# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Consumer data

The consumptions of cauliflower, ginger, kale, cucumber and tomato were surveyed from people living in Suthep sub-districts by specific randomizing sample areas and populations that covered all villages. 244 people divided to 100 males and 144 females, who were about socio-demographic, socio-economic, health, including sex, age, body mass index (BMI), educational level, income, family members, occupation, congenital disease, self-cooking, the most popular vegetable and consumptionform each village to be interviewed. Information data, education and occupational of consumers are shown in Table 4.1, 4.2, and 4.3, respectively. The average age, body weight, body mass index (BMI), income, and family size were found 43.8±7.3 years, 59.0±7.1 kilograms, 21.96±1.28 (normal), 11,415±2,959 bath/person, and 3.27±0.88 person/family, respectively. The highest education levels in bachelor's degree (30.3%) followed by diploma (22.5%), and high school (18.0%), respectively and the most occupation of consumer was trader (34.02%) followed by business owner (24.6%), and general employee (13.5%), respectively. Moreover, it was found that the favorite vegetables of consumers from Pak choy, Chinese White Cabbage, and Kale were 25.4, 16.8, and 12.3%, respectively.

**Table 4.1** Information data of consumers

| **Information** | **Mean ± SD** |
|---|---|
| 1. Age (Year) | 43.8±7.3 |
| 2. Body weight (kg) | 59.0±7.1 |
| 3. Body Mass Index (BMI) | 21.96±1.28 (normal) |
| 4. Income (bath/person) | 11,415±2,959 |
| 5. Family size (person/family) | 3.27±0.88 |

**Table 4.2** Education of consumer

| **Education levels** | **Frequency** (person) | **Percentage (%)** |
|---|---|---|
| 1. Not education | 20 | 8.2 |
| 2. Elementary school | 18 | 7.4 |
| 3. Junior high school | 31 | 12.7 |
| 4. High school | 44 | 18.0 |
| 5. Diploma | 55 | 22.5 |
| 6. Bachelor's degree | 74 | 30.3 |
| 7. Master’s degree | 2 | 0.8 |

**Table 4.3** Occupation of consumer

| **Occupation** | **Frequency** (person) | **Percentage** (%) |
|---|---|---|
| 1. Agricultural worker | 10 | 4.1 |
| 2. General employee | 33 | 13.5 |
| 3. Trader | 83 | 34.0 |
| 4. Office worker | 27 | 11.1 |
| 5. Government officer | 27 | 11.1 |
| 6. Business owner | 60 | 24.6 |
| 7. Other | 4 | 1.6 |

The result study was found that the most eating behaviors of consumers were buying followed by self-cooking meal and generally, the cooking of consumers was 1 meal/day in the evening. The results are shown in Table 4.4, 4.5, and 4.6, respectively.

**Table 4.4** Eating behaviors of consumers

| **Eating behaviors** | **Frequency** (person) | **Percentage** (%) |
|---|---|---|
| Self-cooking meal | 102 | 41.8 |
| Buying | 141 | 57.8 |
| Other | 1 | 0.4 |

**Table 4.5** Rate of self-cooking meal

| **Rate of self-cooking meal** | **Frequency** (person) | **Percentage** (%) |
|---|---|---|
| 1 meal/day | 87 | 46.0 |
| 2 meals/day | 62 | 32.8 |
| 3 meals/day | 40 | 21.2 |

**Table 4.6** Number of meal for Self-cooking

| **Self-cooking meal** | **Frequency** (person) | **Percentage** (%) |
|---|---|---|
| Morning | 107 | 31.9 |
| Afternoon | 46 | 13.7 |
| Evening | 183 | 54.5 |

**4.2 Vegetable consumption**

The consumption of 6 vegetables in area of Suthep sub-district of Chiang Mai city and in area of Thailand studied by the Ministry of Agriculture and Cooperatives (2006) has showed in Table 4.7. It showed that the consumption of cucumber was similar.

**Table 4.7** Food consumption from 6 kinds of vegetables in Thailand and Suthep sub-distric

| **Vegetable samples** (Scientific name) | **Food consumption** (g/day) | |
|---|---|---|
| | Thailand (2007, n = 18,998) | Suthep sub-distric (2013, n = 244) |
| Cauliflower (*Brassica oleracea* L.) | 46.1 | 10.7 |
| Ginger (*Zingiber officinale* Roscoe) | 90.8 | 2.0 |
| Kale (*Brassica albograba* Bailey) | 63.8 | 13.0 |
| Cucumber (*Cucumis sativa* L.) | 55.1 | 63.3 |
| Pepper (*Piper nigrum* L.) | 8.0 | 0.4 |
| Tomato (*Lycopersicon esculentum* Mill.) | 50.0 | 31.0 |

### 4.3 Development of method for determination of carbendazim residue

Sample preparation involves two steps: extraction and clean-up with SPE. The sample was chopped and blended using a blender. Five gram of blended sample was weighed and transferred to the extraction tube followed by the addition of 1 mL of 0.05 M HCl, 0.5 g of sodium acetate and 15 mL of ethyl acetate. Sample was then shaked for 1 min by using a vortex mixer and sonicated in the ultrasonic bath for 15 min. The extract of sample was filtered by vacuum filtration using a buchner funnel. After that 7.5 mL of aliquot was filtered through a SAX/PSA dual-layer cartridge and washed with 5 mL of ethyl acetate and the filtered liquid was collected. The solvent

was evaporated using a rotary evaporator and the dry residue was re-dissolved in 1 mL of HPLC mobile phase (methanol:water, 25:75, v/v). An aliquot 20 µL was injected into the HPLC-UV for carbendazim analysis. Linearity was evaluated using calibration by adding 200 µL of working solutions to 5 g of vegetable samples the concentrations of carbendazim in vegetable samples were 0.05, 0.075, 0.10, 0.20 and 0.30 mg/kg, respectively. The precision was evaluated by relative standard deviation (RSD) of five replications at five concentrations. The accuracy was studied using 0.125 µg/mL (or 0.05 mg/kg) standard analysis on the same day for five replications and was calculated by comparison of concentration measured to the spiking concentration of the standard solution and was expressed as a percentage relative error value. Recovery test at five spiking levels in vegetable samples (0.05, 0.075, 0.10, 0.20 and 0.30 mg/kg) and limit of detection (LOD) were plotted RSD versus concentration and the equation of the calibration curve. Finally, the limit of quantitation (LOQ) was determined as the lowest carbendazim concentration injected that based on a signal-to-noise ratio 10:1. The spiked vegetable samples were allowed to stand for 24 h before the extraction to achieve carbendazim distribution in the samples.

