Effects of Househould Cleaning Methods on Carbendazim Residue in Apples

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Abstract Carbendazim, belonging to fungicides, was commonly used for controlling crop diseases, resulting in frequent residues in fruits and vegetables. Washing was the initial step of household fruit processing for cleaning and reducing pollutant contents. However, the effect of washing on the removal of carbendazim in apples was not systemically studied. Thus, in this study, an analytical method was firstly established for the determination of carbendazim with great accuracy and precision through quick, simple, cheap, effective and safe (QuEChERS) method and ultra-performance liquid chromatography-fluorescence detection (UPLC-FLD). Then, the influencing factors were systematically researched to unveil their effects on the carbendazim residue in apples. The results indicated that water temperature, washing products and washing method could obviously affect the carbendazim content in apples. In addition, the best cleaning scheme for removal of carbendazim was soaking apples for 5 min in a cleaning solution, which was prepared by dissolving 8 g of commercial fruit washing soup with 500 ml of water at 45℃. Under this condition, the removal rate of carbendazim in the washing step reached 73.91%. This study will be helpful for providing guidance for household cleaning methods for controlling pesticide residues.

Key words Carbendazim; Househould cleaning method; Pesticide residue; Pesticide removal

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Carbendazim (methyl benzimidazol-2-yl-carbamate), as one of the benzimidazole fungicides, was applied for control a broad spectrum of fungi pathogens via inhibition of mitotic microtubule formation and cell division[1]. Carbendazim possesses a long half-life with a hundred days in environment, thus it is one of the most frequent detected pesticides in fruits and vegetables[2]. Wang *et al.*[3] investigated carbendazim residue in China plant-based food from 2011 to 2020, and revealed a high detection frequency of carbendazim in fruits (26.4%) and high concentrations in vegetables (110 mg/kg), indicating widespread misuse of the fungicide. Hence, human was exposed to carbendazim through eating contaminated food and vegetables. It has been found out that carbendazim could bring about severe harm to human and other animals, via affecting reproductive, developmental, endocrine and hematological function[4-5].

In consideration of their danger to human health, increasing attention has been paid to reducing pesticide residues in food. Much food possessing treatment have been reported effective in the removal of pesticide residues, such as washing, peeling, canning, etc. Among them, thermal treatment was one of the most efficient methods for decreasing the concentration of pesticides. Kontou *et al.*[6] found that sterilization at 121 ℃ for 15 min could obviously decrease 68% of maneb in tomatoes. Similar result was reported that 40% of fenazaquin residue was removed in okra fruits by boiling[7].

Washing is the initial step in household food processing, in order to eliminate dirt and dust on food products[8]. Washing would contribute to lowering the concentration of pesticide residues to a certain degree, but the removal efficiency depended on varieties factors, including washing methods, the kinds of pesticides and food products. Rawn *et al.*[9] revealed washing could reduce 50% captan residue in apples. Similarly, 25% of azoxystrobin disappeared in grapes after washing[10]. In addition, applying cleaning products during washing would influence the reducing efficiency of pesticides. An experiment was conducted to study the effect of homemade and commercial washing method on reducing the thiabendazole and phosmet pesticides from the apple surface by Yang *et al.*[11]. The results showed that washing products and water temperature would increase the removal rate of pesticides, and one of the most effective methods was washing apple with sodium bicarbonate solution (10 mg/ml). Under this condition, the removal rate was 80% for thiabendazole after 12 min of washing time and 96% for phosmet after 15 min of washing time. The washing with water at 100 ℃ reduced 95% of quinalphos, 84% of diazinon and 100% of fenitrothion, while it with water reduced 34% of quinalphos, 28% of diazinon and 41% of fenitrothion. However, the effect of washing on the removal of carbendazim in apples was not systemically studied. In this study, the influencing factors were systematically investigated to unveil their effects on carbendazim residue in apples.

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Materials and Methods

Materials and reagents

Apples and two common types of fruit washing soup (named as A and B) were purchased from local market. Standard of carbendazim and sodium chloride (NaCl) were purchased from J&K Scientific Ltd. (Beijing, China). Baking soda with food grade was got from Qilu Biotechnology Co., Ltd. (Shandong, China). Acetonitrile and methanol with HPLC-grade were bought from Fisher Scientific (MA, New Jersey, USA). C18 adsorbent (45 μm) was provided from Bonna-Agela Technologies (Tianjin, China). Ultrapure water was obtained from a Milli-Q system by Merck Millipore (Merck KGaA, Darmstadt, Germany).

