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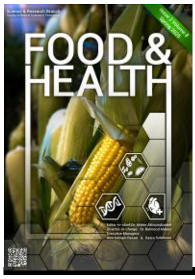

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Investigation of the effect of different processes on the pesticide residues of diazinon, pirimicarb, dimethoate and deltamethrin in wheat

Mahtab Janparvari[1], Leyla Alizadeh[*2]

[1] Department of Food Safety and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2] Department of Food Quality Control and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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ABSTRACT

Cereals, particularly wheat, are staple foods that provide essential nutrients and energy for the human diet. However, the extensive use of pesticides to protect wheat crops against pests raises serious concerns due to the persistence of their residues in food products, especially bread as a high-consumption commodity. This study aimed to evaluate the impact of different processing stages, including milling, dough preparation, and bread baking, on the residues of diazinon, pirimicarb, dimethoate, and deltamethrin in wheat. Wheat samples were collected from a flour factory in Tehran, Iran, and subjected to standard bread-making processes. Residue extraction was performed using the QuEChERS method, followed by quantification with gas chromatography–mass spectrometry (GC-MS). The initial concentrations of diazinon, pirimicarb, dimethoate, and deltamethrin in wheat were 0.862, 0.194, 0.703, and 3.211 mg/kg, respectively. Processing significantly reduced pesticide levels ($p < 0.05$), with overall reductions of 94.55% (diazinon), 90.21% (pirimicarb), 92.32% (dimethoate), and 63.78% (deltamethrin). All processing factors were <1, indicating effective dissipation across the stages, with the greatest reduction observed in diazinon and the lowest in deltamethrin. These findings demonstrate that bread-making substantially decreases pesticide residues, thereby reducing potential dietary exposure risks. Nonetheless, the relatively lower reduction of deltamethrin highlights the need for stricter monitoring and improved pre-harvest management practices to ensure consumer safety.

1. Introduction

Wheat (Triticum aestivum L.) is one of the most important staple crops worldwide, providing a major proportion of calories, proteins, vitamins, and minerals in the human diet (1). Because wheat-based products such as bread are consumed daily in large quantities, even low levels of pesticide residues may contribute significantly to chronic dietary exposure (2,3). Thus, monitoring pesticide residues in wheat and its derived products is essential for ensuring food safety and public health. Pesticides are widely used to protect wheat crops from pre- and post-harvest pests and to maintain yield and quality. However, residues of organophosphates (e.g., diazinon, dimethoate), carbamates (e.g., pirimicarb), and pyrethroids (e.g., deltamethrin) may persist in harvested grains and survive subsequent processing steps (4,5). Their dissipation during processing is influenced by compound-specific physicochemical properties (e.g., thermal stability, solubility, lipophilicity) as well as by matrix composition and processing conditions (6,7). Food processing operations including milling, dough fermentation, and baking may reduce pesticide residues through degradation, volatilization, redistribution, or microbial transformation (8,9). Milling often decreases residue levels in

* Corresponding Author: Leyla Alizadeh
Email: Lm.alizadeh2014@gmail.com

refined flour because pesticides concentrate in bran layers (8). However, the extent of residue reduction is highly variable across compounds: while organophosphates may degrade extensively during thermal processing, pyrethroids such as deltamethrin are more stable and may persist even after baking (10,11). While previous studies have examined the fate of some pesticide residues in various food commodities, comprehensive data mapping the dissipation dynamics of multi-class pesticides (specifically diazinon, pirimicarb, dimethoate, and deltamethrin) across all integrated stages of industrial wheat processing (milling, kneading/fermentation, and baking) is currently lacking. This research is therefore crucial for assessing consumer exposure risk and establishing effective control points within the high-volume wheat supply chain. This study aimed to quantitatively investigate the residual concentrations of diazinon, pirimicarb, dimethoate, and deltamethrin in wheat, flour, dough, and the final baked bread product, assessing the reduction efficiency of each processing step and calculating the corresponding Processing Factor (PF).

2. Materials and Methods

2.1. Sample Collection

This investigation was an applied intervention research project focused on studying the effect of various processes, specifically milling, kneading, and baking, on the remaining residues of four common pesticides in wheat bread. Wheat samples (Triticum aestivum L.) were obtained from a flour factory located in Tehran, Iran. Approximately 5 kg of representative grain samples were collected and were subjected to treatment with the four aforementioned pesticides and stored at 4°C until analysis. For quality control, samples were excluded if they contained foreign grains (such as rye or other seeds), coarse impurities, sand, grit, or if they showed signs of pest damage, such as mold or pest infestation.

