Product Development, Sensory Evaluation and Characterization of Bioactive Isothiocyanates from Broccoli Sprout Powder delivered in Tomato Juice

THESIS

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Abstract

Previous studies have found carotenoids in tomato juice (TJ), and isothiocyanates in broccoli to be associated with positive health outcomes such as bladder cancer prevention. These foods should be investigated further but from previous attempts, there were some reported challenges including lack of flavor and sensory acceptability. Using a sous vide method, with a specific time and temperature, we hypothesized that the development of a product with maximal amount of isothiocyanates and high organoleptic quality was achievable. A multi-characterization approach was employed in analyzing microstructural properties, bioactive present and sensorial acceptability of pretreated broccoli sprout powder (BSP) in TJ. The product was designed to contain 1 gram of broccoli sprout powder which is equivalent to a serving of mature broccoli.

The first group of fresh broccoli sprouts were washed, dried and vacuum sealed, and sous vide (Model SVS-10LS, CO) at 60˚C for 10 minutes. The second group was the raw control broccoli sprouts which were washed, rinsed and patted dry. The third group was the enzyme inactivated steamed broccoli sprouts, this group was washed and placed on a sieve to be steamed for 5 minutes. All the samples were placed on their individual trays, flash frozen with liquid nitrogen and freeze dried for 48 hours. After lyophilization, the samples were pulverized into a powder, placed in labeled plastic bags and stored in a low humidity environment at 4˚C until analyses. Rheological temperature-dependence studies between 25-65 ˚C were conducted to assess impact of 1 gram of BSP in 6 oz of TJ (GFS, Columbus, OH) on the rheological properties including complex viscosity, elastic modulus and loss modulus. The quantification of these rheological properties allows us to have an understanding of how the flow of the TJ will be perceived by humans. HPLC analysis was used to determine and compare levels of glucosinolates as well as

the levels of bioactive isothiocyanates. Particle size, polydispersity, and zeta potential were determined using dynamic light scattering particle analyzer. Sensory panel tests were performed to determine consumer acceptability using hedonic scale, JAR and preference test. A Dynamic Vapor Sorption isotherm was generated to observe water interaction with the powder at different levels of relative humidity for determining optimal storage conditions.

The rheological testing showed that at the proposed dose, there was a not a significant difference in the complex viscosity, elastic and loss modulus values between 6 oz of plain TJ compared to 6 oz TJ with 1 g of BSP. The overlapping of the results demonstrates there was not a detectable difference of the addition of BSP in the viscosity or flow of TJ. Dynamic tests as a function of temperature evidenced the stability and the compatibility between tomato juice and broccoli sprout powder. Levels of glucosinolates by HPLC/MS resulted in 50.79µmol/g in steamed BSP, 1.86µmol/g for the sous vide BSP and 19.75µmol/g for the raw BSP. Levels of isothiocyanates by HPLC/MS resulted in 9.2 µmol/g in steamed BSP, 39.4 µmol/g for the sous vide and 14.2 µmol/g for the raw control. Epidemiology has shown that consumption of raw broccoli seems to have a protective effect from bladder cancer, by consuming sous vide BSP the effect should be almost three times greater. This data serves as indication that level of bioactive isothiocyanates can be maximized with sous vide processing as compared to the raw and steamed BSP.

Dynamic light scattering data shows the sous vide BSP particles in a size range between 600nm and 9000nm with a polydispersity value of 0.513. This polydispersity value is over the 0.5 threshold for achieving a homogeneous particle size, which could be optimized with a better controlled grinding method. Results of a sensory panel showed significantly greater acceptability than past studies with an overall hedonic scale score of 5.2 for canned tomato juice with BSP. Sensory test results from hedonic scale and JAR test were expected to have consistently higher

acceptability from plastic bottled tomato juice with BSP as compared to canned. Furthermore, blinded preference test revealed 65% percent of the panelists preferred the canned TJ with sous vide BSP over plastic bottled TJ with the same sous vide BSP. Results suggest the proposed processing method is effective in achieving a palatable TJ with BSP that delivers a significant concentration of bioactive isothiocyanates. Physicochemical evaluation of TJ with BSP confirmed stability and compatibility between TJ and BSP.

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Chapter 1: Introduction

Phytochemicals have received great attention across research groups since they are known for imparting health benefits to humans beyond nutrition. (Bloch et al., 2003 Liu et al., 2003) Hence, there have been efforts to understand how the consumption of certain foods such as tomatoes, soy or broccoli can have an impact on human health (Canene-Adams et al., 2007; Giovannucci et al, 2003). Researchers suggest future studies concentrate on the early phases of cancer prevention, since it's agreed that phytochemical consumption could potentially impact the early stage of the disease, rather than more advanced metastasized cancer (Abbaoui et al., 2012). Cancer of the urinary bladder is the fourth most common neoplasm in the United States men, with estimates of about 81,190 new cases and approximately 17,240 deaths in 2018, as predicted by the ACS (American Cancer Society, 2018). BC presents a significant health burden as the most expensive cancer to treat and monitor over the lifetime of a person, with an estimated annual cost of 4 billion in 2010 and is expected to rise to 5 billion by 2020 (Mariotto et al., 2011). BC patients face a common problem known as cancer recurrence post-treatment. Healthcare costs for BC treatment are excessive because this type of cancer has been known to have a recurrence rate of about 70%; which means that patients must be on lifelong surveillance, receiving surgical resection and continuous treatment (Muhammad et al., 2014). However, about 75% of BC cases are diagnosed as a low-grade non-invasive (not metastasized) cancer, meaning that it is a superficial tumor that can be removed by surgery (Muhammad er al., 2014). Hence, this presents a good opportunity to use preventative strategies, like functional food consumption, to evaluate further potential protective effects for this BC survivor’s cohort. Some researchers have eluded to the need for innovative, functional food products with bioactive phytochemicals for this specific cohort (Abbaoui et al., 2012). Furthermore, the data available on epidemiology, cell, and mouse studies with broccoli seems promising enough to be used for BC prevention (Verhoeven et al., 1996; Canene-Adams et al., 2007; Abbaoui et al., 2012). Researchers have found that broccoli has significantly suppressed bladder cancer (BC) development in cell and mice studies (Giovannucci et al., 2003; Abbaoui et al., 2012). Moreover, the authors encouraged the development of food products for human consumption incorporating broccoli sprouts, finding them to be innocuous and rich in bioactive isothiocyanates (Abbaoui et al., 2012).

The objective of this work was to develop a functional juice product, using a controlled heat treatment to maximize isothiocyanate (ITC) content in broccoli sprouts. Broccoli sprouts contain 6 to 20-fold more glucosinolate content than mature broccoli (hence, they can form higher amounts of their bioactive ITCs) (Abbaoui et al., 2012). Based on the recommended dietary guidelines for Americans intake, the calculated equivalent to the recommended cup of mature broccoli a day (Office of Disease Prevention and Health Promotion, 2015), is 1 gram of the processed dry broccoli sprout powder (after having accounted for water weight). This 1 gram of dry broccoli sprout powder was the amount added to 6oz of 100% tomato juice. A broccoli sprout powder juice was the product of choice because of the ease of characterization of bioactive analytes, shelf stability, and convenience.

The strategy was to use a multi characterization approach including rheological tests, particle size & surface charge analysis, as well as HPLC to understand qualitative and quantitatively the impact of adding the broccoli sprout powder on the characteristics of the tomato juice. HPLC characterization of a raw control, sous vide, and steamed broccoli sprout powder determined the processing method that significantly increases the level of isothiocyanates (ITCs). Rheological tests provided information on the structural changes in the flow properties of tomato juice after incorporating the broccoli sprout powder (Tiziani et al., 2006). Particle size as well as surface charge analyses determined the powder's size homogeneity and dispersion in the juice. Sensory acceptability was conducted to determine the change in palatability of tomato juice upon broccoli sprout addition and asses preferred packaging of the broccoli-tomato juice. The goal of this project was to formulate and characterize a functional food juice that is rich in bioactive ITCs with good sensory acceptability for future clinical trials.

Chapter 2- Literature Review

2.1 Epidemiological Evidence of Broccoli Bioactivity

Broccoli is a cruciferous vegetable from the Brassica family, known to be one of the richest sources of glucosinolates in the human diet (Bischoff et al., 2016). There have been numerous studies looking at the effect of inhibition of cancer progression in humans by exposure to foods, such as broccoli (Abbaoui et al., 2012; Canene-Adams et al., 2007). But in some epidemiological studies there have been contradicting conclusions regarding the cancer preventative effects of broccoli in people (Verhoeven et al., 1996; Michaud et al., 1999; Tang et al., 2008). The first study (Verhoeven et al., 1996) found results of 7 cohort studies and 87 case-control studies on the association between brassica consumption and cancer risk demonstrating an inverse relationship between these. In a later study (Michaud et al.,) researchers found an inverse association that was not statistically significant between total fruit and vegetable intake and bladder cancer risk. Then a later epidemiological paper (Tang et al., 2008), found protective effects from bladder cancer risk with consumption of cruciferous vegetables that contain isothiocyanates and noted that cooking can substantially reduce or destroy isothiocyanates, and could account for study inconsistencies in the past.

In broccoli, there are certain compounds that may be thermo labile including vitamins, enzymes (usually related to spoilage), and phytochemicals that can be degraded by thermal processing, such as cooking (Matusheski et al., 2001; Dosz et al., 2013). The issue is that studies did not account for factors like the preparation of the broccoli (some ate raw broccoli, others steamed or boiled) that can directly affect the level of phytochemicals delivered; hence, the individuals are receiving different levels of exposure to the ITC bioactive compounds from broccoli.

2.1.1 Factors Affecting Bioavailability in Humans

Thermal processing can have a great impact on bioavailability of phytochemicals (ability to be circulated and absorbed by the body) and bioactivity (active effect) from phytochemicals in food (Manach et al., 2005). Additionally, other variables that could potentially make the consumption of broccoli's phytochemicals effective against disease include intestinal absorption, chemical structure of the bioactive compounds, and the food matrix where they are confined (Palermo et al., 2014). Hence, the sum of these factors could also contribute, to some degree, for the variability in the literature regarding the protective effects of broccoli towards BC. But in the US, where broccoli is commonly consumed as a boiled, steamed or raw vegetable there are some alterations the plant goes through which can begin to account for this variability among epidemiological studies, where some found an inverse relationship between the consumption of broccoli and incidence of bladder cancer (BC), while others did not (Tang et al., 2008).

In a comprehensive epidemiological paper, when consumed raw, cruciferous vegetables (including broccoli, cabbage, cauliflower, and Brussel sprouts) were shown to reduce the risk of BC, an effect attributed to dietary isothiocyanates (ITCs) known to be chemopreventive agents against BC (Tang et al., 2008). The inconsistencies with other studies that did not find this protective effect, was addressed by explaining how thermal processing degrades (Hanschen et al., 2004) the viable content of the bioactive compounds in the consumed broccoli. When the researchers adjusted their data, they observed that people who consumed raw broccoli have a statistical significant protective effect of BC than those who ate broccoli after steaming or boiling (Tang et al., 2008). Therefore, it is imperative to understand the components in the raw plant and the chemical reactions that take place when broccoli is exposed to thermal processing.

2.2 Glucosinolate Chemistry

Glucosinolates are the naturally occurring compounds found in broccoli stored in the plant matrix separately from the enzyme myrosinase; consequently, when the plant matrix is torn, damaged or chewed, myrosinase is released and catalyzes the conversion of glucosinolates to form bioactive isothiocyanates (ITC) (Fahey et al., 2001). In later studies, researchers identified broccoli's intrinsic enzymes, which are responsible for the production and/or inhibition of the synthesis of bioactive ITC's (Matusheski et al., 2004). It is critical to point out that glucosinolates are the precursors to ITCs, and only ITCs are the compounds associated with cancer preventative effects (Matusheski et al., 2001; Abbaoui et al., 2012).

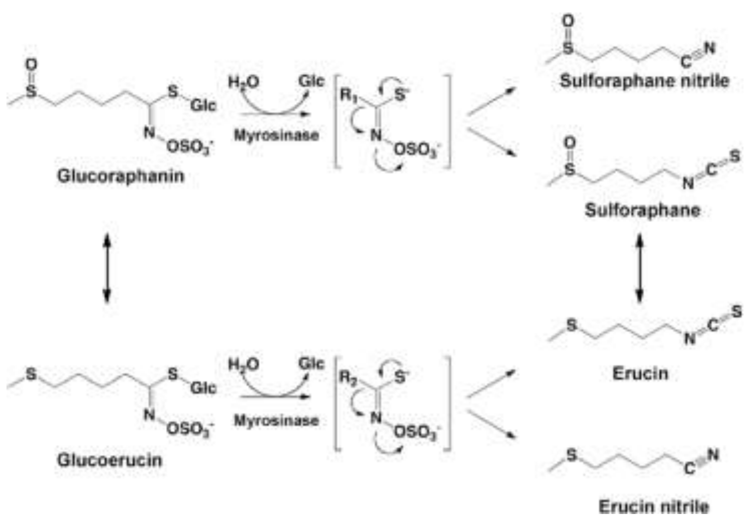


Figure 1 Glucosinolates Conversion to Isothiocyanates by myrosinase vs. ESP enzyme activity. (Kensler et al., 2012)

Thermal exposure to temperatures above 70˚C was found to inactivate broccoli's endogenous enzyme myrosinase (Fahey et al., 2001; Matusheski et al., 2001; Holst et al., 2004) hence, decrease the final levels of bioactive ITCs present (Matusheski et al., 2001).

