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Edamame [Glycine Max (L.) Merrill] Leaf Sulfur Concentration Increased in Response to Increased Sulfur Fertility, While Carotenoids and Sucrose in Bean Tissues Remain Constant

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EDAMAME [*GLYCINE MAX* (L.) MERRILL] LEAF SULFUR CONCENTRATION INCREASED IN RESPONSE TO INCREASED SULFUR FERTILITY, WHILE CAROTENOIDS AND SUCROSE IN BEAN TISSUES REMAIN CONSTANT

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79 Pages

Edamame [*Glycine max* (L.) Merrill] is a specialty soybean that originated from East Asia. It is consumed as a raw vegetable or cooked in various ways. The demand for edamame has rapidly increased in the U.S. due to its beneficial phytonutrient and phytochemical contents. Sulfur fertility is an essential micronutrient for green plants to contribute to the flavor of vegetables. Therefore, the effect of S fertility levels on the biochemical contents and the flavor of edamame in hydroponic systems was investigated. Seeds of the 'Chiba' edamame was sown under greenhouse conditions at 22^{o}C day/ 14^{o}C night in the fall of 2020 and transferred to hydroponic systems containing modified Hoagland's solution containing S treatment concentration of 4, 8, 16, 32, and 64 mg S L^{-1} supplied with magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4). The experimental design was a randomized complete block design and five treatments, each having four replications. Edamame plants (bean, root, and leaf) were harvested at the R6 stage on December 14, 2020. Plant biomass for bean, root, and leaf tissues was measured. Fresh bean biomass and fresh and dry biomass for root and leaf were not significant. Mineral elements for bean, root, and leaf tissues were analyzed using ICP-MS. The accumulation of nutrient elements (K, P, Ca, Mg, Na, Fe, Mn, B, Zn, Cu, Mo, and Se) in bean, root, and leaf was not significant. While S accumulation in bean and root was not affected (P=0.1041 and P=0.3887, respectively) by S treatment, S accumulation in leaf was significantly increased (P=0.0073) in response to increased S treatment levels. Pigment

and carbohydrate contents in edamame bean tissues were analyzed using HPLC methodologies. The concentration of carotenoids (Lutein, Chlorophyll *a*, and Chlorophyll *b*) and saccharides (mono-, di-, tri-, and polysaccharides) remained stable among S treatments. Fatty acids in the bean were analyzed by using gas chromatography. The concentration of fatty acids (monounsaturated omega-9 fatty acids, polyunsaturated omega-6 fatty acids, and saturated fatty acids) remained stable among S treatments. Amino acids in the bean were analyzed by using Near Infra-red Spectroscopy. The concentration of protein and oil and amino acids (Ala, Arg, Asp, Cys, Glu, Gly, His, Hyl, Hyp, Ile, Lan, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, Val) remained stable among S treatment. When S supplementation increased, S accumulation in edamame leaf tissues was significantly increased, while bean and root tissues were not significant. Since S accumulation in bean tissues was not significant, carotenoids, sucrose (a contributor to sweetness), carbohydrates, fatty acids, and amino acid contents were unaffected among S treatment levels.

KEYWORDS: flavor quality, solution culture, macronutrient, micronutrients

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S.S.

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CHAPTER I: THE PROBLEM AND ITS BACKGROUND

Edamame [*Glycine max* (L. Merr.)] is a special soybean type belonging to the bean family. It originated in East Asia (Shurtleff and Aoyagi, 2009) and began on sale in the U.S. market in the late 1990s (Olver, 2015). Its popularity gradually increased as the nutritional and beneficial components of edamame beans became well known (Joshi et al., 2016; Bashi et al., 2019; Wolfe, 2016). The crop contains high phytonutrition and phytochemicals for health (Bennett, 2005). It is attracting customers' attention by providing abundant protein and dietary fiber at a relatively low price. In addition, beneficial phytochemicals have been reported on positive effects on health, such as antioxidants and anticarcinogenic effects (Charron et al., 2005; Lamartiniere, 2000).

Edamame shares many common features with agronomic soybean; however, one of the most different features is its early harvest at the R6 stage. Edamame beans are in the spotlight as the fresh vegetable that can be eaten immediately after harvest or prepared easily and quickly to consume. Edamame beans are reported to be larger in size (Laura et al., 2018), sweeter (Johnson et al., 1999) and nuttier in flavor, and smoother in texture (Wszelaki et al., 2005).

Although its easily accessible to customers (Konovsky et al., 1994), most edamame sold on the market in fresh, frozen, and canned heavily depends on imports from Asia. As market demand increases, research edamame cultivation in the U.S. state becomes proactive to be successful, and it is necessary to consider the needs and expectations of the field and market (Carneiro et al., 2021). Moreover, Carneiro et al. (2021) reported that sensory characteristics in edamame should be considered for the U.S. market.

The flavor of edamame beans is a distinctive combination of sweet, beany, and bitter (Lee and Hwang, 1998; Johnson et al., 1999) and tend to produce buttery (Rackis et al., 1972; Young et al., 2000) and floral flavors (Masuda, 1991). U.S. consumers tend to prefer buttery

and sweet beans (Johnson et al., 1999) while offensive to astringent and bitter taste edamame (Carneiro et al., 2021). Since edamame is a newly adopted crop in the U.S., nutritional, beneficial, and sensory quality studies related to S fertility in edamame are insufficient to accommodate how these factors meet consumer preference (Wszelaki et al., 2005).

Flavor components in vegetables are associated with various factors such as variety, soil minerals, and nutrition contribute. Moreover, Kopsell et al. (2003) reported that S fertility affects the flavor by stimulating S-related components, which cause bitterness in vegetables. In particular, sulfur is a macronutrient for plant cultivation and also constitutes the essential amino acid cysteine (Cys) and methionine (Met). The amino acid profile of the soybean seed protein reported the concentrations of the S-containing amino acids Cys and Met (Rushovich and Weil, 2021), which are the building block of S-containing flavor compounds. Product quality, particularly related to flavor, linked to sulfur affects food purchasing decisions that can be considered objectional to customers (Kopsell et al., 2003). Therefore, manipulating S fertility concentrations during edamame growth and development may change the levels of S accumulation which influence phytonutirtion and biochemical components for beneficial for health and flavor.

CHAPTER II: REVIEW OF LITERATURE

HISTORICAL AND CULTURE

Edamame [*Glycine max* (L.) Merrill] pronounced as "e-dah-mah-may", meaning beans on a branch, can be traced historically from various documents in East Asia, and its name was recorded as early as 1275 in Japan (Konovsky et al., 1994). Known commonly as vegetable soybean, edamame is called "maodou" in China and "poot kong" in Korea in addition to its Japanese name (Kumar et al., 2011). Soybeans are known to originate from China more than 5,000 years ago and it is presumed that edamame was mentioned earlier in a Chinese poem in the 12th century (Shurtleff and Aoyagi, 2009). However, despite its native habitat throughout China, soybean cultivation sites were limited in several states and were not the main diet as it is today (Jain, 1995). Edamame was mainly cultivated on the boundaries between rice paddies but became recognized as a field crop in modern times (Konovsky et al., 1994). In Asia, where the main diet was rice (*Oryza sativa*), soybeans were grown for nitrogen (N) fixation rather than nutritional purposes. Fresh soybeans contain digestive protein inhibitors that cause discomfort for human digestion, so bacterial fermented soy products such as miso, tempeh, soy sauce, and natto that alleviate digestive difficulties have become a part of the diet in Asia (D'Adamo and Sahin, 2014).

Soybeans were first cultivated in the U.S. in the 19th century, and because of digestion difficulty, it was a major food source for livestock rather than humans (Iowa Food and Family Project, 2022). Edamame was introduced to the U.S. media by American Asians in the early 1980s and began selling on the market in the late 1990s (Olver, 2015). As the benefits of edamame beans began to gain popularity, they began to establish themselves as a major vegetable in a typical vegetable bean consumption diet such as salad, soup, and tofu (Zhang and Kyei-Boahen, 2007). In East Asia, it is mainly used as an ingredient for stir-fried dishes, used as an ingredient for steaming with rice, or kneaded with soybean paste to make

thick soup or processed into tofu (Shurtleff and Aoyagi, 2009). In the 1970s, interest in organic agriculture led the Rodale Research Center to research the adaptability and quality of edamame (Hass et al., 1982). Since then, edamame's research has been flushed in the U.S., where Dorsett and Morse have collected extensive germplasm and developed 49 edamame cultivars (Hymowitz, 1984).

BOTANICAL SIGNIFICANCE OF EDAMAME

Edamame is an annual herbaceous plant of the same species as agronomic soybeans which belong to Leguminosae or Fabaceae (Hilderbrand et al., 1986). Edamame is considered to have a superior taste to agronomic soybean because of its large seeds, sweet and nutty flavor, smooth texture, and better digestibility (Weber, 1956; Alleman et al., 2000). Edamame bean size was reported to be as large as 65% to 100% of agronomic soybean (Laura et al., 2018), and bean dry weight was more than 250 mg per bean (Mentreddy et al., 2002). Also, the harvested beans were reported to have high Brix (between 8.5% and 12.0%) (Johnson et al., 1999). Edamame's growth and development is similar to traditional soybeans, usually growing between 60 cm and 90 cm, and usually does not require trellis or stake supports (Zhang and Kyei-Boahen, 2007; Nzaranyimana, 2017). Compound leaves have three leaflets and are densely pubescent, and single or small clusters of flower bloom in the color of white or purple (Nzaranyimana, 2017). Pods are pubescent and shaped, broadly flattened up to 5 cm to 7 cm long to contain 1 to 4 beans (Shanmugasundaram et al., 1989). Each plant produces between 100 and 150 pods (Shurtleff and Aoyagi, 2004). As a legume family it forms a nodule at the root to accommodate N-fixing bacteria which extracts N from the air and fixes it to the soil (Pérez-Pizá et al., 2020). Edamame is mainly planted in Midwest throughout May and June and harvested at the R6 maturity stage and is often sold in intact pods or bundles of plants (Shurtleff and Aoyagi, 2004). Soybean development advances into two

growth phases: vegetative stages (from emergence through flowering) and reproductive stages (to maturation). Agronomic soybeans reach full maturity (moisture levels reduce to less than 15%), while on the early harvest for edamame, green seeds fill only 80% to 90% of the pod cavity (Konovsky et al., 1994; Rao, 2002).

