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Association Mapping of Seed Weight, Protein, and Sucrose Content; and Kinetics of Edamame Under Infrared Treatment

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Association Mapping of Seed Weight, Protein, and Sucrose Content; and Kinetics of Edamame Under Infrared Treatment

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Abstract

Edamame is a specialty large-seeded soybean (*Glycine max L*. Merr.) harvested at an immature stage (R6) that has become the second largest consumed soyfood. Although United States is the largest soybean producer, majority of edamame is imported from Asia, highlighting the importance of developing new edamame varieties. Association mapping (AM) provides an alternative to bi-parental linkage mapping method to detect quantitative trait loci (QTL) adding higher resolution and broader germplasm information. Seed weight, sucrose, and protein are quantitative traits of value when selecting edamame lines. However molecular mechanisms controlling each of these traits are still inconclusive and have not been addressed using edamame accessions. Edamame has not reached its potential due to lack of diversity on edamame-based products. Information regarding edamame processing is limited. Therefore, the objectives were 1) to identify and validate seed weight, sucrose, and protein QTL using single nucleotide polymorphisms (SNP) in an association mapping population, and 2) to determine the kinetics of edamame under infrared heating and evaluate three heating intensity levels on the physicochemical attributes of edamame. For the first objective 378 edamame accessions of different maturity groups (000-IX) were grown in Stuttgart and Fayetteville, AR in 2014 and 2015. A total of 16 SNPs on ten chromosomes were found to be associated (-log P > 2.5) with seed weight. Validating 11 previously reported QTL and identifying three new regions on Gm04, Gm16, and Gm19. For sucrose content 13 SNPs were significantly associated, mainly in Gm08, but also in Gm04, and Gm06. For seed protein content six SNPs were found to be significant on four chromosomes, confirming previously reported QTLs. For the second objective one variety (8080) was used to establish the kinetics of edamame using three IR heating intensities. Temperature, weight, texture, green intensity, and peroxidase activity were measured. Across

heating intensities, treating edamame for 100 and 120 seconds resulted on the best texture, weight reduction, and green intensity. Although 100% of peroxidase inactivation was not achieved, enzyme activity was reduced (62 %). Results indicate the potential of using IR treatment as a drying or pre-drying step in edamame product development.

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Dedication

To God and the angels He has placed upon my path. To Sheny, Nin, Valen, and David.

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Introduction and Literature Review

Introduction

Soybean (*Glycine max L.* Merr.) is a major crop originated in China produced mainly for its high seed protein and oil content (Zhang et al., 2009). Soybeans have been cultivated for multiple purposes, from animal feed to human consumption. Recently, tremendous interest has been generated in soyfoods including tofu, natto, soymilk, and edamame. Shift from animal-based diets to plant-based diets, has led to a higher demand of edamame. Edamame is the Japanese term for soybeans harvested immature (R6). Vegetable soybeans, as grain-type soybeans, have a high protein content ranging from 34 to 48 % (Wilson, 2004). In addition, edamame supplies all essential amino acids and is considered a high-quality protein source (Mebrahtu, 2008). Zhang et al. (2009) report a higher digestibility of protein in edamame when compared to grain-type soybeans.

Some distinguishing features of edamame include large seed and pod size, flavor, light pubescence and hilum color. The two most important components of flavor in edamame are sweet and savory (Konovsky et al., 1994). Sweetness is conferred mainly by sucrose content, which peaks at R6. Sweetness has been reported to be negatively correlated with protein content (Hymowitz, 1970). Therefore, breeders face a challenge when selecting edamame lines that are both nutritious and appealing to the consumers. In addition, edamame varieties adapted to the United States are scarce, limiting the parental material for edamame breeding programs.

Breeders select lines based on plants performance under various environments and enhance their selections utilizing molecular markers. For marker assisted selection to be conducted properly in a breeding program, markers linked to a trait of interest must be identified and validated on different populations. Thus, multiple verification and validation of quantitative trait loci (QTL) is required for molecular markers to be used by breeders.

Association mapping (AM) presents an alternative to traditional linkage mapping to detect QTL regions. AM offers three main advantages over linkage mapping, being an increased mapping resolution, greater allele number, and reduced research time (Zhu et al., 2008). In addition, AM exploits genetic diversity thus, population structure and linkage disequilibrium are important parameters to avoid false marker-trait associations. Moreover, seed weight, sucrose, and protein content have been previously studied on grain-type soybean but the QTL regions identified have not been validated on a food-grade panel.

Currently, the majority of edamame consumed in the United States is imported from China, usually frozen, highlighting the lack of edamame-based products available in the market. Kelley and Sánchez (2005), reported the interest of consumers on edamame-based products, such as freeze dried or patties. Research on the characterization of vegetable soybeans under diverse processing conditions is limited. Except for few studies conducted on blanching and canning, there are limited resources on the kinetics of edamame under any other processing methodology.

Infrared (IR) heating presents an alternative procedure to heat foods and to broaden the current knowledge of edamame. For instance, IR heating has been studied on apples, bananas, and other vegetables to achieve dry blanching and dehydration simultaneously (Pan and Atungulu, 2011). IR heating has also been associated with high energy transfer rate and shorter drying time compared with other convective heated air treatments (Pan et al., 2008). This is the first research to look at the effectiveness of IR heating on drying and blanching edamame; the findings could benefit attempts to develop new edamame-based products.

Edamame, overview

Edamame is the Japanese term for green vegetable soybeans cooked and served in pods (Born, 2006; Shirtleff and Aoyagi, 2009). Edamame is a specialty soybean (*Glycine max L.* Merr.) harvested at an immature stage (R6), when seeds have expanded to fill 80-90 % of the pod width (Konovsky et al., 1994; Shurtleff and Aoyagi, 2009). Distinguishing features of edamame include large pods, 100-seed weight (>30g), green pods without blemishes, two or three seeds per pod, and slight sweetness (Mebrahtu, 2008). Detached pods and shelled beans are used as snacks, or as main dishes cooked with rice, and often used in salads in Asia.

Nutraceutical foods are those with health-giving additives. Wildman (2004) reports edamame as a nutraceutical food, due to nutritional contents. As a result of the awareness of the nutraceutical properties attributed to edamame, demand for this specialty soybean has increased. Of the components that make edamame nutraceutical, proteins are the most abundant compound, ranging from 34 - 48 % (Wilson, 2004). Edamame not only supplies all essential amino acids, but is also considered to be a complete protein source equivalent in quality to that found in meat and dairy products (Mebrahtu, 2008). In addition, edamame contains isoflavones which have been linked with beneficial health effects reducing cancer, cardiovascular disease, and menopausal symptoms (Wildman, 2006). Oil content has been studied extensively in grain-type soybeans, since it is a primary source of oil. However, edamame hasn't been used as a main oil source, yet fat contents range from 15 to 19.5 % (Rao et al., 2002; Born, 2006; Mebrahtu, 2008).

Soybeans are a major crop used for animal feed, human consumption, and biofuels. With a global production of 313.26 million Mt in 2015/ 2016 (Economics et al., 2016) soybeans are a crop that in the United States has a value of $36 billion (National Agriculture Statistics Service [NASS], 2016). As such, extensive resources are continuously invested to improve soybean

production through understanding its physiology, genetics, and developing new technologies for weed and pest management. Likewise, edamame production and research is gaining interest in the United States. Although East Asia remains the leader consumer of edamame, consumption is expanding worldwide. In the United States consumption has increased 9 % in 2013 (SoyFoods, 2015). This notorious increase is due to awareness of the nutritional value and health benefits associated with consumption of edamame. Moreover edamame acreage expanded in past years on western countries, but has not reached its potential due to lack of adapted varieties (Mebrahtu, 2008; Mozzoni et al., 2009; Sato et al., 2014).

Among the characteristics that make edamame varieties different from grain-type soybeans are: larger seed size, ideally >30 g per 100 seeds compared to average 15 g per 100 seeds; flavor, although it is market-dependent; texture as tenderness is desired when cooked; light hilum and pubescence color, pod shape (culture dependent), and nutritional content (Gupta et al., 1976; Young et al., 2000; Carson, 2010; Zhang et al., 2010). Edamame is harvested earlier than grain-type soybeans therefore it is important to consider plant architecture and growth habit. Compared with grain-type soybeans, edamame production practices are far from being completely understood and standardized. Williams (2015a), reported a lower germination and emergence of vegetable soybeans compared to grain-type soybeans, using cultivation practices of grain-type soybeans.

Edamame trials have been conducted in different states over the last 10 years (Duppong and Hatterman-Valenti, 2005; Sánchez et al., 2005; Hunsberger et al., 2007; Williams, 2015b). These field trials have been conducted using the standard grain-type soybean planting times, densities, and irrigation practices. The main difference has been the use of herbicides and pesticides since the majority of chemical products registered for soybeans haven’t been approved or registered

for use in edamame (Williams, 2015b). Also, the varieties used for these trials were mainly grain-type soybeans with few edamame varieties. Currently, only Arkansas has a commercial edamame production and processing facility. One of the main reasons farmers are not growing edamame is the lack of varieties that perform well in their zone, since most varieties have been developed in Asia and few breeding trials have taken place in the United States.

Seed composition

According to Mozzoni et al. (2009), both vegetable soybean and grain-type soybean have an average of 42 % protein and 20 % oil content. Edamame has lower trypsin-inhibitor activity than grain-type soybeans, probably due to harvesting time (R6). Early harvest (i.e.R6, when seeds have fully developed) also results in low indigestible oligosaccharides and higher vitamin content. Gupta et al. (1976) explains that harvesting at the R6 results in higher phytic acid level, which is correlated with tenderness of edamame (Shirtleff and Aoyagi, 2009). The nutritional facts of edamame, based on a serving size of 155 g (one cup), are 189 calories, 8 g of total fat, 0 g of trans-fat, 0 g of cholesterol, 16 g of carbohydrates, 8 g of dietary fiber, 3 g of sugar, and 17 g of protein (Kelley and Sánchez, 2005; Obatolu and Osho, 2006; Wildman, 2006; Shurtleff and Aoyagi, 2009; Carson, 2010).

Protein

Soybean is considered to be a high-quality protein source because it has a digestibility-correlated amino acid score close to one (i.e. one being the best score) (Rao et al., 1998; Stobaugh, 2011). Consequently, soybeans are used extensively as a protein source in animal feed for the production of pork, cattle, and poultry. Soybeans are also used as part of human diet in several supplements, soy protein concentrates, and protein isolates. Soybean protein contains all essential amino acids for humans, namely cysteine, histidine, leucine, isoleucine, lysine,

methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine; yet soybean proteins are low in sulfur-containing amino acids (methionine, cysteine, and threonine) (Wildman, 2006). Soybean protein content has been studied extensively and 146 quantitative trait loci (QTL) have been reported in SoyBase (the USDA, ARS Soybean Genetics and Genomics Database, 2016). These QTL are found mainly in chromosomes 4, 7, 9, 10, 15, 18, and 20. Protein content ranges from 34 - 57 % on a dry weight basis (Mozzoni et al., 2009; Bilyeu et al., 2010). Protein as reported by Zhang et al. (2009) has the highest diversity index (3.47) when compared to seed weight and oil content (3.39 and 2.61 respectively). Protein content has continuously been reported to have a negative correlation with oil content making it harder to breed for lines with high protein and high oil content, which are desired by the industry (Panthee et al., 2005; Carson, 2010; Pathan et al., 2013). In contrast to the strong negative protein-oil association, Eskandari et al. (2013) identified a QTL on chromosome 9 that enhances oil content and is positively correlated with protein content, similarly Hwang et al. (2014) detected seven SNP (single nucleotide polymorphisms) associated with increased protein and oil content in chromosomes 8, 9, and 20. In addition, heritability for protein has been estimated to range from 53 to 83 % on a per entry basis (Panthee et al., 2005; Pathan et al., 2013; Sato et al., 2014).

Carbohydrates

Carbohydrates, the second most abundant component in soybean seeds, are also called saccharides. These are classified as simple (monosaccharides) or complex (disaccharides, oligosaccharides, and polysaccharides). Total sugar content is of interest for the edamame industry, since it confers sweetness and general acceptance of the product. Total sugar content of vegetable soybeans is comparable to grain soybeans. Although Shanmugasundaram (1991) explains that carbohydrate patterns of vegetable soybean are different from those of grain

soybean. His study mainly referred to starch content, which makes up to 10 % of dry weight in vegetable soybean compared to <1 % of dry weight in grain soybean. Total sugar reported was 110.20 mg/g dry weight in vegetable soybeans, while in grain soybeans it was 102.4 mg/g dry weight. Sucrose content differed from 95.14 mg/g dry weight in vegetable soybean to 62.05 mg/g dry weight in grain soybean. These differences are a result of seed development (Min Kuo et al., 1997). Sucrose content changes as the seed develops. However, Mozzoni et al. (2009) demonstrated a moderate positive correlation of sucrose content on seeds harvested at R6 and R8 stages supporting the hypothesis of indirect selection of high-sucrose lines at R8 stage.

Sucrose, of the total sugar profile, is the primary carbon source translocated from leaf tissues to the growing soybean embryos. Sucrose peak is found at R6.2 in grain-type soybeans (Min Kuo et al., 1997). Also, sucrose is a predominant soluble saccharide in soybean seeds (Clevinger, 2006). Moreover, sucrose heritability ranges from 73 - 93 % on a per entry basis (Zeng et al., 2014; Maughan et al., 2000; Yang et al., 2013). In SoyBase a total of 34 QTL for sucrose content have been documented on chromosomes 5, 8, 12, 16, and 19.

Seed weight

Seed weight, also referred to as 100 seed weight, is key for selecting edamame lines. The main differences between edamame and grain-type soybean are seed weight, flavor, and harvesting time. Seed weight is crucial for selecting a desirable variety of edamame as industry looks for 30 g per 100 seeds (Born, 2006; Shirtleff and Aoyagi, 2009). A negative correlation between seed weight and protein was reported by Geater and Fehr (2000), who also reported that seed weight affects the correlation between sugar and oil. In their study, small seeded genotypes decreased the correlation ratio between sugars and oil.

Heritability for seed weight has been reported to range from 50 - 94 % (Hoeck et al., 2003; Huhn, 2003; Bilyeu et al., 2010). Moreover, 230 reported seed weight QTL are distributed across the 20 chromosomes and most of them are found on chromosomes 3, 6, 8, 11, 18, and 19. Unfortunately, most of these QTL have not been validated by other studies or populations.

Variety development

Development of new edamame varieties can be achieved by combining direct selection and marker assisted selection (MAS). Direct selection is the most common and traditional method for selecting new varieties and is based on the performance of lines under certain conditions. Traditional breeding is time consuming and labor intense, since it relies solely on phenotypic characteristics. On the other hand, MAS is a tool that enables breeders to predict the performance of a line without having to grow it. Breeders use molecular markers to verify the presence of a known gene of interest, therefore allowing breeders to select and discard material early in the breeding cycle. MAS is practical and convenient particularly for traits controlled by a single gene. Unfortunately most of the economically important traits are controlled by multiple genes (quantitative traits) which makes marker identification more complex. Nevertheless marker assisted selection maintains its relevance and usefulness.

Molecular markers are DNA sequences in close proximity (i.e. linked) with a gene of interest and therefore predict the performance of a line- in regards to a specific gene. MAS is cost and time-effective when used as aid to pyramid genes or to select backcrosses. Also, as a selection tool for traits which phenotyping is time-consuming and expensive (Mozzoni, 2008). One successful example of MAS is found in wheat, where the screening for resistance to Fusarium head blight in greenhouse conditions is usually inconsistent and not cost effective, but the use of *Fhb1* QTL (after multiple validations) allows now marker-based selection among F_2 plants or F_3

families (Bernardo, 2008). Similarly in soybeans, for soybean cyst nematode, time and resources are saved by using markers to aid selections instead of having to perform multiple phenotypic analysis (Concibido et al., 2004).

