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Full Length Research Paper

Preliminary study on the degradation of seven organophosphorus pesticides in bovine milk during lactic acid fermentation or heat treatment

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Bovine milk was spiked with seven organophosphorus pesticides, denthion, dimethoate, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, and subjected to heat treatment at 63, 80 and 100°C or lactic acid fermentation at 42°C with commercial directed vat set (DVS) starters to investigate the degradation kinetics of the pesticides in bovine milk during heat treatment and yoghurt processing. The pesticides spiked were extracted from the prepared samples, purified and analyzed with gas chromatograph technique. Then the kinetic parameters of degradation of them were calculated accordingly. Comparing this to that of the pesticides in the bovine milk heated at 42°C, the degradation of the pesticides in the bovine milk during yoghurt processing was found to be totally accelerated by the starters of lactic bacteria applied, because the half live periods of six pesticides decreased except for malathion, and two commercial DVS starters which showed different effects somewhat on the degradation of the pesticides. Analysis results also indicated that the pesticides degraded faster at higher temperature in bovine milk during heat treatment; and among the pesticides methyl parathion and phorate were the most unstable and stable one. Heat treatment and lactic acid fermentation could reduce the level of organophosphorus pesticide in dairy products.

Key words: Bovine milk, organophosphorus pesticide, degradation, yoghurt, starter, heat treatment.

INTRODUCTION

Organophosphorus pesticides are widely used in agriculture to protect plants from insects, and hence provide numerous benefits in terms of production and quality. When dairy cows are fed with organophosphorus pesticide polluted forages, or drink polluted water, bovine milk and dairy products could be tainted with pesticide residues (Battu et al., 2004; Fytianos et al., 1985), which might lead to safety risk (Amdur et al., 1991; Russo et al., 2002). Mallatou et al. (1997) investigated thirty-eight samples of bovine milk and twenty-eight samples of cheese, and found that eleven milk samples contained organophosphorus pesticide residues with contents below the maximum permitted amount, and the contents of methyl parathion in two samples were 43 and 280 $\mu g \cdot kg^{-1}$. Pagliuca et al. (2006) also found that among one hundred and thirty-five raw milk samples analyzed, thirty-seven samples were positive for traces and ten samples showed organophosphorus pesticide contamination in the range of 5 to 18 $\mu g \cdot kg^{-1}$. These works demonstrated that the potential existence of organophosphorus pesticides in the milk or dairy products cannot be ignored.

Gas chromatography (GC)-based technique is widely employed to determine the existence or amount of organophosphorus pesticide residues in foods (Ferrer and Thurman, 2007), and special separation or isolation prior to GC analysis might be needed, such as supercritical fluid extraction or solid phase micro-extraction (Poustka et al., 2003; Wang et al., 2008).

Pagliuca et al. (2005) studied the detection method of organo-phosphorus pesticides in matrices of animal origin with dual column capillary GC and nitrogen phosphorus detector. Recently, Uygun et al. (2008) investigated the levels of insecticides residues in wheat,

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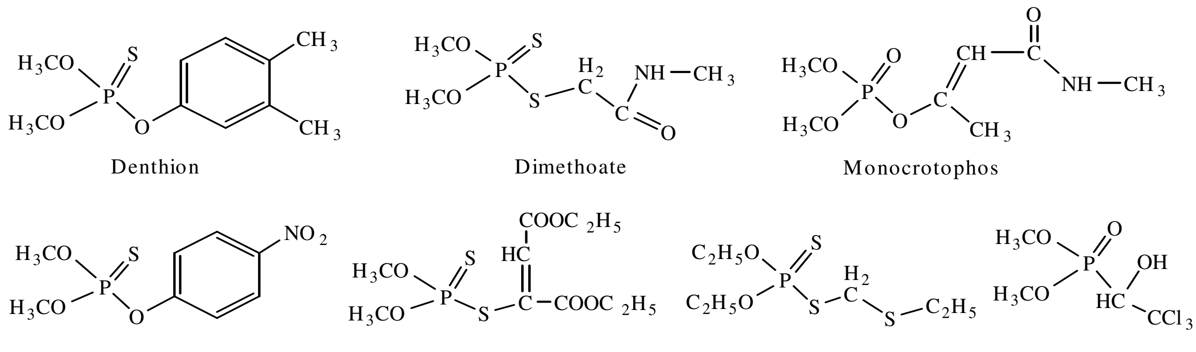

Figure 1. Chemical structures of seven organophosphorus pesticides.

semolina and spaghetti produced from stored wheat with GC analysis. Wang et al. (2008) also employed headspace solid phase micro-extraction coupled with GC and nitrogen phosphorus detector to determine five organophosphorus pesticides in pakchoi samples.

