Biofortification of Selenium in broccoli (*Brassica oleracea* L. var. *italica*) and onion (*Allium cepa* L.)

PRADIP ADHIKARI

NORWEGIAN UNIVERSITY OF LIFE SCIENCES
DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES (IPM)
MASTER THESIS 60 CREDITS 2012

“Biofortification of Selenium in broccoli (*Brassica oleracea* L. var. *italica*) and onion (*Allium cepa* L.)”

Master Thesis in Plant Science

(60 Credits)

By

Pradip Adhikari

Supervisors

Magnor Kåre Hansen

Espen Govasmark

Department of Plant and Environmental Sciences (IPM)

Norwegian University of Life Sciences

PO Box 5003, N-1432

Ås, Norway

June, 2012

ABSTRACT

Selenium (Se) is an essential trace element to animals and human. To increase the consumption of Se in the human, biofortification of Se is usually practiced which is the process of increasing the concentration of Se in the edible portion. The main aim of the study is to investigate the effect of biofortification of Se in onion and broccoli and to evaluate and compare the effect of Se in average yield, dry matter, ascorbic acid, total phenols, antioxidant, Se and S accumulation and accessibility of Se. Four treatments (control, 20 mg, 50 mg and 80 mg) of Se and three treatments (control, 20 mg and 50 mg) of Se were applied in broccoli and onion respectively. Sodium selenate was used as a source of Se. The three varieties Ironman, Lord and Marathon of broccoli and Summit, Hytec and Red Baron of onion were chosen for the experiment. The accumulation of Se concentration was increased with the increase in concentration of Se in the soil in both onion and broccoli without affecting the health related compounds. The accumulation of Se is higher in broccoli than in onion. In broccoli, the highest accumulation of Se concentration was 44.28µg g^{-1} in variety 'Ironman' when treated with 80 mg Se and the accumulation of Se in onion was in the range of 6.11 to 8.31 µg g^{-1} treated with 50 mg Se. The net accumulation of Se lied within the safe limit and thus safe for the consumption for human. The accessibility is higher in onion than in broccoli.

CONTENTS

ACKNOWLEDGEMENT

This master thesis has been written and submitted to Department of Plant and Environment Sciences (IPM) at Norwegian University of Life Science (UMB). First of all, I wish to express my sincere gratitude to my supervisor Associate Professor Magnor Kåre Hansen for his assistance in statistical analysis and valuable guidance. I am grateful and deeply indebted to my co-supervisor Researcher Dr. Espen Govasmark who has been a consistent source of information, and has provided detailed guidance in doing experiments, giving valuable comments and suggestions throughout the research which made the accomplishment of this work possible.

Thanks Karine Svinnset for providing the field information and assistance in doing lab work. Thanks for all of my friends who helped me by giving ideas and providing information in writing this thesis.

Last but not least, I would like to thank my family and especially my wife, Nirmala Adhikari, for their support and love.

Ås, June 2012.

Pradip Adhikari

ABBREVIATIONS

%	Percentage
0C	Degree Celsius
FRAP	Ferric Reducing Ability of Plasma
GPx	Glutathione peroxidase
GSH	Glutathione
GSLs	Glucosinolates
GSSG	Glutathione disulfide
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
LOD	Detection Limit
LOQ	Quantification Limit
OAS	*O*-acetylserine
ROS	Reactive Oxygen Species
S	Sulfur
Se	Selenium
SeCys	Selenocysteine
SeMet	Selenomethionine
SeMSC	Se-methylselenocysteine
SOD	Superoxide dismutases
TPTZ	Tripyridyltriazine

1. INTRODUCTION

Selenium (Se) is an essential trace element to human and animals discovered by Berzelius and Gahn in 1817 while examining the residue from the sulfuric acid plant in Sweden (Whanger 2002). Selenium is important for its antioxidant activity, therapeutic aspects, and chemo protective, anti-inflammatory and antiviral properties (Papp et al. 2007). Selenium is used as the trace element for the genetic code of newly discovered 21st amino acid, selenocysteine (SeCys). (Hardy & Hardy 2004). Diet supplement with a nutritional portion of Se is believed to reduce the incidence of prostate and skin cancer (Witherington et al. 1998). The recommended daily intake value of Se is 40 to 50 µg day^{-1} for adults in Norway (Becker et al. 2008) and daily intakes higher than 400 µg day^{-1} is believed to be toxic (Fordyce 2005).

1.1 Broccoli

1.1.1 History and botany of Broccoli

The genus *Brassica* (family Brassicaceae or Cruciferae) is a rich source of health affecting compounds and is widely considered as a staple food and ideal for plant science research. The genus is characterized into oilseed, forage, condiment, and vegetables crops on the basis of edible portion like buds, inflorescences, leaves, roots, seeds and stems (Cartea et al. 2010). It comprises of leading vegetable species like *B. oleracea* that are used as vegetable and forage crops such as kale, cabbage, broccoli, brussels sprouts, cauliflower and others; *B. rapa* which includes vegetable forms such as pak choi, Chinese cabbage and turnip along with forage and oilseed types; *B. napus* that includes oilseed (rapeseed), forage and vegetable types (leaf rape and nabicols) and mustard groups that are used as a condiment although leaves of *B. juncea* is consumed as vegetables in Asian countries e.g. Nepal (Table 1.1).

Broccoli (*Brassica oleracea* L. var. *italica*) is one of the important members of the Cole crops belonging to the Brassicaceae (=Cruciferae) family which is supposed to be the first of the Cole crops evolved from the wild species of kale or cabbage (Rubatzky & Yamaguchi 1997). It is believed to have evolved in the Eastern coast of the Mediterranean region and was introduced to Europe, especially to Italy, in the medieval times. It means “little sprouts” in Italian language.

Table 1.1 Main vegetable *Brassica* species, crops, and plant parts used for consumption (Cartea et al. 2010)

Species	**Group**	**Common name**	**Edible portion**
Brassica oleracea	*acephala*	Kale, collards	Leaves
	capitata capitata	Cabbage	Terminal leaf buds (heads)
	capitata sabauda	Savoy cabbage	Terminal leaf buds (heads)
	costata	Tronchuda cabbage	Loose heads
	qemmifera	Brussels sprouts	Vegetative buds
	botrytis botrytis	Cauliflower	Inflorescences
	botrytis italic	Broccoli	Inflorescences
	gongylodes	Kohlrabi	Stem
	albogabra	Chinese kale	Leaves
Brassica rapa	*chinensis*	Pak choi, bok choy	Leaves
	dichotoma	Brown sarson, toria	Seeds
	narinosa	Chinese flat cabbage, watacai	Leaves
	nipposinia	Mibuna, mizuna	Leaves
	oleifera	Turnip rape, rapeseed	Seeds
	perkinensis	Chinese cabbage, pe-tsai	Leaves
	perviridis	Komatsuna, Tendergreen	Leaves
	parachinensis	Choy sum	Leaves
	rapa	Turnip, turnip greens, turnip tops	Roots, leaves and shoots
	ruvo	Broccoleto	Shoots
	trilochularis	Yellow sarson	Seeds
Brassica napus	*pabularia*	Leaf rape, nabicol	Leaves
	napobrassica	Swede, rutabata	Roots
Brassica juncea	*rugosa*	Mustard greens	Leaves
	capitata	Head mustard	Heads
	crispifolia	Cut leaf mustard	Leaves

Broccoli was introduced to England in the 18th century as "sprouting cauliflower" or "Italian Asparagus" (Gray 1982). In green sprouting form when the terminal inflorescence is removed, secondary inflorescence will be developed in the axil of the lower leaves (Ockendon 1980); thus edible portion can be harvested over a prolonged time whereas the heading form produced a large, single, terminal curd/head. In Broccoli, the head consists of fully differentiated flower buds (Ockendon 1980).

Broccoli and cauliflower can be distinguished by their comparative morphology during the harvesting period. The head of broccoli is a mass of differentiated floral buds that will terminate less and develop more buds into flower whereas cauliflower head is a mass of proliferated floral tissue that will terminate more and generate few buds (10%) into flower (Gray 1982). Broccoli is genetically more mature than marketable cauliflower. Broccoli is green in color due to chlorophyll within the sepals of the floral buds which contrast with the white or cream color of cauliflower that lacks chlorophyll (Gray 1982). Primary inflorescence is the first harvest in broccoli. As the growth cycle extends, secondary inflorescence will develop in the leaf axil which add up to 30% of the total yield (Rosa et al. 2002).

1.1.2 Importance of broccoli

Broccoli (*Brassica oleracea* L. var. *italica*) is a cruciferous vegetable which is distinguished by the presence of health-promoting compounds like fiber, vitamin C, B1, E, carotenoids, glucosinolate, phenolic and selenium. These compounds are given attention due to their role in the prevention of cancer and cardiovascular diseases (Goncalves et al. 2011). Consumption of 150 gm of broccoli helps in fulfilling the requirement of adult's for vitamins E, A, B1 and C and enhances the immune system (Borowski 2008). Consumption of broccoli has been steadily increased due to its health promoting properties (Herr & Buchler 2010) and conscious of human towards health.

1.2 Onion

1.2.1 History and botany of Onion

Onion is a biennial vegetable crop that belongs to Liliaceae family. Onion develops vegetative growth at the first year of transplanting, and reproductive growth develops in the following year after vernalization. Onion (*Allium cepa* L.) is supposed to be originated in central Asia (Griffiths et al. 2002). The genus *Allium* is very large and includes onion (*Allium cepa* L), garlic (*A.*

sativum L), leek (*A. ampeloprasum* leek group), shallot (*A. cepa* Aggregatum group), multiplier onion, chives, bunching onion and other important species (Table 1.2). Onion (*Allium cepa*) is grown in many parts of the world. The production of onion is extended from the tropics to temperate regions in the countries ranging from the equator to Scandinavia and South Africa (Chope et al. 2011).

Table 1.2 Cultivated *Alliums* (Griffiths et al. 2002)

Species	Sub division	Horticultural name
A. cepa	*cepa*	Bulb Onion
	ascalonicum	Shallot
	aggregatum	Potato multiplier Onion
	proliferum	Tree onion
A. fistulosum		Japanese bunching (Welsh onion)
A. sativum		Garlic
A. ampeloprasum	*porrum*	Leek
	agyptiacum	Kurrat
A. schoenoprasum		Chives
A. chinense		Rakkyo
A. tuberosum		Chinese chives

Onion is a versatile vegetable which is consumed as fresh (bulbs and green salad onion) as well as in the form of processed products. More pungent varieties are dominant over sweet due to its more flavor to cooked dishes, have a longer shelf life and easier to handle (Griffiths et al. 2002). Onion is an important ingredient in all dishes around the world. Onion is usually fried, baked before consumption however sweet onion can be eaten raw due to its mild flavor.

1.2.2 Importance of Onion

Onion is widely used as a flavoring vegetable in different types of food. Onion is used as a source of vitamins and is useful in fever, dropsy, catarrh and chronic bronchitis (Stajner & Varga 2003). They can be used as a poultice to indolent boils, bruises, wounds to relieve burning sensations and applied to the navel for dysentery and fever (Stajner & Varga 2003). Onion is famous for its antimicrobial activity, antioxidant activity, anticarcinogenic and antimutagenic activities, and cardiovascular diseases (Corzo-Martínez et al. 2007). In China, onion along with garlic tea is recommended for fever, headache, cholera and dysentery (Ali et al. 2000).

