CHAPTER 2

METHODOLOGY

2.1 Safety

Arsenic compounds are carcinogenic and must be handled with appropriate safety precaution including the use of plastic gloves, apron or laboratory coat, safety glasses or mask, and a glove box or fume hood. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters (US EPA-Method 1632, 2001).

2.2 Chemicals and materials

All chemicals obtained from Merck, Sigma and Aldrich were of analytical reagent grade and were used without further purification (Appendix B-1). Distilled de-ionized water was used throughout as matrix for reagent and standard solutions. All plasticwares and glasswares were cleaned by soaking in 6% (v/v) nitric acid (HNO_3) for 24 hours and then were rinsed with de-ionized water.

The details of chemical preparation for chemical reagents, stock solutions and arsenic solutions for standard curves are given in Appendix B-2.

2.3 Instrumentation

2.3.1 Flow injection-hydride generation-atomic absorption spectrophotometer (FI-HG-AAS)

An atomic absorption spectrophotometer (AAS), Perkin Elmer Aanalyst 800, equipped with an electrically heated quartz tube furnace was used. An Arsenic electrodeless discharge lamp (As-EDL) was employed as a radiation source. Arsine generation was

performed with a Perkin Elmer Model FIAS-100 Flow-injection system equipped with a PTFE membrane gas-liquid separator (Figure 2-1).

Instrumental parameters were set up as recommended by the manufacturer (Table 2-1). In this system, the solution of acid and reductant are pumped at the rate of 10 and 6 $mlmin^{-1}$, respectively.

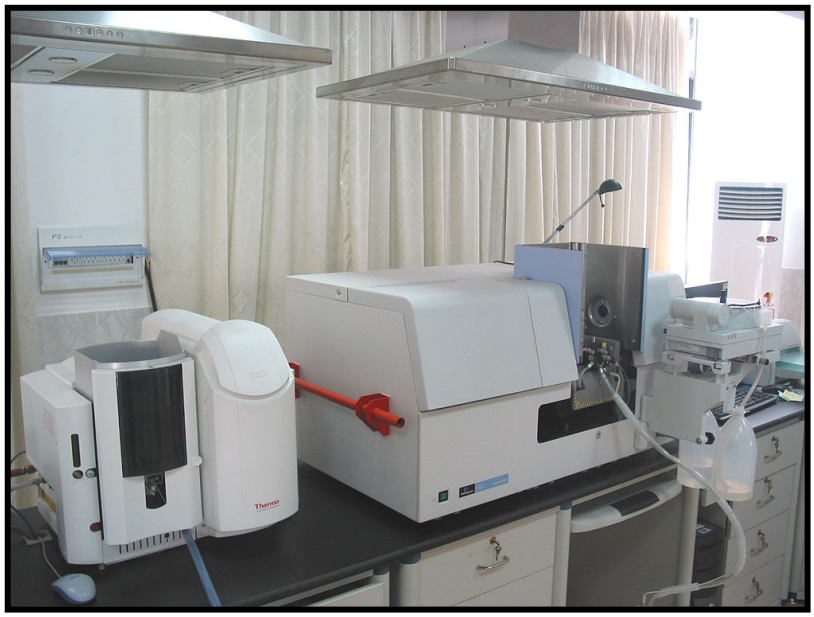


Figure 2-1 FI-HG-AAS system (Perkin Elmer Aanalyst 800)

2.3.2 Square wave cathodic stripping voltammetry (SWCSV)

A potentiostat/ganvanostat AUTOLAB model PG-STAT 100 was used for all voltammetric measurements, interfaced with the multi-mode electrode stand model 663 VA (Metrohm) composed of a hanging mercury drop electrode (HMDE) as working electrode, a Ag/AgCl/3 M KCl as reference electrode and a Pt wire as auxiliary electrode (Figure 2-2).

The electrode cell was equipped with a nitrogen purge tube to remove oxygen prior to sample analysis, as well as to remove gaseous sulfur compounds produced during the reduction step in the total arsenic measurement. Electrochemical parameters for cathodic stripping voltammetry are reported in Table 2-2.

