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Residue levels of malathion and its metabolites and fenitrothion in post-harvest treated wheat during storage, milling and baking

Umran Uygun [a,*], Hamit Koksel [a], Ayhan Atli [b]

[a] Department of Food Engineering, Hacettepe University, 06532 Beytepe, Ankara, Turkey
[b] Department of Food Engineering, Harran University, Sanliurfa, Turkey

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Abstract

In order to investigate residue levels of malathion and its metabolites (malaoxon and isomalathion) and fenitrothion during storage, milling and baking, pesticide-free wheat was treated with the insecticides. Wheat was placed in a sealed plastic container and treated with dust malathion (2%). Fenitrothion emulsion (41.6% wettable powder) was applied onto the wall of a small-scale storage vessels. Residues were determined in wheat, bran, flour, white and bran bread at about one-month intervals during storage. The analysis of the residues was carried out by GC equipped with a NPD. The highest amounts of insecticides and metabolites were present in bran and the least in white bread. Reduction of malathion residues was about 95% in wheat through milling to flour and about 82% in flour through white bread baking. For fenitrothion, the residue levels were also lower in white breads than in bran breads. They generally did not exceed the maximum residue limits, except for the fenitrothion level of bran breads.

Keywords: Malathion; Malaoxon; Isomalathion; Fenitrothion; Wheat; Bran; Flour; Bread

1. Introduction

Malathion and fenitrothion are organophosphorus insecticides, widely used for controlling insects in stored grain. Although malathion is often applied as dust to stored commodities, fenitrothion is used only for treating the surfaces of storage vessels, since it is moderately toxic to mammals (LD_{50} 503 mg/kg). The LD_{50} value (LD_{50} 2100 mg/kg) for malathion can vary according to impurities. The technical grade malathion contains approximately 11 impurities. It is these impurities which scientists conclude are the main toxic ingredients in malathion. The major toxic component has been shown to be as isomalathion, present as a malathion-related impurity and increasing during the storage. Malaoxon, which is an active oxidation metabolite of malathion, is also far more toxic than the parent compound (Cremlyn, 1991; Hassall, 1990; WHO, 1997).

Studies on grain following post-harvest treatments with insecticides have generally shown that residues decline rather slowly. Degradation of residues of insecticides in stored grain is dependent on temperature, water activity, light, and the type of grains stored. In some studies, the residues of the insecticides have also been found to penetrate into the grains during storage and to accumulate with time (Desmarchelier, 1978; Holland, Hamilton, Ohlin, & Skidmore, 1994).

The purpose of this work was to study the fate and magnitude of malathion and fenitrothion in wheat under local conditions of storage, and to investigate reduction and distribution of residues in processed commodities prepared according to standard milling and baking practices. In practice, lack of detailed data on the general effects of processing require more research on pesticide residues in food.

* Corresponding author. Fax: +90 312 299 2123.
E-mail address: umran@hacettepe.edu.tr (U. Uygun).

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For this, wheat of known origin was treated with malathion and fenitrothion, separately. The effects of storage and processing on breakdown of the insecticides and the transformation products of malathion were examined in a variety of samples prepared after different periods of storage. Residues were determined using gas chromatography equipped with an nitrogen–phosphorus detector (NPD).

2. Materials and methods

2.1. Materials

Pesticide and metabolite standards, malathion, malaoxon, isomalathion and fenitrothion were purchased from Promochem Ltd. (Germany). Commercial 2% dust malathion (Hektion 2 Dust) was purchased from Hektas° (Turkey) and 41.6% wettable fenitrothion (Durakil) was obtained from Antec International (UK). The wheat sample (cv. Gun-91) of 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. Sample treatment with malathion

Wheat was treated with malathion (2% dust) according to good application practice which is regulated by the Registered Agrochemicals for Pest Management Warehouse Pest Recommendations of the Turkish Ministry of Agriculture. For this, a small model was chosen for grain storage under laboratory conditions. Wheat (10 kg) was placed in a sealed plastic cylindrical container (50 l) and treated with malathion at the rate of 500 g of dust malathion per tonne of dry grain. Two thirds of the container were empty in order to allow plenty of air in the headspace. The grain was tumbled, end over end, for 1 h and then a time-zero sample was taken for the analysis. The rest of the samples were stored at ambient temperature (20 ± 5 °C) for four months. The grain was analysed at about one-month intervals during storage.