CUSTOMER PREFERENCE

Edamame is a flavorful, nutritious specialty crop consumed in Asian countries for centuries and has consistently attracted the attention of American consumers (Roseboro, 2012; Wolfe, 2016). Consumer interest for healthier, plant-based protein alternatives expressed as edamame has become the second most consumed soybean food after soy milk in the U.S., with 23,000 to 27,000 tonnes per year (CBS News, 2013). According to Johnson's report, edamame demand in the U.S. was expected to be around 13,600 tonnes in 2010, and market demand surged from 60% to 100% over the years (Johnson, 2000). The surging popularity in recent years seems to be due to increased awareness of nutritional and health benefits (Zhang and Kyei-Boahen, 2007). Legume seed products were reported to be high in protein and fiber and low in saturated fat, which naturally sparked interest in consuming vegetables in the U.S. (Joshi et al., 2016). Moreover, U.S. customers acknowledge that a soybean diet has positive health effects (Bashi et al., 2019). For example, the rich linoleic and linolenic acid in soybeans can mitigate the risk of vascular disease (Mateos-Aparicio et al., 2008). Also, interest in soybean products has increased customer preference as the growing Asian population in the U.S. and familiarity with soy-based ingredients, which have been recognized as an alternative protein source (Shim et al. 2021; *Ingredient Insights,* 2020). Besides health issues, consuming meat and other animal products confront ethical questions about meat intake and consumer concerns related to economics, religion, and the environment (Ruby, 2012; Sanchez-Sate and Sabaté, 2019). Amid these social movements, the interest in

vegetable diets aroused in the U.S., and several studies were conducted on edamame that has relatively low production costs and high potential profitability in the market (Sharma, 2013). Moreover, according to Pollack (2001), U.S. customers are prone to consider eating and preparation convenience in food purchasing decisions. Edamame is usually sold fresh, frozen, or canned in the market, which is readily approached to customers (Konovsky et al., 1994). It can be briefly prepared, eaten as a fresh vegetable, or boiled in salt water for 7 to 10 min (Johnson et al., 1999).

NUTRITIONAL AND HEALTH PROFILE

According to the U.S. Department of Agriculture (USDA), fresh agronomic soybean seeds contain 12.35 g of protein, 6.4 g of fat, 11.05 g of carbohydrate, and 4.20 g of fiber per 100 g, while edamame seeds contain 11.90 g of protein, 5.20 g of fat, 8.91 g of carbohydrate, and 5.20 g of fiber per 100 g, in addition, approximately 38% of edamame bean nutrients are proteins, and 400 g to 600 g of edamame intake meets the daily average adult protein requirement of 46 g to 63 g (0.8 grams of protein per kilogram of body weight)(USDA, 2019). Edamame contains rich unsaturated fatty acids, for example, omega 3-fatty acids and alpha-linolenic acid (Mateos-Aparicio et al., 2008), which helps improve fat metabolism and reduce triglycerides to lower blood pressure and cholesterol. Crouse et al. (1999) recommended the intake of soy protein daily to lower the risk of heart disease. Several edamame varieties worldwide are nutrient-rich (Mentreddy et al., 2002) and rich in vitamins, minerals, dietary fiber, and isoflavone (Mebrahtu et al., 2004; Ntatsi et al., 2018). Vitamin C (ascorbic acid) and vitamin E (tocopherol) are abundant even in boiled beans (Johnson et al., 1999). The body readily absorbs minerals in edamame, so mineral loss from excessive sweat can be offset by edamame intake (Stone and Martyn, 2016). Edamame has relatively high antioxidant enzymes such as γ-aminobutyric acid (GABA) compared to agronomic soybeans

(Shiu, 2020) and contains lecithin, which an indispensable enzyme for brain development and improves memory (Chen et al., 2021). Edamame is also easier to digest than other soybeans because of its low levels of trypsin inhibitors that interfere with protein digestion and low undigested oligosaccharides (Rackis, 1972).

Edamame is rich in phytoestrogens (D'Adamo and Sahin, 2014) and beneficial bioactive compounds (Masuda, 1991). Phytoestrogens are derived naturally in many plants, notably soybeans, and have structural and functional similarities to the human estrogen (Patisaul and Jefferson, 2010). Some phytoestrogens have estrogen-like effects and increase estrogen levels in the body, mitigating hot flashes and severity in climacteric women (Lamartiniere, 2000; Bolanos et al., 2010). Moreover, positive clinical outcomes resulted in protective effects of phytoestrogen against breast cancer in both pre-and postmenopausal women (Trock et al., 2000).

A nonsteroidal estrogen group belonging to phytoestrogens is isoflavones, commonly found in legumes, with the highest amount found in soybeans (Merritt and Jenks, 2004). Edamame is already rich in isoflavones and produces more as advances to maturity; however, lost about 50% to 60% during processing (Messina et al., 2006; Simonne et al., 2000).The other health benefits of edamame consumption are reducing the risk of certain types of cancer, including breast cancer and cardiovascular disease, and symptoms of obesity and diabetes (Picherit et al., 2000; Young, 1991).

Nevertheless, concerns raised about imbalanced phytoestrogens and isoflavones include their potential adverse effects on sexual development and reproduction, neurobehavioral development, immune function, and thyroid function (Messina and Redmond, 2006; Conrad et al., 2004; Hamilton-Reeves et al., 2010). In addition, phytoestrogens which mimic its hormonal actions, have been implicated as having both protective and contributory roles in developing hormone-dependent malignancies, including

breast cancer (Adlercreutz and Mazur, 1997), prostate cancer (Ward and Kuhnle, 2014), and hyperthyroid (Conrad et al., 2004). Several studies reported that altered estrogen levels resulted in the consequences of breast cancer in women (especially estrogen receptor-positive breast cancer) (Messina and Redmond, 2006) and hormone-dependent cancer in men, such as prostate cancer and prostate cancer metastasis (Lakshmanet al., 2008). Furthermore, many researchers have found that phytoestrogens can disrupt thyroid function in both men and women, leading to clinical hypothyroidism and goiter development (Doerge and Chang, 2002). High soy isoflavone intake resulted in the risk of cancer recurrence in HER2-positive breast cancer patients (Woo et al., 2012).

The U.S. Food and Drug Administration (FDA) approved that Soy protein is associated with reduced the risk of coronary heart in 1999 (FDA, 1999). Soy protein consumption reduces cholesterol (total and low-density lipoprotein) and fat particle levels such as (hepatic) triglycerides (Bhathena and Velasquez, 2002). Ingestion triggers several mechanisms to modulate cholesterol absorption and metabolism by reducing intestinal cholesterol absorption and increasing fecal bile acid excretion, thereby reducing hepatic cholesterol content and enhancing the removal of LDL (Greaves et al., 2000). In addition, there was *in-vivo* evidence that soy protein may influence lipogenesis in the liver. The soy protein diet consistently increased the degradation of LDL cholesterol by mononuclear cells in patients with hypercholesterolemia even in the presence of an elevated cholesterol intake (Lovati et al., 1987). However, artificial boosting may cause adverse consequences, such as soy protein reducing liver triglycerides or fat by partly inhibiting the hepatic fatty acid synthesis in the liver (Dyck et al., 2000).

High protein content foods can suppress appetite and increase satiety and energy expenditure, which is helpful for the prevention and treatment of obesity and diabetes in human and animal studies (Anderson and Moore, 2004). Regular intake of edamame in diet

can reduce body fat and plasma lipids such as plasma cholesterol and triglyceride, eventually leading to weight loss (Bhathena and Velasquez, 2002). In animal studies of obese Zucker rats (*Rattus sp*.; OZR; Harlan, Indianapolis, IN), a high protein soy diet improved glucose tolerance, insulin resistance, and hepatic cholesterol and triglyceride concentrations (Mezei, 2003). Soy protein also positively affects diabetes by improving insulin resistance and lipid levels by activating peroxisome-proliferator-activated receptors (PPARs) (Mezei, 2003). In addition, soy proteins improve glucose tolerance (Hurley et al., 1998). The positive effect of edamame on diabetes may turn into lowering fasting plasma glucose and insulin concentrations observed in cod protein-fed and soy-protein-fed animal studies (Bhathena and Velasquez, 2002).

Several controversial effects of the consumption of soy products on human health are reported due to an imbalanced amino acid profile (low levels of methionine) or long-term exposure to potentially harmful by-products (hexane-based processing techniques or antinutrients contained in soy) (D'Adamo and Sahin, 2014). In addition, White et al. (2000) reported that long-term consumption of soy products in midlife is correlated to cognitive impairment in the prospective life. Furthermore, soybean is categorized in the eight most common allergenic foods with milk, eggs, fish, crustacea, wheat, peanuts, tree nuts, and soy, accounting for about 90% of food allergies (Cordle, 2004). However, soy protein allergy occurs only in a minority of children with food allergies and is relatively uncommon in adults (Zeiger, 1999).

PRODUCTION PREFERENCE

Consumer interest in edamame, which has been steadily increasing over recent decades, is expected to lead to sales opportunities for U.S. farmers (Lord et al., 2021). Edamame sales have been steadily growing at 12% to15% every year, reaching around $18

million in the early 2000s and rising to $41 million in 2009 (Edamame Production Facts, 2012; Soyatech, 2010). According to Roseboro (2016), a non-GMO edamame breeder, 97% of edamame sold in the U.S. market are frozen products imported from China (15,000 to 20,000 tons). Edamame production lags far behind Asian countries such as China, Japan, Taiwan, and Thailand, while most U.S. production is grown on a small scale or domestically (Roseboro, 2016). Still, American farmers are leading the global field soybean production using about 30 million hectares of land (Roseboro, 2016). Farmers are expected to take advantage of cultivating edamame because it plays a role in crop rotation and increases plant diversity which can alleviate the risk of potential crop failure within the farming system (Mentreddy et al., 2002). Edamame is classified as a specialty or horticultural crop by USDA, but as the same species as agronomic soybean, farmers are readily capable of cultivating it in fields where grain-type beans are grown (Nosowitz, 2016). Edamame is traditionally harvested manually or sold in a bundle by cutting the base of the stem due to its soft pod shell (Shurtleff and Aoyagi, 2009). Fresh edamame beans harvested at the R6 growth stage have a short growing period (approximately 90 to 120 days), a larger size of beans, and a higher sugar content, expecting superior revenue (Rackis, 1972; Rao et al., 2002).

BIOLOGICAL N-FIXATION

As a legume family, edamame roots house Rhizobium bacteria for biological N fixation, synthesizing atmospheric N_2 gas to produce soil organic N (Worwood, 2014). Nitrogen fixation enables N to be used in soybean plants and other crops, enhancing productivity in that it improves soil fertility and cultivation systems (Kebede, 2021). It also can control the N cycle by lessening N fertilizer requirement and contributing to soil health (Beyan et al., 2018). Most of the organic N produced at this time is a significant nutrient for soybean growth and is concentrated between the time when pod development begins and the

maturity stage (Fabre, 2000). Nitrogen assimilated in soybean plants affects nutrient concentration within bean development (Mastrodomenico and Purcell, 2012). Nitrogen availability through symbiotic N fixation increases nutritional accumulation, especially in seed development, in the R6 stage, where carbohydrate synthesis was intensively performed and explains the high amount of significant protein content in edamame beans even at early harvest times (Burias et al., 1990; Til'ba and Sinegovskaya, 2013).

MARKET

Edamame's competitiveness in the domestic market will be a good opportunity for growers and entrepreneurs (Carneiro et al., 2021). For example, soybean sales price peaked at $325 per hectare in 2013 but gradually fell to $268 per hectare in 2015 (Schnitkey, 2016). This trend appeared the same in market prices, which were $13 per bushel in 2012 but gradually fell to $9 per bushel in 2017. It reported that the net return per hectare for growing edamame was $105 for the wholesale market in Kentucky (Ernst and Woods, 2001), $243 to $304 for farmers' markets in Ohio (Bernick, 2009), and overall ranged from $162 to $526 in the U.S. (Binder, 2010) compared with $142 to $243 of the gross income per hectare for growing general-purpose soybean.

