4-21-2017

Determining the effects of sulfur fertility levels on edamame soybean [Glycine max (L.) Merrill] protein components

Theoneste Nzaranyimana
Illinois State University, theokazungu@gmail.com

DETERMINING THE EFFECTS OF SULFUR FERTILITY LEVELS ON EDAMAME SOYBEAN [*Glycine max* (L.) Merrill] PROTEIN COMPONENTS

Theoneste Nzaranyimana

58 Pages

Edamame [*Glycine max* (L.) Merr.] a vegetable type soybean is harvested and consumed before reaching its maturity stage (R6). Its health properties drew attention to U.S. consumers because of its high protein content. Proteins are the major structural component of all cells in the living organisms. They are made of essential and non-essential amino acids which play a big role in growth and development and are source of energy. Proteins act as enzymes, antibodies, and hormones and blood protein. Sulfur (S) is a component of the amino acids cysteine (Cys) and methionine (Met) which are the building blocks for proteins. Little is known about how levels fertility influences protein content in edamame soybean. As result, a solution culture experiment in a protected environment was conducted to evaluate the effect of S fertility concentrations on protein content in edamame. Seeds of the 'Chiba' edamame were allowed to germinate under greenhouse conditions at 22 °C day/14 °C night for 15 days and transplanted in nutrient solution culture in the fall of 2016 using a modified Hoagland's solution containing S treatment concentrations of 4, 8, 16, 32, and 64 mg S L^{-1} was delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4). Treatments were arranged in a randomized complete block design with four replications containing five s treatment levels per replication. The solution was changed every 2 weeks after transplanting until plants reached the R6 maturity stage. Plants were harvested approximately 60 days after planting and weighed for fresh biomass (FM) before tissues were dried at 60 °C prior the extractions and protein content analysis. Elemental S concentrations were measured in oven-dried bean tissue using ICP-MS. Manipulating the S fertility concentrations showed no significant difference on bean accumulation of crude protein (P=0.171), Adjusted protein (P=0.171), ADFNDF (P=0.409), ADFDM (P=0.707), aNDf (P=0.271). The results showed that increasing the S concentration from 4 to 64 mg S L^{-1} in nutrient solution culture did not affect the protein composition in 'Chiba' edamame.

KEYWORDS**:** amino acids, Chiba, ICP, macronutrients, micronutrients

DETERMINING THE EFFECTS OF SULFUR FERTILITY LEVELS ON EDAMAME SOYBEAN [*Glycine max* (L.) Merrill] PROTEIN COMPONENTS

THEONESTE NZARANYIMANA

A Thesis Submitted in Partial
Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Agriculture

ILLINOIS STATE UNIVERSITY

2017

DETERMINING THE EFFECTS OF SULFUR FERTILITY LEVELS ON EDAMAME SOYBEAN [*Glycine max* (L.) Merrill] PROTEIN COMPONENTS

THEONESTE NZARANYIMANA

COMMITTEE MEMBERS:

David E. Kopsell, Co-Chair

Dean A. Kopsell, Co-Chair

Robert L. Rhykerd

ACKNOWLEDGMENTS

It is not easy to say that I could complete this thesis alone without the help from people who had other commitments but managed to find always a time to help me whenever I could run to them. I am mostly thankful to the almighty God and the following people. Dr. Dave Kopsell has been not only my major professor but also a powerful mentor for the rest of my graduate studies. In my life, I have never met a professor whom his desire and determination are to produce the best from his students. The courage, hope and supports you put in me made me to break the odds and now here are the results from your energy spent.

Many thanks to Dr. Robert Rhykerd who has been always there for me in good and bad moments. Indeed you are an outstanding leader which the department of Agriculture has. I appreciate your energy and supports you provided as my thesis committee member. You inspired my life and I feel honored to have met you. My deepest appreciations go to Dr. Dean Kopsell who served on my committee member, your supports and ideas shared. You have contributed tremendously to the success of this thesis. Knowing Michael Ruffatti has contributed a lot to my graduate studies. Your supports during the soil science labs and the statistical analysis for my data played a big role to this thesis.

Many thanks are conveyed to the Wright family, Steve Zwicker's family and the church which supported me financially throughout my studies. I would like to recognize the Illinois soybean association for funding this research. Without your funds, this research could not be known.

I also want to thank Dr. Spaulding Aslihan and Mrs. Aubrey Wagoner who served as my academic advisors for the entire graduate studies. Your love and compassion made me excelling in my studies. Your daily advice turned me into a hard worker and helped me challenging any obstacle I could come across with. I can’t forget motivations, encouragements and supports from Mr. Don Meyer, Kathy Scott, Mary Akers, Dr. Stephen Rickard, Dr. Michelle and Dr. Maria, Mr. Caleb Wrepprecht, Mr. Perry Harlow, Jonah s ‘family, Mr. Peter Tchakatumba, Mr. Bansal, Ms Mitra Ravanan, Mr. Larry Kies and AU Ag department, Ngendakumana’s family, Dr. Bigirimana Stanislas’family, and Ms. Sylvia Mugisha. Your supports and mentoring made me excelling in my studies. Your love and supports are highly appreciated.

T. N.

CONTENTS

TABLES

CHAPTER I: THE PROBLEM AND ITS BACKGROUND

Product quality, particularly related to flavor, affects food purchasing decisions (Farmakalidis, 1999; Glanz et al., 1998; Hilliam,1995). Real or perceived quality shortfalls shape consumer preference to eat fresh produce and food sensory attributes drive immediate and future consumption (Shepherd,1997). Concerns about reductions in taste quality can interfere with the adoption of health diets (Bowman et al., 1998; Glanz et al., 1998), as consumers emphasize sensory experiences during consumption like appearance, texture, aroma, taste, with the pleasure derived from consumption as an important motivator in eating (Westenhoefer and Pudel, 1993). Edamame [Glycine max (L.) Merr.] is a crop that is gaining popularity in American diet. Its increase in consumption in U.S. is due to the health benefits and flavor it provides to its consumers (Gaskell, 2001; Iwata et al., 1982). Edamame is very similar to field soybeans. However, edamame differs from field soy by its clear hilum, its relatively larger seed size, and its unique sensory characteristics. Unlike the field soybean, edamame is harvested at R6 stage (immature) and sold in intact pods.

Marketable edamame pods have to be bright green in color, with no translucent pubescence, and contain at least two beans. Edamame soybeans are grown as a specialty crop, not an agronomic crop. The germplasm enhancement and variety development of edamame type soybeans is relatively new in U.S., as the origin of most varieties can be traced to Asia (Wszelaki et al., 2005). Edamame soybean tends to have a unique mild or neutral flavor, which is caused by the distinctive combination of sweetness, sourness, and bitterness (Lee and Hwang, 1998). Sucrose contributes to sweetness, while saponin, isoflavonoids, and L-arginine add bitterness (Masuda, 1994; Hashizume et al., 1988). Flavor of edamame is due to its nutty characteristic described as buttery, beany, oily, and flowery (Johnson et al., 1999; Rodale Research Center, 1982; Young et al., 2000). Cristo (2002) reported that Chinese consumers of edamame preferred a high sugar content and they were more sensitive to acidity than American consumers. Johnson et al. (1999) reported that the U.S. consumers prefer beans with a buttery flavor and texture while Japanese consumers tend to prefer beans with a sweet, flowery flavor and a crispy texture. Edamame flavor is variety dependent, but soil mineral nutrition also contributes to flavor components, as it does for other vegetable crops. Sulfur (S) is a component of the amino acids cysteine (Cys) and methionine (Met) which are components of proteins, as well as several S-containing coenzymes and secondary plant products derived from the amino acids. These forms of amino acids constitute about 70% of the sulfur content of plants (Haneklaus et al., 2007). Kopsell (2003) reported that S nutrition affects flavor components in vegetables by creating acrid or bitter flavors that can be regarded as objectionable by consumers. Like other vegetables, the influences of production factors such as cultivar and plant management on key sensory quality related properties in edamame and how these factors influence consumer taste preference, particularly in the expanding U.S. market, are poorly understood (Wszelaki et al., 2005). Furthermore, soybean is a source of protein that is essential for human and livestock nutrition (USDA, 2008; Lakins, 1981). Processed seed from soybean provides 31% of vegetable oil and 69% of the protein meal consumed worldwide (Soystats, 2004). Protein and oil from soybeans are major contributors to human nutrition either directly or through use as animal feeds. This crop contains protein, oil, carbohydrates and a variety of minerals, vitamins, and secondary metabolites that contribute to human health (Bennett, 2005). The amino acid profile of soybean seed protein is limited by its concentration of the S-containing amino acids Cys and Met (Duranit and Gius, 1997). Increasing the concentrations of these amino acids in soybean seed would benefit consumers of soybean and could justify a premium price for soybean producers (McVey et al., 1995). Manipulating S fertility concentrations during edamame growth and development may change the levels of protein content and S-containing flavor compounds.

CHAPTER II: REVIEW OF LITERATURE

COMMERCIAL SIGNIFICANCE OF EDAMAME

Edamame [Glycine max (L.) Merr.] has a long history within East Asian culture. The first mention of soybean in Chinese text was before the 7th century B.C. in the Shijing Book of Odes (Shurtleff and Aoyagi, 2009). The word edamame was first documented in Japan in 1275 (Shurtleff and Aoyagi, 2009). However, edamame was virtually unknown until the mid-19th century. Only within the past 30 years has edamame gained widespread acceptance in the U.S. (Shurtleff and Aoyagi, 2009).

In recent years, there has been an increased demand and broader acceptance of edamame in the U.S. Edamame is currently the second most consumed soybean product behind soymilk in the U.S. (Soyfoods, 2014). Edamame can be marketed fresh, frozen, or canned. Informa reported that traditional soy-based, oriental foods such as tofu, tempeh and edamame have grown 9% over the past 5 years to a value of just under $45 million in 2009 (Informa/United Soybean Board, 2010). Soyatech, LLC, a U.S. soybean and oilseed information consultant, reported that sales of frozen edamame shelled or in the pods grew 40% between 2003 and 2007 to $30 million (Bernick, 2009). Ninety-seven percent of the edamame sold to the frozen food market is imported from China and other Asian nations. Roseboro (2012) reported that China exports 13,608 to 18,144 metric tonnes of edamame to the U.S. each year. Nuss (2013) reported that in 2013, the U.S. consumed between 22,700 to 27,270 metric tonnes. Edamame has a great potential to be a profitable crop, especially in the southeastern U.S. The University of Kentucky conducted research which determined that the projected net returns of edamame production could ranges from $640 to $6,177 per ha (Ernst and Woods, 2001; Ernst, 2000; University of Kentucky, 2013). At the lower range of returns, edamame is more profitable than sweet corn

(Zea mays L.) and pumpkin (Cucurbitaceae cucurbita pepo L. At the high end of return, edamame can be more profitable than staked tomatoes (Solanum lycopersicum) and cantaloupe (Cucumis melo var. cantalupensis). In most cases, the breakeven price for edamame is less than $2.20 per kg, much lower than the average wholesale price of $3.30 per kg (Ernst and Woods, 2001; Ernst, 2000). Edamame is of particular interest to soybean farmers due to the similarities in equipment and management practices. This interest may increase in the coming years as commodity soybean prices are projected to decrease (Schnitkey, 2015). While edamame is an excellent source of plant-based protein, it is also a complete protein containing all the essential amino acids in the human diet (Velasquez and Bhathena, 2007). In response to this increasing demand, growers, plant breeders, and food processors have grown increasingly interested in the production of edamame. Furthermore, more than 323 ha of conventional and organic edamame were contracted with Arkansas growers in 2012 (University of Arkansas Extension, 2012). This increase in demand is expected to continue as consumers look for healthier, lower cost sources of protein to add to their diet. Based on current U.S. levels of edamame consumption and expected yields, a large increase in domestic production would be required to meet current domestic demand. This increased demand has prompted food processers and packagers to look to local sources to fill the expanding need.

