The potency of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* to dissipate organophosphorus pesticides in wheat during fermentation

Tijana M. Đorđević¹ · Rada D. Đurović-Pejčev¹

Revised: 26 October 2016 / Accepted: 13 November 2016 / Published online: 8 December 2016
© Association of Food Scientists & Technologists (India) 2016

Abstract The degradation behaviour of pirimiphos methyl with *Saccharomyces cerevisiae* and chlorpyrifos methyl with *Lactobacillus plantarum* in wheat during fermentation was studied. Yeast fermentation was especially effective for reduction of pirimiphos methyl applied at 5 mg kg⁻¹ (maximum residue limit—MRL) causing dissipation for max 48.8%. Pesticide reduction rate decreased with an increase of fortification rate. Thus in samples fortified with 25 and 75 mg kg⁻¹ a reduction up to 27.1%, and 23.7% respectively, was observed. Activity of *L. plantarum* was especially effective for reduction of chlorpyrifos methyl applied at 3 mg kg⁻¹ (MRL) causing dissipation for max 56.7%. This reduction rate decreased with an increase of fortification rate. In samples contaminated with 15 and 45 mg kg⁻¹ dissipation reached up to 38.6% and 34.7% respectively. For both experiments, initial inoculums sizes had no statistically significant effect on pesticides dissipation level, while concerning fermentation temperatures at all fortification levels the highest degradations occurred at 30 °C. Overall, regardless fermentation parameters, the degradation rate constants of pirimiphos methyl fermented with yeast were increased comparing with control samples by 255–573, 56–116 and 119–594% in samples contaminated at MRL, 5MRL and 15MRL of pesticide, while the degradation rate constants of chlorpyrifos methyl fermented with lactobacilli were increased by 74–769, 59–237 and 46–469% respectively. These results evidenced that yeast and lactobacilli played an important role in

promoting pirimiphos methyl i.e. chlorpyrifos methyl dissipation in wheat.

Keywords Pesticides · Reduction · Fermentation · Yeast · Lactobacilli · Wheat

Introduction

Pesticides play important role in the development of the agriculture however the hazards they bring along with them to food safety and human health is becoming the focus of world attention. Chemical pesticides applied at fields might contaminate soil and water thus indirectly causing the food pollution, or could directly pollute the foods. Either way, the frequent presence of residues of various pesticides in commercial food products, as well as their high toxicity, is nowadays an important and real concern. One of the main parts of human diet nowadays is based on wheat flour and its processed products, primary bread, thus, need for tremendous increase of wheat crop cultivation and production is evident in countries world wide. Continues increment of wheat productivity can be attributed not only to planting area increase, but also to yield rise, and without intensive usage of pesticides wheat yield would be unsatisfactory, harvest could be reduced, and loses during storage might be high. In most countries, especially ones in which the production of wheat is one of the main sectors of the economy, stored cereal grain is usually protected from insect attack by various contact and persistent insecticides. Long-term persistence insecticides with an extended insecticidal activity, as organophosphorus pesticides pirimiphos-methyl and chlorpyrifos methyl, are the most commonly used ones for this purpose.

✉ Tijana M. Đorđević
tijana.djordjevic@hotmail.com

¹ Institute of Pesticides and Environmental Protection,
Banatska 31b, Belgrade 11080, Serbia

Pirimiphos methyl (0-(2-diethylamino)-6-methyl-4-pyrimidinyl) 0,0dimethyl phosphorothioate) is insecticide with capacity of controlling a broad spectrum of pests. It can be used as contact poison as well as fumigant. Volatility of this pesticide is low, as well as its water solubility. Hydrolysis of pirimiphos methyl is pH dependent in aqueous solution, whereby at pH 7 it is remarkably slow, somewhat faster although still slow at pH 9, while quite rapid at pH 4. The main hydrolysis products are 2-diethylamino-4-hydroxy-6-methyl-pyrimidine-4-ol, recovered at all three pHs, which do not retain the organophosphate moiety, as well as O-2diethylamino-6-methyl-4-pyrimidinyl-0-methyl-phosphorothioate, recovered at significant amounts in the pH 7 and 9 solutions, which do contain the organophosphate moiety and therefore, may still have significant toxicological activity. However, in metabolism studies of stored grain there were no significant levels of degradation products other than 2-diethylamino-4-hydroxy-6-methyl-pyrimidine-4-ol detected, thus the anticipated residues and dietary exposure analysis for grain include residues of parent only. Chlorpyrifos methyl (O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is also contact broad-spectrum insecticide with respiratory and stomach action as well. In the pesticide classification by hazard, recommended in the WHO, this pesticide is at the list of products with low risk for causing acute toxic effects. Water solubility of chlorpyrifos methyl is very low, as well as its volatility. Its hydrolysis in aqueous solution depends on pH in the way that it is faster at higher pH (pH 8). The main hydrolysis product is 3,5,6-trichloro-2-pyridinol, whereby degradation mechanism involves chemical hydrolysis and microbial activity.

Maximum residue levels (MRLs) for pirimiphos methyl and chlorpyrifos methyl in wheat set by regulatory bodies worldwide are 5 i.e. 3 mg kg⁻¹ respectively. When application of those agrochemicals is in accordance with GLP (good agricultural practices) there are no risks for exceeding MRLs, however incorrect usage of pesticides could lead to accumulation of significant amount of harmful residues in environment. Further, in order to estimate health risks on human from pesticide residues input through food chain, basic established MRLs are not sufficient. Additional information regarding possible residues loss during processing of food commodities need to be taken into consideration in order to accurately regulate acceptable usage of pesticides (Fleurat-Lessard et al. 2007).

Various techniques and methods involved during food processing at the industry or domestically level have been found to play a role in the significant reduction of pesti-

