The Pharma Innovation Journal 2017; 6(3): 209-212

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2017; 6(3): 209-212

www.thepharmajournal.com
Received: 01-01-2017
Accepted: 02-02-2017

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Effect of heat processing on degradation of organophosphorus compounds in milk

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Abstract
In the present scenario, usage of pesticides are inevitable to meet the demands of growing population and to secure the food grain production, there is need for regular screening of milk and milk products for pesticide residues which is being felt in the trade and also at the consumers level. The present work was undertaken to study the effect of heat processing on degradation of various Organophosphorus compounds (Dichlorvos, Dimethoate, Diazinon, Chlorpyrifos, Malathion and methyl Parathion) in both natural and spiked samples of milk were heated to pasteurization temperature (63 ºC for 1/2hrs), sterilization (121 ºC for 15 minutes) and boiling (5min) respectively. Extraction and Cleanup of samples was done by employing QuEChERS method and analysed by GC-FPD. The mean concentration levels of pesticide residues were assessed and degradation percentage of respective pesticide in different food processing methods were calculated. Sterilization and boiling proved to be highly effective on degradation of Organophosphorus compounds than pasteurization temperatures.

Keywords: Organophosphorus residues, QuEChERS method, gas chromatography- flame photometric detector

1. Introduction
Agriculture plays a vital role in India's economy and over 58% of the rural households depend on agriculture as their principal means of livelihood. Usage of pesticides are usually authorized and their toxic pattern is evaluated to determine the acceptable exposure for both farm workers and consumers but excessive use and misuse in developing countries is common practice [1]. Usage of older, non-patented, inexpensive and environmentally stable chemicals were creating serious acute health problems and producing local and global ecological imbalance [2]. Most of the applied pesticides find their way as "residues" in the environment, terrestrial and aquatic food chains where they concentrate and magnify biologically in nature. [3]. The use of Organochlorine compounds (OC) has been replaced by Organophosphorus compounds (OP), which becomes a major public health problem, usually spraying of pesticides and consumption of contaminated feed are main reason for accumulation of OP residues in fat, muscle and also in milk [4]. Since humans occupy the peak of food pyramid and the residue accumulation is more in them. Thus, food, mainly fatty foods, meat, fish and dairy product have been identified at the primary immediate intake route for pesticides in general population [5]. It is necessary to evaluate changes in the nature of the residues during commercial and household food processing and the fate of pesticides in food [6]. The concept of food processing is very much important in the present situation, to study the various metabolites, identify breakdown or reaction products generated by the different food processing methods and to find out the levels of residue in processed products and to evaluate dietary-exposure calculations. Effect of food processing on pesticide residue levels may be influenced by the physical location as well as the physio-chemical properties such as solubility, volatility and thermal degradation of pesticides [7].

2. Materials and Methods
2.1 Collection of samples
Raw Milk samples were collected from local markets of Hyderabad, India. Both natural and spiked milk samples (with respective Organophosphorus compounds) were processed according to the prescribed protocol for Pasteurization (63 ºC for ½ hrs), Boiling (5 min) and Sterilization (121 ºC for 15 mins).

2.2 Chemicals and Reagents

Acetonitrile (ACN), Acetone, n- Hexane, Anhydrous Sodium sulphate, Sodium acetate, Primary Secondary Amines (PSA), C_{18} (octadecylsilane) and Magnesium Sulphate ($MgSO_4$) (Anhyd.) of High Performance Liquid chromatography residue grade obtained from Qualigens and Merck specialities private limited. Analytical pesticide standards with >99% purity were obtained from Dr. Ehrenstosfer, Germany and stored in deep freeze maintained at -40 ˚C.