BOTANICAL CLASSIFICATION AND CULTURAL OF EDAMAME

Edamame is a special soybean type, which belongs to the family of leguminaceae (Fabaceae). Edamame is an annual herbaceous plant that originated in the southern Asia prior to the 7th century B.C. Edamame soybeans are similar to regular soybeans but are planted in intervals throughout May and June and harvested at the R6 maturity stage (Shurtleff & Aoyagi, 2004). Edamame plants are typically 60 to 90 cm in height, but can grow to 2 m tall. Leaves are compound, densely hairy, with three leaflets. The inconspicuous white to purple flowers are borne singly or in small clusters in the axils. The fruit is a broad hairy flattened legume or pod around 10 cm long (Bailey et al. 1976; Ecocrop 2012; Faostat, 2012). Each plant typically bears 100 to 150 pods (Shurtleff & Aoyagi, 2004). This crop has large seeded beans, green in color, which are cooked and served in pods as snacks, similar to peanuts (Shurtleff, 2001). The roots bear nodules, which extract nitrogen (N) from the air and fix it in the soil, where it stimulates the growth of the edamame beans (Shurtleff & Aoyagi, 2004). Unlike field soybean, edamame is harvested at reproductive stage R6 when the seeds are immature and have expended to fill 80% of the pod width. In Asia, where edamame is an important vegetable, farmers harvest stems with fresh green pods before full maturity when pods are fully filled (Kiuchi et al. 1987).

Edamame does well in deep or moderately deep, well-drained, fertile soils with a soil pH of 6.0 to 7.5 and it prefers full sun (Miles, 2000). If crops like legumes have been grown in the field before, fertilization is not generally needed. If edamame is grown in a field without prior legume production, Rhizobia inoculation at the rate of 10 g per kg of seed is sufficient to boost N-fixation (Lal, 2001). Edamame can be direct seeded or transplanted. The transplanted crop tends to have shorter stems and a lighter top weight, but a greater ratio of pods to total weight, which is an advantage for farmers who sell pods at the market off the stem, which some customer prefer for the greater freshness (Kokobun, 1991).

The critical step in growing edamame is the selection of appropriate variety for the appropriate growing environment. Some factors which are considered during variety selection are maturation date, pod characteristics, and cost for the seeds. Edamame seeds are sold based on their maturity group (0 to V) for the region of adaptation for better yield potential and desirable marketable qualities (North Carolina State University, 2007). Japanese classify soybeans as summer or fall types (Kono, 1986). Summer types are planted in spring and harvested immature after 75 to100 days. While fall types are planted in early summer and take 105 days or more to reach harvestable size. About 170 edamame varieties are listed by the Niho Shubyo Kyokai (JSTA, 1987). Edamame seed germination is sensitive to soil temperature. Seeds can fail to emerge if soil is too wet or when the soil temperature is below 18 °C. Soil temperatures can be managed with plasticulture low tunnels during cold periods to increase temperature and by using ventilation during hot periods (Watanabe, 1988). Plant tops in seeded fields are cut after the primary leaf stage to increase plant branching and pod set (Gotoh, 1984; Kokobum, 1991).

The seeding rates of this crop differs from the grain-type soybean. The recommended space between rows is 61 cm and plant spacing within row is 5 to 10 cm apart depending on the region and variety. This spacing will give a plant population of approximately 20,000 to 28,000 plants per ha, which is equivalent to 36 kg to 50 kg of seeds per ha. When germination percentages are below 85%, a higher seeding rate is recommended to ensure a good stand. A seed should be planted between to 2.5 to 3.9 cm deep, however the deeper planting depth beyond this will result in low seedling emergence (Ogles et al., 2015). If seeds are not inoculated, the application of 45 kg of N per ha will be required. Half of the amount of N, potassium (K) and phosphorus (P) are applied at planting time and the remaining half can be applied in a band 10 cm to the side of the crop row during the growing season about 6 weeks after planting. If manure is used, it must be broadcasted before the final field preparation and thoroughly incorporated in the soil (Hemphill and Miles, 1999). Soils which are dry can be irrigated before planting to avoid crust to form on the surface of the soil which can prevent emergence (Monsour, 1998).

Early-season weed control prevents competition of weeds and the crop. Mechanical cultivation between rows and hand cultivation between plants can be used as methods of controlling weeds invasion in the field (Cherly and Kaiser, 2007). Hoes can be used when the crop is still young and weeds are not yet mature. Mulching is used in gardens to suppress the weeds. Hand weeding can also be effective on a small scale (Alabama A&M University Extension, 2015). Edamame is mostly attacked by foliage and stem feeding caterpillars and beetle species. Other edamame insect feeders are soybean loopers (Pseudoplusia includes), fall armyworms (Spodoptera frugiperda), and Kudzu bugs (Megacopta cribraria). Both soybean loopers and fall armyworms can cause serious defoliation in this crop, greatly affecting yield. Kudzu bugs can also cause significant damage with their piercing-sucking mouth parts. The best ways of controlling these insects are hand picking and the use of recommended insecticides. Bacterial pustules (Xanthomonas axonopodis) can be a problem in some varieties, especially those originating from Asia. Rootknot (Meoidogeyne sp.) and cyst (Heterodera sp.) nematodes cause severe reduction in edamame yield when present. Long rotations away from the affected area to reduce nematodes populations may be the most effective management strategy (Alabama A&M University Extension, 2015).

Edamame is harvested at the R6 soybean maturity stage when pods begin to swell and beans almost fill the pod. Pods must be bright green in color. A yellow pod color indicates that edamame is over mature and the beans become starchy and lose their sweet nutty flavor. During harvesting, the majority of the pods must be mature and the whole plant must be harvested (Culbertson, 1999). It is recommended for growers to harvest edamame early in the morning when the pods are still cool to help increase storage life. Immediate precooling is recommended in order to remove field heat and to maintain freshness. Precooling may be accomplished by using forced air, vacuum, or hydrocooling processes (Cheryl and Ernst, 2007).

HEALTH PROPERTIES AND SIDE EFFECTS OF EDAMAME

The health properties of edamame have contributed to its popularity. In a 2009 survey, 84% of consumers said that soy products are healthy, and 31% claimed to know specific health benefits of dietary soy. About a third of Americans also seek out and consume soy products at least once in a month (United State Soybean Board, 2009). The measured nutritional values of soybeans include low fat and high protein (Rao et al., 2002). Edamame also contains amounts of all the essential amino acids, which make it a particularly good meat substitute. Montri et al. (2006) reported that edamame also contained lower levels of trypsin inhibitors, fewer indigestible oligosaccharides, a greater amount of vitamins, and higher levels of phytic acid than agronomic field soybeans. Edamame is also a good source of sodium (Na), iron (Fe), calcium (Ca), K, P, magnesium (Mg), folate, and vitamins A, B1, B2, B3, C, E and K (Nair, 2010). Proximate analysis of seed nutritional composition of edamame grown in Colorado and in Japan indicated that edamame has superior nutritional content than green peas (Pisum sativum). The calorific value of edamane soybean is about 6 times than that of green peas. Edamame also contains 60% more Ca and twice the P and K of green peas. The Na and carotene content of soybean are about one third than that of green peas and have similar quantities of Fe, vitamin B, and vitamin B2. It is also rich in ascorbic acid but low in niacin (Goubran and Garti, 2000).

Other health properties of edamame, beyond mineral nutrition, include antioxidants which prevent the negative effects of free radicals in the body (Achouri et al., 2005; Patel et al., 2001; Messina, 2001). Dietary intake of antioxidants is reported to be associated with reduced incidence of atherosclerosis, cancer risk, neurodegenerative diseases, and improved immune system (Kumar et al., 2009; Patel et al., 2001). Edamame also contains isoflavones which can act as important regulators to maintain health by interfering with proliferation, growth and maturity of cancer cells. Isoflavones present in edamame soybean are the aglycone, beta glycoside, 6-o-maloyl-glycoside or 6-o-acetyl-beta-glucoside forms. Compounds like genistein and daidzein isoflavones also have antioxidant properties, protecting cells from deleterious effects of free radicals that promote aging (Demirbucker and Blomberg, 1990). Genistein inhibits protein tyrosine kinase activity, topoisomerase I and II, ribosomal 6-s kinase and alters cell proliferation. It also has antioxidant properties and suppresses skin tumorigeneses (Demirbucker and Blomberg, 1990). The protective roles associated with soybean isoflavones have received increasing attention by American consumers (Charron et al., 2005).

Some of the adverse health effects found in soybean are attributed to its oxalate content. Saturated oxalate in solution binds to Ca to form crystals that aggregate, often becoming large enough to block the urinary stream (Massey et al. 1993). High level of oxalate in urine increases the risk of calcium oxalate kidney stones in humans. The overconsumption of edamame can lead to over production of estrogen in males and this can contribute to infertility. For females, the overconsumption of edamame estrogenic compounds may stimulate the growth of cancerous cells in the breast [(Messina et al.(2006); Messina and Lopinzi(2001); Ju et al.(2002).

QUALITY AND FLAVOR COMPONENTS OF EDAMAME

The quality and flavor for edamame are influenced by many factors. The larger beans of edamame are considered superior to soybeans in flavor and texture and for their ease of cooking. Edamame soybean is reported to have higher phytic acids levels which give this crop its quality of being tender and easy to cook (Gupta et al., 1976). Hymowitz (1972) reported that the large seeded soybeans (edamame) have nutritionally superior protein. Yen et al. (1970) reported that edamame had higher protein content than the grain soybean in a feeding study done on rats. Edamame also differs from grain soybean with its sweet, mild flavor, nutty texture, and beany taste. Edamame soybean has larger, easily shattered pods and seeds with a fragile coat (Smith andVan Duyne, 1951) and stems that may have several podless nodes (Shanmugasundaram et al. 1989). Hymowitz et al. (1972) reported that high levels of protein in edamame can lead to the lack of sweetness which is an important component of flavor. Quality is also determined by the appearance, aroma, and firm texture after cooking. For good quality of this crop, pods have to have preferably sparse, white soft pubescence (Watanabe, 1988; Sunada, 1986). Edamame pods should be completely green with no hint of yellowing and contain two or three beans (IDA, 1990). Pods should be at least 5 cm long. One hundred seventy-five pods should weigh no less than 500 g and contain no less than 100 beans with a total fresh mass (FM) exceeding 30 g (Shanmugasundaram et al., 1989). According to IDA (1990), A-grade edamame has to have 90% or more of the pods with two or three seeds. Pods have to be perfectly shaped, completely green and show no injury or spotting. B-grade edamame also has to have 90% or more pods with two or three seeds, but can be lighter green in color, and a few pods can be slightly spotted, injured, malformed, short, or have small seeds. In these two grades, pods must not be over mature, diseased, insect damaged, one seeded, malformed, yellowed, split, spotted, or unripen.

Crop management practices that contribute to the quality and flavor of this crop are the variety selection, fertilization application, planting density, the harvesting procedures, and the processing conditions (Masuda, 1989). Excessive N fertility affects pod set and can lead to the emptiness of the pod or one seeded pod. These characteristics lead to the unmarketability of pods. Increases in supply of N after anthesis can increase the amino acids in this crop; however, it can also change the consistence on sugars. The sugar content can decrease from 3.2% to 2.6%, depending on the rate and the timing of N application. Lower plant densities have an effect on darkening pod color and production of consistent higher amino acid levels and sucrose during the maturation period (Chiba et al., 1989). Postharvest handling is also a major influence of quality and flavor. Rapid postharvest cooling helps to slow enzymatic activity and minimizes deterioration. Without proper postharvest temperature management, sugars and amino acids start to deteriorate between 3 to 10 hr after harvesting. If whole plants are packaged and sealed in low density, polyethylene bags, they remain marketable for one week at 20 °C (Iwata et al., 1982). Blanching is an important method to stop the oxidation of fatty acids which change into undesirable tastes in frozen edamame. Both blanching time and temperature must be controlled to reach a balance between the destruction of trypsin inhibitors and the maintenance of good texture.