To identify these molecular markers, researchers conduct studies to first detect quantitative trait loci (QTL). As explained by Collard et al. (2005), QTL analysis is based on the principle of association between genotype and phenotype of populations developed from extreme phenotypes. After identifying these QTL-linked markers and validating them in different populations, markers can be used for assisting breeder selections (Bernardo, 2008).

Linkage mapping has been the most used method for QTL detection, based on the use of one or multiple mapping populations, developed from contrasting parents (i.e. high-oil content x low-oil content) to obtain various contrasting genotypes and polymorphic markers. Linkage mapping (LM) has played a key role on understanding the basic genetic concepts of quantitative traits. However LM usually detects QTL with 10 to 20 cM intervals due to the limited recombination. Large intervals are a result of few opportunities for recombination and the fact that only two parents are usually present on these populations (Gupta et al., 2005a). Another factor that limits the potential outcome of linkage mapping is the need of a large progeny, which sometimes is not achieved due to poor seed development or lack of resources to grow and maintain large populations.

Association mapping

As an alternative and complement to linkage mapping, association mapping (AM) presents a different approach to detect QTL and molecular markers. AM measures the correlation between genotypic and phenotypic variation based on the strength of linkage disequilibrium (LD) across a broad set of germplasm (Gupta et al., 2005b; Zhu et al., 2008). Flint-Garcia et al. (2003) define

linkage disequilibrium (LD) as the nonrandom association of alleles at different loci, or the chances of finding one allele at one locus that is not independent of an allele at a different locus. Linkage disequilibrium is affected by allele frequency, recombination between sites, selection, population matting patterns, and admixture. Generally LD increases on self-pollinating species like soybeans. In cultivated *G. max* groups, LD can extend from 90 to 574 kb, a high LD when compared to autogamous specie *Arabidopsis thaliana* that has LD extending up to 50 kb (Hyten et al., 2007).

Linkage analysis is used to measure the genetic proximity of loci to each other and to map QTL (Flint-Garcia et al., 2003). AM has enabled the identification of novel disease loci in humans that were previously unknown (Flint-Garcia et al., 2003; Soto-Cerda and Cloutier, 2012a). As a result of the cost reduction of genotyping, this approach is now feasible in plant and animal studies. Furthermore, association mapping offers advantages, such as increase of mapping resolution, reduced research time, and greater allele numbers. Thus, providing detailed marker data in a large number of lines which could be of practical application in breeding (Flint-Garcia et al., 2003; Soto-Cerda and Cloutier, 2012a). AM has the potential to identify casual polymorphisms within a gene that are responsible for the difference between two phenotypes (Soto-Cerda and Cloutier, 2012a).

In contrast with linkage mapping, association mapping uses a broad set of germplasm hence resolving complex trait variation by exploiting historical and evolutionary recombination events at the population level (Zhu et al., 2008). As stated previously, due to a high genetic diversity, AM offers three main advantages over linkage mapping: mapping resolution, allele number, and time saving (i.e. compared with time required to develop a F_2 derived population used for linkage mapping). Mapping resolution tends to be higher as a result of the multiple

recombination events that have occurred overtime and have broken the linkage between loci. AM includes a greater allele number since it is not limited to two parents but rather includes a multiple set of parents. AM studies include landraces, public cultivars, and accessions from different breeding programs (Flint-Garcia et al., 2003).

Association mapping of different traits has been conducted in rice, wheat, sorghum, soybean, potato, tomato, common bean, and lettuce (Breseghello and Sorrells, 2006; Agrama et al., 2007; Björn et al., 2008; Casa et al., 2008; Jun et al., 2008; Wang et al., 2008; Zhu et al., 2008; Wen et al., 2009; Upadhyaya et al., 2012). In soybeans, association mapping has focused on iron deficiency chlorosis, aluminum tolerance, biotic stresses (sudden death syndrome, cyst nematode, and sclerotina stem rot) seed size and shape, and protein content. Mostly grain-type soybeans have been studied and only few researchers have included food-grade soybeans (Wang et al., 2008; Shi et al., 2010; Hwang et al., 2014). Similar to other analysis, association mapping has its own limitations. As discussed by Soto-Cerda and Cloutier (2012b), some of AM limitations are population structure, which affects LD distribution, and the constant need for innovative statistical models to better allocate the environmental effect.

Identifying genetic diversity of edamame will enable breeders to select parental material and potentially increase the number of edamame varieties available. According to Sammour (2011), the study of genetic diversity is valuable for efficient utilization, conservation, and management of germplasm collections. Mimura et al. (2007) studied the genetic diversity of 131 edamame accessions and described that genetic diversity was clustered around maturity groups and testa color. In the same study, Japanese accessions were less diverse when compared to Chinese accessions, suggesting they may have different genetic pools. Sammour (2011) analyzed the genetic diversity among and within Asian and North American soybean cultivars using AFLP.

In edamame, two studies have taken the AM approach using both vegetable soybean and food grade soybeans. Food-grade soybeans include varieties suitable for natto, soymilk, and tofu production. Hou et al. (2011) used 323 accessions representing the diversity of germplasm available in China. They focused on pod and seed fresh weight, sucrose, and free amino acid content. Using a set of 101 SSR they detected 79 associations among all four traits, mainly in chromosomes 5 and 2 validating previously reported QTL of grain-type soybeans (Zhang et al., 2008). Shi et al. (2010) conducted an AM study using food-grade soybeans which included edamame. In this study a total of 105 genotypes and 65 SSR were selected to perform the analysis for protein and oil content, resulting in 12 markers associated with both protein and oil, from which four were new in chromosomes 10, 11, and 13.

Due to the continuous interest in edamame, varieties adapted to different environments and selected to satisfy consumer demands are needed. Moreover the type of consumers and their preferences are changing and are affected by cultural influences, resulting in a continuous search for varieties. As stated by Kelley and Sánchez (2005), U.S. consumers preferred a buttery flavor and texture, in contrast to Japanese consumers who are known to prefer a flower-like flavor and texture. Understanding consumers and the diversity of germplasm will enable breeders to better select the parental material for breeding programs. Genetic information about the traits (phenes) will help researchers and breeders to more efficiently select towards the phenes of interest. Recently, research on edamame has started to focus on variety development including seed quality, diversity analysis, production improvement, consuming exploration, and market evaluation (Zhang et al., 2010). As evidence of the potential of edamame and interest that is arising Krinsky et al. (2006), developed a new lexicon consisting of 14 terms to reference the sensory language for frozen vegetable soybean.

As a result of QTL discovery in grain-type soybeans, we now have a better understanding of the form in which genes are inherited as well as the relationship between genes and alleles. Thereby molecular markers are an important tool for breeders when selecting parental material and designing crosses. As stated before, there is an increasing demand of faster and more efficient breeding methodologies but also lack of genotypic information in vegetable soybean. In other words, there is a need for information that will enable breeders to use marker assisted selection on their vegetable breeding programs and therefore develop edamame varieties that can serve market needs.

Food processing

Food processing is any deliberate change in a food that occurs before it is available for consumers to eat. Food processing is defined as "a variety of operations by which raw foodstuffs are made suitable for consumption, cooking, or storage (European Food Information Council, 2010). For centuries humans have processed foods to obtain different flavors, texture, increase nutrient availability, and to preserve foods for longer periods of time. Several technologies have been used including cooking, drying, freezing, salting, fermenting, and blanching.

Blanching

Blanching is a heat treatment utilized to destroy enzymatic activity. Blanching is widely used in fruits and vegetables before they are frozen or dehydrated. Most vegetables, except for onions and green peppers, require blanching to prevent them from suffering considerable loss in quality and sensory characteristics (Fellows, 2009). In general terms, blanching consists of heating the food rapidly to a set temperature for a certain length of time and then cooled rapidly to either ambient or freezing temperature. The two most common commercial methods for blanching use hot water or water steam.

Water blanching uses water at temperatures ranging from 70 °C to 100 °C where raw food is immersed for a pre-determined period of time. Combinations of low-temperature/ long-duration and high-temperature/ short-duration have been studied and standardized on most vegetables (Suwan, 2015). The most common water blanchers consist of a chain conveyor to transport the food to a hot water tank for a certain time. Others use a rotary drum to immerse the product (De Corcuera et al., 2004; Suwan, 2015). For water blanching, water must be food- grade and is usually heated using steam in a heat exchanger. Steam in this case is not required to be food-grade, since it is never in direct contact with water. The use of hot water tends to result in uniform heating but it can lead to leaching of minerals and nutrients, and produces effluents with large biological oxygen demand (De Corcuera et al., 2004). Moreover, water can be recycled without affecting the product quality and yield, but it reduces the volume of effluent produced. If water is recycled, it is mandatory to ensure appropriate hygienic standards for the recycled water, the product, and the equipment, to avoid a possible build-up of bacteria at any stage (Fellows, 2009a).

Steam blanching consists of a conveyor chain or belt that transports food through a chamber where the atmosphere is saturated with food-grade water steam. As summarized by Fellows (2009b), steam blanching results in higher nutrient retention when the cooling stage includes either cold air or cold-water sprays. Though air-cooling causes weight loss of the product reducing total yield. An alternative cooling stage involves the use of cold running water, which counteracts the weight loss but increases leaching losses. Another limitation on the use of steam blanchers is the limited cleanse of foods, thus requiring additional washing. However, steam blanchers tend to have a better energy efficiency when compared to hot water blanchers (De Corcuera et al., 2004; Fellows, 2009a).

New blanching procedures, methods, and technologies have been studied by different industries (Fellows, 2009b; Mujumdar and Law, 2010; Pan and Atungulu, 2011; Chandrasekaran et al., 2013). For instance, microwave heating has various applications in the food industry including blanching, pasteurization, tempering, drying, and baking. Microwave heating is based on the capacity of materials to absorb energy carried by microwaves and convert it into heat. As explained by Chandrasekaran et al. (2013), microwave heating occurs mainly because of ionic and dipolar mechanisms. Ionic mechanism occurs when ions migrate within the food, generating heat. On the other hand, dipolar mechanism takes advantage of the dipolar nature of water molecules present in foods, which after being polarized try to realign in accordance with the electric field creating heat with the movement. This technology was studied by Schirack and Sandeep (2007) as an alternative blanching method for peanuts. Their study focused on the effect of different temperatures and exposure times on the physical and sensory characteristics of peanuts. Microwave blanching presents some advantages over conventional blanchers such as reduced energy costs due to a faster heating, reduced processing time, decreased nutrient losses and uniform heating (Fellows, 2009a). In contrast, microwave heating equipment is more expensive than conventional blanchers, making its use limited to high-value products in the food industry (De Corcuera et al., 2004). The usage of microwave heating is now a commercial practice for blanching mushrooms, effectively inactivating polyphenoloxidase and preventing browning. Though this technology is commercially used in Europe and Japan, its use is still limited in the United States (Fellows, 2009).

Infrared heating technology

Another novel technology that started to gain interest across the food industry is the use of infrared wavelengths to heat food products thereby improving quality and safety. Infrared (IR)

technology has higher energy efficiency, shorter drying time, and better product quality when compared with convective drying methods (Kocabiyik, 2010). As part of the electromagnetic spectrum IR has the capacity to provide high heating and heat transfer rates (Pan and Atungulu, 2010). When IR radiation impinges a food surface, IR energy is absorbed at discrete frequencies -corresponding to the nature of the chemical bonds present in the food- creating heat. Wavelengths of IR fall in the spectrum of 76-1000 μm and are categorized as near infrared (NIR) (0.76-2 μm), medium infrared (MIR) (2-4 μm), and far infrared (FIR) (4-1000 μm). High temperatures associated with NIR can negatively impact color and food quality. Contrarily, low temperatures of FIR may not be enough to achieve the goals of dehydration. In addition, the absorption properties of foodstuffs depend mainly on three factors: water content, physicochemical nature, and thickness of the product (Kocabiyik, 2010).

IR technology has been studied by Pan et al. (2008) and Zhu et al. (2010) to develop a new approach using IR radiation to achieve dry blanching and dehydration at the same time on fruits and vegetables. This approach is intended to replace current blanching methods to produce value-added products such as freeze-dried, frozen, dehydrated, and dried products. The advantages of simultaneous infrared blanching and dehydration as reported by Pan and Atungulu (2011), include uniform heating, which enhances energy efficiency. IR has the capacity of zone heating, and equipment is versatile in terms of the amount of product that can be processed. A typical IR heating unit consists of a variable speed conveyor belt and a heating compartment with the IR emitters (powered with natural gas). IR heating intensity can be modified by either adjusting the gas supply or the product-to-emitter gap size.

Physicochemical characteristics affected by blanching

Blanching, modifies the attributes of any food product. Flavor, texture, color, and nutritional contents are commonly affected. Flavor is altered due to deactivation of enzymes associated with off-flavors, such as lipoxygenase and peroxidase. Texture after blanching results usually in a softer product, and if not done properly softening can reach an undesirable point of "sogginess". Color tends to be affected negatively because soluble compounds tend to leach. Contrarily in some vegetables (i.e. green peas, corn) color intensity improves after blanching. These physicochemical characteristics have a remarkable effect on consumer-purchasing decisions.

Texture

Fellows (2009) summarizes the physical and metabolic changes that occur within food cells when they undergo blanching. First, heat modifies cell membranes which become permeable and lose cell turgor, allowing solutes and water to migrate out of the cell. Likewise, organelles in the cell and their components are altered and become free to interact with contents within the cell. In addition, when exposed to high temperatures the starch granules gelatinize and soluble pectin substances are formed, which also decrease hardness (Song et al., 2003).

Color

Color changes occur due to the leach of pigments and the chemical that these undergo. In edamame the quantities of chlorophylls *a* and *b* where studied by Song et al. (2003). Under different water blanching treatments, greenness was decreased as a result of loses on chlorophylls *a* and *b.* Suwan (2015), also studied the effect of blanching on green intensity on edamame. CaCl was used as a constituent of the blanching solution to prevent color degradation. Under those conditions green intensity peaked after blanching for 5 min, but decreased with further blanching. On the contrary Mozzoni et al. (2009) concluded that after 5 min of water

blanching, green intensity decreased. The difference might be explained by the addition of CaCl to the blanching water (Song et al., 2003). In addition to the changes in chlorophyll content, blanching removes intercellular gases, which alters the wavelength of reflected light of the food, causing an increase of brightness (Fellows, 2009b).

Enzyme activity

Blanching is intended to decrease oxidation rate and deteriorative spoilage, and to improve flavor. Lipoxygenase, an enzyme that catalyzes the bioxygenation of polyunsaturated fatty acids into hydroperoxide and free radicals (Sheu and Chen, 1991; Baysal and Demirdöven, 2007) tends to confer a "beany" or "greasy" taste on soybeans. According to Young et al. (2000) this "beany" or "greasy" taste is undesired by consumers. Lipoxygenase is associated with off-flavors like rancidity. Heat and pH are common methods to inactivate this enzyme and prevent generation of off-flavors (Bahçeci et al., 2005).

Another enzyme usually assayed to ensure blanching effectivity is peroxidase, considered to be one of the most heat stable enzymes in vegetables (Morales-Blancas et al., 2002; De Corcuera et al., 2004; Xu, 2012). Sheu and Chen (1991), studied the effect of using lipoxygenase vs peroxidase as blanching index and concluded that samples where lipoxygenase was used as blanching index had 11.5% peroxidase activity. While samples where peroxidase was used as blanching index had no remaining lipoxygenase activity. Similarly in green beans Bahçeci et al. (2005), concluded that the use of peroxidase as blanching indicator preserved better the quality of the beans. The availability of certain compounds is also enhanced after blanching, for instance trypsin inhibitors reduce digestibility of proteins (Mozzoni, 2009). Heat treatment can destroy trypsin inhibitors activity, thereby increasing protein availability.

Objectives

The first part of this research focused on the identification of genetic diversity among a group of varied vegetable soybean accessions, with maturity groups from 000 to IX and originated on six different countries. Association mapping was used to identify and validate quantitative trait loci (QTL) for seed weight, sucrose, and protein content in edamame. In addition, the heritability of these phenotypic traits was evaluated.

