American Journal of Food Science and Technology, 2018, Vol. 6, No. 6, 290-294
Available online at http://pubs.sciepub.com/ajfst/6/6/9

DOI:10.12691/ajfst-6-6-9

Impact of Cow Milk Manufacturing Processes on the Degradation of Malathion Pesticide Residues

Walid A. S. Gafour*

Food Technology Research Institute, Agricultural Research Center, Giza., Egypt
*Corresponding author: walidg4@yahoo.com

Received October 12, 2018; Revised November 14, 2018; Accepted December 22, 2018

Abstract The main objective of the current research aimed at evaluating the impact of the manufacturing processes of cow milk such as pasteurization, boiling, ultra-heat treatment, yoghurt fermentation, milk skimming, cream, butter, and ghee production on the degradation of an organophosphate pesticide, Malathion. For that purpose, samples of raw cow milk spiked with Malathion at level 0.5 mg/kg were used. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used to extract the malathion-spiked milk samples and HPLC-Photo Diode Array (HPLC-PDA) was used as an analytical tool. The precision of the analytical method was ranged between 90.5-97.8 %. The obtained findings demonstrated that sterilization, and boiling were more effective than pasteurization in Malathion degradation (the degradation efficiency were 91.2, 82.2 and 45.2 %, respectively). Regarding the impact of fermentation, it was found that Malathion degradation increased with the progress of cold storage. It was 56, 80, and 97 % at 1, 7, and 14 days of yoghurt storage. Moreover, a cheese manufacturing also had a positive effect on Malathion degradation where it was 53 and 60.8 % in cheese after 1 and 7 days. The data also displayed that Malathion retained in fatty milk products such as cream (91 %), butter (88 %), and ghee (12.3 %). In view of this, it could be concluded that manufacturing processes such as sterilization, boiling, fermentation and curd coagulation processes had positive impact on Malathion degradation in spiked samples of cow milk.

Keywords: *Malathion, organophosphate, Spiked Cow Milk, heat treatments, Fermentation, Coagulation*

Cite This Article: Walid A. S. Gafour, "Impact of Cow Milk Manufacturing Processes on the Degradation of Malathion Pesticide Residues." *American Journal of Food Science and Technology*, vol. 6, no. 6 (2018): 290-294. doi: 10.12691/ajfst-6-6-9.

1. Introduction

Nowadays, pesticides are commonly used in Egypt for crops treatment for control insects, rodents, and other undesirable animals and plants [1]. According to their chemical nature and structure, pesticides may classify as stated by [2] to four main classes: Organophosphorus, Organochlorine, Pyrethroids, and Carbamates. The Organophosphorus pesticides (OPPs) are esters, amides, or thiol derivatives of phosphoric acid, and they are easily hydrolyzed and therefore does not persist in the environment. However, their toxicity (high or moderate) and the possibility of their accumulation, especially fat-soluble OPs in animal tissues (milk and eggs), represent significant harm to human health [3]. Worldwide, Organophosphorus (OP) compounds such as Malathion, parathion and chlorpyrifos, are commonly used principally as insecticides for over 50 years in agriculture and or household plant care, because of their broad range of action and shorter persistence in the environment than Organochlorine pesticides [4].

Lactating animals can absorb these compounds through all routes including inhalation, ingestion, and dermal absorption, and thus contaminated milk can be secreted [5]. Additionally, it can appear in milk due to several possible causes: (a) use of insecticides directly on dairy cattle for ectoparasite control; (b) pasture, forages, or animal feed manufactured from plant material that has been treated with insecticides; and (c) use of pesticides in stables or dairy factories [6].

Many researchers evaluated the probable harmful impact of the intake of small amounts of pesticides as residues in air, water, crops, and cattle (meat and milk) on the general population health [1]. Thus, the presence of pesticides in meat or dairy represents a significant health concern and, consequently, has many severe health consequences resulted from the accumulation nature of pesticides. This concern and it is related to severe health risks are growing increasingly if this milk used in the diet of infants and children [2]. Therefore, pesticide limits for milk and dairy products are starker than those for other foods. Therefore, the annual reports of the WHO and FAO committee group represent acceptance level and limits for specific pesticides in different food products, including milk and dairy products [7].

Hence, it is of great importance to developing methods that can discover and quantify these toxins to assure the safety of food and dairy products. Besides, several researchers [8,9,10,11] studied the effect of some treatments on pesticide residues in milk and dairy products.

