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Residual Carbaryl in Selected Vegetables

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Abstract: This study developed a quick detection method of 1-naphthyl-methyl carbamate (carbaryl) in fruits and vegetable samples using UV spectrophotometry. The method was validated by establishing limits of detection (0.08 ppm) and quantitation (0.29 ppm), accuracy (91.7-99.6%), recovery (86.0-91.2%) and precision (10.47%) which are all within acceptable ranges. The method was then employed for the analysis of trace carbaryl in experimentally exposed produce. These include ampalaya, tomato, eggplant, petchay and kamote tops. Morphological features like texture and size-to-mass ratio of individual vegetables seem to affect the levels of residue detected. Common methods of washing were then compared in terms of carbaryl residue reduction. Longer and more thorough washing with rinsing was demonstrated to more effectively reduce detected carbaryl. The method was also applied to actual market samples of ampalaya. Out of nine samples tested, four indicated a positive test result for the presence of surface residues.

Key words: carbaryl; pesticide residue in produce; uv spectrophotometric

1. INTRODUCTION

Carbaryl (1-naphthyl methylcarbamate) belongs to the Carbamate family of insecticides which are esters of N-methyl carbamic acid. This pesticide is a class of highly effective commercial pesticide which have been used since the 1960s due to its high insecticide and nematocide effects.[2] However, despite the advantages of using these, carbamate pesticides are suspected carcinogens and mutagens because it is an acetyl cholinesterase inhibitor that disrupts the insect's nervous system. The toxicity, stability and mobility of pesticides demand their monitoring to prevent the harmful effects on animals, humans and the environment.[2,3]

Second hand information indicates an alarming practice of growers of ampalaya. In order to maintain the quality of the crop, the ampalaya is soaked in the commercial pesticide Sevin® upon harvest to protect the produce from pests while in transit to the marketplace. While the risk of poisoning from residue in fruits and vegetables treated with pesticide applied during cultivation exists, this practice, if true, introduces a higher risk to consumers. This study aimed to develop a simple method for the detection of carbaryl, the active ingredient of Sevin, in produce. This study also aimed to evaluate the efficacy of typical washing methods of vegetables in removing or reducing carbaryl from the vegetables.

Various analytical methods are employed for pesticide residue analysis. Most of these involve chromatography because samples are expected to be impure and require separation before detection and quantitation. Detection methods employed include uv spectrophotometry and mass spectrometry, In most cases, methods require homogenization and extraction of samples prior to analysis. In this controlled study, carbaryl is the only pesticide to be

determined and with this limitation, direct UV measurement of washings was employed in the detection and quantitation of carbaryl residues. The proposed analytical method was first validated to ensure that the procedure is suitable for the analysis and that the method will yield accurate and precise results within the scope of its intended use.

2. METHODOLOGY

2.1 Materials and Instrumentation

All chemicals used, except carbaryl, were of analytical reagent grade. Aqueous solutions were prepared using distilled water. Carbaryl was obtained from commercial Sevin® WP 85 manufactured by Bayer, USA. Sevin® is declared in the product label to contain 85% carbaryl. The remainder of the product is presumed to be surfactants that aid in the dispersal of carbaryl in preparations since it is not very soluble in water. Carbaryl was extracted from Sevin using ethanol. The suspension was centrifuged and the supernatant evaporated to yield carbaryl. The carbaryl was confirmed on Agilent Technologies 1200 Series HPLC C18 column with water and acetonitrile as mobile phase (t_r = 5.1 minutes) and Bruker micrOTOF- Q11 Mass Spectrometer (ESI + mode, pseudo molecular ion peak $M+Na^+$ m/z = 224.0678, 201 + Na).

All uv absorption measurements were performed on a double beam HITACHI U- 2900 UV-Visible spectrophotometer using matched quartz cells at 279 nm.

2.2 Sample Collection

Organically grown samples of tomatoes, eggplants and kamote tops for were obtained from Bahay Pag-ibig in San Fernando, Pampanga while ampalaya and pechay were obtained from a wet market in 8th avenue, Caloocan. Bahay Pag-ibig is a Home for the Aged run by the Archdiocese of San Fernando, Pampanga It maintains an organic garden which is a recipient of a grant from the DOST-III Provincial Science and Technology Center. Pechay and ampalaya were sourced from the market in Caloocan and were washed thoroughly prior to use. The market ampalaya samples tested for the presence of carbaryl residue were obtained from Divisoria, Manila and the market near MCU, Caloocan. Masses of all produce were recorded. Untreated samples for each produce as well as blank runs were processed for control purposes.

2.3 Method Development and Validation

A 100 ppm carbaryl standard was prepapred by dissolving 10 mg carbaryl in 100 ml volumetric flask. Dilutions were made in order to solutions for the Beer's Law plots. Validation analysis parameters were Limit of Detection (LOD), Limit of Quantitation (LOQ), Accuracy, Recovery and Precision. All parameters were determined with Sevin pesticide serving as the unknown and carbaryl as the standard.