Standard solution preparation

The carbendazim standard stock solution of 100 mg/L was prepared by dissolving 100 mg of carbendazim standard in 1 L of methanol. Then, a series of working standard solutions were obtained from dilution of stock solution with methanol. All working standard solutions were stored in the dark at 4 ℃ for later UPLC-FLD analysis.

UPLC-FLD analysis

The Ultimate 3000™ HPLC system and a FLD-3000 fluorescence detector (Thermo Fisher Scientific, USA) with an Inertsil ODS-3 column (4.6 mm i.d. ×250 mm, 5 μm particle size) was applied for analyzing carbendazim. The mobile phase was composed of 70% acetonitrile and 30% water. The flow rate was 1 ml/min, and the injection volume was 10 μl. The excitation and emission wavelength of carbendazim were 285 and 315 nm, respectively.

Sample collection and preparation

Fresh apples were purchased from local market, and used as blank matrix. Carbendazim-contaminated apple samples were obtained by steeping blank sample into working standard solutions of 100 mg/L and standing for 2 h, and stirring once on every 10 min. After natural drying, the carbendazim-polluted apple samples were obtained and stored at 4 ℃ for later use. Apple samples were homogenized with a WBL2501B household blender (Midea Kitchen Electrics Manufacturing Company, Guangdong, China) before sample preparation process.

Sample preparation of homogenized apple samples was based on QuEChERS procedure. Briefly, 2 g of apple sample was weighed in a 15 ml centrifuge tube, and mixed with 10 ml of acetonitrile. After 5 min of vortexing, 1 g of NaCl was added, followed by 3 min of sonication. Then, the tube was centrifuged at 4 000 rpm for 3 min, and 1 ml of supernatant was transferred in another 2 ml centrifuge tube containing 50 mg of C18 absorbent. The centrifuge tube was capped and vortexed for 3 min, and centrifuged at 5 000 rpm for 1min. The supernatant was filtered and analyzed with UPLC-FLD.

Method validation

A series of working solutions at different levels (0.5, 1, 5, 10, 15, 25, 50 mg/L) were obtained by diluting stock solution of carbendazim standard with blank sample extracts, which were subsequently determined by UPLC-FLD method. The calibration curves were conducted according to the peak areas of carbendazim and concentrations of working solutions. Then, three concentrations of carbendazim (2.5, 5, 25 mg/L) were spiked in the blank apple samples for evaluating the accuracy and precision of the method, and five replicates were analyzed for every concentration.

Household cleaning processing on apple samples

Household cleaning was the initial step for fruits and vegetables, and many types of cleaning products were frequently applied for household fruit washing. Thus, the effect of cleaning products on the removal of carbendazim residue in apples was studied, including salt, baking soda, and two common types of fruit washing soup. In addition, the washing method, washing time and water temperature were also identified as influencing factors, and related experiments were conducted for the exploration of their effects on carbendazim residue in apples.

Results and Discussion

Method validation

The typical HPLC chromatogram of carbendazim with concentration of 10 mg/L in apple sample was shown in Fig. 1. On the basis of Fig. 1, the peak of carbendazim appeared at 3.588 min, without interference peaks. The calibration curve, recoveries and precision of carbendazim in apple sample are shown in Table 1. As shown, the calibration curve of carbendazim in apple samples was $y = 187\ 775x + 22\ 721$ with the linear correlation coefficient (R^2) as 0.999 9, which indicated that the relation between peak area and carbendazim concentration displayed good linearity. Then, the recoveries of analytes in apple samples spiked with three concentrations of carbendazim ranged from 74.1% to 120.9%. In addition, RSDs of five replicates for various groups were from 4.5% to 13.7%. Hence, the data of recoveries and RSDs elucidated that the analysis method was of great accuracy and precision.