This work was to develop the determination of carbendazim in vegetables. Initially, ethyl acetate was used to extract carbendazim from vegetables according to previous studies experiments (Pan et al., 2008; Bernal et al., 1997 and Garrido et al., 1997) and the extractant was acidified with hydrochloric acid and sodium acetate . The clean-up by passing 7.5 mL of the extractant through the SAX/PSA dual-layer cartridges and the chromatogram of vegetable samples spiked with the standard compound at 0.05 mg/kg.

### 4.3.1 Method Efficiency

Objective of this work was to develop a method for detecting carbendazim residue in vegetables. Initially, ethyl acetate was used to extract carbendazim from vegetables according to previous studies experiments (Pan et al., 2008) and the extract was acidified with hydrochloric acid and sodium acetate. Clean-up of the extract (7.5 mL) was performed by passing through the SAX/PSA dual-layer cartridges and the eluate (20 µL) was injected onto HPLC column for analysis. In this study was extracted under different experimental conditions.

4.3.1.1 Effect of extraction sample weight

In this study, the sample weights in 5 and 10 g were evaluated. The results are shown in Figure 4.1, sample weight had no significant effect on the extraction recovery. Consequently, 5 g of sample was chosen for the experiment.

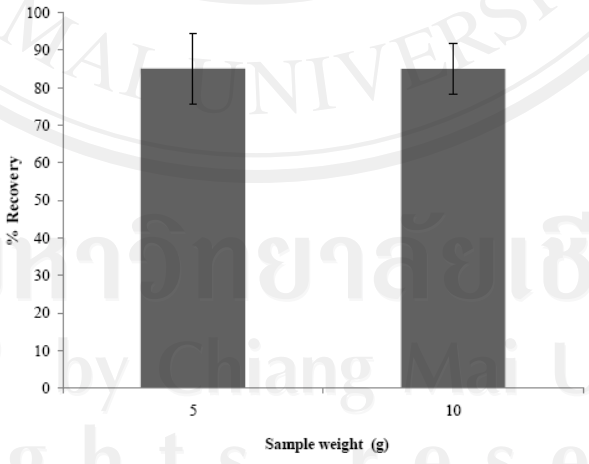



**Figure 4.1** Effect of sample weight

4.3.1.2 Effect of extraction solvent volume

The influence of solvent volume on the extraction recovery by changing its volume from 10, 15, 20 and 25 mL of ethyl acetate, respectively. The results are shown in Figure 4.2, the extraction recovery increased when the volume of ethyl acetate was changed from 10 to 15 mL and then decreased when the volume of ethyl acetate was changed from 20 to 25 mL. Base on the experimental results 15 mL of ethyl acetate was chosen.

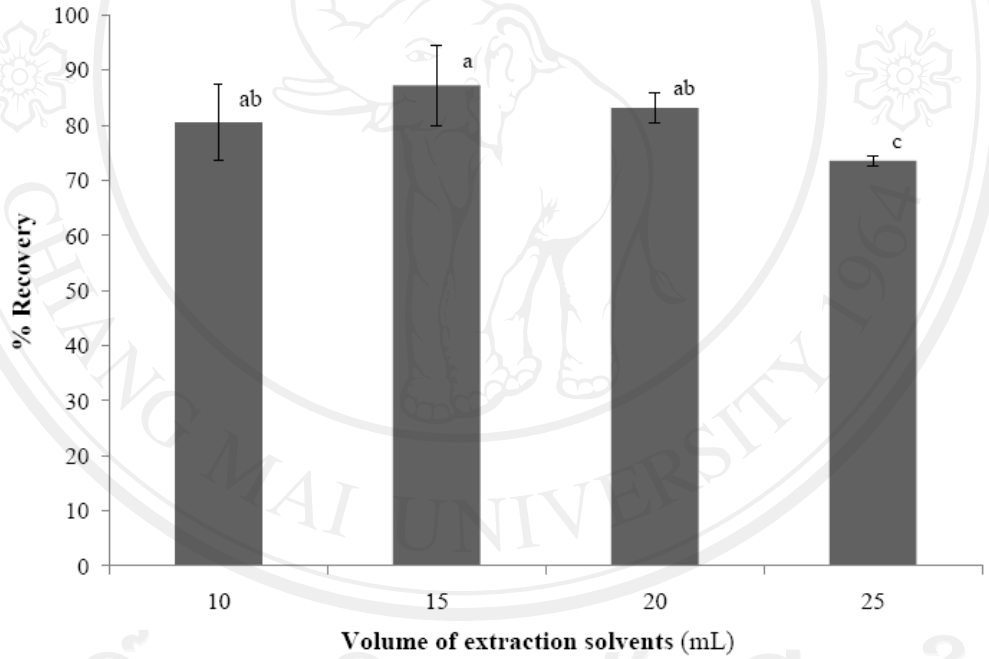



**Figure 4.2** Effect of extraction solvent volume

(Different superscript letters a, b and c in the top indicate significant at $p \leq 0.05$)

4.3.1.3 Effect of extraction time

Figure 4.3 shows the effect of extraction time was studied between 0-55 min and the results indicated that the extraction efficiency no significant on the extraction recovery with increased extraction time. Consequently, short extraction time can be used as long as sufficiently sensitive are achieved. In this method, the extraction was selected as sonicating for 15 min.

