รายงานวิจัยฉบับสมบูรณ์

โครงการ: สารประกอบอะลูมิเนียมในน้ำชากับผลกระทบต่อสุขภาพ

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สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง

สิงหาคม 2555

สัญญาเลขที่ MRG4980049

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

ABSTRACT

Project Code: MRG4980049

Project Title: Aluminum Complexing in Tea: The Origin of the Paradoxical Effect in Relation to Human Health?

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Project Period: 2 years

Abstract:

Four types of dried tea leaves produced from two tea varieties, namely Chinese (*C. sinensis* var. *sinensis*) and Assam (*C. sinensis* var. *assamica*) grown in Chiang Rai, were selected in this study. The total Al concentrations in dried leaves and their infusions were determined by ICP-OES and found in the range of 557 – 829 mg kg^{-1} and 2 – 8 mg L^{-1}, respectively, depending on variety and type of tea. General trend was observed; the largest values were obtained from *C. sinensis* var. *assamica* used to produce green and black tea. The element was also released into the infusions at different percentages. More than 40% of Al was released into *C. sinensis* var. *assamica* infusions, whereas only 16% was leached into *C. sinensis* var. *sinensis* infusions. This suggests that tea variety plays an important role not only for the Al contents present in leaves, but also for the amounts transferred into infusions. Monomeric Al determination using spectrophotometric method has proved to be unsuccessful. The effect of Al^{3+} to human health, thus, is not reported here.

Keywords: Tea, Tea infusions, Chiang Rai tea, Aluminum, Health

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 IMPORTANCE AND RATIONALE

Tea (*Camellia sinensis*) is one of the few plants that accumulate aluminum (Al). Tea contains variable, but often high concentrations of Al. Mature tea leaves may contain up to 30000 mg/kg on a dry weight basis (Matsumoto, Hirasawa et al. 1976) and up to 600 mg/kg in young leaves (Wong, Zhang et al. 1998). Aluminum 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) However this is still open to debate as to whether the relationship is causal (Flaten 2001).

Tea has an attractive aroma, good taste, and health-promoting effects, and these benefits make it one of the most popular drinks in the world, second only to water. 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. However, when discussing the possible negative health effects of tea related to its Al content, it is important to realize that tea is also a rich source of antioxidants, so it may potentially have positive effects on human health. Scientific evidences indicate that tea consumption may protect against cardiovascular diseases and several types of cancers (Weisburger 1999; Trevisanato and Kim 2000). In addition, current studies show that green and black tea may help in improving memory (2005). These beneficial effects may outweigh any negative effects of the Al present in tea.

Aluminum forms in tea may play a vital role in toxicity. The chemical species of Al is linked to the bioavailability of Al. Monomeric form (Al^{3+}) is the most toxic species because it is easily absorbed by biological membranes, thus having high bioavailability. It is also known that Al forms complexes with polyphenols and organic acids in tea, and that these species are accumulated in leaves (French, Gardner et al. 1989). Thus, if these species, which are less bioavailable than the ionic Al, are present in tea, they could prevent the release of ionic, (and thus toxic), Al. This may explain why drinking tea does not seem to have negative effects on memory. This is considered to be a paradoxical effect.

It is therefore of interest to determine the total concentration of Al and that of monomeric Al in various types of tea (green, oolong and black) infusions in order to verify the hypothesis that a high proportion of Al in tea infusions is in complexed forms and has low bioavailability. Thus, human health may not be affected adversely by drinking tea. It is essential to establish this.

1.2 Objectives

1.2.1 To determine the total Al concentrations in dried tea leaves

1.2.2 To investigate the amount of Al transferred into tea infusions

1.2.3 To analyze the total Al and ionic Al contents in tea infusions

1.2.4 To investigate the major Al species present in tea infusions

1.2.5 To verify the hypothesis that the dominant forms of Al in tea infusions are complexes, which have low negative effects on human health

1.3. Literature Review

Tea is an industrial crop widely cultivated in Southeast Asia including India, Sri Lanka, China, and Japan and in central African countries. In Thailand, Chiang Rai is the largest cultivating area, producing 6,677 tons of dried tea leaves, which is approximately 80% of total tea produced in Thailand. Most tea plants grown in Chiang Rai are Assam variety, *Camellia sinensis* var. *assamica*, and China variety, *Camellia sinensis* var. *sinensis.* Tea is generally classified, depending on the method of manufacture, into three major categories: non-fermented green tea, partially fermented oolong tea, and fully fermented black tea. Black tea is consumed worldwide while green and oolong teas are mainly consumed in Asia and Northern Africa. In addition, tea is also produced as various tea products, for example instant tea and tea beverage.

