Angewandten Biologie Forschung
www.ctbscience
ISSN: 2321-1733
Vol-1: No-3: 2013: 29-34.

Study of effect of processing techniques on Endosulphan content in milk and milk products

Anil Kumar[1], Reena Chauhan[1], Priyanaka Dagar[1], Beena Kumari*[2]

[1]Department of Chemistry and Physics, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India
[2]Department of Entomology, CCS Haryana Agricultural University, Hisar-125 004, Haryana, India

Abstracts

In the present work, an attempt has been done to find out the dissipation behaviour and effect of processing like curd formation and butter milk on reduction of endosulfan residue in bovine milk after applying endosulfan. The residue of endosulfan were estimated on GC-ECD system equipped with capillary column. Per cent recoveries in milk, curd and butter milk ranged from 86-91. Limit of detection (LOD) and limit of determination (LODe/LOQ) were 0.003 and 0.01 mg kg^{-1}, respectively. Initial residue of endosulfan were 16.35 mg kg^{-1} which dissipated to 11.14 and 7.85 mg kg^{-1} after 1 and 2 days of spray by showing 31.86 and 51.99 per cent dissipation, respectively. Maximum percentage reduction of total endosulfan was 45 and 88 for curd and butter milk, respectively.

Keyword: Endosulphan, milk and milk products, Endosulphan residue.

Cite this article as
Anil Kumar, Reena Chauhan, Priyanaka Dagar, Beena Kumari*.Study of effect of processing techniques on Endosulfan content in milk and milk products. Angewandten Biologie Forschung. 2013; 1(3):29-34.

1.0 Introduction

Milk is an essential component of our diet due to its nutritional values. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk called “colostrums”, carries the mother's antibodies to the baby and can reduce the risk of many diseases in the baby [1] . World's dairy farms produced about 730 million tonnes of milk in 2011 [2] . India is the world's largest producer and consumer of milk, and became the world leader in milk production with a production volume of 84 million tons. India has about three times as many dairy animals as the USA, which produces around 75 million tons, over 80 percent being kept in herds of 2 to 8 animals [3]. The cow is the principal source of milk for human consumption in many parts of the world but in India milk is generally obtained from buffalo as compared to cow.

Pesticides are one of the major technological development of twentieth century and used globally for the protection of food, fibre, human health and comfort. To protect the crop from severe damage, many pesticides have been used to minimize loses [4-6]. Due to improper and injudicious use of pesticide, besides posing health threat to the farm workers, their residues enters in the food chain. From green plants or fodder it reaches to the milk producing animals and then to milk. Contamination of the milk with pesticides or their residues is a matter of concern as the presence of pesticide residues is a major bottleneck in the international trade of food commodities.

On consumption of this milk, the pesticide ultimately reaches to human beings. The organochlorine insecticides are known to be extremely persistent compounds and are either banned or their uses is severely restricted in most of the developed countries in the world [7] . Dairy milk and its products in particular have been shown to have a high incidence of contamination with residues of persistent organochlorine insecticides in India [8-13]. DDT and HCH constituted the bulk of pesticides used in India for about five decades [14] Food scientists and food processors have long been interested in the effect of processing on pesticide residues in food commodities. The extent to which pesticide residues are removed by processing depends on a variety of factors, such as chemical properties of the pesticides, the nature of food commodity, the processing step and the length of time the compound has been in contact with the food [15, 16].

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3- benzodioxathiepine-3-oxide ($C_9 H_6 Cl_6 0_3$ S)) is a broad spectrum organochlorine insecticide and acaricide. Endosulfan is a mixture of α and β isomers in the ratio 70:30. It is a highly toxic pesticide and suspected be endocrine disrupter [17]. The extensive use of endosulfan and its mammalian toxicity warrant estimation of toxic residues in/on treated crops and other food commodities because it is a biologically active and hazardous to humans, cattle and wild life. The objective of this study is to investigate the assess the effect of some processes on reduction of Endosulphan and its residues in milk and milk products, like curd and butter milk.