On the other hand, results of a human clinical trial found that consuming the fresh broccoli sprouts without any heat treatment resulted in better phytochemical exposure in humans than drinking a commercially sold broccoli supplement (myrosinase inactivated) in capsule form

(Clarke et al., 2011). Previously, a study confirmed that broccoli sprouts, which are the 3 to 4-day old broccoli seedlings, were approximately 20 times more concentrated in glucosinolates, hence, capable of forming 20 times more ITCs (Abbaoui et al., 2010). Researchers then used broccoli sprouts for human consumption and observed a 7- and 12- fold higher bioavailability in human plasma of the main ITCs present in broccoli sprouts, sulforaphane and erucin (Clarke et al., 2011). While the authors of this work might conclude that consuming fresh broccoli sprouts is the way to obtain the greatest health benefit, later studies confirm that inactivating an enzyme called epithiospecifier protein (ESP) with a mild heat treatment at 60°C for 10 minutes resulted in the highest ITC concentration in broccoli (Matusheski et al., 2004; Bricker et al., 2014). In the raw plant the enzyme epitiospecifier protein (ESP), converts glucosinolates to nonbioactive nitrile products (Matusheski et al., 2004; Bricker et al., 2014). The ESP which forms nitrile products instead of ITCs presents an obstacle that may prevent study subjects from obtaining the significantly higher amounts of ITCs from broccoli (Bricker et al., 2014). The data has shown that the mild heat pretreatment obtained near 100% conversion of glucoraphanin (the glucosinolate form) to sulforaphane (the deglycosylated ITC form), consistent with the selective inactivation of ESP (Bricker et al., 2014).

2.2.1 Processing for Maximal Enzymatic Conversion of Glucosinolates to ITC

The mild heat treatment of 60℃ for ten minutes seems like the best option for maximization of ITC levels, compared to raw broccoli sprouts which convert glucosinolates to non-bioactive nitrile products, and steamed group that inactivates myrosinase and prevents glucosinolates from being converted to ITCs. The chemical reaction that happens when the glucosinolate parent structure is deglycosylated to the resulting products is shown in Figure 2.

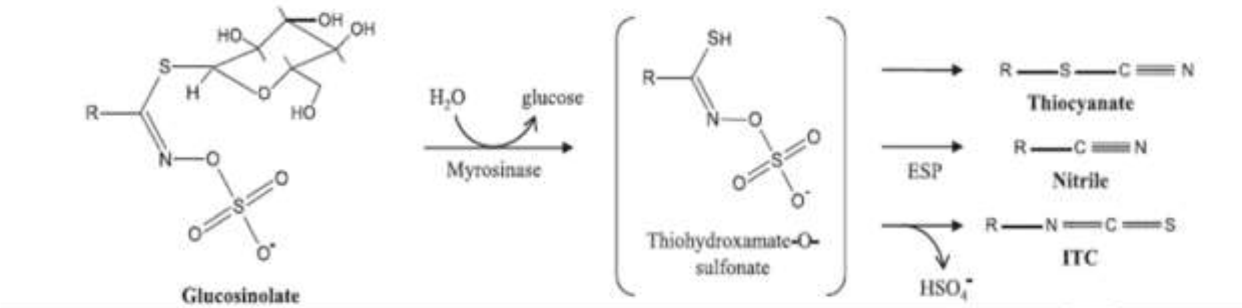


Figure 2 Glucosinolate and ITC metabolism (Bricker et al., 2014)

HPLC analysis confirmed this mild heat processing method selectively inactivated ESP, and retained endogenous myrosinase activity. The process was achieved by submerging the broccoli sprouts in a water bath at 60°C for 10 minutes then freeze drying them to make a dry-shelf stable powder (Bricker et al., 2014). This mild heating was first conducted by placing the broccoli sprouts inside a ziplock bag (so the broccoli is not in contact with the water and glucosinolates do not leach into the water bath (Dosz et al., 2013)) and submerging the bag in a water bath placed on a hot plate. It was hypothesized that this previously reported method (Bricker er al., 2014), could be optimized by using the sous vide method. Sous vide essentially is the cooking of a food item in vaccuum sealed bags, in a water bath with very precise temperature-controlled walls (Baldwin et al., 2012). Based on the previous study by using the standard sous vide method of submerging the broccoli sprouts in vacuum sealed, food-safe bags and exposing them to this time/temperature regimen, it was hypothesized that ESP would be selectively inactivated, consequently augmenting the levels of ITCs in the plant. Thermal processing is therefore a critical step in the broccoli preparation process. The most important steps are therefore, to heat the broccoli sprouts at 60°C and inactivate the enzyme ESP (Matusheski et al., 2004; Bricker et al., 2014) and to take care not to heat over 70°C to retain myrosinase enzymatic activity which converts non- bioactive glucosinolates into bioactive ITCs (Matusheski et al., 2004).

The important factor in the studies where study subjects report broccoli consumption is that very commonly it is eaten as a boiled, blanched, stir-fried or steamed which inhibits glucosinolates from converting to bioactive ITCs (Matusheski et al., 2004; Tang et al., 2008; Bricker et al., 2014). Knowing the health benefit associated with eating broccoli without exposing it to high temperatures, may change the general populations' perception on how to prepare and consume broccoli.

2.2.2 Factors affecting glucosinolate conversion to ITC

Broccoli is also commercially sold as a frozen chopped product in retail stores. This frozen broccoli presents another barrier for people to be able to consume bioactive ITCs from the broccoli plant (Dosz et al., 2013). The common operating procedure for frozen vegetables, like broccoli, is to blanch (at 100°C for approximately 2-3 minutes), inactivating spoilage enzymes (Severini et al., 2016) then the broccoli is frozen. The blanching step process can readily inactivate myrosinase, as well as cause the water-soluble glucosinolates to leach in the water used for blanching (Holst et al., 2004). The freezing step of the broccoli can cause the water inside the broccoli plant to expand, as it is frozen it forms ice crystals which can rupture the plant matrix and release myrosinase prematurely (Dosz et al., 2013). When the plant matrix is ruptured and the released enzymes come into contact with glucosinolates and ITCs are formed. Due to their reactive nature, ITCs can degrade in a short period of time into other non-bioactive degradation products (Hanschen et al., 2004). Hence, consumers will be consuming a very minimal amount, if any, bioactive ITCs (Dosz et al., 2013).

2.3 Human consumption of broccoli sprouts

The sous vide method seems like a viable option to obtain optimal ITC levels and deliver significantly higher doses to humans, but it is imperative to assess for a safe but effective dose. When selecting ingredients with for human consumption, it is important to assert what dose has proven to be innocuous from previous studies. Researchers must search for a dose that could potentially achieve a biological effect while avoiding any negative side effects. The key to obtaining optimal health benefits from broccoli seems to be a combination between the processing method and dosage (Manach et al., 2005).

In a phase one clinical trial, people consumed between 12 and 50 grams of fresh whole broccoli sprouts and reported no significant or consistent subjective or objective abnormal events (toxicities) associated with sprout ingestion (Clarke et al., 2011). In a separate study, the subjects were instructed to consume 4 meals consisting of dry cereal and yogurt with 2 g of broccoli sprouts, 2 g of dry broccoli powder, both, or neither (Jeffery et al., 2010) and a liver function panel indicated no toxicity from any treatment at 24 h. Based on the amount of sprouts consumed in previous human clinical studies, it is estimated that 1 gram of the sous vide, lyophilized powder is safe for human consumption. The broccoli sprout powder will be stored in resealable bags with air, which has been proven to be the optimal storage condition, when compared to controlled atmospheres using CO_2 gas (Hansen et al., 2005).

2.3.1 In vivo studies with broccoli sprouts and exogenous myrosinase

In a study where fresh versus steamed broccoli sprout powders were compared, thermally processed broccoli was found capable of imparting bioactivity by addition of an external myrosinase source from daikon radish (Abbaoui et al., 2012). Seemingly, both diets, fresh sprouts and thermally processed broccoli sprouts with daikon radish, had statistically significant reduction in BC tumors in mice (Abbaoui et al., 2012). The addition of an external myrosinase source is an effective way to convert all the present glucosinolates to bioactive ITCs. Daikon radish addition to broccoli sprout powder imparts a strong bitter flavor to the food (Dubroff et al., 2015).

2.3.2 Product development with broccoli and exogenous myrosinase source

Although broccoli is known to be one of the most abundant sources of glucosinolates in the human diet, integrating it in substantial amounts and into palatable food products is a challenge. Previously, researchers found that a juice with daikon radish was not viable for consumption, as rated by a randomized sensory study where panelists said the juice was much too bitter (Riddle et al., 2011). In this study, the tomato-soy-broccoli-daikon radish product lacked sensory acceptability among panelists due to an off flavor (bitter) likely due to the external source of myrosinase used daikon radish (Riddle et al., 2011). Since daikon radish has been known to impart a characteristic bitter flavor (Johnson et al., 2007) for the present work we opted to leave out this ingredient.

This sensory component brings about big challenges and limitations that product developers face when working to produce functional foods. Even if the product has great potential health benefits, it needs to have acceptable organoleptic properties for people to consume willingly and comply in future clinical trials.

2.4 Tomato Juice and Broccoli Sprout Powder

Based on previous experience and sensory trials, 100% tomato juice as a vehicle for delivery of the broccoli sprout powder seemed to be the best alternative. The principle ITC in broccoli, sulforaphane, as well as the ITC iberin, were found to be most stable at acidic pH, demonstrating the highest stability at a pH of 2 (Janobi et al., 2006). Tomato juice has, on average, an acidic pH of 4, is a great flavor masker, and contains salt which is a known bitterness suppressor in food (Breslin et al., 1997) which makes it an ideal combination for our broccoli sprout powder masking the slight bitter flavor associated to broccoli sprout powder.

2.4.1 Processed Tomato Product's Bioavailability and Epidemiological Evidence

Some studies suggest processed tomatoes have an inverse association to cancer development (Rao et al., 2000; Bohn et al., 2001). The processing of tomatoes, such as the pasteurization of tomato juice, releases lycopene from the plant matrix making it more bioavailable (Rao et al., 2000). While tomatoes have not been tested in BC patients, they are found in some epidemiological studies to have a protective effect for BC risk (Giovannucci et al., 1999). In an in vivo study of prostate cancer in mice, the group fed tomato and broccoli showed to have greater protective effects, than groups fed only tomato or only broccoli (Canene-Adams et al., 2007). The data from this in vivo mouse study showed that the combination of these two vegetables where complimentary and enhance anti tumorigenic effects (Canene-Adams et al., 2007). Hence it seems that by combining these two foods into one product, processed as proposed, there should be a greater health potential than using either food in isolation.

2.5 Characterization Methods

The strategy applied in this study was to use a multi characterization approach including HPLC, testing in a sensory trial, rheological tests, particle size & surface charge analysis, and dynamic vapor sorption analysis to understand the impact of adding the broccoli sprout powder on the characteristics of the tomato juice. This qualitative and quantitative approach was designed to assess whether the sous vide method could enhance the amount of ITC's delivered in the tomato juice by the broccoli powder.

2.5.1 HPLC analysis

Amount of glucosinolates in each of the powder samples was assessed using a previously reported HPLC method (Bricker et al., 2014). This method allows for the quantification of predominant glucosinolates in broccoli sprout powder including glucoraphanin, glucoerucin, and glucoiberin (Bricker et al., 2014). Following the baseline measurement of the glucosinolates precursors, the maximum level of ITC formed were measured. With a previously reported method, ITC extraction can be performed by using water to hydrolyze the glucosinolates then ITC extraction and conjugation was performed with a methanol buffer (Vermeulen and Oliviero et al., 2014)..

2.5.2 Rheological properties of tomato juice

Rheological tests will be conducted to understand the viscoelastic properties of this juice-powder system. The rheological behavior of a fluid food depends on its composition, temperature and shear (Tiziani et al., 2006). Factors such as consistency, thickness, viscosity, and viscoelasticity can provide information and help to characterize behavior of a material, during and after a known applied deformation (Xu et al., 1986; Tiziani et al., 2006). Rheological studies are therefore needed to assess the flow properties of the tomato juice and the influence on this system with the addition of broccoli sprout powder which in turn is related to the perceived mouthfeel of the beverage.

The first step is to find where the linear viscoelastic region for the tomato juice is for our samples to help us bridge the gap between molecular structure and product performance (Fischer et al., 2011). In this way we can compare the plain tomato juice versus the tomato juice with broccoli sprout powder and see if there is a fluctuation in the material's properties like complex viscosity that provides information of the flow and thickness of our juice (Tiziani et al., 2006). This can help characterize the base material (tomato juice) and how broccoli sprout powder influences its structural characteristics resulting from the way in which the elementary units like pectin and water are spatially distributed and interact with the fiber from the broccoli powder. The material sample, in this case tomato juice with and without broccoli sprout powder, is placed in a couette device, which is essentially a pair of concentric cylinders, one of which is fixed (Tiziani et al., 2006). The material is put in the narrow gap between the cylinders, and the free cylinder is rotated. This test allows the quantification of any differences between the structural properties of plain tomato juice versus tomato juice with broccoli sprout powder.