Due to their instabilities, the residue levels of organophosphorus pesticides in foods are affected by a number of physical factors applied in food processing, including fermentation, heat treatment and drying. In addition, the chemical nature of organophosphorus pesticides and some environmental factors such as chemical structure, pH, light, metal ions and ozone, also have impacts on the degradation of pesticide residues (Bogialli et al., 2006; Ozbey and Uygun, 2007). For safety concerns, there exists a need to know the behavior of organophosphorus pesticides in bovine milk during dairy processing. In the presented study, we added seven organophosphorus pesticides (their chemical structures are given in Figure 1) to bovine milk, treated the spiked bovine milk with simulated trials (lactic acid fermentation or heating), employed GC-based method to analyze the pesticides in the prepared samples, and investigated the degradation of the pesticides in the bovine milk during yoghurt processing or heat treatments. The aim was to show the impact of yoghurt fermentation by lactic bacteria and heat temperature on the degradation of the pesticides in dairy products.

MATERIALS AND METHODS

Materials

Bovine milk was collected daily from a dairy farm in Harbin, Heilongjiang Province and stored at 4℃ before use. Seven organophosphorus pesticide standards, denthion, dimethoate, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and stored at -18℃. Two commercial directed vat set (DVS) starters applied in yoghurt preparation were purchased from Rhodia (Melle, France) and Danisco (Copenhagen, Denmark), and stored at -18℃ before use. The chemicals and solvents used were analytical and chromatographic agents. Water used was highly purified water prepared with Milli-Q PLUS (Millipore Corporation, New York, NY, USA).

GC analysis of organophosphorus pesticides

GC analysis of organophosphorus pesticides, either for the standard solutions or the prepared samples, was performed by using an Agilent 7890 GC (Agilent Technologies, Inc., Santa Clara, USA), equipped with a flame photometric detector and a capillary column (DB–1701, 30 m×0.250 mm×0.25 μm), and nitrogen as carrier gas at a flow rate of 3 $mL \cdot min^{-1}$. The analysis followed the method described by Pagliuca et al. (2005) with some modifications. The temperature profile was set as follows: an initial temperature of 100℃ for 1 min, heating from 100 to 195℃ at 30℃ min^{-1}, holding for 8 min at 195℃, heating from 195 to 202℃ at 10℃ min^{-1}, holding for 1 min at 202℃, heating from 202 to 205℃ at 1℃ min^{-1}, and heating from 205 to 240℃ at 15℃ min^{-1}. The injector and detector temperature were set at 200 and 250℃, respectively. Quantification of the pesticides was performed by comparing the peak areas of the pesticides to a calibration curve of the standards, and multitude-point calibration was used.

Preparation of yoghurt

Some organophosphorus pesticides were added to bovine milk, and the spiked bovine milk was shaken vigorously and stood for half of an hour to ensure pesticide distribution. The preparation of yoghurt followed a reported method of Isleten and Karagul-Yuceer (2006). Container of the spiked bovine milk was placed in a water bath and heated to 90℃ for 15 min, then cooled in a water bath to 42℃. Commercial DVS starter was added at recommended level by the producer, 0.6 $g \cdot kg^{-1}$ milk. The inoculated milk was poured into glass cups (each having a capacity of 200 mL) with lids and incubated at 42℃ in an incubator. At regular time intervals, one cup was selected random from the bulk samples as yoghurt sample for GC analysis, and rapidly cooled by an ice water bath. All prepared samples were subjected to extraction and purification treatment immediately. Meanwhile, a control was also prepared with same procedure but without starter addition.

Spiking and heat treatment of bovine milk samples

Bovine milk was spiked with seven organophosphorus pesticides at different addition levels. For recovery study, the addition levels of

