Determination of pesticides residues during milk processing

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ABSTRACT

Twenty-seven different pesticides and one polychlorinated biphenyl (PCB) compound were spiked in milk samples with level of 0.1 mg/kg to study the effect of milk processing (boiling, pasteurization, yoghurt-manufacturing). The residues of the spiked compounds were determined using gas chromatography equipped with tandem mass spectrometer (GC-MS/MS). The calculated processing factor (P.F.) after boiling process was found to be in a range of 53-94 %, while after milk pasteurization the range was 57-98% and for yoghurt-manufacturing process was 22-82% after 3 days of storage. In this study, the results showed that yoghurt-manufacturing process has higher effect than boiling process and pasteurization on the reduction of pesticide residues in milk. This could be explained by the degradation of pesticides during yoghurt-manufacturing that was enhanced by lactic acid fermentation in addition to heating process leading to a conclusion that it would be better to consume milk as processed than raw milk to reduce the possible contamination levels of pesticides.

Key words: Milk Processing, Processing factor, Pesticide Residues, GC-MS/MS.

INTRODUCTION

Milk processing is defined as the manufacture, modification, pasteurization, preparation, reconstitution, packaging or storage of dairy products, and also includes the cleaning and sanitizing of equipment and the dairy product contact surface (NDC, 2011). Pasteurization of another two portions were performed by heating every particle of a dairy product in equipment that is designed and operated to meet or exceed the required time and temperature relationships as specified and yoghurt is the food obtained by lactic acid fermentation through the protosymbiotic mixture action of *Streptococcus thermophilus and Lactobacillus delbruckii subsp.bulgaricus* (NDC, 2011). Langlois *et al.* (1970) investigated the effects of bacteria on DDT and found that there were significant changes, where it degraded DDT into two to eight metabolites. Also, Li-Ying *et al.* (2010) had studied the degradation of organophosphorus (OP) in milk during lactic acid fermentation or heat treatment. They found that heat treatment and lactic acid fermentation could reduce the level of organophosphorus (OP) pesticide in dairy. The effects of processing study and storage of dairy products were studied by Abou-Arab (1999) on lindane residues anu its metabolites and found that heat treatments (pasteurization, boiling) reduced lindane levels. Abou Donia *et al.* (2010) reported that some residues of pesticides were removed during the production of yoghurt and these residues were found to contain low levels of organochlorine (OC) pesticides due to the effect of heat treatment. The present investigation aimed to study the effects of heat treatment (pasteurization, boiling) and yoghurt-manufacturing on the stability of the twenty seven pesticides and one PCB compound. The spiked milk samples were treated with simulated trials (boiling, pasteurization and yoghurt-manufacturing); the degradation of the pesticides in milk during processing was investigated using QuEchERS multiresidue method for extraction followed by GC-MS/MS (Gas Chromatography equipped with tandem Mass Spectrometers) for determination.

MATERIALS AND METHODS

1. Pesticides

Twenty seven pesticides belonging to different chemical groups (OC,OP and synthetic pyrethroids) and one PCB (Polychlorinated Biphenyl) compound (reference standards) were kindly obtained from Dr.

Ehrensdorfer (Augsburg, Germany) with purity >95% and they were used to prepare stock solutions.

1.1. Stock solutions

Reference standard solutions of concentration 1 mg/ml were prepared in toluene as a solvent and kept at 4 ±2 °C

1.2. Spiking mixture solution

Twenty µg/ml of each tested compound was prepared in n-hexane and used as spiking mixture and stored in refrigerator at 4 ±2 °C until usage.

1.3. Calibration curve

For GC-MS/MS calibration curve different concentration levels (0.01, 0.05, 0.1, 0.5 µg/ml) were prepared and stored in n-hexane/acetone mixture (9:1). The prepared levels will cover a broad concentration range that will allow the construction of a linear calibration curve.

1.4. Injection standard solution

Working standard concentration of Aldrin (0.1 µg/ml) was prepared in n-hexane/ acetone (9:1) mixture solution and have been used as injection standard for GC-MS/MS.

2. Blank (raw) milk sample

A sample of 200 g of raw milk was spiked with 28 evaluated compounds to get a concentration of (0.1 mg/kg).

3. Reagents

The chemicals and solvents used in the present study were of analytical and chromatographic grade suitable for QuEchERS method of analysis.

