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FULL REPORT

Effects of Tea Infusion Conditions on Aluminum and Manganese Concentrations

By

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PREFACE

Tea is a rich source of several elements such as aluminum and manganese. These elements can have positive or negative health effects, depending on the amount consumed. Since the amount of elements is likely to vary by preparation conditions; it was thus of interest to determine the elements concentrations in Chiang Rai tea infusions prepared by different conditions that might be encountered among tea drinkers. The amount of exposure to these elements by drinking the beverage was assessed in order to provide information on whether Chiang Rai tea contains the elements in amounts that could be toxic at the normal doses usually consumed as beverages. In addition, the information should be useful to support Chiang Rai tea quality and lead to the development of an element profile for Chiang Rai tea in the future.

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ABSTRACT

Effect of tea infusion conditions, *i.e.* water temperature (80, 90, 100 °C) and infusion time (1, 3, 5, 10 min), on Al and Mn concentrations in infusions prepared from three types of tea (black, green and oolong tea) was studied. Concentrations of Al and Mn were determined using ICP-OES and Flame AAS, respectively. The results indicate that the infusion temperature and time has significantly effect on the element concentrations. The higher the water temperature and the longer infusion time, the higher the Al and Mn concentrations. However, after a certain infusion time, the diffusion rate of the metals became lower due to less difference of metals concentration at the surface of tea leaves and in the bulk solution. The results also demonstrated that the metals contents changed significantly between types of tea. The metals contents were found higher in black and green tea, depending on the investigate metal and infusion conditions, than oolong tea. The amounts of Al and Mn in the later were exceptionally low at all investigated infusion conditions. This may be partially due to the form of tea leaves: its rolled leaves caused the slow water uptake and hence the slower dissolution of the metals.

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Phunrawie Promnart
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CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Tea is the most consumed beverage next to water and is prepared from the leaves of the shrub *Camellia sinensis*. Tea is grown commercially in a large number of countries. The major tea exporting countries of the world are Kenya, China, India, Indonesia and Sri Lanka. In Thailand, Chiang Rai is the largest cultivating area and, therefore, produces most tea products. Most tea plants grown in Chiang Rai are Assam variety *(Camellia sinensis* var. *assamica*), and China variety *(Camellia sinensis* var. *sinensis).* Majority of fresh tea leaves are processed into different types of leaf tea, namely non-fermented green tea, partially fermented oolong tea, and fully fermented black tea, which are classified according to the manufacturing process. The rest are processed into various tea products such as instant tea and ready-to-drink beverages (Extension 2006).

Tea contains polyphenols, minerals and trace elements that are essential to human health. Tea is a rich source of several elements, such as aluminum (Al), calcium (Ca), potassium (K), and manganese (Mn), which are present in mg/g level (Kumar, Nair et al. 2005). Aluminum and Mn were of interest in this work. Aluminum has been implicated in some important human diseases, for example dialysis encephalopathy, Parkinson's disease and Alzheimer's disease (Exley and Korchazhkina 2001). Typical levels of Al in tea infusions are 1 – 6 mg/L (Flaten and Lund 1997; Odegard and Lund 1997), making tea a major potential source of dietary Al intake. High Al content in tea, thus, could have negative health effects, especially in heavy tea drinkers.

Manganese is an essential element; its principal function is to activate numerous essential enzymes (Xie, Bohlen et al. 1998). Tea is a rich source of Mn, found in the range of 0.35 - 2.2 mg/g in tea leaves and about 30% is extracted with water during infusion (Kumar, Nair et al. 2005). However, high dose of dietary Mn could be associated with long-term toxicity. Concentrations of these elements in Chiang Rai tea, thus, were important and studied.

Amounts of elements in tea are affected by many factors, such as age of tea leaves, origin, plantation area, manufacturing process, and tea infusion conditions (Flaten 2002). This work aims to study the effect of infusion conditions, *i.e.* water temperature and infusion times, to the Al and Mn concentrations in the infusions of leaf teas manufactured in Chiang Rai. The data obtained provides information on whether the tea contains the elements in amounts that could be toxic at the normal doses usually consumed as beverages. The information can also be used to support the tea quality and will lead to the development of an element profile for Chiang Rai tea in the future.

1.2 Objectives

1) To determine the Al and Mn concentrations in Chiang Rai Tea

2) To study the effect of tea infusion conditions, namely water temperature and infusion time, to aluminum and manganese concentrations in Chiang Rai tea

3) To obtain the element profile of Chiang Rai tea in the future

1.3 Scope of study

The tea samples selected from a local producer were black tea, green tea, and oolong tea. The infusion conditions, namely water temperature and infusion times, were varied, in order to study the effect of the infusion condition to the elements concentrations. Water temperatures of 80 °C, 90 °C, and 100 °C, and infusion times of 1, 3, 5, and 10 minutes were chosen. Determination of Al quantity was performed using an inductively coupled plasma optical emission spectrometer (ICP OES). The quantity of Mn was analyzed using flame atomic absorption spectrophotometer (FAAS). Elements concentrations in tea samples were compared.

1.4 Benefits

1) Elements (Al and Mn) quantities in Chiang Rai tea infusions were determined. The optimum infusion conditions were established. These results are useful for tea consumers, in order to avoid over-consumption and their cumulative toxicities in long-term use.

2) The data obtained from this work could consequently be extended to the element profile for Chiang Rai tea. The developed element profile of Chiang Rai tea could help promoting the tea to the world market.

3) These results are benefits to consumers, chemists, agriculturists, and manufacturers.

4) This work could be published in an international journal.

CHAPTER 2

LITERATURE REVIEWS

2.1 Tea

Tea (*Camellia sinensis*), which is originated in southern China, is now grown commercially in a large number of countries, the northernmost being Georgia in the former Soviet Union, and the southernmost being South Africa and Argentina. Tea plants are also widely cultivated in Southeast Asia, including China, India, Japan, Taiwan, Sri Lanka, and Indonesia and in central African countries. The major tea exporting countries of the world are Kenya, China, India, Indonesia and Sri Lanka. In Thailand, Chiang Rai has the largest tea plantation area, approximately 60,604 rai. In 2006, Chiang Rai produced 11,286.44 tons of tea leaves, which was approximately 50% of total tea products produced in Thailand (Extension 2006) .

Most tea plants grown in Chiang Rai are Assam variety *(Camellia sinensis* var. *assamica*), and China variety *(Camellia sinensis* var. *sinensis).* Majority of fresh tea leaves are processed into leaf tea. The rest are manufactured into various tea products such as instant tea and ready-to-drink beverages. Leaf teas are generally classified into three major categories: non-fermented green tea, partially fermented oolong tea, and fully fermented black tea, depending on the manufacturing process, but they are made from the same plant. To produce black tea, the leaves are air-dried (withered) before they are bruised through rolling and cutting to activate the endogenous enzyme polyphenol oxidase. This starts the fermentation process, which largely consists of oxidation of the polyphenols present in the tea leaves. When the quality is judged optimal, fermentation is arrested by drying. In contrast, green tea is not (or only very lightly) fermented, but the leaves are treated by steaming or pan firing to inactivate the polyphenol oxidase, thus avoiding oxidation. Oolong tea is an intermediate type of tea, produced employing a shorter fermentation time than for black tea (Flaten 2002).

