, 0.081 and 0.058 ppm of γ HCH, 0.051 and 0.022 ppm of δ HCH, 0.046 and 0.031 ppm of endosulfan sulfate, 0.040 and 0.040 ppm of heptachlor, 0.037 and 0.031 ppm of heptachlor epoxide, 0.023 and 0.020 ppm of aldrin and 0.019 and 0.022 ppm of dieldrin respectively. The levels of Organophosphate (OP) pesticide residues in beef and mutton were 0.020 and 0.031 ppm for methyl-parathion, 0.036 and 0.034 ppm of malathion, 0.035 and 0.023 ppm of chlorpyrifos and 0.034 and 0.035 ppm of methyl-chlorpyrifos respectively. We could not detect the selected synthetic pyrethroids in any of the meat samples. Cooking of aldrin and dieldrin spiked beef resulted in 31.02-35.98% reduction in aldrin and 28.91-34.60% reduction in dieldrin. Among the cooked samples, higher reduction in the residue level was noticed in pressure cooked meat samples, followed by boiling and microwave oven cooked samples.

Keywords: Organochlorines, Organophosphates, Synthetic Pyrethroids, Pesticide residues, Gas Chromatography, Cooking studies

Introduction:

Intensive agriculture with judicious use of inputs like water, fertilizers and pesticides was successful in meeting the goals of self-sufficiency in food grain production through green revolution. Although several eco-friendly technologies like integrated pest management system, use of neem based insecticides and other bio-pesticides are available for pest management, farmers rely mostly on the chemical pesticides of their easy availability, immediate and spectacular effect (Little, 1996). Continued and indiscriminate use of

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pesticides has resulted in some problems viz pest resurgence, pest resistance, adverse effects on some beneficial and non-target species, presence of pesticide residues in food and feed and wide spread contamination of environment. Higher stability and persistence of these chemicals in the environment led to the contamination of foodstuffs, especially those having high fat content such as milk and meat products (Kannan *et. al.,* 1992). Pesticides are classified into 4 major groups as organochlorines (OC), organophosphates (OP), carbamates and synthetic pyrethroids (SYP). Despite ban imposed by WHO on use of certain Organo Chlorine Pesticides (OCP), some of these pesticides are still used in limited quantity in many developing countries including India for agricultural and public health programmes. Nine OCP have been targeted for global elimination under the signed Stockholm Convention on persistent organic pollutants. OCP are highly persistent whereas OP and SYP are less persistant (Moye, 1981). OCP, particularly HCH and DDT have become the universal contaminants found in all segments of the environment and food chain (Kaphalia *et. al.,* 1990). To overcome this, OP have replaced the persistent OCP and now are the most frequently used group of insecticides (Juhler, 1997). Pyrethroids are used to control flies, mosquitoes and other insects as ectoparasiticides.

The OCP are very lipophilic in nature and are not readily excreted by animals except milk, makes more persistent, where as OP and carbamates are less soluble, degradable and excreted in urine, faeces and breathe of animals, less persistent but exhibit higher acute toxicity. Due to lipophilic nature of OCP, these tend to accumulate in the fatty tissues which leads to bio-accumulation and bio-magnification when consumed by the organisms of higher tropic level of food chain in an ecosystem (Gill *et. al.,* 2010).

In the present study, in addition to determination of pesticide residues in beef and mutton samples, the effect of different cooking methods on some of the pesticide residue levels was also performed.

- To estimate certain organochlorine, organophosphate and synthetic pyrethroid pesticide residues in beef and mutton samples collected from different regions of Andhra Pradesh by gas chromatography (GC) equipped with electron capture detector (ECD) and Thermionic Specific Detector (TSD).
- To study the effect of certain heat processing methods on the level of pesticide residues (aldrin and dieldrin) in beef by spiking method.

Materials and Methods:

50g each of beef and mutton (40 each) samples were collected at random from local markets and slaughter houses from different regions of Andhra Pradesh. Extraction of fat and elution of the pesticides from the fat was done by using petroleum ether, acetonitrile and florosil columns as per the procedures given in Pesticide Residue Analysis Manual, ICAR, New Delhi (2007) with slight modifications and the estimation was carried out by GC (ECD and TSD detectors).

Gas chromatograph: A model Varian-450 GC (Germany) with WCOT fused silica 25mx0.25mm ID coating CP-SIL 8CB column for organochlorines and pyrethroids and WCOT fused silica 15mx0.25mm ID coating CP-SIL 5CB column for organophosphates. Electron Capture Detector (ECD) was used for detection of OC and SYPs and Thermionic Specific Detector (TSD) for OPs. The oven temperature programme for GC-ECD: from 80^0C (2 min), 5^0C/min to 150^0C (2 min), 3^0C to 180^0C (3min), 3^0C to 230^0C (5 min) and finally at 6^0C to 260^0C (6 min) for elution of different OC and SYP pesticides at different time and temperature combinations (Fig.1). The carrier gas (N_2) flow rate was kept in constant flow mode at 1.2 ml/min. Sample (1µl) was injected with split ratio of 10 at 260^0C.