Table 1: Gas Chromatography operating conditions

Gas Chromatograph	Shimadzu - 2010
Detector	Electron Capture Detector (ECD) Flame Photometric Detector (FPD)
G C Column	Zebron-ZB-50, Length - 30m, 0.25µm film thickness, internal diameter - 0.25mm
Column Oven(^{0}C)	280 ^{0}C
Detector temperature	300 ^{0} C
Injector temperature	260 ^{0}C
Injector status	Font injector type split, split ratio 1:10
Carrier gas	Nitrogen/Air Purity 99.99%
Carrier gas flow (ml/min)	12.4 ml/min
Total run time (min)	60 min

2.3 Pesticide Analysis

Multiresidue pesticide analysis was done by using Gas Chromatography-Flame Photometric Detector (GC – FPD) and Gas Chromatography – Electron Capture Detector (GC-ECD) by employing AOAC official method 2007.01 (QuEChERS method) for sample extraction and clean up techniques with slight modification [8, 9]. The control samples of milk were fortified with respective OP compounds (Dichlorvos, Diazinon, Dimethoate, Chlorpyrifos, Malathion and methyl parathion) at rate of 1ppm (parts per million) and the method was validated. 15g of milk sample was weighed with 15ml of ACN containing 1% acetic acid followed by addition of 6g of $MgSO_4$(Anhyd) and 1.5g of sodium acetate. Gently shaken by hand. Later, the tubes were centrifuged at 5000 RPM for 1 min. 1 ml of ACN extract will be transferred to mini centrifuge tubes for dispersive solid phase extraction (d SPE) in which 50 mg of PSA, 50 mg of C_{18} and 150mg of anhydrous $MgSO_4$ were added and mixed the extracts for 20s.The tube was mixed for 30s using a vortex mixer and centrifuged again for 5 min at 3000 RPM. Finally 2 ml of clear extract was collected and evaporated under the gentle stream of nitrogen (15psi) using Turbovac LV set at 52 ^{0}C upto dryness for 20min.The dried residue content was reconstituted in 1 ml of n-Hexane and further analysed in GC-ECD. In control samples, pure and fortified milk samples with standard mixtures were analysed using same protocol.

2.3.1 Method Validation

The required quantity of organophosphorus compounds stock standards were obtained from Pesticide Residue analysis laboratory associated with Professor Jayashankar Telangana State Agricultural University (PJTSAU) Rajendranagar. The representative samples of milk were fortified with OP compounds at the rate of 1 parts per million (ppm). The recoveries of residues for 70 to 120% is considered, as acceptable limit to validate the method. The elution pattern of OP standards were analysed on the basis of specific retention time for GC-FPD presented in Fig.1. The limit of detection and limit of quantification of specific OP compounds was 0.05 ppm respectively. The recovery percentage were calculated from the calibration curves constructed from the concentration and peak areas of the obtained chromatograms with standards of OP compounds. Blank sample analysis of milk was also conducted to check the different matrix interferences.

$$\textit{Residues in ppm} = \frac{\textit{Sample peak area}}{\textit{Standard Peak area}} \times \textit{conc. of std (ppm)} \times \frac{\textit{µl std. injected}}{\textit{µl of sample injected}} \times \frac{\textit{Final volume of the sample (2 ml)}}{\textit{Wt of the sample}}$$

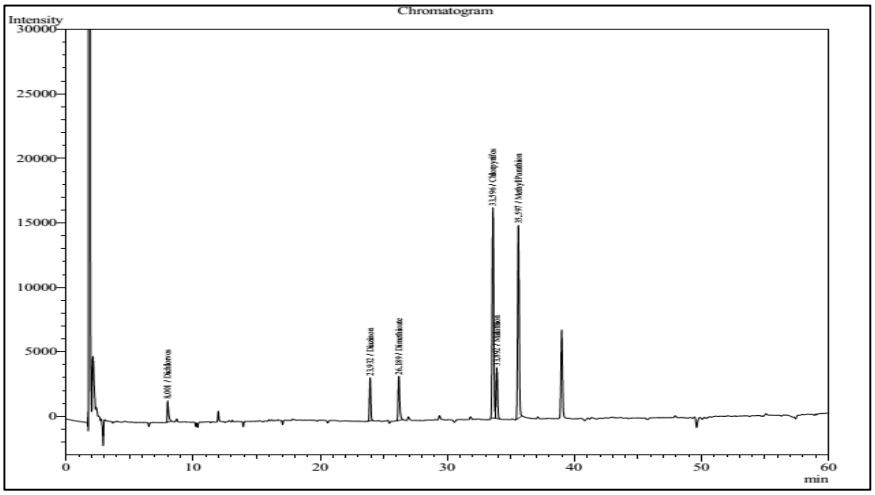

Fig 1: Elution Pattern of Standard mixture of Organophosphorus Compounds in Milk

2.3.2. Statistical Analysis
The data obtained from the studies were statistically analyzed and subjected to one way analysis of variance (ANOVA). The percentage of degradation of Organophosphorus compounds in processed samples of milk were calculated.