1.3 Chemical and biological properties of Se

Chemical behavior of Se is similar with sulfur (S) and exists in the same oxidation states Se^{2-}, Se^0, Se^{4+}, Se^{6+} (Table 1.3). Seleno amino acids such as SeCys and selenomethionine (SeMet) is organic forms of Se which is incorporated to protein to form selenoprotein. Selenomethionine is the major seleno-compound in cereals grains and is stored in the storage proteins whereas Se-methylselenocysteine (SeMSC) is the major selenocompounds in Se enriched plants such as onion, garlic, broccoli florets and sprouts and wild leek (Whanger 2002).

Table 1.3 Chemical forms of selenium in the environment (Fordyce 2005)

Oxidative state	**Chemical forms**
Se^{2-}	Selenide (Se^{2-}, HSe^-, H_2Se_{aq})
Se^0	Elemental selenium (Se^0)
Se^{4+}	Selenite (SeO_3^{2-}, $HSeO_3^{2-}$, H_2SeO_{3aq})
Se^{6+}	Selenate (SeO_4^{2-}, $HSeO_4^{2-}$, H_2SeO_{4aq})
Organic Se	SeMet, SeCys

The chemical forms of Se in soils are presented in figure 1.1. Under oxidising condition, selenite ($Se4^+$) and selenate ($Se6^{+)}$ are the dominant inorganic forms of Se, but the distribution between them is pH dependent. Selenate is less adsorbed to the soil due to its lower affinity towards soil particles than selenite and in neutral to alkaline soils selenite is soluble, mobile and available for plant uptake (Fordyce 2005). In acid and neutral soils, selenite will form insoluble iron oxide and oxy-hydro complex that will bind selenite and thus the plant bioavailability of selenite is low. Elemental Se (Se^0), selenides (Se^{2-}) and Se sulfide salts tend to exist in reducing, acid and organic-rich soil. They have low solubility and oxidation potential which make them unavailable to plants and animals (Fordyce 2005).

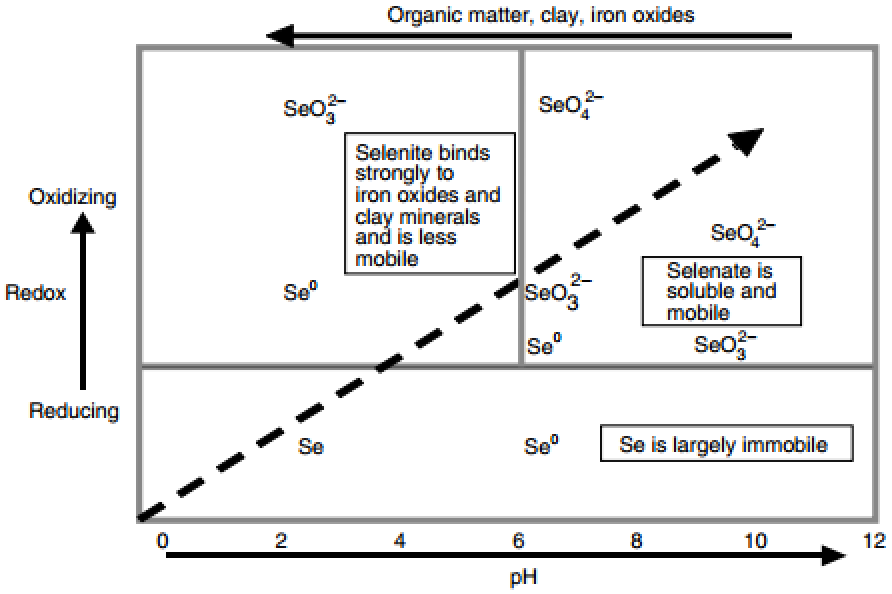


Fig: 1.1 Schematic diagram showing the main controls on the chemical speciation and bio availability of selenium in soils ---▸ increasing mobility (Johnson et al. 2010).

Selenomethionine is two to four times more available to plants than organic selenite whereas SeCys is less bioavailable than SeMet. Presence of clay content decreases the bioavailability of Se as the clay content increased adsorption on fine particles (Fordyce 2005).

1.4 Metabolism of Se and S in plants

The two elements Se and S have similar outer valence-shell electronic configuration. The atomic size, bond energy, ionization potential and electron affinity are almost similar. Selenium compounds are metabolized to more reduced states whereas S compounds are metabolized to more oxidized states (Whanger 2004). Initially in plants, sulfate and selenate share same metabolic pathway for uptake, assimilation and incorporation into *O*-acetylserine (OAS), resulting in the formation of Cys and SeCys, respectively (Fig: 1.2); (Hell 1997; Sors et al. 2005).

Sulfur (S) is an essential element for growth and the physiological function of the plants. Plants will take up S mainly as sulfate (SO_4^{2-}). It is reduced to sulfide (chemical form) and metabolized to organic compounds which will convert to proteins and secondary substances. ATP sulfurylase is the first enzyme in the sulfate assimilate pathway in plants which helps in the formation of adenosine phosphosulfate or 5'adenylylsulfate (APS); (Hell 1997). The reduction of sulfate to sulfide and its subsequent transformation into Cys takes place mainly in the shoot in chloroplast. Root plastids contain all sulfate reduction enzymes (HSU et al. 2011).

Selenium and S share common metabolic pathway in plants due to the same chemical and physical similarity. This affects the uptake, transport and assimilation throughout the plant growth. The main soil-plant available form of Se is selenate which is actively taken up via sulfate transporter (Terry et al. 2000). The assimilation of selenate to form SeCys and SeMet involve enzymes ATP sulfurylase, APS reductase and O-acetyl serine transferase as presented in figure 1.2.

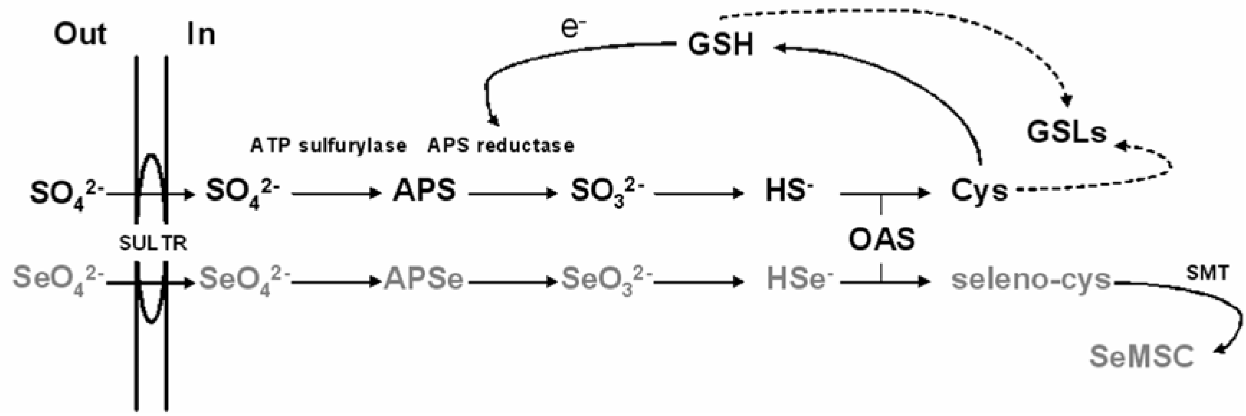


Fig: 1.2 Current models of sulfate and selenate uptake and assimilation pathways in selenate-fertilized broccoli plant. Sulfate transporter (SULTR); 5'adenylylsulfate (APS); 5'adenylylselenate (APSe); O-acetylserine (OAS); cysteine (Cys); glutathione (GSH); glucosinolates (GSLs); selenocysteine methyltransferase (SMT); Se-methylselenocysteine (SeMSC); (HSU et al. 2011).

From the ability to assimilate and accumulate Se, plants can be divided into three groups: non-accumulators, Se-indicators (or secondary Se-accumulators) and Se-accumulators (Rayman 2008). Non-accumulator plants are unable to grow on seleniferous soils, and Se is toxic at tissue concentration as low as 10-100 µg Se g^{-1} dry weight. Cereal crops such as wheat, oats, rye and barley are non-accumulators. Se-indicator (secondary Se-accumulators) plants can colonize both seleniferous and non-seleniferous soils and tolerate Se concentrations pending 1000 µg Se g^{-1} dry weight. Most of *Brassica* species (e.g. Broccoli) and *Allium* species (e.g. Onion) are Se-accumulators. Se-accumulators can accumulate up from 1,000 to 10,000 µg Se g^{-1} dry weight when grown in Se-rich soil. *Bertholletia excelsa* producing Brazil nuts is a Se-accumulators plant.

1.5 Deficiency and toxicity of Se

Selenium (Se) is beneficial to human and other animal health in trace amount but is toxic in excess. Since it has a very narrow range between dietary deficiency (<40 µg day^{-1}) and toxic levels (> 400 µg day^{-1}), it is necessary to control intakes for both human and animals (Fordyce 2005). Selenium is taken up from the soil and enters the food chain through plants. Therefore, the deficiency of Se has been noticed in parts of the world where there is low content of Se in soil (Rayman 2000). Several studies have shown that the deficiency exists even when Se is in an adequate amount. It is expected due to the lack of bio-available form to plants and animals (Fordyce et al. 2000).

Keshan disease is one of the endemic cardiomyopathy (heart disease) caused by the deficiency of Se, which mainly affects children and women of childbearing age in China. The disease derived its name as it was an outbreak in Keshan Country, northeast China in 1935. The visible symptoms of this disease are that the heart cannot function properly or chronic moderate-to-severe heart enlargement and result in death. Keshan disease occurs in areas where the concentration of Se in the soil, food supply and human plasma are all low. The average intake of Se in Keshan disease endemic areas has been estimated at 10µg day^{-1}(Tapiero et al. 2003). The deficiency of Se cause white muscle disease in animals which is characterized by muscular weakness and muscular dystrophy. The other deficiencies include reduces appetite, poor growth and reproductive function and embryonic abnormalities (Fordyce 2005).

At higher dosages Se may be toxic for all organisms. Toxic concentrations cause garlic breath, hair and nail loss, disorders of the nervous system and skin, poor dental health and paralysis in human (Johnson et al. 2010). Excess Se cause death of aquatic birds, malformations of birds embryos and poisoning of fish in Kesterson Wild-life Refuge and Reservoir “in California” (Arteel & Sies 2001).

1.6 Generation and scavenging of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) is composed of free radicals (atom or molecule with one or more unpaired electron such as superoxide ($O_2^{\cdot}$-) and hydroxyl radicals ($OH^{\cdot}$)), and non-radical species such as hydrogen peroxide (H_2O_2). In health, there is a balance between ROS and nonenzymatic antioxidant system that scavenge or reduced ROS concentrations (Gutteridge & Halliwell 2000). ROS is continuously produced in the respiratory chain of mitochondria by one electron reduction of molecular oxygen. NAD(P)H oxidases, xanthine oxidase, lipoxygenase are the major enzymatic sources of ROS in the mammalian cells (Steinbrenner & Sies 2009). Redox imbalance caused by reduced antioxidant reserve or increased ROS production causes oxidative stress. Oxidative stress is the damage of DNA, lipids, proteins and membranes, and early stage for many chronic disease (Shah & Channon 2004). Therefore, increasing the exogenous antioxidants such as carotenoid and tocopherol reduces the risk for chronic diseases. Broccoli is the rich sources of both antioxidant vitamin and nonessential nutrients including polyphenols that have been proposed for the supplement effect of endogenous antioxidant (Prior 2003).

