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Degradation kinetics of seven organophosphorus pesticides in milk during yoghurt processing

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Abstract: Bovine milk spiked with seven organophosphorus pesticides, *i.e.*, dimethoate, fenthion, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, was fermented at 42 °C with commercial directed vat set (DVS) starters to investigate the degradation kinetics of the pesticides during yoghurt processing. The spiked pesticides were extracted from the prepared samples with an organic solvent and analyzed by gas chromatography after purification. Based on published results that the degradation kinetics of pesticides is first order, the rate constant of degradation and the half live period of the pesticides were calculated. The results indicated that degradation of the pesticides in milk during yoghurt processing were enhanced by one or both starters, except for malathion, and the two commercial DVS starters had different influences on the degradation kinetics of the pesticides.

Keywords: bovine milk; organophosphorus pesticide; degradation kinetics; yoghurt; starter.

INTRODUCTION

Organophosphorus pesticides are widely used in agriculture to protect plants from insects. When dairy cows are fed with organophosphorus pesticide polluted forages or drinking water, bovine milk and dairy products might be tainted with pesticide residues,[1,2] which could lead to health risks.[3] Mallatou *et al.* investigated organophosphorus pesticide residues in thirty-eight samples of bovine milk and twenty-eight samples of cheese and found that eleven milk samples contained residues, and the contents of methyl parathion in two samples were 43 and 280 μg kg^{-1}.[4] Pagliuca *et al.* analyzed organophosphorus pesticide contamination in one hundred and thirty-five raw milk samples and found that thirty-seven samples tested positive for traces and ten samples contained residues ranging

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from 5 to 18 μg kg^{-1}.[5] These studies illustrated the existence of organophosphorus pesticides in milk and dairy products.

The residue levels of pesticides in foods are affected by food processing, including fermentation, heat treatment and drying. In addition, the chemical nature of the pesticides and some factors, such as pH, light, metal ions and ozone, also impact the degradation of pesticide residues.[6,7] Uygun and Senoz studied the degradation of five organophosphorus pesticides in wheat during storage.[8] More interesting, the results of Abou-Arab showed that some starters could enhance the degradation of dichlorodiphenyl-trichloroethane and lindane.[9,10] The degradation of organophosphorus pesticides in bovine milk during yoghurt processing has not hitherto been reported. In the present study, seven organophosphorus pesticides, which were or are used in China, were added to bovine milk and the spiked milk was subjected to yoghurt processing with two commercial directed vat set (DVS) starters, in an attempt to study the impacts of the starters on the degradation of the pesticides. A gas chromatography-based method was used to analyze the amount of pesticide residues in the prepared yoghurt samples, and the degradation kinetics of the pesticides were calculated, treating the degradation of organophosphorus pesticides as a first order reaction.

EXPERIMENTAL

Reagents and materials

Seven organophosphorus pesticide standards, dimethoate, fenthion, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with declared purity from 94.5 to 99.5 %, and stored at –18 °C. Initial stock solutions (500 mg L^{-1}) of each pesticide individually were prepared by dissolving the pesticides in acetone. Working stock solutions of the individual pesticides were prepared by diluting the initial stock solutions with acetone to give concentrations ranging from 20 to 40 mg L^{-1}. Working solutions of mixed pesticides were prepared by combining the working stock solutions of the individual pesticides in different ratios and diluting to a fixed volume with acetone. These solutions were used to determine the standard curve or to spike bovine milk. Two commercial DVS starters applied in yoghurt preparation were purchased from Rhodia (Melle, France) and Danisco (Copenhagen, Denmark), and stored at –18 °C before use. The chemicals and solvents used were of analytic and chromatographic grade and procured from Sigma-Aldrich (Schnelldorf, Germany). The water used to prepare all solutions was highly purified using a Milli-Q PLUS unit (Millipore Corporation, New York, NY, USA). Bovine milk used was collected daily from a dairy farm in Harbin, Heilongjiang Province, China, and stored at 4 °C before use.