Infusions derived from tea leaves are one of the most popular beverages in the world as it has an attractive aroma, good taste, and health-promoting effects (Lin, Tsai et al. 2003). The chemical composition of tea leaves and manufactured tea consists of tanning substances, flavonols, alkaloids, proteins and amino acids, enzymes, aroma-forming substances, vitamins, minerals and trace elements (Jha, Mann et al. 1996). Tea is thus an important source of some elements, especially Al, Ca, Na, K, Mg, and Mn that are present in mg/g level (Kumar, Nair et al. 2005). Aluminum and Mn are of interest in this study.

2.2 Aluminum in tea and tea infusion

Tea is well-known to be a typical Al accumulator. It takes up Al throughout its life due to the high solubility of Al in acid soils and can accumulate great quantities of Al in tea leaves, ranging from 8700 to 23000 mg/kg, and even up to 30000 mg/kg in old leaves (Chen, Wang et al. 2006). The total concentration of Al in tea infusion, with very few exceptions, was found in the range 1 - 6 mg/L, and a cup of tea may thus contain approximately 0.2 - 1.0 mg Al, in comparison with the estimated dietary intake of Al of 3 - 10 mg per day (Flaten and Lund 1997). This makes tea a major source of dietary Al intake.

Aluminum is a powerful neurotoxicant, and has a potential for skeletal and haematopoietic toxicity, especially in patients on dialysis due to chronic renal failure (Flaten, Alfrey et al. 1996). High Al levels has been implicated in some important human diseases, for example dialysis encephalopathy (Parkinson, Ward et al. 1981) and Parkinson's disease (Exley and Korchazhkina 2001). Recently, it has been claimed that Al exposure is related to Alzheimer's disease (French, Gardner et al. 1989; McLachlan 1995). Due to a substantial amount of Al in tea leaves and infusions, it has been suggested that consumption of a large quantity of tea may impose health hazards.

A considerable of studies have reported the total concentration of Al in tea infusions (Table 2.1) (Zhou, Wu et al. 1996; Flaten and Lund 1997; Odegard and Lund 1997; Erdemoglu, Pyrzyniska et al. 2000; Nookabkaew, Rangkadilok et al. 2006; Mehra and Baker 2007; Street, Drabek et al. 2007). The total metal content in tea plants depends on many factors, primarily the age of the tea leaves when they are harvested, but also the soil conditions, genetic of the plant, rainfall, altitude, production procedures, etc. This affects the metal concentration in an infusion. The preparation method (infusion time, water temperature, tea-water ratio) also has a large influence (Marcus, Fisher et al. 1996). Despite the variation in infusion conditions (Table 2.1, column 1), the reported Al concentrations in tea infusions are remarkably consistent. The total concentration of Al rarely falls outside the range 1 – 6 mg/L (Flaten 2002). Thus, for the majority of heavy tea drinkers, tea is likely to be the largest single source of Al intake.

Table 2.1 Published concentration of Al in tea infusions, sorted by year of publication

Reference	Infusion conditions	No. of brands or samples analyzed	Mean (mg Al/L)	Range (mg Al/L)	Analytical procedure*
(Zhou, Wu et al. 1996)	1 g/100 mL boiling deionized water, 20 min	4 brands (leaves, 2 green, 1 black, 1 oolong)	2.7	1.3 - 4.1	GF-AAS
(Flaten and Lund 1997)	2 g/200 mL boiling distilled deionized water, 3 min	4 black tea leaves of 4 different nationalities, Twinnings Earl Grey bags	3.2	1.0- 6.3	ICP-AES
(Odegard and Lund 1997)	1 bag/200 mL boiling distilled deionized water, 5 min	1 (Lipton Yellow Label bag)	3.2	-	ICP-MS
(Erdemoglu, Pyrzyniska et al. 2000)	1 g/20 mL hot deionized water, heated to boiling, left to infuse for 10 min	4 Turkish black tea, leaves	12.0	11.0 - 13.0	FAAS
(Mehra and Baker 2007)	5 g/200mL boiling distilled water, 2 min	8 samples (Indian, Sri Lanka, Chinese and	5.16	2.96 - 6.79	ICP-AES

		Tetley teas)			
(Nookabkaew, Rangkadilok et al. 2006)	2 g /100 mL, boiling deionized water, 5 min	17 samples	5.51	1.88 - 16.52	ICP-MS
(Street, Drabek et al. 2007)	1 g/50 mL boiling distilled water, 5 min	29 samples (13 green, 13 black, 2 oolong, 1 white)	2.75	0.279-9.38	ICP-AES

* GF-AAS, graphite furnace atomic absorption spectrometry; FAAS, flame atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; ICP-AES, inductively coupled plasma atomic emission spectrometry.

2.3 Manganese in tea and tea infusion

Manganese is an essential trace element for plants, microorganism and higher animals, including men. Its principal function is as an integral constituent or activator of enzymes (Corrinne, Marilyn et al. 1986). The recommended range of daily dietary intake for an adult is 2 -5 mg of Mn, which appears to be enough to meet daily needs (Heydorn 1988). Manganese deficiency can cause health effects, for example blood clotting, skin problems, lowered cholesterol levels, and other alterations in metabolism (FreeAdvice). The deficiency, however, in humans is unusual. When too high concentration of Mn is consumed, it becomes toxic. Symptoms of Mn poisoning are hallucinations, forgetfulness and nerve damage. Manganese can also cause Parkinson's disease, lung embolism, and bronchitis (Lenntech).

Manganese is present in many foods, including grains and cereals, and is found in high concentrations in many foods, such as tea. Leaf tea from various countries contains about 600 µg/g Mn, ranging from 350 - 900 µg/g (Powell, Burden et al. 1998; Fernandez-Caceres, Martin et al. 2001; Kumar, Nair et al. 2005; Nookabkaew, Rangkadilok et al. 2006), except those from Turkey where much higher Mn contents (1100 - 2205 µg/g) (Ozdemir and Gucer 1998) were found.

Determination of Mn levels in tea infusions have been carried out (Ozdemir and Gucer 1998; Nookabkaew, Rangkadilok et al. 2006; Mehra and Baker 2007).