cides. Reviewing this topic Kaushik et al. (2009) obtained that washing, peeling, drying, pasteurization, sterilization, blanching, steaming, boiling, cooking, frying, roasting, milling, juicing, pureeing, etc. reduced pesticide residues levels in majority of the food commodities, as well as in wheat grain among others. Wheat is a world main cereal crop and one-third of its income is being consumed by milling and baking, thus this food commodity have been widely studied for presence of pesticide residues, and there are few publications dealing with the removal of pesticide residues from wheat during home preparation and commercial processing. Sharma et al. (2005) established the dissipation of endosulfan, chlorpyrifos, malathion, hexaconazole, propiconazole and deltamethrin during bread making, Fleurat-Lessard et al. (2007) studied the durum wheat and distribution of pirimiphos methyl residues in its milling fractions during the processing, while Uygun (2009) investigated degradation of malathion, malaoxon, isomalathion and chlorpyrifos methyl in wheat during cookie processing. Reduction of pesticide residues during fermentation, the oldest plain biotechnological process implemented in production of pastry, dairy, wine, beer and numerous other different foods, has been recorded in majority of tested commodities. González-Rodríguez et al. (2009) established that dissipation rates of pyraclostrobin, famoxadone, benalaxyl-M, and benalaxyl residues reached up to 95% at the end of the vinification. During this technological process a significant decrease in the mepanipyrim and fenhexamid content was observed after fermentation (Noguerol-Pato et al. 2015). Similar was confirmed later, as Noguerol-Pato et al. (2016) obtained more than 68% of fungicide dissipation during the process. Bo et al. (2011) reported that in the process of transformation of bovine milk into yoghurt, as the treatment time progressed content of dimethoate, fenthion, malathion, methyl-parathion, monocrotophos, phorate and trichlorophon decreased, while Ruediger et al. (2005) reported that malolactic fermentation resulted in significant reduction of chlorpyrifos, up to 70%, and Lu et al. (2013) confirmed dissipation behaviour of organophosphorus pesticides during the cabbage pickling process. However, it is shown that some microorganisms were capable of pesticides degradation, there is insufficient knowledge about effect of fermentation processes on OPP residues reduction in cereals. As fermentation is one of the technological processes which is often used to improve product properties by obtaining samples with more complex positive nutritive profile (Salgado et al. 2012; González-Barreiro et al. 2015), the lack of information about pesticide dissipation during cereal fermentation, as the main processing technique during bread baking, was surprising.

In the present study the degradation of pirimiphos methyl in after yeast fermentation and chlorpyrifos methyl after lactic acid fermentation in wheat were studied and compared. First order reaction model was used to calculate degradation rate constants. The aim of the experiment was to evaluate the ability of microorganisms to provoke organophosphate degradation in wheat dough.

Materials and methods

Starter culture and growth conditions

Lactobacillus plantarum (DSMZ 20174) and *Saccharomyces cerevisiae* (WDCM 00058; CBS 2978) (collection of Faculty of Technology and Metallurgy, Belgrade, Serbia, Laboratory of Microbiology) were used for the fermentations. Cells kept at 4–6 °C were activated prior usage by incubation at 30 °C for 24 h in suitable growth substrates (MRS and YPD broth, Torlak Institute, Belgrade, Serbia). For the wheat fermentation, cell harvesting was done by centrifugation at 9580g (10 min; 4 °C) (Velocity 14R, Dynamica, Salzburg-Mayrwies, Austria) after incubation up to the exponential phase of growth at 30 °C (24 i.e. 5 h respectively). Phosphate buffer (50 mmol L⁻¹; pH 7.0) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), was used for cell washing and, prior inoculation into wheat substrate, cells were re-suspended to its original volume in sterile distilled water.

Analytical standard and working solutions

Analytical standards of pirimiphos methyl (Dr. Ehrenstorfer GmbH, Augsburg, Germany, purity 99.2%) and chlorpyrifos methyl (Dow Agro Sciences, Indianapolis, Indiana, USA, purity 99.9%) were dissolved in acetone (J.T. Baker, Deventer, Netherlands) and stored at -18 °C. Working solutions of analytical standards were diluted in sterile distilled water prior usage.

Sample preparation

For the preparation of the wheat substrate, *Triticum spelta* grain produced through organic farming on Jevtić farm (Bačko Gradište, Serbia) was milled, divided into two groups, and one was spiked with 5, 25 and 75 mg kg⁻¹ of pirimiphos methyl while other with 3, 15 and 45 mg kg⁻¹ of chlorpyrifos methyl (chosen concentration were MRL, 5MRL and 15MRL of pesticides for wheat). After homogenization for 24 h, wheat slurry was prepared as previously described (Đorđević and Đurović-Pejčev,

2015). *S. cerevisiae* was inoculated in samples fortified with pirimiphos methyl, while *L. plantarum* was inoculated in samples fortified with chlorpyrifos methyl. Inoculations were performed individually at levels 6, 8 and 10% (v/w). Fermentations were attained in incubators at 23, 30 and 37 °C (FOC 225 I, Velp Scientifica, Usmate, Italy), and sampling was performed after 24, 48 and 72 h. Two controls were used: first comprised sampling a portion directly after sterilization of wheat and prior inoculation to see the effect of high temperature and elevated pressure on pesticides degradation, and second consisted of samples maintained at the same temperatures as for fermentation but without starter and analysed after 24, 48 and 72 h to check the pesticides spontaneous chemical degradation.

Pesticides extraction, purification and detection

For the analyses of OPPs residue, samples were prepared as previously described (Đorđević et al. 2013a, b). For the detection device, a gas chromatograph–mass spectrometer (GC/MS) (CP-3800/Saturn 2200, Varian, Melbourne, Australia) was used, and it was operated under previously described conditions (Đorđević et al. 2013a). Ions (m/z) used for quantification (confirmation) were: 290(233) for pirimiphos methyl and 286(125) for chlorpyrifos methyl.

Calculation of kinetic parameters and statistical analysis

Kinetic parameters of OPPs degradation were calculated by the kinetic equation of a first-order reaction model expressed as below.

$$C_t = C_0 e^{-kt}$$

where, C_t is OPPs concentration (mg kg⁻¹) at time t (hour or day), C_0 is initial OPPs concentration (mg kg⁻¹), k is rate constant (hour⁻¹ or day⁻¹).

All trials were done in triplicates. Statistica 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA) was used for the statistical analysis.

Results and discussion

GC detection of OPPs

Analysis procedure for pesticide extraction and purification used in this study was developed in our previous work as rapid preparation method for GC/MS analysis of pesticides in soil (Đurović and Đorđević 2010). However, this procedure proved to be effective for readily extraction of pirimiphos methyl and chlorpyrifos methyl from unfer-

mented and fermented wheat samples. Thus, recoveries, measured at four levels of fortification (for pirimiphos methyl—1, 5, 10 and 20 mg kg⁻¹ and for chlorpyrifos methyl—0.6, 3, 6 and 12 mg kg⁻¹) ranged for unfermented wheat samples from 86.1 to 90.3% (with RSD% in range 1.1–6.9%), and from 83.9 to 89.7% (with RSD% in range 1.1–8.4%) respectively for those two pesticides. For fermented wheat samples recoveries ranged from 89.9 to 96.0% with RSD% from 3.1 to 7.2% for pirimiphos methyl and from 84.6 to 88.8% with RSD% from 2.4 to 5.2% for chlorpyrifos methyl (Table 1). Limits of detection and quantification (LOD and LOQ), obtained in previous work, were 0.011 and 0.04 mg kg⁻¹ for pirimiphos methyl, and 0.007 and 0.04 mg kg⁻¹ for chlorpyrifos methyl (Đorđević et al. 2013b; Đorđević and Đurović-Pejčev 2015). It is clearly that used method has appropriate sensitivity sufficient for pirimiphos methyl and chlorpyrifos methyl determination at concentration rates far below their maximum residue limits.