2.2. Processing Procedures

Wheat samples were processed into bread through four main steps: milling, dough preparation, fermentation, and baking. Milling was performed with a laboratory mill, dough was prepared with yeast and salt, fermentation lasted 60 min at 30 °C and 85% RH, and baking was performed at 250 °C for 20 min.

2.3. Chemicals and Reagents

Analytical standards of diazinon, pirimicarb, dimethoate, and deltamethrin (≥98% purity) were purchased from Dr. Ehrenstorfer GmbH. Acetonitrile (HPLC grade), $MgSO_4$, NaCl, and PSA were obtained from Merck.

Extraction of Pesticide Residues

The QuEChERS method (12,13) was used. Ten grams of sample was extracted with acetonitrile, salts were added, followed by dispersive solid-phase extraction (SPE) cleanup, and prepared extracts and standard solutions were injected into the GC-MS/MS instrument for quantification.

2.5. GC–MS Analysis

Analyses were conducted using an Agilent 7890A GC coupled to a 5975C MS detector. Separation was on an HP-5MS column with helium as carrier gas, with a purity of 99.999%. The temperature program was as follows:

1. Initial temperature: 50°C, held for 2 minutes.
2. Ramp 1: Increase rate of 25°C/min up to 140°C, held for 2 minutes.
3. Ramp 2: Increase rate of 25°C/min up to 290°C, held for 5 minutes.

2.6. Method Validation

2.6.1. Accuracy, Precision, and Recovery

Method validation was carried out by spiking pesticide-free wheat samples with the four target pesticides at concentrations of 0.1, 0.5, 1.0, and 2.0 mg/kg. The spiked samples were processed through the entire extraction procedure. The recovery percentage was calculated using the Equation 2.1. The test was repeated five times over three consecutive days to ensure precision and accuracy.

Equation 2.1

$$\text{Recovery Percentage } = \left(\frac{\text{Concentration obtained fromsample}}{spiked\ concentration}\right) \times 100$$

2.6.2 LOD and LOQ

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) were determined using the Equation 2.2, where SD is the standard deviation obtained from 10 injections of the blank sample, and B is the slope of the calibration curve.

Equation 2.2

$$\text{LOQ}=\frac{10SD}{\boldsymbol{B}} \qquad \text{LOD}=\frac{3SD}{B}$$

The final pesticide concentration in the samples was calculated taking into account the determined recovery percentage. The reduction percentage after processing was calculated using the Equation 2.3:

Equation 2.3

$$\text{Reduction Percentage} = \left(\frac{\text{Initial Concentration of Pesticide} - \text{Concentration of Pesticide after processing}}{\text{Initial Concentration of Pesticide}}\right) \times 100$$

2.3.1. Calibration Curve

The calibration curve was generated by plotting the ratio of the standard pesticide peak area to the Internal Standard peak area (Y-axis) against the pesticide concentrations (X-axis). The resulting equation of the line and the coefficient of determination (R2) were calculated.

2.3.2. Statistical Analysis

Data analysis was conducted using SPSS software, version 20. The normality of the data distribution was assessed using the Kolmogorov-Smirnov (K-S) test. For comparison between different processing groups, ANOVA (Analysis of Variance) was used, followed by the Duncan's post-hoc test. Statistical significance was established at $P < 0.05$.

3. Results and Discussion

3.1. Analytical Method Validation and Initial Concentrations

The analytical method for quantifying pesticides residues utilized Gas Chromatography-Mass Spectrometry (GC-MS). Method linearity was confirmed by determination coefficients (R2) ranging from 0.9585 for Pirimicarb to 0.9998 for Diazinon (Table 3.1). These strong correlations confirm a reliable relationship between the concentration and the signal response within the tested range. The (LOD) and (LOQ) are essential for verifying the method's capability to detect and measure residues at trace levels. The method showed high sensitivity for all four compounds in the wheat sample (Table 3.1). These low quantification limits confirm that the chosen methodology (QuEChERS extraction followed by GC-MS) is sufficiently sensitive to accurately determine the low residual concentrations remaining after various processing stages. This is crucial for evaluating the effectiveness of the bread-making process in reducing consumer exposure, as the final concentrations are expected to be far below the initial levels.