Ozdermir and Gucer have reported that about 30.0 % of Mn from Turkish black tea leaves was transferred into the water, which was approximately 366 - 421 μg/g on dry basis (Ozdemir and Gucer 1998). Mehra and Baker (Mehra and Baker 2007) have studied the solubility of Mn in green and black tea, which were repeatedly infused in boiling water for different period of times. The results showed that the solubility of Mn in the first infusion (2 min) was the highest followed by the second (5 min) and the third infusion (10 min) in decreasing order for both green and black tea. The Mn content in the first infusion of leaf tea was found in the range 1.76 - 6.24 mg/L, whereas the higher amount 11.61 mg/L was found in the infusion of a tea bag. In addition, the percentage elemental transfer of Mn for green teas was lower than that of black teas for all infusion times. This suggests that the Mn leaching depended on type of leaf tea. The results reported by Matsushima and co-workers (Matsushima, Meshitsuka et al. 1993) were, however, different. The Mn concentrations in infusions were highest in green tea, followed by oolong and black tea, in decreasing order. The contents were found to be 1.75 - 6.67 mg/L in green tea, 0.94 - 4.04 mg/L in oolong tea, and 0.78 - 3.24 mg/L in black tea (Matsushima, Meshitsuka et al. 1993). This difference could be influenced by many factors such as the age of tea leaves, soil conditions, altitude, manufacturing process, and tea infusion conditions (Flaten 2002).

When discussing possible negative health effects of tea related to metals contents, it is important to realize that tea is a rich source of antioxidants, so it may potentially have positive effects on human health. There is a rapidly growing body of scientific evidence indicating that tea consumption may protect against cardiovascular diseases and several types of cancer. In addition, tea may have a positive effect on the intestinal microflora and protect against kidney stones, bacterial infections and dental cavities (Flaten 2002).

The objective of this work is to study the effect of infusion conditions to the Al and Mn concentrations in various types of Chiang Rai tea infusions, using ICP-OES and FAAS, respectively. The amounts of the metals were investigated. The data obtained will provide the information on whether these teas contain metals in amounts that could be toxic at the normal doses usually consumed as beverages.

CHAPTER 3
METHODOLOGY

3.1 Chemicals

All chemicals used were of analytical grade. A standard solution of each element was prepared immediately by dilutions of a 1000 mg/L stock solution (Merck, Germany) prior to use. Water used throughout this experiment was deionized and purified with a Labconco purification system (Labconco, USA). All glassware and equipment were soaked with 10% HNO_3 at least overnight and then rinsed with deionized water prior to use.

3.2 Samples

Three types of leaf tea, namely black tea, green tea, and oolong tea, manufactured by Suwirun factory, labeled as SB, SG and SO, respectively, were collected and analyzed for their Al and Mn concentrations. Suwirun factory is one of the biggest factories in Chiang Rai, Thailand. Its tea was grown in Amphur Mae Lao, which is 500-600 m above the sea level.

3.3 Sample Preparation

The aim of this research is to study the effect of the infusion conditions to the elements concentrations. The conditions, namely infusion temperature and infusion time, were, therefore, varied. Infusion temperatures of 80 °C, 90 °C, and 100 °C, and infusion times of 1, 3, 5, and 10 minutes were chosen. Generally, tea infusion was prepared by adding 100 mL of hot water into a tea sample (2 g) and allowing to infuse for the given time. The extraction was then filtered and acidified with nitric acid. Three replicate infusions were made for each tea. The amount of Al and Mn were determined by ICP-OES and Flame AAS, respectively.

3.4 Instrumentation

An inductively coupled plasma optical emission spectrometer (ICP OES) employed for aluminum determination was a Perkin-Elmer model 4300DV (Perkin-Elmer, USA). The operating parameters are shown in Table 3.1.

Table 3.1 Operating parameters for ICP-OES

Parameters	data
RF frequency	40 MHz
Generator	1400 w
Nebulizer flow	0.8 L/min
Auxilary flow	1.0 L/min
Plasma flow	15 L/min
Sample flow rate	1.5 mL/min
Emission line for Al	369.15 nm

A Hitachi Z5000 Flame Atomic Absorption Spectrophotometer (Hitachi, Japan) interfaced with a computer for data collection was used in the absorbance mode for manganese determination. Absorbances were measured at 279.5 nm. The air-acetylene flow rate and burner height obtained from optimization were 3.0 l/min and 7.5 mm, respectively.

3.5 Statistical analyses

Effect of infusion conditions *i.e.* water temperature and infusion time on the Al and Mn concentrations was carried out using ANOVA analysis (SPSS program, version 14.0, SPSS Inc., Chicago, IL).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Aluminum concentration in tea infusion

The concentrations of Al in the infusions of three types of leaf tea (black tea, green tea, and oolong tea), manufactured by Suwirun factory, were determined using ICP-OES. The concentration of Al, shown in Table 4.1, were reported on the basis of 2 g of leaf tea and 100 mL of water, and leaf teas were infused at different temperatures (80 °C, 90 °C, and 100 °C) for varying periods of time (1, 3, 5, and 10 min). Concentration ranges of Al are illustrated in Figure 4.1.

Table 4.1 Aluminum concentrations (mg/L) in tea infusions as a function of infusion temperature and time

Temperature (°C)	Time (min)	Al concentration (mg/L)*		
		SB	SG	SO
80	1	5.73 ± 0.68	1.62 ± 0.19	0.15 ± 0.03
	3	8.20 ± 0.84	2.34 ± 0.22	0.32 ± 0.03
	5	9.29 ± 1.00	2.82 ± 0.29	0.33 ± 0.08
	10	11.13 ± 0.02	3.30 ± 0.06	0.48 ± 0.08
90	1	7.00 ± 0.43	1.65 ± 0.18	0.22 ± 0.07
	3	9.49 ± 0.40	2.53 ± 0.26	0.49 ± 0.08
	5	10.56 ± 0.35	3.24 ± 0.12	0.64 ± 0.12
	10	10.94 ± 1.01	3.64 ± 0.40	0.81 ± 0.45
100	1	7.30 ± 0.42	1.58 ± 0.10	0.28 ± 0.07
	3	10.00 ± 0.62	2.74 ± 0.14	0.72 ± 0.21
	5	11.07 ± 0.46	3.60 ± 0.29	0.93 ± 0.19
	10	11.87 ± 0.65	3.97 ± 0.14	1.17 ± 0.26

* Data for Al concentrations are average values of three replicates, and are given with SD.