2.3. Sample treatment with fenitrothion

A concrete store (50 × 50 × 50 cm) with a metal cover, which is a small-scale model of commercial storage, was built for laboratory experiments. Fenitrothion emulsion was prepared from 41.6% wettable powder at the dose recommended by the manufacturers' registered label and painted on the surfaces of the store. The application rate was 250 g/10 l for every 200 m^2 of surface area. The insecticide was not sprayed onto the surfaces of the store in order to avoid contamination of the atmosphere of the working area. After standing for a while, to evaporate the excess of solvent, the wheat (10 kg) was placed into it, with mixing, to provide a composite time-zero sample for the analysis. After treatment, the grain was sampled at about one-month intervals during storage at about 30 ± 5 °C.

2.4. Processing

The wheat samples (1 kg) were milled after each storage period in a Buhler laboratory mill to obtain bran consisting mainly of the outer layers of grain and straight-grade flour containing mainly endosperm. Duplicate samples from whole ground grain and milled fractions were used for analysis. The straight-dough bread-making method No. 10–11 (AACC, 2000) was used, with some modifications, to evaluate the baking potential of wheat flours. The baking formula, based on flour weight, was as follows: flour (100 g), fresh compressed yeast (2%), salt (1.5%). In bran breads, bran has been substituted for a portion of wheat flour at the level of 10%. The first and second fermentations were 30 min and the final proof was 55 min. The baking was performed at 230 °C for 25 min. Duplicate samples of bread were used for analysis.

2.5. Extraction

Fifty grammes of wheat and bran samples were ground in a coffee grinder (Moulinex Coffee Mill Model 980). Ground wheat and bran samples, as well as bread and flour samples (50 g), were homogenized with ethyl acetate (50 ml) and anhydrous sodium sulphate (5 g) in a high-speed blender for 2 min. The homogenate was filtered and the residues were extracted twice with ethyl acetate (2 × 50 ml). The combined extracts were concentrated to ca. 20 ml in vacuo at 40 °C using a rotary evaporator. Clean-up by gel permeation chromatography was performed, as previously described (Uygun, 1997).

2.6. Gas chromatography

Gas chromatography was performed using a HP5890 gas chromatograph equipped with a nitrogen phosphorus detector and capillary column (Alltech AT-1, 30 m × 0.32 mm ID, 0.25 μm film thickness) using nitrogen carrier gas at a flow rate of 2 ml min^{-1}. The oven temperature programme was: initial temperature isothermal, at 150 °C, for 5 min, then from 150 to 250 °C at 10 °C min^{-1}, then held for 15 min at 250 °C. Injector and detector temperatures were 250 °C. Quantification of the pesticides was performed by comparing the peak areas to that of a calibration curve of standards. Correlation coefficients were found to be above 0.98 in all cases, indicating a good linearity. Detection limits were calculated by using a signal-to-noise ratio of 3 and determined as 0.001, 0.001, 0.003

and 0.02 mg/kg for malathion, fenitrothion, isomalathion and malaoxon, respectively.

2.7. Statistical analysis

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

3. Results and discussion

3.1. Degradation of malathion in wheat, bran, flour, white and bran breads

The effect of storage on breakdown of malathion was examined at intervals of about one month during four months of storage at ambient temperature (20 ± 5 °C) in a closed container. The compounds were identified from their chromatogram and confirmed by comparison with authentic standards. ANOVA results showed that malathion levels of wheat as well as its milling and baking products, decreased significantly during storage (Table 1). Malathion residues in wheat were dissipated rapidly during the first month of storage and, thereafter, at a slower rate until the third month. Prior to storage (time zero sample), initial concentration of malathion on wheat was 8.89 mg kg^{-1} on dry basis. The original applied dose was at the rate of 500 g of the dust malathion (2%) per tonne of dry grains. A total of 89% of the applied dose was recovered at 1 h. The residue amounts on wheat observed throughout the storage period reflected the minimal losses that occurred in a closed system for the laboratory experiment. The residue was also found to be relatively high in the milled products of wheat treated with malathion, since it was not washed prior to milling.

Most residues were present in the outer portions of the grain, and consequently the determined residue levels in bran were higher than in wheat (Table 1). Although wheat contained of high amount of malathion residues, the residue concentrations greatly reduced during the milling of wheat and baking of bread. Reduction of malathion residues was about 95% in wheat through milling to flour. When flour was converted into white bread, degradation of the remaining residues was about 80%. In bran bread, residue levels were found to be 3–4 times higher than in white bread. In flour and white bread, residue concentrations increased slightly during the first month of storage. This indicated that a proportion of the lipophilic insecticide migrated through the bran and germ, which contained high levels of triglycerides (Fig. 1). In this study the approved dose of malathion was used and the residue levels did not exceed the maximum residue limit (1 ppm) in breads (FAO, 1989).