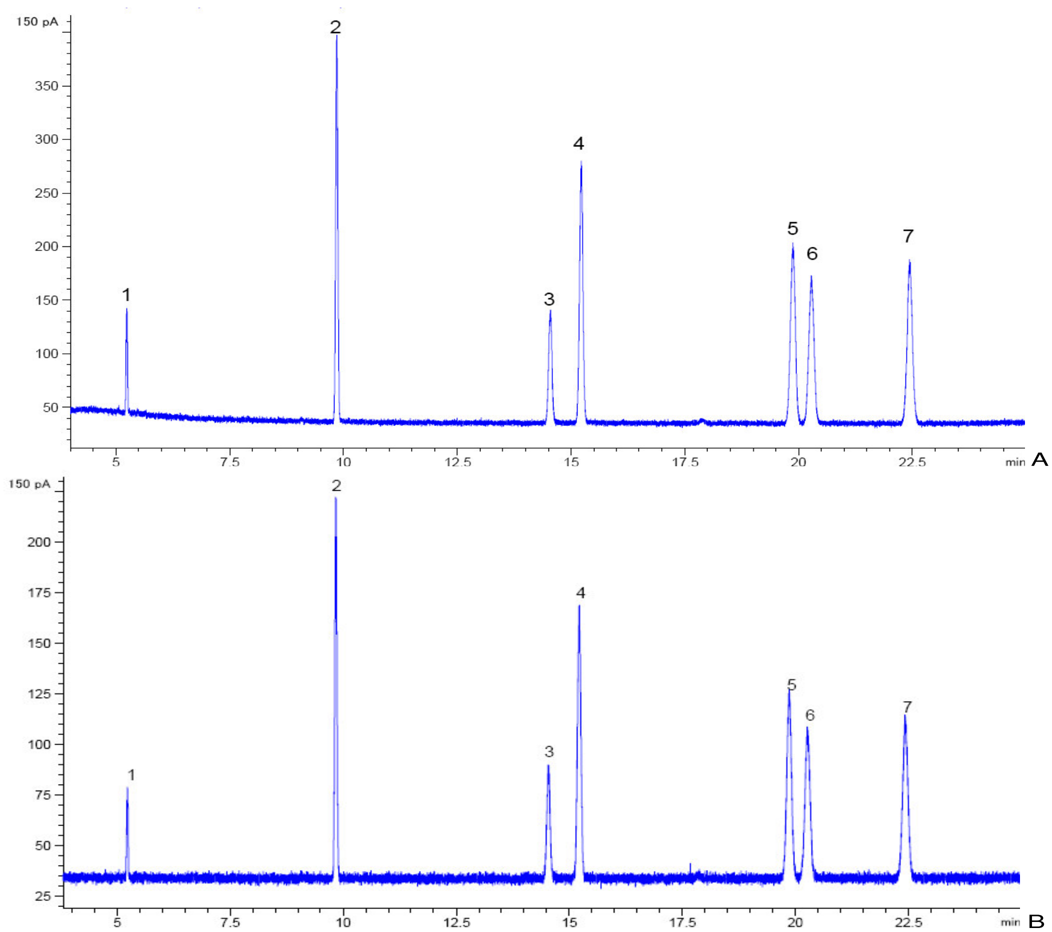

Figure 2. Typical GC profiles of seven organophosphorus pesticides for standard solution (A) and spiked bovine milk sample (B). Peak 1 to 7 represent trichlorfon, phorate, monocrotophos, dimethoate, denthion, malathion and methyl parathion, respectively. The content of the pesticides in milk sample or standard solution was 0.6 or 1.2 mg·kg $^{-1}$.

the pesticides were set at 0.04, 0.1 and 1.0 mg·kg $^{-1}$, respectively. For degradation study, the addition level of the pesticides was fixed at 1.0 mg·kg^{-1}. The spiked bovine milk samples were shaken vigorously and stood for half of an hour to ensure pesticide distribution. Then the spiked samples for degradation study were heated in a water bath to 63, 80 and 100℃ for 3 h. At regular time intervals, 10.0 g portions were separated from the bulk samples and rapidly cooled by an ice water bath. All prepared samples were subjected to extraction and purification treatment before GC analysis.

Extraction and purification of organophosphorus pesticides

The extraction and purification of organophosphorus pesticides followed the methods of Hutton and Heslington (2005) and Giampiero and Teresa (2005) with some modifications. Ten milliliters of the samples were extracted with 20 mL of acetone-acetonitrile (1:4, v/v), shaken vigorously for 2 min, then centrifuged at 6000 r·min^{-1} for 6 min. The liquid phase was transferred to a separatory funnel, and the residue of the samples was collected and re-extracted with 15 mL of acetone-acetonitrile (1:4, v/v) as above. The liquid phases were combined and vigorously shaken with 50 mL dichloromethane for 10 min, and set 20 min for phase separation. Dichloromethane phase was separated and filtered through anhydrous sodium sulphate (2.0 g). The purified extract of 15 mL was measured and concentrated to dryness with nitrogen gas, then measured to 1.0 mL with acetone and filtered through a 0.45 µm film before GC analysis.

Statistical analysis

All data were expressed as means ± SE (standard error) from at least three independent trials. Kinetic parameters were calculated with linear regression analysis. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS AND DISCUSSION

Analysis of organophosphorus pesticides in bovine milk with GC

Seven organophosphorus pesticides could be extracted efficiently from the milk samples and detected accurately with selected analysis procedure. Typical profiles of GC analysis of the pesticides for standard solutions and the samples (for recovery analysis) are shown in Figure 2, which indicates that all pesticides were well-separated by column and the existence of interfering substance could be ignored. Practical GC analysis also showed that the linear range of detection for the pesticides in our study was in the range of 0.1 to 8 $mg{\cdot}kg^{-1}$($R^2 \geq 0.995$), with the lowest detection limits from 0.006 to 0.02 $mg{\cdot}kg^{-1}$ for different pesticides, and the relative standard deviation of detection was smaller than 5.5% (n = 9). When the addition levels of the pesticides were 0.1 or 1.0 $mg{\cdot}kg^{-1}$, the recoveries of the pesticides ranged from 80.8% (trichlorphon, addition level 0.1 $mg{\cdot}kg^{-1}$) to 125.9% (dimethoate, addition level 1.0 $mg{\cdot}kg^{-1}$). These results demonstrate that GC analysis procedure selected can be applied to detect these pesticides in the bovine milk samples.