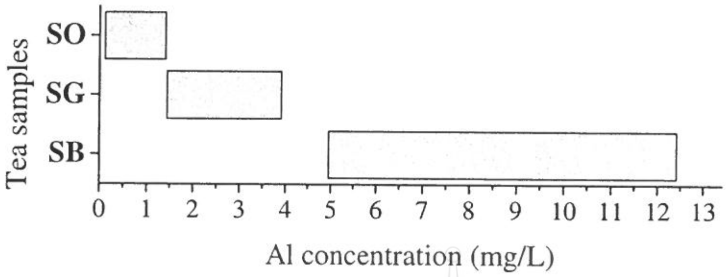


Figure 4.1 Range of Al contents in tea infusions (SB, SG, and SO)

Figure 4.1 shows the concentration ranges of Al in SB, SG and SO, which were 5.73 - 11.87, 1.62 - 3.97 and 0.15 - 1.17 mg/L, respectively, depending on infusion conditions. The results show that the Al concentrations agreed well with the results complied by Flaten (Flaten 2002), who found the values rarely fell outside the range of 1 - 6 mg/L, and by the authors (see Table 2.1), considering that variation of infusion conditions (tea-water ratio, infusion temperature, infusion time) have been used. In addition, the Al levels were found to change greatly with the types of tea. The amounts of Al in black tea infusions (SB) were the highest followed by those in green tea infusions (SG) and oolong tea infusions (SO) in decreasing order, shown in Figure 4.1. Noticeably, the Al contents in SO infusion were remarkably low compared to other samples at all investigated temperature. The form of the leaves may be one of many factors that cause the small amount of Al observed in SO infusion. The leaves of SO were rolled and twisted into round shape, resulting in compacting the particles. The leaves of SB and SG, on the other hand, were surface flat as they were not rolled (Sharma, Gulati et al. 2005). The water uptake of the SO was therefore slower than that of SB and SG, resulting in slow solubilization of Al in the SO sample. Consequently, the Al diffusion rate from SO was slower than that from the other samples. The Al contents in the tea infusion prepared from the SO sample was thus the least among those samples.

Furthermore, the variation in the Al contents was observed between varieties. The Al levels in infusions were found higher in *C. sinensis* var. *assamica*, which was used to produce black and green teas (SB and SG), than those in oolong tea (SO), manufactured from *C. sinensis* var. *sinensis*. The tea production process also influences the Al content. The higher Al concentrations in SB infusions were observed than those in SG infusions. The possible explanation is the contamination during the fermentation process used for the production of the black teas (Erdemoglu, Pyrzyniska et al. 2000).

The Al contents in SB and SG infusions corresponds to the previously published results by Zhou et al. (Zhou, Wu et al. 1996) that the Al levels in black tea infusions were higher than those in green tea infusions. The Al concentrations in oolong tea infusions, however, did not follow a similar trend: the highest amount of Al was found in oolong tea infusion followed by that in black and green tea infusions, respectively (Zhou, Wu et al. 1996). It is of note that the study by Zhou and co-workers was carried out on various Chinese teas, whereas the present study investigated teas from Chiang Rai, Thailand. Additionally, the different in physical appearance of tea leaf may influence the amount of Al released. It has been reported that the variation of metal contents was influenced by the different soil conditions where the tea plants were grown, the different harvesting periods, rainfall, altitude, and production procedures (Marcus, Fisher et al. 1996; Zhou, Wu et al. 1996). Hence, the metal contents in teas from different parts of the world may be different.

4.2 Manganese concentration in tea infusion

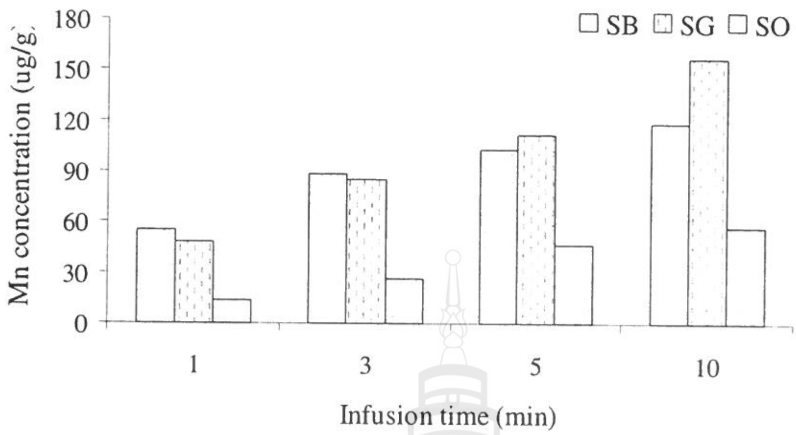
The concentrations of Mn in the infusions of three types of leaf tea (black tea, green tea, and oolong tea), produced by Suwirun factory, were determined using flame AAS. The concentration of Mn in tea infusions prepared by infusing leaf tea at different temperatures (80 °C, 90 °C, and 100 °C) for varying periods of time (1, 3, 5, and 10 min) are reported on dry basis and given in Table 4.2.

Table 4.2 Manganese concentrations (mg/L) in tea infusions as a function of infusion temperature and time

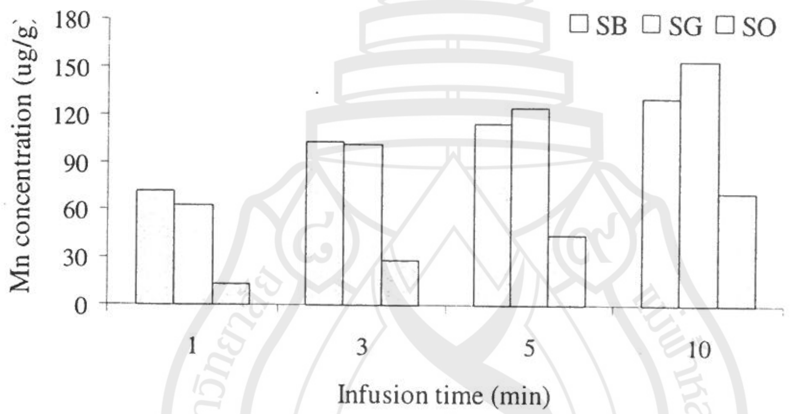
Temperature (°C)	Time (min)	Mn Concentration (μg/g)		
		SB	SG	SO
80	1	55.08 ± 5.07	48.04 ± 5.90	13.30 ± 3.45
	3	88.43 ± 4.97	85.22 ± 6.96	26.72 ± 10.72
	5	102.97 ± 11.39	111.64 ± 2.80	46.60 ± 23.08
	10	118.30 ± 11.23	156.68 ± 6.44	57.13 ± 16.74
90	1	72.22 ± 7.74	62.49 ± 5.15	13.64 ± 4.42
	3	102.71 ± 13.74	101.08 ± 10.07	28.51 ± 10.61
	5	114.58 ± 10.98	124.89 ± 0.13	44.40 ± 20.48
	10	130.75 ± 14.24	154.27 ± 16.33	71.38 ± 29.49
100	1	66.95 ± 6.90	79.04 ± 13.29	17.78 ± 4.78
	3	102.29 ± 9.77	108.17 ± 6.34	45.57 ± 11.65
	5	118.29 ± 10.67	128.81 ± 8.12	58.42 ± 19.75
	10	128.81 ± 11.23	152.22 ± 17.25	77.90 ± 33.61

* Data for Al concentrations are average values of three replicates, and are given with SD.