Table 1
Residue levels of malathion in stored wheat and its products at various times during storage (mg kg^{-1})

Time (days)	Wheat	Bran	Flour	White bread	Bran bread
0	8.89a	9.85a	0.639ab	0.119ab	0.454a
27	7.07b	9.02ab	0.709a	0.129a	0.386ab
41	7.04b	8.16bc	0.570bc	0.098bc	0.306bc
56	6.26bc	7.12c	0.529cd	0.089bc	0.299bc
94	5.60c	5.46d	0.479d	0.070cd	0.209c
127	4.28d	4.80d	0.221e	0.051d	0.177c

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 ($p < 0.05$).

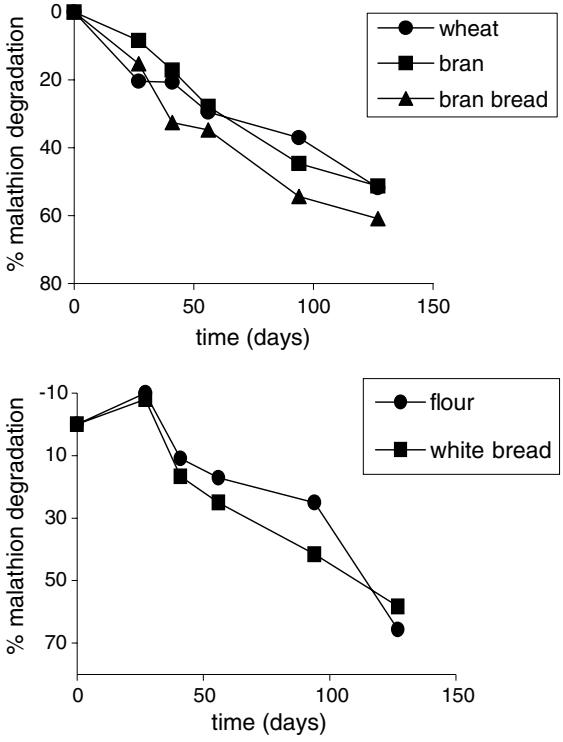

Fig. 1. Percent residue degradation of malathion in wheat, bran, flour and breads.

3.2. Isomalathion and malaoxon residues in samples

Table 2 shows that the isomalathion content in wheat increased significantly with storage time ($p < 0.05$). The isomalathion residue in bran and flour increased significantly ($p < 0.05$) during the first month of storage and remained relatively constant after the third month. An appreciable decline was observed in residues during baking. In preparation of bran bread, approximately 98% of the isomalathion initially present in wheat was lost.

It has been known that oxidizing agents cause malathion to transform into the corresponding P=O analogue, malaoxon, which has a high toxicity.

Table 2
Residue levels of isomalathion in stored wheat and its products at various times during storage (mg kg^{-1})

Time (days)	Wheat	Bran	Flour	White bread	Bran bread
0	0.315e	0.133c	0.016c	0.005c	ND
27	0.360d	0.462a	0.042a	0.006b	0.012a
41	0.385c	0.344ab	0.023bc	0.005c	0.008bc
56	0.429b	0.268bc	0.019c	ND	0.007c
94	0.416b	0.303bc	0.035ab	0.009a	0.010ab
127	0.566a	0.320ab	0.033ab	0.009a	0.011a

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 ($p < 0.05$).
ND, not detectable.

Table 3
Residue levels of malaoxon in stored wheat and its products at various times during storage (mg kg^{-1})

Time (days)	Wheat	Bran	Flour	White bread	Bran bread
0	0.037cd	0.027e	ND	ND	ND
27	0.059ab	0.083a	ND	ND	ND
41	0.031d	0.045de	ND	ND	ND
56	0.033d	0.059cd	ND	ND	ND
94	0.070a	0.070ab	ND	ND	ND
127	0.057ab	0.058cd	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 ($p < 0.05$).
ND, not detectable.

However, the nature of malaoxon breakdown is as yet unknown. The initial malaoxon concentration was low in wheat and bran, as expected. However, it increased significantly ($p < 0.05$) during the first month of storage. Although the levels dropped after the first month, the residue levels generally followed an increasing trend during the last three months of storage (Table 3). An obvious decrease occurred in the last month. Generally, high levels of malaoxon were observed in the wheat and bran samples during storage due to the closed storage system. The residues accumulated in grain were removed by milling and degraded by the baking process. Fortunately, no malaoxon was observed in the flour and bread samples.