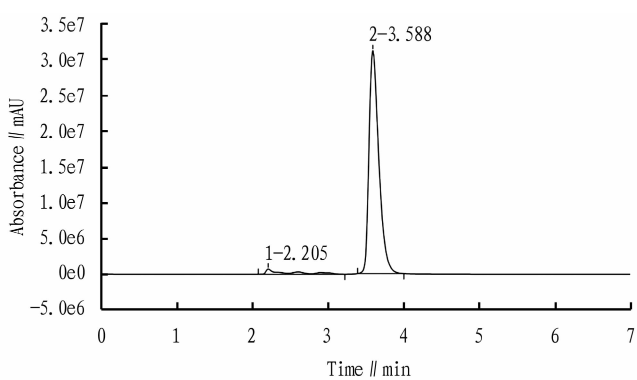

Fig. 1 Typical HPLC chromatogram of carbendazim with concentration of 10 mg/L in apple sample

Household cleaning processing on apple samples

Effect of cleaning products Four cleaning products were chosen for studying the effect on the removal rate of carbendazim, including baking soda, salt and two common commercial fruit washing soup. The results are shown in Fig. 2, which demonstrated that 17.69% -28.12% of carbendazim residue was reduced after washing with different kinds of washing products. Rather than salt, the commercial fruit washing soup and baking soda could obviously increase the removal rate of carbendazim, and A is the most effective cleaning product for decreasing the concentration of carbendazim in apple.

Table 1 Calibration curve, recoveries and precision of carbendazim in apple samples

Pesiticide	Calibration curve	R^2	Concentration of carbendazim // mg/L	Recovery // %	*RSD* // %
Carbendazim	$y = 187\ 775x + 22\ 721$	0.999 9	2.5	120.9	6.1
			5.0	74.1	4.5
			25.0	99.3	13.7

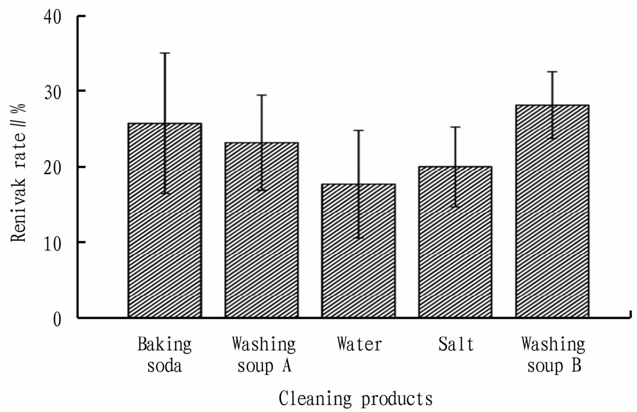

Fig. 2 Effects of cleaning products on the removal of carbendazim in apple

Effect of washing method Different washing methods were applied, such as soaking, rinsing, and soaking assisted with ultrasonic treatment. Thus, washing apples with different methods were conducted for studying the effect of washing method on the removal rate of carbendazim. The results in Fig. 3 showed that soaking was the best method to remove the residue among the three methods, which could lower carbendazim to 79.35% in apples.

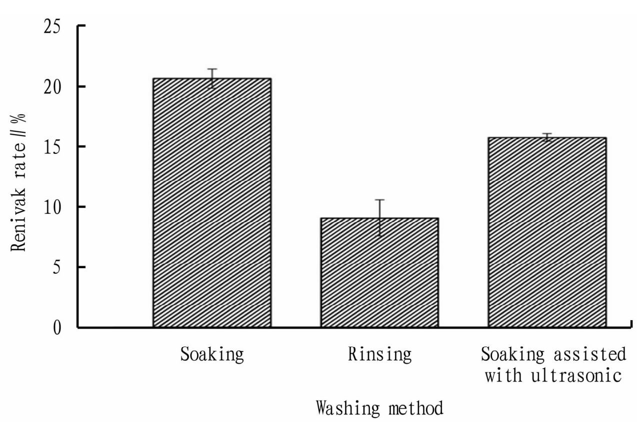

Fig. 3 Effect of washing method on the removal of carbendazim in apples

Effect of washing time Washing time is one of most significant factors which could affect the removal efficiency of pesticides, because enough time is essential for achieving the transfer of pesticides from food to water. Samples with different washing time ranging from 10 s to 5 min were analyzed. It could be seen from Fig. 4 that the removal rate of carbendazim sharply elevated with time increasing from 10 s to 30 s, and remained steady. The reason might be attributed to the great water solubility of carbendazim[12], leading to the fast transfer from food to water.

