การวิเคราะห์ปริมาณออกซาเลตในตัวอย่างชา โดยวิธีโครมาโทกราฟีแบบของเหลวสมรรถนะสูง

นางสาวนันทิชา มหานิล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาอาหารเคมีและโภชนศาสตร์ทางการแพทย์ ภาควิชาอาหารและเภสัชเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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DETERMINATION OF OXALATE CONTENT IN TEA SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Miss Nunticha Mahanin

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Food Chemistry and Medical Nutrition

Department of Food and Pharmaceutical Chemistry

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Thesis Title	DETERMINATION OF OXALATE CONTENT IN TEA SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
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นันทิชา มหานิล : การวิเคราะห์ปริมาณออกซาเลตในตัวอย่างชาโดยวิธีโครมาโทกราฟีแบบของเหลวสมรรถนะสูง. (DETERMINATION OF OXALATE CONTENT IN TEA SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ภญ.ดร.สุญาณี พงษ์ธนานิกร, 93 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์ปริมาณออกซาเลตในชา 120 ตัวอย่างจากชา 40 ยี่ห้อ ประกอบด้วยชา 4 ชนิด ได้แก่ ชาขาว ชาเขียว ชาอู่หลง และชาดำ ทั้งในรูปแบบใบชาและชาซอง และศึกษาถึงผลของระยะเวลาในการแช่ชาต่อปริมาณออกซาเลตในน้ำชา ทำการวิเคราะห์โดยวิธีโครมาโทรกราฟีแบบของเหลวสมรรถนะสูง โดยทำการสุ่มตัวอย่างชาจากร้านค้าในประเทศไทยระหว่างช่วงเดือนกุมภาพันธ์ ถึง มิถุนายน 2554 ผลการวิเคราะห์พบว่า ในรูปแบบใบชานั้น ชาดำมีปริมาณออกซาเลตมากที่สุดคือ 2.32 ± 1.10 มิลลิกรัมต่อชา 1 กรัม รองลงมาได้แก่ ชาอู่หลง ชาเขียว และชาขาว โดยมีปริมาณออกซาเลต 1.62 ± 0.79, 1.41 ± 0.56 และ 1.29 ± 0.33 มิลลิกรัมต่อชา 1 กรัม ตามลำดับ สำหรับรูปแบบชาซองนั้น พบว่าชาดำมีปริมาณออกซาเลตมากที่สุดคือ 2.37 ± 0.80 มิลลิกรัมต่อชา 1 กรัม รองลงมาได้แก่ ชาอู่หลง มีปริมาณออกซาเลต 1.88 ± 1.04 มิลลิกรัมต่อชา 1 กรัม ชาเขียวและชาขาวมีปริมาณออกซาเลตใกล้เคียงกัน คือ 1.24 ± 0.48 และ 1.25 ± 0.45 มิลลิกรัมต่อชา 1 กรัม ตามลำดับ ทั้งนี้พบว่ารูปแบบใบชาและชาซองนั้นให้ปริมาณออกซาเลตไม่แตกต่างกัน ระยะเวลาในการแช่ชายิ่งนานขึ้น ยิ่งส่งผลให้มีปริมาณออกซาเลตในน้ำชาเพิ่มขึ้น ผู้ที่มีความเสี่ยงสูงต่อการเป็นนิ่วไต ควรหลีกเลี่ยงการดื่มชาดำในปริมาณสูงและควรชงชาในระยะเวลาสั้นๆ เพื่อจำกัดปริมาณออกซาเลตที่ได้รับ

ภาควิชา…….....อาหารและเภสัชเคมี……ลายมือชื่อนิสิต…….....
สาขาวิชา..อาหารเคมีและโภชนศาสตร์ทางการแพทย์..ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก…….
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This study was conducted to determine oxalate content in tea samples and study the effect of steeping duration on the oxalate content. One-hundred and twenty samples of both leaf and bag forms of tea-white, green, oolong, and black teas from 40 brands-were purchased from markets in Thailand from February to June 2011. The oxalate content was measured by the high performance liquid chromatography (HPLC) method. The results showed that in leaf form, the highest oxalate content was found in black tea (2.32 ± 1.10 mg/g tea), then in oolong tea (1.62 ± 0.79 mg/g tea), green tea (1.41 ± 0.56 mg/g tea) and lowest in white tea (1.29 ± 0.33 mg/g tea). In bag form, the oxalate content in black tea (2.37 ± 0.80 mg/g tea) was higher than that of oolong tea (1.88 ± 1.04 mg/g tea). The amount of oxalate in green tea (1.24 ± 0.48 mg/g tea) was comparable with that of white tea (1.25 ± 0.45 mg/g tea). The forms (leaf or bag) of tea did not have significant influence on the amount of oxalate. Steeping the tea for a longer duration provided more oxalate content. People with high risk of kidney stones should avoid drinking high amounts of black tea. Steeping tea for a short duration is also recommended for limiting oxalate intake.

Department : ..Food and Pharmaceutical Chemistry…….. Student's Signature…….....
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LIST OF ABBREVIATIONS

C	catechin
°C	degree celsius
EC	epicatechin
ECG	epicatechin gallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
et al.	*et alia* (and others)
FW	fresh weight
g	gram
GC	gallocatechin
h	hour
HPLC	High Performance Liquid Chromatography
i.e.	id est (this is)
l	litre
LDL	low density lipoprotein
mg	milligram
min	minute
ml	millilitre
mmol	millimole
No.	number
µmol	micromole
TFs	theaflavins
TRs	thearubigins

CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Oxalate, a salt form of oxalic acid, is widely distributed in a number of plant tissues (Noonan and Savage, 1999). In general, the oxalate content is high in leaves, seeds and stems respectively (Morrsison and Savage, 2003). There are two forms of oxalate, water-soluble and insoluble forms. Water-soluble oxalate such as sodium, potassium, and ammonium oxalates, can be absorbed in both small and large intestines, while water-insoluble oxalate such as calcium and magnesium oxalates, is unavailable for absorption (Noonan and Savage, 1999; Rinallo and Modi, 2002). Oxalate is not significantly metabolized in human body; therefore, it is the end product of metabolism excreted in urine (Taylor and Curhan, 2008). It was suggested that about 10-20% of urinary oxalate was derived from foods (Williams and Wandzilak, 1989) and the recent study showed that dietary oxalate contributed up to 53% of urinary oxalate (Holmes, Goodman, and Assimos, 2001).

Consumption of foods with high amounts of oxalate may cause adverse health effects. Oxalate combines with several essential minerals including calcium, iron, and magnesium to form insoluble compounds in the gastrointestinal tract; therefore, it may

decrease the bioavailability of these minerals (Morrsison and Savage, 2003; Bohn et al., 2004). A high dietary oxalate intake not only decreases the mineral absorption, but also increases the risk of kidney stone formation (Robertson and Hughes, 1993). Kidney stones are becoming more common in men between the ages of 30 and 50 years in industrialized countries. The most common stones consist of calcium oxalate (80% of stones) (Worcester and Coe, 2010), which the major risk factor of its formation is hyperoxaluria (urinary oxalate excretion above 0.5 mmol/24 h) (Hönow et al., 2010). Thus, it is important to reduce consuming high oxalate foods such as spinach, rhubarb, beetroot and tea. It appears that tea is a significant source of oxalate intake in English diets (Zarembski and Hodgkinson, 1962).

Tea is beverage consumed throughout the world (Khan and Mukhtar, 2007). There are various types of tea depending on botanical varieties, geographical origins and processes (López and Calvo, 2011). White, green, oolong, and black tea are all made by brewing leaves of the tropical evergreen *Camellia sinensis* (L.) O. Kuntze in hot water (Charrier, Savage, and Vanhanen, 2002; Sharangi, 2009). White tea is obtained from steaming and drying buds and young tea leaves with the minimum amount of processing. Green tea is prepared from unfermented leaves, whereas oolong and black tea are partially and fully fermented respectively (Sharangi, 2009). Due to the difference of its fermentation process, tea differs in its appearance, organoleptic taste, chemical content, and flavor (Winyayong, 2008).

There are many previous studies reporting the amount of tea oxalates. Charrier et al. (2002) reported that the soluble oxalate contents from black tea in tea bags and loose tea leaves were 4.68 and 5.11 mg/g tea, respectively. Green tea and oolong tea had lower oxalate contents, ranging from 0.23 to 1.15 mg/g tea. Tsai et al. (2005) measured the oxalate contents in foods and beverages including green tea and black tea. The result showed that the oxalate contents of green tea and black tea were 4.59 mg/100 g tea and 4.82 mg/100 g tea respectively. Recently, Hönow et al. (2010) analyzed the oxalate contents of Chinese green tea. The soluble oxalate contents varied widely from 8.3-139.8 mg/l depending on the origin, the time of harvest and preparation. Moreover, there were many comparisons about oxalate contents among countries. For example, the level of oxalate found in green tea consumed in New Zealand (0.68 mg/g tea) was lower than that reported in Japanese green tea (1.48 mg/g tea) (Kamiya, Ogawa, and Ohkawa, 1991).

Differences in oxalate values may be due to brewing process and/or analytical method. The shorter the tea infusion time, the less oxalate extracted. McKay et al. (1995) reported that 5-minute steeping black tea had more oxalate content (924 μmol/l) than 1-minute steeping black tea (479 μmol/l). Hönow et al. (2010) showed that the steeping duration of green tea leaves between 5 and 10 minutes had no significant influence on the oxalate contents but showed a tendency to lower oxalate concentration when steeping in shorter duration.

Several methods have been used to measure the amount of food oxalate. The oxalate determination by AOAC (2006) method was based on calcium oxalate precipitation. Oxalate was extracted with acid, precipitated as the calcium salt, then analyzed with colorimetric, gas chromatography or atomic absorption. There are some limitations in terms of sensitivity, presicision and time consumption. The other methods include ion electrophoresis, capillary electrophoresis (Holmes and Kennedy, 2000), enzymatic assays using oxalate oxidase (Kasidas and Rose, 1980) and high performance liquid chromatography (HPLC) (Hönow, Bonartz, and Hesse, 1997). The HPLC method was shown to be accurate and reliable for determination of oxalate in plant materials (Brega et al., 1992; Savage et al., 2000).

Although several studies have been published for the amount of oxalate in tea, the data on oxalate content of commercially available tea in Thailand is limited especially in white tea. Therefore, the aims of the present study were to analyze the amount of oxalate in tea samples available in Thailand and to find out the effect of steeping duration on the oxalate content. The results obtained from this study are beneficial for tea drinkers to selecting suitable tea preparation for themselves.

1.2 Objectives of the study

1. To determine the oxalate content in 4 types of tea including white, green, oolong and black both in leaf and bag forms by high performance liquid chromatography (HPLC)

2. To study the effect of steeping duration on the oxalate content in tea samples

CHAPTER II

LITERATURE REVIEW

2.1 Tea

Tea is a popular beverage consumed around the world due to its characteristic aroma, flavor and health benefits (Wu, 2007). Tea is manufactured in many areas over the world such as Japan, Taiwan, India as well as Thailand (Pripdeevech and Machan, 2011). Tea consumption in Thailand is increased about 6.17% per year during 1998-2003 (สุรพล นธการกิจกุล และคณะ, 2551).

2.1.1 Tea plant

Tea plant is a perennial evergreen plant growing into a small- to medium-sized tree (Karak and Bhagat, 2010; Nair, 2010). Tropical and subtropical areas having adequate rainfall, good drainage and acidic soils are appropriate for growing tea plant (Barua, 2008). More than 3 million hectares of the world has been used for planting tea (Ravichandran and Parthiban, 1998). There are approximately 120,000 rais of tea plantation in Thailand, which mostly grown in Chiang Rai Province (กรมส่งเสริมอุตสาหกรรม, 2552). The taxonomy of tea plant is shown as below:

Taxonomic Hierarchy:	Family	*Theaceae*
	Genus	*Camellia*
	Species	*Camellia sinensis* (L.) O. Kuntze

Tea plant grows up to 30 feet tall. It has elliptical dentate leaves with white flowers. There are two major varieties of tea plant (1) *Camellia sinensis* var. *sinensis* (Chinese tea) and (2) *Camellia sinensis* var. *assamica* (Assam tea). The Chinese tree is small with small, dark-green leaves and single flowers. The Assam tree is taller with broad, light-green to yellowish leaves that droop at their ends and have clustered flowers (Nair, 2010). Tea plant should be about 4 years old before harvesting. For the best quality of tea, leaves are picked by hand and only the top two leaves and the buds are plucked from the sprigs of the tree plant (Charrier et al., 2002).

