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Degradation of acephate and its metabolite methamidophos in rice during processing and storage

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ABSTRACT

The degradation of acephate and its metabolite methamidophos during different stages of commercial processing, homing processing, and storage was assessed. Residues were determined by a simple gas-chromatographic method using a flame photometry detector. Acephate and methamidophos mostly remained in rice hull fractions, and hulling significantly reduced acephate and methamidophos in rice. Commercial processing caused the loss of 86% of acephate and 35.9% of methamidophos from rough brown rice to polished rice, whereas home processing caused the loss of 83.9% of acephate and 70% of methamidophos from polished rice to cooked rice. Washing for 5, 15, and 30 min (with tap water, 0.9% NaCl, and 0.1% Na_2CO_3) caused an average loss in the range of 9.8%–35.3% of acephate and 9.7%–45.2% of methamidophos. Extending washing time and adding a small amount of soda into the washing solution can efficiently eliminate acephate and methamidophos. The stability of acephate and methamidophos in polished rice was studied at different storage intervals, from 7 to 42 days at ambient temperatures (25 °C). Methamidophos was found to be more persistent than acephate.

1. Introduction

Rice is the staple food for most Asians, and its production in China accounts for nearly one-third of the world's supply (Maclean, Dawe, Hardy, & Hettel, 2002, chapter 5). Rice is generally cultivated in areas with a warm and humid environment, which attracts a proliferation of insects. Of all possible pest control measures, majority of farmers prefer chemical control methods due to its immediate results. Both acephate and methamidophosare popular products and widely used to protect rice from pests. Meanwhile, acephate can be gradually metabolized to methamidophos. Acephate and methamidophos can cause acute toxic effects by inhibiting acetylcholinesterase (Sultatos, 1994) and may be hazardous to human health (Temerowski & van der Staay, 2005). Hence, monitoring the behavior of acephate and its metabolite residues in rice is important.

Commercial and home processing techniques have been used to transform paddy rice into rice products for human or animal consumption, which may reduce the pesticide content in rice (Saka et al., 2008). Kaushik, Satya, and Naik (2009) reported the effect of parboiling rice exposed to different pesticides. They investigated the significant reduction of residues in parboiled paddy bran. Milling can also decrease the concentration of most pesticides, as these are usually found in bran (Holland, Hamilton, Ohlin, & Skidmore, 1994). The ratio of residue levels in processed products and their respective raw products is called the processing factor (PF) (BfR, 2010). The PFs assist in the dietary intake assessment of related pesticides in processed commodities (Amvrazi & Albanis, 2008; Christensen, Granby, & Rabølle, 2003). They are also used in recommending MRLs for processed products with an existing Codex commodity code, but only if the processing leads to an increase of the residue level (Gonzalez-Rodriguez, Rial-Otero, Cancho-Grande, Gonzalez-Barreiro, & Simal-Gandara, 2011). However, few studies have focused on setting precise values applicable for PFs for rice (Pareja, Fernández-Alba, Cesio, & Heinzen, 2011). Therefore, this study investigates the effect of commercial and home processing as well as the storage of rice on removing the residues of acephate and its metabolite methamidophos.

The objectives of this study were to: (1) investigate the fate of acephate and methamidophos residues in rice from field to the table after being used in paddy situations, (2) determine a more efficient washing method for removing pesticides from rice contaminated with pesticides, which may be used by individual households, and

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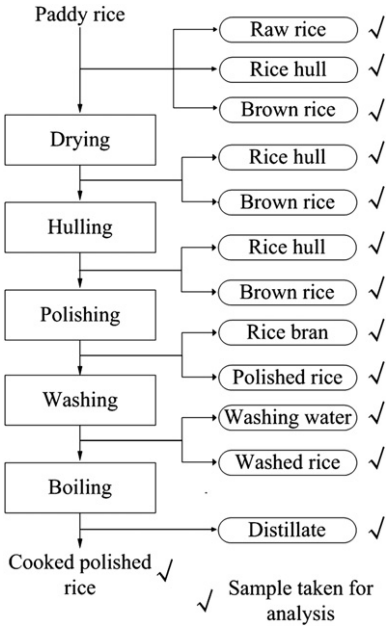

Fig. 1. The chart of traditional commercial processing and home processing of rice.