Milk processing methods

Blank milk samples free from the pesticides residue were used and boiled, pasteurized and yoghurt as different types of processed milk were tested. Milk samples were spiked with the 28 evaluated compounds at a concentration level of 0.1 mg/kg and subjected to the flowing processes:

1. Two portions of the spiked milk sample were analyzed directly without

any processing marked as test [A].

2. Another two portions were boiled for 15 min then left to reach room temperature, analyzed using the reference test method and marked as [B].
3. Pasteurization of another two portions were performed by heating the spiked milk samples to 72 °C for 4 seconds and they were cooled to 4 °C for 30 minutes then left till reached to the room temperature, analyzed using the reference test method and marked as [C].
4. Yoghurt-manufacturing was done by heating the spiked milk samples to 80 °C for 20 minutes on water bath then they were cooled to 40 °C and stored in an incubator (40 °C) for 3 hrs, then stored for 3 days. The effect of storage was studied by testing two portions of yoghurt samples for 3 days to monitor the processing effect during yoghurt-manufacturing. Yoghurt samples were marked as D_0, D_1, D_2, D_3 , where [D_0]= yoghurt at zero time and [D_1],[D_2] and [D_3] are the samples of yoghurt stored for 1,2 and 3 days, respectively (EOS,1970).

Extraction Method

The QuEchERS (ECS, 2008) method was used for pesticides extraction from milk sample. A portion acetonitrile containing the extracted residues was evaporated with rotary evaporator, diluted with injection standard and subjected to GC-MS/MS analysis. The concentration of the residues was calculated from those calibration curves constructed from the standard concentrations and peak areas of the obtained chromatograms with standards of each pesticide. Matrix –matched standard was performed in order to check the matrix effect on each compound. The tested validated data showed mean recoveries ranged from 70 to 120 % with CV% ≤ 20 indicating excellent repeatability.

Conditions of GC-MS/MS

Agilent GC 7980 with 7000 B Quadrupole, El. source was used to perform analysis by injecting 1 µl of aliquot to a column of Agilent HP5MS 5% phenyl methyl siloxane (30 m x 0.52 µm x 0.25 µm).

The temperature profile was set as follows:

The initial oven temperature of 70 °C for 2 min then heating from 70 to 150 °C at 25 °C /min, heating from 150 to 200 °C at 3 °C/ min, heating from 200 to 280 °C at 8 °C /min, holding for 10 minutes. The total running time will be 42 minutes. Quantification of the pesticides was performed by comparing the peak areas of the pesticides to a calibration curve of the standards, and multitude point of calibration was used. Based on the effect on residue levels and the disposition of the residues in the various processed products, processing factors (P.F.) are calculated as follows:

$$\text{Processing factor (P.F.)\%} = \frac{\text{residue level in processed commodity}}{\text{residue level in raw commodity}} \times 100$$

The degradation percent more than 20% considered as significant degradation due to the processing experiments considering that the repeatability of the tested method was within ±20% (CV%).The processing factor is also refer to the percentage of the residues left in the boiled [B], pasteurized [C] and processed milk (yoghurt) at zero time [D_0] or at 1, 2 and 3 days post-processing [D_1], [D_2] and [D_3], respectively. Fat content (%) of each of the tested samples was determined.

RESULTS AND DISCUSSION

The selection of the evaluated pesticides was based on their lipophilicity (Pesticide Manual, 2003) and also on the monitoring data of survey for dairy products. The Joint FAO/WHO Meeting on pesticide residues (JMPR) evaluates food processing data on residue behavior where significant residues occur in plant or plant products which are processed into food (FAO/WHO, 2006). Significant residues are generally defined as >0.1 mg/kg, unless the compound has a high acute or chronic toxicity. Special attention should be given to residues less than 0.1 mg/kg in case of residues concentrate in further processing steps (FAO, 2002).