Pagliuca et al. (2005) isolated the organophosphorus pesticides from animal origin by liquid partition followed by cleanup with solid phase cartridge (SPE C_{18}), and compared the recoveries of the pesticides from two columns (ZB5 and ZB50) with different polarity. For bovine milk samples, the recoveries of acephate, chlorpyriphos, methyl chlorpyriphos, diazinon, methamidophos, methidathion, ethyl parathion, phorate and methyl pirimiphos obtained from ZB50 column ranged from 81% (phorate, addition level 0.01 $mg{\cdot}kg^{-1}$) to 117% (methidathion, addition level 0.05 $mg{\cdot}kg^{-1}$) except for methidathion (59%). But for liver or muscle samples, the recoveries of chlorpyriphos, dimethoate and parathion-ethyl obtained from ZB5 column ranged from 60 to 81%, or 68 to 76%. Their results confirm that the extraction and purification procedure applied in our work is a good selection.

Degradation of organophosphorus pesticides in bovine milk during yoghurt processing

If the spiked bovine milk was fermented with DVS starter to prepare yoghurt samples, it was found that addition of the pesticides to bovine milk had no influence on the fermentation of yoghurt. The variations of the pesticides in the bovine milk during lactic acid fermentation were analyzed and compared with control (the bovine milk heat-treated at 42℃ only), to show the impact of starter on the degradation of the pesticides. The analysis results are given in Table 1, and typical GC profiles of the pesticides for control and yoghurt sample are shown in Figure 3. The kinetic parameters of degradation of seven pesticides in bovine milk subjected to lactic acid fermentation or heat treatment at 42℃ are calculated accordingly and listed in Table 2. Comparing the half live periods of each pesticide, it was found that the pesticides degraded faster when the spiked bovine milk subjected to lactic acid fermentation, except for malathion. The half life periods of the pesticides in the bovine milk subjected to 42℃ heat treatment were in the range of 11.0 to 16.7 h, while yoghurt fermented with Rhodia starter were in the range of 9.6 to 14.6 h, and yoghurt fermented with Danisco starter were in the range of 10.0 to 15.9 h. The decrease of half life period showed the degradation of the pesticides was accelerated totally by the starters added. Two DVS starters gave different impacts on the degradation of single pesticide. Comparing that of the pesticides in the bovine milk subjected to 42℃ heat treatment, the kinetic parameter of degradation of methyl parathion, trichlorfon, phorate, denthion and dimethoate in the yoghurt fermented with Rhodia starter had an increase of 26.1, 24.2, 19.7, 14.4 and 11.7%, respectively, while in the yoghurt fermented with Danisco starter, the kinetic parameter of degradation of dimethoate, trichlorfon and phorate had an increase of 20.4, 18.4 and 5.6%, respectively. The results first revealed that the growth of lactic bacteria in bovine milk could reduce the residue levels of some organo0-phosphorus pesticides, and might decrease the safety risk of the products.

Abou-Arab (2002) studied the effect of starter on dich-diphenyl-trichloro (DDT) and lindane in tryptone soya broth (TSB), mineral salt medium (MSM) and fermented sausage, and found the role of *Lactobacillus plantarum* on the degradation of DDT and lindane. The reductions of DDT at 15 days were about 24.1 and 32.5% in TSB and MSM without nitrite addition, or 37.5 and 46.4% in the same media with nitrite addition. The reductions oflindane were 27.9 and 40.0% in TSB and MSM without nitrite addition, or 38.4 and 48.4% in the same media with nitrite addition. He also found that *Micrococcus varians* had ability to metabolize DDT and lindane. If sausage mixture were cultured with meat starter (*L. plantarum* and *M.varians*) for 72 h, the reduction was 10% in the case of DDT and 18% in lindane. Abou-Arab (1997) also reported that the microorganisms isolated from Ras cheese could reduce the total DDT residues by 10.8, 11.8 and 4.8% for *Streptococci*, *Lactobacilli* and yeasts, respectively, during the incubation period of 10 days. These reports give supports to our result. This is of great interest for yoghurt processing, because it implies that the starter is capable of reducing the toxicological risk of yoghurt.

Degradation of organophosphorus pesticides in bovine milk during heat treatment

When the spiked bovine milk was heated to 63, 80 and 100℃, respectively, proceeding analysis showed that the contents of the pesticides in bovine milk decreased steadily as heat time extended or heat temperature elevated (Table 3). GC profiles of the pesticides for three heat-treated bovine milk samples are given in Figure 4. The decrease of the pesticides in heat-treated bovine milk was the result of degradation of the pesticides. Based on the previous reports that the degradation

Table 1. Contents of seven organophosphorus pesticides in the control and yoghurt samples at different times (mean ± SD, mg.kg^{-1})[a].