The second part of this research focused on developing the kinetics of physicochemical characteristics of edamame under infrared heating, thereby evaluating the effects of three different IR heating intensity levels. The three heating intensities were defined by product-to-emitter gap size (PEG). Temperature, weight reduction, texture, green intensity, and peroxidase activity were measured after exposing edamame under IR treatment.

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Chapter II.

Association Mapping of Seed Weight, Sucrose, and Protein Content in Edamame

Abstract

Edamame is a specialty large-seeded soybean (Glycine max L. Merr.) harvested when pods have filled and remain completely green. This vegetable soybean has gained popularity among western countries. In the United States annual consumption increased by 9 % in 2013. Unfortunately, the lack of adapted varieties limits edamame production in the United States. Breeders use phenotypic and genotypic tools to select and release varieties. To increase selection power, breeders use molecular markers to assist their selections. Discovery of quantitative trait loci (QTL) and marker-trait associations (MTA) will aid the selection of new edamame lines. Association mapping (AM) is an alternative to conventional linkage mapping to detect QTL and MTA. Seed weight, sucrose, and protein content are important quantitative traits for selecting edamame lines. Thus, the objective of this research is to identify and validate seed weight, sucrose, and protein QTL using food-grade soybeans. A total of 378 accessions were selected from six countries and different maturity groups for the mapping panel. The experiment was established as a randomized complete block (RCB) with two blocks per location and accessions nested within maturity group. Plots were harvested at R8 and phenotypic data was used to carry out association analysis using information from 5064 single nucleotide polymorphism markers (SNP) (minor allele frequency > 5%). In order to reduce false positives, five models were performed in TASSEL, GAPIT, and FarmCPU. A total of 16 SNPs on ten chromosomes were found to be significantly associated across models (-log P > 2.5) with seed weight. These results confirm 11 previously reported QTL and identify three QTL regions on Gm04, Gm16, and Gm19 for seed weight. A significant association with sucrose content was detected for 13 SNPs. The majority were located on Gm08 identifying three novel sucrose QTL and validating three previously reported QTL on Gm14, Gm15, and Gm16. For seed protein content six SNPs were

found to be significant on Gm04, Gm05, Gm11, and Gm20, all confirming previously reported QTL regions. These SNPs linked with seed weight, sucrose, or protein content are potential markers for marker assisted selection in edamame breeding programs.

Introduction

Edamame, the Japanese term for soybeans harvested at an immature stage (R6), is gaining popularity among western cultures. SoyFoods (2015) reported that edamame is the second largest consumed soyfood after soymilk. Popularity of this vegetable soybean has increased partially due to nutritional value, but also because of the shift from meat to plant-based diets. Edamame is a good protein source as it contains 34 to 48 % protein and provides all essential amino acids (Wilson, 2004).

Distinguishing features of edamame include large pods and seed size, green pods without blemishes, smooth texture, two or three beans per pod, and light pubescence (Mebrahtu, 2008). Seed weight is one of the most prominent features of edamame. The desired weight is 30 g per 100 seeds, almost twice the weight of an average grain-type soybean (16-18 g per 100 seeds). In addition, sucrose has been reported to be highly associated with consumer preference. Konovsky et al. (1994), suggested that sweet and savory are the two most important components of edamame flavor. Selection for sucrose content is based on market preferences as described by Krinsky et al. (2006), who developed a lexicon for frozen edamame. Though the United States is the largest soybean producer around the world (USDA, 2016), the majority of edamame consumed in the country is imported from China. This as a consequence of the lack of adapted material for edamame production and lack of information about harvesting time or cultivation practices (Mebrahtu, 2008; Mozzoni et al., 2009; Sato et al., 2014).

Varieties can be developed by combining direct selection with marker assisted selection. Direct selection is the most traditional method for breeding, in which lines are tested across different environments and continuously selected based on their performance. Marker assisted selection (MAS) has the potential of aiding breeders to select and discard material early in the breeding

scheme, which can save resources and increase efficiency. Although MAS has been successful mainly for qualitative traits (controlled by one gene), dissecting the genetic basis of quantitative traits is helpful to select and implement MAS.

Genome wide association studies are an alternative to bi-parental linkage methods to detect quantitative trait loci (QTL). Although both of these methodologies are based on the correlation between phenotypic expression of a trait of interest and DNA markers, association mapping uses a diverse set of germplasm for the analysis. Thus, association mapping tends to increase mapping resolution in terms of positioning a QTL closer to the gene of interest. As addressed by Hwang et al. (2014), the main difference between a biparental study and association mapping is the level of linkage disequilibrium (LD). LD is defined as the non-random association of alleles at different loci and is affected by recombination, inbreeding, and selection. Detection of molecular markers is influenced by the level of LD and population structure.

Seed weight, sucrose, and protein content are relevant traits for selecting edamame varieties. These traits are quantitatively inherited and several QTLs have been reported on SoyBase for grain-type soybeans. Multiple marker validation is required to adopt MAS in a breeding program. Thereby this research aims to identify and validate single nucleotide polymorphic (SNP) markers associated with seed weight, protein, and sucrose content in edamame.

Materials and methods

Association mapping panel

A total of 378 accessions were evaluated for seed weight, protein, and sucrose content. These accessions were selected if they had previously been reported as edamame or based on seed weight. Each of the accessions was reported to weigh more than 23 g per 100 seeds on the Germplasm Resources Information Network (GRIN*)* (USDA, 2014) database. From these 378 accessions, 347 were requested from the GRIN and 31 were selected from the University of Arkansas soybean breeding program. This association panel includes advanced breeding lines, landraces, and cultivars. The origin of these accessions is diverse, specifically 47 % from Japan, 37 % from South Korea, 6 % from the Arkansas soybean breeding program, 5 % from China, 2 % from the United States, 1 % from Sweden, 1 % from North Korea, , and 1 % with unknown origin.

All 378 accessions were sent to Costa Rica in February 2014 for seed increase. Accessions were planted in 3 m rows and harvested at maturity (R8). Field experiments were conducted during the 2014 and 2015 cropping seasons at the Rice Research and Extension Center in Stuttgart, AR and the Arkansas Agricultural Research and Extension Center in Fayetteville, AR. Soils in Stuttgart are characterized as silt loam with a silty clay subsoil and in Fayetteville soils are fine sandy loam, with a sandy clay loam subsoil (NRCS, 2016).

Each genotype was planted in a 3 m row. Standard cultural practices were applied throughout both cropping seasons at both locations. The field design was a randomized complete block (RCB) with two blocks per location. Accessions were nested within maturity group. Plots were harvested at maturity and seeds were evaluated for seed weight, sugar, and protein content. Though edamame is harvested at an immature stage (R6) for consumption, it has to be harvested

at maturity (R8) for seed production. Mozzoni (2009), reported a positive correlation between R6 and R8 for protein, iron, texture, sucrose, and calcium content. Therefore evaluating seed quality at R8 should not differ from evaluating it at R6.

Soluble sugars extraction and quantification

Sample preparation was performed using a modified version of Valliyodan et al. (2015). Briefly, a 15 - 20 g seed sample was ground in a coffee grinder. Subsequently, powder was sifted through a 100 µm sieve. Fine powder was lyophilized for 48 hours in a Botanique model 18DX 488A freeze drier (Edwards, Ontario, Canada). A sample of 80.2 (± 0.2) mg were weighed and sent to the Soybean Biotechnology research laboratory at the University of Missouri, Columbia, MO for sugar extraction and analysis. Dry soybean powder was mixed with 900 µL HPLC-grade water in 2 mL centrifuge vials. Vials were then incubated at 55 °C with 250 rpm agitation for 30 min followed by a 30 s high speed vortex. After samples had cooled to room temperature, 900 µL HPLC acetonitrile were added. Suspension was centrifuged for 30 min at 13.3 g × 1000 min $^{-1}$. Supernatant was further diluted five times with acetonitrile: water (65: 35 v/ v) mixture before subjecting the sample to High-Performance Liquid Chromatography (HPLC) analysis.

Seed protein and seed weight determination

Seed protein content was measured using percent total nitrogen, converted to crude protein using the factor of 6.25 (Jones, 1931). Nitrogen was extracted and quantified by combustion using an Elementar rapid N III instrument (Elementar Analysensysteme, Hanau, Germany). Samples were then ground and sifted through a 100 µm mesh. For the analysis 100 - 150 mg were weighed. Samples were analyzed in the Agricultural Diagnostic Laboratory, Fayetteville, AR.

Seed weight was measured by collecting a random sample of 100 seeds per plot and weighing them using a precise scale, the measurement units are g per 100 seeds.

Genotyping

A total of 318 accessions (out of the 342 selected from the GRIN database) were previously genotyped with the SoySNP50K iSelectBeadChip, which consists of 52,041 single nucleotide polymorphisms (SNPs) (Song et al., 2015). The remaining 60 accessions (out of the entire panel, 378 accessions) were planted in a greenhouse located at the Arkansas Agricultural Research and Extension Center in Fayetteville. Leaf tissue was collected from the upper trifoliate and stored at -80 °C, until DNA was extracted following a modified CTAB (cetyltrimethylammonium bromide) method (Doyle, 1991). Frozen leaves were crushed using a mortar. Tissue was placed in a 2 mL tube and 750 µL extraction buffer (2 % CTAB, 100 mM Tris-Cl, 20 mM EDTA pH 8.0, 1.4 M NaCl, and 1 % of volume β-mercaptoethanol) were added. Tubes were incubated at 65 °C for one hour, and cooled to room temperature. Subsequently, 1 mL of chloroform: isoamyl alcohol (24: 1) was added to each tube and centrifuged at 12000 rpm for 15 min at room temperature. The supernatant was transferred to a new tube and 1 mL of 95 % ethanol was added for DNA precipitation. Tubes were centrifuged at 12000 rpm for 5 min and DNA pellets were washed with 75 % ethanol. DNA was dissolved in 200 µL of water (sterilized and distilled). Finally DNA concentration was measured using a NanoDrop™ ND-2000 (Thermo Fisher Scientific, Waltham, MA). Genotyping was performed at the University of Minnesota Genomics Center using the soybean 6K SNP chip.

Statistical analysis

Seed weight, sucrose, and protein content were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc. 2011, Cary, NC). Least square means (LS means) were calculated for each accession and trait with genotype and maturity group considered as fixed effects with accessions nested within maturity group. Year, location, block, and interactions were considered as random

effects. To account for differences between years and locations, BLUPs (best linear unbiased predictor) were also calculated, using accessions as random effect. Pearson's correlation using PROC CORR in SAS was performed to determine correlations between traits. To estimate heritability of each trait, all effects including accessions were considered as random and the covariance parameter estimates were used. Narrow sense heritability on the entry mean basis of seed weight, sucrose, and protein was defined as:

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{\mathrm{GL}}^2}{l} + \frac{\sigma_{\mathrm{GY}}^2}{y} + \frac{\sigma_{\mathrm{GLY}}^2}{ly} + \frac{\sigma^2}{lyr}}$$

Where σ_G^2 is the genetic variance among accessions, σ_{GL}^2 is the variance among locations, σ_{GY}^2 is the variance among years, $\sigma_{\mathrm{G}LY}^2$ is the variance among year × location, and σ^2 is the residual variance.

Association mapping analysis

Though 318 accessions had been genotyped with a 50K SNP Chip, only the common information from the 6K SNP was used for the analysis. From 6000 SNP markers, 5064 were polymorphic and had a minor allele frequency > 5 % with less than 15 % missing data. Selected markers (5064) were used for estimation of population structure using Structure 2.3.4 Software (Pritchard et al., 2000). To obtain reliable estimates, 10 iterations, a burnin period of 10000, and 25000 MCMC (Markov Chain Monte Carlo, Bayesian approach) repetitions after burnin period were used as parameters. To infer the number of clusters, K, that best fit the data Structure Harvester was used. The matrix of population structure was selected at K=8, to achieve the minimum standard error. Another population parameter used as a covariate was the kinship coefficient matrix, estimated through TASSEL Software (Bradbury et al., 2007).

There were five approaches for identification of marker-trait associations (MTA). The mixed linear model (MLM) from TASSEL (Bradbury et al., 2007), which incorporates kinship and population structure in a fixed and random effect mixed linear model. Compressed mixed linear model (CMLM) implemented in the Genomic Association and Prediction Integrated Tool (GAPIT) R package (Zhang et al., 2010), was also used, the model clusters individuals into groups and fits genetic values of groups as random effects. FarmCPU (Fixed and random model Circulating Probability Unification) (Liu, 2015) performs marker tests with associated markers as covariates in a fixed effect model and optimization. Using FarmCPU maximum bin selection and multiple iteration were also included. Only markers that were significant ($-\log P > 2.5$) in at least two models were considered to be MTAs of interest, though regions had to be significant across all models.

Linkage disequilibrium and population structure

Principal component analysis found the first three principal components (PC) to explain 24 % of genotypic variance. Therefore PCs were used as covariates in the association analysis. Plots of the PCs identify population stratification (Figure 1). Linkage disequilibrium (LD) showed rapid decay as genetic distance increased (Figure 2). Population structure matrix was based on $K = 8$, based on the delta K plot (Figures 3 and 4).

Results

From the analysis of variance, accessions nested within maturity group (MG) and the interaction among year × locations × accessions (MG) were significant ($P < 0.0001$) for all traits. For seed weight and sucrose the correlation coefficient was 0.89 across environments, indicating accessions performed similarly across environments. For protein content, the correlation among environments was 0.38, thereby BLUPs were calculated for each year (2014 and 2015). Heritability was estimated on entry mean basis. Seed weight presented the highest heritability (0.90). Heritability for sucrose and protein content was 0.74 and 0.63 respectively.

Correlation coefficients ranged from -0.46 to 0.29 with the greatest correlation observed between protein and sucrose. The correlation coefficient between seed weight and seed sucrose was 0.29 ($P < 0.0001$) across environments, while it was 0.14 ($P = 0.0052$) between seed weight and protein content. For protein and sucrose content, the correlation coefficient was -0.46 ($P < 0.0001$) (Table 1). Seed weight ranged from 8.99 to 40.51 g per 100 seeds across environments. Sucrose content ranged from 2.5 to 9.5% while protein content ranged from 36.4 to 51.8 %.

BLUPs of each accession were used for the marker-trait association analysis. Only markers with LOD (-log P) >2.5 across models were reported. A total of 16 marker-trait associations (MTA) were detected for seed weight (Table 2). These associations appeared on chromosomes Gm04, Gm05, Gm06, Gm10, Gm11, Gm16, Gm17, Gm18, Gm19, and Gm20. For sucrose, 13 MTAs were identified, occurring on chromosomes Gm04, Gm06, Gm08, Gm14, Gm15, Gm16, and Gm17 (Table 3). Furthermore, six MTAs for protein were detected on four chromosomes (Table 4). Overall, detected individual SNPs explained a small percentage of the phenotypic variance with R^2 ranging from 0.01- 0.07.

Discussion

Linkage disequilibrium and population structure

The delta K plot suggests accessions were clustered in eight groups. Clustered appeared both by country and maturity group. Maturity group had a greater effect on clustering accessions, probably because breeders have focused on specific maturity groups. The importance of including population structure as part of the association analysis is discussed by Pritchard et al. (2000) and supported by Earl (2012) among others who report the likelihood of false marker-trait association when population structure is not accounted as a covariate in the association model. For this reason, the Q matrix was used as a covariate on the mixed linear model (MLM). Linkage disequilibrium declined rapidly to 0.5 at 4.5 Mbp (Figure 2), which is in agreement with the LD decay previously reported by Zhang et al. (2016).