Table 2-1 Optimized operating conditions for FI-HG-AAS

Parameters	**Setting**
Spectrophotometer conditions	
Wavelength	193.7 nm
Slit width	0.7 nm
EDL current	300 mA
Energy	35
Integration time	15 s
Read time	20 s
Signal measurement	Peak-area absorbance
Hydride generation	
Quartz cell	15 cm path length x 8 mm i.d.
Heating	Electrothermal
Temperature	900 ^{o}C
Argon flow rate	40 mlmin^{-1}
Reductant concentration ($NaBH_4$)	0.3% (m/v) in 0.05 M NaOH
Reductant flow rate	6 mlmin^{-1}
HCl concentration	5% (v/v)
HCl flow rate	10 mlmin^{-1}

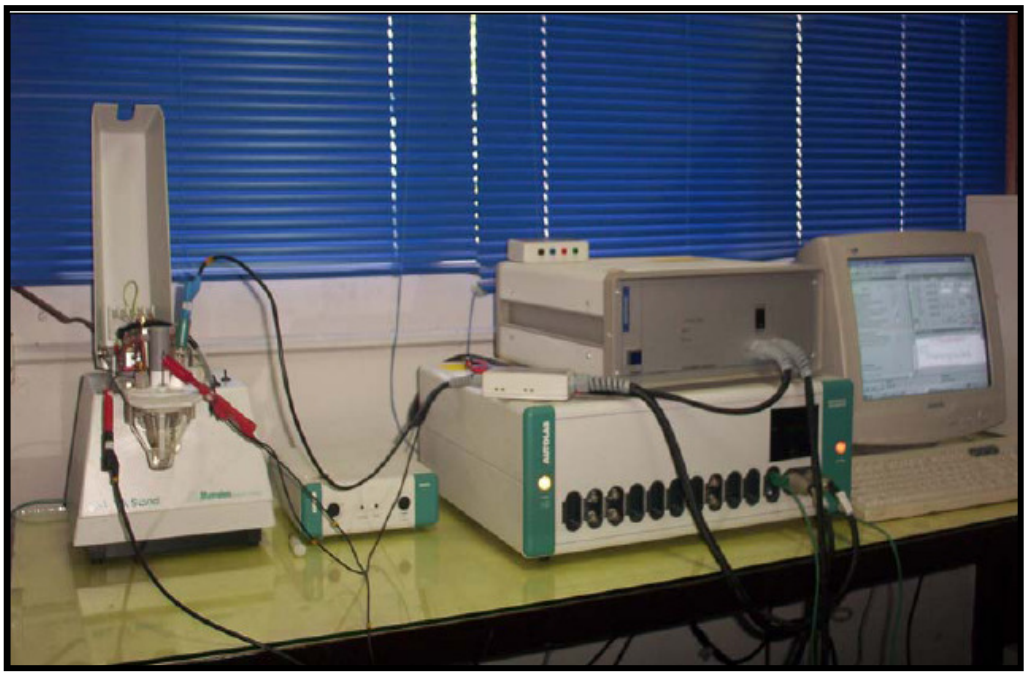


Figure 2-2 Voltammetric equipment (AUTOLAB model PG-STAT 100)

Table 2-2 Optimized operating conditions for SWCSV

Parameters	**As^{III}**	**Total-As_{inorg}**
Deposition potential, (E_{dep})	-0.38 V	-0.39 V
Deposition time, (t_{dep})	30 s	240s
Equilibration time, (t_{eq})	15 s	15 s
Frequency, (f)	200 Hz	200 Hz
Step potential, (E_s)	2 mV	2 mV
Amplitude, (A)	40 mV	40 mV
Stirring	2000 rpm	2000 rpm
Drop size	0.52 mm^2	0.52 mm^2
Cu^{II} concentration	45 mgl^{-1}	600 mgl^{-1}
HCl concentration	2 M	2 M
$S_2O_3^{2-}$ concentration	-	3 mM

2.4 Optimization of operating conditions of FI-HG-AAS

The optimization was carried out by varying the required parameter and keeping other parameters constant. The optimum value was then used for all experiments. The optimization tests were carried out by using 10 μgl^{-1} arsenic stock solution. The following parameters for AAS 800 coupled with FIAS 100 were studied:

2.4.1 Effect of carrier gas flow rate

Argon is needed as carrier gas to transport the formed hydride to the quartz cell atomizer. The carrier gas flow rate generally influences to the sensitivity of the analysis, since increasing the flow rate of carrier gas can affect resting time of arsenic atoms in the atomizer cell (Frank *et al*., 2005). The gas flow rate was investigated within the range 40-100 $mlmin^{-1}$(0, 40, 50, 80, 100 $mlmin^{-1}$) to obtain highest signal intensities for 10 μg As l^{-1} standard solution. Three replicates were performed at each flow rate.

