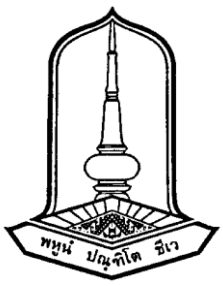

THE PRODUCTION AND SELENIUM SPECIATION OF SELENIUM-ENRICHED KALE SEEDLING (*Brassica oleracea* var. *alboglabra* L.)

SARUNYA MANEETONG

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor Philosophy in Chemistry at Mahasarakham University

October 2013

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Sarunya Maneetong

ชื่อเรื่อง การผลิตและการหาชนิดจำเพาะของซีลีเนียมในต้นกล้าคะน้าที่เสริมซีลีเนียม
ผู้วิจัย นางสาวศรัญญา มณีทอง
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บทคัดย่อ

งานวิจัยนี้ได้เลือกคะน้าสำหรับปลูกเป็นพืชเสริมซีลีเนียมด้วยวิธีการปลูกพืชแบบไร้ดิน ต้นกล้าคะน้าเสริมซีลีเนียมปลูกในสารละลายสูตร Hoagland ที่ความเข้มข้นของซีลีเนียม 5 10 15 30 และ 45 มิลลิกรัมต่อลิตร ในรูปของโซเดียมซีลีไนท์ ที่ระยะเวลาปลูก 15 วัน และเก็บผลผลิตทุก ๆ 5 วัน ต้นกล้าคะน้าที่ปลูกในสารอาหารเลี้ยงที่ความเข้มข้นของซีลีเนียม 45 มิลลิกรัมต่อลิตร พบการเจริญเติบโตและการสะสมของซีลีเนียมถูกยับยั้ง อย่างไรก็ตามความเข้มข้นของซีลีเนียมรวมในพืชทดลองทั้งหมดสูงกว่ากลุ่มควบคุม ปริมาณซีลีเนียมทั้งหมดในต้นกล้าคะน้าเสริมซีลีเนียมพบสูงที่สุดประมาณ 400 มิลลิกรัมต่อกิโลกรัมของน้ำหนักแห้ง ในต้นกล้าคะน้าเสริมซีลีเนียมที่ความเข้มข้นของซีลีเนียม 30 มิลลิกรัมต่อลิตร และระยะเวลาปลูก 15 วัน รูปแบบของซีลีเนียมอินทรีย์ที่พบคือ ซีลีโนเมทไธโอนีน ซีลีโนเมทิลซีลีโนซีสเตอีน และรูปแบบของซีลีเนียมที่ไม่สามารถระบุได้ ด้วยการย่อยใน 0.1 โมลาร์ ไฮโดรคลอริกใน เมทานอล 10 เปอร์เซ็นต์ แยกโดยการจับคู่ไอออนกับเฟสตรงข้ามด้วยเทคนิคคู่ควบระหว่างเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูงและเทคนิคอินดักทีฟลีคัพเพิลพลาสมาแมสสเปกโทรเมทรี

การศึกษาผลของซัลเฟตไอออนในรูปของแมกนีเซียมซัลเฟตต่อการดูดซึมซีลีเนียมของต้นกล้าคะน้า โดยการเปรียบเทียบสาระละลายอาหารที่มีการเติมซัลเฟตไอออนในรูปแมกนีเซียมซัลเฟตและสารละลายอาหารที่ไม่มีซัลเฟตไอออนในรูปแมกนีเซียมคลอไรด์ ที่ใช้ในการปลูกต้นกล้าคะน้า ด้วยวิธีการปลูกพืชแบบไร้ดินต่อการดูดซึมแร่ธาตุต่างๆ และซีลีเนียมของต้นกล้าคะน้า พบว่า ซัลเฟตไอออนไม่มีผลต่อการดูดซึมซีลีเนียมของต้นกล้าคะน้า เพราะซีลีเนียมที่เสริมให้กับคะน้านั้น เป็นซีลีเนียมที่อยู่ในรูปของซีลีไนท์ (SeO_3^{2-}) แต่ซัลเฟตไอออนจะมีผลต่อการดูดซึมซีลีเนียมของคะน้าเมื่อเสริมซีลีเนียมที่อยู่ในรูปของซีลีเนท (SeO_4^{2-}) และแหล่งสารละลายอาหารที่ใช้ในการปลูกต้นกล้าคะน้าทั้งสองไม่แตกต่างกันทางสถิติที่ความเชื่อมั่น 95 เปอร์เซ็นต์ และยังศึกษาผลของการใช้ซ้ำของสารละลายอาหารเสริมซีลีเนียมความเข้มข้น 30 มิลลิกรัมต่อลิตร ต่อการดูดซึมซีลีเนียมและธาตุอาหารอื่นๆ ของต้นกล้าคะน้า พบว่าการใช้ซ้ำของสารละลายอาหาร มีแนวโน้มทำให้ปริมาณซีลีเนียมทั้งหมดและแร่ธาตุต่างๆ ลดลงตามระยะเวลาที่ปลูก ส่งผลให้คะน้าสามารถดูดซึมธาตุต่างๆ น้อยลงด้วย การตรวจวัดหาปริมาณซีลีเนียม ธาตุอาหารหลักและธาตุอาหารรองในต้นกล้าคะน้าได้แก่ แมงกานีส ทองแดง เหล็ก สังกะสี แคลเซียม แมกนีเซียม โซเดียม โพแทสเซียม และฟอสฟอรัส พบว่าเมื่อปลูกครบระยะเวลา 45 วัน ในวันที่ 15 ของรุ่นที่ 1 จะพบปริมาณของซีลีเนียม แมงกานีส ทองแดง เหล็ก สังกะสี แคลเซียม แมกนีเซียม โซเดียม โพแทสเซียม และฟอสฟอรัส สะสมในตัวอย่างคะน้ามากที่สุด

ได้ศึกษาความเสถียรและอายุการเก็บรักษาต้นกล้าคะน้าเสริมซีลีเนียม โดยการเก็บผงแห้งของต้นกล้าคะน้าเสริมซีลีเนียม (ความเข้มข้นของซีลีเนียมที่เสริม 30 มิลลิกรัมต่อลิตร ระยะเวลาปลูก 15 วัน) ในภาชนะบรรจุ (ขวดโพลีสไตรีน (PS), ขวดโพลีเอทิลีน (PE), ขวดโพลีคาร์บอเนต (PC), ถุงซิบใส (LDPE) และถุงอลูมิเนียมฟอยล์) อุณหภูมิ (4 องศาเซลเซียส และอุณหภูมิห้อง) เวลาการเก็บรักษาที่แตกต่างกัน (6, 12, 18 และ 24 เดือน) และเก็บในตู้ดูดความชื้น ผลการทดลองแสดงให้เห็นว่าปริมาณซีลีเนียมทั้งหมดลดลงเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้นและที่ระยะเวลาการเก็บรักษา 6 เดือนพบว่าปริมาณซีลีเนียมทั้งหมดลดลงน้อยที่สุดเมื่อเปรียบเทียบกับระยะเวลาเก็บอื่นๆ ดังนั้นผลการทดลองที่ดีที่สุดสำหรับความเสถียรและอายุการเก็บรักษาต้นกล้าคะน้าเสริมซีลีเนียมคือ เก็บในขวดโพลีสไตรีน, ถุง ซิบใส และถุงอลูมิเนียมฟอยล์ที่อุณหภูมิ 4 องศาเซลเซียส ระยะเวลาเก็บ 6 เดือน

นอกจากนี้ในงานวิจัยได้ศึกษาฤทธิ์ต้านอนุมูลอิสระของต้นกล้าคะน้าเสริมซีลีเนียม โดยใช้วิธียับยั้งอนุมูลอิสระ $DPPH^{\cdot}$ และวิธี FTC จากสารสกัดที่สกัดโดยน้ำและเอทานอล 75 เปอร์เซ็นต์ ผลการศึกษาพบว่า ความสามารถในการยับยั้งอนุมูลอิสระของสารสกัดต้นกล้าคะน้าเพิ่มขึ้น เมื่อความเข้มข้นของซีลีเนียมในอาหารเลี้ยงและระยะเวลาในการปลูกเพิ่มขึ้น ค่าร้อยละการยับยั้งอนุมูลอิสระสูงสุดเท่ากับ 77.30 เวลา 30 นาที ได้จากการสกัดโดยเอทานอลของต้นกล้าคะน้าเสริมซีลีเนียมที่ระดับความเข้มข้นของซีลีเนียมในอาหารเลี้ยง 30 มิลลิกรัมต่อลิตร ระยะเวลาปลูก 15 วัน ฤทธิ์ต้านอนุมูลอิสระจากวิธี FTC มีฤทธิ์ในการต้านอนุมูลอิสระสอดคล้องกับวิธี DPPH

คำสำคัญ: ต้นกล้าคะน้าเสริมซีลีเนียม; รูปแบบของซีลีเนียม; การดักจับอนุมูลอิสระ

TITLE The Production and Selenium Speciation of Selenium-enriched Kale Seedling (*Brassica oleracea* var. *alboglabra* L.)
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ABSTRACT

In this research, Kale (*Brassica oleracea* var. *alboglabra* L.) was selected for cultivation as selenium-enriched vegetable by a hydroponic system. Selenium-enriched kale seedlings were grown in Hoagland's solution supplemented with 5, 10, 15, 30 and 45 mg Se (IV) L^{-1} from sodium selenite for 15 days and harvested every 5 days. Cultivation of kale seedlings with 45 mg Se (IV) L^{-1} inhibited the growth rate and selenium accumulation. However, total selenium concentrations of all selenium-supplemented treatments were higher than that of control treatment. The highest selenium concentration accumulated by kale approximately 400 mg Se kg^{-1} (expressed as selenium in dry matter) was obtained in the kale which was grown with 30 mg Se L^{-1} in solution for 15 days of cultivation time. Selenium Speciation studies indicated that the predominant forms of organic selenium are selenomethionine (SeMet), Se-methylselenocysteine (SeMC) and unknown which were identified in 0.1 M HCl in 10% methanol extracts by ion pairing reversed phase HPLC-ICP-MS.

The study of effect of sulfate ion in nutrient solution on selenium absorption of kale seedlings with hydroponic system were studied. The comparison of nutrients solution between the solution of sulfate ion in form magnesium sulfate and no sulfate ion in form magnesium chloride. It was found that sulfate ion did not affect to selenium absorption and total selenium contents in kale seedling samples. Because, selenium in form of sodium selenite was used for selenium-enriched kale seedling. The selenite absorption was not inhibited by sulfate ion in the nutrient solution. Whereas, the absorption of selenate was clearly inhibited by sulfate ion in the nutrient solution and both nutrient solutions were not significant different ($p>0.05$). The study of reuse of medium solution with 30 mg Se L^{-1} supplemented in nutrients solution on the selenium

and minerals absorption of kale seedling. It was found that total selenium and mineral contents decreased when nutrient solution repeatedly used. The determination of selenium, macronutrients and micronutrients contents in kale seedlings such as manganese (Mn), copper (Cu), iron (Fe), Zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorus (P) were also analyzed. The results completed for 45 days (3 crops), the highest total selenium, manganese, copper, iron, zinc, calcium, magnesium, sodium, potassium and phosphorus were found in 1st generation (crop 1) of selenium-enriched kale seedling.

The stability and shelf life of selenium-enriched kale seedling were studied by storage the dried selenium-enriched kale seedling (30 mg Se L^{-1} for 15 days of cultivation time) powders in the difference container material (polystyrene (PS) bottle, polyethylene (PE) bottle, polycarbonate (PC) bottle, Low-density polyethylene (LDPE) ziplock bag and aluminium foil bag) at difference temperature (4 ^{o}C and room temperature), difference storage time (6, 12, 18 and 24 months) and in desicator. The results show that the total selenium decreased when increase storage time and at storage time 6 months found the lowest decreased when compared with other storage times. Thus, the best results for stability and shelf life of selenium-enriched kale seedling were obtained with PS bottle, LDPE ziplock bag and aluminium foil bag at 4°C for 6 months.

Additionally, the antioxidant activities of selenium-enriched kale seedling using a DPPH radical scavenging and FTC methods were also studied. The extraction were performed with water and 75% ethanol. The result showed that the radical scavenging ability of the kale seedling extracts was increased with increasing of selenium-supplement and cultivation time. The highest inhibition percentage of 77.42 at 30 min were obtained in ethanolic extracts of selenium-enriched kale seedling which grown in the solution supplemented with 30 mg Se L^{-1} and cultivation time of 15 days. The antioxidant activities from FTC method showed almost the same patterns of activities as the DPPH method.

Keyword: Se-enriched kale seedling; Se speciation; radical scavenging

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
cm	Centimeter
°C	Degree Celsius
g	Gram
ICP-MS	Inductively coupled plasma mass spectrometry
L min^{-1}	Liter per minute
MW	Microwave-assisted digestion
mA	Milliamp
µL	Microliter
mg	Milligrame
mg L^{-1}	Milligram per liter
mg kg^{-1}	Milligram per kilogram
min	Minute
mL	Milliliter
mm	Millimater
nm	Nanometer
ppm	Past per million
Psi	Pound-force per square inch
S/A	Ratio of amount of sample to volume of nitric
RSD	Relative standard deviation
Volt	Voltage

CHAPTER 1

INTRODUCTION

1.1 Background

Selenium (Se) is an essential micronutrient for living organisms but its toxic in specific forms and at higher concentrations (Casiot *et al*., 1999). Selenium deficiency is associated to several diseases such as Kashan diseases, Kashin-Beck diseases and hypothyroidism. These diseases usually found in the nonseleniferous area such as China, Tibet and New Zealand (Lavander *et al*., 1997). However, seleniferous areas such as Venezuela demonstrated that the incidence of dermatitis, loose hair and nails disease are usually found in the children (Maurice *et al*., 2006).

Moreover, since in vivo oxygen is efficiently reduced to water in the respiratory chain, only a low percentage (5-10%) is in vivo real eased as the superoxide ion 0_2^- and $OH^{\cdot}$ species, which are dangerous to living organisms (Feroci and Fini, 1998; Battin and Brumaghim, 2009; Ramoutar and Brumaghim, 2010). It is well known that oxidation caused by reactive oxygen species (ROS) is a major cause of cellular damage and death and has been implicated in cancer, neurodegenerative and cardiovascular diseases (Battin and Brumaghim, 2009).

Selenium is one part of the antioxidant enzymes that protect cells against the effects of free radicals (Turło *et al*., 2010; Finley *et al*., 2000). It functions in the active site of glutathione peroxidase (GSH-Px). Several functions of selenium could work as co-worker of thioredoxin reductases, selenoprotein P and selenoprotein W (Burk and Hill, 2005). Selenium has been reported to be associated with its antioxidant activity, anticancer effect and other physiological functions (Xu *et al*., 2003; Zadrożna *et al*., 2009). Recently, selenium has been reported to possess strong free radical scavenging ability and can protect the cell membrane, preventing cells from malignant transformation (Shen *et al*., 2010)

In environmental and biological systems, selenium exist either in inorganic forms or as organic species with direct Se-C bonds. Selenium is primarily present as selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) ions in soil and water. Organic selenium

compounds occur in plants and microorganisms (Zheng *et al*., 2000). The function of selenium depends on each species of selenium (Terry *et al*., 2000). Furthermore, selenium in food is primarily present in an organic form as two modified amino acid, selenomethionine (SeM) and selenocysteine (SeC) (Helen and Mary, 1995), which the selenoamino acid contented proteins are called selenoprotein (Food and Nutrition Board Institute of Medicine, 2000).

Selenium supplemented in plant crops has been used to increase dietary selenium levels in humans and other animals. Growing plants enriched with selenium could be an effective way to reduce dietary deficiencies and increase health benefits. Uptake and accumulation of selenium by plants is determined by selenium form and its concentration, the identity and presence of competing ions and the affinity of a species to absorp and metabolize selenium. Many plant species accumulate higher amounts of selenium in shoot or leaf tissues than root tissues (Chethaka *et al*., 2004). Selenium supplemented plants such as garlic, onion, ramp, broccoli (Kápolna and Fodor, 2006) and kale sprout (Chantiratikul *et al*., 2011) have been achieved. Moreover, cabbage plants like *Brassica* group are perceived as very valuable food products. The research conducted, in many countries, corroborated the nutritive value, high antioxidant and pro-healthy potential of these plants. These plants are a precious source of fiber, mineral compounds, vitamin C, α-tokoferol and carotenoids (β-carotene, lutein). They are abundant of polyphenolic compounds and contain 15-20 different glucosinolates like compounds. Chemical composition decides about the pro-healthy characteristics of these plants, especially as anticancerogenic (Sikora *et al*., 2012; Sugihara *et al*., 2004). Furthermore, *Brassica* family is one of the most of plants which can produce as selenium-enriched food. Several *Brassicaceae* species accumulate selenium into the thousands of ppm (Montes-Bayón *et al*., 2002). Kale (*Brassica oleracea* var. alboglabra L.) is a specie in *Brassica* family which used for selenium-enriched plant production because it can reduce the toxicity of inorganic selenium and transform to several organic species of selenium (Terry *et al*., 2000). In addition, it is the one of the popular plant consumed in Thailand. The primary forms of selenium found in selenium-enriched plants are SeM (Qilin *et al*., 2009), Se-methyl selenocysteine (SeMC) and derivatives like γ-glutamyl-Se-methyl selenocysteine (Montes-Baýon *et al*., 2006). These, γ-glutamyl-Se-methyl selenocysteine serves primarily as a carrier of SeMC. SeMC is a

good precursor for generating methylselenol when enzymes like β-lyase are present. This Se-metabolite seems to be the most active species for cancer reduction. Therefore, SeMC has been widely studied as a potential anticarcinogenic compound (Montes-Baýon *et al*., 2006).

Additionally, selenium-contaminated soils and sand from the selenium-enriched plant production could be an environment pollutant. Hence, cultivation of selenium-enriched plants using hydroponics system was interested to apply for producing selenium-enriched plants because this method is easily controlled production that allows over selenium fertilization without environmental consequences (Sugihara *et al*., 2004; Tsuneyoshi *et al*., 2006). Furthermore, the sulfur (S) as sulfate ion (SO_4^{2-}) have been effected to absorption of selenium in Hoagland solution. Sulfate in an ordinary hydroponic solution inhibited the absorption and assimilation of selenate. A sulfate-free nutrient was used for Se addition. However, the translocation of selenium from root to shoot depend on the form of selenium supplied. Selenite was rapidly converted to organic forms in roots with limited transport to shoots. Selenate was highly mobile in xylem transport but difficult transformed to organic forms (Terry *et al*., 2000). Since the production of selenium-enriched plants with hydroponic system need a lot of water and nutrients and still have many nutrients for the growth of plants after harvesting, the reuse of nutrients solution is one option for saving money and chemicals.

The specific method is necessary for selenium speciation analysis. Therefore, the use of a separation technique combined with a specific and sensitive detection system is very important. Such, traditional elemental techniques as ICP-AES, ICP-MS, ESI-MS (Hymer and Caruso, 2006), HG-AFS and HG-ETAAS are usually used to determine total concentration of selenium in samples with high sensitivity, selectivity and low limit of detection (Johansson *et al*., 2000, Moreno *et al*., 2003). However, ICP-MS technique offers highest sensitivity and relative ease of interfacing with common chromatographic and separation techniques (Hymer and Caruso, 2006). These, several separation technique such as high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and gas chromatography (GC) (Hymer and Caruso, 2006) are used to separate selenium speciation before determining with the traditional elemental techniques (Álvarez-Llamas *et al*., 2005, Waddell *et al*. 2005). HPLC-ICP-MS is a hyphenated technique for selenium speciation analysis. Most separation modes

of HPLC have been utilized for selenium speciation analysis with the ICP-MS including ion-exchange, reversed-phase, ion-pair, size-exclusion and chiral. The separation of selenium species depends on each HPLC mode (Hymer and Caruso, 2006).

1.2 Purposes of the research

The purposes of this research can be summarized as follows;

1.2.1 To study the production of selenium-enriched kale seedling with hydroponic system.

1.2.2 To study the effect of supplemented selenium concentrations in nutrient solution and cultivation time on the selenium accumulation, speciation, biotransformation and growth rate for the production of selenium-enriched kale seedling.

1.2.3 To study the effect of sulfur as sulfate ion in nutrient solution on the selenium concentration in selenium-enriched kale seedling.

1.2.4 To study the effect of reuse of the medium solution (nutrient solution) on the selenium accumulation in selenium-enriched kale seedling.

1.2.5 To study the stability and shelf life of selenium-enriched kale seedling in different container materials.

1.2.6 To study the effect of dried selenium-enriched kale seedling storage conditions on the selenium concentrations in selenium-enriched kale seedling.

1.2.7 To study the antioxidant activity of selenium-enriched kale seedling with using α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging and the ferric thiocyanate (FTC) methods.

1.3 Benefits of the research

This research will obtain the benefits as follows;

1.3.1 Know the optimum supplemented concentration of selenium in nutrient solution and cultivation time for selenium-enriched kale seedling.

1.3.2 Know the selenium species and biotransformation in selenium-enriched kale seedling.

1.3.3 Know the effect of sulfate ion on the selenium accumulation.

1.3.4 Know the stability and shelf life of selenium-enriched kale seedling in different container materials.

1.3.5 Know the effect of reuse of the medium solution (nutrient solution) on the selenium accumulation in selenium-enriched kale seedling.

1.3.6 Know the antioxidant activity of selenium-enriched kale seedling with different selenium supplemented concentrations and cultivation time.

1.4 Scope of the research

The scope of this work has been classified to 5 main parts as follows;

1.4.1 Selenium-enriched kale seedling cultivation with hydroponic system

1.4.1.1 The effect of selenium supplemented concentration in nutrient solution

The selenium as sodium selenite (Na_2SeO_3) was supplemented in 10 L of Hoagland's solution which the concentration of selenium was varied as 0, 5, 10, 15, 30 and 45 mg Se L^{-1} in selenium-enriched kale seedling.

1.4.1.2 The effect of cultivation time

The cultivation time of selenium-enriched kale seedling were designed at 5, 10 and 15 days. The samples were harvested every 5 days of cultivation time for growth rate and selenium accumulation study.

1.4.1.3 The effect of sulfate ion in nutrient solution

The sulfur (S) as sulfate ion (SO_4^{2-}) have been effected to the absorption of selenium from Hoagland's solution to selenium-enriched kale seedling. Thus, $MgCl_2$ was used to replace $MgSO_4$ in Hoagland's solution for selenium absorption study.

1.4.1.4 The effect of reuse of medium solution

The cultivation of kale seedling with the medium solution at 3 crops which each crops were cultivated 15 days and harvested every 5 days of cultivation time for growth rate and selenium accumulation study.

1.4.2 Sample preparation

1.4.2.1 Sample digestion

The dry samples of selenium-enriched kale seedling were digested with 65% nitric acid by closed wet acid method for the concentration of selenium and trace elements study.

1.4.2.2 Sample extraction

The dry samples of selenium-enriched kale seedling were extracted with 0.1M HCl in 10% v/v methanol for selenium speciation study.

1.4.3 Analytical procedures

1.4.3.1 Total selenium

The concentration of selenium was determined with ICP-MS.

1.4.3.2 Selenium speciation

The mixture of kale seedling extracts were determined with ion pairing reversed phase HPLC-ICP-MS.

1.4.3.3 Mineral analysis

The concentration of trace elements in each samples were determined by ICP-MS and AAS techniques.

1.4.4 The optimization of selenium-enriched kale seedling product storage conditions

1.4.4.1 The effect of container materials

The dried selenium-enriched kale seedling products were stored in the different container materials such as polystyrene (PS) bottle, polyethylene (PE) bottle, polycarbonate (PC) bottle, Low-density polyethylene (LDPE) ziplock bag and aluminium foil bag.

1.4.4.2 The effect of storage temperature

The dried selenium-enriched kale seedling products were stored in the different temperature such as 4 oC and room temperature and in desiccator.

1.4.4.3 The effect of storage time

The dried selenium-enriched kale seedling products were stored in the different times such as 6, 12, 18 and 24 months.

1.4.5 The antioxidant activity of selenium-enriched kale seedling

The presence of antioxidant compound in selenium-enriched kale seedling may delay oxidation of linoleic acid and exhibited the antioxidative activity. The FTC method was used to evaluate the antioxidant activity of selenium-enriched kale seedling, only at primary state of oxidation. In addition, the selenium-enriched kale seedling extracts, BHA and Trolox were measured in terms of hydrogen donating or radical scavenging ability by DPPH method.

CHAPTER 2

LITERTURE REVIEW

2.1 History of selenium

Selenium is one of the rarest of the elements. It was discovered by the Swedish Chemist, Jöns Jakob Berzelius, in 1817. Berzelius found the element associated with tellurium. It was discovered as a byproduct of sulfuric acid production. He gave it the name selenium from the Greek is *selene*, which signifies the moon, while *tellus* is the name of earth (Reilly, 1996). Selenium has many uses, in industry, agriculture and health, both animal and human. The employment of selenium in the electronics and electrical industries is based on its unique photoelectric and semiconducting properties. It is used on photoreceptive drums of plain paper copiers and in laser printers, rectifiers, voltage surge protectors, laser windows, infrared detectors, photovoltaic cells (Frankenberger and Benson, 1994) and used as a pigment in plastics, glass and ceramics (Graw-hill, 1982).

The toxicity of selenium was the first mentioned in the 13th century by an Italian scholar, Marco Polo, found problems with the damaged hooves of horses after grazing on certain plants in regions of China (Shao and Zheng, 2008). However, selenium was previously attraced biologic interest in the 1930s when it was found to cause poisoning of livestock in areas with high-selenium soil (Burk and Levander, 2006)

2.2 Chemistry and biochemistry of selenium

2.2.1 Chemistry of selenium

Selenium is a chemical element. It lies between sulfur and tellurium in Group VIA and between arsenic and bromine in Period 4 of the Periodic Table of the elements. Its chemical properties are intermediate between those of sulfur and tellurium and compounds resemble the corresponding sulfur and tellurium compounds in behavior (Reilly, 1996), which the physicochemical properties of selenium are

summarized in Table 2.1 (Graw-hill, 1982). Selenium has a valence of -2 in combination with hydrogen or metals and in oxygen compounds it exists in the +4 and +6 oxidation states. Such, selenium compounds are summarized in Table 2.2 and six stable isotopes of selenium occured with varing degree of abundance as follow: ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%) and ^{82}Se (9.19%) and short-lived isotope is ^{75}Se (Frankenberger and Benson, 1994). Selenium is usually occurred as selenide of heavy elements and selenium minerals such as klockmanite (CuSe), berzelianite (Cu_2Se), clausthalite (PbSe), tiemannite (HgSe), ferroselenite ($FeSe_2$) and crookesite [$(Cu, Tl, Ag)_2Se$] (Kabata-Pendias, 2011).