Tea plant is a well-known Al-accumulator. Tea grows well in strongly acidic soils that contain high levels of soluble Al as Al promotes the growth of the plant. Al is accumulated in tea plants mainly in leaves, especially in old leaves (average 5600 mg Al/kg) and followed by young leaves (average 997 mg Al/kg) (Wong, Zhang et al. 1998). An extreme case has been noted that Al contents in old leaves exceeded 30,000 mg/kg (Matsumoto, Hirasawa et al. 1976). Variation of Al levels in tea leaves are dependent on the tea varieties, soil conditions, and hence Al uptake by tea in different locations (Shu, Zhang et al. 2003).

Aluminum can leach from tea leaves; Al concentrations in brewed tea are in the range of 1 – 6 mg/L and a cup of tea may, thus, contain approximately 0.2 – 1.0 mg of Al (Baxter, Burrel et al. 1989; Flaten and Lund 1997; Odegard and Lund 1997). The maximum recommended standard for Al in drinking water is 0.2 mg/L (Sherlock 1989) and the normal daily intake of Al for an adult is 5 mg (WHO 1997). Tea is, thus, a major potential source of dietary Al intake. Aluminum has no known beneficial effect in humans. Intake of large amounts of Al can lead to a wide range of toxic effects, including microcytic anaemia (Parkinson, Ward et al. 1981), osteomalacia (Starkey 1987), glucose intolerance of uraemia (Banks, Kastin et al. 1987), and cardiac arrest (Starkey 1987). Aluminum may be a contributory factor in certain neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS), Parkinson’s Dementia (Exley and Korchazhkina 2001), and Alzheimer’s disease (French, Gardner et al. 1989; McLachlan 1995). Thus, the high Al content in tea is a concern. The concentration of Al in tea liquor depends on the amount present in the original leaves as well as the time allowed for the infusion process (Erdemoglu, Pyrzyniska et al. 2000).

Drinking tea, however, seems to be beneficial for human health; it may protect against cardiovascular diseases, several types of cancers (Weisburger 1999), kidney stones, and dental cavities (Trevisanato and Kim 2000). Additionally, current studies show that green and black tea may help for improving memory (2005). These positive effects may outweigh any negative effects of the Al present in tea.

The chemical species of Al in tea infusion is also of particular interest, as the forms of Al regulate its solubility, bioavailability, and toxicity. Monomeric forms are the most toxic species because they are easily absorbed by biological membranes, thus having high bioavailability. On the other hand, Al complexes that are bound to the high-molecular-weight organic molecules, which are not readily absorbed, have low bioavailability and, hence, less toxic. Several studies have been carried out to clarify the chemical species of Al in tea infusions. French et al. (French, Gardner et al. 1989) studied Al in tea infusions using a kinetic ion exchange procedure, and found that the Al was mostly bound to organic matter. Aluminum-27 nuclear magnetic resonance (NMR) spectroscopy was employed; Al-oxalate, Al-oxalate-fluoride complexes were identified in tea infusions (Mhatre, Iyer et al. 1993). Owen et al. (Owen, Crews et al. 1992) applied size exclusion chromatography coupled to inductively coupled plasma mass spectrometry to investigate the Al form in tea infusions. It was found that about 14% of the Al existed as 'stable' species (Al associated with high molecular weight species). Zhou et al. (Zhou, Wu et al. 1996) studied the behaviour of leached Al using graphite furnace atomic absorption spectrometry (GF-AAS) and reversed phase high performance liquid chromatography (RP-HPLC) and categorised Al species in tea infusion into three groups, namely large organic compounds, small stable organic compounds, and free form of Al. The compositions of Al species in a tea infusion vary with the methods of the tea production. For green tea, most of the Al is in large and small organic compounds. In oolong and black teas, most of the Al was in free form or in small stable organic compounds. These studies suggested that Al complexes existed exclusively in tea infusions. However, the concentration of the monomeric Al in tea infusions have not been directly determined.

