Using Flavor Chemistry, Sensory, and Texture to Determine Domestic Edamame Quality

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ABSTRACT

Persistent interest in edamame, vegetable soybean (*Glycine max* (L.) Merr.), by U.S. consumers has continued to fuel the development of a domestic edamame supply chain. Studies have shown edamame to be a nutritious specialty crop with potential to provide economic benefit to local growers. Domestically bred and grown edamame has shown to be preferred by growers and consumers with competitive agronomic traits. While domestic varieties of edamame will encourage growers to produce a product catered towards the domestic market, additional considerations of final product quality are necessary to positively influence the market success.

Domestically grown and store-bought edamame samples were utilized to research quality attributes including flavor, taste, and texture of edamame representative of domestic market and supply chain. Solid phase microextraction was utilized for aroma extraction prior to gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) analyses to obtain (1) impactful volatile compounds present, (2) changes in these compounds by stink bug feeding injury, and (3) volatile contributions to sensory characteristics. Sensory methods were utilized to (1) evaluate differences in perception of edamame with and without stink bug feeding injury, and (2) understand important sensory characteristics for domestic edamame.

Volatile analysis recognized 16 volatile compounds when investigating edamame genotypes with 14 compounds having significant differences in contents by genotype. Only 10 compounds were consistently detected through GC-O by panelists, so called aroma-active compounds, and only one compound (E)-2-octenal was significantly different in odor intensities across genotypes. Stink bug injured samples showed dramatic differences in volatile profile compared with the not injured counterpart, from mass chromatogram; however, no noticeable differences were perceived by GC-O or sensory difference testing. An instrumental texture analysis method was proven to be sensitive enough to detect the textural differences of edamame beans after processing. The multi-dimensional sensory characteristics including taste, aroma, and texture, were established showing significant differences by edamame variety and growing location. Domestically bred edamame was found to be sweeter, as is preferred by domestic consumers, confirming encouraging breeding outcome. Despite significant differences in edamame volatile profiles by genotype and stink bug feeding injury, sensory discrimination of these differences seems to be less noticeable than changes from taste and texture. Utilizing our findings toward future research and product development will support the domestic edamame supply chain by providing a foundational understanding of quality attributes and their impacts.

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GENERAL AUDIENCE ABSTRACT

Edamame, or vegetable soybean (*Glycine max* (L.) Merr.), has been gaining popularity in the U.S. as plant based and alternative proteins continue to see increased attention. Research has shown edamame to be a nutritious specialty crop with potential to provide economic benefit to local growers. Edamame developed and grown in the U.S. has been shown to be preferred by growers and consumers. Understanding the quality of these products is important for a positive and lasting presence in the market.

In this work, locally grown edamame as well as storebought edamame were investigated for flavor and texture. Chemistry methods to research volatile compounds were used to determine impactful flavor compounds, changes in these compounds caused by stink bug injury, and specific aroma of these compounds in edamame. Sensory methods were used to determine differences in edamame injured by stink bugs and to determine taste, flavor, and texture terms related to local edamame.

This work identified 16 volatile compounds consistently in edamame samples with 14 being found to vary in amount by edamame genotype. Only 10 volatile compounds were detected through human sniffing results with only one being found to vary in amount of aroma detected by edamame genotype. Edamame showing visual signs of stink bug feeding injury showed different amounts of chemical compounds compared to the uninjured edamame, but aroma detected by human sniffing and sensory evaluation did not show differences. A method using a texture instrument was proven to be sensitive enough to detect even minor differences of edamame beans by texture. Sensory qualities including taste, aroma, and texture, were found to have differences in edamame based on edamame variety and growing location of the edamame. Locally bred and grown edamame was found to be sweeter than comparable edamame, as is preferred by consumer in the U.S. Despite differences in volatile compounds in edamame as identified

in volatile analysis by differences in stink bug feeding injury, edamame genotype, and growing location, detection of these differences through aroma and taste by human panelists is not seen in this work. Providing these understanding of sensory qualities and their impact on the edamame will help support the local edamame supply in decision making and product development.

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List of Abbreviations

ABBREVIATION	FULL NAME
ANOVA	Analysis of variance
ASABE	American Society of Agricultural and Biological Engineers
ASTM	American Society for Testing and Materials
BFT	Blanch freeze thaw
BFT+M	BFT microwave heat
BF+C	BF stove top cooking
BMSB	Brown marmorated stink bug
CATA	Check-all-that-apply
DA	Descriptive analysis
DNW	Declared net weight
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry
IRB	Institutional Review Board
MANOVA	Multivariate analysis of variance
PCA	Principle component analysis
QDA	Quantitative descriptive analysis
RI	Retention index
SD	Standard deviation
SE	Standard error
SPME	Solid phase microextraction
TBSP	Tablespoon
TSP	Teaspoon

Chapter 1

1. Introduction and Justification

Edamame (*Glycine max* (L.) Merr.), also known as vegetable soybean, is a growing vegetable of consumption among Americans as well as a high value specialty crop that has the potential to boost the income of farmers with small to large production goals or acreage (Jiang et al., 2018; Garber & Niell, 2019). As production of tobacco has decreased in Virginia, growers are seeking a viable and economically valuable alternative crop to replace this lost production (Carson, 2010). Edamame is a feasible, efficient crop to implement especially for growers already producing a bean crop (Carson, 2010). The domestic edamame market and product yield has been steadily increasing over the years though dry soybean product is still the dominant soy crop produced (Jiang et al., 2018). A local supply of edamame will allow for growth in the fresh edamame market while expanding the larger soy product market through development of new market potential, including fresh and locally grown markets and further processed products for regional and national distribution. Further processing, including blanching and freezing, is required to create a longer shelf-life for the distribution of edamame prior to commercial packaging and distribution to retail stores to maintain quality (Carson, 2010), which, in turn, increases the final market value of edamame products in the market. This projection would create jobs while boosting income for growers and processors resulting in a positive impact on the regional economy. Moreover, increased domestic production and market demand would also stimulate the development of edamame cultivars exhibiting improved agronomic and quality attributes that are desired by American growers and consumers.

Though some domestic sensory work has been published on edamame (Carneiro et al., 2021; Flores et al, 2019; Ogles, 2016; & Wszelaki et al., 2005), investigation of quality characteristics of domestically grown, commercially available varieties is limited. Understanding the sensory and quality of domestically available edamame is of great importance as the domestic edamame supply chain develops due to the importance of quality and consistency of edamame products to domestic consumers when discussing intent to purchase (Carneiro et al., 2022). Additionally, food purchasing and consumption choices can be based on endless reasons and factors but products with favorable taste and flavor attributes were reported as the top purchase criteria in the U.S. (Statista, 2024); therefore, sensory attributes undoubtably remains as a main research focus for edamame-based foods.

Correlating sensory characteristics to volatile chemical markers to understand the underlying reasons for consumer acceptance has not been studied for the domestic edamame market. Researching connections between sensory attributes and volatile presence in edamame varieties could reduce the need for labor-intensive sensory work in the future as the edamame market and research expands. Though sensory research can well establish human perception of the products, the time and resources needed can be burdensome and could be avoided or at least simplified if predictable chemical markers were identified. This type of research could also improve the efficiency for domestic edamame research.

Soybean crops in the U.S. can be affected by a variety of pests which may lower their economic value. Stink bugs are particularly detrimental to edamame due to the significant physical and visual injury they cause to the beans (Reisig et al., 2019). Stink bugs feed on edamame by inserting their stylet through the pod and into the bean (Kuhar et al., 2012). The resulting visual injury can range from a small gray or black dot to a large brown patch on the bean. Severity of the injury will vary based on numerous factors including pest population, developmental stage of the stink bug, and developmental stage of the edamame (Corrêa-Ferreira & De Azevedo, 2002). The resulting injury to the edamame beans from stink bug feeding cannot be accurately measured before the beans are removed from the pods. Economic loss due to stink bug feeding injury is of great concern for edamame due to the impact on crop yield as well as consumer response to the visual defects in edamame beans caused by stink bug feeding injury.

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly called the brown marmorated stink bug (BMSB), has caused significant feeding injury to crops, including soybean and edamame, in the U.S. as the population of the invasive species has continued to rise, especially in the mid-Atlantic region (Owens et al. 2013; Venugopal et al., 2014; Bakken et al., 2015). Additionally, several native stink bug species including the green stink bug, *Chinavia hilaris* (Say), and the southern green stink bug, *Nezara viridula* (L.), can also cause feeding injury to soybeans (Kamminga et al., 2012).

While the visual impact of stink bug feeding on edamame is obvious, the flavor and sensory impact has not yet been investigated. The enzymes injected into the beans by way of the saliva during stink bug feeding and the possible transmission of plant pathogens during the process may impact taste and aroma compounds as well as texture of the edamame beans (Daugherty, 1967). If affected, it is unclear if the changes would be significant enough for consumers to distinguish. In any case, understanding these possible changes will be important for overall quality and consistency of edamame as is important for buyers and consumers.

Studies have shown edamame to be a nutritious plant-based food providing dietary fiber, protein and numerous vitamins and minerals (Miles et al., 2018) as well as being an advantageous specialty crop for growers with promising economic benefit. Domestically bred and grown edamame has shown to be preferred by growers and consumers with competitive agronomic traits. While domestic varieties of edamame will encourage growers to produce a product catered towards the domestic market, additional considerations of final product quality are necessary to positively influence the market success.

Establishing methods to determine edamame quality will guide future research and development of competitive edamame products. Currently, research on edamame quality attributes and consumer priorities is limited, leaving knowledge gaps around indicators of quality, consumer acceptability, and specifications and methods to determine acceptable and unacceptable products especially for domestically bred and produced products. Understanding how quality and perception of quality is affected from growth to consumption will provide guidelines for stakeholders throughout the supply chain.

The objective of this work is to identify and develop methods to assess quality attributes, with the goal of providing better guidance to breeders, growers, and processors and supporting the domestic edamame industry. More specifically, this research works to understand quality of domestic edamame by determining volatile compounds and their relation to consumer acceptability in domestic edamame (Chapter 3); understanding the effect of stink bug feeding on edamame flavor chemistry (Chapter 4); quantitatively analyzing texture of edamame and two other legumes (Chapter 5); and identifying the sensory descriptors and aroma-active compounds in the U.S. edamame supply chain (Chapter 6).

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Chapter 2

2. Review of Literature

2.1 Domestic Edamame

Vegetable soybean [*Glycine max (L.)* Merr.], also commonly known as edamame, has been gaining acceptance in North America in recent years though it has been grown and consumed in Asia for centuries (Jiang et al., 2018; Zeipina et al., 2017). In the United States, edamame sales saw a $23 million increase from 2003 to 2009, likely due to the high nutritional value and health benefits of soy-based food products, including edamame (Carson, 2010). Soy, including edamame, is an exceptional plant-based protein, providing consumers with all essential amino acids; a complete protein (Young, 1991). Along with nutritional value, general globalization has also likely contributed to the increased interest and demand for edamame in the United States (Jiang et al., 2018).

Edamame is harvested around the R6 stage of maturity, while the soybean plant does not reach full maturity until R8 (Fehr et al., 1971; Guo et al., 2020). Harvesting at R6 is characterized by a green pod with 85-90% seed fill, around 65% moisture content and highest sweetness which results in the desired quality attributes for consumption (Yu et al., 2022). Edamame production is less prevalent in the United States compared to dry soybean crop but has high market prices making it a good option for growers, especially small-scale or urban farmers looking for high net returns (Jiang et al., 2018). With the decline of tobacco crop, Virginia growers have needed alternative high-value crops to replace the previous tobacco production (Carson, 2010). Coupled with growing demand for edamame due to the high nutritional value of the product, an opportunity has arisen for Virginia farmers to grow this profitable crop (Garber & Neill, 2019). Growers producing other vegetable bean crops such as snap beans are likely to find the transition to growing edamame relatively smooth and beneficial due to the similar harvest demands.

An estimation shows that 70% of edamame in the United States is imported from Asia (Nolen et al., 2016). This is likely due to the more recent interest in edamame domestically with local edamame production lagging despite increased local demand. Consumers are motivated to purchase and consume edamame due to nutritional and sensory properties requiring commercial edamame to be of high sensory quality for increased acceptability in the market (Ogles et al., 2016). Edamame varieties from Asia and imported products have been shown to display inferior agronomic qualities including less desirable plant structure and disease susceptibility in the U.S. (Jiang et al., 2018). Breeding programs will find these agronomic characteristics to be important factors as well as sensory attributes and consumer acceptability to be vital considerations for successful domestic cultivar development (Carneiro et al., 2020a).

Edamame has a relatively short harvest period, varying by variety, and growing conditions, with harvest windows being sometimes 4 days (Moseley, 2018). Processing and storage of edamame also can be extremely challenging to produce and retain the quality of products from harvest through handling, processing, distribution, and storage. Furthermore, even in proper storage conditions, flavor and appearance of fresh edamame deteriorate quickly (Saldivar et al., 2010). Freezing edamame is a common practice to ensure higher quality and longer shelf life. However, frozen edamame must first be blanched to destroy enzymes (e.g. lipoxygenases), which would otherwise cause flavor deterioration during processing and storage (Saldivar et al., 2010).

Edamame can be purchased as intact pods or as beans, without the pods (Miles et al., 2018). The beans of the edamame are the only portion of the plant that is consumed. Even when purchased as an intact pod, the beans are consumed, and the pods are discarded. In addition to in-shell and shelled edamame, various edamame products have been appearing on store shelves including dry roasted edamame (The Only Bean, Grand Rapids, MI, USA; Seapoint Farms, Huntington Beach, CA, USA) in various flavor combinations and edamame pasta (Seapoint Farms, Huntington Beach, CA, USA; Explore Cuisine, Red Bank, NJ, USA). These products are often free of additional allergens including peanuts and wheat or gluten making these products nutritional alternatives to allergen containing products. The additional processing needed to create them also extends the shelf life of edamame compared to fresh or frozen edamame.

2.2 Edamame Quality

2.2.1 Expectations

Though expectations of edamame in terms of quality may vary by region, parameters can be separated into 5 categories of visual quality, taste, flavor, texture, and nutrition (Zeipina et al., 2017).

2.2.2 Visual Expectations

Pods are generally expected to be bright green with 2 to 4 beans per pod with 3 to 4 beans per pod being preferred (Masuda, 1991; Miles, et al., 2018). Overall, pods should have a crescent shape being 5cm or longer with fine hairs covering the surface, called pubescence (Miles, et al., 2018). These hairs can be different colors depending on the edamame variety, but do not impact overall bean quality (Miles, et al., 2018). However, sensory work in Alabama (USA) found participants to have strong opinions and responses to edamame based on the color of the pubescence with participants being averse to darker pubescence (Ogles, 2016). This concern would not apply to edamame sold as beans without the pod.

To sell edamame in the pod, pod quality, such as color, size, and shape, is of extreme importance including showing no visible signs of damage or injury to showcase the high quality of the product (Konovsky et al., 1994; Miles, et al., 2018; Moseley, 2018). Pods showing any sign of yellowing are less desirable for consumers as they indicate lower quality and diminished freshness (Carneiro et al., 2022; Flores et al., 2019; Zeipina et al., 2017).

2.2.3 Taste and Flavor Expectations

Sweet taste and sugar content is an important quality attribute among U.S. consumers (Carneiro et al., 2021a; Flores et al., 2019; Wszelaki et al., 2005). Conversely, bitter has been highly associated with lower overall liking of edamame (Carneiro et al., 2021a). Savory, or umami, has also been identified as an import taste attribute in edamame (Konovsky et al., 1994). Beany flavor has been identified as an undesired off-flavor in the U.S., but is appreciated by consumers in Japan (Carneiro et al., 2021a; Konovsky et al., 1994). Other flavors, such as nutty and buttery, have been identified in edamame as desirable (Johnson et al., 1999; Miles et al, 2018).

2.2.4 Texture Expectations

Vegetable texture has been identified as extremely important for consumer acceptance and overall perception of quality (Arntfield & Maskus, 2011). Changes in texture occur from the many factors that can indicate lower quality including early or late harvest, moisture migration, oxidation, microbial actions (Lu & Abbott, 2004). Starchy, mealy, and chewy textures have been found to be undesirable in edamame (Carneiro et al., 2021a; Ogles, 2016), and, firm edamame beans are much more preferred (Flores et al., 2019; Konovsky, 1994).

2.2.5 Nutrition Expectations

Edamame has been proven to be a highly nutritious vegetable providing protein/amino acids, lipids, dietary fiber, vitamins A, C and E, calcium, isoflavones, antioxidants (Johnson, 2000; Miles, et al., 2018; Simonne et al., 2000; Young, 1991). While most vegetables provide various essential amino acidsto the consumer, soy and edamame provide all essential amino acids making these foods a complete protein, comparable to an animal protein (Rigo et al., 2015; Young, 1991). At 38% protein, edamame has been gaining popularity, in part, due to the interest in edamame as a plant-based protein rich food (Miles, et al., 2018). However, soy is one of 9 major food allergens recognized by the U.S. Food & Drug Administration (FDA) as causing allergic reactions to those impacted by a soy allergy and requiring specific labeling of the allergen within the ingredients list of a food product (FDA, 2024). Allergic reactions to food can vary in symptoms from mild to severe including anaphylaxis and death (FDA, 2024). Despite the nutritional benefits soy and edamame can offer, an allergic individual should avoid soy and soy products as the negative effects would greatly outweigh any possible benefit.

2.3 Quality Factors

The quality of any crop or product can vary due to innumerable factors. As a specialty crop with high earning potential for growers, producing high quality edamame is often viewed as a priority for growers and producers as it is a main purchasing factor for consumers. Countless decisions and factors from planting to consumption will impact the overall quality of edamame or edamame products at the time of consumption. Understanding a consumer’s definition of high-quality edamame can help lead to better growing and processing decisions to ensure a high-quality final product a consumer will enjoy.

Generally, quality of vegetables, including edamame, often refers to a combination of numerous physiochemical characteristics including but not limited to color, taste, size, and shape (Biddle, 2017). Visual appearance of vegetables can cause consumers to infer texture attributes and overall appeal (Lawless and Heymann, 2010). Consumers are known to desire bright green edamame and see this vibrant color as an indicator of quality (Carneiro et al., 2022; Flores et al., 2019). Domestic consumers have also been found to value sweetness and sugar content in edamame contributing to overall taste and acceptance (Carneiro et al., 2021a; Flores et al., 2019; Wszelaki et al., 2005). Some primary factors influencing edamame quality include cultivar, growing location and soil composition, management practices, and processing and storage conditions.

Cultivar, or variety, selection greatly impacts the overall final edamame product. Numerous studies have shown variations in the final quality of the edamame pods and beans including nutritional value, flavor and volatile composition, and other quality attributes including color and texture (Carneiro et al., 2021a; Flores et al., 2019; Guo et al, 2022; Moseley et al., 2021; Wszelaki et al., 2005; Xu et al., 2015; Yu et al., 2021; Yuan et al. 2021). Breeding new edamame cultivars for the domestic edamame market is required to provide growers with acceptable agronomic traits while also breeding for consumer desired quality characteristics (Zhang et al., 2021). To provide better options and opportunities for domestic growers, edamame breeding has been ongoing for over 20 years with 5 varieties having been developed and released in addition to other commercial varieties that have been utilized domestically (Table 2-1). Edamame research has shown that overall sweetness is extremely important to consumer acceptability and overall liking of the product in the U.S. and should be considered as a quality characteristic of high priority (Carneiro et al., 2021a; Johnson et al., 1999). Breeding cultivars can be time and resource intensive, but it is a sensible place to focus since many attributes, including final product quality, starts with the cultivar. Proper edamame cultivar selection will have a major impact on the final product characteristics despite any attempts to change, cover, or improve quality characteristics. This is especially true for minimally processed crop products such as fresh edamame.

Growing location has also been shown to cause sensory and chemical variations in edamame (Carneiro et al., 2021a; Yu et al., 2021). Soil composition will often be dependent on growing location outside of farmer intervention. Soil composition can impact sweetness and other quality attributes of the final edamame product (Hung et al., 1991). Similarly, application of chicken manure or fermented pig dung have been shown to not only improve yield but also the overall quality of edamame including pod color, taste, and sweetness (Hung et al., 1991). Should a grower choose to utilize fertilizers, final edamame product could be positively impacted if fertilizer is utilized appropriately.

Environmental factors are also determined by growing location including rainfall and temperature. As these factors are almost impossible to control in traditional agriculture, growing techniques and management practices are important to produce the best possible crop. Planting early can cause exposure to frost and cold that can negatively impact plant growth. Similarly, harvest time will greatly impact edamame quality. The best harvest time for edamame is at the R6 stage of maturity, which is characterized by having edamame pods at 85%-90% seed fill with around 65% moisture content (Konovsky et al., 1994; Moseley et al., 2021; Yu et al., 2022). Harvesting at R6 helps achieve the highest edamame quality attributes for consumption including the highest sweetness (Yu et al., 2022). Early harvest can result in low bean weight and size, low sugar content, and higher moisture content while late harvest can also result in low sugar content but low moisture content (Yu et al., 2022). As sugar and sweetness are a major focus for consumer acceptability, these attributes are of great importance, but color can also indicate high quality. Late harvest can impact the color of edamame beans since they will begin to yellow and dry out as they mature past the R6 stage and towards full maturity at R8. Unfortunately, edamame harvest windows are often extremely short, sometime less than 5 days, making it sometimes difficult to complete harvest at the best time (Miles et al., 2018; Moseley, 2018).

Edamame processing and handling can be utilized to help extend the short shelf life of edamame. Without additional processing, fresh edamame will only maintain quality for a few days. Blanching edamame has been established as an effective method to maintain green coloring of edamame and deactivate enzymes to increase shelf life especially prior to freezing (Carneiro et al., 2020b). Blanching can also cause a reduction in sweetness possibly due to the leaching of the sugar when blanching is done in water rather than steam (Saldivar et al., 2010). Processing, such as blanching, can also influence vegetable texture which is extremely important for consumer acceptance and overall quality (Arntfield & Maskus, 2011). Structural changes in edamame beans during cooking, including starch gelatinization and pectin solubilization, will result in a softer final product compared to the raw beans. Cold storage of edamame has been shown to help improve shelf life and was found to be more important than atmospheric condition when evaluating overall edamame quality during storge (Saldivar et al., 2010). The impact of cooking and storage on moisture content will also impact overall quality of edamame as moisture is a driving force of texture in foods (Ilker & Szczesniak, 1990).

2.4 Off-Flavors and Aromas

Beany flavor tends to be a main off-flavor of focus in edamame for domestic consumers. These flavors have been reported to be a result of a variety of volatile compounds (Vara-Ubol et al., 2004). Though some compounds have been found to exhibit a beany aroma at low concentrations, research has found that multiple compounds and sensory attributes usually combine to produce a beany characteristic (Fischer et al., 2022; Vara-Ubol et al., 2004). Sensory work completed by Vara-Ubol et al. (2004) found a combination of musty/earthy and/or musty/dusty along with green, nutty, or brown attribute came together to create beany. Compounds found to have beany aromas independently included 3-methyl-1-butanol (at 1 ppm), acetophenone (at 10 ppm), 3-isopropyl-2-methoxypyrazine (at 10 ppm), 1-octen-3-ol (at 100 ppm), 2,4-heptadienal (at 100 ppm), 1-octen-3-one (at 10 ppm), and pentanol (at 1 ppm) (Vara-Ubol et al., 2004). Additional compounds often associated with beany aroma, including hexanal, pentanal, and hexanol, may contribute to beany off-flavor even though they have not been shown to provide the aroma on their own (Carneiro et al., 2021b; Fischer et al., 2022; Vara-Ubol et al., 2004).

Compounds associated with the beany off-flavor are formed through lipid oxidation or amino acid degradation (Fischer et al., 2022). Oxidation of linolic acid can result in the formation of a variety of compounds including hexanal, pentanal, hexanol, pentanol, 1-octen-3-ol, and 1-octen-3-one (Fischer et al., 2022). Degradation of amino acids can also result in a variety of compounds which are known to contribute a beany flavor including 3-methyl-1-butanol, and 3-isopropyl-2-methoxypyrazine (Fischer et al., 2022).

To reduce development of off-flavor compounds in edamame through lipid oxidation and amino acid degradation, storage and processing techniques are the main consideration. Off-flavors can be produced during storage as the edamame deteriorates or oxidizes. Blanching is often recommended to improve shelf life but improper blanching, when there is not enough heat, can cause an increase in off-flavor development during storage (Masuda, 1991). Improper blanching can cause plant cells to rupture without enough heat to properly inactive lipoxygenase (LOX) resulting in increased lipid peroxides production after the product is frozen (Masuda, 1991).

2.5 Stink Bugs and Edamame

As with all agriculture, pest management can require a lot of attention at times as the stress pests cause to the plants can impact final edamame product quality. Though pests can impact growers in most any location, some locations might be more vulnerable to certain pests. Travel and feeding patterns of pests can also determine the amount of damage created. Pests of concern and the impact of their presence can vary based on many factors including the growing location, year, and crop. Soybean and edamame have numerous pests that often cause issues throughout the growing season including soybean aphid (*Aphis glycines* Matsumura), potato leafhopper, Mexican bean beetle, and multiple varieties of stink bugs (*Euschistus servus* Say, *C. hilaris*, and *H. halys* (Pentatomidae)) (Lord et al., 2021).

Stink bugs are a major pest concern for a variety of crops around the world including soybean crops (Sosa-Gómez et al., 2020). Stink bugs are economically detrimental due to the variety of and extensive damage they cause to a wide range of plants and crops (Schaefer & Panizzi, 2000, p. 421). Overall crop yield, quality, and germination can be negatively impacted by stink bug feeding, with different varieties of stink bugs causing varied amounts of injury to the crops (Turnipseed & Kogan, 1976). Crop damage can also vary based on insect population density and plant development stage with longer infestation times being correlated to higher yield loss (Corrêa-Ferreira & De Azevedo, 2002). Stink bugs are typically controlled with the use of insecticide applications (Temple et al., 2013). Often multiple applications of insecticides are needed on fruit and vegetable crops because of the continuous re-invasion of adult stink bugs from surrounding crops and wild vegetation (Kuhar & Kamminga, 2017).

Stink bugs feed on the plant material by piercing through the plant material with their stylets, injecting digestive enzymes, and sucking the nutrients out of the plant (Lomate & Bonning, 2018; Schaefer & Panizzi, 2000, p. 421). Soybean injury resulting from stink bug feeding has been attributed to saliva components. Delayed plant maturity can be a result of stink bug feeding but also can be attributed to nutritional imbalance, drought or excessive moisture during different stages of growth, and nematode infestation (Sosa-Gómez & Moscardi, 1995; as interpreted by Sosa-Gómez et al., 2020). Saliva components are not consistent among all species of stink bugs resulting in varied amounts of damage to the affected crop though amylase activity has been confirmed in most studies on stink bugs in the Pentatomidae family (Sosa-Gómez et al., 2020).

2.5.1 Brown Marmorated Stink Bugs

Halyomorpha halys (Stål), commonly called the brown marmorated stink bug (BMSB), is an invasive species of stink bug originally from east Asia (Hoebeke & Carter, 2003). The species was first identified in Pennsylvania in 1996 and has since spread through the country (Hoebeke & Carter, 2003). BMSB vary in size and color when full grown but tend to be 12-17mm long on average with a marbled brown coloring and light-colored underside with dark banding on the antenna distinguishing them from other brown stink bugs (Hoebeke & Carter, 2003). BMSB have been recorded to reduce soybean crop yields of up to 50% in the Mid-Atlantic region of the United States (Leskey et al., 2012). Physical injury occurs to the plant due to the physical breach of the plant material while chemical injury in the plant tissues result from the injection of the digestive enzymes during feeding (Owens et al. 2013). To feed on edamame beans, BMSB will insert their stylet through the pod leaving the bean with visible signs of injury (Kuhar et al., 2012). This feeding location does not cause noticeable injury to the pod but will be apparent when edamame beans are removed from the pod, causing great concern for economic loss due to diminished quality of the edamame beans (Owens et al. 2013).

In general, digestion is a complex process of breaking down food into small particles for absorption and use by the body. Saliva is an important facilitator of this process, starting the digestion process with enzymes to break down larger molecules. Water, along with many enzymes, can be found in saliva with variations found in stink bug saliva based on bug species. α-Amylase, chymotrypsin-, trypsin-, and aminopeptidase contribute to saliva of some stink bug species (Lomate & Bonning, 2018). α-Amylase breaks down complex starches into smaller molecules, α-maltose and oligosaccharides, causing a rapid reduction in viscosity of the food matrix (Parkin, 2008).

Chymotrypsin, trypsin, and aminopeptidase hydrolyze proteins (Parkin, 2008; Terra & Ferreira, 1994). Chymotrypsin and trypsin specifically cleave the protein chains on the carboxyl side (Terra & Ferreira, 1994) while aminopeptidase hydrolyze single amino acids from peptide chains (Terra & Ferreira, 1994).

Lomate and Bonning (2018) found the saliva of BMSB were higher in chymotrypsin- and trypsin-like activities when compared to the gut. Additionally, aminopeptidase activity was higher in the saliva when compared to the gut (Lomate & Bonning, 2018). These enzymes contribute to the visual defect that results in plant tissues (Lomate & Bonning, 2018). These injuries are more significant when feeding occurs on younger fruit and seeds and can result in abnormal plant growth, discoloration of seeds or fruit, deformed growth of seeds or fruit, abortion of seed, delayed maturing, transmission of plant pathogens, or possibly plant death (Koch et al., 2017). Yeast spot disease, caused by *Nematospora coryli* Peglion, is a fungal disease that is known to be transmitted through stink bugs (Daugherty, 1967).

As the BMSB continues to infest crops across the country, research investigating changes in crop and product quality due to these infestations is becoming increasingly important to fully understand the impact of this invasive species. Research investigating strawberries infested with BMSB found visual differences in darker color and swollen flesh of the damaged berries as well as chemical differences including decreased sugar content and off aromas deduced to be a result of trans-2-octenal and trans-2-decenal presence (Weber et al., 2021). Similarly, research has identified olives, apples, and peppers to be negatively impacted by BMSB infestation identifying secondary metabolites (phenols) to be impacted from BMSB infestation and feeding (Ivancic et al., 2022; Zamljen et al., 2021a; Zamljen et al., 2021b).