1.7 Protective role of Se

Superoxide dismutases, catalase and glutathione peroxidases (GPx) are the important antioxidant selenoenzymes in humans (Steinbrenner & Sies 2009). One of the important ROS scavenging mechanisms of Se is the catalysis reaction of the antioxidant enzyme, GPx which is shown in the Equation 1. In the first step, SeCys R-SeH (selenol) reacts with organic hydrogenperoxide (ROOH) to form selenenic acid (R-SeOH) and the corresponding alcohol (ROH). Selenenic acid produced in equation 1 (a) reacts with glutathione (GSH) and produce selenosulphide (R-Se-SG) and water. Selenosulphide reacts with GSH and produce selenol and Glutathione disulfide (GSSG). The overall reaction was carried out by GPx. Another antioxidant protein GSSG reductase (GR) in the GSR system reduced GSH with producing $NADPH^+$.

Equation1. ROS scavenging mechanism catalyses by GPx (Arteel & Sies 2001)

a) $R-SeH + ROOH \longrightarrow R-SeOH + ROH$

b) $R-SeOH + GSH \longrightarrow R-Se-SG + H_2O$

c) $R-Se-SG + GSH \longrightarrow R-SeH + GSSG$

d) $ROOH + 2GSH \xrightarrow{GPx} R-OH + H_2O + GSSG$

e) $GSSG + NADPH \xrightarrow{GR} 2GSH + NADP^+$

1.8 Health related compounds in broccoli and onion

1.8.1 Vitamin C

Vitamin C is the name given for all compounds exhibiting the biological activity of L-ascorbic acid (Lee & Kader 2000). The ascorbic acid cannot synthesize by our human body. Therefore, it is essential for human (Benzie 2000). Fruits and vegetables are the major dietary source of vitamin C for the human body. More than 90% of vitamin C in the human diet is supplied by fruits and vegetables. It is water soluble and is present as L-ascorbic acid and its oxidation product dehydroascorbic acid (Podsedek 2007).

Ascorbic acid is antioxidant vitamin. It helps in synthesis of collagen which is the main component of blood vessel, bone, tendon and ligament. It is found throughout the body and helps to separate the skeletal and smooth muscle cells. The deficiency of ascorbic acid leads to anemia, poor healing and fragility of blood vessels. Other deficiency includes bleeding of gums, swollen and painful joints and rough skin (Lee & Kader 2000).

Adverse handling during harvesting and storage cause destruction of vitamin C. Increasing storage time, higher temperatures, low relative humidity, physical damage and chilling injury cause increase loss of vitamin C (Lee & Kader 2000). Vallejo et al. (2002) found that there was a significant difference in vitamin C among different varieties in broccoli. In an experiment to show the effect of selenium spraying on green tea quality, vitamin C content of green tea was significantly increased by Se spraying (Hu et al. 2001).

1.8.2 Antioxidant

Reactive oxygen species (ROS) or free radicals contain one or more unpaired electrons. ROS is generated because of aerobic respiration or from external sources such as pollution or chemicals. Free radicals react quickly with other compounds and gain electrons and become stable. When the molecules loose electron it becomes free radical itself and start a chain reaction. The result is the disintegration of cell membrane, oxidation of cellular components like DNA and proteins (Kaur & Kapoor 2001).

Antioxidant neutralizes the effect of free radicals by offering one of its own electrons. Antioxidants are safe in either forms, the contribution of electron to free radical will not lead antioxidant to free radical. Antioxidants act as scavengers and protect the body from oxidative damage. Thus, antioxidants are defined as the substance that are capable of stabilizing free radicals either donating a single electron or receiving a single electron from the free radical (Kaur & Kapoor 2001). Antioxidants are classified into two types according to mechanistic categories. They are preventive antioxidants and chain-breaking antioxidants. The preventing antioxidant inhibits formation of ROS. This group of antioxidants includes catalase, peroxidase, SOD. Chain-breaking antioxidants scavenge oxygen radicals and start oxygen radical sequence. Common antioxidants in this group include vitamin C, vitamin E, polyphenols, GSH (Ou et al. 2002).

1.8.3 Phenolic compounds

Phenolic compounds are large and chemically diverse group of secondary metabolites in plants. They are composing of one or more aromatic rings bearing one of more hydroxyl groups that act as protection role against oxidative damage diseases like coronary heart disease, stroke and cancer (Bravo 1998). In plants, they act as an antioxidant, defense response against insects, fungi, virus and bacteria, mechanical support, attractants for insects which assist in pollination and seed dispersal and protect cell from UV radiation (Parr & Bolwell 2000). The content of phenolic and the level of consumption influence the total phenolic in human (Parr & Bolwell 2000). According to Chu et al. (2002) broccoli and onion rank first and third position respectively in the total phenolic content among the 10 most vegetables consume in United states. Genetic and environmental conditions affect the content of phenols (Robbins et al. 2005).

Flavonoids are the extensively studied group of phenolic compounds. Flavonoids are often built upon a C6-C3-C6 flavone skeleton (Fig: 1.3).

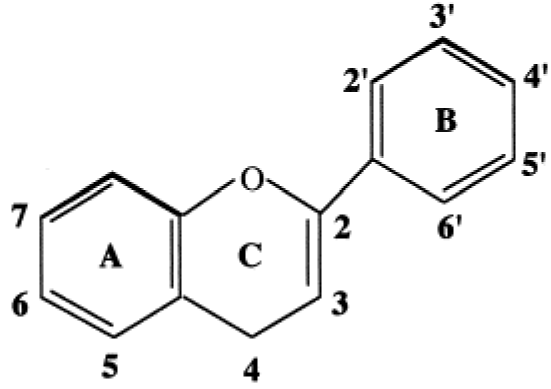
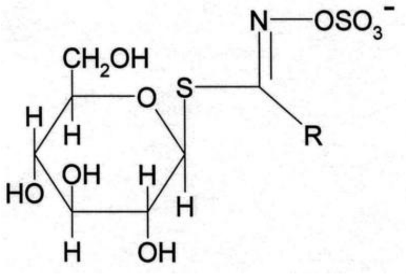


Fig: 1.3 Basic flavonoid structure (Aherne & O’Brien 2002)

Flavonoids act as disease resistance and UV-B (280-315 nm) radiation protection to plant and plant tissue respectively (Harborne & Williams 2000). Common flavonoids that are found in dry peel of onion are quercetin, quercetin glycoside (Singh et al. 2009). Quercetin and kaempferon are found in outer and aerial tissue of skin and leaves of onion and broccoli (Manach et al. 2004). Anthocyanin is the important flavonoid which is responsible for red, blue and purple pigments in plant. Anthocyanin is present in leaves, stems, seeds and root tissue. Cyanidin is found in broccoli (Manach et al. 2004). Finley et al (2000) observed that Se content greatly decreased total phenolic acid production in broccoli.

1.8.4 Glucosinolates

Glucosinolates is nitrogen and sulfur containing natural compounds found in the *Brassicas* (Fig: 1.4). At least 120 chemically distinct glucosinolates are identified in plants (Fahey et al. 2001). Glucosinolates consist of the core structure containing a β-D-thioglucose grouping linked via a sulfur atom to a (Z-N-hydroximio sulfate ester. They are distinguishing from each other by a variable R group. Glucosinolates is hydrolyzed following the destruction of plant tissue. The hydrolyzed product consists of thiocyanate, iso thiocyanate and nitriles (Brown et al. 2003), (Fig:1.5) which has anticarcinogenic properties, and contribute to the characteristic odor and flavor in many *Brassicaceae* (Baik et al. 2003).



¨

Fig: 1.4 Structure of glucosinolate (Wold 2004)

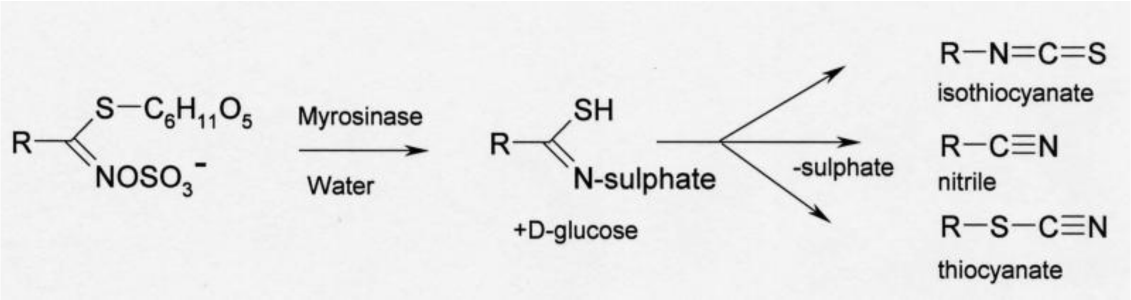


Fig: 1.5 Schematic diagram of hydrolysis of glucosinolates (Verkerk et al. 2001).

Brassica vegetables, including broccoli, are the important dietary source for glucosinolates. Induction of phase II enzymes glutathione S-transferase and quinone reductase are involved in detoxification of carcinogens. Studies have shown that the breakdown products sulphoraphane and indole-3-carbimol which are released by the glucosinolates glucoraphanin and glucobrassicin respectively are strong phase II enzyme inducers (Verkerk et al. 2001).

1.9 Biofortification

There is variability of Se content in soil in a different part of the world. Large areas of Middle and Northern Europe have soils with low Se concentrations or low Se-bioavailability (Stadlober et al. 2001). The concentration of Se in Norwegian farmland soils is generally very low (0.3 ppm; Wu & Låg 1988). If the soil contains low Se, the growth and development of the plant will not be affected but will absorb low to no nutritionally significant content of Se for human. (Spallholz et al. 2004). Biofortification with Se is the process of increasing the Se concentration in edible portions through Se fertilization (Zhu et al. 2009). Biofertification of crops with Se supplemented fertilizers has been practiced successfully from 1984 in Finland in order to increase the human Se intake (Eurola et al. 1991). Biofortification of crops are usually done by selenate fertilizers rather than selenite because selenate is taken up by roots of the plants ten times more effectively than selenite due to its high bioavailability of soils (Stadlober et al. 2001).

1.10 Reasons for choosing broccoli and onion for biofortification

Broccoli and onion are the major vegetables than can be eaten as raw as well as in processed form. Broccoli is rich in health promoting compounds like glucosinolate, antioxidant, phenolic compounds (Goncalves et al. 2011) whereas onion possesses antioxidants, flavonoids like quercetin, kaempferol, myricetin and catechin (Yang et al. 2004). These compounds assist in reducing the ROS that is generated inside the body and protect the body from oxidative damage. Broccoli and onion are efficient in accumulation of Se in the plants when they are grown in seleniferous soil. Therefore, biofortification of Se is a good strategy to increase the Se content of broccoli and onion to give them additional health benefits.