Pyrocatechol violet method has been used extensively for spectrophotometric determination of Al^{3+}, but has not been employed to determine the species in tea infusion. In this study, the total Al concentrations in dried tea leaves and tea infusions will be determined using atomic absorption spectrometry (AAS). The monomeric Al contents in tea infusions will be determined using the pyrocatechol violet method. The content of Al complexes present in tea infusions can, thus, be calculated.

CHAPTER 2

METHODOLOGY

2.1 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.

2.2 SAMPLE COLLECTION

Tea products, grown and manufactured in Chiang Rai, were selected for the study. Four types of leaf tea, namely black tea (*C. sinensis* var. *assamica*), green tea (*C. sinensis* var. *assamica*), green-oolong tea (*C. sinensis* var. *sinensis*) and oolong tea (*C. sinensis* var. *sinensis*), were collected from different factories and further analyzed for their Al contents in leaf tea and their infusions.

2.3 SAMPLE DIGESTION USING MICROWAVE DIGESTION METHOD FOR TOTAL Al DETERMINATION IN TEA LEAVES

The sample digestion was operated using a microwave system following the method previously described. (Nookabkaew, Rangkadilok et al. 2006) Tea leaves were oven dried at 70 ^{o}C, cooled to room temperature and then powdered. Tea powders, 0.25 g, were weighed into PTFE vessels. Two milliliters of H_2O_2 and 6.0 mL of concentrated HNO_3 were added into the vessels. The vessels were closed and placed on the rotating turntable of the microwave oven, and then the digestion process was started. The digestion was allowed to 11.72 bar and 190 ^{o}C over 30 min and then maintained at 190 ^{o}C for 40 min. After microwave digestion, the digested solutions were filtered through filter paper (Whatman no. 42) and diluted to 50 mL with deionized water. Three replicates were made for each tea. The total Al was determined by ICP OES (see operating conditions below).

2.4 Tea Infusions Preparation

Tea infusions were prepared as follows (Street, Drábek et al. 2007): 2 g of tea leaves (2 g) was carefully weighed out into glass beakers. Boiled at 100 ^{o}C of 100 mL distilled water was poured into the glass beakers, after which they were covered with watch glasses for 45 min. After the given time, the extracted solution (tea infusion) was filtered through filter paper and subsequently determined for its total Al concentrations using ICP-OES with 3 replicates for each sample. The long period of infusing ensures the maximum amount of soluble Al in the infusion.

2.5 Ionic Al determination Using Spectrophotometric Method

Ionic Al determination was performed using spectrophotometric method followed Dougan and Wilson's method (Dougan and Wilson 1974). The sample in polyethylene bottle was added 1,10-phenanthroline solution and mix by swirling. The solution was then added catechol violet solution, then add the hexamine buffer. Hydrochloric acid was added to a sample bottle, and shake it well. After addition of the buffer, the pH of the solution should be in the range 6.0 to 6.2; check that this pH is achieved each time a fresh buffer solution is prepared. Between 10 and 20 minutes after adding the hexamine buffer solution, measure the optical density of the solution at 585 nm using 10-mm cuvettes, the reference cuvette being filled with water.

2.6 INSTRUMENTATION

The digestion was carried out in a HP-500 MARS 5 (CEM Corporation, Mathews, NC) poly(tetrafluoroethylene) (PTFE) advanced composite vessel with 100 mL capacity. 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 1.

TABLE 1: Operating parameters for ICP-OES

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

2.7 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 3

RESULTS AND DISCUSSION

Chiang Rai is the largest tea producer in Thailand. Two varieties commonly grown in Chiang Rai are Chinese (*C. sinensis* var. *sinensis*) and Assam (*C. sinensis* var. *assamica*). The Chinese, the most popular variety, is generally used to produce green (non-fermented) and oolong (semi-fermented) teas, while the Assam is manufactured as green and black (fermented) tea products. These are common tea products found in Chiang Rai. Al contents in tea leaves and infusions have been widely studied, but none was performed on Chiang Rai tea. These were, therefore, chosen for the present study.

