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The effectiveness of chemical solutions on the removal of carbaryl residues from cucumber and chili presoaked in carbaryl using the HPLC technique

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ABSTRACT

This research aimed to compare the efficiency of five washing solutions (0.9% NaCl, 0.1% $NaHCO_3$, DI water, 0.001% $KMnO_4$, and 0.1% acetic acid) for removing carbaryl residues from cucumber and chili. The vegetables were soaked in 10 mg/L of carbaryl solution for 30 min and then washed for 30 min in one of the five washing solutions and the results compared. Each experiment was performed in triplicate and the amounts of carbaryl residues remaining were determined using high-performance liquid chromatography (HPLC). The results showed that 0.001% $KMnO_4$ was the most effective at removing carbaryl from both vegetables. Washing with 0.001% $KMnO_4$ reduced carbaryl residues to 64% and 28%, respectively, of the original concentrations. Washing with DI water was the least effective method of removing carbaryl residues. Hopefully, the results will encourage further research, into reducing carbaryl contamination by washing with chemical solutions, which will enable producers to reduce pesticide residues.

1. Introduction

Thailand is an agrarian country, where many people rely on income from agricultural produce (Thai Organic Trade Association, 2011). In farming-based economies, the use of pesticide is unavoidable because growers are under pressure to increase yields by controlling pests. The amounts of pesticides imported by Thailand have increased dramatically from about 110,000 tonnes (14,000 million Baht) in 2007 to 172,000 tonnes (24,000 million Baht) in 2013 (Tawatsin, Thavara, & Siriyasatien, 2015), and their use has had a significant effect on consumers and the environment. There are around 49,000 to 61,000 cases of pesticide intoxication reported each year in Thailand with a mortality rate of between 76 and 97 per 100,000 population (Panuwet et al., 2012; Tawatsin et al., 2015).

Carbamate pesticides are acetylcholinesterase inhibitors, which cause the accumulation of acetylcholine in synapses and neuromuscular junctions, resulting in symptoms associated with interruption of normal muscarinic and nicotinic acetylcholine receptor functions (Lerman, Hirshberg, & Shteger, 1984; Roldan-Tapia et al., 2006). Also, research has indicated that acetylcholinesterase inhibitors, and oxidative stress of carbamate, might have a role in the induction of mutations in gene coding, resulting in carcinogenesis (Dhouib et al., 2014; Jorsaraei, Maliji, Azadmehr, Moghadamnia, & Faraji, 2014; Mahajan et al., 2007). The third most popular insecticide used by Thai farmers for pest control (Panuwet et al., 2012) is carbaryl (Sevin®) or 1-naphthyl methylcarbamate, which belongs to the carbamate pesticide class. It is a contact, non-systemic insecticide that is retained on the surface of fruit and vegetables. It is also acid-stable but degrades after hydrolysis. Carbamate poisoning is a major health issue for Thai farmers, which is caused by lack of knowledge and wide-ranging use of the insecticide (Tawatsin et al., 2015).

Various methods for reducing pesticide residue on fruits and vegetables, such as washing, cooking, peeling, and ultrasound, have been studied (Andrade et al., 2015; Klinhom, Halee, & Methawiwat, 2008; Liang, Wang, Shen, Liu, & Liu, 2012; Osman, Al-Humaid, Al-Redhaiman, & El-Mergawi, 2014; Satpathy, Tyagi, & Gupta, 2012; Zhang, Liu, & Hong, 2007). However, the most simple and practical method is washing and a variety of washing solutions, such as sodium bicarbonate, vinegar, and potassium permanganate, have been used to remove pesticide residue (Andrade et al., 2015; Klinhom et al., 2008). Previous studies have indicated that the amounts of pesticide removed is dependent on factors including amounts present to begin with, physiochemical properties of the pesticide, wash times, washing processes, and the types of solution used.