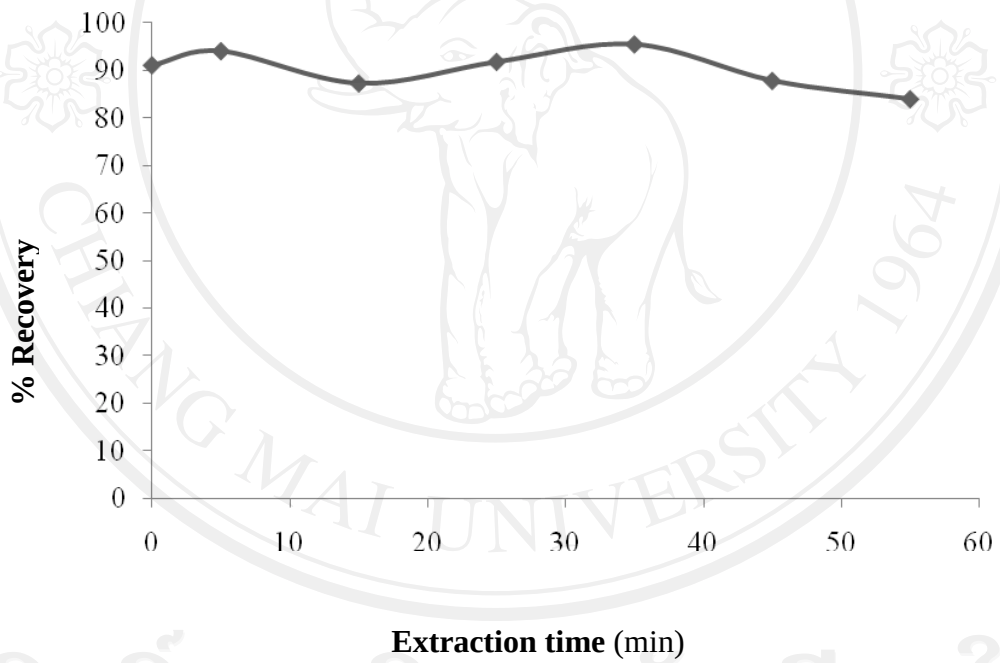



**Figure 4.3** Effect of extraction time

4.3.1.4 Effect of washing solvent volume

The influence of ethyl acetate volume was washed a SAX/PSA dual-layer cartridge after that the sample extracted solution was filtered. Figure 4.4 shows that the extraction recovery increased as the ethyl acetate volume increased from 10 to 15 mL, but unchanged between 10 and 15 mL. Therefore, 10 mL of the ethyl acetate for washing a SAX/PSA dual-layer cartridge was chosen for the experiment.

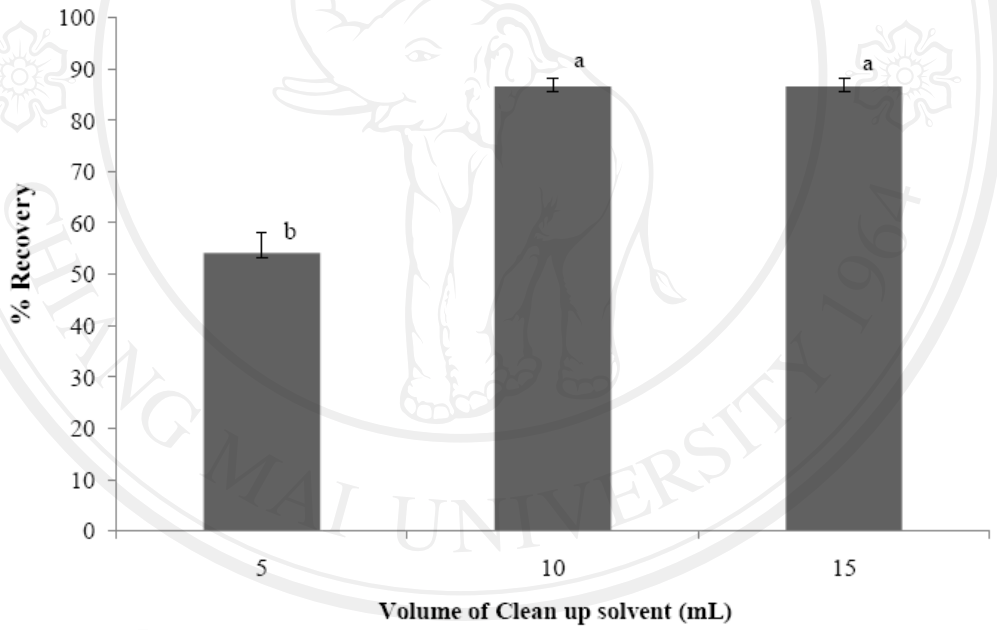



**Figure 4.4** Effect washing solvent volume

(Different superscript letters a and b in the top of results indicate significant at p≤ 0.05)

### 4.3.2 Calibration curve, reproducibility, limit of detection and limit of quantitation

The methods quality parameters (i.e. linearity, detection limits, recovery and precision) were studies under conditions using methanol standard solution of the spiking with concentration ranging from 0.05 to 0.30 mg/kg and are shown in Table 4.1. Calibration graph was obtained by plotting the peak area versus concentration and calibration curve linear was from 0.05 to 0.30 mg/kg. The correlation coefficient was 0.999 and the equation of the calibration curve was y = 193.79x-0.3122. Typical chromatograms of blank vegetable samples, vegetable samples spiked with carbendazim at a concentration of 0.1 mg $kg^{-1}$ and an actual vegetable samples are shown in Figure 4.5.