This work was divided into three parts. The first was to determine the total Al in tea leaves. The amount of Al contents in tea infusions were to be measured in the second part. The transferred amounts of Al from leaves to infusions were to be calculated. The last part was to determine the amount of monomeric Al ion, which is supposed to be toxic to health, in tea infusions as to establish the effect of drinking tea toward human health.

3.1. TOTAL CONTENT OF Al IN TEA LEAVES

The determination of total Al content in tea has been a subject of numerous studies. The great majority of these studies were carried out on Al in tea because of their accumulation in tea plants compared to others.

In the present study, the Al concentrations were determined in four types of Chiang Rai tea products; namely black (var. *assamica*), green (var. *assamica*), green (var. *sinensis*) and oolong (var. *sinensis*). Each type is different in varieties and degree of fermentation. The Al concentrations in dried tea leaves varied among these tea samples as shown in Table 2.

TABLE 2: Aluminum concentrations in dried tea leaves determined by ICP-OES

Tea Samples	Al concentrations (mg kg^{-1})
Black (var. *assamica*)	829 ± 62
Green (var. *assamica*)	779 ± 35
Green (var. *sinensis)*	557 ± 100
Oolong (var. *sinensis*)	607 ± 121

(mean ± standard deviation, n = 3 with 3 replicates)

The highest concentration of Al in Chiang Rai tea products was found in black tea leaves, followed by those of green (var. *assamica*), oolong and green (var. *sinensis*) teas in decreasing order. The Al contents range was 557 – 829 mg kg^{-1}, which were in agreement with those previously reported. Matsushima et. al. (Matsushima, Meshitsuka et al. 1993) reported Al contents in green tea and black tea were 520 and 576 mg kg^{-1}, respectively. Another study reported Al levels of 919 mg kg^{-1} in green tea and 759 mg kg^{-1} in black tea (Wróbel, Wróbel et al. 2000). Higher amount of Al were found in black tea (1070 mg kg^{-1}) and green tea (1340 mg kg^{-1}) imported to Czech Republic (Street, Drábek et al. 2007). Fernandez et. al. (Fernández-Cáceres, Martín et al. 2001) determined Al concentrations in 23 black and 21 green tea from different countries of origin. The results showed that the Al contents were greatly varied from 474 - 1341 mg kg^{-1} for black tea and 465 – 2560 mg kg^{-1} for green tea. Zhou and co-workers (Zhou, Wu et al. 1996) have found the results differently. The highest value of Al content of 1943 mg kg^{-1} was found in Shuihsien (Oolong tea), followed by Pu-Erh (black tea). The least Al content was found in green tea. A high Al content in most of the tea samples analyzed is expected, as the tea plant is known to be an Al accumulator. Moreover, concentrations as high as 23,000 mg kg^{-1} (Coriat and Gillard 1986) and 30,000 mg kg^{-1} (Matsumoto, Hirasawa et al. 1976) have been reported in tea leaves, which are extremely high.

It was previously proposed (Müller, Anke et al. 1998) that most investigated foodstuffs (vegetable, meat, and dairy products) contained less than 5 mg kg^{-1} of Al (fresh weight), and high Al concentrations were found in cocoa and cocoa products (33 mg kg^{-1}), spices (145 mg kg^{-1}), and black tea leaves (899 mg kg^{-1}). These authors indicated that in general, the Al content of frequently consumed food increased in the following order: beverages > food of animal origin > food plant origin.

From the present results, the wide-ranging amount of Al in tea was observed. Street and et.al (Street, Drábek et al. 2007) suggested that the difference in the total Al content could be influenced by many aspects, primarily the age of the tea leaves, but also the genetic make up of the plant soil conditions, rainfall and altitude, but did not find any statistically significant differences in total Al contents between different prevailing soil units. Another study (Fung, Zhang et al. 2003) found that Al concentrations in individual parts of the tea plant (young leaves, old leaves, branches) were different at different locations. It was also stated that metal contents in tea leaves differ according the type of tea (green or black) and geological conditions, tea varieties, soil conditions, and hence Al uptake by tea in different locations (Marcos, Fisher et al. 1998) (Shu, Zhang et al. 2003).