2.5.2 Green Stink Bugs

Chinavia hilaris (Say), or the green stink bug, is native to North America and causes economic injury to a variety of agricultural commodities including soybean crop (Kamminga et al., 2012). Green stink bugs are generally 14 to 19mm long with bright green coloring and are visually similar to *Nezara viridula* (L.), the southern green stink bug, which is also native to the region (Gomez & Mizell, 2008). The green stink bug was one of the most common stink bug species in soybean crop fields in southeast Virginia in

2005 and 2006, but has been joined by the invasive BMSB, which has since outnumbered native stink bugs in most crops in much of the mid-Atlantic region (Nielsen et al. 2011; Bakken et al., 2015).

2.6 Domestic Edamame Sensory and Flavor Research

Table 2-1: Characteristics of vegetable soybean (edamame) varieties developed and released in the United States.

VARIETY[1]	DAYS TO R6 AFTER PLANTING[2]	CHARACTERISTICS	DEVELOPERS	REFERENCE
MOON CAKE	124[3]	White flowers; Gray pubescence	United States Department of Agriculture Agricultural Research Services (USDA-ARS)	Devine et al., 2006
UA KIRKSEY	115	White flowers; Gray pubescence; Tan pod walls	University of Arkansas	Chen et al., 2014
UA MULBERRY	115	Purple flowers; Tawny pubescence; Tan pod walls	Arkansas Agricultural Experiment Station	Moseley et al., 2018
OWENS	89	White flowers; Tawny pubescence; Brown pod walls	Virginia State University, Agricultural Research Station and the USDA-ARS	Mebrahtu et al., 2007
VT SWEET	129	Purple flowers; Gray pubescence; Tan pod wall	Virgina Tech	Zhang et al., 2021

[1] Select edamame varieties developed in the U.S.; [2] Approximation from literature; [3] Data from Zhang & Kyei-Boahen (2007).

2.6.1 Sensory Research

Sensory research on edamame is limited, especially within the American market and with a focus on the American consumer. Published research within this realm varies in methods and objectives. Consumer acceptability and hedonic scales have been reported to determine desired quality attributes and overall acceptability in regions throughout the country (Carneiro et al., 2021a; Flores et al, 2019; Ogles, 2016; Wszelaki et al., 2005). Ogles (2016) completed sensory evaluation of 6 edamame varieties (Sayamusume, Midori Giant, Chiba Green, Owns, Mojo Green, and Gardensoy 51) grown and evaluated in Alabama (USA), across 2 growing years (2015, 2016) to identify preference across the varieties. The research determined Sayamusume, Midori Giant, Chiba Green, and Mojo Green to be the preferred edamame varieties as panelists found Owens and Gardensoy 51 to be difficult to peel, lacking in sweet taste, and ‘hard’, ‘starchy’, and ‘mealy’ in texture (Ogles, 2016). Carneiro et al. (2021a) reported successfully guiding domestic edamame breeders towards varieties consumers found more acceptable based on sensory evaluation of edamame. This work utilized 24 edamame genotypes in year one (2018) and 10 edamame genotypes in year two (2019) finding ‘salty’ and ‘sweet’ sensory attributes to be highly associated with high consumer acceptability while ‘bitter’ was highly associated with low consumer acceptability (Carneiro et al., 2021a).

Applications of descriptive sensory methods for edamame have been reported using various methods including check-all-that-apply (CATA), free choice profiling, Quantitative Descriptive Analysis (QDA), and Sensory Spectrum technique to better understand the sensory attributes of edamame (Carneiro et al., 2021a; Flores et al., 2019; Guo et al., 2022; Krinksy et al., 2006; Wszelaki et al., 2005). Studies have consistently demonstrated noticeable differences in sensory characteristics exist in edamame based on various factors including edamame variety, harvest time, and cooking methods (Carneiro et al., 2021a; Flores et al., 2019; Guo et al., 2022; Krinksy et al., 2006; Ogles, 2016; Wszelaki et al., 2005).

Though published descriptive sensory research is currently minimal in literature for edamame, descriptive analysis (DA) research focused on domestic edamame is even more scarce. Wszelaki et al. (2005) was able to complete affective testing for 6 edamame varieties (Sapporo Midori, White Lion, Early Hakucho, Sayamusume, Misono Green, Kenko) as well as a DA panel with all 6 edamame varieties grown in Ohio (USA). This work showed preliminary evidence of sensory differences including consumer preferences based on edamame variety, but the DA panel only measured 4 characteristics (‘beaniness’, ‘chewiness’, ‘nuttiness’, ‘sweetness’) in the study. Krinksy et al. (2006) developed a lexicon for edamame which consisted of 14 terms but utilized edamame that was grown and processed in Aisa. The study recognized their lexicon would likely be insufficient after market changes and continued product development occurred but provided a previously undeveloped starting point (Krinksy et al., 2006). Carneiro et al.

(2021a) utilized this lexicon in a CATA to collect information on descriptive terms to describe domestically bred and grown edamame for 10 edamame genotypes grown in 4 locations (Blacksburg, VA; Painter, VA; Portageville, MO; and Stoneville, MS) but did not include quantifiable results in conjunction with the CATA terms. However, both Krinksy et al. (2006) and Carneiro et al. (2021a) were able to show that the 14 term CATA is not in excess but may be lacking to fully describe the domestic edamame market especially as the supply chain continues to develop. Recent advances in edamame breeding and implementation into local agriculture has created an expansion of the domestic edamame market, which has not yet been investigated in descriptive sensory.

Flores et al. (2019) utilized 3 edamame varieties (Giant Midori, Kuroshinja, ButterBean) and free choice profiling to evaluate quality of the varieties after growing in Northern California. The study identified flavor to be the leading trait to ensure overall liking and purchasing intent with sweet taste being the main factor (Flores et al., 2019). Of the 3 edamame varieties included, Giant Midori was concluded to be the most liked variety with highest sweet taste, free sugar content, and harder texture compared to Kuroshinja and ButterBean (Flores et al., 2019).

Guo et al. (2022) completed QDA along with volatile analysis through head space solid-phase micro-extraction and gas chromatography with mass spectrometry (HS-SPMEGC-MS), amino acid, chemical, and electronic tongue analysis for 3 edamame varieties (Taiwan 292, Xin 3, Suxin 6) in China to investigate the sensory properties and flavor composition of commonly grown edamame varieties. The research identified forty-one volatile compounds with major flavor components identified from 7 volatile compounds (1-octen-3-ol, hexanal, (Z)-2-heptenal, 2-octene, nonanal, (Z)-2-decenal, and 3,5-octadien-2-one) (Guo et al., 2022). Sensory evaluation in the study found taste and texture to be the most important sensory attributes for panelists while aromas were not found to be significantly different across the edamame varieties (Guo et al., 2022).

Though sensory research to understand edamame quality characteristics and consumer acceptability is still somewhat limited, published work has started to show trends that will be helpful to guide future work. Sensory attributes have been reported to be noticeably different among varieties, harvest time, and cooking methods and hedonic results often report a favored variety among participants, indicating additional sensory testing to investigate consumer preferences, consumer experience, and edamame quality will be useful in guiding the domestic edamame market to higher quality and overall acceptable edamame crop and products (Carneiro et al., 2021a; Flores et al., 2019; Guo et al., 2022; Krinksy et al., 2006; Ogles, 2016; Wszelaki et al., 2005).

2.6.2 Flavor Research

Pairing sensory research with flavor analysis, researching volatile compounds, can provide additional insight into complex food matrixes that a single analysis or approach might miss. QDA along with volatile analysis through head space solid-phase micro-extraction and gas chromatography with mass spectrometry (HS-SPMEGC-MS) of 3 edamame varieties from China found that while 7 volatile compounds were identified as major flavor components of the edamame samples, sensory panelists did not find aroma to be significantly different across the samples (Guo et al., 2022). In fact, taste (defined as the sensations of sweet, sour, salty, bitter, and umami detected on the tongue) and texture were identified as the most important sensory attributes for panelists (Guo et al., 2022).

Current research into volatile and aroma active compounds in edamame is also extremely limited making understanding the role these volatiles play in the quality attributes of soybean and edamame difficult. Solid phase microextraction (SPME) with gas chromatography-mass spectrometry (GC-MS) has been utilized in various studies to explore volatile compounds in soybean varieties including mature soybean, roasted soybean, and vegetable soybean, or edamame (Boué et al., 2003; Cai et al., 2021; Guo et al., 2022; Kim et al., 2020). Of these, Boué et al. (2003) is the only published research on volatile compounds in soybean that utilized domestically grown samples (New Orleans, LA, USA). This work investigated soybean harvested at R6, R7, and R8 maturity finding 49 volatile compounds and concluding hexanal, (E)-2-heptenal, (E)-2-octenal, ethanol, and 1-hexanol to be indicators of seed maturity (Boué et al., 2003).

Soybean samples from the United States and Canada were included in a study utilizing SPME-GC-MS to identify volatile compounds in soybean from various countries (Kim et al., 2020). The research team utilized 36 soybean samples from Korea, China, and North America and were able to identify 146 volatile compounds which were analyzed to find that the volatile compounds present in soybean can indicate the geographical origin of the soybean samples through multivariate data analysis (Kim et al., 2020). Furthermore, research has shown that some volatile compounds could be determined by genes potentially acting as flavor fingerprints for the variety (Yuan et al., 2021). Yuan et al. (2021) discovered this using headspace-gas chromatography-ion mobility spectrometry, analyzed 30 edamame varieties from China. Knowing genetic factors and geographical origin of varieties can impact final product quality in such drastic ways further confirms the importance of variety selection. Selecting edamame varieties suitable for planting will be crucial to the future of edamame. However, research on edamame varieties will be necessary to understand the quality characteristics associated with each variety.

Though identification of volatile compounds has been completed in various studies (Guo et al., 2022; Wu et al., 2009; Yuan et al., 2021), research on domestically bred and grown varieties remains under published even though understanding the quality attributes of any edamame variety has been shown to be necessary for effective and efficient implementation into the supply chain. Investigation into aroma active compounds of domestic edamame varieties would support local breeding programs and growers to understand the volatile compounds presences along with the impact they may have on sensory attributes of the final product. Investigation of domestic commercial edamame varieties would be the first of its kind. Though commonly planted edamame varieties vary by region throughout the country and by grower preferences, researching quality characteristics of a selection of these varieties will provide growers and breeders with details around sensory attributes not previously studied. In addition, understanding the implications of insect damage to edamame flavor quality is needed to assist in guiding integrated pest management of domestic edamame production and establishing pest damage criteria for processors.

2.6.3 Future Research

Though the domestic edamame market has continued to grow with consumer interest and demand (Jiang et al., 2018; Zeipina et al., 2017), research to support the supply chain is lacking. Though sensory and flavor work for soybean and edamame can be found in literature, findings confirm that cultivar, growing location, harvest time, and other factors will greatly influence quality characteristics and volatile compounds presence meaning research findings are not always transferable when changing variables such as variety, location, and harvest time. Published literature utilizing varieties from Asia or research completed internationally provides important insight into the complexities of soybean and vegetable soybean as commodity and specialty crops but highlights the need for additional research focused on the domestic varieties, consumers, and supply chain. Sensory and flavor analysis of domestically bred and grown edamame varieties will be crucial to fully understand the quality attributes associated with a developed variety or growing location and will be necessary for continued improvement of the domestic edamame market (Carneiro et al., 2021a). Volatile analysis and sensory evaluation of domestic edamame varieties, especially VT Sweet, is currently limited to initial sensory acceptability testing (Carneiro et al., 2021a). Further research into volatile compounds and sensory attributes of VT Sweet and other commonly utilized varieties will provide further insight into the sensory differences across edamame that has not been previously completed.

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Chapter 3

3. Determining volatile compounds and their relation to consumer acceptability in domestic edamame

This chapter was in preparation for submission in *ACS Food Science and Technology*.

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Abstract: Edamame is highly nutritious and of high economic value. While it has the potential to be integrated into local, domestic agriculture, current U.S. consumption of edamame relies heavily on imported varieties and products. Domestic edamame breeding has been working to develop varieties that have both satisfactory agronomical traits and consumer expectations. Two years of sensory testing was conducted to gauge consumer acceptability of locally grown edamame. The second-year work included 10 genotypes grown in 2019 at 4 U.S. locations using commercial variety UA Kirksey as a reference. The aroma attributes of these samples were analyzed for volatile compounds using solid-phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS) and investigated for correlation with sensory attributes from consumer acceptability research. Significant differences in compound relative content were found to be significant ($p<0.05$) by edamame genotype and growing location. Correlation of volatile compounds with sensory data shows limited strong correlations ($>|0.5|$) with sensory attributes and only one significant compound, 1-octanol, shows strong correlation with consumer liking. While differences among edamame genotypes exist in volatile compounds, results in this study do not suggest volatile compounds correlate to consumer liking or disliking based on the presence or content of the compounds included in this research.

Keywords: edamame, vegetable soybean, volatile analysis, consumer liking, plant breeding, food development

3.1 Introduction

Vegetable soybean [*Glycine max (L.)* Merr.], also commonly known as edamame, has been consumed in Asia for centuries and increasing in popularity in North America in recent years (Jiang et al., 2018). Globalization as well as the high nutritional value has likely contributed to the rising interest in edamame (Jiang et al., 2018; Ogles et al., 2016; Garber & Neill, 2019). This increased demand for edamame has added strain on the Asian edamame supply and created opportunity for domestic edamame production to develop and alleviate this strain while supporting development of edamame varieties more suited to the needs of local growers and consumers (Garber & Neill, 2019).

Edamame cultivars from Asia and imported edamame products display inferior agronomic qualities including less desirable plant structure, disease susceptibility, and poor performance for growers and consumers in the U.S. (Jiang et al., 2018; Zhang et al., 2021). Breeding programs will find these agronomic characteristics important factors as well as sensory attributes and consumer acceptability to be vital considerations for successful cultivar development (Carneiro et al., 2020).

Sensory work has proven helpful in plant breeding through identifying flavor and sensory attributes to help guide breeding programs. Carneiro et al. (2021) was able to successfully utilize consumer acceptability testing of edamame genotypes and create a decision tree to guide domestic edamame breeding programs. Though proven successful, sensory work often requires resources that can be difficult to obtain, such as a large number of participants and a substantial amount of sample required. Finding alternatives to sensory work could help support breeding programs by finding physical or chemical traits that relate to sensory attributes. Testing or analyzing crops for the specified traits would often be less time and resource intensive.

Identifying chemical compounds correlating with sensory attributes has potential to provide more continuous support of these breeding programs with less demand for resources and time required for sensory work. When looking into flavor and consumer liking, identification of volatile compounds to indicate consumer liking has not been explored for domestic edamame as far as the authors can find. If volatile compounds could be identified and correlated with sensory data, favorable and unfavorable compounds could be identified and used as chemical indicators of consumer acceptability during breeding and genotype analysis.

Release of new breeding lines often lacks detailed data on quality attributes including volatile analysis. With the domestic edamame market growing with new breeding lines and products, exploration of the volatile compounds in these products would help support the sensory and consumer experience while identifying quality attributes of developing or newly released edamame varieties.

Volatile analysis of edamame varieties has been previously completed across numerous studies with focuses on volatile formation, sensory attribute investigation, and volatile fingerprints in edamame varieties from Asia (Arikit et al., 2011; Guo et al., 2022; Wu et al., 2009; Yuan et al., 2021). Guo et al. (2022) researched 3 edamame varieties (Taiwan 292, Xin 3, Suxin 6) popular in China, researching volatile compounds and sensory proprieties of the edamame varieties. The work identified 41 volatile compounds, concluding 7 volatile compounds to be main flavor contributors (1-octen-3-ol, hexanal, (Z)-2-heptenal, 2-octene, nonanal, (Z)-2-decenal, and 3,5-octadien-2-one) and each variety to have significant differences (Gu et al., 2022). Similarly, Yuan et al. (2021) researched 30 edamame varieties from Tiawan detecting 93 volatile compounds, concluding that the composition and concentration of volatiles were different by variety. Arikit et al. (2011) and Wu et al. (2009) researched edamame varieties from across Asia and identified differences in genes that will contribute to differences in volatile compound formations.

Volatile analysis of domestic soybean has also been completed by Boué et al. (2003) who specifically focused on volatile changes throughout maturity (R6, R7, R8), using a soybean variety, Pioneer 95B41, grown in New Orleans, LA (USA) for their work. An additional study by Ravi et al. (2019) used an electronic nose to identify volatile compounds in domestic soybean seed. Of the 5 soybean seed varieties included in the study (UA5014C, UA5414RR, JTN-5503, JTN-5110, JTN-5203), the researchers concluded UA5014C and UA5414RR to have comparable volatile profiles while JTN-5503, JTN-5110, JTN-5203 each had distinct compositions of volatile compounds (Ravi et al., 2019). Investigation of volatile profiles of domestic edamame varieties has not yet been published in literature. However, based on previous findings, differences in volatile compounds are expected to be seen across domestic edamame varieties.

This work looked to determine volatile compound presence across edamame varieties and to determine correlations with consumer sensory acceptability data conducted in previous studies to determine possible volatile compounds as indicators of sensory attributes including overall liking. Similar work has been previously published but has focused on domestic and foreign soybean varieties and foreign edamame varieties. Research into volatile compounds of domestic edamame varieties has not been previously published in literature. Flavor chemistry methods were used to analyze volatile compounds for qualitative and quantitative presence in the edamame varieties to identify differences among varieties. Previously published consumer acceptability sensory data, by Carneiro et al. (2021), of the selected genotypes was utilized to determine possible correlations in compound presence and consumer acceptability.

3.2 Methods

3.2.1 Samples

Ten edamame genotypes were utilized for this study including R14-16195; R14-6238; R14-6450; R15-10280; R16-5336; V10-3653; V16-0524 (VT Sweet); V16-0528; V16-0547 and UA Kirksey (Lord et al., 2021). Other than UA Kirksey, a commercially available edamame variety, edamame genotypes included were part of breeding programs at Virginia Tech (genotypes named with ‘V’) and University of Arkansas (genotypes named with ‘R’). UA-Kirksey was used as a reference in the study due to its presence in the commercial market while the remaining genotypes were selected based on agronomic characteristics, breeder input, and consumer acceptability data. Edamame pods were grown in Blacksburg, VA; Painter, VA; Portageville, MO; and Stoneville, MS by growers with comparable growing and harvesting experiences about edamame. Harvested occurred between August and October of 2019 when the edamame was appropriately mature, and weather and resources allowed for harvest and processing to occur. Edamame harvest is most optimal during the early R6 stage which is characteristic of bright green pods and beans with peak pod fill and sweetness (Xu et al., 2015; Yu et al., 2022). Edamame pods were separated from stems and leaves before transportation in coolers with ice packs to be delivered within 24 hours from harvest to the pilot plant in the department of Food Science and Technology at Virginia Tech, Blacksburg campus. Samples were processed timely upon arrival to ensure processing was completed within 48 hours of cold storage followed by harvest. Edamame processing was performed according to our published protocol with modifications (Carneiro et al., 2021). Specifically, pods were first rinsed in tap water (Blacksburg, VA, USA) to remove dirt and soil from the pods as well as removing any remaining leaves or excess stems, followed by a second rinse with distilled water. Edamame pods were then transferred to metal baskets, blanched in water (98 ± 1°C, 1 minute) in a steam kettle (Legion Utensils Co., Long Island City, NY, USA), and then chilled in an ice bath (4 ± 1°C, 2 minutes) to stop the cooking process. Edamame pods were then dried using salad spinners, manually peeled, and stored at -80°C prior to volatile analysis.

3.2.2 Volatile Analysis

Volatile analysis was conducted using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) to determine key volatile compounds and differences in the samples by genotype. A total of 40 edamame samples were analyzed using 10 edamame genotypes (R14-16195; R14-6238; R14-6450; R15-10280; R16-5336; V10-3653; V16-0524 (VT Sweet); V16-0528; V16-0547; UA-Kirksey) grown in 4 locations (Blacksburg, VA; Painter, VA; Portageville, MO; Stoneville, MS). Edamame was grown in 2019, by growers with comparable growing and harvesting experiences about edamame, and harvested and processed as described above. For each edamame sample, about 20 grams of frozen (-80°C) edamame beans were ground in edamame dedicated Krups Coffee and Spice Grinders (Krups, Solingen, Germany) with around 40 grams of dry ice to create a free-flowing powder. About 2grams of ground samples were weighed into each clean, clear, 20mL SPME vials (Supelco, Bellefonte, PA, USA) in triplicates. Vials were then capped with a PTFE-lined cap (Supelco, Bellefonte, PA, USA). Sample weighing and vial capping occurred in a glovebox flushed with nitrogen to reduce oxygen exposure and relative humidity in the sampling environment. Prior to analysis, 1μL of 8.03 μg/μL of 3-hexanol (Sigma-Aldrich, St. Louis, MO, USA) in methanol was added to each vial, directly through the cap as an appropriately selected internal standard.

An Agilent Gas Chromatography 6890N equipped with 5975B Mass Spectrum Detector (Agilent Technologies, Santa Clara, CA, USA) and Leap Technologies CTC PAL Autosampler (Trajan Scientific and Medical, Ringwood, Vic 3134, Australia) was used for analysis. Samples were incubated at 45ºC with 30 minutes of adsorption time at a rotation speed of 250 rpm. Dedicated 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers (Stableflex, Supelco, Bellefonte, PA, USA) were used for extraction for 30 minutes. The GC injection was completed in hot splitless mode to increase the signal abundance of the analysis. The oven temperature was programmed for an initial temperature of 40ºC was held for 5 minutes, then increased by 5ºC per minute for 20 minutes until a temperature of 225ºC was reached. Separation of volatile compounds occurred using a DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) using helium at 2.2mL per minute as the carrier gas. Volatile compounds were initially identified by the NIST library and quantified through manual integration of peak areas on ChemStation in the Enhanced Data Analysis view (Agilent Technologies, Santa Clara, CA, USA). These compounds were further confirmed through calculation of the retention index of each compound using *n*-alkane (C_7-C_{30}) (Sigma-Aldrich, St. Louis, MO, USA) diluted to 10µg/mL in hexane and the formula derived by Van den Dool and Kratz (1963). Peak area ratios of each volatile compound were calculated by dividing each peak area by the peak area of the internal standard. Relative content was calculated by multiplying the peak area ratio by the ratio of the amount of internal standard to that of the sample then multiplying by one thousand.

$$Relative\ content\ \left(\frac{\mu g}{kg}\right) = \left(\frac{Peak\ Area_{target\ compound}}{Peak\ Area_{corresponding\ IS}}\right) \times \left(\frac{amount\ of\ internal\ standard\ (\mu g)}{amount\ of\ sample_{fresh\ weight}\ (g)}\right) \times 1000$$

3.2.3 Sensory Analysis

Acceptability was assessed for previously mentioned 10 genotypes from 4 growing locations across 3 states in 2018 and 2019. Samples were presented in a balanced incomplete block design allowing for direct comparison between the genotypes. Prior to sensory evaluation, samples were prepared as described by Carneiro et al. (2021). Explicitly, frozen edamame beans were moved to a refrigerator (4°C) for 4-6 hours and then microwaved in a glass container covered with a paper towel for 1.5 minutes and then rested for one minute. Upon cooling, cooked beans were distributed to plastic sensory cups marked with 3-digit codes and refrigerated until served to panelists, who were recruited from the Virginia Tech community and surrounding area. A 9-point hedonic scale was used for overall-liking, aroma, appearance, taste, and texture, followed by check all that apply (CATA) approach for further investigation. Panelists were only allowed to participate and provide ratings once a week for 4 consecutive weeks.

3.2.4 Statistical Analysis

Statistical analysis was completed in JMP Pro 16.0.0 (SAS Institute Inc., Cary, North Carolina, U.S.) and R Studio. Multivariate analysis of variant (MANOVA) was used to determine any significant effects across edamame genotypes ($p<0.05$) using growing location as additional replications. Analysis of variance (ANOVA) was used to determine volatile compounds of significance followed by Tukey's HSD procedure to identify differences across samples ($p<0.05$) as well as across locations by genotype and volatile compound. Principal component analysis (PCA) was then used to visualize the distinctions of volatile compounds, sensory scores by edamame genotype. Pearson's correlation was employed to identify correlations between volatile compounds and sensory attributes.

3.3 Results

GC-MS identified 16 volatile compounds in the domestic edamame samples which may contribute to aroma and flavor of edamame genotypes (Figure 3-1)(Table 3-1). The majority of the compounds centers around descriptors such as “green”, “fatty”, “fruity” and “mushroom”, indicating those attributes being primary volatile descriptors in our measured edamame breeding lines. These compounds had been previously identified in volatile analysis of edamame and soy in numerous studies (Boué et al., 2003; Cai et al., 2021; Guo et al., 2022; Kim et al., 2020; Ravi et al., 2019; Yuan et al., 2021). Of these compounds, 14 compounds were significantly different ($p<0.05$) across the 10 edamame genotypes analyzed in this work (Table 3-2). Pentanal and 2-pentylfuran were the only compounds not found to be significantly different by edamame genotype.

Figure 3-1: Volatile compounds of interest identified in this work with aroma descriptors for each compound, color coded by compound classification.
Descriptors obtained from https://www.thegoodscentscompany.com

Table 3-1: Volatile compounds of interest and aroma descriptors identified through GC-MS in edamame in this work using ten edamame genotypes (R14-16195; R14-6238; R14-6450; R15-10280; R16-5336; V10-3653; V16-0524 (VT Sweet); V16-0528; V16-0547; UA-Kirksey) each grown at four growing locations (Blacksburg, VA; Painter, VA; Portageville, MO; Stoneville, MS).

Calculated RI*	**Reference RI**	**Compound**	**Descriptors[1]**
~	935[2]	Pentanal	Fermented, Bready
1073	1084[2]	Hexanal	Fresh, Green
1180	1174[2]	Heptanal	Fresh, Green
1229	1227[3]	2-Pentylfuran	Fruity, Green
1257	1255[2]	1-Pentanol	Pungent, Fermented
1289	1280[2]	Octanal	Waxy, Citrus
1325	1305[4]	(E)-2-Heptenal	Pungent, Green
1339	1332[5]	6-Methyl-5-hepten-2-one	Citrus, Green
1357	1360[2]	1-Hexanol	Green, Fruity
1393	1385[2]	Nonanal	Citrus, Cucumber
1396	1395[5]	3-Octanol	Earthy, Mushroom
1428	1408[2]	(E)-2-Octenal	Fatty, Green
1452	1451[2]	1-Octen-3-ol	Mushroom, Earthy
1563	1553[2]	1-Octanol	Waxy, Green
1617	1620[6]	(E)-2-Octen-1-ol	Green, Citrus
1644	1630[7]	(Z)-2-Decenal	Tallow, Fatty

*Using a DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) with helium (2.2mL per minute) as the carrier gas.
[1]https://www.thegoodscentscompany.com/; [2]https://www.flavornet.org/flavornet.html; [3]Jakobsen, H.B., Hansen, M., Christensen, M.R., Brockhoff, P.B., & Olsen, C.E. (1998). Aroma Volatiles of Blanched Green Peas (Pisum sativum L.). *J. Agric. Food Chem.* 46, 9, 3727–3734 https://doi.org/10.1021/jf980026; [4]Rychlik, M., Schieberle, P., and Grosch, W. (1998). Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Ordorants. TUM. Garching, Germany; [5]Kesen, S., Kelebek, H., Sen, K., Ulas, M., & Selli, S. (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Research International, 54 (2), 1987-1994. https://doi.org/10.1016/j.foodres.2013.09.005; [6]Cho, I.H., Choi, H.-K., & Kim, Y.-S. (2006). Difference in the Volatile Composition of Pine-Mushrooms (Tricholoma matsutake Sing.) According to Their Grades. *J. Agric. Food Chem,* 54, 13, 4820–4825. https://doi.org/10.1021/jf0601416; [7]Lin, J. and Rouseff, R.L. (2001), Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time–intensity GC–olfactometry and GC–MS. *Flavour Fragr. J.*, 16: 457-463. https://doi.org/10.1002/ffj.1041

Table 3-2: Relative content (µg/kg), expressed as mean ± SE, of each volatile compound of interest by edamame genotype.