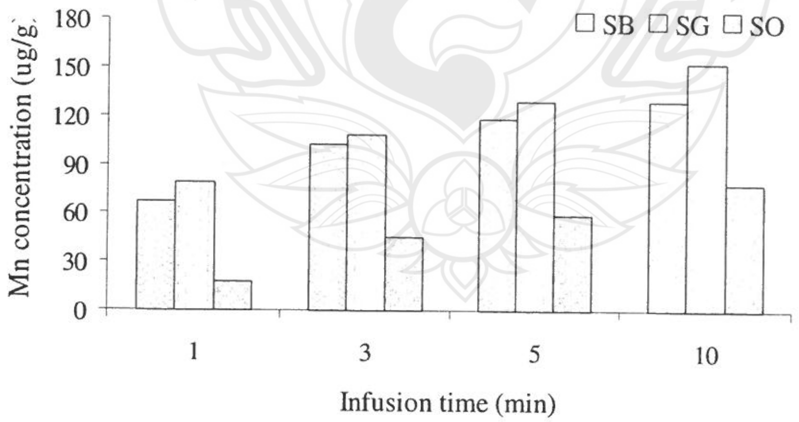
The results showed that concentration ranges of Mn in SB, SG and SO infusions were found to be 55.08 - 128.81, 48.04 - 156.68, and 13.30 - 77.90 μg/g, respectively, depending also on infusion temperature and time. Figure 4.2 illustrates the variation of Mn contents in investigated tea infusions at different infusion conditions. At infusion temperature of 80 and 90 °C, the Mn levels in SB were found to be the highest followed by those in SG and SO in decreasing order. However, at 100 °C, the Mn contents in SG became higher than those in SB and SO. Once again, the exceptionally small amount of Mn in SO infusion was observed at all investigated temperature. This supports the assumption that the form of tea leaves affected the diffusion of the element, as mentioned in the previous section.



(a) 80 °C



(b) 90 °C



(c) 100 °C

Figure 4.2 Manganese concentrations (μg/g) in SB, SG and SO infusions at varying period of infusion time at infusion temperature of (a) 80 °C, (b) 90 °C, and (c) 100 °C

The Mn contents in SB and SG agreed with a study reported by Matsushima and co-workers (Matsushima, Meshitsuka et al. 1993) in which the Mn levels in green tea infusions were higher than those in black tea infusions. However, the Mn levels in SO agreed less well with the previously mentioned study (Matsushima, Meshitsuka et al. 1993) in which the amount of Mn in oolong tea infusion was higher than that in black tea infusion *i.e.* Mn contents in green tea were more than those in oolong tea and black tea, respectively. The Mn contents in oolong tea infusions (SO) determined in this study, on the other hand, were found to be the lowest. Apart from the different in structure of the leaf form, this may be due to many factors such as the different in origin and age of tea leaves, the soil conditions where the tea plants were grown, rainfall, altitude, and production procedures (Marcus, Fisher et al. 1996; Zhou, Wu et al. 1996).

The present study showed that the Mn concentrations in tea infusions were comparable to the previous studies in which the concentrations ranges were 68.5 - 370.5 μg/g for *C. sinesis* infusions (Nookabkaew, Rangkadilok et al. 2006) and 70.4 - 249.6 μg/g in infusions prepared from black and green teas from various countries (Mehra and Baker 2007). They were, however, far less than those in Turkish black tea infusions (366 - 421 μg/g) (Ozdemir and Gucer 1998), even the same type of tea was compared. This could result from the different tea infusion preparation: a long period of time (15 min) was used to prepare the Turkish black tea infusions (Ozdemir and Gucer 1998), whereas this work and the previous studies (Nookabkaew, Rangkadilok et al. 2006; Mehra and Baker 2007) used a shorter period of time, ranging from 1 to 10 min. The origin of the plant may also play a role in the metal variation.

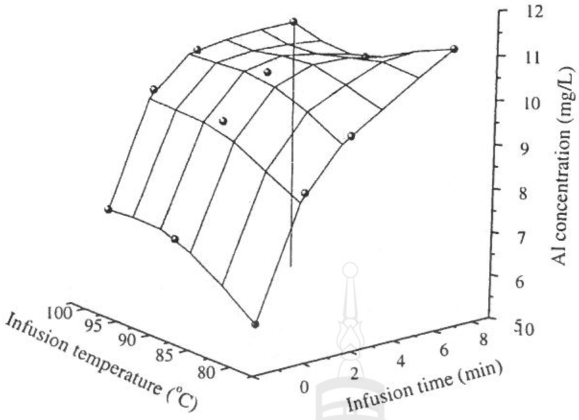
The varieties of tea were shown to influence the metal contents in this work. The Mn concentrations were lower in *C. sinensis* var. *sinensis*, which was used to make oolong tea, than those in *C. sinensis* var. *assamica*, used to produced green and black teas. Additionally, on comparing tea samples produced with the same varieties, it can be seen that the Mn levels were higher in tea types that did not go through the fermentation process *i.e.* green tea and oolong-green tea. This may be attributed to the processing of the green teas being different from black tea, which results in green tea being different both technologically and chemically from black tea (Mehra and Baker 2007)

4.3 Effect of infusion conditions

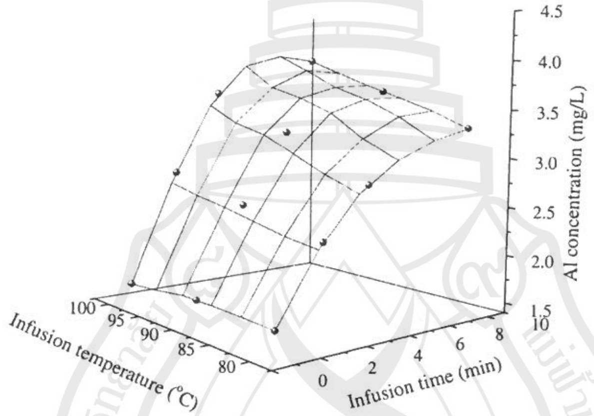
The tea infusion conditions *i.e.* tea-water ratio, infusion temperature and infusion time have been reported to affect the metal concentration in tea infusions (Marcus, Fisher et al. 1996). Demonstrated here is the influence of the infusion temperature and infusion time to the Al and Mn contents in Chiang Rai tea infusions. Results obtained are expected to provide useful information for preparing tea.

The infusion temperature and infusion time were varied for the tea infusion preparation and then the influence of these parameters on the Al and Mn contents was assessed. Tea infusions were prepared by infusing leaf tea in 100 mL water at different temperature (80 °C, 90 °C, and 100 °C) for varying periods of time (1, 3, 5, and 10 min). The conditions used were chosen to reflect the various conditions that might be encountered among tea drinkers. The variation of the Al and Mn contents in tea infusions due to infusion conditions is given in Table 4.1 and Table 4.2, respectively.