OPPs degradation in wheat dough

Bacteria, yeast and moulds are constitution of natural microbial population on cereal grains (Russo et al. 2010) thus flour and flour-based products (basically yeast-raised dough) is normally contaminated with several of those microorganisms. As regularly, in bakery, flour is not subjected to sterilization, the complexity and number of so called sourdough microbiota depend on a number of determinants, which include not just environmental microbiota, but also specific technological parameters. Because of that, in order to isolate the effects of *L. plantarum* and *S. cerevisiae* on residues of pesticides, autoclaving of milled fortified wheat samples was performed prior preparation of fermentation substrate, with aim to eliminate presence and metabolic activities of wild strains. Thermal processing has long been perceived to generally led to the reduction of the nutritional value of product by diminishing various heat labile nutrients. However, recent studies reported that autoclaving, among other tested stabilization

treatments of whole wheat flour, improved digestibility of proteins, phytate content, minerals and dietary fibres and intensify antioxidant activity of flour by increasing total phenolic content (Demir and Elgün 2014). As it was recorded that during thermal treatments involving pasteurization and/or sterilization (autoclaving) degradation of pesticides may occur at different rates (Lalah and Wandiga 2002; Kontou et al. 2004), possibility that applied sterilization may affect residues of pirimiphos methyl and chlorpyrifos methyl in wheat was taken into consideration in this study. As can be seen from obtained results presented in Table 2, the concentration of chlorpyrifos methyl in the samples after thermal processing for 15 min at 121 °C (103 kPa) was drastically reduced at all fortification levels, reaching 77.6% for sample spiked with MRL, 75.8% for one spiked with 5MRL and 75.1% for sample fortified with 15MRL of pesticide. The concentration of pirimiphos methyl was reduced at somewhat lower level, reaching 38.0, 42.6 and 48.5% respectively for those fortification levels. This is in correlation with earlier established observations, as Uygun et al. (2009) reported that volatilization and/or degradation of OPPs residues occurred during heat treatments, with significant reduction in pesticides concentration levels not just after exposing to extremely high temperatures (205 °C), but also after treatment with lower ones (40 °C). Clearly, volatilization of pesticides by itself increases throughout heating, thus reducing residue amounts of those agrochemicals. Heat as well can boost hydrolysis and other chemical degradation with the same results concerning pesticide residues concentration rates. The main physico-chemical processes responsible for pesticide loss during thermal treatments of various substrates are co-distillation, thermal degradation and/or evaporation, and the chemical nature of specific pesticides dictate which of those will prevail (Sharma et al. 2005). Additionally, if sample which undergo heating contains significant amount of water, it may penetrate the molecules of the pesticides and provoke co-distillation along with thermal degradation and evaporation (Cabras et al. 1998). Considering all this, high reduction of chlorpyrifos methyl

Table 1 Recoveries of two organophosphorus pesticides from wheat and wheat dough

Pesticide	Fortification rate (mg kg ⁻¹)	Recoveries (%)	
		Wheat	Wheat dough
Pirimiphos methyl	1	90.3 ± 6.9	89.9 ± 7.2
	5	90.2 ± 2.3	91.3 ± 5.4
	10	87.6 ± 1.1	93.0 ± 3.1
	20	86.1 ± 3.0	96.0 ± 3.5
Chlorpyrifos methyl	0.6	83.9 ± 8.4	86.6 ± 5.2
	3	89.7 ± 2.5	88.8 ± 5.5
	6	86.8 ± 5.5	84.6 ± 2.4
	12	85.1 ± 1.1	86.7 ± 3.8

Table 2 Effect of sterilization (at 121 °C for 15 min) on pirimiphos methyl and chlorpyrifos methyl residues in wheat prior to fermentation

Pesticide	Fortification level (mg kg ⁻¹)	Residual concentration (mg kg ⁻¹)	
		Sterilization ^A	Control ^B
Pirimiphos methyl	5.36	3.32 ± 0.14 ^a (38.0) ^C	4.80 ± 0.15 ^b (10.5)
	25.58	14.68 ± 0.26 ^a (42.6)	21.90 ± 0.33 ^b (14.4)
	75.20	38.73 ± 0.73 ^a (48.5)	63.32 ± 0.65 ^b (15.8)
Chlorpyrifos methyl	2.99	0.67 ± 0.03 ^a (77.6)	2.61 ± 0.05 ^b (12.6)
	15.81	3.83 ± 0.22 ^a (75.8)	13.22 ± 0.24 ^b (16.4)
	45.14	11.24 ± 0.96 ^a (75.1)	37.47 ± 0.88 ^b (17.0)

^A Remaining pirimiphos methyl and chlorpyrifos methyl (mg kg⁻¹) after sterilization in autoclave (n = 3). Results are not corrected for recovery

^B Pirimiphos methyl and chlorpyrifos methyl (mg kg⁻¹) extracted from samples prior sterilization in autoclave was determined at each fortification levels as analytical recovery rates (n = 3)

^C Data presented in parenthesis is the percent loss of pesticide during the process

Values with different superscripts (a, b) within each fortification level were significant different ($p = 0.05$)

and somewhat lower thus still high reduction of pirimiphos methyl are not surprising, especially knowing that those pesticides have significantly high vapour pressure.

Results presented in Table 3 indicated that biodegradation of pirimiphos methyl during wheat fermentation by *S. cerevisiae* occur red at all three fortification level. Pirimiphos methyl extracted from sterilized samples immediately after inoculation (day zero) was determined at each fortification level as recovery for correction of pesticide concentration level at the beginning of incubation. In order to point out the effect of *S. cerevisiae*, separately, on pirimiphos methyl residues, determination of chemical degradation of pesticide which occurred spontaneously in the control wheat substrate maintained at the same temperatures as for fermentation without yeast inoculations was performed. Obtained results showed that there were reductions of 10.2%, 21.1%, respectively in sample spiked with MRL, 5MRL and 15MRL of pirimiphos methyl during the incubation, though statistically significant differences were not suggested between variations in incubation parameters ($\alpha = 0.1$, $p < 0.1$). Commonly, during storage, grains over an extended period exposed to ambient temperature and humidity and pirimiphos methyl is generally more persistent insecticide. However, concerning chemical nature of this pesticide, its obtained low degradation in present experiment could be caused by elevated temperature and humidity. Further, during yeast fermentation, pirimiphos methyl degradation in wheat was significantly impacted by the inoculated *S. cerevisiae*. The highest overall dissipation of pirimiphos methyl, up to 48.8% comparing with control and considering dissipation during sterilization, occurred in samples fortified with the lowest used concentration (5 mg kg⁻¹), and pesticide reduction rate decreased with an increase of fortification rate. Thus in samples fortified with 25 mg kg⁻¹ reduction was up to