Phenotypic data

Heritability of seed weight was 90 % in agreement with previous studies, as it typically ranges from 50 to 94 % (Hoeck et al., 2003; Huhn, 2003; Kato et al., 2014). On the GWAS conducted by Zhang et al. (2016) heritability of seed weight was 97 %. For sucrose content heritability on a per entry basis was 74 % consistent with previously reported, being 73 to 93 % (Maughan et al., 2000; Yang et al., 2013; Zeng et al., 2014). Likewise, for protein content heritability was 63 % in correspondence with previous research that reported heritability ranges from 53 to 83 % (Panthee et al., 2005; Pathan et al., 2013; Sato et al., 2014). The analysis of variance for seed weight, sucrose, and protein revealed accessions were significantly different ($P < 0.0001$). Although the interaction year x location x accession (mg) was significant for all traits, correlation among environments was high ($R^2 = 0.89$) for seed weight and sucrose, but was low for protein content ($R^2 = 0.38$). For seed weight and protein BLUPs across years were used for the association

analysis. For protein BLUPs by year and across years were used. Protein content was also found to be negatively correlated with sucrose content, in agreement with Hymowitz et al. (1971), who reported sucrose and oil content tend to be negatively correlated with protein content.

Association analysis for seed weight, sucrose and protein content

Seed weight is a relevant selection component for edamame lines and due to diversity of the 378 accessions, a total of 16 marker-trait associations (MTA) were found to be significant with a LOD (-log P) value higher than 2.5. These MTAs were distributed across 10 chromosomes. On Gm04 two markers were located at the 36-36.5 Mbp region. Conversely in a GWAS performed by Zhang et al. (2016) two markers were associated with seed weight at 42 Mbp. On chromosome five, one marker was consistent among association models at 40 Mbp. Zhang et al. (2004) describe the presence of a QTL on Gm05 located at 30Mbp. Though the QTL reported by Zhang et al. (2004) is 10 Mbp away from the marker identified by our panel, differences could be due to the type of markers used (SSR vs SNP) and the use of a biparental population compared with the association mapping approach. Moreover this region has also been reported by Han et al. (2012a).

The seed weight MTA identified on Gm06 confirms a previously reported QTL by Rossi et al. (2013). Furthermore, two MTAs were identified on Gm10 located on the 45-46 Mbp region. These validate two QTLs reported by Han et al. (2012b) and Zhang et al. (2016). Also, on chromosome 11 the MTA positioned at 36 Mbp has been reported previously (Han et al., 2012a; Zhang et al., 2016). Likewise, two MTAs on Gm16 located at 29 and 5.9 Mbp respectively confirm the existence of a QTL at 27 Mbp reported by Kim et al. (2010) and Han et al. (2012a). Yet no QTL has been reported at 5.9 Mbp. In addition, on Gm17 one marker was found to affirm the previously reported QTL at 10 Mbp by Zhou et al. (2015) and Kim et al. (2010). Two regions

on Gm18 present significant MTAs, one at 5.3 Mbp, in agreement with Mian et al. (1996). The other MTA is located at 6.1 Mbp which validates a QTL reported by Zhang et al. (2016). Conversely on Gm19 two MTAs at 43 and 45 Mbp where identified to differ from the results of Zhang et al. (2016) in which MTAs were located at 41 - 42 Mbp. However on Gm19 Mian et al. (1996) reported a QTL at the 46 Mbp, suggesting this region could contain several minor QTLs. Lastly, on Gm20 the two MTAs were located at 26 Mbp validating previously reported QTL (Han et al., 2012a).

For sucrose content, a total of 13 MTAs were identified on seven different chromosomes. Though four were clustered on Gm08, distributed one at 40 Mbp, two at 41 Mbp, and one at 46 Mbp. Previous studies report three QTL on Gm08, but locate them at 8 – 20 Mbp (Maughan et al., 2000; Kim et al., 2005a). Previous studies used recombinant inbred lines and SSR for their analysis. This region on Gm08 (40 – 46 Mbp) indicates a putative novel QTL for sucrose. In addition, two MTAs on Gm04 at 36 Mbp and 40 Mbp represent a new QTL. Similarly, on Gm06 two MTA detected at 7 and 20 Mbp, and two MTA located at 36 and 40 Mbp on Gm17 are novel QTL for sucrose content. On the other hand MTAs identified on Gm14, 15, and 16 confirm previously QTL detected by linkage mapping (Kim et al., 2005b; Zeng et al., 2014).

Protein content, is a major trait of interest for edamame consumers. Six MTAs for protein content were identified on six chromosomes. On Gm04 and Gm05 two MTAs confirm previously reported QTL by Lee et al. (1996). In addition, on Gm11 we confirm the QTL reported by Gai et al. (2007). On Gm20 one MTA confirms a previously reported QTL (Wang, 2013; Hwang et al., 2014).

Conclusions

A total of 35 marker-trait association (MTA) were identified as significant across models for seed weight, sucrose, and protein content. Association mapping for seed weight successfully defined 16 MTAs. From which 11 markers validate previously reported seed weight quantitative trait loci (QTL). Thus, for seed weight novel QTLs were identified on Gm04, Gm16, and Gm19.

For sucrose content, a total of 13 MTAs were detected as significant. The majority of MTAs were located on Gm08 suggesting a new QTL located at 40 – 46 Mbp. Likewise on Gm04, Gm06, and Gm17 three putative novel QTL were detected. Three MTAs validate previously reported QTLs on Gm14, Gm15, and Gm16. Similarly, a total of six MTAs for protein content were distributed on four chromosomal regions, Gm04, Gm05, Gm11, and Gm20. All of which validate previously reported QTL and provide details to further investigate and identify genes on these regions.

Moreover, this was the first study aiming to evaluate the genetic diversity for seed weight, sucrose, and protein using edamame accessions. Resulting on novel QTL, mainly on Gm04, could aid breeders on releasing varieties with larger seed size and modified sucrose content.

Table 1. Analysis of variance and heritability of seed weight, sucrose, and protein content.

		Seed weight		Sucrose		Protein	
Source	DF[Ŧ]	MS[ǂ]	Pr > F	MS[ǂ]	Pr > F	MS[ǂ]	Pr > F
Accession (MG)	366	105.67	<0.0001	3.28	<0.0001	165.26	<0.0001
MG	11	1295.48	<0.0001	92.37	<0.0001	16.92	<0.0001
Year (Yr)	1	244.41	0.4892	180.74	0.2044	1228.70	0.2857
Location (loc)	1	4634.56	0.0611	130.69	0.2668	1198.86	0.2933
Block (loc)	2	270.73	0.0199	1.98	0.6429	4.89	0.6945
Yr × loc	1	229.88	0.0025	20.19	0.1281	283.58	0.0197
Block(year×loc)	2	5.48	0.1665	3.56	<0.0001	11.12	0.0004
Yr × loc× Accession (MG)	1024	7.49	<0.0001	0.66	<0.0001	4.97	<0.0001
Error	1211	3.05		0.21		1.42	
Heritability		0.90		0.74		0.63	

[Ŧ]Degrees of freedom.
[ǂ]Mean square.

Table 2. Summary of marker-trait associations for seed weight in 378 edamame accessions using 5064 SNP markers.

Models[ǂ]	Marker name	Chr.[ǂ]	Position	SNP	-log (P)[£]	Maf[ǂ]	Allele effect	R^2
5	Gm0436357346	4	36357346	C/T	3.6 - 7.9	0.05	-4.3	0.05
2	Gm0436604337	4	36604337	A/G	3.8 - 4.1	0.06	2.0	0.05
3	Gm0540517408	5	40517408	C/T	2.4 - 5.1	0.37	4.2	0.07
3	Gm0613990118	6	13990118	C/T	3.4 - 4.6	0.11	1.0	0.01
2	Gm1045332216	10	45332216	A/G	2.8 - 3.2	0.17	0.5	0.05
5	Gm1046008769	10	46008769	A/G	2.8 - 5.9	0.2	-0.9	0.02
4	Gm1135710195	11	35710195	A/G	3.0 - 4.9	0.08	-1.0	0.01
4	Gm1629181015	16	29181015	T/G	2.6 - 6.6	0.15	1.3	0.01
2	Gm165998589	16	5998589	A/G	2.9 - 3.5	0.21	0.8	0.01
2	Gm1710541830	17	10541830	G/T	2.8 - 3.1	0.20	2.2	0.05
5	Gm1861265863	18	61265863	C/T	5.1-8.0	0.12	1.5	0.01
2	Gm1853147357	18	53147357	A/C	3.6 -3.7	0.38	-0.6	0.01
3	Gm1943013222	19	43013222	A/C	3.0 - 3.3	0.39	-1.2	0.04
3	Gm1945525374	19	45525374	A/G	2.6 - 3.3	0.09	1.4	0.04
3	Gm2026092580	20	26092580	A/G	2.9 - 3.1	0.07	1.2	0.04
3	Gm2026500747	20	26500747	A/G	2.5 - 3.3	0.10	1.3	0.01

[ǂ]Models, number of models where the marker-trait association was significant,
[ǂ]Chr. Chromosome number,
[£]-log (P) Negative logarithm of the P-value,
[ǂ]Maf. Minor allele frequency.

Table 3. Summary of marker-trait associations for sucrose content in 378 edamame accessions using 5064 SNP markers.

Models[ǂ]	Marker name	Chr.[ǂ]	Position	SNP	-log (P)[£]	Maf[ǂ]	Allele effect	R^2
4	Gm0436357346	4	36357346	C/T	2.6 - 5.7	0.05	-0.4	0.01
4	Gm0440668983	4	40668983	A/C	3.2 - 6.49	0.06	0.3	0.05
5	Gm0620739900	6	20739900	C/T	2.9 - 4.48	0.06	0.3	0.03
4	Gm067756576	6	7756576	A/G	3.6 - 5.8	0.26	-0.2	0.03
2	Gm0840589333	8	40589333	C/T	2.4 - 2.6	0.43	-1.2	0.02
4	Gm0841693863	8	41693863	A/G	3.4 - 6.1	0.21	-0.3	0.01
3	Gm0841798055	8	41798055	A/G	2.4 - 2.5	0.41	2.3	0.05
3	Gm0846434966	8	46434966	G/T	2.5 - 3.4	0.24	1.0	0.04
4	Gm1445637666	14	45637666	A/G	2.4 - 2.7	0.34	-0.1	0.01
4	Gm1510665966	15	10665966	A/G	3.2 - 4.6	0.39	0.5	0.01
5	Gm168120136	16	8120136	A/G	2.7 - 4.9	0.05	0.4	0.01
2	Gm1736453965	17	36453965	A/G	2.7 - 2.9	0.20	0.4	0.04
5	Gm1740393123	17	40393123	A/G	3.0 - 4.9	0.06	0.3	0.01

[ǂ]Models, number of models where the marker-trait association was significant,
[ǂ]Chr. Chromosome number,
[£]-log (P) Negative logarithm of the P-value,
[ǂ]Maf. Minor allele frequency.

Table 4. Summary of marker-trait associations for seed protein content in 378 edamame accessions using 5064 SNP markers.

Env.[ǂ]	Marker name	Chr.[ᵀ]	Position	SNP	-log (P)[£]	Maf[ǂ]	Allele effect	R^2
2014	Gm0410868076	4	10868076	A/G	2.2 - 2.5	0.37	-0.45	0.03
14/15	Gm0412283176	4	12283176	C/T	2.4 - 2.5	0.06	-0.20	0.01
14/15	Gm0535518408	5	35518408	C/T	2.3 - 2.7	0.06	0.10	0.01
2014	Gm0537305888	5	37305888	A/G	3.0 - 3.2	0.12	1.30	0.01
2015	Gm0537305888	5	37305888	A/G	3.0 - 3.2	0.12	1.34	0.01
2014	Gm1114704799	11	14704799	C/T	2.5 - 2.6	0.10	-1.32	0.02
2015	Gm1114704799	11	14704799	C/T	2.5 - 2.6	0.10	-1.33	0.02
14/15	Gm2043713385	20	43713385	A/G	2.5 - 4.2	0.07	1.92	0.02
2014	Gm2043713385	20	43713385	A/G	2.4 - 2.8	0.07	1.91	0.02
2015	Gm2043713385	20	43713385	A/G	2.5 - 2.8	0.07	1.92	0.02

[ǂ]Env. Environment, 2014 and 2015 indicates significance was found using LS Means by year, 14/15 indicates significance using BLUPs,
[ᵀ]Chr. Chromosome number,
[£]-log (P) Negative logarithm of the P-value,
[ǂ]Maf. Minor allele frequency.

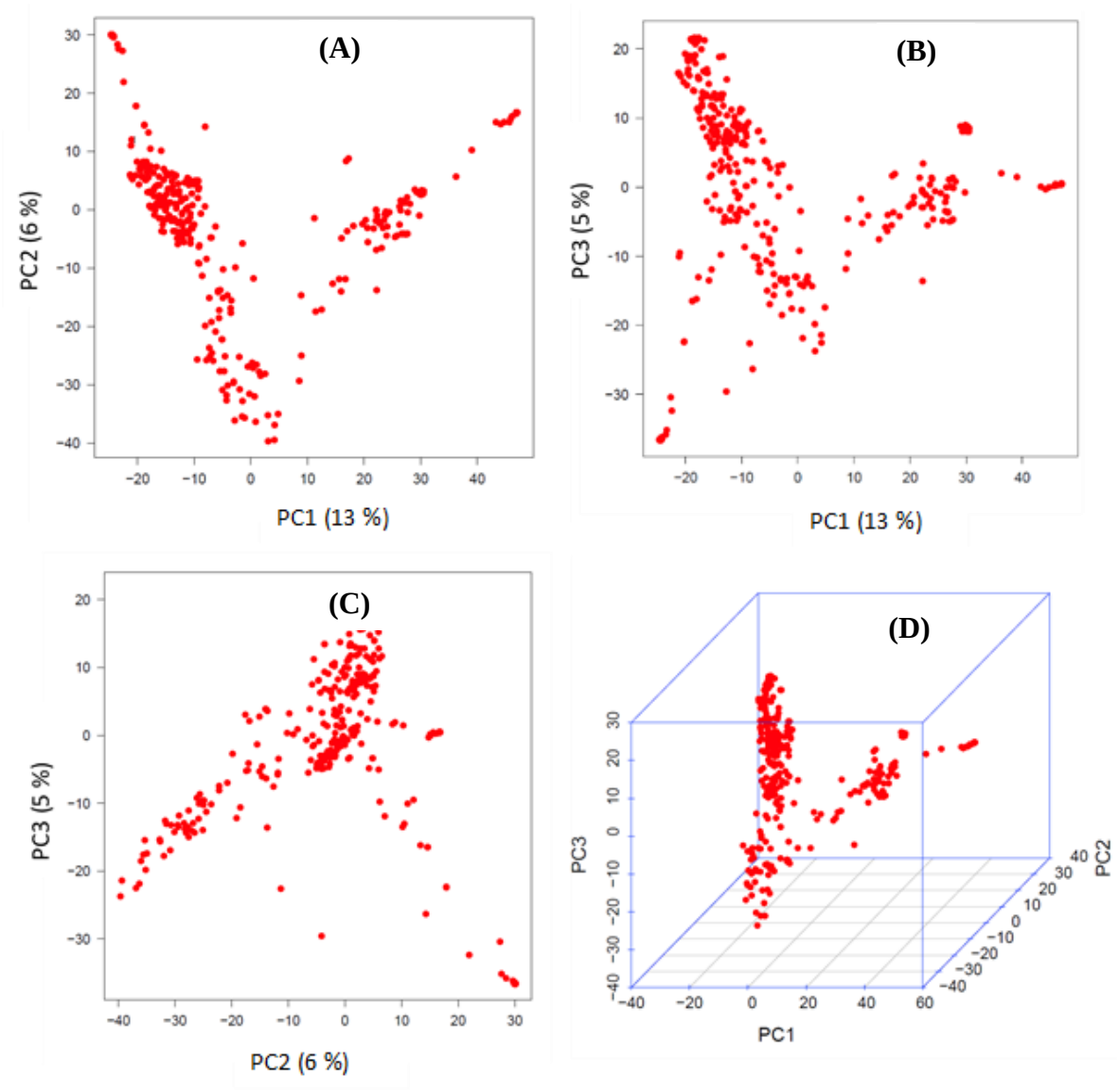

Figure 1. Principal component analysis for population structure represents the stratification due to the principal components (PC) **(A)** one and two, **(B)** one and three, **(C)** two and three, and **(D)** 3D interaction of the three PCs.