2.4.2 Effect of HCl concentrations

The HCl concentrations are the important parameters because they significantly influence the HG efficiency. (Frank *et al.*, 2005) The concentration of HCl, acting as a carrier solution, was investigated within the range 1-20 % v/v (1, 5, 10, 20 % v/v) to obtain highest signal intensities for 10 μg As l^{-1} standard solution. Three replicates were performed at each concentration.

2.4.3 Effect of $NaBH_4$ concentrations

The $NaBH_4$ concentrations are also considered to be the important parameters that significantly influence the HG efficiency. Optimization of $NaBH_4$ concentration was carried out between 0.1-0.7 % m/v (0.1, 0.3, 0.5, 0.7 % m/v). Then the optimized HCl concentration was

used to obtain highest signal intensities for 10 μg As l^{-1} standard solution. Three replicates were performed at each concentration.

2.4.4 Effect of As^{V} over As^{III} detection

Interference of As^{V} on the As^{III} signal under the analytical conditions selected in this work was evaluated. As^{V}/As^{III} concentration ratios were varied within the range 0.1-1.0, corresponding to 1/10 to 10/10 μg As l^{-1}.

2.4.5 Effect of KI/ascorbic acid concentration

Although the direct determination of As^{V} is desirable, its determination suffers from high detection limits. Additionally, the accuracy and precision for the direct determination of As^{V} by HG-AAS in acid digests of plant samples was only about 20-40% much lower compared to the target values (Frank *et al*., 2005).

As the direct determination of As^{V} failed, quantitative pre-reduction of As^{V} to As^{III} is necessary to obtain accurate results and optimum sensitivity. The most popular pre-reductant is potassium iodide (KI) in combination with ascorbic acid, with the latter preventing the oxidation of iodide by air (Chen *et al*., 1992 cited in Frank *et al*., 2005). The reduction of As^{V} with KI prior to the reaction with $NaBH_4$ occurs according to the scheme:

$$AsO_4^{3-} + 2I^- + 2H^+ \rightarrow AsO_3^{3-} + I_2 + H_2O \qquad (2\text{-}1)$$

Various concentrations of KI (0, 1, 3, 5, 7, 9 %, w/v) stabilized with ascorbic acid (5%, w/v) were tested for their potential to quantitatively reduce As^{V} to As^{III}. Three replicates were performed at each concentration.

2.4.6 Effect of reducing time

The pre-reduction times for the optimized KI/ascorbic acid concentration were also investigated to completely reduce As^{V} to As^{III}. Various times (0, 15, 30, 45, 60, 75 min) were tested to obtain highest signal intensities for 10 μg As l^{-1} standard solution into three replicates each.

2.4.7 Efficiency of reduction of As^{V} to As^{III}

With the optimized KI/ascorbic acid concentration, the efficiency of reduction of As^{V} to As^{III} was evaluated. The efficiency of conversion were tested by adding the pre-reductant to various concentrations of As^{V} standard solution (2, 4, 6, 8, 10, 15, 20 μg l^{-1}) and the % recoveries of As^{V} were observed.

2.5 Optimization of operating conditions of CSV

2.5.1 Optimization of conditions for the determination of As^{III}

Since only As^{III} is electroactive during the SWCSV procedure, optimization procedures for all parameters were carried out using As^{III} solution. Each parameter was optimized by varying it while keeping others constant. The optimum value was then used for all experiments.

In the optimization of the instrumental and the chemical conditions, an appropriate amount of As^{III} standard solutions were added to the analysis vessel containing de-ionized water, making the total volume of 10 ml, followed by the addition of 12 M HCl to provide a 1 M HCl supporting electrolyte and 1000 mgl^{-1} Cu^{II} to provide the final concentration of 45 mgl^{-1} (Ferreira and Barros, 2002). A purge of N_2 for 30 s was applied after varying the parameters. The HMDE was scanned from -0.50 V to -1.0 V with square wave pulse with frequency of 200 Hz, a potential step of 2 mV and an amplitude of 100 mV, with an equilibrium time of 15 s after stirring. The following parameters for SWCSV (AUTOLAB model PG-STAT 10) were studied:

2.5.1.1 Deposition potential

To achieve maximum sensitivity in the voltammetric response, first of all the deposition potential was be examined. With standard solution containing 20 $\mu gl^{-1} As^{III}$ and 45 mgl^{-1} Cu^{II} , the deposition potential was varied from -0.30 to -0.50 V versus reference electrode to obtain the highest signal. Three replicates were performed for each.