[12] and [9] reported that thermal treatment and lactic acid fermentation resulted in a reduction of Organophosphorus pesticide levels in dairy products.

Because of those as mentioned earlier, the objective of this research was to study the effect of different food process technology such as heat treatments pasteurization (65ºC/30 min), sterilization (121°C / 15 min) and boiling (for 5, 10, 15, and 20°C), fermentation and separation for making cream, butter & ghee, and the coagulation for cheese processing on the degradation of Malathion residue in spiked cow milk.

2. Materials and Methods

2.1. Materials

Fresh Cow's milk was obtained from the farm of Faculty of Agriculture, Moshtohor, Benha, University, Egypt. Organophosphate pesticide standards Malathion and acetonitrile of high-performance liquid chromatography (HPLC) - grade were obtained from Sigma Aldrich (Inter lab A.S., Istanbul, Turkey). Water used was highly Purified Milli-Q water was used throughout the whole experiment prepared with Milli-Q PLUS (Millipore Corporation, New York, NY, USA). Pure mixed starter cultures of yogurt (YO-MIX505LOY100DCU) containing *Streptococcus thermophilus and Lactobacillus delbruckii sub sp. bulgaricus* were obtained from Danisco, Denmark. Microbial granulate Rennet was obtained from Hansen, Denmark L3000 Granulate NB microbial rennet, and was used in dosage 1-2 gm /100 liters of milk.

2.2. Methods

2.2.1. Chemical Analysis of Raw Cow Milk

Fat, total solids content, treatable acidity contents were determined according to [13]. The PH values was measured using a pH meter with a combined glass electrode Sentix ® 41 (Electric Instruments Limited). The raw cow milk samples were extracted and cleaned up according to QuEChERS method [14]) for the determination of its Malathion content by HPLC-PDA.

2.2.2. Malathion Extraction and Efficiency of Recovery (ER %)

Efficiency of Recovery (ER %) of Malathion from milk samples were assessed using spiked cow milk samples with three different concentrations (0.5, 1, and 2 mg/kg milk). The spiked milk samples were extracted, and cleaned up according to QuEChERS method ([14]) and then analyzed using HPLC-PDA.

2.2.3. Effect of Manufacturing Processes on Malathion Pesticides Residues in Spiked Cow Milk

The collected cow milk samples were spiked with (0.5 mg/kg milk) of Malathion and shacked vigorously for 30 min to ensure pesticide distribution. Briefly, the spiked samples were divided into seven portions: The first portion was served as a control Raw Milk Spiked (Without treatment). The second portion was laboratory pasteurized at (65°C/30 min) and then cooled to 5°C. The third portion was sterilized at (121°C/15min) and then cooled to 5°C. The fourth portion was heated with stirring to boiling point for 5, 10, 15, and 20 min and then cooled to 5°C. The fifth portion was fermented to produce yoghurt by heating the spiked milk up to 90°C /10 min and then cooled up to 42º C. The starter culture was added (2 %) and incubated at 42°C until pH reach 4.6, Then, the resultant yoghurt samples were transferred to the refrigerator and kept at 5°C for 14 days. The sixth portion the spiked milk was processed to soft cheese according to [15] and cheese sample (25 g) were extracted at 1 and 7 days of cheese processing for Malathion analysis. The seventh portion of milk was heated up to 40°C and separated by a separator to obtain cream. Part of the obtained cream was churned to obtain butter and a part of the obtained butter was processed to obtain the ghee. Cream and butter samples (250 g) was warmed to about 50°C until fat separation and transferred through dry filter paper No.10 While ghee sample (750 g) was given through dry filter paper No. 10. Three grams of cream, butter, and ghee samples were used for the pesticide residues analysis (Figure 1. flow diagram of the processing steps conducted in this research study).