Edamame at the peak of ripeness has higher levels of (Z)-3-hexenyl acetate, linalool, acetophenone, and jasmore (Sugawara et al., 1988). Other several components which offer edamame beany odor are hexanal, 1-hexanol, (E)-2-hexanal, l-octen-3-Ol, and 2-pentylfuran. Finally Cis-jasmone is found in this crop only at peak of ripeness and may be the key component of aroma.

Edamame gets its popularity from its sweet and savory flavor. Edamame soybean tends to have a mild or neutral unique flavor, which is caused by the distinctive combination of sweetness, sourness, and bitterness (Lee and Hwang, 1998). The sweet taste is determined by the sucrose content and its savory taste by amino acids such as glutamic acid (Masuda et al., 1988). Beany flavor increases with maturity and this can be divided into two components which are beany and bitter (Rackis et al., 1972). The beany taste is thought to originate from linolenic acid oxidized by lipoxygenase and the bitter taste from lipoxygenase itself. Sucrose contributes to sweetness, while saponin, isoflavonoids, and l-arginine add bitterness (Masuda, 1994; Masuda et al., 1988). Flavor of edamame is also due to its nutty characteristic, butter, beany, oily, and flowery (Johnson et al., 1999; Rodale Research Center, 1982; Young et al., 2000).

Other factors that influences flavor are amino acids and γ-aminobutyric acid (GABA), which contribute to the sensory and flavor characteristics (Abe and Takeya, 2005). The increase of phenylalanine and tryptophan caused by the high temperature during the storage changes the sweet flavor of edamame bitter (Kirimura, et al., 1969). Leucine contributes to a bitter taste (Kirimura et al., 1969), while alanine contributes to sweetness which is especially important for the overall taste of edamame (Masuda, 1991). Edamame contains anticarcinogenic compounds which are sulfur-based (isothiocyanates). The isothiocyanates form the group of hot and bitter compounds in vegetables.

Cristo (2002) reported that Chinese consumers of edamame preferred high sugar and they were more sensitive to acidity than American consumers in taste preference. Johnson et al. (1999) reported that U.S. consumers prefer beans with a buttery flavor and a crispy texture. The increase of S fertility may increase the bitterness flavor and reduce sweetness flavor in edamame.

SULFUR

Sulfur is a component to all forms of the living organisms. With an atomic radius of 1.04 angstroms (Å), S exhibits nonmetallic chemical properties (Woollins, 2006). Sulfur plays a major role in plant growth. This element is considered as an essential secondary macronutrient in plant nutrition. Sulfur deficiency impairs basic plant metabolic functions, thus reducing both crop yield and quality. Sulfur deficiencies and responses have been reported in crops worldwide (Tisdale et al., 1986; McGrath and Zhao, 1995; Scherer, 2001) and are becoming more common (Haneklaus et al., 2008). In general, oilseed crops and legumes including soybean have a higher requirement for S than the small grains and maize (Zea mays L.) (Duke and Reisenourer, 1986). Sulfur is also a component of amino acids Cys and Met which are components of proteins, as well as several S-containing coenzymes and secondary plant products derived from the amino acids. These forms of amino acids constitute about 70% of the S content of plants (Haneklaus et al., 2007). Plant absorbs S in form of the sulfate (SO42-) ion along the plasma membrane of root cells and transport it by xylem to vegetative portions for growth (Haneklaust et al., 2007). Most of the S absorbed by plants is converted into Cys which leads to the formation of other organic S compounds in plants (Hell, 1997; Leustek and Saito, 1999; Said, 2003). Sulfur also plays a role in enzymes, coenzymes, an in thiol (-SH) functional groups which are essential to make disulfide bridges needed for proper protein structure (Marschner, 1995). Sulfur forms the sulfonatedoxime component common to all GS compounds as well the side chain of some GS with methionine in their structure (Fenwick et al., 1983). The increase of S gives Brassica and Allium vegetables their characteristic pungent, bitter flavors.

PROTEINS

Protein is the major structural component of all cells in living organisms. Proteins are composed of chains of N-containing amino acids, which are generally divided into two categories: the essential amino acids and non-essential amino acids. These essential amino acids are particularly important during growth and development and are a source of energy. Grain crops constitute approximately 18% of plant protein consumption in the human diet (Smit, 1999). These proteins act as enzymes, antibodies, and structural components of tissues, hormones and blood protein. Protein composition in soybean depends upon various environmental factors including temperature, photoperiod, and nutrition during the seed development (Arslanoglu et al., 2011; Kumar et al., 2013). Changes in the nutritional conditions may cause a wide range of morphological and biochemical changes by modification of genes and enzymes expressions, thus affecting protein synthesis and activity (Fabre and Planchon, 2000). The high synthesis of proteins in soybean seeds is reported to be associated with the application of minerals like P, K, N, and S (Peak et al., 1997; Utsumi et al., 2002; Mahmood et al., 2001).

Due to the crucial role S plays in protein formation, balance is needed between S:N because S is both a building block for proteins and enzymes, and S-containing amino acids are important in forming the high quality glutenins and gliadins (Alberta Agriculture, Food and Rural Development, 2006).

Soybean protein meal and soybean oil accounted for 69% and 30% of the world's supply of protein meal and edible oil in 2006 and 2007, respectively (USDA, 2008). Many international and domestic soybean processors prefer at least 340 g kg-1 protein and 190 g kg-1 oil assuming a seed moisture content of 130 g kg-1 (Hurburgh et al., 1990). There are two factors that are considered to influence the quality of soybean. These are the soybean quantity and quality of protein and oil composition. More than 70% of protein consumed by humans comes from legumes and cereals. Legumes accumulate more proteins compared to the cereals. Soybean contain high amounts of protein, varying between 35% to 50%. This protein percentage depends on the variety or cultivar and the growing conditions. Soybean proteins serve as important source of vegetable protein in the diets of many humans and animals (Lakins, 1981). The amino acid profile of soybean seed protein is limited by its concentration of the S containing amino acids Cys and Met (Duranit and Gius, 1997). Increasing the concentrations of these amino acids in soybean seed would benefit consumers of soybean and could justify a premium worth millions of dollars annually to producers (McVey et al., 1995).

The rapid N2 fixation during pod fill for soybean (stages R5 to R6) has been shown to contribute to increased edamame seed yield and seed protein content (Waterer, 2003). Pod fill NO3 assimilation is necessary to achieve higher seed yield and seed protein content (Waterer, 2003; Rao and Mohamed, 2002). Kandapal and Chandel (1993) reported that the application of S consistently increased the protein and oil contents in grain of soybean resulting in maximum content of protein and oil at 30 kg gypsum per ha. Singh and Aggarwal (1998) also reported that among the sources of S, gypsum produced significantly higher pods per plant and seed per pod of black gram (Vigna mungo).

The National Health and Nutrition Examination Survey (NHANES) reported that women aged 20 and older should consume about 67 g of protein per day. Research suggest that high intakes may be beneficial for various health outcomes, such as weight management, maintaining muscle mass, preventing osteoporosis, and reducing the risk of cardiovascular disease (Rodriguez, 2008; Fulgoni, 2008). Several studies have been performed to examine relationships between plant protein intake and risk factors for coronary heart disease containing 25 g of soy protein per day and may reduce the risk of heart disease (FDA, 1999). Other reports have shown significant reduction in cholesterol (Harland, 2008; Zhan, 2005; Reynolds, 2006; Jenkins, 2010). Furthermore, other discoveries showed that the uptake of high quality protein led to adequate bone strength and density during aging (Wolf, 2006). Kim (2008) reported that in women, soy protein was associated with reduced breast cancer. Though there are more health benefits related to protein consumption, there are some side effects for overconsumption for protein products. It was discovered that, diets which are high in protein generate a large amount of acid in body fluids (Barzel and Massey, 1998). Kidneys respond to this dietary acid challenge with net acid excretion, and concurrently the skeleton supplies buffer by active resorption of bone resulting in excessive Ca loss (Barzel and Massey, 1998). The overconsumption of protein is believed to be associated by the increase in risk of forearm fracture for women who consume more than 95 g per day compared to those who consume less than 68 g per day (Reddy et al., 2002). Increases in protein intake from 48 to 141 g significantly elevated urinary calcium values from 175 to 338 mg in humans (Johnson et al., 1970). The relationship of animal protein rich diet to Ca metabolism demonstrated that the increase in urinary Ca excretion has risk factor for the development of osteoporosis (Breslau et al., 1988). Increasing the ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in post-menopausal women. The animal foods provide predominantly acid precursors, whereas protein in vegetable foods is accompanied by base precursors not found in animal foods. Imbalance between dietary acid and base precursors leads to a chronic net dietary acid load that may have adverse consequences on bone. An increase in vegetable protein intake and a decrease in animal protein intake may decrease bone loss and the risk of hip fracture (Sellnoyer et al., 2001). The high protein intake induces changes in urinary uric acid and citrate excretion rates and a decrease in the ability of urines to inhibit calcium oxalate monohydrate crystal agglomeration (Kok et al., 1990). High protein intake can cause intermittent abdominal pain, transient elevations in transaminases, and hyperalbuminemia without there being any identifiable cause (Mutlu et al., 2006). The composition of dietary proteins has the potential to influence the balance of glucagon and insulin activity.

Soy flour is the basic soy-protein product produced from soybean flakes. This product is grounded from high quality, clean, dehulled soybeans after most of the oil has been removed. It contains at least 50% protein on a dry mass (DM) basis (Waggle and Kolar, 1979). Next, the soy-protein concentrate is prepared from high quality, clean, dehulled soybeans by removing most of the oil and water soluble nonprotein constituents. This product is made of 65% protein on a DM basis (Campbell et al., 1991). Furthermore, the isolated soy protein is the major proteinaceous fraction prepared from high-quality, dehulled soybeans by removing most nonprotein components; it contains not less than 90% protein on a DM basis (Kolar et al., 1985). Oilseeds add important nutritional value to the diet due to high quality protein and or vegetable oil, together with oil soluble vitamins like vitamin A. It was found that oilseed meals from soybean and other oilseeds type are rich in protein and when they are mixed up with cereals, they provide a balance nutrition (Sarwar et al., 2004b; 2011a).

EXPERIMENTAL PERSPECTIVE

This study of S fertility concentrations on edamame soybean (green soybean) is needed to determine any influence on protein content in this crop. This information will identify the influence of production factors on edamame quality and can be used to enhance marketing and increasing consumer preference. There has been some research on S and its influence on protein content, taste, and flavor in cruciferous species. However, there is little information on the effect of S fertility on edamame protein content. By using edamame soybean, a crop which is currently among the emerging crop in U.S., the study will help to determine the production practices which can increase potential health benefits and economic advantages which this crop can contribute to the Americans. Thus, the objectives of this study were: 1) to determine if S fertilization influences the accumulation of bean protein content of edamame soybean; and 2) to identify the S fertilization concentration that maximizes bean protein content.

REFERENCES

Abe, I., Y. Okuda, T. Iwata, and K. Chachin. 1985. Kikai dakkyo edamame no sendo hoji gijutsu ni kansuru kenkyu. Engei Gakkai Happyo Yoshi, Fall S60:494-495.

Achouri, A., J.I. Boye, and D. Belanger. 2005. Soybean isoflavones: efficacy of extraction conditions and effect of food type on extractability. Food Res. Intl. 38:1199-1204.

Alabama A&M University Extension. 2015. <http://www.aces.edu/pubs/docs/A/ANR-2231/ANR-2231-low.pdf>.

Alberta Agriculture, Food and Rural Development. 2006. wheat nutrition and fertilizer requirements: Sulfur. [Online]

Arslanoglu, F., Aytac, S. and Oner, E. K. 2011. Effect of genotype and environment interaction on oil and protein content of soybean (*Glycine max* (L.) Merrill) seed. *African JournalofBiotechnology,*10:1840918417.Available:http://www1.agric.gov.ab.ca/$department/deptdocs. nsf/all/crop1297.dry matter yield and quality of soybean (*Glycine max*). *Indian J. of Agron.,* 38: 137-139.