Preparation of yoghurt

Seven organophosphorus pesticides were added to bovine milk at a level of about 1.5 mg kg^{-1}. The spiked milk was shaken vigorously and stood at ambient temperature for 30 min. The procedure of yoghurt preparation followed the method reported by Isleten and Karagul-Yuceer.[11] A stainless steel vessel containing the spiked milk was placed in a water bath and heated to 95 °C for 15 min with continuous gentle stirring, and then cooled in a water bath to 42 °C. A commercial DVS starter was added to the milk at 0.6 g kg^{-1} milk, recommended by the producer. The inoculated milk was poured into glass cups (each had a capacity of 200 mL) with lids, and incubated at 42 °C in an incubator for 8 h. At regular time intervals, three cups were selected at random from the bulk samples, rapidly cooled in an ice water bath and subjected to pesticide extraction and purification. A control was also prepared by the same procedure but without starter addition.

Extraction and purification of organophosphorus pesticides

The extraction and purification of organophosphorus pesticides from the yoghurt samples followed the methods of Pagliuca *et al.*[12] The sample (10.0 g) was extracted with 20 mL of a 1:4, v/v acetone–acetonitrile mixture, shaken vigorously for 2 min and centrifuged at 400× *g* for 6 min. The liquid phase was transferred to a separation funnel, and the residue of the sample was collected and re-extracted with 15 mL of the acetone–acetonitrile mixture as above. The obtained liquid phases were combined and vigorously shaken with 50 mL dichloromethane for 10 min, and left for 20 min for phase separation. The dichloromethane phase was separated, filtered through anhydrous sodium sulfate (2.0 g) and collected as a purified pesticide extract. A sample of the purified extract (15.0 mL) was measured and evaporated to dryness at 30 °C with nitrogen gas in an evaporation station. The residue was reconstituted to a volume of 1.0 mL with acetone and filtered through a 0.45 μm microporous membrane filter before GC analysis.

GC analysis of organophosphorus pesticides

The GC analysis of organophosphorus pesticides for the standard solutions or the samples was performed using an Agilent 7890 gas chromatography (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 the carrier gas at a flow rate of 3 mL min^{-1}. The method described by Pagliuca *et al.* was employed.[12] The temperature profile was set as follows: initial temperature 100 °C for 1 min, heating from 100 to 195 °C at 30 °C min^{-1}, holding for 8 min at 195 °C, heating from 195 to 202 °C at 10 °C min^{-1}, holding for 1 min at 202 °C, heating from 202 to 205 °C at 1 °C min^{-1} and heating from 205 to 240 °C at 15 °C min^{-1}. The temperatures of the injector and detector were set at 200 and 250 °C, respectively. Quantification of the pesticides was performed by comparing the peak areas of the pesticides to a calibration curve of the standards. A multitude-point calibration was used.

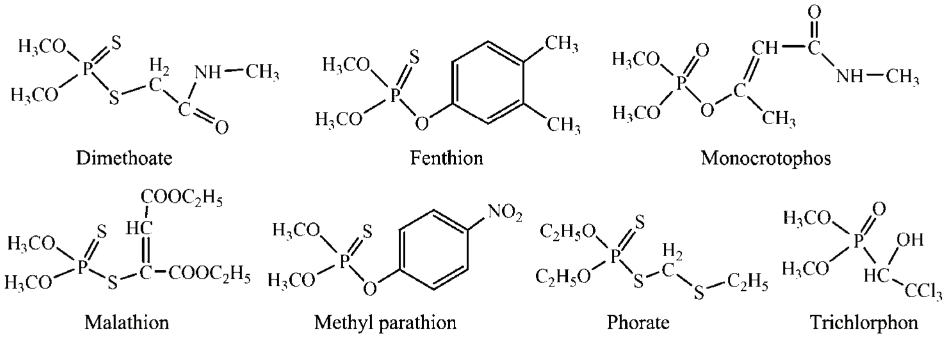
Statistical analysis

All data are expressed as means ±*SD* (standard deviation) from at least three independent trials. The rate constant of pesticide degradation was calculated by linear regression analysis. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data.