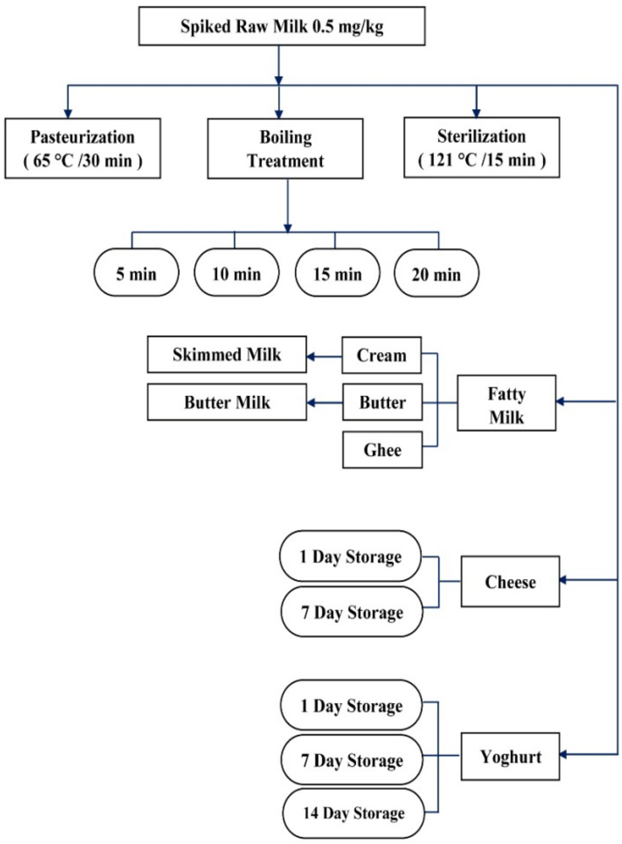

Figure 1. Flow Diagram of the Processing Steps Conducted in this Research Study

2.2.4. Chromatographic Analysis

In this study, chromatographic analysis has been done using a liquid chromatography-photodiode array detector (HPLC-PDA). The HPLC-PDA system was consisted of a water HPLC Model Alliance 2695 equipped with an auto sampler, a column oven and a water 996PDA detector (34Maple street, Milford, MA, USA). Column: Stainless steel, lichrosopher 60 RP- select B (125mm X 4mm X sum, Merck), mobile phase: acetonitrile / water 55/45 V/V in elution at 1 ml /min., column temperature at 25°C. Detection was done using PDA at 220 nm.

2.2.5. Statistical Analysis

The obtained data were statistically analyzed with one-way ANOVA to identify the significant differences between the means of samples. All data were expressed as a mean ± standard deviation of three replicates. The means of results were compared by the Tukey test with a confidence interval set at 95%.

3. Results and Discussion

Currently, pesticides are widespread in agriculture for control insects, rodents and other undesirable animals and plants. The various pesticides are well known for its toxicity and their potential harmful effect on the general population from the ingestion of small quantities of these pesticides is chemicals as residues in food, water and air, or from cattle (fat tissue and/or milk) fed by pesticide-treated crops. While pesticides provide efficient and functional control of disease vector carrying and insect-destroying crops, the need to use these toxic materials on humans or their immediate environment has created potential hazards of great public health significance [2].

3.1. Chemical Composition of Raw Cow Milk and its Content of Malathion

Fat, total solids content, acidity and pH of milk samples were determined. All samples have normal composition as shown in Table 1. In addition, the data revealed that Malathion content in raw cow milk is not detected. Similar results found by [8] reported that none of the raw milk samples contains any of the Organophosphorus pesticides including Malathion; and this may be due to the lower sensitivity of method of analysis. In this sense, reports obtained from the Central Lab of residue analysis of pesticides and heavy metals in food, Agriculture Research.

Center, Ministry of Agriculture, indicated that buffalo milk samples collected at (December 2011) were free from four hundred and two of different classes of pesticides including Dimethoate and Malathion. On the other hand, [11] reported that pesticide residue presented in Egyptian buffalo milk collected from different locations ranged between 0.022-0.040 mg / 100 g.

3.2. Recovery of Malathion Pesticide

As can be seen in Table 2, the recovery percentage of Malathion present in the spiked milk samples at levels of 0.5, 1 and 2 mg/kg were 97.8, 90.5 and 94.8 %. Thus, the data displayed some loss in the added Malathion due to the loss during extraction, handling, and purification. Several researchers previously reported the loss of pesticides during extraction or purification [16,17]. [11] Obtained lower value (84.75 %) of recovery for Malathion residue in spiked cow milk.