3. Results & Discussion
The present study was undertaken to estimate the levels of various Organophosphorus Compounds (Dichlorvos, Diazinon, Dimethoate, Chlorpyrifos, Malathion, Methyl Parathion) in milk and the effect of heat processing methods on the residue levels were analysed by testing both natural as well as spiked samples of milk. The levels of Dichlorvos, Dimethoate, Diazinon, Chlorpyrifos and methyl-parathion compounds were recorded at below detectable level in the samples while malathion concentration levels were high i.e. 0.1398 ppm in milk presented in Table no. 2.

Table 2: Residual levels of Organophosphorus compounds after heat processing in milk

Name of the Pesticide		Raw Milk (ppm)	Pasterization (ppm)	Deg (%)	Boiling (ppm)	Deg (%)	Sterilization (ppm)	Deg (%)
Dichlorvos	Natural	-	-	-	-	-	-	-
	Spiked	0.8263 ± 0.04^{a}	0.1409± 0.007^{b}	82.94	0.0409± 0.018^{c}	95.05	0.05185± 0.017^{c}	93.73
Dimethoate	Natural	-	-	-	-	-	-	-
	Spiked	0.8236 ±0.042^{a}	0.2664± 0.01b^{b}	67.65	0.2181± 0.013^{b}	73.51	0.187± 0.056^{c}	77.29
Diazinon	Natural	BDL	-	-	-	-	-	-
	Spiked	0.8260 ±0.023^{a}	0.1810± 0.006^{c}	78.08	0.4280 ±0.0141^{b}	48.18	0.1619 ±0.031^{c}	80.39
Chlorpyrifos	Natural	BDL	-	-	-	-	-	-
	Spiked	0.8834 ±0.025^{a}	0.3445 ±0.007^{c}	61.00	0.4932± 0.022^{b}	44.17	0.2790± 0.0206^{c}	68.41
Malathion	Natural	0.1398±0.043^{a}	0.0829 ±0.0025^{b}	40.70	0.0524 ±0.0024^{c}	62.51	0.0556 ±0.014^{c}	60.22
	Spiked	0.8379 ±0.05^{a}	0.2421 ±0.009^{b}	71.10	0.1746 ±0.008^{c}	79.16	0.2681 ±0.012^{b}	68.00
methyl Parathion	Natural	BDL	-	-	-	-	-	-
	Spiked	0.8742 ±0.054^{a}	0.3545 ±0.017^{b}	59.44	0.3484 ±0.002^{b}	60.14	0.3742 ±0.005^{b}	57.19

Residue levels with different superscripts a, b, c and d were differed significantly (P<0.01)

The initial residue levels of Dichlorvos in milk was below detectable level (BDL), while in spiked milk (1ppm) the residue levels was 0.8263 ppm, reduced to 0.1409ppm on pasteurization, 0.0409 ppm on boiling and 0.05185 ppm on sterilization, accounting 82.94, 95.05 and 93.73% of degradation respectively. The Dichlorvos content in spiked milk differed significantly (p<0.01) with the treatments, but it was non-significant between boiling and sterilization. Heat treatment reduces the Dichlorvos content by 84 - 90% in preparation of Tofu [10]. Dichlorvos is an unstable pesticide and very volatile, it may get vapourized by heat during cooking stages [11].