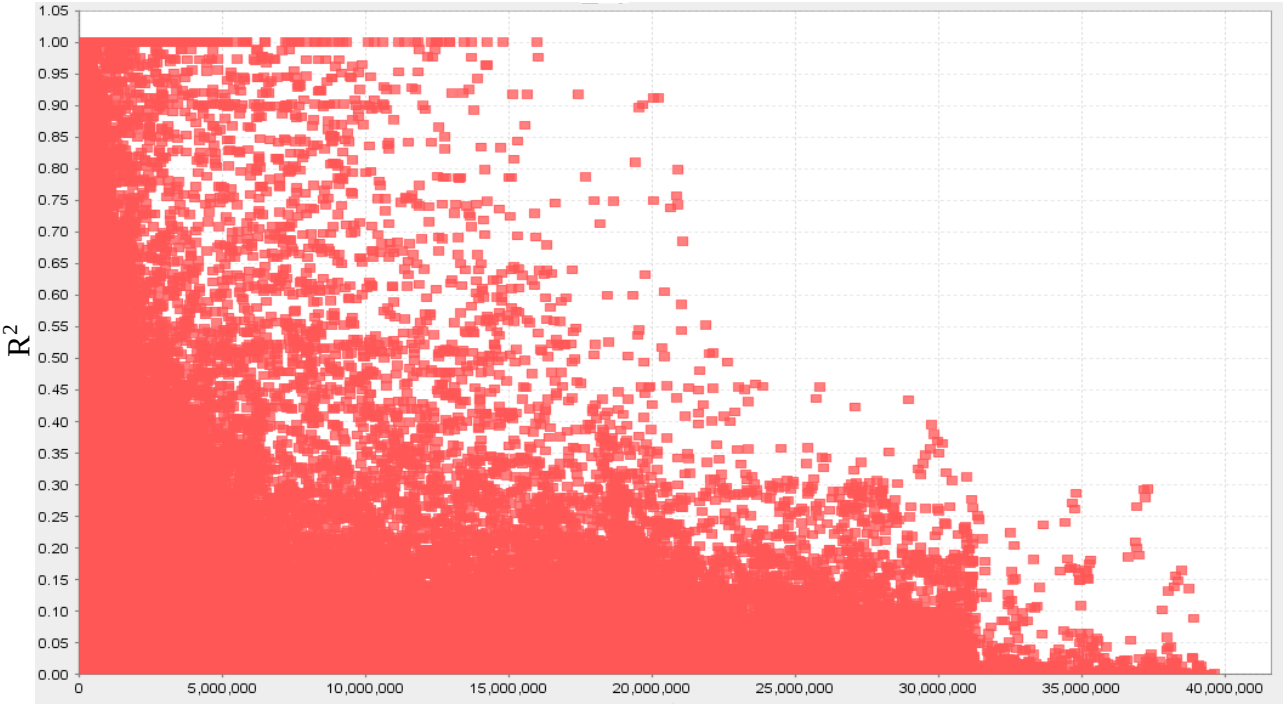

Figure 2. Linkage disequilibrium decay for all chromosomes, using GAPIT.

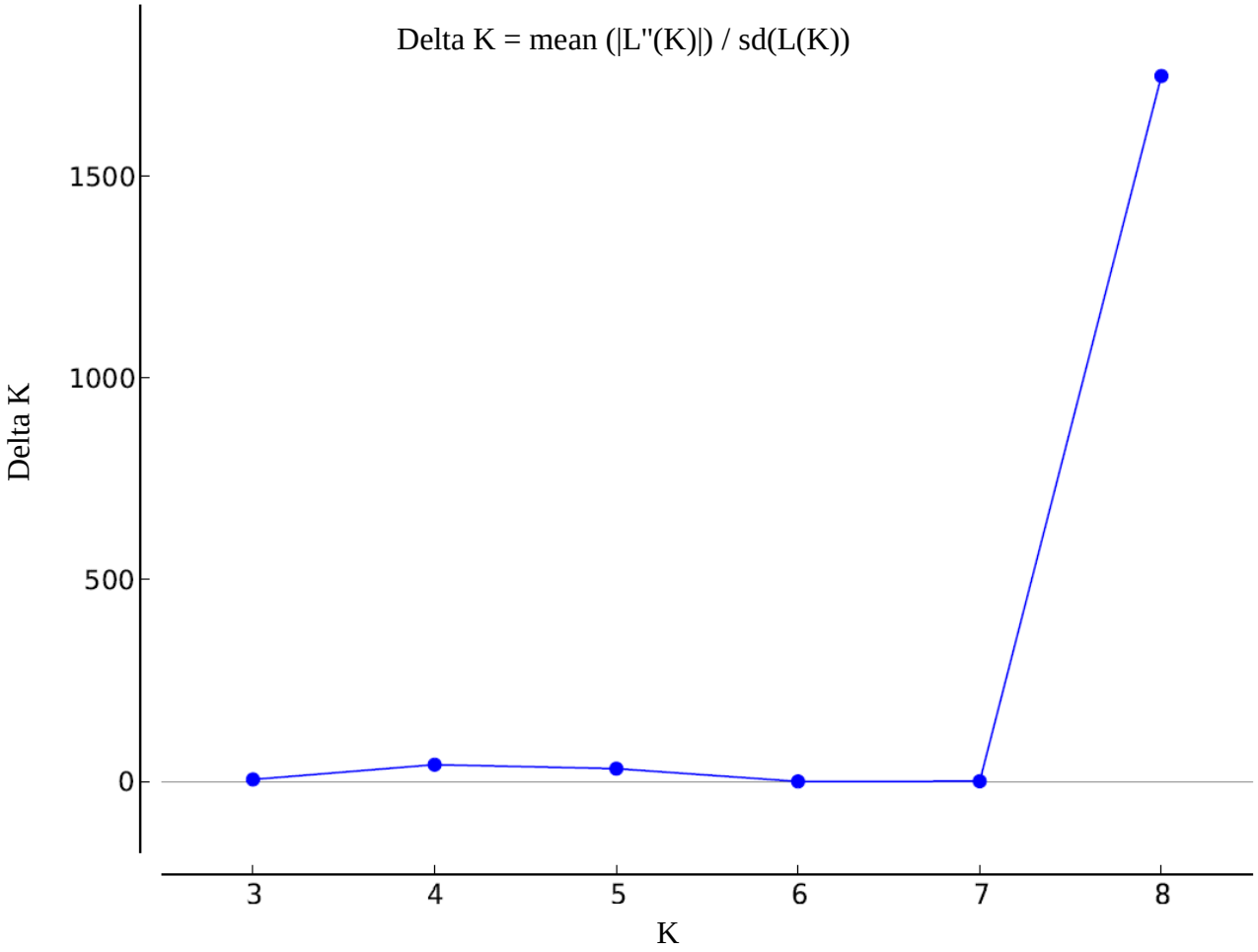

Figure 3. DeltaK plot indicating the highest DeltaK value was obtained at K=8 (K=clusters).

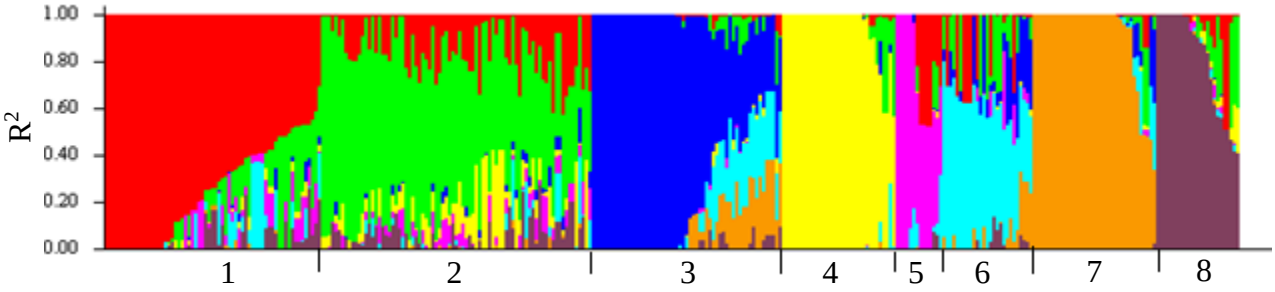

Figure 4. Estimated membership proportions of the 378 accessions, under eight clusters. Each column represents an accession and each color a cluster.

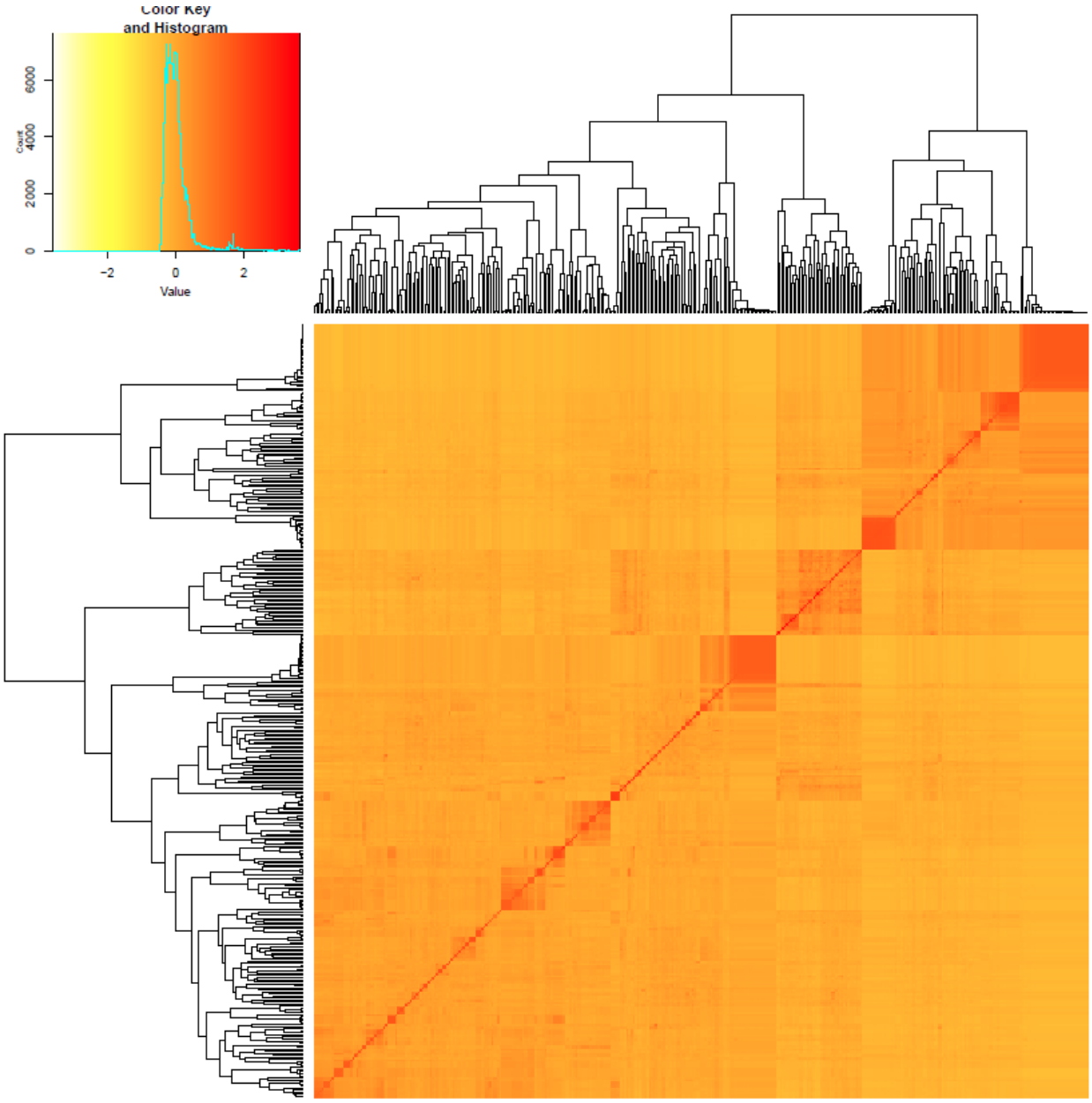

Figure 5. Heat map of the kinship relationship among 378 accessions using 5064 SNPs.

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Supplementary table 1. Accessions included on the association mapping panel.

Accession	MG	Accession	MG	Accession	MG	Accession	MG
PI 506697	0-0-0	PI 518758	1	PI 593949 A	1	PI 416892	3
PI 593979	0-0-0	PI 532468	1	PI 594304 A	1	PI 423899	3
FC 30685	0-0	PI 532469	1	PI 594319	1	PI 504508	3
PI 194626	0-0	PI 538403	1	PI 181534	2	PI 506592	3
PI 194647	0-0	PI 538405	1	PI 196151	2	PI 506637	3
PI 196486	0-0	PI 538407	1	PI 227213	2	PI 506790	3
PI 538408	0-0	PI 538409	1	PI 416929	2	PI 506799	3
PI 549054	0-0	PI 538410 A	1	PI 417436	2	PI 506800 A	3
PI 567283	0-0	PI 538410 B	1	PI 417455	2	PI 506800 B	3
FC 21340	0	PI 540740	1	PI 532472	2	PI 506801 A	3
JYC-2	0	PI 549057 A	1	PI 532473	2	PI 506801 B	3
PI 181531	0	PI 549067	1	PI 549071	2	PI 506982	3
PI 243547	0	PI 549068	1	PI 561234	2	PI 506987	3
PI 248512	0	PI 549072	1	PI 561236	2	PI 507226 A	3
PI 361082	0	PI 561232	1	PI 567151	2	PI 507226 B	3
PI 416845	0	PI 561235	1	PI 567152	2	PI 507273	3
PI 417095	0	PI 561241	1	PI 567153	2	PI 507487	3
PI 507038	0	PI 561295	1	PI 80485	2	PI 507523	3
PI 507351	0	PI 561345	1	PI 86137 -1	2	PI 507570	3
PI 549069	0	PI 561348	1	PI 196149	3	PI 536547 B	3
PI 549070	0	PI 567155 A	1	PI 196162	3	PI 536547 C	3

Supplementary Table 1. (Cont.

Accession	MG	Accession	MG	Accession	MG	Accession	MG
PI 567177	0	PI 567193	1	PI 342438	3	PI 548361	3
PI 548624	3	PI 398222	4	PI 398879	4	PI 408334	4
PI 561292 A	3	PI 398256	4	PI 398891	4	PI 416888	4
PI 80459	3	PI 398293	4	PI 398904	4	PI 417006	4
PI 87165	3	PI 398319	4	PI 398925	4	PI 417021	4
PI 89162	3	PI 398342	4	PI 399048	4	PI 417086 B	4
FC 19976 -2	4	PI 398379	4	PI 399053	4	PI 417163	4
PI 124871	4	PI 398401	4	PI 399069	4	PI 417233	4
PI 157419	4	PI 398450	4	PI 408033	4	PI 417238	4
PI 157424	4	PI 398531	4	PI 408058	4	PI 417301	4
PI 157442	4	PI 398532	4	PI 408064	4	PI 417339	4
PI 19986	4	PI 398615	4	PI 408065	4	PI 417468	4
PI 229343	4	PI 398735	4	PI 408072	4	PI 423739	4
PI 243519	4	PI 398738	4	PI 408076 B	4	PI 423740	4
PI 243527	4	PI 398744	4	PI 408109 A	4	PI 423750	4
PI 243529	4	PI 398759	4	PI 408125 B	4	PI 423763	4
PI 243545	4	PI 398767	4	PI 408179	4	PI 423777	4
PI 243551	4	PI 398801	4	PI 408228 B	4	PI 423830 B	4
PI 248514	4	PI 398802	4	PI 408233 B	4	PI 423980	4
PI 274210	4	PI 398854	4	PI 408263	4	PI 424151	4
PI 339983	4	PI 398872	4	PI 408291	4	PI 424189	4

Supplementary Table 1 (Cont.)