The oven temperature programme for GC-TSD was from 100^0C (1 min), 3^0C/min to 150^0C (1 min), 3^0C to 180^0C (3min), 3^0C to 230^0C (5 min) and finally at 4^0C to 180^0C (2 min) for elution of different OP pesticides (Fig.2). The carrier gas (N_2) flow rate was kept in constant flow mode at 1.4 ml/min. Sample (1µl) was injected with split ratio of 10 at 260^0C. The analysis of extracts was performed in the Gas Chromatography Laboratory, National Research Centre on Meat (NRCM), Hyderabad.

Recovery Studies: Six samples each of beef and mutton samples which did not contain any of the residues were fortified with the working standards (0.01 and 0.1 ppm) to estimate the mean recovery percentage.

Spiking and cooking studies: Minced beef was spiked with aldrin and dieldrin at 1.5 ppm and subjected to different cooking methods like pressure cooking, boiling and microwave cooking. Six replications were done for this purpose.

RESULTS AND DISCUSSION

To know the accuracy of the extraction and detection methods, recovery studies were carried out where the recovery percentage ranged from 84.46% - 103.42% for OCP and SYPs and 88.52% - 98.56% for OP residues. The recovery percent of more than 70% is said to be satisfactory (Garrido-Frenich *et. al.,* 2006). The elution pattern and mean retention time for each of the selected pesticide residue in spiked samples are shown in Fig.1 & 2. The limits of

detection (LOD) and quantification (LOQ) were calculated for each of the selected pesticide and the LOD values were ranging from 0.001 to 0.02 ppm and LOQ from 0.01 to 0.025 ppm. The LOD and LOQ values in the present study were well below their respective MRLs indicating that this method was able to detect the given pesticides at sufficiently low level (Bedi *et. al.,* 2005; Jadhav, 2008 and Stefanelli *et. al.,* 2009).

The mean residual levels of different pesticide residues detected in beef and mutton samples are given in Table.1, 2 & 3. In the present study, the high incidence and concentration of DDT metabolites (p,p'- DDT, DDE and DDD) indicates the continuous exposure to DDT (Sallam and Morshedy, 2008) and also indicates the metabolism of DDT inside animal body and environment (Kannan *et. al.,* 1997). Among all the HCH isomers, there was high incidence and concentration of γ-isomer, it might be due to the fact that only γ-HCH (lindane) is legally permitted for agriculture use (Bedi *et. al.,* 2005). Aldrin rapidly gets metabolized to dieldrin in soil, plants and animals, so high incidence and persistence of dieldrin was detected in the environment (Jorgneson, 2001) and so is the case with heptachlor epoxide which is the metabolic product of heptachlor and is more persistent in the environment (Howard, 1989). Also α and β isomers of endosulfan get metabolized to endosulfan sulfate and get accumulated in plants and animals (Gupta, 1999).

Though the OP pesticides are less persistent when compared to others, some of the meat samples were found to be containing OP residues. It may be due to the regular use of OP compounds for insect and vector control in animal houses. No SYPs were detected in the samples. The levels of contamination of all the residues detected were quite low and well below the maximum residue levels (MRLs) specified by different national and international regulatory bodies.

Among the cooked samples, higher reduction in the residue levels of aldrin and dieldrin were noticed in pressure cooked meat samples, followed by boiling and microwave oven cooked samples (Table.4).

Table.1 Mean residual levels (ppm) of DDT metabolites and HCH isomers in beef and mutton samples

Sample	p,p'-DDT	p,p'-DDD	p,p'-DDE	α-HCH	β-HCH	γ-HCH	δ-HCH
Beef	0.107 (± 0.003)	0.077 (± 0.005)	0.086 (± 0.003)	0.074 (± 0.011)	0.058 (± 0.010)	0.081 (± 0.013)	0.051 (± 0.010)
Mutton	0.092 (± 0.003)	0.058 (± 0.004)	0.067 (± 0.003)	0.039 (± 0.007)	0.046 (± 0.007)	0.058 (± 0.012)	0.022 (± 0.004)

Table 2. Mean residual levels (ppm) of different cyclodiene pesticide and SYP residues in beef and mutton samples

Sample	Aldrin	Dieldrin	Endo. sulfate	Heptach-lor	Hepta. epoxide	Cyper-methrin	Deltame-thrin
Beef	0.023 (± 0.004)	0.019 (± 0.004)	0.046 (± 0.007)	0.040 (± 0.007)	0.037 (± 0.011)	-	-
Mutton	0.020 (± 0.004)	0.022 (± 0.004)	0.031 (± 0.005)	0.040 (± 0.008)	0.031 (± 0.009)	-	-