Compound	**UA Kirksey**	**R14-16195**	**R14-6238**	**R14-6450**	**R15-10280**	**R16-5336**	**V10-3653**	**V16-0524**	**V16-0528**	**V16-0547**
Pentanal*	168.34 ± 10.93	222.90 ± 36.58	189.71 ± 16.65	381.90 ± 164.07	237.47 ± 28.49	344.38 ± 123.10	515.93 ± 225.75	316.50 ± 23.32	337.43 ± 15.02	285.94 ± 35.55
Hexanal	687.26 ± 45.07^{c}	1877.08 ± 435.49^{abc}	1119.01 ± 98.74^{bc}	2443.95 ± 406.19^{ab}	1702.74 ± 161.33^{abc}	1442.25 ± 201.71^{bc}	2950.89 ± 506.25^{a}	2077.21 ± 212.57^{ab}	1923.52 ± 166.56^{abc}	2232.56 ± 362.02^{ab}
Heptanal	14.43 ± 1.99^{c}	39.22 ± 7.63^{abc}	29.35 ± 2.92^{bc}	64.29 ± 7.00^{a}	43.63 ± 4.58^{abc}	37.21 ± 9.18^{abc}	60.22 ± 9.52^{a}	50.71 ± 5.68^{ab}	48.78 ± 2.73^{ab}	60.59 ± 8.80^{a}
2-Pentylfuran*	6.13 ± 2.33	5.41 ± 2.83	7.47 ± 3.91	17.59 ± 5.49	62.22 ± 36.13	38.27 ± 35.17	23.50 ± 10.50	4.37 ± 2.29	5.30 ± 2.78	33.52 ± 12.12
1-Pentanol	404.69 ± 38.40^{d}	501.05 ± 38.20^{cd}	511.24 ± 44.49^{bcd}	724.15 ± 54.67^{a}	652.24 ± 37.95^{abc}	508.02 ± 52.27^{bcd}	677.51 ± 53.98^{abc}	644.71 ± 31.94^{abc}	611.98 ± 31.20^{abc}	710.43 ± 54.72^{ab}
Octanal	67.63 ± 7.13^{d}	130.92 ± 23.11^{bcd}	107.72 ± 15.92^{cd}	243.21 ± 14.17^{ab}	170.45 ± 18.73^{abcd}	171.10 ± 26.07^{abcd}	185.58 ± 38.36^{abcd}	232.02 ± 27.24^{abc}	223.21 ± 18.81^{abc}	272.38 ± 59.25^{a}
(E)-2-Heptenal	641.00 ± 47.23^{c}	838.51 ± 135.33^{bc}	1032.74 ± 107.66^{abc}	1329.59 ± 173.83^{abc}	1222.51 ± 121.18^{abc}	1450.66 ± 205.91^{abc}	1193.80 ± 250.87^{abc}	1851.38 ± 229.00^{a}	1647.66 ± 204.16^{ab}	1411.55 ± 276.76^{abc}
6-Methyl-5-hepten-2-one	20.96 ± 2.38^{c}	40.88 ± 6.39^{bc}	48.37 ± 6.94^{bc}	92.99 ± 6.36^{a}	71.87 ± 7.99^{ab}	59.69 ± 12.26^{abc}	59.96 ± 11.47^{abc}	62.91 ± 6.90^{ab}	63.15 ± 6.41^{abc}	97.70 ± 15.99^{a}
1-Hexanol	8.79 ± 2.80^{b}	21.51 ± 6.68^{b}	23.08 ± 4.84^{b}	140.74 ± 42.83^{a}	35.59 ± 11.43^{b}	66.80 ± 18.11^{ab}	50.57 ± 18.13^{b}	37.97 ± 7.72^{b}	30.33 ± 6.72^{b}	39.91 ± 7.67^{b}
Nonanal	21.75 ± 1.67^{b}	48.04 ± 8.10^{ab}	44.95 ± 6.33^{ab}	76.25 ± 7.93^{a}	70.17 ± 6.23^{a}	55.00 ± 11.28^{ab}	65.44 ± 12.92^{a}	62.48 ± 11.84^{a}	48.22 ± 5.22^{ab}	66.86 ± 8.68^{a}
3-Octanol	0.000 ± 0.00^{b}	0.000 ± 0.00^{b}	7.94 ± 4.21^{b}	0.000 ± 0.00^{b}	4.15 ± 2.20^{b}	41.00 ± 15.50^{a}	8.45 ± 4.44^{b}	8.06 ± 4.22^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
(E)-2-Octenal	90.94 ± 10.57^{d}	144.04 ± 27.67^{bcd}	103.98 ± 12.99^{cd}	205.92 ± 18.76^{abcd}	121.97 ± 15.89^{cd}	213.44 ± 30.13^{abcd}	249.21 ± 45.92^{abc}	296.23 ± 39.85^{a}	276.08 ± 30.14^{ab}	286.13 ± 56.51^{ab}
1-Octen-3-ol	687.38 ± 78.44^{d}	1152.92 ± 107.87^{cd}	1378.27 ± 108.55^{bcd}	1591.65 ± 278.46^{abc}	1440.39 ± 137.09^{abcd}	2251.55 ± 335.08^{a}	1383.05 ± 131.17^{bcd}	1860.39 ± 156.63^{abc}	1562.99 ± 178.04^{abc}	2180.83 ± 177.34^{ab}
1-Octanol	27.42 ± 5.74^{c}	73.48 ± 6.90^{bc}	141.17 ± 33.28^{ab}	133.95 ± 15.95^{ab}	194.40 ± 20.32^{a}	103.49 ± 17.34^{bc}	93.66 ± 16.94^{bc}	112.61 ± 11.83^{b}	98.08 ± 14.60^{bc}	138.69 ± 15.74^{ab}
(E)-2-Octen-1-ol	182.69 ± 17.48^{c}	260.23 ± 32.43^{bc}	467.46 ± 76.69^{abc}	436.84 ± 79.04^{abc}	596.03 ± 52.29^{a}	470.19 ± 68.61^{ab}	354.45 ± 69.47^{abc}	458.97 ± 63.49^{abc}	410.58 ± 74.37^{abc}	553.41 ± 63.33^{a}
(Z)-2-Decenal	8.08 ± 2.33^{b}	26.05 ± 3.31^{ab}	39.94 ± 7.18^{ab}	37.35 ± 8.59^{ab}	52.48 ± 2.44^{a}	35.16 ± 9.03^{ab}	31.52 ± 7.95^{ab}	30.52 ± 9.32^{ab}	37.70 ± 7.46^{ab}	49.45 ± 11.73^{a}

Results within each compound with different letters indicate significant differences between genotypes ($p<0.05$) based on Tukey's HSD.
*Compounds not found to be significantly different across genotypes.

By edamame genotype, hexanal, 1-octen-3-ol, and (E)-2-heptenal were consistently found to have the three highest relative contents across genotypes. Hexanal had the highest relative content in 7 of the edamame genotypes (R14-16195; R14-6450; R15-10280; V10-3653; V16-0524; V16-0528; V16-0547), second highest relative content in 2 genotypes (UA-Kirksey; R14-6238), and the third highest relative content in one genotype (R16-5336) ranging from 687.25 to 2950.89 μg/kg. 1-Octen-3-ol, similarly, had the highest relative content in 3 edamame genotypes (UA-Kirksey; R14-6238; R16-5336), the second highest relative content in 6 genotypes (R14-16195; R14-6450; R15-10280; V10-3653; V16-0524; V16-0547) ranging from 687.38 to 2251.55 μg/kg. (E)-2-Heptenal had the second highest relative content for 2 genotypes (R16-5336; V16-0528), and the third highest relative content in 8 genotypes (UA-Kirksey; R14-16195; R14-6238; R14-6450; R15-10280; V10-3653; V16-0524; V16-0547) ranging from 641.00 to 1851.39 μg/kg. UA-Kirksey consistently had the lowest relative content compared to the other 9 edamame genotypes for all significant compounds in the study. These results suggest the significant influence of genotype on volatile profile of edamame.

Of the 16 compounds identified in this study, 15 compounds were found to be significantly different ($p<0.05$) in relative content based on growing location (Table 3-3). Pentanal was the only compound found to not be significantly different in relative content by growing location. By growing location, hexanal had the highest relative content in Painter, VA; Portageville, MO; and Stoneville, MS with 1-octen-3-ol having the second highest and €-2-heptenal having the third highest relative content. In Blacksburg, the same compounds had the highest relative content with 1-octen-3-ol having the highest followed by hexanal a€(E)-2-heptenal. Location effects are not uncommonly observed in volatile profiles of fresh produce, because many factors will impact volatile generation pathways. For example, the climate, soil, latitude, daylight, and more, could all have contributed to the differences.

Sensory acceptability results from Carneiro et al. (2021) (Table 3-4) found edamame genotypes to be significantly different ($p<0.05$) in 'liking', 'sweetness', 'appearance', 'taste', and 'texture' by edamame genotype but did not find significant differences based on 'aroma'.

Table 3-3: Relative content (μg/kg), expressed as mean ± SE, of edamame genotypes for each compound of interest by growing location.

Compound	Blacksburg, VA	Painter, VA	Stoneville, MS	Portageville, MO
Pentanal*	340.90 ± 80.33	222.23 ± 16.80	330.96 ± 62.13	306.11 ± 22.07
Hexanal	1278.71 ± 96.27^{c}	2068.63 ± 243.87^{ab}	1542.82 ± 108.85^{bc}	2492.43 ± 250.94^{a}
Heptanal	28.03 ± 2.90^{c}	54.39 ± 4.601^{ab}	39.30 ± 2.71^{bc}	57.66 ± 5.47^{a}
2-Pentylfuran	29.56 ± 14.87^{a}	8.35 ± 3.13^{a}	3.17 ± 1.20^{a}	40.39 ± 14.68^{a}
1-Pentanol	577.79 ± 22.48^{b}	601.95 ± 33.65^{ab}	505.15 ± 16.95^{b}	693.53 ± 39.24^{a}
Octanal	127.98 ± 10.12^{b}	178.36 ± 16.36^{ab}	166.10 ± 11.59^{b}	249.24 ± 28.61^{a}
(E)-2-Heptenal	851.37 ± 75.06^{b}	1527.95 ± 125.80^{a}	1221.63 ± 84.55^{ab}	1446.82 ± 151.55^{a}
6-Methyl-5-hepten-2-one	48.90 ± 5.40^{b}	62.48 ± 6.15^{ab}	56.72 ± 4.16^{ab}	79.28 ± 8.17^{a}
1-Hexanol	35.06 ± 3.88^{b}	82.11 ± 19.19^{a}	33.19 ± 3.70^{b}	31.74 ± 11.38^{b}
Nonanal	36.69 ± 3.11^{b}	68.88 ± 5.94^{a}	41.76 ± 2.47^{b}	76.33 ± 6.81^{a}
3-Octanol	0.79± 0.47^{b}	2.83 ± 1.58^{b}	24.22 ± 4.66^{a}	0.000 ± 0.00^{b}
(E)-2-Octenal	117.33 ± 7.81^{b}	205.82 ± 20.02^{a}	198.31 ± 15.17^{a}	273.72 ± 30.59^{a}
1-Octen-3-ol	1470.99 ± 137.16^{ab}	1940.60 ± 167.84^{a}	1298.72 ± 74.96^{b}	1485.46 ± 108.40^{ab}
1-Octanol	84.17 ± 10.65^{b}	124.65 ± 11.16^{ab}	85.24 ± 5.38^{b}	152.73 ± 17.16^{a}
(E)-2-Octen-1-ol	326.38 ± 25.00^{b}	528.22 ± 45.36^{a}	324.62 ± 24.77^{b}	497.11 ± 45.19^{a}
(Z)-2-Decenal	14.38 ± 3.28^{b}	47.56 ± 4.13^{a}	38.88 ± 2.41^{a}	38.47 ± 6.67^{a}

Results within each compound with different letters indicate significant differences between genotypes ($p<0.05$) based on Tukey's HSD.

*Compounds not found to be significantly different across locations.

Table 3-4: Sensory scores (mean ± SD) by attribute for each edamame genotype from Carneiro et al. (2021).

Attribute	UA Kirksey	R14-16195	R14-6238	R14-6450	R15-10280	R16-5336	V10-3653	V16-0524	V16-0528	V16-0547
Liking	5.9 ± 1.7^{b}	6.3 ± 1.8^{ab}	6.2 ± 1.7^{ab}	6.1 ± 1.6^{ab}	6.4 ± 1.7^{a}	6.2 ± 1.6^{ab}	5.8 ± 1.6^{b}	6.1 ± 1.7^{ab}	5.8 ± 1.7^{b}	6.2 ± 1.7^{ab}
Sweetness Intensity	1.8 ± 0.9^{cd}	1.9 ± 0.9^{bcd}	2.0 ± 1.0^{bc}	2.1 ± 0.9^{bc}	2.4 ± 1.2^{a}	2.0 ± 1.0^{bc}	1.6 ± 0.8^{d}	2.0 ± 0.9^{bc}	1.8 ± 0.8^{bcd}	2.1 ± 0.9^{b}
Aroma	5.7 ± 1.5	5.8 ± 1.7	5.7 ± 1.6	5.6 ± 1.7	5.9 ± 1.7	5.8 ± 1.6	5.8 ± 1.5	5.7 ± 1.5	5.6 ± 1.6	5.8 ± 1.6
Appearance	6.0 ± 1.6^{abc}	6.3 ± 1.6^{ab}	6.5 ± 1.7^{a}	5.8 ± 1.7^{bc}	6.1 ± 1.6^{abc}	5.9 ± 1.7^{bc}	6.0 ± 1.6^{bc}	6.1 ± 1.5^{abc}	5.6 ± 1.7^{c}	6.1 ± 1.6^{abc}
Taste	5.7 ± 1.7^{bc}	6.0 ± 1.8^{abc}	6.1 ± 1.7^{ab}	5.9 ± 1.6^{abc}	6.3 ± 1.8^{a}	6.1 ± 1.5^{abc}	5.5 ± 1.6^{c}	5.9 ± 1.6^{abc}	5.6 ± 1.7^{bc}	6.1 ± 1.6^{ab}
Texture	5.8 ± 1.7^{bc}	6.3 ± 1.6^{ab}	6.2 ± 1.8^{abc}	6.3 ± 1.6^{abc}	6.3 ± 1.7^{a}	6.2 ± 1.6^{abc}	5.8 ± 1.6^{c}	6.3 ± 1.6^{ab}	5.9 ± 1.7^{abc}	6.3 ± 1.5^{abc}
Final Determination	Control	Accepted	Accepted	Accepted	Accepted	Accepted	Not Accepted	Accepted	Accepted	Accepted

Attributes were rated by panelists on a scale of 1-9 with the exception of sweetness intensity which was on a scale of 1-5 (Carneiro et al., 2021).
Different letters by column indicate significance ($p<0.05$)

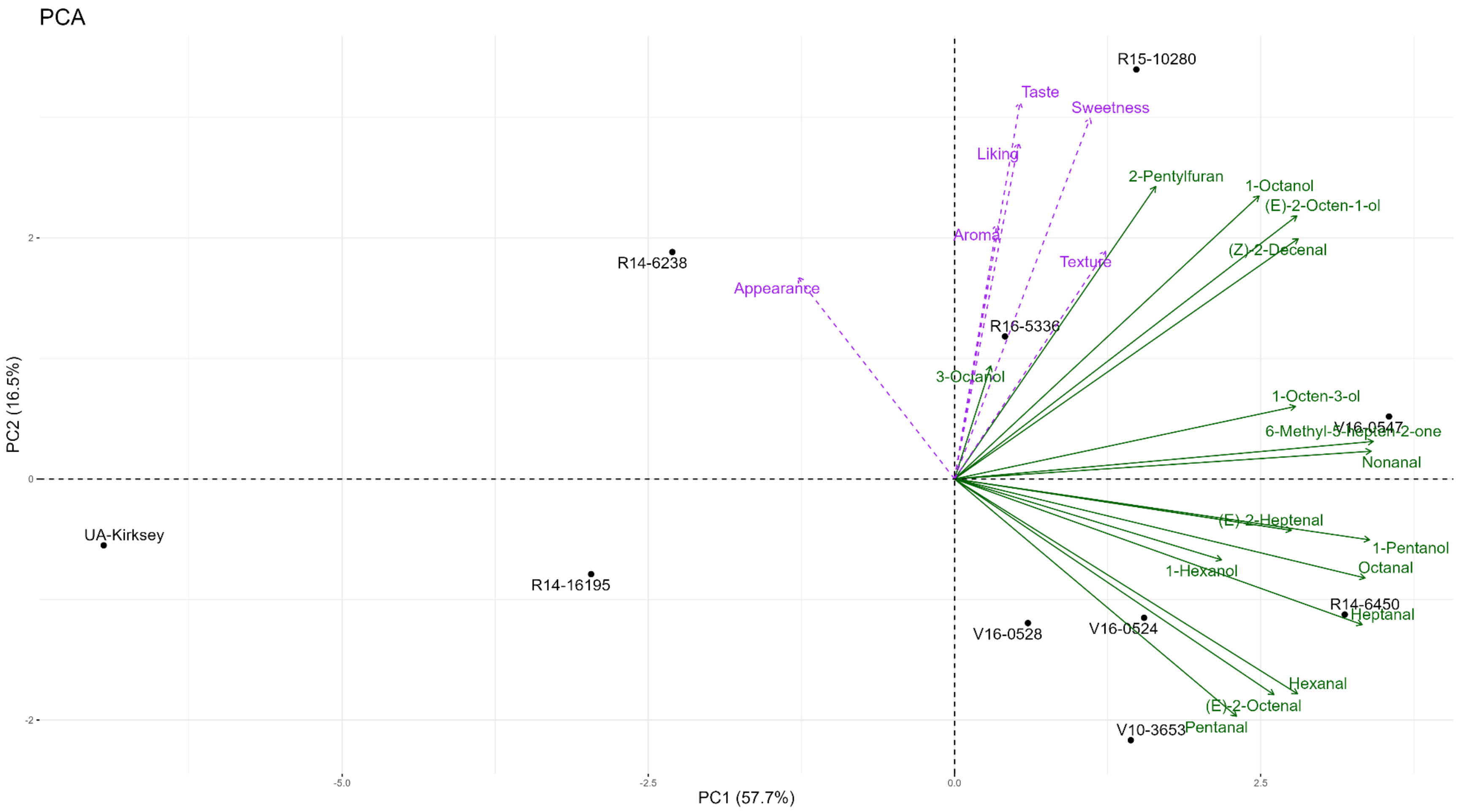

Figure 3-2: Principal component score and loading biplot of edamame genotypes based on relative content of volatile compounds included in the study of edamame on dimensions 1 and 2 with sensory data projected onto the plot.

Solid green lines indicate active variables; Dashed purple lines indicate supplementary variables

Dimension 1 of the PCA plot of the volatile compounds with the sensory attributes projected onto the plot as supplementary data accounts for 57.7% of the variation while dimension 2 accounts for 16.5% (Figure 3-2). The plot shows the sensory attributes to be clustered close together with ‘taste’, ‘liking’, and ‘aroma’ to be especially tightly clustered. Of the 16 volatile compounds, 3-octanol, 2-pentylfuran, 1-octanol, (E)-2-octen-1-ol, (Z)-2-decenal are tightly clustered towards the sensory attributes indicating a likelihood of potentially utilizing those volatile compounds to predict the sensory outcome. Pentanal, (E)-2-octenal, heptanal, and hexanal are clustered almost opposite ‘appearance’ and further away from the remaining sensory attributes than any other compounds. Edamame samples are largely spread across the plot with UA-Kirksey being the furthest from sensory attributes and volatile compounds as well as R14-16195, indicating little association between the 2 edamame genotypes and the attributes. R14-6238 is most closely associated with ‘appearance’ and least associated with pentanal, (E)-2-octenal, and hexanal. R15-10280 and R16-5336 are the most closely associated with the remaining sensory attributes (‘liking’, ‘aroma’, ‘taste’, texture’, ‘sweetness’) and 3-octanol. V16-0547 is plotted closely to 1-octen-3-ol and 6-methyl-5-hepten-2-one while R14-6450, V10-3653, V16-0524, and V16-0528 are somewhat clustered together with the remaining volatile compounds indicating associations. Additionally, all breeding lines from Virginia (naming started with “V”) were more closely associated to each other, while breeding lines from Arkansas (varieties named with ‘R’) were spread across the plot.

Correlations of the 14 volatile compounds identified in the study with and found to be significantly different across edamame genotypes with the significant and relevant sensory attributes (‘liking’, ‘sweetness intensity’, and ‘taste’), found limited correlations greater than 0.5 or less than -0.5 (Table 3-5). 1-Octanol showed the most significant correlation with ‘liking’ at 0.583. 1-Octanol, (E)-2-octen-1-ol, and (Z)-2-decenal were all found to be positive correlated with ‘sweet’ (0.787, 0.724, 0.652) and ‘taste’ (0.657, 0.621, 0.566). Only 2-pentylfuran is somewhat strongly correlated (0.721) to the perceived “aroma” from sensory panel, which might indicate a possibility only semi-quantify this compound to provide a quick estimate on how the aroma intensity of harvested samples is. But on the other hand, there is no difference in content of this compound between treatments, which made it harder to draw conclusions.

Table 3-5: Correlations of significant volatile compounds and sensory attributes of edamame.

	HEXANAL	HEPTANAL	1-PENTANOL	OCTANAL	(E)-2-HEPTENAL	6-METHYL-5-HEPTEN-2-ONE	1-HEXANOL	NONANAL	3-OCTANOL	(E)-2-OCTENAL	1-OCTEN-3-OL	1-OCTANOL	(E)-2-OCTEN-1-OL	(Z)-2-DECENAL	LIKING	SWEETNESS	TASTE
HEXANAL	1.000	0.941	0.847	0.740	0.465	0.648	0.497	0.785	-0.159	0.692	0.365	0.264	0.254	0.371	-0.194	-0.148	-0.293
HEPTANAL	0.941	1.000	0.950	0.902	0.613	0.858	0.615	0.884	-0.151	0.748	0.552	0.450	0.479	0.561	-0.068	0.091	-0.107
1-PENTANOL	0.847	0.950	1.000	0.885	0.617	0.902	0.565	0.911	-0.238	0.654	0.510	0.626	0.630	0.674	0.005	0.281	0.010
OCTANAL	0.740	0.902	0.885	1.000	0.811	0.900	0.524	0.783	-0.074	0.865	0.738	0.451	0.598	0.594	-0.031	0.226	0.005
(E)-2-HEPTENAL	0.465	0.613	0.617	0.811	1.000	0.610	0.310	0.570	0.243	0.840	0.773	0.404	0.615	0.486	-0.085	0.174	-0.001
6-METHYL-5-HEPTEN-2-ONE	0.648	0.858	0.902	0.900	0.610	1.000	0.643	0.877	-0.063	0.591	0.710	0.695	0.760	0.784	0.218	0.485	0.280
1-HEXANOL	0.497	0.615	0.565	0.524	0.310	0.643	1.000	0.662	0.152	0.268	0.373	0.296	0.267	0.251	0.028	0.218	0.025
NONANAL	0.785	0.884	0.911	0.783	0.570	0.877	0.662	1.000	0.038	0.505	0.604	0.737	0.713	0.735	0.302	0.458	0.278
3-OCTANOL	-0.159	-0.151	-0.238	-0.074	0.243	-0.063	0.152	0.038	1.000	0.084	0.534	0.019	0.211	0.046	0.156	0.015	0.234
(E)-2-OCTENAL	0.692	0.748	0.654	0.865	0.840	0.591	0.268	0.505	0.084	1.000	0.692	0.081	0.323	0.280	-0.345	-0.195	-0.317
1-OCTEN-3-OL	0.365	0.552	0.510	0.738	0.773	0.710	0.373	0.604	0.534	0.692	1.000	0.473	0.728	0.624	0.258	0.330	0.362
1-OCTANOL	0.264	0.450	0.626	0.451	0.404	0.695	0.296	0.737	0.019	0.081	0.473	1.000	0.929	0.931	0.583*	0.787	0.657
(E)-2-OCTEN-1-OL	0.254	0.479	0.630	0.598	0.615	0.760	0.267	0.713	0.211	0.323	0.728	0.929	1.000	0.937	0.491	0.724	0.621
(Z)-2-DECENAL	0.371	0.561	0.674	0.594	0.486	0.784	0.251	0.735	0.046	0.280	0.624	0.931	0.937	1.000	0.480	0.652	0.566
LIKING	-0.194	-0.068	0.005	-0.031	-0.085	0.218	0.028	0.302	0.156	-0.345	0.258	0.583*	0.491	0.480	1.000	0.825	0.962
SWEETNESS	-0.148	0.091	0.281	0.226	0.174	0.485	0.218	0.458	0.015	-0.195	0.330	0.787	0.724	0.652	0.825	1.000	0.885
TASTE	-0.293	-0.107	0.010	0.005	-0.001	0.280	0.025	0.278	0.234	-0.317	0.362	0.657	0.621	0.566	0.962	0.885	1.000

Includes significant compounds ($p<0.05$) and significant and relevant sensory attributes based on differences across edamame genotype. *Indicates significant correlation (>|0.5|) of the volatile compound and 'liking'.

3.4 Discussion

Variations in relative content of the 16 volatile compounds identified in this work are significant based on edamame genotype and growing locations.

n-Nonanal and 2-decenal were detected in research by Ravi et al. (2019), who used an electronic nose to identify volatile compounds in domestic soybean seed using 5 soybean genotypes (UA5014C, UA5414RR, JTN-5503, JTN-5110, JTN-5203). Though the research identified over 20 volatile compounds, ethyl-2-methyl butyrate, 2-methyl propanal, 2-propanol, and dimethyl sulfide were found to be the main volatile contributors with n-nonanal and 2-decenal being the only compounds detected by Ravi et al. (2019) that were also detected in this work on domestic edamame. Though the results are vastly different, these variations are not surprising as Ravi et al. (2019) used soybean seed while this study used domestic edamame beans.

Though this work, to the author's knowledge, will be the first publication researching volatile compounds of domestic edamame genotypes, previous research by Boué et al. (2003) investigated volatile compounds of domestic soybean harvested at R6 maturity, the same maturity recommended for edamame harvest. Of the 16 compounds of focus in this study, Boué et al. (2003) identified 12 (pentanal, hexanal, heptanal, 2-pentylfuran, 1-pentanol, octanal, (E)-2-heptanal, 1-hexanol, nonanal, 3-octanol, (E)-2-octenal, 1-octen-3-ol) in their study. Of these, 1-hexanol was noted to be consistently high at R6 maturity, which differs from the findings in our study which shows 1-hexanol to be present but has a low relative content compared to many of the other compounds included. Our work showed hexanal, 1-octen-3-ol, and (E)-2-heptenal to be the volatile compounds consistently the highest in relative content across edamame genotypes while Boué et al. (2003) found multiple compounds, including 3-hexanone, 1-hexanol, and 3-octanone, to have higher content in their samples. Of these, only 1-hexanol was included in our study. These differences could be due to the variety used by Boué et al. (2003), the growing location and agronomical practices, and other variations. Overall, volatile variation among samples from the same species but different studies is commonly observed, as volatile emission from plants is highly impacted by environment.

Recent research completed outside of the U.S. into edamame volatile compounds has previously identified 12 of the 16 compounds identified in our research (Guo et al., 2022; Yuan et al., 2021). Guo et al. (2022) researched volatile compounds in 3 edamame varieties from Asia identifying hexanal, octanal, 1-hexanol, nonanal, 1-octen-3-ol, (E)-2-octen-1-ol, and (Z)-2-decenal and concluded hexanal, 1-octen-3-ol, nonanal, and (Z)-2-decenal to be the main flavor compounds (Guo et al., 2022). Yuan et al. (2021) researched 30 edamame varieties from Taiwan detected 93 volatile compounds including 8 (pentanal, hexanal, heptanal, 1-pentanol, (E)-2-heptenal, 1-hexanol, (E)-2-octenal, 1-octen-3-ol) of the 16 compounds identified in this study and concluded 1-pentanol, (E)-2-hexenol, (E)-2-heptenal, and 1-octen-3-ol to be high in content across all 30 edamame varieties.

While Guo et al. (2022) found nonanal and (Z)-2-decenal to be major flavor compounds in the edamame varieties studied, Yuan et al. (2021) did not identify either of these compounds in their work. While our study did detect both nonanal and (Z)-2-decenal, neither were found to be in particularly high relative content across edamame genotypes. However, 1-octen-3-ol was identified as a compound of high content across edamame varieties by Guo et al. (2022), Yuan et al. (2021) and in our study of domestic edamame volatile compounds. Additionally, hexanal was found to be a compound of high content in our study of domestic edamame as well as by Guo et al. (2022) and (E)-2-heptenal was found to be a compound of high content in our study of domestic edamame as well as by and Yuan et al. (2021). The 16 volatile compounds identified in the domestic edamame genotypes in this study show there may be differences in volatile compound presence between domestic and Asian edamame genotypes. These differences include the identification of 2-pentylfuran, 6-methyl-5-hepten-2-one, and 3-octanol in this work which were not reported in similar analysis of Asian edamame varieties in work by Gu et al. (2022) and Yuan et al. (2021). However, 2-pentylfuran and 3-octanol were identified in similar volatile work with domestic edamame by Boué et al. (2003). Of these, in this study, 2-pentylfuran was found to be positively correlated with ‘liking’, ‘sweetness intensity’, ‘aroma’, and ‘taste’ (>0.500) for each but was not found to be significantly different across the edamame genotypes (α=0.05). As 2-pentylfuran is often described as “fruity”, it is possible the compound continues a flavor or aroma that is associated with ‘sweet’ and therefore indicative of overall liking. This finding may indicate the significance of 2-pentylfuran in domestic edamame varieties or in domestically grown and processed edamame when compared to internationally bred or grown varieties.

Results of this work showed no strong correlations (>│0.75│) between the volatile compounds and the sensory attributes that are most reasonably impacted by aroma active compounds including ‘liking’, ‘sweet’, and ‘taste’ (Table 3-5). However, a PCA plot of the volatile compounds identified with the sensory attributes shows the sensory attributes to be clustered together along 3-octanol indicating similarities (Figure 3-2).

Previous research on domestic edamame has shown that overall sweetness is extremely important to consumer acceptability and overall liking of the product (Carneiro et al., 2021; Yu et al., 2021). As strong correlations between volatile compounds in the edamame and sensory attributes are not observed, managing sweetness to gauge possible consumer liking might be more realistic than modifying volatile profiles of beans. However, large changes in quality attributes including volatile compound presence in edamame varieties could cause changes in consumer preferences and acceptability including the importance of sweetness. Additional research would be required to investigate these possibilities.

Since the start of this work, edamame genotype V16-0524 was released as edamame variety VT Sweet due to its agronomic and sensory characteristics (Zhang et al., 2021). The 16 volatile compounds analyzed in this study showed VT Sweet (V16-0524) to have no significant differences from V10-3653, the only edamame genotype found to be unacceptable due to sensory qualities by Carneiro et al. (2021). Volatile differences from VT Sweet and UA Kirksey, which had been previously released and used commercially in the US, were significant in hexanal, heptanal, 1-pentanol, octanal, (E)-2-heptenal, nonanal, (E)-2-octenal, 1-octen-3-ol, and 1-octanol between the 2 varieties (Table 3-2). These similarities and differences between VT Sweet, V10-3653, and UA Kirksey may further indicate that, for the domestic edamame genotypes utilized, domestic consumer acceptance might be more heavily influenced by sweetness, texture, appearance and other characteristics over volatile compounds and aromas.

Quality characteristics of edamame including sensory and flavor attributes can vary due to numerous factors including growing location, soil composition, harvest time, post-harvest processing, and storage conditions (Carneiro et al., 2021; Hung et al., 1991; Yu et al., 2021; Yu et al., 2022). Though this work utilized four growing locations as replications and worked to minimize variations from other specified factors, variations from year to year along with other factors will cause variations in volatile compound presence and development in edamame as well as different sensory and quality characteristics. The aroma activity of the compounds in the samples used in this study were not evaluated but may need to be considered in future work.