The U.S. is a major soybean producer, and the constant increase in domestic demand for edamame can benefit farmers seeking emerging most profitable crops. (Ernst and Woods, 2001). Moreover, the reality that most edamame's domestic consumption relies on frozen imports and consumers' positive views on domestic edamame production could be an opportunity for existing U.S. soybean growers (Renata et al., 2022). Edamame is more valuable than most agricultural products previously studied for regional characteristics, given the dominance of imports to meet East Asian origin and U.S. demand (Wang, 2018).

The young generation (Millennials and Generation Z) are the leading customer of plant-based protein, and their purchasing decisions are more sensitive to new trends (Tyson Foods, 2019). In addition, the rapid growth of Asian populations in the U.S. promises to enhance new market opportunities for specialty Asian produce (Sciarappa et al., 2016; Kaiser and Ernst, 2020).

Challenges that farmers or entrepreneurs may face include the lack of reliable profitability estimates for new crops and the high instability of fluctuations in the prices of vegetables due to the global situation. To reduce financial risks, it is reported that using existing market channels already used for similar products is more effective for marketing and profitability than finding completely new market channels (Ernst and Woods, 2001).

CROP MANAGEMENT

Crop management practices contribute to quality and taste through comprehensive seedling, fertilizer, pest control, harvesting, and post-harvesting processing (Masuda, 1989). The edamame quality assessment process is done by observing the physical characteristics of the edamame beans based on predetermined criteria. The harvested edamame pods show preferably sparse, white soft pubescence and are utterly green with no signs of stains (Watanabe, 1988; Sunada, 1986). Although each country has different factors that determine the quality of edamame, according to Iwata Prefecture in Japan (IDA 1990), the shape of the pod is perfectly shaped, green, and there are no wounds or stains; it is judged by grade A, but if the color is light, wounds or deformities, and the size of the seed or pod is small, it is judged as grade B. At least 90% of the yield, the pod should be more than 5 cm long and contain two or three beans (Shanmugasundaram et al., 1989). Moreover, the edamame grade determination process considers various parameters, including pests and diseases, shape, number of pods per 500 g, mechanical damage, pod fiber, standard pods, appearance, pod

length, thickness, pod color, cleanliness, pods maturity, diseased, insect-damaged, seeded, deformed, yellow, cracked, stained, or ripe (Wibowo, 2020).

Edamame plants grow to be about the same size as agronomic soybeans and thrive in similar environments. However, poor germination or lack of plant populations can also be attributed to inadequate soil temperature and depth planting (Pearson, 2001). Edamame seeds are larger than agronomic soybeans but tend to be environmentally sensitive to soil temperature, pH, and humidity prior to being sown (Mentreddy et al., 2002). The soil temperature must be at least 16 to 21 °C, and when the temperature drops below 13 °C, the possibility of germination is significantly diminished (Didier, 2018). Weak acidic soil pH (pH 6.0) is preferred because nitrogen fixation occurs more actively and meets the demand for N fertilizers (Alleman et al., 2000). In addition, when the ground is damp, difficulty in germination is bolstered, and the ability to absorb nutrients through roots is hindered. Excessive moisture can also increase the likelihood of causing soil disease.

Edamame is a short-day plant where reproductive growth is more active when the amount of light per day is longer than darkness, and flower expression is induced when this is reversed (Garner and Allard, 1920; Shanmugasundaram, 1989). In addition, as the relative maturity increases, the soybean reproductive growth stages become increasingly more sensitive to long nights (Johnson et al., 1999). Therefore, photoperiodism is a significant factor in yield potential since the plant will reach maximum yield only if it has produced enough photosynthetic material to produce maximum seed potential.

Although it varies depending on the soil condition and environment, edamame satisfies the N needs through symbiosis with N-fixed bacteria. However, a recent study had posed an issue of the cultivation of edamame, which used more synthetic fertilizers (3 to 4 times the recommended amount) than soybeans need (Astuti et al., 2021). Sudiarti (2019) warned malpractice could damage agricultural land and disturb the balance of nutrients in the

soil. Excess N concentration would not affect yield but defer the N fixation rate in the soil and inflorescence timing (Johnson et al., 2015). Increasing the supply of N after anthesis can increase the amino acids; however, it can also change the consistency of sugars. Depending on the N application's rate and timing, the sugar content can decrease from 3.2% to 2.6% in soybeans (Chiba et al., 1989). The insect pest and diseases of edamame are generally the same as those of conventional soybeans (Kaiser and Ernst, 2020).

Harvesting edamame is crucial because the optimal texture and flavor are only retained for a short period of time (Johnson, 2000; Wszelaki et al., 2005). The harvest period of edamame is when the seeds in pods are grown fully, reproductive stage 6 (Rao et al., 2002). At this time, the pods are bright green, and the beans can almost be touched within the pods; if the pods begin to turn yellow, the harvest period has passed, and the quality of the beans also turns less sweet and nutty flavor (Alleman et al., 2018). The harvest period for edamame is very narrow and labor-intensive, about three to four days (Lard et al., 2019; Mebrahtu and Mullins, 2007). Small-scale farms or family gardens usually harvest edamame by hand; however, mechanical harvest is also possible. Existing bean harvesting equipment can also be used, but edamame bean pods are weaker than fully matured agronomic soybeans; thus, modified equipment or hand harvesting must be employed (Neill et al., 2021). A 0.4 hectare edamame farm in Virginia that employed hand-harvesting was found unprofitable (Lord et al., 2019). Personnel expenses for manual harvesting consisted of more than 62% of operation cost (Garber et al., 2019). If less than 20% of the yield is damaged during harvesting, mechanical harvesting is economically feasible (Garber et al., 2019).

The quality of edamame beans, which are fully stacked in the shell during harvest, should be stored at refrigerated temperatures (~5ºC) for deterring quality deterioration (Sugimoto et al., 2010). In addition, since edamame has a high respiratory rate, harvesting at low temperatures, such as early morning, can preclude its rapid quality declines (Su et al.,

2020). the storage period longer. Lowering the storage temperature after rapid collection dulls enzyme activity and minimizes molder, maintaining quality and flavor. Improper temperature management begins to deteriorate sugar and amino acids between 3 and 10 h after harvest (Chiba et al., 1988). Edamame is slightly blanched in boiling water before freezing, preventing fatty acid oxidation and minimizing taste changes. It also destroys trypsin, which can cause indigestion while maintaining a good texture.

FLAVOR OF EDAMAME

Taste preferences for fruits and vegetables among different ethnic groups have been reported in several studies. For example, Chinese consumers favored sweetness and were more sensitive to acidity in grapes than American consumers (Crisosto, 2002). Japanese consumers preferred sweet, crispy texture and flowery taste, while American consumers preferred more mature beans that tasted like butter (Johnson et al., 1999). A study of soybean product preferences among ethnic groups reported the same inclination for sweet and sweet aroma among Asian, Caucasian, and African American females; however, Asians were more objectional about beany and astringent taste than Americans (Lawrence and Drake, 2016). Carneiro et al. (2021) reported that sensory characteristics in edamame should be considered for the U.S. market: edamame genotypes representing salt, sweet, umami, nutty, cooked bean, and chewy quality showed higher acceptability, while associations with starch, raw bean, metallic, grassy, astringent, sour, sulfur, and bitter attributes were confirmed undesirable for the consumers in the U.S. In particular, the chewiness of edamame according to genetic predisposition is an important attribute in consumer preference, which depends on the maturity of pods and beans (Wszelaki et al., 2005). Excessive maturation for soybean products was reported to have a negative influence as the visual effect of browned pods and

beans and the olfactory effect of sour scent decreased consumer preference (Vara-Ubol et al., 2004).

Edamame beans are characterized by a unique combination of sweet, beany, and bitter flavors (Lee and Hwang, 1998) and tend to produce buttery (Rackis et al., 1972; Young et al., 2000) and floral flavors (Masuda, 1991). The most influential factor in the sweetness of soybeans is the content of sucrose, a disaccharide (Masuda et al., 1988). The undesirable flavor of soybeans was reported with high oil content (Konovsky et al., 1994; Wszelaki et al., 2005; Young et al., 2000), and soy proteins (saponin, isoflavones, and arginine) contribute to the bitter taste (Shiu et al., 2020). Genetic information and harvest timing affected edamame's beany and bitter flavor, and linoleic acid produced by the lipoxygenase gene (Lox) has been reported as a major cause of aroma (Lenis et al., 2010; Lv et al., 2011). In addition, as edamame approaches its ripeness, the elements that affect taste and aroma change, and the lipoxygenase itself before linoleic acid is oxidized, gives a bitter taste (Rackis et al., 1972). Cultural management can overcome bitter taste, and lipoxygenase can be deactivated by blanching soybean with heat (Rhee et al., 1989).

Soy products reported beany flavor and aroma compounds, including hexanal, hexanol, 2-nonenal, 1-octen-3-ol, (E, E)-2,4-decadienal, benzaldehyde, 2-pentyl furan, 1-octen-3-one, (E, E)-2,4-nonadiena (Yu et al., 2018), while cis-jasmone and (Z)-3-hexenyl-acetate have been reported to confer desirable flavor (Masuda 1991).

Amino acids, a protein component, also affect the taste and aroma of edamame beans, in which glutamic acid has a savory flavor (Masuda et al., 1988) and alanine is sweetness (Masuda, 1991). Phenylalanine and tryptophan affect bitterness and lowering storage temperature slows enzyme changes and prevents them from becoming bitter (Kirimura et al., 1969). Geisenhoff (2009) refuted that leucine and phenylalanine, a typical partial enzyme hydrolyzed soybean protein, showed no significant effect on bitterness

astringent taste in soybeans. In most cases, these bitter flavors adversely affect food consumption as the total amount of protein in the bean affects the carbohydrate content that produces a sweet taste, resulting in a lack of sweetness. Surprisingly, edamame, highlighted for its high protein content, is deficient in sweetness (Hymowitz et al. 1972); on the contrary, oil content and total sugar are positively linked, but the excessively greasy taste is still not appreciated. In most cases, these bitter flavors adversely affect food consumption. The total amount of protein in the bean affects the carbohydrate content that produces a sweet taste, resulting in a lack of sweetness. On the contrary, oil content and total sugar are positively linked, but the excessively greasy taste is not appreciated.

PROTEIN

Soybean is the main crop utilized for plant-based protein production accounting for 70% of global meal consumption (USDA, 2019), while 18% of protein sources for the human diet come from grain crops (Smit, 1999). Not only do soy products serve as vegetable protein for humans and livestock, but also an abundant amount of essential amino acids and other macronutrients are nutritionally valuable (Cober and Voldeng, 2000; Ebert et al., 2017). Therefore, edamame is an excellent source of protein and oil, which provides estimating 33.3% to 38.6%% of the protein (dry weight basis) and 5.0% to 6.9% of vegetable oil (fresh weight basis) (Mentreddy et al., 2002). Edamame is also an excellent source of plant-based protein to replace soybean containing all the essential amino acids, isoflavones, and sucrose in the human diet (Velasquez and Bhathena, 2007). In addition, the high seed protein concentration is valuable for animal feeding due to the low cost of production and a relatively complete amino acid profile (Medic et al., 2014).