Cucumber and chili are vegetables that are commonly consumed either cooked or raw. They are eaten raw in green papaya salad, one of the most popular Thai dishes. Many studies have acknowledged the presence of pesticides in vegetables including cucumber and chili

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(Liang et al., 2012; Pakakasama, Suwannee, & Soramon, 2016). In Thailand, research has demonstrated that fruit and vegetables sold in supermarkets have levels of pesticide residue that are above the Maximum Residue Limit (MRL). Among the vegetables sampled, cucumber and chili were found to have high concentrations of pesticide residues (Thai PAN, 2016). However, research on the effects of washing, to reduce carbaryl contamination on these vegetables, is limited.

Carbaryl residue can be determined using a variety of methods such as spectrophotometry (Sastry, Vijaya, & Rao, 1986), ELISA (Wang, Yu, & Wang, 2005), chlolinesterase-based biosensors (Skladal, Nunes, Yamanaka, & Ribeiro, 1997), and chromatography. For chromatography, high-performance liquid chromatography (HPLC) with fluorescence detection has been used widely for the analysis of carbaryl. However, this technique is time-consuming and requires sample derivatization to detect fluorescence compounds (Koc, Yigit, Das, Gurel, & Yarali, 2008; Sabala, Portillo, Broto-Puig, & Comellas, 1997). In previous research, reverse-phase HPLC with ultraviolet detection has been modified to overcome these problems (Duck & Woolias, 1985).

In this research, the effect of washing with five different chemical solutions on cucumber and chili presoaked in carbaryl was investigated to discover the most effective method of reducing the residues. The quantity of carbaryl residue remaining on the vegetable was determined using high-performance liquid chromatography (HPLC) with an isocratic mobile phase and UV detection was at 220 nm.

2. Materials and methods

2.1. Chemical and reagents

Methanol (PubChem CID:887) and acetonitrile (PubChem CID:6342) used in the experiment were high-performance liquid chromatography (HPLC)-grade obtained from Merck, (Darmstadt, Germany). Other chemicals (NaCl, $NaHCO_3$, $KMnO_4$, acetic acid) were analytical grade and purchased from Sigma (St. Louis, MO, USA). Carbaryl (PubChem CID: 6129; Sevin85®, Bayer Crop Science, North Carolina, USA), an agricultural pesticide imported to control pests, was used to prepare standard solutions. Washing solutions were prepared from analytical grade chemicals, as described in Satpathy et al. (2012).

2.2. Standard preparation

A stock standard solutions of carbaryl (Sevin85®, 1.0 g/L) was prepared by dissolving 1.176 g of carbaryl in methanol and adjusting the final volume with methanol to 1 L in a volumetric flask. The standard working solutions for the calibration were prepared by diluting the stock standard with methanol to five concentrations (0.1, 0.5, 1.0, 5.0, and 10.0 mg/L).

2.3. Sample collection and preparation

Cucumber and chili were purchased from Mueng Ake market (Pathumthani, Thailand). 120 g of cucumber and 70 g of chili were sliced into small pieces and soaked in 10 mg/L carbaryl solution for 30 min, before being air-dried under a fume hood for one hour. More cucumber than chili was required for the experiment, because cucumber loses more water than chili when it is air-dried.

The washing solutions were prepared based on studies by Klinhom et al. (2008) and Satpathy et al. (2012). Presoaked cucumber and chili were divided into six groups (accurately weighed at 10 g each). The control group was not washed (the unwashed group) and used as the reference value equal to 100% carbaryl retention. The remaining five groups were washed with shaking for 30 min in 9% NaCl, 0.1% $NaHCO_3$, 0.001% $KMnO_4$, 0.1% acetic acid, and DI water, respectively. Washed samples were removed from solutions, placed in sieving baskets and air-dried in a hood cabinet for one hour prior to extraction.

