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Dissipation of organophosphorus pesticides in wheat during pasta processing

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Abstract

For investigating the carryover of some organophosphorus pesticide residues in the cereal food chain from grain to consumer, a study was set up on durum wheat, semolina and pasta. Pesticide-free durum wheat was placed into a small-scale model of a commercial storage vessel and treated with pesticides (malathion, fenitrothion, chlorpyrifos methyl, and pirimiphos methyl) according to the raw material legislation of Turkey. The residue levels of insecticides were determined in wheat, semolina, and spaghetti produced from stored wheat at various time intervals during five months of storage. A multiresidue analysis was performed using GC equipped with an NPD. The confirmation was performed by GC–MS. The residue levels of insecticides in wheat exceeded the maximum residue limits (MRLs) for wheat. The storage period was generally not effective enough to reduce the residues in wheat to levels below the MRLs. Although a considerable amount of the insecticides remained in the semolina, spaghetti processing significantly reduced residue concentrations in general. Pirimiphos methyl was the most persistent of the insecticides and comparatively less substantial loss occurred during milling and spaghetti processing due to its physicochemical properties.

Keywords: Malathion; Fenitrothion; Chlorpyrifos methyl; Pirimiphos methyl; Wheat; Semolina; Spaghetti

1. Introduction

Organophosphorus insecticides are widely used for protecting stored commodities from pests. They are mainly used to treat the stores but some can be applied directly to the commodity. Postharvest applied pesticides have been attracting much attention because residues in stored cereal grain may be hazardous to human health (Nakamura et al., 1993; Sharma, Satya, Kumar, & Tewary, 2005; Uygun, Ozkara, Ozbey, & Koksel, 2007). The concern regards the possibilities that the cereals may contain a number of pesticide residues.

Cereal grains are raw materials for the main foods at the basis of all of the regional diets in the world. Pasta is a major candidate, following bread, as regards the intake of residues through cereal-based products. Turkey is the third largest durum wheat producer in the world, next to the EU and Canada, with the production averaging 3.0 Mt over the past 5 years. In addition, Turkey has a large pasta industry and is a major exporter of pasta (Anonymous, 2005). In the present study, pasta was selected for investigation because it is a widely consumed foodstuff. Furthermore, pasta processing is highly standardized and the results obtained can be easily generalized. Durum wheat processing is represented by milling of durum wheat into semolina and pasta manufacturing, which involves the use of only two basic ingredients: semolina and water.

For investigating the carryover of some organophosphorus pesticide residues in the cereal food chain from grain to consumer, a study was set up on durum wheat, semolina, and pasta. The levels of pesticide residues in the final products are affected by a number of physical and chemical transformations that the raw material undergoes

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during milling as well as further processing of wheat into its products. However, limited information about the effect of these processes on the dissipation of pesticides in wheat is available (Balinova, Mladenova, & Obretenchev, 2006; Fleurat-Lessard, Chaurand, Marchegay, & Abecassis, 2007; Sharma et al., 2005; Uygun, Koksel, & Atlı, 2005).

In a previous study, residue levels of malathion and its metabolites (malaoxon and isomalathion) and fenitrothion during storage, milling and baking were investigated. Residues were determined in wheat, bran, flour, white, and bran bread at about one-month intervals during storage (Uygun et al., 2005). In the present study, we extend the previous study to investigate the residue levels of two additional pesticides for different pesticide application methods and storage types. The final product was spaghetti instead of bread.

In this study, the effect of storage, milling and pasta processing on the residues of four organophosphorus pesticides in pilot scale storage and processing conditions were investigated. For this, malathion, fenitrothion, chlorpyrifos methyl, and pirimiphos methyl were applied on pesticide-free durum wheat. The residue levels of insecticides were determined in wheat, semolina and spaghetti produced from stored wheat at various time intervals during five months of storage. The storage period continued until no detectable residues were found in spaghetti samples.

2. Materials and methods

2.1. Materials

Pesticide standards, malathion, fenitrothion, chlorpyrifos methyl, and pirimiphos methyl were purchased from Dr. Ehrenstorfer (Germany). Commercial chlorpyrifos methyl (Reldan 2E, Dow Agro Science, UK), pirimiphos methyl (Actellic 50 EC, Zeneca, UK), Malathion (20 EC, Safa Tarım, Turkey) and fenitrothion (Sathion 3 dust, Safa Tarım, Turkey) were purchased from local distributors. The durum wheat sample (cv. Kızıltan) with known origin was supplied by Field Crops Improvement Center, Ankara, Turkey. The sample was cleaned on a Carter Dockage Tester before the treatment.