Protein formation in soybeans is characterized by various genetic and environmental factors, including cultivar, temperatures, photoperiods, and available nutrients (Arslanoglu et

al., 2011; Kumar et al., 2013). For example, the availability of organic NO_3 through the N-fixation during pod-fill periods (R5-R6) can affect edamame yield and protein content in bean tissues (Waterer, 2003; Mentreddy et al., 2002). Furthermore, according to Fabre (2000), accessibility to nutrients affected soybeans' genetic and enzyme expression, further affecting the process and amount of protein synthesis. In addition to N, macronutrients such as P, K, and S are associated with the amount of protein synthesis in soybean seeds, especially the application of S fertilizer has a positive effect on soybean protein and oil content (Peak et al., 1997).Sulfur is a crucial component in the protein synthesis in plants, synthesizing essential amino such as cysteine (Cys) and methionine (Met), in which the amount of S determines the production of Cys and Met (Rushovich and Weil, 2021). The concentration of S-related amino acids is associated with constant S concentration as well as S assimilation rate and N:S ratio (Wilcox and Shibles, 2001). Even though the considerable protein content in soybean is a valuable target for crop improvement because of its economic and nutritional importance, the S-rich proteins are often regarded as suboptimal due to the low concentration of Cys and, particularly, Met (Hell, 1977). Therefore, there is a critical need to investigate the relationship between seeds produced and sulfur fertility to improve the nutritional quantity and quality, especially S-containing proteins (Patil et al., 2017). Increased quantity and quality of nutrients in soybean seed would benefit consumers and producers (McVey et al., 1995).

SULFUR

Sulfur nutrition in crops is vital for developing plants and the food quality, while it is contained in the vegetative parts of crops between 0.1% and 2% of dry weight (Mahler, 2004). Sulfur is actively absorbed through the plant's roots and leaf systems. In the soil, sulfate (SO_4^{2-}) ion-type S is absorbed across the roots, and gaseous S, such as sulfur dioxide

and hydrogen sulfide in the air, is absorbed through leaves (Haneklaus et al., 2007, Schnug et al., 1995).

Severe S deficiency reduces crop productivity, diminishes crop quality, and affects plant health and environmental conditions (Schnug, 1984). Without adequate S, crops were under the influence at the beginning of development and could not reach their full development, which leads to physical disability makes less expression in traits (Haneklaus et al., 2008). In addition, excess S causes the same problem as its deficiency: premature leaf defoliation hinders plant growth and, therefore, lowers production (Kowalenko, 2004). Furthermore, S deficiency limits N fixation for legumes, uptake, and assimilation by the plant, leading to nitrogen losses from the soils through volatilization and leaching (Schnug, 1991). Moreover, the ecologically relevant impact of S deficiency triggers a minor attraction of flowers to honeybees (Sabbahi, 2005). As maldevelopment of the features of flowers (scent, color, size, and shape) due to sulfur deficiency, the number of visiting honeybees on oilseed rape petals is less likely ensued (Schnug and Haneklaus, 2005).

The crucial role of S in protein formation is a building block for proteins and enzymes and S-containing amino acids (Chowdhury, 2020). For example, sulfate is reduced to sulfide and subsequently incorporated into Cys, a precursor to the formation of other organic S compounds in plants (Leustek and Saito, 1999). Besides the structural role of Cys, it contributes to synthesizing various other compounds such as glutathione, phytochelatins (formation of chlorophyll), and secondary sulfur compounds present in plants. More importantly, Cys donates the sulfur group to synthesize Met via the trans-sulfurization pathway (Haneklaus et al., 2007 Wüthrich et al., 2018). Methionine biosynthesizes glucosinolates (GSs) from the chain elongation pathway, where GSs are a group of about 130 various sulfur-rich plant secondary protein metabolites (Halkier and Gershenzon, 2006). These could be synthesized from other amino acids, for example, alanine, leucine, isoleucine,

or valine. When cells are disrupted by chopping or chewing, myrosinase comes in contact with GSs and catalyzes their hydrolysis (Barickman et al., 2014). Kopsell et al. (2003) reported that these S-derived proteins attribute distinctive pungent and bitter flavor to vegetative crops like Brassica and Allium could impart an objectionable flavor to customers often regard it distasteful.

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CHAPTER III: EDAMAME [*GLYCINE MAX* (L.) MERRILL] LEAF SULFUR CONCENTRATION INCREASED IN RESPONSE TO INCREASED SULFUR FERTILITY, WHILE CAROTENOIDS AND SUCROSE IN BEAN TISSUES REMAIN CONSTANT

ABSTRACT

Edamame [*Glycine max* (L.) Merrill] is a desirable food in East Asia and has gained in popularity in the U.S. to become the second-largest consumed soy food. Consumer demand is due to the flavor and nutritional content of this specialty crop. Edamame is a dietary source of vitamins, minerals, complete protein, and phytonutrients, as well as providing a sweet flavor. A study was undertaken to determine the effects of increasing sulfur (S) fertility in solution culture on the flavor and phytonutrient quality characteristics of 'Chiba' edamame. Plants were hydroponic greenhouse-grown (lat. 40° 30'N; 22°C day/14°C night temperatures) in the fall of 2020. Fifteen days after germination, seedlings were transferred to twenty 30 L tubs holding four plants each and filled with a modified Hoagland's nutrient solution containing S treatment levels of 4, 8, 16, 32, and 64 mg S L^{-1}. Sulfur treatments were delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4) to balance the other essential nutrients. Treatments were arranged in a randomized complete block design with four replications. Nutrient solutions were replaced with their original nutrient concentrations every 2 weeks, and the photoperiod was reduced to 10 h to induce flowering. Edamame pods were harvested at the R6 maturity stage, and fresh (FM) and dry mass (DM) was taken from pods, beans, leaves, and roots. Mineral elements were analyzed using inductively coupled plasma (ICP), and carotenoids, chlorophylls, and carbohydrates were analyzed using high-performance liquid chromatography (HPLC). Gas chromatography (GS) for fatty acids analysis and near-infrared spectrometer (NIR) for amino acid analysis were conducted. Fresh

mass and DM of bean, leaf, and root tissues were not affected by increasing S treatments. Sulfur content increased linearly in leaf tissue (P=0.073), while S in bean tissue (P=0.1041) and root (P=0.3887) tissue increased, then decreased quadratically in response to increasing S fertility while all other essential elements remained unchanged. Sucrose bean content remained (P=0.2793) from 63.97 mM to 61.20 mM when S was increased from 4 to 64 mg S L^{-1}. Sulfur fertility concentration resulted in no significant effect on bean accumulation of carbohydrates, fatty acids, and amino acids in bean tissue. Lutein bean content averaged 1.80 mg g^{-1} DM and chlorophyll *a* and *b* bean content averaged 39.61 mg g^{-1} and 20.54 mg g^{-1} DM, respectively, and did not change in response to increasing S treatments. These results will impact production practices aimed at optimizing the flavor and nutritional quality of edamame for consumer preference and consumption.

KEYWORDS: flavor quality, solution culture, macronutrient, micronutrients

INTRODUCTION

Edamame [*Glycine max* (L.) Merrill] is an annual bean plant originating in South Asia (Shurtleff, 2001). It is a specialty crop known worldwide by its Japanese term for "branched beans" (Miles et al., 2000). Edamame is the same species as agricultural soybeans (*Glycine max*), but its bean seeds are larger and harvested earlier (Fehr et al., 1971). Edamame is harvested at the R6 maturity stage when seeds are immature, and 80% to 90% of the pods are filled (Zhang and Kyei-Boahen, 2007; Tsindi and Tukamuhabwa, 2019). Edamame is also called vegetable soybean because of its fresh, green, soft bean seeds (Zhang et al., 2017). In addition, edamame beans have a more potent sweetness than agronomic soybeans and a taste that appeals to consumers, such as flowery, nutty, and buttery flavors (Masuda, 1991).

Soybeans are important crops grown worldwide to supply vegetable oils and proteins as food for animals and humans (Bennett, 2005). In the U.S., the consumption of edamame is reported as the second most consumed soy food (Soyfoods, 2014). However, the history and the cultivational practices of edamame in the U.S. are relatively new compared to Asia (Shurtleff and Aoyagi, 2009). Moreover, since small farms and household gardens are the primary sources of domestic cultivation, most domestic edamame demand is maintained by imports (Zhang et al., 2017). Nevertheless, as consumer interest in edamame grows in the U.S., cultivations and research have increased (Lee et al., 2018).

Edamame is an excellent source of vegetable protein, and it is considered a complete protein food containing all amino acids essential to the human diet and animal feed (Hertamawati et al., 2021). Therefore, it earns expectations as an excellent alternative to meat protein amid personal health consciousness (Sanchez-Sabate and Sabaté, 2019). Moreover, consumers have begun to look for more nutritional benefits in diets such as high fiber, high protein, and low sodium food sources (Carneiro et al., 2020; Wilson et al., 2013). Legume plants, including edamame, are good sources of fiber and vegetable protein, which can help alleviate chronic diseases such as type 2 diabetes by lowering blood sugar levels at ingestion (Mezei et al., 2003). In addition, low-fat and high-protein-focused diets have reduced obesity and diabetes rates (Rao et al., 2002). The relationship between dietary protein in food intake and chronic diseases such as coronary artery disease is of great interest (Trumbo et al., 2002), and there is a growing scientific consensus that soy protein can lower the risk of dietary-linked diseases (Tova-Palacio et al., 1998). Furthermore, edamame contains antioxidant components, isoflavone and saponin, that alleviate prostate cancer (Charron et al., 2005), breast cancer (Lamartiniere, 2000), degenerative immune diseases, and mild menopausal symptoms (Kumar et al., 2009). Lecithin in edamame is an essential nutrient for the central nerve system, which contributes to brain development, memory, and intelligence (Chen et al.,

2021). Foods rich in dietary fiber are capable of lowering blood pressure and cholesterol and improving diarrhea and constipation (Mateos-Aparicio et al., 2008).