RESULTS AND DISCUSSION

Analysis of organophosphorus pesticides by GC

With the selected analysis procedure, seven organophosphorus pesticides, the chemical structures of which are shown in Fig. 1, could be extracted efficiently from the prepared yoghurt or milk samples and measured accurately. Typical GC profiles of the seven pesticides for standard solutions and the samples are given in Fig. 2, which shows that seven pesticides were well-separated by the column. Practical GC analysis found that the linear range of detection for the pesticides was from 0.1 to 8 mg kg^{-1} ($R^2 \geq 0.995$). The data listed in Table I demonstrate that the detection limits for the pesticides ranged from 0.01 to 0.02 mg kg^{-1}, and the recovery of the pesticides at two addition levels ranged from 80.26 (fenthion, at 0.1 mg kg^{-1}) to 125.9 % (dimethoate, at 1.0 mg kg^{-1}). The relative standard deviation of the recovery of monocrotophos at an addition level of 0.1 mg kg^{-1} was the largest, but less than 7.0 %. This means that the applied extraction and purification procedure appeared to be efficient and the GC analysis procedure was suitable for the detection of the pesticide residues in the prepared samples.



Dimethoate Fenthion Monocrotophos

Malathion Methyl parathion Phorate Trichlorphon

Fig. 1. Chemical structures of the seven studied organophosphorus pesticides.

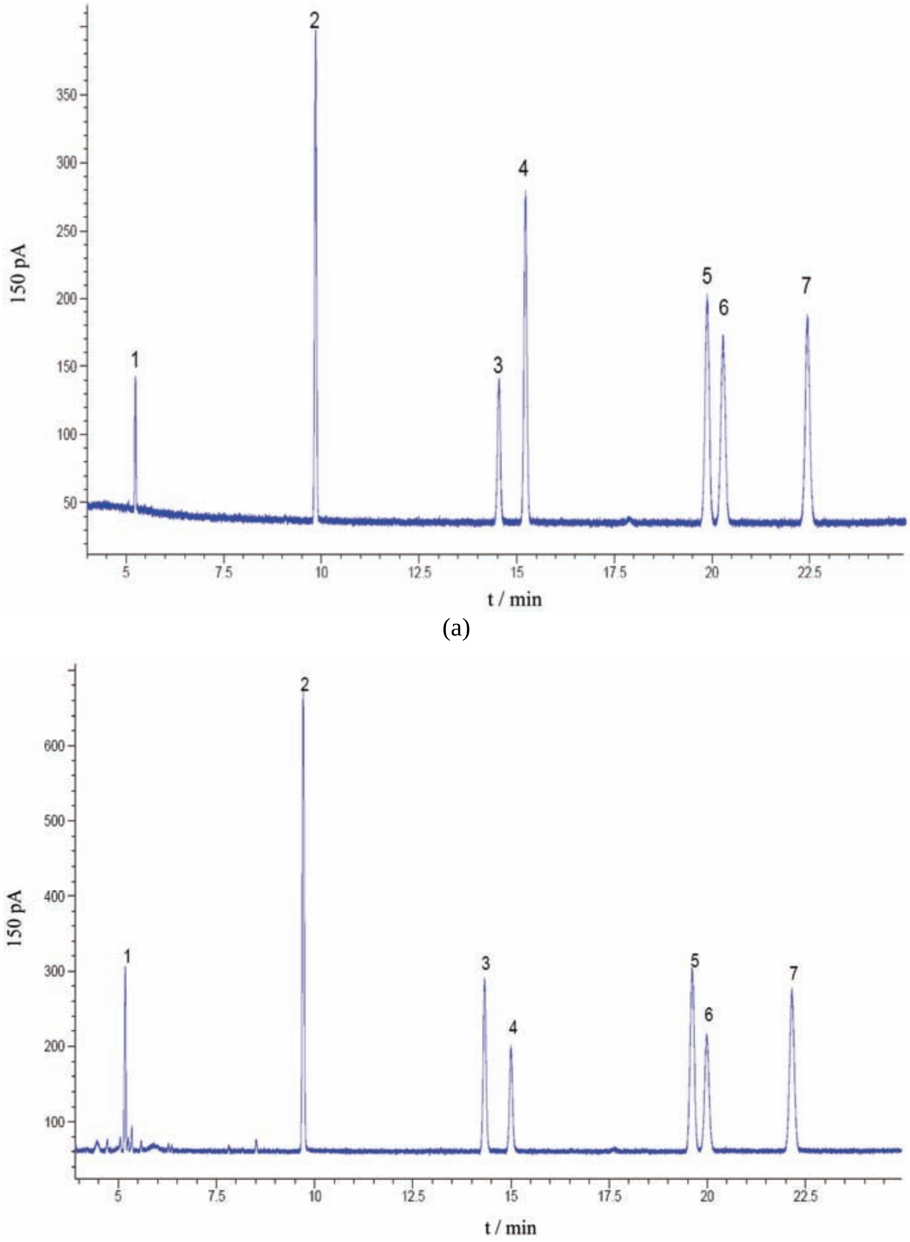
Degradation parameters of organophosphorus pesticides in bovine milk during yoghurt processing