27.1%, while in samples with 75 mg kg⁻¹ it was 23.7%. Lower pesticide dissipation, as a result of biodegradation by yeast, in samples contaminated at higher rate with pirimiphos methyl is most likely consequence of pesticide toxicity to yeast, as numerous authors previously found that some strains cannot normally grow in substrates contaminated with various pesticides (Shin et al. 2003; Čuš and Raspor 2008; Bi Fai and Grant 2009; Noguerol-Pato et al. 2014). Concerning differences in reduction rate among variations of incubation parameters, results indicated that initial inoculums size had no statistically significant effect on pesticide dissipation level while at all fortification levels the highest degradations, mentioned previously, occurred at 30 °C. Somewhat lower, thus not significantly, pirimiphos methyl degradation occurred at 37 °C (max 41.5, 24.2 and 22.0% respectively in samples fortified with 5, 25 and 75 mg kg⁻¹), while the lowest reduction (max 28.3, 11.0 and 14.8% respectively in those samples) was obtained during yeast fermentation at 23 °C. As can be seen from results and degradation rate constants in Table 3, residual pirimiphos methyl in fermented samples, as well as in control, showed decreasing trend as fermentation time progressed. Usually, the highest degradation initially occurred during first 24 h, continuing more or less equally over 48 and 72 h. Exception was the degradation trend in samples fortified with highest pesticide concentration (15MRL) incubated at the lowest temperature (23 °C), where practically at day one there was no pesticide dissipation whatsoever. Earlier in studies it was noticed that presence of various pesticides affect *S. cerevisiae* growth in the way that growth rate was affected, while the final biomass production was not. Thus pesticide contamination at higher level temporary reduced cell growth rate, while after a period of inactivity microorganisms encountered growth resumption (Jawich et al. 2006; Braconi et al. 2006;

Table 3 Dissipation of pirimiphos methyl residues during yeast fermentation with *S. cerevisiae* in wheat substrate

Fortification level (mg kg ⁻¹)	Residual concentration after sterilization (mg kg ⁻¹) ^A	Samples ^B	Temperature (°C)	Residual concentration (mg kg ⁻¹) at different times (days)				k ^D (×10 ⁻¹) day ⁻¹
				0 ^C	1 (24 h)	2 (48 h)	3 (72 h)	
5.01	3.22 ± 0.12	Control	23	2.85 ± 0.15	2.62 ± 0.50	2.56 ± 0.03	2.56 ± 0.07	0.58
		Sample I		2.87 ± 0.03	2.10 ± 0.04	2.09 ± 0.06	1.80 ± 0.05	2.09
		Sample II		2.86 ± 0.04	2.11 ± 0.03	2.11 ± 0.05	1.76 ± 0.06	2.06
		Sample III		2.88 ± 0.06	2.08 ± 0.06	2.02 ± 0.06	1.79 ± 0.04	2.20
		Control	30	2.85 ± 0.15	2.64 ± 0.33	2.61 ± 0.19	2.60 ± 0.18	0.50
		Sample I		2.87 ± 0.03	2.11 ± 0.06	1.77 ± 0.05	1.25 ± 0.07	2.75
		Sample II		2.86 ± 0.04	2.09 ± 0.05	1.70 ± 0.05	1.24 ± 0.05	2.84
		Sample III		2.88 ± 0.06	2.08 ± 0.04	1.60 ± 0.03	1.22 ± 0.07	3.02
		Control	37	2.85 ± 0.15	2.63 ± 0.28	2.61 ± 0.29	2.60 ± 0.53	0.52
		Sample I		2.87 ± 0.03	1.75 ± 0.05	1.56 ± 0.06	1.50 ± 0.05	3.39
		Sample II		2.86 ± 0.04	1.72 ± 0.06	1.54 ± 0.05	1.42 ± 0.08	3.50
		Sample III		2.88 ± 0.06	1.72 ± 0.04	1.56 ± 0.05	1.49 ± 0.04	3.47
25.11	14.56 ± 0.21	Control	23	14.31 ± 0.12	11.71 ± 0.14	11.60 ± 0.10	11.29 ± 0.13	1.28
		Sample I		14.25 ± 0.10	10.04 ± 0.25	9.96 ± 0.38	9.66 ± 0.22	2.20
		Sample II		14.13 ± 0.11	10.38 ± 0.29	10.12 ± 0.27	9.74 ± 0.28	2.00
		Sample III		14.53 ± 0.14	10.44 ± 0.26	10.07 ± 0.23	9.87 ± 0.23	2.14
		Control	30	14.31 ± 0.12	11.78 ± 0.15	11.70 ± 0.10	11.50 ± 0.13	1.23
		Sample I		14.25 ± 0.10	9.93 ± 0.18	9.10 ± 0.30	7.89 ± 0.33	2.61
		Sample II		14.13 ± 0.11	10.06 ± 0.20	9.35 ± 0.25	7.62 ± 0.24	2.51
		Sample III		14.53 ± 0.14	10.60 ± 0.15	9.44 ± 0.22	7.74 ± 0.21	2.47
		Control	37	14.31 ± 0.12	11.83 ± 0.11	11.37 ± 0.12	11.35 ± 0.15	1.28
		Sample I		14.25 ± 0.10	9.77 ± 0.18	9.43 ± 0.38	8.16 ± 0.27	2.57
		Sample II		14.13 ± 0.11	9.55 ± 0.28	9.47 ± 0.32	7.96 ± 0.25	2.61
		Sample III		14.53 ± 0.14	9.64 ± 0.27	9.38 ± 0.20	8.01 ± 0.25	2.76
75.56	39.29 ± 0.25	Control	23	38.16 ± 0.54	37.81 ± 0.47	36.55 ± 0.55	36.40 ± 0.54	0.16
		Sample I		38.72 ± 0.61	38.46 ± 0.72	33.26 ± 0.61	32.76 ± 0.74	0.46
		Sample II		39.04 ± 0.52	37.90 ± 0.93	33.45 ± 0.57	31.47 ± 0.68	0.60
		Sample III		37.76 ± 0.58	37.86 ± 0.88	33.60 ± 0.60	32.55 ± 0.63	0.35
		Control	30	38.16 ± 0.54	37.32 ± 0.63	37.32 ± 0.58	36.49 ± 0.50	0.16
		Sample I		38.72 ± 0.61	34.04 ± 0.76	32.74 ± 0.64	29.92 ± 0.91	1.00
		Sample II		39.04 ± 0.52	34.56 ± 0.67	31.98 ± 0.75	28.07 ± 0.54	1.11
		Sample III		37.76 ± 0.58	33.42 ± 0.68	30.89 ± 0.61	28.30 ± 0.55	1.06
		Control	37	38.16 ± 0.54	36.78 ± 0.66	35.94 ± 0.64	35.79 ± 0.67	0.29
		Sample I		38.72 ± 0.61	34.65 ± 0.82	30.66 ± 0.94	29.49 ± 0.65	1.06
		Sample II		39.04 ± 0.52	32.58 ± 0.96	29.76 ± 0.65	28.02 ± 0.68	1.42
		Sample III		37.76 ± 0.58	31.89 ± 0.67	29.56 ± 0.69	29.44 ± 0.76	1.25