3.2. Effect of Processing on Malathion Residue spiked in Cow Milk

3.2.1. Effect of Thermal Treatments

The data showed in Table 3 exhibited the degradation percentage of Malathion pesticide residue spike in raw cow milk as affected by different heat treatments including pasteurization (65°C/30 min), boiling point for 5, 10, 15, and 20 min, sterilization (121°C for 15 min). These data showed significant degradation percentage of Malathion (45.2 %) due to pasteurization process while boiling had more significant effect on the Malathion degradation than pasteurization treatment, and the degradation increased by increasing the boiling time. The significant degradation in Malathion content was 53, 65.8, 77.6 to 82.2 after 5, 10 and 15 and 20 min of boiling, respectively. In addition, sterilization treatment had a higher impact significant effect on the Malathion degradation and the percent of reduction was 91.2 %. Similar results obtained by [12] reported that sterilization (121°C for 15 mints) and boiling (5 mints) had stronger impact than pasteurization (63°C for 30 min) on degradation of organophosphorus compounds residues in milk. In addition, [9] reported that pesticides degraded faster at a higher temperature in bovine milk during heat treatment. [11] demonstrated that Malathion was completely degraded and not detected in milk sample while sterilization process had strong impact on Malathion degradation where 95.99 % of the pesticide was reduced.

3.2.2. Effect of Fermentation and Coagulation

As can be seen in Table 4, it could be noted that fermentation (yoghurt processing) and coagulation (cheese processing) had a significant effect on Malathion reduction. During yoghurt processing, the concentration of Malathion significantly reduced to be 0.220 mg/kg at the first day after fermentation, 0.098 mg/kg after 7 days and 0.015 mg/kg after 14 days, by reduction percentage 56, 80 and 97% at 1, 7, and 14 days, respectively. These results are in general agreement with those found by [11], [9], [18], they exhibited that dimethoate and methyl parathion were more stable but Malathion was the most labile. [9] reported that the lactic bacteria could participate in the degradation of pesticides in bovine milk. This is of great notice for yogurt processing because it suggests that the starter is capable of reducing the toxicological risk of yogurt. Additionally, [19] and [20] revealed that fermentation, heat treatment, and drying have an effect on organophosphorus pesticides in foods.

As for the effect of coagulation process including heating, the data in a Table 4 obtained showed that residue levels of Malathion were 0.235 mg/kg and 0.195 mg/kg at the first and the seventh days after cheese processing, respectively. The data also revealed that whey obtained from cheese processing had 0.071 mg/kg. Malathion residue was degraded by 52 and 60 % at the first and seventh days of cheese processing, respectively, which may be attributed to heating or binding of small amount of pesticide to the rennet enzyme or the chemical nature of the pesticide. [21] and [22] obtained similar results. Moreover, [23] found a significant reduction in pesticide levels during food processing and they mention that the reduction of pesticides in cheese may be due to the microorganisms in ripening cheese as well as the absorption of Malathion residue and interference with the cellular metabolism of Microorganisms.

On the other hand, [24] reported that the yogurt processing technology (including the heat treatment and microorganism's growth during processing and storage) could participate in reduction of pesticide levels in yoghurt. [9] displayed that lactic acid bacteria growth can reduce the safety risks related to dairy products which can destroy some organophosphorus pesticides. In addition, [10] used two direct vat set starters to enhance organophosphorus degradation in whole milk in different extents.

Table 1. Chemical Composition and Malathion Content of Raw Cow Milk

Parameter	Fat (%)	Total solids	Acidity	pH	Malathion concentration (mg/kg)
Value	3.23 ± 0.23	12.30 ± 0.42	0.16 ± 0.04	6.78 ± 0.23	ND*

ND*: not detected
The values are means ± SD of three independent replicates.

Table 2. Recovery of Malathion Pesticide Spiked in Raw Cow Milk

Pesticide	Spiked (mg/kg)	Determined(mg/kg)	Recovery (%)
Malathion	0.5	0.489±0.003	97.80 %
	1	0.905±0.01	90.50 %
	2	1.896±0.02	94.80 %

The values are means ± SD of three independent replicates.

Table 3. Effect of Different Processes on Degradation of Malathion Spiked in Cow Milk

Process		Malathion concentration (mg/kg)	Malathion degradation (%)
Spiked raw milk (Without treatment)		0.5 ± 0.003^{a}	-
Pasteurization		0.269 ± 0.003^{b}	45.2 %
Boiling	**5 min**	0.235 ± 0.002^{c}	53 %
	10 min	0.171 ± 0.003^{d}	65.8 %
	15 min	0.112 ± 0.0030^{e}	77.6 %
	20 min	0.089 ± 0.007^{f}	82.2 %
Sterilization	**15 min**	0.044 ± 0.002^{g}	91.2 %

The values are means ± SD of three independent replicates. The values in the same column with different superscript letters are significantly different ($p<0.05$).