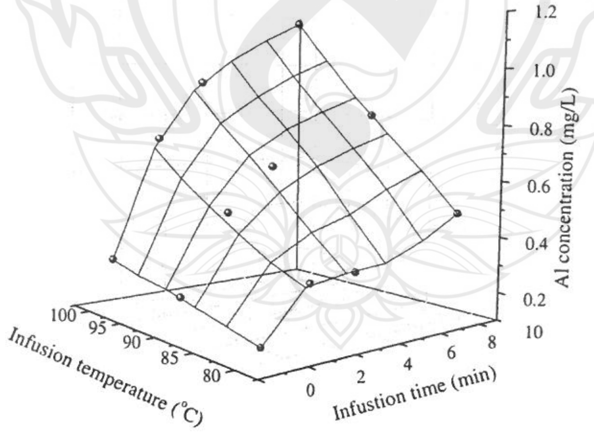
The results demonstrated that the infusion temperature and infusion time had major influences on the Al and Mn concentrations, shown in Figure 4.3 and 4.4, respectively ($P < 0.001$; see Appendix A and B). The results showed that the metals concentrations increased as the infusion temperature rose from 80 °C to 100 °C. At 80 °C, the Al content in SB infusion was 8.20 mg/L and increased until a value of 10.00 was reached at 100 °C after 3 min infusion. This effect may be caused by an increase in energy facilitating the metal diffusion and consequently the component extraction. A rise of temperature increases the component mobility and thus accelerates the diffusion speed of the species (Labbe, Tremblay et al. 2006).



(a) SB

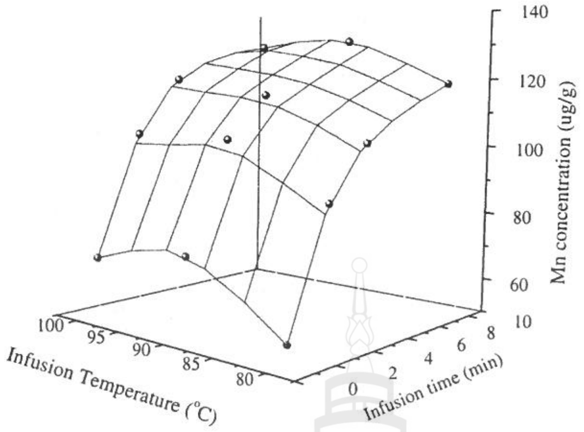


(b) SG

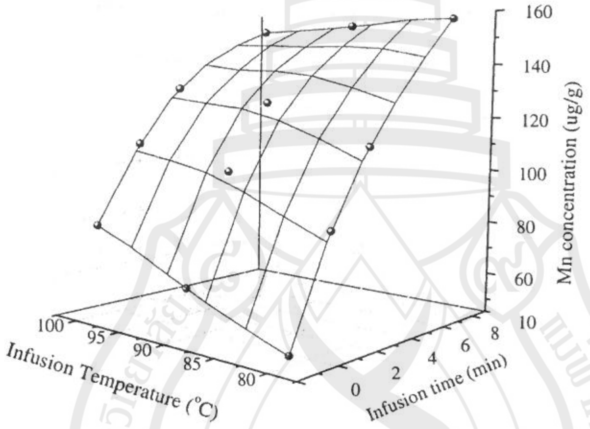


(c) SO

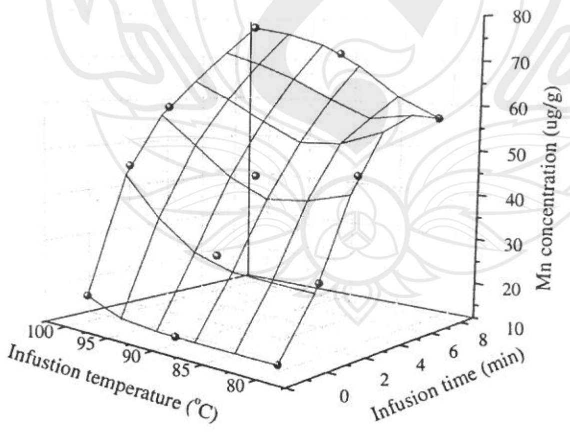
Figure 4.3 Alumimum concentrations (mg/L) in tea infusions, (a) SB; (b) SG; and (c) SO, as a function of infusion temperature and time



(a) SB



(b) SG



(c) SO

Figure 4.4 Manganese concentrations (μg/g) in tea infusions, (a) SB; (b) SG; and (c) SO, as a function of infusion temperature and time

Figure 4.3 and 4.4 show the amount of Al and Mn released increased with time, respectively. Tea infused for 10 min gave the higher levels of the metals than did the tea infusion prepared at shorter period of time. The metals were rapidly diffused in the infusions at the highest rate from 1 min to 3 min, after that (3 min to 5 min) the lower rate was observed. From 5 min to 10 min, the metals were released at the lowest rate and practically remained constant with time. For example, Al contents in SB increased dramatically from 7.30 to 10.00 mg/L after only 3 min in 100 °C infusing water, after that the Al content of 11.07 and 11.87 mg/L were determined after 5 and 10 min of infusion, respectively. This is because at shorter time the initial concentration of the metals at the surface of leaf was high, resulting in the high mass transfer rate. At longer time, the initial concentration at the leaf was low due to the on-going diffusion of the metals to the bulk solution. The rate of mass transfer was thus low and the metals concentrations, finally, reached equilibrium.

The results clearly demonstrated that the higher infusion temperature and the longer infusion duration promoted the Al extraction. All types of tea showed the effect of the infusion conditions with the similar trend.

CHAPTER 5

CONCLUSIONS

Effect of tea infusion conditions, *i.e.* water temperature and infusion time, on Al and Mn concentrations in infusions prepared from three types of tea (black, green and oolong tea) was studied. Concentrations of Al and Mn were determined using ICP-OES and Flame AAS, respectively. The results showed that Al and Mn diffusion was dependent of water temperature and infusion time ($P < 0.001$). The higher the water temperature and the longer infusion time, the higher Al and Mn levels. The higher water temperature influenced the metals diffusion rate, resulting in higher dissolution of the metals. The infusion time related to the mass transfer of the metals due to the initial gradient concentration on the surface of the leaves. Most of the soluble metals were noticeably extracted in the infusion within the first 5 minutes. For shorter infusion time, the rate of mass transfer was high due to the high initial concentration on the surface of the leaves. This resulted in the initial rapid increase in the metals contents. The diffusion rate of the metals became lower after a certain time due to less difference of the metals concentration at the surface of tea leaves and in the bulk solution.