In order to apprehend the effect of tea variety to the amount of Al, data were thus divided into two groups according to the variety (Figure 1). The Al concentrations of *C. sinensis* var. *assamica,* black (B) and green (G), and *C. sinensis* var. *sinensis*, green (GO) and oolong (O), were treated statistically. It was shown that Al concentrations between these two varieties were significant different (95% confidence level; $P < 0.01$). This strongly suggests that tea variety has a great effect to the Al concentrations in tea leaves. This is in line with the work of Ruan and Wong (Ruan and Wong 2001), who suggested that that the concentrations of Al in tea plants were significantly different among the varieties.

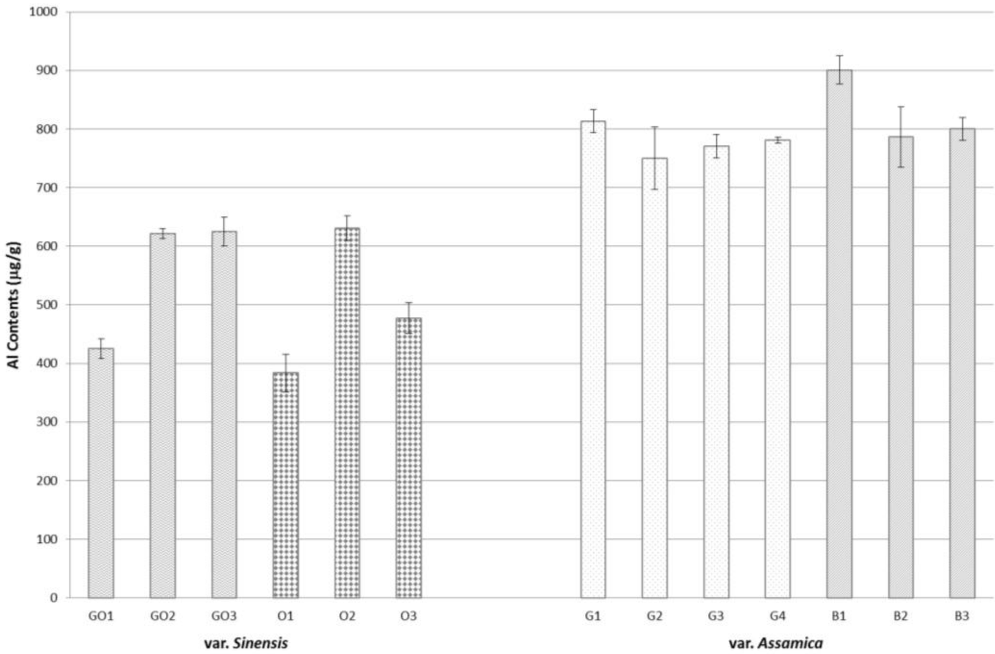

FIGURE 1: Amount of Al contents in Chiang Rai tea leaves

When comparing the tea type *i.e*. degree of fermentation, the differences in Al contents between black and green (var. *assamica*) teas were not statistically significant (95% confidence level), shown in Figure 2. This showed that degree of fermentation in the process of producing tea product does not affect the amount of Al in tea leaves. This corresponds to the previous report, indicating that there were no clear differences between the metal contents of green and black teas (Fernández-Cáceres, Martín et al. 2001).