There was no effect of presence of organophosphorus pesticides on yoghurt formation from bovine milk. This indicated that the microbial cultures of both commercial starters were tolerant to the added pesticides. To insure more pesticides remained in the milk after heat treatment at 95 °C and to obtain higher extraction efficiencies of the pesticides, the seven pesticides were added at a higher level of 1.5 mg kg^{-1}. The pesticides residues remaining in the milk at different treatment times were analyzed. The results are given in Table II. The contents of the pesticides in the milk subjected to fermentation or heat treatment showed a decreasing trend with progressing treatment time, indicating degradation of the pesticides.

The degradation of organophosphorus pesticides was reported to be a first order reaction.[13,14] This means that the content of each pesticide in the milk at different times would follow Eq. (1):

$$\frac{\mathrm{d}c}{\mathrm{d}t} = kt \qquad (1)$$

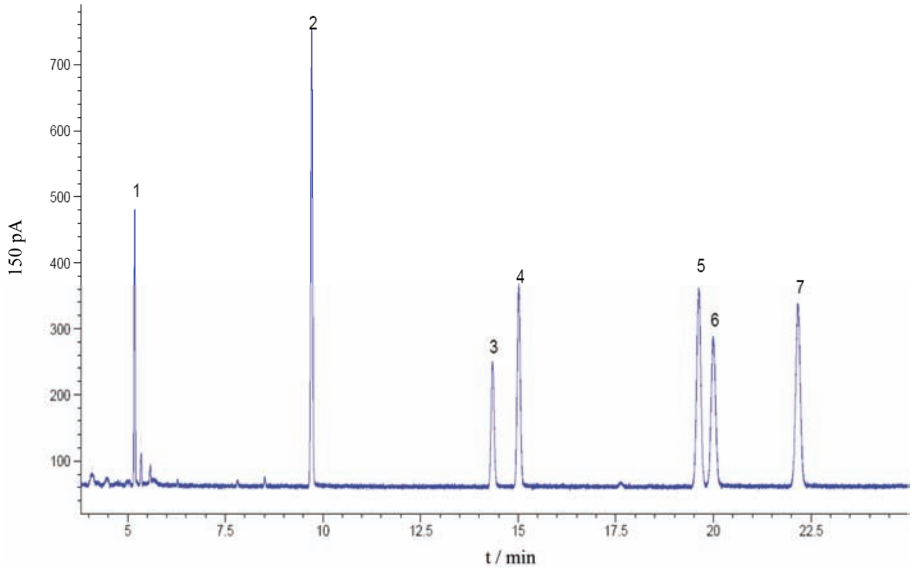
where c is the content of the studied pesticide, t is the treatment time and k is rate constant of degradation of the pesticide.