Barzel, U.S. and Massey,L.K. 1998. "Excess dietary protein may can adversely affect bone," J. Nutr. 128(6):1051–1053.

Bennet, J.O. 2005. Soybean seed components as affected by nodal position, environmental conditions, and irrigation. https://mospace.umsystem.edu/xmlui/handle/10355/4240. Accessed on 21st, April, 2017 at 12:30 pm.

Bernick, K. 2009. Edamame takes root in U.S. corn and soybean dig. Penton Media, Overland Park, KS.

Bowman, S.A., Lino, M., Gerrior, S.A., & Basiotis, P.P. 1998. The healthy eating index 1994–1996. Washington, D.C.: USDA Center for Nutrition Policy and Promotion Publ. CNPP-5.

Breslau, N.A., L. Brinkley, K.D. Hill, and C.Y.C. Pak. 1988. Relationship of animal protein-rich diet to kidney stone formation 6 ISRN Nutrition and calcium metabolism. J. Clinic. Endocrinology Metabol. 66(1):140–146.

Campbell, C.A., M. Schnitzer, G.P. Lafond, R.P. Zentner, and J.E. Knipfel. 1995. Thirty-year crop rotations and management practices effects on soil and amino nitrogen. Soil Sci. Soc. Amer. J. 55:739-745.

Carrão-Panizzi, M.C. and J.B. Silva. 2011. Soy in food: Quality in grain production with added value. *Proc. Congreso De La Soja Del Mercosur* - Mercosoja, Rosário.

Charron, C.S., F.L. Allen, R.D. Johnson, V.R. Pantalone, and C.E. Sams. 2005. Correlations of oil and protein with isoflavone concentration in soybean [*Glycine max* (L.) Merr.]. J. Agric. Food Chem. 53:7128–7135.

Chiba, Y., S. Yaegashi, and H. Sato. 1989. Cultivation method of green soybean I: Effect of plant density on quality and yield. Tohoku Nogyo Kenkyu 42:279-80.

Crisosto, C.H., & Crisosto, G.M. 2002. Understanding American and Chinese consumer acceptance of Redglobe table grapes. Postharvest Biology and Technology, 24, 155–162.

Culbertson, E. 1999. Pachamama Organic Farm, Longmont, Colorado. http://www2.hawaii.edu/~theodore/Images/edamame.pdf

D. E. Sellmeyer, K. L. Stone, A. Sebastian, and S. R. Cummings, “A high ratio of dietary animal to vegetable protein increases the rate of bone loss and the risk of fracture in postmenopausal women,” *American Journal of Clinical Nutrition*, vol. 73, no. 1.

D. J. Kok, J. A. Iestra, C. J. Doorenbos, and S. E. Papapoulos. 1990. "The effects of dietary excesses in animal protein and in sodium on the composition and the crystallization kinetics of calcium oxalate monohydrate in urines of healthy men," *Journal of Clinical Endocrinology and Metabolism*, vol. 71, no. 4, pp. 861– 867.

Demirbuker M. and L.G. Blomberg. 1990. Group separation of triacyl glycerols on micropacked argentation columns using supercritical media as mobile phase. Journal of Chromatographic Science 28:67-72.

Duke, S.H. and H.M. Reisenauer. 1986. Roles and requirements of sulfur in plant nutrition. p. 123–168. In: M.A. Tabatabai (ed.). Sulfur in agriculture. Agron. Monogr. 27. ASA, CSSA, and SSSA, Madison, WI. Edamame and acceptance of edamame based patties. HortScience 41:1616-1622.

Ernst, M. 2000. Industry Focus: Soy Foods Explosion. PROFILES in Agricultural Entrepreneurship: Iowa Soy Specialties, LLC. University of Kentucky Cooperative Extension Publication AEC 2000-01. <http://www.uky.edu/Ag/AgEcon/publications/case_IowaSoy.pdf>.

Ernst, M. and T. Woods. 2001. Marketing challenges for emerging crops in Kentucky: Vegetable soybean. Cooperative extension service. University of Kentucky.

Ernst, M., 2000. Marketing new crops. Cooperative extension service. University of Kentucky.

Ernst, M. and T. Woods. 2001. Marketing challenges for emerging crops in Kentucky: Vegetable Soybeans, p. 35–38. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). *2nd Intl. Veg. Soybean Conf.* Washington State Univ, Pullman, WA.

Fabre, F. and Planchon, C. 2000. Nitrogen nutrition, yield and protein content in soybean. *Plant Science,* 152: 51-58.

Farmakalidis, E. 1999. Consumer issues in relation to antioxidants, p. 423–435. In: N.J. Temple and M.L. Garg (eds.). Antioxidants in human health and disease. New York: CABI Publishing.

Fenwick, G.R., K. Heaney, and W. Mullin. 1983. Glucosinolates and their breaking down products in food and food plants. Critical reviews in Food Science and Nutrition 18:123-201.

Food and Drug Administration. 1999. Soy Protein and heart disease health. <http://www.soyfoods.org/nutrition-health/soy-for-healthy-living/soy-for-heart-disease/soy-protein-and-heart-disease-health-claim>.

Fulgoni, III, V.L. Current protein intake in America: Analysis of the National Health and Nutrition Examination Survey, 2003–2004. Am. J. Clin. Nutr. 2008, 87, 1554–1557.

Gaskell, M. 2001. Field Trials with ‘‘Edamame’’ Vegetable Soybeans. <http://www.sbceo.k12.ca.us/~uccesb1/ sfoct01.htm>.

Glanz, K., Basil, M., Maibach, E., Goldberg, J., & Snyder, D. 1998. Why Americans eat what they do: Taste, nutrition, cost, convenience, and weight control concerns as influences on food consumption. Journal of the American Dietetic Association, 98, 1118–1126.

Gotoh, K. 1984. Historical review of soybean cultivation in Japan. Tropical Agriculture Research Series 17:135-142.

Goubran, R. and N. Garti. 2000. Stability of water in oil emulsions using high molecular weight emulsifires. J. Disper. Sci. Tech. 9:131-148.

Guan, P.Z. 1977. Vegetable bean, p. 333-338. In: S.X. Li (ed.). Vegetable Cultivation, South China. Chinese Agricultural Publishers, Beijing.

Gupta, A.K., M. Kapoor, and A.D. Deodhar. 1976. Chemical composition and cooking characteristics of vegetable and grain-type soybeans. Journal of Food Science and Technology 13:133-37.

Haneklaus S., E. Bloem, and E. Schnug. 2007. Sulfur and Plant Disease, p. 101-118. In: L.E. Datnoff, W.H. Elmer, and D.M. Huber (eds.). Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN.

Haneklaus, S., E. Bloem, and E. Schnug. 2008. History of sulfur deficiency in crops. p. 45–58. In: J. Jez (ed.). Sulfur: A missing link between soils, crops, and nutrition. Agron. Monogr. 50. ASA, CSSA, and SSSA, Madison, WI.

Haneklaus, S., E. Boem, E. Schnug, L.J. de Kok, and I. Stulen. 2007. Sulfur, p.183-238. In: A.V. Barker and D.J. Pilbeam (eds.). Handbook of plant Nutrition. CRC press, Boca Raton, FL.

Harland J.I. and T.A. Haffner. 2008. Systematic review, meta-analysis and regression of randomised controlled trials reporting an association between an intake of circa 25 g soya protein per day and blood cholesterol. Atherosclerosis 200(1):13-27.

Hell, R. 1997. Molecular Physiology of Plant Sulfur Metabolism. Planta 202:138-148.

Hemphill, D.D. and C. Miles. 1999. Effects of between row spacing and rate of applied nitrogen on yield of two cultivars of edamame. Vegetable research at North Willamette Research and Extension Center, 1999. Special Report, OSU Ag. Expt. Sta.

Hilliam, M. 1995. Functional foods: The western consumer viewpoint. Nutrition Review, 54, S189–S194.

Hurburgh, C.R., Brumm, T.J., Guinn, J.M. and Hartwig, R.A. 1990. Protein and oil patterns in U.S. and world soybean markets. *Journal of the American Oil Chemists' Society*, **67**, 966-973.

Hurburgh, C.R., Brumm, T.J., Guinn, J.M. and Hartwig, R.A. 1990. Protein and oil patterns in U.S. and world soybean markets. *Journal of the American Oil Chemists'*.

Hymowitz, T., F.I. Collins, J. Panczner, and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in the soybean seed. Agronomy Journal 64:613-16.

IDA. 1990. Iwate-ken Seikabutsu To Shukka Kikaku Shido Hikkei. Iwate-ken Department of Agriculture, Morioka.

Informa/United Soybean Board. 2010. Farmer-driven for every U.S. soybean farm's bottomline. 13 April 2016. <http://unitedsoybean.org/wpcontent/uploads/41191_FINAL_FY2010AnnualReport.pdf>.

Iwata, T., Sugiura, H., & Shirahata, K. 1982. Keeping quality of green soybeans by whole plant packaging. J. Japanese Soc. Hort. Sci. 51, 224–230.

Jenkins, D.J.A., A. Mirrahimi, K. Srichaikul, C.E. Berryman, L. Wang, A. Carleton, S. Abdulnour, J.L. Sievenpiper, C.W. Kendall, and P.M. Kris-Etherton. 2010. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. J. Nutr. 140(12):2302-2311.

Johnson, D., S. Wang, and A. Suzuki. 1999. Edamame: A vegetable soybean for Colorado, p. 385–387. In: J. Janick (ed.). Perspectives on new crops and uses. ASHS Press, Alexandria, VA.

JSTA. 1987. Yasai Hinshu Meikan. Japanese Seed Trade Association, Tokyo.

Kaiser, C. and M. Ernst. 2007. <http://www.uky.edu/Ag/CCD/introsheets/edamame.pdf>.

Kaiser, C. and M. Ernst. 2013. Estimating the Economic Viability of a New Crop Alternative for the U.S. Organic Market: Edamame - A Vegetable Soybean.

Kandpal, B.M. & Chandel, A.S. 1993. Effect of gypsum and pyrites as sources of sulphur on nitrogen fixation.

Kim, M., J. Kim, S. Nam, S. Ryu, and G. Kong. 2008. Dietary intake of soy protein and tofu in association with breast cancer risk based on a case-control study. Nutr. Cancer 60:568-576.

Kirimura, J., A. Shinizu, A. Kimizuka, T. Ninomiya, and N. Katsuya. 1969. The contributions of peptides and amino acids to the taste of food stuff. J. Agric. Food Chem. 17:689-695.

Kiuchi, Y., H. Ishikawa, N. Nitta, and T. Sasaki. 1987. Collection of crop genetic resources and their characteristics in Iwate Prefecture I: Characteristics of local soybean varieties. Tohoku Nogyo Kenkyu 40:139- 40.

Kokobun, M. 1991. Cultural Practices and Cropping Systems for Vegetable Soybean in Japan. Vegetable Soybean, Research Needs for Production and Quality Improvement. Nat. Agri. Res. Center, Japan.

Kopsell, D.E., D.A. Kopsell, W.M. Randle, T.W. Coolong, C.E. Sams, and J. Celentano. 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. J. Agri. Food Chem. 51:5319-5325.

Kumar, V., A. Rani, A.K. Dixit, D. Bhatnagar, and G.S. Chauhan. 2009. Relative changes in tocopherols, isoflavones, total phenolic content, and antioxidative activity in soybean seeds at different reproductive stages. J. Agr. Food Chem. 57:2705-2710.

Lee, J.D., and Y.H. Hwang. 1998. Quality evaluation for vegetable use in local soybean cultivars with various seed coat color. Korean J, Crop Sci. 43, 83–88.

Leustek, T. and K. Saito. 1999. Sulfate transport and assimilation in plants. Plant Physiol. 120:637-643.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, U.K.

Massey, L.K., H. Roman-Smith, and R.A.L. Sutton. 1993. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. J. Amer. Diet. Assoc. 93(8):901-906.

Masuda, A. 1994.What is the quality of vegetable soybeans? Soybean Monthly Report January 1994.