Table 2.1 Physicochemical properties of selenium

Symbol	Se
Atomic number	34
Atomic mass	78.96
Electronic configuration	$[Ar]3d^{10}4s^24p^4$
Density, g/cm^3	4.79[a]
Melting point, ^{o}C	217
Boiling point, ^{o}C	685.4
Atomic radius, µm	0.117
Hardness, relative units	2[a]
Electronegativity, relative units (Li = 1)	2.4
Latent heat of fusion, J/g	6.91
Heat of vaporization, J/g	272.98

[a]Hexagonal modification

Source: Modified from Frankenberger and Benson, 1994

2.2.2 Biochemistry of selenium

Twenty-five selenoprotein genes have been identified in the human genome by bioinformatics methods. The selenoproteins that result from expression of these genes are responsible for the biochemical function of selenium. Therefore, their properties and function were described in this work.

2.2.2.1 Selenoamino acids

Selenoamino acids are defined as those amino acids where selenium has been substituted for sulfur. These include selenocysteine, selenohomocysteine and selenomethionine. Selenocysteine is an important selenoamino acid obtained in mammalian which contains in several proteins such as selenoprotein P, selenoprotein W, glutathione peroxidases, thioredoxin reductases and iodothyronine deiodinases (Stadtman, 1996). Selenocysteine is encoded by a UGA codon in mRNA, is specific for the element and regulated physiologically. These specific selenocysteine-containing proteins are referred to selenoproteins, in contrast to other types of selenium-containing proteins which lack specificity for the element and are not encoded by a unique codon in mRNA (Reilly, 1996). Selenocysteine is located in active site of selenoproteins in which this residue is essential for catalytic activity (Kim and Gladyshev, 2005).

Several selenoamino acid were found in both bacteria and higher plants such as selenomethionine, selenocysteine and Se-methlyselenocysteine. Predominated forms of selenium in plants are selenomethionine in cereal grains and legumes seeds and Se-methlyselenocysteine in vegetables (Kabata-Pendias, 2011). Furthermore, selenoamino acids were identified in plant such as γ-glutamyl-Se-methylselenocysteine and γ-glutamyl-Se-methylselenomethionine are γ-glutamyl derivative which were observed in some plants especially *Allium* family such garlic and onion (Larsen *et al*., 2006). However, the most efficiency selenoamino acid in selenium metabolism partway are selenocysteine and selenomethionine. They were presented in tissue or animal fluid with incorporated in peptide chain of selenoprotein (Behne *et al*., 1998).

2.2.2.2 Selenoproteins

The first selenoproteins were discovered in 1973, several other selenoproteins have been identifiled. Selenoproteins have been found in all kingdoms of life, but certain organisms like yeast or higher plants, lack selenoproteins. The fact that selenocysteine is encoded by the stop codon UGA, probably postponed the identification of many selenoproteins and there are cases where enzymes were identified and cloned as truncated products, due to misinterpreted UGA codons (Johansson, 2005). Selenoprotein could be classified for two types as selenomethionine-containing proteins and selenocysteine-containing proteins. In plants and microorganisms found selenomethionine-containing proteins but the function is not known (Reilly, 1996).

Selenomethionine can be converted into selenocysteine by the transsulphuration enzymatic pathway, as has been shown in rat liver (Esaki *et al*., 1981)

However, selenoprotein-containing proteins are the major selenoprotein in higher animals, were found at least 25 specific (Jing, 2010). This review would be described some selenoproteins which known the property and function.

2.2.2.3 Glutathione peroxidases

Glutathione peroxidase (GPx) was the first specific mammalian selenoprotein identified (Schwarz and Foltz, 1957; Stadtman, 1980). It protects the cell from oxidative damage by catalyzing the reduction of hydrogen peroxide, lipid hydroperoxides and other organic peroxides. There are five types of mammalian selenium-containing glutathione peroxidase such as cytosolic glutathione peroxidase (GPx1), gastrointestinal glutathione peroxidase (GPx2), plasma glutathione peroxidase (GPx3), phospholipid hydrogenperoxidase PHGPx (GPx4) and sperm nuclei glutathione peroxidase (snGPx) (Behne, 2001). All five types have a selenocysteine residue in the catalytic active site. There is also least one non-selenium glutathione peroxidase (GPx5), which has a cysteine residue in the active site (Ghyselinck *et al*., 1991). However, they exist in different parts of a cell or tissue using glutathione or other thiols to reduce a variety of organic hydrogenperoxides produced during normal cellular metabolism or upon exposure to environmental carcinogens, which otherwise damage the structure of macromolecules and consequently interfere with the function of enzymes(El-Bayoumy, 2001). Therefore, they play an important role in the body's antioxidant defence system.

Table 2.2 Some inorganic and organic selenium compounds

Chemical name	Formula
Selenide (-2)	Se^{2-}
Dimethylselenide (DMSe)	$(CH_3)_2Se$ (volatile)
Dimethyldiselenide (DMdSe)	$(CH_3)_2Se_2$ (volatile)
Dimethylselenone/methylmethylselenite	$(CH_3)_2SeO_2$ (volatile)
Hydrogen selenide	H_2Se (volatile)
Elemental Selenium (0)	Se^0
Selenite (+4)	SeO_3^{2-}
Trimethylselenonium ($TMSe^+$)	$(CH_3)_3Se^+$
Selenous acid	$H_2SeO_3^-$
Selenium dioxide	SeO_2
Selenate (+6)	SeO_4^{2-}
Selenic acid	$H_2SeO_4^-$
Selnocyanate	HSeCN
Dimethylseleniumsulfide	$(CH_3)Se\text{-}S(CH_3)$ (volatile)
Dimethylseleniumdioxide	$(CH_3)SeO_2$
Methylselenol	CH_3SeH
Selenocysteine	$HOOCCH(NH_2)CH_2\text{-}Se\text{-}H$
Selenomethylcysteine	$HOOCCH(NH_2)CH_2\text{-}Se\text{-}CH_3$
Selenocystine	$HOOCCH(NH_2)CH_2\text{-}Se\text{-}Se\text{-}CH_2CH(NH_2)COOH$
Selenomethionine	$HOOCCH(NH_2)CH_2CH_2\text{-}Se\text{-}CH_3$
Selenoethionine	$HOOCCH(NH_2)CH_2CH_2\text{-}Se\text{-}CH_2CH_3$
□-Glutamyl-Se-methylselenocysteine	$H_2NCH_2CH_2\text{-}CO\text{-}NHCH(COOH)CH_2\text{-}Se\text{-}CH_3$
Slenohomocysteine	$HOOCCH(NH_2)CH_2CH_2\text{-}Se\text{-}H$
Se-adenoxylselenohomocysteine	$HOOCCH(NH_2)CH_2CH_2\text{-}Se\text{-}CH_2C_4H_5C_5N_4NH_2$

Source: Modified from Frankenberger and Benson, 1994; Hymer and Caruso, 2006

2.2.2.4 Thioredoxin reductases

Thioredoxin reductase (TrxR) is a flavoprotein belonging to a family of homodimeric pyridine nucleotidedisulfide oxidoreductases, which includes glutathione reductase, lipoamide dehydrogenase and mercuric ion reductase (William *et al*., 1992). Thioredoxin reductases have evolved from bacterial, plant and yeast thioredoxin reductases are homodimers of around 35 kDa and are different from the large thioredoxin reductase, which has a subunit size between 55-60 kDa and is present in higher eukaryotes (Williams *et al*., 2000). The function of thioredoxin reductases are also play a role in protection against oxidant injuly, cell growth transformation and the recycling of ascorbate from its oxidized form. Sinc thioredoxin reductases are able to reduce a number of substrates other than thioredoxin. It is likely that additional biological effects will be discovered for thioredoxin reductases. Furthermore, inhibiting thioredoxin reductases with drugs may lead to new treatments for human diseases such as cancer, AIDS and autoimmune diseases (Mustacich and Powis, 2000).

2.2.2.5 Selenoprotein P

Selenoprotein P (SelP) is among the first discovered selenoproteins but its function has not fully understood. It is the major selenoprotein in plasma, which represents 44-70% of the total plasma selenium (Hill *et al*., 1996). Human selenoprotein P contains 10 selenocysteine residuces per polypeptide chain. Therefore, it may function primarily as a selenium transport protein. Selenoprotein P is synthesized mostly in the liver and released in to blood circulation, then degraded to liberate selenium for the synthesis of novel selenoprotein in target tissue. Selenoprotein P may be required to retain selenium and Se-dependent antioxidant system in the brain as it can also be expressed locally and not lost into general circulation (Scharpf *et al*., 2007). Selenoprotein P may also function as a plasma antioxidant and heavy metal antidote as it contributes to the destruction of peroxynitrite, an important factor in inflammatory toxicity (Arteel *et al*., 1998).

2.2.2.6 Selenoprotein W

Selenoprotein W (SelW) has been known for years as a protein enriched in skeletal and heart muscle, which was related to the white muscle disease in Se-deficient lambs. It is also found widely distributed in other tissue, including brain, heart

and kidney (Gu *et al*., 2000). Selenoprotein W is present in the cytosol as well as being membrance-associated. It has a potential function in muscular function and redox metabolism (Behne and kyriakopoulos, 2001)

2.3 Metabolism of selenium

2.3.1 Metabolism of selenium in humans

2.3.1.1 Forms of selenium in human diet

In human diet, two naturally occurring selenoamino acids make up the bulk of the element such as selenomethionine, the predominant form in food plants and selenocystein, mainly found in food from an animal source. There are other organic selenocompounds present in very small quantities in human diet such as Se-methyl- selenocysteine, γ-glutamyl Se-meyhyl selenocysteine, selenocystathionine, selenohomocysteine, γ-glutamyl selenocystathione and methyl selenol (Reilly, 1996). Selenite and selenate are two inorganic forms of selenium that are often used as dietary supplements in humans.

2.3.1.2 Absorption and transportation

Selenium in foods is readily absorbed with apparently no physiological control over the absorption. In general, the absorption of the organic forms of selenium is more efficient than that of inorganic forms with uptake from the gastrointestinal tract of more than 90% of selenomethionine compared with about 60% of selenite (Reilly, 1996; Finley *et al*., 2004). The uptake of selemethionine appears to utilize the active transport process using the same system as methionine. Competition for uptake occurs between methionine and its seleno analogue. The inorganic selenium and other organic forms of selenium including selenocysteine are absorbed from the gastrointestinal tract via a normal concentration gradient which is a passive process and competes with inorganic sulphur compounds for absorption (Jing *et al*., 2009). Selenomethionine is initially incorporated non-specifically into tissue proteins including red cell haemoglobin and plasma albumin and transported around the body before being metabolized (Wolffram *et al*., 1989). When selenomethionine is the main form of the element in people diet, nearly 50% of the selenium in plasma is associated with albumin (Burk, 2002).

2.3.1.3 Metabolism pathway

The metabolism of selenium in human depend on the chemical form and selenium status, the ingested selenium could follow different metabolic pathways in the body. In general, all absorbed selenium would either be metabolised in the biologically active pool, which provides the basis for metabolism and synthesis of all the functionally important selenoproteins and seleno-metabolises for excretion or enter the body Se-binding protein pool, which derives from non-specific incorporation of exogenous selenomethionine into tissue proteins in place of methionine. The later is considered as a selenium store in the body (Reilly, 1996). Figure 2.1 shows the proposed metabolic pathways for different forms of selenium. Most forms of selenium are efficiently absorbed but subsequent metabolism depends on the form in which they are present in plasma. Selenomethionine, selenocysteine, selenate and selenite enter the selenide pool and from here the selenium is either used for selenoprotein synthesis or excreted in the urine as a selenosugar. Selenomethionine can be incorporated directly (and nonspecifically) into proteins through the replacement of methionine. A separate pathway is followed by the organic compound, γ-glutamyl methylselenocysteine, found in brassica and allium vegetables, whereby it is first converted to Se-methyl-selenocysteine and then transformed by β-lyase into methylselenol, which is primarily excreted in breath and urine but may also enter the selenide pool (Fairweather-Tait *et al*., 2010).

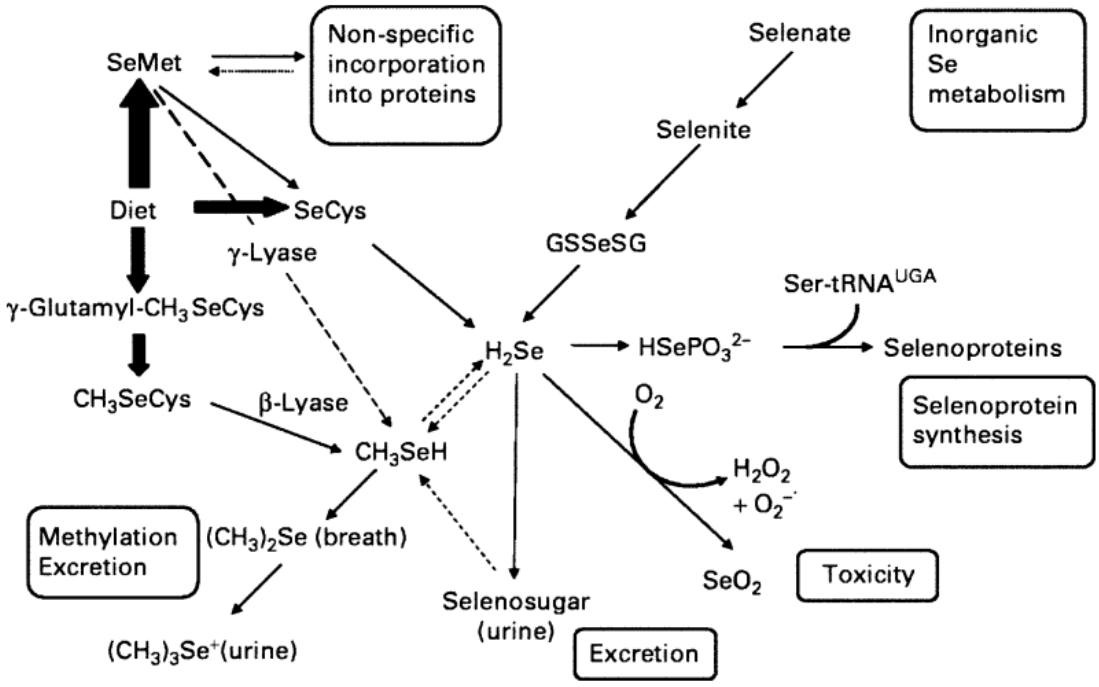

Figure 2.1 Metabolic pathway of dietary selenium in humans (Fairweather-Tait *et al*., 2010)

2.3.2 Metabolism of selenium in animals

Selenium distribution within in the body and also its absorption and excretion depend on several factors particularly on the chemical forms or speciation and the total quantity of the element in the diet. In addition, in take can be affected by the presence of certain other components of food including sulphur, heavy metals and vitamins (Reilly, 1996). The metabolic pathways for selenium in animals are shown in Figure 2.2 Organic selenium such as selenomethionine or inorganic selenium can be converted to a common intermediate H_2Se (Ip, 1998).

There are two possible pathways for the catabolism of selenomethionine. One is the transsulfuration pathway via selenocystathionine to produce selenocysteine which in turn is degraded to H_2Se by the enzyme β-lyase. The other pathway is the transamination–decarboxylation pathway (Mitchell and Benevenga, 1978). It was estimated that 90% of methionine is metabolized through this pathway and thus could be also the major route for selenomethionine catabolism. Se-methylselenocysteine is the predominant seleno-compound formed in selenium-enriched garlic at relatively low concentrations, but γ-glutamyl-Se-methylselenocysteine is the predominant form at

high selenium concentrations. Even though this glutamyl derivative may be the predominant form it is hydrolysed in the intestinal tract and the absorbed Se-methylselenocysteine cleaved by a lyase to form methylselenol (Dong *et al*. 2001). Thus, this glutamyl derivative is metabolized like Se-methylselenocysteine at the tissue level. Se-methylselenocysteine is converted to methylselenol directly when cleaved by β-lyase and unlike selenomethionine it cannot be incorporated non-specifically into proteins. Since Se-methylselenocysteine can be converted directly to methylselenol this explains why it is more efficacious than other forms of selenium in cancer prevention (Whanger, 2004).

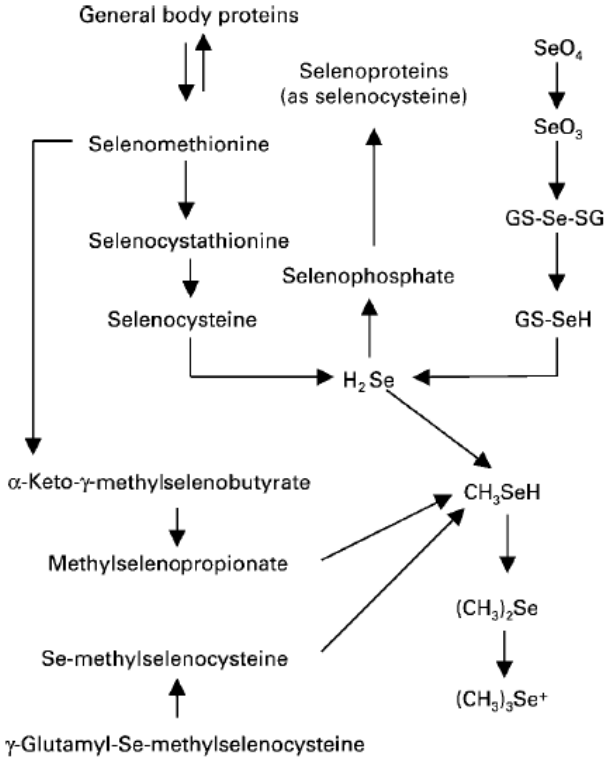

Figure 2.2 Proposed pathways for the metabolism of selenium in animals (Whanger, 2003)

2.3.3 Metabolism of selenium in plants

Plants differ in their ability to accumulate selenium in their tissues. Certain native plants are able to hyperaccumulate selenium in their shoots when they growon seleniferous soils. These species are called selenium accumulators and include a number of species of *Astra-galus, Stanleya, Morinda, Neptunia, Oonopsis* and *Xylorhiza*. They

can accumulate from hundreds to several thousand milligrams of Se kg^{-1} dry weight in their tissues. Although selenium accumulators grow on seleniferous soils not all plant species on seleniferous soils are selenium accumulators, some plants accumulate only a few milligrams of Se kg^{-1} dry weight. For example, the genus *Astragalus* contains both Se-accumulating species and nonaccumulating species (Terry *et al*., 2000).

The translocation of selenium from root to shoot is dependent on the form of selenium supplied. Selenate is transported much more easily than selenite or organic selenium, such as selenomethionine (Zayed *et al*., 1998). The selenate uptake across the root plasma membrane is mediated by the high-affinity sulfate transporter. The expression of the high-affinity sulfate transporter is regulated positively by O-acetylserine, and negatively by sulfate and glutathione. Selenite is a dominant form of selenium in aerobic soils. However, unlike selenate, the mechanism of selenite uptake by plants remains unclear. Selenite uptake is an active process likely mediated at least partly by phosphate transporters. Selenite and selenate differ greatly in the ease of assimilation and xylem transport. Selenite was rapidly converted to organic forms in roots, with limited transport to shoots. Selenomethionine, selenomethionine Se-oxide, Se-methylselenocysteine and several other unidentified selenium species were obtained in the root and sylem sap from selenite-treated plants. Selenate was highly mobile in sylem transport but little was transformed to organic forms (Terry *et al*., 2000).

Figure 2.3 shows the proposed pathways for the metabolism of selenium in plants. Selenate (SeO_4^{2-}) is reduced to selenite (SeO_3^{2-}) by ATP sulfurylase. Then, selenite is reduced to selenide (Se^{2-}) by a number of steps that involved reduced glutathione. Selenide reacts with O-acetylserine to form selenocysteine in a manner directly analogous to S metabolism. The S-amino acid cysteine is the starting point for a series of reactions that lead to the synthesis of methionine and it has been postulated. Cysteine is mostly due to lack of experimental evidence to the contrary that selenocysteine is also metabolized by this same pathway. Selenium enters the food chain through incorporation into plant proteins, mostly as selenocysteine and selenomethionine at normal Se levels. However, with elevated selenium levels, Se-methylselenocysteine can be the predominant selenocompound (Whanger, 2004). Several selenocompounds have been identified in plants, but their concentrations are usually very low. Indicator plants (called Se-accumulators) can accumulate extremely

large amounts of selenium, ranging from 1000 to 10000 mg Se g^{-1}, because they synthesize mostly non-protein selenoamino acids (Brown and Shrift, 1981). Furthermore, Se-methylselenocysteine concentrarion in accumulator plants is higher than non-accumulator plants (Ellis and Salt, 2003).

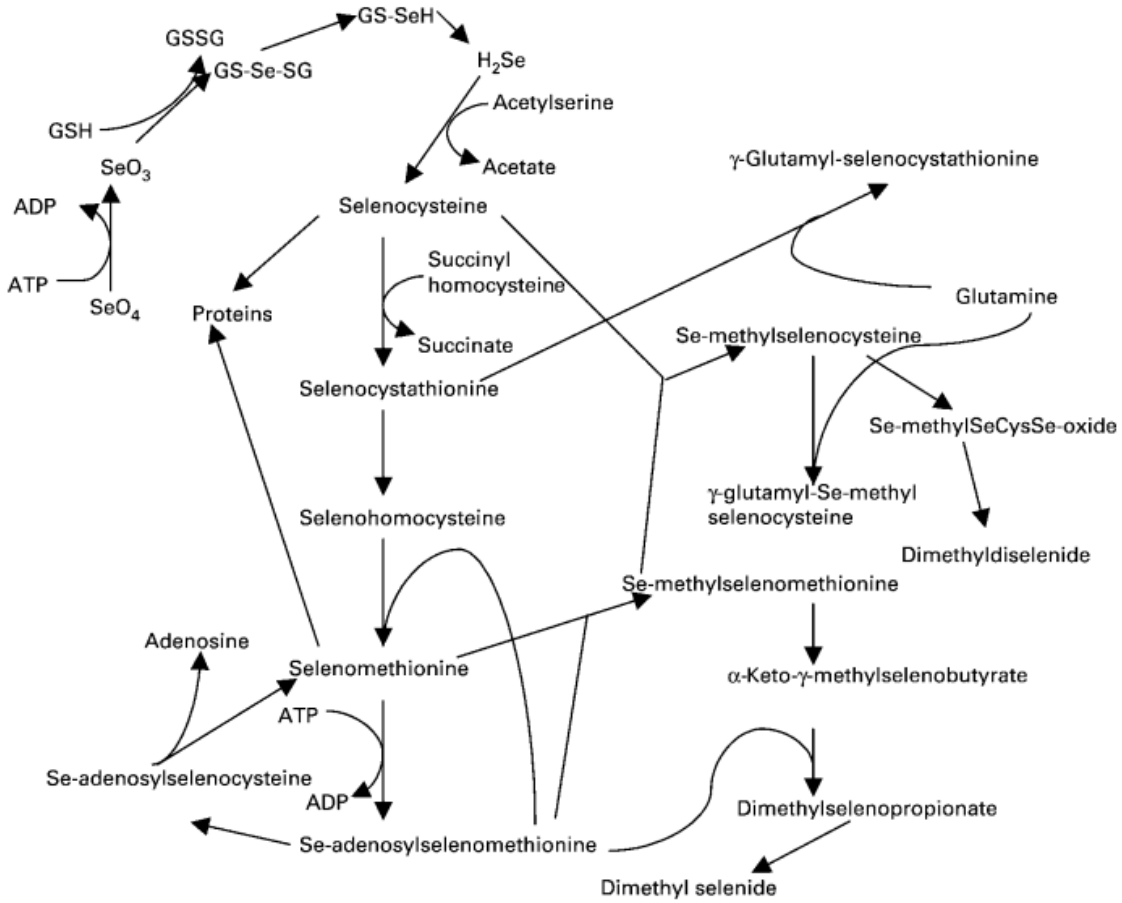

Figure 2.3 Proposed pathways for the metabolism of selenium in plants (Whanger, 2003)

2.4 Selenium in foods

The selenium content of foods reflects the selenium content of the soil in which the plants consumed were grown or on which the animals consumed were raised. The relationship between soil content and a food selenium content is not always straightforward. Therefore, selenium content in foods also varied by the region (Table 2.3). Plant foods are the major dietary sources of selenium and transfers to another

living organism in food chain. Animals that eat grains or plants, grown in selenium-rich soil, have higher levels of selenium in their muscle. In addition, people living in the high levels of selenium region have higher selenium intakes than people living in the lower levels of selenium region (Gutric and Picciano, 1995). For example, in the United States, pasta tends to be high selenium because the durum wheat used tents to come from high selenium soils in the Dakotas (Wardlaw and Hampl, 2004). Table 2.4 shows the selenium content in various Thai foods which the selenium concentration were varied by geographical location of plants and season of the year (Sirichakwal *et al*., 2005)

Table 2.3 Comparison of selenium data of some foods in various countries

Food	Source of data and selenium content (µg/100 g)					
	UK	USA	New Zealand	Irland	China	Thailand
Rice	10, 13	31.9	7.3	-	2	5
Beef	3, 7.6	19.9	1.0, 1.2	8.1	5.3	15.4
Pork	14	23.9	5.7	10.4	13.8	18
Egg, chicken, whole	-	-	24	-	13	32.7
Egg, chicken, white	6	5.1	5.5	6.4	7	18.8
Egg, chicken, yolk	20	18.3	48	24.1	27	50.6
Chicken, breast	-	11.6	-	11.5	10.5	22.3
Chicken, leg	-	13.6	-	-	12.4	22.9
Oyster	-	65.3	46, 82	-	47	29.3
Shrimp	-	58.8	-	-	33.7	35.5
Milk, powder, infant	8	-	-	-	8	5.7
Milk, whole	1.5	1	0.3, 0.4	0.3	1.9	2.8
Peanut	3	-	-	-	3.9	11.1
Soybean	-	-	-	-	6.2	-
Tomato	<1	0.5	0.1, 0.2	-	0.1	1.2
Carrot	<1	2.2	-	-	0.6	2.2
Cabbage	<1	2.3	0.07, 0.08	-	1	0.2
Garlic	2	24.9	-	-	3.1	3.4
Corn, fresh	-	0.4	-	-	3.5	0.3
Cauliflower	-	0.7	0.3	-	0.7	0.7
Lettuce	-	0.8	-	-	0.8	0.2
Onion	-	1.5	2.1, 0.2	-	0.9	1.1
Green pepper	-	0.7	-	-	0.4	0.1
Mushroom	8, 10	13.2	-	3.3	vary	1.2
Orange	<1	13.2	-	-	0.3	1.2
Banana	-	1	-	-	0.9	0.3
Pineapple	-	0.6	-	-	0.2	0.2

Source: Modified from Sirichakwal *et al*., 2005

Table 2.4 Selenium content in Thai foods

Food item	Selenium (µg/100 g)
Vegetable	
Acacia pennata (Cha-om)	12.7
Asparagus	3.5
Banana flowers/blossoms,	3.5
Broccoli	0.6
Brussels sprouts	5.7
Cabbage, Chinese/flowering white cabbage	0.3
Cabbage, Chinese, green	0.3
Cabbage, Chinese, white	1.4
Carrot	3.9
Cauliflower	0.7
Celery	0.3
Chinese convolvulus	0.3
Chive, Chinese, flowers	0.4
Chive, Chinese, leaves	0.3
Coriander	0.7
Corn, baby	0.3
Cucumber	0.4
Eggplant/aubergine, green, long	0.3
Eggplant/brinjal	1.3
Garden peas, pods	0.3
Gourd, wax/winter melon	0.5
Ivy gourd	0.3
Kale, Chinese	0.3
Lettuce	0.3
Mung bean sprout	1.1
Mushroom, Chinese or straw	1.2
Mushroom, Indian oyster	1.5
Mushroom, Jew's ear, fresh	0.8
Neem, leaves and tips	5.5
Papaya, raw	0.6
Pumpkin	0.9
Solanum	1.4
Spinach	0.6
Spring onion/Welsh onion	0.6
Swamp morning glory, red stems	0.3
Tomato, cherry, small	1.2

Source: Modified from Sirichakwal *et al*., 2005

Table 2.4 Selenium content in Thai foods (cont.)