2.1.2 Tea fermentation

Fermentation is the process in which the polyphenols in the tea leaves are oxidized due to the presence of the enzymes, resulting in the change of chemical composition of tea (Kim et al., 2011). Major enzymes in tea fermentation process consist of polyphenol oxidase (PPO), catalase (CAT), peroxidase (PO) and ascorbic acid oxidase (AAO) (Lee, 2009). Major reactions occurring with polyphenol during tea processing are composed of oxidation, hydrolysis, polymerization and transformation (Xu and Chen, 2002).

2.1.3 Types of tea

Tea is classified into four main types based on the degree or period of fermentation: white tea (non-fermented or semi-fermented), green tea (non-fermented),

oolong tea (semi-fermented) and black tea (fully-fermented) (สุรพล นธการกิจกุล และคณะ, 2551; de Mejia, Ramirez-Mares, and Puangpraphant, 2009).

2.1.3.1 White tea

White tea is made from the buds and young tea leaves plucked before the buds have fully opened. Then the leaves are streamed and dried with the minimum amount of processing (Figure 1). Some white tea processes are very slightly fermented. For this reason white tea retains the greatest level of polyphenol than any other teas from *Camellia sinensis* (Sharangi, 2009). When infused, white tea has a pale yellow color and a delicate flavor.

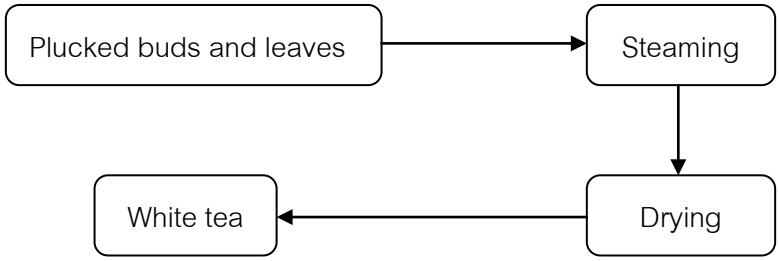


Figure 1 White tea processing

2.1.3.2 Green tea

Green tea is consumed in many Asian and North African countries. It is produced by using young tea leaves. The enzyme activities in plucked tea leaves need to be inactivated. There are two ways to deactivate the enzymes in the fresh leaves, i.e. pan-firing and steaming, resulting in pan-fired and steamed tea respectively (Wang, You, and Chen, 2005). The pan-fired green tea is made from pan-firing fresh tea leaves

at 300-350°C (สุรพล นธการกิจกุล และคณะ, 2551). Then, tea leaves are rolled to shape the green tea leaves, followed by drying (Xu and Chen 2002). Pan-fired green tea is mainly production in China. The production of the steamed green tea is characterized by an initial steaming process, which inactivates the enzyme polyphenol oxidase. The condition for steaming is setting at 100°C for 0.7 minutes (สุรพล นธการกิจกุล และคณะ, 2551). The other important process is rolling, in which leaves are cut and twisted. Drying is the last process to reduce moisture. Steamed green tea is mainly production in Japan. Green tea processing is shown in Figure 2 (Cabrera, Artacho, and Giménez, 2006; สุรพล นธการกิจกุล และคณะ, 2551).

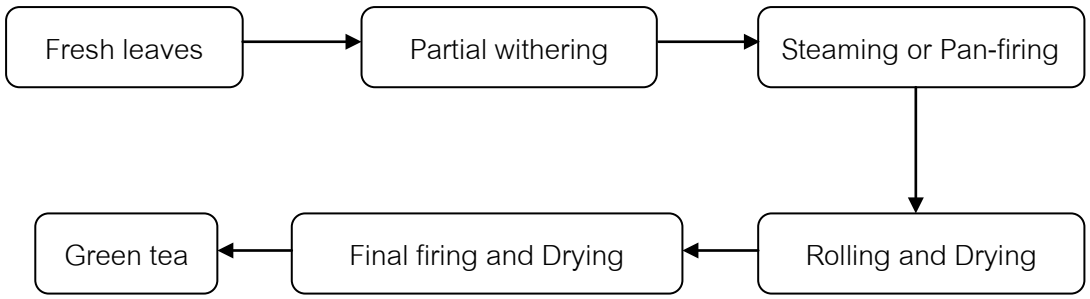


Figure 2 Green tea processing

2.1.3.3 Oolong tea

Oolong tea is generally refered to semi-fermented tea which widely consumed in China and Taiwan (Charrier et al., 2002). The characteristics of oolong tea are between black and green teas (Wang, Provan, and Helliwell, 2000). It has a rich

flowery (fragrant) and fruity aroma (Kan et al., 2005).The processing of oolong tea requires a partial oxidation of the leaves. After the leaves are plucked, they are sun dried or air dried to reduce the moisture content in the withering step. Then, the leaves are shaken or rolled in order to bruise the edges of the leaves. Bruising process causes the tea leaves to partial oxidation. Once the desired level of fermentation is reached, the oxidation process must be terminated by pan-firing the leaves at high temperature. After that, a final drying takes place (Wang et al., 2000). Oolong tea processing is shown in Figure 3.

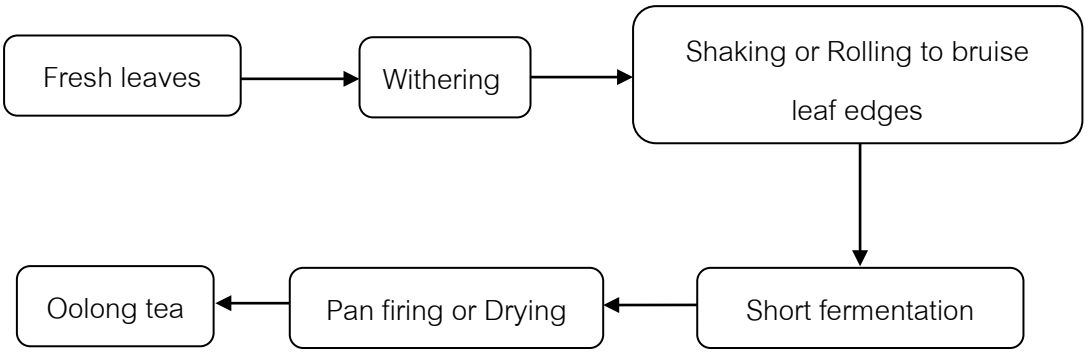


Figure 3 Oolong tea processing

2.1.3.4 Black tea

Black tea is commonly consumed in Western countries. Its processing composed of a few steps as shown in Figure 4. There are two types of black tea processing, conventional method and CTC (cut, tear, curl) method. The difference between two methods is the rolling step. The conventional method involved hand rolling of the tea leaves while the CTC method uses a machine to tear the leaves (Peterson et

al., 2004). Thus, the CTC method uses a shorter duration for rolling tea leaves. After the membranes of the tea leaves have been broken by rolling process, the fermentation process starts. Tea leaves are allowed to ferment for several hours to undergo a full oxidation. This step causes the leaves coppery brown color and gives them the typical flavor of the tea (Bhattacharyya et al., 2007). When the aroma and flavor have fully developed, tea leaves are dried to stop the fermentation process.

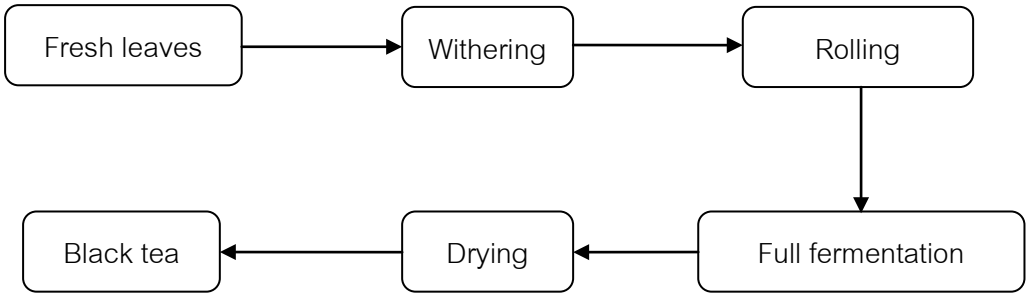


Figure 4 Black tea processing

2.1.4 Chemical constituents of tea

The major components of tea leaves include polyphenols (catechins, flavonoides), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, carbohydrates, lipids, amino acids, vitamins, etc. (Khan and Mukhtar, 2007; Sharangi, 2009) as illustrated in Table 1. Catechins, the flavan-3-ols, are members of a general class of flavonoid. Green tea contains six primary catechin compounds namely (+)-catechin (C), (+)-gallocatechin (GC), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG) and (–)-epigallocatechin gallate (EGCG) (Sharangi, 2009).

EGCG is the major catechin and may account for 50-80% of the total catechins in tea (Sang et al., 2011). In the manufacture of black tea, catechins can be oxidized to theaflavins (TFs) and thearubigins (TRs) (Wang et al., 2000).

Table 1 Chemical constituents of tea (สุรพล นธการกิจกุล และคณะ, 2551)

Components	Dry weight (%)
Soluble in water	
Flavonol	
(–)-EGCG	9-14
(–)-EGC	4-7
(–)-ECG	2-4
(–)-EC	1-3
(+)-GC	1-2
(+)-C	0.5-1
Minor catechin	0.4-1
Flavonol glucosides	3-4
Proanthocyanidins	2-3
Caffeine	3-4
Amino acids	2-4
Carbohydrates	3-5
Organic acids	0.5-2
Saponins	0.04-0.07
Pigments	0.5-0.8
Vitamins	0.6-1
Soluble minerals	2-4
Insoluble or slightly soluble in water	
Cellulose	6-8

Table 1 Chemical constituents of tea (สุรพล นธการกิจกุล และคณะ, 2551) (continued)

Components		Dry weight (%)
	Lignin	4-6
	Polysaccharides	4-10
	Lipids	2-4
	Insoluble pigments	0.5
	Insoluble minerals	1.5-3
Volatiles		0.01-0.02

2.1.5 Health benefits of tea consumption

There are several animal and clinical studies reported the health benefits of tea consumption. Chemical constituents in tea especially polyphenols play an important role to human health (McKay and Blumberg, 2002; Sharangi, 2009). The potentially beneficial effects of tea are summarized below.

2.1.5.1 Antioxidant

In vitro study, tea flavonoids can chelate metal ions to prevent the participation in Fenton and Haber-Weiss reaction (Miller et al., 1996; van Acker et al., 1998). In humans, the plasma antioxidant activities have been increased after the consumption of green tea catechins (Higdon and Frei, 2003). Tea catechins have been found to be better antioxidants than vitamin C, vitamin E, tocopherol, and carotene (Sharangi, 2009).

2.1.5.2 Cardiovascular disease

Green tea is beneficial for lowering cholesterol and preventing platelet clumping (Sharangi, 2009). Ingestion of a green tea extract high in catechins led to a reduction in body fat, systolic blood pressure, and low density lipoprotein (LDL) cholesterol (Nagao, Hase, and Tokimitsu, 2007). Black tea has shown to reduce LDL cholesterol in mildly hypercholesterolemic adults (Davies et al., 2003).

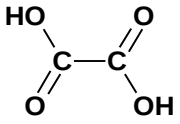
2.1.5.3 Cancer

Green tea extracts and tea polyphenols inhibit tumorigenesis in animal models for different organs including the lung, oral-digestive tract, skin, liver, pancreas, bladder, prostate and mammary glands (Yang et al., 2011). Possible mechanisms of inhibition of carcinogenesis by tea polyphenols have been proposed. Green tea polyphenols induced apoptosis and cell cycle arrest, inhibited cell proliferation, angiogenesis and metastasis (Chen et al., 2008; Sharangi, 2009). Studies have shown that green tea extract acted as direct antioxidant and increased endogenous antioxidant enzymes such as superoxide dismutase and catalase (Lambert and Elias, 2010).

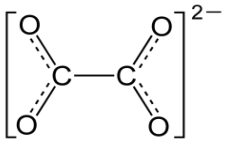
Other benefits of tea consumption are to prevent diabetes (Fu et al., 2011), prevent influenza infection (Matsumoto et al., 2011), improve oral health (Awadalla et al., 2011), treat arthritis (Singh, Akhtar, and Haqqi, 2010), and burn fat (Rains, Agarwal, and Maki, 2011).