Masuda, R., K. Hashizume, and K. Kaneko. 1989. Effect of holding time before freezing on the constituents and the flavor of frozen green soybeans. Nihon Shokuhin Kogyo Gakkaishi 35:763-70.

Mausda, R. 1991. Quality Requirement and Improvement of Vegetable Soybean. Vegetable Soybean, Research Needs for Production and Quality Improvement. National Food Research Institute, Ministry of Agriculture, Forest and Fisheries, Japan.

McVey, M.J., Pautsch, G.R. and Baumel, C.P. 1995. Estimated domestic producer and end user benefits from genetically modifying U.S. soybeans. J. Prod. Agric. 8: 209-214.2, pp. 265–274, 2002.

Messina, M. 2001. An overview of the health effects of soyfoods and soybean isoflavones, p. 117-122. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). Second Int. Vegetable. Soybean Conference, Tacoma, W. A. 10-12 Aug.2001.Washington State Univ., Pullman, WA.

Miles, C.A. and C. Chen. 2000. Washington State University edamame trials 2000. <http:agsyst.wsu.edu/Edamame.Report2000.pdf>.

Montri, D.N., K.M. Kelley, and E.S. Sanchez. 2006. Consumer interest in fresh, in-shell edamame and acceptance of edamame based patties. Hortscience 41: 1616-1622.

Mutlu, E., A. Keshavarzian, and G.M. Mutlu. 2006. Hyperalbuminemia and elevated transaminases associated with high-protein diet. Scandinavian J. Gastroenterology 41(6):759–760.

Nair, R., S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida, and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. Plant Sci. 179:154–163.

Nuss, J. 2013. US soybean farmers see growth potential in edamame. 21 Oct. 2015. <http://bigstory.ap.org/article/us-soybean-farmers-see-growth-potential-edamame>.

Ogles, C.Z., Guertal, E.A., Weaver, D.B., and C. Mitchell. 2015. Edamame in the home garden.University of Alabama Cooperative Extension Fact Sheet ANR-2231. <http://www.aces.edu/pubs/docs/A/ANR-2231/ANR-2231-low.pdf

Organic Edamame Production. 2007. Center for Environmental Farming Systems, North Carolina State University, Raleigh, NC. <http://www.cefs.ncsu.edu/resources/fieldnotesforfarmers/20070103organicedamame.pdf >.

Peak, N.C., J. Imsande, R.C. and Shoemaker. 1997. Nutritional control of soybean seed storage protein. Crop Sci. 37:498-503.

Rackis, J.J., D.H. Hoing, D.S. Sessa, and H.A. Moser. 1972. Lipoxygenase and peroxidase activities of soybeans as related to flavor profile during maturation. Cereal Chem. 49:586-597.

Rao, M.S.S., A.S. Bhagsari, and A.I. Mohamed. 2002. Fresh green seed yield and seed nutritional traits of vegetable soybean genotypes. Crop Sci. 42: 1950-1958.

Reddy, S.Y., Wang,C.-Y., Sakhaee,K., Brinkley, L., and Pak,C.Y.C. 2002. Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. Amer. J. Kidney Dis. 40(2):265–274.

Reynolds, K., A. Chin, K.A. Lees, A. Nguyen, D. Bujnowski, and J. He. 2006. A meta-analysis of the effect of soy protein supplementation on serum lipids. Amer. J. Cardiol. 98, 633–640.

Rodale Research Center. 1982. Fresh green soybeans: Analysis of field performance and sensory qualities. Rodale Press, Kutztown, PA.

Rodriguez, N. and P. Garlick. 2008. Introduction to Protein Summit 2007: Exploring the impact of high quality protein on optimal health. Amer. J. Clin. Nutr. 87:1551S-1553S.

Roseboro, K. 2016. Edamame Offers Good Non-GMO Opportunities to US Farmers. <http:www.nongmoreport.com/articles/april2012/edamame-non-gmo-us-farmers.php>. (accessed on 15 March 2016).

Saito, K. 2003. Molecular and metabolic regulation of sulfur assimilation: Initial approach by the post-genomics strategy, p.11-20. In: J.C. Davidian, D. Grill, L.J. de Kok, I. Stulen, M.J. Hawkesford, E. Schnug, and H. Rennenberg (eds.). Sulfur Transport and Assimilation in Plants: Regulation, Interaction and Signaling. Backhuys Publishers: Leiden, The Netherlands.

Scherer, H.W. 2001. Sulphur in crop production—Invited paper. Eur. J. Agron. 14:81–111.

Schnitkey, G. 2015. Revenue and costs for corn, soybeans, wheat, and double-crop soybeans, actual for 2009 through 2014, projected 2015 and 2016. Dept. of Agri. and Con. Econ. Farm Business Management.

Shanmugasundaram, S., T.C.S. Tsou, and S.H. Cheng. 1989. Vegetable soybeans in the East, p.1978-1987. In: A.J. Pascale (ed.). World Soybean Research Congress IV: Actas proceedings.

Shepherd, R. 1997. The psychology of food choice: The influence of attitudes and flavour perception, p.157–172. In: F.A. Toma´s-Barbera´n and R.J. Robins (eds.). Phytochemistry of fruit and vegetables. Clarendon Press, Oxford, U.K.

Shurtleff, W. and A. Aoyagi. 2004. History of World Soybean Production and Trade. Soyfoods Center, Lafayette, California.

Shurtleff, W. and A. Aoyagi. 2009. History of edamame, green vegetable soybeans, and vegetable-type soybeans (1275 - 2009): Extensively annotated bibliography and sourcebook. Soyinfo Center, Lafayette, CA.

Shurtleff, W. 2001. On the English-language etymology of "green vegetable soybeans," "edamame," "vegetable-type soybeans," and "food-grade soybeans", p.179–181. In: T. Lumpkin, ed. 2001. Second International vegetable Soybean Conference. Washington State University, Pullman, WA.

Singh, Y.P. and Aggarwal, R.L. 1998. Effect of sulphur sources and levels on yield, nutrient uptake and quality. Soil Sci. Soc. Am. J. Society 67:966-973.

Smit, E., J. Nieto, C. Crespo, and P. Mitchell. 1999. Estimates of animal and plant protein intake in U.S. adults: Results from the Third National Health and Nutrition Examination Survey, 1988-1991. J. Amer. Diet. Assoc.99:813-820.

Smith, J.M. and F.O. Van Duyne. 1951. Other soybean products, p.1055-1078. In: K.S. Markley (ed.). Soybeans and Soybean Products. Interscience, New York, NY.

Soybean Oil Consumption 2007/2008, United Soybean Board Market View Database. 2009. <http://usb.adayana.com:8080/usb/jsp/login.jsp>.

Soyfoods. 2014. Soy foods and supplementation: a review of commonly perceived health benefits and risks. <https://www.ncbi.nlm.nih.gov/pubmed/24473985>.

SoyStats American Soybean Association, http://www.soystats.com/2004

Soystats. 2009. Ending Stocks. American Soybean Board. <http://soystats.com/2009/Default-frames.htm>.

Sugawara, E., T. Ito, S. Odagiri, K. Kubota, and A. Kobayashi. 1988. Changes in aroma components of green soybeans with maturity. Nihon Nogei Kagaku Kaishi 62:148-55.

Sunada, K. 1986. Methods of bean production in (Hokkaido No Mame Saku Gijutsu). Sapporo: Nogyo Gijutsu Fukyu Kyokai.

Tisdale, S.L., R.B. Reneau, Jr., and J.S. Plateau. 2000. Atlas of sulfur defencies. P 295-322. In M.A Tabatabai. Report 2000 pdfs.

United States Department of Agriculture, Foreign Agricultural Service. 2008. Oilseeds: World markets and trade. Report, Circular Series FOP 11–08.USDA-FAS, Washington, DC.

University of Arkansans Extension, 2012. Edamame production facts for Arkansas's emerging industry. University of Arkansas Cooperative Extension Service Printing Services, Fayetteville, AR.

Velasquez, M.T. and S.J. Bhathena. 2007. Role of dietary soy protein in obesity. Int. J. Med. Sci. 4:72–82.

Watanabe, M. 1988. Tunneru saibai--Sapporo-midori. Nogyo Gijutsu Taikei (yasai) 13:1-8.

Westenhoefer, J. and V. Pudel. 1993. Pleasure from food: Importance for food choice and consequence of deliberate restriction. Appetite 20:246–249.

Wolf, R. 2006. The underappreciated role of muscle in health and disease. Amer. J. Clinic. Nutr. 84:475-482.

Woollins, J.D. 2006. Sulfur: Inorganic chemistry. In: R.B. King (ed.). Encyclopedia of inorganic chemistry. John Wiley and Sons, Inc., Chichester, NY.

Wszelaki, A.L., J.F. Delwiche, S.D. Walker, R.E. Liggett, J.C. Scheerens, and M.D. Kleinhenz. 2005. Sensory quality and mineral and glycoalkaloid concentrations in organically and conventionally grown redskin potatoes (*Solanum tuberosum*). J. Sci. Food Agri. 85(5):720–726.

Young, G., T. Mebrahtu, and J. Johnson. 2000. Acceptability of green soybeans as a vegetable entity. Plant Foods Human Nutr. 55:323–333.

Zhan S. and S. Ho. 2005. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Amer. J. Clin. Nutr. 81(2):397-408.

CHAPTER III: DETERMINING THE EFFECTS OF SULFUR FERTILITY LVELS ON EDAMAME SOYBEAN [*Glycine max* (L.) Merrill] PROTEIN COMPONENTS

ABSTRACT

Edamame [*Glycine max* (L.) Merr.] a vegetable type soybean is harvested and consumed before reaching its maturity stage (R6). Its health properties drew attention to U.S. consumers because of its high protein content. Proteins are the major structural component of all cells in the living organisms. They are made of essential and non-essential amino acids which play a big role in growth and development and are source of energy. Proteins act as enzymes, antibodies, and hormones and blood protein. Sulfur (S) is a component of the amino acids cysteine (Cys) and methionine (Met) which are the building blocks for proteins. Little is known about how levels fertility influences protein content in edamame soybean. As result, a solution culture experiment in a protected environment was conducted to evaluate the effect of S fertility concentrations on protein content in edamame. Seeds of the 'Chiba' edamame were allowed to germinate under greenhouse conditions at 22 °C day/14 °C night for 15 days and transplanted in nutrient solution culture in the fall of 2016 using a modified Hoagland's solution containing S treatment concentrations of 4, 8, 16, 32, and 64 mg S L^{-1} was delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4). Treatments were arranged in a randomized complete block design with four replications containing five s treatment levels per replication. The solution was changed every 2 weeks after transplanting until plants reached the R6 maturity stage. Plants were harvested approximately 60 days after planting and weighed for fresh biomass (FM) before tissues were dried at 60 °C prior the extractions and protein content analysis. Elemental S concentrations were measured in oven-dried bean tissue using ICP-MS.

Manipulating the S fertility concentrations showed no significant difference on bean accumulation of crude protein (P=0.171), Adjusted protein (P=0.171), ADFNDF (P=0.409), ADFDM (P=0.707), aNDf (P=0.271). The results showed that increasing the S concentration from 4 to 64 mg S L^{-1} in nutrient solution culture did not affect the protein composition in 'Chiba' edamame.

Keywords: amino acids, Chiba, ICP, macronutrient, micronutrients

INTRODUCTION

Product quality, particularly which related to flavor, affects food purchasing decisions (Farmakalidis, 1999; Glanz et al., 1998; Hilliam, 1995) as real or perceived quality shortfalls shape consumer prefer to eat fresh produce and food sensory attributes drive immediately and future consumption (Shepherd, 1997). Concerns about reduction in taste quality can interfere with the adoption of health diets (Bowman et al., 1998; Glanz et al., 1998), as consumers emphasize sensory experiences during consumption like appearance, texture, aroma, taste, with the pleasure derived from consumption as an important motivator in eating (Westenhoefer and Pudel, 1993).