2.5.1.2 Deposition time

To increase peak current and improve method sensitivity, the influence of deposition time was investigated. For As concentration 20 μgL^{-1} and 45 mgL^{-1} Cu^{II}, deposition time between 0-240 s (0, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 s) were evaluated and three replicates were performed for each time.

2.5.1.3 HCl concentration

In previous studies of several researchers, it was found that chloride plays an important role in the process of deposition of an intermetallic compound (Cu_xAs_y) on the electrode. The function of chloride is to stabilize Cu^{I} formed in an intermediate step, while As^{III} is reduced to As according to the following reaction:

Accumulation step: $As + 3CuCl_3^{2-} + 3e^- \rightarrow Cu_3As + 9Cl^-$ (2-2)

Stripping step: $Cu_3As + 3H^+ + Hg + 3e^- \rightarrow Cu(Hg) + AsH_3$ (2-3)

The high concentration of chloride at the electrode surface can increase the amount of As-Cu compound deposited, whereas too much concentration of chloride can cause positive shift of peak current because it favors the reductions of As^{III} and Cu^{II} (Smart *et al*., 1996; Ferreira *et al*., 2002 and Profumo *et al*., 2005). In this work the concentrations of HCl as supporting electrolyte were examined. HCl concentrations from 0-5 M (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 M) were experimented and three replicates were performed for each.

2.5.1.4 Copper (II) concentration

Arsenic cannot be directly electrolytically deposited onto a Hg electrode. Normally, As^{III} reacts with Cu^{II} to form an intermetallic compound (Cu_xAs_y) that is deposited onto the HMDE and is subsequently stripped cathodically. The amount of Cu^{II} was investigated by varying concentration between 0-120 mgl^{-1} (0, 1, 15, 30, 45, 60, 75, 90, 105 and 120 mgl^{-1}) to obtain the highest signal and three replicates were performed for each.

2.5.2 Optimization of conditions for the determination of Total-As (TAs)

The previous conditions were optimized for TAs, with the difference that As^{V} was used instead of As^{III}. Each parameter was optimized by varying it while keeping others constant. The optimum value was then used for all experiments.

In the optimization of the instrumental and the chemical conditions, an appropriate amount of As standard solutions was added to the analysis vessel containing de-ionized water, making the total volume 10 ml, followed by addition of 12 M HCl to provide a 2 M HCl supporting electrolyte, 1000 mgl^{-1} Cu^{II} to provide 400 mgl^{-1} and 0.5 M thiosulfate to provide 3.2 mM (Ferreira and Barros, 2002). A purge of N_2 for 30 s was applied after varying the parameters. The HMDE was scanned from -0.50 V to -1.0 V under the following condition: deposition potential, -0.38 V versus Ag/AgCl/KCl(3M); deposition time, 120 s; square wave pulse frequency, 200 Hz; potential step, 2 mV; square wave amplitude, 100 mV and equilibrium time of 15 s after stirring. The following parameters for SWCSV (AUTOLAB model PG-STAT 100) for the determination of total inorganic arsenic were studied:

2.5.2.1 Deposition potential

To achieve maximum sensitivity in the voltammetric response, first of all the deposited potential was examined. For As^{V} standard solution containing 20 μgl^{-1} and 400 mgl^{-1}

Cu^{II} , the deposition potential was varied from -0.37 to -0.42 V versus the reference electrode to obtain the highest signal. Three replicates were performed for each.

2.5.2.2 Deposition time

The influence of deposition time on the current signal was investigated. For As^{V} concentration 20 μgl^{-1} and 400 mgl^{-1} Cu^{II}, deposition time between 30-390 s (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 and 390 s) were evaluated and three replicates were performed for each.