Accession	MG	Accession	MG	Accession	MG	Accession	MG
PI 548587	3	PI 398198	4	PI 408299	4	PI 424237 A	4
PI 339990	4	PI 398201	4	PI 408298 A	4	PI 424233	4
PI 424238	4	PI 424537	4	PI 506789	4	PI 86134 -1	4
PI 424250 A	4	PI 424546 A	4	PI 506903	4	PI 86134 -4	4
PI 424255 B	4	PI 424558 A	4	PI 506937	4	PI 91684	4
PI 424282	4	PI 424564	4	PI 506993	4	PI 96118	4
PI 424292	4	PI 424571	4	PI 507123	4	PI 96550	4
PI 424313	4	PI 424574	4	PI 507179	4	PI 96783	4
PI 424314	4	PI 424590 A	4	PI 507309	4	R07-589	4
PI 424322	4	PI 424590 B	4	PI 507445	4	R08-4011	4
PI 424364 A	4	PI 438300	4	PI 507449	4	MFL-159	5
PI 424364 B	4	PI 442007 B	4	PI 507564	4	PI 398238	5
PI 424377	4	PI 458021	4	PI 548350	4	PI 398240	5
PI 424379	4	PI 458031	4	PI 548351	4	PI 398263	5
PI 424385	4	PI 458036	4	PI 548408	4	PI 398779	5
PI 424450	4	PI 458055	4	PI 548559	4	PI 407748	5
PI 424459	4	PI 458086	4	PI 549063	4	PI 417159	5
PI 424470	4	PI 458118	4	PI 549065	4	PI 417205	5
PI 424484 B	4	PI 458125	4	PI 561288	4	PI 417322	5
PI 424492	4	PI 458136	4	PI 561292 B	4	PI 417491	5
PI 424493 A	4	PI 458141	4	PI 561293	4	PI 423743 B	5

Supplementary Table 1 (Cont.)

Accession	MG	Accession	MG	Accession	MG	Accession	MG
PI 424516	4	PI 458203 B	4	PI 81029 -1	4	PI 424312	5
PI 424513	4	PI 458203 A	4	PI 70243	4	PI 423823	5
PI 424518	4	PI 506560	4	PI 85441	4	PI 424337 -1	5
PI 424576	5	R10-2929	5	PI 424333	6	R07-10396	6
PI 424586	5	R10-9266	5	PI 424375	6	R07-10397	6
PI 424593	5	R11-3743	5	PI 424439	6	R08-4014	6
PI 437734	5	R11-3765	5	PI 458196	6	R09-345	6
PI 458159	5	R11-3896	5	PI 458219	6	RANDOLPH	6
PI 458174	5	R12-2728	5	PI 458245	6	V94-3168T	6
PI 506594	5	R12-2766	5	PI 458251	6	RM-9508	6
PI 506730	5	R12-2800	5	PI 506530	6	PI 408254	6
PI 506746	5	R12-2953	5	PI 506569	6	PI 416876	6
PI 506752	5	R12-2982	5	PI 506593	6	PI 417099	6
PI 506797	5	R12-3127	5	PI 506606	6	PI 424139	6
PI 506890	5	R12-9730	5	PI 506744	6	PI 408051	7
PI 507031	5	R12-9752	5	PI 506754	6	PI 416813	7
PI 507121	5	R12-9774	5	PI 507208	6	PI 416928	7
PI 507135	5	UA Kirksey	5	PI 507428	6	PI 416947	7
PI 507433	5	V95-7456	5	PI 507438	6	PI 417047	7
PI 509081	5	V96-7198	5	PI 507469	6	PI 417206	7
PI 567764	5	Ozark	5	PI 509086	6	PI 417270	7

Supplementary Table 1 (Cont.)

Accession	MG	Accession	MG	Accession	MG	Accession	MG
R07-7722	5	UA 5612	5	PI 548457	6	PI 458242	7
R07-7628	5	Osage	5	PI 509093	6	PI 423909	7
R08-4004	5	R05-1947	5	PI 548486	6	PI 506475	7
R10-2867	5	PI 424185	6	PI 578467	6	PI 506555	7
PI 578470	7	PI 506570	7	PI 506877	7	PI 506679	8
PI 181569	7	PI 506603	7	PI 506990	7	PI 506680	8
PI 187154	7	PI 506616	7	PI 507042	7	PI 507018	8
PI 200544	7	PI 506618	7	PI 507336	7	PI 587587 B	8
PI 248510	7	PI 506735 A	7	PI 507359	7	PI 445847	9
PI 181565	7	PI 506735 B	7	PI 181564	8		
PI 506556	7	PI 506756	7	PI 506579	8		

Chapter III.

Kinetics of Physicochemical Characteristics of Edamame under Infrared Treatment

Abstract

Edamame is a specialty soybean harvested when pods are completely filled and beans are completely green. This vegetable soybean is popular in Asia and is gaining popularity among western countries. Traditionally, edamame is blanched using hot water for 2 or 3 minutes before being frozen. The objective of this research was to provide the kinetics of edamame under infrared (IR) heating and to evaluate the impact of three intensity levels on physicochemical attributes of edamame. This research is the first to evaluate the effectiveness of IR heating on drying and blanching edamame. Heating intensities – high, medium, and low- correspond to product-to-emitter gap (PEG) sizes of 6, 8, and 11 cm respectively. Temperature, weight, texture, green intensity, and peroxidase activity were analyzed under various treatment durations to characterize the effects of IR heating. Temperature increased rapidly with a high heating intensity yet it showed a similar pattern across heating intensities. In general weight decreased during IR heating. The largest weight reduction (9.5 %) was achieved after 100 seconds under high heating intensity. Hardness was reduced alongside treatment duration, reaching the lowest values at 100 or 120 seconds despite heating intensity. Similarly, at 100 or 120 seconds, the highest green intensity was recorded. After treating edamame for 100 or 120 seconds, peroxidase presented the lowest activity. Yet only 62 % peroxidase inactivation was achieved indicating edamame must be treated for longer periods of time to reach the 90 % inactivation required by the industry. Thereby further studies are required to establish a protocol to inactivate 90 % of peroxidase activity on edamame under IR heating. However, this study indicates the potential use of IR heat to dry edamame as well as to improve texture and green intensity.

Introduction

Edamame, a popular vegetable in East Asia has started to gain popularity among western countries. For instance, Montri et al. (2006) reported that in Philadelphia, PA consumers are interested in purchasing fresh, in-shell edamame, and edamame-based patties from supermarkets. In addition, edamame ranked as the second most consumed soy-food after soymilk with sales scaling up to $85 million in 2013 (SoyFoods, 2015). This vegetable soybean is usually served as part of a dish or in salads, though it is also consumed as a snack. Simonne et al. (2000), suggested American consumers are likely to accept edamame as a snack after being freeze dried. Kelley and Sánchez (2005) reported U.S. consumers prefer a buttery flavor and texture, in contrast to Japanese consumers who prefer a flower-like and beany flavor. Currently the majority of edamame consumed in the U.S. is imported from China, usually marketed frozen, thereby indicating the need of diverse edamame-based products.

Increase in popularity of edamame is probably due to its nutritional value. Edamame does not only supply the essential amino acids, but is considered to be a complete protein source (Mebrahtu, 2008). In addition to high quality and quantity of protein content, edamame also contains isoflavones, which have been reported to provide health benefits (Wildman, 2006). On average the nutritional contents of edamame, based on a serving size of 155 g (one cup) are 189 calories, 8 g of fat, 16 g of carbohydrates, 3 g of sugar, and 17 g of protein (Song et al., 2003; Hu et al., 2007; Carson, 2010; Xu et al., 2012; Suwan, 2015).

Traditionally, edamame undergoes blanching before freezing and storing. Blanching is a heat pretreatment used to deactivate enzymes that accelerate food spoilage. This procedure consists of heating the food rapidly to a set temperature for a certain period of time. Typically hot water (70 – 100 °C) is used for 2 – 5 min to blanch edamame. However, Song et al. (2003) report

blanching for 10 – 30 min. Blanching not only deactivates enzymes thus prolonging shelf life, but also improves flavor and texture. Song et al. (2003) describe the effect of blanching at various times on nutrients, proteins, and vitamins in edamame. Overall, amino acids, vitamins, and soluble sugars tend to decrease with water blanching; less reduction occurs if treated for short periods of time. Edamame blanched with hot water absorbs water, which increases product weight and could be a negative aspect when developing dry snack products.

Development of new products utilizing edamame has been limited due to lack of information on characterization of vegetable soybeans under diverse processing conditions. Besides blanching, Mozzoni et al. (2009) also evaluated the feasibility of canning edamame. Qing-guo et al. (2006) describe the kinetics of edamame under vacuum drying, hot air drying, and freeze drying. These have been the few resources focused on understanding the impact of diverse processing methodologies on edamame. IR heating presents an alternative procedure to heat foods and to broaden the categories of products that could be made from edamame. IR wavelength ranges from 76 – 1000 μm. When IR radiation impinges the food surface, IR energy is absorbed at discrete frequencies thereby heating the food product. IR heating process is reported to have higher energy efficiency and shorter drying duration when compared to other drying methods (Kocabiyik, 2010). For instance, IR has been studied for apples, bananas, and other vegetables to achieve dry blanching and dehydration simultaneously; it has also been reported for use on rice and corn tempering (Pan and Atungulu, 2011; Wilson et al., 2015; Wilson, 2016). IR has also been used in combination with hot air drying to improve product quality, increase energy efficiency, and reduce the duration of the treatment (Kocabiyik, 2010).

The aim of this research is to provide the kinetics of physicochemical characteristics of edamame under IR heating, and evaluate the impact of three different intensity levels on edamame beans.

This is the first research to look at the effectiveness of IR heating on drying and blanching edamame; the findings could benefit attempts to develop new edamame-based products.

Materials and methods

Fresh vegetable soybeans were supplied by a commercial edamame processing company AVS (American Vegetable Soybean & Edamame, Inc.). The variety used for the experiment was 8080. Edamame was shelled and stored at 4 ˚C until processing (less than a week). Samples were standardized based on size using two meshes of 30.48 × 1.91 cm and 40.64 × 1.91 cm oblong (12 × ¾ " and 16 × ¾ ") to use a more homogeneous sample for treatment.

Infrared equipment and treatments

A laboratory-scale IR equipment with two catalytic IR emitters provided by Catalytic Industrial Group (Independence, KS, USA) was used to conduct this research. This equipment generates IR radiation energy by catalyzing natural gas to produce heat along with small amounts of water vapor and carbon dioxide as by-products.

Once samples were screened and standardized by size, they were divided into subgroups (40 g) and randomly assigned to one of the following treatments:

1. High IR heating intensity: Sample placed at 6 cm from the IR emitter.
2. Medium IR heating intensity: Sample placed at 8 cm from the IR emitter.
3. Low IR heating intensity: Sample placed at 11 cm from the IR emitter.

To determine the temperature profile of edamame, two T-type- thermocouples (time constant of 0.15 s) were embedded in the beans to record internal temperature of edamame. Samples were placed under the IR emitter for various durations (20, 40, 60, 80, 90,100, and 120 s). Once samples were removed from the IR equipment they were placed in a re-sealable zipper storage

bag and cooled immediately in iced water. Furthermore, all the samples remained for the same period of time under ice, before storing them at 4 ˚C.

Green intensity, texture, and weight were measured from each subsample within a day of treatment. Peroxidase was analyzed after samples had been frozen. The design of experiment was two-factor factorial, with IR heating intensity and heating duration as fixed factors. The experiment was conducted four times (blocks). There were five missing treatment combinations: High - 90 s, High - 120 s, Medium - 100 s, Medium -120 s, and Low - 90 s, due to the negative impact these treatments had on edamame. For instance, at high heating intensity samples tend to burn after 100 s, but at medium heating intensity burning of the skin occurred at 90 s.

Color determination

Color was measured using Hunter's scale. 'L', 'a', and 'b' values were determined with a HunterLab ColorFlex EZ Spectrophotometer (Hunter Associates Laboratory, Inc. Virginia, USA). L^* is the degree of lightness / darkness, a^* the degree of redness (+) and greenness (−); and b^* the degree of yellowness (+) and blueness (−). The instrument was calibrated on a white card with values L = 93.6, a = 0.6, and b = -2.3. Each sample was measured two times and results were averaged. Green intensity was calculated as y = -a/b.

Texture analysis

Texture of edamame is complex and due to lack of a standard protocol for measuring texture in edamame there is inconsistency on the methodology and units reported (Xu et al., 2012; Suwan, 2015). Therefore texture was assessed based on the protocol reported by Suwan (2015), where texture is measured as hardness, using a Texture Analyzer TA.XT Plus (Texture Technologies by Stable Micro Systems LTD, Hamilton, MA) and a compression test utilizing two compression platens of 7.6 cm diameter. A loading weight of 5000 g and a distance of 5 cm were selected as

parameters for the test. For each run, five beans were selected and the average force of four runs was the hardness value expressed in g_f.

Weight reduction

To account for weight reduction, weight was measured for each sample. Weight was recorded before treatment and once the sample had cooled to room temperature after the treatment. Percentage of weight reduction was calculated from:

$$\text{Weight reduction (\%)} = \frac{\text{Initial weight (g)-final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Peroxidase (POD) activity

Enzyme extraction was performed using a modified version of Sheu and Chen (1991) reported by Xu et al. (2012). A sample, 2 g, of shelled beans was homogenized with 10 mL of distilled water at 4 ˚C using a mortar to grind the mixture for 2 min. Slurry was filtered through three layers of cheesecloth and centrifuged at 13000 rpm for 30 min at 4 ˚C. Resultant supernatant was the enzyme extract, which was stored on ice until evaluated (< 3 hrs). The substrate solution was prepared by mixing 0.1 mL guaiacol (0.1 M), 0.1 mL hydrogen peroxide (30 %), and 99.8 mL potassium phosphate buffer (pH 6.5), homogenized for 30 s. Then 1 mL of the POD substrate was transferred into a 3 cm cuvette. The reaction was initiated by adding 0.2 mL of crude enzyme extract. Peroxidase activity was recorded from the absorbance at 420 nm. When POD reacts with guaiacol (colorless) and hydrogen peroxide, tetraguaiacol (brown) is formed. The reaction is:

$$\underset{\text{Colorless}}{[4\,H_2O_2 + 4C_7H_8O_2\ (\text{guaiacol})]} + \text{POD} \longrightarrow \underset{\text{Brown}}{\text{tetraguaiacol} + 8\,H_2O}$$

POD was measured using a spectrophotometer (Beckman DU-250, Fullerton, CA), where activity is defined as the increase in absorbance at 420 nm at 1, 10, and 120 s after the reaction had started. For the purpose of this study, only POD activity measured immediately after guaiacol and hydrogen peroxide were added to the enzyme extract is included on the discussion. The authors took this approach because POD is temperature labile and ambient temperature could have an effect on measurements (Figure 3). POD extract was kept on ice until the reaction with guaiacol and hydrogen peroxide started but the spectrophotometer was kept at room temperature, which affected the POD activity overtime. In addition, POD was measured on edamame from the industry (bought frozen) and used to compare blanching effectiveness of the IR treatments.

Results

Temperature profile at three different heating intensities

The internal temperature of edamame beans was recorded and temperature profile for each heating intensity is illustrated in Figure 1. As expected the lower the heating intensity, the slower the rate of temperature rise.

Weight reduction

The interaction between heating intensity and IR heating duration was significant for weight reduction with a P-value of <0.0001 (Table 1). Accordingly, Tukey's HSD analysis was conducted to determine differences among LS Means (Table 2). The largest weight reduction (9.5 %) was achieved when edamame was treated under high heating intensity for 100 s.