Edamame [*Glycine max* (L.) Merr.] is an annual herbaceous plant originating from Southern Asia. This crop has large green beans which are served in pods as snacks similar to peanuts (Shurtleff, 2001). Unlike field soybean, edamame is harvested at reproductive stage R6 when the seeds are immature and have expended to fill 80% of the pod with. This emerging crop in U.S. is counted among the vegetable crops that have economical and health contributions to the American society. Edamame is currently the second most consumed soybean product behind soymilk in the U.S. (Soyfoods, 2014). Soyatech, LLC, a U.S. soybean and oilseed information consultant reported that sales of frozen edamame shelled or in the pods grew 40% between 2003 and 2007 to $30 million (Bernick, 2009). Rosboro (2012) reported that 97% of edamame sold to the frozen market was imported from China and other Asian nations. China only exports 13,608 to 18,144 metric tonnes of edamame to the U.S. each year. Nuss (2013) reported that U.S. consumed 22,700 to 27,270 metric tonnes of edamame. This increase in demand is expected to continue as consumers look for healthier, low cost sources of protein to add to diet. The projected net returns for this crop range from $640 to $6,177 per ha, depending on the market and harvesting method (Ernst Woods, 2001; Ernst, 2000). At lower range of return, edamame is more profitable than sweet corn and pumpkin. At the high end of return, edamame can be more profitable than staked tomatoes and cantaloupe. In most cases, the breakeven price for edamame is less than $2.20 per kg, much lower than the average wholesale price of $3.30 per kg. Interest for this crop may increase in the coming years as commodity soybean prices are projected to decrease (Schnitkey, 2015).

During harvesting, the majority of the pods have to be mature and the whole plant has to be harvested (Culbertson, 1999). The measured nutritional value of soybeans include low fat and high protein (Rao et al., 2002). Edamame contains all the essential amino acids which can be used particularly as a good meat protein substitute. Montri et al. (2006) reported that edamame contains lower levels of trypsin inhibitors, fewer indigestible oligosaccharides, a greater amount of vitamins, and higher levels of phytic acid than agronomic soybeans. These factors make edamame more nutritious than agronomic soybeans. Edamame is a good source of sodium (Na), iron (Fe), calcium (Ca), potassium (K), phosphorus (P), magnesium (Mg), folate, and vitamins A, B_1, B_2, B_3, C, E and K (Nair, 2010). The other health properties beyond the normal nutrition include the antioxidants isoflavones and saponins which prevent the negative effects of free radicals in the body (Achouri et al., 2005; Patel et al., 2001). The dietary intake of antioxidants is associated with the reduction of incidence of atherosclerosis, cancer risk, neurodegenerative diseases, and improved immune system (Kumar et al., 2009; Patel et al., 2001). Edamame also contains also isoflavones that can interfere in the physiology of the cells, as in the proliferation, growth, and maturity, acting as important regulators to maintain health. The isoflavones are the phytohormones which reduce prostate, breast cancer, cholesterol level and lighter menopausal symptoms (Messina, 2001). The protective roles associated with soybean isoflavones have received increasing attention by consumers (Charron et al., 2005). While edamame is an excellent source of plant-based protein, it is also a complete protein containing all the essential amino acids in the human diet (Velasquez and Bhathena, 2007).

Edamame tends to have a mild or neutral flavor, which is caused by the distinctive combination of sweetness, sourness, and bitterness (Lee and Hwang, 1998). Sucrose contributes to sweetness, while saponin, isoflavonoids, and l-arginine add bitterness (Masuda, 1994; Hashizume et al., 1988). Flavor of edamame is due to its nutty, buttery, beany, oily, and flowery characteristics (Johnson et al., 1999; Rodale Research Center, 1982; Young et al., 2000). Johnson et al. (1999) reported that U.S. edamame consumers prefer beans with buttery sweet, flowery flavor and crisp texture. Cristo (2002) reported that the Chinese consumers of edamame showed that they prefer high sugar content and were more sensitive to acidity than American consumers (Cristo, 2002). Hymowitz et al. (1972) reported that too high protein in edamame can lead to the lack of sweetness which is an important component of flavor. Postharvest handling is among the major influence of quality and flavor. Postharvest management such as rapid precooling helps to slow enzymatic activity and minimizes deterioration. Blanching helps to stop the oxidation of fatty acids into undesirable tastes. Kopsell (2003) reported that the sulfur components in vegetable such as acrid flavors are regarded as objectionable by consumers. The lowering of sulfur fertility in edamame is likely to decrease the levels of negative flavors of glucosinolates and sulfur methylcysteine sulfoxide.

Sulfur (S) is an essential macronutrients for plant growth. Its deficiencies and responses have been reported in crops worldwide (Tisdale et al., 1986; McGrath and Zhao, 1995; Scherer, 2001) and are becoming more common (Hanecklaus et al., 2008). In general, the oilseed crops and legumes including soybean have a much higher requirement for S than the small grains and maize (*Zea mays* L.) (Duke and Reisenourer, 1986). Sulfur is a component of the amino acids cysteine (Cys) and methionine (Met) which are components of proteins, as well as several sulfur-containing coenzymes and secondary plant products derived from the amino acids. These forms of amino acids constitute about 70% of S in plant tissues (Haneklaus et al., 2007). Most of the S absorbed by plants is converted into Cys which goes on to form other organic S enzymes, coenzymes, an in thiol (-SH) functional groups essential to make disulfide bridges for proper protein structure (Marschner, 1995). Sulfur forms the sulfonatedoxime component common to all glucosinolates (GS) compounds as well the side chain of some GS with methionine in their structure (Fenwick et al., 1983). Kopsell et al. (2003) reported altering the level of S in solution culture significantly affected the flavor and bitterness compounds in kale (*Brassica oleracea* var. *acephala*).

Protein is the major structural component of all cells in the animal body. Protein are made up of chains of N-containing amino acids, which are generally divided into two categories: the essential amino acids and non-essential amino acids. In soybean, the rapid N_2 fixation during pod fill (R5-R6) contributes to the increase of seed yield and seed protein content (Waterer, 2003). About 18% of grains contribute the most to plant protein consumption (Smit, 1999). Research suggests that higher intakes of protein may be beneficial for various health outcomes, such as weight management, maintaining muscles mass, preventing osteoporosis, and reducing the risk of cardiovascular disease (Rodriguez, 2008; Fulgoni, 2008). Kim (2008) reported that in women, soy protein was associated with reduced breast cancer. Oilseeds add important nutritional value to the diet due to high quality protein and vegetable oil, together with oil soluble vitamins like vitamin A. As S is the major component for soy protein, it is believed that manipulating the levels of S fertility during the growth and development of edamame may have an effect on protein content.

MATERIALS AND METHODS

Plant Culture.

'Chiba' edamame soybean seeds (Burpee Selected Seeds, Warminster, PA) were sown into 2.5 x 2.5 cm growing cubes (Grodan A/S, DK-2640, Hedehusense, Denmark) on 3 Oct. 2016 and germinated in a greenhouse in Normal, IL (lat. 35°30'S at 22 °C day/15 °C night temperature under a 13 h photoperiod). Peter's 20N-4.4P-16.6K water-soluble fertilizer was applied twice a week at the rate of 200 mg L^{-1} N. Fifteen days after planting (DAP), seedlings were transferred to tubs (Rubermaid Inc., Wooster, OH) and filled with 30 L of a modified Hoagland's nutrient solution in deionized water (Hoagland and Arnon, 1950). Elemental nutrient concentration of the nutrient solution were (mg L^{-1}): N (105), P (15.3), K (117.3), Ca (40.0), Mg (24.3), Fe (0.5) B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025). The S treatment concentrations were as follow: 4, 8, 16, 32, and 64 mg S L^{-1} delivered using magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2S0_4). Magnesium (Mg) levels were maintained at 24.3 mg L^{-1} across all treatments using magnesium chloride ($MgCl_2$) additions to the 4, 8, and 16 mg S L^{-1} treatments. Each container held four individual edamame plants placed into 2 cm round holes set at 15 cm x 9 cm spacing. The experimental design was a random complete block design with five S treatments and four replications. Solutions were aerated with an air blower (Model VB-007S, Sweetwater, Ft. Collins, CO) connected to an air stone in each container. Deionized water was added every 2 days to maintain initial solution volumes in each container. Nutrient solutions were replaced at 14 days after transferring to hydroponic culture to refresh the solution to the initial nutrient concentrations. Photoperiod was reduced to 10 h on 8 Nov. 2016 to induce flowering. Plants were harvested at the R6 growth stage, approximately 60 days after transferring to hydroponic growing tubs. At harvest, pods, shoot and root tissues were collected from each container (4 plants each) and fresh mass (FM) for pods, beans, shoots and roots of each treatment replication was recorded. Beans, shoots and roots were dried at 60 °C until a constant weight was achieved. Dried bean tissue was sent to Cumberland Valley Analytical Services, Inc. Labs (Waynesboro, PA) for elemental composition, protein content and plant biomass analysis.

Elemental Determination.

At Cumberland Valley Labs, dried soybeans were ground so they would pass through a 0.5 mm screen in a sample mill grinder (model 1093, Cyclotec-Tector, Höganäs, Sweden). A 0.35 g of edamame was dried for 1 hr at 535 °C. The ash was digested in an open crucibles for 20 min in 15% nitric acid on hot plate. The sample was diluted to 50 ml and analyzed by inductively coupled argon plasma atomic emission spectrometry (ICP-AES; model Vista AX, Varian, Inc., Palo Alto, CA).

Sulfur Analysis. The S analysis followed the Leco Organic Application Note "Sulfur and Carbon in Plant, Feed, Grain, and Flour" procedure from 203-821-321, 5/08-REV1. Approx. 0.15 g sample was introduced to a Leco S632 Sulfur Combustion Analyzer (Leco Corporation, St. Joseph, MI) with the use of tungsten oxide as a combustion aid.

Tissue Analysis: Plant Biomass.

Dry matter analysis was conducted using procedures adapted from the Forage Fiber Analysis (USDA Agricultural Research Service Handbook # 379; Goering and Van Soest, 1970), modified to 105 °C for 3 hr per National Forage Testing Association recommendations, and the Official Methods of Analysis (Association of Official Analytical Chemists, 2000).

Tissue Analysis: Acid Detergent Fiber (ADF) Analysis.

The analysis followed this procedure: Fiber (Acid Detergent) and lignin in animal Feed (973.18). Official Methods of Analysis, 17th edition. 2000. Association of Official Analytical Chemists. (Modifications: Whatman 934-AH glass micro-fiber filter with 1.um particle retention used in place of fritted glass crucible.) ADF method only.

Tissue Analysis: Ash Analysis.

The ash analysis followed the Ash of Animal Feed (942.05) (AOAC, 2000), modified to a 1.5 g sample weight and a 4 hr ash time.

Neutral Detergent Fiber (NDF) analysis

The NDF analysis followed Van Soet et al., (1991), modified using Whatman 934-AH glass micro-fiber filters with 1.5 um particle retention.

Tissue Analysis: Soluble Protein Analysis.

The soluble protein analysis followed Krishnamoorthy et al. (1982).

Tissue Analysis: Statistical Analysis.

Data were analyzed by the GLM procedure of SAS (Cary, NC). The relationships between experimental dependent variables and S treatments were determined by regression analysis. Orthogonal polynomials were used to study changes associated with increasing S fertility concentrations by portioning the sums of squares into components associated with linear and quadratic terms (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Plant Biomass.

Increasing S fertility levels had no significant effect on pods, beans, shoots, and roots FM accumulation in edamame. The total number of pods (P=0.979), total amount of pod weight (P=0.543), total amount of bean FM (P= 0.342), total amount of bean DM (P=0.404), total FM of shoots (P=0.215), total FM of the root (P=0.450) did not significantly differ in response to increasing S fertility (Table 1). There was also no significance quadratic relationships observed between dependent variables and S treatment concentration (Table 1). Kandapal and Chandel (1993) reported that, the application for S improved nitrogenase activity, nitrogen fixation, plant dry matter and quality of soybean grain in a sulfur deficient soil. Kedar and Rajendra (2003) found that the application of S at the rate of 30 kg per ha to pea (*Pisum sativum*) resulted in higher number of grains per plant which was 24.18% higher than the control treatment. Singh and Singh (1995) reported that the application of 20 kg zinc oxide per ha and 30 kg S per ha significantly increased the number of pods per plant and number of seeds per pod of soybean.