2.2. Application of the insecticides

A small-scale model of a commercial storage vessel (65 × 45 × 46 cm) was built for laboratory experiments. The inner surfaces of stores were covered with thin metal sheet. Malathion, chlorpyrifos methyl and pirimiphos methyl were prepared at approved doses (190 g/l, 227 g/l and 500 g/l) and then sprayed onto the surfaces of the stores (650 ml/100 m^2, 425 ml/100 m^2 and 300 ml/100 m^2). The wheat samples (50 kg each) were placed into storage vessels by mixing to provide a homogenous distribution of pesticide on grain. Dust fenitrothion (3%) (132.2 g/ton) was directly applied on wheat (50 kg). Time-zero samples were taken for the analysis after 2 h. The rest of the samples were stored at ambient temperature for about five months. The grain was analysed at various time intervals during storage.

2.3. Milling

The durum wheat samples were tempered to moisture content of 16.5% and left overnight. A Buhler pneumatic laboratory mill (Model MLU 202D, Uzwil, Switzerland) with three break and three sizing passages was used to obtain semolina from the durum wheat samples. The semolina was purified using a laboratory purifier (Namad, Italy).

2.4. Spaghetti processing and drying

The semolina was processed into spaghetti separately using the micromethod of D'Egidio et al. (1982). Water was added to obtain total water content of 30%. The samples were mixed in a premixer (Namad, Italy) at room temperature for 15 min. The dough was extruded in an extruder (Namad, Italy) under vacuum at a pressure of 400–600 mmHg. The extruder temperature was kept below 45 °C by water circulation. The spaghetti samples (1.7 mm thick) were dried in a ventilated static drying system (Namad, Italy) working at 40 °C. During drying, the relative humidity was decreased gradually from 90% to 60% over 20 h. The final dried spaghetti contained a maximum of 12% moisture.

2.5. Extraction

Fifty grams of wheat samples were ground in a coffee grinder (Moulinex Coffee Mill Model 980). Ground samples (50 g) were homogenized with ethyl acetate (100 ml) and anhydrous sodium sulphate (5 g) and left overnight for the static extraction. The extract was filtered and concentrated to 2 ml in vacuo at 40 °C using a rotary evaporator. The samples were applied onto GC-NPD and GC–MS without clean up (Anonymous, 1996).

2.6. GC-NPD and GC–MS

Gas chromatography was performed using an Agilent 6890 gas chromatograph equipped with a nitrogen phosphorus detector and capillary column (DB-5, 30 m × 0.25 mm ID, 0.25 μm film thickness) using helium carrier gas at a flow rate of 1 ml min^{-1}. The oven temperature program was: initial temperature at 50 °C, then from 50 °C to 150 °C at 25 °C min^{-1}, 150 °C to 170 °C at 10 °C min^{-1}, then 170 °C to 200 °C at 2 °C min^{-1} and 200 °C to 250 °C at 10 °C min^{-1}, held for 2 min at 250 °C. Injector and detector temperatures were 250 °C and 300 °C, respectively. Gas chromatography–mass spectrometry was performed using an Agilent 5973 mass detector under the same conditions of gas chromatography.

Identification and confirmation of the pesticides were based on their GC retention times and comparison of their sample mass spectrum with the characteristic ions in the

Table 1
Recoveries and standard deviations of the pesticides in wheat samples at different fortification levels ($n = 6$)

Pesticides	Fortification (mg kg^{-1})	Mean recovery ± SD (%)
Malathion	16	79 ± 1.63
	8	84 ± 2.11
	4	86 ± 3.53
Average		83 ± 3.87
Chlorpyrifos methyl	6	79 ± 3.01
	3	78 ± 2.00
	1.5	80 ± 3.69
Average		79 ± 3.12
Pirimiphos methyl	10	93 ± 1.29
	5	95 ± 0.81
	2.5	96 ± 0.81
Average		95 ± 1.59
Fenitrothion	10	81 ± 1.29
	5	83 ± 3.05
	2.5	84 ± 2.11
Average		83 ± 2.62

reference standards mass spectra. Single-point calibration was used to estimate pesticide concentrations (SANCO, 2003). Detection limits were calculated by using a signal-to-noise ratio of 3 and determined as 0.02, 0.05, 0.01 and 0.01 mg kg^{-1} for malathion, fenitrothion, chlorpyrifos methyl and pirimiphos methyl, respectively.