Texture

The analysis of variance indicated the statistical model for texture was significant with R^2 value of 0.88, suggesting a good fit of the general model for this study. IR heating duration was the

only effect that presented significant differences (Table 1). Tukey's HSD was used to determine mean differences (Table 3). Texture decreased as IR heating duration increased despite heating intensity.

Green Intensity

The general statistical model for green intensity presented R^2 value of 0.87. IR heating intensity and heating duration were both significant main effects at a P-value of 0.0204 and <0.0001, respectively. Conversely, the interaction between main effects was not significant (Table 1). Hence, mean comparison was performed independently for IR heating duration and heating intensity (Table 4). The highest green intensity (0.3275) was achieved by treating edamame for 120 s. To better understand the progress of green intensity with time, figure 2 is used to illustrate the effects of the three studied heating intensity levels.

Enzyme activity

Peroxidase (POD) activity was recorded from 1 to 120 s after reaction with guaiacol and hydrogen peroxide (Figure 3). Data was selected from 1, 10, and 120 s after reaction for analysis. The analysis of variance is reported in Table 5. For POD activity measured immediately (1 s) after the reaction, the interaction between heating intensity and IR heating duration was found to be significant with a p-value of 0.0409. However, for POD activity at 10 and 120 s, IR heating intensity and heating duration were both significant, but their interaction was not. For POD at 1 s, Tukey's HSD test was performed to find the significant differences among LS means (Table 6). For POD at 10 and 120 s, means were separated using Tukey's HSD test (Figures 6 and 7). In addition, POD from the industry sample was 0.10 au (absorbance unit). Dunnett's test identified three treatments to generate comparable POD values to the ones found on the industry sample (High IR heating for 100 s, low IR heating for 100 s, and low IR heating for 120 s).

Discussion

Temperature profile

To determine the duration of the treatment for each heating intensity, preliminary studies were performed and a temperature profile for each heating intensity was developed (Figure 1). Duration of IR treatment was constrained by two main factors, damage on the quality of edamame (roasting/ burning), or if based on the temperature profile the inner temperature of the beans had reached at least 75 °C. Ghaemmaghami et al. (2010), reported peroxidase was less active at temperatures above 75 °C, but no temperature of POD inactivation is reported for edamame. Therefore 75 °C was used as targeted temperature. The three intensity levels were based on the equipment configuration. Traditionally, edamame has been blanched using hot water or water steam for 120 – 180 s (Mozzoni et al., 2009; Xu et al., 2012; Suwan, 2015) hence treatment duration in this study did not exceed 120 s, mainly due to skin damage on edamame.

Temperature presented a similar trend between the three levels, yet less differences were observed among high and medium heating intensities when compared to low heating intensity. Samples treated under medium heating intensity had a different behavior than samples from other intensities, for instance at 100 s samples tend to burn, consequently 90 s was selected as the longest duration for medium intensity. Wilson (2016), reported a similar temperature rise on corn kernels treated under IR with a PEG of 11 cm, yet for the authors' experiment temperature was measured at the external surface of corn resulting on higher temperatures.

Weight reduction

Percentage of weight reduction increased as time under treatment increased, achieving 9.5 % when heated for 100 s under a high heating intensity (PEG size of 6 cm). The observed reduction on weight is probably associated with a decrease on moisture content, due to membrane

disruption caused by high temperatures. These results support the concept of utilizing IR heat to dry or as a pre-drying step to produce freeze dried products. Pan et al. (2008) and Zhu et al. (2010) already used IR radiation as drying technology for bananas and apples. IR has also resulted on a rapid drying method for corn kernels with potential benefits of microbial decontamination (Wilson, 2016).

Moisture content was not measured on this study, but it normally ranges around 60- 72 % (Qing-guo et al., 2006), assuming weight reduction was caused by moisture loses. Theoretically moisture content was reduced from 66 to 56 % in 100 s under a high IR heating intensity. IR could be used as part of the drying methodology, combining IR with conventional hot air. Hebbar et al. (2004) reported increased energy efficiency when combining IR and hot air, compared with either process alone, on potatoes and carrots.

Texture

Hardness values decreased as treatment duration increased. The softest texture was obtained when edamame was treated for 100 s under both high and low IR heating intensity treatment. Results indicated a major effect of IR treatment duration over heating intensity. Pohl et al. (1988), reported hardness is due to starch and pectin, which under heat are gelatinized and some pectin substances become soluble hence softening the product. Additionally, disruption of the middle lamella and softening of the protein matrix of the beans contributed to reduced hardness (Whistler and BeMiller, 1997). Low and medium heating intensities demonstrate similar patterns on hardness reduction (Figure 4). Under high heating intensity edamame showed a different behavior. Probably due to the sudden increase of internal temperature at a high heating intensity.

Reduced hardness has also been reported by blanching, and canning (Mozzoni, et al.2009; Suwan, 2015). Yet hardness achieved in this study is higher than previous reports (Qing-Guo et

al., 2006; Mozzoni et al., 2009; Xu et al., 2012); difference is likely due to the use of fresh samples rather than frozen-thawed (used in previous studies). Hardening of edamame is not desired by consumers. Suwan (2015) reported a negative correlation (-0.482) between hardness and acceptability by a sensory panel, which indicates a soft texture might increase consumer preference.

Green intensity

Green intensity followed the same pattern among heating intensities across treatment duration. Thus, reinforcing the null interaction between main effects (IR heating duration and intensity). The highest values for green intensity were recorded when edamame was exposed to IR heating for 100 and 120 s. Results are in agreement with Xu et al. (2012) who used hot water with calcium chloride for blanching treatment which increased green intensity of edamame. Conversely, Mozzoni et al. (2009) reported green intensity decreased as duration of blanching increased. Differences are caused by the addition of CaCl (Xu et al., 2012). Industry samples sourced from the commercial edamame processing facility (water blanched) had a green intensity that ranged from 0.32 – 0.38; similar values were achieved when edamame was treated under IR for 100 or 120 s.

Peroxidase activity

In general, peroxidase activity decreased as treatment duration increased. POD increased after 20 s of IR heating, because small doses of heat increases POD activity (Bahçeci et al., 2005; Xu et al., 2012). After this increase on enzyme activity, POD activity started to decline. The lowest POD activity was obtained when edamame was treated for 100 and 120 s, under both low and high heating intensity respectively. For medium IR heating intensity, the lowest POD activity was recorded after 90 s of treatment (Table 6).

Frozen edamame (from the industry, sourced frozen from a local store) was used to compare POD activity. Edamame treated under high IR heating intensity after 100 s or under low IR heating intensity after 100 and 120 s resulted on comparable (no significant difference) POD activity to the industry sample (Dunnett's value = 0.1068). Despite this comparison, the food industry targets enzyme inactivation of 90 % to consider a product as completely blanched. POD inactivation in this study reached 62 % at 100 and 120 s. Thus indicating longer IR treatments or higher heating intensities are required to achieve complete POD inactivation using IR radiation.

Conclusions

Infrared heating is a technology with potential use for dehydrating and blanching edamame. IR heating edamame for 100 or 120 s, resulted in weight reduction, texture and color improvement, and reduction of peroxidase. Weight decreased consistently across treatments indicating moisture loses. Under high heating intensity (PEG size of 6 cm) the highest weight reduction was achieved (9.5 %) when samples had been treated for 100 s. Texture, measured as hardness, decreased consistently among IR heating intensities. Despite of the heating intensity, treating edamame for 100 or 120 s resulted on the lowest hardness (11172.9 - 10847 g_f). Green intensity increased alongside treatment duration. The largest green intensity was achieved after treating edamame for 100 or 120 s. POD decreased as duration of the treatment increased and the lowest peroxidase activity was recorded after treating edamame for 100 or 120 s. Peroxidase activity was decreased only to 62 % thereby not achieving the industry standard for complete enzyme inactivation (90 %). Longer IR heating duration or higher IR heating intensities may be required to completely deactivate POD in edamame.

Overall, treating edamame under IR heat for 100 or 120 s resulted in the highest percentage of weight reduction, lowest hardness, highest green intensity, and lowest peroxidase activity. Further research is recommended to develop a blanching protocol using IR heat for complete enzyme inactivation. Overall, the demonstrated the potential of IR heating as processing methodology to dry and blanch edamame.

Table 1. Analysis of variance, P- values of weight reduction, texture, and green intensity of edamame under infrared heating.

Source	Degrees of freedom	Weight reduction	Texture	Green intensity
Heating intensity	2	< 0.0001	0.2311	0.0204
Infrared (IR) heating duration	6	< 0.0001	< 0.0001	< 0.0001
Heating intensity × IR heating duration	7	< 0.0001	0.0845	0.9179
Blocks	3	0.2079	0.8802	0.4121
Error	45			

Table 2. Percentage of weight reduction on edamame under different IR heating durations and intensities.

Infrared heating duration (s)	Weight reduction (%) under three IR heating intensities[Ŧ]		
	High[◦]	Medium[◦]	Low[◦]
20	0.99 ± 0.29^{I}	1.29 ± 0.29^{HI}	1.29 ± 0.29^{HI}
40	2.10 ± 0.29^{GHI}	2.59 ± 0.29^{FGH}	2.23 ± 0.29^{GHI}
60	4.03 ± 0.29^{DEF}	3.38 ± 0.29^{EFG}	3.76 ± 0.29^{EF}
80	6.79 ± 0.29^{BC}	5.46 ± 0.29^{CD}	4.77 ± 0.29^{DE}
90		6.42 ± 0.29^{BC}	
100	9.56 ± 0.29^{A}		6.38 ± 0.29^{BC}
120			7.85 ± 0.29^{B}

[Ŧ] LS Means followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.

[◦]High, medium, and low represent heating intensity based on product-to-emitter gap sizes of 6, 8, and 11 cm respectively.

Table 3. Comparison of edamame texture means under seven infrared heating durations.

Infrared heating duration (s)	Sample size	Mean (g_f)[Ŧ]	SD[ǂ]
20	12	20372.89[A]	1904.85
40	12	18904.33[A]	1832.44
60	12	14911.68[B]	1785.79
80	12	12783.33[BC]	1603.79
90	4	12517.35[BC]	903.50
100	8	10847.03[C]	1254.05
120	4	11172.90[C]	590.87

[Ŧ] Means followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.
[ǂ] Standard deviation.

Table 4. Comparison of green intensity means under seven infrared heating durations.

Infrared heating duration (s)	Sample size	Mean[Ŧ]	SD[ǂ]
20	12	0.2025[C]	0.0209
40	12	0.2183[C]	0.0170
60	12	0.2333[C]	0.0202
80	12	0.2958[AB]	0.0202
90	4	0.2900[B]	0.0337
100	8	0.3063[AB]	0.0239
120	4	0.3275[A]	0.0189

[Ŧ] Means followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.
[ǂ] Standard deviation.

Table 5. P-value of peroxidase (POD) activity at 1, 10, and 120 s recorded after reaction with guaiacol and hydrogen peroxide.

Source	Degrees of freedom	POD 1[‡]	POD 10[‖]	POD 120[‾]
Heating intensity	2	0.2009	0.0380	< 0.0001
Infrared (IR) heating duration	6	< 0.0001	< 0.0001	0.0097
Heating intensity × IR heating duration	7	0.0409	0.1348	0.3324
Blocks	3	0.5874	0.5905	0.0551
Error	45			

[‡] Peroxidase activity absorbance at 1 s (immediately) after reaction with guaiacol and hydrogen peroxide.
[‖] Peroxidase activity absorbance at 10 s after reaction with guaiacol and hydrogen peroxide.
[‾] Peroxidase activity absorbance at 120 s after reaction with guaiacol and hydrogen peroxide.

Table 6. Multiple comparison of peroxidase activity of edamame after infrared treatment.

Infrared heating duration (s)	Peroxidase activity (au)[Ŧ]		
	High	Medium	Low
0	0.37 ± 0.0495^{AB}	0.37 ± 0.0495^{AB}	0.37 ± 0.0495^{AB}
20	0.4439 ± 0.0285^{A}	0.3485 ± 0.0285^{ABC}	0.4091 ± 0.0285^{AB}
40	0.3054 ± 0.0285^{CD}	0.2980 ± 0.0285^{CD}	0.3374 ± 0.0285^{BC}
60	0.1434 ± 0.0285^{F}	0.2190 ± 0.0285^{DEF}	0.2606 ± 0.0285^{CDE}
80	0.2232 ± 0.0285^{DEF}	0.1839 ± 0.0285^{EF}	0.1838 ± 0.0285^{EF}
90		0.1984 ± 0.0285^{EF}	
100	0.1475 ± 0.0285^{F}		0.1442 ± 0.0285^{F}
120			0.1442 ± 0.0285^{F}

[Ŧ] LS Means followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test. High, medium, and low represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively.

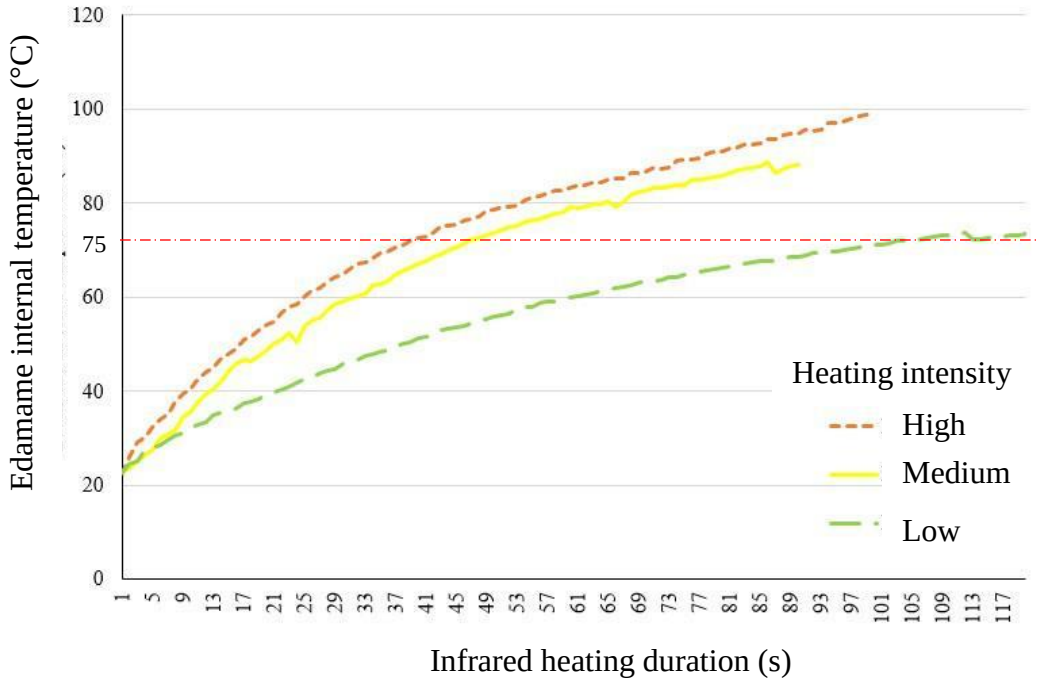

Figure 1. Temperature profile of edamame beans during infrared heating under three heating intensities. Horizontal red dashed line represents targeted temperature (75°C). Heating intensity is based on product-to-emitter gap (PEG). High heating intensity represents samples with PEG size of 6 cm, similarly medium is 8 cm, and low is 11 cm.