3.5 Conclusions

This work shows variations in volatile compound presence in edamame based on genotype as well as growing locations occur in a statistically significant ($p<0.05$) way. Sixteen volatile compounds were characterized in this study. Volatile differences by edamame genotype, growing location, and growing year have the potential to impact the sensory attributes for crops including edamame (Hung et al., 1991; Yu et al., 2021; Yu et al., 2022). However, no direct correlation between individual volatiles and sensory attributes were observed in this study. To our knowledge, our work was the first study investigating the volatile profiles of newly released VT Sweet, an edamame cultivar developed specifically for the domestic edamame market (Zhang et al., 2021). This variety was found to be favorable by agronomic characteristics and through sensory work (Carneiro et al., 2021; Zhang et al., 2021). The volatile profile of VT Sweet were found to be clearly discriminated from UA Kirksey, a commercial edamame cultivar developed domestically and used as control in our sensory work. Future work may benefit from evaluating the odor activity values of volatiles and their contribution to the overall perception.

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Chapter 4

4. Effect of stink bug feeding on edamame flavor chemistry

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Abstract: As a high value and nutritive crop, edamame (*Glycine max* (L.) Merr) can boost the income of farmers of varying production capacities. A local supply of edamame is necessary for fresh market sales as the product shelf life is extremely short and often requires freezing for distribution. The brown marmorated stink bug *Halyomorpha halys* (Stål), has been a major pest concern for edamame as feeding on the pods during growing causes significant visual defects on the beans without leaving noticeable injury to the pods. To evaluate possible flavor changes to the edamame beans due to this stink bug feeding, two growing seasons (2020; 2021) were used to produce edamame samples including both injured and not injured (control) edamame. Edamame was harvested around the R6 growth stage, processed for storage, and sorted based on the presence of stink bug injury, characterized by having a small gray or black dot or a large brown patch on the bean. Headspace solid-phase microextraction was used for volatile extraction and characterization followed by hot spitless injection for gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O) to analyze volatile compounds. A tetrad sensory test between injured and control samples was conducted to further evaluate flavor differences with visual appearance of the edamame being masked for evaluation. Comparing volatile compounds showed significant differences ($p<0.05$) in volatile profiles between the stink bug feeding injury and control edamame through GC-MS but detection of these differences in GC-O were not significant. Sensory results also concluded differences were not consistently detected between treatments. Though differences are present based on injury, consumer detection of these taste and aroma differences are unlikely. Use of injured edamame in further processed products should be appropriate to avoid crop loss from stink bug feeding injury.

Keywords: edamame, stink bug, flavor analysis, sensory, aroma, quality, crop damage, vegetable soybean

4.1 Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly called the brown marmorated stink bug (BMSB), is an invasive species originally from east Asia (Hoebeke & Carter, 2003). The species was first reported in Pennsylvania in the 1990's and has since spread through the United States (Hoebeke & Carter, 2003). For soybean (*Glycine max* (L.) Merr.) crops, including vegetable soybean, or edamame, BSMB can cause reduced quality and yield due to feeding injury on seeds (Owens et al., 2013). BMSB has been recorded to reduce soybean crop yields up to 50% in the Mid-Atlantic region of the United States (Leskey et al., 2012) and insecticide applications are often required to control infestations (Cissel et al., 2015; Kuhar & Kamminga, 2017).

Stink bugs feed on soybean by inserting their stylets through the pod and into the bean (Kuhar et al., 2012). The resulting injury can range from a small gray or black dot to a large brown patch on the bean (Figure 4-1) due to the physical breach of the plant as well as the chemical changes from the injection of digestive enzymes into the bean for feeding (Owens et al., 2013). Severity of the feeding injury will depend on a variety of factors including pest population, developmental stage of both the stink big and the soybean pod (Corrêa-Ferreira & De Azevedo, 2002). Feeding injury to the beans cannot be accurately detected until removed from the pod, which makes early detection of injury to fresh product difficult and especially problematic for edamame, which is often consumed as whole beans. Economic loss is of great concern due to the possible impact on crop yield due to undeveloped beans or aborted pods as well as consumer response to the visual defects of stink bug feeding on beans.

Figure 4-1: Edamame bean samples along a ruler (cm) with 3 beans down the left side showing no stink bug feeding injury and 5 beans across the bottom showing varying amounts of injury due to stink bug feeding.

Lomate and Bonning (2018) found the saliva of BMSB was higher in chymotrypsin- and trypsin-like activities as well as aminopeptidase activity when compared to the gut. These enzymes contribute to the visual changes that results in the edamame bean (Lomate & Bonning, 2018). This injury is more significant when feeding occurs on younger fruit and seeds and can result in abnormal plant growth, discoloration of seeds or fruit, deformed growth of seeds or fruit, abortion of seed, delayed maturing, transmission of plant pathogens, or possibly plant death (Koch et al., 2017). Research into crop damaged by BMSB have shown chemical changes due to infestation and damage in olives, apples, and peppers with researchers identifying changes specifically in phenolic compounds (Ivancic et al., 2022; Zamljen et al., 2021a; Zamljen et al., 2021b). Weber et al. (2021) also research BMSD damaged strawberries finding damaged strawberries to have decreased sugar content and off aromas concluded to be a result of trans-2-octenal and trans-2-decenal.

As edamame continues to increase in popularity in the United States, domestic growers have an opportunity to meet demands while building a local edamame supply chain with profitable returns (Jiang et al., 2018; Garber & Neill, 2019). This will also require managing the BMSB to protect the edamame from emergence to harvest. Though visual injury caused by stink bug feeding is less than favorable for edamame from an aesthetic standpoint, understanding the full effect of this injury on the edamame bean is important to assessing the impact on crop quality. As zero tolerance for stink bug injured edamame is probably an unrealistic expectation for production, understanding the quality impact outside of visual blemishes is necessary. Understanding possible flavor and aroma changes that may occur in the injured edamame beans could support further edamame market development. The objective of this project was to determine if notable differences in flavor chemistry or sensory exist between stink bug injured and non-injured edamame beans.

4.2 Materials and Methods

4.2.1 Samples and Processing

Year 1 (2020) of the study included one edamame genotype (R15-10280), that was planted in 3-m plots in a randomized completed block design with four replications as part of a separate edamame variety trial investigation conducted at the Virginia Tech Kentland Farm in Whitethorne, Virginia, USA; 37.198236 N, -80.582786 W; see Lord et al. (2021) for additional details on the crop production. Edamame plots were not treated with any insecticides throughout the growing season. Harvest occurred in September when the plants had reached R6 maturity, which has been established as the ideal time for harvest due to the bright green color, pod fill, and sweetness (Xu et al., 2015; Yu et al., 2022). Pods were separated from the stems and leaves then immediately transported to the Virginia Tech Food Processing Pilot Plant (Blacksburg, VA, USA) in coolers with ice packs. Samples arrived within 24 hours of harvest to be processed within 48 hours of harvest.

Edamame processing followed the established method described by Carneiro et al. (2021) which utilized washing, blanching, and peeling of the edamame to prepare for storage. Edamame pods were first rinsed in tap water (Blacksburg, VA, USA) to remove remaining soil from the pods then received a final rinse in distilled water. Edamame pods were then transferred to metal baskets for the blanching, which occurred in a steam kettle

(Legion Utensils Co., Long Island City, NY, USA), using distilled water (98 ± 1°C, 1 minute) before moving to an ice bath of distilled water (4 ± 1°C, 2 minutes). After chilling in the ice bath, the edamame was dried using salad spinners and peeled by hand to reveal the edamame beans. The resulting beans were then inspected for probable stink bug feeding injury and sorted based on injury presence. Stink bug feeding injury was identified as small pin prick markings and brown or yellow discoloration spots on the beans (Kuhar et al., 2015). Samples were stored at -80°C to preserve volatile compounds until analysis.

Year 2 (2021) of the study included one edamame variety, ‘VT-Sweet’, which was provided by the soybean breeding program at Virginia Tech, Blacksburg, VA (USA) (Zhang et al., 2021). Edamame was planted in June, spaced apart from each other by approximately two feet, and covered with cages consisting of 114 × 43 × 51 cm PVC frames covered in typical fiberglass window screen (18 × 16 weave fine mesh design) in September, when the plants were at R4 maturity. Treatment groups assigned at random with the control (no injury) plants having no stink bugs in their cages during the growing season, while the treatment plants had 10 wild-caught BMSB adults in the cages to ensure feeding injury to the edamame beans. Cages were removed from the plants in October, after 20 days, for harvest, when the plants were at R6 maturity and processed as described for year 1. Processing occurred at Virginia Tech Kentland Farm (Whitethorne, Virginia, USA) following the process used in year 1 of the study (Carneiro et al., 2021).

4.2.2 GC-MS Volatile Analysis

Solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) was utilized to analyze volatile compounds and determine differences in the injured and not injured edamame samples. Analysis included edamame samples from year 1, which utilized one edamame genotype (R15-10280) grown in 4 replications and divided into 2 treatment groups (injured and control (not injured)) for each growing replication, and edamame samples from year 2, which included one edamame variety (VT-Sweet) grown without growing replications and divided into 2 treatment groups.

For volatile analysis, edamame samples were ground into free-flowing powder using around 20 grams of frozen (-80°C) edamame beans with around 40 grams of dry ice in edamame dedicated Krups Coffee and Spice Grinders (Krups, Solingen, Germany). The resulting powder was weighed into clean, 20mL SPME vials (Supelco, Bellefonte, PA, USA), adding 2.0g ± 0.3g of sample per vial before capping with a clean a PTFE-lined cap (Supelco, Bellefonte, PA, USA). Weighing and capping occurred in a glove box that had been flushed with nitrogen to reduce oxygen and humidity exposure. One microliter of 8.03 μg/μL of 3-hexanol (Sigma-Aldrich, St. Louis, MO, USA) in methanol was added into each vial, through the cap as an internal standard as it was not found in previous edamame or soy volatile research (Boué et al., 2003; Cai et al., 2021; Guo et al., 2022; Ravi et al., 2019; Yuan et al., 2021).

GC-MS was completed on an Agilent Gas Chromatography 6890N equipped with 5975B Mass Spectrum Detector (Agilent Technologies, Santa Clara, CA, USA) and Leap Technologies CTC PAL Autosampler (Trajan Scientific and Medical, Ringwood, Vic 3134, Australia). Samples were incubated at 45^{o}C with 30 minutes of adsorption time at a rotation speed of 250 rpm using a dedicated 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Stableflex, Supelco, Bellefonte, PA, USA). A hot, splitless GC injection was used with an oven temperature programed for an initial temperature of 40^{o}C for 5 minutes followed by an increase of 5^{o}C per minute until 225^{o}C was reached. A DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) with helium flowing at 2.2mL per minute was used for volatile compound separation. Compounds then were identified by a NIST library and quantified through manual integration of peak areas on ChemStation in the Enhanced Data Analysis view (Agilent Technologies, Santa Clara, CA, USA) and further confirmed through calculation of the retention index using *n*-alkane (C_7-C_{30}) (Sigma-Aldrich, St. Louis, MO, USA) diluted to 10µg/mL in hexane and the formula derived by Van den Dool and Kratz (1963). Relative content of each compound was calculated by multiplying the peak area ratio by the ratio of the amount of internal standard to that of the sample then multiplying by one thousand.

$$Relative\ content\ \left(\frac{\mu g}{kg}\right) = \left(\frac{Peak\ Area_{target\ compound}}{Peak\ Area_{corresponding\ IS}}\right) \times \left(\frac{amount\ of\ internal\ standard\ (\mu g)}{amount\ of\ sample_{fresh\ weight}\ (g)}\right) \times 1000$$

4.2.3 GC-O Volatile Analysis

Gas chromatography-olfactometry (GC-O) was utilized to identify aroma active compounds of the identified key volatile compounds to further identify sensory differences in the injured and control edamame samples. Edamame samples were prepared as described for GC-MS, but did not utilize an internal standard.

Agilent Gas Chromatography 6890N equipped with 5975B Mass Spectrum Detector (Agilent Technologies, Santa Clara, CA, USA) and Leap Technologies CTC PAL Autosampler (Trajan Scientific and Medical, Ringwood, Vic 3134, Australia) and PHASER GLS-G300021 Olfactory Detection Port system was used for analysis along with 6 panelists (1 male; 5 female) trained on 12 volatile compounds found to be important aroma compounds through GC-MS and preliminary sensory work (Table 4-1).

Table 4-1: Volatile compounds with their aroma descriptors and retention index numbers used to train GC-O panelists.

Calculated RI	**Reference RI**	**Compound**	**Description**[1]
~	935[2]	Pentanal	Fermented, Bready
1072	1084[2]	Hexanal	Fresh, Green
1163	1157[3]	1-Penten-3-ol	Horseradish, Green
1289	1280[2]	Octanal	Waxy, Citrus
1304	1295[4]	1-Octen-3-one	Herbal, Mushroom
1325	1305[4]	(E)-2-Heptenal	Pungent, Green
1339	1332[3]	6-Methyl-5-hepten-2-one	Citrus, Green
1357	1360[2]	1-Hexanol	Green, Fruity
1393	1385[2]	Nonanal	Citrus, Cucumber
1428	1408[2]	(E)-2-Octenal	Fatty, Green
1452	1451[2]	1-Octen-3-ol	Mushroom, Earthy
1456	1467[2]	1-Heptanol	Musty, Leafy

[1]https://www.thegoodscentscompany.com/; [2]https://www.flavornet.org/flavornet.html; [3]Kesen, S., Kelebek, H., Sen, K., Ulas, M., & Selli, S. (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Research International, 54 (2), 1987-1994. https://doi.org/10.1016/j.foodres.2013.09.005.; [4]Rychlik, M., Schieberle, P., and Grosch, W. (1998). Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Ordorants. TUM. Garching, Germany

Panelists completed 3 to 4, 30-minute training sessions, depending on the panelists' comfort and confidence with identifying the compounds. During training sessions, panelists sniffed a chemical solution of aroma compounds through the GC-O port. Each session utilized an injection of 1µL of solution into the GC which included 3.5µL of pentanal (97%), 2.5µL of hexanal, 1.0 µL of octanal (95%), 0.5 µL of 1-octen-3-one (≥96%), 1.0µL of 2-heptenal (≥95%), 1.0 µL of 6-methyl-5-hepten-2-one (≥98%), 7.0 µL of 1-hexanol (≥99.5%), 1.0 µL of nonanal (97%), 0.9 µL of 1-octen-3-ol (≥98%), and 1.5 µL of 1-heptanol (≥97%) which were purchased from Sigma Aldrich (St. Louis, MO, USA). 3.5µL of 1-penten-3-ol (99%), and 0.8 µL of (E)-2-octenal (96%) were also included in the solution and were kindly provided by Bedoukian Research Inc. (Danbury, CT, USA). The specified amounts of each standard were added into 10mL of methanol to create the standard training solution.

For training sessions, panelists were provided with the list of compounds including the name, aroma descriptors, and the retention time of each compound in the mixture for each training session. Once panelists felt comfortable with identifying each aroma, panelists were tested on the same aroma compounds in the same mixture used for training sessions. During this, panelists were expected to sniff compounds, recording aroma descriptors, retention times, and the aroma intensity of each compound (very weak; weak; moderate; strong; very strong) of each compound. Panelists needed to be able to detect at least 11 of the 12 compounds to participate in sample evaluation. All 6 panelists (1 male; 5 female) who started training were able to pass the test and participated in evaluation sessions.

For GC-O evaluation, sample vials were incubated at 45^{o}C with 30 minutes of adsorption time at a rotation speed of 250 rpm. Dedicated 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers (Stableflex, Supelco, Bellefonte, PA, USA) were used for extraction for 30 minutes. The oven temperature was programmed for an initial temperature of 40^{o}C for 5 minutes, then increased by 5^{o}C per minute for 18 minutes until the temperature reached 130^{o}C. The temperature then increased by 15^{o}C per minute for around 21 minutes, reaching 225^{o}C. Separation of volatile compounds occurred using a DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) using helium at 2.2mL per minute as the carrier gas. During the GC run, a panelist was stationed at the olfactory detection port, recording aroma descriptors, retention time, and odor intensity (very weak; weak; moderate; strong; very strong) throughout the GC run. Each edamame sample was evaluated by 3 of the trained panelists, one per vial, with no panelists evaluating a single sample twice. Panelists were selected for samples based on their availability during the scheduled evaluations. Reported odor intensities were converted to ordinal data (0 (not detected) to 5 (very strong)) for data analysis.

4.2.4 Sensory Discrimination Testing

A tetrad discrimination test was conducted for year 1 of the study at the Human and Agricultural Biosciences Building 1 (HABB1) at 1230, Washington St. SW, Virginia Tech, Blacksburg, VA 24061 in the sensory booths of the sensory laboratory. The research was approved by the Virginia Tech Institutional Review Board for Research Involving Human Subjects (IRB 18-310).

Untrained panelists were recruited from the Blacksburg community and surrounding area to participate in the discrimination testing. Panelists were required to be at least 18 years of age with no known allergies to soy or soy products. Participating panelists did not receive financial compensation but were compensated with light refreshments.

Of the 4 growing replications from year 1 of the study, 3 growing replications were included in sensory testing. The fourth growing replication from year 1 and samples from year 2 were not included due to limited sample amounts. Edamame samples were prepared for sensory using the method described by Miller et al. (2023), which utilized microwaving thawed edamame beans for 40 seconds in a 1L Pyrex glass measuring container, covered with a paper towel, in a carousel microwave (model R-2W38, 120 VAC, 60Hz, 1200 watts, Sharp Corporation, Thailand). Samples were then refrigerated overnight in black plastic sample cups with lids and labeled with blinded with 3-digit codes.

For evaluation, panelists were presented with 4 sample cups, each with unique 3-digit codes, along with water and unsalted saltines. Panelists were asked to taste the samples one at a time, cleansing their palate with water and unsalted saltines between samples, and group the samples into two groups of two based on similarities. Red lights were on in the sensory booths to reduce the panelists' awareness of visual differences in the samples. Each panelist evaluated 2 of the 3 replicates included in the sensory portion of the study with 46 panelists participating in the study. The combination of replicates evaluated by each panelist was random. Data was collected through Compusense Sensory Management Software (Compusense Inc., Ontario, Canada).

4.2.5 Statistical Methods

To determine significant differences between the two edamame treatments, t-tests were performed ($p<0.05$) on GC-MS and sensory tetrad discrimination data from year 1 of the study. Year 1 GC-O data underwent Wilcoxon test to show significant variability ($p<0.05$) of volatile compounds between edamame treatments. Data collected in year 2 of the study was not included in statistical analysis due to limitations with growing replications. Data analysis was performed using JMP Pro 15 (SAS, Cary, NC) and R Studio (R version 4.0.5).

4.3 Results

GC-MS identified 17 compounds in the edamame samples that may contribute to edamame aroma and flavor (Table 4-2). These compounds were also identified in literature as compounds identified in volatile analysis of edamame and soy products (Boué et al., 2003; Cai et al., 2021; Guo et al., 2022; Han et al., 2022; Kim et al., 2020; Miller et al., unpublished; Ravi et al., 2019; Yuan et al., 2021). Of these, 10 compounds (pentanal, hexanal, heptanal, octanal, 6-methyl-5-hepten-2-one, 1-hexanol, nonanal, 3-octanol, (E)-2-octenal, and 1-octanol) were found to be significantly different ($p<0.05$) by treatment in year 1 of the study with all of the compounds being significantly higher in the injured treatment compared to the control with the exception of 3-octanol, which was higher in the control (Table 4-3). Since year 2 of the study was not able to include multiple growing replications, statistical significance cannot be determined, but data can be seen to follow similar trends for each of the statistically significant compounds shown in year 1 of the study (Table 4-3; Figure 4-2).

Table 4-2: Volatile compounds of interest identified in GC-MS of edamame.

Calculated RI	Reference RI	Compound	Description[1]
~	935[2]	Pentanal	Fermented, Bready
1072	1084[2]	Hexanal	Fresh, Green
1180	1174[2]	Heptanal	Fresh, Green
1229	1227[3]	2-Pentylfuran	Fruity, Green
1257	1255[2]	1-Pentanol	Pungent, Fermented
1289	1280[2]	Octanal	Waxy, Citrus
1325	1305[4]	(E)-2-Heptenal	Pungent, Green
1339	1332[5]	6-Methyl-5-hepten-2-one	Citrus, Green
1357	1360[2]	1-Hexanol	Green, Fruity
1386	1389[4]	(Z)-3-Hexen-1-ol	Green, Grassy
1393	1385[2]	Nonanal	Citrus, Cucumber
1396	1395[5]	3-Octanol	Earthy, Mushroom
1428	1408[2]	(E)-2-Octenal	Fatty, Green
1456	1451[2]	1-Octen-3-ol	Mushroom, Earthy
1563	1553[2]	1-Octanol	Waxy, Green
1617	1620[6]	(E)-2-Octen-1-ol	Green, Citrus
1644	1630[7]	(Z)-2-Decenal	Tallow, Fatty

[1] https://www.thegoodscentscompany.com/; [2] https://www.flavornet.org/flavornet.html; [3] Jakobsen, H.B., Hansen, M., Christensen, M.R., Brockhoff, P.B., & Olsen, C.E. (1998). Aroma Volatiles of Blanched Green Peas (Pisum sativum L.). *J. Agric. Food Chem.* 46, 9, 3727–3734 https://doi.org/10.1021/jf980026; [4] Rychlik, M., Schieberle, P., and Grosch, W. (1998). Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Ordorants. TUM. Garching, Germany; [5] Kesen, S., Kelebek, H., Sen, K., Ulas, M., & Selli, S. (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Research International, 54 (2), 1987-1994. https://doi.org/10.1016/j.foodres.2013.09.005; [6] Cho, I.H., Choi, H.-K., & Kim, Y.-S. (2006). Difference in the Volatile Composition of Pine-Mushrooms (Tricholoma matsutake Sing.) According to Their Grades. *J. Agric. Food Chem,* 54, 13, 4820–4825. https://doi.org/10.1021/jf0601416; [7] Lin, J. and Rouseff, R.L. (2001), Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time–intensity GC–olfactometry and GC–MS. *Flavour Fragr. J.*, 16: 457-463. https://doi.org/10.1002/ffj.1041

Table 4-3: Relative content (µg/kg) of volatile compounds (mean ± SE) in edamame by year and treatment.

	Year 1		**Year 2**	
Compound	**Injured**	**Control**	**Injured**	**Control**
Pentanal*	163.10 ± 8.42	90.97 ± 6.83	154.07 ± 4.78	26.25 ± 4.06
Hexanal*	2923.69 ± 381.01	1135.77 ± 97.05	8642.30 ± 140.11	127.67 ± 30.11
Heptanal*	88.77 ± 2.95	21.18 ± 1.81	136.27 ± 14.89	15.32 ± 2.29
2-Pentylfuran	6.94 ± 3.63	28.78 ± 16.78	53.04 ± 1.17	10.43 ± 4.44
1-Pentanol	819.66 ± 49.53	682.67 ± 39.08	496.80 ± 4.79	192.50 ± 33.28
Octanal*	163.19 ± 6.27	71.96 ± 8.28	147.27 ± 5.73	13.39 ± 0.75
(E)-2-Heptenal	1048.45 ± 178.96	714.48 ± 115.04	503.77 ± 221.94	193.73 ± 86.45
6-Methyl-5-hepten-2-one*	64.88 ± 8.59	40.42 ± 4.37	21.33 ± 1.06	7.09 ± 1.39
1-Hexanol*	109.75 ± 9.01	62.09 ± 10.26	78.33 ± 4.15	146.07 ± 26.74
(Z)-3-Hexen-1-ol	9.95 ± 3.71	12.33 ± 2.27	11.72 ± 0.77	13.91 ± 2.71
Nonanal*	124.58 ± 4.92	32.63 ± 3.23	215.21 ± 11.79	14.69 ± 4.07
3-Octanol*	0.00 ± 0.00	7.45 ± 2.05	0.00 ± 0.00	0.00 ± 0.00
(E)-2-Octenal*	137.13 ± 14.00	76.34 ± 11.33	145.02 ± 5.00	69.34 ± 40.79
1-Octen-3-ol	1733.18 ± 150.47	1449.24 ± 110.32	3934.26 ± 112.90	2028.75 ± 248.67
1-Octanol*	123.73 ± 11.03	69.09 ± 7.37	100.02 ± 2.59	17.51 ± 1.21
(E)-2-Octen-1-ol	586.76 ± 78.93	402.95 ± 47.45	352.34 ± 9.54	126.43 ± 22.79
(Z)-2-Decenal	68.14 ± 28.59	15.32 ± 3.35	29.13 ± 12.67	0.00 ± 0.00

*Indicates compounds found to be significantly different ($p<0.05$) through t-test by treatment in year 1 of the study. Year 1 control and injured data consisted of 12 replicates per treatment; Year 2 control and injured data consisted of 3 replicates per treatment; 'Injured' indicates edamame beans had visual injury consistent with stink bug feeding; 'Control' indicates edamame beans did not show visual injury consistent with stink bug feeding

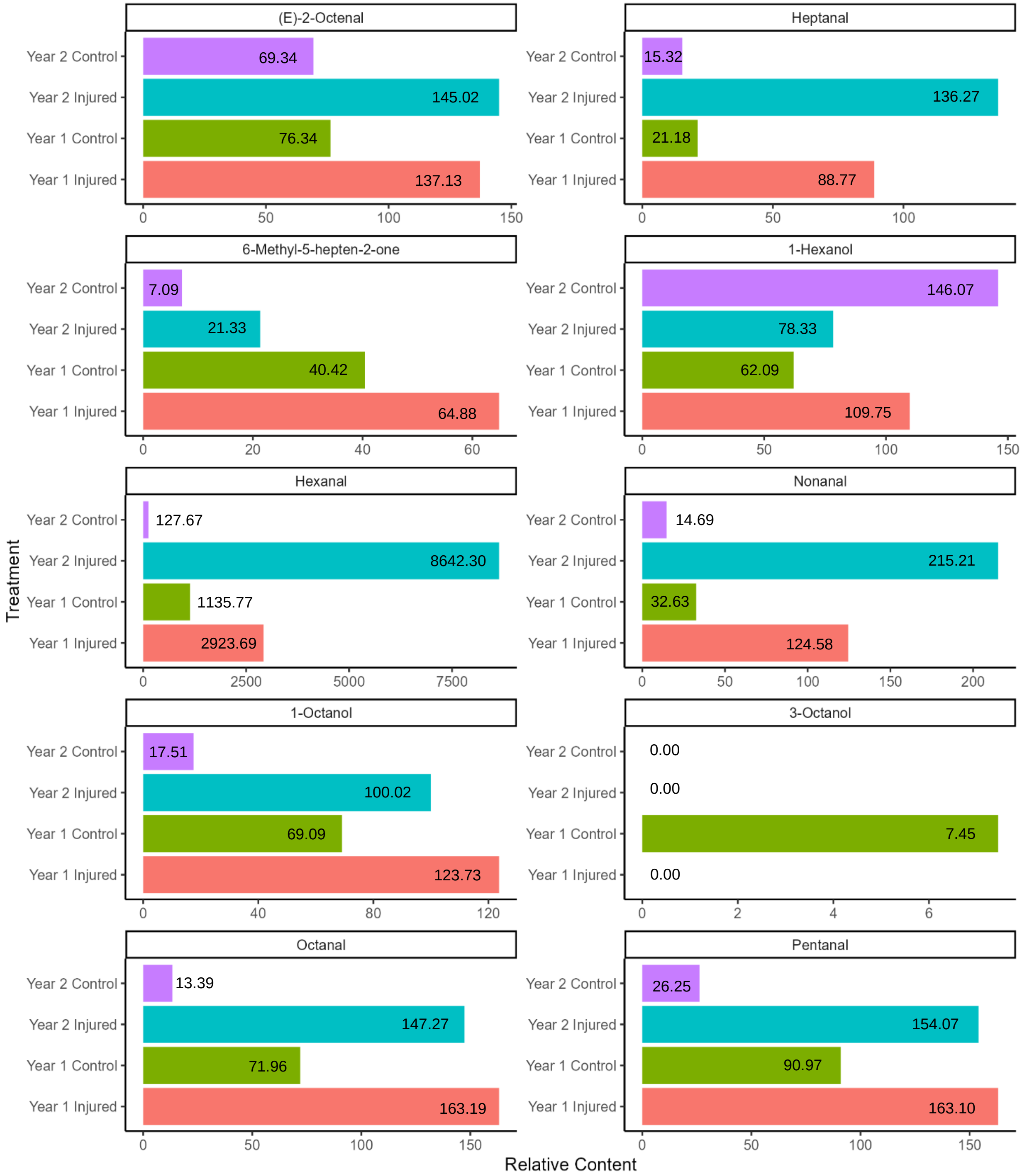

Figure 4-2: Relative content (μg/kg) of volatile compounds found to be significant ($p<0.05$) by treatment in year 1 of the study by year and treatment in the study. Bars labeled with relative content mean.
Year 1 control and injured data consisted of 12 replicates per treatment; Year 2 control and injured data consisted of 3 replicates per treatment; 'Injured' indicates edamame beans had visual injury consistent with stink bug feeding; 'Control' indicates edamame beans did not show visual injury consistent with stink bug feeding.

GC-O results showed 5 compounds (hexanal, 1-octen-3-one; 6-methyl-5-hepten-2-one, (E)-2-octenal, and 1-octen-3-ol) to be consistently detected by panelists (Table 4-4). The Wilcoxon test of the GC-O odor intensity data showed none of the volatile compounds detected by panelists to be significantly different ($p<0.05$) by treatment. Sensory results from the tetrad discrimination test also showed panelists were not able to detect the stink bug injured edamame from the control edamame ($p=0.329$).

Table 4-4: Volatile compounds included in GC-O and their detection status based on year and treatment.