2.2 Oxalate

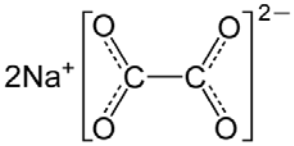
Oxalate, a salt form of oxalic acid, can be found in small amount of many plants. Oxalic acid is an organic acid (dicarboxylic acid; HOOC-COOH). There are two forms of oxalate, water-soluble and water-insoluble oxalate. Oxalic acid binds with sodium (Na^{+}), potassium (K^{+}) and ammonium (NH_4^{+}) ions to form water-soluble oxalate and binds with calcium (Ca^{2+}), Ferrous (Fe^{2+}) and magnesium (Mg^{2+}) ions to form water-insoluble oxalate (Noonan and Savage, 1999). The structure of oxalic acid and its salts are shown in Figure 5.



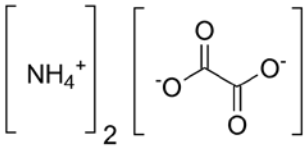
Oxalic acid



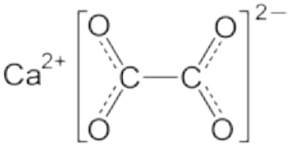
Oxalate anion



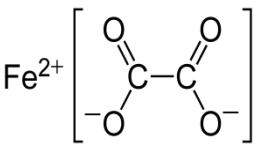
Sodium oxalate



Ammonium oxalate



Calcium oxalate



Iron oxalate

Figure 5 Oxalic acid and its salt forms

2.2.1 Oxalate in foods

In general, oxalate is found in small amounts of human edible foods. It is sometimes important in seasonal foods in some areas of the world. Oxalate rich foods are shown in Table 2. Plants and plant products are the primary sources of dietary oxalate. The oxalate content in plants is highest in the leaves, then the seeds and is lowest in the stems (Noonan and Savage, 1999). It has been suggested that factors such as the age, the climate, the season cultivar, and the type of soil influence the oxalate content of plants (Caliskan, 2000; Morrison and Savage, 2003). The minor amount of oxalate is found in foods of animal origin.

Table 2 Moderate and high oxalate containing foods (Verhulst and De Broe, 2008)

Moderate oxalate content	High oxalate content
beans, green or wax	beet greens
blackberries	chocolate, cocoa
carrots	figs
celery	lamb's quarters
coffee	pepper, black
currants	poppy seeds
wheat	purslane
gooseberries	rhubarb
grapes, Concord	sorrel
green pepper	spinach
lemon peel	Swiss chard
Okra	tea (black)

2.2.2 Synthesis of oxalate in plant

Oxalate can be synthesized in a number of plants. The amount of oxalate varies from a few percents up to 80% of the total weight of the plant. Oxalate is accumulated within the vacuoles of plant cells (Wagner, 1981). It is suggested that oxalate occurs as metabolic end products of the oxaloacetate, glycolate and glyoxylate pathways (Franceschi and Loewus, 1995). Glyoxylate, glycolate and L-ascorbic acid appear to be the major precursors of oxalate in many plants (Seal and Sen, 1970; Yang and Loewus, 1975; Williams, Saito, and Loewus, 1979; Chang and Huang, 1981). Several studies reported that oxalate formation was related to photosynthetic process (Kitchen, Burns, and Langston, 1964; Caliskan, 2000). The biosynthesis of oxalate in plants is shown below (Hodgkinson, 1977; Noonan and Savage, 1999).

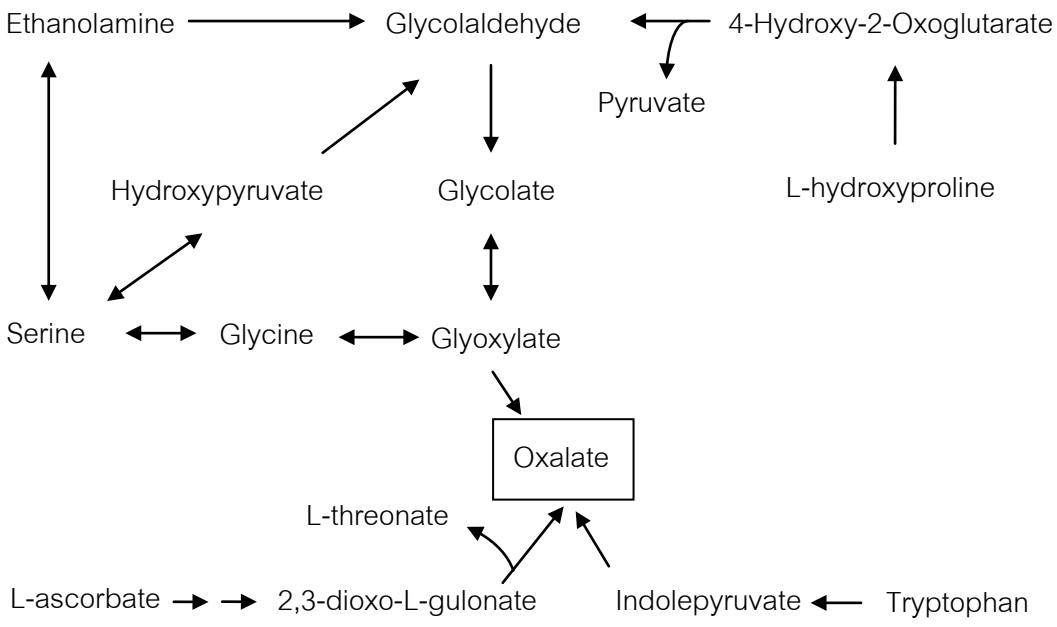


Figure 6 Biosynthesis of oxalate in plants

Oxalate may act as a buffer system in plant cells (Noonan and Savage, 1999). It regulates charge balance, metal detoxification and calcium activity. Oxalate also protects the plants from diseases and grazing animals. Prasad, Sahay, and Masood (1994) found that calcium oxalate could inhibit aflatoxin growth and biosynthesis in *Aspergillus flavus*.

2.2.3 Absorption and excretion of oxalate in mammals

Oxalate can be absorbed in the stomach, small and large intestines (Hatch and Freel, 2005). Some studies indicated that soluble oxalate is absorbed in the colon via passive diffusion (Dobbins and Binder 1977; Modigliani et al., 1978; Hughes and Norman, 1992). Plasma oxalate increased within 1 hour and peaked at 4-6 hours after the oxalate load (Holmes, Ambrosius, and Assimos, 2005). There are three major factors influencing the amount of oxalate absorbed from food: the amount and form of oxalate in the food as consumed; the amount of calcium and magnesium in the oxalate containing food and/or meal; the presence or absence of oxalate-degrading bacteria (Massey, 2007).

The percentage of oxalate absorption in human varied from 1% for rhubarb and spinach to 22 % for tea (Finch, Kasidas, and Rose, 1981) as shown in Table 3. It was suggested that foods containing small amounts of oxalate gave the highest absorption values (Finch et al., 1981; Hanson, Frankos, and Thompson, 1989).

Table 3 Intake and absorption of oxalate from various foods

Food	Oxalate intake (mg/day)	Mean absorption (%)
Rhubarb	1518 - 1718	1.33 ± 0.39
Spinach	1359 - 1498	1.51 ± 0.45
Beetroot	1138 - 1349	2.74 ± 0.75
Peanut	373 - 420	8.28 ± 1.11
Tea	48 - 55	22.22 ± 8.97

Soluble oxalate seems to be the major form of oxalate absorbed by human while insoluble oxalate is very difficult to be absorbed. Thus, foods with high amount of soluble oxalate significantly affected human health.

Oxalate-containing foods will be slightly absorbed when consumed with high amount of calcium and magnesium. Voss et al. (2004) reported that a 10 mmol supplementation of calcium and magnesium ions in human decreased the oxalate absorption significantly and showed that calcium was twice as effective as magnesium.

The presence of intestinal oxalate-degrading microorganisms also affects oxalate bioavailability. One of the best known oxalate-degrading microorganisms is *Oxalobacter formigenes* (Liebman and Al-Wahsh, 2011) which is an obligate anaerobic bacterium found in the lumen and hindgut of human (Allison et al., 1986). The colonization of this bacterium begins in infancy, and by the age of 6-8 year. It is found that only 60-80% of adults but almost all children test positive for this bacterium (Sidhu et al., 1997). *O. formigenes* degrades oxalate by the two enzymes using oxalate as the

substrate for biosynthesis: formyl-CoA transferase and oxalyl-CoA decarboxylase (Hoppe et al., 2005). The products of oxalate degradation are CO_2 and formate, of which the latter is a less toxic acid (Lane, 1994) and will be further metabolized and excreted via the feces (Sidhu et al., 1999).

Oxalate is excreted primarily by the kidney. Urinary oxalate is derived from three sources: hepatic synthesis (40~50%), breakdown of ascorbic acid in the body (40~50%) and diet (10~20%) (William and Wandzilak, 1989; Verhulst and De Broe, 2008). However, the recent study showed that dietary oxalate contributed up to 53% of urinary oxalate (Holmes et al., 2001). Oxalate is excreted in the urine almost immediately after oral load and peaked at 3-6 hours (Hautmann, 1993; Holmes et al., 2005). Oxalate excretion can occur by both glomerular filtration and tubular secretion.

Urinary oxalate excretion in normal adults varies from 28 to 43 mg/day (311-478 μmol/day). Men have slightly higher average oxalate excretion than women. If urinary oxalate is excreted above 40 to 45 mg/day (444-500 μmol/day), it will be defined as hyperoxaluria which is a major risk factor of calcium oxalate stone formation (Marengo and Romani, 2008; Hönow et al., 2010).

Jiang et al. (2011) studied the impact of calcium and oxalate, and *O. formigenes* colonization on urinary oxalate excretion in healthy subjects. The results showed that urinary oxalate excretion was 19.5% lower in *O. formigenes* colonized subjects than in noncolonized subjects on the low calcium (400 mg daily) and moderate oxalate diet

(250 mg daily). Thus, *O. formigenes* has ability to decrease oxalate excretion during periods of low calcium and moderate oxalate intake.

2.2.4 Toxic and adverse effects of oxalate

There have been many published studies reporting about toxic of consuming oxalate. Small doses of oxalate may cause headache, pain and twitching in muscles, and cramps. If consumed in larger doses, oxalate may cause an irregular heartbeat, a drop in blood pressure and signs of heart failure (Tsai et al., 2005). High dose oxalate ingestion may also interfere carbohydrate metabolism, especially by succinic dehydrogenase inhibition (James, 1968) and may cause convulsion, coma and possibly death (Tsai et al., 2005). The mean lethal dose of oxalate is about 15 to 30 g in adult (Tsai et al., 2005), but it has been demonstrated that ingestion of 4-5 g of oxalate may lead to death in adults (Figdor, 1961; Gontzea and Sutzescu, 1968). The other toxic effects of oxalate ingestion are mouth and gastrointestinal tract corrosion, gastric hemorrhage, renal failure, hematuria (Concon, 1988) and a functional hypocalcemia with tetany which results from the binding of oxalate to calcium ions (Hughes and Norman, 1992).

A number of plants contain calcium oxalate crystals which are not absorbed in gastrointestinal tract. These sharp crystals can penetrate to the tissue of the mouth and cause uncomfortable. The plants having calcium oxalate crystals are usually the member of the arum family (Osweiler et al., 1985).

Free oxalate can form insoluble salt with calcium ions and precipitate in the urine, resulting in kidney stones or nephrolithiasis. Nephrolithiasis is a worldwide problem commonly seen between the ages of 30-50 years (Hughes and Norman, 1992). The male to female ratio of the incidence is approximately 3:2 (Scales et al., 2007). Nephrolithiasis has a recurrent rate of 50% in 5-10 years and 75% in 20 years (Sutherland, Parks, and Coe, 1985; Trinchieri et al., 1999). The stone composition is summarized in Table 4. The most common stones are calcium-based including calcium oxalate stones (Menon and Koul, 1992).

Table 4 Composition of kidney stones (Moe, 2006)

Kidney stones	Percentage
Crystal	
Calcium oxalate-monohydrate	40–60
Calcium oxalate-dihydrate	40-60
Calcium phosphate (apatite; $Ca_{10}[PO_4]_6[OH]_2$)	20-60
Calcium phosphate (brushite; $CaHPO_4 \cdot 2H_2O$)	2-4
Uric acid	5-10
Struvite (magnesium ammonium phosphate)	5-15
Cystine	1-2.5
Ammonium urate	0.5-1
Mixed stones	
Mixed calcium oxalate-phosphate	35-40
Mixed uric acid-calcium oxalate	5

The prevalence of calcium oxalate stones varies according to environmental factors, especially dietary intake. There are many dietary factors influenced the risk of calcium oxalate stones. For example, dietary oxalate may increase urinary oxalate excretion, and vitamin C increases oxalate generation and excretion. Urivetzky, Kessaris, and Smith (1991) reported that consumption of vitamin C 1000 mg/day would increase urinary oxalate excretion 6-13 mg/day. On the other hand, the dietary factors that decrease risk of calcium oxalate stones are dietary calcium which binds dietary oxalate in gut, and phytate which inhibits calcium oxalate absorption and calcium oxalate crystal formation (Saxena and Sharma, 2010).