2.7. Recovery

The recovery studies were performed by using blank samples of untreated wheat grain. The weights of blanks were the same as the weights of analytical samples of treated grains. Fortifications were carried out by the addition of calculated volumes of working standard solutions in order to obtain three concentration levels of the insecticides studied; at MRLs, half of MRLs and twice of MRLs. The fortified samples were analysed according to the method described above. Data derived from these experiments are presented in Table 1.

2.8. Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA) procedure. When significant differences were found, the LSD (least significant difference) test was used to determine the differences among means.

3. Results and discussion

3.1. Degradation of the pesticides in wheat during storage

The effect of storage on breakdown of the pesticides was examined at various times during five months of storage at ambient temperature. The results of pesticide degradation during storage are presented in Table 2. All residues in wheat were dissipated relatively faster during the first week of storage except chlorpyrifos methyl and thereafter at a slower rate until the end of the second month. This was less evident for pirimiphos methyl. The residues declined rapidly during the third month of storage except pirimiphos methyl, since the ambient temperature had gradually increased. The average temperatures in the store were 15 ± 5 °C in the first two months (April–May) and 25 ± 5 °C in June–August, respectively.

Table 2
Residue levels of the pesticides in stored wheat at various times during storage (mg kg^{-1})

Time (days)	Malathion	Fenitrothion	Chlorpyrifos methyl	Primiphos methyl
0	43.2a	11.8a	38.3a	24.3a
7	30.1b	8.46b	37.6a	19.9b
14	28.8b	6.29c	32.5b	18.0bc
21	20.8c	6.96c	24.4c	17.4c
60	20.9c	4.98d	19.3d	10.8d
90	9.60d	3.62e	9.23e	8.44d
120	6.58de	1.95f	6.75f	8.78d
150	5.09e	1.63f	6.05f	5.73e

Data are the means of four replicates and expressed on a dry basis. Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

During the storage period, malathion, fenitrothion, chlorpyrifos methyl and pirimiphos methyl in wheat decreased by 88%, 86%, 84% and 76%, respectively (Fig. 1). The most persistent of the pesticides was pirimiphos methyl. The present results agree with the results of the long residual effectiveness of post-harvest applied insecticides on storage pests (Balinova et al., 2006; Holland, Hamilton, Ohlin, & Skidmore, 1994). Chlorpyrifos methyl was found to be less persistent than pirimiphos methyl under the same conditions that corresponds with the result of Balinova et al. (2006). Similar to the results of the present study, it was reported that chlorpyrifos methyl and pirimiphos methyl were more persistent than malathion in stored grains (Anonymous, 1990).

3.2. Pesticide residue levels in semolina and spaghetti produced from stored wheat

Tables 3 and 4 show the residue levels of pesticides in semolina and spaghetti produced from stored wheat at the beginning of storage and various times during storage. A considerable amount of the insecticides remained in the semolina. After two months of storage, malathion and chlorpyrifos methyl residues in semolina declined significantly with a similar rate of dissipation to that of the insecticides in wheat during the same period. This is mainly due to the changes in temperature as explained previously. However, fenitrothion and pirimiphos methyl residues decreased slowly indicating that the increasing temperature did not affect them during storage.

Malathion, fenitrothion, chlorpyrifos methyl and pirimiphos methyl in semolina produced from the wheat stored