2.5.3 Dynamic Light Scattering - Phase Analysis Light Scattering ζ Potential

The dynamic light scattering (DLS) phase analyzer (PALS) has become a commonly used instrument for characterizing the size, surface charge and distribution of particles in solution (Koszak et al. 2012). It provides information for the hydrodynamic diameter (d_H) of the particles in solution, assuming these particles are perfect spheres (Betts et al. 2013). This technique also has the potential to report the zeta potential, or surface charge, of the particles in solution, which is an indicator for the stability of the particles or colloids solution (Kosak et al. 2012). If a value of above |30| is obtained, then the particles in solution are considered to be stable (Pate et al., 2016). With this information it is possible to characterize the size of the broccoli sprout powder in solution, as well as the stability of this powder in solution.

2.5.4 Dynamic Vapor Sorption

A Dynamic Vapor Sorption analyzer can quickly determine the sorption equilibrium and behavior of broccoli sprout powder with as little as 5 mg of sample. Specifically, for powdered products, this data serves to understand ideal storage conditions and shelf stability based on the material's interaction with water. The dynamic vapor sorption, or DVS, method has become one of the standard laboratory methods for studying drying and dehydration behavior (Arlabosse et al., 2003) with a wide range of applications from pharmaceuticals to food products. In this case for broccoli sprout powder, moisture sorption isotherms can examine the behavior of the solid state of our powder and how it uptakes water stored at different relative humidity's. For a powder product, excess water uptake could result detrimental to the product causing off flavors (oxidation), agglomeration/ caking or even mold growth (Singh et al., 2003.

In order to study the optimal conditions for storage stability of a product, knowing the interaction between the moisture content (MC) in the plant material and the relative humidity (RH) of the surrounding air at a given temperature is essential to prevent decline of the product quality. The moisture sorption data helps further understand the theoretical interpretation of food microstructure and physical interaction between water molecules and the solid matter of a food (Arlabosse et al., 2003).

2.5.5 Sensory panel testing

Sensory evaluation has become another key part of product development projects because the wealth of information gathered from a sensory trial helps better understand the intrinsic characteristics of a food product which makes it more appealing and acceptable to consumers (Rousseau et al., 2004). For a product to succeed in the food industry, sensory evaluation is a critical component for this process (Sidel et al., 1992). A sensory evaluation panel can help provide information that would be difficult to measure otherwise, because it involves the 5 senses of humans (Rosseau et al., 2004). The 9-point hedonic scale is a very straightforward and easy to use tool that helps panelist rate their level of satisfaction with a product, rating it from 1- dislike extremely to 9- like extremely (Rosseau et al., 2004). Another type of hedonic scale that provides additional information on the intensity of certain attributes of the product is the Just-About-Right or JAR scale (Rosseau et al., 2004). Both tests are complimentary to gather information of the acceptability of the product being evaluated. For example, the hedonic scale gives you information of how acceptable the perceived saltiness of the product is, and the JAR scale determines if the saltiness level was too little, too much, or just about right. This way it is easier to assert if the product level of saltiness was not liked because it was bland or way too salty, resulting in a more complete assessment for future product development efforts. The next test that could be useful for comparing two products where you want to test which of the two is better accepted, is a preference test. Using a preference test can help identify if there is an affinity for one over the other product or if there truly is no difference between the two samples tested (Rosseau et al., 2004).

Broccoli sprout powder in tomato juice has presented challenges for sensory acceptability in the past when combined with other functional foods like soy and daikon radish (Riddle et al., 2011).

It was hypothesized that by eliminating the soy protein element that forms a connected network structure with the tomato pectin (Tiziani et al., 2006), the juice should flow more similarly to 100% tomato juice. And by using the sous vide method to retain myrosinase enzymatic activity, there is no need for the daikon radish component which brings additional bitterness to the product (Riddle et al., 2011). Additionally, previous studies reported tomato juice having a metallic off-taste in their sensory panel with canned tomato juice (Riddle et al., 2011). Therefore, the current study will evaluate a plastic bottled versus canned tomato juice with and without broccoli sprout powder. A preference test can evaluate if a specific kind of packaging influences consumer acceptability. Previous efforts with less than subpar sensorial results have provided some insight on the challenges of this product formulation. This sensory study was an opportunity to understand if the broccoli sprout powder in tomato juice has improved acceptability, impacting future indications for this formulation.

2.5.6 Shelf stability Markers

The last parameter that is important for the successful development of proposed food product is conducting shelf stability studies. Previous studies have found that short-term storage is correlated to increased glucosinolate content in broccoli; while in long term storage glucosinolate levels appear to decrease (Cavaiuolo and Ferrante, 2014).

A shelf stability study will be conducted through 28 days to assess ITC bioactive levels in the broccoli sprout powder, along with colorimeter measurements L, a and b as indicators of senescence (Curia et al., 2009). 'L' serves as an indicator of how light or dark is the sample, with a range from 0- 50 is representative of a dark sample, while 51-100 is representative of a light-colored sample (Hunter et al., 1987). The 'a' scale measures how red or green is the sample being tested. This range just has positive numbers to indicate the redness, and negative values for samples that are detected to be green (Hunter et al., 1987). The last parameter measured is ‘b’ which provides information on how yellow or blue the samples are, with positive and negative values respectively (Hunter et al., 1987). With this data it was possible to approximate the amount of viable bioactive phytochemicals in the sous vide broccoli sprout powder.

Chapter 3: Research Plan

Research Plan

Data from in vivo and in vitro studies have documented isothiocyanates found in broccoli to be bioactive and non-toxic, while alluding to the need for products incorporating this vegetable in substantial amounts for human consumption. (Abbaoui et al., 2012) The combination of broccoli and tomato has been found to have increased anti cancerous effect in mice, than either food alone. (Canene-Adams et al., 2007; Grainger et al., 2008) However previous attempts have failed to meet sensory acceptability among consumers which is essential for long term consumption, for clinical adherence to be able to be further tested in clinical trials or for potential commercialization. The selected source of bioactive isothiocyanates was chosen to be broccoli sprouts since these contain on average 20 times more glucosinolates than mature broccoli (Abbaoui et al., 2012). By exposing the broccoli sprouts to a slight heat treatment in a temperature controlled sous vide, the level of bioactive isothiocyanates could be increased compared to raw or steamed sprouts (Bricker et al., 2014). The goal of this work is to deliver the maximum amount of bioactive isothiocyanates in a tomato juice that has acceptable sensorial properties.

Since it has been found that isothiocyanates are highly reactive and degrade into other products when they are in an aqueous medium (Hanschen et al., 2004), they were freeze dried and ground into a broccoli sprout shelf stable powder. Right before consumption, the powder was added to tomato juice for hydrolysis of glucosinolates and formation of isothiocyanates which are known to be stable up to a pH of 3 (Bertelli et al., 1998).

Objectives

1. Using HPLC/MS determine if the sous vide method can maximize the level of isothiocyanates in broccoli sprout powder compared to raw and steamed sprout powders.
2. Examine the effect on the chemical and structural properties of the tomato juice upon addition of broccoli sprout powder by assessing changes in the complex viscosity as well as particle size, dispersion and zeta potential.
3. Conduct a sensory panel study to ascertain the overall acceptability of tomato juice compared to tomato juice with 1 gram of broccoli sprout powder.
4. Dynamic vapor sorption test can be used to examine the behavior of the powder at different relative humidity's to identify optimal storage conditions. While colorimeter measurements of L, a, and b can serve as a shelf stability indicator.

Upon completion of these tests, the aim is to obtain a phytochemical-rich functional juice with high quality organoleptic properties.

Chapter 4: Methods

4.1 Chemicals

Optima-grade solvents were used for all analyses involving MS and HPLC-grade solvents were used for all other analyses (Fisher Scientific; Pittsburgh, PA, USA). 2-mercaptoethanol, TFA, triethylamine, 1-butanethiol and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucoraphanin and glucoerucin standards were purchased from the Royal Veterinary and Agricultural University (Copenhagen, Denmark). Sulforaphane and Iberin standards were obtained from LKT Laboratories (St. Paul, MN, USA).

4.2 Materials

Both canned and plastic bottled tomato juice (Gordon Food Service Columbus, OH) were used for product development, rheology, and sensory analysis. Locally grown broccoli sprouts of the calabrese variety seeds, lot number SBC2-7BD from Sunsprouts Farms of Central Ohio (Whole Foods Columbus, OH) were picked on delivery date after approximately 4 days of sprouting.

4.3 Development of the raw, steamed and pretreated broccoli sprout powders

Fresh broccoli sprouts (Whole Foods Columbus, OH) were washed following the recommended protocol for fruits and vegetables, 1 oz. of Fruit and Vegetable Wash (Ecolab, MN) was added to 1 gallon of deionized water. The sprouts were placed in this wash water for 5 minutes (recommended contact time), rinsed and patted dry. The raw control sprouts were placed on their labeled tray after being rinsed and dried. The sous vide experimental group were washed and patted dry then vacuum sealed (without crushing the sprout's structure) in a food grade plastic bag and placed under water in a Sous-vide (Model SVS-10LS, CO) at 60º C for 10 minutes. The package was partially opened and cooled in a water ice bath for 5 minutes. The steamed broccoli sprouts were placed in a steaming basket for steaming in an 8-inch teflon pot for 5 minutes, and then transferred to a labeled tray to cool off before the next step. All the trays of sprouts were flash frozen with liquid nitrogen and immediately placed in the freeze dryer Freeze Zone 12 Plus (Labconco, MO) which had been preequilibrated and ran at 15℃, and the sprouts were lyophilized for 48 hours, until completely dry. After, all samples were ground into a fine powder with a Secura Coffee & Spice grinder (Secura Model SP-7412, China), then the powder was stored in a ziplock bag inside a 4ºC refrigerator.

4.4 Glucosinolates HPLC Analysis

The level of both glucosinolates and ITCs were measured immediately at day 1 after the freeze drying and pulverization was done. The ITC level over a 4-week period was also obtained to determine shelf stability of these bioactive compounds. The glucosinolates were extracted with a water extraction method developed by Dr. Ken Riedl who extracted the highly polar glucosinolates with a water extraction method and analyzed them via HPLC (Bricker et al., 2014). As per the described extraction method for glucosinolates (Bricker et al., 2014), for each of the broccoli sprout powders 40 mg were weighed and added to a glass vial. To inactivate myrosinase (inactivation temperature is 80℃) 3 mL boiling water were added to denature endogenous myrosinase and preserve the glucosinolates in their native structures. The vial was loosely capped and placed in a 100℃-water bath for 5 minutes. After this, the vial was simultaneously vortexed using a Vortex Genie Pulse (Scientific Industries, NY) for 5 min to ensure leaching of the glucosinolates into the water extract. The samples were then centrifuged for 5 min at 600×g, and then the aqueous extract was pipetted into a clean glass vial, labeled and set aside. The pellet remaining in the centrifuge tube was resuspended in 3mL of water at room temperature (23°C) then simultaneously vortexed and sonicated for 10 min, followed by centrifugation and decanting twice more. The three water extract supernatants were added to the same tube resulting in a 9-mL aqueous extract. To this extract 1 mL of deionized water was added resulting in 10 mL of the aqueous extract for analysis. Using glucoraphanin, glucoerucin and glucoiberin, the three major glucosinolates found in broccoli, the powder extracts were analyzed by HPLC. Glucoraphanin and glucoerucin were separated on a Zorbax SB-CN RP column (4.6×250 mm; 5 μm; Agilent Technologies, Santa Clara, CA, USA) with an Agilent 1100 Series HPLC. A binary gradient, consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), was used at a flow rate of 1.5 mL/min. Initial conditions were 0% B for 3 min, followed by linear increases to 10% B by 4 min, 50% B by 8 min, and 95% B by 9 min, after which the column was equilibrated at 0% B for 3 min. An AB Sciex QTrap 5500 mass spectrometer (Concord, Ontario, Canada), operated in electrospray negative-ion mode, was used for quantitation.

4.5 Isothiocyanates HPLC Analysis and Colorimeter Analysis and Shelf Stability

The extraction if isothiocyanates was as described in the methods for ITC extraction published by *Oliviero 2014*. For each of the powders, 1 gram of dry powder was weighed in an analytical balance and placed in a centrifuge tube with 18 mL of water at 40°C. The tubes were vortexed for 2 minutes then centrifuged at 3000 RPM for 5 minutes. Then 1 mL of this water was separated from the solid broccoli put in a micro centrifuge tube and this vial was placed on ice. In separate centrifuge tubes, 1 gram of broccoli solids was flash frozen with liquid nitrogen, mixed with a methanol buffer which inhibits myrosinase activity from hydrolyzing glucosinolates any further. The ground frozen broccoli (1 g) was extracted with 16 mL of buffer (formic acid 0.08 M, triethylamine 15.8 mM in methanol), was centrifuged, and the supernatant was analyzed. From this buffer solution 100 µL was mixed with 100 µL of the water extract kept on ice and 800 µL of then methanol buffer was added. As a final step, 12 µL of 1-butanethiol (99%) was added for conjugation to the mixture in a centrifuge tube which was placed in a warm water bath at 50˚C for 2 h.