1.11 Bioaccessibility of Se

The chemical forms of Se present in the edible portion of broccoli and onion affects its bioaccessibility. The determination of the total content of Se is not enough to evaluate its bioavailability. *In vitro* experiments are alternative to human studies and provide a good estimation. *In vitro* experiments are faster, cheaper and simpler than *in vivo* experiments (Pedrero et al. 2006) but they need to be validated by *in vivo* experiments in order to verify the results.

1.12 Objective

The aim of the present study is to evaluate and compare the effectiveness of biofortification of Se on the yield and on the content of ascorbic acid, total phenols, antioxidant effect measured with FRAP methods, accumulation of Se and S and to estimate the bioavailability of Se in onion and broccoli by *in vitro* experiment, commonly named the accessibility of Se.

2. MATERIALS AND METHODS

2.1 Field experiment

2.1.1 Site description and experimental design

Field experiments were conducted at Vollebekk, UMB, Ås Norway on a loamy soil classified as a Typic cryaquept in soil taxonomy (Sogn et al. 2007). The experimental sites were situated at ($59^{o}39^{'}$ N) latitude and ($10^{o}44^{'}$E) longitude with an altitude of 70 m above sea level. Experiments were carried out according to factorial complete block design with four replications and free randomization of varieties and Se levels within blocks. There are four treatments in broccoli and three treatments in onion. The outdoor growth season lasted from May 25, 2010 to September 3, 2010.

The three varieties Ironman, Lord and Marathon of broccoli along with Summit, Hytec and Red Baron of onion were chosen for the experiment as they are common cultivars in Norway.

2.1.2 Weather data

Monthly average temperature (^{o}C) and total monthly rainfall (mm) from the transplanting to harvest are presented in the table 2.1. The monthly average of rainfall for the location was 67.28 mm during the year 2010. The average rainfall during the growing season was 99.46 mm which is higher than the average rainfall during the year 2010 (Hansen & Grimenes 2011).

Table 2.1 showing monthly average temperature and total rainfall recorded at the local weather station during the field experiment.

Month	Average temperature (^{o}C)	Total rainfall (mm)
May	9.4	91
June	13.9	62.6
July	16.8	100.7
August	15.3	149.5
September	10.3	93.5

2.1.3 Transplants, planting distance and plot size

Broccoli transplants were raised in a green house in trays and transplanted when they had reached 5-7 leaves stage with a density of 3.84 plants m^{-2}. The individual plot size was 1.95 m x 4.4 m = 8.58 m^2. The distance between rows was 0.65 m with 0.40 m between plants within a row.

The onion sets were directly planted in the field. The individual plot size was 1.95 m x 1.1 m= 8.58 m^2 with a density of 15.38 plants m^{-2}. The distance between rows was 0.65 m with 0.10 m between plants within a row.

There were 3 rows in each plot and 10 plants in each row. The experimental field of broccoli and onion are shown in figure 2.1 and 2.2 respectively.

Fig: 2.1 Experimental field of broccoli during growing

Fig: 2.2 Experimental field of onion during growing

2.1.4 Fertilization, herbicide treatment and irrigation

The broccoli field was applied a basal dose of 1 kg solubor/daa, 160 kg dolomittkalk/daa and 130 kg N-P-K 11-5-18/daa prior to planting. Side dressing with 20 kg $Ca(NO_3)_2$ /daa (15.5 % N) was applied 4 weeks after transplanting. It rained heavily on the same day of side dressing therefore it was sprayed 20 kg $Ca(NO_3)_2$/daa after 5 weeks of transplanting. The field was treated with lentagran as herbicide 4 weeks after transplanting (Table 3.1).

The onion field was applied a basal dose of 100 kg/daa of N-P-K 11-5-18 prior to planting. Side dressing of fertilizer was done by 40 kg $CaNO_3$/daa 2 and half month after transplanting.

Irrigation was given every 15 days intervals and was stopped before 1 weeks of harvesting.

2.1.5 Selenium treatments

Selenium was supplied as sodium selenate in the experimental fields.

In broccoli, Se was applied at the concentration of 0, 20 mg, 50 mg and 80 mg per meter square. Selenium solution of 500 ml was prepared and was sprayed in each plot six weeks after transplanting.

In onion, Se was applied at the concentration of 0, 20 mg and 50 mg per meter square in each plot one and half month after sowing. Selenium solution of 1.2 l was prepared in the garden pot and was applied in each plot one and half month after sowing.

2.1.6 Harvesting and Sampling procedure

The plants of broccoli did not mature evenly so they were harvested on different days. The harvesting started in August on day 51 of growth after transplanting and was completed on day 58 after transplanting. The head of the broccoli before harvesting is shown in figure 2.3. The harvesting of onion was in September after 109 and 110 days of growth. The summary of the all the cultural practices are shown in table 2.2.

Fig: 2.3 Broccoli head during the time of harvest

Table 2.2 Seed sowing, planting date, intercultural operations, Se treatment and harvesting date in broccoli and onion during the year 2010

Crop	Seed Sowing	Planting	Side dressing with fertilizer	Herbicide treatment	Selenium treatment	Harvesting
Broccoli	7 June	1 July	July 27; 6 August	27 July	12 August	20-27 August
Onion	-	25 May	11 Aug.	-	9 July	2-3 September

Eight plants of broccoli and onion in the middle rows were harvested for chemical analysis and yield determination. The two heads of broccoli and three bulbs of onion were randomly selected and cut into equal halves. The first halves were frozen in liquid nitrogen and stored at -80^{o}C prior to analysis of Se and S for the assessment of bioaccessibility of Se. The other halves were stored at -50^{o}C for the analysis of L-ascorbic acid, dry matter, FRAP and total phenols in broccoli and dry matter, FRAP, total phenols in onion.

2.2 Chemical Analysis

2.2.1 Selenium and Sulfur

2.2.1.1 Instrumentation

Inductively coupled plasma – mass spectrometry (ICP-MS) (Perkin Elmer – SCIEX ELAN 6000) was used for the determination of total Se in plant tissue and in the enzymatic extracts. The Se analyses were performed in a 5% acid and 2% ethanol solution. An ICP-Optical emission spectrometer (Perkin Elmer Optima 5300 DV) was used for total S measurements in a 10% HNO_3 solution.

2.2.1.2 Sample preparation

The onion and broccoli were freeze dried, grinded and homogenized prior to analysis. Approximately 0.2 g dry sample were mineralized in 5ml HNO_3 at 250^{o}C for 15 min in a microwave (Ultra CLAVE, Milestone) for the determination of total plant element concentrations. The temperature and time relationship inside the microwave is presented in the table 2.3. De-ionized water (18 MΩcm^{-1}) was used throughout the analysis. All samples were added 250 µg of an internal standard prior to acid mineralization to a concentration of 20 µg Tellur (Te) L^{-1} in the analyzed sample. Mineralized samples were diluted to 50 ml prior to analysis.

Table 2.3 for Ultra Clave procedure

Temperature (^{o}C)	Time (min)
Up to 50	5
50	10
Up to 105	10
105	10
Up to 250	32
250	15

2.2.1.3 Quality assurance

The three standard reference materials NIST 1567a Wheat Flour and 1570a Spinach (from the National Institute of Standards and Technology, Gaithersburg, MD) and NCS ZC73013 Spinage (from the China National Analysis Center for Iron and Steel 2010) were used for method verification. Six blanks were run for the determination of the detection limit (LOD) and quantification limit (LOQ) of the methods used. Tellur was used for the instrument performance control for the analysis on ICP-MS.

2.2.2 Enzymatic extraction

Precisely 0.5 g of grinded sample of broccoli and onion were added with 10 ml of 100 mM Tris-HCl; pH 7.5 and 0.5 mg of 20 mg of protease XIV from *Streptomyces griseus* (Sigma-Aldrich) and 10 mg of lipase VII from *Candida rugosa* (Sigma-Aldrich). The samples were placed in a water bath at 37^{o}C for 6 hr and continuously shaken. The samples were centrifuged with Beckman JC-MC centrifuge at 10,000 x g for 10 min at 5^{o}C. Four to five ml of supernatant was sampled for determination of total Se in the enzymatic extracts. One ml of extract was added 5 ml of HNO_3 and 250 µl of internal standards and heated to 80^{o}C for 48 h. The samples were diluted to 50 ml prior to analysis.

2.2.3 Analysis of L-Ascorbic acid

Fifty g of frozen samples were made up to 150 g after adding 1% oxalic acid. The samples were homogenized for 1 min with hand blender and filtered through Whatman folded filter paper (112

V, Maidstore, England) and applied onto a Sep-pack C-18 column (Waters, Ireland). It was activated using 5 ml methanol followed by 5 ml Milli-Q water and washed with 2 ml of sample. The samples were filtered through a 0.45µm syringe filter (Millipore, Carrigtwohill, Ireland) before injection. HPLC analysis was performed as described by (Williams et al. 1973) using an Agilent Technologies comprising HP1100 liquid chromatography, auto sampler and UV detector (Agilent Technologies, Oslo Norway). Monitoring of the chromatography and data processing were performed by means of Chemstation software. Separation was achieved by using a 250 x 4.6 mm Zorbax SB-C18 5 µm column (Agilent technologies). The mobile phase was 0.05 M KH_2PO_4 for isocratic elution at 1 mL min^{-1} and 25^oC. The injection volume was 5 µl and the time was set to 5 min. L-ascorbic acid was measured at 254 nm. The calibration was performed by a reliable standard of L-ascorbic acid in 1% of oxalic acid.

2.2.4 Ferric Reducing Ability of Plasma (FRAP)

The Ferric Reducing Ability of Plasma (FRAP) assay is used to measure the concentration of total antioxidant. The method is based on the colour changes appear when the TPTZ-Fe^{3+} (2, 4, 6-tripyridyl-s-triazine) complex is reduced to TPTZ-Fe^{2+} form in the presence of plasma antioxidant. An intense blue color with the absorption maximum at 593 nm develops. The measurements were made at 600 nm. An aqueous solution of 500µM $FeSO_4$ x $7H_2O$ was used for the calibration of the instrument (Benzie & Strain 1996). 2,4,6-tri-pyridyl-s-triazine (TPTZ) Sodium acetate, acetic acid glaciale, $FeCl_3$ x $6H_2O$, $FeSO_4$ x $7H_2O$, MilliQ water, Trolox and methanol of HPLC- grade was used for all extractions.

Analysis of the antioxidant was determined using a FRAP (Ferric Reducing Ability of Plasma) assay on a Konelab 30i (Thermo Electron Corp. Vantaa, Finland). Three g of frozen samples were homogenized, and 30 ml of 10 mM HCl was added. The samples were sonicated on a water bath at 0^o C for 15 min after it was vortexed for 30 seconds. Two ml of samples were centrifuged at 13,200 (rpm) for 2 min at 4^oC. The concentration of total antioxidant was measured in triplicates and was expressed in µmol g^{-1} dry weight.

2.2.5 Total Phenols

The amount of total phenolics in broccoli and onion was determined with the Folin-Ciocalteu's reagent (FCR) on a konelab 30i (Thermo Electron Corp. Vantaa, Finland) using gallic acid as a standard.