2.5.2.3 Copper (II) concentrations

In previous study found that in the presence of thiosulfate (pre-reduction process), Cu^{II} was less efficient in promoting the accumulation of As at the electrode, a situation that required the use of much higher concentration of Cu^{II} (Ferreira and Barros, 2002). Therefore, the effect of Cu^{II} was investigated by varying its concentrations between 100-800 mgl^{-1} (100, 200, 300, 400, 500, 600, 700 and 800 mgl^{-1}) to obtain the highest signal.

2.5.2.4 Reducing agent concentrations

In general, the determination of total arsenic by using electrometric techniques requires a preliminary step to reduce As^{V} to As^{III}, followed by the determination of this last species. Several reducing agents, such as hydroxylamine, oxalic acid, hydrazinium dichloride, potassium iodide, bromidic acid, potassium dissulfite, sodium sulfate and sodium thiosulfate, have been tested to reduce As^{V} to As^{III}. The more convenient reductant was found to be sodium thiosulfate and it was used through this work (Ferreira and Barros, 2002). The reduction of As^{V} with thiosulfate prior to the formation of intermetallic compound with Cu^{II} occurs according to the scheme:

$$AsO_4^{3-} + 2S_2O_3^{2-} + 2H^{+} \rightarrow AsO_3^{3-} + S_4O_6^{2-} + H_2O \qquad (2\text{-}4)$$

Various concentrations of sodium thiosulfate (0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.0, 3.2, 3.6 and 4.0 mM) were tested to quantitatively reduce As^{V} to As^{III} with three replicates for each.

2.5.2.5 Reducing time

The pre-reduction time for the optimized thiosulfate concentration was also investigated to find the optimum time to completely reduce As^{V} to As^{III}. Various time periods (0, 5, 10, 20 and 30 min) were tested to obtain the highest signal intensities for 20 μgl^{-1} As^{V} standard solution with three replicates for each.

2.5.2.6 Efficiency of reduction of As^{V} to As^{III}

With the optimized thiosulfate concentration, the efficiency of reduction of As^{V} to As^{III} was evaluated. The efficiency of conversion was tested by comparing the results obtained in the analysis of five solutions with the same total arsenic concentrations (20 μgl^{-1}) but different ratios of As^{III}/As^{V}. Three compositions of 20 μgl^{-1} As (total) solutions (20/0, 10/10, and 0/20 μgl^{-1} for As^{III}/As^{V} ratios) were tested with three replicates for each.

2.6 Analytical performances of FI-HG-AAS and CSV methods

2.6.1 Linear range

Concentrations of arsenic standard in the range of 0-80 μgl^{-1} and 0-150 μgl^{-1} for FI-HG-AAS and CSV techniques were studied for investigating the linearity and range which were obtained by making a calibration curve to get the corresponding correlation coefficient.

2.6.2 Limit of detection (LOD)

The limit of detection (IUPAC definition) is expressed as the smallest concentration that can be detected with a certainty of more than 95%. Determination of the limit of detection for FI-HG-AAS and CSV techniques in this work were studied based on $3\sigma/m$ (Coelho *et al*., 2002) where:

σ = the standard deviation of 10 measurements of blank signal

m = the slope of the calibration graphs

2.6.3 Limit of quantification (LOQ)

The limit of quantification is expressed as the smallest concentration that can be quantified with suitable precision and accuracy. Usually the limit of quantification is evaluated as the signal-to-noise ratio that equivalent to 10 times the standard deviation of the noise ($S/N=10\sigma$). However, the determination of the limit of quantification for FI-HG-AAS and CSV techniques in this work were studied based on $10\sigma/m$ (Coelho *et al*., 2002) where:

σ = the standard deviation of 10 measurements of blank signal

m = the slope of the calibration graphs

2.6.4 Precision

Precision is the measure of the degree of and analytical method under the same conditions. Normally it is expressed as a percentage of the relative standard deviation (%RSD) for a statistically significant number of samples. The calculation of %RSD is given as:

$$\%\text{RSD} = \frac{S}{\overline{X}} \times 100 \quad ; \quad S = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \overline{x})^2}{(n-1)}} \qquad (2\text{-}5)$$

Where:

S = standard deviation

n = total number of values

x_i = each individual value used to calculate mean

$\bar{x}$ = mean of n values

In this study, the precisions were investigated to measure the degree of repeatability and were used for the analyses of samples. The experiments for the edible plant samples were repeated 10 times.