Pesticide	Samples [b]	Contents of the pesticides at different treatment times (h)				
		0	2	4	6	8
Denthion	Control	1.121 ± 0.036	0.994 ± 0.028	0.942 ± 0.045	0.901 ± 0.025	0.852 ± 0.020
	Yoghurt 1	1.152 ± 0.040	1.023 ± 0.035	0.974 ± 0.042	0.921 ± 0.038	0.842 ± 0.025
	Yoghurt 2	1.133 ± 0.027	1.024 ± 0.031	0.954 ± 0.032	0.932 ± 0.026	0.853 ± 0.019
Dimethoate	Control	1.073 ± 0.045	0.964 ± 0.046	0.938 ± 0.036	0.874 ± 0.031	0.831 ± 0.030
	Yoghurt 1	1.163 ± 0.040	1.042 ± 0.047	0.987 ± 0.023	0.943 ± 0.046	0.892 ± 0.044
	Yoghurt 2	1.171 ± 0.013	1.034 ± 0.027	0.972 ± 0.018	0.923 ± 0.011	0.881 ± 0.035
Malathion	Control	1.023 ± 0.030	0.931 ± 0.051	0.863 ± 0.050	0.832 ± 0.042	0.814 ± 0.045
	Yoghurt 1	1.134 ± 0.025	1.043 ± 0.031	0.992 ± 0.032	0.953 ± 0.025	0.942 ± 0.031
	Yoghurt 2	1.231 ± 0.020	1.142 ± 0.036	1.094 ± 0.027	1.062 ± 0.033	1.053 ± 0.029
Methyl parathion	Control	1.051 ± 0.030	0.983 ± 0.035	0.934 ± 0.035	0.873 ± 0.045	0.832 ± 0.051
	Yoghurt 1	1.214 ± 0.030	1.124 ± 0.055	1.042 ± 0.050	0.981 ± 0.045	0.940 ± 0.042
	Yoghurt 2	1.312 ± 0.023	1.251 ± 0.031	1.184 ± 0.024	1.122 ± 0.021	1.081 ± 0.033
Monocrotophos	Control	1.082 ± 0.055	1.033 ± 0.035	0.954 ± 0.031	0.895 ± 0.025	0.872 ± 0.025
	Yoghurt 1	1.031 ± 0.040	0.983 ± 0.040	0.912 ± 0.046	0.833 ± 0.062	0.814 ± 0.063
	Yoghurt 2	1.192 ± 0.017	1.093 ± 0.020	1.042 ± 0.011	0.963 ± 0.013	0.951 ± 0.033
Phorate	Control	1.131 ± 0.026	1.093 ± 0.016	1.031 ± 0.046	0.983 ± 0.020	0.952 ± 0.026
	Yoghurt 1	1.101 ± 0.032	1.052 ± 0.036	0.983 ± 0.035	0.914 ± 0.035	0.890 ± 0.031
	Yoghurt 2	1.215 ± 0.016	1.142 ± 0.013	1.103 ± 0.020	1.054 ± 0.023	1.012 ± 0.019
Trichlorfon	Control	1.036 ± 0.012	0.933 ± 0.016	0.892 ± 0.011	0.871 ± 0.015	0.860 ± 0.015
	Yoghurt 1	1.071 ± 0.015	0.953 ± 0.010	0.914 ± 0.015	0.873 ± 0.025	0.854 ± 0.030
	Yoghurt 2	1.131 ± 0.023	1.022 ± 0.013	0.991 ± 0.017	0.952 ± 0.014	0.921 ± 0.020

a: The number of trial times is three.
b: Yoghurts 1 and 2 were produced with commercial DVS starter of Rhodia and Danisco.