(3) study the stability of acephate and methamidophos residues in rice storage under ambient temperature conditions.

2. Materials and methods

2.1. Materials

The analytical standard acephate (99.9% purity) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and methamidophos (99% purity) was obtained from the National Institute of Metrology (Beijing, China). Commercial 75% acephate soluble powder (SP, containing 0.5% methamidophos) was obtained from Sanonda Co., Ltd. (Hubei, China). Acetone, acetonitrile, methanol, dichloromethane, anhydrous magnesium sulfate and sodium chloride were analytical grade and were obtained from Beijing Chemical and Reagent (Beijing, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA).

2.2. Field trials

The trials were conducted in experimental fields located in Beijing, China, which were investigated and were determined free of acephate and methamidophos prior to the experiment. The fields were divided into 30 m^2 blocks for the control. Acephate 75% SP was applied at the fivefold of highest dosage recommended by the manufacturer of 5077.5 g active ingredient $hectare^{-1}$ to ensure sufficient pesticide primary deposit for the following processing and storage studies. The rice was sprayed twice at 37 days before harvest, with interval of 7 days. The samples of paddy rice were placed in polyethylene bags and were transported to the laboratory. All the subsamples were kept deep-frozen (−20 °C) until analysis.

2.3. Sample preparations

2.3.1. Rice from field to the table

In general, the processing procedures of rice include five stages, as shown in Fig. 1.

Process 1. Harvest. A bulk sample of rough rice (50 kg) was collected from the field, with 2 kg reserved for analysis as raw samples for field-incurred residues.

Process 2. Drying. The remaining 48 kg of rice was sun-dried for two days to remove excess moisture from the grains. The 2 kg rice sample was used for the pesticide residue analysis.

Process 3. Hulling and polishing. A total of 46 kg of rice was hulled using the shelling machine and the brown rice was polished. Brown rice, polished rice, rice hull, and rice bran were obtained from the machine and the concentrations of the pesticides were determined. About 80%–90% of the kernel hulls were removed during this process.

Process 4. Washing. The obtained polished rice (300 g) was washed with 500 mL of tap water by shaking thrice for 5 min.

Process 5. Boiling. The washed rice (250 g) obtained from Process 4 was cooked with 500 mL of tap water in a 1000 mL two-neck round-bottom flask and was allowed to stand for 20 min. The flask was connected to a condenser and steam distillate was collected in a 100 mL receiving flask while the rice was boiling. After 20 min of distillation, 435 g of cooked rice and 41 mL of distillate were obtained. The samples after commercial and home processing were homogenized for analysis.

2.3.2. Removal effect with different washing solutions and times

The 300 g of polished rice was washed with gentle rotation by hand for 5, 10, and 30 min in tap water, 0.9% NaCl solution, and 0.1% Na_2CO_3 solution, respectively. The two factors estimated were washing solution and washing time.

2.3.3. Storage stability

The polished rice obtained from Process 3 was stored under ambient conditions (25 °C) for six weeks. Dissipation rates were calculated based on the residues of acephate and methamidophos in treated rice at weekly intervals during the storage period.

2.4. Extraction and purification procedure

2.4.1. Extraction and purification for rice samples

Homogenized samples (10 g rice or 5 g rice husk/bran) were weighed into a 50 mL PTFE centrifuge tube; 4 mL pure water was added, and then shaked for 1 min, After that, 25 mL acetonitrile was added and the mixture was placed on a Geno/Grinder mechanical shaker (SPEX SamplePrep, USA) for 4 min at 1200 strokes min^{-1}. A

Table 1
Recoveries and RSDs of acephate and methamidophos in samples at different fortification levels ($n = 5$).