Table 3.1. Calibration Curve Equation, Determination Coefficient, Limit of Detection (LOD), and Limit of Quantification (LOQ) in Wheat Sample

Pesticide	Determination Coefficient (R^2)	Calibration Curve Equation	LOD (mg/kg)	LOQ (mg/kg)
Diazinon	0.9998	y = 4612.8 x + 9503.3	0.002	0.005
Pirimicarb	0.9585	y = 0.1561 x + 0.9115	0.001	0.004
Dimethoate	0.9752	y = 0.1058 x + 0.677	0.002	0.006
Deltamethrin	0.9980	y = 1475.3 x + 3014.5	0.003	0.006

3.2. Assessment of Accuracy and Precision

The accuracy and precision of the analytical method are paramount for ensuring the reliability of residue data in complex food matrices like wheat. Accuracy was established through recovery tests across four spiking levels of certified standards of Diazinon, Pirimicarb, Dimethoate, and Deltamethrin ((0.01, 0.05, 0.5, and 1.0 mg/kg) to blank wheat sample. The overall mean recovery percentages for Diazinon, Pirimicarb, Dimethoate, and Deltamethrin were 96.93%, 92.85%, 107.01%, and 98.40%, respectively (Table 3.2). These recovery results confirm the successful implementation of the analytical method. These results fall within the acceptable criteria (64% to 140%) established for pesticide residue analysis (14). Furthermore, the low standard deviation values observed across all spiking concentrations indicate excellent precision and repeatability of the extraction and quantification process over the duration of the validation study.

Table 3.2. Mean ± Standard Deviation of Pesticides Recovery at Spiking Levels of 0.01, 0.05, 0.5, and 1.0 (mg/kg) Added to Wheat Sample

	Spiked Concentration (mg/kg)				
Pesticides	0.01	0.05	0.5	1.0	Overall Mean
Diazinon	103.15 ± 0.8	93.67 ± 1.02	95.32 ± 0.87	95.58 ± 1.02	96.93
Pirimicarb	102.62 ± 1.007	90.17 ± 0.92	92.91 ± 1.09	86.16 ± 0.98	92.85
Dimethoate	101.08 ± 1.004	96.28 ± 1.011	108.22 ± 1.008	114.45 ± 1.05	107.01
Deltamethrin	98.39 ± 0.95	101.79 ± 1.003	91.54 ± 0.997	101.89 ± 1.004	98.40

3.3. Reduction of Pesticide Residues During Processing

Initial residual concentrations in the raw wheat samples (pre-processing) were measured: 3.211 mg/kg for Deltamethrin (highest), 0.862 mg/kg for Diazinon, 0.703 mg/kg for Dimethoate, and 0.194 mg/kg for Pirimicarb (Table 3.3). The conversion of raw wheat into bread involved three key processes, milling, kneading (fermentation), and baking, all of which resulted in a statistically significant reduction ($P < 0.05$) in the concentration of all four pesticides at every stage. The Processing Factor (PF), calculated as the ratio of the residue concentration in the processed product to the raw material, was less than one for all compounds at every stage, confirming a net decrease in residue load throughout the bread-making process. The overall percentage reduction achieved from raw wheat to final bread product ranged from 63.78% to 94.55% (Table 3.3).

Table 3.3. Mean Concentration, Processing Factor, and Reduction Percentage of pesticides Residues During Different Stages of Bread Preparation

Pesticide	Process	Mean Concentration ± SD (mg/kg)	% Reduction Compared to Wheat	Process Factor
Diazinon	Wheat	0.862 ± 0.03^{a}	–	–
	Flour	0.273 ± 0.04^{b}	68.33	0.32
Diazinon	dough	0.130 ± 0.02^{c}	84.92	0.15
	Bread	0.047 ± 0.03^{d}	94.55	0.05

Table 3.3 (continued). Mean Concentration, Processing Factor, and Reduction Percentage of pesticides Residues During Different Stages of Bread Preparation

Pesticide	Process	Mean Concentration ± SD (mg/kg)	% Reduction Compared to Wheat	Process Factor
Pirimicarb	Wheat	0.194 ± 0.03 [a]	–	–
	Flour	0.103 ± 0.03 [b]	46.91	0.53
	dough	0.039 ± 0.03 [c]	79.90	0.20
	Bread	0.019 ± 0.03 [d]	90.21	0.10
Dimethoate	Wheat	0.703 ± 0.03 [a]	–	–
	Flour	0.150 ± 0.03 [b]	78.66	0.21
	dough	0.084 ± 0.04 [c]	88.05	0.12
	Bread	0.054 ± 0.03 [d]	92.32	0.08
Deltamethrin	Wheat	3.211 ± 0.08 [a]	–	–
	Flour	2.905 ± 0.04 [b]	9.53	0.90
	dough	2.041 ± 0.07 [c]	36.44	0.64
	Bread	1.163 ± 0.06 [d]	63.78	0.36