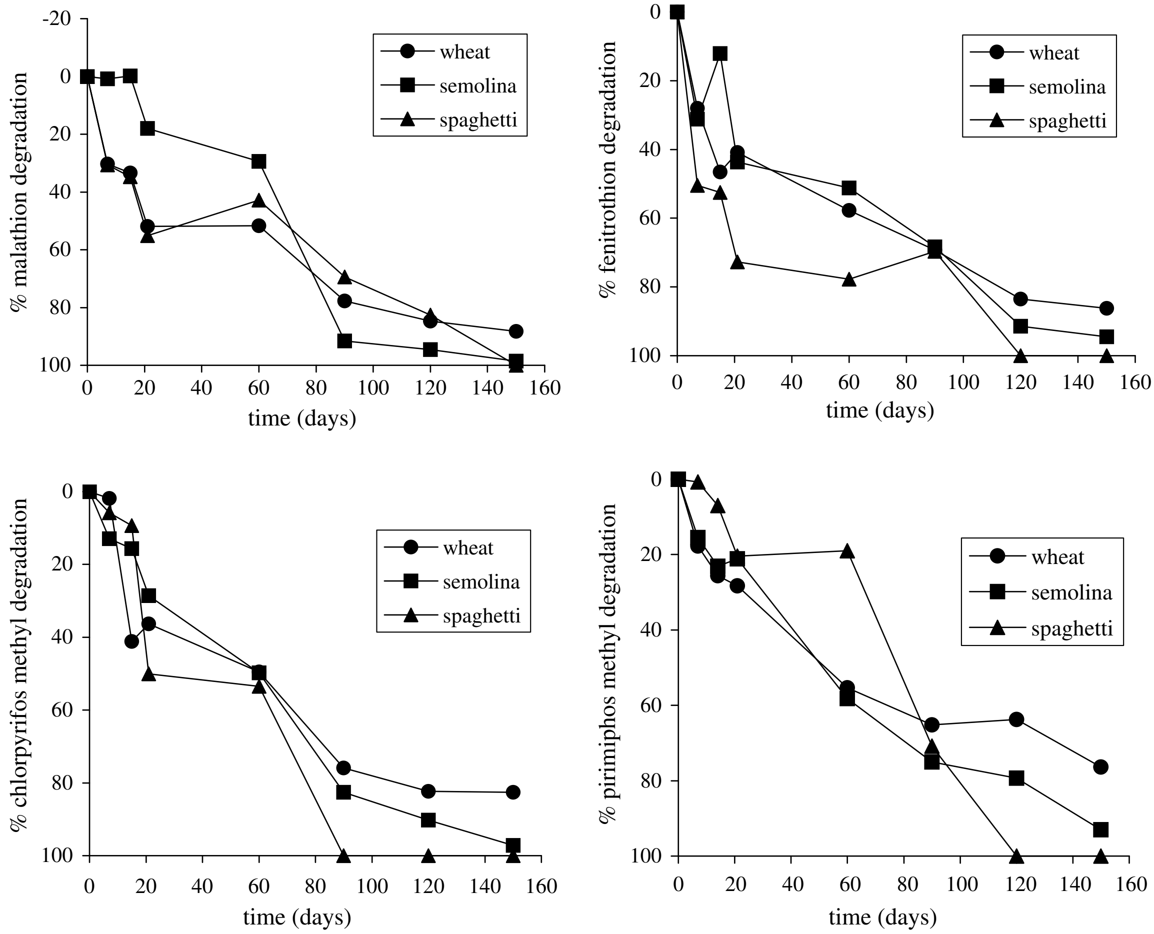

Fig. 1. Percent residue degradation of malathion, fenitrothion, chlorpyrifos methyl and pirimiphos methyl in wheat, semolina and spaghetti.

Table 3
Residue levels of the pesticides in semolina produced from stored wheat at various times during storage (mg kg^{-1})

Time (days)	Malathion	Fenitrothion	Chlorpyrifos methyl	Primiphos methyl
0	7.10a	2.00a	2.87a	6.05a
7	6.96b	1.38bc	2.50ab	5.12ab
14	7.03a	1.75ab	2.43ab	4.95b
21	5.75b	1.12c	2.06b	4.77b
60	4.95c	0.97cd	1.45c	2.53c
90	0.59d	0.63d	0.51d	1.51cd
120	0.39d	0.18e	0.29d	1.25cd
150	0.11d	0.11e	0.09d	0.42d

Data are the means of four replicates and expressed on a dry basis.
Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Table 4
Residue levels of the pesticides in spaghetti produced from stored wheat at various times during storage (mg kg^{-1})

Time (days)	Malathion	Fenitrothion	Chlorpyrifos methyl	Primiphos methyl
0	0.50a	0.99a	0.86a	1.38a
7	0.35ab	0.50b	0.82a	1.36a
14	0.33abc	0.47bc	0.79a	1.25a
21	0.23bcd	0.28bc	0.44b	1.09a
60	0.28bcd	0.22cd	0.40b	1.12a
90	0.15cde	0.31bc	0.01c	0.40b
120	0.09de	ND	0.01c	0.11bc
150	ND	ND	ND	ND

Data are the means of four replicates and expressed on a dry basis.
Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

for 150 days decreased by 98%, 95%, 97% and 93%, respectively (Fig. 1). The lowest decrease in residue levels was observed for pirimiphos methyl in semolina samples.

The rates of degradation and volatilisation of the residues were increased by the heat and ventilation involved in pasta processing especially during drying. Malathion, chlorpyrifos methyl and pirimiphos methyl residues in spaghetti samples produced from four months stored wheat were not detectable. Fenitrothion residues were not determined after three months. Although, chlorpyrifos methyl was detected at 90 and 120 days, the residue levels were quite low (Table 4, Fig. 1).

3.3. Carryover percentages of the pesticides from wheat to pasta

Carryover percentages of the pesticides from wheat to semolina and spaghetti are presented in Table 5. The carryover values were calculated until the end of two months since the residue levels were very low after two months.