Insecticides	Fortification (mg kg^{-1})	Recoveries (%)									
		Rice	RSD	Cooked rice	RSD	Rice hull	RSD	Rice bran	RSD	Water	RSD
Acephate	1	77.4	3.4	112	8.8	87.6	4.4	101	4.8	102	3.3
	0.2	86.6	5.2	92.0	7.8	97.3	5.6	89.4	5.4	82.7	8.1
	0.04	104	4.8	108	7.9	101	9.6	95.1	5.2	76.5	12.5
Methamidophos	0.5	83.2	4.1	92.4	5.3	82.2	5.4	79.5	3.8	106	1.7
	0.1	82.7	3.5	81.4	4.0	86.8	5.1	89.6	4.3	87.1	8.7
	0.02	96.7	11.7	99.6	4.2	94.5	7.1	88.3	3.8	86.2	8.6

Table 2
Amount of acephate and methamidophos residues (mg kg^{-1}) recovered from rice grains after various commercial processes (mean ± SD).

Name	Paddy rice			Drying		Hulling		polishing	
	Raw rice	Rice hull	Brown rice	Rice hull	Brown rice	Rice hull	Brown rice	Rice bran	Polished rice
Acephate	3.71 ± 0.04	11.7 ± 0.2	0.92 ± 0.04	9.42 ± 0.2	0.84 ± 0.01	8.92 ± 0.3	0.8 ± 0.02	1.26 ± 0.07	0.51 ± 0.02
Methamidophos	0.61 ± 0.04	0.88 ± 0.02	0.54 ± 0.02	0.8 ± 0.02	0.43 ± 0.02	0.75 ± 0.01	0.42 ± 0.02	0.6 ± 0.03	0.31 ± 0.02

total of 4 g of NaCl was added and vortexed at full speed for 4 min and centrifuged for 5 min at 2077×g. Then, 10 mL upper layer (acetonitrile) was collected in a round-bottom flask concentrated almost to dryness using a rotary evaporator (Yarong Machiners, China) at 35 °C. The residue was reconstituted in 2 mL of acetone and was filtered with 0.22 μm syringe filters for gas chromatography (GC) analysis.

2.4.2. Extraction and purification for water

10 mL samples were weighed into a 50 mL PTFE centrifuge tube; 30 mL acetonitrile was added and then vortexed at full speed for 1 min, After that, 4 g of NaCl and 5 g anhydrous $MgSO_4$ was added and vortexed at full speed for 1 min and then centrifuged for 5 min at 2077×g; 3 mL upper layer (acetonitrile) was collected in a centrifuge tube and evaporated to dryness with weak nitrogen stream without disturbing the surface of the solution. The residue was reconstituted in 1 mL of acetone and was filtered with 0.22 μm syringe filters for GC analysis.

2.5. GC analysis

The GC analysis was conducted with an Agilent GC 7890A (Agilent Technologies, USA) equipped with flame photometry detector (FPD). Separations were carried out using fused-silica capillary column DB-17 (methyl 50% phenyl polysiloxane; 30 m × 0.53 mm I.D., 1 μm film thickness) from J&W Scientific (Folsom, CA, USA). Ultra-pure quality nitrogen (purity 99.999%) was employed as carrier gas at a constant flow-rate of 10 mL min^{-1}, detector temperature was 250 °C, and inlet temperature was 220 °C, and the column temperature was programmed as follows: 120 °C for 1 min and ramped to 250 °C at 30 °C min^{-1}, and holding for 2 min, 1.0 μL was injected in the splitless mode.

Identification and quantification of the pesticides were based on their GC retention times and the peak areas were compared with those of a calibration curve of standards. Limits of detection (LOD) was calculated using a signal-to-noise ratio of 3 and determined as <10 of acephate and <5 ng g^{-1} of methamidophos in different rice and water samples.

2.6. Recovery assay

Recovery was performed on spiked blank rice samples with mutually independent replicates at three different concentration levels of acephate and methamidophos reported in Table 1, with five replicates for each level. Prior to the extraction step, the fortified samples were allowed to settle for 30 min and then processed according to the described procedure. The recoveries obtained with the extracted spiked samples were compared with that of the matrix-matched calibration solutions. Calibration curves in the matrix, which was prepared using this method, automatically corrected the data for analytical recovery.

2.7. Statistical analysis

Significant differences were accepted at $p < 0.05$*, $p < 0.01$**, or $p < 0.001$***. All values reported are means ± standard deviation (SD) of five replicates and statistics were calculated using SPSS base 11.0 software.