The n-butanethiol conjugates of SR and IB were analyzed using LC-MS/MS (TSQ Quantum, Thermo Instruments) with an XBridge RP18 column (3.0 × 100 mm, 5 m). SR (LKT laboratories/Biomol S8040 177.29 g/mol) and IB (LKT laboratories/Biomol I0416, IB 163 26 g/mol) conjugates were prepared by incubation with n-butanethiol at 50˚C for 2 h with SR and IB, and were used for quantification by external calibration. Each of the BSP samples including raw, sous vide and steamed were tested at day 1, 7, 14 and 28 for ITC levels. The powders were also subjected to a colorimeter MiniScan EZ 4500L (Hunter Associates Lab, VA) measurement of L, a, and b as part of shelf stability studies, using color as an indicator of senescence. All measurements were taken as triplicates for each sample on the day of color measurement.

4.6 Rheological Analysis of Broccoli Sprout Powder in Tomato Juice

For the rheological test, samples of 100% tomato juice (Whole Foods Columbus, OH) were used as the control, and a sample of 1 gram of broccoli sprout powder added in 6 oz. of tomato juice was tested to evaluate the flow of the juice and temperature effects using a strain-controlled rheometer (RFS II Rheometrics system, Rheometrics Inc., Piscataway, NJ). A concentric cylinder or couette geometry was used (diameter of cup, 34 mm; diameter of bob, 32 mm; length of bob, 33.3mm) because tomato juice is a liquid fluid, considered to be a low viscous material, and this geometry helps minimize evaporation effects enhancing the surface of contact between the fluid and the apparatus geometry. Rheological temperature-dependence studies between 25°C -65°C were conducted to assess impact of the powder on tomato juice flow properties. The tomato juice was agitated several times and then left to rest for 1 min at room temperature to limit serum- pulp separation.

4.6.1 Tomato Juice Solids

A handheld refractometer (Fisher Scientific, PA) was used to determine the total percent soluble solids (˚Brix).

4.6.2 Tomato Juice pH

The pH (Orion Research, Beverly, MA) of the tomato juice without and with broccoli sprout powder was measured.

4.7 Dynamic Light Scattering (DLS) - Phase Analysis Light Scattering (PALS) ζ Potential

To characterize the particle size of the sous vide broccoli sprout powder, 5 mg of powder was combined with 1 mL of deionized water. Using a syringe with a 0.1 µm aqueous membrane the sample was filtered and transferred to a disposable cuvette. Dynamic Light Scattering (DLS) and Phase Analysis Light Scattering (PALS) measurements of the average effective diameter, ds, and polydispersity index, µ, values were obtained using a ZetaPALS instrument (Brookhaven Instruments, Holtsville, NY) at 25 °C. The run's duration was 3 minutes long using a 90º angle laser source. After the effective diameter was measured a second disposable cuvette was filled with the prepared sample and placed on the zeta, ζ, potential electrode for analysis of the surface charge.

4.8 Dynamic Vapor Sorption

For the dynamic vapor sorption analysis, a DVS Advantage (Surface Measurement Systems Ltd., London, UK) was used for a sample of 5 mg BSP. The closed chamber where the sample plate was held at the constant temperature of 25℃ was monitored by an ultra-sensitive microbalance that measured changes in the mass of the BSP sample. Nitrogen gas was used as the flowing carrier gas for the microbalance chamber at a specified relative humidity (or partial pressure) over the sample suspended on the sample plate using the mass flow controllers. With the partial pressure of water at equilibrium the apparatus and the water uptake was plotted experimentally as a function of time. When the changes in mass with time (dm/dt) was under 0.001 for 5 consecutive minutes the sample was considered to have reached equilibrium.

4.9 IRB approved Sensory Panel

4.9.1 Recruitment

As part of the IRB approved document #2018E0038l, 75 subjects age 18 and older were recruited. Eligible participants were not taking prescription medications; not be allergic to crucifers, tomatoes, ascorbic acid, citric acid, as well as wheat or gluten-free crackers; not be diabetic or have altered glucose metabolism; and also have no visual or sensory impairments. We used the sensory evaluation center database to recruit participants. Recruited subjects were provided with the informed consent form prior to testing. Once written consent was obtained, they were offered a copy of the consent and permitted to participate in this study.

4.9.2 Approval of Institutional Review Board (IRB)

The sensory trial was held following the protocols described in the IRB approved document 2018E0038.

4.9.3 Sensory analysis

Tomato juice (3 oz serving) was provided alone then with 0.5 g of broccoli sprout powder previously measured on analytical scale. This study was conducted in a space where respondents evaluated the products in individual sensory booths, under white light. The panelists were blinded to the packaging of the juice to avoid potential bias against one packaging or the other. This study consisted of three parts described in detail below. Sensory evaluation was conducted in the Department of Food Science Sensory Testing Facility and using ISO protocols for sensory evaluation (ISO 2007). All samples were labeled with randomized three-digit numbers. Randomization and counterbalancing was performed by Compusense and samples were presented in a serial monadic presentation scheme. Sample ballots were provided to the 75 panelists enrolled (in Appendix A). To minimize bias, the sensory tests were administered in the following order:

1. Acceptability tests were conducted to obtain the likability or palatability of 2 juices- one tomato juice from a plastic bottle and one tomato juice sample from a can, in 3 oz. portions, stored at 4˚C until serving time. Each of the 2 juices was tested plain versus the same juice with 0.5g broccoli sprout powder, for a total of 4 samples. A 9-point Hedonic scale was used to describe overall acceptability, appearance, aroma, flavor and viscosity

for each sample. The panelists rated the juices using the scores from a scale of 1- dislike extremely to 9- like extremely.

2. A 5-point Just About Right (JAR) scale was used to obtain information enabling optimization of the formulation of tomato juice with the broccoli sprout powder. The attributes examined were as follows: aroma, thickness (viscosity), saltiness and bitterness. The Just About Right (JAR) scale ranges from 1- Much too little, 2- Too Little, 3- Just About Right, 4- Too Much 5- Much Too Much. Penalty Analysis was used to analyze the JAR data obtained. By calculating the results of the JAR that deviated from a score of 3 (Just About Right) in the categories of Too Little or Too Much it was possible to determine what attributes received a significant penalty;
3. Panelists completed a paired preference test between the tomato juices from a can or plastic bottle with broccoli sprout powder. To avoid potential bias, consumers were blinded to whether the juice is from a can or plastic bottle source.

Participants were given water and either wheat or gluten-free water crackers to cleanse their palates in between each juice they taste.

4.9.4 Statistical methods for Sensory Test Results

Means ± SD (standard deviation) were used to express the results from acceptability tests, and differences in acceptability were determined using the SPSS statistical software package (IBM, New Castle, NY). One-way ANOVA was employed for all sensory tests, with Tukey's post-hoc test, and all results were considered significant when $P<0.05$. Error bars represent 95% confidence interval. a-d Letters represent significant differences at α (0.05) level. For the JAR tests penalty scores were calculated to see significant penalties and for the preference test

Binomial Probability was used to determine if there was a significant difference between sample preference.

Chapter 5: Results & Discussion

5.1 HPLC

5.1.1 Glucosinolates HPLC: raw, steamed and sous vide broccoli sprout powders

With the quantification of the level of glucosinolates in each of the broccoli sprout powders it was possible to have a baseline estimate of the levels of ITC's that can be formed. Figure 3 shows the level of glucosinolates in the raw and treated broccoli sprout powder. As was expected, the level of glucosinolates was lowest in the sous vide powder, highest in the steamed and intermediate for the raw group.

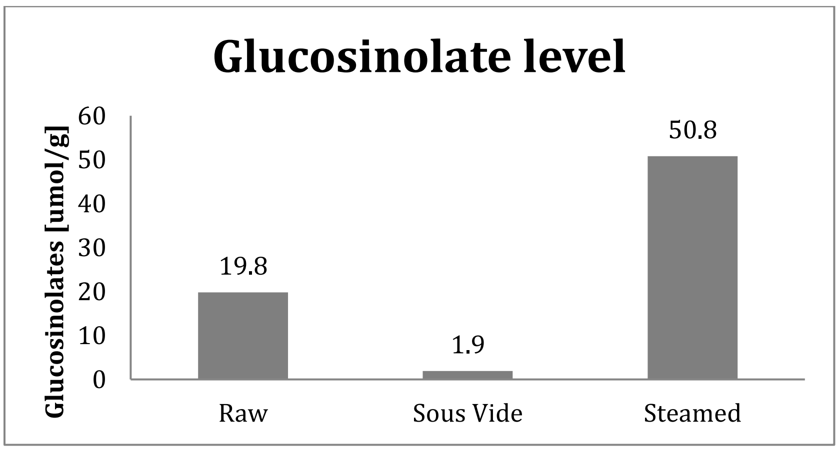


Figure 3 Glucosinolate HPLC Analysis

According to previous studies, (Matusheski et al., 2001; Bricker et al., 2014) the steamed broccoli sprout powder should have the highest level of glucosinolates since both myrosinase and ESP enzymes are inactivated and therefore the glucosinolates should remain in their native structures. Glucosinolate level in this study was 50.8 µmol/g for steamed broccoli sprouts which resulted, similar to a previous work done using the same extraction method that reported a glucosinolate level of 46.7 µmol/ g (Bricker et al., 2014). A different study also found a similar level of glucosinolates in steamed broccoli sprouts of 65.8 µmol/ g (Abbaoui et al., 2012). The differences in the observed levels could be due to variations in the seeds (Fahey et al., 2001) or freshness of the sprouts since younger broccoli sprouts 2-3 days seem to be more concentrated than more mature broccoli sprouts 10 days (Fahey et al., 2001). The broccoli sprouts used by prior studies (Abbaoui et al., 2012; Bricker et al., 2014) are from the same brand and company used in this present study, but variations in seeds have been shown to have an effect on the observed levels of glucosinolates in the plant (Matusheski et al., 2004). For the present study, the company, Sun Sprout Farms, where the broccoli sprouts were purchased from provided this important piece of information stating that the seed variety is Calabrese and they specifically germinate the seeds for 4 days before retail. The raw broccoli sprout powder resulted in 19.8 µmol/g glucosinolates. The lower level was expected since this group has epithiospecifier protein enzymatic activity competing with myrosinase activity, thus, small ITC conversion was expected. Comparing to previous glucosinolate data for raw broccoli sprouts the level was found to be at 41.2 µmol/g (Bricker et al., 2014) (the results from the present study was almost half the amount), and a different study found glucosinolate in raw broccoli sprouts to range from 0.8 µmol/g to 21.6 µmol/g (Kushad et al., 1999) likely due to differences in seed variety of sprouts used (Fahey et al., 2001). The latter study examined over 50 different varieties of broccoli, finding this 26-fold difference in glucosinolate content among the broccoli seed varieties used (Kushad et al., 1999). Lastly, the sous vide broccoli sprout powder was expected to have the lowest level of glucosinolates, since they are expected to be deglycosylated by myrosinase and converted to bioactive ITCs. The glucosinolate level in this present study for the 60℃ sous vide sprouts was 1.9 µmol/g, while the 60℃-pretreated group of sprouts from a previous study reported a 30.2 µmol/g glucosinolate level previously reported (Bricker et al., 2014). For the present study, these results confirm the hypotheses that by using the sous vide method, which efficiently exposes all sides of the broccoli sprouts in the vacuum-sealed package to a very precise 60°C for ten minutes. Based on the ITC levels obtained, ESP seems to have been inactivated and myrosinase activity was not in competition and able to convert the maximum amount of glucosinolates to bioactive ITCs. The difference in the levels of the 60°C treated broccoli sprouts was also attributed to the uneven heating of the broccoli sprouts in the previous study (Bricker et al., 2014) where the heat source was a hot plate which exposes only the bottom side of the bagged sprouts to the temperature. The fluctuation in temperature is also more difficult to control by a hot plate than a sous vide which has temperature controlled walls constantly monitored by thermocouples integrated in this chamber's walls resulting in an even heat by convection (Baldwin et al., 2012).