The results also demonstrated that the metals concentrations depended on type of tea. The amounts of Al in black tea infusion were the highest, followed by those in green tea infusion and oolong tea infusions in decreasing order. The lowest amount of Mn was found in oolong tea infusions. The Mn contents in black tea infusion were the highest at the lower infusion temperature. At 100 °C, green tea infusion contained the highest amount of Mn.

Moreover, it was found that the form of tea leaves played a role in the infusion of the metals from tea samples. The metals could probably be released from the rolled (oolong tea) leaves slower than the unrolled (black and green teas) leaves as the leaves of the former needed more time for extraction.

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APPENDICES

APPENDIX A

Statistical analysis of effects of tea infusion conditions on Al concentration

The factorial designs of 3 x 3 x 4 with three replications were examined, with the total of 108 experimental observations. There were three independent variables (Tea samples, Temperature, and Time), being tested in a Factorial ANOVA. There are also three *two-way* interactions (Tea samples X Temperature, Tea samples X Time, and Temperature X Time), and one *three-way* interaction (Tea samples X Temperature X Time).

Case Processing Summary

	Cases			
	Included		Total	
Al (mg/L) * Tea samples * Temperature * Time * Replication	N Percent	N Percent	108 100.0%	108 100.0%

Tests of Between-Subjects Effects (ANOVA table)

Dependent Variable: Al (mg/L)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1673.893(a)	35	47.826	278.324	.000
Intercept	1922.126	1	1922.126	11185.929	.000
Tea	1525.948	2	762.974	4440.174**	.000
Temp	11.317	2	5.658	32.930**	.000
Time	86.701	3	28.900	168.187**	.000
Tea * Temp	4.321	4	1.080	6.287**	.000
Tea * Time	42.068	6	7.011	40.802**	.000
Temp * Time	1.106	6	.184	1.073 ^{ns}	.387
Tea * Temp * Time	2.432	12	.203	1.180 ^{ns}	.314
Error	12.372	72	.172		
Total	3608.391	108			
Corrected Total	1686.265	107			

a R Squared = .993 (Adjusted R Squared = .989)

b ** = significant at 1% level, ^{ns} = not significant.

c *cv* = 9.8%

Descriptive Statistics

Temperature	Time (min)	Al concentration (mg/L)								
		SB			SG			SO		
		Mean	S.E.	N	Mean	S.E.	N	Mean	S.E.	N
80 °C	1	5.76	0.41	3	1.62	0.11	3	0.15	0.02	3
	3	8.20	0.48	3	2.34	0.13	3	0.32	0.02	3
	5	9.29	0.58	3	2.82	0.17	3	0.33	0.05	3
	10	11.13	0.01	3	3.30	0.03	3	0.48	0.05	3
	Total	8.59	0.61	12	2.52	0.19	12	0.32	0.04	12
90 °C	1	7.00	0.25	3	1.65	0.10	3	0.22	0.04	3
	3	9.49	0.23	3	2.53	0.15	3	0.49	0.05	3
	5	10.56	0.20	3	2.94	0.31	3	0.64	0.07	3
	10	10.94	0.60	3	3.64	0.23	3	0.81	0.26	3
	Total	9.50	0.49	12	2.69	0.24	12	0.54	0.09	12
100 °C	1	7.30	0.24	3	1.58	0.05	3	0.28	0.04	3
	3	10.00	0.36	3	2.74	0.08	3	0.72	0.12	3
	5	11.07	0.26	3	3.60	0.17	3	0.93	0.11	3
	10	11.87	0.38	3	3.97	0.08	3	1.17	0.15	3
	Total	10.06	0.54	12	2.97	0.28	12	0.78	0.11	12
Total	1	6.69	0.28	9	1.62	0.05	9	0.22	0.02	9
	3	9.23	0.33	9	2.54	0.08	9	0.51	0.07	9
	5	10.30	0.33	9	3.12	0.17	9	0.63	0.10	9
	10	11.31	0.25	9	3.63	0.12	9	0.82	0.13	9
	Total	9.38	0.32	36	2.73	0.14	36	0.55	0.06	36

These are all the Means, Std. Errors of Mean, and Numbers of cases.

Appendix B

Statistical analysis of effects of tea infusion conditions on Mn concentration

The factorial designs of 3 x 3 x 4 with three replications were examined, with the total of 108 experimental observations. There were three independent variables (Tea samples, Temperature, and Time), being tested in a Factorial ANOVA. There are also three *two-way* interactions (Tea samples X Temperature, Tea samples X Time, and Temperature X Time), and one *three-way* interaction (Tea samples X Temperature X Time).

Case Processing Summary

	Cases			
	Included		Total	
ug Mn/1 g * Tea samples * Temperature * Time * Replication	N Percent	N Percent	108 100.0%	108 100.0%

Tests of Between-Subjects Effects (ANOVA table)

Dependent Variable: Mn (μg/g))

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	175488.681	35	5013.962	28.168	0.000
Intercept	757670.029	1	757670.029	4256.536	0.000
Time	68540.583	3	22846.861	128.352**	0.000
Temp	3884.746	2	1942.373	10.912**	0.000
Tea	96707.651	2	48353.825	271.648**	0.000
Time * Temp	262.544	6	43.757	0.246 ^{ns}	0.959
Time * Tea	4235.892	6	705.982	3.966**	0.002
Temp * Tea	517.930	4	129.483	0.727 ^{ns}	0.576
Time * Temp * Tea	1339.335	12	111.611	0.627 ^{ns}	0.812
Error	12816.112	72	178.002		
Total	945974.822	108			
Corrected Total	188304.793	107			

a R Squared = .932 (Adjusted R Squared = .899)

b ** = significant at 1% level, ^{ns} = not significant.

c *cv* = 15.9%

Descriptive Statistics

Temperature	Time (min)	Mn concentration (µg/g)								
		SB			SG			SO		
		Mean	S.E.	N	Mean	S.E.	N	Mean	S.E.	N
80 °C	1	55.08	2.93	3	48.04	3.41	3	15.51	0.09	3
	3	88.43	2.87	3	85.22	4.02	3	28.97	0.18	3
	5	102.97	6.58	3	111.64	1.62	3	44.68	4.79	3
	10	118.30	6.48	3	156.68	3.72	3	68.08	5.99	3
	Total	91.20	7.37	12	100.40	12.02	12	39.31	6.12	12
90 °C	1	72.22	4.47	3	62.49	2.97	3	14.57	0.69	3
	3	102.71	7.93	3	101.08	5.81	3	36.82	4.47	3
	5	114.58	6.34	3	124.89	0.08	3	55.48	4.14	3
	10	130.75	8.22	3	154.27	9.43	3	70.10	6.73	3
	Total	105.07	7.10	12	110.68	10.42	12	44.24	6.56	12
100 °C	1	66.95	3.98	3	79.05	7.67	3	21.41	1.38	3
	3	102.29	5.64	3	108.17	3.66	3	44.13	1.07	3
	5	118.29	6.16	3	128.81	4.69	3	55.17	4.58	3
	10	128.81	6.48	3	152.22	9.96	3	71.08	4.15	3
	Total	104.09	7.46	12	117.06	8.64	12	47.95	5.62	12
Total	1	64.75	3.18	9	63.19	5.16	9	17.16	1.16	9
	3	97.81	3.75	9	98.16	4.10	9	36.64	2.56	9
	5	111.95	3.93	9	121.78	2.97	9	51.78	2.87	9
	10	125.95	4.05	9	154.39	4.15	9	69.75	2.90	9
	Total	100.12	4.24	36	109.38	5.97	36	43.83	3.48	36

These are all the Means, Std. Errors of Mean, and Numbers of cases.