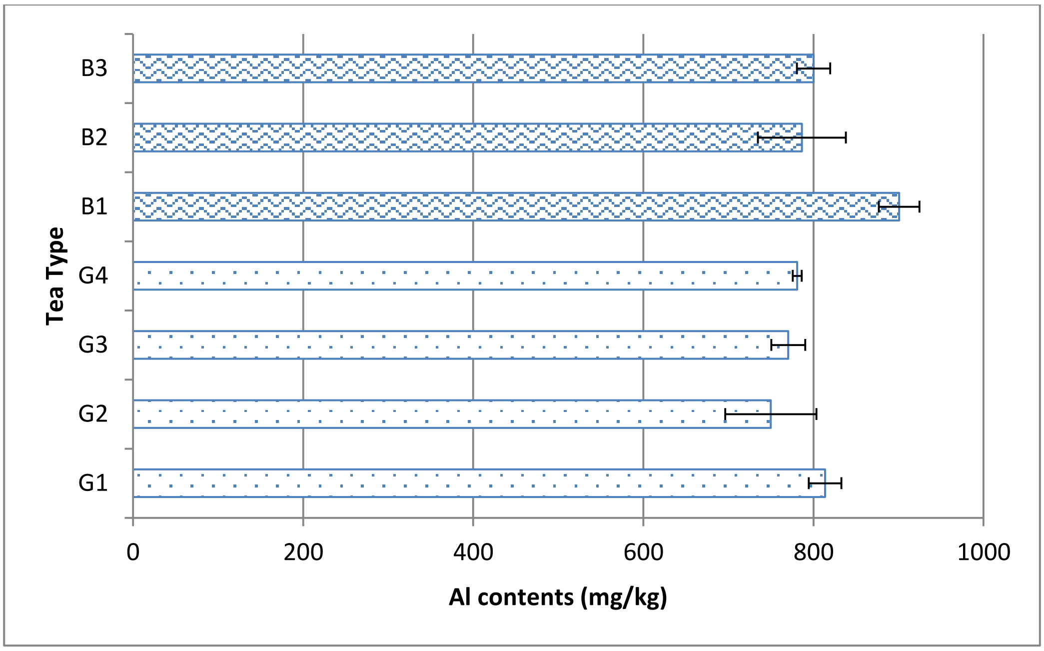

FIGURE 2: Al contents in black and green teas (var. *assamica*)

The amounts of Al in tea leaves were noticeably high, which was expected as tea plants accumulate Al. Most plants are quite sensitive to Al as its toxicity is probably the major factor limiting crop productivity on acid soils. In contrast, Al stimulates the growth of tea plants. Old tea leaves may contain up to 2-3% Al (dry weight), but the leaves are usually harvested long before they reach such levels. (Flaten 2002) If the total Al contents were transferred to tea infusions, it might implicate with health problem because Al has no known beneficial effect in humans. Intake of large amounts of Al can lead to a wide range of toxic effects, including microcytic anaemia (Parkinson, Ward et al. 1981), osteomalacia (Starkey 1987), glucose intolerance of uraemia (Banks, Kastin et al. 1987), cardiac arrest (Starkey 1987), and in certain neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS), Parkinson's Dementia (Exley and Korchazhkina 2001), and Alzheimer's disease (French, Gardner et al. 1989; McLachlan 1995). Thus, the high Al content in tea is a concern. It was, therefore, important to determine the amount of Al in tea infusions in Chiang Rai tea products to assess the actual amount of exposure to this element by drinking tea.

3.2. Total Content of Al in Tea Infusion

To determine the amounts of Al leached from tea leaves, two grams of Chiang Rai tea products were infused in boiled at 100 ^{o}C water (100 mL) for 45 minutes. Normally, tea leaves would only be infused in water for 1-3 minutes before drinking. However, in this study, the long infusion period was to ensure the maximum amount of Al leached from tea leaves. It was from our experiment that the concentrations of Al in the infusion were not significantly changed after 30 minute of infusion time. The amount of Al were determined by ICP-OES and reported in ppm as shown in Table 3.

Table 3: Aluminum concentrations in tea infusions determined by ICP-OES

Tea Infusions	Al concentrations ($mg\ L^{-1}$)
Black (var. *assamica*)	7.30 ± 1.00
Green (var. *assamica*)	6.73 ± 1.75
Green (var. *sinensis)*	1.96 ± 1.36
Oolong (var. *sinensis*)	1.90 ± 0.49

(mean ± standard deviation, n = 3 with 3 replicates)

The Al concentrations in Chiang Rai tea infusions were 2 – 8 mg L^{-1}; this corresponds to the results of 36 studies complied by Flaten (Flaten 2002), who found that the values rarely fell outside the range 1 – 6 mg L^{-1}. An extremely high amount of Al found at 40 - 100 mg L^{-1} was published in Nature in 1986. These data have not been fully published reporting analytical methods and quality control procedure, so results may have been in error (Coriat and Gillard 1986). The results demonstrated clearly that the amounts of Al were considerably higher in var. *assamica* compared to those in var. *sinensis*, which is consistent with the amount of Al in tea leaves. The higher the Al contents in tea leaves, the higher Al amounts in tea infusions.