Understanding the food choices of consumers can aid producers in offering more suitable food options. Sevenhuysen and Gross (2003) reported that the dietary patterns of respondents were considered personal perceptions and the interaction between their social environment and themselves. Moreover, personal perceptions of a specific food differ because of individual characteristics and genetic uniqueness of sensitivity to taste; for example, bitter-sensitive individuals tend to be more objectionable to bitter-tasting food and prefer sweet-tasting food. The association between bitter-taste phenotype and preference for bitter-tasting vegetables resulted in a weak relationship and needed more evidence for analysis (Bawajeeh et al., 2020). In addition, pleasant sensory experiences through aroma, flavor, and texture strongly motivate shoppers to reinforce their purchase intention (Biswas and Szocs, 2019; Bleasdale et al., 2021). For example, U.S. consumers preferred buttery beans with a fragrant taste and crunchy texture, while Chinese consumers preferred sweet and low-acidic flavor beans (Johnson et al., 1999; Christo, 2002). Edamame beans tend to have mild or moderate flavors due to their unique combinations of sweetness, sourness, and bitterness (Lee and Hwang, 1998; Nzaranyimana, 2017). Sweetness in edamame is conferred by sucrose content (Santisteban, 2016). Otherwise, S produces organic compounds in vegetables to provoke a repulsive taste such as pungent and objectional to consumers (Kopsell et al., 2003).

Sulfur is an essential nutrient for plant growth and development and a component of the amino acids cysteine (Cys) and methionine (Met; Takahashi et al., 2011). Among the S-derived organic S enzymes and coenzymes, are compounds that form the taste of vegetables (Fenwick et al., 1983). The enzymatic reaction of S compounds stands out prominent in allium species to form a unique flavor (Lee et al., 2009). Kopsell et al. (2003) reported that

the flavor of kale (*Brassica oleracea* L. Acephala Group) and S fertility were strongly correlated and as the levels of S increased, the flavor of kale became more bitter. Therefore, a study was undertaken to determine the effect of S fertility on the important quality and flavor components of edamame.

MATERIALS AND METHODS

Plant Culture

'Chiba' edamame seeds (Johnny's Selected Seeds, ME, USA) were sown on September 21, 2020, in a greenhouse in Normal, IL (lat. 40.51 °N, 88.99 °W). Edamame seeds were sown into 2.5 x 2.5 cm growing cubes (Grodan A/S, DK-2640, Hedehusense, Denmark), and the surface was covered with vermiculite (Therm-O-Rock East, PA, USA). The growing cube was soaked with regular tap water daily. To help germinate, seed cubes were placed on a heat mat set at 24 ℃. One week after germination, Peter's 20N-4.4P-16.6K fertilizer (1010g to 10L of water; 1:100 with deionized water) was applied. The greenhouse conditions were 24 °C under a 13-h photoperiod supplemented by 600W HPS lighting.

A modified nutrient solution (Hogland and Arnon, 1950) was prepared for hydroponic cultivation systems. The solution consisted of: ammonia phosphate ($NH_4H_2PO_4$), potassium nitrate (KNO_3), calcium nitrate [$Ca(NO_3)_2$] (Fisher Scientific), iron (Fe) (Sprint 330), and a micronutrition solution. The micronutrient solution was composed of: boric acid (H_3BO_3) (Acros), manganese (II) chloride ($MnCl_2$) (Acros), molybdic acid (MoO_3H_2O) (Acros), sulfuric acid ($ZnSO_4$), cupric sulfate **(**$CuSO_4$) (Acros). The S treatments were 4, 8, 16, 32, and 64 mg S L^{-1} supplied with magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4). Magnesium chloride ($MgCl_2$) was added to the 4, 8, and 16 mg S L^{-1} treatment to maintain the same level of magnesium in all treatments. Each element nutrient solution was prepared in a 1 M solution with deionized water. The concentrations of element nutrients

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 (0.05).

On October 13, 2020, three weeks after sowing, "Chiba" Edamame plants were transferred to the hydroponic solution. Twenty 32 L containers (Rubbermaid, Atlanta, GA) were connected to air tubes. The air tube was aerated from the air blower (Model VB-007S, Sweetwater, Ft. Collins, CO), and each tube end had attached an air stone. Each container held four edamame plants, and each plant was fixed onto the opening hole about 2 cm round at 15 cm x 9 cm spacing in the lid. The growing cubes where the plants were rooted and intertwined aided to float in the solution. The experimental design was a randomized complete block design with five S treatments, each having four replications. Each container was filled with Hogland's nutrient solution and 30 L of deionized water, and the containers were emptied every 2 weeks and replenished with a new stock solution until harvest. To stimulate flowering, the photoperiod was reduced to 10 h on Nov. 8, 2020.

Plant Harvest

On December 14, 2020, edamame was harvested at the R6 maturity stage approximately 60 days after being transferred to hydroponic cultivation. At the time of harvest, pod, leaf, and root tissues were collected, and fresh mass for beans, leaves, and roots of each replication was measured. Edamame bean tissues were stored at -80 °C freezer, while leaf and root tissues were dried at 60 °C oven. Dry mass (DM) of the tissues were recorded at harvest.

Tissue Analysis

Edamame beans were stored in a -80 °C freezer. Root and leaf tissues were dried in an oven at 60 °C or 4 days. The dried tissues were pulverized. Frozen bean tissues and dried

leaf and root tissues were shipped to the University of Tennessee for elemental and metabolite composition analyses.

Mineral Nutrient Analysis

The plant tissue samples were analyzed for nutrient elements S, K, P, Ca, Mg, Na, Fe, Mn, B, Zn, Cu, Mo, and Se using inductively-coupled plasma-mass spectroscopy (ICP-MS). A 100 mg of subsampled lyophilized plant tissue and 10 ml of 70% nitric acid (HNO_3) were blended for a microwave digestion unit (Model: Ethos, Milestone Inc., Shelton, CT, USA). The microwave condition initiated at 40 °C for 5 min at 1000W and 2000 kPa and then increased to 210 °C for 10 min at 1000W and 3000 kPa. It remained at 210 °C for 10 min at 1000W and 4000 kPa. After that, the digest was cooled for 10 min at 0W and 2000 kPa and then cooled to 20 °C. Inductively coupled plasma mass spectroscope (ICP-MS; Agilent Technologies, Inc., Wilmington, DE, USA) analyzed the subsample of the digest that was diluted with 9900 µl a matrix (2% HNO_3 and 0.5% hydrochloric acid (HCl) (v/v). ICP-MS was equipped with an octapole collision/reaction center, Agilent 7500 ICP-MS ChemStation software, a Micromist nebulizer, a water-cooled quartz spray chamber, and an ASX-510 (CETAC, Omaha, NE, USA) autosampler. ICP-MS had optimized its sensitivity by using a tuning solution containing 1 µg L^{-1} of lithium (Li), yttrium (Y), thallium (Tl), oxide, and doubly charged cesium (Ce), and cobalt (Co) in a 2% HNO_3 matrix.

Pigment Content Analysis

According to Kopsell et al. (2012), pigment extraction and analysis were conducted. 0.1g of lyophilized edamame bean tissue sample was immersed in 0.8mL of reverse osmosis (RO) water for 20 min for rehydration. 0.8 mL of the internal standard ethyl-β-8′-apo-carotenoate (Sigma-Aldrich, St. Louis, MO) was added to determine extraction

efficiency. The hydrated samples were added with 2.5 mL of tetrahydrofuran (THF). The samples were homogenized in a tissue grinding tube (Potter-Elvehjem; Kimble Chase-Kontes Glass, Vineland, NJ) which carried out a drill press (at 540 rpm) of ~ 25 insertions of a pestle. The tube was placed in ice to dissipate heat from the homogenization process. The tube was centrifuged for 3 min at 500 rpm. The supernatant was discarded, and the sample pellet was re-suspended in mL THF. The re-suspended sample was processed through the same homogenization and extraction process again. To obtain a colorless supernatant, the procedure was repeated. The colorless supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation, Berlin, MA) and were combined with acetone to a final volume of 5 mL.

By using a 5mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ), a 20mL aliquot was filtered through a 0.2-µm polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20, Agilent Technologies)

High-performance liquid chromatography (HPLC) analysis was conducted using a photodiode array detector (Agilent 1200 series, Agilent Technologies) for pigment separation. Analytical scale (4.6 i.d. x 250 mm) 5 µm and 200 Å polymeric RP-C30 column (ProntoSIL, MAC-MOD Analytical, Chadds Ford, PA) effectively separated chemically similar pigment compounds such as lutein, chlorophyll a and chlorophyll b. The polymeric column was equipped with a 5 µm guard cartridge (4.0 i.d. x 10 mm) and holder (ProntoSIL, MAC-MOD Analytical), while a heated column compartment maintained its condition at 30 ºC. All separations were achieved isocratically using a binary mobile phase of 11% methyl tert-butyl ether (MTBE), 88.99% MeOH, and 0.01% triethylamine (TEA) (v/v/v). The flow rate was 1.0 mL·min-1, with a run time of 58 min. Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array

detection using external standards [antheraxanthin (ANT), BC, Chl a, Chl b, LUT, neoxanthin (NEO), violaxanthin (VIO), ZEA from ChromaDex, Irvine, CA].

Carbohydrate Content Analysis

According to Muir et al. (2009) and Thavarajah et al. (2016), an analysis of nonstructural water-soluble carbohydrates was conducted. A 0.1 g of edamame bean tissues were extracted by adding 2 mL of RO water heated to 80 °C. Samples in 15 mL test tubes were rocked for 15 min at 300 rpm on a shaker and then vortexed. The tubes were centrifuged at 3,000 rpm for 20 min.A 1 mL aliquot of the supernatant was transferred into a new 15 mL test tube. Samples were reduced to dryness under a stream of nitrogen gas and added to 2.5 ml RO water for rehydration. By using a 5mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ), samples were filtered through a 0.2 μm polypropylene filter (Captiva Econofilter; Agilent Technologies, Santa Clara, CA). Effluents were collected in a 2 mL vial for HPLC analysis.

An HPLC unit (Agilent 1200 series; Agilent Technologies) equipped with an evaporative light scattering (ELS) detector (1290 Infinity II; Agilent Technologies) conducted separation parameters and carbohydrate quantification. The ELS detector had a nitrogen gas flow rate of 1.6 L min-1, while the evaporative gas temperature was at 80ºC, and the nebulizer gas temperature was at 50ºC. Rezex RCM Monosaccharide Ca+2 (8%) 300 x 7.8mm i.d., 8 μm analytical scale column (Phenomenex, Torrance, CA) allowed for effective separation of chemically similar carbohydrate compounds. The analytical scale column was equipped with a Carbo-Ca 4 x 3.0 mm i.d. security guard cartridge and holder (Phenomenex), while a heated column compartment maintained its condition at 80ºC. All separations were achieved isocratically using a mobile phase of 100% RO water. The flow rate was set at 0.6 mL min-1, with a run time of 20 min, followed by a 2 min equilibration prior to the next

injection. Eluted compounds from a 5.0 µL injection were detected, and data was collected using ChemStation Software (Agilent Technologies). Peak assignment for individual carbohydrates (mono-, di-, tri-, and polysaccharide) was performed by comparing retention times from the ELS detector using external standards of fructose, glucose, and sucrose (Sigma-Aldrich, St. Louis, MO).