Singh and Aggarwal (1998) reported that among different sources of S, gypsum ($CaSO_4$) produced significantly higher pods per plant and seed per pod of black gram (*Vigna mungo* (L.) Hepper). Fismes et al. (2000) reported that low supply of S leads to abortion of pods in rapeseed (*Brassica napus* L.), but increases in S combined with inoculum increased soybean seed yield by 20%. Furthermore, Matula and Zukalová (2001) reported oilseed rape dry matter was not affected by the increasing $MgSO_4$ fertility in potted soil culture. Hara and Sonoda (1981) also observed no differences in FM or DM in cabbage (*Brassica oleracea* L. Capitata Group) grown in nutrient solution culture at 10 mg and 100 mg of S L^{-1}.

Proteins Content.

The protein accumulation in 'Chiba' edamame was not affected by increasing S treatments. The total dry matter (P=0.360), crude protein (P=0.171), adjusted protein (P=0.171), ADFNDF (P=0.409), ADFDM (P=0.707), aNDf (P=0.271), ash (P=0.690) were also not affected by S treatment (Table 2). Havlin et al. (1999) reported that S was involved in the synthesis of fatty acids and the increase of protein quality through the synthesis of certain amino acids such as Cys and Met. Kandpal and Chandel (1993) reported that the application of S consistently increased the protein and oil contents in grain of soybean, resulting in a maximum content of protein and oil content at 30 kg gypsum per ha. Jamal et al. (2006 and 2010a) and Ahmad et al. (2007) reported that S availability had a role in regulating nitrate reductase and ATP-sulphurylase and increases protein and chlorophyll content in legumes. However, Paek et al. (2000) reported that S treatments during both vegetative and reproductive growth stages did not affect total protein content in soybean beans cv. Kenwood. The storage protein fraction of the total bean protein did not vary and appeared to be conserved across the N and S treatments (Paek et al., 2000). Other authors reported that S and N sources interact to determine soybean seed protein quality (Holowach et al.,1984 a,b; Grabau et al.,1986; Ohtake et al.,1996; Paek et al., 1997; Sexton et al., 1998). However, these sources emphasized that soybean has a limited capacity to utilize oxidized sources of S and N for storage protein synthesis during seed fill.

Elemental Accumulation.

The accumulation of macro- and microelements in 'Chiba' edamame was not affected by S treatments. Plant macroelements evaluated included Ca (P=0.339), P (P=0.530), Mg (P=0.493), K (P=0.764), S (P=0.127), Na (P=0.518) (Table 3). Micronutrients evaluated included Fe (P=0.561), Mn (P=0.305), Zn (P=0.544), Cu (P=0.489) (Table 4). Though there was no statistical significance in S (P=0.127), S displayed the highest P-value significance when compared to other factors analyzed (Table 3). Kopsell et al. (2003) reported the accumulation of S percentage in kale (*B. oleracea* L. Acephala Group) leaves responded significantly to S treatment concentration, cultivar and the interaction of S treatment and cultivar. The leaf %S increased linearly for all cultivars in response to increasing S content in nutrient solution (Kopsell et al., 2003). The increase of concentration of S in the nutrient solution from 4 to 64 mg S L^{-1} increased leaf tissue S levels of kale 716% in 'Winterbor', 622% in 'Redbor', and 638% in 'Toscano' (Kopsell et al., 2003). Kastori et al. (2000) reported that when S level was increased from 0 to 96 mg S L^{-1} in nutrient solution culture, the level of leaf tissue S of sugar beet (*Beta vulgaris* L.) increased 1,100%. Ganeshamurthy (1996) reported that S significantly increased the S uptake. Furthermore, Kopsell et al. (2000) reported that Mg and Ca were significantly affected by S availability in the nutrient solutions. Millis et al. (2000) reported that the concentrations of other mineral elements were slightly above sufficiency ranges in leaves of mature, field grown kale.

CONCLUSION

The goal of this study was to determine if protein content and quality in edamame could be affected by varying S fertility concentrations and also to determine the S fertilization rate that maximized protein contents. Based on the results obtained, there were no significant effects of the S treatments on plant biomass, protein content, and both macro- and microelements. While biomass, protein content, macro- and microelement levels were not affected by the S treatments in ‘Chiba’ edamame, there was a significant taste difference as the S levels increased when the edamame beans were evaluated for flavor (personal communication). It is clear that there might be other factors involved in increasing or decreasing protein content in combination with S nutrient, such as inoculation and genotype. Lowering S fertilization will not affect yield or nutrient composition for this crop, instead it might be a way of changing undesirable flavor characteristics in edamame which are not acceptable to consumers. This benefit can be used by growers to supply what consumers prefer without affecting nutrient composition and yield for edamame.

Table 1. Mean values[a] for fresh (FM) and dry (DM) biomass for 'Chiba' edamame [*Glycine max* (L.) Merr.] grown in solution culture at Illinois State University, Normal, IL under increasing sulfur treatments (S Level).

		Plant biomass[b] (g)				
S-Level[c]	Pod#	Pod FM	Bean FM	Bean DM	Shoot FM	Root FM
4	71.25	74.74	85.43	20.69	370.52	215.46
8	73.50	188.61	87.20	21.70	378.52	222.31
16	68.00	164.38	79.53	19.07	352.89	200.51
32	75.25	181.09	86.22	21.12	366.18	208.01
64	70.50	168.27	79.51	19.28	345.89	211.84
Contrast[d]						
Linear	P=0.979	P=0.543	P=0.342	P=0.404	P=0.215	P=0.450
Quadratic	P=0.911	P=0.749	P=0.863	P=0.826	P=0.739	P=0.488

[a] Mean composition of 4 replications with five S treatments are 4 plants per treatment.
[b] FM=fresh mass, DM=DM. Standard error estimates: Pod# = 3.12, Pod FM = 8.25, Bean FM = 4.05, Bean DM = 1.23, Shoot FM = 17.36, Root FM = 20.28.
[c] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[d] Significance for linear and quadratic orthogonal contrasts.

Table 2. Mean values[a] for bean protein content for 'Chiba' edamame [*Glycine max* (L.) Merr.] grown in solution culture at Illinois State University, Normal, IL under increasing sulfur treatments (S Level).

				Protein[b] content (g)			
S-Level[c]	Dry Matter	Crude Protein	Adjusted Protein	ADFNDF	ADFDM	aNDf	Ash
4	96.65	35.78	35.78	66.48	8.20	5.35	7.62
8	96.68	35.88	35.88	65.33	8.53	5.35	7.03
16	96.65	35.83	35.83	71.45	7.98	5.70	7.82
32	96.73	36.45	36.45	71.08	8.60	6.10	7.79
64	96.68	36.30	36.30	67.60	8.33	5.60	7.46
Contrast[d]							
Linear	P=0.360	P=0.171	P=0.171	P=0.409	P=0.707	P= 0.271	P=0.690
Quadratic	P=0.685	P=0.887	P=0.887	P=0.339	P=0.978	P=0.458	P=0.806

[a] Mean composition of 4 replications with five S treatments are 4 plants per treatment;
[b] Abbreviation definitions of Acid Detergent Fiber-Neutral Detergent Fiber (ADFNDF), Acid Detergent Fiber Dry Matter (ADFDM), and amylase Neutral Detergent Fiber (aNDF). Standard error estimates: Dry Matter = 0.16, Crude Protein = 0.47, Adjusted Protein = 0.47, ADFDM = 0.53, aNDf = 0.30, Ash = 0.36.
[c] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[d] Significance for linear and quadratic orthogonal contrasts.

Table. 3 Mean values[a] for bean macroelement content for 'Chiba' edamame [*Glycine max* (L.) Merr.] grown in solution culture at Illinois State University, Normal, IL under increasing sulfur treatments (S Level).

	Macroelement concentrations[b] (% dry weight)					
S-Level[c]	Ca	P	Mg	K	S	Na
4	0.19	0.68	0.30	2.58	0.37	0.01
8	0.17	0.62	0.28	2.49	0.37	0.01
16	0.17	0.60	0.28	2.58	0.38	0.01
32	0.17	0.61	0.27	2.52	0.38	0.01
64	0.18	0.66	0.29	2.58	0.38	0.01
Contrast[d]						
Linear	P=0.339	P=0.530	P=0.493	P=0.764	P=0.127	P=0.518
Quadratic	P=0.216	P=0.179	P=0.193	P=0.459	P=0.667	P=0.582

[a] Mean composition of 4 replications with five S treatments are 4 plants per treatment.
[b] Standard error estimates: Ca = 0.01, P = 0.02, Mg= 0.01, K = 0.04, S = 0.01, Na = 0.001.
[c] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[d] Significance for linear and quadratic orthogonal contrasts.

Table 4. Mean values[a] for bean microelement content for 'Chiba' edamame [*Glycine max* (L.) Merr.] grown in solution culture at Illinois State University, Normal, IL under increasing sulfur treatments (S Level).

	Microelement[b] concentrations (µg g^{-1} DM)			
S-Level[c]	Fe	Mn	Zn	Cu
4	111.25	53.00	49.25	5.25
8	101.25	49.50	48.75	6.75
16	122.50	56.50	46.25	3.75
32	91.75	54.25	47.25	6.50
64	104.50	55.75	52.50	7.00
Contrast[d]				
Linear	P=0.561	P=0.305	P=0.544	P=0.489
Quadratic	P=0.887	P=0.946	P=0.168	P=0.500

[a] Mean composition of 4 replications with five S treatments are 4 plants per treatment;
[b] Standard error estimates: Fe = 7.09, Mn = 3.09, Zn = 2.98, Cu = 0.87.
[c] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[d] Significance for linear and quadratic orthogonal contrasts.

CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

The main objectives of this experiment were to find out if protein content in edamame is affected by the application for different concentrations of S fertility. To achieve this goal, the report given by Kandpal and Chandel (1993) was given preference. Data collected in the greenhouse at Illinois State University may have been impacted by fluctuating environmental conditions. This was likely the cause for the lack significance in protein content across all treatments. Also the fact that only one cultivar was used, and data were collected in the beans not in the whole plant may have also impacted protein content. Furthermore, there was no inoculation of the seed, yet this also influences protein content in the seeds. All these factors probably contributed to the lack of significance in this study.

Based on the results obtained in this experiment, the lack of significance across all treatments concluded that for maximizing protein content in edamame soybean, S nutrient cannot be the sole factor to use during the process. Instead, there might be other factors which need to be considered which influence the formation of protein content. Therefore, there is a need of further investigations to determine these other factors which interact with S nutrient to influence the soybean protein content.

REFERENCES

Achouri, A., J.I. Boye, and D. Belanger. 2005. Soybean isoflavones: efficacy of extraction conditions and effect of food type on extractability. Food Res. Intl. 38:1199-1204.

Ahmad, S.1., I.S. Fazli, A. Jamal, M. Iqbal and M.Z. Abdin. 2007. Interactive effect of sulfur and nitrogen on nitrate reductase and ATP-sulfurylase activities in relation to seed yield from *Psoralea corylifolia* L. J. Plant Biol. 50:351-357.

AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th Edition, Washington D.C.

Bernick, K. 2009. Edamame takes root in U.S. corn and soybean dig. Penton Media, Overland Park, KS.

Bowman, S.A., M. Lino, S.A. Gerrior, and P.P. Basiotis. 1998. The healthy eating index 1994–1996. Washington, D.C.: USDA Center for Nutrition Policy and Promotion Publ. CNPP-5.

Charron, C.S., F.L. Allen, R.D. Johnson, V.R. Pantalone, and C.E. Sams. 2005. Correlations of oil and protein with isoflavone concentration in soybean [*Glycine max* (L.) Merr.]. J. Agric. Food Chem. 53:7128–7135.