	Year 1		**Year 2**		
Compound	**Injured**	**Control**	**Injured**	**Control**	**Description[1]**
Pentanal	+	+	-	+	Fermented, Bready
Hexanal	+	+	+	+	Fresh, Green
1-Penten-3-ol	-	-	-	-	Horseradish, Green
Octanal	+	+	+	-	Waxy, Citrus
1-Octen-3-one	+	+	+	+	Herbal, Mushroom
2-Heptenal	+	+	+	-	Pungent, Green
6-Methyl-5-hepten-2-one	+	+	+	+	Citrus, Green
1-Hexanol	+	-	-	-	Green, Fruity
Nonanal	+	+	-	-	Citrus, Cucumber
(E)-2-Octenal	+	+	+	+	Fatty, Green
1-Octen-3-ol	+	+	+	+	Mushroom, Earthy
1-Heptanol	-	+	-	-	Musty, Leafy

'+' indicates the compound was detected; '-' indicated the compound was not detected. [1]https://www.flavornet.org/flavornet.html. Wilcoxon test of the GC-O odor intensity data showed none of the volatile compounds to be significantly different ($p<0.05$) by treatment in year 1; Year 1 control and injured data consisted of 12 replicates per treatment; Year 2 control and injured data consisted of 3 replicates per treatment; 'Injured' indicates edamame beans had visual injury consistent with stink bug feeding; 'Control' indicates edamame beans did not show visual injury consistent with stink bug feeding

4.4 Discussion

Volatile differences identified in the GC-MS analysis are likely due to the degradation and oxidation occurring within the injured edamame. Edamame is known to be rich in fatty acids including linoleic acid, linolenic acid, oleic acid, stearic acid, and palmitic acid and have found to have more unsaturated fatty acids (linoleic, linolenic, and oleic acid) than saturated fatty acids (Agyenim-Boateng et al., 2022). Though these fatty acids can bring positive health benefits, they can also contribute to production of off-flavors and aromas through lipid oxidation (Fischer et al., 2022).

Lipoxygenase and autoxidation of linoleic, linolenic, and oleic acid can produce various volatile compounds including pentanal; hexanal; 1-hexanol; (Z)-3-hexen-1-ol; 2-octenal; and 1-octanol; all of which, except (Z)-3-hexen-1-ol, were identified has being significantly more present in stink bug injured edamame (Fischer et al., 2022). Amino acid degradation can also contribute to off-flavor and aroma development (Fischer et al., 2022). These processes are likely initiated when the BMSB feeds on the edamame beans, causing both physical and chemical damage through the physical invasion into the bean and the injection of digestive saliva and enzymes (Lomate & Bonning, 2018).

Yeast has also been known to be transmitted to fruits and vegetables due to BMSB feeding including *Nematospora coryli* Peglion and *Eremothecium coryli* which can cause yeast spot disease (Daugherty, 1967; Brust & Rane, 2021). Yeast transmission could further damage the fruit and vegetables infected but are unlikely to be the primary cause of the visual injuries resulting from stink bug feeding (Brust & Rane, 2021). The presence of yeasts, such as those that cause yeast spot disease, could also impact flavor chemistry of edamame but was not investigated in this study.

Though volatile chemical differences were shown to exist in the GC-MS results based on presence of stink bug feeding injury, GC-O and discrimination sensory testing showed these volatile differences were not detected through human participants. Though the visual defects caused by stink bug feeding are still less than favorable, this work indicates edamame with visual marks due to stink bug feeding could be used in products that diminish the visual appearance of the edamame or eliminate the ability to detect the visual defects. As various edamame products, such as roasted edamame or edamame pasta, which require additional processing which could hide a visual defect, become more popular, utilization of the stink bug feeding injury edamame should become increasingly easier for growers dealing with stink bug injured crop.

4.5 Conclusions

Differences in volatile contents were found between stink bug injured edamame beans and non-injured beans. However, these differences were not detected by panelists in sensory testing through a discrimination test nor through GC-O analysis. This finding indicated opportunities for product developers to sustainably utilize slightly injured beans on snacks such as energy bars, flavored crunchy edamame. Though the influence of visual defects of beans was eliminated in our sensory experimental design, future research evaluating the impact of visual defects on consumer acceptance will be needed to establish quality specifications.

Though controlling the BMSB will still need to rely on currently accepted methods such as pesticides, late infestations of the BMSB into edamame crop could be evaluated and addressed on a case-by-case basis. Knowing that taste and flavor of "stink bug injured" edamame is not detected by human subjects, treatment plans for the crop can be determined based on the crop destination and visual requirements for the edamame after harvest.

Additional investigation into pathogen transmission due to stink bug feeding should also be completed to evaluate the safety of stink bug injured beans. Though pathogenic bacteria have not yet been recovered from BMSB feeding sites, understanding this possibility is necessary to properly prevent food safety issues (Brust & Rane, 2021). Possible transmission of pathogenic bacteria would require additional processing steps to ensure the stink bug injured edamame beans are safe to consume.

The edamame samples in this work included full-size beans with various levels of feeding injury from stink bugs (Figure 4-1), but did not include extremely damaged product. Edamame that has been fed upon by stink bugs early in seed development may produce extremely small, brown edamame beans that are unlikely to be included in processing or packaged products due to the bean size. Beans with this level of extreme damage might show different sensory and GC-O results in a similar study. Though this work shows that stink bug injured edamame does not provide off flavors or aromas that would be consistently detected by a consumer, utilizing highly damaged, brown, small edamame beans may show different results. Additionally, this work looked at edamame beans by stink bug injury presence per bean. This design does not account for changes that may occur across the plant due to pest response.

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Chapter 5

5. Quantitative texture analysis comparison of three legumes

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Abstract: A validated texture-analysis method to evaluate product quality in frozen or cooked legumes is needed to support high-quality vegetable production but is not currently established in the literature. Peas, lima beans, and edamame were investigated in this study due to similar market use as well as growth in plant-based protein consumption in the United States. These three legumes were evaluated after three different processing treatments (blanch/freeze/thaw (BFT); BFT+microwave heat (BFT+M); BF+stove-top cooking (BF+C)), using both compression and puncture analysis following an American Society of Agricultural and Biological Engineers (ASABE) texture analysis method and moisture testing following an American Society for Testing and Materials (ASTM) standard method. Texture analysis results showed differences between legumes and processing methods. Compression analysis identified more differences between treatments within product type than puncture for both edamame and lima beans indicating compression might be more sensitive to texture changes in these products. Implementation of a standard texture method for legume vegetables for growers and producers would provide a consistent quality check to support efficient production of high-quality legumes. Due to the sensitivity obtained from the compression texture method in this work, compression should be considered for future research into a robust method to evaluate edamame and lima bean textures throughout the growing and production processes.

Keywords: texture, legume, vegetables, quality, compression, puncture

5.1 Introduction

Consumer acceptance, market value, and overall usefulness of food ingredients including vegetables and products are dependent on texture, which is highly influenced by growing and storage conditions as well as processing methods and techniques (Arntfield & Maskus, 2011). Properties of processed and raw fresh foods, such as legume vegetables, change over time due to microbial actions, oxidation, moisture migration and other factors, making accurate and complete understanding of the mechanical properties of foods, especially raw foods, difficult (Lu & Abbott, 2004). Quantification of quality attributes, such as texture, allows for a better understanding of the impact of changes occurring during production or storage of vegetable products prior to consumption. Additionally, plant foods, such as peas, lima beans, and vegetable soybean (*Glycine max L.* Merr.), commonly called edamame, have textural attributes based in their tissue structure, which could indicate textural differences between crops and other factors (Ilker & Szczesniak, 1990). Developing a method to quantify texture differences in vegetable legumes will result in a better understanding of the differences between varieties, growing conditions, postharvest factors and other variables experienced in agricultural production of plant foods.

Food texture measurements can be accomplished using destructive or non-destructive methods (Lu & Abbott, 2004). Destructive methods are more closely related to sensory evaluation methods, making destructive methods preferred over nondestructive methods, despite the limitation of collecting measurements from a sample instead of all products (Lu & Abbott, 2004). Of the many destructive methods, puncture and compression via texture analyzer can be used to understand the force required to penetrate and compress the food sample, respectively (Lu & Abbott, 2004). Texture research on convex, starchy vegetables, such as legumes, after processing is limited. Due to the varying consumption options of these products, puncture and compression methods are both relevant measurements to relate consumer consumption of peas, lima beans, and edamame to the analytical measurements.

Puncture analysis uses a probe smaller than the product being tested, resulting in the probe pushing through the surface and into the center of the product similar to a sensory experience of biting into the product using an incisor or canine tooth (Lu & Abbott, 2004). These results provide information on the force needed to break through the skin or outer surface of the product. Measurements can continue through the center and out the other side of the product resulting in a hole cored out of the sample. This provides measurements of force needed to penetrate both surfaces of the product.

Compression analysis uses a flat probe with enough surface area to fully cover the product being analyzed. The product is placed on a flat plate and as the probe comes into contact with the sample, the entire sample is flattened (Lu & Abbott, 2004; Wilhelm et al., 2004) similar to using molars to compress and consume a food. The force required to reach the point of rupture is measured. There is limited information on texture analysis through puncture or compression analysis of processed vegetable legumes such as peas, lima beans, and edamame or other vegetables of similar texture or use by manufacturers or consumers.

Demand for vegetable-sourced protein and protein products, including protein from legumes, has continued to diversify product options (Biddle, 2017). Frozen vegetables provide access to high quality protein vegetable sources year-round. About 70% of the peas grown domestically are processed and frozen for sale (Biddle, 2017). Quality of vegetables and legume vegetables often refers to characteristics such as color, palatability, taste, size, and shape of the product (Biddle, 2017), all of which cause consumer inference on texture attributes resulting in overall appeal (Lawless and Heymann, 2010).

More than 70% of the edamame sold in the United States are imported from Asia (Yu et al., 2021) after being processed and frozen for distribution. Currently, edamame imported from Asia are of cultivars considered inferior in agronomic quality and consumer acceptability in the domestic market (Jiang et al., 2018). Domestic edamame crop production is a feasible addition for growers producing other bean crops and looking to diversify their field production to add economic value (Carson, 2010). With the growing consumer demand for edamame as a highly nutritional product, local farmers have an opportunity to grow and profit from edamame production (Garber & Neill, 2019). Carneiro et al. (2022) found consumers were willing to pay $0.77 more for dark green edamame beans compared to light green beans, indicating consumers are willing to pay more for what they perceive as higher quality edamame. Increasing the domestic production of edamame will result in fresh market potential for this vegetable and expand available product for incorporation of edamame into a variety of fresh and processed products. Recently released edamame varieties, such as VT Sweet (Zhang et al., 2021), are developed with consideration for quality and consumer acceptability, thus honed to the American palate. Edamame is harvested prior to full maturity, during the R6 development stage, when the pods are full of bright green beans (Fehr et al., 1971; Guo et al., 2020). However, harvest timing is challenging and can readily result in differences in edamame quality. Edamame product quality is measured on a combination of agronomic conditions and postharvest characteristics such as sensory, and nutritional composition (Carneiro et al., 2020).

The popularity of edamame is increasing in the United States due to the nutritional value provided to consumers and economic value it can add to growers, producers, and processors of varying production capacity (Jiang et al., 2018; Garber & Neill, 2019). Edamame can be easily substituted for peas and lima beans (Klausner, 2004). All of these legumes can be added to salads, soups, stir-fries, or served as a side dish as an added component of flavor, texture, and nutrition. The versatility of these vegetables is a driving factor of their success in the market.

Peas and lima beans are already commonly produced in the United States. Peas are also grown and processed in Canada, France, China, and Russia and lima beans are also produced in Latin America and Canada (Arntfield & Maskus, 2011). As the edamame market and availability in the United States grow, quality specifications are needed for efficient production and higher consumer satisfaction. Many methods and approaches for texture analysis can be applied for specific purposes or products (Wilhelm et al., 2004). The American Society of Agricultural and Biological Engineers (ASABE S368.4) published a standard method for food materials with convex shape, such as edamame (ASAE, 2017a). Validating and standardizing a method specific to edamame texture will reduce variability in production and increase product quality in the market.

The goal of this study was to evaluate texture analysis methods for sensitivity to detect changes in texture of vegetable legumes. Texture analysis was completed in reference to three different preparation treatments (blanch/freeze/thaw (BFT) as a control; BFT+microwave heat (BFT+M); BF+stove-top cooking (BF+C)) and comparison of competitive protein-rich legume vegetables, peas, lima beans, and edamame, all of which have similar potential in salads, stir fries and other uses in the market (Klausner, 2004). Moisture was also evaluated as a driving force of texture making it appropriate to relate these attributes while understanding many additional factors could potentially impact texture (Ilker & Szczesniak, 1990). Application of ASABE S368.4 texture analysis method for vegetable legumes has previously been reported on lima beans (Aghkhani et al., 2012) but has not been used to evaluate peas or edamame. This applies the standard texture method to each product providing a previously unreported application and the ability to compare textures across product types.

5.2 Materials and Methods

5.2.1 Product

Products of commercially processed (blanched and frozen) edamame, lima beans, and peas were purchased as shelled and frozen product from supermarkets (Krogers, Cincinnati, OH) in the local area (Blacksburg and Christiansburg, Virginia) (Table 5-1). Two lot numbers were obtained for each product brand. Two bags of each vegetable, brand, and lot number, except the Brand P lima beans, were purchased and transported in coolers with icepacks to the Virginia Tech Food Science and Technology building and stored in a freezer (True Manufacturing Company Inc., O’Fallon, Missouri) at -15°C prior to sample preparation and analysis.

Table 5-1: Product information of samples used in the study.

Sample Type	Brand 1				Brand 2			
	Brand Name*	**Lot Number***	**DNW**	**Best By Date**	**Brand Name***	**Lot Number***	**DNW**	**Best By Date**
Peas	Brand S[1]	L737[1]	340g	Apr 2023	Brand B[2]	L520[1]	283.5g	Sept 1, 2022
		L454[2]	340g	Apr 2023		L420[2]	283.5g	Sept 30, 2022
Lima Beans	Brand K[1]	L161[1]	340g	Oct 26, 2022	Brand P[2]	L031[1]	567g	Apr 13, 2023
		L991[2]	340g	Oct 9, 2022		L661[2]	567g	Jun 15, 2023
Edamame	Brand P[1]	L680[1]	227g	Jun 16, 2022	Brand S[2]	L452[1]	340g	Apr 2023
		L871[2]	227g	Jul 6, 2023		L890[2]	340g	Apr 2023

*Brand identity and lot number has been coded for this study. [1]Notates brand or lot 1 as appropriate. [2]Notates brand or lot 2 as appropriate.

5.2.2 Product Preparation

Three separate treatments were tested: (1) blanch/freeze/thaw (BFT); (2) BFT+microwave (BFT+M) (3) BF+stove-top cooking (BF+C). BFT products were thawed at refrigeration temperature (2° C) in a refrigerator (True Manufacturing Company Inc., O’Fallon, Missouri) overnight prior to analysis. These blanched, frozen, and thawed (BFT) products did not undergo any additional heat treatment and served as the control treatment. Microwave heated (BFT+M) products were processed in a modified method seen in previous studies (Carneiro et al., 2021) to represent the typical cooking method. Products were allowed to thaw in refrigeration temperatures for 8 to 12 hours, microwaved in 50-gram batches in a 1L Pyrex glass measuring container, covered with a paper towel, for forty seconds in a carousel microwave (model R2W38, 120 VAC, 60Hz, 1200 watts, Sharp Corporation, Thailand), and refrigerated overnight. Carneiro et al. reported a similar process with microwaving occurring for 4min in polyethylene plastic bags (2021). This length of heating time was determined to be inappropriate in this study as only 50-gram batches of products were prepared at a time.

Cooked products (BF+C) were prepared following stovetop cooking instructions for the entire package contents (Table 5-1) on Brand B (pea) and Brand P packaging (edamame, lima bean). Pea products were put in a 2.7L pan (Tefal, Rumilly, Haute-Savoie, France) with 118.3 mL of water, covered with a lid and cooked over medium heat on a gas stove (Southbend, Fuquay Varina, NC) for 5 min., then removed from heat and allowed to stand, covered, for 2min. Peas were then drained and allowed to cool at room temperature. Lima beans were covered with water, brought to a boil for 3min, then covered and reduced to a simmer for 25min before draining and cooling. Edamame beans were covered with water and brought to a boil for 3min before draining and cooling. Listed instructions were applied by sample type to recreate the texture to be closest to appropriate and acceptable texture by the producers and consumers for each sample type. All product treatments were stored in the refrigerator for 4 to 24 hours prior to texture and moisture analyses to ensure consistent temperature across products and treatments. Though BF+C treatments varied by product, the preparation methods represented the intended product texture for each legume.

While peas, lima beans, and edamame can be consumed hot or cold, products were tested at refrigeration temperature to mimic the sensory attributes of items on a salad bar. The cool refrigeration temperature also allows greater control in product temperature during testing and relates to ‘salad bar conditions’ when comparing to sensory data.

5.2.3 Analysis

Two brands were used for each vegetable for replication with two lots from each brand (Table 5-1). Experiment was designed to compare 3 different vegetables (edamame, pea, lima bean) with 3 different preparation treatments (BFT; BFT+M; BF+C) from 2 different brands each with 2 lots within the brand and 20 beans, with minor exceptions (Table 5-2) per treatment lot.

Puncture and compression testing were both completed following the ASABE S368.4, Compression Test of Food Materials of Convex Shape, guidelines using the TA XT Plus by Texture Technologies Corporation (Hamilton, MA) (ASAE, 2017a). Lima beans and edamame were oriented horizontally on the texture analyzer surface in reference to the hilum for consistency; though results will vary depending on vertical or horizontal orientation, one is not superior to the other (Paulsen, 1978). Products were positioned under the probe to ensure the tallest part of the vegetable made contact with the center of the probe. This alignment reduced sample movement and breakage during testing before the point of rupture was reached. Products with broken skin or damaged exteriors were excluded from the study. For puncture testing, beans were tested individually, arranged on a plate with hole, allowing the probe to completely penetrate the vegetable calculating surface strength as the probe entered (force 1) and exited (force 2) the sample. For compression testing, each individual bean was tested with the force-deformation curve recorded through the point of rupture. Puncture and compression analysis aimed to analyze twenty individual beans from each lot and treatment. Puncture testing used a 2mm puncture probe moving at 2.00mm/min. Compression used a flat plat probe moving at 2.00mm/min with 70% strain.

Moisture content of each sample and treatment was determined based on the American Society for Testing and Materials (ASTM) standard method S352.2 designed to measure moisture of unground grains and seeds (ASAE, 2017b). Legumes were prepared following the processing described and weighed (15 g). Testing was completed in triplicate. Products were placed in a hot air-drying oven (model OV702F, Thermo Scientific, Waltham, MA) at 105°C until a consistent weight was reached at approximately 72 hrs. Products were then brought to room temperature in a desiccator before being weighed. Products were weighed again, and moisture was calculated by dividing the sample weight loss (g) by the original sample weight (g) and multiplying by 100 ((loss in weight(g)/initial weight (g)) x 100) (ASAE, 2017b).

Statistical analysis of puncture, compression, and moisture data was completed using JMP Pro 15 (SAS, Cary, NC). Mixed model ANOVA was conducted followed by Tukey's HSD.

5.3 Results

5.3.1 Puncture

Mixed model ANOVA of these results showed significant interaction between product type and treatments in both force 1 ($p<0.05$) and force 2 ($p<0.05$). Tukey's HSD results (Figure 5-1) of a fixed model ANOVA show the BFT and BFT+M treatments to have similar impacts on each product type while the BF+C treatment reduced the force required for puncture which is likely due to structural changes which occur during cooking such as cell breakdown and starch gelatinization.

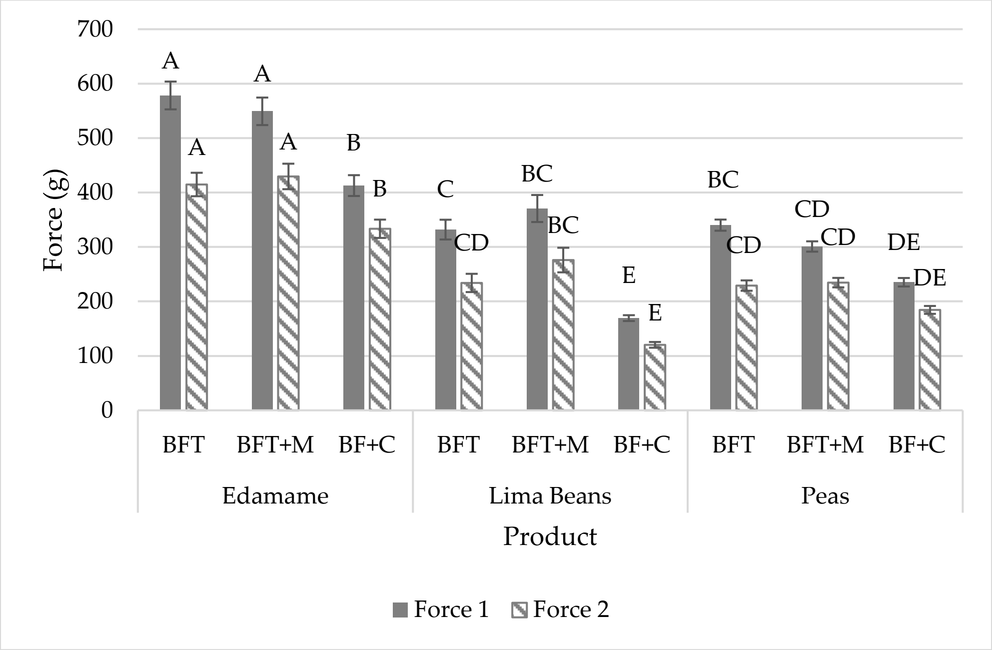

Figure 5-1: Puncture results (mean) of force 1 (g) and force 2 (g) by product (edamame; lima beans; peas) and treatment (blanch/freeze/thaw (BFT); BFT+microwave (BFT+M); BF+stove-top cooking (BF+C)).
Error bars were constructed using 1 standard error from the mean. Tukey's HSD connecting letters indicate similarities within force 1 and force 2 respectively and were calculated with a fixed effects model.

Edamame required more force to puncture the product across treatments with overall results of force 1 at 513.45 ± 14.30 (mean ± SE (g)) and force 2 at 392.62 ± 12.26 (mean ± SE (g)) as compared to the lima beans with force 1 at 290.68 ± 11.79 (mean ± SE (g)) and force 2 at 210.10 ± 10.40 (mean ± SE (g)) and peas with force 1 at 292.12 ± 6.00 (mean ± SE (g)) and force 2 at 216.07 ± 5.12 (mean ± SE (g)) indicating edamame is a firmer legume compared to peas and lima beans (14) (Figure 5-1).

Puncture analysis was unable to differentiate between BFT and BFT+M treatments of the three products from force 1 or force 2. However, puncture results indicated higher forces required by edamame than lima beans and peas in all cooking treatments (BFT, BFT+M, BF+C).

5.3.2 Compression

Mixed model ANOVA showed significant interaction between product type and treatments ($p<0.05$). Tukey's HSD results (Figure 5-2) of a fixed model ANOVA showed each treatment of peas to be similar texture among the product type while the treatments of edamame and lima beans showed similarities based on treatment type within the two products. Across treatments, peas required less force to cause sample rupture, with an overall mean force of 837.25 ± 68.59 (mean ± SE (g)), than both edamame and lima beans which required 3402.08 ± 68.02 (mean ± SE (g)) and 3598.94 ± 68.02 (mean ± SE (g)) respectively. These force values indicated that processed peas persisted less hardness than both processed edamame and lima beans (Lu & Abbott, 2004).

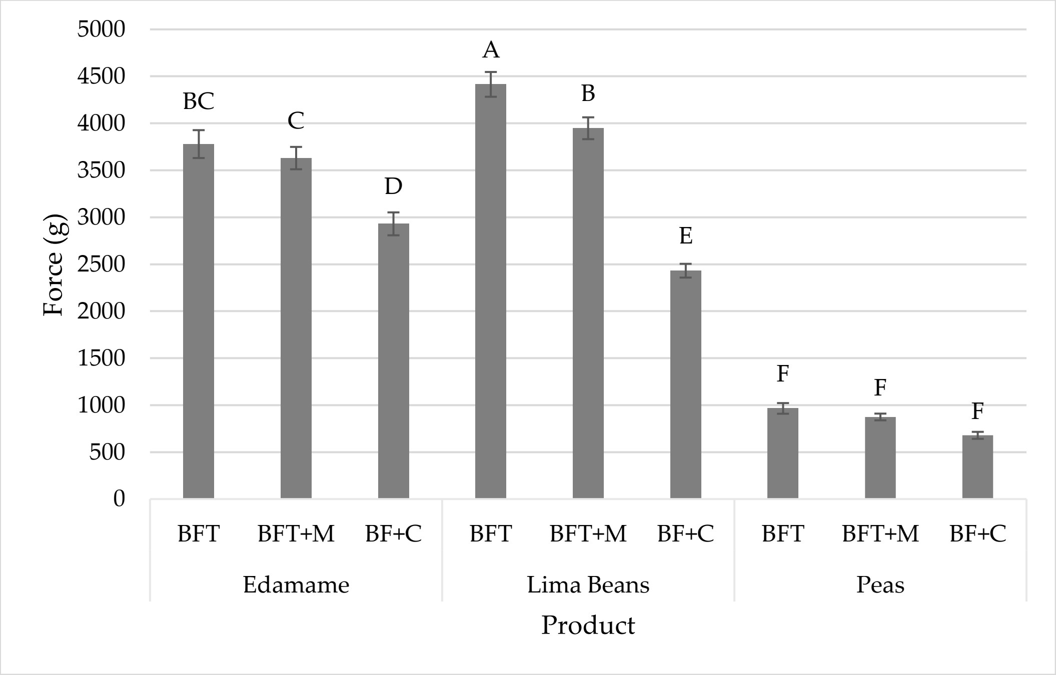

Figure 5-2: Compression results (mean) of force (g) by product (edamame; lima beans; peas) and treatment (blanch/freeze/thaw (BFT); BFT+microwave (BFT+M); BF+stove-top cooking (BF+C)).
Error bars were constructed using 1 standard error from the mean. Tukey's HSD connecting letters indicate similarities within force 1 and force 2 respectively and were calculated with a fixed effects model.

Compression analysis differentiated between treatments implemented for both edamame and lima beans but was unable to differentiate any of the treatments of peas. All treatments across peas (BFT, BFT+M, BF+C) were found to be similar in Tukey's HSD connecting letters.

5.3.3 Moisture

Mixed model ANOVA of the moisture results showed significant interaction between sample type and treatments ($p<0.05$). Results showed both sample type ($p<0.05$)

and treatments ($p<0.05$) to have at least one difference. Tukey's HSD results showed vegetables were similar within sample type except lima beans which showed higher moisture content in the BF+C treatment group (Figure 5-3). Across treatments, peas had higher moisture content at 78.00 ± 0.53 (mean ± SE (%)) than both edamame and lima beans at 69.47 ± 0.53 (mean ± SE (%)) and 67.82 ± 0.53 (mean ± SE (%)) respectively. This difference may explain the lower compression force required to rupture the products when compared to edamame and lima beans products.

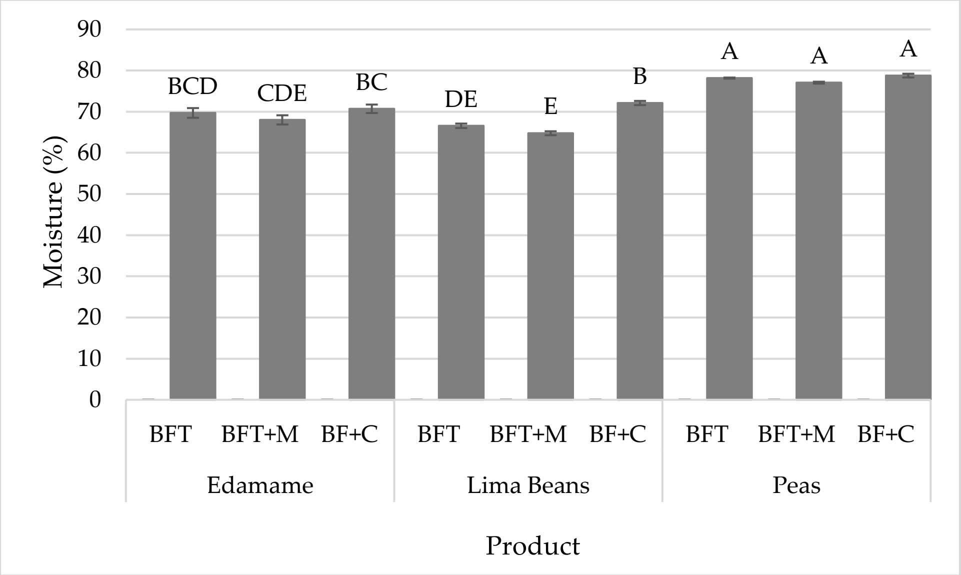

Figure 5-3: Results (mean) of moisture (%) by product (edamame; lima beans; peas) and treatment (blanch/freeze/thaw (BFT); BFT+microwave (BFT+M); BF+stove-top cooking (BF+C)).
Error bars were constructed using 1 standard error from the mean. Tukey's HSD connecting letters indicate similarities within force 1 and force 2 respectively and were calculated with a fixed effects model.

Table 5-2: Means (+/- SE) for compression (force (g)), puncture (force 1 (g); force 2 (g)), and moisture (%) evaluation of three vegetables (edamame, lima beans, peas) as affected by preparation method (blanched, frozen, thawed (BFT); BFT+microwave (BFT+M); BF+cooking (BF+C)) by lot and replication.