5.1.2 Isothiocyanate HPLC: raw, steamed and sous vide broccoli sprout powders

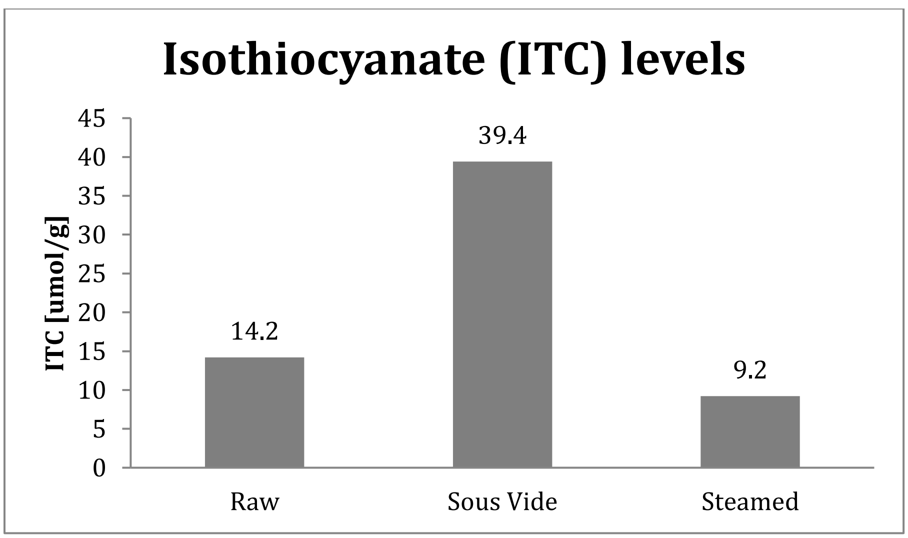


Figure 4 Isothiocyanate Levels from HPLC Analysis Day 1

The effect observed from processing the broccoli sprouts with the sous vide method was that it seemed to convert more glucosinolates than the raw or steamed sprout powders. Compared to the raw, sous vide powder contain 2.8 times more ITCs and compared to steam powder contain 4.2 times more ITCs. The only ITCs measured are 2 of the most abundant or major ITCs found in broccoli including sulforaphane and iberin. Erucin could not be quantified due to lack of a standard, thus, the actual level of ITCs might be slightly higher. Nonetheless, the effect of maximizing the ITC level through sous vide processing can still be appreciated in Figure 4. The initial study that found the 60℃-heat treatment increased formation of ITCs, with a simultaneous decrease in the nitrile product tested temperatures from 20 ℃ up to 100℃ (Matusheski et al., 2004). Yet this study found that with the treatment of 60℃ the lowest level of the nitrile product was observed at 0 µmol/g and approximately 8 µmol/g of the sulforaphane ITC were observed (Matusheski et al., 2004). However, in their methods section, the description of their ITC extraction included an 8-hour incubation step (Matusheski et al., 2004), which could have resulted in ITC degradation (Hanschen et al., 2004). Comparing to past results of a different study which used the same HPLC extraction method and experimental groups used in this present work, reported ITC levels in raw, steamed and 60℃ treated broccoli sprout powder at 5.3, 0.94, and 22.1 µmol/g respectively (Bricker et al., 2014). As in this current study, the highest ITC level in broccoli sprout powder was the sample with the 60℃-heat treatment. However, the discrepancy in the observed levels of ITC's between the two studies may be attributed to the fact that the previous study only accounted for the main ITC sulforaphane (Bricker et al., 2014), whilst in the present study both sulforaphane and iberin were quantified for the quantification of ITC levels. The quantified levels of sulforaphane obtained in the present study were 26.45µmol/g for the 60℃ sous vide broccoli sprouts, which seems to be in range with the previous reported ITC level of 22.1 µmol/g (Bricker et al., 2014). Another factor that could also be affecting the results observed is the differences in the processing apparatus used. A sous vide can provide a more uniformed and controlled heat over all the surface of the submerged vacuum sealed broccoli sprouts (Baldwin et al., 2012), inactivating close to all ESP present and enhancing further myrosinase activity, converting more efficiently the glucosinolates to bioactive isothiocyanates. And while the broccoli sprouts were obtained from the same store and manufacturer, the previous study (Bricker et al., 2014) did not report the broccoli seed variety used, introducing another element of variability (Fahey et al., 2001).

The conversion from glucosinolates to bioactive ITCs can be better appreciated in Figure 5. The effect of ESP in the raw group and lack of myrosinase in the steamed group likely resulted in the low conversion to ITCs in each of these groups.

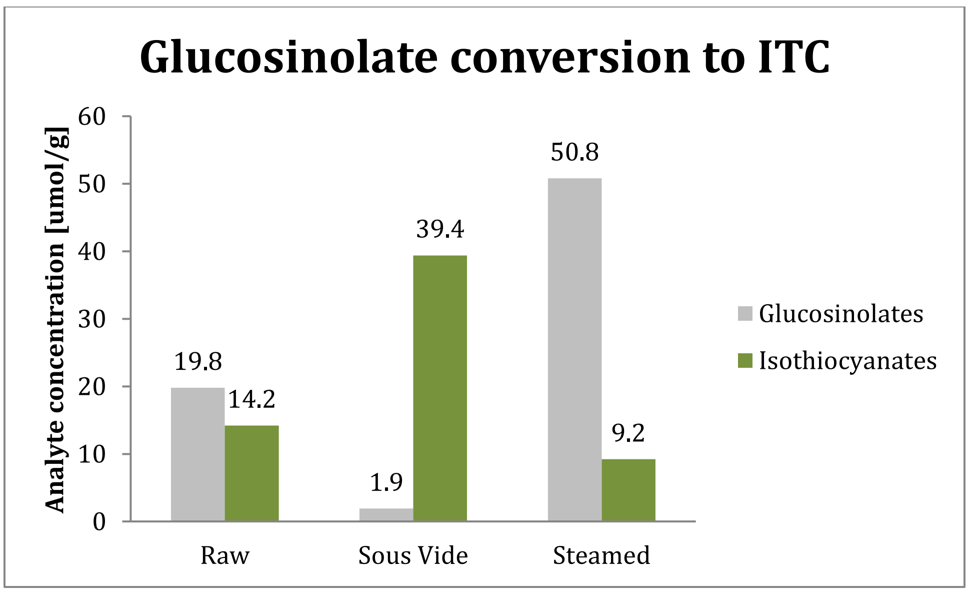
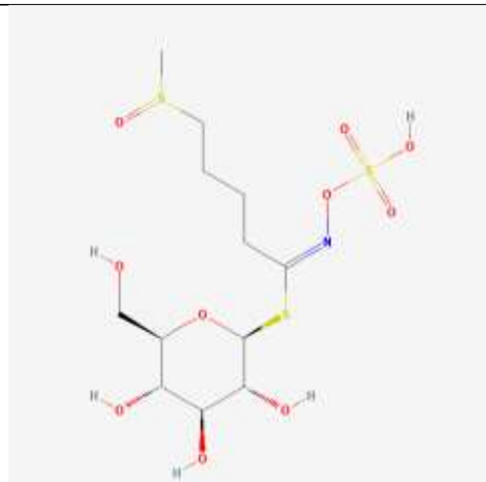
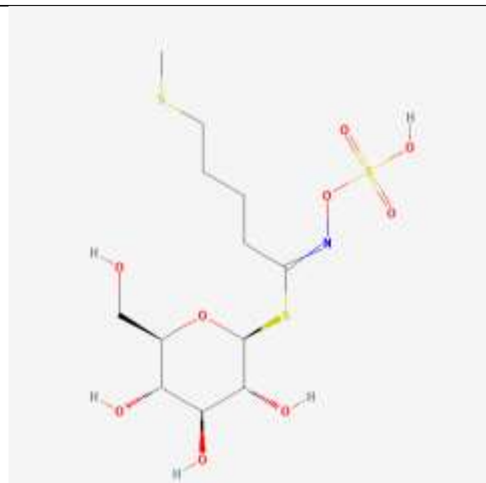
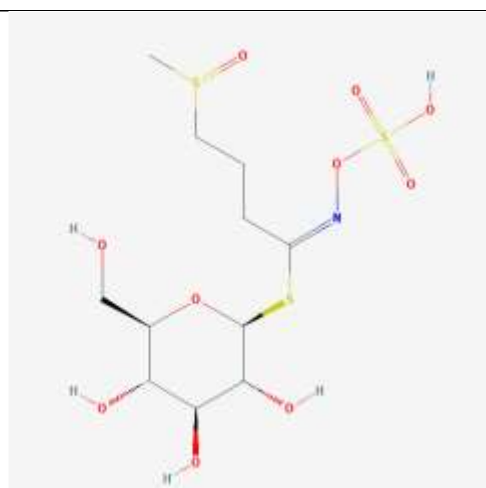


Figure 5 Glucosinolate conversion to ITC in Raw, Sous Vide and Steamed powders

It is imperative to point out that for the quantification of glucosinolates 3 standards of the most abundant glucosinolates were used including glucoraphanin, glucoerucin and glucoiberin whose structures can be found in Table 1 (National Center for Biotech. Info).

Table 1 Most Abundant Glucosinolate Structures in Broccoli Sprouts (Fahey et al., 2001)

Glucoraphanin	
Glucoerucin	
Glucoiberin	

For the quantification of ITCs only sulforaphane and iberin levels could be measured due to lack of a standard for erucin, another of the principle ITCs in broccoli sprouts. Consequently, the actual levels of ITC could be higher than the reported, but for the purpose of comparison between processing methods, the effects of using the sous vide as the best alternative could still be appreciated.

5.1.3 Isothiocyanate HPLC: 28-day Analysis

ITC stability during 28-day storage is presented in Figure 6 for the three powders: raw, sous vide and steamed.

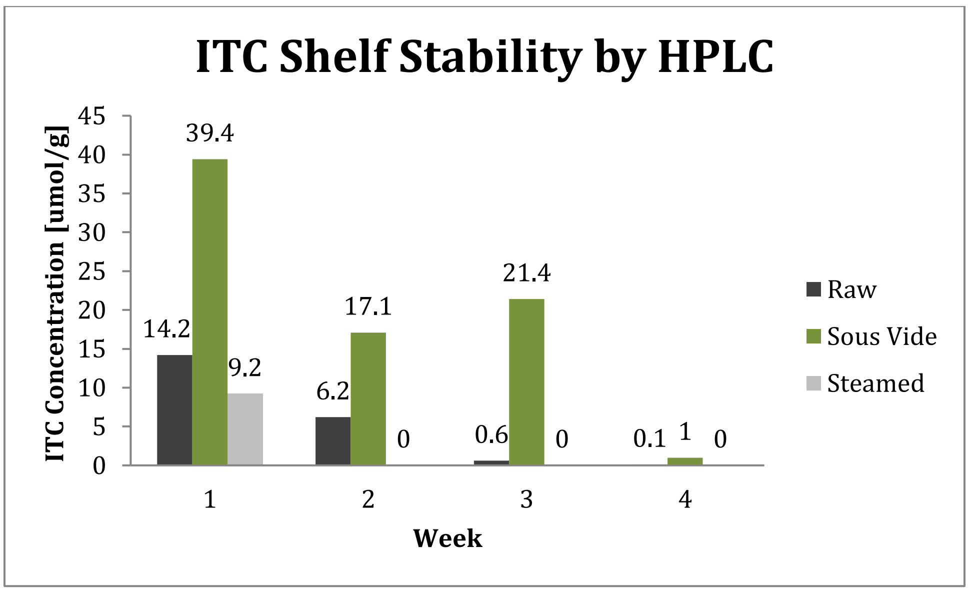


Figure 6 HPLC ITC levels from 28-day study in Raw, Sous vide and Steamed broccoli sprout powders

In the first three weeks, the sous vide broccoli sprout powder consistently resulted in the highest level of ITC detected. On the fourth week the levels dropped dramatically which could be due to the powder picking up humidity from the environment. Since all these dry powders are ground and placed in a zip lock back, as soon as humidity enters the package, myrosinase activity can deglycosylate the glucosinolates converting them to ITCs (Abbaoui et al., 2012) which are highly reactive then what we saw the fourth week could be a process attributed to ITC degradation (Hanschen et al., 2004). For the creation of a functional beverage using broccoli sprout powder, this means the powder needs to be stored completely sealed from exposure to humidity up to the point of consumption. Therefore, the broccoli sprout powder needs to be separate from the tomato juice up to the time of consumption. In this way it is possible to ensure the highest conversion of glucosinolates to ITCs therefore maximal exposure to the bioactive analytes.

5.2 Rheology

One way to have a correlation between the perceived mouthfeel of a juice is by studying the rheological properties, like viscosity, of a fluid.

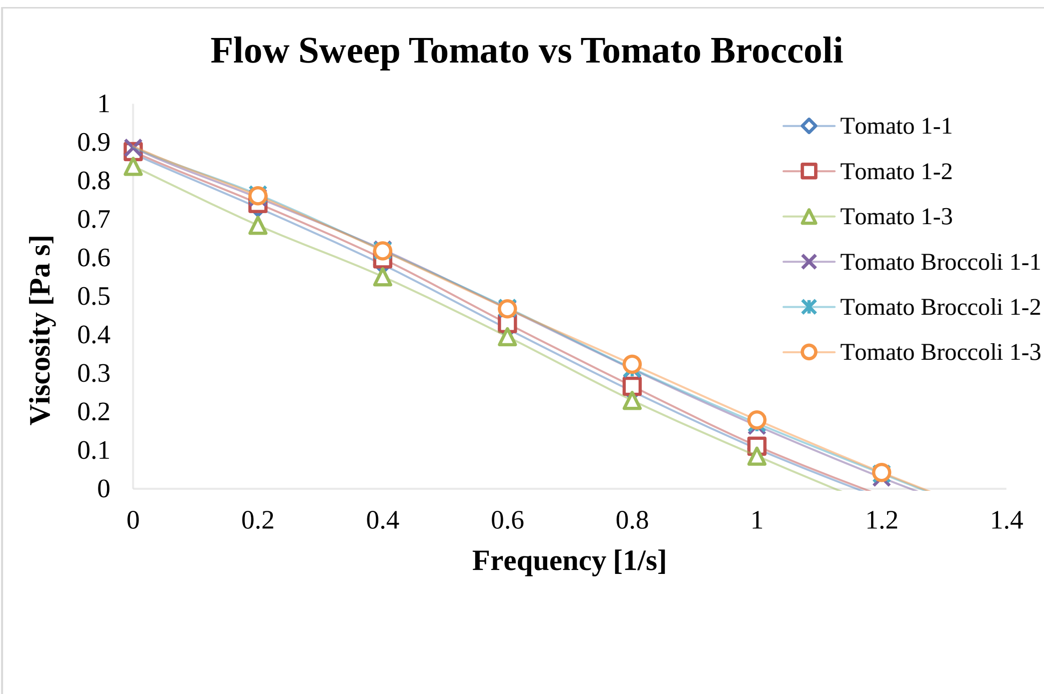


Figure 7 Triplicates of Rheological Flow Sweep TJ vs. TJ + BSP at Room Temperature 25°C

Rheological dynamic testing showed stability and compatibility between TJ and BSP. The complex viscosity, overlapped for both the tomato juice and tomato with added BSP as seen if

Figure 7. In a dynamic time sweep, (results not shown) the material response is monitored at a constant frequency, amplitude and temperature. This way we can correlate the deformation of the juice with the amount of applied shear as a viable system in order to model the flow behavior of our sample (which for tomato juice is known to be shear thinning) (Tiziani et al., 2005). Temperature is known to have an important influence on the flow behavior of liquids. In Figure 8, the same trend of complex viscosity versus frequency could be observed for tomato juice and tomato juice with added BSP, collected in steps in isothermal conditions at the different tested temperatures from 25℃ - 65℃.