Some of the calcium oxalate crystals precipitated in renal vasculature causing renal tubular obstruction, vascular necrosis and haemorrhage, resulting in anuria, uremia, electrolyte disturbances or rupture (Noonan and Savage, 1999).

2.2.5 The effect of oxalate on mineral bioavailability

Anti-nutrients such as dietary fiber, phytate and oxalate have a toxic or anti-nutritional action (Sotelo et al., 2010). Oxalate binds with several essential minerals including calcium, iron, and magnesium to form insoluble compounds in the gastrointestinal tract, making these minerals unavailable to human. The ability of oxalate to bind with minerals and form insoluble salt is dependent on its solubility product constant (Ksp). The smaller the Ksp value, the lower the solubility. The Ksp values of key minerals are shown in Table 5 (Budavari et al., 1989).

Table 5 The Ksp values of example minerals

Minerals	Ksp (Oxalate salt)
Calcium	2.7×10^{-9}
Zinc	1.7×10^{-9}
Iron	1.5×10^{-6}
Magnesium	2.2×10^{-5}

There are many factors affecting bioavailability of calcium such as phytate and oxalate (Hambidge, 2010). Calcium oxalate is insoluble in neutral or alkaline pH but freely dissolves in acidic condition (Morrison and Savage, 2003). The binding capacity of calcium and oxalate is high at pH above 4 (Siener, Heynck, and Hesse, 2001). High-oxalate foods such as spinach and rhubarb are high in calcium but are poor sources of calcium because of the presence of oxalates. The oxalate to calcium ratio in certain foods is classified into three groups: plants with an oxalate to calcium ratio greater than two, approximately one, and less than one. The oxalate and calcium contents, as well as oxalate to calcium ratio in some foods are shown in Table 6. Foods with oxalate to calcium ratio higher than two contain excess oxalate that can bind calcium in other foods eaten at the same time while foods with oxalate to calcium ratio about one do not inhibit calcium absorption from other foods (Noonan and Savage, 1999).

High oxalate foods also have negative effect on iron absorption (Noonan and Savage, 1999). It is suggested that the oxalate may impair the absorption of non-heme iron (Chawla, Saxena, and Seshadri, 1988).

Table 6 Oxalate, calcium and oxalate/calcium ratio of some common foods

Foodstuff	Oxalate (mg/100 g FW)		Calcium (mg/100 g FW)		Oxalate/Ca (mEq)
	Range	Mean	Range	Mean	
Group 1					
Rhubarb (*Rheum rhaponticum*)					
Victoria, forced, stewed	260		12.4		9.32
raw	275-1336	805	40-50	45	7.95
Common sorrel (*Rumex acetosa*)	270-730	500	35-45	40	5.56
Red beetroot (*Beta vulgaris*)	121-450	275	121-450	275	5.09
Garden sorrel (*Rumex patientia*)	300-700	500	40-50	45	4.94
Purslane (*Portulaca oleracea*)	910-1679	1294	13-236	125	4.60
Spinach (*Spinacia oleracea*)	320-1260	970	80-122	101	4.27
Garden orach (*Atriplex hortensis*)	300-1500	900	100		4.00
Coffee (*Coffea arabica*)	50-150	100	10-15	12	3.70
Cashew (*Anacardium occidentale*)	231		41		2.50
Cocoa (*Theobroma cacao*)	500-900	700	100-150	125	2.49
Beet leaves (*Beta vulgaris* var. cicla)	300-920	610	100-120	110	2.46
Rhubarb (*Rheum rhaponticu*)					
Crimson, end of season, stewed	460		91.5		2.23
Group 2					
Potato (*Solanum tuberosom*)	20-141	80	10-34	22	1.62
Amaranth (Amaranthus polygonoicles)	1586		595		1.18
Tea (*Thea chinesis*)	300-2000	1150	400-500	450	1.14
Amaranth (*Amaranthus tricolor*)	1087		453		1.07
Rhubarb (*Rheum rhaponticum*)					
Victoria, end of season, stewed	620		266		1.04
Group 3					
Apple (*Malus* spp.)	0-30	15	5-15	10	0.67
Blackcurrant (*Ribes nigrum*)	2-90	50	19-50	35	0.63
Tomato (*Licopersicum esculentum*)	5-35	20	10-20	15	0.58
Parsley (*Petroselinum sativum*)	140-200	170	180-290	235	0.32

From Noonan and Savage, 1999; FW = fresh weight

Oxalate and zinc taken together slightly interferes zinc absorption (Morrison and Savage, 2003). The precipitation of zinc oxalate was inhibited when magnesium presented (Faboya, 1990).

2.2.6 Methods of oxalate determination

There have been several methods for oxalate determination. Beutler et al. (1980) analyzed oxalate by using an enzyme oxalate decarboxylase from aspergillus species to decarboxylate oxalic acid to formic acid. The formate form could be determined by photometric method. The oxalate determination by AOAC (2006) method was based on calcium oxalate precipitation. Oxalate was extracted with acid, precipitated as the calcium salt, then determined by permanganate titration or atomic absorption. The alternate methods for determining precipitated calcium were colorimetric method and gas chromatography (Massey, 2007). This precipitation method had some limitations in terms of sensitivity, presicision and time consumption. Enzymatic assay using oxalate oxidase is one of the methods used for determining oxalate. This method is based on oxidation of oxalate by oxalate oxidase and detection of hydrogen peroxide (H_2O_2) produced during this reaction (Okombo and Liebman, 2010). Other methods for analyzing extracted oxalate were ion electrophoresis, capillary electrophoresis (Holmes and Kennedy, 2000), and high performance liquid chromatography (HPLC) (Hönow et al., 1997). The HPLC method was shown to be accurate and reliable for determinations of oxalate in plant materials (Brega et al., 1992; Savage et al., 2000).

For HPLC method, there are two methods for oxalate analysis. One is by ion-exchange and the other is by anion exclusion. Ion-exchange chromatography consists of anion exchange column which can be used to separate oxalate from other anions. In anion exclusion method, the cation exchange column is used as the separator column. Both a conductivity detector and UV detector can be used for detection of oxalate.

2.3 Oxalate in tea

Tea is a principal source of oxalate intake of English diets (Zarembski and Hodgkinson, 1962). Previous studies showed that black tea contained much higher soluble oxalate content than green tea and oolong tea. Reduction of black tea consumption by both stone formers and normal individuals is able to decrease the risk of kidney stones.

2.3.1 The studies related to oxalate content in tea

McKay et al. (1995) measured the oxalate contents of black tea, green tea and herbal tea. All teas were steeped in 250 ml of boiling tap water for 5 minutes without stirring. Samples of black tea were also steeped for 1 minute. A standard clinical diagnostic kit was used to determine the oxalate content of samples. The results showed that the oxalate contents of black tea ranged from 7 to 32 times and 1.5 to 3 times more than those of herbal tea and green tea respectively. The oxalate content of black tea steeped for 1 minute was less than that when steeped for 5 minutes. This study

suggested that herbal tea was the alternative hot beverages for people who tend to form calcium oxalate stones.

Charrier et al. (2002) measured the oxalate content of tea and herbal tea available in New Zealand. Thirty-two samples of green, oolong and black teas, and fifteen herbal teas were analyzed. Each sample of tea in tea bag was steeped in 245 ml of 90°C nanopure water for 5 minutes. Each tea bag was stirred two times. After 5 minutes for tea infusion, the tea bag was removed and squeezed two times. The samples of tea leaves were prepared by adding nanopure water to two loosely heaped teaspoons of tea leaves. Tea leaves were removed by filtering the solution through a tea strainer after 5 minutes of infusion. The soluble oxalate content of tea sample was determined by HPLC. The results showed that the total amount of soluble oxalate extracted from black tea in leaf form and bag form were 5.11±0.51 mg/g tea and 4.68±0.38 mg/g tea, respectively. The soluble oxalate content of green tea was 0.68±0.19 mg/g tea, while 0.23 mg/g tea was obtained from oolong tea. Herbal tea contained very low level of soluble oxalate ranged from not detected to 3.00 mg/g tea. When calculated as a cup of tea, soluble oxalate content ranged from 0.58 to 16.43 mg/cup of tea. The consumption of six cups of tea (a moderated daily consumption for a tea drinker) provided 3.5 to 98.6 mg soluble oxalate/day.

Savage, Charrier, and Vanhanen (2003) measured bioavailability of soluble oxalate from two samples of black tea in tea bag. Ten healthy non-stone-forming

subjects were recruited to a randomized double-blind crossover study. Each subject consumed six cups of tea per 24 hours. Tea sample was prepared by infusing tea bag in 245 ml of 90°C nanopure water for 5 minutes. The soluble oxalate was determined by HPLC. The results showed that the mean soluble oxalate content of two samples of black tea were 6.07 and 6.22 mg/g tea respectively. Urine samples were collected for the initial 6 hours followed by a further 18 hours after consuming the first cup of tea. The availability of oxalate consumed from the black tea without added milk over 6 hours ranged from 1.9 to 4.7% for the two tea samples. No oxalate in the urine appeared to be derived from the tea when consumed with milk. Over 24 hours, the availability of oxalate consumed from the black tea without added milk ranged from -3.0 to 2.3% for the two tea samples. When milk was consumed with the black tea, the oxalate output was significantly reduced.

Tsai et al. (2005) measured the oxalate contents in foods and beverages including green tea and black tea in Taiwan. Samples were prepared in different ways according to the different textures of the materials. Solid sample was made into juice with distilled water. The oxalate concentration of each sample was analyzed by the oxalate kit. The result showed that the oxalate contents of packaged green tea and black tea were 4.59 and 4.82 mg/100 g tea, respectively. This study suggested that patients who need to restrict their intake of oxalate should replace tea with plain water.

Hönow et al. (2010) analyzed the oxalate content of green tea in different origin, quality, preparation, and time of harvest. Fifty-two samples of Chinese green tea were prepared by steeping 3.5 g of tea leaves in 200 ml of 90°C water for 5 or 10 minutes, and analyzed by HPLC-enzyme-reactor method. The results indicated that the soluble oxalate content of Chinese green tea varied widely from 8.3 to 139.8 mg/l. Leaves harvested in autumn had more oxalate content than leaves harvested in spring. Steeping duration of green tea leaves between 5 and 10 minutes had no significant effect on oxalate content. However, the longer steeping duration appeared to slightly increase the oxalate content. This study suggested that patients at risk for recurrent stone formation should take into account the oxalate content of green tea.

Beata and Grzegorz (2010) studied the effect of extraction conditions on the soluble oxalate content in water infusions of green and herbal teas. Tea samples were prepared by four different extraction methods: a traditional extraction procedure with boiling water of 100°C, a microwave radiation supported extraction at 80°C, and an ultrasound supported extraction at 40°C and at 60°C. The results showed that the highest level of soluble oxalate obtained from the microwave procedure providing the soluble oxalate ranged from 7.73 to 14.89 mg/g tea for green tea and from 3.53 to 18.11 mg/g tea for herbal tea. On the other hand, the lowest level of soluble oxalate obtained from ultrasound supported extraction at 40°C procedure; the soluble oxalate ranged from 5.06 to 10.88 mg/g tea for green tea and from 1.5 to 11.03 mg/g tea for herbal teas.

CHAPTER III

MATERIALS AND METHODS

3.1 Instruments

HPLC system was composed of a model 626 pump, model 630 autosampler, a model 650 conductivity detector with a model 640 suppressor, all from Alltech, USA. Chromatographic separation was carried out using anion exchange column (Allsep™, 7 µm, 100 x 4.6 mm, USA). PeakSimple chromatography data system SRI model 302 was used for data integration. Analytical balance (Sartorius CP, Scientific Promotion) was used for measuring tea leaves. Syringe (3 ml and 5 ml, Terumo®, Philippines) and filter membrane (Cellulose acetate, Pore size 0.45 µm, USA) were used for filtering tea samples and standard oxalate solutions.

3.2 Reagents

The chemicals used for this study consisted of oxalate standard solution (IC grade, Alltech, USA), sodium carbonate (Analytical grade, Prolabo®, Belgium), sodium bicarbonate (Analytical grade, BDH chemicals, UK), ultrapure water (ω-18, Maxima ultrapure water, ELGA, France) and water (Coolly fresh®, filtered and reverse osmosis, Thailand). Tea samples were prepared using filtered water in order to relate real life situation.