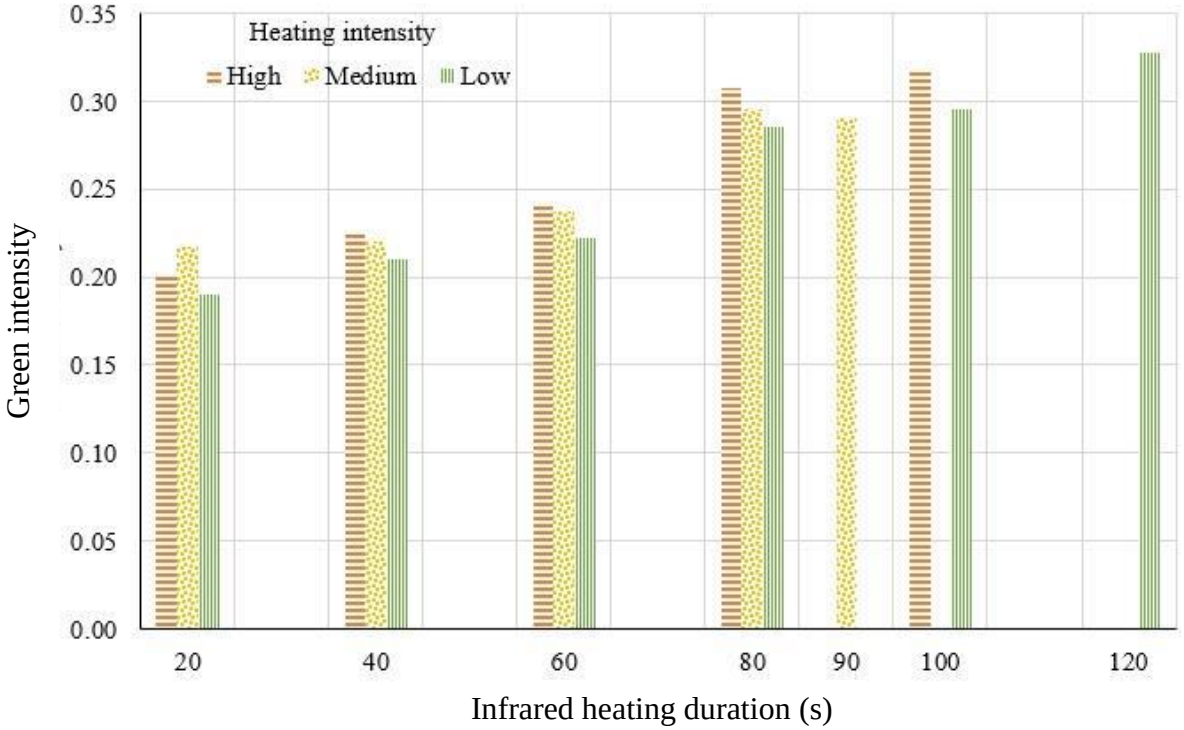

Figure 2. Green Intensity of edamame over infrared heating duration for three heating intensity levels. High, medium, and low represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively.

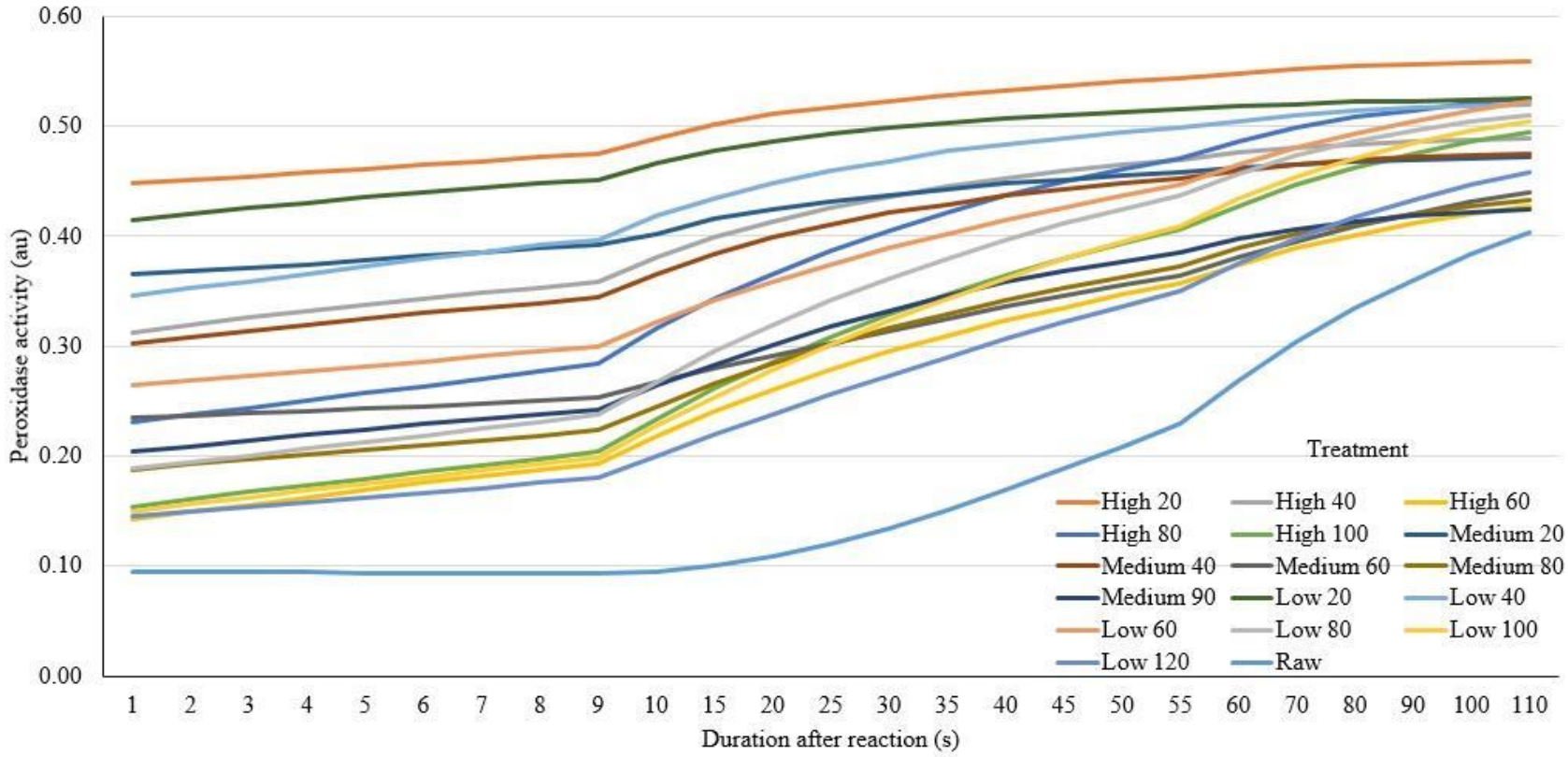

Figure 3. Peroxidase activity of edamame under different infrared treatments, measured continuously after reaction with guaiacol had started. Treatment includes heating intensity and duration of the treatment. High, medium, and low are the heating intensity levels, representing three product-to-emitter gap sizes (6, 8, and 11 cm).

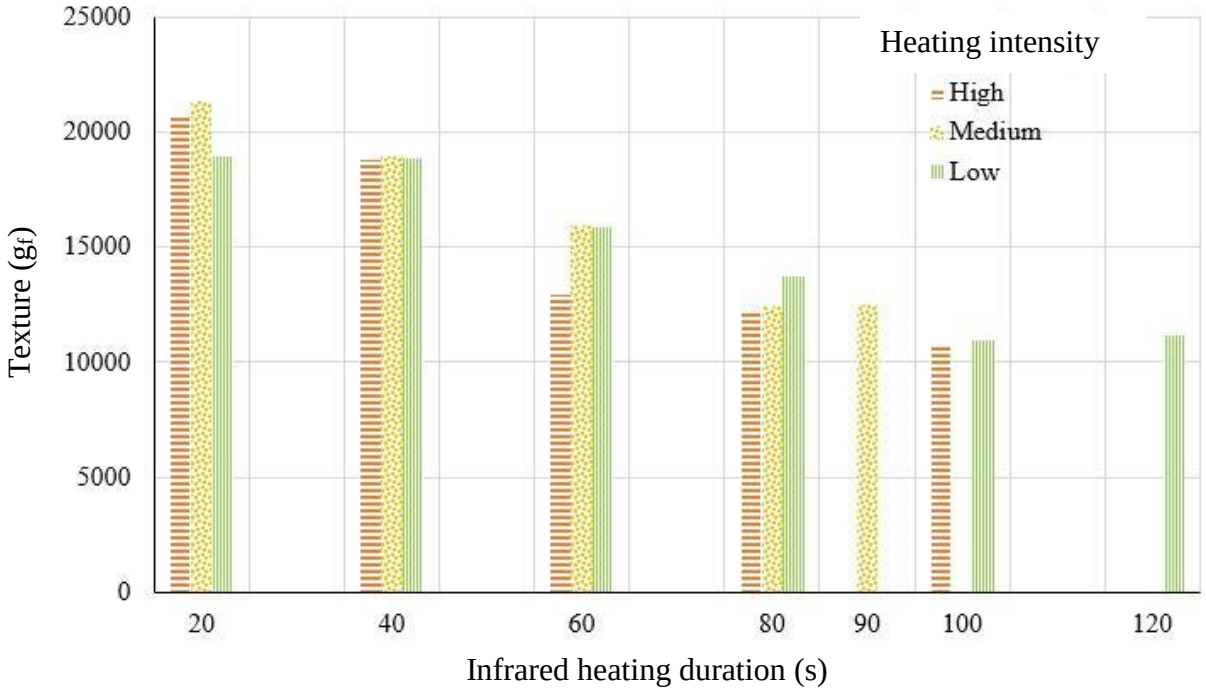

Figure 4. Texture of edamame under different infrared heating intensities and duration. High, medium, and low represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively.

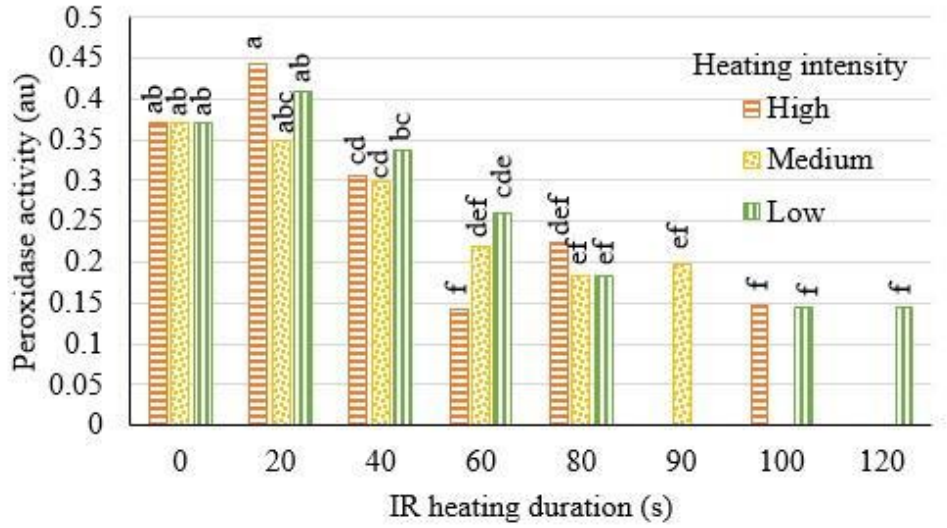

Figure 5. Peroxidase activity measured at 1 second (immediately) after reaction started. High, medium, and low heating intensities represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively. Bars followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.

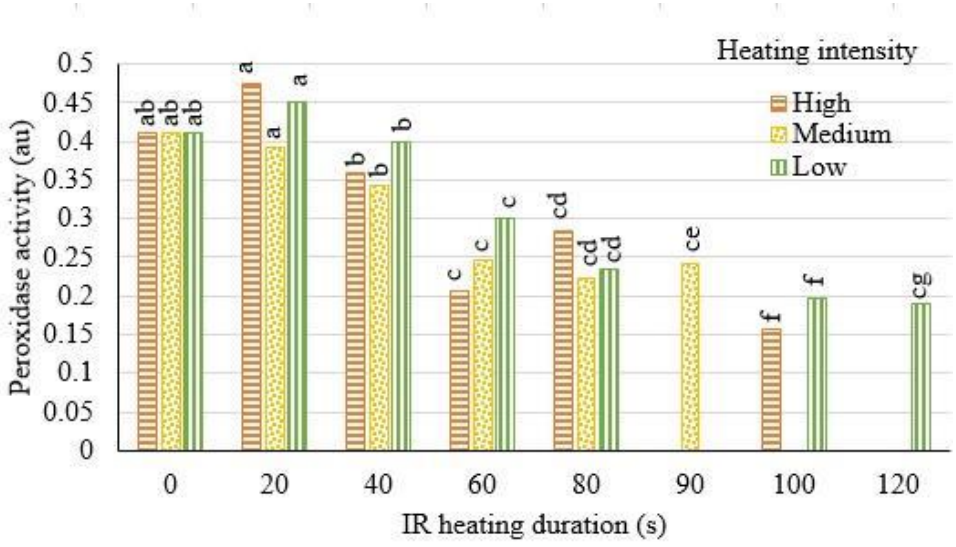

Figure 6. Peroxidase activity measured at 10 s after reaction started, for three IR heating intensities. High, medium, and low heating intensities represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively. Bars followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.

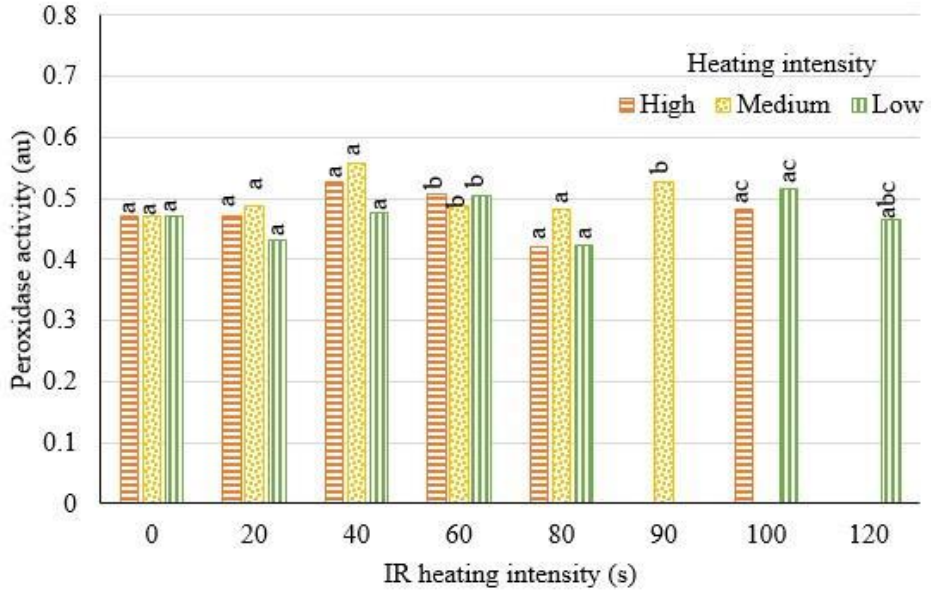

Figure 7. Peroxidase activity measured after 120 s of reaction on edamame treated with three levels of infrared intensity. High, medium, and low represent different product-to-emitter gap sizes of 6, 8, and 11 cm respectively. Bars followed by the same letter are not significantly different at the 0.05 level by the Tukey's HSD test.

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Overall conclusions

A novel QTL on Gm04 was identified to be associated with seed weight and sucrose content. A region that could be of interest for edamame breeders. Marker-trait associations identified in this study validate previously reported quantitative trait loci (QTL) from grain-type soybeans. Yet for seed weight and sucrose seven novel QTL were detected, likely due to the uniqueness of the association panel. On Gm04, Gm16, and Gm19, significant associations were identified as novel for seed weight. For sucrose, novel QTL were located on Gm04, Gm06, Gm08, and Gm17. In addition, six previously reported QTL for protein content were validated on Gm04, Gm05, Gm11, and Gm20. Thus, providing breeders with molecular markers (SNPs) that could aid their selections.

Infrared (IR) heating is a promising technology for new product development. For edamame, across three heating intensities, texture and green intensity were improved after 100 s of treatment. Although 100 % inactivation of peroxidase was not achieved in this research, a decrease on peroxidase activity (62 %) was observed. In addition, up to 9.5 % weight reduction was noted after only 100 s of IR treatment at high intensity. The foregoing observation indicate the potential of using IR treatment as a drying or pre-drying step in edamame product development.