3.3. Degradation of fenitrothion in wheat, bran, flour, white and bran bread

The initial residue of fenitrothion in wheat was high in accordance with storage treatment (Table 4). This amount declined rapidly during the first month. In wheat, the rate of degradation during the second month was slower than that of the previous month. Statistical results showed that fenitrothion levels of milling and baking products generally decreased during storage of wheat ($p < 0.05$).

Table 4
Residue levels of fenitrothion in stored wheat and its products at various times during storage (mg kg^{-1})

Time (days)	Wheat	Bran	Flour	White bread	Bran bread
0	24.5a	34.3a	2.35a	0.275a	1.43a
26	18.1b	23.3b	2.17a	0.295a	0.765b
55	17.1b	19.5c	0.355b	0.140b	0.353c

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

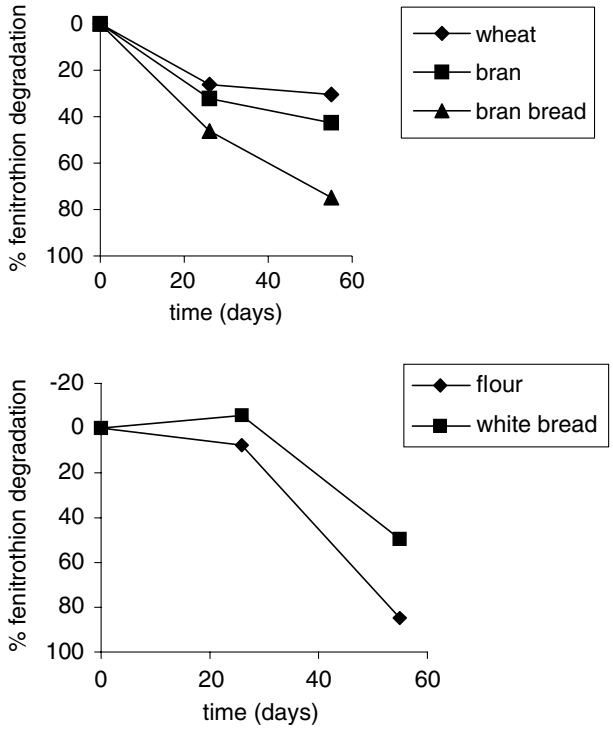

Fig. 2. Percent residue degradation of fenitrothion in wheat, bran, flour and breads.

The losses observed in the milling (wheat to flour) and baking (flour to bread) processes were about 90%. In general, the baking of bread affected the residue levels to an extent, as did milling. A large amount of applied fenitrothion accumulated in the bran fraction and a small amount in the flour. Fig. 2 shows that the fenitrothion in all samples gradually decreased with time. After the first month of storage, while the residues in wheat decreased significantly, the level of residue in flour and white bread remained relatively constant. This may be attributed to penetration of the insecticide from the seed coat into the endosperm with time. Although, in this study, the approved dose of fenitrothion was used, the residue level in bran bread exceeded the maximum residue limit (0.3 ppm) in breads (FAO, 1989).

4. Conclusions

It is note worthy that the rate of penetration of fenitrothion into wheat grain is relatively high in comparison with that of malathion into wheat at the end of the first month. This might be related to the more lipophilic character of fenitrothion than that of malathion. The octanol–water partition coefficients for fenitrothion and malathion are 3.43 and 2.75, respectively (Tomblin, 1994).

A significant role in determining the rate and extent of breakdown of the insecticide applied to grain was played by storage conditions and pesticide applications. In application of dusts or liquids, considerable amounts of the insecticides remain on the surface of wheat and some penetrate to internal parts of wheat. Consequently, residue levels in bran were found to be higher than in wheat. Fenitrothion, malathion and its degradation product, isomalathion, were found in stored wheat and in all of its products during the storage period. In this study, the approved doses of insecticides for stored grain were used. The residue levels were lower in white breads than in bran breads. They generally did not exceed the maximum residue limits (MRLs), except for the fenitrothion level of bran breads. MRLs for malathion and fenitrothion in breads are 1 and 0.3 ppm, respectively (FAO, 1989). MRLs are a basis for calculating human dietary consumption of pesticides, over-estimating actual intakes by one to three orders of magnitude (Holland et al., 1994). However, these do not reflect any information about metabolites. Since some of the metabolites could be more toxic than the parent compounds, concerns have been expressed about the lack of MRLs on metabolite residues in foods established by international authorities. This lack of knowledge causes difficulties in the evaluation of data on pesticide metabolite residues in food.

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