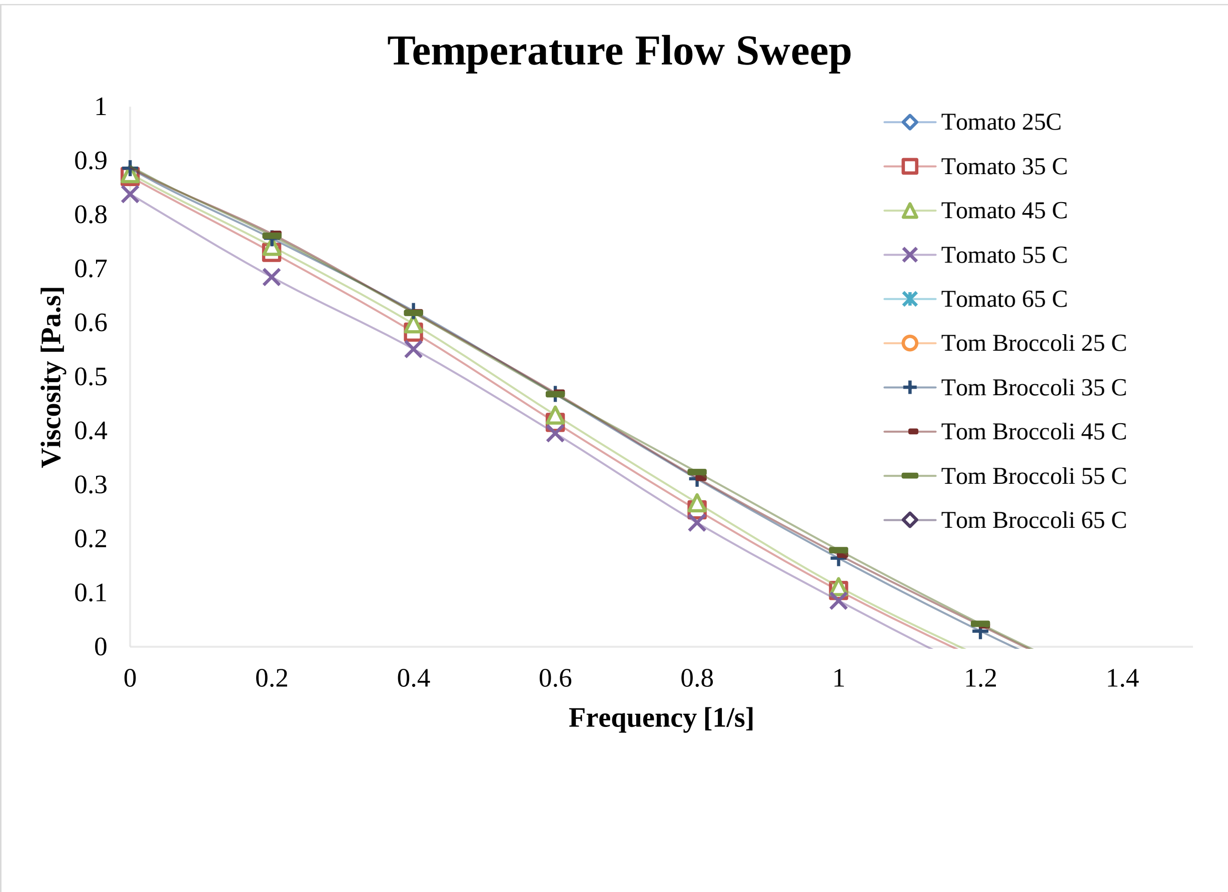


Figure 8 Rheological Tests Using Temperature Gradient

In the graph there was an overlap of the results for the complex viscosity, with a minimal degree of separation between each of the runs. A slight increase when adding the broccoli sprout powder was expected since this is a system similar to that of a food hydrocolloid solution (Tiziani et al., 2005). The addition of broccoli sprout powder, into a tomato juice system was thought to have a slight effect. Therefore, we hypothesized that the addition of 1 gram of broccoli sprout powder was not expected to significantly change the mouthfeel when added to 6 oz. of plain tomato juice. In a prior study the viscosity of tomato juice seemed to have been affected by the addition of soy protein (Tiziani et al., 2005), but in the absence of the tomato pectin- soy protein interactions, the BSP fibers did not seem to alter the structure of tomato juice.

5.2.1 Tomato Juice Solids

The ˚Brix was measured as 5.8% and 6% for plain tomato juice and tomato juice with broccoli sprout powder respectively.

5.2.2 Tomato Juice Solids

At time 0 the pH= 4.30 and the sample with the broccoli sprout powder in the tomato juice was pH = 4.31. After 24 hours the samples were measured again, and results showed no change in pH levels measured to be at pH= 4.30 and pH= 4.30 for plain tomato juice and tomato juice with the broccoli sprout powder respectively. The results were in accordance to previously measured pH of tomato juice where, depending on the tomato variety and processing the pH has been found to be between 4.1 and 4.5 (Yoon et al., 2004; Anderson et al., 1984; Tiziani et al., 2005).

5.3 Dynamic Light Scattering - Phase Analysis Light Scattering ζ Potential

Dynamic light scattering data in Figure 9 shows the sous vide broccoli sprout powder particles to be in a size range between 700nm and 1,770nm with a polydispersity value of 0.513. This indicates that there are two populations of sizes in the powder which is thought to be due to lack of a milling machine for grinding the powder to a homogenous size. The value for polydispersity above 0.5 indicates that that there is a broad/ wide range of sizes in our powder (Mirhosseini et al. 2008). For future endeavors a better milling method is recommended for better particle size and polydispersity results.

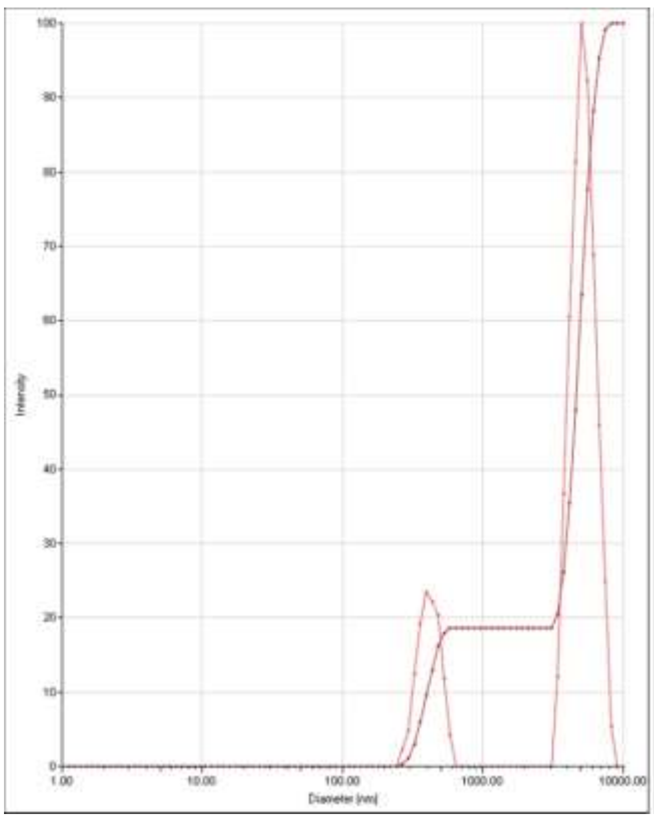


Figure 9 DLS- PALS sous vide broccoli sprout powder

The zeta potential was -40.99 which represents a powder- liquid system that indicated fairly good stability and has even distribution (Riddick et al., 1968). According to previous studies, values more electronegative than -30 mV generally represent sufficient mutual repulsion to result in stability, without agglomeration (Sarkar et al., 2016).

5.4 Dynamic Vapor Sorption- DVS

For the many food and powder products, stability is dependent on their behavior at different levels of humidity. Water sorption isotherms are therefore critical for proper development, packaging and storage conditions of these products. Water uptake can cause microbiological damage in food as well as ruin its organoleptic properties. In the case of broccoli sprout powder moisture, uptake of water can also degrade the bioactive compound and have an overall detrimental effect on the BSP.

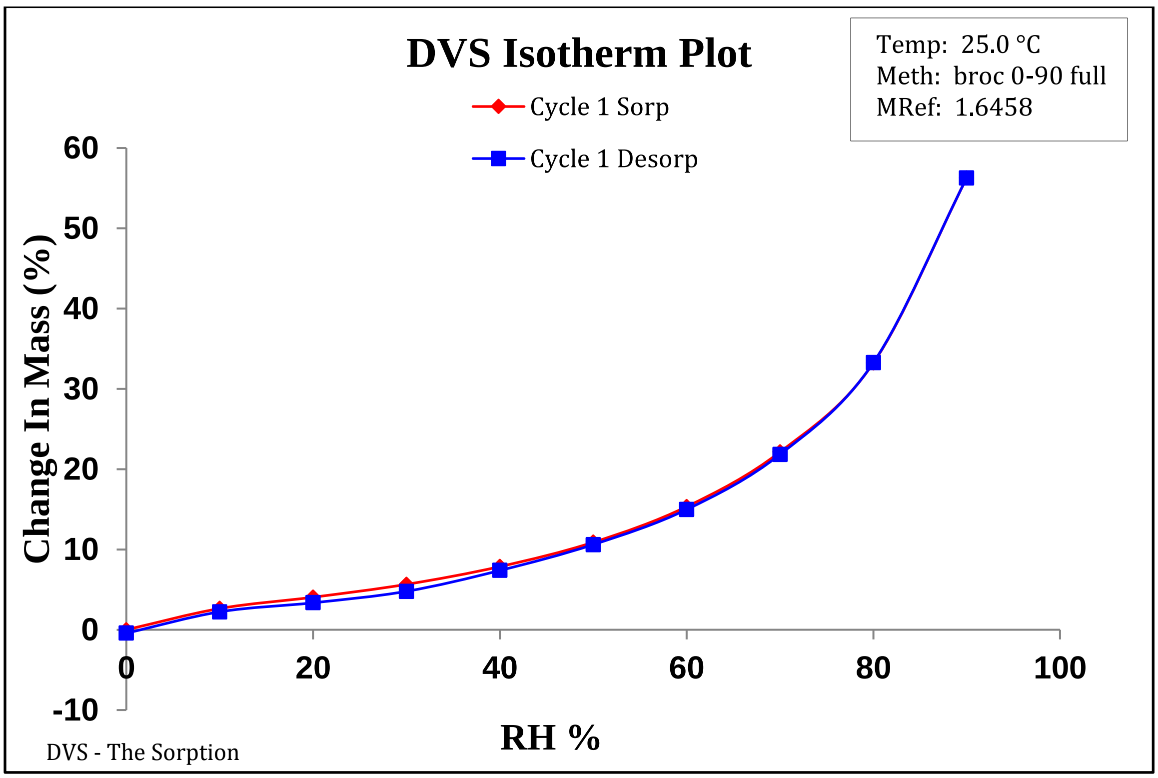


Figure 10 Sous Vide Broccoli Sprout Powder Isotherm at 25℃ constant Temperature

The sous vide broccoli sprout powder absorption and desorption isotherms are shown in Figure 10, based on the van der Waals adsorption of gases of solid substrates this graph clearly displays a type 3 adsorption isotherm plot (Brunauer et al., 1940; Masclaux et al., 2010). The first portion of the curve where the relative humidity (RH %) is less than 20%, is the region that represents strongly bound water. Between the 20 - 60% relative pressure is the region that represents the water molecules that are less firmly bound to the powder, also known as available water which can act as a solvent or take part in biochemical reactions (Muhtaseb et al., 2002)). Above the 60% relative pressure is the excess water which is commonly found in high moisture materials, which can act as a solvent as well but it is a major concern for microbial growth (Muhtaseb et al., 2002). This curve shape is commonly obtained for many hydrophilic materials and foods rich in soluble components (Masclaux et al., 2010; Gocho et al., 2000; Gouanve et al., 2006). The Brunauer-Emmett-Teller (BET) equation,

$$Mw = \frac{aw\ Mo\ C}{(1 - aw)(aw(C - 1) + 1)}$$

would be the most appropriate for the interpretation of a sigmoidal sorption isotherm as the one in Figure 10, where Mw is the amount of water and Mo is the monolayer content, and C is an energetic constant (estimated for each temperature) (Staudt et al., 2013). This curve is indicative of a strong attractive interaction between the powder and water and it resembles the expected shape of a dry starchy food (Muhtaseb et al., 2002). According to this equation the parameters for the DVS isotherm have been calculated as shown in Table 2.