^A Pirimiphos methyl (mg kg⁻¹) extracted from samples after sterilization in autoclave was determined at each fortification levels for correction of pesticide concentration level at the beginning of incubation (n = 3)

^B Control wheat sample has no yeast addition, fermented wheat sample I, II and III were inoculated at levels 6, 8 and 10% (v/w) respectively

^C Pirimiphos methyl (mg kg⁻¹) extracted from samples at day zero was determined as analytical recovery rates (n = 3)

^D Degradation rate constant

Santos et al. 2009). Considering this, and optimal temperature growth of *S. cerevisiae* being higher than 23 °C, obtained exception in the degradation trend was not surprising. As for samples fortified with 15 MRL and

incubated at 30 and 37 °C it was most likely that negative impact of pesticide at *S. cerevisiae* growth rate was overcome by optimal temperature conditions, i.e. yeast required shorter period of adaptation, thus degradation rates were

equally distributed through time. Overall, regardless fermentation parameters, the degradation rate constants of pirimiphos methyl in fermented wheat samples were comparable with control samples, increased by 255–573, 56–116 and 119–594% respectively in samples fortified with MRL, 5MRL and 15MRL. These results evidenced that yeast played an important role in promoting pirimiphos methyl dissipation in wheat dough.

Ability of *S. cerevisiae* cells to adsorb various contaminants from surrounding, whether organic or inorganic, were determined earlier (Razmkhab et al. 2002; Goyal et al. 2003). This implied possibility for adsorption of pesticide molecules, as well. During the fermentation procedures behaviour of present pesticide depended on its physical–chemical properties as well as on the type and character of the process (Regueiro et al. 2015). Sharma et al. (2005) demonstrated the considerable degradation of different groups of pesticides (endosulfan, deltamethrin, malathion, propiconazole, chlorpyrifos and hexaconazole) during bread-making process, whereby the degradation of all tested pesticides varied with their concentrations present in the samples in the way that the loss of residue decreases as residue concentration in the sample increases. Ruediger et al. (2005) considered that when it comes to pesticide residue loss during fermentation, polysaccharides in the microbe's cell wall might be responsible vectors. They revealed a decrease in pesticide by microorganisms resulted from the adsorption on the cell walls, and biological degradation less likely the key mechanism for the same. However, in present study the pesticide extraction was done from fermented wheat samples coupled with yeast cells i.e. the yeast cells were not removed from samples prior extraction. Thus, during sample preparation, procedure allowed for molecules of pesticides possibly

adsorbed on the cell wall extracted. The dissipation of pirimiphos methyl may also be the consequence of microbial enzymatic activity, which may have caused biological degradation instantaneously, but also could require an adaptation stage for microbes in the surrounding contaminated with agrochemicals (Boethling 1993). During the most microbial degradation of organophosphates, metabolic pathway is similar, if not identical, for all pesticides belonging to this group, whereby the first step is always catalysed by enzyme phosphotriesterase or organophosphate hydrolase produced by microorganisms (Singh and Walker 2006). Considering that it might be assumed that degradation pathway of pirimiphos methyl by *Saccharomyces cerevisiae* that occurred in this experiment was same or similar.

Results of chlorpyrifos methyl biodegradation in wheat during fermentation with *L. plantarum* are presented in Table 4. For this part of experiment pesticide extracted from sterilized samples immediately after inoculation (day zero) was also determined at each fortification level as recovery for correction of chlorpyrifos methyl concentration level at the beginning of incubation. The individual effect of *L. plantarum* on chlorpyrifos methyl residues was separated by determination of chemical degradation of pesticide which occurred spontaneously in the control wheat substrate maintained at the same temperatures as for fermentation yet without lactobacilli inoculations. As can be seen from results, when held on different temperatures for distinct time and without lactobacilli, there were reductions of chlorpyrifos methyl up to maximum 13.8% in sample fortified with MRL, 23.1% in one spiked with 5MRL and 13.1% in sample fortified with 15MRL. The highest chlorpyrifos methyl dissipations obtained at 30 °C and residual pesticide in samples showed decreasing trend

Table 4 Dissipation of chlorpyrifos methyl residues during lactic acid fermentation with *L. plantarum* in wheat substrate

Fortification level (mg kg ⁻¹)	Residual concentration after sterilization (mg kg ⁻¹) ^A	Samples ^B	Temperature (°C)	Residual concentration (mg kg ⁻¹) at different times (days)				k ^D (×10 ⁻¹) day ⁻¹
				0 ^C	1 (24 h)	2 (48 h)	3 (72 h)	
3.06	0.67 ± 0.06	Control	23	0.65 ± 0.05	0.62 ± 0.05	0.62 ± 0.03	0.62 ± 0.07	0.29
		Sample I		0.61 ± 0.03	0.57 ± 0.04	0.48 ± 0.02	0.45 ± 0.02	0.96
		Sample II		0.64 ± 0.04	0.60 ± 0.03	0.51 ± 0.09	0.41 ± 0.02	1.09
		Sample III		0.61 ± 0.03	0.58 ± 0.02	0.54 ± 0.05	0.38 ± 0.03	0.90
		Control	30	0.65 ± 0.04	0.61 ± 0.07	0.59 ± 0.09	0.56 ± 0.08	0.54
		Sample I		0.61 ± 0.03	0.33 ± 0.02	0.29 ± 0.04	0.19 ± 0.04	4.58
		Sample II		0.64 ± 0.04	0.35 ± 0.06	0.27 ± 0.02	0.21 ± 0.05	4.69
		Sample III		0.61 ± 0.03	0.36 ± 0.02	0.27 ± 0.04	0.25 ± 0.04	4.11
		Control	37	0.65 ± 0.04	0.65 ± 0.08	0.61 ± 0.09	0.60 ± 0.03	0.19
		Sample I		0.61 ± 0.03	0.58 ± 0.03	0.56 ± 0.04	0.53 ± 0.04	0.47
		Sample II		0.64 ± 0.04	0.64 ± 0.03	0.56 ± 0.07	0.53 ± 0.03	0.43
		Sample III		0.61 ± 0.03	0.61 ± 0.02	0.55 ± 0.04	0.53 ± 0.02	0.33