Effect of tea variety: The Al concentrations in green tea infusions prepared from var. *sinensis* and var. *assamica* were 1.96 ± 1.36 and 7.30 ± 1.00 mg L^{-1}, respectively. Data were treated statistically and found that Al concentrations in these solutions were significantly different (99% confidence level). This suggests that tea variety has a great effect to the Al concentrations in tea infusions. This agrees to the results reported by Shu and co-workers (Shu, Zhang et al. 2003) that Al contents were varied among different tea varieties. However, it should be noted that the difference in the content could be influenced by many aspects, primarily the age of tea leaves, the genetics of the plant, but also tea origin e.g. cultivation method, soil conditions, altitude and climate). Further work could be done in order to shed light on the effect of tea origin by using the different-variety tea samples that grown in the same area to illustrate.

Effect of tea type: The Al concentrations in tea infusions prepared from different type of tea (green and oolong tea produced from *C. sinensis* var. *sinensis*; 1.96 $\pm$ 1.36 and 1.90 $\pm$ 0.49 mg L^{-1}, respectively, and green and black tea made from *C. sinensis* var. *assamica*; 7.42 $\pm$ 2.18 and 7.30 $\pm$ 1.00 mg Al /L, respectively) were statistically compared. It was found that Al contents in these infusions were not significantly different (95% confidence level) between the different types of tea produced from the same variety. The results agree with the findings from Street and co-workers (Street, Drábek et al. 2007), who reported that the amounts of Al in green and black tea infusions are not significantly different. The results from the present work, however, quite the opposite of the results reported by Zhou and co-workers (Zhou, Wu et al. 1996) who found that the metal content in tea infusions varied greatly among different type of tea: oolong tea contained the highest amount of Al, followed by black and green tea, respectively. However, the variety used to produce these tea samples in the literature was not accounted; therefore the metal contents between types of tea cannot be comparable. In the present work, the different-type of tea samples were made certain that they were produced from the same variety. Thus, the Al concentration is evidently independent to the type of tea.

The Al concentrations were also calculated on dry weight. The percent releases of Al from tea leaves to infusions for var. *assamica* were 40 – 45 %, while those of var. *sinensis* was only 16%, as shown in Table 4. Studies (Wróbel, Wróbel et al. 2000), (Erdemoglu, Pyrzyniska et al. 2000) showed that the percentage release of Al was about one-third of the total element content leached into the infusion, while other report (Street, Drábek et al. 2007) found 11% Al leached from black and green tea.

TABLE 4: Al contents in tea leaves, tea infusions and the percent of Al released into infusion

Tea samples	Al contents		% Release
	Dried tea leaves (mg kg^{-1})[a]	Tea infusions (mg kg^{-1})[a]	
Black[c]	829 ± 62	364 ± 50	45.6 ± 4.2
Green (var. *assamica*)[c]	779 ± 35	335 ± 85	40.9 ± 8.8
Green (var. *sinensis*)	557 ± 100	96 ± 66	16.6 ± 9.8
Oolong	607 ± 121	94 ± 24	16.4 ± 4.2

[a] data calculated on dry basis

The results showed that the amounts of Al were released into the infusions at different percentages. Tea from *C. sinensis* var. *sinensis*, markedly, had lower percent releases than those from *C. sinensis* var. *assamica*. This is in line with a report from Costa et al. (Costa, iacute et al. 2002), who found that the extraction of Al in black teas was higher than that observed in green teas, owing to the more stable high molecular masses of compounds present in this latter sample. It was explained that in non-fermented green tea most of the leached Al is bound to large and small organic compounds, while in fully-fermented black tea Al is mainly present as both free and bound forms to small stable organic compound. Wong et al. (Wong, Zhang et al. 1998), however, reported that in Chinese tea products the highest percentage Al solubility was observed in green tea followed by Oolong tea, black tea and Puerh tea. It was reported that teas from different locations in the world can be significantly different in terms of their components associated with trace metals, thus affecting the solubility of metals from these teas. Hence, the difference in the works may be attributed to the speciation of the metals in the different tea infusions. Black tea contains predominately polymeric polyphenols which may form soluble and insoluble complexes with the different metals. (Mehra and Baker 2007)