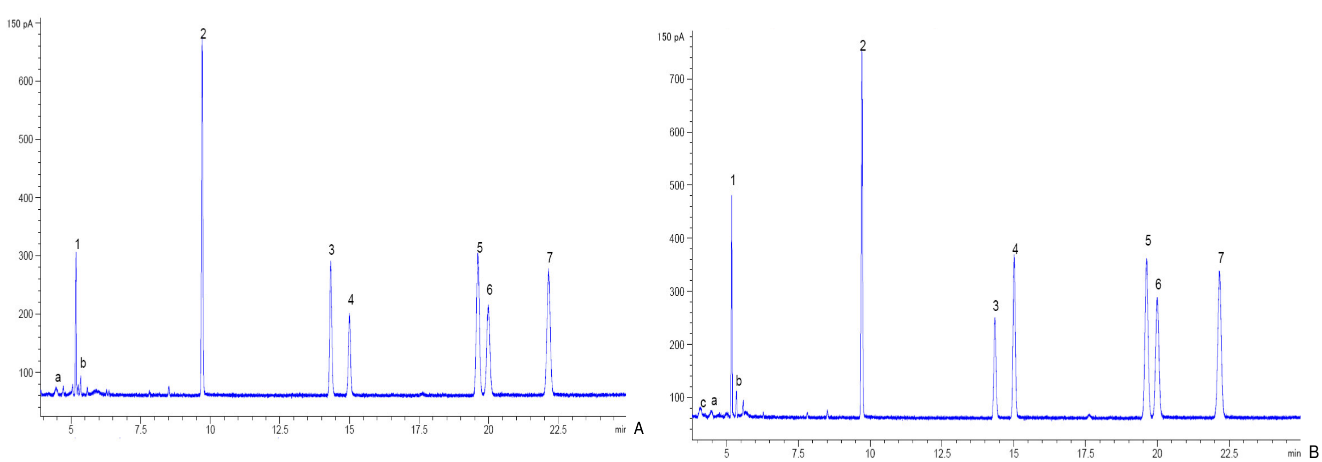

Figure 3. Typical GC profiles of seven organophosphorus pesticides of the heat-treated bovine milk (A) and prepared yoghurt (B) at 42℃. Peak 1 to 7 represent trichlorfon, phorate, monocrotophos, dimethoate, denthion, malathion and methyl parathion, respectively.

Table 2. Degradation kinetic parameters of seven organophosphorus pesticides in the bovine milk subjected to 42°C heat treatment (control) and lactic acid fermentation (yoghurt).

Pesticide	Kinetic parameters [a]			
	Samples [b]	k (h^{-1})	R^2	$t_{1/2}$ (h)
Denthion	Control	0.0631	0.936	11.0
	Yoghurt 1	0.0722	0.966	9.6
	Yoghurt 2	0.0652	0.956	10.6
Dimethoate	Control	0.0574	0.957	12.1
	Yoghurt 1	0.0641	0.950	10.8
	Yoghurt 2	0.0691	0.933	10.0
Malathion	Control	0.0517	0.915	13.4
	Yoghurt 1	0.0474	0.912	14.6
	Yoghurt 2	0.0436	0.900	15.9
Methyl parathion	Control	0.0548	0.994	12.6
	Yoghurt1	0.0691	0.979	10.0
	Yoghurt 2	0.0591	0.993	11.7
Monocrotophos	Control	0.0558	0.974	12.4
	Yoghurt1	0.0584	0.974	11.8
	Yoghurt 2	0.0612	0.949	11.3
Phorate	Control	0.0468	0.989	14.8
	Yoghurt 1	0.0560	0.981	12.4
	Yoghurt 2	0.0494	0.987	14.0
Trichlorfon	Control	0.0414	0.940	16.7
	Yoghurt 1	0.0514	0.900	13.5
	Yoghurt 2	0.0490	0.916	14.1

a: k, rate constant of degradation; R, coefficient of regression; $t_{1/2}$, half life period.
b: Yoghurts 1 and 2 were produced with commercial DVS starters from Rhodia and Danisco.

kinetics of organophosphorus pesticides is a first order reaction (von Götz et al., 1999; Vanclooster et al., 2000) and the data listed in Table 3, kinetic parameters of degradation of seven pesticides in bovine milk are calculated accordingly and listed in Table 4. Half life periods of the pesticides in the bovine milk at 63°C are in the range of 6.6 to 10.3 h, at 80°C in the range of 4.8 to 8.6 h, and at 100°C in the range of 4.0 to 6.0 h, indicating the pesticides degraded faster at higher temperature. According to the values of half life period, the instability of the pesticides is in the order of methyl parathion > denthion > malathion > trichlorfon > dimethoate > monocrotophos > phorate.

The degradation of organophosphorus pesticides in bovine milk during heat treatment had not reported yet, but Uygun and Senoz (2008) had studied the degradation of methyl chlorpyrifos, fenitrothion, malathion and methyl pirimiphos in wheat stored at ambient temperature for five months. They found that the pesticides gave different degradation behaviors at different storage periods, but storage period was generally not effective enough to reduce the residues in wheat to the levels below the maximum residue limits. During the storage period, malathion, fenitrothion, methyl chlorpyrifos and methyl pirimiphos in wheat decreased by 88, 86, 84 and 76%, respectively, indicating that methyl pirimiphos and malathion was most stable and unstable. Unfortunately, degradation kinetics of the pesticides was not studied.

Conclusions

Bovine milk was spiked with seven organophosphorus pesticides, denthion, dimethoate, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, and then subjected to commercial DVS starter fermentation or

Table 3. Contents of seven organophosphorus pesticides in the bovine milk subjected to heat treatment at three temperatures (mean ± SD, mg·kg $^{-1}$) [a].