Fatty Acid Content Analysis

According to Spencer et al. (2003), fatty acid gas chromatography was conducted. Freeze-dried samples were pulverized and then placed with a 2.5 mL mixture of chloroform, hexane, and methanol (8:5:2 v/v/v) in a test tube. The test tubes were fully capped to be awaited for at least four hours. Following extraction, 100 µl of the oil sample was placed in a 1.5 mL autosampler vial. Then, 75 µl of methylation reagent [sodiummethoxid:methanol:petroleum ether:ethyl ether (1:4:2 v/v/v)] and 0.75 mL of hexane were added to each vial before capping. The fatty acid compositions were determined by a Hewlett Packard HP 6890 series gas chromatograph (Palo Alto, CA) system set using a model 7683 autosampler and a model 7673 flame ionization detector, and an immobilized 30 mm x 0.53 mm inner diameter, Alltech AT-Silar capillary column with 0.5 µm fused stationary phase. Analysis was conducted with a carrier, Helium (20 ml/min), and 20:1(v/v) split injection, while injection temperature was at 250 °C; detector temperature was 275 °C, and the column temperature was 230 °C. The RM-1 standard (appropriate for measuring soybean oil) was used to calibrate and determine the relative fatty acid content (monounsaturated omega-9 fatty acids, polyunsaturated omega-6 fatty acids, and saturated fatty acids) of the experimental samples.

Amino Acid Content Analysis

A FOSS 6500 near-infrared spectrometer (NIR) was conducted to analyze amino acid components in edamame bean tissues. The NIR was warmed up for two h after turning on the lamp, and a dehumidifier reduced the humidity to 40% throughout the analysis. The room temperature was maintained at approximately 20ºC. Auto diagnostics were run for instrument response, wavelength accuracy, and NIR repeatability. Edamame been tissues were pulverized by using a water-cooled Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 s to homogenize in their particle size. Using ISIscan (System II version 2.80 software (FOSS, State College, PA), the concentration of protein and oil and amino acids (%) (Ala, Arg, Asp, Cys, Glu, Gly, His, Hyl, Hyp, Ile, Lan, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, Val) were recorded. Each amino acid sample was corrected as a percentage of overall crude protein content to report values as g of the amino acid per kg of crude protein.

STATISTICAL ANALYSIS

The Proc mixed procedure of SAS analyzed Data (Cary, NC). Regression analysis determined the relationships between experimental dependent variables and the S fertility levels.

RESULTS AND DISCUSSIONS

Plant Biomass

The increase of S fertility in solution culture from 4 to 64 mg S L^{-1} had no significant effect on the FM or DM of bean, root, and leaf tissue of 'Chiba' edamame. Edamame plant biomass exhibited no difference in bean FM (BFM; F=1.17 and P=0.3860), root FM (RFM; F=1.25 and P=0.3450), root DM (RDM; F=0.19 and P=0.9405), and leaf FM (RFM; F=1.22

and P=0.3517) when S in nutrient solution was increased from 4 to 64 mg S L^{-1} (Table 1). The leaf DM (RDW; F=3.1 and P=0.0571) was statistically insignificant for the given S treatments; however, it was close to the critical value (α=0.05).

Sulfur fertility is an essential component of plant growth and development and the relationship between S fertility and biomass has been studied extensively. Several studies on the legume families reported that S fertility improved soybean quality (Kandapal and Chandel, 1993) and quantity by up to 20% (Fismes et al., 2000). Grains of pea (*Pisum sativum* var. *Arvense*; Kedar and Rajendra, 2003) and black gram (*Vigna mungo* L.; Ramamoorthy, and Ariraman, 2021). resulted in a significant increase in production yield under the application of S. The biomass of oilseed rape (*Brassica napus* L.) was not significantly affected by increasing S fertility (Matula and Zukalova, 1998). Sulfur fertility displayed a significant impact on Leucaena [*Leucaena leucocephala* (Lam.) de Wit.] plant development, whereas the leaf yield was not affected (Chotchutima et al., 2015). However, increases in S fertility levels paralleled increases in aloe (*Aloe vera* L.) leaf biomass up to 47.5% (Chowdhury et al., 2020). There was no change in the biomass for edamame when increasing S fertility among the ranges provided in this study, but further studies may be needed to determine whether S concentration impacts the biomass of beans, roots, and leaves under different concentrations or cultural conditions.

Mineral Elements

The increase of S fertility had no statistically significant effect on the nutrient element accumulation in 'Chiba' edamame. The S accumulation in leaf tissue was significant (F=8.78, P=0.0073) in response to increase S treatments, while the S accumulation of edamame plant tissues for bean and root (F=2.9, P=0.1041 and F=1.28, P=0.3887, respectively) were statistically indifferent (Table 2). As a result, the edamame leaf tissue

accumulated S more readily with the increase in S fertility. The average accumulation of S in leaf tissue was the highest at 0.38% in the range of 4 to 64 mg S L^{-1} while 0.16% for bean tissue and 0.23% for root tissue. The amount of S accumulation in leaf tissue was the highest at 0.48% at 64 mg S L^{-1}. In South Korea, soybean leaf consumption is common. The benefit of soybean leaf consumption has been studied in that it contains pterocarpans (antifungal and anti-inflammatory properties) to reduce the risk of diabetes and dyslipidemia in Korean obese subjects (Ryu et al., 2016). The correlation between the health benefit and the effect of S fertility is yet to be determined; however, adjusting the flavor of soybean leaf by changing S fertility may boost consumption among Koreans.

The S concentration in bean tissues fluctuated when the S level increased at 8 and 32 mg S L^{-1}; however, it declined in response at 16 and 64 mg of S L^{-1} (Table 2-1a). Root and leaf S accumulation increased linearly in response to increasing S levels in nutrient solution (Table 2-1b and Table 2-1c). The linear regression of root and leaf tissue was statistically significant (P=0.0316 and P=0.0312, respectively). Sulfur accumulation increased 52.63% in root tissue and 182.35% in leaf tissue from 4 mg to 64 mg S L^{-1}.

The S concentration range of soybeans is between 0.20% and 0.40%, and the S content range of most leaves is reported to be between 0.15% and 0.5% (Mills and Jones, 1996). The accumulation of S is positively related to S availability in plants (Kopsell et at., 2003; Ganeshamurthy, 1996). Depending on the concentration of S in the given range, the range of S accumulation in edamame beans was not statistically significant, but the S accumulation in the roots and leaves was significant. In a similar study, Thomas et al. (2000) reported that the accumulation of S in the leaves of sugar beet (*Beta vulgaris*) increased to 1,100%, while the S level increased from 0 to 96 mg of S L^{-1}. Angiosperms (species of flowering plants) are also known to have distinct S accumulation capacities; for example,

brassica and alliums deposit organic S compounds in the tissues more than other plants in the same environment (Mengel and Kirkby, 2001; Willey and Wilkins, 2006).

Carotenoid and Chlorophyll Compounds

There was no statistical significance among carotenoid or chlorophyll compounds accumulation in edamame bean tissue when S in solution culture was increased from 4 mg to 64 mg S L^{-1}. Lutein, chlorophyll *a*, and chlorophyll *b* phytochemicals in edamame bean tissues were analyzed (F =0.87 and P=0.5074, F =0.46 and P =0.7615, F = 0.29 and P =0.8819, respectively; Table 4). Lutein is a dietary carotenoid that accumulates in the human retina and lens to protect eye tissue from oxidation (Edge et al., 1997). Lack of lutein leads to a cessation of antioxidant protection and aging-related malady (Humphries and Khachik, 2003). Consumption of various vegetables and fruits containing carotenoids is associated with delayed cataracts and macular degeneration (Mares-Perlman et al., 2001) and reduced risk of cancer (Kim et al., 2019).

As a result, decreasing S concentrations in nutrient solutions had no effect on the concentration of carotenoid and chlorophylls accumulation in edamame tissues. Studies on the content of carotenoids associated with S fertilizers have also been reported on other crops. The content of carotenoids in beet leaves showed no change in the condition of 32 mg and 96 mg S L^{-1} (Thomas et al., 2000), and similarly, other studies showed no change in beet leaf chlorophyll content between 24 mg and 48 mg S L^{-1} (Bone et al., 1997).

Sucrose and Brix

In edamame bean tissues, sucrose and acid (F =1.44 and P=0.2793, F =1.83 and P =0.1887, respectively) remained constant as S was increased from 4 mg to 64 mg S L^{-1} in solution culture (Table 3). The Brix of edamame beans also remained unchanging in response

to increasing S treatment levels. Sucrose is known to be a significant contributor to sweet flavor in edamame. Although the amount of sucrose was measured to be stable among S treatment levels, the organoleptic bitterness of edamame beans was enhanced as the level of S treatment increased (data not shown).

Sulfur fertility has been reported to influence flavor potential in onion (*Allium cepa* L.) (Randle et al., 1995). While the bitter, astringent, and pungent flavors of vegetables are cited as unpleasant or unpalatable (Drewnowski and Gomez-Carneros, 2000), S fertility changes would be less objectionable flavor in edamame while stabilizing beneficial dietary phytonutrients.

Fatty Acids

Fatty acid contents for edamame bean tissue resulted in no statistical difference in response to increasing S levels (Table 7). Even though S affects the lipid and protein formation of seeds, the amount of polyunsaturated omega-6, monounsaturated omega-9, and saturated fatty acid contained in edamame bean tissue did not change in response to increasing S treatments. Moreover, the deficiency of S could reduce fatty acid accumulation and alter lipid composition in plants (D'Hooghe et al., 2014). For example, field mustard (*Brassica campestris*) has been reported to have a positive correlation with lipid content and S application from the early stages of seed formation (Ahmad and Abdin, 2000).

Amino Acids

The amino acid accumulation in edamame bean tissue was not affected by increasing S treatments. The S-related amino acids, Cys ($F=0.83$ and $P=0.5699$) and Met ($F=0.12$ and $P=0.968$) were not affected by S treatment levels (Table 8). The S-related amino acids are dietarily essential because humans cannot synthesize them. Several studies reported that S

fertility increased the protein contents in soybean (Kandpal and Chandel, 1993; Arshad et al., 2010; Ahmad et al., 2007). Moreover, Brassica oilseed species with S fertilization reported a significant increase in protein concentration in the seed (Malhi et al., 2007). However, Paek et al. (2000) refuted that soybean protein contents were influenced by S availability during vegetative and reproductive growth stages.

CONCLUSION

Plant biomass, mineral elements, and nutrient levels remained stable regardless of S fertilization in 'Chiba' edamame bean, leaf, and root tissues, while there was a significant S element augment in edamame leaf tissues in response to the increase of S fertility. This study aimed to evaluate the consequence of S fertility in edamame in terms of nutritional, health benefits, and sensory quality. As a result, edamame beans produced a constant number of nutritional compositions and beneficial biochemicals unaffected by S fertility levels. This is an advantage for producers to supply stable nutritional and healthy quality beans without yield difference. However, sensory quality in bean tissues was affected by S fertility changes even though sucrose levels were constant. The negative effect of S treatment on flavor is that it is a precursor of stringency in many vegetables. Therefore, complex interactions S fertility and flavor of edamame must be determined the optimal rate of fertilization for desired taste quality for target customers. Moreover, the accumulation of S in leaf tissues was affected by the S fertility levels. There was a statistically significant increase. Based on the result, further analysis must be determined any quality changes in edamame leaf tissues in response to S fertility changes.