(a)

(b)

Fig. 2. Typical GC profiles of the seven studied organophosphorus pesticides for the standard solution (a) and the spiked milk sample heated at 42 °C (b). Peaks 1–7 represent trichlorphon, phorate, monocrotophos, dimethoate, fenthion, malathion and methyl parathion, respectively. The concentration of the pesticides standard solution was 0.6 mg kg^{-1}. The control or yoghurt samples were spiked with pesticides at 1.5 mg kg^{-1} and heated or fermented for 2 h.



(c)

Fig. 2 (continued). Typical GC profiles of the seven studied organophosphorus pesticides for the prepared yoghurt sample (c). Peaks 1–7 represent trichlorphon, phorate, monocrotophos, dimethoate, fenthion, malathion and methyl parathion, respectively. The concentration of the pesticides standard solution was 0.6 mg kg^{-1}. The control or yoghurt samples were spiked with pesticides at 1.5 mg kg^{-1} and heated or fermented for 2 h.

TABLE I. Recovery and limits of detection (*LOD*) of the seven studied organophosphorus pesticides during the preparation of yoghurt in GC analysis (the number of trials was nine)

Pesticide	Spiking levels and recovery (mean±*SD*, %)		*LOD* / mg kg^{-1}
	0.1 mg kg^{-1}	1.0 mg kg^{-1}	
Dimethoate	114.0±3.7	125.9±2.3	0.01
Fenthion	80.26±4.6	118.7±3.3	0.01
Malathion	87.24±3.6	98.90±5.0	0.01
Methyl parathion	89.85±5.2	94.40±4.1	0.01
Monocrotophos	108.7±7.5	115.7±2.8	0.02
Phorate	81.47±3.5	95.87±3.8	0.01
Trichlorphon	80.75±4.3	96.53±4.3	0.01

The rate constant of degradation and half-life period of the seven pesticides in the milk during yoghurt processing or heat treatment are thus calculated from the data listed in Table II using, respectively, Eqs. (2) and (3):

$$\ln c = -kt + A \tag{2}$$

$$t_{1/2} = \frac{0.693}{k} \tag{3}$$

where A is a constant and $t_{1/2}$ is half-life period of the pesticide. The obtained values are listed in Table III.

TABLE II. Contents of seven organophosphorus pesticides in the control and yoghurt samples after different treatment times (mean±SD, mg·kg^{-1}; the number of trials was three)

Pesticide	Sample[a]	t / h				
		0	2	4	6	8
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
Fenthion	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
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
Trichlorphon	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] Yoghurts 1 and 2 were produced with commercial DVS starter of Rhodia and Danisco, respectively, at 42 °C. The control was bovine milk subjected to heat treatment at 42 °C

Compared to the degradation of seven pesticides in the control, the degradation of the pesticides in the milk during yoghurt processing was impacted by the applied starters. Based on the corresponding rate constants listed in Table III, the degradation of dimethoate, fenthion and trichlorphon in the milk during yoghurt processing increased when either of the two starters was added, leading to a larger rate constant of degradation or a shorter half-live period of these pesticides. The starter from Rhodia also accelerated the degradation of methyl parathion, monocrotophos and phorate but the starter from Danisco had little or no impact on the degradation of these three pesticides. The two starters had different influences on the degradation of the six pesticides in the milk during yoghurt processing. The half-live period of fenthion was the shortest, indicating fenthion was the most unstable pesticide. It was unexpected that the degradation of malathion in the milk was not enhanced by the starters, because its rate constant of degradation in the milk fermented with two starters was lower than that in the control.

The reason for this unusual result remains unclear and needs further study. In addition, studies of the degradation products and of the impact of other microbial starters on the degradation of the pesticide are required.