3. Results and discussion

3.1. Recoveries of acephate and methamidophos

The recovery of acephate was within 76.5%–112% and 79.5%–106% for methamidophos at various concentration levels (Table 1), which were within the range expected for residue analysis. The reproducibility of the recovery results, as indicated by relative standard deviations (RSDs), confirmed that the method is sufficiently reliable for pesticide analysis in this study.

3.2. Degradation of acephate and methamidophos in rice during commercial and home processing

The changes in concentrations of acephate and methamidophos during commercial and home processing are presented in Tables 2 and 3. Acephate was degraded into methamidophos, resulting in a considerable amount of methamidophos remaining in the rice. The residues analysis demonstrated that 9.8% of acephate was degraded into methamidophos in the paddy rice, similar to the results obtained in another study that only approximately 5%–10% of acephate degraded to methamidophos in crops (Downing, 2000). In paddy rice, 92.7% of acephate and 62% of methamidophos were found in the rice hull; hence, hulling is a major method of reducing pesticide residues on cereal grains. After drying in the sun, acephate and methamidophos were reduced by 8.7% and 20.4% in brown rice, respectively, which may be due to solar energy and heat. Chevron reported that acephate does not undergo photolysis

Table 3
Amount of acephate and methamidophos residues (mg kg^{-1}) recovered from rice samples after home processes (mean ± SD).

Treatment	Acephate	Methamidophos
Polished rice	0.51 ± 0.02	0.31 ± 0.02
Water from washing		
First time	0.084 ± 0.002	0.055 ± 0.002
Second time	0.024 ± 0.002	0.029 ± 0.0005
Third time	0.022 ± 0.001	0.014 ± 0.0007
Washed polished rice	0.33 ± 0.009	0.2 ± 0.008
Cooked polished rice	0.082 ± 0.006	0.093 ± 0.006
Distillate	0.078 ± 0.004	0.061 ± 0.002

Table 4
PFs in rice samples.

Processed commodity	PF	
	Acephate	Methamidophos
Rice sun-dried	0.23	0.70
Rice hulled	0.21	0.69
Rice polished	0.14	0.51
Rice bran	0.34	0.98
Polished rice washed	0.09	0.33
Polished rice cooked	0.02	0.10

Table 5
The *F*-values of intuitive analysis and variance analysis by washing treatment.

Pesticides	Factors							
	Time (average value)			*F* value	Washing solution (average value)			*F* value
	5 min	10 min	30 min		tap water	0.1% NaCl	0.9% Na_2CO_3	
Acephate	9.8%	15.7%	35.3%	1748[a]	17.6%	19.6%	23.5%	109[a]
Methamidophos	9.7%	19.4%	45.2%	3348[a]	22.3%	25.8%	29%	53[a]

[a] *F*-values with the level of significant.

(Chevron Chemical Company-Ortho Division, 1972), however, methamidophos will break down in the presence of sunlight (Athanasopoulos, Pappas, Kyriakidis, & Thanos, 2005).

Home processing for rice varies in different countries (Lee, Mourer, & Shibamoto, 1991). The most commonly used method is to wash rice with water several times, after which it is boiled with an appropriate amount of water. The results (Table 3) indicate that approximately 35.3% of acephate and 35.5% of methamidophos were removed by washing with water. Repeated washing decreased residues in rice grains. The residue concentrations of acephate and methamidophos were greatly reduced by about 75.2% and 53.5%, respectively, from the washed polished rice to the cooked polished rice during boiling (Table 3). Approximately 23.6% of acephate and 30.5% of methamidophos were removed from rice grains in water vapor (distillate in Table 3), possibly due to the acephate and methamidophos readily dissolving or evaporating in water. However, it is possible that this amount is retained in cooked rice because most rice grains are boiled in a sealed pot.