Table 3. Mean residual levels (ppm) of different organophosphate pesticide residues in beef and mutton samples

Sample	Methyl parathion	Malathion	Chlorpyrifos	Methtl-chlorpyrifos
Beef	0.020 (± 0.005)	0.036 (± 0.005)	0.035 (± 0.006)	0.034 (± 0.005)
Mutton	0.031 (± 0.007)	0.034 (± 0.006)	0.023 (± 0.004)	0.035 (± 0.004)

Table.4: Effects of cooking methods on the levels of aldrin and dieldrin in spiked beef samples

Treatment	Control	Heat treatment		
		Pressure cooking (121^0C)	Boiling (100^0C)	Microwave cooking (2450 MHz /10 min)
Levels of aldrin (ppm)	1.412 ± 0.012^{d}	0.904 ± 0.023^{ab}	0.946 ± 0.010^{bc}	0.974 ± 0.012^{c}
Reduction levels of aldrin (%)	-	35.98	33.20	31.02
Levels of dieldrin (ppm)	1.425 ± 0.012^{d}	0.932 ± 0.011^{ab}	0.981 ± 0.023^{bc}	1.013 ± 0.006^{c}
Reduction levels of dieldrin (%)	-	34.60	31.16	28.91

Means bearing different superscripts (a,b,c,d,) differ significantly between the cooking methods ($p<0.05$)

Fig. 1: Elution pattern and mean retention time of organochlorine and synthetic pyrethroid pesticides from standard pesticide mixture using ECD detector (0.01 ppm)