2.6.5 Accuracy

The accuracy term is the measurement of exact value of the analyte concentration or the agreement between measured value and certified value or an accepted reference value. Normally, the accuracy value is expressed as the relative percent error term. The following calculation of relative percent error is given below: (http://www.jesuitnola.org/upload/clark/labs/PerError.htm).

$$\%Error = \frac{(Measured\ value - Real\ value)}{Real\ value} \times 100 \qquad (2\text{-}6)$$

The accuracy from this research was studied by using Certified Reference Material (CRM), Virginia Tobacco leaves (CTA-VTL-2) from Poland, following the same method as used for plant samples extraction and determination (three replicates). The difference in values between the measured value and certified value was compared and the percent error was calculated.

2.6.6 Recovery

The terms recovery (*R*) is used to indicate the yield of an analyte in an extraction stage in an analytical method. Generally, the recovery value is presented as a percent recovery (%R) and can be calculated from the equation below.

$$\%R = \frac{Measured\ value}{\mathrm{Re}\,al\ value} \times 100 \qquad (2\text{-}7)$$

In this study, since no reference material was available for As^{III}, recovery studies on spiked samples were carried out. The % recovery from edible plant samples were spiked with 1, 10 and 20 μg As l^{-1} for HG-AAS method and 20 and 30 μg As l^{-1} for CSV method. All spiked samples were left overnight before the extraction stage was performed. Three replicates were carried out for each.

2.7 Application to edible plant samples (arsenic speciation analysis)

2.7.1 Sample collection

According to previous work (i.e. Na Chiangmai, 1999 and Rakwong, 1999) on a risk assessment study of arsenic in Ron Phibun sub-district, Ron Phibun district in Nakhon Si Thammarat province, it was concluded that village 1, 2, 12 and 13 were identified as high risk areas, whereas village 8, 9, 11 and 14 were low risk areas (Na Chiangmai, 1999 and Rakwong, 1999). In this work the study area was restricted to the high risk arsenic-contaminated villages (village 1, 2, 12 and 13; Ban Hudan, Ban Ronna, Ban Talardronphibun and Ban Salakheleg), where the agriculture production is for self-consumption and trading, of Ron Phibun sub-district, Ron Phibun district in Nakhon Si Thammarat province. However, the sample from village 12 was not included in this case because this area has no agriculture activity.

The sampling sites are illustrated in Figure 2-3. Edible plant samples were taken according to the type of harvest. Table 2-3 and Figure 2-4 show the common vegetables for

consumption and trading at the local market of Ron Phibun sub-district, Ron Phibun district in Nakhon Si Thammarat province.