Table 4 continued

Fortification level (mg kg ⁻¹)	Residual concentration after sterilization (mg kg ⁻¹) ^A	Samples ^B	Temperature (°C)	Residual concentration (mg kg ⁻¹) at different times (days)				k ^D (×10 ⁻¹) day ⁻¹
				0 ^C	1 (24 h)	2 (48 h)	3 (72 h)	
15.81	3.79 ± 0.15	Control	23	3.76 ± 0.12	3.65 ± 0.15	3.52 ± 0.12	3.46 ± 0.16	0.30
		Sample I		3.64 ± 0.17	3.23 ± 0.24	3.21 ± 0.17	2.54 ± 0.28	1.01
		Sample II		3.61 ± 0.11	3.31 ± 0.23	3.20 ± 0.14	2.57 ± 0.23	0.87
		Sample III		3.63 ± 0.22	3.54 ± 0.24	3.20 ± 0.17	2.55 ± 0.29	0.69
		Control	30	3.76 ± 0.12	3.13 ± 0.18	2.92 ± 0.09	2.89 ± 0.17	1.33
		Sample I		3.64 ± 0.17	2.13 ± 0.21	1.73 ± 0.17	1.45 ± 0.11	4.05
		Sample II		3.61 ± 0.11	2.21 ± 0.24	1.79 ± 0.15	1.40 ± 0.25	3.86
		Sample III		3.63 ± 0.22	2.28 ± 0.23	1.78 ± 0.15	1.39 ± 0.21	3.80
15.81	3.79 ± 0.15	Control	37	3.72 ± 0.12	3.65 ± 0.15	3.58 ± 0.12	3.18 ± 0.14	0.37
		Sample I		3.64 ± 0.17	3.20 ± 0.18	3.07 ± 0.04	2.73 ± 0.25	1.03
		Sample II		3.61 ± 0.11	3.29 ± 0.14	3.18 ± 0.14	2.75 ± 0.24	0.82
		Sample III		3.63 ± 0.22	3.45 ± 0.12	3.42 ± 0.11	2.72 ± 0.21	0.59
45.21	11.30 ± 0.25	Control	23	11.24 ± 0.53	10.73 ± 0.67	10.73 ± 0.54	10.64 ± 0.71	0.29
		Sample I		10.26 ± 0.49	9.64 ± 0.54	9.62 ± 0.54	7.09 ± 0.37	0.73
		Sample II		10.34 ± 0.32	9.66 ± 0.50	9.54 ± 0.50	7.11 ± 0.32	0.78
		Sample III		10.26 ± 0.68	9.61 ± 0.52	9.58 ± 0.36	6.99 ± 0.35	0.76
		Control	30	11.24 ± 0.67	10.09 ± 0.63	9.77 ± 0.58	9.77 ± 0.50	0.75
		Sample I		10.26 ± 0.49	8.74 ± 0.32	6.73 ± 0.50	5.56 ± 0.45	1.92
		Sample II		10.34 ± 0.32	8.99 ± 0.54	6.81 ± 0.51	5.61 ± 0.48	1.84
		Sample III		10.26 ± 0.68	9.04 ± 0.50	6.82 ± 0.53	5.36 ± 0.57	1.82
		Control	37	11.24 ± 0.52	10.63 ± 0.47	10.59 ± 0.55	10.40 ± 0.54	0.37
		Sample I		10.26 ± 0.49	9.64 ± 0.31	9.17 ± 0.32	8.99 ± 0.38	0.54
		Sample II		10.34 ± 0.32	9.48 ± 0.36	9.05 ± 0.37	8.95 ± 0.33	0.67
		Sample III		10.26 ± 0.68	9.37 ± 0.38	9.15 ± 0.63	8.94 ± 0.31	0.65

^A Chlorpyrifos methyl (mg kg⁻¹) extracted from samples after sterilization in autoclave was determined at each fortification levels for correction of pesticide concentration level at the beginning of incubation (n = 3)

^B Control wheat sample has no lactobacilli addition, fermented wheat sample I, II and III were inoculated at levels 6, 8 and 10% (v/w) respectively

^C Chlorpyrifos methyl (mg kg⁻¹) extracted from samples at day zero was determined as analytical recovery rates (n = 3)

^D Degradation rate constant

as incubation time progressed, still statistically there were no significant differences between variations in incubation parameters ($p < 0.1$). Results recorded in this study correspond with previously reported chlorpyrifos methyl stability, tested during wheat storage at ambient conditions over an extended period. The half life for this insecticide on 30 °C and 50% relative humidity is established to be 120–150 days. In surrounding with higher humidity degradation of chlorpyrifos methyl slightly increase thus the half-life in this condition is 60 days (Fleurat-Lessard et al. 1998). During lactic acid fermentation of wheat, the inoculated *L. plantarum* significantly affected chlorpyrifos methyl degradation. The highest overall dissipation of this OPP was 56.7% (comparing with control and after adjusting for dissipation during sterilization) in samples contaminated with lowest used concentration (3 mg kg⁻¹).

Chlorpyrifos methyl reduction rate decreased with an increase of fortification rate, reaching 38.6% in samples fortified with 15 mg kg⁻¹ and 34.7% in samples with 45 mg kg⁻¹. This lower pesticide dissipation in samples contaminated at higher rate is in accordance with numerous indications of various authors regarding negative impact of pesticides especially at extreme high concentrations on lactobacilli growth (Abou-Arab 2002; Cho et al. 2009; Abou Ayana et al. 2011; Clair et al. 2012). Variations of initial *L. plantarum* inoculums size had no statistically significant effect on pesticide dissipation level. Concerning fermentation temperature, the highest chlorpyrifos methyl dissipations, mentioned previously, obtained at 30 °C at all fortification levels, and somewhat lower in samples fermented at 23 °C (max 33.1, 22.2 and 26.9% respectively in samples fortified with 3, 15 and 45 mg kg⁻¹), although

statistically there were no significant differences between those groups ($\alpha = 0.1$, $p < 0.1$). Significantly lower dissipation of pesticide influenced by *L. plantarum* obtained in samples fermented at 37 °C (max 9.8, 9.7 and 5.9% in samples spiked with 3, 15 and 45 mg kg⁻¹, respectively). This could be explained by most adequate growth temperature for this lactobacilli being 15–35 °C. Calculated degradation rate constants (Table 4) indicated that residual chlorpyrifos methyl in fermented and control samples showed decreasing trend as fermentation time progressed. As can be seen from results, during wheat fermentation by *L. plantarum* chlorpyrifos methyl degradation rate was equal throughout fermentation time. Overall, regardless fermentation parameters, the degradation rate constants of chlorpyrifos methyl in fermented wheat samples were, comparing with control samples, increased by 74–769, 59–237 and 46–469% in samples contaminated with pesticide at MRL, 5MRL and 15MRL levels. These results evidenced directly that *L. plantarum* play an important role in promoting chlorpyrifos methyl dissipation in wheat dough.