2.4 Analysis of Trace Carbaryl and Methods of Removal

All produce were initially weighed before storing in the refrigerator prior to analysis. Two sets of samples were prepared, the experimentally exposed samples and the market samples. Exposed samples were soaked in Sevin prepared according to instructions on the packaging, and then subjected to air drying. These were then subject to washing with varied treatment in fixed volumes of 30% MeOH:water. The methods of removal were soaking for 1 minute, soaking the samples with rinsing and soaking for 15 minutes.

The treatment of methods of removal for each produce was applied using 250 ml solvent in a 400 ml beaker. After the first washing, the vegetables were air dried then a second washing was employed by soaking them again in 200 ml solvent for 2 minutes. Both washings were separately collected and transferred to a 60-ml brown bottle with cap. A funnel and a clean filter paper was used during the transfer to ensure that no solid components were included with the collected washing. The absorbance of the washings were then read at 279 nm. The absorbances were interpolated to mg/L concentrations using the calibration curves. Total volumes and mass of vegetable samples were then used to calculate mg/kg concentrations in the produce. The first washing gives an indication of the

levels of residue on the vegetables while the second washing served to check the removal efficiency of the washing method as the procedure employed for the second washing was similar across all the samples.

Real ampalaya samples from the market were also subjected to analysis to determine whether the produce from the market contained pesticide residue and to verify the account on the farmers practice of soaking their ampalaya harvest. Nine samples were collected from Divisoria, Manila and MCU, Caloocan. All nine samples were subjected immediately to the developed method.

3. RESULTS AND DISCUSSION

3.1 Method Development Validation

Calibration standards at 1, 3,5,7,9 ppm and 0.1, 0.3, 0.5, 0.7, 0.9 ppm concentrations for both upper and lower limits were obtained and yielded linear regression equations of the line at $y= 0.0326x - 0.0002$ with a correlation of determination of 0.9997 and $y= 0.034x + 0.0016$ with $R^2 = 0.994$ respectively.

Method validation parameters obtained for limit of detection (LOD) is 0.08 ppm and limit of quantitation (LOQ) of 0.29 ppm. Both were obtained by calculating the unknown in seven trials. LOD was calculated using the formula 3×SD and LOQ was calculated using 10×SD with SD as the standard deviation obtained for all seven trials. Accuracy was obtained via % recovery using three trials of standard addition method. 91.72%, 99.64% and 97.44% were the obtained accuracy % recoveries. Recovery level was also prepared via three trials of standard addition method both in upper and lower limits. 88.76%, 98.82% and 86.01% were the % recovery obtained in upper limit analysis and 89.49%, 80.00% and 88.39% were the % recovery value obtained in lower limit analysis. The acceptable % recovery for ppm level is from 80-110 % for both accuracy and recovery test.[7] Lastly, precision test were prepared via interday analysis of three trials of homogeneous test samples, analyzed for three consecutive days. The precision value in terms of % relative standard deviation (%RSD) obtained were 13.75%, 7.14%, 10.52% for three trials. The accepted precision test result should not be greater than 16%.[7] Table 1 summarizes the validation parameters of this study. All values obtained in the validation analysis were within acceptable limits of a valid method.

Table 1. Results of Validation Parameters

Parameter	Value
Regression equation (upper stds)	y= 0.0326x – 0.0002
Regression equation (lower stds)	y= 0.034x + 0.0016
LOD	0.083
LOQ	0.29
Accuracy	$96.27 \pm 4.09 \times 10^{-2}$
Precision (Average RSD)	10.47%
Upper limit recovery	91.20%
Lower limit recovery	85.96%

3.3 *Sample Analysis*

Ten samples were subjected to analysis for each of the selected produce. The ten samples were distributed for three trials in each method of removal. One control sample for each was also analyzed.

Table 2. Carbaryl concentrations (mg/L) in washings

	1 min Soaking		Soaking/ Rinsing		15 mins soaking	
	1st	2nd	1st	2nd	1st	2nd
Ampalaya	1.38	1.57	2.73	0.84	2.82	0.78
Tomato	0.8	0.74	1.68	0.58	1.68	0.54
Eggplant	1.22	0.64	1.95	0.46	1.79	0.48
Pechay	2.48	1.80	5.94	0.40	5.14	0.37
Kamote tops	8.25	4.46	10.74	4.61	8.45	3.34