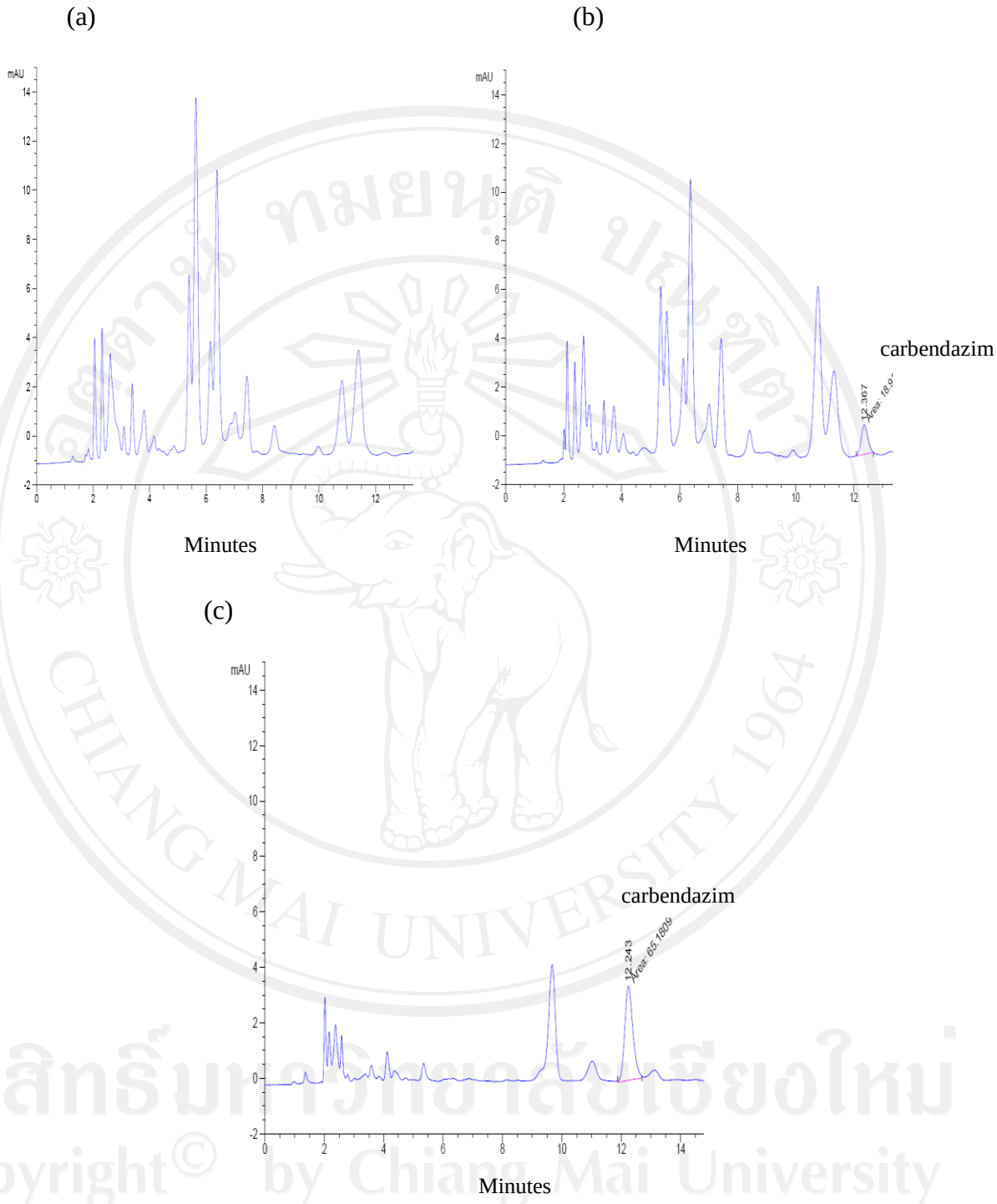



**Figure 4.5** Chromatograms of (a) blank vegetable samples, (b) vegetable samples spiked with carbendazim at a concentration of 0.1 mg $kg^{-1}$ and (c) an actual vegetable samples.

Recovery values were calculated as the ratio of the peak areas of the analytes from the spiked vegetable to the peak areas of standard solution. Recoveries were good, more than 90% at 0.05-0.30 mg/kg spiking levels and limit of detection (LOD) were plotted RSD versus concentration of spiked carbendazim show equation curve was y = 0.0056x + 0.003 thus the determined LOD value was 0.003 mg/kg. The limit of quantitation (LOQ) define as 10 times of LOD was 0.008 mg/kg. The precision of quantitative measurements was checked at each concentration level of five levels by measuring five replications standard solutions. The results (as in Table 4.8) and RSD of the precision test was low than 8.0%, for each of the concentrations.

**Table 4.8** Recovery and precision (RSD) of carbendazim from spiked vegetables

| **Spiked levels** (mg/kg) | **%Recovery±SD** | **Precision** (%RSD) |
|---|---|---|
| 0.050 | 92.6±5.8 | 6.3 |
| 0.075 | 94.1±7.0 | 7.5 |
| 0.100 | 94.5±2.8 | 3.0 |
| 0.200 | 96.0±2.0 | 2.1 |
| 0.300 | 95.6±2.6 | 2.7 |

### 4.3.3 Carbendazim residues in 8 kinds vegetable samples from Chiang Mai city

The validated method was applied to a survey of carbendazim residues in vegetable samples. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.), ginger (*Zingiber officinale* Roscoe), kale (*Brassica albograba* Bailey), cucumber (*Cucumis sativa* L.), yard long bean (*Vigna sesquipedalis* Koprn), guinea-pepper (*Capsicum frutescens* L.), chili (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) were purchased from the four markets in Chiang Mai city and analysed using the present developed method. The results are presented in Table 4.9. Results of the study showed that carbendazim was detected in 24 samples including 3 cauliflowers, 2 gingers, 2 kales, 2 cucumbers, 3 cow-peas, 3 guinea-peppers, 3 chilies and 3 tomatoes. Interestingly, carbendazim was detected in 66% (21 samples) of the vegetable samples although the concentrations were lower than the MRLs established.