Three g of frozen samples were homogenized, and 30 ml of 10 mM HCl was added. The samples were sonicated on a water bath at 0^o C for 15 min after it was vortexed for 30 seconds. Two ml of samples were centrifuged at 13,200 (rpm) for 2 min at 4^oC.

Samples (2 ml) were introduced into test cuvettes, and then 100 µl FC- reagents and 80 µl of sodium carbonate solution (7.5%) were added. The absorbance of all the samples was measured at 765 nm after incubating at 37^oC for 60 s. The result of total phenols was expressed as milligrams of gallic acid equivalent (GAE) per 100 gram of fresh weight.

2.2.6 Dry matter

Dry matter was determined by drying 6 gm of homogenized material for 24 h at 100^oC and weighing after become stabilizing at room temperature in an exsiccator. The dry matter is presented in percentage.

2.3 Data analysis

Data were analyzed by analysis of variance (ANOVA) using Minitab statistical software version 16 and differences among treatment means were tested by general linear model at $P=0.05$. All results were expressed as means with corresponding standard errors.

3. RESULTS

3.1 Broccoli

3.1.1 Fresh weight (yield) m^{-2} and dry matter

There were no significant differences in the total yield m^{-2} between the varieties (Table 3.1). Variety 'Ironman' had a tendency of highest yield m^{-2} when it was treated with 50 mg Se; 'Marathon' had a tendency of highest yield m^{-2} in the control and 80 mg Se and 'Lord' had a tendency of highest yield in the 20 mg Se treatment. The treatment of Se did not affect the total yield m^{-2} in the varieties.

Table 3.1 Total yield m^{-2} in g in three different varieties of broccoli at four different treatments of Se

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
	Fresh weight (yield) m^{-2}			
Ironman	770.2 ± 57.8a	820.4 ± 106.5a	920.5 ± 99.1a	797.6 ± 70.4a
Lord	755.4 ± 51.8a	947 ± 216a	819.0 ± 52.4a	851.2 ± 88.3a
Marathon	880.7 ± 93.9a	862.3 ± 184.4a	802.1 ± 26.8a	913.9 ± 144.0a

Error bars are the standard error of means (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

There was no significant difference in the dry matter between the varieties (Table 3.2).

Table 3.2 Dry matter content in percentage in three varieties of broccoli at four treatments of Se

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
	Dry matter in percentage (%)			
Ironman	9.29 ± 0.54a	8.89 ± 0.55a	9.19 ± 0.21a	9.21 ± 0.55a
Lord	8.86 ± 0.54a	8.55 ± 0.49a	8.62 ± 0.70a	8.47 ± 0.43a
Marathon	8.57 ± 0.18a	8.48 ± 0.41a	8.98 ± 0.26a	8.90 ± 0.19a

Error bars are the standard errors of means (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

The variety 'Ironman' had the inclination of highest content of dry matter when it was treated with 20 mg Se and 80 mg Se. The variety 'Lord' had the highest inclination when it was treated with 50 mg Se. The Se treatment did not affect the dry matter of broccoli.

3.1.2 Selenium

The Se concentration increased with increasing Se fertilization for all varieties (Fig 3.1). There was a significant difference in the Se concentration between the varieties with the 80 mg Se treatment. The variety 'Ironman' had the highest concentration of Se when it was treated with 20 mg and 80 mg of Se being 9.19 and 44.28 µg g^{-1} respectively whereas variety 'Marathon' had highest Se concentration in the control and 50 mg Se being 0.27 and 22.25 µg g^{-1}.

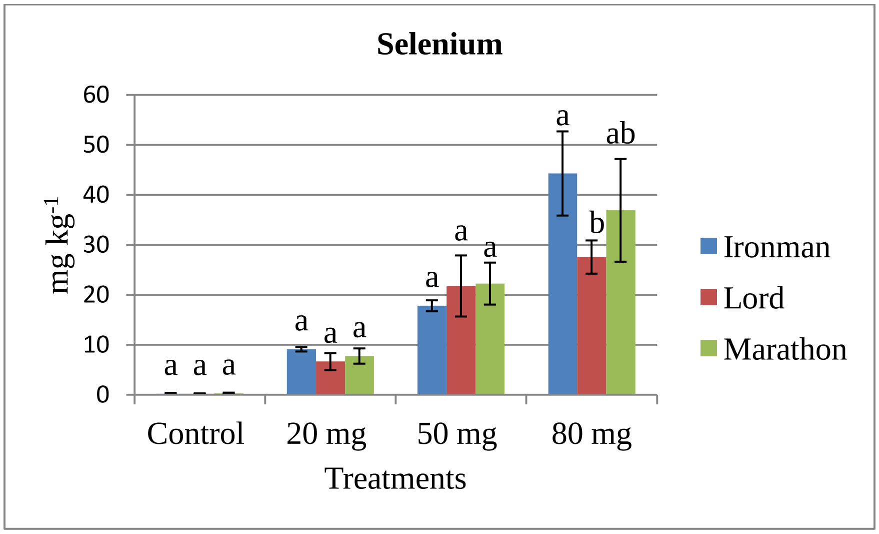


Fig: 3.1 Diagram showing Se concentration between treatments in broccoli varieties. N=4. Error bars indicate standard error of the mean. The mean values within one treatment followed by the same letter are not significantly different at P=0.05.

3.1.3 Sulfur

There was no significant difference in the concentration of S between the varieties (Table 3.3). The variety 'Ironman' had a tendency of highest concentration of S when it was treated with 20 mg Se; variety 'Marathon' had the tendency of highest S concentration in 50 mg Se whereas variety 'Lord' had a tendency of highest S concentration when it was treated with 80 mg Se and in control. The treatment with Se did not affect the concentration of S.

Table 3.3 Concentration of S in μg g^{-1} in different varieties of broccoli at different Se treatments

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
		μg S g^{-1}		
Ironman	36.50 ± 1.73a	39.00 ± 4.24a	39.50 ± 4.80a	37.50 ± 5.07a
Lord	35.70 ± 3.9a	34.50 ± 1.73a	37.00 ± 2.94a	39.00 ± 5.72a
Marathon	37.29 ± 2.20a	37.00 ± 3.27a	35.25 ± 5.12a	34.75 ± 3.59a

Error bars are the standard error of means (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.1.4 L-Ascorbic acid

There was a significant difference in the content of ascorbic acid between the varieties (Fig: 3.2). The variety 'Ironman' had significantly higher content of ascorbic acid in all treatments. The treatment of Se did not affect the content of L-ascorbic acid in the varieties. The ascorbic acid content did not increase in all the treatments of Se. The variety 'Ironman' had significantly higher content of ascorbic acid in all treatments. The treatment of Se did not affect the content of L-ascorbic acid in the varieties. The ascorbic acid content did not increase in all Se treatments.

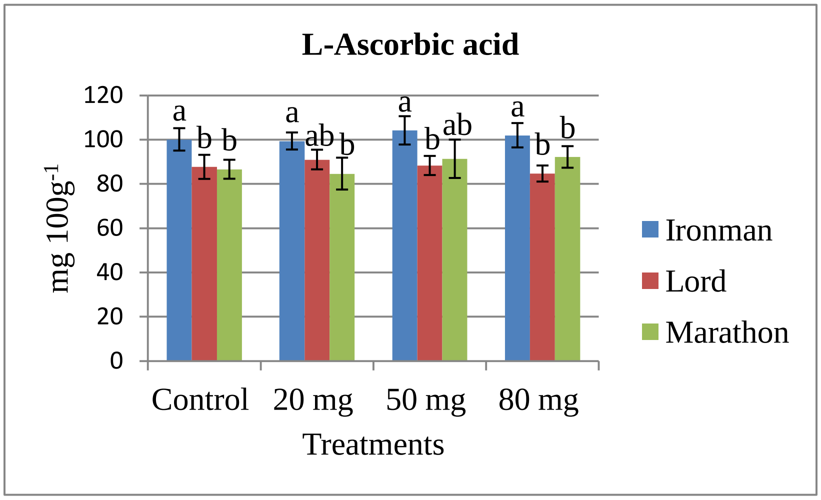


Fig: 3.2 Ascorbic acid content between treatments of broccoli varieties. (N=4). Error bars indicate standard error of the mean. The mean values within one treatment followed by the same letter are not significantly different at P=0.05.

3.1.5 Total Phenols

There was no significant difference in the content of total phenols between the varieties (Table 3.4). The variety 'Lord' had the inclination of highest content of total phenols in the control and the treatments with 20 mg Se and 50 mg of Se whereas variety 'Ironman' had the inclination of highest content of total phenol when it was treated with 80 mg of Se. The treatment of Se did not affect the content of total phenols in all treatments.

Table 3.4 Total phenols in mg GAE $100g^{-1}$ in three different varieties of broccoli at four different treatments of Se

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
	Total phenols in mg GAE $100g^{-1}$			
Ironman	85.04 ± 5.80a	82.48 ± 8.31a	84.58 ± 8.56a	79.54 ± 16.36a
Lord	80.32 ± 13.79a	82.83 ± 12.43a	75.75 ± 14.10a	72.56 ± 7.47a
Marathon	74.99 ± 9.09a	73.10 ± 7.94a	78.05 ± 11.55a	68.69 ± 5.41a

Error bars are the standard error of the mean (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.1.6 FRAP

There was no significant difference in FRAP assay between the varieties (Table 3.5).

Table 3.5 FRAP assay in µmol $100g^{-1}$ in three different varieties of broccoli at four different treatment level of Se

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
	FRAP in µmol $100g^{-1}$			
Ironman	0.89 ± 0.09a	0.85 ± 0.15a	0.92 ± 0.19a	0.78 ± 0.25a
Lord	0.84 ± 0.24a	0.88 ± 0.15a	0.71 ± 0.18a	0.70 ± 0.09a
Marathon	0.74 ± 0.13a	0.72 ± 0.15a	0.78 ± 0.21a	0.59 ± 0.05a

Error bars are the standard error of mean (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

The variety ‘Ironman’ had comparatively higher inclination value of antioxidant measured by FRAP when it was treated with 80 mg Se. The variety ‘Lord’ had highest inclination value of FRAP in the control and variety ‘Marathon’ had the highest inclination value of FRAP when it was treated with 50 mg Se. The treatment with Se did not differ significantly in the value of FRAP.

3.1.7 Accessibility of Se

There was no significant difference in the accessibility of Se in broccoli between the varieties (Table 3.6). The variety ‘Marathon’ had a tendency of higher accessibility in the 20 mg and 50 mg Se treatments whereas variety ‘Lord’ tended to have highest accessibility in the 80 mg Se treatment. The accessibility was not significant different between the treatments. In case of control, we could not determine the accessibility as the Se concentration in the extracts was below the limit of quantification for the ICP-MS with the method used.

Table 3.6 Accessibility of Se in three different varieties of broccoli in four different treatments of Se

	Treatments			
Varieties	Control	20 mg Se	50 mg Se	80 mg Se
	Accessibility of Se in percentage (%)			
Ironman	ND	75.92 ± 15.10a	56.93 ± 15.53a	67.59 ± 10.20a
Lord	ND	69.73 ± 4.37a	55.35 ± 26.45a	80.15 ± 2.10a
Marathon	ND	79.49 ± 4.96a	77.96 ± 4.16a	77.62 ± 2.79a

Error bars are the standard error of mean (n=4); ND= not defined and the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.2. Onion

3.2.1 Fresh weight (Yield m^{-2}) and dry matter

There were no significant differences in the total yield m^{-2} between the varieties (Table 3.7). The variety ‘Hytec’ had a tendency of highest yield m^{-2} when it was treated with of 20 mg Se and in the control variety ‘Summit’ had a tendency of highest yield m^{-2} in the treatment of 50 mg Se. The treatment of Se was significant in the total yield m^{-2} in the variety ‘Summit’.