3.3 Methods

3.3.1 Sample Collection

Tea samples (white, green, oolong and black tea) were purchased from markets from February to June 2011. Teas both in leaf and bag forms were collected. Five brands of leaf and bag forms were collected for each tea type (total=40 brands). Three samples of each brand were selected. Therefore, one-hundred and twenty samples were used to analyze the oxalate content.

3.3.2 Research procedure

The experimental procedures for determining the oxalate content in tea samples were shown in Figure 7. There were 2 phases of the experiment. Phase I, all tea samples were prepared by steeping in hot water and were analyzed by HPLC method. The goal in this phase was to obtain the oxalate content in 40 brands of tea samples. Phase II, the 2 of 5 brands of each tea type were selected and steeped in hot water in different steeping times to study the effect of steeping duration on the oxalate contents.

3.3.3 Preparation of tea samples (modified form Hönow et al., 2010)

Tea samples in leaf form

Tea leaves (3.50 g) were accurately weighed into a 250-ml beaker. The sample was cleaned by rinsing in hot water (100°C, 100 ml). After cleaning, 200 ml of hot filtered water (80±2°C) was added into the beaker. The steeping times for phase I was 3 minutes and for phase II were 0.5, 1, 3, 5 and 7 minutes. Then the solution was filtered

through a tea strainer to remove tea leaves. Each tea sample was cooled to room temperature. For analysis, 20 ml of tea infusion was pipetted into a 100-ml volumetric flask and made up to volume with filtered water. After that, the sample was filtered through a 0.45 µm cellulose acetate membrane syringe filter and then analyzed by HPLC method.

Tea samples in bag form

Each tea bag was placed into the 250-ml beaker. 200 ml of hot filtered water (80±2°C) was added for infusion. After 3 minutes of infusion in phase I and 0.5, 1, 3, 5 and 7 minutes of infusion in phase II, the tea bag was taken out. When the tea solution reached room temperature, the 20 ml of tea solution was pipetted in a volumetric flask to make up 100-ml volume. After that, each tea sample was filtered through a cellulose acetate membrane syringe filter with a pore size of 0.45 µm and then analyzed by HPLC method.

3.3.4 Preparation of standard solutions

A 100 µg/ml of oxalate standard stock solution was prepared in ultrapure water and stored at 4°C. For analysis, 1, 5, 10, 20 and 30 µg/ml of standard solutions were prepared from stock oxalate standard solution by appropriate dilution with ultrapure water. All standard solutions were filtered through a 0.45 µm cellulose acetate membrane syringe filter and protected from light.

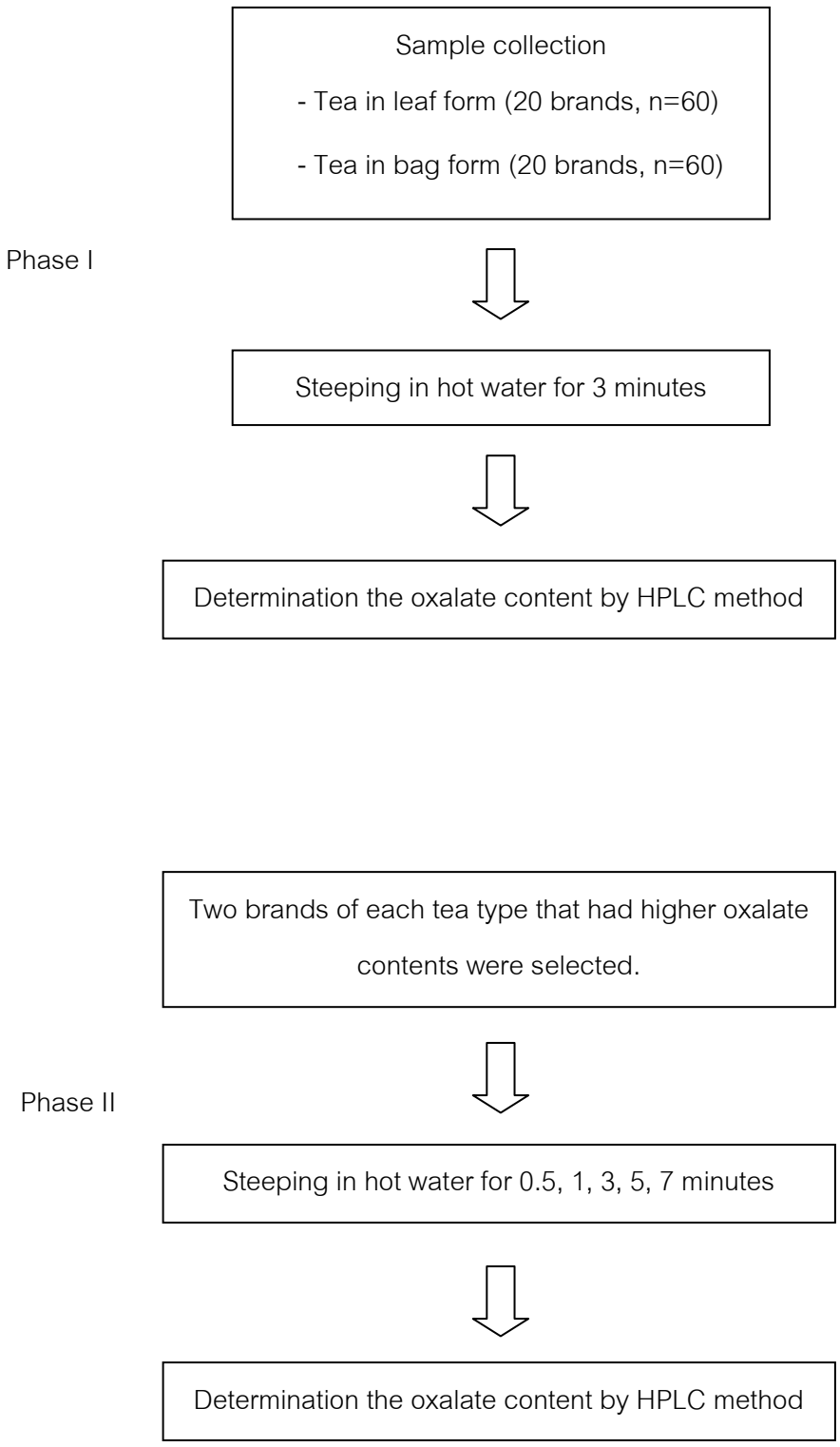


Figure 7 Research procedure

3.3.5 Determination of oxalate content in tea samples by HPLC

Plant tissues are the main source of oxalate. There are two forms of oxalate occurred in plant tissue, soluble and insoluble oxalate (Hönow and Hesse, 2002). For determination of oxalate, soluble oxalate and total oxalate are analyzed. Insoluble oxalate is obtained from deducting soluble oxalate from total oxalate (Liebman and Okombo, 2009). In this study only soluble oxalate was analyzed because tea drinkers generally do not consume whole tea leaves. Moreover, soluble oxalate is an important form of oxalate absorbed in the gut and has an influence on human body. High performance liquid chromatography (HPLC) method was used for analyzing oxalate because it was rapid, sensitive, accurate and reliable (Wilson, Shaw, and Knight, 1982; Judprasong et al., 2006).

The chromatographic conditions were set up according to the manufacturer's recommendations. Tea samples and standard solutions were analyzed by anion exchange column using 0.85 mM sodium bicarbonate and 0.9 mM sodium carbonate as a mobile phase prepared as followed: 0.07 g of sodium bicarbonate and 0.095 g of sodium carbonate were accurately weighed and transferred into a beaker. The reagents were mixed with 1,000 ml of ultrapure water. The mobile phase was filtered to remove any particulate matters and other impurities. It was also degassed by sonication prior to use. The oxalate anion was detected with a conductivity detector. The HPLC column was run at a flow rate of 1 ml/minute. The volume for each injection was 20 µl.

3.4 Method validation

3.4.1. Precision and accuracy

The precision was investigated by five injections of each oxalate standard solution (1, 5, 10, 20 and 30 µg/ml) in the same day (Intraday-precision) and the analysis was continued for 3 consecutive days (Interday-precision). Precision could be expressed as the standard deviation (SD) and relative standard deviation (RSD) shown in Equation 3.1 and 3.2. The smaller the RSD value, the greater precision of the analysis.

$$S.D = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{n-1}} \quad (3.1)$$

$$R.S.D = \frac{S.D}{\bar{X}} \quad (3.2)$$

The accuracy was determined by standard addition technique. Known oxalate standard solutions containing 1, 5, 10, 20 and 30 µg/ml were added to know aliquot of sample solution. Oxalate in the added sample was analyzed in triplicate by HPLC. The accuracy was expressed as % recovery shown in Equation 3.3

$$\%\, Recovery = \frac{conc.recovered \times 100}{conc.added} \quad (3.3)$$

The standard calibration curve had a correlation coefficient of 0.9999. The relative standard deviations (RSD) were 0.59% and 1.37% for Intraday-precision and

Interday-precision respectively. Percentage of the recovery was 94.41. HPLC chromatogram of oxalate standard solution and tea sample were shown in Figure D-1 and D-2 (Appendix D).

3.5 Data analysis

The oxalate standard calibration curve was obtained by plotting the peak area against the concentration of oxalate standard. The linear regression equation and the correlation coefficient (r^2) were calculated from calibration curve. The data were analyzed using SPSS (version 17.0, SPSS Inc. Chicago, IL, USA). The effect of tea type was calculated by Kruskal-Wallis H test and the effect of tea form was calculated by Mann-Whitney U test.

CHAPTER IV

RESULTS

4.1 Oxalate content in tea samples

One-hundred and twenty samples of tea-white, green, oolong and black teas from 40 brands-were determined for the oxalate content by high performance liquid chromatography. The values of oxalate were presented as range and mean ± SD (Table 7 and 8). In leaf form, the highest oxalate content was found in black tea (2.32 ± 1.10 mg/g tea), then in oolong tea (1.62 ± 0.79 mg/g tea), green tea (1.41 ± 0.56 mg/g tea) and lowest in white tea (1.29 ± 0.33 mg/g tea). In bag form, the oxalate content in black tea (2.37 ± 0.80 mg/g tea) was higher than that of oolong tea (1.88 ± 1.04 mg/g tea). The amount of oxalate in green tea (1.24 ± 0.48 mg/g tea) was comparable with that of white tea (1.25 ± 0.45 mg/g tea). The oxalate contents of black tea both in leaf and bag forms were significantly different from those of white tea and green tea. Only bag form, oxalate contents in black tea differed from those in oolong tea significantly (Table 9 and 10). The amount of oxalate found in leaf and bag forms were not different (Table 11). Oxalate contents in tea samples were also calculated and compared between products made in Thailand and other countries as illustrated in Table 12. The oxalate content of tea samples produced in others countries was slightly higher than that made in Thailand (p-value>0.05).