Once microbes get in surroundings contaminated with pesticides, they will utilize those chemicals as a nutrition source in order to provide sufficient amount of carbon, nitrogen and phosphorus required for cell growth, or they will simply, striving for environmental decontamination, produce pesticide degrading enzymes responsible for biological degradation (Singh et al. 2004; Bhalariao and Puranik 2009). Thus, lactic acid bacteria is able to produce gene encoding organophosphorus hydrolase for which Islam et al. (2010) proved to be the main enzyme responsible for degradation of nine organophosphorus insecticides. Ability of lactic acid bacteria to reduce pesticides as contaminants is determined in several works. Bo et al. (2011) established that, during yoghurt production from bovine milk, applied starters containing lactobacilli due their activity enhanced degradation of organophosphorus pesticides dimethoate, fenthion, malathion, methyl parathion, monocrotophos, phorate and trichlorphon. This was confirmed by Zhao and Wang (2012) who, testing the fermentation efficiency for dissipation of same pesticide in skimmed milk, obtained positive effect of the inoculated *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus paracasei* or *Lactobacillus plantarum* on the kinetics of degradation. Besides milk, degradation of various pesticides (chlorpyrifos, bifenthrin, metalaxyl azoxystrobin, cyprodinil, fludioxonil, mepanipyrim, pyrimethanil, tetraconazole, DDT, lindan) by numerous lactic acid bacteria (*Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus bulgaricus*, *Leuconostoc oenos* and *Streptococcus thermophilus*) as well occurred in different substrates like kimchi (Cho et al. 2009; Jung et al. 2009), vine (Cabras

et al. 1999; Ruediger et al. 2005), cheese (Abou-Arab 1997) and fermented sausages (Abou-Arab 2002).

The results of yeast biodegradation of pirimiphos methyl and lactobacilli biodegradation of chlorpyrifos methyl in wheat reported in this work correspondence with significant number of previously published studies regarding microbiological capacity for reduction of pesticide contamination in various food commodities. It could be cautiously concluded that fermentation as food processing technique can be used as a mean for significant reduction of excess amounts of pesticide residues in food.

Conclusion

Yeast *Saccharomyces cerevisiae* and lactic acid bacteria *Lactobacillus plantarum* showed ability for significant reduction of pirimiphos methyl i.e. chlorpyrifos methyl in wheat. Yeast fermentation was especially effective for reduction of pirimiphos methyl applied at MRL concentration, causing dissipation for max 48.8% after 72 h at 30 °C, while pesticide reduction rate decreased with an increase of fortification rate at same temperature (reaching 27.1 and 23.7%). Lactic acid fermentation, activity of *L. plantarum* was, as well, especially effective for reduction of chlorpyrifos methyl applied at MRL concentration, causing dissipation for max 56.7% after 72 h at 30 °C, and reduction rate of this pesticide also decreased with an increase of fortification rate (reaching up to 38.6 and 34.7%).

Sourdough fermentation with microorganisms, as basic wheat processing technique, might be a mean for significant decrement of pesticide contamination in food product.

Acknowledgements This study was carried out as a part of the Project No TR31043, supported by the Ministry of Education and Science of the Republic of Serbia.

References

- Abou Ayana IAA, Gamal El Deen AA, El-Metwally MA (2011) Behavior of certain lactic acid bacteria in the presence of pesticides residues. *Int J Dairy Sci* 6:44–57
- Abou-Arab AAK (1997) Effect of Ras cheese manufacturing on the stability of DDT and its metabolites. *Food Chem* 59:115–119
- Abou-Arab AAK (2002) Degradation of organochlorine pesticides by meat starter in liquid media and fermented sausage. *Food Chem Toxicol* 40:33–41
- Bi Fai P, Grant A (2009) A comparative study of *Saccharomyces cerevisiae* sensitivity against eight yeast species sensitivities to a range of toxicants. *Chemosphere* 75:289–296
- Bo L-Y, Zhang Y-H, Zhao X-H (2011) Degradation kinetics of seven organophosphorus pesticides in milk during yoghurt processing. *J Serbian Chem Soc* 76:353–362
- Boethling RS (1993) Biodegradation of xenobiotic chemicals. In: Corn M (ed) *Handbook of hazardous materials*. Academic, New York, pp 55–67