Table 3.7 Total yield m^{-2} in g in three different varieties of onion at three different Se treatments

	Treatments		
Varieties	Control	20 mg Se	50 mg Se
	Fresh weight (yield m^{-2}) in g		
Summit	634.6 ± 27.2a	492 ± 95.5a	719.9 ± 108.8a
Hytec	703.2 ± 88.8a	736.9 ± 183.7a	698.1 ± 101.5a
Red Baron	644.0 ± 38.0a	695.6 ± 61.1a	637.6 ± 32.1a

Error bars are the standard error of mean (n=4); the mean values within one treatment followed by the same letter are not significantly different at P=0.05.

There was a significant difference in the dry matter between the varieties (Fig 3.3). The variety ‘Red Baron’ had highest content of dry matter in all treatments. The treatment with Se was significant in the content of dry matter only in the variety ‘Summit’.

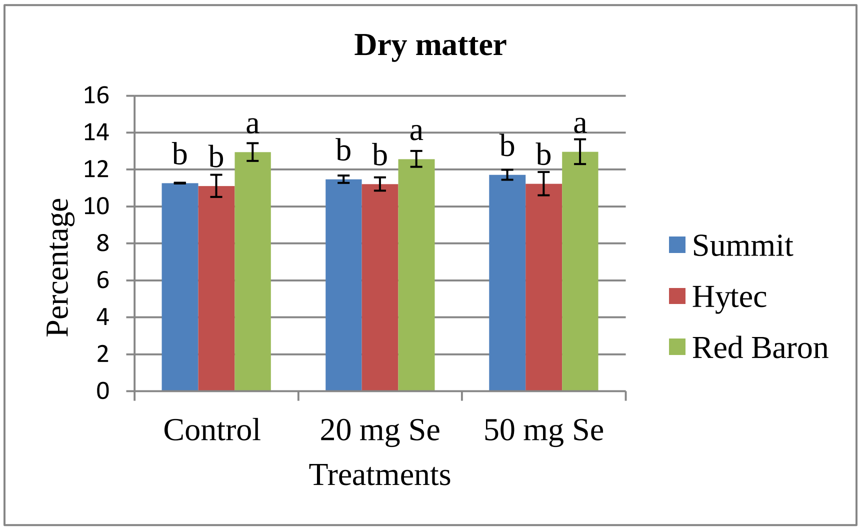


Fig: 3.3 Diagram showing dry matter content between treatments of onion varieties. N=4. The mean values within one treatment followed by the same letter are not significantly different at P=0.05.

3.2.2 Selenium

The Se concentration increased with increasing Se fertilization for all varieties (Table 3.8). There was no significant difference in the Se concentration between the varieties. Variety ‘Red Baron’ had the tendency of highest Se concentration when it was treated with 50 mg of Se.

Table 3.8 Concentration of Se in mg kg^{-1} in three different varieties of onion at three different treatments of Se

		Treatments	
Varieties	Control	20 mg Se	50 mg Se
		mg Se kg^{-1}	
Summit	0.07 ± 0.06a	2.95 ± 0.48a	6.11 ± 1.98a
Hytec	0.04 ± 0.02a	2.66 ± 1.15a	7.46 ± 1.60a
Red Baron	0.05 ± 0.02a	2.65 ± 1.19a	8.31 ± 4.86a

Error bars are the standard error of the mean (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.2.3 Sulfur

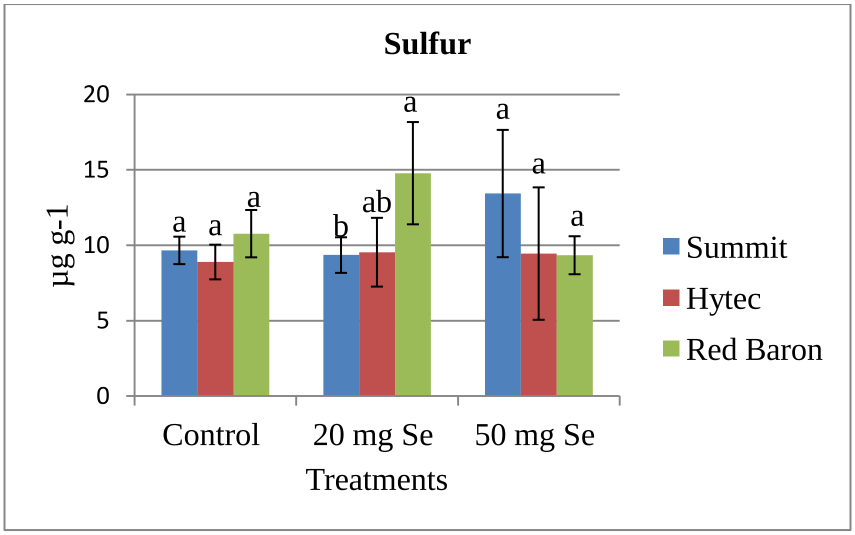


Fig: 3.4 Diagram showing S concentration between treatments in onion varieties. N=4. Error bars indicate standard error of the mean. The mean values within one treatment followed by the same letter are not significantly different at P=0.05.

There was a significant difference in the concentration of S between the varieties in 20 mg of Se (Fig: 3.4). The variety ‘Red Baron’ had the highest inclination on concentration of S in the treatments of 20 mg Se and variety ‘Summit’ and ‘Hytec’ had the higher inclination on concentration of Se in the treatment of 50 mg of Se. The treatment with Se was significant in the concentration of S only in the variety ‘Red Baron’.

3.2.4 Total Phenols

There was no significant difference in the content of total phenol between the varieties (Table 3.9). The variety ‘Hytec’ had a tendency of higher total phenols in the control whereas variety ‘Red Baron’ had a tendency of higher content of total phenols the treatments of 20 mg and 50 mg Se. The treatment with Se did not affect the content of total phenols.

Table 3.9 Total phenols in mg GAE 100g^{-1} in three different varieties of onion at three different treatments of Se.

		Treatments	
Varieties	Control	20 mg Se	50 mg Se
		Total phenols in mg GAE 100g^{-1}	
Summit	90.93 ± 19.47a	94.69 ± 15.05a	89.90 ± 20.90a
Hytec	81.50 ± 22.30a	92.66 ± 12.59a	95.38 ± 16.69a
Red Baron	103.99 ± 16.73a	105.9 ± 22.0a	112.9 ± 61.9a

Error bars are the standard error of the mean (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.2.5 FRAP

There was no significant difference in the FRAP assay between the varieties (Table 3.10). The variety ‘Red Baron’ had the inclination of highest value of FRAP in all treatments. The treatment of Se did not affect the value of FRAP.

Table 3.10 FRAP value in µmol $100g^{-1}$ in three different varieties at three different treatments of Se.

	Treatments		
Varieties	Control	20 mg Se	50 mg Se
	FRAP in µmol $100g^{-1}$		
Summit	0.64 ± 0.16a	0.67 ± 0.14a	0.66 ± 0.20a
Hytec	0.59 ± 0.18a	0.69 ± 0.11a	0.69 ± 0.18a
Red Baron	0.85 ± 0.25a	0.90 ± 0.26a	0.96 ± 0.63a

Error bars are the standard error of the mean (n=4); the mean values within one column followed by the same letter are not significantly different at P=0.05.

3.2.6 Accessibility of Se

There was significant different in the accessibility of Se between the varieties when it was treated with 50 mg Se (Table 3.11). The variety 'Summit' had a tendency of higher accessibility the 20 mg Se treatments whereas variety 'Hytec' tended to have highest accessibility in the 50 mg Se treatment. There was no significant difference between the treatments of 20 mg and 50 mg Se. In the control group, we could not determine the accessibility as the concentration in the extracts was below the LOQ for the ICP-MS with the method used. The concentration of Se in the extract was higher than that in the sample which is the reason for the higher accessibility in the variety 'Red Baron'. In the real condition, this is not possible.

Table 3.11 Accessibility of Se in percentage between three varieties of onion at three different treatments

	Treatments		
Varieties	Control	20 mg Se	50 mg Se
	Accessibility of Se in percentage (%)		
Summit	ND	84.75 ± 12.42a	98.1 ± 4.40a
Hytec	ND	91.18 ± 7.75a	79.5 ± 13.11b
Red Baron	149.26 ± 31.82	93.38 ± 8.70a	75.95 ± 8.58 b

Error bars are the standard error of the mean (n=4); ND= not determined and the mean values within one column followed by the same letter are not significantly different at P=0.05.

4. DISCUSSION

The results revealed that there were no influence of Se fertilization on the yield per meter square between the varieties of broccoli and onion. Selenium is not an essential trace element in onion and broccoli, and will therefore not affect on the growth performance unless it is at toxic concentrations. The Se fertilization levels were not in the toxic range for the plants in this experiment. All the plants were normal and healthier. The results are in agreement with Hu et al. (2002) in rice where no difference in yield was found between Se treated and untreated rice. Broadly et al. (2010) did not find any difference in yield among the selenate treatments of 0, 1, 5, 10, 15, 20, 50 and 100 g Se ha^{-1} in wheat. HoMin et al. (2000) did not find any significant difference on yield in *Coriandrum sativum* when 2, 4, 6 and 8 mg Se L^{-1} as sodium selenate was applied hydroponically. According to Rani et al. (2005) the critical level of Se in plant above which significant reduction in yield was found to be 76.9 µg g^{-1} in maize (*Zea mays*) and 104.8 µg g^{-1} in rayo (*Brassica juncea*) shoots, which is higher than the Se concentration obtained in this experiment. According to Euliss and Carmichael (2004) Se treated plants showed slower growth and delayed in flowering and fruit set, but the final yield was higher during harvesting when *Brassica napus* was grown in hydroponically on 2 mg kg^{-1} selenate. According to Germ and Stilbilj (2007), there was uncertainty of essential of Se in higher plants, Se induce plants to tolerance to oxidative stress, delay senescence, promote the development of ageing plants, delay senescence and adjust the water status of plants under drought condition.

The results revealed that Se treatment had no effect on dry matter content between the varieties of broccoli. Se treatment showed the significant difference in dry matter content between varieties of onion. Dry matter content was found to be higher in onion than in broccoli. According to Nenad et al. (2007) genetic differentiation affected the dry matter content in onion. According to Bansal et al (2012) difference in the concentration of Se affects vegetative and reproductive growth in *Brassica napus* compare to control which lead to the difference in the content of dry matter. According to Rani et al. (2005) significant decrease in the dry matter yield was found above the level of 5 µg Se g^{-1} soil in maize (*Zea mays*) and raya (*Brassica juncea*); 4 µg Se g^{-1} soil in wheat (*Triticum aestivum*) and 10 µg Se g^{-1} in rice (*Oryza sativa*) shoots when individual level of Se was treated from 1-25 µg Se g^{-1} soil. These levels are below the concentration that we used in our experiments in both onion and broccoli.