Table 1. Mean values[a] for fresh (FM) and dry (DM) biomass for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

S-Level[d]	Plant biomass[c](g)				
	Bean FM	Root FM	Leave FM	Root DM	Leave DM
4	107.12 ± 5.58	94.03 ± 16.02	36.59 ± 0.97	6.93 ± 0.86	8.94 ± 0.52
8	91.42 ± 11.61	93.92 ± 10.71	37.21 ± 2.75	7.14 ± 0.81	8.95 ± 1.00
16	97.17 ± 5.89	124.30 ± 26.82	43.10 ± 4.76	6.66 ± 0.70	7.53 ± 0.58
32	91.17 ± 7.99	119.88 ± 7.30	34.86 ± 2.35	6.91 ± 0.85	7.66 ± 0.22
64	90.86 ± 3.93	75.52 ± 3.71	34.00 ± 2.50	7.17 ± 0.78	7.83 ± 0.25
Contrast[e]					
Linear	*P*=0.2060	*P*=0.5516	*P*=0.2358	*P*=0.8204	*P*=0.181
Quadratic	*P*=0.3936	*P*=0.1039	*P*=0.4467	*P*=0.9337	*P*=0.131

[a] Mean composition of 4 replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[c] FM=fresh mass, DM=dried mass.
[d] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[e] Significance for linear and quadratic orthogonal contrasts.

Table 2. Mean values[a] for macronutrient concentration in bean tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

S-Level [c]	Macronutrient (%)				
	Sulfur (S)	Potassium (K)	Phosphorus (P)	Calcium (Ca)	Magnesium (Mg)
4	0.11 ± 0.01	0.62 ± 0.05	0.26 ± 0.02	0.07 ± 0.01	0.09 ± 0.01
8	0.20 ± 0.01	0.70 ± 0.03	0.29 ± 0.02	0.08 ± 0.00	0.09 ± 0.00
16	0.17 ± 0.02	0.69 ± 0.04	0.28 ± 0.02	0.08 ± 0.01	0.09 ± 0.01
32	0.19 ± 0.01	0.70 ± 0.03	0.29 ± 0.01	0.07 ± 0.00	0.09 ± 0.00
64	0.14 ± 0.02	0.62 ± 0.05	0.25 ± 0.02	0.07 ± 0.01	0.08 ± 0.01
Contrast [d]					
Linear	*P*=0.4322	*P*=0.1895	*P*=0.2763	*P*=0.6282	*P*=0.4065
Quadratic	*P*=0.1589	*P*=0.2316	*P*=0.4750	*P*=0.7254	*P*=0.5672

[a] Mean composition of 4 replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 contrast.

Table 3. Mean values[a] for macronutrient concentration in root tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Macronutrient (%)				
S-Level [c]	Sulfur (S)	Potassium (K)	Phosphorus (P)	Calcium (Ca)	Magnesium (Mg)
4	0.19 ± 0.06	4.00 ± 0.82	2.40 ± 0.59	3.57 ± 1.93	2.56 ± 1.07
8	0.17 ± 0.01	2.37 ± 0.34	1.51 ± 0.22	2.52 ± 0.39	1.73 ± 0.27
16	0.24 ± 0.06	2.71 ± 0.36	1.76 ± 0.21	3.69 ± 0.64	2.12 ± 0.30
32	0.28 ± 0.00	3.10 ± 0.67	2.06 ± 0.41	2.60 ± 0.66	1.93 ± 0.45
64	0.29 ± 0.04	2.56 ± 0.28	1.47 ± 0.22	2.37 ± 0.49	1.49 ± 0.19
Contrast [d]					
Linear	P=0.0316	P=0.3933	P=0.3151	P=0.423	P=0.2817
Quadratic	P=0.0629	P=0.6608	P=0.6005	P=0.732	P=0.5689

[a] Mean composition of 4 replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 macronutrient concentration in leaf tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Macronutrient (%)				
S-Level [c]	Sulfur (S)	Potassium (K)	Phosphorus (P)	Calcium (Ca)	Magnesium (Mg)
4	0.17 ± 0.05	3.76 ± 1.19	0.68 ± 0.20	6.21 ± 1.77	0.69 ± 0.21
8	0.33 ± 0.04	2.43 ± 0.64	0.56 ± 0.19	4.01 ± 0.97	0.44 ± 0.10
16	0.43 ± 0.05	2.71 ± 0.35	0.43 ± 0.04	4.21 ± 0.77	0.42 ± 0.05
32	0.42 ± 0.07	2.91 ± 0.48	0.58 ± 0.12	4.45 ± 0.75	0.51 ± 0.11
64	0.48 ± 0.03	2.57 ± 0.43	0.50 ± 0.09	4.06 ± 0.86	0.43 ± 0.10
Contrast [d]					
Linear	*P*=0.0312	*P*=0.5225	*P*=0.5657	*P*=0.4278	*P*=0.3983
Quadratic	*P*=0.0513	*P*=0.7712	*P*=0.7910	*P*=0.6163	*P*=0.4672

[a] Mean composition of 4 replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 5. Mean values[a] for micronutrient concentration in bean tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal IL

	Micronutrient (ppm)					
S-Level[b]	Iron (Fe)	Manganese (Mn)	Boron (B)	Zinc (Zn)	Copper (Cu)	Molybdenum (Mo)
4	47.19 ± 16.92	17.56 ± 3.45	5.41 ± 0.45	14.93 ± 0.90	2.71 ± 0.40	3.92 ± 0.38
8	40.09 ± 4.45	18.67 ± 0.84	6.16 ± 0.62	15.09 ± 0.84	2.61 ± 0.24	4.40 ± 0.34
16	36.62 ± 2.15	17.95 ± 1.93	6.55 ± 0.74	15.37 ± 1.29	2.62 ± 0.30	4.48 ± 0.45
32	35.76 ± 2.31	17.28 ± 1.04	6.01 ± 0.32	16.58 ± 0.56	3.36 ± 0.65	4.46 ± 0.58
64	33.48 ± 3.54	17.34 ± 1.59	5.65 ± 0.31	16.30 ± 1.36	3.03 ± 0.27	4.11 ± 0.32
Contrast[d]						
Linear	*P*=0.2843	*P*=0.7218	*P*=0.7288	*P*=0.2239	*P*=0.3069	*P*=0.9264
Quadratic	*P*=0.4698	*P*=0.9356	*P*=0.5471	*P*=0.3597	*P*=0.4141	*P*=0.6160

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 6. Mean values[a] for micronutrient concentration in root tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Micronutrient (ppm)					
S-Level [b]	Iron (Fe)	Manganese (Mn)	Boron (B)	Zinc (Zn)	Copper (Cu)	Molybdenum (Mo)
4	11409.40 ± 5948.34	1359.24 ± 702.99	50.73 ± 20.50	89.18 ± 40.30	71.39 ± 32.53	33.19 ± 7.22
8	7822.21 ± 1016.61	954.38 ± 143.91	32.43 ± 4.60	77.82 ± 9.16	44.42 ± 4.67	20.64 ± 2.91
16	11634.45 ± 2072.72	1396.85 ± 217.59	60.16 ± 16.89	89.19 ± 11.46	70.04 ± 8.01	39.00 ± 12.76
32	7926.90 ± 1710.29	1019.17 ± 246.73	38.18 ± 7.39	70.03 ± 12.31	57.25 ± 14.21	21.83 ± 2.18
64	7610.61 ± 1461.47	938.49 ± 178.69	28.77 ± 3.12	72.21 ± 16.68	62.70 ± 25.93	21.57 ± 3.67
Contrast [d]						
Linear	*P*=0.4199	*P*=0.4643	*P*=0.2511	*P*=0.5321	*P*=0.9809	*P*=0.2992
Quadratic	*P*=0.7230	*P*=0.7710	*P*=0.4860	*P*=0.7984	*P*=0.7570	*P*=0.5927

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 7. Mean values[a] for micronutrient concentration in leaf tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Micronutrient (ppm)					
S-Level [b]	Iron (Fe)	Manganese (Mn)	Boron (B)	Zinc (Zn)	Copper (Cu)	Molybdenum (Mo)
4	224.45 ± 43.15	486.98 ± 125.20	127.52 ± 40.15	42.44 ± 10.09	7.87 ± 3.44	0.96 ± 0.28
8	229.89 ± 131.73	298.48 ± 67.27	85.19 ± 22.89	33.44 ± 6.75	na	0.58 ± 0.13
16	193.64 ± 70.27	361.62 ± 84.04	96.70 ± 17.22	27.56 ± 3.19	2.54 ± 0.34	0.60 ± 0.08
32	157.47 ± 109.76	375.20 ± 51.17	103.56 ± 20.08	34.93 ± 4.39	5.32 ± 2.33	0.93 ± 0.13
64	169.81 ± 48.68	367.57 ± 63.20	99.72 ± 25.29	29.45 ± 6.74	2.46 ± 1.32	0.76 ± 0.23
Contrast [d]						
Linear	*P*=0.5427	*P*=0.7633	*P*=0.8219	*P*=0.3890	*P*=0.1963	*P*=0.8765
Quadratic	*P*=0.7545	*P*=0.8550	*P*=0.9244	*P*=0.6194	*P*=0.3741	*P*=0.9842

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 8. Mean values[a] for sugar and acid content of bean tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

S-Level [c]	Sugar content			Brix/Acid Ratio
	Sucrose (mM)	Brix (%)	Acid	
4	63.97 ± 1.17	13.33 ± 0.44	0.34 ± 0.02	39.73
8	62.68 ± 0.65	12.57 ± 0.52	0.27 ± 0.01	46.44
16	61.10 ± 1.46	13.93 ± 0.18	0.32 ± 0.03	44.42
32	61.72 ± 1.00	13.43 ± 0.43	0.33 ± 0.01	41.14
64	61.20 ± 1.20	13.42 ± 0.61	0.32 ± 0.01	42.09
Contrast [d]				
Linear	*P*=0.1704	*P*=0.6198	*P*=0.6195	
Quadratic	*P*=0.2321	*P*=0.6888	*P*=0.8662	

[a] Mean composition of 4 replications per S treatment, four plants per replication.
[b] Greenhouse conditions of 24°C and extended photoperiod for 14 hours until blooming.
[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 9. Mean values[a] for pigment concentration of bean tissue for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Pigment (ug g^{-1} DW)		
S-Level [c]	Lutein	Chlorophyll *a*	Chlorophyll *b*
4	1.64 ± 0.53	37.54 ± 8.38	19.39 ± 3.62
8	2.23 ± 0.46	44.63 ± 4.25	21.76 ± 2.40
16	1.75 ± 0.20	37.29 ± 2.18	19.60 ± 1.18
32	1.74 ± 0.30	38.75 ± 4.09	20.86 ± 1.59
64	1.65 ± 0.18	39.86 ± 2.01	21.09 ± 0.50
Contrast [d]			
Linear	*P*=0.5712	*P*=0.9395	*P*=0.7216
Quadratic	*P*=0.8558	*P*=0.9602	*P*=0.9397

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 10. Mean values[a] for bean protein content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Percent (%)[c]						
S-Level[d]	Moisture	Protein Dry	Oil Dry	Fiber Dry	ADF	NDF	Ash Dry
4	10.48 ± 0.23	37.72 ± 0.32	19.30 ± 0.38	5.85 ± 0.02	12.90 ± 0.02	14.30 ± 0.15	5.75 ± 0.01
8	10.49 ± 0.47	37.65 ± 0.19	19.81 ± 0.33	5.67 ± 0.05	12.59 ± 0.29	15.07 ± 0.03	5.78 ± 0.02
16	10.61 ± 0.21	37.82 ± 0.32	19.48 ± 0.10	5.76 ± 0.07	12.89 ± 0.36	14.95 ± 0.11	5.74 ± 0.01
32	10.76 ± 0.01	37.91 ± 0.33	19.78 ± 0.27	5.52 ± 0.18	12.84 ± 0.01	14.52 ± 0.14	5.76 ± 0.06
64	10.66 ± 0.23	38.22 ± 0.66	19.32 ± 0.67	5.66 ± 0.10	12.79 ± 0.15	14.20 ± 0.06	5.79 ± 0.02
Contrast[e]							
Linear	*P*=0.4912	*P*=0.1989	*P*=0.7359	*P*=0.2794	*P*=0.9480	*P*=0.1144	*P*=0.3265
Quadratic	*P*=0.6146	*P*=0.4635	*P*=0.6677	*P*=0.1961	*P*=0.9699	*P*=0.1840	*P*=0.5527

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[c] ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber.
[d] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[e] Significance for linear and quadratic orthogonal contrasts.