Table 7 Oxalate content in tea samples (leaf form)

No.	Type of tea	Brands	Oxalate content (mg/g tea)	
			Range	Mean ± SD
1	White (W)	W1	1.41 - 1.43	1.42 ± 0.01
2		W2	1.57 - 1.69	1.64 ± 0.05
3		W3	0.85 - 0.90	0.88 ± 0.02
4		W4	1.43 - 1.65	1.58 ± 0.10
5		W5	0.91 - 0.94	0.92 ± 0.01
			0.85 - 1.69	**1.29 ± 0.33**
6	Green (G)	G1	1.32 - 1.35	1.33 ± 0.01
7		G2	2.11 - 2.18	2.16 ± 0.03
8		G3	1.42 - 1.45	1.43 ± 0.01
9		G4	1.63 - 1.76	1.71 ± 0.05
10		G5	0.44 - 0.45	0.44
			0.44 - 2.18	**1.41 ± 0.56**
11	Oolong (O)	O1	0.74 0.79	0.76 ± 0.02
12		O2	2.26 - 2.28	2.27 ± 0.01
13		O3	2.79 - 2.82	2.80 ± 0.01
14		O4	1.34 - 1.42	1.38 ± 0.03
15		O5	0.86 - 0.97	0.91 ± 0.05
			0.74 - 2.82	**1.62 ± 0.79**
16	Black (B)	B1	2.29 - 2.37	2.33 ± 0.03
17		B2	1.48 - 1.66	1.58 ± 0.08
18		B3	4.40 - 4.42	4.41 ± 0.01
19		B4	1.36 - 1.49	1.40 ± 0.06
20		B5	1.84 - 1.87	1.85 ± 0.02
			1.36 - 4.42	**2.32 ± 1.10**

Table 8 Oxalate content in tea samples (bag form)

No.	Type of tea	Brands	Weight/bag (g)	Oxalate content (mg/g tea)	
				Range	Mean ± SD
1	White (WB)	WB1	1.5	1.37 - 1.47	1.43 ± 0.04
2		WB2	2	0.40 - 0.45	0.42 ± 0.02
3		WB3	1.8	1.58 - 1.63	1.60 ± 0.02
4		WB4	1.8	1.43 - 1.79	1.60 ± 0.15
5		WB5	1.8	1.19 - 1.23	1.21 ± 0.02
				0.40 - 1.79	**1.25 ± 0.45**
6	Green (GB)	GB1	2	0.55 - 0.60	0.58 ± 0.02
7		GB2	2	1.18 - 1.27	1.22 ± 0.04
8		GB3	2	0.87 - 0.93	0.90 ± 0.02
9		GB4	2	1.56 - 1.78	1.63 ± 0.10
10		GB5	2	1.75 - 1.99	1.87 ± 0.10
				0.55 - 1.99	**1.24 ± 0.48**
11	Oolong (OB)	OB1	2	1.13 - 1.22	1.18 ± 0.04
12		OB2	2.5	1.00 - 1.17	1.07 ± 0.07
13		OB3	1.5	1.85 - 1.87	1.86 ± 0.01
14		OB4	2	3.82 - 3.94	3.89 ± 0.05
15		OB5	2	1.40 - 1.44	1.42 ± 0.02
				1.00 - 3.94	**1.88 ± 1.04**
16	Black (BB)	BB1	3.125	1.45 - 1.50	1.47 ± 0.02
17		BB2	1.5	2.37 - 2.39	2.37 ± 0.01
18		BB3	2	1.72 - 1.86	1.81 ± 0.06
19		BB4	1.75	3.79 - 3.82	3.81 ± 0.01
20		BB5	2	2.37 - 2.43	2.41 ± 0.03
				1.45 - 3.82	**2.37 ± 0.80**

Table 9 Multiple comparisons of oxalate content between white tea, green tea, oolong tea and black tea (leaf form)

	Type of tea	White tea	Green tea	Oolong tea	Black tea
p-value	White tea				
	Green tea	0.455			
	Oolong tea	0.756	0.604		
	Black tea	0.001*	0.010*	0.059	

* Statistical significant differences were calculated using Mann-Whitney U test.

Table 10 Multiple comparisons of oxalate content between white tea, green tea, oolong tea and black tea (bag form)

	Type of tea	White tea	Green tea	Oolong tea	Black tea
p-value	White tea				
	Green tea	0.901			
	Oolong tea	0.384	0.120		
	Black tea	0.000*	0.000*	0.031*	

* Statistical significant differences were calculated using Mann-Whitney U test.

Table 11 Multiple comparisons of oxalate content in tea samples between leaf and bag forms

	Forms		Bag			
		Type of tea	White tea	Green tea	Oolong tea	Black tea
p-value	Leaf	White tea	0.983			
		Green tea		0.395		
		Oolong tea			0.373	
		Black tea				0.350

Table 12 Oxalate content in tea samples between products made in Thailand and other countries

Products	No. of brand	Oxalate content (mg/g tea)	
		Range	Mean ± SD
Thailand	19	0.44 - 4.41	1.58 ± 0.84
Other countries	21	0.42 – 3.89	1.76 ± 0.87

The oxalate contents of tea samples were also calculated in a cup of tea (200 ml) in order to estimate the amount of oxalate consumed per day (Table 13 and 14). For leaf form, oxalate content of white tea was between 3.08 and 5.72 mg/cup. Green tea contained oxalate content between 1.55 and 7.55 mg/cup. Oolong tea and black tea contained higher oxalate level. Oxalate level of oolong tea ranged from 2.66 to 9.81 mg/cup while for black tea was between 4.90 and 15.40 mg/cup. For bag form, the oxalate contents of white tea, green tea, oolong tea and black tea were 0.84 to 2.89, 1.15 to 3.74, 2.36 to 7.78 and 3.56 to 6.67 mg/cup, respectively. The oxalate contents in six cups of tea (moderate consumption per day for a tea drinker) were also calculated.

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Table 13 Oxalate content in a cup and six cups of tea samples (leaf form)

No.	Type of tea	Brands	mg oxalate/cup of tea[a]	mg oxalate/six cups of tea[b]
1	White (W)	W1	4.97 ± 0.03	29.82
2		W2	5.72 ± 0.18	34.32
3		W3	3.08 ± 0.08	18.48
4		W4	5.51 ± 0.35	33.06
5		W5	3.24 ± 0.04	19.44
		RANGE	3.08-5.72	18.48-34.32
6	Green (G)	G1	4.67 ± 0.05	28.02
7		G2	7.55 ± 0.11	45.30
8		G3	5.01 ± 0.05	30.06
9		G4	5.97 ± 0.19	35.82
10		G5	1.55 ± 0.01	9.30
		RANGE	1.55-7.55	9.30-45.30
11	Oolong (O)	O1	2.66 ± 0.08	15.96
12		O2	7.94 ± 0.03	47.64
13		O3	9.81 ± 0.03	58.86
14		O4	4.83 ± 0.11	28.98
15		O5	3.19 ± 0.17	19.14
		RANGE	2.66-9.81	15.96-58.86
16	Black (B)	B1	8.16 ± 0.11	48.96
17		B2	5.53 ± 0.27	33.18
18		B3	15.40 ± 0.04	92.40
19		B4	4.90 ± 0.21	29.40
20		B5	6.48 ± 0.06	38.88
		RANGE	4.90-15.40	29.40-92.40

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

Table 14 Oxalate content in a cup and six cups of tea samples (bag form)

No.	Type of tea	Brands	Weight/bag (g)	mg oxalate/ cup of tea[a]	mg oxalate/ six cups of tea[b]
1	White (WB)	WB1	1.5	2.14 ± 0.07	12.84
2		WB2	2	0.84 ± 0.04	5.04
3		WB3	1.8	2.88 ± 0.04	17.28
4		WB4	1.8	2.89 ± 0.26	17.34
5		WB5	1.8	2.18 ± 0.03	13.08
			RANGE	0.84-2.89	5.04-17.34
6	Green (GB)	GB1	2	1.15 ± 0.04	6.90
7		GB2	2	2.44 ± 0.07	14.64
8		GB3	2	1.81 ± 0.05	10.86
9		GB4	2	3.26 ± 0.20	19.56
10		GB5	2	3.74 ± 0.19	22.44
			RANGE	1.15-3.74	6.90-22.44
11	Oolong (OB)	OB1	2	2.36 ± 0.08	14.16
12		OB2	2.5	2.68 ± 0.17	16.08
13		OB3	1.5	2.79 ± 0.02	16.74
14		OB4	2	7.78 ± 0.11	46.68
15		OB5	2	2.84 ± 0.03	17.04
			RANGE	2.36-7.78	14.16-46.68
16	Black (BB)	BB1	3.125	4.59 ± 0.06	27.54
17		BB2	1.5	3.56 ± 0.01	21.36
18		BB3	2	3.62 ± 0.12	21.72
19		BB4	1.75	6.67 ± 0.02	40.02
20		BB5	2	4.81 ± 0.05	28.86
			RANGE	3.56-6.67	21.36-40.02

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

4.2 Oxalate content in tea samples in various steeping times

Two brands of each tea type, which had the highest amount of oxalate, were selected and steeped in hot water in different steeping times. The oxalate content was found to increase with longer steeping duration as shown in Table 15 and 16.

Table 15 Oxalate content in tea samples (leaf form) in various steeping times

Type of tea	Brands	Time (min)	Oxalate content (mg/g tea)	
			Range	Mean ± SD
White (W)	W2	0.5	0.69 - 0.71	0.70 ± 0.01
		1	1.33 - 1.35	1.34 ± 0.01
		3	1.57 - 1.69	1.64 ± 0.05
		5	2.26 - 2.31	2.29 ± 0.03
		7	2.41 - 2.43	2.42 ± 0.01
	W4	0.5	0.83 - 0.86	0.85 ± 0.02
		1	1.04 - 1.18	1.12 ± 0.06
		3	1.43 - 1.65	1.58 ± 0.10
		5	1.88 - 2.02	1.93 ± 0.06
		7	2.28 - 2.59	2.42 ± 0.13
Green (G)	G2	0.5	1.52 - 1.65	1.56 ± 0.06
		1	2.06 - 2.12	2.09 ± 0.03
		3	2.11 - 2.18	2.16 ± 0.03
		5	4.52 - 4.69	4.62 ± 0.07
		7	5.12 - 5.17	5.14 ± 0.02
	G4	0.5	1.28 - 1.41	1.33 ± 0.05
		1	1.53 - 1.59	1.57 ± 0.03
		3	1.63 - 1.76	1.71 ± 0.05
		5	2.26 - 2.37	2.33 ± 0.05
		7	2.36 - 2.42	2.39 ± 0.02

Table 15 Oxalate content in tea samples (leaf form) in various steeping times (continued)

Type of tea	Brands	Time (min)	Oxalate content (mg/g tea)	
			Range	Mean ± SD
Oolong (O)	O2	0.5	0.44	0.44
		1	0.79 - 0.82	0.80 ± 0.01
		3	2.26 - 2.28	2.27 ± 0.01
		5	2.42 - 2.43	2.42 ± 0.01
		7	2.62 - 2.67	2.65 ± 0.02
	O3	0.5	1.40 - 1.42	1.41 ± 0.01
		1	1.87 - 1.98	1.93 ± 0.04
		3	2.79 - 2.82	2.80 ± 0.01
		5	3.57 - 3.61	3.60 ± 0.01
		7	3.64 - 3.66	3.65 ± 0.01
Black (B)	B1	0.5	1.84 - 1.87	1.86 ± 0.01
		1	2.25 - 2.27	2.26 ± 0.01
		3	2.29 - 2.37	2.33 ± 0.03
		5	2.90 - 2.96	2.93 ± 0.02
		7	2.92 - 2.96	2.94 ± 0.02
	B3	0.5	2.33 - 2.36	2.35 ± 0.01
		1	3.71 - 3.75	3.73 ± 0.02
		3	4.40 - 4.42	4.41 ± 0.01
		5	4.98 - 5.00	4.99 ± 0.01
		7	5.43 - 5.47	5.46 ± 0.01

Table 16 Oxalate content in tea samples (bag form) in various steeping times

Type of tea	Brands	Time (min)	Oxalate content (mg/g tea)	
			Range	Mean ± SD
White (WB)	WB3	0.5	0.49 - 0.52	0.51 ± 0.01
		1	0.65 - 0.70	0.67 ± 0.02
		3	1.58 - 1.63	1.60 ± 0.02
		5	1.63 - 1.64	1.63
		7	1.97 - 2.00	1.99 ± 0.01
	WB4	0.5	0.64 - 0.66	0.65 ± 0.01
		1	0.74 - 0.76	0.75 ± 0.01
		3	1.43 - 1.79	1.60 ± 0.15
		5	1.94 - 1.99	1.96 ± 0.02
		7	2.53 - 2.57	2.55 ± 0.02
Green (GB)	GB4	0.5	0.24 - 0.30	0.26 ± 0.03
		1	0.77 - 0.78	0.78 ± 0.01
		3	1.56 - 1.78	1.63 ± 0.10
		5	2.33 - 2.34	2.34
		7	2.35 - 2.41	2.38 ± 0.02
	GB5	0.5	0.83 - 0.90	0.87 ± 0.03
		1	1.28 - 1.32	1.30 ± 0.02
		3	1.75 - 1.99	1.87 ± 0.10
		5	3.50 - 3.51	3.50
		7	3.58 - 3.63	3.61 ± 0.02

Table 16 Oxalate content in tea samples (bag form) in various steeping times (continued)

Type of tea	Brands	Time (min)	Oxalate content (mg/g tea)	
			Range	Mean ± SD
Oolong (OB)	OB3	0.5	0.41 - 0.43	0.42 ± 0.01
		1	0.90 - 0.92	0.91 ± 0.01
		3	1.85 - 1.87	1.86 ± 0.01
		5	2.44 - 2.46	2.45 ± 0.01
		7	2.51 - 2.52	2.52
	OB4	0.5	0.92 - 0.98	0.94 ± 0.03
		1	1.80	1.80
		3	3.82 - 3.94	3.89 ± 0.05
		5	4.61 - 4.62	4.61 ± 0.01
		7	4.43 - 4.86	4.71 ± 0.20
Black (BB)	BB4	0.5	0.94 - 0.96	0.95 ± 0.01
		1	1.89 - 1.92	1.91 ± 0.01
		3	3.79 - 3.82	3.81 ± 0.01
		5	4.89 - 4.93	4.91 ± 0.02
		7	5.22 - 5.27	5.24 ± 0.02
	BB5	0.5	0.84 - 0.85	0.84 ± 0.01
		1	1.60 - 1.63	1.62 ± 0.01
		3	2.37 - 2.43	2.41 ± 0.03
		5	3.08 - 3.10	3.09 ± 0.01
		7	3.38 - 3.40	3.39 ± 0.01

The oxalate contents of tea samples in various steeping times in a cup and six cup of tea were shown in Table 17 and 18. The highest amount of oxalate was found in black tea (19.09 ± 0.05 mg/cup, leaf form) steeped at 7 minutes.