Broccoli and onion are the crops that are widely consumed in parts of the world. The capacity of broccoli and onion to accumulate Se in the edible part (head and bulb respectively) is crucial for determining the effectiveness of biofortification program. The results revealed that Se content varied significantly between the treatments in broccoli and onion. The total accumulation of Se markedly increased in the edible portion as the concentration of Se increases in the soil. The increase of Se is higher in broccoli than in onion. Biofortification of broccoli and onion could be the appropriate strategy for increasing the concentration of Se in the Se deficient areas. There is a large variation in human response with the Se supplement. Selenium intake greater than 400 µg day^{-1} is believed to be toxic (Fordyce 2005). Sakurai and Tsuchya (1975) recommended that the maximum acceptable daily intake of Se is 500 µg day^{-1}. Longnecker et al. (1991) did not find any evidence of toxicity when the intake of Se was 724 µg day^{-1} in South Dakota. In this experiment, the maximum accumulation of Se is 44.28µg g^{-1} in variety 'Ironman' of broccoli and 8.31µg g^{-1} in variety 'Red Baron' of onion. The Se concentration is found below the toxic level in human when consuming around 400 gm fresh weight of onion per day treated with 50 mg Se. This is based on the recommended by Fordyce (2005) that diet above 400 µg day^{-1} is toxic. The results are in the same direction reported by (Frías et al. 2010) in garden cress.

The result showed that there was no effect of Se in the concentration of S for all treatments in broccoli. In case of onion, Se influence S concentration when it was treated with 20 mg. Sulfate and selenate are absorbed by the sulfate transporter in plants. Sulfate will compete with selenate for the uptake and transport. There was no significant correlation between S and Se in broccoli (r = 0.132) and onion (r = 0.055). Lower concentration of selenate (0.5, 1.0 1.5 and 2.0 mg L^{-1}) increases uptake of sulfate in onion (Kopsell & Randle 1997). The result of broccoli is inconsistent as reported by (HSU et al. 2011) where the treatment of 0, 2 and 20 mg Se increased the concentration of S in broccoli. The reason behind this is that Se fertilization increases the concentration of sulfur transporters and increases the root-shoot transfer. Sulfate assimilation is regulated by the sulfur status of the plant. It is induced by sulfur deficiency (Kopriva & Koprivova 2004).

The determination of the total concentration of Se in the head and bulb of broccoli respectively is not enough to determine its bioaccessibility. The Se concentration and its speciation in the enzymatic extracts should be determined in order to know about the accessibility. There is a negative correlation between accessibility and Se concentration in both broccoli ($r = -0.611$) and onion ($r = -0.087$). The percentage of Se in the enzymatic extract was found in the range of 55-80% and is statistically no significant difference in broccoli. The enzymatic extract of onion was in the range of 79.5 to 98% and is statistically significant when it was treated with 50 mg Se. There were no symptoms of toxicity in the present study.

The result revealed that the content of ascorbic acid was not affected by the Se treatments. There was significant different in ascorbic acid content between varieties. In this experiment, variety 'Ironman' contained significantly higher ascorbic acid than other varieties in all the treatments of Se. Choosing the variety containing high content of ascorbic acid could be the main strategy for increasing the consumption of ascorbic acid. The results are in the same direction as reported by Lee et al. (2008) where no significant difference in ascorbic acid content was observed in broccoli when treated with 1,2,5 and 20 mg L^{-1} sodium selenate; Kaur et al. (2007) where there was a significant difference between the varieties of broccoli and Xu et al. (2003) where there was no significant difference between Se treated and untreated green tea. The results are contradictory as reported by Hu et al. (2001) where vitamin C content increased significantly with the treatment of Se. According to Murcia et al. (2000) the percentage distribution of ascorbic acid content is superior in the florets than in the stalk. Consuming of only the florets could also increase the ascorbic acid consumption. One of the possible reasons of increasing the ascorbic acid is by crossing with a variety containing high content of ascorbic acid.

The results revealed that there were no increase in the total phenol content due to Se treatments in broccoli and onion due to Se. The total phenol was higher in onion than in broccoli which is contradictory to the result shown by Chu et al. (2002) while analyzing 10 different vegetables. According to Finley et al. (2005) the phenolic acid is significantly decreased due to Se fertilization. It might be because of stress. According to Robbins et al. (2005) total phenolic increased with Se fertilization up to 100 µg Se g^{-1} of dry matter but decreased with a further increase of Se up to 1,000 µg Se g^{-1}. It could be because at a certain point Se concentration in a plant exceeds the ability of the plant to detoxify. Further growth will cause toxic stress and reduced production of metabolites such as phenolic components.

There was no significant difference in the total antioxidant capacity measured by the FRAP assay between the varieties and the treatments in broccoli and onion. Broccoli is a rich source of antioxidants comprising ascorbic acid, tocopherol, phenolics and carotenoids. Onion is rich in flavonoids which act as antioxidant. There is a strong correlation between total phenols and antioxidants in both broccoli ($r = 0.922$) and onion ($r = 0.976$) in this experiment. It showed that total phenol is contributing the antioxidant, which is in accordance with the purpose of the method, namely to determine the antioxidant capacity of food extracts rich in phenols. The results are in the same directions as reported by Ramos et al. (2011) who had found that there was no significant different in FRAP in half of the experiments between Se treated and untreated leaf in the different varieties of broccoli. The results are inconsistent as reported by (Rios et al. 2008) in lettuce and (Frías et al. 2010) in garden cress where there was a significant difference in FRAP assay. In this experiment, Se biofortification of broccoli and onion did not affect the antioxidant potential and thus do not alter the content of antioxidants in the plants.

5. CONCLUSION

In the present study of biofortication of Se in broccoli and onion, there was a difference in the accumulation capacity of Se when grown in a soil with high levels of Se. Broccoli accumulates more Se than onion. They did not show any difference in yield along with antioxidants like ascorbic acid, total phenols and FRAP assay. The strategy to increase the health benefits by adding value was found to be effective in our experiments. The concentration of Se is within the safe limit in both onion and broccoli. The accessibility is higher in onion than in broccoli. Hence, biofortification of Se in broccoli and onion are potentially suitable for use as Se supplement in the diets of humans in the regions with the deficiency of Se.

6. REFERENCES

Aherne, S. A. & O’Brien, N. M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition*, 18 (1): 75-81.

Ali, M., Thomson, M. & Afzal, M. (2000). Garlic and onions: their effect on eicosanoid metabolism and its clinical relevance. *Prostaglandins, leukotrienes and essential fatty acids*, 62 (2): 55-73.

Arteel, G. E. & Sies, H. (2001). The biochemistry of selenium and the glutathione system. *Environmental Toxicology and Pharmacology*, 10 (4): 153-158.

Baik, H. Y., Juvik, J., Jeffery, E., Wallig, M., Kushad, M. & Klein, B. (2003). Relating glucosinolate content and flavor of broccoli cultivars. *Journal of food science*, 68 (3): 1043-1050.

Bansal, A., Sharma, S., Dhillon, S. & Dhillon, K. (2012). Selenium Accumulation and Biochemical Composition of *Brassica* Grains Grown in Selenate-or Selenite-Treated Alkaline Sandy Loam Soil. *Communications in Soil Science and Plant Analysis*, 43 (9): 1316-1331.

Becker, W., Lyhne, N., Pedersen, A. N., Aro, A., Fogelholm, M., Phórsdottir, P., Alexander, J., Anderssen, S. A., Meltzer, H. M. & Pedersen, J. I. (2008). Nordic Nutrition Recommendations2004-integrating nutrition and physical activity. *Food & Nutrition Research*, 48 (4): 178-187.

Benzie, I. F. F. & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" : the FRAP assay. *Analytical biochemistry*, 239 (1): 70-76.

Benzie, I. F. F. (2000). Evolution of antioxidant defence mechanisms. *European journal of nutrition*, 39 (2): 53-61.

Borowski, J.-S., Agnieszka - Borowska, Eulalia J. - Ciska, Ewa - Zieliński, Henryk. (2008). Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.) *European Food Research and Technology* 226 (3): 459-465.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, 56 (11): 317-333.

Broadley, M. R., Alcock, J., Alford, J., Cartwright, P., Foot, I., Fairweather-Tait, S. J., Hart, D. J., Hurst, R., Knott, P. & McGrath, S. P. (2010). Selenium biofortification of highyielding winter wheat (*Triticum aestivum* L.) by liquid or granular Se fertilisation. *Plant and Soil*, 332 (1): 5-18.

Brown, P. D., Tokuhisa, J. G., Reichelt, M. & Gershenzon, J. (2003). Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry*, 62 (3): 471-481.

Cartea, M. E., Francisco, M., Soengas, P. & Velasco, P. (2010). Phenolic compounds in *Brassica* vegetables. *Molecules*, 16 (1): 251-280.

Chope, G. A., Cools, K. & Terry, L. A. (2011). *Alliums* (Onion, Garlic, Leek and Shallot). In Terry, L. A. (ed.) *Health-promoting Properties of Fruits and Vegetables*, pp. 5-28: CAB International, Wallingford, UK.

Chu, Y. F., Sun, J., Wu, X. & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of agricultural and food chemistry*, 50 (23): 6910-6916.

Corzo-Martínez, M., Corzo, N. & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends in Food Science & Technology*, 18 (12): 609-625.

Euliss, K. W. & Carmichael, J. S. (2004). The effects of selenium accumulation in hydroponically grown canola (*Brassica napus*). *Biological & Biomedical Sciences*, 1 (10).

Eurola, M. H., Ekholm, P. I., Ylinen, M. E., Varo, P. T. & Koivistoinen, P. E. (1991). Selenium in Finnish foods after beginning the use of selenate-supplemented fertilisers. *Journal of the Science of Food and Agriculture*, 56 (1): 57-70.

Fahey, J. W., Zalcmann, A. T. & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56 (1): 5-51.

Finey, J., Davis, C. & Feng, Y. (2000). Selenium from high selenium broccoli protects rats from colon cancer. *The Journal of nutrition*, 130: 2384-2389.

Finley, J. W., Sigrid-Keck, A., Robbins, R. J. & Hintze, K. J. (2005). Selenium enrichment of broccoli: interactions between selenium and secondary plant compounds. *The Journal of nutrition*, 135 (5): 1236-1238.

Fordyce, F., Ohnson, C., Navaratne, U. B., Appleton, D. a. & Dissanayake. (2000). Selenium and iodine in soil, rice and drinking water in relation to endemic goiter in Sri Lanka. *The Science of the Total Environment*, 263: 1-3.

Fordyce, F. (2005). Selenium deficiency and toxicity in the environment. In Selinus, O. & Alloway, B. J. (eds) *Essentials of medical geology*, pp. 373-415. Amsterdam: Elsevier Academic Press.

Frías, J., Gulewicz, P., Martinez-Villaluenga, C., Peñas, E., Piskula, M. K., Kozlowska, H., Ciska, E., Gulewicz, K. & Vidal-Valverde, C. (2010). Changes in nutritional value and cytotoxicity of garden cress germinated with different selenium solutions. *Journal of agricultural and food chemistry*, 58 (4): 2331-2336.

Germ, M. & Stibilj, V. (2007). Selenium and plants. *Acta agriculturae Slovenica*, 89 (1): 65-71.