Degradation of pesticides in boiled milk [B]

When the spiked milk was heated for 15 minutes, proceeding analysis showed that the contents of pesticide residues in milk decreased with boiling (Table 1). The processing factor (P.F.) was calculated after milk boiling for four organophosphorus pesticides (Pirimiphos-methyl 88%, Chlorpyrifos-methyl 88%, Diazinon 88 % and Chlorpyrifos 86%). The results indicate that there was no significant degradation of these compounds. The processing factor (P.F.) for 17 OC compounds can be arranged in a descending order as follows: Heptachlor (94%)> HCH-β isomer (93%)> HCH-α isomer (87%), HCH-δ isomer (87%)> HCH-γ isomer (86%)> Endosulfan-sulfate (85%), Endosulfan-β isomer (85%)> Heptachlor-exo-epoxide (84%)> Chlordane-trans (83%), Endosulfan-α isomer (83%), DDT- o, p` (83%), DDD- p, p` (83 %)> Endrin (82%), DDE- p, p` (82%), DDD-o, p` (82%)> Dieldrin (81%)> Chlordane- cis (79%). This finding is in harmony with those results obtained by Abou Donia *et al.* (2010). For the synthetic pyritheroids compounds, the processing factor (P.F.) was Deltamethrin (88%)> Cypermethrin (87%) and Fenvalerate (87%). The processing factor (P.F.) for other evaluated compounds was for Diphenylamine (85%), PCB (82%), Biphenyl (72%) and (53%) for Dichlofluanid. These results proved the efficient role of heat treatments on the degradation of some pesticides like Biphenyl, Dichlofluanid and Chlordane-cis. These differences in the reduction levels may be due to their differences in degradation by temperature. It could be said that milk boiling can reduce the residues of certain pesticides but to less extent.

Table (1): Residues of 28 evaluated compound in raw and processed milk (mg/kg) and their processing factor (P.F. %)

		Raw milk	Boiled milk		Pasteurized milk		Yoghurt							
	Fat %	3.05	1.25		2.55		6.95							
No.	Compound	[A]*	[B]**	P.F.	[C]	P.F.	[D_0]	P.F.	[D_1]	P.F.	[D_2]	P.F.	[D_3]	P.F.
1	Biphenyl	0.082	0.067 ± 4	72	0.081 ± 1	88	0.096 ± 15	105	0.074 ± 0	80	0.075 ± 5	81	0.065 ± 4	71
2	Chlordane-cis	0.091	0.072 ± 10	79	0.078 ± 6	85	0.093 ± 6	102	0.078 ± 6	85	0.072 ± 3	79	0.071 ± 6	78
3	Chlordane-trans	0.090	0.075 ± 3	83	0.079 ± 8	88	0.092 ± 4	102	0.078 ± 2	87	0.072 ± 3	81	0.070 ± 7	79
4	Chlorpyrifos	0.121	0.104 ± 9	86	0.108 ± 3	89	0.093 ± 5	77	0.076 ± 2	62	0.074 ± 9	61	0.074 ± 8	61
5	Chlorpyrifos-methyl	0.126	0.111 ± 9	88	0.113 ± 6	89	0.096 ± 5	76	0.079 ± 3	63	0.078 ± 7	61	0.079 ± 7	63