Theoretically, the more efficient methods of removal would have a higher pesticide concentration in the first washings. It would also have a lower concentration in the second washing, since most of the carbaryl residue were already previously washed away. These are reflected in the results summarized in Table 2 where generally the first wash values are greater than the second. Comparison of 1 minute soaking against 15 minutes also show that washings from the longer period remove more residue, and that soaking with rinsing produces relatively the same effect as soaking for a longer period of time. Ampalaya, however, produced a contrary result in that the second washing had a higher concentration than the first in the 1 minute soaking. The two other more extensive washing methods were consistent with the rest of the samples. This result may be due to the warty surface of ampalaya which may have

allowed residue to deposit in the indentations of the surface. The effect of the morphology on the washed carbaryl can also be inferred from a comparison of the samples studied. Among the fruits, ampalaya has the highest levels, while tomato has the lowest. Eggplant values lie in between but are closer to ampalaya. Tomatoes have very smooth surfaces, and thus retain little residue. While eggplants are likewise smooth, the calyx which is retained as the fruit develops is rough. Residue might also penetrate the space between the calyx and the fruit. Leafy vegetables retain more residue than fruit due to the larger surface area available for the same mass. Between pechay and kamote tops, the kamote gave up to almost double the washed residue from pechay. This is probably due to the more velvety form of the young shoots of sweet potato as compared to pechay, which allow it to retain more of the pesticide on which it was soaked.

If the concentrations in the washings are converted into amounts of carbaryl in the vegetables, data after the first washing can be interpreted as change in concentration effected by the washing procedure. These are summarized in Table 3.

Table 3. Concentration (mg/kg) removed from vegetable after one washing

	1 min Soaking	Soaking/ Rinsing	15 mins soaking
Ampalaya	6.21	14.64	14.04
Tomato	4.14	9.04	12.12
Eggplant	13.93	16.48	16.82
Pechay	173.4	349.1	334.6
Kamote tops	199.4	259.0	212.2

If it is assumed that the second washing, which was the same treatment for all samples, removed all residual carbaryl after one washing, an expected minimum concentration of carbaryl in the produce after one washing can be calculated. These are summarized in Table 4.

Table 4. Minimum Concentration (mg/kg) in vegetable after one washing

	1 min Soaking	Soaking/ Rinsing	15 mins soaking
Ampalaya	5.36	3.77	3.00
Tomato	2.94	2.49	3.03
Eggplant	5.65	3.21	3.61
Pechay	96.05	18.91	20.45
Kamote tops	85.16	91.01	65.85

Together the two concentrations may estimate the amount of carbaryl residue that is deposited in the vegetables if they are dipped in carbaryl preparations after harvest. The method of extraction as already mentioned above, obviously affects the amounts indicated. These are summarized in Table 5.

Table 5. Estimated minimum residual concentration (mg/kg) in vegetable

	1 min Soaking	Soaking/ Rinsing	15 mins soaking
Ampalaya	11.57	18.41	17.04
Tomato	7.08	11.53	15.15
Eggplant	19.58	19.69	20.43
Pechay	269.5	368.0	355.1
Kamote tops	284.6	350.0	278.1

For smooth skinned vegetables like tomato and eggplant, soaking with rinsing makes little difference from soaking for 15 minutes that is why both methods gave very close results for these vegetables. But for the textured ampalaya and the leafy vegetables, soaking with rinsing is more effective in removing residual pesticide, as it is this method which gave the highest levels detected. However, from these controlled experiments, it is seen that levels of residue on the vegetables if so treated with Sevin, are all significantly higher than maximum residue levels deemed safe. Tomato has the highest MRL at 5 mg/kg (Krol, 2011), followed by petchay at 3 ppm (EU MRL), ampalaya at 2 ppm (Nollet and Rathore, 2010) and eggplant (Krol, 2011) and leafy greens at 1 ppm (EU MRL).

3.3.2 Analysis of Market Samples

Of the nine market samples of ampalaya studied, four gave positive results ranging from 1.3 to 3.7 mg/kg or from slightly less to slightly more than the maximum residue limit for carbaryl of 2 ppm. The results of the analysis could possibly mean that pesticides may be present in the ampalaya samples tested. While the method detects carbaryl, other substances that have similar absorption characteristics in the uv region could be giving false positives for carbaryl. Nevertheless, chemicals absorbing at the λmax of carbarly was detected.

4. Summary and Conclusion

For the purposes of detecting carbaryl in controlled experimental samples, the present study was able to validate a quick UV spectrophotometric method of carbaryl detection in selected produce.

Samples including kamote tops, petchay, ampalaya, tomato and eggplant exposed to Sevin by soaking as an alleged practice for ampalaya, obtained estimated minimum residue way beyond their maximum residue limits. Prudent washing practices like extended soaking or rinsing, however were demonstrated to effectively remove residue.

Although this study experimentally exposed the produce to Sevin, the analytical results support quantitatively what is intuitively expected in terms of pesticide residue and their removal.

It is recommended that farmers be educated on the heightened risk involved in the practice of applying pesticide post harvest. Similarly, the public must be constantly reminded of the precautionary measure of intensive washing of produce to avoid the risk of pesticide residue contamination in fresh fruits and vegetables.

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