Pesticide	Temperature (℃)	Contents of the pesticide in the milk at different times (h)					
		0.5	1	1.5	2	2.5	3
Denthion	63	0.950 ± 0.021	0.890 ± 0.031	0.739 ± 0.013	0.644 ± 0.018	0.531 ± 0.011	0.456 ± 0.024
	80	0.998 ± 0.011	0.877 ± 0.019	0.654 ± 0.015	0.494 ± 0.031	0.410 ± 0.023	0.360 ± 0.032
	100	0.986 ± 0.011	0.852 ± 0.022	0.681 ± 0.027	0.491 ± 0.028	0.274 ± 0.015	0.148 ± 0.018
Dimethoate	63	0.979 ± 0.029	0.875 ± 0.020	0.796 ± 0.030	0.72 ± 0.013	0.670 ± 0.025	0.596 ± 0.041
	80	0.986 ± 0.027	0.891 ± 0.017	0.774 ± 0.016	0.639 ± 0.020	0.551 ± 0.022	0.490 ± 0.038
	100	0.928 ± 0.017	0.794 ± 0.039	0.663 ± 0.031	0.549 ± 0.011	0.418 ± 0.036	0.302 ± 0.023
Malathion	63	0.952 ± 0.023	0.843 ± 0.017	0.755 ± 0.038	0.693 ± 0.021	0.560 ± 0.025	0.471 ± 0.028
	80	1.01 ± 0.012	0.843 ± 0.032	0.673 ± 0.038	0.523 ± 0.010	0.424 ± 0.033	0.370 ± 0.022
	100	0.971 ± 0.028	0.86 ± 0.029	0.725 ± 0.021	0.562 ± 0.031	0.377 ± 0.021	0.181 ± 0.08
Methylparathion	63	0.932 ± 0.015	0.875 ± 0.017	0.794 ± 0.019	0.716 ± 0.014	0.653 ± 0.025	0.593 ± 0.024
	80	1.04 ± 0.037	0.817 ± 0.015	0.682 ± 0.018	0.605 ± 0.011	0.543 ± 0.015	0.499 ± 0.011
	100	0.968 ± 0.013	0.836 ± 0.010	0.675 ± 0.019	0.487 ± 0.029	0.282 ± 0.010	0.113 ± 0.012
Monocrotophos	63	0.959 ± 0.017	0.871 ± 0.018	0.786 ± 0.016	0.691 ± 0.016	0.638 ± 0.027	0.58 ± 0.016
	80	0.948 ± 0.010	0.842 ± 0.018	0.747 ± 0.017	0.685 ± 0.014	0.631 ± 0.011	0.592 ± 0.010
	100	0.932 ± 0.013	0.848 ± 0.011	0.741 ± 0.012	0.623 ± 0.019	0.491 ± 0.012	0.329 ± 0.018
Phorate	63	0.965 ± 0.012	0.891 ± 0.025	0.821 ± 0.020	0.767 ± 0.014	0.690 ± 0.025	0.627 ± 0.023
	80	0.970 ± 0.017	0.852 ± 0.012	0.793 ± 0.024	0.722 ± 0.020	0.651 ± 0.018	0.552 ± 0.017
	100	0.945 ± 0.018	0.867 ± 0.013	0.755 ± 0.015	0.623 ± 0.011	0.512 ± 0.028	0.378 ± 0.019
Trichlorfon	63	0.914 ± 0.016	0.877 ± 0.022	0.818 ± 0.018	0.763 ± 0.014	0.697 ± 0.011	0.559 ± 0.019
	80	0.968 ± 0.034	0.924 ± 0.018	0.836 ± 0.032	0.710 ± 0. 011	0.592 ± 0.022	0.543 ± 0.015
	100	0.946 ± 0.019	0.878 ± 0.017	0.685 ± 0.010	0.571 ± 0.011	0.475 ± 0.015	0.345 ± 0.013

a: The number of trial times is three.

heat treatment at 42, 63, 80 and 100℃, respectively, to study the degradation of the pesticides during lactic acid fermentation or heat treatment. The pesticides were extracted from the prepared samples and purified with organic solvents, and determined by gas chromatography with flame photometric detector and a capillary column with selected conditions. The analysis results revealed that the lactic bacteria in two starters could accelerate the degradation of the pesticides in bovine milk totally. The kinetic parameter of degradation of methyl parathion, trichlorfon, phorate, denthion and dimethoate in the bovine milk fermented with Rhodia starter had an increase of 26.1, 24.2, 19.7, 14.4 and 11.7%, respectively, while that of dimethoate, trichlorfon and phorate in the bovine milk fermented with Danisco starter had an increase of 20.4, 18.4 and 5.6%, respectively. The results also showed that the pesticides in the bovine milk all decreased steadily during the heat treatment and gave faster degradation at higher temperature. The half life periods of the pesticides at 63℃ were in a range of 6.6 to 10.3 h, at 80℃ in a range of 4.8 to 8.6 h, and at 100℃ in a range of 4.0 to 6.0 h. Based on the values of the half life period, phorate and methyl parathion are most stable and unstable pesticides studied. These results indicate that both heat treatment and lactic acid fermentation might reduce the level of organophosphorus pesticides in bovine milk.