At present, the World Health Organization (WHO) guideline for the maximum level of Al in drinking water is 0.2 mg total Al per liter; therefore concentration in tea infusion is 10-100 times more than that in drinking water. This does not mean that tea infusion is 10-100 times more toxic because toxicity is dependant on Al speciation. (Street, Drábek et al. 2007) The chemical species of Al in tea is likely to play a vital role in toxicity. The monomeric form (Al^{3+}) is the most toxic Al species and suggested to be present in only small amounts in tea infusions with the remainder present as an organic complex. The proportions of monomeric and complexed Al in tea infusions are consequently of particular interest in trying to understand the positive and negative health effects of drinking tea. In order to shed some lights, monomeric Al (Al^{3+}) contents in tea infusions were, thus, to be determined.

3.3. Ionic Al Determination Using Spectrophotometric Method

The attempt to determine the ionic Al contents in tea infusions was unsuccessful. The method of Dougan and Wilson proved to be non-applicable to ionic Al determination. Other analytical techniques, namely Flow Injection Analysis (FIA) and electrochemistry, have been used in attempts to determine the amount of monomeric Al. All have proved to be successful so far. This is in agreement with Street et al. (Street, Drábek et al. 2007), who did the Al speciation in tea infusions using High Performance Liquid Chromatography/Ion Chromatography (HPLC/IC). It was found that the Al^{3+} species were not detected in any sample. This observation confirms the fact that Al present in tea infusions does not exist in "free" forms of hexahydrates or in form of hydroxypolymers. Another (Alberti, Biesuz et al. 2003) reported that the concentration of free metal ion was found to be very low, at the nM-level. It was due to a large fraction of Al(III) linked to very strong ligands that do not compete with the resin for the metal complexation.

The results revealed that free form of aluminum from tea leaves extracted using spectrochemical method could not be detected. This could address in term of plant defense respond since aluminum cation Al^{3+} has been reported which is toxic to many plants at a low concentration by limiting plant growth and development (Kochian 1995).

Many works have been shown that plants respond to heavy metal toxicity in different ways to evolve some mechanisms for detoxifying Al both internal and external process (Ma, Ryan et al. 2001).

For example, tea roots showed a limit amount of aluminum both by secrete anionic (e.g. malate, oxalate and citrate) to bind with aluminum and limit to uptake to plant cell during grown in the present of aluminum. In addition, the high levels of Al-oxalate complexes in response to an increase in the Al level also found in tea roots. This suggested that oxalate is a key Al-chelating compound in the mechanism of Al detoxification in the tea root which is required for transport organic acid anions out of the root cells that is mediated by aluminum-activated anion channels in the plasma membrane (Morita, Horie et al. 2004) (Morita, Yanagisawa et al. 2008). Consistent with other plants including hydrangea, buckwheat (Ma, Hiradate et al. 1997) and Melastoma malabathricum (Watanabe, Osaki et al. 1998), Al–citrate, Al–oxalate and Al–oxalate complexes were detected in xylem and leaves, respectively. Alternatively, plant also accumulates the metal ion in vacuole of epidermal cell both roots and leaves. Recently, Nagata suggested that Al is mainly present as Al–catechin complexes in tea leaves (Nagata, Hayatsu et al. 1992). It suggested that free Al is likely to be complex with other molecules rather than free Aluminum

To conclude, analysis of aluminum in tea leave is not only free aluminum but also Al-complexes. Identification and quantification of amount of Al-complex in tea leaves can use LC-MS that will show more detail about free aluminum and Al-complex profiles.

OUTPUT

Poster Presentations

1. Phunrawie Promnart,[*] J. Keith Syers, Determination of Aluminium in Chiang Rai Tea Products and their Infusions, Pure and Applied Chemistry International Conference 2009 (PACCON 2009), 14-16 January 2009, Phitsanulok, Thailand.

2. Phunrawie Promnart*, Siripat Suteerapataranon[1], and J. Keith Syers, Aluminum Concentrations in Chiang Rai Tea Infusions: Effects of Tea Variety and Type, 35th Congress on Science and Technology of Thailand (STT35), 15-17 October 2009, Chonburi, Thaland.

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