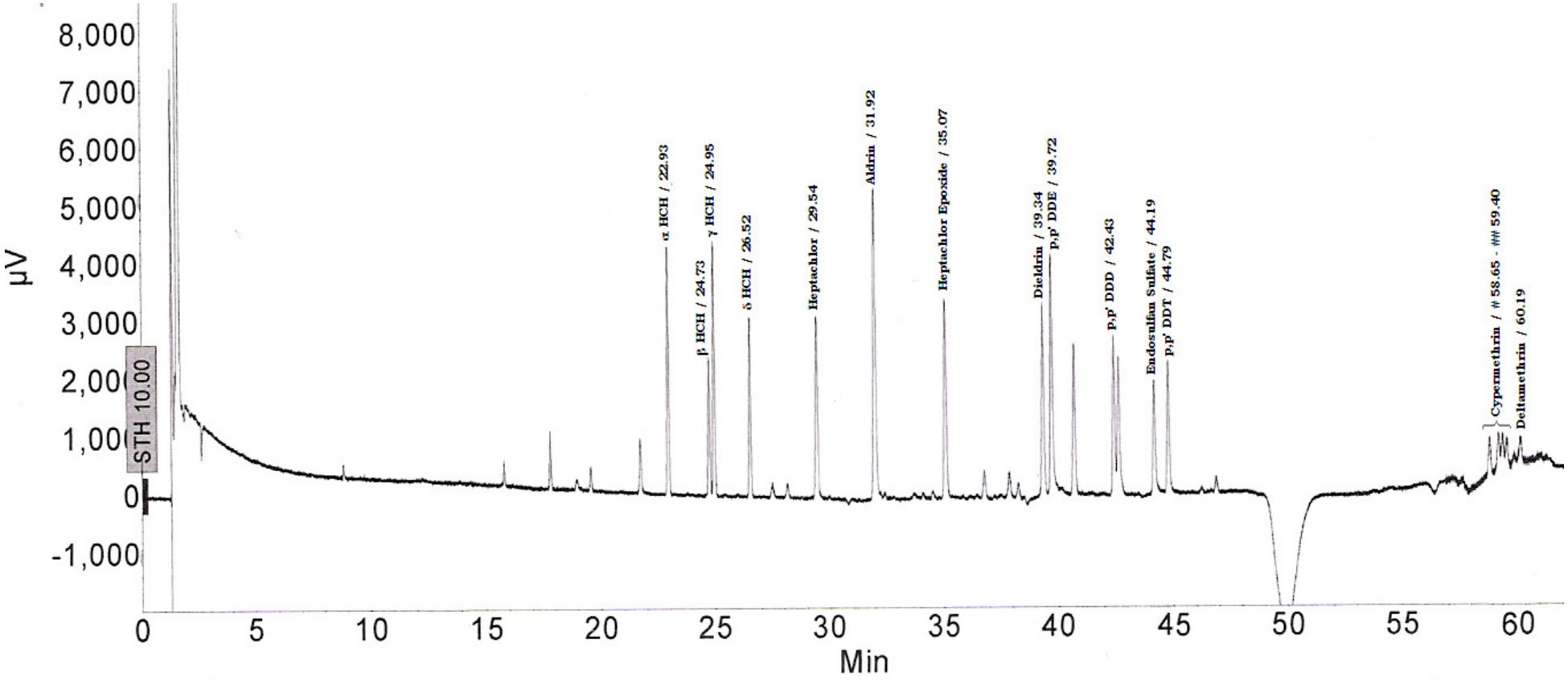
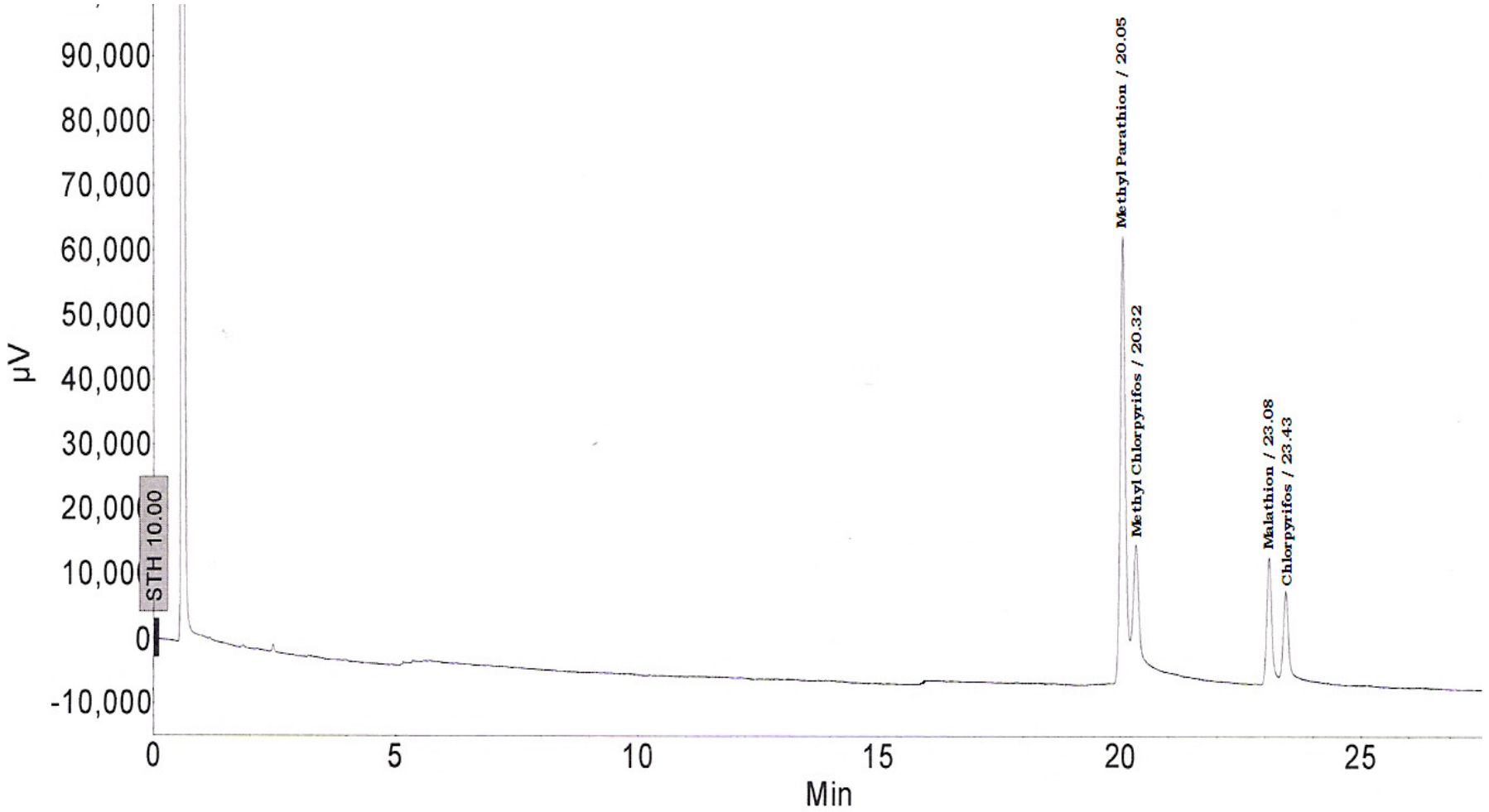


Fig. 2: Elution pattern and mean retention time of organophosphorous pesticides from standard pesticide mixture using TSD detector (0.01 ppm)



CONCLUSION

In the present study, most of the commonly used pesticides were found in beef and mutton samples as residues but their levels of contamination were quite low and well below the MRLs specified by different national and international regulatory bodies. More over proper washing and adequate cooking of the food stuffs further decreases the residual level making the livestock products more safe.

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