2.0 Materials and methods

2.5 litre milk was procured from the local market, out of which 50 ml was kept as control. Milk sample was treated with the commercial sample of Endosulphan (Thiodan 35% EC @ 20 µg ml^{-1} , aqueous solution of Endosulphan was uniformly added into milk). Milk treated with Endosulphan was divided into two lots. Lot one was kept at room temperature and the second lot was subjected for processing. Samples of lot two were divided into two groups. Group one was kept as it is and group two was converted to curds by standard procedures. Samples from treated lots were processed in triplicates on 0 (2 h after spray), 1 and 2 days after treatment for knowing persistence of endosulfan and the effects of curd formation and butter milk on reduction of Endosulphan.

Extraction cum clean-up

All reagents and solvents were of analytical grade and distilled before use. Anhydrous sodium sulphate was activated by keeping in hot air oven at 180 °C for 6 h before use. The utensils and glass wares were washed properly and sterilized each time before use. Samples of milk, curd and butter milk were extracted as per method of Kathpal et al [18,19] . Representative samples of milk (10 ml), curd and butter milk were taken and mixed properly with 10 g of silica gel (40–60 mesh size), 10 g of anhydrous sodium sulfate and 5 g Florisil (60–100 mesh size) until it was flow able. The flow able sample was packed compactly in a glass column (60 cm x 20 mm i.d.) and the elution was done with 150 ml of acetone and dichloromethane mixture (1:1 v/v) at the flow rate of 4 ml min^{-1}. The eluate was concentrated to near dryness on rotary vacuum evaporator followed by gas manifold evaporator. Meticulous care was taken to remove traces of dichloromethane. The residue was re-dissolved in n-hexane and final volume was made to 2 ml.

Tabl-1: % recovery of endosulfan in milk, curd and butter milk

Level of fortification (mg kg^{-1})	Milk	Curd	Butter Milk
0.01	87.40±2.45	86.00±1.22	89.20±1.78
0.10	88.10±1.44	88.05±0.78	90.00±1.40
0.25	90.84±1.22	89.60±1.24	91.08±1.94

*** Mean ± Standard deviation based on three replications**

Table-2: Persistence of Endosulfan residue in milk, curd and butter milk

Days	Milk		Curd		Butter milk	
	Average Residue*	Dissipation (%)	Average Residue*	Dissipation (%)	Average Residue*	Dissipation (%)
0	16.35±0.072	-	10.67±0.004	-	2.46±0.078	-
1	11.14±0.024	31.86	6.93± 0.029	35.04	1.54 ±0.054	37.39
2	7.85±0.006	51.99	4.31 ±0.011	59.60	0.94 ±0.022	61.79

*** Average of three replicates**

Estimation

Residual Endosulphan (obtained in the above process) was estimated using gas liquid chromatography (Shimadzu GC–2010). Determination parameters are

1. Column: SPB-5 of 5 % diphenyl and 95 % dimethyl fused silica capillary column (30 m, 0.32 mm i.d., 0.25 µm film thickness) with split system.
2. Detector: Electron Capture Detector (ECD) Ni ^{63}.
3. Temperatures: Injection Port–280 °C, Oven–150 °C (5 min) @ 8 °C min^{-1} to 190 °C (2 min) @ 15 °C min^{-1} to 280 °C (10 min), Detector–300 °C.
4. Carrier gas: Nitrogen, flow rate 60 ml min^{-1}, 2 ml through column, split ratio 1:10.

The retention times for α-endosulfan, β-endosulfan and endosulfan sulfate were 13.97, 15.30 and 16.14 min, respectively with above GC conditions.

Recovery experiment

Recovery experiments were carried out at different fortification levels to establish reliability and validity of analytical method and to know the efficiency of extraction-cum clean-up procedures. The control samples of milk, curd and butter milk were spiked at 0.01, 0.10 and 0.25 mg kg^{-1} and processed by following the methodology as described above. The percent recoveries of endosulfan were 87.40-90.84 in milk, 86.00-89.60 in curd and 89.20 -91.08 in butter milk at 0.01, 0.10 and 0.25 mg kg^{-1} level, respectively (Table-1). The average recovery values from the fortified samples were found to be more than 85 per cent. Therefore, the results have been presented as such without applying any correction factor. Limit of detection was 0.003 and limit of determination/ quantification was 0.010 mg kg^{-1}.