Goncalves, E. M., Abreu, M., Brandao, T. R. S. & Silva, C. L. M. (2011). Degradation kinetics of colour, vitamin C and drip loss in frozen broccoli (*Brassica oleracea* L. ssp. *italica*) during storage at isothermal and non-isothermal conditions. *International Journal of Refrigeration*, 34 (8): 2136-2144.

Gray, A. R. (1982). Taxonomy and evolution of broccoli (*Brassica oleracea* var. *italica*). *Economic Botany*, 36 (4): 397-410.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. & Smith, B. (2002). Onions—a global benefit to health. *Phytotherapy Research*, 16 (7): 603-615.

Gutteridge, J. & Halliwell, B. (2000). Free radicals and antioxidants in the year 2000: a historical look to the future. *Annals of the New york Academy of Sciences*, 899 (1): 136-147.

Hansen, V. T. & Grimenes, A. A. (2011). *Meteorologiske data for Ås 2010*: Institute for matematiske realfag og teknologi Universitetet for miljø- og biovitenskap.

Harborne, J. B. & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55 (6): 481-504.

Hardy, G. & Hardy, I. (2004). Selenium: the Se-XY nutraceutical. *Nutrition*, 20 (6): 590-593.

Hell, R. (1997). Molecular physiology of plant sulfur metabolism. *Planta*, 202 (2): 138-148.

Herr, I. & Buchler, M. W. (2010). Dietary constituents of broccoli and other cruciferous vegetables: Implications for preventation and therapy of cancer. *Cancer Treat Rev.*, 36: 377-383.

HoMin, K., MoonJung, L. & KuenWoo, P. (2000). Effects of selenium on growth, storage life, and internal quality of coriander (*Coriandrum sativum* L.) during storage. *Journal of the Korean Society for Horticultural Science*, 41 (5): 490-494.

HSU, F. U. C., WIRTZ, M., HEPPEL, S. C., BOGS, J., KRÄMER, U., KHAN, M. S., BUB, A., HELL, R. & RAUSCH, T. (2011). Generation of Se fortified broccoli as functional food: impact of Se fertilization on S metabolism. *Plant, cell & environment*, 34 (2): 192-207.

Hu, Q., Xu, J. & Pan, G. (2001). Effect of selenium spraying on green tea quality. *Journal of the Science of Food and Agriculture*, 81 (14): 1387-1390.

Hu, Q., Chen, L., Xu, J., Zhang, Y. & Pan, G. (2002). Determination of selenium concentration in rice and the effect of foliar application of Se enriched fertiliser or sodium selenite on the selenium content of rice. *Journal of the Science of Food and Agriculture*, 82 (8): 869-872.

Johnson, C. C., Fordyce, F. M. & Rayman, M. P. (2010). Symposium on'Geographical and geological influences on nutrition': Factors controlling the distribution of selenium in the environment and their impact on health and nutrition. *The Proceedings of the Nutrition Society*, 69 (1): 119-132.

Kaur, C. & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables–the millennium's health. *International journal of food science & technology*, 36 (7): 703-725.

Kaur, C., Kumar, K., Anil, D. & Kapoor, H. (2007). Variations in antioxidant activity in broccoli (*Brassica oleracea* L.) cultivars. *Journal of food biochemistry*, 31 (5): 621-638.

Kopriva, S. & Koprivova, A. (2004). Plant adenosine 5′-phosphosulphate reductase: the past, the present and the future. *Journal of Experimental Botany*, 55 (404): 1775-1783.

Kopsell, D. A. & Randle, W. M. (1997). Selenate concentration affects selenium and sulfur uptake and accumulation by Granex 33'onions. *Journal of the American Society for Horticultural Science*, 122 (5): 721-726.

Lee, S. J., Kang, H. M. & I.S., K. (2008). Effect of Sodium Selenate Supplied Condition by Fertigation of the Growth and Content of Minerals, Ascorbic acid, Nitrate, and Selenium of some Western Vegetables. *Journal of Bio-Environment Control*, 17 (1): 43-50.

Lee, S. K. & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest biology and technology*, 20 (3): 207-220.

Longnecker, M. P., Taylor, P. R., Levander, O. A., Howe, M., Veillon, C., McAdam, P., Patterson, K., Holden, J., Stampfer, M. & Morris, J. (1991). Selenium in diet, blood, and toenails in relation to human health in a seleniferous area. *The American journal of clinical nutrition*, 53 (5): 1288-1294.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition*, 79 (5): 727-747.

Murcia, M. A., Lopez-Ayerra, B., Martinez-Tome, M., Vera, A. & Garcia-Carmona, F. (2000). Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *Journal of the Science of Food and Agriculture*, 80 (13): 1882-1886.

Nenad, P., Dejan, C., Milan, Z., Radiša, Đ. & Slaven, P. (2007). Variability and heritability coefficient of average dry matter content in onion (*Allium cepa* L.) bulbs. *Genetika*, 39.

Ockendon, D. (1980). Distribution of S-alleles and breeding structure of Cape broccoli (*Brassica oleracea* var.*italica*). *TAG Theoretical and Applied Genetics*, 58 (1): 11-15.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A. & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of agricultural and food chemistry*, 50 (11): 3122-3128.

Papp, L. V., Lu, J., Holmgren, A. & Khanna, K. K. (2007). From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxidants & redox signaling*, 9 (7): 775-806.

Parr, A. J. & Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, 80 (7): 985-1012.

Pedrero, Z., Madrid, Y. & Cámara, C. (2006). Selenium species bioaccessibility in enriched radish (*Raphanus sativus*): a potential dietary source of selenium. *Journal of agricultural and food chemistry*, 54 (6): 2412-2417.

Podsedek, A. (2007). Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. *LWT-Food Science and Technology*, 40 (1): 1-11.

Prior, R. L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *The American journal of clinical nutrition*, 78 (3): 570S-578S.

Ramos, S. J., Yuan, Y., Faquin, V., Guilherme, L. R. G. & Li, L. (2011). Evaluation of genotypic variation of broccoli (*Brassica oleracea* var. *italica*) in response to selenium treatment. *Journal of agricultural and food chemistry*, 59: 3657-3665.

Rani, N., Dhillon, K. & Dhillon, S. (2005). Critical levels of selenium in different crops grown in an alkaline silty loam soil treated with selenite-Se. *Plant and Soil*, 277: 367-374.

Rayman, M. P. (2000). The importance of selenium to human health. *The Lancet*, 356 (9225): 233-241.

Rayman, M. P. (2008). Food-chain selenium and human health: emphasis on intake. *British journal of nutrition*, 100 (2): 254-268.

Rios, J., Rosales, M., Blasco, B., Cervilla, L., Romero, L. & Ruiz, J. (2008). Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulturae*, 116 (3): 248-255.

Robbins, R. J., Keck, A. S., Banuelos, G. & Finley, J. W. (2005). Cultivation conditions and selenium fertilization alter the phenolic profile, glucosinolate, and sulforaphane content of broccoli. *Journal of medicinal food*, 8 (2): 204-214.

Rosa, E. A. S., Haneklaus, S. H. & Schnug, E. (2002). Mineral content of primary and secondary inflorescences of eleven broccoli cultivars grown in early and late seasons. *Journal of plant nutrition*, 25 (8): 1741-1751.

Rubatzky, V. & Yamaguchi, M. (1997). Cole crops, other *Brassica* and other crucifer vegetables. In: World Vegetables: Principles, Production and Nutritive Values. *2nd ed. New York: Chapman and Hall*: 371-417.

Sakurai, H. & Tsuchya, K. (1975). A tentative recommendation for the maximum daily intake of selenium. *Environmental physiology & biochemistry*, 5 (2): 107.

Shah, A. & Channon, K. (2004). Free radicals and redox signalling in cardiovascular disease. *Heart*, 90 (5): 486-487.

Singh, B. N., Singh, B., Singh, R., Prakash, D., Singh, D., Sarma, B., Upadhyay, G. & Singh, H. (2009). Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology*, 47 (6): 1161-1167.

Sogn, T. A., Govasmark, E., Eich-Greatorex, S., Falk-Øgaard, A. & MacLeod, J. A. (2007). Assessment of seafood processing wastes as alternative sources of selenium in plant production. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, 57 (2): 173-181.

Sors, T., Ellis, D. & Salt, D. (2005). Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research*, 86 (3): 373-389.

Spallholz, J. E., Mallory Boylan, L. & Rhaman, M. (2004). Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Science of the total environment*, 323 (1-3): 21-32.

Stadlober, M., Sager, M. & Irgolic, K. J. (2001). Effects of selenate supplemented fertilisation on the selenium level of cereals--identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chemistry*, 73 (3): 357-366.

Stajner, D. & Varga, I. S. (2003). An evaluation of the antioxidant abilities of *Allium* species. *Acta Biologica Szegediensis*, 47 (1-4): 103-106.

Steinbrenner, H. & Sies, H. (2009). Protection against reactive oxygen species by selenoproteins. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1790 (11): 1478-1485.

Tapiero, H., Townsend, D. & Tew, K. (2003). The antioxidant role of selenium and seleno-compounds. *Biomedicine & pharmacotherapy*, 57 (3): 134-144.

Terry, N., Zayed, A., De Souza, M. & Tarun, A. (2000). Selenium in higher plants. *Annual review of plant biology*, 51 (1): 401-432.

Vallejo, F., Tomás-Barberán, F. A. & García-Viguera, C. (2002). Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *Journal of the Science of Food and Agriculture*, 82 (11): 1293-1297.

Verkerk, R., Dekker, M. & Jongen, W. M. F. (2001). Post-harvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. *Journal of the Science of Food and Agriculture*, 81 (9): 953-958.

Whanger, P. (2002). Selenocompounds in plants and animals and their biological significance. *Journal of the American College of Nutrition*, 21 (3): 223-232.

Whanger, P. (2004). Selenium and its relationship to cancer: an update. *British journal of nutrition*, 91 (1): 11-28.

Williams, R., Baker, D. & Schmit, J. (1973). Analysis of water-soluble vitamins by high-speed ion-exchange chromatography. *Journal of Chromatographic Science*, 11 (12): 618-624.

Witherington, R., Janosko, J. H. H. E., Borosso, D. C. C., Falk, S. & Rounder, J. (1998). Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. *British Journal of Urology,* 81: 730-734.

Wold, A. B. (2004). *Antioxidant activity in tomato (Lycopersicon esculentum Mill.) and cabbage (Brassica oleracea L.), and the content of individual glucosinolates in cabbage: effects of maturity, fertilisation and storage conditions*: Agricultural University of Norway, Department of Plant and Environmental Sciences. 1-27 pp.

Xu, J., Zhu, S., Yang, F., Cheng, L., Hu, Y., Pan, G. & Hu, Q. (2003). The influence of selenium on the antioxidant activity of green tea. *Journal of the Science of Food and Agriculture*, 83 (5): 451-455.

Yang, J., Meyers, K. J., Van Der Heide, J. & Liu, R. H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of agricultural and food chemistry*, 52 (22): 6787-6793.

Zhu, Y. G., Pilon-Smits, E. A. H., Zhao, F. J., Williams, P. N. & Meharg, A. A. (2009). Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. *Trends in Plant Science*, 14 (8): 436-442.