Table 2. DVS Isotherm Parameters with BET model

Parameters	**Values**
Temp. °C	**25**
aw	**0.1**
Mo	**2.23**
C	**1.98**
Mw	**0.448**

To our knowledge this is the first report on DVS Isotherm values for broccoli sprout powder which is characteristic for a dry solid powder that did not undergo any polymorphisms in its structure (Muhtaseb et al., 2002). Thus, the information obtained from the DVS isotherm serves as an indication that the BSP must be stored in a completely sealed package to prevent water uptake.

5.5 Sensory acceptability of tomato juice with broccoli sprout powder

Previous work on of the development of a functional soy to tomato juice showed that rheology of the juice can be affected (Tiziani et al. 2005) likely impacting the sensory experience. However, the tomato juice with broccoli sprout powder flows similarly to the 100% tomato juice, because of the lack of the tomato pectin- soy protein interactions (Tiziani et al. 2005). To verify the instrumentation results, sensory analysis was conducted.

Additionally, since the sous vide method was used to treat the broccoli sprouts, there would not be a need for an external myrosinase source such as daikon radish, which has been used in previous products but received poor sensory scores due to excessive bitterness issue as stated by the sensory panel (Riddle et al. 2011).

Previous studies found that panelists detected an off metallic taste in the canned tomato juice product (Riddle et al. 2011). This is an issue with canned products and metallic packaging in general, which hinders the liking of the taste of food items like orange juice because of the detection of an odor of a tin can and metallic taste (Luckow et al., 2004). Another group of trained panelists described a functional carob canned juice as having a flat chemical feeling factor stimulated on tongue and teeth (Rababah et al.,2013). By having panelists taste canned tomato juice versus plastic bottled tomato juice it was possible to account for packaging effects on the acceptability of the product.

5.5.1 Hedonic scale Results

One-way ANOVA was employed for all sensory tests, with Tukey's post-hoc test, and all results were considered significant when $P<0.05$. Error bars represent 95% confidence interval. a-d Letters represent significant differences at α (0.05) level.

In Figure 11, the overall liking scores are seen to have a statistically significant difference between plastic bottled tomato juice and canned tomato juice with p-values < 0.05.

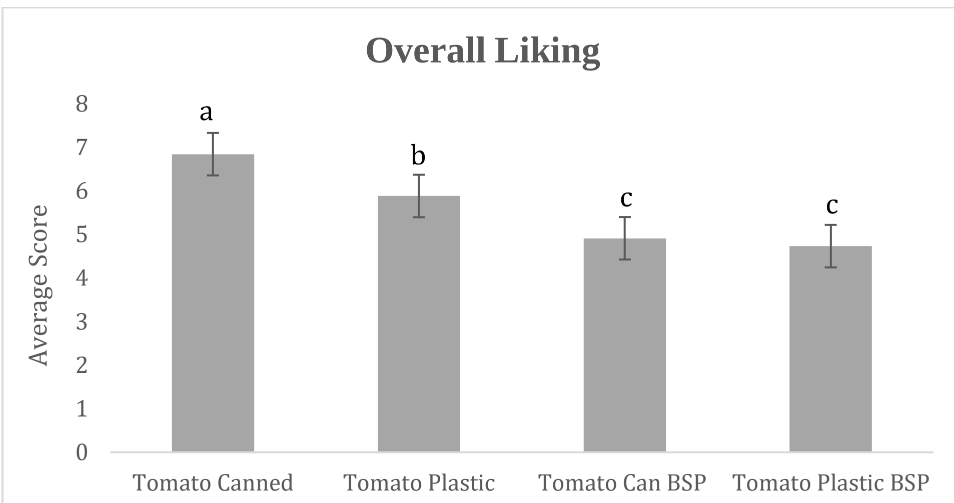


Figure 11 Hedonic Scale Score: Overall Liking

On average the results from the 75 panelists indicated that they seemed to prefer the canned tomato juice best. However, when tested with broccoli sprout powder added inside the juice samples, there was not a significant difference between canned tomato juice and plastic bottled tomato juice. When broccoli sprout powder was added overall liking of the juice decreased. Some panelists commented they did not like the green particles in the juice, but they would be more open to consume the product a a smoothie, not as a juice. On the other hand, some panelist commented they appreciated the extra healthy component in the juice and they did like the small green broccoli particles throughout the red juice. The variability in the comments and results is part of the nature of consumer surveys (Amerine et al., 2013).

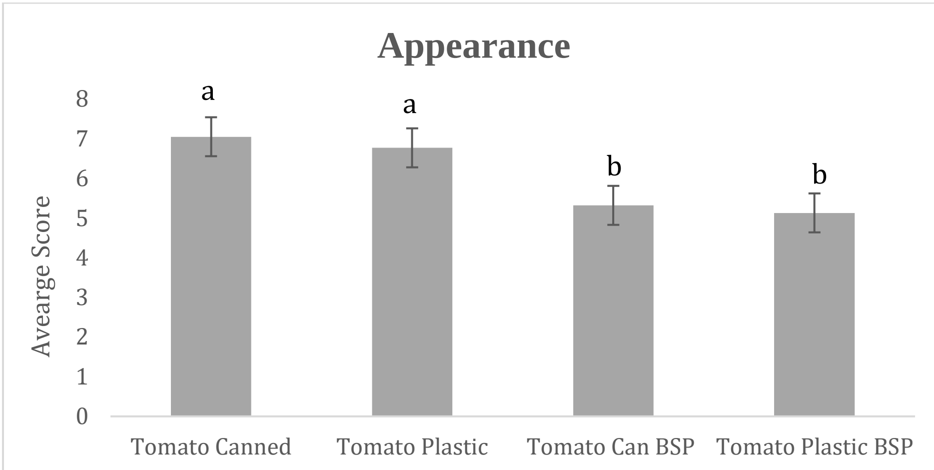


Figure 12 Hedonic Scale Score: Appearance

Appearance liking results of the BSP and TJ in plastic and canned bottles are shown in Figure 12. No difference was observed between the plastic bottled tomato juice and canned BSP tomato juice $p > 0.05$. Yet the appearance of canned and plastic bottled tomato juice was significantly higher than both juices with the broccoli sprout powder $p < 0.05$. Then it was observed that canned TJ with broccoli sprout powder was not significantly higher in appearance liking than the plastic bottled TJ with broccoli sprout powder. In the comment section some predominant comments stated that it reminded the consumer of marinara sauce or pizza sauce, more than a juice. Some comments liked the ‘spice’ added to the juice, while most said it was a bit off what they expect when they think of consuming tomato juice.

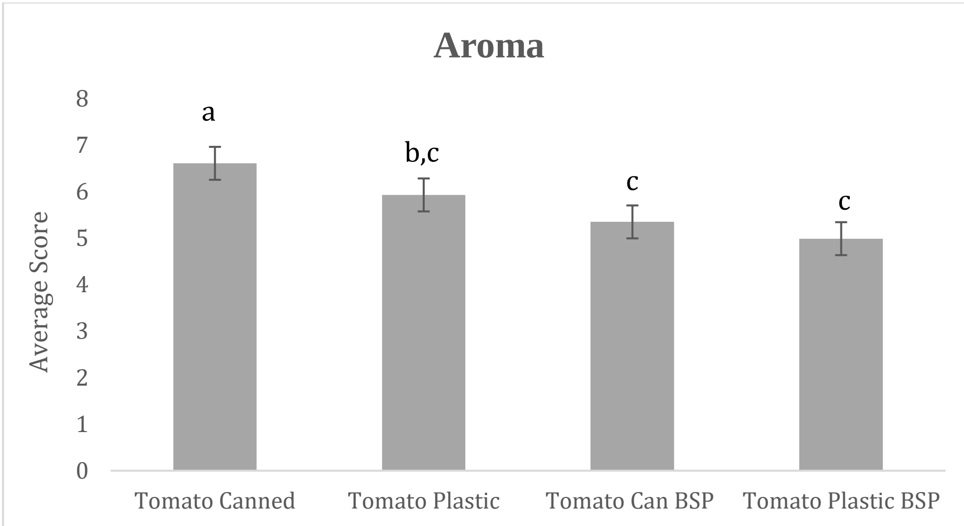


Figure 13 Hedonic Scale Score: Aroma

Subjects preferred the canned tomato juice aroma best as seen in Figure 13. Interestingly, there did not seem to be a significant difference between the plastic bottled tomato juice and both juices with added broccoli sprout powder. When asked to comment about the samples, some panelists stated the combination with the broccoli was a pleasant herb-like aroma, while others said they would prefer the original aroma characteristic of plain tomato juice.

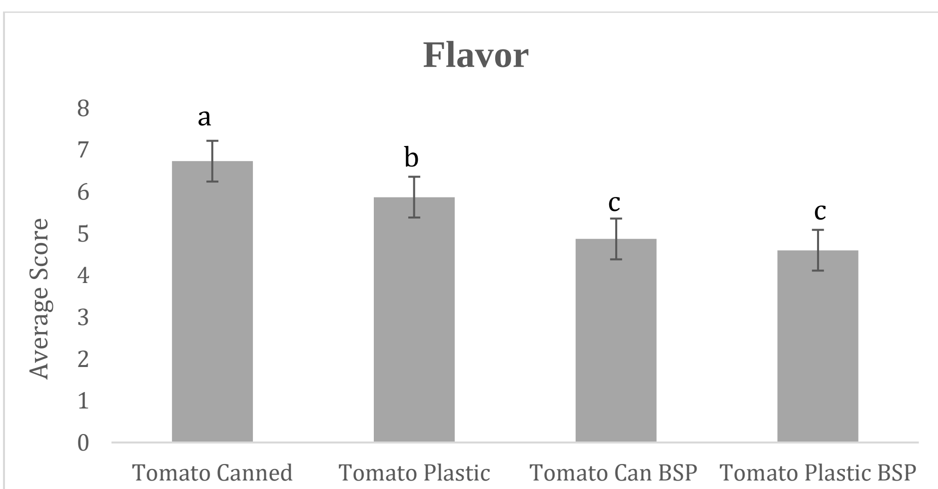


Figure 14 Hedonic Scale Score: Flavor

Flavor liking of each of the juice samples as shown in Figure14, displayed a similar pattern. Panelists scored their liking of the flavor of the canned tomato juice higher than the plastic bottled TJ. No difference was observed, with broccoli sprout powder added. This is very interesting because previous sensory panel results (Riddle et al., 2011), had demonstrated a dislike for the flavor of canned tomato juice and subjects complained about perceiving a metallic flavor. In this case, they consistently appear to like canned tomato juice over plastic bottled. As for the samples containing BSP, the predominant comment was that it had a resemblance to bloody Mary, enjoyed the veggie flavor and thought there was a good amount of broccoli added which did not over power the tomato juice flavor. But others commented they did not like the strong broccoli flavor they sensed and it reminded them of tomato soup more so than tomato juice.

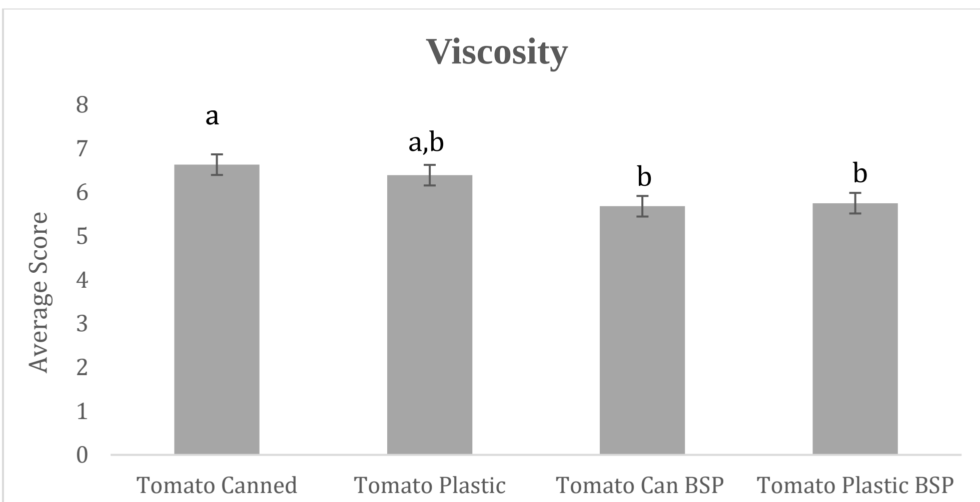


Figure 15 Hedonic Scale Score: Viscosity

When asked about the viscosity (flow) of the juices panelists preferred tomato canned juice better as observed in Figure 15, but these scores were not significantly different than the plastic bottled plain tomato juice. Additionally, there was not a significant difference in liking between the plastic bottled plain tomato juice, compared to both canned and plastic bottled with added broccoli sprout powder. There does not seem to be a significant difference perceived by the panelists in the flow of the plastic bottled and canned tomato juice with 1 gram of added broccoli sprout powder; although panelists consistently seem to prefer canned tomato juice over all other samples. These results agree with another study which found the addition of 1% of soy germ did not significantly affect the viscosity or flow of 100% tomato juice (Tiziani et al., 2005), since an addition of 1% soy germ is equivalent to 1 gram of BSP in a serving of 6 oz of tomato juice.