Table 17 Oxalate contents in a cup and six cups of tea samples (leaf form) in various steeping times

Type of tea	Brands	Time (min)	mg oxalate/ cup of tea[a]	mg oxalate/ six cups of tea[b]
White (W)	W2	0.5	2.46 ± 0.03	14.76
		1	4.70 ± 0.04	28.20
		3	5.72 ± 0.18	34.32
		5	8.03 ± 0.09	48.18
		7	8.46 ± 0.03	50.76
	W4	0.5	2.98 ± 0.05	17.88
		1	3.92 ± 0.20	23.52
		3	5.51 ± 0.35	33.06
		5	6.76 ± 0.22	40.56
		7	8.46 ± 0.45	50.76
Green (G)	G2	0.5	5.47 ± 0.21	32.82
		1	7.30 ± 0.10	43.80
		3	7.44 ± 0.08	44.64
		5	16.17 ± 0.25	97.02
		7	17.98 ± 0.08	107.88
	G4	0.5	4.67 ± 0.19	28.02
		1	5.49 ± 0.09	32.94
		3	5.97 ± 0.19	35.82
		5	8.15 ± 0.17	48.90
		7	8.38 ± 0.07	50.28

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

Table 17 Oxalate contents in a cup and six cups of tea samples (leaf form) in various steeping times (continued)

Type of tea	Brands	Time (min)	mg oxalate/ cup of tea[a]	mg oxalate/ six cups of tea[b]
Oolong (O)	O2	0.5	1.53	9.18
		1	2.82 ± 0.05	16.92
		3	7.94 ± 0.03	47.64
		5	8.47 ± 0.02	50.82
		7	9.27 ± 0.07	55.62
	O3	0.5	4.93 ± 0.02	29.58
		1	6.77 ± 0.15	40.62
		3	9.81 ± 0.03	58.86
		5	12.58 ± 0.05	75.48
		7	12.78 ± 0.03	76.68
Black (B)	B1	0.5	6.50 ± 0.04	39.00
		1	7.90 ± 0.02	47.40
		3	8.26 ± 0.03	49.56
		5	10.25 ± 0.08	61.50
		7	10.29 ± 0.06	61.74
	B3	0.5	8.21 ± 0.04	49.26
		1	13.07 ± 0.06	78.42
		3	15.44 ± 0.04	92.64
		5	17.45 ± 0.03	104.70
		7	19.09 ± 0.05	114.54

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

Table 18 Oxalate contents in a cup and six cups of tea samples (bag form) in various steeping times

Type of tea	Brands	Weight/ bag(g)	Time (min)	mg oxalate/ cup of tea[a]	mg oxalate/ six cups of tea[b]
White (WB)	WB3	1.8	0.5	0.91 ± 0.02	5.46
			1	1.20 ± 0.04	7.20
			3	2.88 ± 0.04	17.28
			5	2.94	17.64
			7	3.58 ± 0.02	21.48
	WB4	1.8	0.5	1.16 ± 0.01	6.96
			1	1.35 ± 0.02	8.10
			3	2.89 ± 0.26	17.34
			5	3.54 ± 0.04	21.24
			7	4.59 ± 0.03	27.54
Green (GB)	GB4	2	0.5	0.52 ± 0.05	3.12
			1	1.55 ± 0.01	9.30
			3	3.26 ± 0.20	19.56
			5	4.68 ± 0.01	28.08
			7	4.76 ± 0.05	28.56
	GB5	2	0.5	1.74 ± 0.06	10.44
			1	2.59 ± 0.03	15.54
			3	3.74 ± 0.19	22.44
			5	7.01 ± 0.01	42.06
			7	7.22 ± 0.04	43.32

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

Table 18 Oxalate contents in a cup and six cups of tea samples (bag form) in various steeping times (continued)

Type of tea	Brands	Weight/ bag(g)	Time (min)	mg oxalate/ cup of tea[a]	mg oxalate/ six cups of tea[b]
Oolong (OB)	OB3	1.5	0.5	0.62 ± 0.01	3.72
			1	1.37 ± 0.01	8.22
			3	2.79 ± 0.02	16.74
			5	3.68 ± 0.01	22.08
			7	3.77	22.62
	OB4	2	0.5	1.88 ± 0.05	11.28
			1	3.60	21.60
			3	7.78 ± 0.11	46.68
			5	9.23 ± 0.01	55.38
			7	9.43 ± 0.40	56.58
Black (BB)	BB4	1.75	0.5	1.66 ± 0.01	9.96
			1	3.34 ± 0.02	20.04
			3	6.67 ± 0.02	40.02
			5	8.60 ± 0.03	51.60
			7	9.17 ± 0.03	55.02
	BB5	2	0.5	1.69 ± 0.01	10.14
			1	3.24 ± 0.03	19.44
			3	4.81 ± 0.05	28.86
			5	6.18 ± 0.02	37.08
			7	6.78 ± 0.01	40.68

[a] Data was expressed as mean ± SD of triplicate extraction; [b]estimated value

CHAPTER V

DISCUSSION

5.1 Oxalate content in tea samples

Consumption of tea, which is made from the leaves of *Camellia sinensis* plants, has shown many health benefits because of its potent ingredients such as polyphenols (Serafini et al., 2011). Polyphenols, particularly catechins act as antioxidants scavenging free radicals both *in vitro* and *in vivo*, reduce the risk of cardiovascular diseases, fight against various types of cancer, and prevent diabetes (Sharangi, 2009). However, oxalate in tea is thought to be a major risk factor for kidney stones. Kidney stones are correlated with increased urinary oxalate. High urinary oxalate results from increased dietary oxalate intake. Gasinska and Gajewska (2007) found that the main sources of food oxalate in patients with kidney oxalate stones were tea and coffee (80-85%). One of them drank 10 cups of black tea per day. Moderate consumption of tea is considered as a tea drinker who consumes six cups of tea per day (Charrier et al., 2002). In the result of present study, if people consume in moderate, they will consume oxalate 21.36-92.40 mg/day for black tea while consumption of oolong tea, green tea and white tea will result in an intake of oxalate 14.16-58.86, 6.90-45.30 and 5.04-34.32 mg/day respectively. The American Dietetic Association (ADA) (2005) recommended that patients who had kidney

stones should avoid consuming dietary oxalate more than 40-50 mg/day. If six cups of tea are consumed per day, this may cause recurrent stone formation, especially consumption of black tea. In fact, people not only consume oxalate in tea, but also consume oxalate from other foods. If patients having kidney stones consume one cup of black tea per day, they should avoid consuming oxalate from other foods more than 34.60 mg/day. They should not consume oxalate from other foods more than 40.19, 42.45 and 44.28 mg/day when consume one cup of oolong, green and white tea respectively.

In this study, the highest of oxalate content both in leaf and bag forms was found in black tea (1.36-4.42 mg/g tea), followed by oolong tea (0.74-3.94 mg/g tea), green tea (0.44-2.18 mg/g tea) and the lowest was found in white tea (0.40-1.79 mg/g tea). The amount of oxalate in black tea was less than that (1.5-6.9 mg/g tea) reported by Charrier et al. (2002) who determined oxalate in tea sold in New Zealand and also less than those in black tea bags (6.07-6.22 mg/g tea) reported by Savage et al. (2003). The oxalate levels of oolong tea and green tea in present study were higher than those (0.23 mg/g tea, 0.24-1.15 mg/ g tea respectively) in the study of Charrier et al. (2002). Nevertheless, the amount of oxalate in green tea found in this study was lower than that (2.78 mg/g tea) reported in Japan by Morita and Tuji (2002).

The present results indicated that the amount of oxalate found in black tea was significantly higher than that in the other types of tea. These differences may result from

the degree or period of fermentation (de Mejia et al., 2009). Black tea is made from tea leaves that are allowed to ferment for several hours to undergo a full oxidation. So, it is confirmed that oxalate occurs as metabolic end products of the oxaloacetate, glycolate and glyoxylate pathways in many plants (Franceschi and Loewus, 1995).

The oxalate content in tea samples made in Thailand (1.58 ± 0.84 mg/g tea) was lower than that made in others countries (1.76 ± 0.87 mg/g tea). The differences of oxalate content may be result from cultivation condition and time of harvest. Hönow et al. (2010) found that green tea harvested in autumn had more oxalate than green tea harvested in spring.

When considering the form of tea (leaf or bag), there was no significant difference in the oxalate content. It indicated that the form of tea did not influence the amount of oxalate. The result of this study was similar to the study of Charrier et al. (2002).

Consumption of foods high in oxalate not only increases risk of kidney stones but also decreases mineral absorption. People who require high amount of minerals should aware of mineral insufficiency. For example, pregnant women requiring high amount of calcium for fetal growth (Sanders et al., 2009) are at high risk of calcium deficiency and post-menopause women tend to have high risk of osteoporosis, if they consume high amount of dietary oxalate.

5.2 Effect of steeping duration on oxalate content in tea samples

Steeping duration had an influence on the amount of oxalate in tea samples, especially for first 3 minutes and slightly increases after 5 minutes. The longer the tea infusion time, the more oxalate extracted. These results were similar to the study of McKay et al. (1995). It was reported that 5-minutes steeping black tea (83.16 mg/l) had more oxalate content than 1-minute steeping black tea (43.11 mg/l). According to the results of this study, tea drinkers who have kidney stones should concern not only about tea types as mention above, but also the duration of tea infusion. Sometimes they should realize the brand of tea as well. For example of consumption tea in moderate, tea drinkers having kidney stones should not steep white tea leaves more than 3 minutes but they can steep white tea bags up to 7 minutes. In black tea, tea drinkers should not steep black tea leaves more than 30 seconds in brand 1 (B1) and they should not select brand 3 (B3) for daily consumption in order not to consume oxalate more than 40-50 mg/day.

This study consisted of simple methods for preparing tea samples. The process of stirring tea leaves or squeezing tea bags were not included. As the previous study, stirring the tea leaves leads to significant increases in the oxalate extraction (McKay et al., 1995). Thus, the result of this study showed the minimal amount of oxalate. Tea drinkers could adjust suitable tea preparation for themselves.

CHAPTER VI

CONCLUSION

This study aimed to determine the soluble oxalate content in tea samples available in Thailand and to study the effect of steeping duration on oxalate content. The results showed that different types of tea and steeping durations had an influence on the amount of oxalate in tea. Black tea contained the highest amount of oxalate while white tea contained the lowest. The amount of oxalate in black tea was significant higher than oolong tea (bag form), green tea (leaf and bag forms) and white tea (leaf and bag forms). The highest level of oxalate was found in black tea in leaf form while the lowest level was found in white tea in bag form. The form of tea between leaf and bag did not significantly influence on the amount of oxalate. People who have risk of kidney stones or risk of mineral insufficiency should avoid drinking high amounts of black tea. Steeping tea for a short duration is also recommended.