Table 4. Effect of Fermentation and Coagulation on Degradation of Malathion Spiked in Cow Milk

Process		Malathion concentration (mg/kg)	Malathion degradation (%)
Spiked raw milk (without treatment) Sampling time		0.5 ± 0.003^{a}	-
Fermentation	1 day	0.220 ± 0.003^{b}	56 %
	7 day	0.098 ± 0.004^{c}	80 %
	14 day	0.015 ± 0.002^{d}	97 %
Coagulation	1 day	0.235 ± 0.005^{b}	53 %
	7 day	0.196 ± 0.006^{c}	60.8 %
Whey		0.071 ± 0.004^{d}	

The values are means ± SD of three independent replicates. The values in the same column with different superscript letters are significantly different ($p<0.05$).

Table 5. Effect of Cream, Butter and Ghee Processing on Retaining of Malathion Spiked in Cow Milk

Process		Malathion concentration (mg/kg)	Malathion retaining (%)
Spiked raw milk (without treatment)		0.5 ± 0.003^{a}	-
Skimming	**Skimmed milk**	0.045 ± 0.001^{f}	9 %
	Cream	0.455 ± 0.01^{b}	91 %
Churning	**Butter milk**	0.06 ± 0.003^{e}	12 %
	Butter	0.440 ± 0.009^{c}	88 %
Ghee		0.064 ± 0.001^{d}	12.8 %

The values are means ± SD of three independent replicates. The values in the same column with different superscript letters are significantly different ($p<0.05$).

3.2.3. Effect of Butter, Cream and Ghee Processing on Malathion Residues Retaining

The obtained findings presented in Table 5 showed that only 9 % of Malathion retained in skim milk while a higher amount (91 %) retained in the obtained cream. Data obtained exhibited that 12 % of Malathion retained in buttermilk while the higher amount (88 %) retained in the obtained butter. The production of ghee from butter had high ability to reduce the Malathion retained in ghee to be 12.8 % and the concentration of Malathion reduced from 0.440 to be 0.064 mg/kg in the obtained ghee. [8] obtained similar results indicated that pesticide residues might be found in higher concentration (on a fat basis) in milk products as compared to the material from which these were derived. This might be attributed to the affinity of pesticides residues for the lipoprotein portion of the products.

4. Conclusion

Although the severe consequences of pesticide intake on human health, many processing technologies can be utilized to destroy pesticide residues or reduce its concentration in food and dairy products through its degradation. In the dairy industry field, it was found that heat treatments such as pasteurization, boiling, and sterilization had a significant positive impact on Malathion degradation as well as, the process of fermentation and coagulation had reduced the concentration of Malathion in yogurt and cheese, and this effect is improved during products storage. The current data revealed that a higher amount of Malathion retained in cream and butter while the lower amount present in ghee. Thus, conducting this process of technology assures the safety of dairy products and complies with good manufacture practices (GMP).

References

[1] Iftikhar, B., S. Siddiqui and S. Rehman, 2014. Assessment of the dietary transfer of pesticides to dairy milk and its effect on human health. Afr. J. Biotechnol., 13: 476-485.

[2] Shaker E.M., E.E., 2015. Elsharkawy Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt. Environmental Toxicology and Pharmacology. 39, pp. 433-440.

[3] Fagnani, R., V. Beloti, A.P.P Battaglini, K.S. Dunga, R.Tamanini, 2011.Organophosphorus and carbamates residues in milk and feedstuff supplied to dairy cattle. Pesq. Vet. Bras. 31(7): 598-602.

[4] Cannon J.M., V. Reddy, E. Murrill, 1996. Characterization of Malathion residues in dairy goats and poultry. J. Agric. Food Chem., 44, 3365-3373. 6.

[5] Gazzotti, T., P. Sticca, E. Zironi, B. Lugoboni, A. Serraino and G. Pagliuca, 2009. Determinatio of 15 organophosphorus pesticides in Italian raw milk. Bull. Environ. Contaminat. Toxicol., 82: 251-254.