Food item	Selenium (µg/100 g)
Water mimosa	0.4
Wild betal, leaves	0.8
Winged beans, pods, Yard long bean, green	0.5
Cereals, legumes and seeds	
Rice, polished, raw	5.4
Rice, jasmine variety, polished, raw	4.6
Cow pea, seeds, dried	5.8
Mung bean, seeds, dried	12.3
Peanut/groundnut, seeds, dried	11.1
Rice bean, seeds, dried	15.7
Soybean, seeds, dried	12.7
Sesame seeds, black, dried	23.0
Sesame seeds, white, dried	15.6
Fruits	
Banana, apple, common (Nam-wa)	0.3
Banana, rice (Klouy Khai)	0.6
Durian, Mon-thong/golden pillow variety	0.7
Jackfruit, Nung variety	1.1
Litchi/Ly chee, Hong-houy variety	0.1
Litchi/Ly chee, Jom-jai-jakapat variety	2.8
Litchi/Ly chee, Jom-jai-kuang-joa variety	0.3
Longan, Beaw-keaw variety	2.4
Longan, E-daw variety/Heaw variety	0.3
Longan, See-chompu variety	0.5
Mango, Kaew variety, unripe	0.6
Mango, Kiew-sa-weya variety, unripe	0.7
Mango, Num-dok-mai variety, ripe	0.8
Mango, Okrong variety, ripe	1.0
Mango, Rad variety, unripe	1.1
Papaya, ripe	1.2
Rambutan, pink variety	0.5
Rambutan, Rong-rean variety	0.6
Meat and poultry	
Beef, lean, raw	15.8
Beef, Longissimus dorsi, raw	12.3
Beef, tenderloin, raw	15.3
Chicken, breast, raw	22.3

Table 2.4 Selenium content in Thai foods (cont.)

Food item	Selenium (µg/100 g)
Chicken, drumstick, raw	22.9
Pork, lean, raw	17.2
Pork, Longissimus dorsi, raw	18.2
Pork, tenderloin, raw	18.7
Fish and sea foods	
Batrachian walking catfish, raw	47.3
Short-bodied mackerel, raw	88.1
Silver pomfret, raw	52.3
Squid, splendid, raw	41.0
Striped snake-head fish, raw	33.5
Crab, serraed mud, meat, boiled	46.1
Cockle/ark shell, fresh	44.0
Mussel, green, fresh	42.6
Oyster, Pacific	29.3
Prawn, green tiger, raw	35.4
Eggs	
Egg, hen, whole	32.7
Egg, hen, white	18.8
Egg, hen, yolk	50.6
Egg, duck, whole	48.5
Egg, duck, white	36.9
Egg, duck, yolk	53.4
Milk and milk products	
Milk, powder, full cream	6.4
Milk powder, infant formula	5.7
Milk, whole, UHT, natural	2.8

2.5 Selenium requirement for human health

The first Recommended Dietary Allowances (RDA) for selenium was announced in 1989 (Drummond and Brefere, 2004). The minimum selenium requirement is determined by comparing dietary intakes in geographical areas in which deficiency occurs with intakes in those areas without such deficiency (Table 2.5). As indicated by the dietary intake in areas where Keshan disease is not found at least 19 and 13 µg/day for adult Chinese males and females respectively (Yang *et al*., 1988).

In New Zealand, intakes of 33 µg/day by male and 23 µg/day by female are not associated with deficiency. Although, several selenium balance studies have been conducted to investigate selenium requirements. However, The homeostatic mechanisms appear to ensure that intake of selenium are in balance with losses over a wide range of intake from 9 to 80 µg/day which selenium requirements depend on age and sex as Table 2.6 (Gutric and Piccano, 1995).

Table 2.5 Representative mean serum selenium concentrations from selected studies

Country or Area	Sample serum selenium concentration (mol L^{-1})
Pathologic subjects	
Keshan disease (China)	0.15 - 0.25
Kashin-Beck disease (China)	0.22 - 0.03
Myxedematous cretins (Zaire)	0.26 - 0.12
HIV and AIDS	0.36 - 0.54
Normal subjects	
Bulgaria	0.66 - 0.72
Hungary	0.71 - 0.13
New Zealand	0.69
Norway	1.52 - 1.69
Serbia and Croatia	0.63 - 0.85
United state, Maryland	1.69 - 2.15
United state, South Dakota	2.17 - 2.50

Source: WHO/WFO, 2004

Table 2.6 Recommended dietary intakes for selenium

Age (years)	Amount (µg/day)
Infants	
< 1.1	10 - 15
1.1 - 9	20
6 - 9.9	30
Males	
10 - 11.9	40
12 - 17.9	50
18 - 51+	70
Females	
11 - 14.9	45
15 - 18	50
19 - 51+	55

Source: Gutric and Picciano, 1995

2.6 Antioxidant activity of selenium compounds

The body contains complex antioxidant systems that require adequate intake of selenium for normal physiological function; the RDA for selenium is approximately 55 µg/day and selenium can be incorporated into the body by ingesting foods such as carrots, cabbage, garlic, mushrooms, cheese, meats, and grains and selenium-containing supplements (Foster, 1995; Hawkes et al., 2008;). Selenium, in the form of selenocysteine, is a constituent of 25 classes of selenoproteins, including GPxs, selenoproteins P, W, and R, and thioredoxins (Diwadkar-Navsariwala and Diamond, 2004; Tapiero *et al*., 2003). There is evidence that several of these selenoproteins have antioxidant activities. However, the functions of most have not been determined. Early observations linking selenium and pathogenesis started an intense investigation into the role of selenium in antioxidant defense and disease treatment, and many selenium compounds have been investigated for their antioxidant properties (Figure 2.4, Table 2.7).

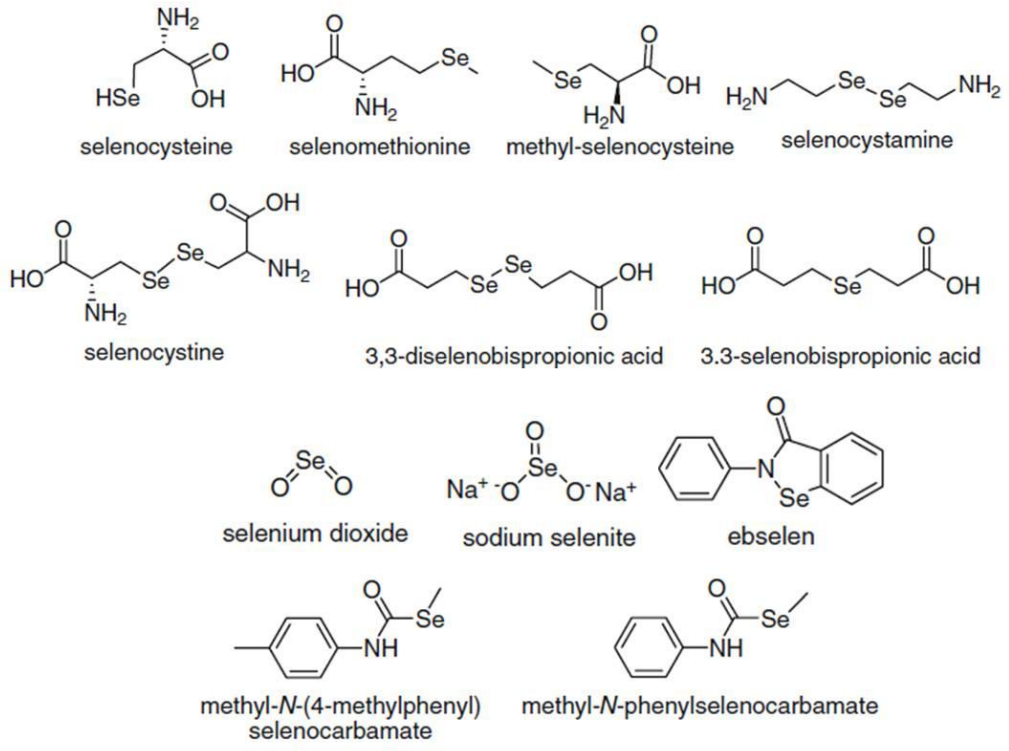

Figure 2.4 Antioxidant selenium compounds (Battin and Brumaghim, 2009)

Table 2.7 Sources and activities of selenium compounds

Selenium	Compound source	Activity
Selenocysteine	Diet	Antioxidant
Selenomethionine	Diet	Antioxidant
Methyl-selenocysteine	Diet	Antioxidant
Selenocystamine	Endogenously synthesized	Antioxidant
Selenocystine	Diet/endogenously synthesized	Antioxidant
3,3-Diselenobispropionic acid	Synthetic	Antioxidant
3,3-Selenobispropionic acid	Synthetic	Antioxidant
Selenium dioxide	Synthetic	Antioxidant/pro-oxidant
Sodium selenite	Environmental/diet	Antioxidant/pro-oxidant
Ebselen	Synthetic	Antioxidant
Methyl-N-(4-methylphenyl) Selenocarbamate	Synthetic	Antioxidant
Methyl-N-phenylselenocarbamate	Synthetic	Antioxidant

Source: Battin and Brumaghim, 2009

2.7 Antioxidant activity of selenium-enriched plants

Finding an effective way of increasing the production of selenium-enriched dietary sources is necessary because selenium supplementation from natural food materials is considered safer than directly ingesting inorganic selenium (Liu *et al*., 2012). Most common selenium supplements are selenite yeasts whose major selenocompound is selenomethionine (SeMet) (Kotrebai *et al*., 1999). Other materials such as selenite supplement in *Allium fistulosum* (Kápolna and Fodor, 2006), *Allium sativum*, and *Brassica juncea* (Montes-Bayon *et al*., 2006) have been reported recently. Growing evidence shows that selenium supplementation is able not only to improve people's dietary selenium, but also plays a role in protecting from oxidative damage by free radicals. Recently, some selenium-enriched food materials and selenium-containing compounds extracted from these materials such as selenium-enriched green tea (Xu *et al*., 2003), selenium-enriched mushroom (Zhao *et al*., 2008), selenium-containing phycocyanin isolated from selenium-enriched *Spirulina platensis* (Chen *et al*., 2008), selenium-containing proteins (Se-Ps) isolated from selenium-enriched bifidobacteria (Shen *et al*., 2010) and mushroom (Zhao *et al*., 2008) have been reported to possess strong antioxidant activities. It have been reported that the primary forms of selenium

found in selenium-enriched plants are Se-methyl selenocysteine, selenomethionine and derivatives like γ-glutamyl-Se-methylselenocysteine. Se-methyl selenocysteine is a good precursor for generating methylselenol when enzymes like β-lyase are present. This Se-metabolite seems to be the most active species for cancer reduction. Therefore, Se-methyl selenocysteine has been widely studied as a potential anticarcinogenic compound (Montes-Bayón *et al*., 2006)

2.8 Selenium-enriched plant production

Plants may be used both to provide dietary selenium in areas of selenium deficiency and to clean up selenium pollution from seleniferous areas. Therefore, selenium-enriched plant production was interested for supplementation dietary because plants are known to convert inorganic forms of selenium to organic selenium compounds following the sulfur assimilatory pathway (Pilon-Smits and LeDue, 2009; Terry *et al*., 2000). The organic selenium compounds such as Se-methylselenocysteine, selenomethionine selenocysteine and γ-glutamyl-Se-methylselenocysteine which these compounds are the major selenium compounds in plant (Whanger, 2004). The ability of several plants to accumulate and transform inorganic forms of selenium into bioactive organic compounds has important implications for human nutrition and health (Pyrzynska, 2009).

The production of high-selenium vegetables requires particular soil with a high selenium concentration. High-selenium soil must be prepared by fertilizing with selenite or selenate. This selenium fertilization of soil may cause environmental pollution by selenium. However, selenium in the forms of selenate and selenite is readily absorbed by the plant and converted metabolically in the chloroplast to organic selenium compounds (Terry *et al*., 2000), which are a component of protein in plant tissues (Leustek and Saito, 1999; Tinggi, 2003). Numerous studies revealed that selenium-enriched plants could be successfully produced for human nutrition using edible plants such as broccoli sprouts (Finley *et al*., 2001), green onions (*Allium fistulosum*), chives (*Allium schoenoprasum*), ramp, cabbage (Kapolna and Fodor, 2006), garlic (Tsuneyoshi *et al*., 2006), kaiware radish sprout (Yoshida *et al*., 2007) and sprouts of several plants (Lintschinger *et al*., 2000; Sugihara *et al*., 2004). Therefore, a

closed system is necessary to produce high-selenium vegetables. In Japan, the sprouts of several plant species are utilized in foodstuffs. The unique *Brassica* plant in Japan, nozawana (*Brassica rapa* var. hakabura) and komatsuna (*Brassica rapa* var. peruviridis) show the same selenium accumulating ability as Indian mustard (Yawata *et al*., 2010). Thereby, the *Brassica* family has a high potentiality to provide as the seleniume-enriched diet. These sprouts are cultivated in a closed system which is considered a relatively easy method to produce selenium-enriched sprouts (Sugihara *et al*., 2004).

Hydroponics is a method of growing plants using mineral nutrient solutions in water without soil. Terrestrial plants may be grown with their roots in the mineral nutrient solution only or in an inert medium such as perlite, gravel, mineral wool or coconut husk. Moreover, hydroponic system is easy cultivation method for selenium-enriched plant production with closed system and easy control production. Table 2.8 shows plant cultivation for several plant species and their selenium concentration and selenium compounds obtained which was reported by Sugihara *et al*., 2004.

2.9 Uptake and transport of sulfate ion in plants

The transportation of sulfate ion (SO_4^{2-}) occurs across several membrane systems as it enters and is distributed throughout the plant and within cells. Transport across the plasma membrane occurs with protons at a ratio of $1SO_4^{2-}$:3 H^+ (symport) and is driven by a proton gradient maintained by a proton ATPase. Transport across the tonoplast membrane is mediated by an unknown mechanism that is driven by the electrical gradient between the vacuole sap and cytoplasm. The phosphate/triose phosphate translocator of the inner chloroplast membrane or a proton/SO_4^{2-} symporter may mediate SO_4^{2-} transport into chloroplasts (Leustek and Saito, 1999)

The plasma membrane transporters of plants have been characterized. The sequences of cDNAs cloned from *Stylosanthes hamata*, Arabidopsis, soybean, barley, maize, resurrection grass and Indian mustard showed that the plasma membrane transporters of plants are most closely related to fungal and animal proton/SO_4^{2-} cotransporters. Hydropathy analysis revealed that the plant transporters may span the

membrane 12 times, a structural feature that is typical of many types of solute symporters (Smith *et al*., 1997).

Moreover, the role of the sulfate transporter in selenate uptake have undertaken studies to determine whether overexpression of either the high-affinity (*SHST1*) or low-affinity (*SHST3*) transporter genes from *S. hamata* increases the uptake of selenium in Indian mustard. Overexpression of *SHST1* increased selenate accumulation up to twofold in transgenic plants compared to wild type. On the other hand, transgenic plants overexpressing *SHST3* did not differ significantly from wild type in their accumulation of selenate. These data support the view that the high-affinity sulfate transporter is involved in selenate uptake (Huang and Wu, 1991).

Unlike selenate, there is no evidence that the uptake of selenite is mediated by membrane transporters. Selenite uptake was inhibited by only 20% by the addition of a respiratory inhibitor, hydroxylamine, to nutrient solution. Selenate uptake was inhibited by 80% (Arvy, 1993). A study by Asher *et al*., showed that although the selenium concentration in the xylem exudate of selenate-supplied detopped roots exceeded that of the external medium by 6 to 13 times, selenium concentration was always lower in the xylem exudate than outside when selenite was supplied.

Table 2.8 Total selenium and selenium species concentrations in various plants

Plant		Period of cultivation (day)	Selenium		Ration of selenium species (%)				
Common name	Scientific name		Whole content (μg g^{-1} wet weight)	Proportion of 0.2 M HCl extractable (%)	Se (IV)	Unknown 1	SeMC	SeMet	□-glu SeMC
Spinach	*Spinaia oleracea*	8	25.6	75.0	n.d.	n.d.	96.7	3.3	n.d.
Tallfescue	*Festuca arundinacea*	8	11.8	65.7	13.2	15.1	56.6	15.1	n.d.
Barley	*Hordeum vulgare*	8	16.7	74.9	5.1	32.3	40.4	22.2	n.d.
Rice	*Oryza sativa*	20	24.1	75.2	4.4	33.6	44.2	17.7	n.d.
Astragal	*Astragarus sinicus*	8	15.0	81.7	10.7	20.2	69.1	n.d.	n.d.
Soy bean	*Glycine max*	5	7.8	85.9	n.d.	30.9	63.2	5.9	n.d.
Kidney bean	*Phaseeous vulgaris*	6	9.5	82.6	3.1	14.1	79.7	3.1	n.d.
Red clover	*Trifolium pretense*	8	14.4	70.9	11.9	10.4	77.6	n.d.	n.d.
Onion	*Allium cepa*	8	17.8	98.3	n.d.	n.d.	100.0	n.d.	n.d.
Edible burdock	*Arctium lappa*	12	11.7	78.1	0.3	n.d.	91.7	n.d.	n.d.
Garland chrysanthemum	*Chrysanthemum coronarian*	8	17.5	69.4	11.3	n.d.	88.7	n.d.	n.d.
Qin gin cai	*Brassica campestris; chinese group*	8	19.7	92.5	3.2	2.1	94.7	n.d.	n.d.
Chinese cabbage	*Brassica campestris; pekinensis group*	7	36.6	86.9	6.1	3.0	90.9	n.d.	n.d.
Turnip	*Brassica campestris; rapifera group*	8	37.7	77.7	n.d.	5.5	94.5	n.d.	n.d.

n.d. = not detected
Source: Modified from Sugihara *et al*., 2004

Table 2.8 Total selenium and selenium species concentrations in various plants (cont.)

Plant		Period of cultivation (day)	Selenium		Ration of selenium species (%)				
Common name	Scientific name		Whole content (μg g^{-1} wet weight)	Proportion of 0.2 M HCl extractable (%)	Se (IV)	Unknown 1	SeMC	SeMet	□-glu SeMC
Welsh onion	*Allium fistulosum*	8	27.1	98.3	n.d.	n.d.	100.0	n.d.	n.d.
Chinese chieve	*Allium tuberosum*	8	25.8	92.3	n.d.	n.d.	90.6	n.d.	9.4
Buck wheat	*Fagopyrum esculentum*	7	8.5	72.4	n.d.	19.4	80.6	n.d.	n.d.
Eggplant	*Solonum melongena*	15	22.0	94.0	n.d.	n.d.	96.9	3.1	n.d.
Tossa jute	*Corchorus olitorius*	8	16.2	87.0	3.2	6.5	90.3	n.d.	n.d.
Broccoli	*Brassica campestris; italic group*	7	32.1	77.8	1.9	3.8	94.3	n.d.	n.d.
Mitsuba	*Crytotaenia japonica*	15	29.2	75.8	1.7	n.d.	98.3	n.d.	n.d.
Carrot	*Daucus catota*	8	29.0	86.6	3.0	n.d.	93.4	3.6	n.d.
Nozawana	*Brassica campestris; rapifera group*	8	46.7	80.0	1.9	1.9	96.2	n.d.	n.d.
Kintoki (japan carrot)	*Daucus catota*	8	41.0	87.1	7.1	n.d.	92.9	n.d.	n.d.
Parsley	*Petroselinium cripum*	15	46.3	98.2	n.d.	n.d.	100.0	n.d.	n.d.
Kaiware daikon	*Rephanus sativus; daikon group*	8	2.6	95.1	n.d.	n.d.	100.0	n.d	n.d.

2.10 Toxicity of selenium

Toxicity of selenium depends not only on the chemical form and quantity of the element consumed but also on individual variations such as age, physical state, liver weight, nutrition and diet of the person (Reilly, 1996). Both organic and inorganic forms of selenium are toxic at high dose. Organic selenocompounds such as selenomethionine are usually lower in acute toxicity than inorganic selenocompounds as they are mostly food-bound and incorporation into tissue proteins removes a significant portion from circulation, thereby attenuating the adverse effects. However, excessive inorganic selenium that cannot be used derectly for selenoprotein synthesis must remain in circulation until it can be methylated and excreted (Jing, 2010).

The biochemical basis of selenium toxicity at high levels of intake is not fully understood. It is possible that excess substitution of methionine residues with selenomethionine may alter the physiochemical properties of structural protein because the seleno moiety of selenomethionine is more hydrophobic than the thiol moiety of methionine (Hatfield and Gladyshev, 2002). The formation of hydrogen selenide extracellularly may be the key event in selenocompound metabolism that induces oxidative stress and toxicity through overt consumption of intracellular reduced glutathione (Tarze *et al*., 2007). It is also possible that selenium interferes with enzyme activity, particularly by catalytic oxidation of sulphyhydryl (thiol) groups involved in oxidative metabolism within cells and thereby interfere with biosynthesis of essential molecules and DNA repair (Reilly, 1996). Furthermore, exceeding the tolerable upper intake level of 400 micrograms per day can lead to selenosis (Gutric and Piccano, 1995).

2.11 Determination of total selenium, selenium species and trace elements

The determination of selenium and trace elements in biological materials is a particular challenge because of its often very low concentrations and the ease with which it can be lost during sample preparation (Reilly, 1996). However, the commonly techniques for determination of selenium and trace elements in biological samples with high sensitivity and selectivity such as electrothermal atomic absorption spectrometry

(ETAAS), hydride generation absorption spectrometry (HG-AAS) (Hegedűs *et al*., 2008), graphite furnace atomic absorption spectrometry (GF-AAS) (Zheng *et al*., 2000), inductively couple plasma atomic emission spectroscopy (ICP-AES) (Iwashita *et al*., 2006), gas chromatography atomic emission detector (GC-AED) (Kahakachchi *et al*. 2004) and electrospray ionization mass spectrometry (ESI-MS) (Hymer and Caruso, 2006). However, they can determine only the total concentration of selenium which this information is not sufficient for identify the real toxicity or biological function of selenium because selenium was occurred in several species in environmental and biological system (Robles *et al*., 1999). Thus, the higher efficiency analytical technique was developed for selenium speciation analysis by using several separation techniques such as gas chromatography, high performance chromatography, field flow fractionation and capillary electrophoresis combine with the traditional elemental techniques such as HG-AFS, ICP-OES and ICP-MS (Gómez-Ariza *et al*., 1998; Michalke and Schramel, 1998; Łobínski and Szpunar, 1999). Although, several couple techniques such as HPLC-HG-AAS, HPLC-ICP-AES, GC-ICP-MS, CE-ICP-MS, FFF-ICP-MS and other techniques were successfully developed for selenium speciation analysis. Several reports suggest that, the coupling between HPLC and ICP-MS or HPLC-ICP-MS by using HPLC as separation unit and ICP-MS as detection unit could determine selenium species with high sensitivity, selectivity and low limit of detection. Therefore, HPLC-ICP-MS is more wildly used to determined selenium species and other element species in various samples (Sutton and Caruso, 1999; Waddell *et al*., 2005).

2.12 Selenium-enriched plants product storage

The most research about selenium-enriched plants studies in the part of total selenium, selenium species and antioxidant of selenium. The selenium-enriched plants product storage have been very few studies. However, the storage condition is important for the stability of organic selenium in plants. Thus, the stability and shelf life of selenium-enriched plant were studied. The most common indicators of selenium-enriched plants product storage influenced by storage time (expressed in days) and conditions (temperature and container materials).