3.0 Results and discussion

The experimental data (as shown in Table-2) indicate that the application of endosulfan on milk in the laboratory resulted in an initial deposits of 16.35 mg/kg on 0 (2h after application) day after treatment showing percent dissipation of 31.86 and 51.99 on 1 and 2 day after treatment at room temperature.

Table-3: Effect of processing on reduction of endosulfan residue curd and butter milk

Days	Milk	Curd		Butter milk	
	Average Residue*	Average Residue*	Reduction (%)	Average Residue*	Reduction (%)
0	16.35±0.072	10.67±0.004	34.74	2.46±0.078	84.95
1	11.14±0.024	6.93±0.029	37.79	1.54±0.054	86.17
2	7.85±0.006	4.31±0.011	45.09	0.94±0.022	88.02

*** Average of three replicates**

Effect of Processing on milk products

Curd

The milk was processed to find out to the extent of reduction of endosulfan residues. Data reveal that the average residue of 16.35 mg kg^{-1} in milk reached to 10.67 mg kg^{-1} on initial day in curd which further reached to the levels of 6.93 and 4.31 mg kg^{-1} after 1 and 2 day of application. On assessing the reduction of residue by curd formation, it has been observed that residues reduced by 34.74 per cent on 0 day which further reduced by 37.79 and 45.09 per cent after 1 and 2 days, respectively (Table-3).

Butter milk

In butter milk, residue on 0, 1 and 2 days were 2.46, 1.54 and 0.94 mg kg^{-1}, respectively. Respective per cent reduction of residue in butter milk was observed to be 84.95, 86.17 and 88.02 on 0, 1 and 2days (Table-3). It has been observed that in these processes, i.e. curd and butter milk formation, residue of endosulfan reduced to great extent.

Ali *et al* (1993) studied organochlorine pesticide residues in buffalo milk samples and observed that p' -p'- DDT, p'-p'-DDE, p'-p'-DDD, lindane and aldrin concentrations did not exceed the tolerance limits established by FAO/WHO in the different studied samples. In another study carried out by Mukherjee and Gopal [11] in buffalo milk, residue levels of HCH and DDT isomer often exceeded the limits recommended by FAO/WHO. Donia *et al* [20] reported that organochlorine pesticides were detected at a value exceeding the tolerance levels of FAO/WHO. The present study was in agreement with Donia *et al;* 2010 who reported that the manufacture of yoghurt from buffalo's and cow's milk resulted in a decreased levels of pesticides. Ali *et aI* [21,22] reported that pesticide concentration cannot only be considered during the processes of manufacturing and ripening of cheese, since other alternatives to the processing of milk can reduce their concentration; for example, yoghurt manufacture using buffalo milk reduces pesticides residue contents. The present study was not in agreement with Chaudhary *et al;* [23] who reported that raw milk show 100% contamination while the processing (heating effect) also does not show any large effect of reduction of endosulfan residue after processing, residue remain above maximum residue level even after boiling. Pasteurisation (62.8 QC/0.5 h), or heating to the boiling point for 5 minutes, reduced p'-p'-DDT, p' -p' -DDE, diazinon and malathion concentrations, according to the studies carried out by EI- Hoshy [24]. Madan and Kathpal [25] reported that processing of milk showed prominent reduction in residue of HCH and its isomers, DDT and its analogues in the order of malai removal> lassi> boiled milk.

4.0 Conclusion

In the present work, an attempt has been done study the effects of processing techiniques like converting milk to curds and buttermilk on Endosulphan content of the milk. Results of the study indicate that when the milk is coverted into curds and buttermilk, the content of Endosulphan decreased to greater extent. However, further studies are required to confirm the finding of the study.

Acknowledgements

The authors wish to express their gratitude to the Head, Department of Entomology for providing research facilities.