Different letters indicate a significant difference in each group ($P < 0.05$)

3.3.1 Effect of Milling

Milling is a physical separation process, crucial for separating the nutrient-rich bran (exosperm) and germ from the endosperm. Pesticide residues, particularly those with strong lipophilic characteristics, tend to accumulate and concentrate superficially on the grain kernel or specifically within the outer layers, known as the bran. The bran layer contains high levels of triglycerides and lipids, which enhances the accumulation and retention of fat-soluble pesticides. The milling process removes the bran and husk layers from the inner endosperm to produce refined flour, thereby physically separating the bulk of the pesticide contamination. Previous studies on various grains confirm that residue levels in the separated bran are typically 2 to 6 times higher than those found in the raw wheat (15,16). The high efficacy of milling was observed particularly for the organophosphates: Dimethoate and Diazinon. These compounds showed substantial reduction percentages (78.66% and 68.33%, respectively) during the conversion of wheat to flour. This finding strongly suggests that the majority of the organophosphate residues were located externally or superficially on the wheat kernel, making them highly susceptible to removal via physical separation during milling. This is consistent with literature findings regarding other organophosphates like Malathion and Fenitrothion, which have been shown to experience high rates of removal during the milling (17,18). Conversely, Deltamethrin, a pyrethroid, exhibited the lowest reduction during milling, at only 9.53%. This lower rate of reduction, despite the physical removal of the bran, indicates that Deltamethrin may possess characteristics, such as greater chemical stability or deeper penetration into the endosperm of the wheat kernel, that reduce its susceptibility to purely mechanical removal (19-21). This observation aligns with other studies noting that compounds exhibiting high thermal stability and persistence, often demonstrate lower initial reductions during physical processing stages (22). However, it is crucial to acknowledge a methodological limitation: the samples used in this study were spiked (artificially treated post-harvest) rather than containing residues that were naturally field-incurred. While this approach confirms the potential for physical removal, the superficial location of spiked residues may lead to an overestimation of the milling efficiency when compared to residues that accumulate naturally over the growing season and may penetrate deeper into the endosperm of the wheat kernel. Therefore, the generalizability of these particularly high reduction percentages to field-contaminated wheat must be considered with caution.

3.3.2 Effect of Kneading and Fermentation

The kneading and fermentation stage, mediated by yeast (Saccharomyces cerevisiae), represents a simultaneous opportunity for biological and chemical reduction. This process resulted in notable further reduction, achieving cumulative reductions from raw wheat of 88.05% for Dimethoate and 84.92% for Diazinon. The reduction achieved during fermentation is primarily mediated by two integrated mechanisms: microbial/enzymatic degradation and chemical hydrolysis (23,24). Yeast is known to degrade various pesticides, including organophosphates. Furthermore, fermentation causes the dough pH to drop (typically 5–6). This acidic environment promotes chemical hydrolysis, especially for compounds like Diazinon (pKa = 2.6) and Pirimicarb (pKa = 4.4), which are susceptible to chemical breakdown in this pH range, thereby explaining their continued strong reduction. Conversely, Deltamethrin, which lacks significant dissociation capability in this pH range, is less affected by hydrolysis during fermentation (25). The PF at the dough stage ranged from 0.12 (Dimethoate) to 0.64 (Deltamethrin).

3.3.3 Effect of Baking

Baking, the final high-temperature process, facilitates maximum overall residue removal primarily through thermal degradation, volatilization, and co-distillation (26,27). Diazinon demonstrated the highest total reduction (94.55%). As an organophosphate with a relatively high vapor pressure (approx. 11 MPa), Diazinon is considered highly volatile (25). This volatility allows for rapid vaporization and removal from the food matrix during the elevated oven temperatures. This high degradation aligns with previous thermal studies on organophosphates during cooking (28,29). In contrast, Deltamethrin showed the lowest overall reduction (63.78%). Pyrethroids are characteristically more thermally stable and less volatile than organophosphates. The persistence of Deltamethrin may also be linked to its chemical stability, low water solubility, and potential concentration in high-bran products (30). The final Processing Factors ranged from 0.05 for Diazinon to 0.36 for Deltamethrin.

Conclusion

The observed significant reductions (P< 0.05) demonstrate that the combined industrial and domestic processes of bread making, milling (physical removal), kneading/fermentation (biological/chemical degradation), and baking (thermal removal) act synergistically to drastically decrease consumer exposure to these four pesticide residues. This intervention study underscores the necessity of food processing as a critical step in reducing the health risks associated with pesticide contamination in widely consumed staple foods.

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