A study by Zheng *et al*. (2002) demonstrated that the stability of five selenium compounds such as selenate, selenourea, trimethylselenonium ion, selenomethionine and selenoethionine stored at -20 °C, 4 °C and ambient temperature (ca. 25 °C) in the dark without addition of any stabilizing reagent. These results show that the general trend is the lower temperature used for storage found the higher stability of selenium species when other conditions such as light, acidity and container material are kept constant.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research designs

In this research, the experiments was designed as Figure 3.1

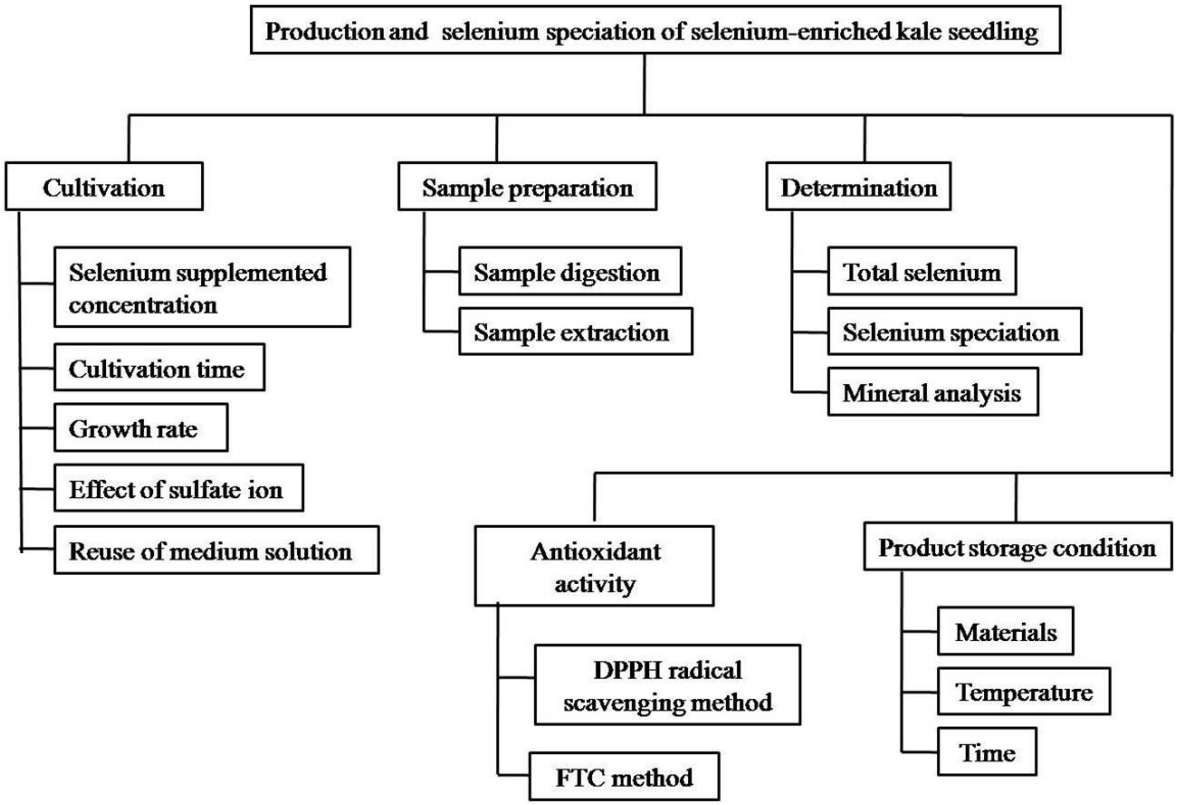

Figure 3.1 The schematic of this research

3.2 Materials, instruments and apparatus

Kale (*Brassica oleracea* var. alboglabra L.) was used for selenium-enriched plant samples. The materials were used in this work as follows:

3.2.1 Materials and apparatus for cultivation

3.2.1.1 Chinese kale seeds (Chia Tai Company Limited, Thailand)

3.2.1.2 Shelf (shown in Figure 3.2)

3.2.1.3 Plastic pot (35 x 40 x 30 cm)

3.2.1.4 Sponge (2.5 x 12 x 30 cm)

3.2.1.5 Air pump (Yamano AP-30)

3.2.1.6 Fluorescent lamp (36 W, Toshiba)

3.2.2 Instruments and apparatus for digestion

3.2.2.1 Water bath (maximum temperature 99.99 ^{o}C, Model TW 12, Julabo, Germany)

3.2.2.2 Micropipette (100-1000 μL, Gilson, France)

3.2.2.3 Analytical balance (TC 254, Denver Instrument Company)

3.2.2.4 Filter paper No.1 (particle size 11 μm, Whatman, England)

3.2.3 Instruments and apparatus for extraction

3.2.3.1 Water bath (temperature 70 ^{o}C, Model TW 12, Julabo, Germany)

3.2.3.3 Analytical balance (TC 254, Denver Instrument Company)

3.2.3.4 Filter paper No.1 (particle size 11 μm, Whatman, England)

3.3 Chemicals and reagents

All chemicals and reagents used in this work were of AAS grade and AR grade. The description of all chemicals and reagents used are shown in Table 3.1

Table 3.1 The description of chemicals and reagents used in this work

Name	Formula	Molar mass ($g\ mol^{-1}$)	Density ($g\ mL^{-1}$)	Grade	Company
Copper standard solution	Cu	63.54	-	AAS	Carlo Erba
Ammonium thiocyanate	NH_4SCN	76.122	-	AR	Univar
α,α-diphenyl-β-picylhydrazyl	$C_{18}H_{12}N_5O_6$	394.32	-	AR	Sigma
Boric acid	H_3BO_3	61.83	-	AR	Carlo Erba
Butylated hydroxyanisol	$C_{11}H_{16}O_2$	220.35	-	AR	Fluka
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	236.15	-	AR	BDH
Copper chloride	$CuCl_2.2H_2O$	170.49	-	AR	Carlo Erba
Deionized water	H_2O	18.20	-	18.2 $M\Omega cm^{-1}$	-
Ethanol	C_2H_6O	46.07	0.789	AR	BDH
Ethylenediaminetetraacetic acid disodium salt	$[CH_2N(CH_2COOH)CH_2COONa]_2.2H_2O$	372.24	-	AR	Carlo Erba
Ferrous chloride	$FeCl_2$	126.751	-	AR	Fluka
Hydrochloric acid	HCl	36.46	1.18	AR	BDH
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid	$C_{14}H_{18}O_4$	250.29	-	AR	Fluka
Iron (III) chloride hexahydrate	$FeCl_3.6H_2O$	270.29	-	AR	BDH
Iron standard solution	Fe	55.84	-	AAS	Carlo Erba
Linoleic acid (ca. 99%)	$C_{18}H_{32}O_2$	280.44	-	AR	Fluka
Manganese chloride	$MnCl_2.4H_2O$	203.31	-	AR	Carlo Erba
Magnesium sulphate	$MgSO_4.7H_2O$	246.48	-	AR	BDH
Manganese standard solution	Mn	54.93	-	AAS	Carlo Erba

Table 3.1 The description of chemicals and reagents used in this work (cont.)

Name	Formula	Molar mass ($g\ mol^{-1}$)	Density ($g\ mL^{-1}$)	Grade	Company
Nitric acid	HNO_3	63.01	1.4	AR	Carlo Erba
Potassium nitrate	KNO_3	101.10	-	AR	Carlo Erba
Se-methylselenocysteine	$C_4H_9NO_2Se$	192.08	-	AR	Fluka
Selenomethionine	$C_5H_{11}NO_2Se$	196.11	-	AR	Acros organics
Selenocysteine	$C_6H_{12}N_2O_4Se_2$	334.09	-	AR	Acros organics
Sodium molybdate	$Na_2MoO_4.2H_2O$	241.95	-	AR	Carlo Erba
Sodium selenite	Na_2SeO_3	172.94	-	AR	Fluka
Zinc chloride	$ZnCl_2$	136.30	-	AR	Carlo Erba
Zinc standard solution	Zn	65.38	-	AAS	Carlo Erba

3.4 Instruments and apparatus

The inductively coupled plasma-mass spectrometer (Perkin-Elmer SCIEX, Norwalk, USA) with a double-pass Scott spray chamber fitted with a cross-flow nebulizer were used for the determination of Se, Cu and Mn in samples. The operating parameters of ICP-MS using for standard and DRC mode are shown in Table 3.2 and Table 3.3, respectively. The chromatographic system was consisted of a Dual pump KP-11 (Ogawa & Co., Ltd., Kobe, Japan), a syringe-loading injector (Model 9725i, Rheodyne six-port inject valve) with a 120 µL sample loop. The reversed phase column is Inertsil® C18 (Tokyo, Japan, 250 x 4.6 mm I.D., 5 µm particle size). The chromatographic system was connected to ICP-MS instrument with 300 mm of PEEK (polyether ether ketone) capillary tubing (0.25 mm I.D.). All types mobile phase were filtered through nylon membrane filters (0.45 µm, 47 mm, Whatman International Ltd., UK) and sample extracts were filtered through nylon syringe filter (0.45 µm, 13 mm I.D., Agela Technalogy, USA) prior to use. The flame atomic absorption spectrometer (FAAS) coupled with a 10 cm long slot-burner head, a lamp and an air-acetylene flame were used for Zn, Fe, Ca, Mg, Na and K determination. The operating parameters for AAS are shown in Table 3.4

Table 3.2 The operating parameters of ICP-MS using for standard mode

Parameters	Setting
Instrument	Elan DRC-e (Perkin–Elmer SCIEX. Norwalk, CT, USA)
Standard mode	External standard
Nebulizer gas flow	0.95 L min^{-1}
Auxilary gas flow	1.10 L min^{-1}
Plasma gas flow	17 L min^{-1}
Lens voltage	5 Volt
ICP RF power	1200 W
Pulse state voltage	1200 Volt
Interface	Ni cones
Mass analyzer	Quadrupole

Table 3.3 The operating parameters ICP-MS using for dynamic reaction cell mode

Parameters	Setting
Instrument	Elan DRC-e (Perkin–Elmer SCIEX. Norwalk, CT, USA)
Standard mode	External standard
Nebulizer gas flow	0.95 L min^{-1}
Auxilary gas flow	1.10 L min^{-1}
Plasma gas flow	15 L min^{-1}
Lens voltage	5 Volt
ICP RF power	1200 W
Pulse state voltage	800 Volt
Interface	Ni cones
Mass analyzer	Quadrupole
RPa	0.10
RPq	0.70
Cell gas A	0.60

Table 3.4 The operating parameters of flame atomic absorption spectrometer (FAAS) for determination of Zn, Fe, Ca, Mg, Na and K

Parameters	Zn	Fe	Ca	Mg	Na	K
Wavelength (nm)	219.3	248.3	422.7	285.2	589.0	766.5
HC lamp current (mA)	4	8	6	4	6	5
Slit width (nm)	0.5	0.2	0.5	0.5	0.5	0.5
Fuel gas flow rate	2	2	2	1.6	1.6	1.9
1% absorption concentration (mg/L)	0.02	0.1	0.08	0.007	0.02	0.04
Type of flame	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2	Air/C_2H_2

3.5 Experimental

The experimental of this work has been classified to 4 main parts as follows;

3.5.1 Selenium-enriched kale seedling cultivation with hydroponic system

The hydroponic system was used for cultivation of selenium-enriched kale seedling. The system consists of shelf, pot, air pump, sponge, fluorescent lamp and timer as shown in Figure 3.2

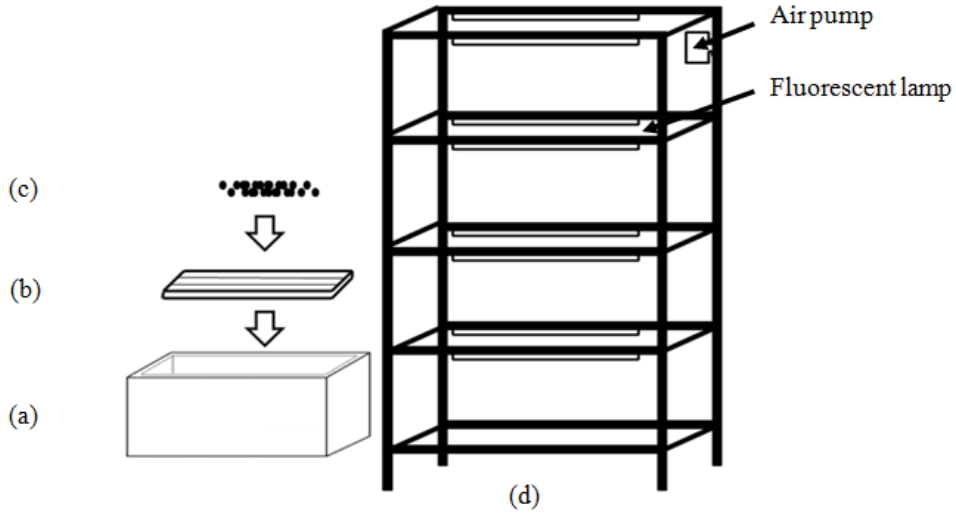

Figure 3.2 The schematic of hydroponic system; (a) plastic pot, (b) sponge, (c) kale seeds and (d) shelf

3.5.1.1 The effect of selenium supplemented concentration in nutrient solution

Kale seeds (*Brassica oleracea* var. alboglabra L.) were selected for selenium-enriched kale seedling production. The cultivation was begun by soaking the kale seeds in tap water about 15 hours before planting. After that, kale seeds were planted into sponges and watered daily with tap water. After 1 week, kale sprouts were attained optimal growth, selenium supplementation were started. The selenium in form of sodium selenite (Na_2SeO_3) was supplemented in 10 L of Hoagland's solution which was prepared as described in Table 3.5-3.7. Hoagland's solution were prepared with deionized water. The concentration of selenium in solution was varied as 0, 5, 10, 15, 30 and 45 mg Se L^{-1}. After 15 days of cultivation time, selenium-enriched kale seedling

were harvested and washed with deionized water to exclude contamination from the surface. Then, selenium-enriched kale seedling were dried at 70 ^{o}C in oven to a constant weight and grind. The dried selenium-enriched kale seedling were milled with grinder and stored at 4 ^{o}C before determination. The suitable selenium supplemented concentration was gave the highest selenium accumulation and good growth rate in kale seedling when compare with other selenium supplemented concentration.

Table 3.5 The preparation of Hoagland's solution (adapted) in 100 L

Chemical	Stock Solution (g)	Stock Solution (mL)
$MgSO_4.7H_2O$	49.30	-
$Ca(NO_3)_2.4H_2O$	118.60	-
KH_2PO_4	13.60	-
KNO_3	50.56	-
Micronutrient	Table 3.6	100
Fe.EDTA	Table 3.7	200

Table 3.6 The preparation of Micronutrient solution in 1 L

Chemical	Stock Solution (g)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnCl_2$	0.11
$Na_2MoO_4.2H_2O$	0.025
$CuCl_2.2H_2O$	0.05

Table 3.7 The preparation of Fe-EDTA solution in 1 L

Chemical	Stock Solution (g)
$FeCl_3.6H_2O$	13.5
EDTA	22.4

3.5.1.2 The effect of cultivation time

The cultivation time of selenium-enriched kale seedling were designed at 5, 10 and 15 days of cultivation time. The samples were harvested in 5, 10 and 15 days of cultivation time. After that, the selenium accumulation, selenium species, biotransformation and growth rate were studied. The cultivation time suitable was found the highest selenium accumulation, selenium species and growth rate when compare with other cultivation time.

3.5.1.3 The effect of sulfate ion to selenium-enriched kale seedling

The chemical properties of selenium are intermediate between those of sulfur (S) and tellurium (Te) and compounds resemble the corresponding sulfur and tellurium compounds in behavior (Reilly, 1996). Thus, the sulfur as sulfate ion (SO_4^{2-}) have been effected to the absorption of selenium in Hoagland's solution. In this work was assigned to use $MgCl_2$ replace $MgSO_4$ in Hoagland's solution to control the amounts of sulfur in solution. The concentration of $MgSO_4$ and $MgCl_2$ were fixed before selenium supplemented in Hoagland's solution. Then, the suitable concentration of selenium (30 mg Se L^{-1}) was supplemented in Hoagland solution. The harvesting of kale seedling were designed at 5, 10 and 15 days of cultivation time.

3.5.1.4 The effect of reuse of medium solution (Hoagland's solution)

The selenium supplemented concentration (30 mg Se L^{-1}) in Hoagland's solution was reused for cultivation of selenium-enriched kale seedling. In this work, cultivated kale seedling with the medium solution at 3 crops which each crops were cultivated for 15 days and harvested every 5 days of cultivation time. In addition, the dilution of Hoagland's solution was varied at ½ and ¼ formulas for compare the selenium absorption with the reuse of medium solution.

3.5.2 Sample preparation

3.5.2.1 Sample digestion

The selenium-enriched kale seedling sample were prepared by closed wet acid digestion method using home-made digestion apparatus (Thai Petty Patent No. 6432, 2011) (Figure 3.3). The accurate weight of 0.1 g of dry samples were placed into the digestion vessel. Then, 3 mL of 65% nitric acid was added (Sittipout, 2012). The mixture were heated in water bath for 40 minutes and cooled down at room temperature for 20 minutes. After that, the mixture were made up to 20 mL with deionized water in

PE bottle and filtered through a filter paper. Finally, the concentration of selenium and trace elements in each samples were determined with ICP-MS and AAS.

3.5.2.2 Sample extraction

The sample extraction is an important procedure for selenium speciation as well as HPLC-ICP-MS system. A suitable extraction method should not destroy selenium species in sample and give high extraction yield. Thus, the extraction with 0.1 M HCl in 10% v/v methanol for selenium speciation in selenium-enriched kale seedling sample was performed (Thosaikham, 2009). The accurate weight of 0.1 g of dry samples were placed into the digestion vessel and added 10 mL of 0.1 M HCl in 10% v/v methanol. Then, the mixture were mixed and sonicated in ultrasonic bath for 10 min. After that, incubated at 60 ^{0}C for 8 hours and centrifuged at 5000 rpm for 30 min. The supernatant of the mixture were transferred into polyethylene bottle and diluted with mobile phase solution. Finally, sample extracts were injected to the introduction system of ion-paired reversed phase HPLC-ICP-MS.

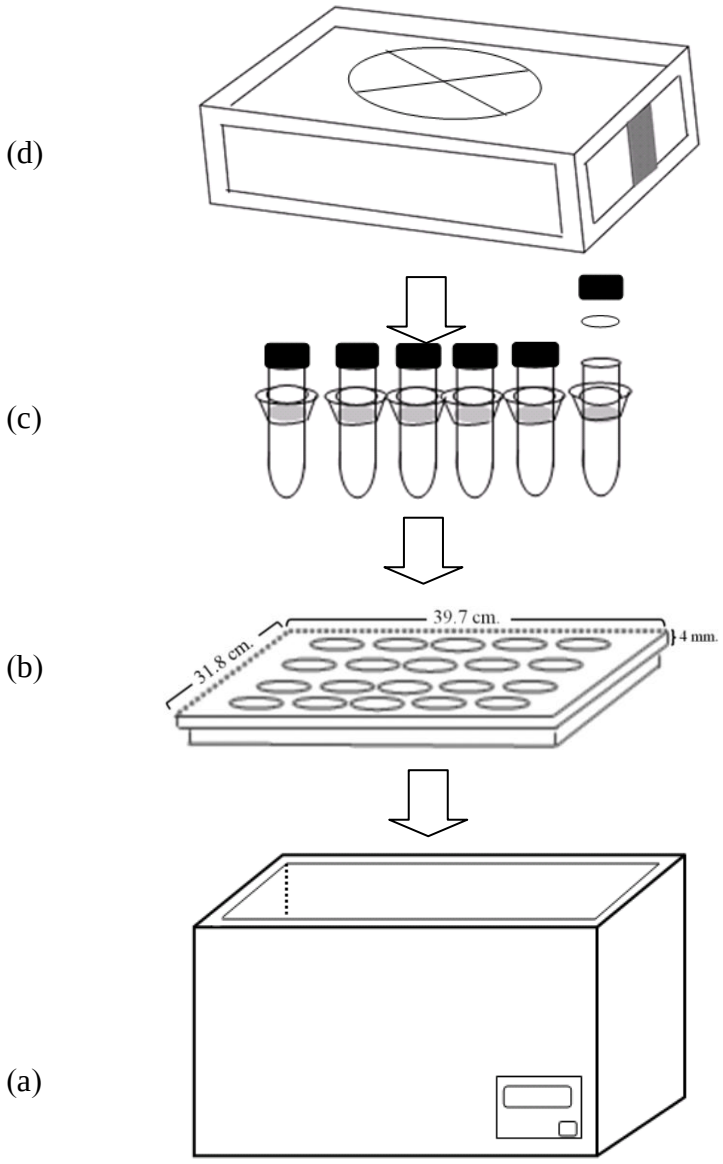

Figure 3.3 The schematic of home-made digestion apparatus comparison; (a) heating source, (b) water stream controller system, (c) sample and chemical container and (d) cooling system (Thai Petty Patent No. 6432, 2011)

3.5.3 The optimization of selenium-enriched kale seedling product storage conditions

The dried selenium-enriched kale seedling (30 mg Se L^{-1} for 15 days of cultivation time) powders were stored in the difference container material (polystyrene (PS) bottle, polyethylene (PE) bottle, polycarbonate (PC) bottle, Low-density polyethylene (LDPE) ziplock bag and aluminium foil bag) at difference temperature (4 ^{o}C and room temperature) and difference storage time (6, 12, 18 and 24 months). The stability and shelf life of selenium-enriched kale seedling were studied.

Furthermore, the dried sample powders were stored in desiccator with difference container material (polystyrene (PS) bottle, polyethylene (PE) bottle, polycarbonate (PC) bottle, Low-density polyethylene (LDPE) ziplock bag and aluminium foil bag) and difference storage time (6, 12, 18 and 24 months).

3.5.4 Antioxidant activity of selenium-enriched kale seedling

3.5.4.1 Preparation of selenium-enriched kale seedling extracts

The extraction procedure was adapted from Xu *et al*. (2004). The accurate weight of 1 g of dried sample was extracted with 30 mL of distilled water or 30 mL of 75% ethanol. The extractions were conducted in a water bath with a constant temperature of 60 ^{o}C for 3 h. Each sample was extracted three times with the same volume of solvents. The mixture was filtered and combined. Finally, the filtrate was evaporated to dryness in vacuum and kept frozen for antioxidative assays.

3.5.4.2 Determination of DPPH radical scavenging activity

The DPPH radical scavenging method was adapted from Xu *et al*. (2003). The antioxidant activities of selenium-enriched kale seedling extracts, BHA and Trolox were measured in terms of hydrogen donating or radical scavenging ability. Three mL of sample extracts, BHA and Trolox were placed in cuvettes. After that, 1 mL of a 2×10^{-4} mol L^{-1} ethanolic DPPH solution was added. The decrease in absorbance was determined at 517 nm and continuously measured for 10 min until the reaction reached steady state. The percentage of DPPH remaining at the steady state was calculated as a function of the molar ration of antioxidant to DPPH. The IC_{50} value, can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. All of the tests were performed in triplicate and the results were averaged.

3.5.4.3 Determination of antioxidant activity with the FTC method

The FTC method was adapted from Xu *et al*. (2004). Two mL of 1000 mg L^{-1} sample extracts and 2 mL of 200 mg L^{-1} antioxidants, 2 mL of 2.51% (w/v) linoleic acid in ethanol, 4 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.0) and 2 mL of distilled water were mixed in a 10 mL vial with a screw cap. Then, the mixture was kept in a 40 °C water bath in the dark. After that, 0.1 mL of the above mixture was added to the mixture of 9.7 mL of 75% (v/v) ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 mL of 0.02 mol L^{-1} ferrous chloride in 3.5% (v/v) hydrochloric acid was added to above mixture and then kept in a 40 °C water bath in the dark. The absorbance of the mixture was measured every 24 h at 500 nm until an unchangeable absorbance value arrived. All of the tests were performed in triplicate, and the results were averaged.

3.6 Data analysis

The chromatographic results were calculated using Tablecurve 2D software version 5.01 (Hearne Scientific Software Pty. Ltd. Melbourne, Victoria, Australia) for peak smoothing and Origin 8.1 software (OriginLab, Northampton, Massachusetts, USA) for chromatograph integration.

All experimental data were expressed as mean ± SD. The data of samples preparation part (fresh weight, dried weight, trace elements concentration, total selenium accumulation and selenium species) were analyzed for variance (ANOVA) and significant different among the means from triplicate analysis were set at $p < 0.05$ by using Duncan's new multiple range test. These were determined by General Linear Model multivariate range test using the statistical program for social science (SPSS, Chicago, IL, USA) Version 16.0 for windows.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Hydroponic system

The plant cultivation in soils has been achieved to produce selenium-enriched plant (Sugihara *et al*., 2004). Nevertheless, this method is an open system and selenium contaminated soils from the selenium-enriched production could be an environment pollution. Hence, the closed system is necessary to produce selenium-enriched plants. Hydroponic is an interesting method for selenium-enriched plant production by using mineral nutrient solutions in water or without soil. Terrestrial plants may be grown with their roots in the mineral nutrient solution only or in an inert medium such as perlite, gravel, mineral wool or coconut husk (Sugihara *et al*., 2004; Tsuneyoshi *et al*., 2006).

In this work, the hydroponic system apparatus was specially designed. It was constructed with suitable materials and consisted of 6 components; shelf, plastic pot, sponge, timer, fluorescent lamp and air pump (Figure 3.2). The plant seeds were holded in styrofoam and directly floated on the nutrient solution. The air pump was used for supplying the air in the nutrient solution which oxygen could be diffuse to the roots of plant.

Consequently, the cultivation of selenium-enriched plants in the hydroponic system was successfully applied for the producing of selenium-enriched kale seedling because of its easy controlled production.

4.2 The production of selenium-enriched kale seedling

4.2.1 Effect of selenium supplemented concentration and cultivation time

4.2.1.1 Total selenium in selenium-enriched kale seedling

Different cultivation periods and selenium concentrations supplemented in the medium solution affecting the growth rate of selenium-enriched kale seedlings were studied. Selenium-enriched kale seedlings cultivated in solutions containing 5-30 mg Se L^{-1} did not affect the growth rate of the kale seedlings (Table 4.1) while the level

of 45 mg Se L^{-1} in the solution inhibited the growth rate (Figure 4.1) and the abnormalities in the shape or color were apparent in the kale seedlings. A study by Lintschinger *et al*. (2006), demonstrate that the selenium-enriched plants found that the total selenium and germination decreased at high selenium concentrations or over dose. However, high enrichment is not restrained by any saturation phenomenon but limited by the lethal selenium concentration. In addition, when plants are exposed to high concentration of selenium in their root medium, they may exhibit symptoms of injury including stunting of growth, chlorosis, withering and drying of leaves, decreased protein synthesis and premature death of the plant (Terry *et al*., 2000).

The selenium uptake from different selenium concentrations in the solutions was determined by measuring the total selenium content in kale seedling samples. The total selenium content in dried samples with different selenium concentrations in the solution and cultivation period is presented in Table 4.2. The results indicated that the total selenium concentration in the kale seedlings increased with an increase in selenium concentration in the solution and the cultivation period. The highest total selenium concentration was found in kale seedlings cultivated for 15 days in a solution containing 30 mg Se L^{-1} (386.18±8.16 mg Se kg^{-1}). The selenium concentration in selenium-enriched kale seedling was similar to that in broccoli (Finley *et al*., 2000; Finley *et al*., 2001), but higher than that in carrot, parsley, barley, soybean, buck wheat (Sugihara *et al*., 2004; Chan *et al*., 2009) and malt (Jiakui and Xiaolong, 2004). However, the kale seedling supplemented with 45 mg Se L^{-1} showed that the total selenium decreased when compared with the solution supplemented with 30 mg Se L^{-1}. These results indicated that kale is tolerant to the presence of inorganic selenium up to 30 mg Se L^{-1} and when cultivation goes beyond 15 days the total selenium concentration also decreases.

Selenium concentrations in the nutrient medium were also studied by sampling the solutions every 5 days of the cultivation period. It was shown that the selenium content in Hoagland's solution decreased with an increase of supplemented selenium concentration and cultivation period. The greatest decrease of selenium in the solution was found in the selenium supplemented 30 mg Se L^{-1} (50.49 mg) for 15 days (Table 4.3). These results indicated that kale seedlings can uptake the selenium in Hoagland's solution and accumulate in the roots, stems and leaves. Consequently, the

optimum selenium-supplemented concentration was 30 mg Se L^{-1} for the 15 day of cultivation time.