**Table 4.9** Carbendazim residue in 8 kinds of vegetable samples from four fresh markets in Chiang Mai city

| **Vegetable samples** (Scientific name) | **No. of samples analyzed** | **No. of samples detected** (%) | **Residue level range** (mg/kg) | **Mean ± SD** |
|---|---|---|---|---|
| Cauliflower (*Brassica oleracea* L.) | 4 | 3(75) | 0.020-0.056 | 0.032±0.021 |
| Ginger (*Zingiber officinale* Roscoe) | 4 | 2(50) | 0.009-0.011 | 0.010±0.001 |
| Kale (*Brassica albograba* Bailey) | 4 | 2(50) | 0.034-0.119 | 0.077±0.060 |
| Cucumber (*Cucumis sativa* L.) | 4 | 2(50) | 0.013-0.20 | 0.016±0.005 |
| Yard long bean (*Vigna sesquipedalis* Koprn) | 4 | 3(75) | 0.008-0.334 | 0.118±0.187 |
| Guinea-pepper (*Capsicum frutescens* L.) | 4 | 3(75) | 0.011-0.081 | 0.036±0.039 |
| Chili (*Capsicum annuum* L.) | 4 | 3(75) | 0.038-0.040 | 0.039±0.001 |
| Tomato (*Lycopersicon esculentum* Mill.) | 4 | 3(75) | 0.014-0.064 | 0.035±0.026 |
| **Total** | **32** | **21(66)** | **0.008-0.334** | **0.045±0.043** |

The present method has been developed for the determination of carbendazim residue and vegetables the use of ethyl acetate is appropriate to be the extractant. The procedure relies on chromatographic equipment and the separation of carbendazim was achieved on a common $C_{18}$ reverse-phase HPLC column with isocratic elution using methanol-water (25:75). The results indicate that the combination of solvent extraction and then cleaned up by passing through the SAX/ PSA dual-layer cartridges provides satisfactory recovery values and very low limit of detection and quantitation lower than Thailand maximum residue limits (MRLs of Thailand). The

used method in this study needed only 5 g sample that was less than the method from the other research that used 10 g sample (QuEChERS method, 2008). It achieved more recovery compared to the other (Pan et al., 2008). Most research used Liquid chromatography–mass spectrometry (LC-MS) that was an expensive equipment, so it was advantage to use a simple method and cheaper equipment by using high performance liquid chromatography-UV detection (HPLC-UV) in this study.

### 4.4 Development of method for determination of mancozeb residue

#### 4.4.1 Optimization of extraction

To study the effect of sample weight the following condition were used 5 and 10 g. The result found that the peak area of 10 g more than 1.5 times of 5 g, so 5 g was selected as sample weight for extraction and has showed in Figure 4.6.

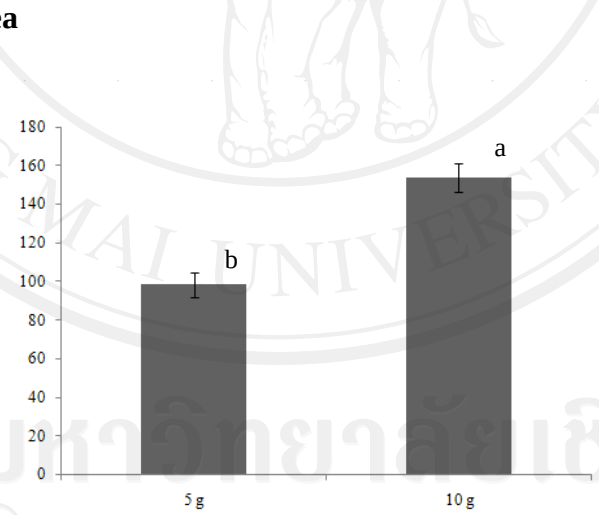



**Figure 4.6** Effect sample weight

(Different superscript letters a and b in the top of results indicate significant at p≤ 0.05)

The optimum concentration of the stannous chloride was studied at 0.1, 0.5, and 1.0% w/v in HCl 5 M. It was found that peak area increase by increasing the concentration of the stannous chloride from 0.1% to 0.5% w/v and becomes constant at 1.0% w/v and peak area are shown in Figure 4.7.

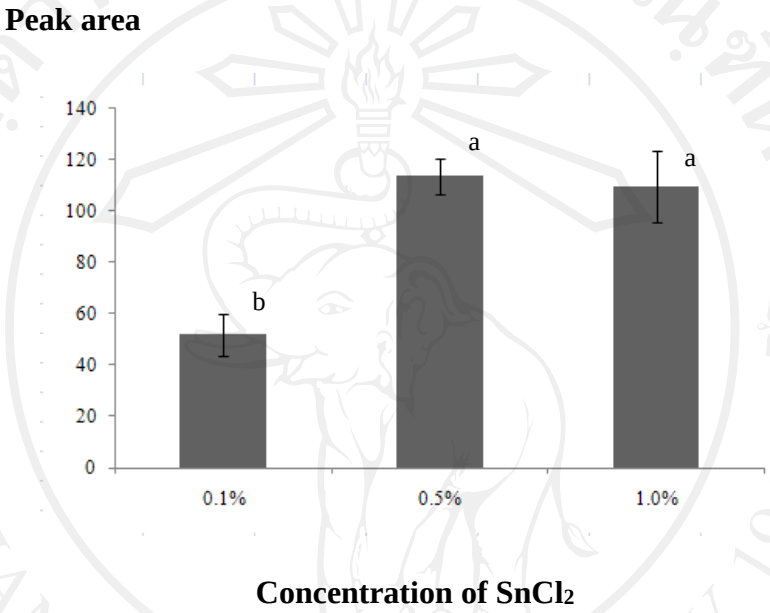
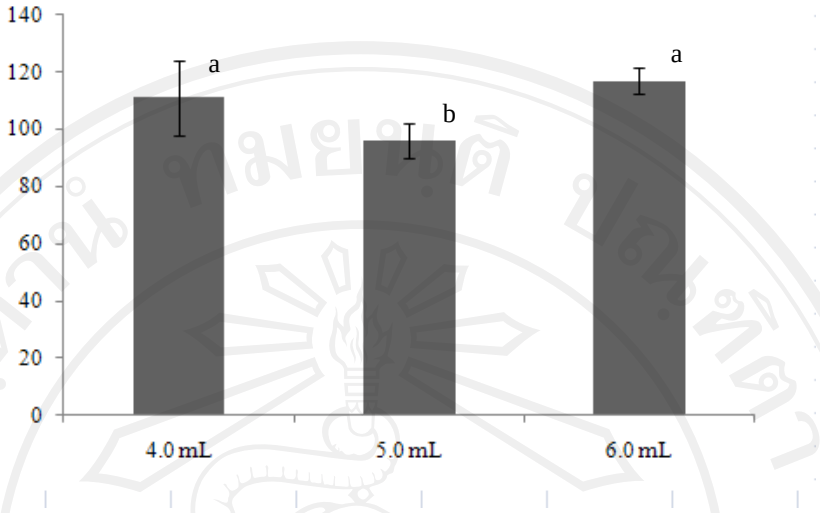



**Figure 4.7** Effect concentration of stannous chloride

(Different superscript letters a and b in the top of results indicate significant at p≤ 0.05)

Next, to determine the volume of the stannous chloride was added with 4, 5, and 6 mL. From this study, appropriate volume was added with 4 mL. Thus, 0.5 % w/v and 4 mL were selected as the concentration and volume of the stannous chloride and peak area of determine the volume of the stannous chloride are shown in Figure 4.8.