Crisosto, C.H. and G.M. Crisosto. 2002. Understanding American and Chinese consumer acceptance of Redglobe table grapes. Postharvest Biol. Tech. 24:155-162.

Culbertson, E. 1999. Pachamama Organic Farm, Longmont, Colorado. http://www2.hawaii.edu/~theodore/Images/edamame.pdf.

Duke, S.H. and H.M. Reisenauer. 1986. Roles and requirements of sulfur in plant nutrition. p. 123–168. In: M.A. Tabatabai (ed.). Sulfur in agriculture. Agron. Monogr. 27. ASA, CSSA, and SSSA, Madison, WI. Edamame and acceptance of edamame based patties. HortScience 41:1616-1622.

Effect of sulfur on nitrate reductase and ATP sulfurylase activities in groundnut (*Arachis hypogea* L.). *Plant Biol.,* 49: 513–517.

Ernst, M. and T. Woods. 2001. Marketing challenges for emerging crops in Kentucky: Vegetable soybean. Cooperative Extension Service. University of Kentucky, Lexington, KY.

Farmakalidis, E. 1999. Consumer issues in relation to antioxidants, p.423-435. In: N.J. Temple and M.L. Garg (eds.). Antioxidants in human health and disease. CABI Publishing, New York, NY.

Fenwick, G.R., K. Heaney, and W. Mullin. 1983. Glucosinolates and their breaking down products in food and food plants. Critical Rev. Food Sci. Nutr. 18:123-201.

Fismes, J., P.C. Vong, A. Guckert and E. Frossard, 2000. Influence of sulphur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. European J. Agron. 12:127-141.

Ganeshamurthy, A.N. 1996. Critical plant S content and effect of sulphur application on grain and oil yield of rainfed soybean in vertic ustochrepts. J. Indian Soc. Soil Sci. 44:290-294.

Haneklaus S., E. Bloem, and E. Schnug. 2007. Sulfur and Plant Disease, p. 101-118. In: L.E. Datnoff, W.H. Elmer, and D.M. Huber (eds.). Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN.

Hara, T. and Y. Sonado. 1981. The role of macronutrients in cabbage head formation. Soil Sci. Plant Nutr. 27:45-54.

Havlin, L.J., D.J. Beaton, L.S. Tisdale and L.W. Nelson. 1999. Soil fertility and fertilizers. 6th ed. Prentice Hall.

Hoagland, D.R. and D.I. Arnon.1950. The water-culture method for growing plants without soil. Calif. Agri. Expt. Stat. Circ. 347, Berkeley, CA.

Hymowitz, T., F.I. Collins, J. Panczner, and W.M. Walker. 1972. Relationship between the content of oil, protein, and sugar in the soybean seed. Agronomy J. 64:613-16.

Jamal A, Fazli IS, Ahmad S, Abdin MZ (2006a) Interactive effect of nitrogen and sulphur on yield and quality of groundnut (Arachis hypogea L.). Korean J Crop Sci 51(6): 519-522

Jamal, A., Y.S. Moon and M.Z. Abdin. 2010a. Enzyme activity assessment of peanut (*Arachis hypogea* L.) under slow release sulfur fertilization. Australian J. Crop Sci. 4: 169-174.

Johnson, D., S. Wang, and A. Suzuki. 1999. Edamame: A vegetable soybean for Colorado, p. 385-387. In: J. Janick (ed.). Perspectives on new crops and uses. ASHS Press, Alexandria, VA.

Ju YH Doerge DR Allred KF. 2002. Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice. Cancer Res. 62 (9): 2474 – 2477.

Kandpal, B.M. and A.S. Chandel. 1993. Effect of gypsum and pyrites as sources of sulphur on nitrogen fixation.

Kastori, R.: Plesnicar, M.: Arsenijevic-Maksimovic, I.; Petrovic, N.: Pankovic, D.: Sakac, Z. 2000. Photosynthesis, chlorophyll fluorescence, and water relations in young sugar beet plants as affected by sulfur supply.J.Plant Nutr.23,1037-1049.

Kedar, P. & Rajendra, P. 2003. Effect of varieties and sulphur on yield and yield attributes of field pea(*Pisum.sativum var. arvense*). *Crop Res*., 25: 419-422.

Kim, M., J. Kim, S. Nam, S. Ryu, and G. Kong. 2008. Dietary intake of soy protein and tofu in association with breast cancer risk based on a case-control study. Nutr. Cancer 60:568-576.

Kopsell, D.E., D.A. Kopsell, W.M. Randle, T.W. Coolong, C.E. Sams, and J. Celentano. 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. J. Agri. Food Chem. 51:5319-5325.

Krishnamoorthy, U., T.V. Muscato, C.J. Sniffen, and P.J. Van Soet. 1982. Borate phosphate procedure as detailed in Nitrogen Fractions in Selected Feedstuffs. J. Dairy Sci. 65:217-225.

Kumar, V., A. Rani, A.K. Dixit, D. Bhatnagar, and G.S. Chauhan. 2009. Relative changes in tocopherols, isoflavones, total phenolic content, and antioxidative activity in soybean seeds at different reproductive stages. J. Agr. Food Chem. 57:2705-2710.

Lee, J.D., and Y.H. Hwang. 1998. Quality evaluation for vegetable use in local soybean cultivars with various seed coat color. Korean J, Crop Sci. 43, 83–88.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London, U.K.

Masuda, A. 1994.What is the quality of vegetable soybeans? Soybean Monthly Report January 1994.

Matula. J. ; Zukalova´.H. 2001. Sulphur concentrations and distribution in three varieties of oilseed rape in relation to Sulphur fertilization at a maturity stage . Rostl.Vyroba, 14-17.

Messina M McCaskill-Stevens W Lampe JW. 2006. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. J Natl Cancer Inst. 98 (18): 1275 – 1284.

Messina MJ Loprinzi C. 2001. Soy for breast cancer survivors: a critical review of the literature. J Nutr. 131 (11 Suppl): 3095S – 3108S.

Messina, M. 2001. An overview of the health effects of soyfoods and soybean isoflavones, p. 117-122. In: T.A. Lumpkin and S. Shanmugasundaram (eds.). Second International Vegetable Soybean Conference, Tacoma, WA. Washington State Univ., Pullman, WA.

Mills, H. A. and J.B. Jones, Jr. 1996. Plant analysis handbook II: A practical sampling, preparation, analysis, and interpretation guide. MicroMacro Publishing, Athens, GA.

Montri, D.N., K.M. Kelley, and E.S. Sanchez. 2006. Consumer interest in fresh, in-shell edamame and acceptance of edamame based patties. HortScience 41:1616-1622.

Nair, R., S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida, and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. Plant Sci. 179:154–163.

Nuss, J. 2013. US soybean farmers see growth potential in edamame. 21 Oct. 2015. <http://bigstory.ap.org/article/us-soybean-farmers-see-growth-potential-edamame>.

Paek, N.C., P. J. Sexton, S. L. Naeve, and R. Shibles. 2000. Differential accumulation of soybean seed storage protein subunits in response to sulfur and nitrogen nutritional sources. Plant Pod. Sci. 3:268-274.

Rao, M.S.S., A.S. Bhagsari, and A.I. Mohamed. 2002. Fresh green seed yield and seed nutritional traits of vegetable soybean genotypes. Crop Sci. 42: 1950-1958.

Rodriguez, N. and P. Garlick. 2008. Introduction to Protein Summit 2007: Exploring the impact of high quality protein on optimal health. Amer. J. Clin. Nutr. 87:1551S-1553S.

Roseboro, K. 2016. Edamame Offers Good Non-GMO Opportunities to US Farmers. <http:www.nongmoreport.com/articles/april2012/edamame-non-gmo-us-farmers.php>. (accessed on 15 March 2016).

Schnitkey, G. 2015. Revenue and costs for corn, soybeans, wheat, and double-crop soybeans, actual for 2009 through 2014, projected 2015 and 2016. Dept. of Agri. and Con. Econ. Farm Business Management.

Shepherd, R. 1997. The psychology of food choice: The influence of attitudes and flavour perception, p.157-172. In: F.A. Tomaś-Barberań and R.J. Robins (eds.). Phytochemistry of fruit and vegetables. Clarendon Press, Oxford, U.K.

Shurtleff, William. 2001. On the English-language etymology of "green vegetable soybeans," "edamame," "vegetable-type soybeans," and "food-grade soybeans". In: T. Lumpkin, ed. 2001. Second International vegetable Soybean Conference. Pullman, Washington: Washington State University. 202p. See p.179–181.

Singh, D. and V. Singh. 1995. Effect of potassium, zinc and sulphur on growth characters, yield attributes and yield of soybean (*Glycine max*). Indian J. of Agron. 40:223-227.

Singh, Y.P. and R.L. Aggarwal. 1998. Effect of sulphur sources and levels on yield, nutrient uptake and quality. Soil Sci. Soc. Am. J. 67:966-973.

Smit, E., J. Nieto, C. Crespo, and P. Mitchell. 1999. Estimates of animal and plant protein intake in U.S. adults: Results from the Third National Health and Nutrition Examination Survey, 1988-1991. J. Amer. Diet. Assoc.99:813-820.

Soyfoods. 2014. Soy foods and supplementation: a review of commonly perceived health benefits and risks. <https://www.ncbi.nlm.nih.gov/pubmed/24473985>.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill: New York, NY.

Tisdale, S.L., R.B. Reneau, Jr., and J.S. Plateau. 2000. Atlas of sulfur defencies. P 295-322. In M.A Tabatabai. Report 2000.pdfs.

Van Soet, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-starch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583-3597.

Velasquez, M.T. and S.J. Bhathena. 2007. Role of dietary soy protein in obesity. Int. J. Med. Sci. 4:72–82.

Waterer D. 2003.Yield and economics of high tunnel for production of warm-season vegetable crops. HortTechnology 13:339-343.

Westenhoefer, J. and V. Pudel. 1993. Pleasure from food: Importance for food choice and consequence of deliberate restriction. Appetite 20:246–249.

APPENDIX: CUMBERLAND VALLEY ANALYTICAL SERVICES, INC. PROCEDURES

REFERENCES

Acid Insoluble Ash

Sand and Silica in Plants, Gravimetric method (920.08). Official Methods of Analysis, 17^{th} edition. 2000. Association of Official Analytical Chemists.

ADF

Fiber (Acid Detergent) and Lignin in Animal Feed (973.18). Official Methods of Analysis, 17^{th} edition. 2000. Association of Official Analytical Chemists. (Modifications: Whatman 934-AH glass micro-fiber filters with 1.5 µm particle retention used in place of fritted glass crucible) ADF method only.

ADF-ash free

ADF procedures as listed under ADF. Final glass fiber filter and sample ashed in 535 °C furnace for 2 hours.

ADF Nitrogen (ADIN, ADIP)

Total residue from ADF procedure pressed and wrapped in foil. Analyzed for nitrogen: Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085.

Ash

Ash of animal Feed (942.05). Official Methods of Analysis, 17^{th} edition. 2000. Association of Official Analytical Chemists. (Modifications: 1.5g sample weight, 4 hour ash time, hot weigh).

Chloride

Sample is extracted with 0.5% nitric acid and analyzed by potentiometric titration with silver nitrate using Brinkman Metrohm 848 Titrino Plus. Brinkmann Instruments Inc., One Cantiague Rd P.O. Box 1019, Westbury NY 11590.

Corn Silage Processing Score (CSPS)

Mertens, D.R. Determination of Starch in Large Particles, Ro-tap Shaker Method. U.S. Dairy Forage Research Center. 2002. Particle size analysis of greater than 4.75 mm, 4.75 -1.18, and less than 1.18. Starch analysis on particles greater than 4.75 mm.

Crude Fiber

Fiber (Crude) in Animal Feed and Pet Food (978.10). Official Methods of Analysis, 17th edition.2000. Association of Official Analytical Chemists.

Degradable Protein (Strep. griseus)

Krishnamoorthy, U., C. J. Sniffen, M.D.Stern, and P.J. VanSoest. 1983. Evaluation of a mathematical.