- Braconi D, Sotgiu M, Millucci L, Paffetti A, Tasso F, Alisi C, Martini S, Rappuoli R, Lusini P, Sprocati AR, Rossi C, Santucci A (2006) Comparative analysis of the effects of locally used herbicides and their active ingredients on a wild-type wine *Saccharomyces cerevisiae* strain. *J Agric Food Chem* 54:3163–3172
- Cabras P, Angioni A, Garau VL, Menelli EV, Cabitza F, Pala M (1998) Pesticide residue in raisin processing. *J Agric Food Chem* 46:2309–2311
- Cabras P, Angioni A, Garau VL, Piris FM, Farris GA, Madau G, Emonti G (1999) Pesticides in fermentative processes of wine. *J Agric Food Chem* 47:3854–3857
- Cho KM, Math RK, Islam SM, Lim WJ, Hong SY, Kim JM, Yun MG, Cho JJ, Yun HD (2009) Biodegradation of chlorpyrifos by lactic acid bacteria during kimchi fermentation. *J Agric Food Chem* 57:1882–1889
- Clair E, Linn L, Travert C, Amiel C, Séralini G-E, Panof J-M (2012) Effects of roundup and glyphosate on three food microorganisms: *Geotrichum candidum*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus delbrueckii subsp. Bulgaricus*. *Curr Microbiol* 64:486–491
- Čuš F, Raspor P (2008) The effect of pyrimethanil on the growth of wine yeasts. *Lett Appl Microbiol* 47:54–59
- Demir MK, Elgün A (2014) A Comparison of autoclave, microwave, IR and UV-C stabilization of whole wheat flour branny fractions upon the nutritional properties of whole wheat bread. *J Food Sci Technol* 51:59–66
- Đorđević T, Đurović-Pejčev R (2015) Dissipation of chlorpyrifos methyl by *Saccharomyces cerevisiae* during wheat fermentation. *LWT Food Sci Tech* 61:516–523
- Đorđević T, Šiler-Marinković S, Đurović-Pejčev R, Dimitrijević-Branković S, Gajić-Umiljendić J (2013a) Efficiencies of different methods for determination of organophosphate pesticide residues in fermented wheat substrate. *Pestic Phytomed* 28:133–140
- Đorđević T, Šiler-Marinković S, Đurović-Pejčev R, Dimitrijević-Branković S, Gajić-Umiljendić J (2013b) Dissipation of pirimiphos methyl during wheat fermentation by *Lactobacillus plantarum*. *Lett Appl Microbiol* 57:412–419
- Đurović R, Đorđević T (2010) Liquid-solid extraction method for pesticides determination in soil samples. In: *Zbornik rezimea X Savetovanja o zaštiti bilja, Zlatibor, RS*, pp 135–136 (in serbian)
- Fleurat-Lessard F, Vidal M-L, Budzinski H (1998) Modelling biological efficacy decrease and rate of degradation of chlorpyrifos methyl on wheat stored under controlled conditions. *J Stored Prod Res* 34:341–354
- Fleurat-Lessard F, Chaurand M, Marchegay G, Abecassis J (2007) Effects of processing on the distribution of pirimiphos methyl residues in milling fractions of durum wheat. *J Stored Prod Res* 43:384–395
- González-Barreiro C, Rial-Otero R, Cancho-Grande B, Simal-Gándara J (2015) Wine aroma compounds in grapes: a critical review. *Crit Rev Food Sci* 55:202–218
- González-Rodríguez R, Cancho-Grande B, Simal-Gándara J (2009) Efficacy of new commercial formulations to control downy mildew and dissipation of their active fungicides in wine after good agricultural practices. *J Sci Food Agric* 89:2625–2635
- Goyal N, Jain SC, Banerjee UC (2003) Comparative studies on the microbial adsorption of heavy metals. *Adv Environ Res* 7:311–319
- Islam SMA, Math RK, Cho KM, Lim WJ, Hong SY, Kim JM, Yun MG, Cho JJ, Yun HD (2010) Organophosphorus hydrolase (OpdB) of *Lactobacillus brevis* WCP902 from Kimchi is able to degrade organophosphorus pesticides. *J Agric Food Chem* 58:5380–5386
- Jawich D, Lteif R, Pfohl-Leszkowicz A, Strehaiano P (2006) Effects of penconazole on two yeast strains: growth kinetics and molecular studies. *Mol Nutr Food Res* 50:552–556
- Jung J-K, Park S-Y, Kim S-H, Kang J-M, Yang J-Y, Kang S-A, Chun H-K, Park K-Y (2009) Removal effects of Bifenthrin and Metalaxyl pesticides during preparation and fermentation of *Baechu kimchi*. *J Korean Soc Food Sci Nutr* 38:1258–1264
- Kaushik G, Satya S, Naik SN (2009) Food processing a tool to pesticide residue dissipation—a review. *Food Res Int* 42:26–40
- Kontou S, Tsipi D, Tzia C (2004) Stability of the dithiocarbamate pesticide maneb in tomato homogenates during cold storage and thermal processing. *Food Addit Contam* 21:1083–1089
- Lalah JO, Wandiga SO (2002) The effect of boiling on the removal of persistent malathion residues from stored grains. *J Stored Prod Res* 38:1–10
- Lu Y, Yang Z, Shen L, Liu Z, Zhou Z, Diao J (2013) Dissipation behavior of organophosphorus pesticides during the cabbage pickling process: residue changes with salt and vinegar content of pickling solution. *J Agri Food Chem* 61:2244–2252
- Noguerol-Pato R, Torrado-Agrasar A, González-Barreiro C, Cancho-Grande B, Simal-Gándara J (2014) Influence of new generation fungicides on *Saccharomyces cerevisiae* growth, grape must fermentation and aroma biosynthesis. *Food Chem* 146:234–241
- Noguerol-Pato R, Sieiro-Sampedro T, González-Barreiro C, Cancho-Grande B, Simal-Gándara J (2015) Evaluation of the effect of fenhexamid and mepanipyrim in the volatile composition of Tempranillo and Graciano wines. *Food Res Int* 71:108–117
- Noguerol-Pato R, Fernández-Cruz T, Sieiro-Sampedro T, González-Barreiro C, Cancho-Grande B, Cilla-García DA, García-Pastor M, Martínez-Soria MT, Sanz-Asensio J, Simal-Gándara J (2016) Dissipation of fungicide residues during winemaking and their effects on fermentation and the volatile composition of wines. *J Agric Food Chem* 64:1344–1354
- Razmkhab S, López-Toledano A, Ortega JM, Mayen M, Merida J, Medina M (2002) Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. *J Agric Food Chem* 50:7432–7437
- Regueiro J, López-Fernández O, Rial-Otero R, Cancho-Grande B, Simal-Gándara J (2015) A review on the fermentation of foods and the residues of pesticides—biotransformation of pesticides and effects on fermentation and food quality. *Crit Rev Food Sci* 55:839–863
- Ruediger GA, Pardon KH, Sas AN, Godden PW, Pollnitz AP (2005) Fate of pesticides during the winemaking process in relation to malolactic fermentation. *J Agric Food Chem* 53:3023–3026
- Russo P, Beleggia R, Ferrer S, Pardo I, Spano G (2010) A polyphasic approach in order to identify dominant lactic acid bacteria during pasta manufacturing. *LWT Food Sci Technol* 43:982–986
- Salgado JM, González-Barreiro C, Rodríguez-Solana R, Simal-Gándara J, Domínguez JM, Cortés S (2012) Study of the volatile compounds produced by *Debaryomyces hansenii* NRRL Y-7426 during the fermentation of detoxified concentrated distilled grape marc hemicellulosic hydrolysates. *World J Microb Biot* 28:3123–3134
- Santos PM, Simões T, Sá-Correia I (2009) Insights into yeast adaptive response to the agricultural fungicide mancozeb: a toxicoproteomics approach. *Proteomics* 9:657–670
- Sharma J, Satya S, Kumar V, Tewary DK (2005) Dissipation of pesticides during bread making. *Chem Health Safety* 12:17–22
- Shin J-H, Kim Y-M, Park J-W, Kim J-E, Rhee I-K (2003) Resistance of *Saccharomyces cerevisiae* to fungicide chlorothalonil. *J Microbiol* 41:219–222
- Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. *FEMS Microbiol Rev* 30:428–471
- Singh BK, Walker A, Morgan AW, Wright DJ (2004) Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in

- bioremediation of contaminated soils. Appl Environ Microb 70:4855–4863
- Uygun U, Senoz B, Ozturk S, Koksel H (2009) Degradation of organophosphorus pesticides in wheat during cookie processing. Food Chem 117:261–264
- Zhao X-H, Wang J (2012) A brief study on the degradation kinetics of seven organophosphorus pesticides in skimmed milk cultured with *Lactobacillus* spp. at 42 °C. Food Chem 131:300–304