**Peak area**




**Volume of $SnCl_2$**

**Figure 4.8** Effect volume of stannous chloride

(Different superscript letters a and b in the top of results indicate significant at $p \leq 0.05$)

The volume of isooctane was added with 0.75, 1.0, and 1.5 mL. The result was found that peak area decrease by increasing the volume of isooctane from 0.75, 1.0, and 1.5 mL, respectively (Figure 4.9). Thus, 1.0 mL was selected as the optimum volume of isooctane because 1 µL aliquots from 1.0 mL of the isooctane layer easy than 0.75 mL of the isooctane layer.

Under the effect of sonication time can be found in Figure 4.10. When the sonication time increased from 10 to 40 min, the peak area of all analytes were increased and no apparent change occurred when the sonication time increased from 40 to 60 min. Thus, 40 min was selected as the optimum sonication time.

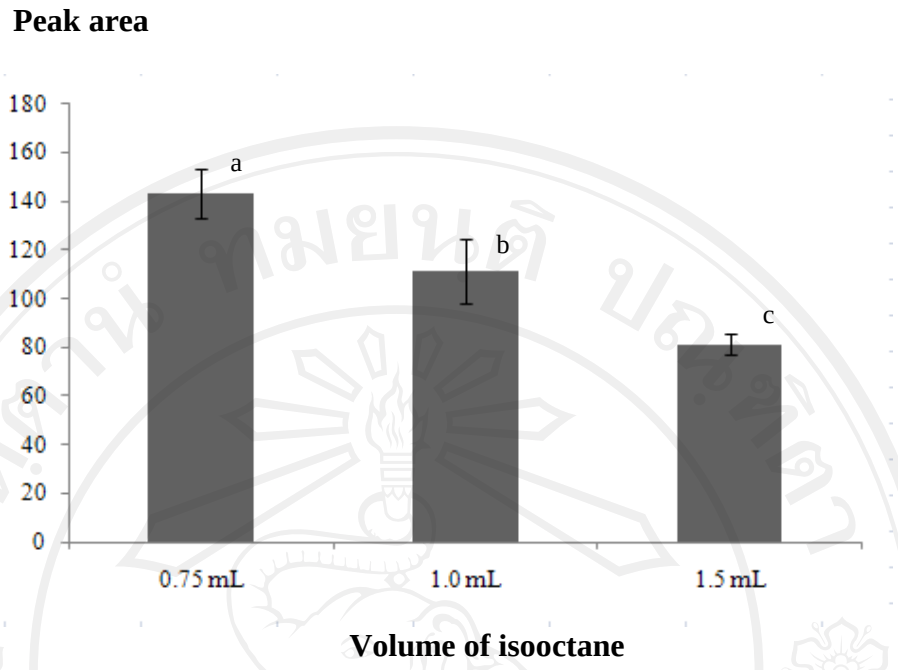



**Figure 4.9** Effect volume of isooctane

(Different superscript letters a, b and c in the top of results indicate significant at $p \leq 0.05$)