Cooking Method	**Brand**	**Edamame**						**Lima Beans**						**Peas**					
		Compression (Force(g))																	
		Lot 1			Lot 2			Lot 1			Lot 2			Lot 1			Lot 2		
		mean	±	SE	mean	±	SE	mean	±	SE	mean	±	SE	mean	±	SE	mean	±	SE
BFT	1	4158.96	±	330.14	2789.59	±	156.39	4913.60	±	259.39	5096.65	±	235.67	1184.51	±	94.36	903.06	±	149.73
	2	4041.92	±	236.13	4134.36	±	328.01	3641.53	±	192.75	4019.05	±	228.57	917.99	±	105.03	851.94	±	77.33
BFT+M	1	4057.37	±	240.20	2663.99	±	176.54	4574.49	±	192.64	3969.20	±	265.73	1015.37	±	60.05	699.74	±	76.34
	2	3606.32	±	205.97	3643.73	±	223.76	3721.71	±	147.65	3530.43	±	249.66	911.61	±	67.47	870.82	±	67.98
BF+C	1	3282.18	±	246.18	1994.95	±	108.21	2427.86	±	95.49	2847.56	±	138.23	908.34	±	63.17	519.30	±	31.85
	2	3039.67	±	188.09	3411.94	±	273.74	2322.14	±	131.88	2127.88	±	170.33	777.19	±	92.89	509.94	±	56.59
		Puncture (Force 1 (g))																	
BFT	1	695.94	±	60.16	345.59	±	21.69	319.61	±	38.72	411.17	±	52.77	403.46	±	20.25	283.90	±	19.59

	2	630.25 ± 40.36	641.50 ± 34.92	319.87 ± 22.28	277.18 ± 13.58	344.38 ± 17.55	328.77 ± 16.20
BFT+M	1	645.36 ± 62.98	345.68 ± 20.16	353.40 ± 43.17	448.17 ± 56.94	341.61 ± 21.26	259.03 ± 17.38
	2	576.67 ± 34.11	625.34 ± 45.94	327.43 ± 51.85	353.41 ± 44.54	311.17 ± 17.84	292.02 ± 15.25
BF+C	1	420.83 ± 44.21	277.20 ± 61.97	164.36 ± 13.41	192.14 ± 13.10	257.81 ± 12.54	247.33 ± 16.61
	2	496.85 ± 39.22	456.41 ± 30.96	154.96 ± 4.91	166.09 ± 7.76	236.27 ± 17.68	199.61 ± 12.93
Puncture (Force 2 (g))							
BFT	1	556.54 ± 52.72	278.40 ± 26.01	234.29 ± 28.50	299.26 ± 54.49	263.85 ± 23.94	197.32 ± 12.61
	2	459.30 ± 35.87	641.50 ± 26.70	230.81 ± 19.53	171.64 ± 9.91	211.17 ± 19.39	244.23 ± 16.13
BFT+M	1	573.74 ± 56.35	268.70 ± 29.08	272.82 ± 33.17	369.92 ± 68.62	263.45 ± 23.53	229.47 ± 10.06
	2	464.37 ± 43.28	412.38 ± 28.65	220.63 ± 33.94	240.76 ± 28.88	210.80 ± 17.66	234.66 ± 14.63
BF+C	1	409.38 ± 38.99	196.97 ± 20.28	112.96 ± 10.40	142.45 ± 13.17	204.27 ± 14.67	201.39 ± 12.54
	2	381.35 ± 30.63	346.23 ± 20.48	116.35 ± 8.11	109.33 ± 9.22	155.16 ± 13.42	177.07 ± 15.15
Moisture (%)							

BFT	1	63.20 ± 0.31	73.25 ± 0.48	65.770 ± 0.31	64.40 ± 0.25	78.40 ± 0.22	78.37 ± 0.28
	2	71.19 ± 0.45	71.16 ± 0.59	68.498 ± 0.63	67.62 ± 0.86	77.97 ± 0.35	77.93 ± 0.17
BFT+M	1	61.77 ± 0.38	71.09 ± 0.29	62.811 ± 0.22	63.98 ± 0.37	77.21 ± 0.25	77.60 ± 0.24
	2	70.24 ± 0.12	68.95 ± 0.39	65.965 ± 0.82	66.22 ± 0.72	75.94 ± 0.15	77.50 ± 0.42
BF+C	1	65.09 ± 0.25	74.07 ± 0.29	71.62 ± 0.33	69.85 ± 0.12	78.27 ± 0.13	78.60 ± 0.32
	2	71.85 ± 0.47	71.80 ± 0.51	72.63 ± 0.41	74.33 ± 0.44	77.09 ± 0.31	81.15 ± 0.20

[1] Edamame Brand 1: Brand P (lot 1:L680; lot 2:L871), Brand 2: Brand S (lot 1: L452; lot 2: L890); Lima Beans Brand 1: Brand K (lot 1: L161; lot 2: L991), Brand 2: Brand P (lot :L031; lot 2:L661); Peas Brand 1: Brand S (lot 1: L737; lot2: L454), Brand 2: Brand B (lot 1: L520; lot 2: L420). [2] The BFT peas during compression analysis only collected 19 data points from lots L737 and L520 and only 18 from L420. Similarly, the MH lima beans compression only collected 19 data points from lot L161. The puncture testing of edamame collected an extra data point resulting in a total of 21 from the BFT+M treatment of L890. These variations were due to data transfer issues. [3] blanch/freeze/thaw (BFT); BFT+microwave heat (BFT+M); BF+stove-top cooking (BF+C).

5.4 Discussion

Though edamame, peas, and lima beans all have similarities in use and interest in the domestic market, texture of these vegetable legumes vary based on product type and preparation. Mean force required to puncture through edamame products were consistently higher than both lima beans and peas. Using compression, peas required less force to rupture compared to edamame and lima beans. Based on our results, generally, lima beans and peas are more similar to each other than edamame based on puncture while edamame and lima beans are more similar to each other than peas through compression.

Compression analysis more successfully differentiated between treatments implemented for both edamame and lima beans. However, compression was unable to differentiate between any of the implemented treatments of peas. Puncture analysis was more successful differentiating the processing treatments of peas but was not precise enough to fully differentiate each treatment entirely. This was also true for the BFT and BFT+M treatments of edamame and lima beans. Puncture analysis was able to differentiate the BF+C treatments of edamame and lima beans, however.

BF+C treatment methods varied by product to better evaluate the intended state of each individual product. This variation limits the ability to compare this treatment method across product types while also providing a baseline specific to each product. Differences across products within the BF+C treatments show the presumably intended texture for these products as established by the brands. The BF+C treatment also consistently required less force for each legume over BFT and BF+M with edamame and lima beans showing this with statistical significance. This variation from edamame and lima beans to peas is likely due to not only the intrinsic structure differences between legumes, but also the longer cooking time required to prepare these products, which caused structural changes such as further protein denaturation or breakdown of starch granules caused by heat.

Total starch content and fiber in legumes varies by legume type and variety. Edamame nutritional content also varies by maturity at harvest (Yu et al., 2021). Nikolopoulou et al. found variety to impact nutrition of peas as well as environmental factors and growing year (2007). It is likely that variety, environment, year, and location can also impact nutritional content and other attributes of crops including legume vegetables.

When harvested at optimal maturity, Yu et al. found edamame to be around 12% starch and 6% fiber while noting higher starch content in edamame is often preferred to achieve softer edamame after cooking caused by starch gelatinization as well as pectin solubilization (2021). Lima beans tend to be higher in starch with 35-40% starch and 6-7% fiber on a wet basis reported while peas have the highest starch of these three legumes with 55-68% starch and 3-7% fiber reported also on a wet basis (Arntfield & Maskus, 2011). These numbers help explain the low forces required for both puncture and compression of peas. As they have the highest starch content, they would also likely soften due to starch gelatinization during any heat process. As the compression data of peas shows the force required for each processing method is the lowest of all vegetables, peas also have the highest moisture contents. This moisture content likely contributes to the low forces required to reach the rupture point as the water present in the sample could provide less resistance to the probe than the starch, protein and fiber that are more prominent in the edamame and lima beans. These patterns may change when analyzing samples which were not previously frozen and is a path for additional research in this area. Additional research into a standardized texture analysis for legume vegetables, fresh or frozen, will be needed to conclude either of these methods to be appropriate for implementation into quality control programs.

While lima beans and peas both have a thicker skin around the starchy center holding the vegetable together and creating contrast in texture, edamame was observed to have less texture variation through the structure of the bean. Though this observed lack of texture contrast was not measured in this work, the observation helps explain additional differences among the products researched. The methods employed in this researched does not specifically measure this characteristic however, these similarities and differences may be considered when selecting ingredients for a new or reformulated food or food product.

Our knowledge around the vegetable growing and processing conditions including varieties produced, processing by brand, and vegetable type is a significant limitation of this research. Variations in data by lot within brand for lima beans and peas are not easily explained as physical appearance of products and moisture results do not support any obvious conclusions. These variations between lots within sample types and brands maybe due to differences in growing and/or processing conditions that are not known by the researchers in this study. Edamame products had statistical differences in results of brand 1 (Brand P) edamame lots which were likely due to poor quality of edamame in the lot (L680). Half of the edamame in the packages were yellow, indicating late harvest of the product which also implies lower moisture content compared to edamame harvested on time. The higher force required, and lower moisture content of these products confirm their maturity and inferior quality initially inferred based on the vegetable color. Continuing this research with additional brands or products grown and processed in controlled environments may result in a better understanding of method outcomes as related to the growing and processing conditions of which the products were subjected.

The results of this study do not relate to any product evaluation of warm product. Storage and analysis at refrigeration temperature was chosen to reduce variability in analysis and relates directly to cold consumption uses of these products such as how they are on a salad bar or other cold foods. Consumer perception of products will vary based on temperature due to influence on flavor perception and needs to be considered for research relating to consumers.

Development and implementations of an instrumental-based texture method and quality standards for legumes quality control readily adopted by growers and producers are of vital importance for plant-based market. This study showed compression texture analysis could be useful when determining maturity of edamame as well as processing changes in edamame and lima beans. Setting standards could more easily guide and determine appropriate quality specifications for products through the processing plans.

For texture testing by growers and producers, the compression method may be more sensible for implementation due to the ease of data analysis and relation to chewing with molars as these products would often be crushed in the mouth more than punctured. Additionally, puncture analysis was not able to distinguish processing differences in edamame, lima beans, or peas while compression analysis was sensitive enough to detect these distinctions in edamame and lima beans.

Continuing research focused on the compression method researched here would be advised over the puncture method due to the ease of analysis and sensitivity of analysis for both edamame and lima beans. While the puncture method results in two forces, the compression method gives a single force output. Puncture analysis was able to distinguish treatments of peas better than compression analysis, but this was not true of lima beans and edamame. However, this work has shown utilizing the ASABE S368.4, Compression Test of Food Materials of Convex Shape, is an effective method which can be applied further to determine specific standard methods for legumes (ASAE, 2017a).

Additional research should be completed to better relate this resulting force to texture attributes. Specific quality parameters would need to be established based on the individual product, desired quality and sensory attributes, moisture content, and other variations among vegetables, locations, and facilities. Cooking methods impact product characteristics differently due to unique product attributes. Texture changes can also be seen due to storage conditions and time, moisture migration, and general degradation of the food structure throughout the processing and shelf life.

5.5 Author Contributions

RM, SD, BZ, and YY contributed to conception and design of the study. RM collected the data. RM, SD, and JL performed the statistical analysis. RM wrote the first draft of the manuscript. All authors contributed to manuscript revision. All authors read and approved the submitted version with the exception of SD who passed prior to the final version.

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Chapter 6

6. Identification of sensory descriptors and aroma-active compounds in the U.S. edamame supply chain

This chapter was in preparation for submission to *Food Quality and Preference*.

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Abstract: A continued increase in U.S. edamame consumption has created opportunities for domestic edamame development and production. Domestically bred and produced edamame, as they adapt to growing environment, are known to be more advantageous to the local growers and consumers due to favorable and recognized traits. Research into the sensory and flavor attributes of domestic edamame will support further development of edamame products. This project worked to develop a list of sensory descriptors important to the domestic edamame market and identify aroma-active compounds contributing to these characteristics. Four edamame varieties were grown in 3 locations for this study. Two additional samples were purchased from a local grocery store. A generic descriptive analysis (DA) sensory panel with 8 trained panelists developed a list of 27 edamame characteristics including taste, aroma, and texture attributes. Gas chromatography-olfactometry (GC-O) was completed by 7 additional panelists who were trained on 12 identified aroma-active compounds in edamame from literature and preliminary research. Eleven of the identified characteristics through DA were significantly different across edamame samples including 2 taste attributes (“sweet”, “bitter”), 6 aroma attributes (“fresh”, “salad”, “grassy”, “vegetable oil”, “alcohol”, “burnt toast”), and 3 texture attributes (“hardness”, “crunchy”, “juicy”). GC-O results found (E)-2-octenal as the only compound displaying significant differences across edamame samples. Significant differences identified in taste, aroma, and texture suggest a diversity of sensory characteristics currently present in the domestic edamame market. The application of these sensory findings could help support further breeding and additional product development as domestic edamame production continues to flourish.

Keywords: edamame, descriptive analysis, sensory, volatile analysis, flavor, aroma

6.1 Introduction

As an interest in plant-based foods and healthy living continue to draw focus in the United States (U.S.), vegetable soybean [*Glycine max* (L.) Merr.], commonly known as edamame, is seeing an increase in popularity. Along with being high in protein, edamame can provide dietary fiber, vitamins C and E, isoflavones and carotenoids (Johnson, 2000; Simonne et al., 2000). Though soy is a common crop in U.S., edamame is less commonly grown with an estimated 70% of domestic edamame being imported from Asia (Jiang et al., 2018). While soybeans are allowed to grow to full maturity, referred as R8 stage of maturity, edamame is harvested around the R6 stage of maturity that is characterized by a green pod with 85-90% seed fill, around 65% moisture content and highest sweetness (Fehr et al., 1971; Yu et al., 2022).

Edamame has been established as a feasible crop for growing across the U.S. (Garber & Neill, 2019; Jiang et al., 2018; Zhang et al., 2021), with increasing interest from breeders and growers to expand the domestic edamame supply chain especially as consumer interest continues to rise. With other specialty crops such as tobacco continuing to lose demand, growers may find edamame to be a good replacement crop to support economic goals. To better meet domestic needs, edamame breeding programs have been continuously developing edamame varieties well suited for various growing regions accounting for climate and preferred agronomic traits of the crop. Consumer acceptability and priorities must also be evaluated to ensure domestically bred varieties not only grow well but create products of appreciated quality and value.

Descriptive sensory methods are beneficial when researching multiple complex sensory attributes (Lawless & Heymann, 2010a). Applications of descriptive sensory methods for edamame have been reported using check-all-that-apply (CATA), free choice profiling, Quantitative Descriptive Analysis (QDA), and Sensory Spectrum technique (Flores et al, 2019; Guo et al., 2022; Krinksy et al., 2006; Wszelaki et al., 2005). Studies have shown noticeable differences in sensory characteristics exist in edamame based on variety, harvest time, and cooking methods (Carneiro et al., 2021; Flores et al., 2019; Krinksy et al., 2006; Wszelaki et al., 2005). However, there have been very few investigations of the flavor and texture of edamame grown in the U.S. from domestically available edamame products. Wszelaki et al. (2005) conducted a descriptive analysis (DA) panel with edamame grown in Ohio (USA), but only measured 4 characteristics ("beaniness", "chewiness", "nuttiness", "sweetness") in the study. Krinksy et al. (2006) developed a 14-term lexicon for edamame ("raw bean"; "cooked bean"; "green complex"; "fruity complex"; "nutty/almond"; "brothy"; "sulfur"; "salty"; "sweet"; "sour"; "bitter"; "astringent"; "umami"; "metallic") but utilized edamame that was harvested in China, Taiwan, and Japan. Carneiro et al. (2021) utilized a 15 term CATA to collect information on descriptive terms and to describe domestically bred and grown edamame that included the 14 terms described by Krinksy et al. (2006), but combined "brothy" and "umami" into one term ("brothy/umami") and included "chewy" and "starchy" as well.

A recent study by Gu et al. (2022) conducted a detailed study in China on volatile compounds and sensory properties of 3 edamame varieties (Taiwan 292, Xin 3, Suxin 6) grown in Nanjing (China) from the local edamame market. The varieties selected had varying origins and history despite all being popular varieties to grow in China at the time of the research. The study included electronic tongue analysis, volatile analysis using solid phase microextraction and gas chromatography with mass spectrometry (SPME-GC-MS), and sensory QDA conducted in Nanjing (China). Forty-one volatile compounds were identified in the edamame, but the main sensory results focused on texture and taste characteristics as panelists struggled to differentiate edamame varieties by aroma (Gu et al., 2022).

Recent advances in U.S. edamame breeding and implementation into local agriculture have created an expansion of the domestic edamame market. Investigating volatile organic compounds contributing to aroma would bring an understanding of which compounds most contribute to overall edamame flavor. Research focused on the sensory and flavor characteristics of domestic edamame is currently lacking in literature but will be beneficial to this industry; therefore, our study will fill in this knowledge gap by understanding the edamame quality and current state of the supply chain.

This project worked to identify the sensory characteristics of edamame varieties commonly used in U.S. edamame production and volatile compounds which may contribute to sensory experiences during consumption. We compared these sensory and chemical characteristics across multiple varieties grown in multiple locations to determine which attributes vary amongst domestically grown edamame and how. Descriptive sensory methods paired with flavor chemistry to determine quality attributes and aroma-active compounds has not been completed with domestic edamame but will add valuable information understanding, communicating, and utilizing important descriptors to support the development of the domestic edamame industry.

6.2 Materials and Methods

6.2.1 Samples

Four commercially available U.S. edamame cultivars were grown each in Blacksburg, VA; Painter, VA; and Portageville, MO by experienced growers and 2 additional samples were purchased from local grocers (Blacksburg, VA, USA). Each growing location grew a total of 4 cultivars (Chiba Green, VT Sweet, UA Kirksey, and Midori Giant) during the 2022 growing season. Cultivars were chosen based on availability and representation in domestic production at the time of this research. Chiba Green seeds were provided by the Eastern Shore Agricultural Research and Extension Center (Virginia Tech, Painter, VA), and VT Sweet and UA Kirksey seeds were provided by the School of Plant and Environmental Sciences (Virginia Tech, Blacksburg, VA). Midori Giant seeds were purchased from Wannamaker Seeds (Saluda, NC). Chiba Green and Midori Giant plants grown in Blacksburg were damaged by deer early in the growing season and were unable to be used in the study.

Edamame samples were harvested around R6 maturity as is most optimal for edamame based on pod fill, sweetness, and color (Xu et al., 2015; Yu et al., 2022). At harvest, pods were separated from stems and leaves before transportation in coolers with ice packs to the pilot plant in the Department of Food Science, Virginia Tech (Blacksburg, VA). Samples were delivered within 24 hours of harvest to ensure processing and storage could be completed within 48 hours of harvest time. Edamame processing was followed by the protocol developed by Carneiro et al. (2021) with modifications. Specifically, edamame pods were thoroughly rinsed in tap water to remove remaining dirt, debris, and leaves then rinsed in distilled water before blanching. Edamame pods were then put into metal baskets and blanched in a steam kettle (Legion Utensils Co., Long Island City, NY, USA) at 98 ± 1°C for 1 min. Immediately after blanching, edamame was plunged into an ice bath (4 ± 1°C, 2 minutes) to chill; pods were dried in salad spinners before being peeled by hand and stored at -20°C.

Two additional edamame samples were purchased as frozen beans from Kroger (Cincinnati, OH) in the local area (Blacksburg, VA, USA) to provide additional representation of the domestic edamame market. Store bought edamame was stored with the edamame previously described at -20°C.

6.2.2 Descriptive Analysis

To prepare edamame for the descriptive analysis training and evaluation sessions, tap water (Blacksburg, VA, USA) was brought to a rolling boil before adding frozen edamame beans to continue to boil for 5 minutes. The edamame was then strained out of the boiling water and cooled under cool running tap water for 1 minute allowing the beans to cool quickly and reduce visual wrinkles appearing on the surface of the beans. Edamame was then drained on a paper towel and placed into black plastic ramekins topped with a clear plastic lid. Ramekins were labeled with a random 3-digit code to de-identify the samples. Samples were then stored at refrigeration temperature (4°C) overnight. Around 1 hour prior to DA panel training or evaluation sessions, samples were removed from the refrigerator and conditioned to room temperature.

The descriptive analysis panel was completed at Virginia Tech following the general method outlined by Lawless and Heymann (2010a). Panelists were recruited from the Blacksburg community and compensated for their time with snacks and meals at the conclusion of each training and evaluation session. Eight panelists (2 male, 6 female) completed the study with each attending 9 training sessions and 6 evaluation sessions. All sessions occurred in the Human and Agricultural Biosciences Building 1 (HABB1) at 1230, Washington St. SW, Virginia Tech, Blacksburg, VA 24061 with training sessions occurring in the atrium and evaluation sessions occurring in the sensory lab booths. Ethical approval for the involvement of human subjects in this study was granted by the Virginia Tech Institutional Review Board (IRB-23-064).

Training sessions were led by the panel leader, with 9 1-hour training sessions occurring over 3 weeks (3 sessions per week for 3 weeks). Each training session utilized 4 edamame samples, chosen at random, in plastic ramekins with random 3-digit codes to de-identify samples. Across the 9 training sessions, panelists saw each of the 12 samples 3 times. In the first training sessions, panelists were given written and verbal instructions on sample evaluation including directions on smelling, tasting, and evaluating each sample. After panelists evaluated each sample, recording descriptors in the provided worksheet, the panel leader led discussion inviting panelists to share the attributes identified, possible meaning and references for each attribute, and general discussion to build understanding and consensus among the panelists. Panelists were provided with written evaluation instructions, training worksheets, pencils, samples, references for identified characteristics, and water and saltines to cleanse palates.

Evaluation sessions were scheduled based on individual participant availability, starting one day after training was completed and ending within 3 weeks. Panelists were allowed to schedule up to 2 evaluation sessions per day with at least 1 hour between sessions to avoid fatigue. Evaluation sessions required panelists to evaluate 6 edamame samples using the established evaluation methods, attributes, and references established and practiced in training sessions. Panelists were given all references at the time of evaluation for their use as desired. Panelists also had water and unsalted saltines to cleanse their palate during training and evaluation sessions.

Evaluation sessions utilized Compusense Sensory Management Software (Ontario, Canada) for data collection with panelists responding to each established attribute using a line scale. Panelists practiced with Compusense Sensory Management Software in the last 2 training sessions and line scales in the last 4 training sessions. Panelists worked together to determine a preferred line scale determining labeled endpoints (“low” on the left, “high” on the right) with the midpoint marked but not labeled would be most preferred for taste and flavor attributes. Texture and mouthfeel attributes used a similar line scale with end points labeled as deemed appropriate by attribute (Table 6-1) with the midpoint marked, but not labeled.

Table 6-1: End point labels for the line scales of each texture and mouthfeel attribute for edamame descriptive analysis evaluation sessions.

Texture and Mouthfeel Attribute	Left Label	Right Label
Hardness	Soft	Firm
Crumble	No crumble	High crumble
Crunchy	No crunch	High crunch
Chalky	Not chalky	High chalky
Juicy	Not juicy	High juicy
Coating	No coating	High coating
Buttery	Not buttery	High buttery

Note: the midpoint of the line scale was marked but left unlabeled for all attributes.

6.2.3 GC-O Volatile Analysis

Gas chromatography-olfactometry (GC-O) was utilized to identify key volatile compounds in the edamame samples. GC-O analysis was completed on an Agilent Gas Chromatography 6890N equipped with 5975B Mass Spectrum Detector (Agilent Technologies, Santa Clara, CA, USA) and Leap Technologies CTC PAL Autosampler (Trajan Scientific and Medical, Ringwood, Vic 3134, Australia) and PHASER GLS-G300021 Olfactory Detection Port system. Nine panelists (2 male; 7 female) were trained on 12 volatile compounds (Table 6-2) found to be important aroma compounds through GC-MS and preliminary GC-O. Eight panelists (2 male; 6 female) successfully completed training and testing to continue with GC-O sample evaluations.

Table 6-2: Volatile compounds used to train GC-O panelists for edamame GC-O sample evaluations.

Calculated RI	**Reference RI**	**Compound**	**Description[1]**
~	935[2]	Pentanal	Fermented, Bready
1072	1084[2]	Hexanal	Fresh, Green
1163	1157[3]	1-Penten-3-ol	Horseradish, Green
1289	1280[2]	Octanal	Waxy, Citrus
1304	1295[4]	1-Octen-3-one	Herbal, Mushroom
1325	1305[4]	(E)-2-Heptenal	Pungent, Green
1339	1332[3]	6-Methyl-5-hepten-2-one	Citrus, Green
1357	1360[2]	1-Hexanol	Green, Fruity
1393	1385[2]	Nonanal	Citrus, Cucumber
1428	1408[2]	(E)-2-Octenal	Fatty, Green
1452	1451[2]	1-Octen-3-ol	Mushroom, Earthy
1456	1467[2]	1-Heptanol	Musty, Leafy

[1]https://www.thegoodscentscompany.com/; [2]https://www.flavornet.org/flavornet.html; [3]Kesen, S., Kelebek, H., Sen, K., Ulas, M., & Selli, S. (2013). GC–MS–olfactometric characterization of the key aroma compounds in Turkish olive oils by application of the aroma extract dilution analysis. Food Research International, 54 (2), 1987-1994. https://doi.org/10.1016/j.foodres.2013.09.005.; [4]Rychlik, M., Schieberle, P., and Grosch, W. (1998). Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Ordorants. TUM. Garching, Germany

Training for GC-O included 3 or 4, 30-minute training sessions, depending on panelists comfort and confidence identifying the aromas, where panelists sniffed 1.0 µL of a solution of known aroma compounds prepared from chemical standards through the GC-O port. The solution included 3.5µL of pentanal (97%), 2.5µL of hexanal, 1.0 µL of octanal (95%), 0.5 µL of 1-octen-3-one (≥96%), 1.0µL of 2-heptenal (≥95%), 1.0 µL of 6-methyl-5-hepten-2-one (≥98%), 7.0 µL of 1-hexanol (≥99.5%), 1.0 µL of nonanal (97%), 0.9 µL of 1-octen-3-ol (≥98%), and 1.5 µL of 1-heptanol (≥97%) which were purchased from Sigma Aldrich (St. Louis, MO, USA). 3.5µL of 1-penten-3-ol (99%), and 0.8 µL of (E)-2-octenal (96%) were also included in the solution and were kindly provided by Bedoukian Research Inc. (Danbury, CT, USA). The specified amounts of each standard were added into 10mL of methanol to create the standard training solution. The amount of each compound was chosen based on preliminary GC-O of the solution to ensure each compound was detectable in solution and a variety of aroma strengths were present in the solution.

During training sessions, panelists received a reference document showing the retention time, compound name, and descriptors for each compound they could expect to detect. Panelists were able to take notes and ask questions before, during, and after each training session. The number of training sessions completed depended on the individual panelists comfort with the aroma compounds. They were asked to complete at least three training sessions but were allowed to decide if they were ready to test after the third training session or if additional training sessions were desired. After the panelists were comfortable with the training progress, they were tested using the same process and aroma solution used in the training sessions. The test session required panelists to provide the aroma descriptors and retention times without reference materials. Only panelists able to detect at least 11 of the 12 compounds were selected to continue in the study for sample evaluation resulting in 8 panelists (2 male; 6 female) for evaluation sessions.

The edamame samples were first prepared as they were for descriptive analysis, boiling for 5 minutes followed by cooling for 1 minute before refrigeration storage overnight. Samples for GC-O were then prepared for analysis by grinding around 10 grams of prepared edamame beans with around 20 grams of dry ice to a free-flowing powder in a dedicated Krups Coffee and Spice Grinders (Krups, Solingen, Germany) that were thorough cleaned with diluted dish soap and water, rinsed, and allowed to dry completely between uses. The ground samples were then weighed into clean, 20mL SPME vials (Supelco, Bellefonte, PA, USA), adding 2.0g ± 0.4g of sample per vial, preparing three vials from each grind to result in laboratory triplicates. The headspace of each vial was flushed with nitrogen then capped with a PTFE-lined cap (Supelco, Bellefonte, PA, USA).

For sample evaluation, sample vials were incubated at 45^{o}C with 30 minutes of adsorption time at a rotation speed of 250 rpm. Dedicated 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers (Stableflex, Supelco, Bellefonte, PA, USA) were used for extraction for 30 minutes. The oven temperature was held an initial temperature of 40^{o}C for 5 minutes, then increased by 5^{o}C per minute for 18 minutes until the temperature reached 130^{o}C. The temperature then increased by 15°C per minute for 21 minutes, reaching 225°C. Separation of volatile compounds occurred using a DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) (Supelco, Bellefonte, PA, USA) using helium at a flow rate of 2.2mL per minute as the carrier gas. Each edamame sample required 3 panelists (1 male; 2 female), one panelist per vial, with each vial being a triplicate for the edamame sample. Panelists were selected for each evaluation session based on schedule availability. During sample evaluation, panelists recorded aroma descriptors and the corresponding retention time and odor intensity (“very weak”; “weak”; “moderate”; “strong”; “very strong”) for the duration of the GC run. Odor intensities were converted to ordinal data (0-5) for analysis.

6.2.4 Statistical Analysis

Results obtained through descriptive analysis (DA) were analyzed through multivariate analysis of variance (MANOVA) by panelist, replication, and variety as well as by panelists, replication, and location to identify significant differences ($p<0.05$). To identify differences among edamame samples by sensory attributes, pseudomixed ANOVA and Tukey’s HSD was used accounting for sample and panelist interactions. GC-O data underwent Kruskal-Wallis test to show significant variability ($p<0.05$) of volatile compounds across edamame varieties with the Dunn post-hoc test with the Benjamini-Hochberg FDR correction. Principal component analysis (PCA) based on the significant DA attributes, nonsignificant sensory attributes, and the GC-O data projected as supplemental data was used to provide a visual representation of the results and relationships among the edamame samples, sensory attributes, and GC-O compounds. To determine differences among the edamame varieties and growing locations, data from the store-bought samples were excluded for an additional type III sum of squares ANOVA analysis followed by Tukey’s HSD to identify differences by edamame varieties and growing locations by significant sensory attributes. Data analysis was completed using a combination of JMP Pro 15 (SAS, Cary, NC) and R Studio (R version 4.0.5).

6.3 Results

6.3.1 Sensory Attributes

Table 6-3: Sensory descriptors and references developed during the edamame descriptive analysis training sessions involving 8 panelists and 12 edamame samples.