The initial residue levels of Dimethoate in milk was BDL, while in spiked milk (1ppm) the residue levels was 0.8236, reduced to 0.2664, 0.2181 and 0.187ppm, accounting 67.65, 73.51 and 77.29% of degradation on pasteurization, boiling and sterilization respectively. The Dimethoate content in spiked milk differed significantly with three treatments while, pasteurization and boiling residue levels were non-significant. Degradation of 73.42% and 75.72% in Dimethoate content were seen on pasteurization and sterilization respectively in spiked milk [12], which were almost similar to the present study, but higher 100% of degradation on boiling was also recorded [13].

The initial residue levels of Diazinon in milk was BDL, while in spiked milk (1ppm), the residue levels was 0.8260 ppm, reduced to 0.1810, 0.4280 and 0.1619 ppm accounting 78.08, 48.18 and 80.39% of degradation on pasteurization, boiling and sterilization respectively. The Diazinon content in spiked milk differed significantly (p<0.01) with the treatments but, pasteurization and sterilization were non-significant. 70.54% of degradation on pasteurization was observed in spiked milk samples [14] which was in coordination with the present study findings. Higher degradation may be attributed to easily biodegradable nature of Organophosphorus compounds chemically derived from phosphoric acid [15].

The initial residue levels of Chlorpyrifos in milk was BDL, while in spiked milk (1ppm) the residue levels was 0.8834 ppm, reduced to 0.3445 ppm, 0.4932 ppm and 0.2790 ppm, accounting 61.0, 44.17 and 68.41% degradation on pasteurization, boiling and sterilization respectively. The Chlorpyrifos content in spiked milk and in treated milk significantly (P<0.01) differed, but levels on boiling differed significantly (p<0.01) with other treatments. The degradation of 44.68% [14] and less degradation was observed [16] on pasteurization in contrast with present study findings while 100% degradation in boiled milk were recorded [13]. In present study, the effective degradation of pesticide residues by sterilization may be due to higher temperature and pressure resulted in more volatility of the compound [17]. The organophosphorus pesticides in foods were affected by level of heat treatments [18, 19].

The initial residue levels of Malathion in raw milk was 0.1398 ppm, degraded to 0.0829 ppm on pasteurization, 0.0524 ppm on boiling and 0.0556 on sterilization, accounting 40.70, 62.51 and 60.22% of degradation respectively. In spiked milk (1ppm) it was 0.8379 ppm, reduced to 0.2421 ppm on pasteurization, 0.1746 ppm on boiling and 0.2681 ppm on sterilization, accounting 71.10, 79.16 and 68.0% of degradation. The Malathion content in raw milk and in spiked milk significantly (P<0.01) differed with processing methods, but between boiling and sterilization, in natural and pasteurization and sterilization in spiked samples were non-significant. 51.94% of degradation during pasteurization [14], which was less than the present study, while 100% degradation in spiked milk samples was observed [13]. In the present study, boiling proved to be more effective than sterilization and pasteurization temperatures. The initial residue levels of Methyl Parathion in raw milk was BDL, while in spiked milk (1ppm) it was 0.8742, reduced to 0.3545 ppm on pasteurization,0.3484 ppm on boiling and 0.3742 ppm on sterilization accounting 59.44, 60.14 and 57.19% of degradation respectively. The methyl Parathion content in spiked milk differed significantly (P<0.01) with processing methods but non-significant among the three processing methods. Reduction of 80.0, 75.0 and 83.75% in spiked (1, 2,

3 ppm) samples respectively [20], which was higher than the present study on pasteurization. Methyl parathion is more stable compound than other organophosphorus compounds might be the reason for lesser percentage of degradation [15, 21, 22].

In the present study, sterilization and boiling proved very much effective than pasteurization temperatures on degradation of OP residues in milk. The food processing like heat processing methods are adequately balance the food quality and food safety aspects of food if the application of pesticides are in compliance with good agricultural practices.

4. Acknowledgements

All the authors were highly thankful to Pesticide Residue analysis laboratory associated with Professor Jayashankar Telangana Agriculture University, Rajendranagar, Hyderabad, Telangana for providing financial support to accomplish the research work. We want to extend our heartfelt thanks to the Associate Dean, College of Veterinary Science, Rajendranagar, Hyderabad for providing the necessary facility to complete the research work in stipulated period.

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