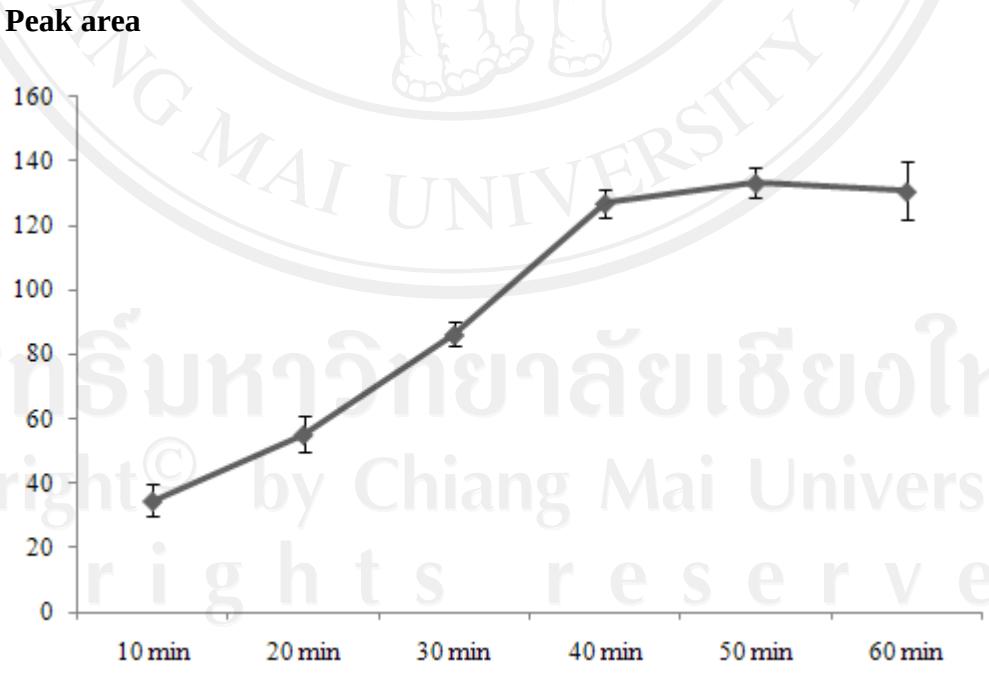



**Figure 4.10** Effect of extraction times

**4.4.2 Calibration curve, reproducibility, limit of detection and limit of quantitation**

In this study, the construction of calibration curve was carried out by mixture sample spiked six concentration of mancozeb (Figure 4.11) and peak area of mancozeb from spiked vegetable samples is shown Table 4.10. Calibration curve was linear in the range 0.25- 2.50 mg/kg and the equation was $y = 100.8x$, with a correlation coefficient $r^2$ = 0.9984. The intra-day precision (repeatability) of the method was assessed by analyzing on the same day five replicated standard at 1.0 mg/kg of mancozeb. The relative standard deviation (RSD) value was 5.84%. The inter-day precision (reproducibility) of the method was checked by analyzing five replicated standard at 1.0 mg/kg of mancozeb for five consecutive days. The RSD value was 5.76%. The limit of detection (LOD) were plotted RSD versus concentration and the equation of the calibration curve was $y = 0.013x + 0.0103$ thus the determined LOD value was 0.01 mg/kg. The limit of quantitation (LOQ) based on a signal-to-noise ratio 10:1 calculation was 0.03 mg/kg.

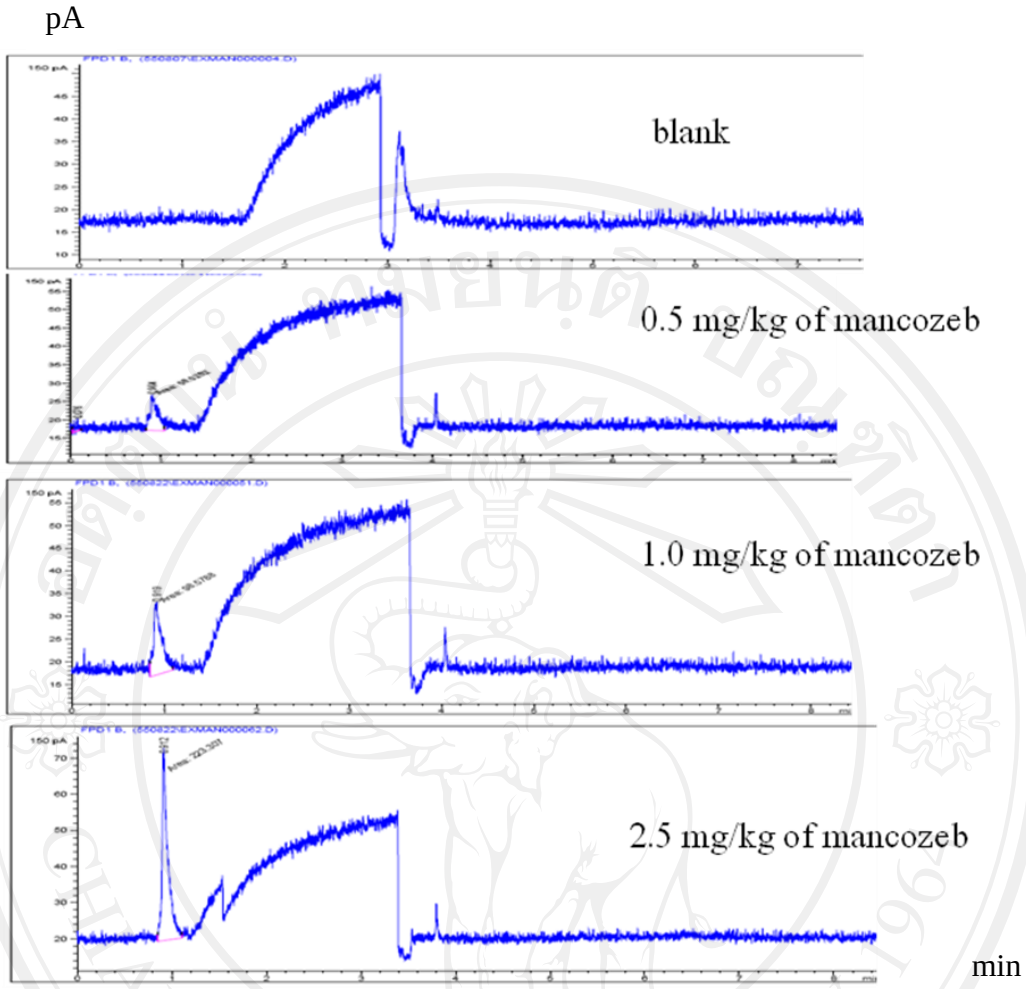



**Figure 4.11** Chromatograms of $CS_2$ from spiking mancozeb in vegetable samples

**Table 4.10** Peak area of mancozeb from spiked vegetable samples

| **Spiked levels** (mg/kg) | **Peak area±SD** |
|---|---|
| 0.25 | 27.4±3.5 |
| 0.50 | 53.6±5.3 |
| 1.00 | 96.8±9.7 |
| 1.50 | 152.2±4.6 |
| 2.00 | 207.3±10.5 |
| 2.50 | 247.3±6.0 |

**4.4.3 Mancozeb residues in 3 kinds of vegetable samples from Chiang Mai city**

An application, we evaluated concentration of mancozeb in 54 samples (3 vegetables: ginger, cucumber, and pepper) from five the fresh markets in Maung, Chiang Mai Province and determination of samples were used optimum extraction. Figure 4.12 shows chromatograms of blank and a mancozeb exposed sample (decompose to $CS_2$). The $CS_2$, which eluted at ~0.9 min, is well resolved from interferences. Positive values were obtained in determining mancozeb residue in pepper and others were all negative. Sample data from this work are shown in Table 4.11. The residue level found in these sample range from 0.12-30.64 mg/kg of mancozeb. Mancozeb was detected in 10 of 54 samples (18.5%), with the high levels (0.14-30.6 mg/kg) found 7 of 14 pepper samples. No residues were found in ginger samples and only 3 of 20 cucumber samples were found rang 0.12-0.27 mg/kg.