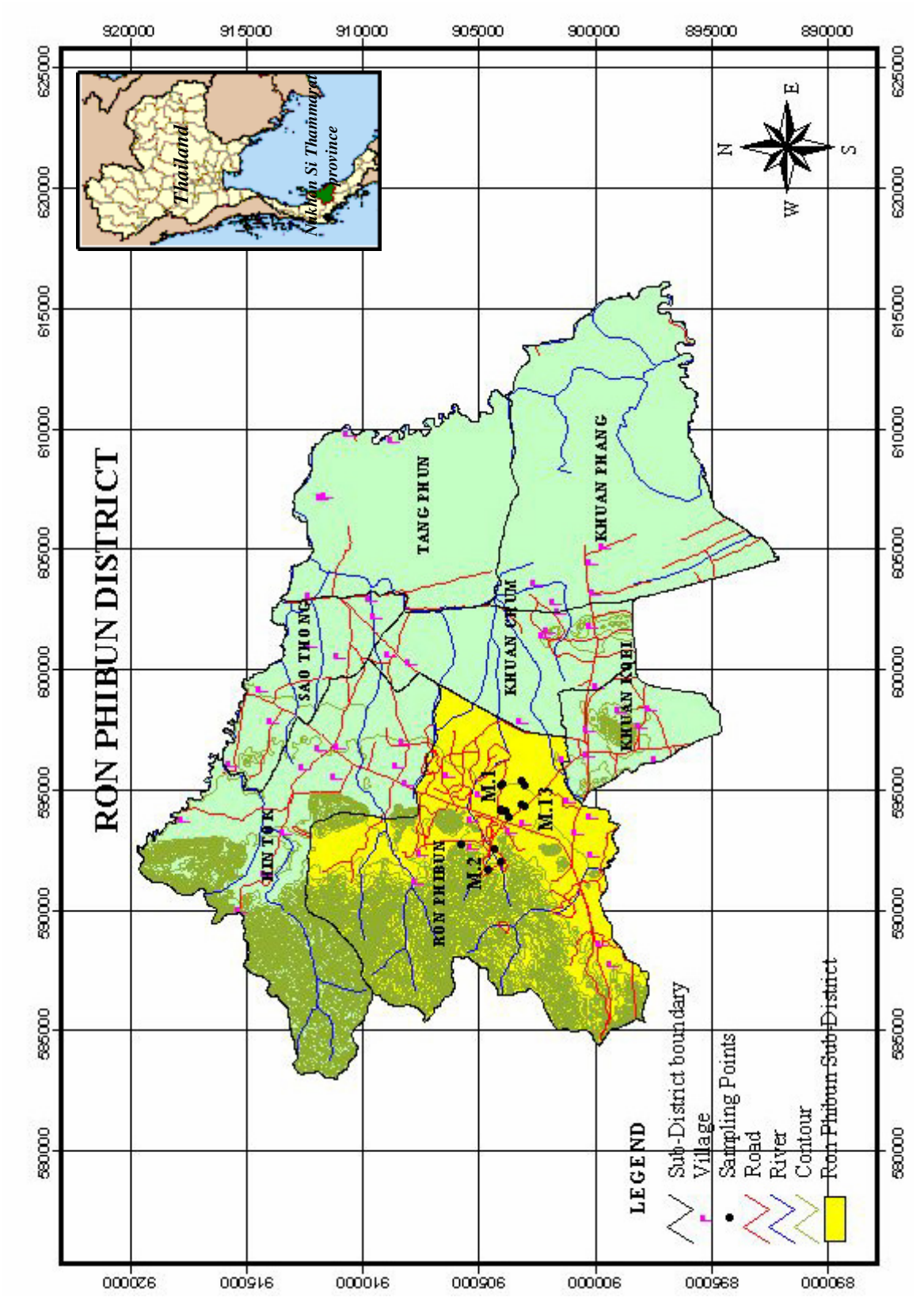
Table 2-3 Common vegetables for direct consumption and trading at the local market

Common name	**Scientific name**	**Code of samples**
Sugar Apple, Sweetsop	Annona squamosa Linn. Ann L.	
Lemongrass, Lapine	Cymbopogon citratus (DC.)	Stapf. Cym S.
Turmeric, Curcuma	Curcuma Longa Linn.	Cur L.
Galanga, Greater Galangal	Alpinia galanga Stunz.	Alp S.

Source: www.samunpri.com/modules.php?name=Herb&file=Herbs_kitchen(11/1/06)

Fifteen samples of Lemongrass and fifteen of samples of Turmeric were sampled in the field (high risk area) with a random sampling procedure and each sample consisted of a number of sub-samples taken within an area of 100 x 100 m or less. Each sampling site was identified by using the global positioning system (GPS) are shown in Appendix C-1. Two types of edible plants were sampled by hands protected with plastic gloves. Each sample was selectively chopped to obtain the part which is normally consumed (stem for Lemongrass and tuber for Turmeric) and then carefully packed into plastic bags after being cleaned with de-ionized water and weighed in situ. The samples were stored in a cold box at 4 ^{o}C and transported to the laboratory.

At the laboratory, the samples were washed briefly again with de-ionized water prior to ambient air dried and then homogenized with a high speed homogenizer. All the samples were frozen at -20 ^{o}C. After that the freeze-drying was performed at 22 ^{o}C with the final pressure of 6.2 x 10^{2} mbar. The dried samples were ground to powder with mortar and pestle. The water was driven away by freeze-drying because the water removed by an oven is not recommended for arsenic speciation analysis (heat increased may lead to the transformation of arsenic species). Water content values were in the 84-90% range for Lemongrass and 83-91% for Turmeric (Appendix C-2).



Source: Southern Remote Sensing & GIS Centre, Prince of Songkla University

Figure 2-3 Map showing the location to collect samples

Sugar Apple

Annona squamosa Linn.