[6] Salsa, H., M. M. Gonzalez, M. Noa, N. A. Perez, G. Díaz, R. Gutierrez , H. Zazueta and I. Osuna, 2003. Organophosphorus Pesticide Residues in Mexican Commercial Pasteurized Milk J. Agric. Food Chem., 51, 4468-4471.

[7] FAO and WHO. 2008. Joint FAO / WHO food standards programme: Codex alimentarius commission. Pesticide Residues in Food, Volume, 11, Rome, Italy.

[8] AbouDonia, M.A., A.A.K. Abou-Arab, A. Enb, M.H. El-Senaity and N.S. Abd-Rabou, 2010. Chemical composition of raw milk and the accumulation of pesticide residues in milk products. Global Veterinaria, 4: 6-14.

[9] Bo, L.Y. and X.H. Zhao, 2010. Preliminary study on the degradation of seven organophosphorus pesticides in bovine milk during lactic acid fermentation or heat treatment. Afr. J. Microbial. Res., 4: 1171-1179.

[10] Bo, L.Y., Y.H. Zhang and Zhao, X.H., 2011. Degradation kinetics of seven organophosphorus pesticides in milk during yoghurt processing. J. Serbian Chem. Soc., 76: 353-362.

[11] Abd-Rabo, F.H., H. Elsalamony and Sally S. Sakr, 2016. Reduction of pesticide residues in Egyptian buffalo milk by some processing treatments. Int. J. Dairy Sci., 11: 75-80.

[12] Singh S.; N. Krishnaiah; T. M. Rao; R. Pushpa and G. Reddy, 2017. Effect of heat processing on degradation of organophosphorus compounds in milk, The Pharma Innovation Journal; 6(3): 209-212.

[13] AOAC.2003. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed. Association of Official Analytical Chemists, Washington, DC. USA.

[14] AOAC, 2007.07. Official Method Pesticide Residues in Food by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry, First Action. Association of Official Analytical Chemists, Washington, DC. USA.

[15] Fahmi, A.H. and Sharara, H.A. (1950).Studies on Egyptian Domiaticheese.J.Dairy Res.17 (3), 312-328.

[16] Abou-Arab, A.A., 1987. Effect of microbial fermentation on pesticide residues in milk. M.Sc. Thesis, Faculty of Agriculture, Food Science Department, Ain-Shams University.

[17] Abd-Rabo, F.H., N.S. Ahmed, A.E. Abou-Dawod and F.A. Hassan, 1989. Organochlorine, Organophos-phorus and Carbamate pesticides cause changes in some properties of buffalo's milk. Egypt. J. Dairy Sci., 17: 105-114.

[18] Zhao, X.H. and J. Wang, 2012. A brief study on the degradation kinetics of seven organophosphorus pesticides in skimmed milk cultured with Lactobacillus spp. At 42EC. Food Chem., 131: 300-304.

[19] Bogialli, S., R. Curini, A. Di Corcia and M. Nazzari, 2005. Analysis of Urea Derivative Herbicides in Water and Soil. In: Chromatographic Analysis of the Environment, Nollet, L.M.L. (Ed.). CRC Press, Boca Raton, ISBN-13: 9781420027983, pp: 935-975.

[20] Ozbey, A. and U. Uygun, 2007. Behaviour of some organophosphorus pesticide residues in peppermint tea during the infusion process. Int. J. Food Sci. Technol., 104: 237-241.

[21] Abdou, S.M., S.M. Abd-El-Hady, M.B. El-Alfy, A.A. Abd-El-Gawaad and E. Abo-El-Amaim, 1983. Organochlorine pesticide residues in buffalo's milk in kalubia province and the effect of the presence of insecticides on the coagulation time. Egypt. J. Dairy Sci., 11: 197-203.

[22] Pagliuca, G.,A. Serraino, T.Gazzotti, E. Zironi, A. Borsari and R. Rosmini, 2006. Organophosphorus pesticides residues in Italian raw milk. J. Dairy Res., 73: 340-344. January 15, 2016

[23] Kaushik, G., S. Satya and S.N. Naik, 2009. Food processing a tool to pesticide residue dissipation-a review. Food Res. Int. J., 42: 26-40.

[24] Zhang, H., Z.F. Chai, H.B. Sun and J.L. Zhang, 2006. A survey of extractable persistent organochlorine pollutants in Chinese commercial yogurt. J. Dairy Sci., 89: 1413-1419.