6	Cypermethrin	0.109	0.095 ± 4	87	0.099 ± 12	91	0.117 ± 7	108	0.099 ± 13	91	0.098 ± 5	90	0.089 ± 11	82
7	DDD- o,p`	0.102	0.084 ± 6	82	0.088 ± 8	87	0.101 ± 8	99	0.087 ± 4	85	0.079 ± 4	78	0.077 ± 9	75
8	DDD- p,p`	0.098	0.082 ± 2	83	0.086 ± 5	88	0.091 ± 10	93	0.081 ± 7	82	0.074 ± 5	75	0.071 ± 10	73
9	DDE- p,p`	0.082	0.068 ± 3	82	0.069 ± 3	84	0.079 ± 1	96	0.062 ± 4	76	0.060 ± 1	73	0.059 ± 5	72
10	DDT- o,p`	0.099	0.082 ± 2	83	0.087 ± 5	88	0.091 ± 10	92	0.081 ± 7	82	0.074 ± 5	75	0.071 ± 10	72
11	Deltamethrin	0.114	0.101 ± 2	88	0.104 ± 11	91	0.102 ± 9	89	0.097 ± 17	84	0.093 ± 1	81	0.087 ± 11	76
12	Diazinon	0.127	0.111 ± 11	88	0.119 ± 4	93	0.108 ± 5	85	0.085 ± 4	67	0.086 ± 9	68	0.084 ± 6	66
13	Dichlofluanid	0.104	0.055 ± 8	53	0.059 ± 1	57	0.03 ± 13	29	0.026 ± 7	24	0.024 ± 9	23	0.023 ± 6	22
14	Dieldrin	0.106	0.085 ± 5	81	0.089 ± 7	84	0.111 ± 3	105	0.089 ± 7	84	0.087 ± 3	82	0.085 ± 8	80
15	Diphenylamine	0.123	0.104 ± 9	85	0.110 ± 3	90	0.107 ± 14	87	0.099 ± 10	81	0.09 ± 10	74	0.081 ± 5	66
16	Endosulfan- α isomer	0.103	0.086 ± 4	83	0.087 ± 10	84	0.113 ± 8	110	0.090 ± 3	88	0.088 ± 1	86	0.084 ± 7	82
17	Endosulfan- β isomer	0.114	0.097 ± 6	85	0.104 ± 4	91	0.123 ± 12	108	0.098 ± 0	86	0.094 ± 8	83	0.089 ± 15	78
18	Endosulfan-sulfate	0.126	0.107 ± 2	85	0.111 ± 7	88	0.126 ± 13	101	0.108 ± 9	86	0.103 ± 10	82	0.095 ± 9	76
19	Endrin	0.112	0.092 ± 3	82	0.098 ± 2	87	0.096 ± 17	85	0.08 ± 9	71	0.077 ± 9	68	0.070 ± 14	62
20	Fenvalerate	0.108	0.095 ± 2	87	0.098 ± 11	90	0.072 ± 8	67	0.063 ± 10	58	0.060 ± 3	56	0.057 ± 10	52
21	HCH- α isomer	0.113	0.098 ± 5	87	0.106 ± 2	94	0.122 ± 7	108	0.100 ± 5	88	0.097 ± 6	86	0.092 ± 8	81
22	HCH- β isomer	0.121	0.113 ± 5	93	0.114 ± 1	95	0.119 ± 12	99	0.098 ± 7	81	0.094 ± 11	78	0.086 ± 12	71
23	HCH- δ isomer	0.119	0.103 ± 5	87	0.107 ± 0	90	0.126 ± 9	107	0.102 ± 6	86	0.100 ± 6	84	0.093 ± 8	79
24	HCH- γ isomer	0.106	0.091 ± 2	86	0.103 ± 8	97	0.122 ± 10	115	0.099 ± 8	94	0.097 ± 8	91	0.085 ± 12	80
25	Heptachlor	0.094	0.088 ± 6	94	0.092 ± 7	98	0.074 ± 14	79	0.066 ± 7	70	0.061 ± 6	65	0.057 ± 13	61
26	Heptachlor-exo-epox.	0.106	0.09 ± 6	84	0.092 ± 3	86	0.109 ± 8	103	0.092 ± 1	87	0.087 ± 4	82	0.080 ± 8	75
27	PCB 101	0.069	0.056 ± 4	82	0.058 ± 4	84	0.068 ± 1	98	0.054 ± 2	78	0.054 ± 1	78	0.053 ± 4	77
28	Pirimiphos-methyl	0.126	0.111 ± 9	88	0.114 ± 5	90	0.107 ± 6	84	0.088 ± 4	69	0.085 ± 9	67	0.086 ± 6	68