Category	Attribute	Reference
Taste	Bitter	1 piece of pith from a grapefruit, rinsed in water
	Sweet	7 g sucrose dissolved in 500 mL water
	Umami	1/4 tsp monosodium glutamate dissolved in 1 L water
	Salty	1.5 g NaCl dissolved in 500 mL water
	Sour	0.75 g citric acid dissolved in 500 mL water
Aroma	Grassy	2 Tbsp cut grass with 1/2 Tbsp dry grass, stored at 40° C for 2 days
	Fresh	2-inch piece crushed cucumber
	Herbs	1 tsp fresh parsley leaves with 1/2 leaf of fresh cilantro and 3-4 leaves fresh oregano
	Soy beany	2-3 Tbsp drained and rinsed canned chickpeas with 1-2 Tbsp prepared dried soybeans (soaked overnight in excess water and cooked over medium heat with excess water for 60-90 min.)
	Bell pepper	1 inch piece green bell pepper
	Celery	1 inch piece of celery
	Salad	1 tsp frozen peas, 2, 3-inch pieces of ripped romaine lettuce, 4-5 leaves of ripped, slightly crushed spinach, 1-inch piece of fresh broccoli stem microwaved in water for 2.5 min.
	Nutty	6-8 smashed raw almonds with 3-4 pieces of hazelnuts, 3 smashed walnuts, 1 smashed chestnut
	Vegetable oil	2 tsp vegetable oil with 1 teaspoon flax seed oil
	Buttery	1-2 Tbsp of Country Crock Original Spread
	Sulfur	1/4 egg boiled in water for 14 min.
	Wet soil	1 Tbsp Miracle-Grow potting mix
	Alcohol	1 tsp 70% isopropyl alcohol with 3 tsp water
	Metallic	7 pennies
	Burnt toast	1/2-inch piece of burnt toast
Texture	Buttery	1 Tbsp pad of butter
	Crumble	Mid: Queso fresco cheese
		High: Athenos feta cheese
	Harness	Firm: Brugge comtesse cheese
		Soft: Slightly green banana slice
	Crunchy	Roasted cashews, unsalted
	Coating	Olive oil
	Chalky	1/2 cup butter and herb mashed potato mix with 1 cup hot water
	Juicy	Red seedless grapes

DA panelists developed a list of 27 characteristics with references for the edamame samples (Table 6-3). Characteristics were categorized as taste, aroma, or texture. Multivariate analysis of variance (MANOVA) identified significant differences ($p<0.05$) in sensory attributes of the 12 edamame samples. Of the 27 sensory attributes determined by DA panelists, 11 were identified to be significantly different ($p<0.05$) by pseudomixed ANOVA between all 12 samples including 2 taste attributes (“sweet”, “bitter”), 6 aroma attributes (“fresh”, “salad”, “grassy”, “vegetable oil”, “alcohol”, “burnt toast”), and 3 texture attributes (“hardness”, “crunchy”, “juicy”) (Table 6-4). The pairwise differences in these 11 significant attributes based on Tukey’s HSD can be seen in Figure 6-1.

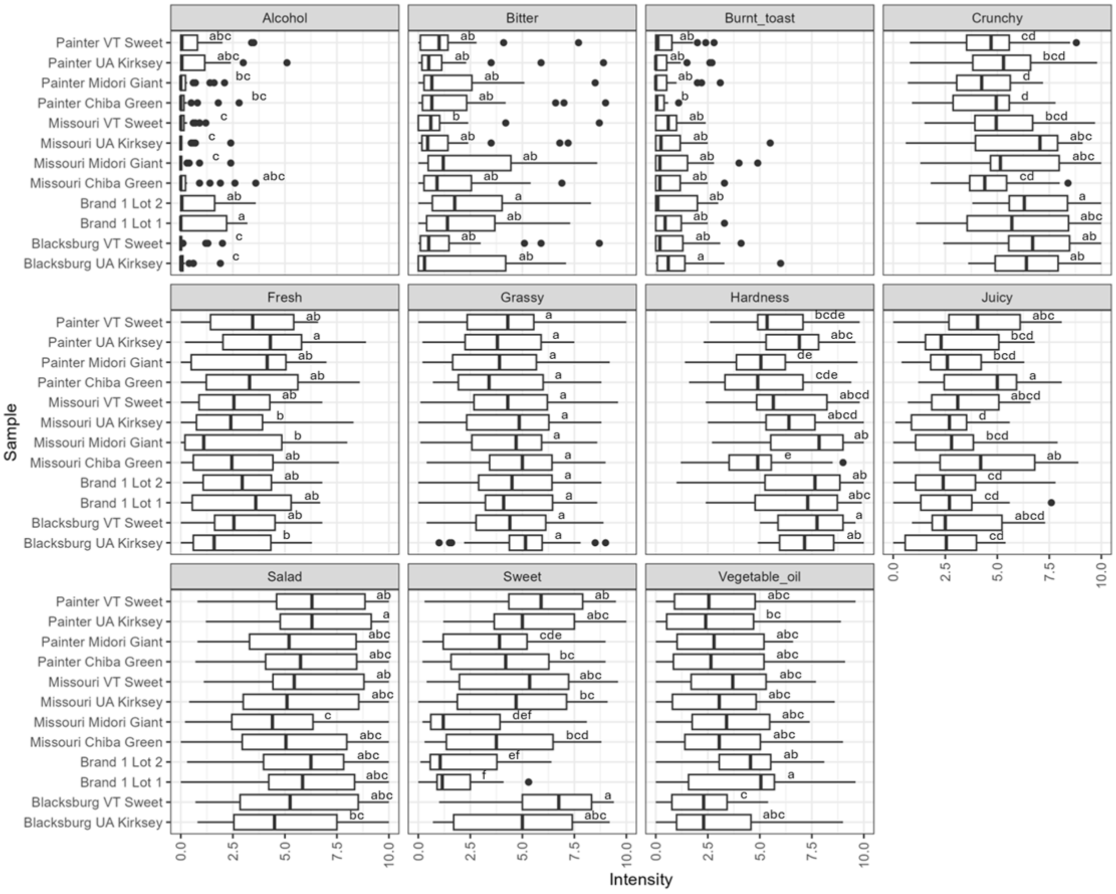

Figure 6-1: Boxplot of intensity ratings for each significant descriptive analysis attribute ($p<0.05$) by edamame sample through pseudomixed ANOVA.
Tukey’s HSD connecting letters indicate similarities by attribute.

UA Kirksey edamame grown in Painter, VA was perceived to be the most “fresh” sample, significantly higher than the lowest “freshness” ratings from UA Kirksey grown in Blacksburg, VA, and UA Kirksey and Midori Giant grown in Portageville, MO. UA Kirksey grown in Blacksburg, VA had the highest mean for “grassy”, but post hoc testing did not show significant differences from other samples, likely indicating insufficient power for discrimination. UA Kirksey grown in Painter, VA had significantly higher mean for “salad” than the lowest “salad” rating seen in UA Kirksey grown in Blacksburg, VA and Midori Giant grown in Portageville, MO. Above observations confirmed the location effect. Similarly, lot 1 store sample had significantly higher “vegetable oil” ratings, than UA Kirksey grown in Painter, VA and VT Sweet grown in Blacksburg, VA. Lot 1 store sample also had significantly higher mean for “alcohol” than Chiba Green grown in Painter, VA and Portageville, MO, VT Sweet grown in Portageville, MO and Blacksburg, VA, UA Kirksey grown in Portageville, MO and Blacksburg, VA, and Midori Giant grown in Painter, VA, and Portageville, MO. However, this attribute was not used by all panelists. UA Kirksey grown in Blacksburg, VA had the highest mean for “burnt toast”, significantly higher than Chiba Green grown in Painter, VA.

VT Sweet grown in Blacksburg, VA had the highest mean “sweet” ratings, significantly higher than Chiba Green grown in both Painter, VA and Portageville, MO, UA Kirksey grown in Portageville, MO, Midori Giant grown in both Painter, VA and Portageville, MO and both store-bought samples. The lot 2 store sample had the highest “bitter” ratings, significantly higher than the lowest “bitter” from VT Sweet grown in Portageville, MO.

VT Sweet grown in Blacksburg, VA had the highest mean “hardness” ratings, significantly higher than VT Sweet and Chiba Green grown in Painter, VA as well as Chiba Green grown in Portageville, MO. The lot 2 store sample had the highest “crunchy” ratings, significantly higher than all 4 edamame varieties grown in Painter, VA as well as Chiba Green and VT Sweet grown in Portageville, MO. Chiba Green grown in Painter, VA had the highest mean “juicy” ratings, significantly higher than UA Kirksey and grown in Blacksburg, VA, Painter, VA and Portageville, MO as well as Midori Giant grown in both Painter, VA and Portageville, MO and both store-bought edamame samples . Texture attributes are notoriously difficult to quantify in sensory work with “juicy” being especially difficult due to the reference being a grape (Table 6-3) requiring panelists to rate beans individually for this attribute instead of using multiple beans as in other attributes.

The first two dimensions of the PCA explained 69.3% of the variability in the 11 DA attributes with significant differences across the 12 edamame samples. Dimension 1 accounts for 44.1% of the variation, opposing “crunchy”, “burnt toast”, “hardness”, and “grassy” from “fresh”, and “salad” (Figure 6-2). Dimension 2 accounts for 25.2% variation. The PCA plot shows “sweet” and “bitter” almost directly opposite each other. The 6 aroma attributes found to be significantly different (“fresh”, “salad”, “grassy”, “vegetable oil”, “alcohol”, “burnt toast”) seem to be used distinctly, with the exception of “fresh” and “salad” which are overlapping on dimensions 1 and 2. As the references for both of these terms contained green vegetables, the distinction may have been difficult to distinguish (Table 6-3).

The PCA plot shows edamame sample to be largely clustered by growing location with the store samples being the most different from the others, highly associated with “vegetable oil” and “bitter” attributes (Figure 6-2). Edamame samples grown in Painter, VA seem to be associated with “fresh” and “salad” sensory attributes. Across growing locations, VT Sweet is most associated with “sweet”. UA Kirksey grown in Blacksburg, VA and Portageville, MO are closely associated with “grassy”. Dimension 3 of the PCA explains an additional 15% of the variation, discriminating Chiba Green and Midori Giant edamame varieties.

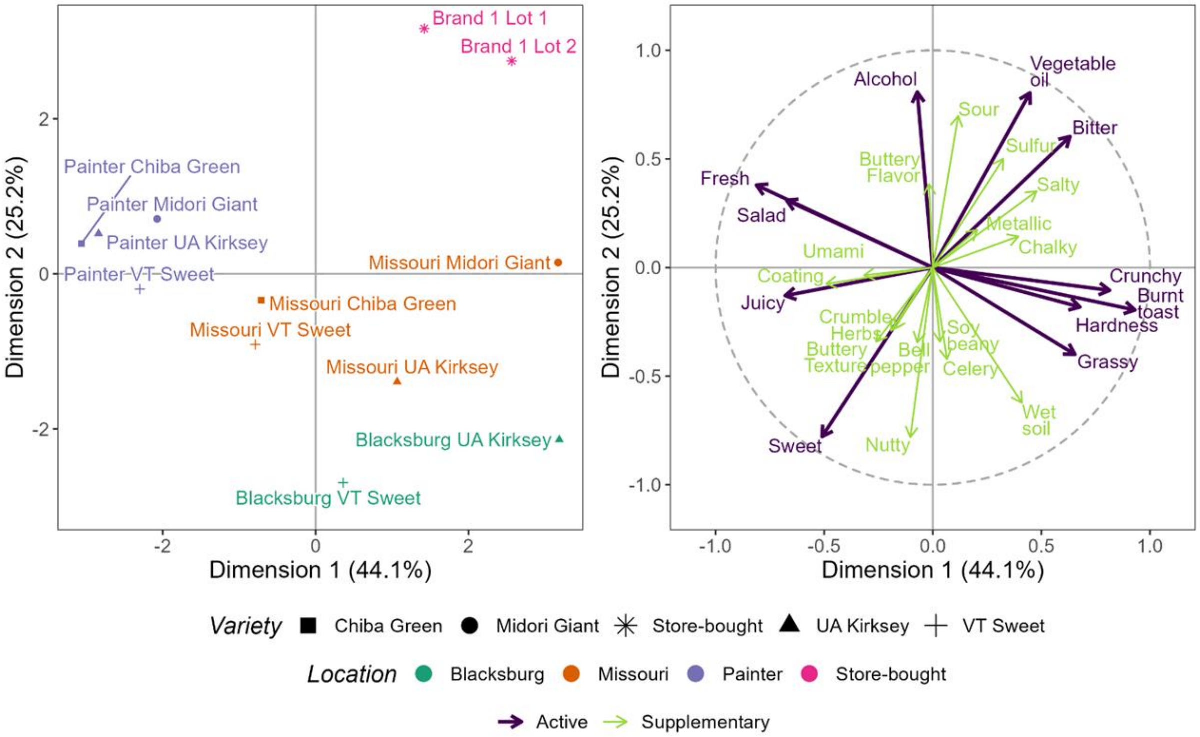

Figure 6-2: Principal component analysis (PCA) of edamame samples in DA panel based on sensory characteristics (left) with sensory characteristics identified in edamame DA panel (right).

6.3.2 Aroma Compounds

GC-O results were converted to ordinal data (0-5) and underwent Kruskal-Wallis test revealing (E)-2-octenal as the sole compound that was significantly different ($p<0.05$) between edamame samples. The Dunn post-hoc test with the Benjamini-Hochberg FDR correction could not identify the specific edamame varieties that were significantly higher or lower in (E)-2-octenal aroma intensity, likely due to insufficient power.

Of the 12 volatile compounds investigated in GC-O, pentanal, hexanal, octanal, 1-octen-3-one, 2-heptenal, 6-methyl-5-hepten-2-one, (E)-2-octenal, and 1-octen-3-ol were consistently detected across edamame samples (Table 6-4). 1-Hexanol was detected in Chiba Green edamame and store samples but was not detected in additional cultivars in this study. Nonanal was detected in Chiba Green edamame but was not detected in other edamame cultivars in this study. 1-Penten-3-ol and 1-heptanol were included in the study due to preliminary work and previously published work suggesting their importance in the overall aroma of edamame but were not consistently detected in this study (Fischer et al., 2022; Lee& Shibamoto, 2000; Ravi et al. 2019; Rosario et al., 1984).

Table 6-4: Detection status of volatile compounds evaluated in GC-O by edamame variety.

	Chiba Green	**VT Sweet**	**UA Kirksey**	**Midori Giant**	**Store**
Pentanal	+	+	+	+	+
Hexanal	+	+	+	+	+
1-Penten-3-ol	-	-	-	-	-
Octanal	+	+	+	+	+
1-Octen-3-one	+	+	+	+	+
2-Heptenal	+	+	+	+	+
6-Methyl-5-hepten-2-one	+	+	+	+	+
1-Hexanol	+	-	-	-	+
Nonanal	+	-	-	-	-
(E)-2-Octenal	+	+	+	+	+
1-Octen-3-ol	+	+	+	+	+
1-Heptanol	-	-	-	-	-

'+' indicates the compound was detected; '-' indicated the compound was not detected; Chiba Green data consists of 6 replications; Midori Giant data consists of 6 replication; UA Kirksey data consists of 9 replication; VT Sweet data consists of 9 replications; store data consists of 6 replications.

6.3.3 Relationships Between Sensory and Chemistry Characteristics

The PCA plot of the GC-O data shows (E)-2-octenal to be closely associated with "grassy" and edamame samples grown in Portageville, MO and Blacksburg, VA (Figure 6-2; Figure 6-3). The plot also shows pentanal to be most closely associated with edamame samples grown in Painter, VA as well as "fresh" and "salad" attributes while 1-octen-3-ol looks to be most closely associated with the edamame store samples and "vegetable oil". However, as pentanal and 1-octen-3-ol were not significant effects with the Kruskall-Wallis test, follow up studies would be necessary to confirm these potential associations.

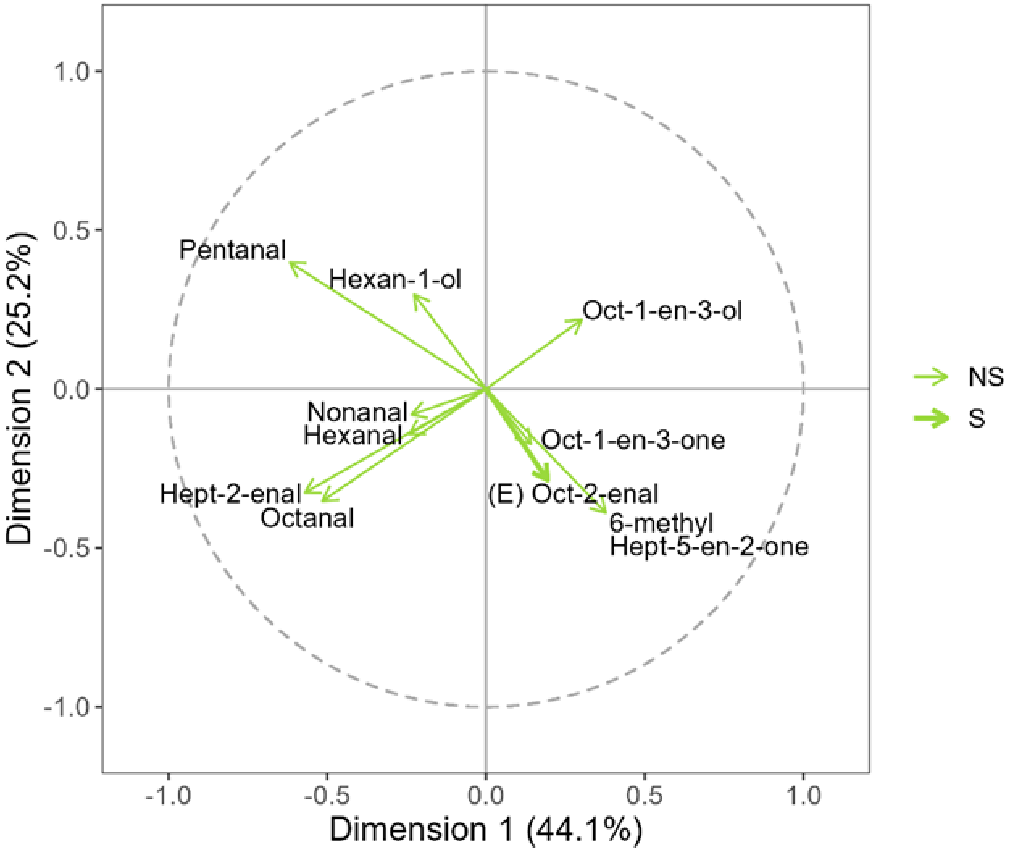

Figure 6-3: Compounds detected in GC-O analysis of edamame samples projected onto principal component analysis (PCA) of edamame DA results.

6.3.4 Sensory Differences by Variety and Location

When looking at only the edamame grown for this experiment, ANOVA by edamame variety showed that “sweet” and “bitter” taste, as well as “hardness”, “crunchy”, and “juicy” texture, were significantly different across edamame varieties ($p < 0.05$) (Figure 6-4). None of the 15 aroma attributes were found to be significantly different between edamame varieties. Tukey’s HSD post hoc testing showed VT Sweet to have the highest mean “sweet” rating, significantly higher than Chiba Green and Midori Giant. Midori Giant was found to be the least sweet edamame variety. Though “bitter" was found to be a significant attribute by edamame variety, post hoc testing did not show differences among varieties indicating insufficient power to determine varieties being higher or lower in “bitter” intensity. VT Sweet, UA Kirksey, and Midori Giant were all found to be the highest in “hardness” and “crunchy” while Chiba Green was significantly juicier than UA Kirksey samples, with the other varieties somewhere in-between.

Excluding the store samples, ANOVA by growing location showed “sweet” to be the only significant ($p<0.05$) taste attribute, “fresh”, “grassy”, and “burnt toast” to be significant aroma attributes, and “hardness” and “crunchy” to be significant texture attributes (Figure 6-5). Tukey’s HSD post hoc testing showed Blacksburg, VA to be the highest location for “sweet”. Edamame grown in Blacksburg, VA and Portageville, MO were the highest in “grassy” attribute. Blacksburg, VA was identified as the highest in “burnt toast” attribute but Tukey’s HSD shows no significant difference between Blacksburg, VA and Painter, VA. Additionally, Painter, VA was identified as the highest in “fresh” attribute, but Tukey’s HSD showed no significant difference between Painter, VA and Blacksburg, VA. These are likely due to having fewer samples from Blacksburg, VA due to field damaged and therefore lower confidence. Furthermore, edamame samples grown in Blacksburg, VA were found to be the highest in “hardness” and “crunchy” attributes.

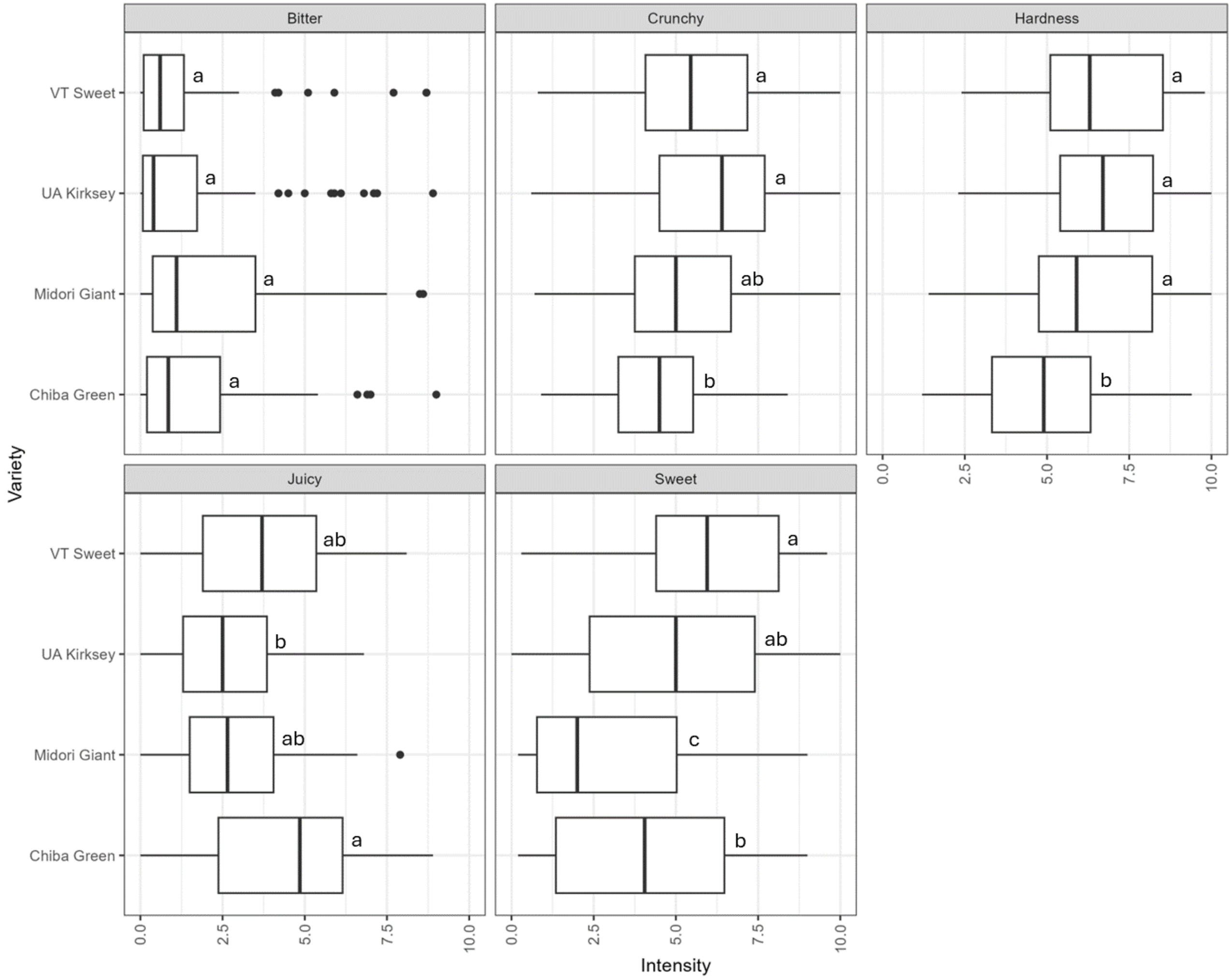

Figure 6-4: Boxplot of intensity ratings for each significant attribute ($p<0.05$) through ANOVA by edamame variety. Tukey's HSD connecting letters indicate similarities by attribute.

Store edamame samples included in the study were not included in this analysis.

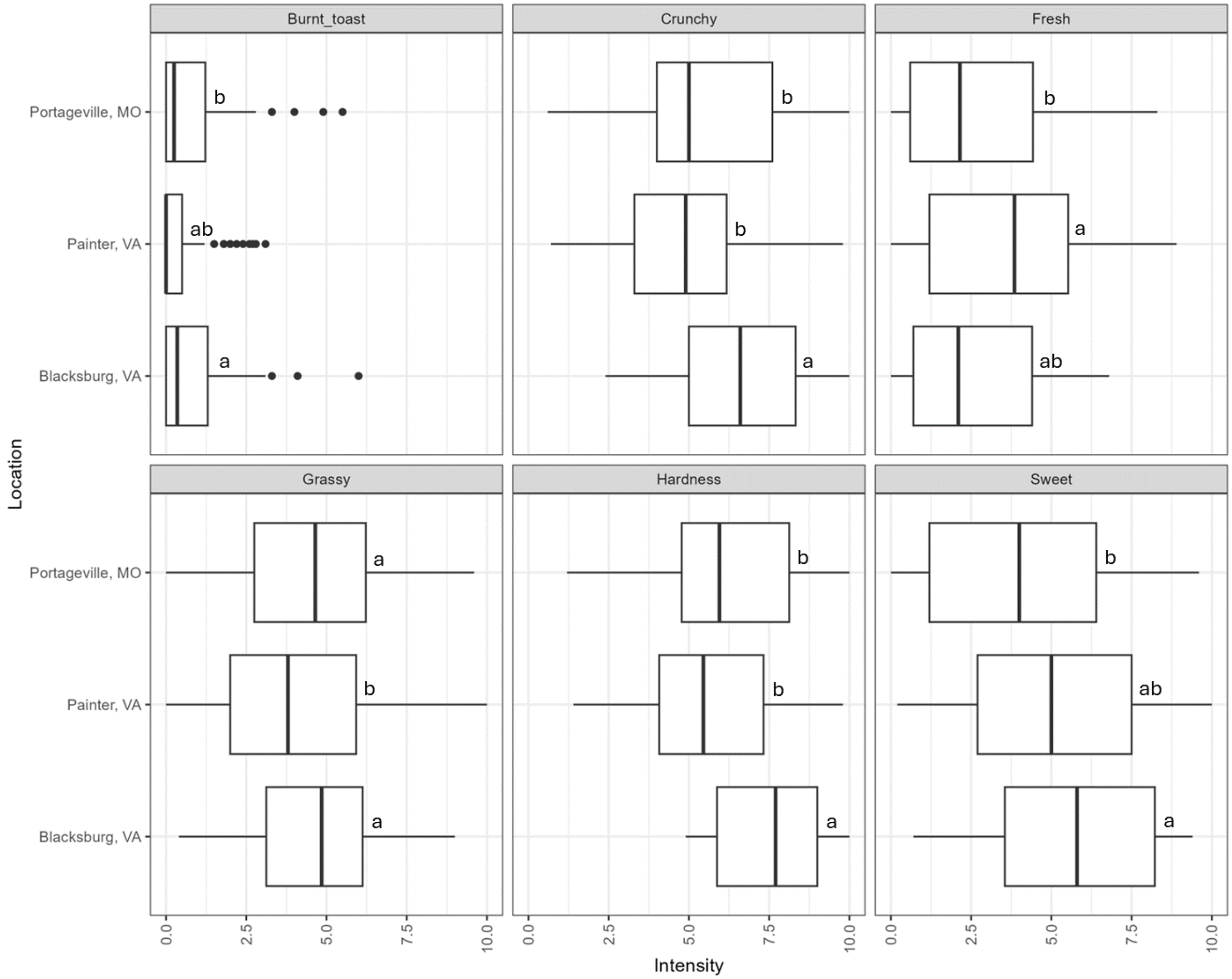

Figure 6-5: Boxplot of intensity ratings for each significant attribute ($p<0.05$) through ANOVA by edamame growing location. Tukey’s HSD connecting letters indicate similarities by attribute.
Store edamame samples included in the study were not included in this analysis.

6.4 Discussion

Carneiro et al. (2021) identified sweetness as a key characteristic of consumer liking of edamame in the U.S. Previous sensory research conducted by Wszelaki et al. (2005) also found a distinct preference for edamame varieties based on taste and textural characteristics. While the study found "sweetness" and "nuttiness" to be positive taste and flavor attributes with "beaniness" being a negative attribute, "nuttiness" and "beaniness" were not found to be significant by edamame variety and additional taste and flavor characteristics were not explored during the study to specifically identify taste attributes of preference. Similarly, the study only looked into "chewiness" for texture but did not explore additional textural attributes and concluded that multiple texture attributes as well as "chewiness" are likely continued to overall liking and preferences by edamame varieties (Wszelaki et al., 2005). Like findings from Wszelaki et al. (2005), our study identified "soy beany" and "nutty" as sensory characteristics through the DA panel but neither of these attributes were found to be significantly different across edamame samples, varieties, or growing locations.

Flores et al. (2019) utilized free choice profiling for edamame varieties and identified flavor to be the leading trait to indicate overall liking noting "sweet" to be the main factor with notes of "fresh" and finally "earthy" and "grassy", but this study did not differentiate between taste and aroma when discussing flavor attributes. Additionally, free choice profiling does not use a common vocabulary and the research did not use common references or term definitions making it impossible to say with certainty that "fresh" or "grassy" identified have the same definitions as "fresh" or "grassy" in this study. However, Flores et al. (2019) did include Midori Giant in their study, growing samples in Northern California (USA) and found "fresh" to be associated with Midori Giant, similar to the findings in this study showing Midori Giant grown in Painter, VA to be highly associated with "fresh" through PCA (Figure 6-1; Figure 6-2). Midori Giant grown in Portageville, MO did not see this association in PCA, showcasing the impact of growing location on sensory characteristics.