2.4. Sample extraction

Samples from each group were extracted using 40 mL of methanol under sonication for 30 min. Next, the extracted samples were passed through Whatman™ Qualitative Filter Paper No.1 (GE Healthcare, Chicago, USA) and concentrated using a vacuum rotator evaporator (Rotavapor@ R-210/215; BUCHI Labortechnik AG, Meierseggstrasse, Flawil, Switzerland). Then, the volume in a volumetric flask was adjusted with methanol to 25 mL. Prior to HPLC, extracted samples were passed through a 0.45 µm nylon syringe filter (VertiClean™ syringe filter; Ligand Scientific Co., Ltd., Nonthaburi, Thailand). Finally, the carbaryl residues found in the extractions were analyzed, based on the retention time for the carbaryl. Three replicates were analyzed to ensure reproducible data.

2.5. Chromatographic conditions

HPLC spectra were recorded using a Shimadzu LC-10 system (Shimadzu Corp., Kyoto, Japan). Carbaryl separation was performed on a reversed-phase column, COSMOSIL 5C18-MS-II Packed (4.6 mm × 250 mm I.D. 5 µm), purchased from Nacalai Tesque, Inc. (Kyoto, Japan). The mobile phase consisted of a mixture of water and acetonitrile at a ratio of 55:45 (v/v). Distilled water was obtained using Milli-Q purification apparatus (Millipore, Billerica, MA, USA). The flow rate and UV absorbance were set at 1.0 mL/min and 220 nm, respectively. The temperature of the column was 40 °C (Duck & Woolias, 1985; Falah, 2009). The method was simple and rapid, because it incorporated UV detection at 220 nm without the need for post-column derivatization.

2.6. Statistical analysis

The mean and standard deviation (Mean ± SD) were calculated using Microsoft Excel version 2010 (Microsoft Corporation, Redmond, WA, USA). Differences between percentages of carbaryl reduced by the different chemical solutions were assessed using the Student's *t*-test. A statistically significant difference was considered when the *p*-value was ≤ 0.05.

3. Results and discussion

3.1. Retention time and the standard calibration curve

The reverse-phase HPLC process used was adapted from previous studies (Duck & Woolias, 1985; Falah, 2009) and was used to determine amounts of carbaryl residues in extracts from cucumber and chili pretreated with carbaryl. The retention times and peak areas of carbaryl at various concentrations are shown in Table 1 and Fig. 1. The retention time was 6.4 min (ranging from 6.346 to 6.396 min). Concentrations of the standard in the range 0.1 to 10 mg/L exhibited excellent linearity when peak area was plotted against concentration [coefficient (R^2) was 0.999, Fig. 2]. The pesticide was not 100% pure, but could be separated from potentially interfering substances and had a sharp peak. Furthermore, the method was less complicated compared with other methods (Koc et al., 2008; Sabala et al., 1997), meaning this method might be suitable for detecting carbaryl residues in other fruits and vegetables.

Table 1
Retention time and peak area of standard carbaryl.

Concentration (mg/L)	Retention time (min)	Peak area (Arbitrary Unit)
0.1	6.346	49,852
0.5	6.356	268,389
1.0	6.375	518,154
5.0	6.386	2,496,293
10.0	6.396	4,813,821