All data were expressed as means from at least two independent trails.
*[A] = raw milk, [B]= boiled milk for 15 min, [C]= pasteurized milk, [D_0]= yoghurt at zero time and [D_1],[D_2] and [D_3]= yoghurt stored for 1,2 and 3 days respectively.
** mean number ± CV

Degradation of pesticides in pasteurized milk [C]

When the spiked milk was pasteurized, proceeding analysis showed that the contents of pesticide residues (mg/kg) in milk decreased with pasteurization and the processing factor (P.F.) was calculated (Table 1). Pesticide which was most affected by pasteurization was Dichlofluanid with P.F. of 57% during pasteurization. The results indicated that pasteurization has a lower effect on the reduction of the tested pesticides compared with boiling process. These results proved the efficiency role of milk boiling on the degradation of Dichlofluanid than in pasteurization, as example among the tested compounds, Dichlofluanid was the most susceptible compound to be affected either by boiling or pasteurization.

Degradation of pesticides in manufactured yoghurt [D]

Concerning the fresh processed yoghurt [D_0], the P.F. of Heptachlor was (79%), while for Fenvalerate was (67%), Dichlofluanid was (29%), Chlorpyrifos was (77%) and Chlorpyrifos-methyl was (76%). For the samples of yoghurt of 1-day old post-manufacturing [D_1], the processing factor was ranging between 24% (as for Dichlofluanid) and 94 % (as for HCH-γ isomer). The residues and P.F. of the 2-day old of yoghurt [D_2] were little lower than those of 1-day old [D_1], giving a range of 23-91%. Three days post-manufacturing [D_3], the residues of the evaluated pesticides in yoghurt samples were declined and the processing factor pronounced a range of 22-80%. It would be noticed that the tested polychlorinated biphenyl (PCB 101) was declined after the first day of yoghurt storage to 78% and it was stable after that giving the same level of residues in those samples of [D_1], [D_2] and [D_3].The results show that the content of the pesticides in the milk all decreased gradually as the treatment progressed. According to the results, the decrease in the level of pesticides in yoghurt samples after 24 hrs. Post-period fermentation showed that Dichlofluanid (with P.F. 29%) was the most susceptible tested pesticide and the more stable pesticide was HCH-γ isomer (with P.F. 115%). For the 1st, 2nd and 3rd day of storage, the most affected pesticide was Dichlofluanid (with P.F. 24%, 23%, 22%, respectively), while for HCH-γ isomer P.F. was 94%, 91% and 80%, respectively, which was agreed with the results of Li-Ying *et al.* (2010) who studied the degradation kinetics of pesticides in milk during yoghurt processing and also with those of Abou-Arab (1998) who studied the effects of processing and storage of dairy products on lindane residues and its metabolites.

As indicated, food processing will usually result in a decrease in pesticide or contaminant levels Petersen *et al.* (1996) and FAO/WHO (1999). However, in some cases, residue levels may increase in the final product due to the concentration factors of raw commodities in the process of the final product. This concentration effect can be related to the accumulation of lipophilic materials in the fatty phase of a food such as butter compared to milk or vegetable oils Petersen *et al.* (1996), Hamilton *et al.* (2004) and Geetanjali *et al.* (2009). For compounds like some oraganochlorine, the processing factor was higher than 100% during yoghurt-manufacturing which was in harmony with those results of Gonzalez *et al.* (2011).

CONCLUSION

Literature review demonstrates that in most cases the processing leads to large reductions in residue levels in the processed milk, particularly through boiling, pasteurization, and yoghurt-manufacturing operations. The presented results showed that Dichlofluanid was the most susceptible compound affected during all processing steps. Most of the tested compounds showed significant degradation after yoghurt-manufacturing especially (Chlorpyrifos, Chlorpyrifos-methyl, Dichlofluanid, Heptachlor and Fenvalerate) indicating that the degradation of the pesticides was enhanced by the applied starter. This lead to the conclusion that the reduction of pesticide residues show that yoghurt-manufacturing process has higher effect than boiling and pasteurization processes and therefor it would be better to consume milk as processed type than raw milk to reduce the possible contamination levels of pesticides.

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الملخـــــص العربـــــي

تقدير متبقيات المبيدات خلال عمليات تصنيع الحليب المختلفة

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تناولت الدراسة تأثير عمليات تصنيع الحليب (الغليان و البسترة و تصنيع الزبادي) علي متبقيات المبيدات المتواجدة في الحليب و ذلك باختيار 27 مبيد و مركب واحد من ثنائي الفينيل متعدد الكلور و تتبع تأثير عمليات تصنيع الحليب المختلفة علي مدي ثبات و متبقات هذه المركبات . تم حساب معامل التصنيع (الذي يعبر عن النسبة المئوية لمتبقيات المبيدات المختبرة في العينة بالمقارنة مع تلك المتبقيات في الحليب الخام) و ذلك بعد عملية الغليان و تراوح ما بين53 -94% بينما كان هذا المعامل للحليب المبستر ما بين 57 - 98% و بعد عملية تصنيع الزبادي بثلاثة أيام تراوح معامل التصنيع ما بين 22 -82 %. أظهرت النتائج أن عملية تصنيع الزبادي كان لها التأثير الأعلي في خفض متبقيات المبيدات في الحليب مقارنة بعمليتـــي الغليان و البسترة . و تشير الدراسة إلي إنخفاض متبقيات المبيدات في الحليب أثناء عملية تصنيع الزبادي يّعزي إلي كلاً من عمليتي التخمر و الغليان و لذلك ينصح بإستهلاك الحليب في صوره المــصنعة عــن إستخدامة في صورة الحليب الخام لتجنب التلوث المحتمل من المبيدات.