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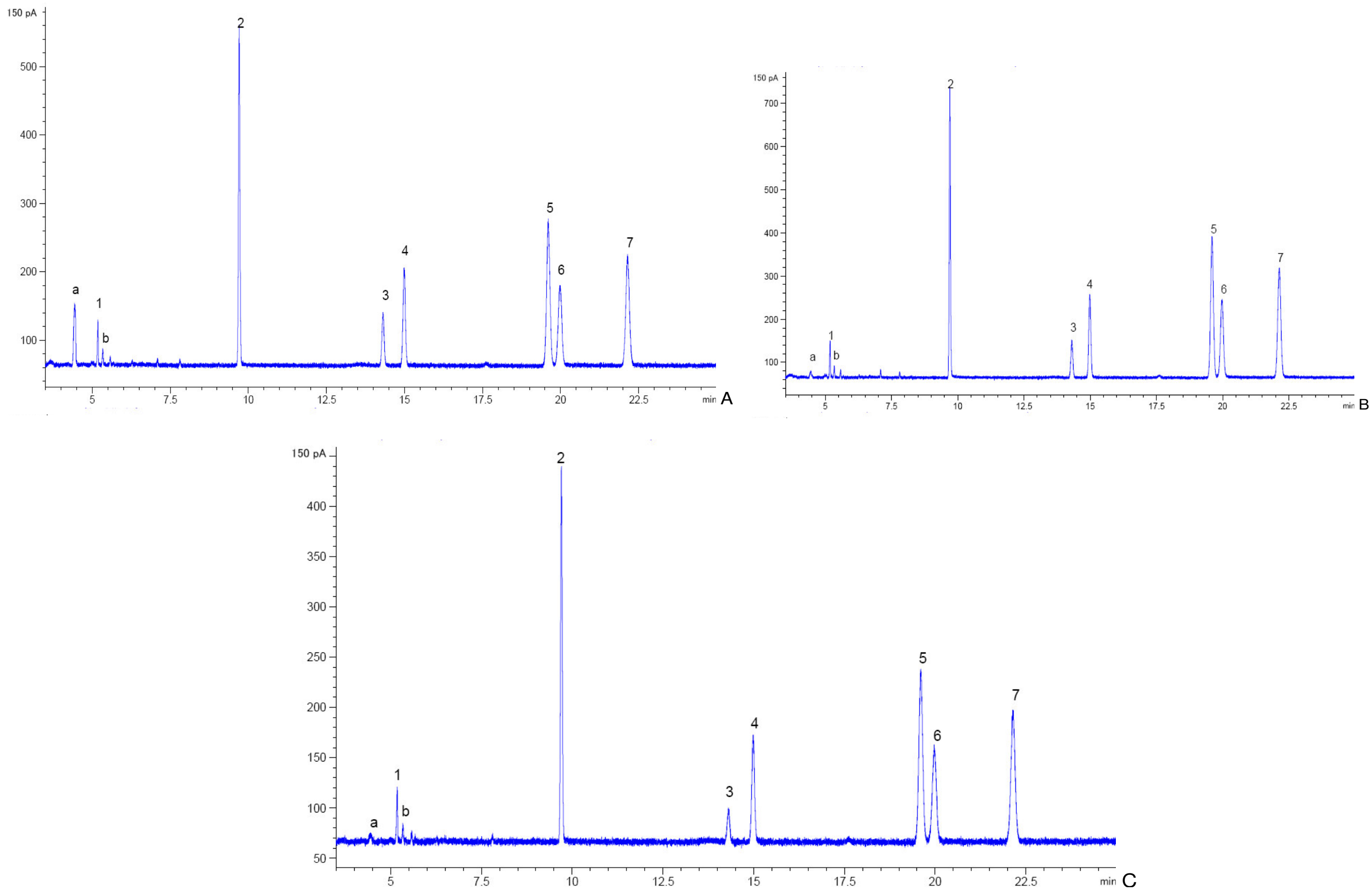

Figure 4. Typical GC profiles of seven organophosphorus pesticides in bovine milk subjected to heat treatment at 100°C (A), 80°C (B) or 63°C (C). Peak 1 to 7 represent trichlorfon, phorate, monocrotophos, dimethoate, denthion, malathion and methyl parathion, respectively.

Table 4. Degradation kinetic parameters of seven organophosphorus pesticides in the bovine milk subjected to different heat treatments.

Pesticide	Temperature (°C)	Kinetic parameters [a]		
		k (h^{-1})	R^2	$t_{1/2}$ (h)
Denthion	63	0.105	0.994	6.6
	80	0.144	0.971	4.8
	100	0.175	0.995	4.0
Dimethoate	63	0.0848	0.971	8.2
	80	0.0996	0.989	7.0
	100	0.125	0.999	5.5
Malathion	63	0.0944	0.996	7.3
	80	0.139	0.979	5.0
	100	0.159	0.990	4.4
Methyl parathion	63	0.0756	0.988	9.2
	80	0.115	0.937	6.0
	100	0.175	0.996	4.0
Monocrotophos	63	0.0765	0.993	9.1
	80	0.0806	0.960	8.6

Table 4. Contd.

	100	0.120	0.990	5.8
Phorate	63	0.0673	0.999	10.3
	80	0.0843	0.988	8.2
	100	0.115	0.995	6.0
Trichlorfon	63	0.0712	0.969	9.7
	80	0.0924	0.987	7.5
	100	0.124	0.989	5.6

a: k, rate constant of degradation; R, coefficient of regression; $t_{1/2}$, half life period.

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