Table 11. Mean values[a] for bean carbohydrate content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State, Normal IL

	Saccharide (%)					
	Monosaccharides		Disaccharide	Trisaccharide		Polysaccharide
S-Level[c]	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Starch
4	0.66 ± 0.01	0.92 ± 0.01	7.69 ± 0.23	0.89 ± 0.04	4.22 ± 0.07	4.61 ± 0.31
8	0.67 ± 0.03	0.90 ± 0.03	7.56 ± 0.04	0.79 ± 0.01	4.28 ± 0.03	4.12 ± 0.07
16	0.67 ± 0.01	0.95 ± 0.00	7.77 ± 0.06	0.92 ± 0.04	4.33 ± 0.12	4.79 ± 0.01
32	0.66 ± 0.01	0.91 ± 0.02	7.57 ± 0.19	0.85 ± 0.03	4.28 ± 0.02	4.19 ± 0.03
64	0.67 ± 0.00	0.91 ± 0.01	7.69 ± 0.26	0.86 ± 0.01	4.28 ± 0.18	4.19 ± 0.15
Contrast[d]						
Linear	*P*=0.6865	*P*=0.8288	*P*=0.9276	*P*=0.9739	*P*=0.8763	*P*=0.2814
Quadratic	*P*=0.8494	*P*=0.8152	*P*=0.9587	*P*=0.9906	*P*=0.8875	*P*=0.5825

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 12. Mean values[a] for bean fatty acid content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Fatty Acid (%)					
	Polyunsaturated Omega-6 Fatty Acids		Monounsaturated Omega-9 Fatty Acids		Saturated Fatty Acids	
S-Level[c]	Linoleicacid1	Linolenicacid2	Oleicacid	OleicacidHOLLDRY	Palmiticacid	Stearicacid
4	53.94 ± 0.61	10.06 ± 0.40	18.20 ± 1.04	36.71 ± 1.27	11.05 ± 0.17	3.82 ± 0.14
8	53.97 ± 2.43	9.90 ± 0.53	17.92 ± 3.05	37.50 ± 0.82	11.45 ± 0.00	3.72 ± 0.03
16	51.73 ± 0.62	10.54 ± 0.27	19.20 ± 1.59	36.21 ± 1.01	11.53 ± 0.13	3.86 ± 0.16
32	53.97 ± 1.05	10.48 ± 0.14	18.07 ± 1.46	35.92 ± 0.25	11.10 ± 0.15	3.78 ± 0.03
64	52.71 ± 3.67	10.66 ± 0.21	18.81 ± 4.30	38.98 ± 3.42	11.23 ± 0.20	3.78 ± 0.04
Contrast[d]						
Linear	*P*=0.7444	*P*=0.1304	*P*=0.8573	*P*=0.3469	*P*=0.6927	*P*=0.9031
Quadratic	*P*=0.9487	*P*=0.2629	*P*=0.9850	*P*=0.3622	*P*=0.9143	*P*=0.9799

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[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 13. Mean values[a] for bean amino acid content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Amino Acid (%)						
S-Level[d]	Lysine	Cysteine	Methionine	Threonine	Tryptophan	Isoleucine	Leucine
4	2.53 ± 0.01	0.61 ± 0.01	0.55 ± 0.01	1.49 ± 0.01	0.47 ± 0.01	1.81 ± 0.00	2.93 ± 0.00
8	2.52 ± 0.01	0.62 ± 0.02	0.55 ± 0.01	1.47 ± 0.01	0.47 ± 0.01	1.79 ± 0.01	2.91 ± 0.02
16	2.52 ± 0.02	0.64 ± 0.01	0.55 ± 0.01	1.48 ± 0.01	0.50 ± 0.02	1.81 ± 0.01	2.91 ± 0.00
32	2.51 ± 0.02	0.61 ± 0.01	0.55 ± 0.00	1.48 ± 0.02	0.46 ± 0.01	1.78 ± 0.02	2.90 ± 0.05
64	2.54 ± 0.04	0.60 ± 0.03	0.55 ± 0.02	1.49 ± 0.03	0.47 ± 0.02	1.81 ± 0.05	2.94 ± 0.06
Contrast[e]							
Linear	*P*=0.5932	*P*=0.4495	*P*=0.8933	*P*=0.8012	*P*=0.5751	*P*=0.9411	*P*=0.6237
Quadratic	*P*=0.5491	*P*=0.5459	*P*=0.9272	*P*=0.7419	*P*=0.8093	*P*=0.7436	*P*=0.5366

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[c] ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber.
[d] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[e] Significance for linear and quadratic orthogonal contrasts.

Table 13 (cont.). Mean values[a] for bean amino acid content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Amino Acid (%)						
S-Level[d]	Histidine	Phenylalanine	Valine	Alanine	Arginine	Aspartic acid	Glutamic acid
4	1.00 ± 0.00	1.93 ± 0.01	1.89 ± 0.00	1.63 ± 0.00	2.70 ± 0.02	4.29 ± 0.01	6.74 ± 0.01
8	0.99 ± 0.01	1.91 ± 0.01	1.88 ± 0.01	1.62 ± 0.01	2.70 ± 0.00	4.26 ± 0.03	6.67 ± 0.04
16	0.99 ± 0.00	1.92 ± 0.00	1.88 ± 0.00	1.62 ± 0.00	2.71 ± 0.02	4.26 ± 0.03	6.74 ± 0.06
32	0.99 ± 0.02	1.91 ± 0.03	1.87 ± 0.03	1.62 ± 0.02	2.68 ± 0.05	4.26 ± 0.07	6.67 ± 0.13
64	1.00 ± 0.02	1.95 ± 0.04	1.89 ± 0.04	1.64 ± 0.03	2.74 ± 0.06	4.29 ± 0.09	6.73 ± 0.17
Contrast[e]							
Linear	*P*=0.6583	*P*=0.3354	*P*=0.8362	*P*=0.4254	*P*=0.3525	*P*=0.7294	*P*=0.9385
Quadratic	*P*=0.5224	*P*=0.4089	*P*=0.7375	*P*=0.5225	*P*=0.4813	*P*=0.7874	*P*=0.9164

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[c] ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber.
[d] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[e] Significance for linear and quadratic orthogonal contrasts.

Table 13 (cont.) Mean values[a] for bean amino acid content for 'Chiba' edamame [*Glycine max* (L.) Merr.] cultivated in solution culture with varying S levels in protected culture[b] at Illinois State University, Normal, IL

	Amino Acid (%)						
S-Level[d]	Glycine	Proline	Serine	Tyrosine	Hydroxylysine	Hydroxyproline	Lanthionine
4	1.62 ± 0.01	1.84 ± 0.01	1.72 ± 0.02	1.45 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.03 ± 0.00
8	1.61 ± 0.01	1.83 ± 0.01	1.69 ± 0.00	1.45 ± 0.01	0.05 ± 0.01	0.07 ± 0.00	0.02 ± 0.00
16	1.60 ± 0.01	1.85 ± 0.02	1.69 ± 0.01	1.45 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
32	1.61 ± 0.02	1.84 ± 0.02	1.71 ± 0.02	1.43 ± 0.01	0.06 ± 0.01	0.07 ± 0.00	0.03 ± 0.01
64	1.63 ± 0.04	1.86 ± 0.06	1.70 ± 0.03	1.45 ± 0.02	0.06 ± 0.00	0.06 ± 0.01	0.03 ± 0.01
Contrast[e]							
Linear	*P*=0.4841	*P*=0.4518	*P*=0.8623	*P*=0.8006	*P*=0.0341	*P*=0.6605	*P*=0.9125
Quadratic	*P*=0.6427	*P*=0.7656	*P*=0.9857	*P*=0.4349	*P*=0.1085	*P*=0.5070	*P*=0.7889

[a] Mean composition of four replications per S treatment, four plants per replication.
[b] Greenhouse conditions at 24°C and extended photoperiod for 14 hours until blooming.
[c] ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber.
[d] Sulfur treatment level measured in mg L^{-1} and delivered as magnesium sulfate ($MgSO_4$) and sodium sulfate (Na_2SO_4).
[e] Significance for linear and quadratic orthogonal contrasts.

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CHAPTER IV: CONCLUSION AND RECOMMENDATIONS

Edamame is in the spotlight in the U.S. for its nutritional and health properties. To satisfy market expectations, this experiment focused on the effect of S fertility on the nutritional, beneficial, and sensory quality attributes of edamame. The hydroponic systems in a greenhouse at Illinois State University were designed to measure the effects of S fertility on edamame biomass production and bean nutritional quality. Different tissue parts were analyzed to summarize S accumulation data. Essential dietary nutrients (amino acids, carbohydrates, fats) and beneficial phytochemicals in edamame bean tissues were not impacted by S fertility treatments. Based on the results, it gives an insight into edamame production related to S fertility. However, since the study was conducted with only one cultivar, small-sized samples, and in hydroponic culture, the lack of significance does warrant further investigation using more cultivars and different production system to come to a definitive conclusion.

Even with stable sucrose contents in bean tissues, it provoked a change in the taste of beans. Sucrose is well-known to contribute significantly to sweetness, and in addition, S is the primary component of sensorial compounds that induce bitterness in vegetables. Moreover, the significant increased S in edamame leaf tissues requires further analysis of its nutritional and beneficial compositions. Because bean leaf is also a common dietary vegetable in South Korea, it can be excellent as an exported leafy crop. Therefore, understanding the effects of sensory compounds in edamame beans and leaves related to S fertility will satisfy the taste preferences of the target customer, leading to a purchase decision.