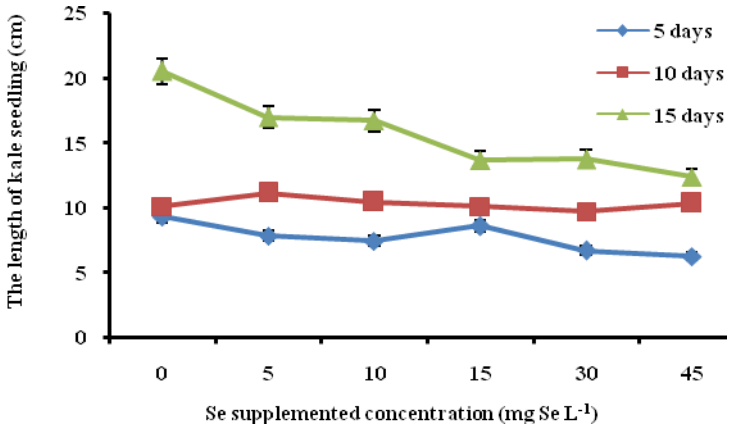

Figure 4.1 The length of kale seedling for different selenium supplemented concentrations and cultivation time

Table 4.1 Fresh weight and dried weight of kale seedling with different selenium concentrations and cultivation time

Se concentration in solution (mg Se L^{-1})	Samples (g ± sd)					
	Fresh weight			Dried weight		
	5 days	10 days	15 days	5 days	10 days	15 days
5	$120.69^{c, A}$ ± 2.12	$140.07^{b, C}$ ± 0.29	$186.92^{a, AB}$ ± 0.29	$5.69^{c, A}$ ± 0.72	$6.87^{b, B}$ ± 0.18	$9.49^{a, AB}$ ± 0.69
10	$118.32^{c, B}$ ± 0.65	$150.01^{b, B}$ ± 1.36	$184.28^{a, B}$ ± 0.05	$5.48^{c, A}$ ± 0.56	$7.97^{b, A}$ ± 0.46	$9.00^{a, B}$ ± 0.21
15	$84.27^{c, D}$ ± 0.87	$151.67^{b, B}$ ± 3.46	$184.28^{a, B}$ ± 3.08	$4.04^{c, B}$ ± 0.39	$7.86^{b, A}$ ± 0.64	$9.72^{a, AB}$ ± 0.32
30	$87.50^{c, C}$ ± 0.77	$156.65^{b, A}$ ± 1.59	$187.67^{a, A}$ ± 1.18	$4.46^{c, B}$ ± 0.15	$8.08^{b, A}$ ± 0.05	$9.90^{a, A}$ ± 0.10
45	$80.44^{c, E}$ ± 0.86	$116.67^{b, D}$ ± 0.34	$136.21^{a, C}$ ± 0.77	$4.25^{b, B}$ ± 0.30	$5.40^{a, C}$ ± 0.53	$6.17^{a, C}$ ± 0.52

* $^{a, b, c}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C, D, E}$ The difference was significant in column ($p < 0.05$)

Table 4.2 Total selenium contents in dried samples with different selenium concentrations and cultivation time

Se concentration in solution (mg Se L^{-1})	Se in dried samples (mg Se kg^{-1} ± sd)		
	5 days	10 days	15 days
5	$35.12^{b, D}$ ± 4.35	$38.28^{ab, E}$ ± 2.83	$44.87^{a, E}$ ± 5.61
10	$48.73^{c, C}$ ± 5.97	$63.15^{b, D}$ ± 1.86	$84.93^{a, D}$ ± 6.86
15	$61.77^{b, B}$ ± 6.36	$119.86^{a, C}$ ± 4.67	$113.40^{a, C}$ ± 3.74
30	$104.07^{c, A}$ ± 6.91	$338.29^{b, A}$ ± 9.55	$386.18^{a, A}$ ± 8.16
45	$102.32^{b, A}$ ± 1.69	$212.76^{a, B}$ ± 28.65	$207.11^{a, B}$ ± 11.12

* $^{a, b, c}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C, D, E}$ The difference was significant in column ($p < 0.05$)

Table 4.3 The selenium concentration decreasing in Hoagland's solution

Se-enriched solutions (mg Se L^{-1})	Se concentration (mg Se/10 L ± sd)		Decreasing amounts of Se (mg)
	Before cultivation	After cultivation	
0	n.d.	n.d.	n.d.
5	45.34 ± 2.83	33.41 ± 3.45	11.93
10	95.76 ± 5.15	66.39 ± 3.17	29.39
15	158.31 ± 1.63	139.19 ± 1.13	19.12
30	301.80 ± 8.99	251.31 ± 6.42	50.49
45	451.18 ± 13.89	412.22 ± 5.14	38.88

* n.d. = not detected

4.2.1.2 Selenium speciation in selenium-enriched kale seedling

The ion pairing reversed phase HPLC-ICP-MS was applied to study selenium distribution and accumulation in kale seedling. The chromatogram of the standard selenium species is shown in Figure 4.2-4.4 (a). The predominant form of selenium in selenium-enriched kale seedling are SeMet, SeMC, unknown 1 (U1) and unknown 2 (U2), respectively (Figure 4.2-4.4 (b-f)). Unknown 1 content in dried samples is shown in Table 4.6. Unknown 2 was detected in kale seedlings cultivated in the solution containing 15 and 30 mg Se L^{-1} for 15 days as 4.95 and 8.12 mg Se kg^{-1}, respectively. SeMet, SeMC and two types of unknown were not obtained in the control group. A study by Kahakachchi *et al.* (2004), demonstrated that the concentration of SeMet was higher than that of other organic selenium, and most of the inorganic selenium was transformed to organoselenium species. The unknown selenium compounds (U1 and U2) detected in kale seedling may be an intermediate in the pathway from SeC to SeMet. SeMet and SeMC were the major organic species in kale seedling cultivated for 5, 10 and 15 days (Tables 4.4-4.5). However, the selenium-enriched kale seedling cultivation period extending beyond 15 days found that the SeMet and SeMC decreased and may be transformed to the other organic selenium species. Furthermore, the inorganic selenium was not obtained in selenium-enriched kale seedling for the 15 day cultivation time.

Consequently, the ratios of each selenium species in selenium-enriched kale seedlings were calculated and are shown in Table 4.7. It shows that almost 80% of selenium in selenium-enriched kale seedlings were found in SeMC and SeMet forms. Moreover, most of the previous reports found that SeMC or SeMet was the primary selenium form in broccoli, garlic (Finley *et al*., 2001; Cai *et al*., 1995), Indian mustard (Yawata *et al*., 2010; Kahakachchi *et al,*. 2004), Chinese chives and several plant sprouts (Sugihara *et al*., 2004). The present results may be inconsistent with other results probably due to the different species of plants, selenium source and concentration, and cultivation conditions.

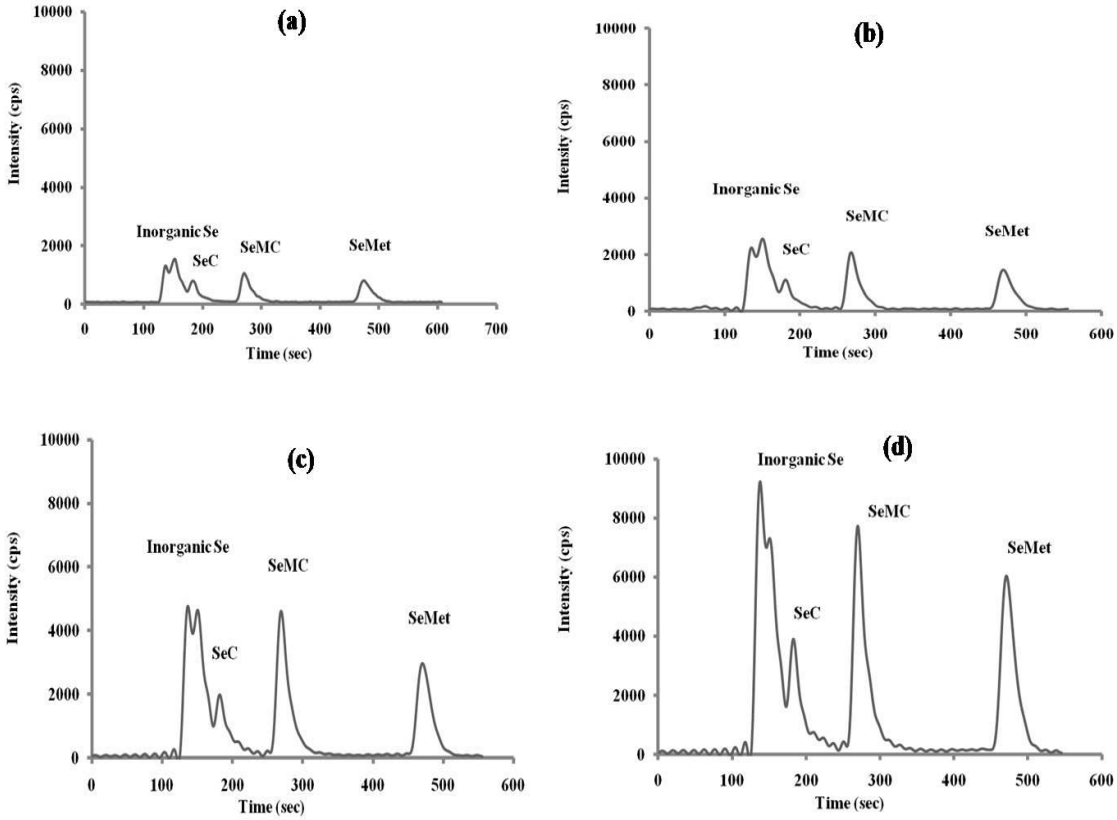

Figure 4.2 Chromatograms of standard selenium species; (a) 25 µg Se L^{-1} ; (b) 50 µg Se L^{-1} ; (c) 100 µg Se L^{-1} and (d) 200 µg Se L^{-1}

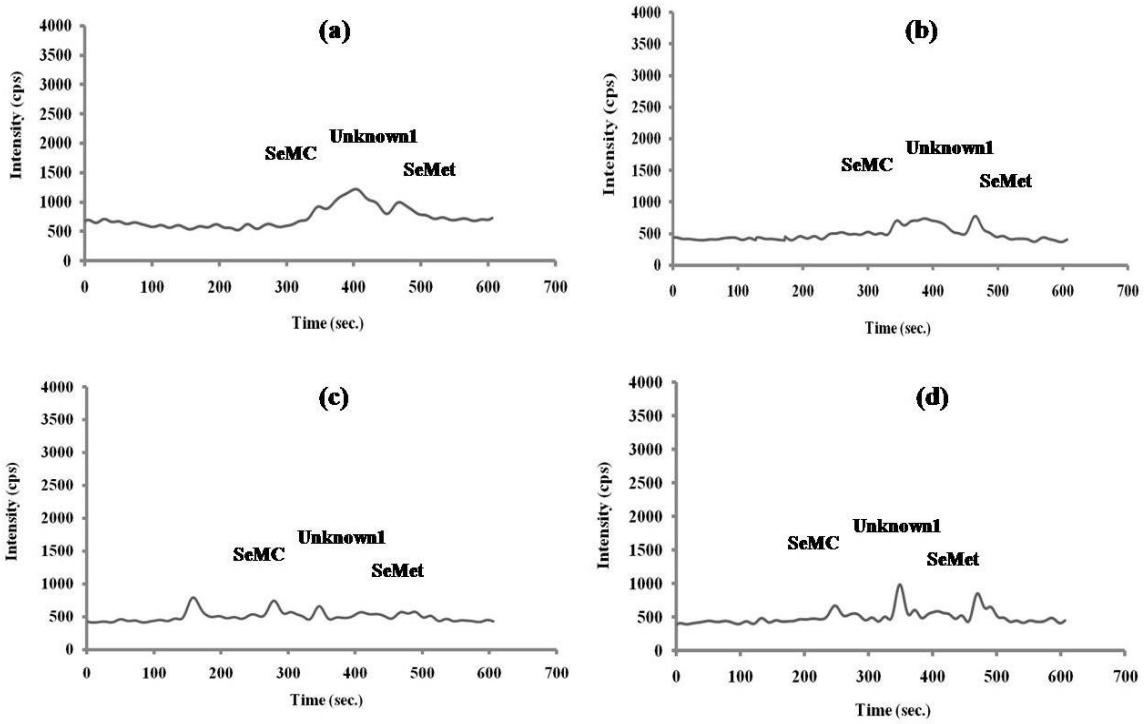

Figure 4.3 Chromatograms of selenium species in selenium-enriched kale seedling from different enrichment solutions for 5 days of cultivation time; (a) 5 mg Se L^{-1}, (b) 10 mg Se L^{-1}, (c) 15 mg Se L^{-1} and (d) 30 mg Se L^{-1}

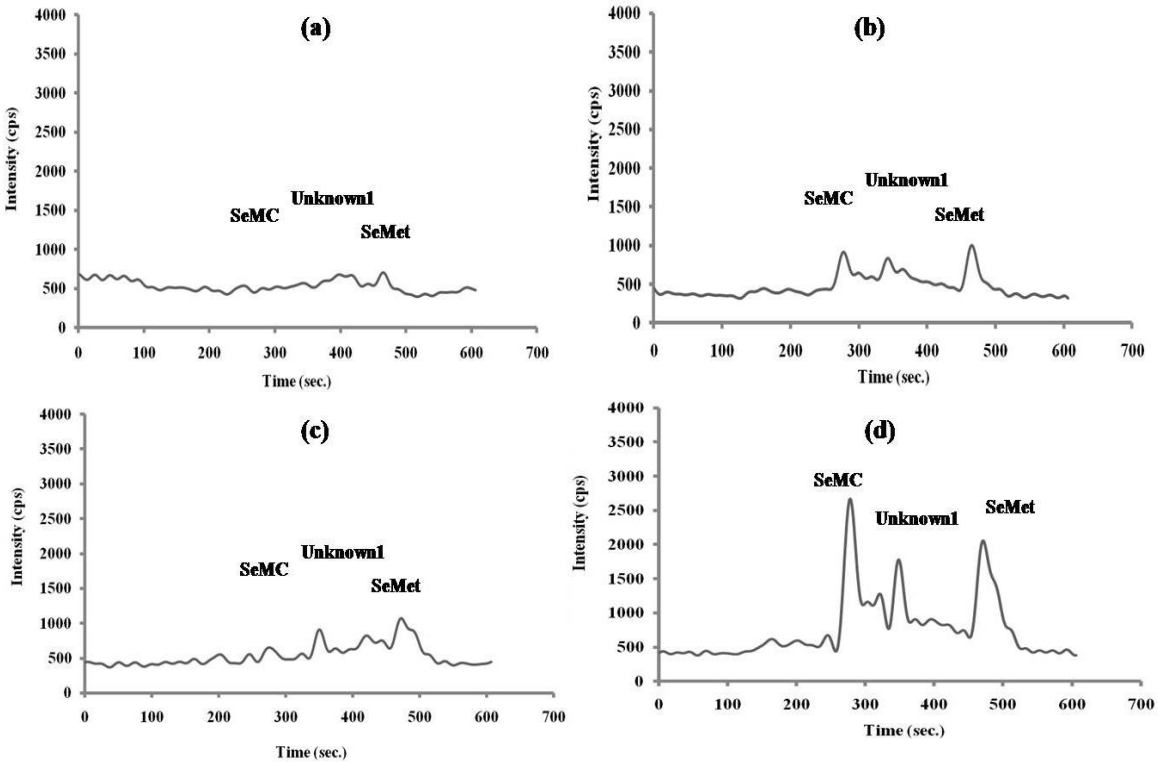

Figure 4.4 Chromatograms of selenium species in selenium-enriched kale seedling from different enrichment solutions for 10 days of cultivation time; (a) 5 mg Se L^{-1}, (b) 10 mg Se L^{-1}, (c) 15 mg Se L^{-1} and (d) 30 mg Se L^{-1}

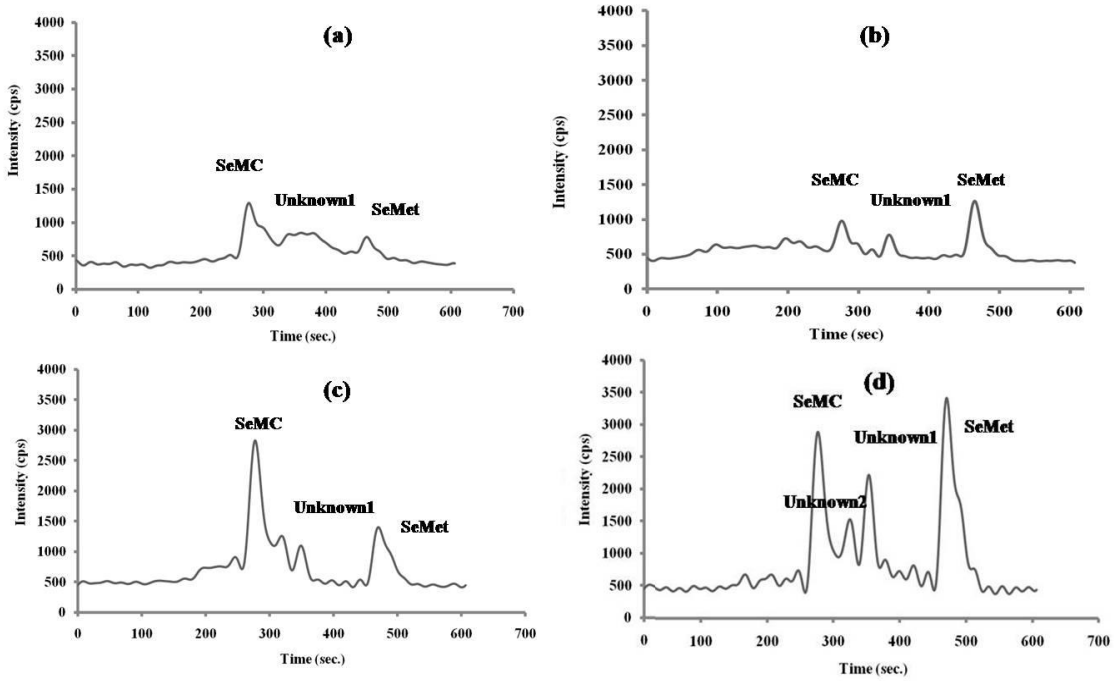

Figure 4.5 Chromatograms of selenium species in selenium-enriched kale seedling from different enrichment solutions for 15 days of cultivation time;
(a) 5 mg Se L^{-1}, (b) 10 mg Se L^{-1}, (c) 15 mg Se L^{-1} and (d) 30 mg Se L^{-1}

Table 4.4 Selenomethionine content in dried samples for different selenium concentrations and cultivation time

Se concentration in solution (mg Se L^{-1})	SeMet in dried samples (mg SeMet kg^{-1} ± sd)		
	5 days	10 days	15 days
5	$3.91^{a, A} \pm 0.34$	$2.17^{b, D} \pm 0.17$	$2.35^{b, D} \pm 0.38$
10	$2.40^{b, B} \pm 0.38$	$7.22^{a, C} \pm 0.35$	$7.39^{a, C} \pm 0.74$
15	$1.31^{c, C} \pm 0.20$	$4.95^{b, D} \pm 0.34$	$6.56^{a, CD} \pm 0.23$
30	$2.51^{c, B} \pm 0.17$	$29.51^{b, A} \pm 1.59$	$38.98^{a, A} \pm 5.65$
45	$2.80^{b, B} \pm 0.14$	$20.53^{a, B} \pm 2.18$	$17.34^{a, B} \pm 2.13$

$*^{a, b, c}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C, D}$ The difference was significant in column ($p < 0.05$)

Table 4.5 Se-methylselenocysteine content in dried samples for different selenium concentrations and cultivation time

Se concentration in solution (mg Se L^{-1})	SeMC in dried samples (mg SeMC kg^{-1} ± sd)		
	5 days	10 days	15 days
5	-	$0.90^{b, C} \pm 0.04$	$5.99^{a, C} \pm 0.09$
10	-	$3.65^{b, B} \pm 0.06$	$4.95^{a, C} \pm 0.11$
15	$1.47^{c, C} \pm 0.09$	$3.38^{b, B} \pm 0.14$	$5.34^{a, C} \pm 0.26$
30	$1.96^{c, B} \pm 0.07$	$17.86^{b, A} \pm 0.55$	$24.38^{a, A} \pm 2.16$
45	$1.27^{b, A} \pm 0.14$	$3.17^{b, B} \pm 0.18$	$13.70^{a, B} \pm 1.46$

$*^{a, b, c}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C,}$ The difference was significant in column ($p < 0.05$)

Table 4.6 Unknown 1 content in dried samples for different selenium concentrations and cultivation time

Se concentration in solution (mg Se L^{-1})	Unknown 1 in dried samples (mg U1 kg^{-1} ± sd)		
	5 days	10 days	15 days
5	$0.29^{b, C}$ ± 0.04	$1.14^{ab, E}$ ± 0.09	$1.83^{a, D}$ ± 0.84
10	$1.18^{b, BC}$ ± 0.39	$2.79^{a, D}$ ± 0.38	$3.14^{a, CD}$ ± 0.17
15	$1.84^{b, B}$ ± 0.32	$4.20^{a, C}$ ± 0.39	$4.38^{a, C}$ ± 0.62
30	$6.11^{c, A}$ ± 0.67	$12.04^{b, A}$ ± 0.78	$14.82^{a, A}$ ± 1.82
45	$5.16^{b, A}$ ± 0.87	$9.68^{a, B}$ ± 0.75	$9.66^{a, B}$ ± 0.61

∗ $^{a, b, c}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C, D, E}$ The difference was significant in column ($p < 0.05$)

Table 4.7 Total selenium concentration and ratio of selenium species in selenium-enriched kale seedling with selenium supplemented 30 mg L^{-1}

Items	
Total Se concentration (mg Se kg^{-1})	386.18
Ratio of Se species (% of total Se)	
Selenomethionine	41.56
Se-methyselenocysteine	34.70
Unknown 1	15.82
Unknown 2	7.91

4.2.1.3 Macronutrient and Micronutrient in selenium-enriched kale seedling

The macronutrient and micronutrient were essential for growth rate of kale seedling such as photosynthesis of plants, absorption of plants food and the disease resistance of plants. Determination of macronutrient (Ca, Mg, Na and K) and micronutrient (Cu, Zn, Mn and Fe) in selenium-enriched kale seedling were done by ICP-MS and FAAS technique. Cu and Mn were determined by ICP-MS with DRC mode and other elements were determined by FAAS technique.

The result showed that all elements concentrations were increased when compared between before and after cultivated excepted Zn (Table 4.8). The study by Kabata-Pendias (2011) and Chan *et al*. (2009), demonstrated that the high selenium concentrations inhibit the absorption of metals, mainly Zn and Cd. These relationships are dependent on the ratio between the elements and therefore stimulating effects of high selenium levels on uptake of some trace metals may sometimes be expected. The Zn decreasing have effected to growth rate of kale seedling. Because, Zn is required in a large number of enzymes and plays an essential role in DNA transcription. A typical symptom of Zn deficiency is the stunted growth of leaves. These cause found that the growth rate of selenium-enriched kale seedling was inhibited at high selenium concentration supplemented in nutrient solution.

Table 4.8 Trace elements concentration in selenium-enriched kale seedling

Elements	Elements concentration (mg kg^{-1} ± sd)	
	Before cultivation	After cultivation
Macronutrients		
Ca	26.12^{b} ± 1.59	46.00^{a} ± 3.43
P	18.96^{b} ± 1.13	59.45^{a} ± 0.93
Mg	9.04^{b} ± 0.30	10.48^{a} ± 0.26
Na	17.10^{b} ± 1.11	19.20^{a} ± 0.96
K	16.29^{b} ± 0.89	57.74^{a} ± 2.00
Micronutrients		
Cu	4.22 ± 0.52	4.20 ± 0.16
Zn	0.073^{a} ± 0.004	0.063^{b} ± 0.004
Mn	77.41^{b} ± 2.18	176.01^{a} ± 4.85
Fe	0.081^{b} ± 0.007	0.207^{a} ± 0.012

* $^{a, b}$ The difference was significant in row ($p < 0.05$)

4.2.2 The effect of sulfate ion in nutrient solution

Sulfur (S) is one of the six micronutrients required by plants and is found in the amino acids such as cysteine and methionine. When considers that sulfur in plants is only 3% to 5% as abundant as nitrogen. The transport of sulfur as sulfate ion (SO_4^{2-}) occurs across several membrane systems and distributed within cells of plant (Leustek and Saito ,1999). In this work, magnesium sulfate was the one of nutrient used in nutrient solution. It consists of magnesium and sulfur and both chemicals are micronutrients of plants. Thus, the effect of sulfate ion was studied by comparison the magnesium sulfate with magnesium chloride in nutrient solution.

4.2.2.1 The growth rate of kale seedling

The growth rate of kale seedling when supplemented with 30 mg Se L^{-1} in nutrient solution was studied. The nutrient solution was prepared base on two sets of mixed solution of magnesium sulfate and magnesium chloride. The magnesium salts were selected for the preparation of nutrient solution. The growth rate of kale seedling as average fresh weight per 1 tree of selenium-enriched kale seedling (n=20) with different corps and nutrient solutions are shown in Table 4.9. In Table 4.10 show the fresh weight, dried weight and percentage moisture of different corps and nutrient solutions. In Table 4.9 and Table 4.10 indicated that the growth rate of kale seedling in the solution of magnesium sulfate and magnesium chloride did not significant different at $p < 0.05$. In this work, selenium in form of sodium selenite was used for selenium-enriched kale seedling. The selenite absorption was not inhibited by sulfate ion in the nutrient solution. Whereas, the absorption of selenate was clearly inhibited by sulfate ion in the nutrient solution (Tsuneyoshi *et al*. 2006). Thus, in the solution of magnesium sulfate and magnesium chloride did not effect to growth rate of kale seedling.