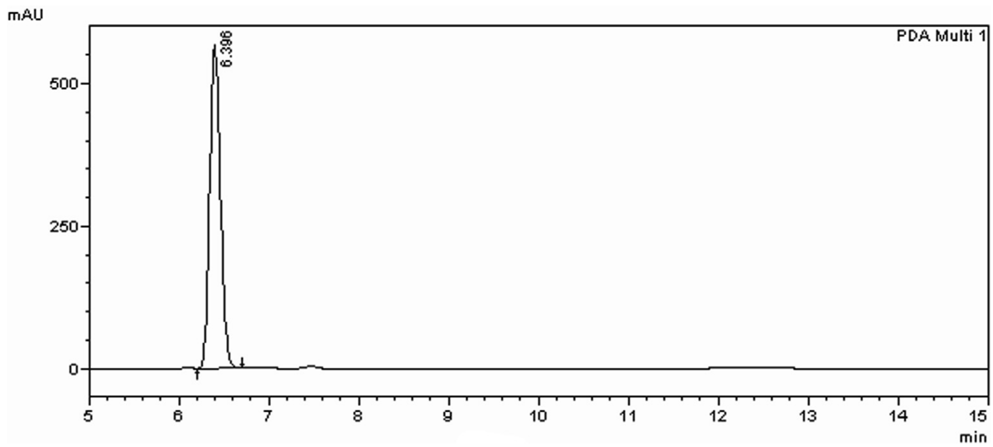

Fig. 1. HPLC Chromatogram of carbaryl (Retention time 6.396 min).

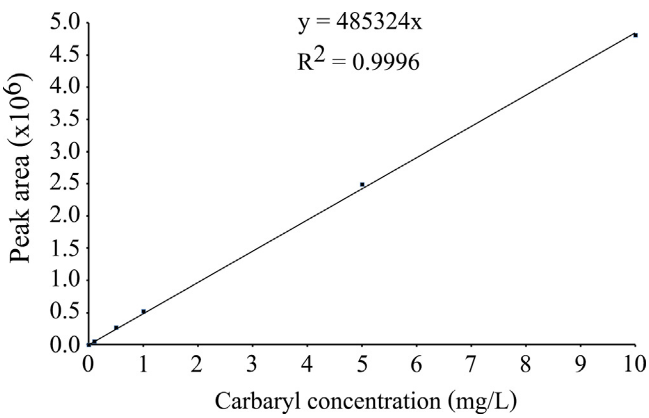

Fig. 2. Standard curve of carbaryl.

3.2. Effects of different washing solutions on the removal of carbaryl from cucumber

After washing with one of five different solutions, carbaryl residue reduction in cucumber samples were in the range 54–64% of the original value. The results showed an increase in the percentage of carbaryl reduced across the groups, such that 0.001% $KMnO_4$ > 0.1% acetic acid > 0.1% $NaHCO_3$ > 9% NaCl > DI water (Fig. 3). 0.001% $KMnO_4$ was the most effective washing solution, because it reduced carbaryl residues in presoaked cucumber by 64% (p = 0.05). Conversely, DI water was the least effective (54% reduction).

3.3. Effects of different washing solutions on the removal of carbaryl from chili

After washing, carbaryl residue reduction in the chili was in the range 17–28% (Fig. 4). As with the cucumber, 0.001% $KMnO_4$ and DI water were the most and the least effective at removing carbaryl residue, respectively. The effects of washing exhibited a statistically significant difference across groups. The percentage of carbaryl reduction increased across the groups, such that 0.001% $KMnO_4$ > 9% NaCl > 0.1% acetic acid > 0.1% $NaHCO_3$ > DI water. The percentage of carbaryl residue reduction with 0.001% $KMnO_4$ and 0.1% acetic acid were statistically different when compared with DI water, such that *p*-values were less than 0.05 and 0.01, respectively.

Washing is the most common method of reducing pesticide contamination in agricultural produce. The effects of washing presoaked cucumber and chili with five solutions studied and showed that some solutions were more effective than others at reducing the percentage of carbaryl residue remaining. Washing in 0.001% $KMnO_4$ was the most

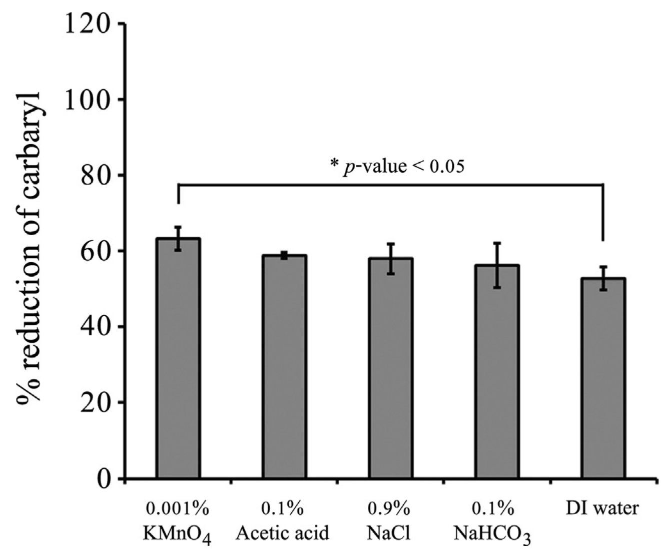

Fig. 3. Percent carbaryl reduction by different washing solutions from cucumber.