Lemongrass

Cymbopogon citratus (DC.)

Turmeric

Curcuma Longa Linn.

Galanga

Alpinia galanga Stunz.

Figure 2-4 Common vegetables for consumption and trading at the local market of Ron Phibun district, Nakhon Si Thammarat province

2.7.2 Water extraction for arsenic speciation

A critical requirement for obtaining accurate arsenic speciation information is maintaining the concentration and chemical forms of the original species through the sample extraction and preparation. The correct sample preparation procedure is essential to obtain accurate speciation analysis data (Gong *et al*., 2002).

Freeze-dried samples were ground to powder with mortar and pestle. A portion of the freeze-dried sample (*ca* 100 mg) was weighed into a centrifuge tube and nanopure water (5.00

ml) was added. The sample was extracted by placing the tubes in an ultrasonic bath for 1.5 h. The mixture was centrifuged (50,000 g, 30 min), and the supernatant decanted from the pellet. The above extraction procedure was carried out on the pellet two more times. The three supernatants were combined just before arsenic speciation analysis (As^{III} and As^{V}) by FI-HG-AAS and CSV, respectively (Khokiattiwong, S., 2001). In the case that inorganic arsenic concentration can not be detected in samples, the increase of the sample amount was necessary.

2.7.3 Sample digestion for total arsenic determination

A sample of 250 mg (dry weight) was transferred into 50 ml beaker, than 5 ml HNO_3, 1 ml $HClO_4$ and 0.5 ml H_2SO_4 were added and a watch glass was covered the beaker. The mixture was heated at 100 ^{0}C to drive away the acid and concentrated to about 1 ml. The clear solution was diluted to 25 ml with de-ionized water before analysis (Zhao *et al*., 2006). The total arsenic content was then measured by FI-HG-AAS and CSV, respectively. In the case that total acid-digested arsenic concentration can not be detected in sample, the increase of the sample amounts was necessary.

2.7.4 Determination of inorganic arsenic species

The inorganic arsenic species in edible plant samples were evaluated following optimization of the two methods. Speciation of inorganic arsenic (As^{III}, As^{V}) was carried out in two stages, estimation of total water-extracted inorganic As (TAs) followed by As^{III}. As^{V} was determined by subtraction ($As^{V} = TAs - As^{III}$).

2.7.4.1 Determination of arsenic species by FI-HG-AAS

For the determination of As^{III} by HG-AAS, an aliquot (1 ml) of the extracted samples was transferred into a 10 ml PTFE tube and then diluted to 10 ml with 5% v/v HCl.

For the determination of total water-extracted inorganic arsenic (TAs), an aliquot (1 ml) of the water-extracted samples was transferred into a 10 ml PTFE tube and then 1 ml conc.

HCl and 1 ml of a solution containing 3% m/v KI/ascorbic acid were added. After 45 minutes at ambient temperature, it was diluted to 10 ml with 5% v/v HCl.

In case of the determination of total acid-digested arsenic, an aliquot (1 ml) of the acid digestion solution was transferred into a 10 ml PTFE tube and then 1 ml conc. HCl and 1 ml of a solution containing 3% m/v KI/ascorbic acid were added. After 45 minutes at ambient temperature, it was diluted to 10 ml with 5% v/v HCl.

2.7.4.2 Determination of arsenic species by CVS

For the determination of As^{III} by SWCSV, an appropriate amount of standard or extracted sample was transferred to the analysis vessel containing de-ionized water, making the total volume 10 ml, followed by addition of conc. HCl to provide a 2 M HCl supporting electrolyte. Optimized amount of the 1000 mgl^{-1} Cu^{II} solution was also added to produce concentration of 45 mgl^{-1}. Sample was purged for 30 s with N_2 before SWCSV analysis was performed.

For the determination of TAs by SWCSV, an appropriate amount of standard or extracted and digested sample was transferred to the analysis vessel containing de-ionized water, making the total volume 10 ml, followed by the addition of conc. HCl to provide a 2 M HCl supporting electrolyte and of 0.5 M thiosulfate to yield a 3 mM of the reducing agent. Optimized amount of the 1000 mgl^{-1} Cu^{II} solution was also added to produce the concentration of 600 mgl^{-1}, the sample was purged for 30 s with N_2 before SWCSV was performed.