Our study found (E)-2-octenal to be the only compound, out of 12 included in the study, that was significantly different across edamame varieties based on GC-O results. Of the 6 aroma attributes ("fresh", "salad", "grassy", "vegetable oil", "alcohol", "burnt toast") found to be significantly different across edamame varieties in the DA panel, 4 attributes are consistent with the typical aroma of (E)-2-octenal which has been described as fatty or green (Table 6-2) as well as fresh, and sweet (TGSC, 2021). "Fresh", in this study, used cucumber as the sensory reference which is an aroma descriptor for (E)-2-octenal. Additionally, "salad" and "grassy" used a combination of vegetables including romaine lettuce and cut grass respectively. The resulting aromas may have mimicked the green or cucumber aroma of (E)-2-octenal. The reference for "vegetable oil" was a mixture of vegetable oil and flaxseed oil. Flaxseed oil can have various aroma descriptions based on processing methods, packaging, and storage choices but can be described as "nutty" and "green" (Ma et al., 2023; Sun et al., 2023; Wei et al., 2019). Additionally, the "burnt toast" reference used a burnt piece of bread for the reference which can also have a nutty aroma component (Pico et al., 2015). However, based on the PCA plots (Figure 6-2; Figure 6-3), (E)-2-octenal is most associated with "grassy" and therefore most likely contributing to the "grassy" sensory attribute in this study. The remaining significant aroma attributes cannot be concluded to be associated with a volatile compound included in this study.

None of the volatile compounds of focus in the GC-O portion of this study could account for the "alcohol" sensory attribute found to be significant in the DA portion of the study. Though "alcohol" is most closely correlated with 1-hexanol on the PCA plot, 1-hexanol is unlike to contribute to an alcohol flavor or aroma as it is most commonly described as green or fruity (Table 6-2). Additional volatile work would need to be conducted to further investigate possible volatile compounds causing the alcohol aroma. For example, the "alcohol" note might be perceived from a few volatiles combined. Additionally, the significant differences found in taste attributes of "sweet" and "bitter" across edamame samples were not surprising based on previously published work identifying differences in taste in edamame (Gu et al., 2022). However, since this study only measured volatile and aroma-active compounds, the GC-O data cannot explain the differences perceived through taste receptors in the mouth instead of the olfactory receptors in the nasal cavity (Lawless & Heymann, 2010b). Volatile compounds, however, can enhance, suppress, or interfere the tasting perceptions. Sweet taste in edamame is known to be due to presence of soluble sugars including sucrose, glucose, and fructose, as well as alanine, a sweet tasting amino acid (Konovsky et al., 1994; Song et al., 2013; Yu et al., 2021). Bitter taste in edamame is known to be caused by various compounds including saponins, isoflavones, and some aromatic amino acids including phenylalanine and tryptophan (Konovsky et al., 1994; Mentreddy, et al., 2002; Sugimoto et al., 2010). Researching and quantifying these compounds would be needed to better understand the taste variations across edamame samples.

Similarly, the texture attributes identified to be significantly different across edamame samples ("hardness", "crunchy", "juicy") in the DA panel are not explainable by volatile compounds. Texture is perceived through a combination of visual appearance, physical touch, and auditory perception of the food before and during consumption (Lawless & Heymann, 2010c). The texture of a food, including edamame, can be an extremely important quality attribute for consumers (Lawless & Heymann, 2010c). While the preparation for the edamame samples remained consistent, the edamame beans often varied in size by multiple millimeters which would result in texture variations due to sample preparation. Consistent sample preparation was chosen to better reflect the cooking instructions a consumer might follow when preparing edamame in their own kitchen. Vegetable packaging does not change cooking times based on bean size, so we chose a method that would reflect the texture differences in differently sized edamame beans being prepared at-home with a consistent cooking time.

1-Hexanol and nonanal were previously identified as main components of the volatile compounds present in soybean at the R6 maturity (Boué et al., 2003). In their work, gas chromatography-mass spectrometry (GC-MS) was used to research volatile compounds in soybean harvested at R6, R7, and R8 maturity using Pioneer 95B41 soybean variety grown in New Orleans, LA (USA). As Boué et al. (2003) utilized a soybean variety over an edamame variety, differences in volatile findings compared to this work were expected. However, research identifying volatile compounds and sensory attributes of domestic vegetable soybean is currently limited. Similar work completed by Guo et al. (2022) utilized methods including Quantitative Descriptive Analysis (QDA) with GC-MS to identify flavor compounds and sensory attributes for 3 edamame varieties concluding 1-octen-3-ol, hexanal, (Z)-2-heptenal, 2-octene, nonanal, (Z)-2-decenal, and 3,5-octadien-2-one were the main volatile compounds in their edamame varieties.

However, this work utilized edamame varieties, growing locations, and panelists in China; therefore, similarities and differences in findings were observed compared to our study due to geographical discrepancy.

Though Boué et al. (2003) found 1-hexanol and nonanal to be main volatile compounds in vegetable edamame, the study did not include gas chromatography olfactometry (GC-O) or other testing to confirm aroma activity of these volatile compounds. While our GC-O work did not consistently detect 1-hexanol and nonanal, 1-hexanol was detected in Chiba Green edamame and store-bought edamame while nonanal was detected in Chiba Green edamame. Similarly, Guo et al. (2022) identified numerous compounds to be main flavor compounds in edamame but did not include GC-O or other testing to confirm aroma activity of the volatile compounds. Of the compounds they identified, our work also found 1-octen-3-ol and hexanal to contribute to the aroma profiles of all edamame samples included in the study. (Z)-2-Heptenal, 2-octene, (Z)-2-decenal, and 3,5-octadien-2-one were also identified by Guo et al. (2022), but were not found in this study.

VT Sweet grown in Painter, VA and Portageville, MO showed higher levels of sweetness compared to others, indicating the particular advantage of high sweetness of this cultivar. It was also possible that the Blacksburg VT Sweet sample experienced the shortest period between harvest and processing, which preserved some of the sugar content in beans from extended respiration. Overall, VT Sweet and UA Kirksey were found to be sweeter than Chiba Green, Midori Giant, or the store-bought edamame in this study. Both VT Sweet and UA Kirksey were cultivars developed in the U.S. specifically for commercial production of edamame in the mid-Atlantic region (Zhang et al., 2021). As sweetness is a driving characteristic of domestic consumer liking, this attribute is an important consideration for growers and a sign of success for breeders who brought VT Sweet and UA Kirksey to the market.

Growing location may need to be considered when preparing for edamame production. Like many other factors, growing location can impact overall quality and sensory characteristics including sweetness and textural properties of “crunchy” and “hardness” as identified in this study. With sweetness being a highly important characteristic for consumer liking as well as textural properties, changing growing locations can impact edamame quality but specific details on how these changes may occur will require additional research over multiple growing years to account for changes seen from year to year.

In this study, the store-bought samples were found to be lower in “sweet” taste compared to the edamame grown specifically for this research. Though the specific information on edamame variety, growing location, and processing steps associated with these samples is unknown, recognizing notable difference in sweetness is important to understand the edamame currently available to domestic consumers. Further research into processing and storage impacts on quality characteristics such as sweetness should be considered in later research to better understand the changes occurred from field to store.

Significant differences identified in taste, flavor, and texture in the DA results suggest a diversity of sensory characteristics currently present in the domestic edamame market. Our study concurred previously published studies that suggested edamame varieties have unique characteristics that can indicate overall acceptability (Carneiro et al., 2021; Flores et al., 2019; Wszelaki et al., 2005). Future research areas are broad, which include but certainly not limited to, fully quantitative GC results to explore additional volatile compounds contributing to edamame aroma, chemical analysis to better understand taste attributes, and reliable texture analysis to appropriately reflect differences in edamame texture.

6.5 Conclusions

This work identified differences in sensory attributes across domestic edamame samples including 4 edamame varieties (Chiba Green, VT Sweet, UA Kirksey, and Midori Giant) grown in 3 locations (Blacksburg, VA; Painter, VA; and Portageville, MO) as well as two store-bought samples. Out of the 27 sensory attributes identified in descriptive analysis, the U.S.-grown edamame samples differed in sweetness, bitterness, “fresh”, “salad”, “grassy”, “vegetable oil”, “alcohol”, and “burnt toast” as well as hardness, juiciness, and crunchiness. This work identified VT Sweet and UA Kirksey, both domestically bred edamame cultivars, as the sweetest varieties in the study which has been identified as an important attribute for overall liking by domestic edamame consumer (Carneiro et al., 2021). Chiba Green was the least crunchy, least hard, and most juicy variety. Six attributes were found to be significantly different based on growing location with Blacksburg, VA growing the sweetest edamame with the highest crunchy and hard textures.

Of the 12 volatile compounds investigated for aroma contributions to the edamame samples, only one compound, (E)-2-octenal, was identified as significantly different among the varieties. Principal component analysis shows (E)-2-octenal to be associated with UA Kirksey edamame grown in Blacksburg, VA, and Portageville, MO. Overall, this work has provided additional understanding of sensory attributes associated with the edamame varieties and has indicated diversity of sensory characteristics present in the domestic edamame supply chain but aroma active compounds detected through GC-O did not drive these detected differences.

6.6 Funding

This research was funded by the Specialty Crop Research Initiative (SCRI) program of the United States Department of Agriculture (USDA) (Grant No: 2018-51181-28384; Accession No. 1016465).

6.7 Acknowledgements

The authors would like to thank the edamame team (faculty, staff, graduate students, undergraduate students) for their dedication and support. The authors would also like to acknowledge (in no significant order) Jingsi Liu, Tianyou Xu, Ann Sandbrook, Kim Waterman, Jian Wu, Sofia Little, Mckenna Helder, Megan Mershon, Isabelle Haser, Jordan Allen, Jessica Wilbur, Virell To, Mary Salinas, and Jacob DeGregoris for their technical support and encouragement.

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Chapter 7

7. Conclusions and Future Work

7.1 Conclusions

Overall, this work aimed to identify and develop methods to assess quality attributes of domestic edamame, providing information and methods for breeders, growers, and producers to utilize in their work with edamame.

Solid phase microextraction with gas chromatography-mass spectrometry (GC-MS) and GC-olfactometry (GC-O) were used to identify volatile compounds in domestic edamame and determine their potential contribution to aroma and flavor through human detection. Significant differences in volatile compounds were identified based on edamame genotype or variety, growing location, and presence of stink bug feeding injury. However, human detection through GC-O and sensory analysis methods did not find volatile compounds to be the attribute detected identifying differences. GC-O and sensory discrimination testing found panelists were unable to detect the volatile differences in the stink bug injured and not injured edamame samples. Additionally, while sensory panelists were able to identify numerous flavor/aroma sensory characters as significantly different by edamame sample in descriptive analysis panel, GC-O of these edamame samples found only one volatile compound, out of 12, to be significantly different by sample. These results indicate taste and texture may be more important sensory attributes over aroma but may also indicate aroma needs to be researched in combination with all sensory properties to fully understand the impact.

Instrumental texture analysis as well as sensory methods were used to identify texture and flavor differences in domestic edamame. This research was able to investigate texture analysis for possible implementation into the domestic edamame supply chain as a quality check point concluding the ASABE S368.4, Compression Test of Food Materials of Convex Shape, method to be an effective method to detect differences in texture of edamame beans based on processing changes. Texture was also evaluated through sensory methods finding ‘hardness’, ‘crunchy’, and ‘juicy’ to be significant sensory attributes through descriptive analysis.

Chapter 3 utilized 10 edamame genotypes grown in 4 locations. GC-MS of these samples identified 16 volatile compounds including 6 alcohols, 8 aldehydes, one ketone, and one furan. Of these 16 volatile compounds identified, 14 were found to have significant differences across the 10 edamame genotypes in the study. Correlation of the relative content of these compounds to sensory attributes found no strong correlations ($>|0.75|$) between the volatile compounds and ‘liking or ‘aroma’, the sensory attributes most likely to be impacted by volatile compounds. Additionally, 15 of the 16 volatile compounds were found to be significant by growing location.

Chapter 4 utilized 2 growing years to investigate flavor differences in edamame based on the presence of stink bug feeding injury. Though differences in relative content of volatile compounds were identified based on stink bug feeding injury, detection of these changes through GC-O and sensory difference testing was not identified. Though the stink bug feeding injury leaves a visual defect on edamame beans, utilizing these beans in more processed products should not impact overall flavor quality based on these findings.

Chapter 5 investigated the ASABE S368.4, Compression Test of Food Materials of Convex Shape, method for instrumental texture analysis of edamame. This work found compression to be a consistent method to identify changes in processing of edamame and could be implemented as a quality check point in the edamame supply chain by growers or processors.

Chapter 6 further investigated domestic edamame involving 4 edamame varieties used in the local market including 2 varieties bred in the U.S. and additional store-bought samples. Descriptive analysis sensory paired with GC-O found 27 total sensory attributes with 11 of the attributes being significant by sample. Of the 11 significant attributes, 6 were flavor/aroma attributes. GC-O consistently detected 8 volatile compounds across all edamame samples with only one compound being significantly different by sample. The chapter further outlines the major differences in sensory attributes across domestic edamame samples and recognizes that taste and texture are more influential in the sensory experiences than aroma alone. The combination of sensory proprieties provides a more complex but accurate understanding of the edamame samples.

These chapters included many edamame varieties including VT Sweet and UA Kirksey. Both of these varieties were developed in the U.S. to have favorable agronomic characteristics. VT Sweet was also bred to have superior sensory qualities, including higher sweetness, than other varieties as can be seen in this research. The identification of differences in volatile compound presence and sensory attributes based on edamame genotype or variety also indicates successful breeding and production of varied edamame to provide diverse options in the domestic edamame market.

7.2 Future Work

Additional research continuing the investigation of quality characteristics of edamame will be needed as the domestic edamame supply chain continues to grow. Conducting research projects to understand the full sensory profile of domestic edamame utilizing instrumental techniques as well as sensory methods should be considered.

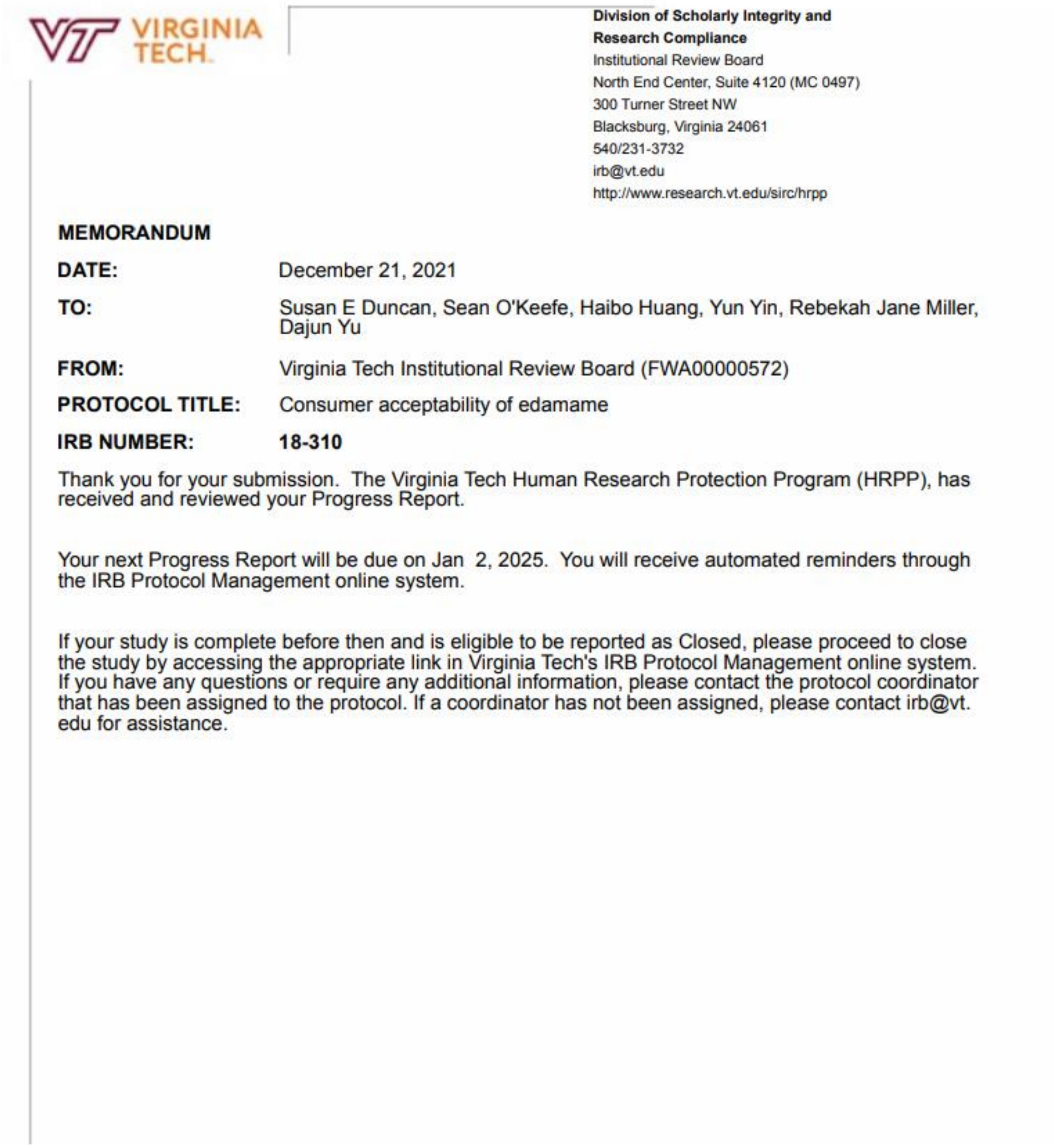
Research including fully quantitative GC to explore all volatile compounds which may be contributing to edamame aroma and flavor would be beneficial to better understand edamame aroma. Chemical analysis of edamame geared towards taste attribute investigation would be needed to understand the taste attributes of edamame. Finally, instrumental texture analysis of edamame varieties, researching differences and changes based on variety and harvest or ripeness would be beneficial for implementation of quality checks and methods in the supply chain.

Additional research into consumer perfection of visual damage or injury to edamame will be necessary to further edamame quality as perceived by consumers. Literature does not currently include investigations into consumer tolerance of blemishes or marks on edamame beans but would be needed to establish quality specifications for edamame growers, producers, and retailers. Understanding the level of visual defect consumers tolerate in edamame will be necessary to understand consumer perception of quality. Research designed to establish quality specifications for visual defects and injury, such as the visual defect caused by stink bug feeding, should be established to support domestic producers and processors of edamame.

Future research should include multiple edamame varieties, growing locations, and growing years to account for variations in edamame quality based on these variables. As the number of cultivars continues to increase in the domestic market, additional research should be included to establish data around the cultivars, noting quality relevant results for marketing and further product development. Additional work investigating the impact of processing and storage on quality characteristics such as sweetness should also be considered to better understand the changes that occur from field to store.

Appendix A: IRB 10-310

A.1 Approval Letter



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irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

MEMORANDUM

DATE:	December 21, 2021
TO:	Susan E Duncan, Sean O'Keefe, Haibo Huang, Yun Yin, Rebekah Jane Miller, Dajun Yu
FROM:	Virginia Tech Institutional Review Board (FWA00000572)
PROTOCOL TITLE:	Consumer acceptability of edamame
IRB NUMBER:	**18-310**

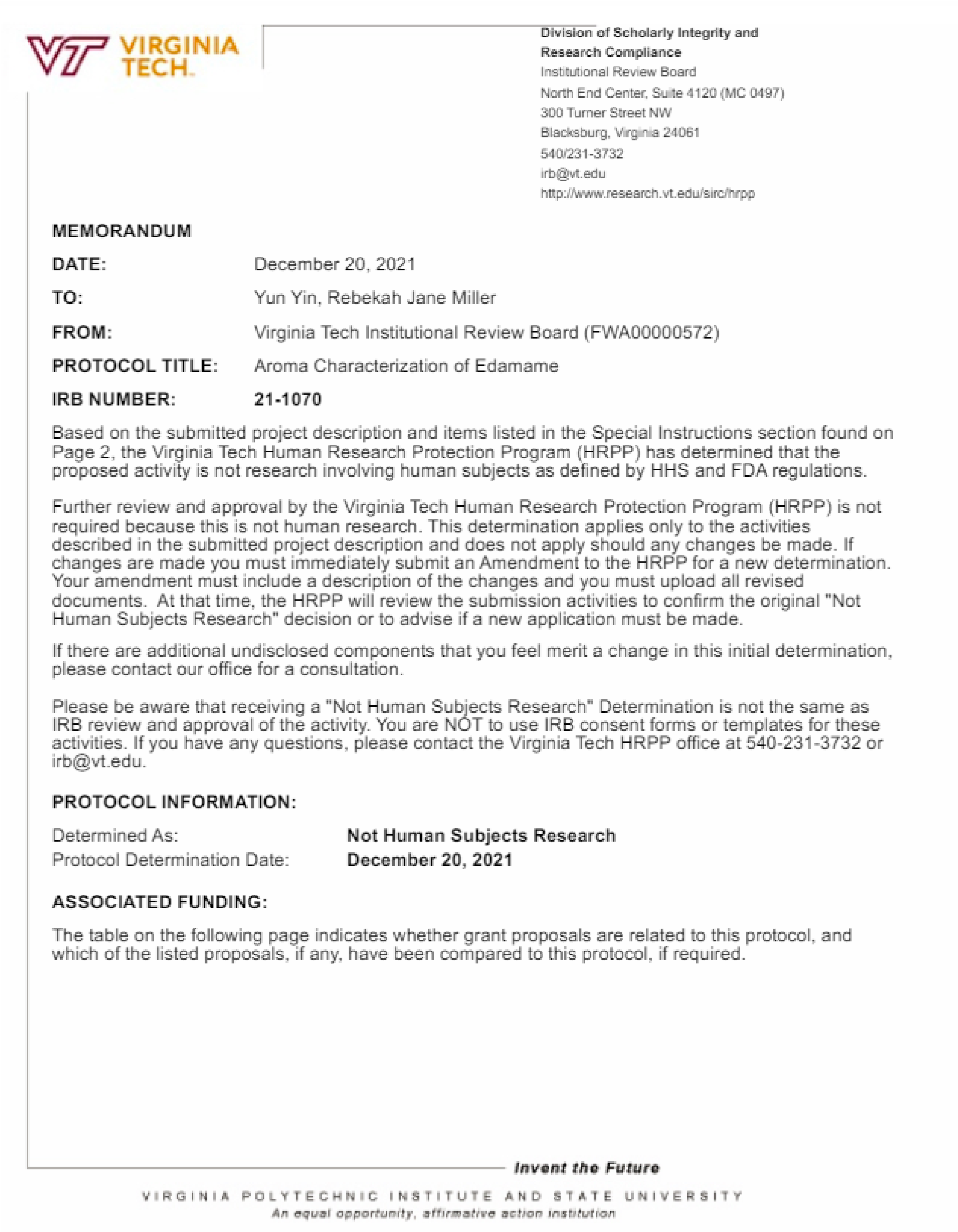
Thank you for your submission. The Virginia Tech Human Research Protection Program (HRPP), has received and reviewed your Progress Report.

Your next Progress Report will be due on Jan 2, 2025. You will receive automated reminders through the IRB Protocol Management online system.

If your study is complete before then and is eligible to be reported as Closed, please proceed to close the study by accessing the appropriate link in Virginia Tech's IRB Protocol Management online system. If you have any questions or require any additional information, please contact the protocol coordinator that has been assigned to the protocol. If a coordinator has not been assigned, please contact irb@vt.edu for assistance.

Appendix B: IRB 21-1070

B.1 Approval Letter



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MEMORANDUM

DATE: December 20, 2021

TO: Yun Yin, Rebekah Jane Miller

FROM: Virginia Tech Institutional Review Board (FWA00000572)

PROTOCOL TITLE: Aroma Characterization of Edamame

IRB NUMBER: **21-1070**

Based on the submitted project description and items listed in the Special Instructions section found on Page 2, the Virginia Tech Human Research Protection Program (HRPP) has determined that the proposed activity is not research involving human subjects as defined by HHS and FDA regulations.

Further review and approval by the Virginia Tech Human Research Protection Program (HRPP) is not required because this is not human research. This determination applies only to the activities described in the submitted project description and does not apply should any changes be made. If changes are made you must immediately submit an Amendment to the HRPP for a new determination. Your amendment must include a description of the changes and you must upload all revised documents. At that time, the HRPP will review the submission activities to confirm the original "Not Human Subjects Research" decision or to advise if a new application must be made.

If there are additional undisclosed components that you feel merit a change in this initial determination, please contact our office for a consultation.

Please be aware that receiving a "Not Human Subjects Research" Determination is not the same as IRB review and approval of the activity. You are NOT to use IRB consent forms or templates for these activities. If you have any questions, please contact the Virginia Tech HRPP office at 540-231-3732 or irb@vt.edu.

PROTOCOL INFORMATION:

Determined As: **Not Human Subjects Research**
Protocol Determination Date: **December 20, 2021**

ASSOCIATED FUNDING:

The table on the following page indicates whether grant proposals are related to this protocol, and which of the listed proposals, if any, have been compared to this protocol, if required.

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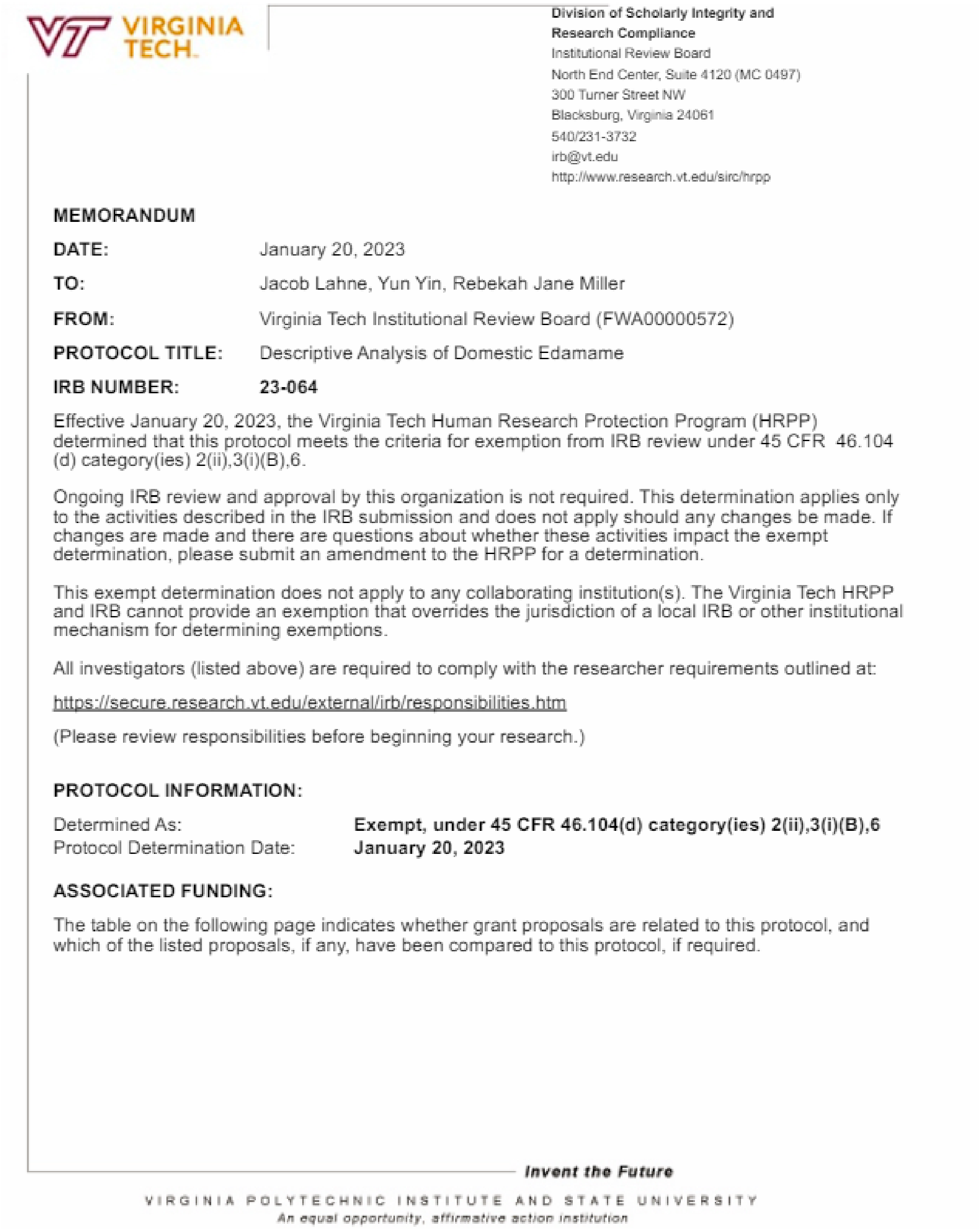
Appendix C: IRB 20-064

C.1 Approval Letter



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MEMORANDUM

DATE:	January 20, 2023
TO:	Jacob Lahne, Yun Yin, Rebekah Jane Miller
FROM:	Virginia Tech Institutional Review Board (FWA00000572)
PROTOCOL TITLE:	Descriptive Analysis of Domestic Edamame
IRB NUMBER:	**23-064**

Effective January 20, 2023, the Virginia Tech Human Research Protection Program (HRPP) determined that this protocol meets the criteria for exemption from IRB review under 45 CFR 46.104 (d) category(ies) 2(ii),3(i)(B),6.

Ongoing IRB review and approval by this organization is not required. This determination applies only to the activities described in the IRB submission and does not apply should any changes be made. If changes are made and there are questions about whether these activities impact the exempt determination, please submit an amendment to the HRPP for a determination.

This exempt determination does not apply to any collaborating institution(s). The Virginia Tech HRPP and IRB cannot provide an exemption that overrides the jurisdiction of a local IRB or other institutional mechanism for determining exemptions.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

https://secure.research.vt.edu/external/irb/responsibilities.htm

(Please review responsibilities before beginning your research.)

PROTOCOL INFORMATION:

Determined As:	**Exempt, under 45 CFR 46.104(d) category(ies) 2(ii),3(i)(B),6**
Protocol Determination Date:	**January 20, 2023**

ASSOCIATED FUNDING:

The table on the following page indicates whether grant proposals are related to this protocol, and which of the listed proposals, if any, have been compared to this protocol, if required.

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