TABLE III. Kinetic parameters for degradation of seven organophosphorus pesticides in the bovine milk during yoghurt processing or heat treatment

Pesticide	Sample[b]	Kinetic parameters[a]		
		k / h^{-1}	R^2	$t_{1/2}$ / h
Dimethoate	Control	0.0305	0.969	22.7
	Yoghurt 1	0.0315	0.965	22.0
	Yoghurt 2	0.0343	0.951	20.2
Fenthion	Control	0.0324	0.955	21.4
	Yoghurt 1	0.0366	0.976	18.9
	Yoghurt 2	0.0331	0.966	20.9
Malathion	Control	0.0285	0.930	24.3
	Yoghurt 1	0.0231	0.925	30.0
	Yoghurt 2	0.0192	0.905	36.1
Methyl parathion	Control	0.0293	0.997	23.6
	Yoghurt1	0.0324	0.987	21.4
	Yoghurt 2	0.0248	0.996	27.9
Monocrotophos	Control	0.0287	0.978	24.1
	Yoghurt1	0.0319	0.975	21.7
	Yoghurt 2	0.0289	0.958	24.0
Phorate	Control	0.0225	0.991	30.8
	Yoghurt 1	0.0283	0.983	24.5
	Yoghurt 2	0.0223	0.992	31.1
Trichlorphon	Control	0.0221	0.857	31.4
	Yoghurt 1	0.0270	0.910	25.7
	Yoghurt 2	0.0241	0.932	28.8

[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 starter of Rhodia and Danisco, respectively, at 42 °C. The control was bovine milk subjected to heat treatment at 42 °C

Uygun and Senoz[8] studied the degradation of methyl chlorpyrifos, fenitrothion, malathion and methyl pirimiphos in wheat stored at ambient temperature for five months and found the levels of malathion, fenitrothion, methyl chlorpyrifos and methyl pirimiphos were decreased by 88, 86, 84 and 76 %, respectively. Unfortunately, the rate constant of degradation of these pesticides was not given. In practical yoghurt processing, the milk is fermented with a starter for about 4 h at 42 °C. According to the present results, the decrease in the level of seven pesticides in yoghurt samples with fermentation time of 4 h would range from 9.2 (phorate) to 17.1 % (dimethoate).

CONCLUSIONS

The degradation of seven organophosphorus pesticides, dimethoate, fenthion, malathion, methyl parathion, monocrotophos, phorate and trichlorphon, in bovine milk during yoghurt processing was studied in the present work. The bovine milk was spiked with the pesticides and fermented with two commercial DVS starters at 42 °C. The pesticide residues were extracted from the prepared samples and measured by GC. The content of the pesticides in the milk all decreased as the treatment time progressed, indicating degradation of the pesticides. The calculated rate constant of degradation or half-live period of the pesticides revealed that the degradation of the selected pesticides, except for malathion, was enhanced by one or both of the applied starters and the two starters had different impacts on the degradation of the pesticides.

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ИЗВОД

КИНЕТИКА РАЗГРАДЊЕ СЕДАМ ОРГАНОФОСФОРНИХ ПЕСТИЦИДА У МЛЕКУ ТОКОМ ПРОЦЕСА ПРОИЗВОДЊЕ ЈОГУРТА

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Крављем млеку је додато седам органофосфорних пестицида и ферментисано је на 42 °C у присуству комерцијалних стартера (DVS), у циљу испитивања кинетике разградње пестицида током процеса производње јогурта. Додати су следећи пестициди: диметоат, фентион, малатион, метил-паратион, монокротофос, форат и трихлорфон. Пестициди су изоловани из узорака млека органским растварачем и анализирани гасном хроматографијом након пречишћавања. Узевши у обзир да је кинетика разградње пестицида првог реда, израчунате су константе брзине разлагања и време полуживота пестицида. Резултати су показали да је разлагање пестицида (осим малатиона) у млеку током процеса производње јогурта убрзано стартерима и да комерцијани DVS стартери имају различит утицај на кинетику разлагања.

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