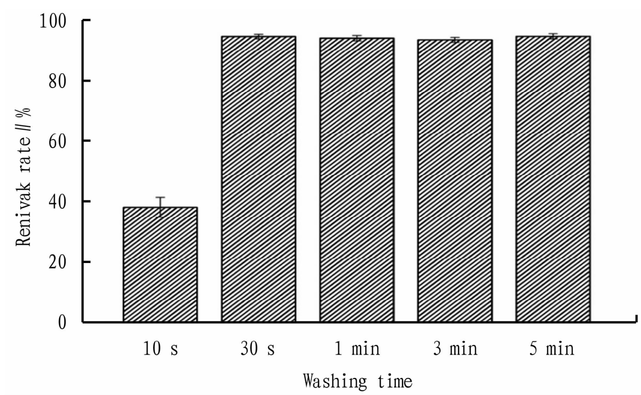

Fig. 4 Effect of washing time on the removal of carbendazim in apples

Effect of water temperature Temperature is of great significant for pollutant removal, which has been proved by various studies[13-14]. Water with different temperature was used to wash apples, and the concentration of carbendazim was detected. The results are shown in Fig. 2, which displayed that 49.73% -66.99% of carbendazim residue was reduced after washing. The removal efficiency was obviously improved with the increasing temperature, which is consistent with above reported research. However, the removal rate of sample by washing with water at 60 ℃ was lower than the rate of group with water at 45 ℃, the reason of which might be excessive temperature could hinder the function of washing soup.

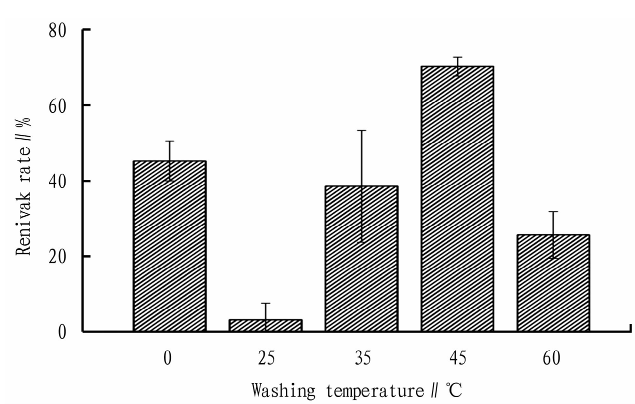

Fig. 5 Effect of washing temperature on the removal of carbendazim in apples

Effect of dosage of washing products The dosage of washing product is another factor which greatly influences the washing performance which is easily ignored during household washing process. Hence, this study investigated the effect of dosage of commercial washing soup (1 – 10 g) on the removal rate of carbendazim in apples. The results in Fig. 6 indicated that the best cleaning solution was dissolving 8 g of washing soup with 500 ml of water for reducing carbendazim residue in water, and 73.91% of carbendazim could be removed from apples.

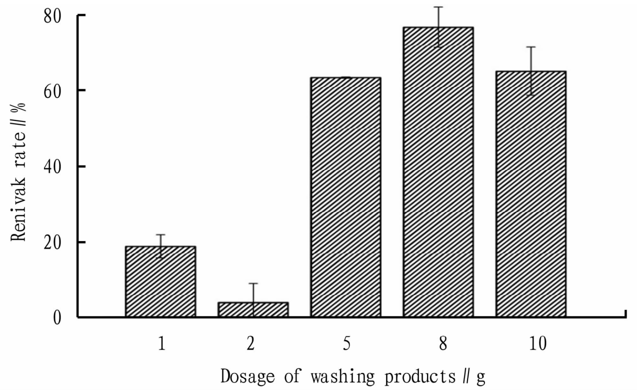

Fig. 6 Effect of dosage of washing products on the removal of carbendazim in apples