Table 4.9 The average fresh weight per 1 tree of selenium-enriched kale seedling (n=20) with different solutions and crops

Crops	Cultivation time (day)	Average fresh weight (g/1 tree ± sd)	
		$MgSO_4$	$MgCl_2$
1	5	$0.1082^{a,E} \pm 0.0124$	$0.1059^{b,D} \pm 0.0151$
	10	$0.1327^{a,B} \pm 0.0133$	$0.1312^{a,B} \pm 0.0132$
	15	$0.1425^{b,A} \pm 0.0103$	$0.1452^{a,A} \pm 0.0163$
2	5	$0.1129^{a,D} \pm 0.0123$	$0.1069^{b,D} \pm 0.0171$
	10	$0.1267^{a,C} \pm 0.0159$	$0.1255^{a,C} \pm 0.0182$
	15	$0.1387^{a,AB} \pm 0.0165$	$0.1395^{a,B} \pm 0.0114$
3	5	$0.1088^{a,E} \pm 0.0174$	$0.1072^{a,D} \pm 0.0124$
	10	$0.1268^{a,C} \pm 0.0116$	$0.1257^{a,C} \pm 0.0192$
	15	$0.1343^{b,B} \pm 0.0122$	$0.1432^{a,A} \pm 0.0163$

$*^{a, b}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C, D, E}$ The difference was significant in column ($p < 0.05$)

Table 4.10 The fresh weight, dried weight and percentage moisture of selenium-enriched kale seedling which grown in $MgSO_4$ and $MgCl_2$ and different crops

Crops	Cultivation time (day)	Selenium-enriched kale seedling					
		$MgSO_4$			$MgCl_2$		
		Fresh weight (g ± sd)	Dried weight (g ± sd)	% moisture	Fresh weight (g ± sd)	Dried weight (g ± sd)	% moisture
1	5	51.14 ± 2.54	2.14 ± 0.09	95.82	52.94 ± 2.21	2.29 ± 0.15	95.67
	10	53.35 ± 2.73	2.23 ± 0.13	95.82	53.38 ± 2.63	2.45 ± 0.08	95.41
	15	54.19 ± 2.98	2.43 ± 0.12	95.52	54.53 ± 2.52	2.56 ± 0.13	95.31
2	5	49.35 ± 2.69	1.89 ± 0.14	96.17	50.99 ± 2.48	1.98 ± 0.12	96.12
	10	50.54 ± 2.93	1.96 ± 0.11	96.12	51.23 ± 2.62	2.16 ± 0.14	95.78
	15	51.87 ± 2.74	2.19 ± 0.12	95.78	53.78 ± 2.85	2.37 ± 0.09	95.59
3	5	49.89 ± 1.93	1.94 ± 0.16	96.06	50.27 ± 1.83	2.16 ± 0.17	95.70
	10	50.65 ± 2.89	2.19 ± 0.14	95.68	51.46 ± 2.11	2.21 ± 0.08	95.71
	15	52.12 ± 2.35	2.28 ± 0.08	95.63	53.23 ± 2.65	2.33 ± 0.18	95.62

4.2.2.2 Effect of sulfate ion to total selenium contents in selenium-enriched kale seedling supplemented with 30 mg Se L^{-1}

Selenium is intermediate between those of sulfur (S) and tellurium (Te) and compound resemble the corresponding sulfur and tellurium compounds in behavior (Reilly, 1996). Thus, the sulfur as sulfate ion has been affected to the absorption of selenium in nutrient solution. The selenium uptake in selenium-enriched kale seedling with different nutrient solution (solution of sulfate ion in form magnesium sulfate and no sulfate ion in form magnesium chloride) was determined by measuring the total selenium contents in kale seedling samples. The results indicated that sulfate ion did not affect to selenium (sodium selenite) absorption and total selenium contents in kale seedling samples. Furthermore, a study by Tsuneyoshi *et al*. (2006), demonstrated that selenite uptake was not inhibited by sulfate ion in the nutrient solution. Whereas, the absorption of selenate was clearly inhibited by sulfate ion in the nutrient solution. Thus, the magnesium sulfate can be used to prepare the nutrient solution for selenium-enriched kale seedling.

4.2.3 The reuse of medium solution

The cultivation of kale seedling with the medium solution supplemented with 30 mg Se L^{-1} at 3 crops which each crops were cultivated for 15 days and harvested every 5 days of cultivation time was studied. The total selenium, macronutrient and micronutrient contents in Hoagland's (medium) solution for 15 days of cultivation time with different crops are shown in Table 4.11. In Table 4.11 found that the total selenium, macronutrient and micronutrient contents in Hoagland's (medium) solution decreased when compared with each crops. Whereas, the total selenium, macronutrient and micronutrient contents in dried samples for 15 days of cultivation time decreased when compared with each crops (Table 4.12) and the decreasing of elements showed almost the same patterns of Hoagland's (medium) solution. These results indicated that the reuse of medium solution found the essential elements for the plants growth decreased and inappropriate for selenium-enriched kale seedling cultivation.

Table 4.11 Total selenium, macronutrient and micronutrient contents in Hoagland's solution for 15 days of cultivation time with different crops

Elements	Elements concentration (mg L^{-1} ± sd)		
	Crop 1	Crop 2	Crop 3
Se	22.43 ± 1.51	18.02 ± 1.29	12.54 ± 1.14
Mn	0.51 ± 0.03	0.45 ± 0.03	0.38 ± 0.02
Cu	0.39 ± 0.03	0.29 ± 0.03	0.21 ± 0.02
Fe	2.23 ± 0.04	1.95 ± 0.06	1.31 ± 0.12
Zn	0.15 ± 0.02	0.14 ± 0.01	0.12 ± 0.01
Ca	444.52 ± 19.64	310.39 ± 7.36	224.98 ± 13.00
Mg	90.50 ± 5.19	62.77 ± 1.64	36.51 ± 2.26
Na	179.67 ± 8.78	147.57 ± 8.95	124.59 ± 12.35
K	173.33 ± 11.41	155.50 ± 9.57	122.64 ± 11.99
P	63.14 ± 3.03	55.84 ± 2.21	47.04 ± 4.38

Table 4.12 Total selenium, macronutrient and micronutrient contents in dried selenium-enriched kale seedling for 15 days of cultivation time with different crops

Elements	Elements concentration (mg kg^{-1} ± sd)		
	Crop 1	Crop 2	Crop 3
Se	668.96 ± 10.98	576.51 ± 38.70	491.28 ± 21.65
Mn	89.34 ± 4.43	76.45 ± 4.57	61.67 ± 6.09
Cu	145.65 ± 5.33	127.87 ± 3.68	108.73 ± 10.26
Fe	148.00 ± 4.90	140.34 ± 7.41	117.59 ± 5.11
Zn	262.51 ± 6.03	220.68 ± 3.35	175.98 ± 5.55
Ca	35.12 ± 1.40	28.94 ± 9.84	25.50 ± 16.10
Mg	18.31 ± 0.97	15.03 ± 1.12	11.93 ± 0.90
Na	41.51 ± 1.09	34.34 ± 1.60	30.24 ± 0.60
K	47.85 ± 0.99	39.42 ± 0.88	29.15 ± 2.11
P	6.69 ± 0.38	5.59 ± 0.19	4.77 ± 0.35

4.3 The stability and shelf life of selenium-enriched kale seedling in different container materials

The stability and shelf life of selenium-enriched kale seedling were studied. Dried selenium-enriched kale seedling (supplemented with 30 mg Se L^{-1} for 15 days of cultivation time) powders were used. The sample powders were stored in different container materials (polystyrene (PS) bottle, polyethylene (PE) bottle, polycarbonate (PC) bottle, Low-density polyethylene (LDPE) ziplock bag and aluminium foil bag), temperature (4 ^{o}C and room temperature), storage time (6, 12, 18 and 24 months) and in desiccator.

The effect of the container material at different storage temperature and times was evaluated. The loss of selenium amounts are illustrated in Figure 4.5. The results indicated that the PE and PC bottle induced severe losses of the total selenium and organic selenium after the storage time only 6 months. Whereas, PS bottle, LDPE ziplock bag and aluminium foil bag found the amounts of organic selenium higher than PE and PC bottle (Table 4.13-4.21). These results can be related to the possible adsorption of organic selenium on the container walls. This behavior is only apparent in the polyethylene containers (Olivas *et al*., 1998).

The effect of temperature on the stability and shelf life of selenium-enriched kale seedling was studied. Table 4.13-4.21 shown the amounts of selenium species and unknown with different temperature and storage in desiccator. The results indicated that at temperature 4 °C found the loss of organic selenium and unknown lower than room temperature and in desiccator which decreased regularly when increased the storage time. A study by Olivas *et al*. (1998), indicated that the samples kept frozen at -20°C in the polyethylene vials displayed a loss of organic selenium similarly to the other temperature conditions (4°C and 20°C) along with decrease of selenoamino acid. Furthermore, a study by Hwang *et al*. (2012), demonstrated that the results of refrigeration at 4°C can help preserve the nutritive value of Korean cabbage (*Brassica campestris* L. ssp. *pekinensis*) by maintaining high levels of glucosinolates and other bioactive compounds.

Finally, the container material, temperature and storage time are the most influential parameter. The results indicated that the different storage times, container

materials, temperature and in desiccator affected to the total selenium, stability and shelf life of organic selenium species decreased. It shows that the total selenium decreased when increased the storage time (Table 4.22-4.24), at storage time for 6 months found the total selenium decreased lesser than other storage times. The percentage decreasing of total selenium with difference container materials, temperature and storage time shown in Table 4.25-4.27. The percentage decreasing of total selenium increased when increased the storage time. These results relationship with organic selenium species decreased in dried sample. A study by Olivas *et al*. (1998), demonstrated that the rapid loss of SeC, SeMet and $TMSe^{+}$ was observed under any conditions in polyethylene vials at high temperature. Thus, the optimum condition for the stability and shelf life of selenium-enriched kale seedling were obtained with PS bottle, LDPE ziplock bag and aluminium foil bag at 4°C for 6 months.

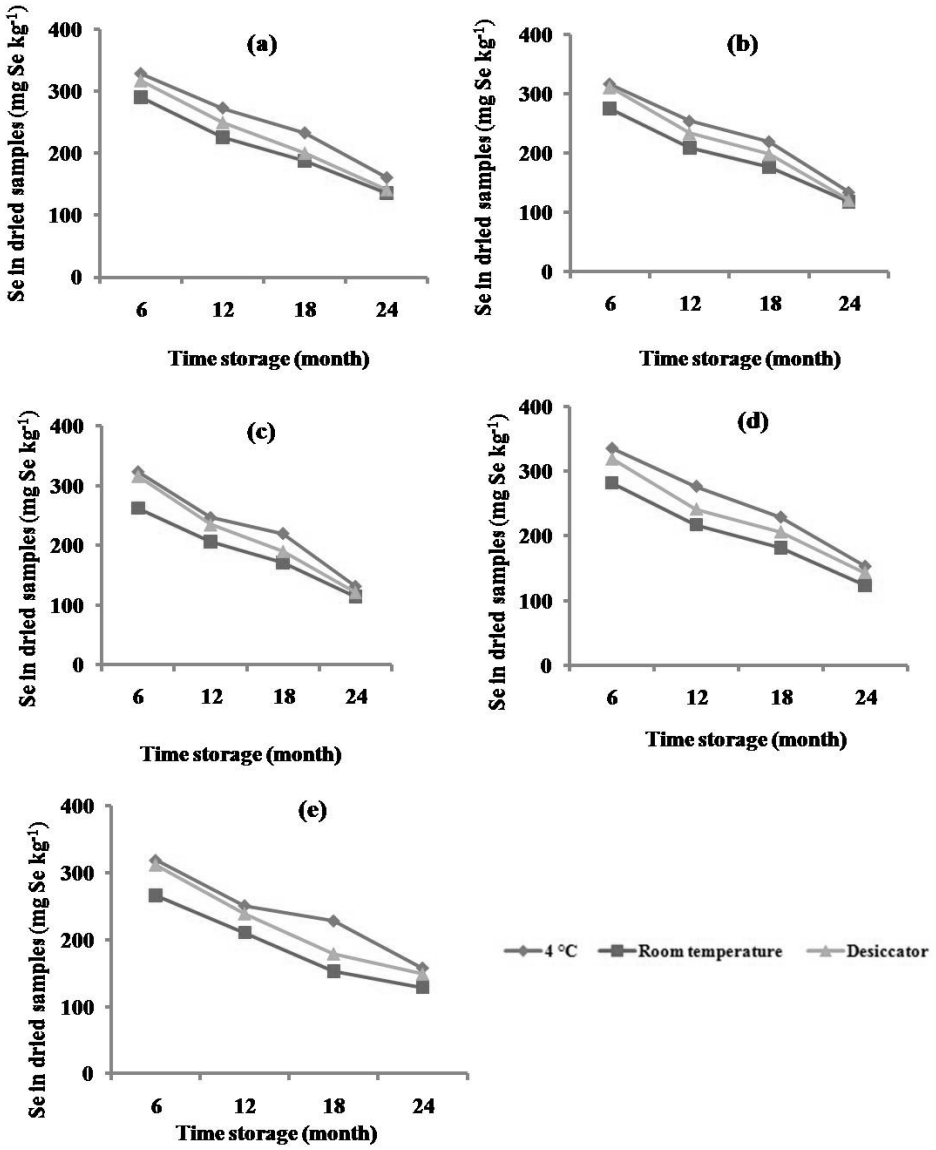

Figure 4.6 Total selenium decreasing in dried samples with different container materials and times storage; (a) PS bottle, (b) PE bottle, (c) PC bottle, (d) LDPE ziplock bag and (e) Aluminium foil bag

Table 4.13 Selenomethionine content in dried samples with different container materials and times storage at 4 ^{o}C

Materials	SeMet in dried samples (mg SeMet kg $^{-1}$ ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$25.69^{a,AB}$ ± 0.12	$20.69^{b,B}$ ± 0.72	$16.07^{c,B}$ ± 0.29	$8.87^{d,AB}$ ± 0.18
PE bottle	$24.32^{a,C}$ ± 0.65	$19.48^{b,C}$ ± 0.56	$15.01^{c,C}$ ± 1.36	$7.97^{d,B}$ ± 0.46
PC bottle	$23.27^{a,D}$ ± 0.87	$18.04^{b,D}$ ± 0.39	$15.67^{c,BC}$ ± 3.46	$7.86^{d,B}$ ± 0.64
LDPE ziplock bag	$25.50^{a,B}$ ± 0.77	$20.46^{b,B}$ ± 0.15	$15.65^{c,BC}$ ± 1.59	$8.08^{d,B}$ ± 0.05
Aluminium foil bag	$26.44^{a,A}$ ± 0.86	$21.25^{b,A}$ ± 0.30	$17.67^{c,A}$ ± 0.34	$9.40^{d,A}$ ± 0.53

* $^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.14 Selenomethionine content in dried samples with different container materials and times storage at room temperature

Materials	SeMet in dried samples (mg SeMet kg $^{-1}$ ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$22.09^{a,AB}$ ± 0.32	$17.19^{b,B}$ ± 0.2	$13.34^{c,A}$ ± 0.22	$5.82^{d,A}$ ± 0.45
PE bottle	$21.12^{a,B}$ ± 0.35	$15.40^{b,C}$ ± 0.56	$11.01^{c,B}$ ± 1.36	$4.97^{d,B}$ ± 0.96
PC bottle	$21.27^{a,B}$ ± 0.87	$15.04^{b,C}$ ± 0.39	$10.23^{c,C}$ ± 1.16	$4.86^{d,B}$ ± 0.44
LDPE ziplock bag	$21.50^{a,B}$ ± 0.17	$14.16^{b,D}$ ± 0.19	$10.65^{c,C}$ ± 1.09	$5.08^{d,AB}$ ± 0.15
Aluminium foil bag	$23.44^{a,A}$ ± 0.86	$18.25^{b,A}$ ± 0.41	$11.21^{c,B}$ ± 0.14	$5.40^{d,AB}$ ± 0.13

* $^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.15 Selenomethionine content in dried samples with different container materials and times storage in desiccator

Materials	SeMet in dried samples (mg SeMet kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$23.32^{a,AB}$ ± 0.18	$19.69^{b,A}$ ± 0.52	$15.27^{c,B}$ ± 0.21	$6.57^{d,B}$ ± 0.23
PE bottle	$22.32^{a,B}$ ± 0.35	$17.08^{b,B}$ ± 0.43	$13.65^{c,D}$ ± 1.06	$5.56^{d,C}$ ± 0.51
PC bottle	$22.57^{a,B}$ ± 0.18	$16.04^{b,C}$ ± 0.19	$14.67^{c,C}$ ± 1.16	$5.36^{d,BC}$ ± 0.39
LDPE ziplock bag	$22.46^{a,B}$ ± 0.38	$17.56^{b,B}$ ± 0.35	$15.65^{c,B}$ ± 0.34	$7.08^{d,AB}$ ± 0.28
Aluminium foil bag	$23.64^{a,A}$ ± 0.36	$19.25^{b,A}$ ± 1.30	$16.67^{c,A}$ ± 0.18	$7.36^{d,A}$ ± 0.87

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.16 Se-methylselenocysteine content in dried samples with different container materials and time storage at 4 ^{o}C

Materials	SeMC in dried samples (mg SeMC kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$16.32^{a,B}$ ± 0.52	$14.28^{b,A}$ ± 0.52	$10.07^{c,A}$ ± 0.29	$4.14^{d,A}$ 0.56
PE bottle	$15.51^{a,C}$ ± 0.35	$14.26^{a,A}$ ± 0.64	$8.11^{b,B}$ ± 0.73	$2.19^{c,C}$ ± 0.35
PC bottle	$14.17^{a,D}$ ± 0.42	$13.04^{a,B}$ ± 0.79	$7.67^{b,B}$ ± 0.46	$2.86^{c,BC}$ ± 0.84
LDPE ziplock bag	$17.50^{a,A}$ ± 0.39	$14.16^{b,A}$ ± 0.37	$9.67^{c,A}$ ± 0.61	$3.05^{d,B}$ ± 0.63
Aluminium foil bag	$17.41^{a,A}$ ± 0.56	$13.68^{b,AB}$ ± 0.27	$9.27^{c,A}$ ± 0.41	$4.40^{d,A}$ ± 0.33

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.17 Se-methylselenocysteine content in dried samples with different container materials and time storage at room temperature

Materials	SeMC in dried samples (mg SeMC kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$11.12^{a,A}$ ± 0.34	$8.41^{b,A}$ ± 0.12	$5.11^{c,A}$ ± 0.19	$3.10^{d,A}$ ± 0.16
PE bottle	$10.08^{a,B}$ ± 0.76	$6.11^{b,B}$ ± 0.32	$4.10^{c,B}$ ± 0.13	$1.26^{d,C}$ ± 0.18
PC bottle	$9.26^{a,C}$ ± 0.41	$6.04^{b,B}$ ± 0.21	$3.67^{c,B}$ ± 0.32	$1.87^{d,BC}$ ± 0.54
LDPE ziplock bag	$10.38^{a,AB}$ ± 0.35	$7.81^{b,A}$ ± 0.22	$3.97^{c,B}$ ± 0.53	$2.11^{d,B}$ ± 0.42
Aluminium foil bag	$11.37^{a,A}$ ± 0.32	$7.61^{b,A}$ ± 0.24	$4.27^{c,AB}$ ± 0.21	$2.42^{d,AB}$ ± 0.21

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.18 Se-methylselenocysteine content in dried samples with different container materials and time storage in desiccator

Materials	SeMC in dried samples (mg SeMC kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$12.41^{a,A}$ ± 0.22	$10.52^{b,A}$ ± 33	$7.12^{c,A}$ ± 0.15	$4.11^{d,A}$ ± 0.23
PE bottle	$11.08^{a,B}$ ± 0.43	$8.19^{b,B}$ ± 0.53	$5.10^{c,B}$ ± 0.43	$2.08^{d,C}$ ± 0.28
PC bottle	$9.53^{a,C}$ ± 0.32	$8.08^{a,B}$ ± 0.51	$4.65^{b,B}$ ± 0.41	$2.01^{c,C}$ ± 0.14
LDPE ziplock bag	$11.62^{a,AB}$ ± 0.31	$9.72^{b,A}$ ± 43	$6.53^{c,A}$ ± 0.13	$2.86^{d,BC}$ ± 0.18
Aluminium foil bag	$12.57^{a,A}$ ± 0.51	$9.87^{b,A}$ ± 0.32	$6.37^{c,A}$ ± 0.36	$3.27^{d,B}$ ± 0.11

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.19 Unknown1 content in dried samples with different container materials and time storage at 4 ^{o}C

Materials	Unknown1 in dried samples (mg kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$10.19^{a,A}$ ± 0.52	$8.59^{b,A}$ ± 0.31	$5.17^{c,A}$ ± 0.31	$2.77^{d,A}$ ± 0.15
PE bottle	$9.41^{a,AB}$ ± 0.53	$6.40^{b,C}$ ± 0.42	$3.86^{c,B}$ ± 0.28	$1.97^{d,B}$ ± 0.46
PC bottle	$8.68^{a,B}$ ± 0.51	$6.04^{b,C}$ ± 0.21	$2.97^{c,C}$ ± 0.16	$1.80^{d,B}$ ± 0.36
LDPE ziplock bag	$9.85^{a,A}$ ± 0.34	$7.53^{b,B}$ ± 0.23	$4.35^{c,B}$ ± 0.09	$2.18^{d,AB}$ ± 0.14
Aluminium foil bag	$10.42^{a,A}$ ± 0.51	$8.20^{b,AB}$ ± 0.26	$5.07^{c,A}$ ± 0.14	$2.32^{d,A}$ ± 0.13

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.20 Unknown1 content in dried samples with different container materials and time storage at room temperature

Materials	Unknown1 in dried samples (mg kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	$8.31^{a,A}$ ± 0.12	$6.59^{b,A}$ ± 0.24	$5.32^{c,A}$ ± 0.11	$2.03^{d,A}$ ± 0.20
PE bottle	$7.32^{a,B}$ ± 0.32	$4.86^{b,BC}$ ± 0.12	$3.06^{c,C}$ ± 0.18	$1.23^{d,B}$ ± 0.06
PC bottle	$6.75^{a,C}$ ± 0.30	$4.24^{b,C}$ ± 0.13	$2.66^{c,C}$ ± 0.12	$1.10^{d,B}$ ± 0.11
LDPE ziplock bag	$7.93^{a,AB}$ ± 0.26	$5.34^{b,B}$ ± 0.21	$4.05^{c,B}$ ± 0.13	$1.67^{d,AB}$ ± 0.13
Aluminium foil bag	$8.35^{a,A}$ ± 0.20	$5.20^{b,B}$ ± 0.30	$3.87^{c,B}$ ± 0.06	$1.92^{d,A}$ ± 0.15

$*^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.21 Unknown1 content in dried samples with different container materials and time storage in desiccator

Materials	Unknown1 in dried samples (mg kg^{-1} ± sd)			
	Times (month)			
	6	12	18	24
PS bottle	9.52 ^{a,A} ± 0.31	6.76 ^{b,A} ± 0.31	5.46 ^{b,A} ± 0.13	3.43 ^{c,A} ± 0.18
PE bottle	7.67 ^{a,C} ± 0.12	5.34 ^{b,B} ± 0.32	3.74 ^{c,C} ± 0.27	1.87 ^{d,C} ± 0.05
PC bottle	7.66 ^{a,C} ± 0.26	4.65 ^{b,C} ± 0.21	2.86 ^{c,D} ± 0.31	1.79 ^{d,C} ± 0.11
LDPE ziplock bag	8.53 ^{a,B} ± 0.31	5.04 ^{b,B} ± 0.11	4.55 ^{b,B} ± 0.23	2.37 ^{c,B} ± 0.09
Aluminium foil bag	9.42 ^{a,A} ± 0.25	5.43 ^{b,B} ± 0.17	4.27 ^{c,BC} ± 0.18	1.95 ^{d,BC} ± 0.21

∗ $^{a, b, c, d}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.22 Total selenium contents in dried samples with different container materials and times storage at 4 ^{o}C

Materials	Se in dried samples (mg Se kg $^{-1}$ ± sd)				
	Times (month)				
	0	6	12	18	24
PS bottle	$386.18^{a,A}$ ± 8.16	$328.32^{b,AB}$ ± 7.07	$272.93^{c,A}$ ± 7.69	$232.78^{d,A}$ ± 13.83	$160.51^{e,A}$ ± 5.58
PE bottle	$386.18^{a,A}$ ± 8.16	$316.22^{b,B}$ ± 1.87	$253.37^{c,B}$ ± 13.17	$218.69^{d,B}$ ± 20.58	$133.21^{e,B}$ ± 5.60
PC bottle	$386.18^{a,A}$ ± 8.16	$323.42^{b,B}$ ± 9.10	$246.58^{c,B}$ ± 9.42	$219.78^{d,B}$ ± 14.61	$131.22^{e,B}$ ± 4.61
LDPE ziplock bag	$386.18^{a,A}$ ± 8.16	$334.68^{b,A}$ ± 16.14	$276.35^{c,A}$ ± 21.25	$229.00^{d,A}$ ± 7.10	$153.36^{e,A}$ ± 8.76
Aluminium foil bag	$386.18^{a,A}$ ± 8.16	$318.60^{b,B}$ ± 14.32	$250.54^{c,B}$ ± 7.58	$227.74^{d,A}$ ± 15.31	$157.14^{e,A}$ ± 1.06

$*^{a, b, c, d, e}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.23 Total selenium contents in dried samples with different container materials and time storage at room temperature

Materials	Se in dried samples (mg Se kg^{-1} ± sd)				
	Times (month)				
	0	6	12	18	24
PS bottle	$386.18^{a,A}$ ± 8.16	$290.55^{b,A}$ ± 19.73	$225.61^{c,A}$ ± 12.63	$187.86^{d,A}$ ± 15.36	$135.47^{e,A}$ ± 6.81
PE bottle	$386.18^{a,A}$ ± 8.16	$274.53^{b,AB}$ ± 2.57	$208.76^{c,C}$ ± 2.19	$176.50^{d,AB}$ ± 10.88	$117.53^{e,BC}$ ± 1.24
PC bottle	$386.18^{a,A}$ ± 8.16	$262.27^{b,C}$ ± 17.45	$205.76^{c,C}$ ± 4.95	$170.84^{d,B}$ ± 2.44	$114.13^{e,C}$ ± 1.04
LDPE ziplock bag	$386.18^{a,A}$ ± 8.16	$281.76^{b,A}$ ± 13.54	$216.93^{c,B}$ ± 5.82	$181.14^{d,A}$ ± 7.63	$123.06^{e,B}$ ± 17.99
Aluminium foil bag	$386.18^{a,A}$ ± 8.16	$266.03^{b,C}$ ± 2.05	$210.37^{c,BC}$ ± 1.24	$152.37^{d,C}$ ± 8.81	$128.56^{e,AB}$ ± 5.36

$*^{a, b, c, d, e}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.24 Total selenium contents in dried samples with different container materials and times storage in desiccator