Table 5
Carryover percentages of the pesticides from wheat to semolina and spaghetti

Time (day)	Pesticides	From wheat to semolina (%)	From semolina to spaghetti (%)	From wheat to spaghetti (%)
0	Malathion	16	7	1
7		23	5	1
14		25	5	1
21		28	4	1
60		24	6	2
0	Fenitrothion	17	49	8
7		16	36	6
14		22	34	7
21		16	25	4
60		19	23	4
0	Chlorpyrifos methyl	8	21	2
7		7	33	2
14		7	32	2
21		8	21	2
60		7	28	2
0	Primiphos methyl	25	23	6
7		26	27	7
14		26	21	7
21		28	23	6
60		23	44	10

Although the initial concentration of malathion in wheat was relatively higher than that of the other pesticides, the residue levels greatly reduced during milling and pasta processing. Only around 1% of the residues transferred into the spaghetti samples.

The transportation of fenitrothion residues from wheat to semolina increased with time until the end of the second week. Malathion residues also followed a similar trend but the increase was less obvious (Fig. 1). Residues of the more lipophilic materials tend to remain on the seed coat, although a proportion can migrate through to the bran and germ which contain high levels of triglycerides (Holland et al., 1994).

The carryover percentages of malathion residues from semolina to spaghetti were relatively lower than that of the residues from wheat to semolina. Taking into consideration that volatilisation is one of the basic processes acting on pesticide residues, this fact could be explained by the higher vapour pressure of malathion. Due to physicochemical properties of malathion, it was the most affected pesticide during spaghetti processing, particularly in the drying stage at 40 °C for 20 h. For other pesticides, carryover percentages from semolina to spaghetti were found to be relatively higher as compared to malathion.

Fenitrothion residues transferred into the spaghetti samples to a higher extent during spaghetti processing. Although fenitrothion is not as persistent as pirimiphos methyl in wheat during storage, carryover percentages of fenitrothion from semolina to spaghetti were generally higher than that of pirimiphos methyl. This might be due to their differences in their application methods and physicochemical properties. Direct application of pesticides on grain is generally expected to result in higher residue levels (Hassall, 1990).

Chlorpyrifos methyl residues were carried over from wheat to semolina and spaghetti samples less than pirimiphos methyl residues. Pirimiphos methyl undergoes comparatively less substantial loss during milling and spaghetti processing due to its physicochemical properties. The highest residues remained in spaghetti samples were of pirimiphos methyl, which has the lowest vapour pressure (2 mPa at 20 °C) among the insecticides studied.

4. Conclusion

In this study, the approved doses of insecticides for stored grain were applied on a small-scale storage vessel. However, the residue levels of insecticides in wheat exceeded the MRLs established by Codex Alimentarius Commission for wheat (Anonymous, 2003). The storage period was generally not effective enough to reduce the residues in wheat to the levels at or below the MRLs of the insecticides. Malathion residue levels were below the MRL (8 mg kg^{-1}) in wheat after 90 days of storage and for fenitrothion (5 mg kg^{-1}) after 60 days of storage. During the whole storage period (150 days) chlorpyrifos methyl and pirimiphos methyl did not decline to the MRL levels of 3 mg kg^{-1} and 5 mg kg^{-1}, respectively (Table 2).

There are no official limits for pesticide residues in semolina and spaghetti. For white flour, the MRLs of malathion, fenitrothion, chlorpyrifos methyl and pirimiphos methyl are 2 mg kg^{-1}, 0.2 mg kg^{-1}, 2 mg kg^{-1} and 2 mg kg^{-1}, respectively (Anonymous, 2003). If the MRLs for white flour were considered, the residue levels in time-zero samples of semolina exceeded the limits excessively. This might be due to application of pesticides on wheat at pilot scale. Dissipation behavior of pesticides at the commercial scale may follow a somewhat different trend then the one observed in the present study. The residues in the semolina samples produced from the stored wheat at 60 or 90 days of storage were below the limits (Tables 3 and 4). The residue level in semolina could be higher than in white flour since the semolina might contain more bran particles than white flour.

In general, the spaghetti processing significantly reduced the concentrations of the insecticides. If the MRLs for the white flour were applied, the residue levels of the pesticides in spaghetti samples were below the limits except fenitrothion. The remaining fenithrothion in spaghetti could endanger consumer health. Utilization of fenitrothion was authorised when the present study was initiated. The European Commission also decided to withdraw authorizations for plant protection products containing fenitrothion by 25 November 2007 (Anonymous, 2007).

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