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APPENDICES

APPENDIX A

TEA SAMPLES

Figure A-1 White tea leaves

Figure A-2 Green tea leaves

Figure A-3 Oolong tea leaves

Figure A-4 Black tea leaves

Figure A-5 White tea in tea bags

Figure A-6 Green tea in tea bags

Figure A-7 Oolong tea in tea bags

Figure A-8 Black tea in tea bags

APPENDIX B

PEAK AREA OF TEA SAMPLES

Table B-1 Peak area of tea samples (leaf form)

No.	Type of tea	Brands	Peak area		
			sample 1	sample 2	sample 3
1	White (W)	W1	1882.16	1902.57	1912.42
2		W2	2090.67	2201.25	2246.91
3		W3	1151.14	1206.59	1215.31
4		W4	2194.64	2193.84	1915.90
5		W5	1231.14	1266.24	1253.54
6	Green (G)	G1	1761.99	1800.79	1795.85
7		G2	2882.30	2805.26	2896.34
8		G3	1894.92	1908.02	1938.81
9		G4	2348.91	2290.42	2177.93
10		G5	622.84	622.92	615.82
11	Oolong (O)	O1	1076.47	1025.26	1003.84
12		O2	3018.48	3015.72	2991.72
13		O3	3694.16	3700.56	3724.53
14		O4	1891.67	1793.83	1854.93
15		O5	1315.24	1158.80	1224.42
16	Black (B)	B1	3039.76	3135.19	3098.19
17		B2	2129.55	2217.74	1973.58
18		B3	5789.70	5821.37	5823.19
19		B4	1983.49	1817.69	1812.95
20		B5	2443.42	2454.20	2492.88

Table B-2 Peak area of tea samples (bag form)

No.	Type of tea	Brands	Weight/ Bag (g)	Peak area		
				sample 1	sample 2	sample 3
1	White (WB)	WB1	1.5	847.87	866.26	807.01
2		WB2	2	348.69	377.99	339.38
3		WB3	1.8	1134.30	1101.69	1115.86
4		WB4	1.8	1004.75	1108.13	1246.12
5		WB5	1.8	840.89	870.20	855.66
6	Green (GB)	GB1	2	491.40	467.03	453.51
7		GB2	2	989.68	942.64	924.99
8		GB3	2	693.72	736.25	717.32
9		GB4	2	1206.18	1205.86	1367.82
10		GB5	2	1443.93	1526.6	1349.22
11	Oolong (OB)	OB1	2	885.38	953.47	930.37
12		OB2	2.5	979.20	1129.47	1015.47
13		OB3	1.5	1076.49	1083.39	1090.25
14		OB4	2	2961.27	2893.48	2987.38
15		OB5	2	1116.00	1106.34	1086.27
16	Black (BB)	BB1	3.125	1737.43	1742.58	1790.13
17		BB2	1.5	1377.57	1367.62	1368.49
18		BB3	2	1328.48	1432.58	1419.35
19		BB4	1.75	2519.26	2539.11	2536.34
20		BB5	2	1811.95	1847.27	1858.04

Table B-3 Peak area of tea samples (leaf form) in various steeping times

Type of tea	Brands	Time (min)	Peak area		
			sample 1	sample 2	sample 3
White (W)	W2	0.5	942.69	968.60	966.00
		1	1778.33	1800.01	1810.59
		3	2090.67	2201.25	2246.91
		5	3068.38	3060.17	2992.12
		7	3192.68	3192.23	3219.70
	W4	0.5	1170.40	1127.05	1169.76
		1	1403.76	1584.72	1531.58
		3	2194.64	2193.84	1915.90
		5	2682.71	2498.69	2515.40
		7	3152.97	3027.68	3429.26
Green (G)	G2	0.5	2025.28	2032.74	2196.75
		1	2762.48	2731.02	2817.06
		3	2882.30	2805.26	2896.34
		5	6118.79	6177.40	5954.75
		7	6741.69	6802.09	6739.66
	G4	0.5	1757.88	1881.82	1719.18
		1	2115.41	2115.94	2041.60
		3	2348.91	2290.42	2177.93
		5	3199.84	3178.10	3133.41
		7	3137.48	3125.55	2998.06

Table B-3 Peak area of tea samples (leaf form) in various steeping times (continued)

Type of tea	Brands	Time (min)	Peak area		
			sample 1	sample 2	sample 3
Oolong (O)	O2	0.5	610.49	609.94	613.26
		1	1092.75	1071.51	1115.28
		3	3018.48	3015.72	2991.72
		5	3215.90	3199.70	3203.19
		7	3515.02	3471.16	3532.37
	O3	0.5	1883.89	1895.00	1874.19
		1	2491.96	2588.34	2627.66
		3	3694.16	3700.56	3724.53
		5	4755.70	4758.90	4716.48
		7	4798.33	4824.74	4823.07
Black (B)	B1	0.5	2448.85	2484.05	2475.59
		1	2984.74	2994.06	3003.72
		3	3039.76	3135.19	3098.19
		5	3865.21	3906.46	3836.67
		7	3857.53	3885.67	3914.55
	B3	0.5	3120.90	3090.64	3117.79
		1	4895.02	4945.63	4932.23
		3	5789.70	5821.37	5823.19
		5	6548.25	6563.78	6575.33
		7	7148.65	7190.91	7188.22

Table B-4 Peak area of tea samples (bag form) in various steeping times

Type of tea	Brands	Weight/ bag (g)	Time (min)	Peak area		
				sample 1	sample 2	sample 3
White (WB)	WB3	1.8	0.5	373.11	382.22	387.67
			1	487.22	508.77	476.16
			3	1134.00	1101.69	1115.86
			5	1141.17	1138.66	1137.84
			7	1387.33	1378.75	1365.09
	WB4	1.8	0.5	471.97	472.64	481.79
			1	545.67	554.58	536.78
			3	1004.75	1108.13	1246.12
			5	1362.64	1378.40	1344.74
			7	1740.90	1760.01	1770.59
Green (GB)	GB4	2	0.5	262.00	221.76	217.70
			1	623.91	622.55	613.12
			3	1206.18	1205.86	1367.82
			5	1785.06	1790.43	1788.89
			7	1818.44	1840.94	1795.58
	GB5	2	0.5	700.40	713.58	663.69
			1	1000.26	1002.70	1026.72
			3	1443.93	1526.60	1349.22
			5	2655.71	2658.43	2664.20
			7	2717.03	2750.02	2754.39

Table B-4 Peak area of tea samples (bag form) in various steeping times (continued)

Type of tea	Brands	Weight/ bag (g)	Time (min)	Peak area		
				sample 1	sample 2	sample 3
Oolong (OB)	OB3	1.5	0.5	280.36	269.22	270.24
			1	554.52	550.41	547.33
			3	1076.49	1083.39	1090.25
			5	1409.45	1414.5	1421.48
			7	1449.43	1451.81	1452.26
	OB4	2	0.5	773.43	726.63	734.09
			1	1388.57	1385.10	1386.87
			3	2961.27	2893.48	2987.38
			5	3494.59	3482.45	3490.21
			7	3671.24	3665.46	3351.89
Black (BB)	BB4	1.75	0.5	667.43	657.63	655.60
			1	1277.95	1291.32	1295.90
			3	2519.26	2539.11	2536.34
			5	3237.26	3262.20	3264.44
			7	3484.23	3458.95	3455.97
	BB5	2	0.5	666.53	678.20	668.69
			1	1261.40	1237.42	1255.78
			3	1811.95	1847.27	1858.04
			5	2339.38	2347.51	2360.86
			7	2575.40	2567.15	2580.54

APPENDIX C

PEAK AREA OF STANDARD OXALATE SOLUTION AND

STANDARD CALIBRATION CURVE OF OXALATE

Table C-1 Peak area of standard oxalate solution

Concentration of oxalate (µg/ml)	Peak area				
	1	2	3	Mean	SD
1	374.07	354.11	371.23	366.47	8.82
5	1955.00	1904.74	1895.25	1918.33	26.22
10	3650.50	3792.18	3966.40	3803.03	129.19
20	7567.88	7461.48	7709.07	7579.48	101.41
30	11201.05	11282.61	11119.41	11201.02	66.63

Standard calibration curve of oxalate

The oxalate standard calibration curve was obtained by plotting the peak area against the concentration of oxalate standard as shown in Figure C-1. The standard calibration curve had a correlation coefficient of 0.9999.

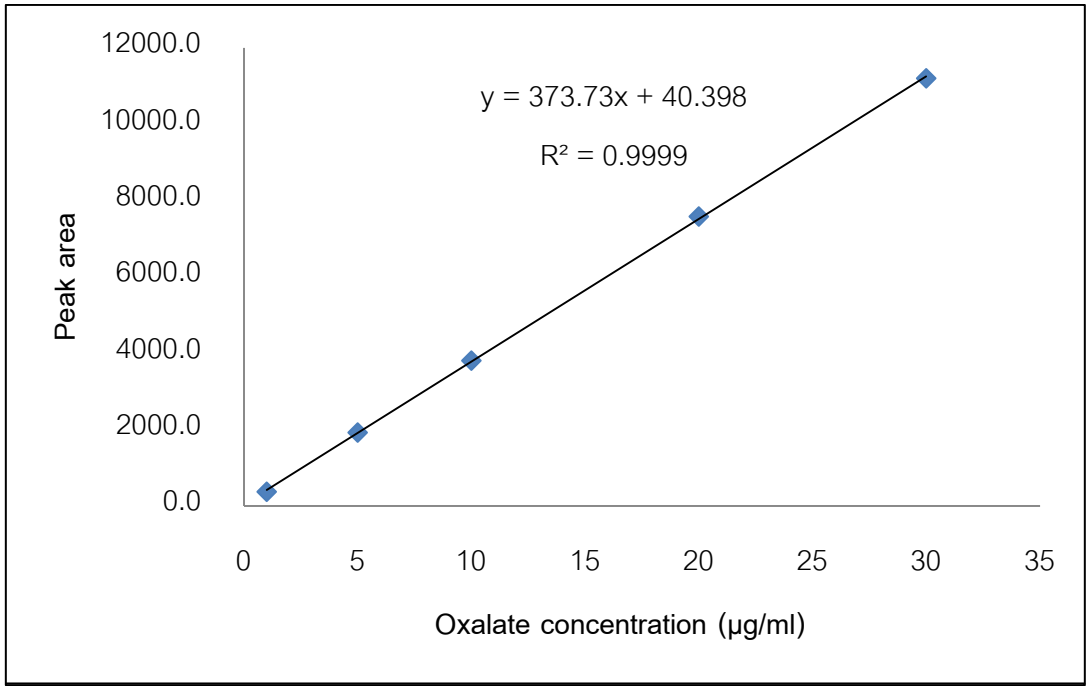

Figure C-1 Standard calibration curve of oxalate

APPENDIX D

EXAMPLES OF CHROMATOGRAMS

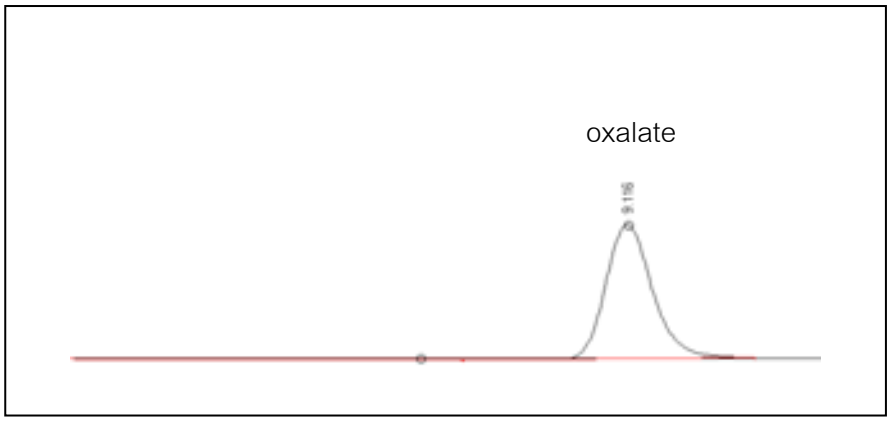

Figure D-1 Chromatogram of standard oxalate solution

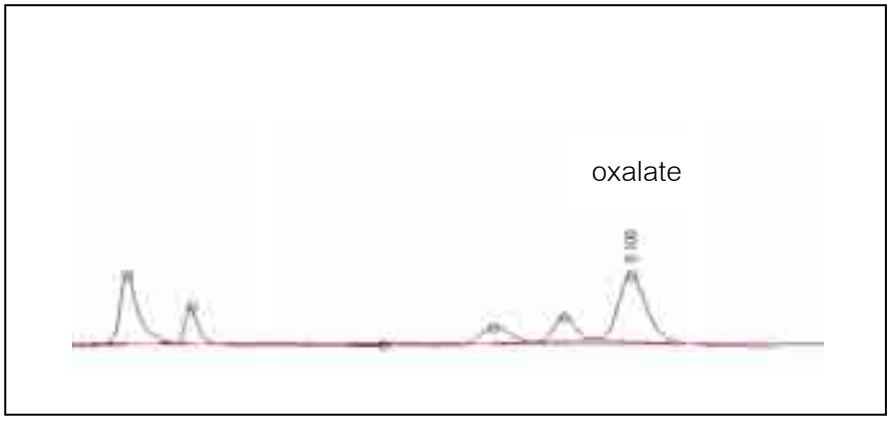

Figure D-2 Chromatogram of tea sample

BIOGRAPHY

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