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EDUCATION

1997	B.Sc., 2nd Hon., (Chemistry)	Chiang Mai University
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1993 – 2002	Recipient of the Development and Promotion of Science and Technology Talented Student Program (DPST) scholarship from the Royal Thai Government
1998	Awarded £280 to attend the 2nd International Symposium on Phthahlocyanine from Research Conference Grant Committee, Birkbeck University of London
1998	Awarded a student bursary £105 from Royal Society of Chemistry 1997 Second Class Honours B.S. degree in Chemistry from Chiang Mai University
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EXPERIENCES

2003 – present	Lecturer, School of Science, Mae Fah Luang University, Chiang Rai, Thailand
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PUBLICATIONS

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2. Peter J. Heard, Paul M. King, **Phunrawie Sroisuwan**, Nikolas Kaltsoyannis, *Tricarbonylrhenium(I) Halide Complexes of Chiral Non-Racemic 2-(dioxolanyl)-6-(dioxanyl)pyridine Ligands: Synthesis, NMR and DPT Calculations*. Polyhedron, 2003, **22**, 3371 - 3378.

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1996	Tab Nelaniti Honor Medal for outstanding student in Industrial Chemistry of Chiang Mai University, awarded by Dr.Tab Nelaniti Foundation.
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EXPERIENCES

2000-2003	Teaching assistant in chemical laboratory courses, Chiang Mai University
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Analytical Chemistry, Flow-based Analysis, Electrochemistry

PUBLICATIONS

1. J. Jakmunee, **S. Suteerapataranon**, Y. Vaneesorn, and K. Grudpan. *Determination of Cadmium, Copper, Lead and Zinc by Flow Voltammetric Analysis*, Analytical Sciences **17** (2001) i399-i401.
2. **S. Suteerapataranon**, J. Jakmunee, Y. Vaneesorn, and K. Grudpan *Exploiting Flow Injection and Sequential Injection Anodic Stripping Voltammetric Systems for Simultaneous Determination of Some Metals*, Talanta **58**:6 (2002) 1235-1242.
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31 October – 2 November 2008 at Queen Sirikit National Convention Center, Bangkok

Effect of Tea Infusion Conditions on Aluminum/Manganese Concentrations

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Abstract: Concentrations of Al and Mn in tea infusions were affected by the tea infusion conditions, *i.e.* water temperature and infusion time. The higher the water temperature and the longer the infusion time, the higher the elements concentrations. Amounts of the metals also varied significantly between types of tea.

Introduction:

Tea is a processed product from the leaves of tea plant (*Camellia sinensis*). Tea products are commonly categorized as black tea, green tea and oolong tea, according to the manufacturing process. Tea is a rich source of several elements, such as Al, Ca, K and Mn, which are present in mg/g level. Demonstrated here is the influence of the infusion condition to the Al and Mn contents in tea infusion.

Methodology:

Different types of tea (black, green and oolong teas) were infused in water at different temperatures (80, 90, and 100 °C) for varying periods of time (1, 3, 5, and 10 min). Concentrations of Al and Mn were determined using ICP-OES and Flame AAS, respectively. The conditions used were chosen to reflect the various conditions that might be encountered among tea drinkers.

Results, Discussion & Conclusion:

The results demonstrated that the infusion temperature and time had major influences on the Al and Mn concentrations (Figure 1 and 2). The higher infusion temperature and the longer infusion duration promoted the metals extraction. Tea infused in 100 °C water for 10 min gave the highest metals levels. All types of tea exhibited the effect of the infusion conditions with the similar trend. Moreover, it should be noted that high doses of Al and Mn could be associated with long-term toxicity and may cause health risks. Therefore, in order to prevent the exceeding metals average daily intakes, tea drinkers should avoid using extremely hot water and brewing tea for too long. For general practices, the local tea producers in Chiang Rai recommend brewing tea in boiling water for 1 min. The results also showed that the metals concentrations depended on type of tea. The amounts of metals in black and green tea infusions were higher than those in oolong tea.

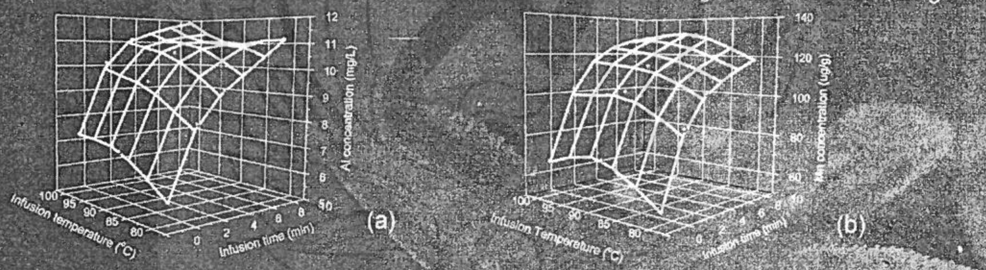

Figure 1: Al concentrations (mg/L) in (a) black tea infusion (b) green tea infusion

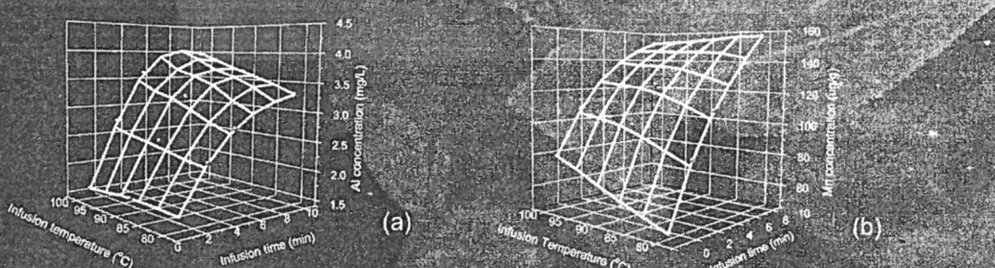

Figure 2: Mn concentrations (ug/g) in (a) black tea infusion (b) green tea infusion

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1. Kumar, A., et al., Food Chemistry, 2005, 89(3), 441-448.
2. Flaten, T.P., Coordination Chemistry Reviews, 2002, 228 (2), 385-395.

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