3.3. PFs of acephate and methamidophos with commercial and home processing

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluates food processing data on residue behavior where significant residues occur in plant or plant products which are processed into food (FAO/WHO, 2006). Based on the effect on residue levels and the disposition of the residues in the various processed products, processing factors (PFs) are calculated and considered by JMPR as follows:

$$\mathrm{PF} = \frac{\text{residue concentration in the processed commodity}\left(\text{mg kg}^{-1}\right)}{\text{residue concentration in the raw commodity}\left(\text{mg kg}^{-1}\right)}$$

The PF values of <1(=reduction factor) indicate a reduction of the residue in the processed commodity, whereas the values of >1(=concentration factor) indicate a concentration effect of the processing procedures (BfR, 2010).

The results (Table 4) suggested that PF was generally less than 1, indicating that the acephate and methamidophos were lower in the final products. However, the PFs for rice bran are markedly high, 0.34 for acephate and 0.98 for methamidophos, which might be related to their hydrophilic properties. Rice bran is the outer layer of brown rice and is usually used as a common ingredient in livestock, industrial, household, or food products (Ravindran, Ravindran, & Sivalogan, 1994). Therefore, if rice bran is contaminated with pesticides, then these practices should be discouraged.

3.4. Removal effect of acephate and methamidophos in polished rice with different washing style

The *F*-values of the analysis of variance (ANOVA) data in Table 5 indicate that the effect of time on removing acephate and methamidophos residues in polished rice was statistically the most significant ($F = 1748.03^{***}$). An average of 35.3% of acephate and 45.2% of methamidophos residues were eliminated from the samples during three aqueous washing solutions in 30 min. The results of the intuitive analysis (Table 5) indicated that the sodium carbonate solution was most effective, and eliminated 23.5% of acephate and 29% of methamidophos residues. These results are related to the physicochemical properties of pesticides such as stability. Acephate and methamidophos are more stable in acidic conditions and least stable in alkaline conditions (Downing, 2000; Gammon, Kellner, & Morris, 2005). Some pesticides can possess the ability to translocate into internal plant tissues under field conditions (Kin & Huat, 2010; Ling et al., 2011). Extending the washing time, which helps the washing solution penetrate the internal parts of rice, will improve the capability of the solution to eliminate residues.

3.5. Stability of acephate and methamidophos in rice during storage

The residues of acephate and methamidophos in polished rice were dissipated quickly during the first two weeks of storage (Fig. 2), and then which decreased at a slower rate until the end of storage. Acephate declined rapidly with 56.9% of the original residue after four weeks of storage. As such, the calculated half-life of acephate was 22.6 days with the dynamic degradation equation $C_t = 0.4648e^{-0.0229t}$. The methamidophos was found to be more persistent than acephate, with 39.9 days of half-life and a degradation equation $C_t = 0.2798e^{-0.0148t}$. Similar results were reported by Tao et al. (2010).

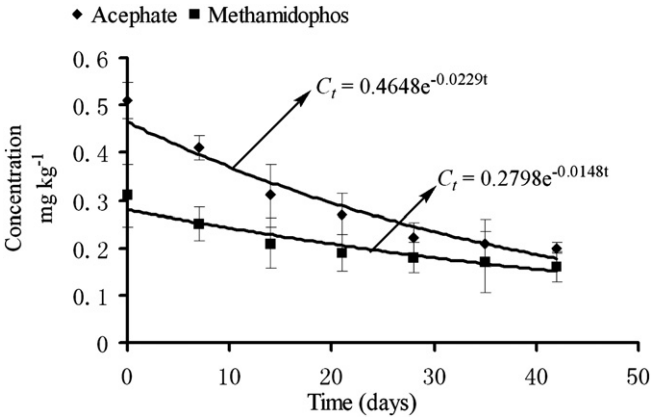

Fig. 2. Degradation of acephate and methamidophos in polished rice at ambient temperatures.

4. Conclusion

The concentration of acephate and methamidophos residues significantly decreased after commercial and home processing. Acephate and methamidophos were partitioned between husk, bran, and polished rice. However, during hulling, polishing, washing, and boiling, a large percentage of the acephate and methamidophos was removed. The results indicate that PFs of rice were generally less than 1. In households, extending the washing time and adding a small amount of home soda into the tap water can efficiently eliminate pesticide residue. During storage, methamidophos was more persistent than acephate and had comparatively lower substantial loss.

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