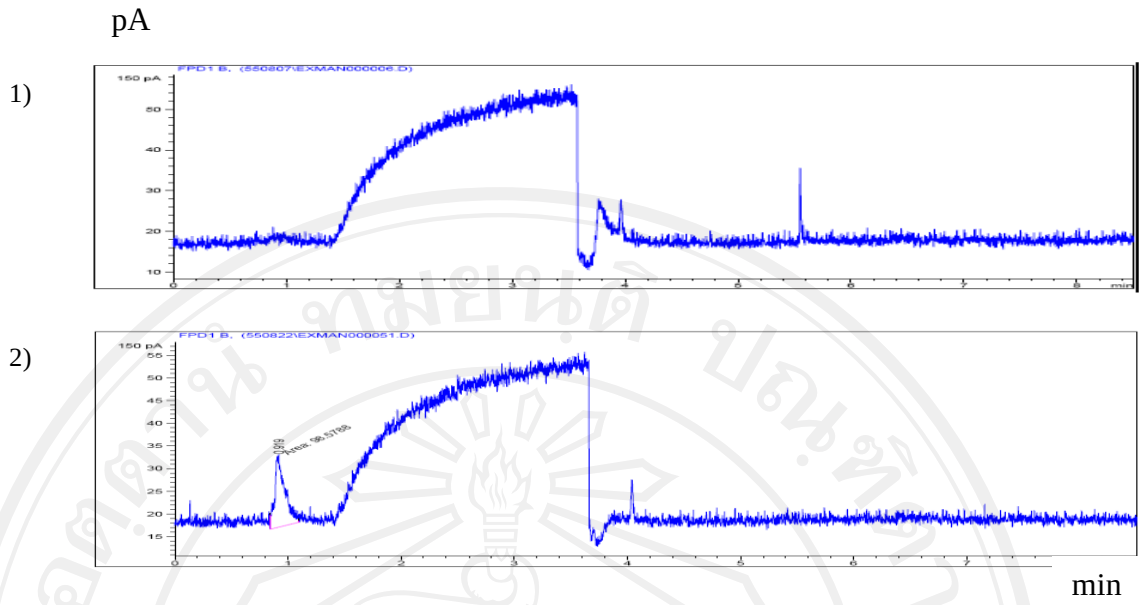



**Figure 4.12** Chromatogram 1) shows blank and chromatogram 2) a mancozeb exposed sample (decompose to $CS_2$).

**Table 4.11** Mancozeb residue in 3 kinds of vegetable samples from five fresh markets in Chiang Mai city

| **Vegetable samples** (Scientific name) | **No. of samples analyzed** | **No. of samples detected** (%) | **Residue level range** (mg/kg) | **Mean±SD** |
|---|---|---|---|---|
| Ginger (*Zingiber officinale* Roscoe) | 20 | - | ND | ND |
| Cucumber (*Cucumis sativa* L.) | 20 | 3(15) | 0.12-0.27 | 0.08±0.11 |
| Guinea-pepper (*Capsicum frutescens* L.) | 14 | 7(50) | 0.14-30.6 | 5.89±11.13 |
| **Total** | **54** | **10(19)** | **0.12-30.6** | **2.99±4.11** |

ND = not detectable

**4.5 Health risk assessment**

The exposure assessment of carbendazim from intake cauliflower, ginger, kale, cucumber and tomato were lower than 100% ADI (0.03 mg/kg) as shown in Table 4.12. As a result the consumption of those 5 vegetables had no effect to the consumer health. Moreover, the exposures of carbendazim from intake of cauliflower, ginger, kale, cucumber, and tomato were 19.3, 0.9, 56.7, 59.6, and 64.1 % of ADI, respectively. However, the exposure of carbendazim from intake tomato was the highest (64.1 % of ADI) and the studied people used them for most cooking, so giving knowledge to the consumers about food safety to choose vegetables from a safe source to avoid the accumulating of carbendazim in their bodies is needed. The exposure assessment of pesticide from consumption food is beneficial data for human health so it should have been up to date and accurate. Therefore, the consuming of tomato is a matter of concern and it is necessary to choose the vegetables come from a safe source to avoid the accumulating of carbendazim in their bodies.

**Table 4.12** Exposure assessment of carbendazim from 5 kinds of vegetables compared to ADI

| **Vegetable samples** (Scientific name) | **CDI ± SD** (mg/kg bw/day) | **%ADI** | **CDI range** (mg/kg bw /day) |
|---|---|---|---|
| Cauliflower (*Brassica oleracea* L.) | 0.006±0.008 | 19.3 | 0.000-0.088 |
| Ginger (*Zingiber officinale* Roscoe) | 0.000±0.001 | 0.9 | 0.000-0.006 |
| Kale (*Brassica albograba* Bailey) | 0.017±0.016 | 56.7 | 0.000-0.090 |
| Cucumber (*Cucumis sativa* L.) | 0.018±0.021 | 59.6 | 0.000-0.154 |
| Tomato (*Lycopersicon esculentum* Mill.) | 0.019±0.021 | 64.1 | 0.000-0.105 |

The exposure assessment of mancozeb from was pepper consumption was lower than 100% ADI (0.03 mg/kg) but cucumber was much higher than 100% ADI and as shown in Table 4.13. The result of consumption of ginger was no effect to the consumer health. The CDI of mancozeb from cucumber, ginger, and pepper consumption were 293, 0.0, and 93.3% of ADI, respectively. The CDI of mancozeb from cucumber consumption was the highest (293 % of ADI) and it was more than has also been reported in previous papers (Caldas et al., 2001).

**Table 4.13** Calculated daily intake (CDI) of mancozeb from 3 kinds of vegetables compared to ADI that set at 0.03 mg/kg bw/day (FAO/WHO, 2005)

| **Vegetable samples** (Scientific name) | **CDI ± SD** (mg/kg bw/day) | **%ADI** | **CDI range** (mg/kg bw /day) |
|---|---|---|---|
| Cucumber (*Cucumis sativa* L.) | 0.088±0.101 | 293 | 0.000-0.105 |
| Ginger (*Zingiber officinale* Roscoe) | 0.000±0.000 | 00.0 | 0.000-0.000 |
| Pepper (*Piper nigrum* L.) | 0.028±0.079 | 93.3 | 0.000-0.090 |