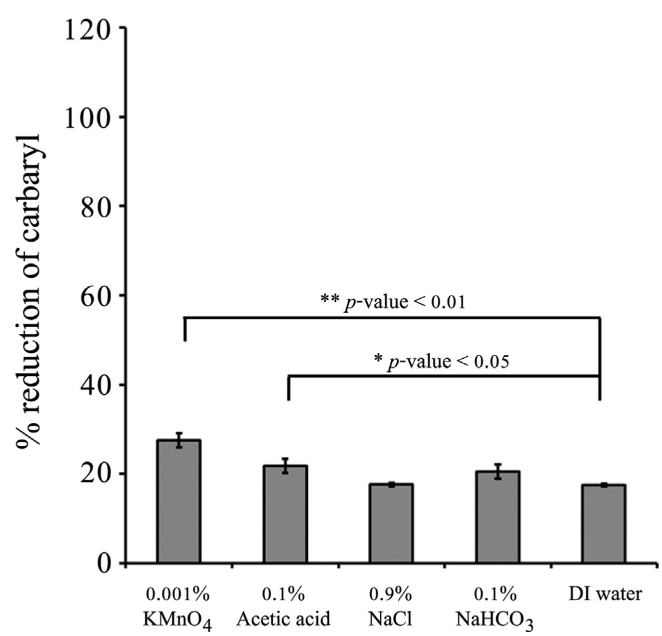

Fig. 4. Percent carbaryl reduction by different washing solutions from chili.

effective method, whereas washing in DI water was the least effective. These results agree with Klinhom et al. (2008) who found that, among various solutions, 0.001% $KMnO_4$ was the most effective at removing carbaryl from Chinese-kale and concluded this might be due to its oxidative properties.

Potassium permanganate or 0.001% $KMnO_4$ is a strong oxidizing agent with high redox potential. It may be effective at reducing carbaryl residue due to its oxidative and alkaline properties, which increase degradation of carbaryl, as described previously (Klinhom et al., 2008), in addition to physically washing away some residues. Carbaryl is stable in acid solutions, but it is unstable at alkaline pHs. Hydroxyl radical (*OH), produced by oxidation, e.g·H_2O_2/UV photolysis, has been reported to react with carbaryl and have an important role in its degradation (Wang & Ann, 2003).

The initial concentration of presoaked carbaryl (10 mg/L) was relatively high and, therefore, washing with different solutions did not remove the residue completely from either product. After the vegetables had been washed, the percentage of carbaryl still present in extracts from the cucumber and chili were in the range 54–64% and 17–28%, respectively. More carbaryl residues were removed from cucumber than chili. This might be due to differences in the chemical composition of the vegetables, e.g. water content and surface area.

That DI water was the least effective method of removing carbaryl residue suggests solubility was not the main factor influencing removal of the residues. Also, this study showed that washing alone could not remove carbaryl residue from cucumber and chili entirely.

4. Conclusion

Washing vegetables with a variety of chemical solutions including water before consumption is still recommended, because reduces pesticide residues, especially in vegetables that are consumed raw. However, 0.001% $KMnO_4$ was significantly more effective in reducing carbaryl residues than water alone, which might be due to its oxidative properties. More research into the effectiveness of chemical solutions on the removal of carbaryl residues is required. A study with a larger sample size that determines MRLs prior to presoaking should be carried out to compare existing washing methods, which could be removing all or none of the residues.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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