References

1. www.wikipedia.org/wiki/Milk.
2. http://www.fao.org/docrep/O 15/aI98ge/aI98geOO.pdf.
3. www.fao.org/ag/anainfo.
4. Krishnaiah K, Mathur AC, Mohan NJ, Tondon PL. Evaluation of insecticides for the control of major insect pests of okra. Ind J Agric Sci. 1976; 46(4): 178-186.
5. Mishra PN, Singh MP. Chemical control of insect pests of okra in the Terai region of Uttar Pradesh. Ind J Entomol. 1976; 45(2): 152-158.
6. Mishra HP. Field evaluation of some newer insecticides against aphids (Aphis gossypii) and jassids (Amrasca biguttula biguttula) on okra. Ind J Entomol. 2002; 64(1): 80-84.
7. Battu RS, Singh B, Kang BK. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. Ecotoxicol Environ Saf. 2004; 59(3):324-31.
8. Kalra R.L, Chawla, R.P., Sharma, M.L., Battu, R.S., Gupta, S.C.1983. Residues of DDT and HCH in butter and ghee in India 1978-1981. Environ Pollut. 6 (Series B), 195-206.
9. Kalra RL, Chawla RP. Pesticidal contamination in food in the year 2000 AD. Proc. Ind Nat Sci Acad Part B. 1985;52: 186-204.
10. Kannan K, Tanabe S, Ramesh A, Subramanian A, Tatsukawa R. Persistent organoclorine residues in food stuffs from India and their implications on human dietary exposure. J Agric Food Chem. 1992; 40:518-524.
11. Mukherjee I, Gopal M. Organochlorine pesticide residues in dairy milk in and around Delhi. J. AOAC lnt., 1983; 76(2):283-286.
12. Battu RS, Singh Balwinder, Kalra RL. Seasonal variation in residues of DDT and HCH in dairy milk in Punjab, India. Pestic Res J. 1996; 8:32-37.
13. Battu RS, Singh PP, Joia BS, Kalra RL. Contamination of bovine (buffalo, Bubalus bubalis (L.)) milk from indoor use of DDT and HCH in malaria control programmes. Sci Total Environ. 1989; 86(3):281-287.
14. Parmar BS, Dureja P. Minimizing environmental hazards of agrochemicals Indian approach and status Soc. Pestic Sci. New Delhi. 1990.

15. Farris GA, Cabras P, Spanedda L. Pesticide residues in food processing. Italian J Food Sci. 1992; 3:149–169.
16. Holland PT, Hamilton D, Ohlin B, Skidmore MW. Effects of Storage and Processing on Pesticide Residues in Plant Products. Pure Appl. Chem. 1994; 66,:335-356.
17. http://en.wikipedia.org/wiki/Endosulfan
18. Kathpal TS, Kumari Beena, Singh Shashi, Singh Jagdeep. Multiresidue analysis of bovine and human milk in cotton growing belt of Haryana. In: Pesticide: Environment and Food Security. (Eds P.Dureja, D.B.Saxena,Jitender Kumar, Madhuban Gopal, Shashi Bala Singh and R.S.Tanwar) Society of Pesticide Science, India, New Delhi. 2004. 140-148.
19. Donia MAA, Abou-Arab AAK, Enb A, Senaity El, Abd-Rabou NS. Chemical composition of raw milk and the accumulation of pesticide resdues in milk products. Global Vet. 2010; 4(1): 06-14.
20. Donia MAA, Abou-Arab AAK, Enb A, Senaity El, Abd-Rabou NS. Chemical composition of raw milk and the accumulation of pesticide resdues in milk products. Global Vet. 2010; 4(1): 06-14.
21. Ali AA, Metwally MM. Ahmed NS, Anb AK. Pesticide residues in milk and their distribution among dairy products. Egy J Dairy Sci. 1003; 21(2): 291-299.
22. EI-Hoshy SM. Insecticide residues in milk and influence of heat treatments and bacterial fermentation as safeguard against these pollutants. Assiut Vet Med J. 1997; 37(73): 141-155.
23. Choudhary PL, Sharma RS, Borkartria VN. Effect of chilling and heating on aflatoxin M1 content of contaminated Indian cow's milk. Egy J Dairy Sci. 1998; 26:223–229.
24. EI-Hoshy SM. Insecticide residues in milk and influence of heat treatments and bacterial fermentation as safeguard against these pollutants. Assiut Vet Med J. 1997; 37(73): 141-155.
25. MadanVK, Kathpal TS. Decontamination of pesticide residues in milk by different kitchen processes. In: Int. Conf.: Pesticide, Environment, Food Security. 2001; 267-268.