Conclusions

Carbendazim, as one of the benzimidazole fungicides, was applied for control a broad spectrum of fungi pathogens via inhibition of mitotic microtubule formation and cell division, which is frequently detected in fruits and vegetables. Washing is the initial step in household food processing, which can reduce the concentration of pesticides residue in a certain degree. However, the effect of washing on the removal of carbendazim in apples was not systemically studied. Hence, the QuEChERS and UPLC-FLD method for determination of carbendazim was established. Then, the influencing factors were systematically researched to unveil their effects on the carbendazim residue in apples. The results demonstrated the best cleaning scheme for removal of carbendazim was soaking apples for 5 min in a cleaning solution, which was prepared by dissolving 8 g of commercial fruit washing soup with 500 ml of water at 45 ℃, which could reduce 73.91% of carbendazim in apples. This study will provide guidance for providing household cleaning methods for controlling pesticide residues.

References

[1] MAGNUCKA EG, SUZUKI Y, PIETR SJ, *et al*. Action of benzimidazole fungicides on resorcinolic lipid metabolism in rye seedlings depends on thermal and light growth conditions[J]. Pestic Biochem Physiol, 2007, 88(2): 219 – 25.

[2] YE M, NIE J, LI Z, *et al*. Health risks of consuming apples with carbendazim, imidacloprid, and thiophanate-methyl in the Chinese population: Risk assessment based on a nonparametric probabilistic evaluation model [J]. Human and Ecological Risk Assessment: An International Journal, 2016, 22(4): 1106 – 1121.

[3] WANG D, YANG G, YUN X, *et al*. Carbendazim residue in plant-based foods in China: Consecutive surveys from 2011 to 2020[J]. Environ Sci Ecotechnol, 2024(17): 100301.

[4] LU SY, LIAO JW, KUO ML, *et al*. Endocrine-disrupting activity in carbendazim-induced reproductive and developmental toxicity in rats[J]. Journal of Toxicology and Environmental Health, Part A, 2004, 67(19): 1501 – 1515.

[5] YOON CS, JIN JH, PARK JH, *et al*. Toxic effects of carbendazim and n-butyl isocyanate, metabolites of the fungicide benomyl, on early development in the African clawed frog, *Xenopus laevis*[J]. Environ Toxicol, 2008, 23(1): 131 – 144.

[6] KONTOU S, TSIPI D, TZIA * C. Stability of the dithiocarbamate pesticide maneb in tomato homogenates during cold storage and thermal processing[J]. Food Additives & Contaminants, 2004, 21(11): 1083 – 1089.

[7] DUHAN A, KUMARI B, GULATI R. Effect of household processing on fenazaquin residues in okra fruits[J]. Bulletin of Environmental Contamination and Toxicology, 2010, 84(2): 217 – 220.

[8] CÁMARA M A, CERMEñO S, MARTíNEZ G, *et al*. Removal residues of pesticides in apricot, peach and orange processed and dietary exposure assessment[J]. Food Chem, 2020(325): 126936.

[9] RAWN DFK, QUADE SC, SUN WF, *et al*. Captan residue reduction in apples as a result of rinsing and peeling[J]. Food Chem, 2008, 109(4): 790 – 796.

[10] LENTZA-RIZOS C, AVRAMIDES EJ, KOKKINAKI K. Residues of azoxystrobin from grapes to raisins[J]. J Agric Food Chem, 2006, 54 (1): 138 – 141.

[11] YANG T, DOHERTY J, ZHAO B, *et al*. Effectiveness of commercial and homemade washing agents in removing pesticide residues on and in apples[J]. J Agric Food Chem, 2017, 65(44): 9744 – 9752.

[12] XU R, DU Y, WANG J, *et al*. Solubility modelling, solvent effect and preferential solvation of carbendazim in aqueous co-solvent mixtures of N, N-dimethylformamide, methanol, ethanol and n-propanol[J]. The Journal of Chemical Thermodynamics, 2019(128): 87 – 96.

[13] LOZOWICKA B, JANKOWSKA M. Comparison of the effects of water and thermal processing on pesticide removal in selected fruit and vegetables[J]. J Elementol, 2016, 21(1).

[14] LOZOWICKA B, JANKOWSKA M, HRYNKO I, *et al*. Removal of 16 pesticide residues from strawberries by washing with tap and ozone water, ultrasonic cleaning and boiling[J]. Environ Monit Assess, 2016 (188): 1 – 19.

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