Materials	Se in dried samples (mg Se kg^{-1} ± sd)				
	Times (month)				
	0	6	12	18	24
PS bottle	$386.18^{a,A}$ ± 8.16	$317.05^{b,A}$ ± 13.80	$249.80^{c,A}$ ± 1.39	$200.39^{d,AB}$ ± 2.79	$141.11^{e,A}$ ± 11.25
PE bottle	$386.18^{a,A}$ ± 8.16	$310.56^{b,A}$ ± 23.90	$232.91^{c,B}$ ± 6.99	$197.70^{d,B}$ ± 8.35	$120.11^{e,B}$ ± 12.12
PC bottle	$386.18^{a,A}$ ± 8.16	$316.03^{b,A}$ ± 6.24	$234.79^{c,B}$ ± 11.13	$190.24^{d,B}$ ± 20.65	$121.57^{e,B}$ ± 1.24
LDPE ziplock bag	$386.18^{a,A}$ ± 8.16	$319.22^{b,A}$ ± 14.79	$241.35^{c,AB}$ ± 15.09	$206.16^{d,A}$ ± 19.87	$143.10^{e,A}$ ± 10.86
Aluminium foil bag	$386.18^{a,A}$ ± 8.16	$311.17^{b,A}$ ± 11.38	$238.55^{c,B}$ ± 11.94	$178.94^{d,C}$ ± 12.27	$148.62^{e,A}$ ± 10.99

$*^{a, b, c, d, e}$ The difference was significant in row ($p < 0.05$)

$^{A, B, C}$ The difference was significant in column ($p < 0.05$)

Table 4.25 Percentage decreasing of total selenium in dried samples with different container materials and time storage at 4 ^{o}C

Materials	% Decreasing			
	Times (month)			
	6	12	18	24
PS bottle	14.92	29.33	39.72	58.44
PE bottle	18.12	34.39	43.34	65.51
PC bottle	16.25	36.15	43.09	66.02
LDPE ziplock bag	13.34	28.44	40.70	60.29
Aluminium foil bag	17.50	35.12	41.03	59.31

Table 4.26 Percentage decreasing of total selenium in dried samples with different container materials and time storage at room temperature

Materials	% Decreasing			
	Times (month)			
	6	12	18	24
PS bottle	24.76	41.58	51.35	64.92
PE bottle	28.91	45.94	54.30	69.57
PC bottle	32.09	46.72	55.76	70.45
LDPE ziplock bag	27.04	43.83	53.09	68.14
Aluminium foil bag	31.11	45.53	60.54	66.71

Table 4.27 Percentage decreasing of total selenium in dried samples with different container materials and time storage in desiccator

Materials	% Decreasing			
	Times (month)			
	6	12	18	24
PS bottle	17.90	35.32	48.11	63.46
PE bottle	19.58	39.69	48.81	68.90
PC bottle	18.16	39.20	50.74	68.52
LDPE ziplock bag	17.34	37.50	46.62	62.94
Aluminium foil bag	19.42	38.23	53.66	61.52

4.4 The antioxidant activity of selenium-enriched kale seedling

4.4.1 Antioxidant activity of selenium-enriched kale seedling extracts assessed by scavenging DPPH radical method

The DPPH radical is considered to be a model of a stable lipophilic radical. Antioxidants react with DPPH reducing a number of DPPH molecules equal to their number of available hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual $DPPH^{\bullet}$ (Xu & HU, 2004). The antioxidant activity of selenium-enriched kale seedling extracts determined using the DPPH radical scavenging method was compared with BHA and Trolox (Figure 4.6). The results showed that the kinetic classification according to the time at the steady state has been reported as follows: rapid < 5 min; intermediate 5-30 min; and slow > 30 min. During the first 15 min, however, the reaction between DPPH radical and ethanolic extracts (Figure 4.6 (a-c)) were rapidly decreased and reached to the steady state in 15 min. Nevertheless, the reaction of ethanolic extracts reached a plateau in 15 min. The inhibition percentage of selenium-enriched kale seedling extracts are shown in Table 4.28. The higher inhibition rate is the greater hydrogen-donating ability and thus the higher antioxidant activity of the selenium-enriched kale seedling extracts. It is optimal

to calculate the inhibition percentage of antioxidant activity on DPPH at 30 min. It shows that selenium-enriched kale seedling (30 mg Se (IV) L^{-1} for 15 days) provided the highest inhibition percentage of 77.42% at 30 min. However, the inhibition percentage of selenium-enriched kale seedling is lower than Trolox (94.78%) but higher than BHA (73.09%).

The half maximal inhibitory concentration (IC_{50}) is a parameter used to measure the antiradical efficiency. The lower IC_{50} is the higher antioxidant power. The IC_{50} values of selenium-enriched kale seedling extracts, BHA and Trolox were compared and shown in Table 4.29. The results showed that all of the ethanolic extracts possessed lower IC_{50} than aqueous extracts. In addition, selenium-enriched kale seedling ethanolic extracts (30 mg Se (IV) L^{-1} for 15 days) was the most efficient by the lowest IC_{50} values of 1.07 mg Se mL^{-1} among all of the extracts. Thus, the results indicated that selenium-enriched kale seedling exhibited selenium concentration dependent scavenging effect on DPPH radical but lower than that of BHA and Trolox. Therefore, the scavenging effect increased with the increase of selenium concentrations.

4.4.2 Antioxidant activity of selenium-enriched kale seedling extracts assessed by FTC method

The antioxidant activity of selenium-enriched kale seedling extracts determined using the FTC method was compared with BHA and Trolox. The results are shown in Figure 4.7. The individual activity of selenium-enriched kale seedling showed low absorbance values which indicated a high level of antioxidant activity. All of the selenium-enriched kale seedling extracts delayed oxidation of linoleic acid. The basis of low absorbance values exhibited higher activity than control. Furthermore, lipid inhibitive activities of selenium-enriched kale seedling ethanolic extracts (Figure 4.7 (a-c)) were higher than aqueous extracts (Figure 4.7 (d-f)). Selenium-enriched kale seedling extracts with 15 and 30 µg Se mL^{-1} for 15 days of cultivation time displayed significantly higher activity than control (without antioxidants) and other selenium-supplemented concentrations but not significantly different with BHA and Trolox. In addition, the antioxidant activities from FTC method showed almost the same patterns of activities as the DPPH method.

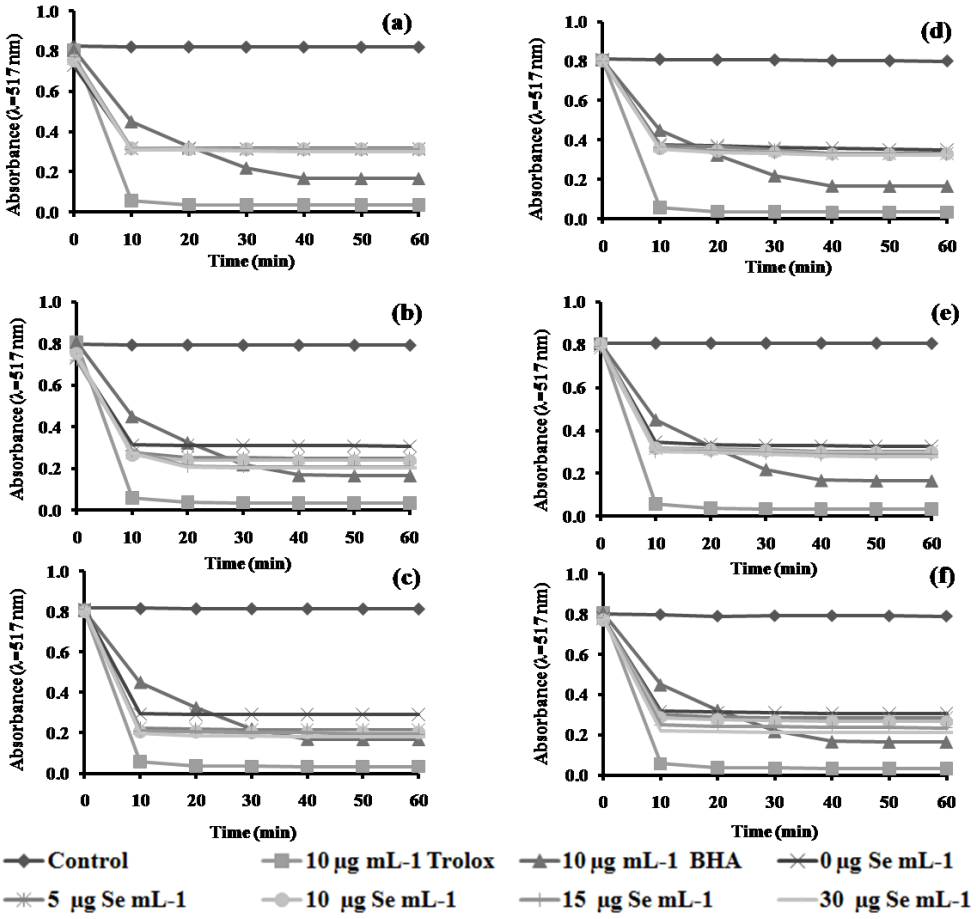

Figure 4.7 Kinetic behaviors of radical scavenging activity of selenium-enriched kale seedling ethanolic extracts (a-c) and aqueous extracts (d-f) with different selenium concentrations and cultivation time and two antioxidants as measured by DPPH method; (a,d) 5 days, (b,e) 10 days, and (c,f) 15 days.

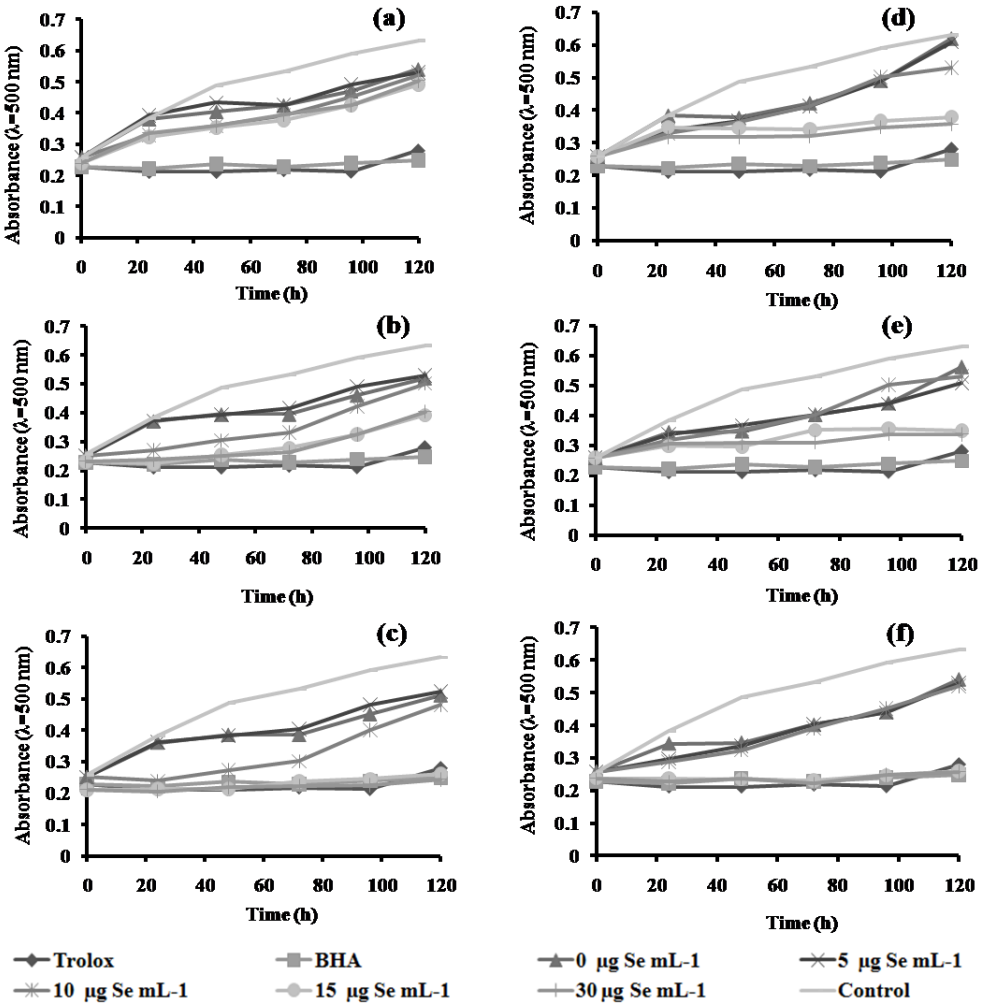

Figure 4.8 Antioxidant activity of selenium-enriched kale seedling ethanolic extracts (a-c) and aqueous extracts (d-f) with different selenium concentrations and cultivation time and two antioxidants as measured by FTC method; (a,d) 5 days, (b,e) 10 days, and (c,f) 15 days.

Table 4.28 Antioxidant activity of selenium-enriched kale seedling with different selenium concentrations and cultivation time and assessed with the DPPH method

Treatments	Inhibition (%), at 30 min					
	Aqueous extract			Ethanolic extract		
	Cultivation time			Cultivation time		
	5 days	10 days	15 days	5 days	10 days	15 days
Kale (control)	54.14 ± 1.00	58.24 ± 1.08	60.45 ± 0.39	61.68 ± 0.07	60.77 ± 0.19	64.17 ± 0.21
Kale (5 mg Se L^{-1})	55.17 ± 1.24	61.01 ± 0.56	63.26 ± 0.98	61.64 ± 0.19	67.30 ± 1.79	70.67 ± 0.49
Kale (10 mg Se L^{-1})	57.07 ± 1.50	61.42 ± 0.88	65.07 ± 1.03	61.81 ± 0.19	68.27 ± 1.58	71.90 ± 0.37
Kale (15 mg Se L^{-1})	57.28 ± 1.87	62.91 ± 0.50	69.15 ± 0.60	62.37 ± 0.19	73.58 ± 0.18	74.64 ± 0.43
Kale (30 mg Se L^{-1})	57.69 ± 1.58	63.28 ± 0.59	72.51 ± 0.50	62.70 ± 0.32	74.21 ± 0.18	77.42 ± 0.17
Trolox	94.78 ± 1.48					
BHA	73.09 ± 0.43					

Table 4.29 Radical scavenging activities of selenium-enriched kale seedling with different selenium concentrations and cultivation time and antioxidants expressed by IC_{50}

Treatments	IC_{50} (mg Se mL^{-1})					
	Aqueous extract			Ethanolic extract		
	Cultivation time			Cultivation time		
	5 days	10 days	15 days	5 days	10 days	15 days
Kale (control)	6.51	7.81	7.01	5.28	3.93	2.69
Kale (5 mg Se L^{-1})	3.32	4.03	4.77	2.18	2.85	2.24
Kale (10 mg Se L^{-1})	3.94	4.16	3.88	2.04	2.44	1.74
Kale (15 mg Se L^{-1})	3.71	2.27	2.37	2.09	2.12	1.46
Kale (30 mg Se L^{-1})	3.32	2.11	2.04	1.93	1.87	1.07
Trolox	0.0041					
BHA	0.0059					

CHAPTER 5

CONCLUSIONS

5.1 The production and determination of selenium-enriched kale seedling

Hydroponic enrichment of selenium in kale seedlings could be a safe and efficient system of producing organic selenium compounds in kale seedlings. The ICP-MS and ion pairing reversed phase HPLC-ICP-MS were used for investigation of total selenium and selenium species in kale seedlings enriched with Se (IV), respectively. The results from total selenium determination indicated that kale seedlings enriched with 30 mg Se L^{-1} Se for 15 days of cultivation found the highest selenium accumulating up to 386.18 mg Se kg^{-1}. Speciation study revealed that kale seedlings enriched with Se (IV) by hydroponic cultivation could be transformed to anticarcinogenic organic selenium like SeMet and SeMC by almost 80%.

5.2 The effect of sulfate ion in nutrient solution

The study of effect of sulfate ion in nutrient solution on selenium absorption of kale seedlings with hydroponic system. The comparison of nutrients solution between the solution of sulfate ion in form magnesium sulfate and no sulfate ion in form magnesium chloride were studied. It was found that sulfate ion did not affect to selenium (sodium selenite) absorption and total selenium contents in kale seedling samples. Since, selenium in form of sodium selenite was used for selenium-enriched kale seedling. The selenite absorption was not inhibited by sulfate ion in the nutrient solution. Whereas, the absorption of selenate was clearly inhibited by sulfate ion in the nutrient solution.

5.3 The reuse of medium solution (nutrient solution)

The study of reuse of medium solution with 30 mg Se L^{-1} supplemented in nutrient solution on the selenium and minerals absorption of kale seedling. It was found that total selenium and mineral contents decreased when nutrient solution repeatedly used. The results completed for 45 days (3 crops), the highest total selenium, manganese, copper, iron, zinc, calcium, magnesium, sodium, potassium and phosphorus were found in 1st generation (crop 1) of selenium-enriched kale seedling.

5.4 The stability and shelf life of selenium-enriched kale seedling in different container materials

The determination of total element concentrations in selenium-enriched kale seedling is analyzed immediately after collection with difference conditions. In this work, the stability and shelf life of selenium species with different temperatures, container materials and storage time by HPLC–ICP-MS and ICP-MS methods. It is better to stored the samples in the dark, in the PS bottle, LDPE ziplock bag and aluminium foil bag containers, at 4 °C. Under these conditions the selenium species (SeMet and SeMC) were stable within a period of 6 months.

The PE bottle and PC bottle containers induced severe losses of the total selenium and organic selenium after only 6 months of storage time. PS bottle, LDPE ziplock bag and aluminium foil bag appears to be losses slightly superior to PE bottle and PC bottle at 4°C for 6 months of storage time. Thus, the best results for stability and shelf life of selenium-enriched kale seedling were obtained with container material of PS bottle, LDPE ziplock bag and aluminium foil bag at 4°C for 6 months.

5.5 The antioxidant activity of selenium-enriched kale seedling

The antioxidant activity of selenium-enriched kale seedlings were studied by DPPH radical scavenging and FTC methods. The DPPH assay showed that the kinetic behaviors of ethanolic extracts reacted quickly while aqueous extracts were complex and slow with DPPH radical. However, aqueous or ethanolic extracts of selenium-enriched kale seedling displayed significantly higher antioxidant activity against lipid peroxidation. Ethanolic extracts of selenium-enriched kale seedling exhibited higher antiradical efficiencies than aqueous extracts, and selenium-enriched kale seedling (30 µg Se (IV) mL^{-1} for 15 days) presented the lowest IC_{50} values of 1.07 mg Se mL^{-1}. Furthermore, selenium-enriched kale seedling obtained by ethanolic extracts exhibited the highest inhibition percentage of 77.42% at 30 min. However, the inhibition percentage of selenium-enriched kale seedling is lower than Trolox (94.78%) but higher than BHA (73.09%).

REFERENCES

REFERENCES

Álvarez-Llamas, G., Rosario, M., Campa, F. & Sanz-Medel, A. (2005) ICP-MS for specific detection in capillary electrophoresis. *Trends in Analytical Chemistry*, 24, 28-36.

Aras, N.K. & Ataman, O.Y. (2006) *Trace Element Analysis of Food and Diet.* The Royal Society of Chemistry, United Kingdom.

Arteel, G.E., Mostert, V., Oubrahim, H., Briviba, K., Abel, J. & Sies, H. (1998) Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. *Biological Chemistry*, 379, 1201-1205.

Arvy, M.P. (1993) Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*). *Journal of Experimental Botany*, 44, 1083-87.

Battin, E.E. & Brumaghim, J.L. (2009) Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochemistry and Biophysics*, 55, 1-23.

Baylan, M., Canogullari, S., Ayasan, T. & Copur, G. (2011) Effects of dietary selenium source, storage time, and temperature on the quality of quail eggs. *Biology Trace Element Research*, 143, 957-964.

Behne, D., Hammel, C., Pfeifer, H., Röthlein, D., Gessner, H. & Kyriakopoulos, A. (1998) Speciation of selenium in the mammalian organism. *Analyst*, 123, 871-3.

Behne, D. & Kyriakopoulos, A. (2001) Mammalian selenium-containing proteins. *Annual Review of Nutrition*, 21, 453-473.

Brown, T.A. & Shrift, A.A. (1981) Exclusion of Selenium from Proteins of Selenium-Tolerant *Astragalus* Species. *Plant Physiology*, 67, 1051-1053.

Burk, R. (2002) Selenium, an antioxidant nutrient. *Nutrition in Clinical Care*, 5, 75-79.

Burk, R. & Hill, K. (2005) Selenoprotein P: an extracellular protein with unique physical charanteristics and a role in selenium homeostasis. *Annual Review of Nutrition*, 25, 215-235.

Burk, R. & Levander, O.A. (2006) *Selenium in modern nutrition in health and disease*. Lippincott Williams & Wilkins, Philadelphia.

Burnett, J.H. & Trinci, A.P.J. (1978) (eds) *In: Fungal walls and hyphal growth*. Handbook for the British Mycological Society. Cambridge University Press, Cambridge, England, 2-8.

Cai, X.J., Block, E., Uden, P.C., Zhang, X., Quimby, B.D. & Sullivan, J.J. (1995) Allium chemistry: identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas chromatography with atomic emission detector. *Journal of Agricultural and Food Chemistry*, 43, 1754-1757.

Cao, G., Sofic, E. & Prior, R.L. (1996) Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry*, 44, 3426-3431.

Casiot, C., Szpunar, J., Lobinski, R. & Potin-Gautier, M. (1999) Sample preparation and HPLC separation approaches to speciation analysis of selenium in yeast by ICP-MS. *Journal of Analytical Atomic Spectrometry*, 14, 645-650.

Chan, Q., Afton, S.E. & Caruso, J.A. (2009) Selenium speciation profiles in selenite-enriched soybean (GlycineMax) by HPLC–ICPMS and ESI-ITMS. *Metallomics*, 2, 147-153.

Chantiratikul, A., Chinrasri, O., Pakmaruek, P., Chantiratikul, P., Thosaikham, W. & Aengwanich, W. (2011) Responses of growing Japanese quails that received selenium from selenium enriched kale sprout (*Brassica oleracea* var. alboglabra L.). *Biological Trace Element Research*, doi:10.1007/s12011-011-9112-z.

Chen, T.F., Zheng, W.J., Wong Y.F. & Yang, F. (2008) Selenium-induced changes in activities of antioxidant enzymes and content of photosynthetic pigments in Spirulina platensis. *Journal of Integrative Plant Biology*, 50(1), 40-8.

Chethaka, K., Boakye, H.T., Uden, P.C. & Tyson, J.F. (2004) Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating Brassica juncea (Indian mustard) and in selenized yeast. *Journal of Chromatography A*, 1054, 303-312.

Diwadkar-Navsariwala, V. & Diamond, A.M. (2004) The link between selenium and chemoprevention: A case for selenoproteins. *Journal of Nutrition*, 134, 2899-2902.

Dong, Y., Lisk, D.J., Block, E. & Ip, C. (2001) Characterization of the biological activity of γ-glutamyl-Se-methylselenocysteine: A novel, naturally occurring anticancer agent from garlic. *Cancer Research*, 61, 2923-2928.

Drummond, K.E. & Brefere, L.M. (2004) *Nutrition for food service and Culinary Professionals*. Hoboken: John Wiley and Son.

Ebdon, L., Evans, E.H., Fisher, A. & Hill, S.J. (1998) *An Introduction to Analytical Atomic Spectrometry*. John Wiley & Sons Ltd. England.

El-Bayoumy, K. (2001) The protective role of selenium on genetic damage and on cancer. *Mutation Research*, 475, 123-139.

Ellis, D.R. & Salt, D.E. (2003) Plants, selenium and human health. *Current Opinion in Plant Biology*, 6, 273-279.

Esaki, N., Nakamura, T., Tanaka, H., *et al*. (1981) Enzymatic synthesis of selenocysteine in rat liver. *Biochemistry*, 20, 4492-4500.

Evans, E.H., Giglio, J.J, Castillano, T.M. & Caruso, J.A. (1995) *Inductively Coupled and Microwave Plasma Sources for Mass Spectrometry*. Cambridge, England: :Royal Society of Chemistry.

Fairweather-Tait, S. J., Collings, R. & Hurst, R. (2010) *Selenium bioavailability: current knowledge and future research requirements*. New York: American Society for Nutrition, United States of America.

Feroci, G. & Fini, A. (1998) Study of the antioxidant effect of several selenium and sulphur compounds. *Journal of Trace Eelements in Medicine and Biology*, 12, 96-100.

Finley, J.W., Davis, C.D. & Feng, Y. (2000) Selenium from high selenium broccoli protects rats from colon cancer. *Journal of Nutrition*, 130, 2384-2389.

Finley, J.W., Ip, C., Lisk, D.J., Davis, C.D., Hintze, K.J. & Whanger, P.D. (2001) Investigation on the cancer-protective properties of high-selenium broccoli. *Journal of Agricultural and Food Chemistry*, 49, 2679-2683.

Finley, J.W., Grusak, M.A., Keck, A.S & Gregoire, B.R. (2004) Bioavailability of selenium from meat and broccoli as determined by retention and distribution of 75Se. *Biological Trace Element Research*, 99, 191-209.

Food and Nutrition Board Institute of Medicine. (2000) *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoides*. National Academics Press, Washington DC, United States of America.

Foster, L.H. (1995) Selenium in the environment, food and health. *Nutrition and Food Science*, 95, 17-23.

Frankenberger, J.R. & Benson, S. (1994) *Selenium in the Environment*. Marcel Dekker, Inc. New York: United States of America.

Gómez-Ariza, J.L., Pozas, J.A., Girldez, I. & Morales, E. (1998) Speciation of volatile forms of selenium and inorganic selenium in sediments by gas chromatography-mass spectrometry. *Journal of Chromatrography A*, 823, 259-277.

Graw-Hill, M. (1982) Encyclopedia of Chemistry. Mc Graw-Hill, New York, United States of America.

Gu, Q.P., Sun, Y., Ream, L.W. & Whanger, P.D. (2000) Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. *Molecular Cell Biochemistry*, 204, 49-56.

Gupta, U., Cheema, G.S., Sodhi, H.S. & Phutela, R.P. (1997) Protoplast isolation and regeneration in Agaricus bisporus strain MS. *Mushroom Research*, 6, 59-62.

Gutric, H.A. & Picciano, M.F. (1995) *Human Nutrition*. New York: Mobby Year Book.

Hatfield, D.L. & Gladyshev, V.N. (2002) How selenium has altered our understanding of the genetic code. *Molecular and Cellular Biology*, 3565-3576.

Hawkes, W.C., Richter, B.D., Alkan, Z., Souza, E.C., Derricote, M., Mackey, B.E., et al. (2008) Response of selenium status indicators to supplementation of healthy north American men with high-selenium yeast. *Biological Trace Element Research*, 122, 107-121.

Hegedűs, O., Hegedűsová, A., Šimková, S., Pavlík, V. & Jomová, K. (2008) Evaluation of the ET-AAS and HG-AAS methods of selenium determination in vegetables. *Journal of Biochemical and Biophysical Methods*, 70, 1287-1291.

Helen, A.G. & Mary, F.P. (1995) *Human Nutrition*. Mogby-Year Book, Inc.

Hibbert, B. (2007) *Quality assurance for the analytical chemistry laboratory*. New York: Oxford University Press, 241-245.

Hill, K.E., Xia, Y., Akesson, B., Boeglin, M.E. & Burk, R.F. (1996) Selenoprotein P Concentration in Plasma Is an Index of Selenium Status in Selenium-Deficient and Selenium-Supplemented Chinese Subjects. *Human and Clinical nutrition*.

Hoenig, M. (2001) Preparation steps in environmental trace element analysis facts and traps. *Talanta*, 54,1021-1038.

Huang, Z.Z. & Wu, L. (1991) Species richness and selenium accumulation of plants in soils with elevated concentration of selenium and salinity. *Ecotoxicology and Environmental Safety*, 22, 251-266.

Hwang, E.S., Jang, M.R. & Kim, G.H. (2012) Effects of storage condition on the bioactive compound contents of Korean cabbage. *Food Sci. Biotechnol*, 21(6), 1655-1661.

Hymer, C.B. & Caruso, J.A. (2006) Selenium speciation analysis using inductively coupled plasma-mass spectrometry. *Journal of Chromatography A*, 1114, 1-20.

Ip, C. (1998) Lessons from basic research in selenium and cancer prevention. *The Journal of Nutrition*, 128, 1845-1854.

Iwashita, A., Nakajima, T., Takanashi, Hirokazu., Ohki, A., Fujita, Y. & Yamashita, T. (2006) Effect of pretreatment conditions on the determination of major and trace elements in coal fly ash using ICP-AES. *Fuel*, 85, 257-263.

Jarvis, K.E., Gray, A.L., & Houk, R.S. (1992) *Handbook of Inductively Coupled Plasma Mass Spectrometry*. New York: Blackie Academic., USA.

Jiakui, L. & Xiaolong, W. (2004) Effect of dietary organic versus inorganic selenium in laying hens on the productivity, selenium distribution in egg and selenium content in blood, liver and kidney. *Journal Trace Elements in Medicine and Biology*, 18, 65-68.

Jing, W., Lyons, G.H., Graham, R.D. & Fenech, M.F. (2009) The effect of selenium, as selenomethionine, on genome stability and cytotoxicity in human lymphocytes measured using the cytokinesis-block micronucleus cytome assay. *Mutagenesis*, 24, 225-232.

Jing, W. (2010) *The effect of selenomethionine and wheat biofortified with selenium on DNA damage and cell death in human lymphocytes*. University of Adelaide.

Johansson, L. (2000) *Selenocysteine in proteins*. Stockholm, Sweden: Karolinska University Press,

Johansson, L., Gafvelin, G. & Arnèr, E.S.J. (2005) Selenocysteine in proteins-properties and biotechnological. *Biochimica et Biophysica Acta*, 1726, 1-13.

Kabata-Pendias, A. (2011) *Trace Elements in Soils and Plants*. Taylor & Francis Group, United States of America.

Kahakachchi, C., Boakye, H. T., Uden, P.C. & Tyson, J.F. (2004) Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating *Brassica juncea* (Indian mustard) and in selenized yeast. *Journal of Chromatography A*, 1054, 303-312.

Kápolna, E. & Fodor, P. (2006) Speciation analysis of selenium enriched green onions (*Allium fistulosum*) by HPLC-ICP-MS. *Microchemical Journal*, 84, 56-62.

Kápolna, E., Shaha, M., Carusoa, J.A. & Fodorb, P. (2007) Selenium speciation studies in Se-enriched chives (*Allium schoenoprasum*) by HPLC-ICP-MS. *Food Chemistry*, 101, 1398-1406.

Kim, H.Y. & Gladyshev, V. (2005) Difference catalytic mechanisms in mammalian selenocysteine and cysteine-containing methionine-R-sulfoxide reductases. *Physiology Biology*, 3, 2080-2088.

Kotrebai, M., Bird, S.M., Tyson, J.F., Block, E. & Uden, P.C. (1999) Characterization of selenium species in biological extracts by enhanced ion-pair liquid chromatography with inductively coupled plasma-mass spectrometry and by referenced electrospray ionization-mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 54, 1573-1591.

Larsen, E.H., Lobinski, R., Burger-Meÿer, K., Hansen, M., Ruzik, R., Mazurowska, *et al.* (2006) Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate. *Analytical Bioanalytical Chemistry*, 385, 1098-1108.

Leustek, T. & Saito, K. (1999) Sulfate transport and assimilation in plants. *Plant Physiology*, 120, 637-643.

Levander, O.A. (1997) Nutrition and newly emerging viral disease. *Nutrition*, 127, 948-950.

Lindemann, D.M., Cho, H.J., & Wang, M.Q. (2009) Chromium: An essential mineral. *Revista Colombiana de Ciencias Pecuarias*, 22, 339-345.

Lintschinger, J., Fuchs, N., Moser, J., Kuehnelt, D. & Goessler, W. (2000) Selenium-enriched sprouts: a raw material for fortified careal-based diets. *Journal of Agricultural and Food Chemistry*, 48, 5362-5368.

Liu, K., Zhao, Y., Chen, F., Gu, Z. & Bu, G. (2012) Purification, identification, and in vitro antioxidant activities of selenium-containing proteins from selenium-enriched brown rice. *European Food Research and Technology*, 234, 61-68.

Łobínski, R. & Szpunar, J. (1999) Biochemical apeciation analysis by hyphenated techniques. *Analytical Chimica Acta*, 400, 321-323.

Maneetong, S., Chookhampaeng, S., Chantiratikul, A., Chinrasri, O., Thosaikham, W., Sittipout, R. & Chantiratikul, P. (2013) Hydroponic cultivation of selenium-enriched kale (*Brassica oleracea* var. *alboglabra* L.) seedling and speciation of selenium with HPLC–ICP-MS. *Microchemical Journal*, 108, 87-91.

Matthews, D., Millam, S. & Wilkinson, M.J. (1999) *Plant Cell Re, p*18, 786–790

Matusiewicz, H. (2003) Wet digestion methods. *Comprehensive Analytical Chemistry*, 41, 193-233.

Maurice, E.S., Shike, M., Ross, A.C., Caballero, B. & Cousins, R.J. (2006) *Modern Nutrition in health and desease*. Lippincott Williams & Wikins, United States of America, 315.

Melaku, S., Dams, R. & Moens, L. (2005) Determination of trace elements in agricultural soil samples by inductively coupled plasma-mass spectrometry: Microwave acid digestion versus aqua regia extraction. *Analytica Chimica Acta*, 543, 117-123.

Mester, Z. & Sturgeon, R. (2003) *Sample Preparation for Trace Element Analysis*. Ottawa, Canada: John Wiley & Sons, Inc.

Mitchell, A.D. & Benevenga, N.J. (1978) The role of transamina in methionine oxidation in the rat. *The Journal of Nutrition*, 108, 67-78.

Michalke, B., & Schramel, P. (1998) Application of capillary zone electrophoresis-inductively coupled plasma mass spectrometry and capillary isoelectric focusing- inductively coupled plasma mass spectrometry for selenium speciation. *Journal of Chromatrography A*, 807, 71-80.

Montes-Bayón, M., Yanes, E.G., de León, C.P., Jayasimhulu, K., Stalcup, A., Shann, J., & Caruso J.A. (2002) Initial Studies of Selenium Speciation in *Brassica juncea* by LC with ICPMS and ES-MS Detection: an Approach for Phytoremediation Studies. *Analytical Chemistry*, 74, 107-113.

Montes-Baýon, M., JośeDíaz Molet, M., González, E.B. & Sanz-Medel, A. (2006) Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum and Brassica juncea*) and Se speciation by HPLC-ICP-MS. *Talanta*, 68, 1287-1293.

Moreno, E., Pérez-Code, C. & Cámara, C. (2003) The effect of the presenc of volatile organoselenium compuunds on the determinationof in organic selenium by hydride generation. *Analytical and Bioanalytical Chemistry*, 375, 666-672.

Mustacich, D. & Powis, G. (2000) Thioredoxin reductase. *Journal of Biochemistry*, 346, 1-8.

Olivas, R.M., Quevauviller, P. & Donard, O.F.X. (1998) Long term stability of organic selenium species in aqueous solutions. *Fresenius Journal of Analytical Chemistry*, 360, 512-519.

Oliver, M.A. (1997) Soil and human health: A review. *European Journal of Soil Science*, 48, 573-592.

Parr, R.M., Aras, N.K., & Iyengar, G.V. (2006) Dietary intakes of essential trace elements: Results from total diet studies supported by the IAEA. *Journal of Radioanalytical and Nuclear Chemistry*, 270, 155-161.

Pilon-Smits, E.A.H. & LeDuc, D.L. (2009) Phytoremediation of selenium using transgenic plants. *Current Opinion in Biotechnology*, 20, 207-212.

Polkowska-Motrenko, H., Danko, B., Dybczy´nski, R., Koster-Ammerlaan, A. & Bode, P. (2000) Effect of acid digestion method on cobalt determination in plant materials. *Analytica Chimica Acta*, 408, 89-95.

Pyrzynska, K. (2009) Selenium speciation in enriched vegetables. *Food Chemistry*, 114, 1183-1191.

Qilin, C., Afton, S.E. & Caruso, J.A. (2009) Selenium speciation profiles in selenite-enriched soybean (Glycine Max) by HPLC-ICPMS and ESI-ITMS. *Metallomics*, 2, 147-153.

Ramoutar, R.R. & Brumaghim, R.L. (2010) Antioxidant and anticancer properties and mechanisms of inorganic selenium, oxo-sulfur, and oxo-selenium compounds. *Cell Biochemistry and Biophysics*, 58, 1-23.

Reilly, C. (1996) *Selenium in food and health*. Chapman & Hall, London.

Robles, L.C., Feo, J.C., De Celis, B. Lumbreras, J.M., Garcia-Olalla, C. & Aller, A.J. (1999) Speciation of selenite and selenate using living bacteria. *Talanta*, 50, 307-325.

Scharpf, M., Schweizer, U., Arzberger, T., Roggendorf, W., Schomburg, L. & Köhrle, J. (2007) Neuronal and ependymal expression of selenoprotein P in the human brain. *Jounal of transmission*, 114, 877-884

Schwarz, K. & Foltz, C. (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society*, 79, 3292.

Shao, S. & Zheng, B. (2008) The biogeochemistry of selenium in Sunan grassland, Gansu, Northwest China, casts doubt on the belief that Marco Polo reported selenosis for the first time in history. *Environt Geochem Health*, 30, 307-314.

Shen, Q., Zhang, B., Xu, R., Wang, Y., Ding, X. & Li, P. (2010) Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched *Bifidobacterium animalis* 01. *Anaerobe*, 16, 380-386.

Sikora, E. & Bodziarczyk, I. (2012) Composition and antioxidant activity of kale (*Brassica oleracea* L. var. *acephala*) raw and cooked. *Acta Scientiarum Polonorum*, 11(3), 239-248.

Sirichakwal, P.P., Puwastien, P., Polngam, J. & Kongkachuichai, R. (2005) Selenium content of Thai foods. *Journal of Food Composition and Analysis*, 18, 47-59.

Sittipout, R. (2012) *Development of home-made digestion apparatus for trace metal analysis in biological samples*. A thesis submitted in partial fulfillment of the requirement for the Master of Science degree in Chemistry, Mahasarakham University.

Smith, F.W., Hawkesford, M.J., Ealing, P.M., Clarkson, D.T., Vanden Berg, P.J., Belcher, A.R., Warrilow, A.G. (1997) Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter. *Plant Journal*, 12, 875-884.

Soylak, M., Saraçoğlu, S., Tüzen, M. & Mendil, D. (2005) Determination of trace metals in mushroom samples from Kayseri, Turkey. *Food Chemistry*, 92, 649-652.

Stadtman, T.C. (1980) Selenium-Dependent Enzymes. *Annual Review of Biochemistry*, 49, 93-110.

Stadtman, T. C. (1996) Selenocystein. *Annual Review of Biochemistry*, 63, 83-100.

Subramanian, K.S. (1996) Determination of metals in biofluids and tissues: sample preparation methods for atomic spectroscopic techniques. *Spectrochimica Acta*, 51, 291-319.

Sugihara, S., Kondo, M., Chihara, Y., Yuji, M., Hattori, H. & Yoshida, M. (2004) Preparation of selenium-enriched sprouts and identification of their selenium species by high-performance liquid chromatography-inductively couple plasma mass spectrometry. *Bioscience Biotechnology and Biochemistry*, 68, 193-199.

Sutton, K.L. & Caruso, J.A. (1999) Liquid chromatrography- inductively coupled plasma mass spectrometry. *Journal of Chromatrography A*, 856, 243-258.

Tapiero, H., Townsend, D.M. & Tew, K.D. (2003) The antioxidant role of selenium and seleno-compounds. *Biomedicine & Pharmacotherapy*, 57, 134-144.

Tarze, A., Dauplais, M., Grigoras, I., Lazard, M. & Ha-Duong, N.T. (2007) Extracellular production of hydrogen selenide accounts for Thiol-assisted toxicity of selenite against *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, 282, 8759-8767.

Taylor, H.E. (2001) *Inductively Coupled Plasma-Mass Spectrometry*. Academic Press, California, America.

Terry, N., Zayed, A.M., De Souza, M.P., & Tarun, A.S. (2000) Selenium in higher plants. *Plant Molecular Biology*, 52, 401-432.

Thomas, R. (2001) *A Beginner's Guide to ICP-MS Part I*. Spectroscopy. 26.

Thosaikham, W., Phansi, P., Chantiratikul, P., Maneechote, U. & Chantiratikul, A. (2009) Determination of selenium content in watermeal (*Wolffia globosa*) supplemental sodium selenate using ICP-MS technique. *Pure and Applied Chemistry .International Conference*. 14-16th January 2009. Naresuan University, Phisanulok, Thailand, 151-152.

Tinggi, U. (2003) Essentiality and toxicity of selenium and its status in Australia: a review. *Toxicology Letters*, 137, 103-110.

Tsuneyoshi, T., Yoshida, J. & Sasaoka, T. (2006) Hydroponic cultivation offers a practical means of producing selenium-enriched garlic. *Journal of Nutrition*, 136, 870S-872S.

Turło, J., Gutkowska, B. & Herold, F. (2010) Effect of selenium enrichment on antioxidant activities and chemical composition of *Lentinula edode*s (*Berk*.) *Pegl*. mycelial extracts. *Food and Chemical Toxicology*, 48, 1085-1091.

Turunen, M., PerZniemi, S., Markku, A. & Westerholm, H., (1995) Determination of trace elements in heavy oil samples by graphite furnace and cold vapour atomic absorption spectrometry after acid digestion. *Analytica Chimica Acta*, 311, 85-91.

Waddell, R., Lewis, C., Hang, W., Hassell, C. & Majidi, V. (2005) Inductively coupled plasma mass spectrometry for elemental speciation: Applications in the New Millennium. *Applied Spectroscopy Review*, 40, 33-69.

Wardlaw, G.M. & Hampl, J.S. (2004) *Perspectives in Nutrition*. New York: Mcgraw Hill Companies.

Whanger P.D. (2003) Metabolic pathways of selenium in plants and animals and their nutritional significance. *In Nutritional Biotechnology in the Feed and Food Industries*, 51-58.

Whanger, P.D. (2004) Review article: Selenium and its relationship to cancer: an update. *British Journal of Nutrition*, 91, 11-28.

WHO/WFO (2004) *Vitamin and mineral requirements in human nutrition*. 2nd, Rome: WHO/WFO.

Williams, C.H.J., Zanetti, G., Arscott, L.D. & Mcallister, J.K. (1992) *Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase-A family of flavoenzyme transhydrogenases*. New York: *CRP Press*.

Williams, C.H.J, Arscott, L.D, Müller, S., Lennon, B.W., Ludwig, M.L., Wang, P.F., Veine, D.M., Becker, K. & Schirmer, R.H. (2000) Thioredoxin reductase two modes of catalysis have evolved. *Journal of. Biochemistry*, 267, 6110-6117.

Wolffram, S., Berger, B., Grenacher, B. & Scharrer E. (1998) Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. *Journal of Nutrition*, 119, 706-712.

Wróbel, K., Wróbel, K., Kannamkumarath, S.S., Caruso, J.A., Wysocka, I.A., Bulska, E., Świątek, J. & Wierzbicka, M. (2004) HPLC–ICP-MS speciation of selenium in enriched onion leaves-a potential dietary source of Se-methylselenocysteine. *Food Chemistry*, 86, 617-623.

Xu, J. & Hu, Q. (2004) Effect of foliar application of selenium on the antioxidant activity of aqueous and ethanolic extracts of selenium-enriched rice. *Journal of Agricultural and Food Chemistry*, 52, 1759-1763.

Xu, J., Yang, F., Chen, L., Hu, Y. & Hu, Q. (2003) Effect of selenium on increasing the antioxidant activity of tea leaves harvested during the early spring tea producing season. *Journal of Agricultural and Food Chemistry*, 51, 1081-1084.

Yang, G.Q., Ge, K.Y., Chen, J.S. & Chen, X.S. (1988) Selenium-related endemic diseases and the daily selenium requirement of humans. *World Review of Nutrition and Dietetics*, 55. 98-152.

Yang, L., Sturgeon, R.E., McSheehy, S. & Mester, Z. (2004) Comparison of extraction methods for quantitation of methionine and selenomethionine in yeast by species specific isotope dilution gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1055, 177-184.

Yasumoto, K., Suzuki, T. & Yoshida, M. (1988) Identification of selenomethionine in soya-bean protein. *Journal of Agricultural and Food Chemistry*, 36, 463-467.

Yawata, A., Oishi, Y, Anan, Y., & Ogra, Y. (2010) Comparison of selenium metabolism in three *Brassicaceae* plants. *Journal of Health Science*, 56, 699-704.

Yoshida, M., Okada, T., Namikawa, Y., Matsuzaki, Y., Nishiyama, T. & Fukunaga, K. (2007) Evaluation of nutritional availability and anti-tumor activity of selenium contained in selenium-enriched *Kaiware* radish sprouts. *Bioscience Biotechnology Biochemistry*, 71, 2198-2205.

Zadrożna, M., Gawlik, M., Nowak, B., Marcinek, A., Mrowiec, H., Walas, S., Wietecha-Posłuszny, R. & Zagrodzki, S. (2009) Antioxidants activities and concentration of selenium ,zinc and copper in preterm and IUGR human placentas. *Journal of Trace Elements in Medicine and Biology*, 23, 144-148.

Zayed, A. Lytle, C.M. & Terry, N. (1998) Accumulation and volatilization of different chemical species of selenium by plants. *Planta*, 206, 284-92.

Zhao, L., Zhao, G., Du, M., Zhao, Z., Xiao, L. & Hu X. (2008) Effect of selenium on increasing free radical scavenging activities of polysaccharide extracts from a Se-enriched mushroom species of the genus *Ganoderma*. *European Food Research and Technology*, 226, 499-505.

Zheng, J., Ohata, M., Furuta, N. & Kosmus, W. (2000) Speciation of selenium compounds with ion-pair reversed-phase liquid chromatography using inductively coupled plasma mass spectrometry as element-specific detection. *Journal of Chromatography A*, 874, 55-64.

Zheng, J., Shibata, Y. & Tanaka, A. (2002) Study of the stability of selenium compounds in human urine and determination by mixed ion-pair reversed-phase chromatography with ICP–MS detection. *Analytical and Bioanalytical Chemistry*, 374, 348-353.

APPENDICES

APPENDIX A

The percentage relative standard deviation

The percentage relative standard deviation (%RSD) is referred to the precession of the measurements or the method. It can be calculated using the equation as follow:

$$\%RSD = \frac{SD}{\bar{x}} \times 100$$

Where %RSD = percentage relative standard deviation
SD = standard deviation
$\bar{x}$ = mean of data measurements

The standard deviation (SD) can be calculated using the equation as follow:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n - 1}}$$

Where x_i = the individual measurements
n = the number of measurements

APPENDIX B

Cultivation of selenium-enriched kale seedling

(a)

(b)

(c)

(d)

Figure B The cultivation of selenium-enriched kale seedling with hydroponic system; (a) kale sprouts for 3 days of cultivation time; (b) kale sprouts for 7 days of cultivation time; (c) kale seedling for 15 days of cultivation time; (d) hydroponic system

BIOGRAPHY

BIOGRAPHY

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OUTPUT OF THE RESEARCH

PUBLICATIONS

1. **Maneetong, S**., Chookhampaeng, S., Chantiratikul, A., Chinrasri, O., Thosaikham, W., Sittipout, R. & Chantiratikul, P. (2013) Hydroponic cultivation of selenium-enriched kale (*Brassica oleracea* var. *alboglabra* L.) seedling and speciation of selenium with HPLC–ICP-MS, *Microchemical Journal*. 108, 87-91.

2. Thosaikham, W., Jitmanee, K., Sittipout, R., **Maneetong, S.**, Chantiratikul, A. & Chantiratikul, P. (2014) Evaluation of selenium species in selenium-enriched pakchoi (*Brassica chinensis Jusl var parachinensis (Bailey) Tsen & Lee*) using mixed ion-pair reversed phase HPLC–ICP-MS, *Food Chemistry*. 145, 736-742.

3. Chantiratikul, A., Chinrasri, O., Jaichong, D., Laodim, T., Changkhunthod, S., Chookhampaeng, S., **Maneetong, S.** & Chantiratikul, P. (2013) Selenium concentration in tissues of laying quails fed selenium from hydroponically produced Se-enriched kale sprout (*Brassica oleracea* var. *alboglabr*a L.). *Agricultural Science Journal*. 44 (1), 111-114.

4. Chinrasri, O., Chantiratikul, P.**, Maneetong, S.**, Chookhampaeng, S., & Chantiratikul, A. (2013) Productivity and selenium concentrations in egg and tissue of laying quails fed selenium from hydroponically produced selenium-enriched kale sprout (*Brassica oleracea* var. *alboglabra* L.). *Biological Trace Element Research*. DOI 10.1007/s12011-013-9824-3.

5. **Maneetong, S.**, Chantiratikul, P., Chookhampaeng, S., Chantiratikul, A., Chinrasri, O. & Jitmanee, K. (2012) The production and selenium speciation of Se-enriched kale seedling (*Brassica oleracea* var. *alboglabr*a L.). The 8th Mahasarakham University Research Conference. 8th - 9th November 2012, Mahasarakham, Thailand.

6. Pakmaruek, P., Chinrasri, O., Aengwanich, W., Chantiratikul, P., Chookhampaeng, S., **Maneetong, S.** & Chantiratikul, A., Utilization of Se-enriched kale sprout (*Brassica oleracea* var. *alboglabr*a L.) as selenium source in broiler diets. The 8th Mahasarakham University Research Conference. 8th - 9th November 2012, Mahasarakham, Thailand.

7. Thosaikham, W., **Maneetong, S.**, Sittipout, R., Chantiratikul, A. & Chantiratikul, P. Extraction method for extracting selenium from Se-enriched plants. The 8th Mahasarakham University Research Conference. 8th - 9th November 2012, Mahasarakham, Thailand.

8. Chinrasri, O., Pakmaruek, P., Chookhampaeng, S., **Maneetong, S.**, Chantiratikul, P. & Chantiratikul, A. (2013) Supplementing selenium from selenium-enriched kale sprout (*Brassica oleracea* var. *alboglabr*a L.) in broiler diets. The 4th International Conference on Sustainable Animal Agriculture for Developing Countries. 27th - 31st July 2013, Lanzhou, China.

9. Chantiratikul, A., Chinrasri, O., Pakmaruek, P., Chookhampaeng, S., **Maneetong, S.** & Chantiratikul, P. (2013) Organic selenium from selenium-enriched kale sprout increased tissue selenium concentrations of broilers. The 4th International Conference on Sustainable Animal Agriculture for Developing Countries. 27th - 31st July 2013, Lanzhou, China.

CONFERENCES

1. **Maneetong, S.** & Chantiratikul, P. (2012) The production and selenium speciation of Se-enriched kale seedling (*Brassica oleracea* var. *alboglabr*a L.). The 7th Conference on Science and Technology for Youths. Poster Presented. 2nd - 4th May 2012, Bangkok International Trade and Exhibition Centre, Bangkok, Thailand.

2. **Maneetong, S.**, Chantiratikul, P., Chookhampaeng, S., Chinrasri, O., Jitmanee, K. & Chantiratikul, A. (2012) Se-enriched kale seedling production by hydroponic cultivation. Private Public Partnership, The First National Conference on 3P (Private Public Partnership) Research & Technology licensing Office (TLO). 28th - 30th July 2012, Phitsanulok, Thailand.

3. **Maneetong, S.** & Chantiratikul, P. (2011) The production of Se-enriched kale sprout (*Brassica oleracea* var. *alboglabr*a L.). The 6th Conference on Science and Technology for Youths. Poster Presented. 18th - 19th March 2011, Bangkok International Trade and Exhibition Centre, Bangkok, Thailand.

4. **Maneetong, S.**, Thosaikham, W.**,** Sittipout, R. & Chantiratikul, P. (2010) Speciation and determination of selenium in selenium-enriched duckweed (*Lemna minor*). Pure and Applied Chemistry International Conference (PACCON). Poster Presented. 21st - 23rd January 2010, Ubon Ratchathani, Thailand.

5. Sittipout, R., Thosaikham, W., **Maneetong, S.** & Chantiratikul, P. (2010) Sample preparation methods for selenium determination with ICP-MS technique. Pure and Applied Chemistry International Conference (PACCON). Poster Presented. 21st - 23rd January 2010, Ubon Ratchathani, Thailand.

6. Thosaikham, W., Jitmanee, K., Sittipout, R., **Maneetong, S.**, Chantiratikul, A. & Chantiratikul, P. 2012. Ion-paired reversed phase HPLC-ICP-MS for selenium speciation analysis in Se-enriched yeast. Pure and Applied Chemistry

International Conference (PACCON). Oral presentation. 11st-13rd January 2012. The Empress Convention Center, Chiang Mai, Thailand.

7. Thosaikham, W., Jitmanee, K.**,** Chantiratikul, A., Sittipout, R., **Maneetong, S.** & Chantiratikul, P. (2010) Selenium extraction using mixture of pepsin and cellulase for selenium-enriched Chinese kale (*Brassica alboglabra* L.H. Bailey). Pure and Applied Chemistry International Conference (PACCON). Oral presentation. 21st - 23rd January 2010, Ubon Ratchathani, Thailand.