5.5.2 JAR Scale Results

Just About Right (JAR) evaluations were performed to assess what changes, if any, need to be made to the product to make it acceptable.

The panelists were asked to rate the juices' aroma, thickness, saltiness and viscosity using the JAR scores. JAR evaluation can give us further information on an attribute's intensity and if it needs to be increased or decreased to maximize consumer acceptability (Cadot et al., 2010). In this project, the JAR scale results are nonparametric (the data does not rely on numbers, but on ranking). Furthermore, for a penalty to be considered significant, if the penalty calculates was above a value of 1 more than 20% of the panelists had to have rated the attribute (too little or too much) and in this way, it was possible to identify attributes that are hindering product performance (Narayanan et al., 2014).

For the first sample, canned tomato juice, there were no significant penalties calculated (graph not shown). The penalty values were either less than one, or if the penalty score was above one there were not enough panelists that thought the attribute was not JAR (less than 20%). In all the categories canned tomato juice without broccoli sprout powder was the sample that scored highest on average on the hedonic scale, therefore we did not expect to see any parameters that deviated from a JAR score for aroma, thickness, saltiness and bitterness. This result could be attributed to panelists' comments that this is the sample perceived as the known 100% tomato juice they are used to drinking.

The next penalty analysis results are plotted in Figure 16, where 4 attributes seem to have negatively impacted consumer acceptability.

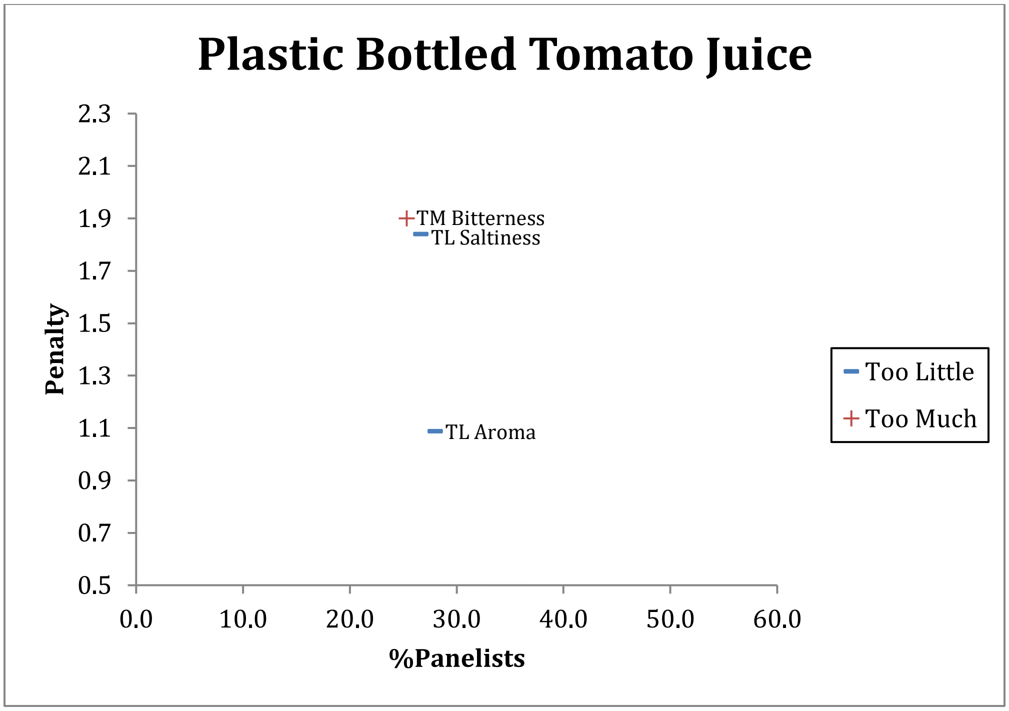


Figure 16 JAR Penalty Mean Drops for Plastic Bottle Tomato Juice

Results indicate that plastic bottle tomato juice led to tomato juice that was too bitter, not salty enough and lacked aroma. Such information is critical during the formulation of the tomato juice with broccoli sprout powder since the starting material (tomato juice) was not JAR in various attributes and therefore may influence final sensory results. Potential improvements to the current tomato juice include adding salt to enhance flavor and suppress the perceived bitterness (Breslin et al., 1995). The lack of salty taste was surprising since both packaged juices contained equal salt levels. However, packaging material has been shown previously to impact flavor attributes (Willige et al., 2006). The juices used for the sensory panel were purchased from the same manufacturer on the same day with equal amounts of calories, salt and pH. As future work, it would be of value to test what flavor compounds are observed in the canned tomato juice versus plastic bottled tomato juice related to product acceptability.

Penalty scores of canned tomato juice with broccoli sprout powder are plotted in Figure 17. The attributes that negatively impacted this sample were too little saltiness, too much aroma and too much bitterness. The results for bitterness are of concern, since for the plain canned tomato juice there was not a statistically significant penalty for bitterness indicating panelists detected this change once BSP was added. It is worth mentioning that a total of 13.3% of panelists had rated canned tomato juice (graph not shown) to have too much bitterness, which according to penalty analysis calculations anything below 20% is not important (Narayanan et al., 2014). The can tomato juice might introduce a source of bitterness detected by a minority, but the level of bitterness was exacerbated by the addition of BSP as seen in Figure 17. According to literature, this is characteristic of broccoli and brassica vegetables in general (Bongoni et al., 2014). It has also been found that people with a genetic sensitivity, to bitter taste have an enhanced effect when tasting broccoli, than the general population (Ly et al., 2001). While it is hard to account for genetic factors when doing a sensory panel, it is important to point out that people with the ability to taste 6-*n*-propylthiouracil (PROP) has been linked with lowered acceptance of some bitter foods (Ly et al., 2001). Another reason for the too much bitterness results could be because many phytochemicals including glucosinolates, phenols, and flavonoids are known to be bitter tasting which makes it a challenge to incorporate them in significant amount in food products despite having health benefits (Jones et al., 2006). In a different study, authors found that sulfur-containing compounds could be responsible for bitterness in cooked broccoli (Baik et al., 2003).

But further work needs to be done with a flavor-omics approach to be able to understand what are these compounds and is there a way of mitigating their bitterness without affecting ITC bioavailability.

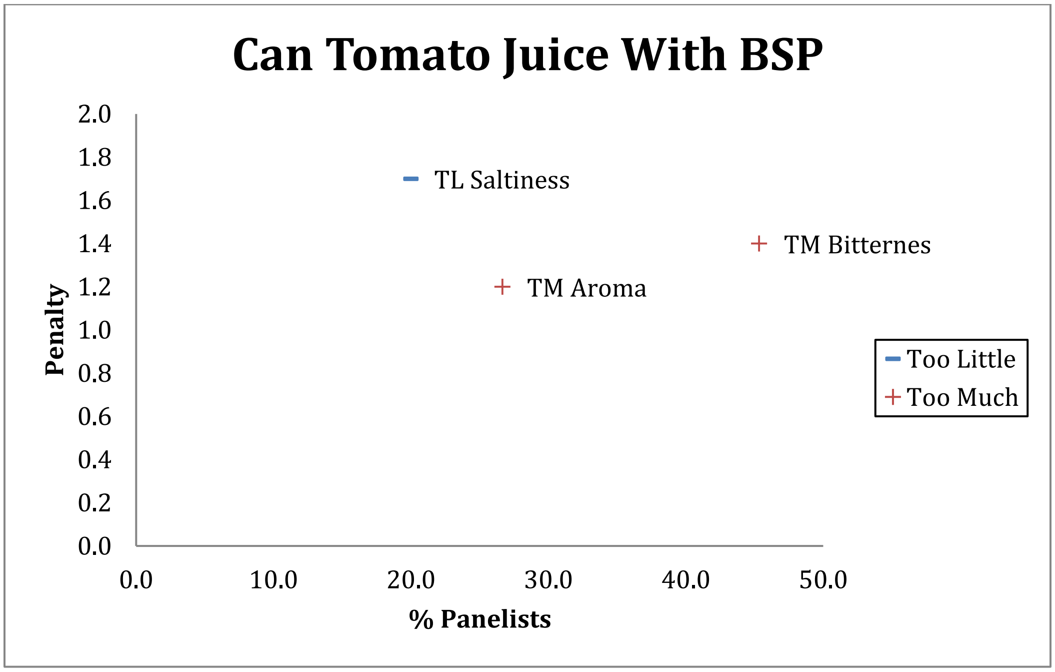


Figure 17 JAR Penalty Mean Drops for Can Tomato Juice with BSP

As for the high aroma value, there is a need to investigate which compounds might result from the mixture of tomato juice and BSP and find out how to mitigate the undesirable compounds from the formulation.

Results of the penalty analysis for plastic bottle tomato juice with BSP are plotted in figure 18. An excess of aroma and bitterness were detected by the panelists, with about 21% finding there was too much aroma and 44% finding too much bitterness in this sample. This sample received the highest penalty for bitterness amongst all and had the largest number of panelists rating it as having too much bitterness. This was expected since initially the plastic bottle tomato juice without BSP received a penalty of 1.9 for too much bitterness by 25% of panelists. And the panel was designed in this way to account for the disliking of the juice with and without BSP. In this way we have a clear suggestion as to which juice should use for future product development efforts.

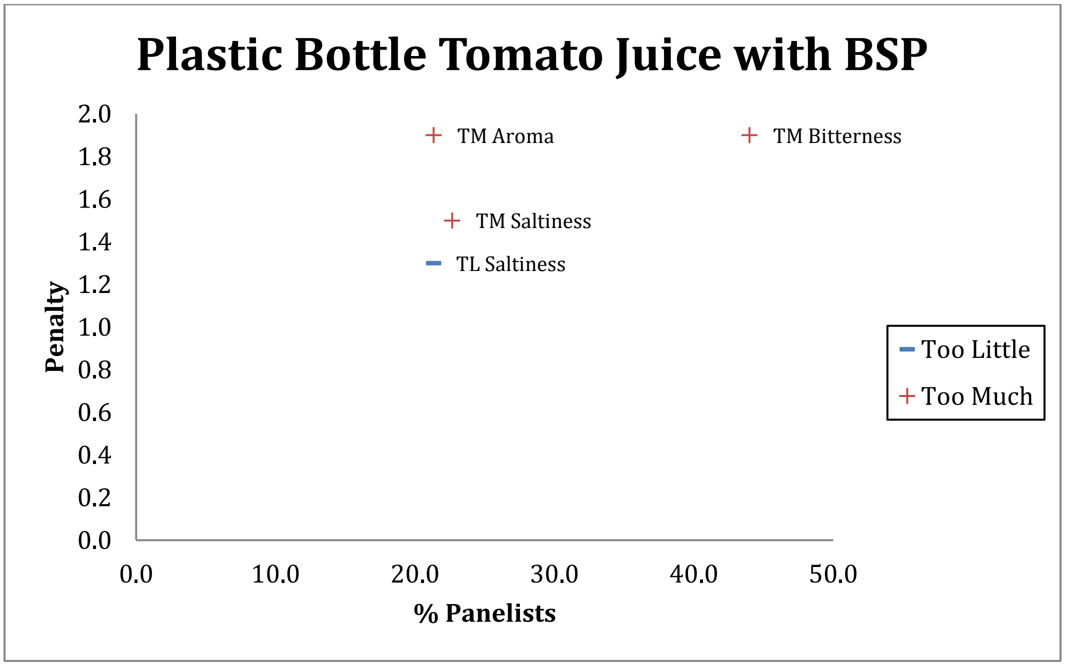


Figure 18 JAR Penalty Mean Drops for Plastic Bottle Tomato Juice with BSP

Of interest is that both too much saltiness and too little saltiness were factors in this sample. Taking into consideration the results from the plain plastic bottle tomato juice, (Figure 16) lack of saltiness may be more relevant especially in the context of saltiness affects bitterness perception (Breslin et al., 1995). Overall the canned tomato juice may prove the better option for future studies. Even though it seems to have some challenges in acceptability with the BSP, the best option from a product development standpoint is to modify and test different amounts of salt too observe if that helps raise the perceived saltiness and bitterness scores. In the comment section the predominant response for canned tomato juice was that it was perceived more as a full-bodied tomato juice than the plastic bottled tomato juice. The conclusion of this portion of the test is that the most commonly sold tomato juice is the canned version which has been studied and optimized for the last 64 years (Rice et al., 1954), therefore perceived as the standard for most tomato juice drinkers, as commented by some panelists.

5.5.3 Preference Test Results

For the last part of the sensory trial there was a preference test just between the two possible products that could continue to further development. Canned tomato juice with broccoli sprout powder was compared to the plastic bottled tomato juice with broccoli sprout powder and the panelists had to choose which they preferred. As observed in Figure 19, the results of a binomial probabilities tests showed the panelists preferred significantly better the canned tomato juice with broccoli sprout powder 65%.

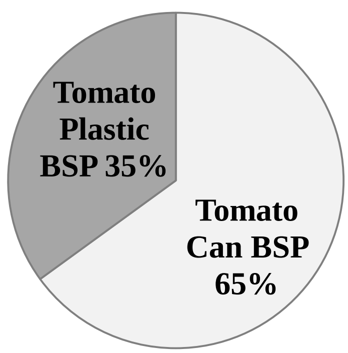


Figure 19 Preference Test Results Canned Juice with BSP vs. Plastic Bottled Juice with BSP

The results on Figure 19, from this preference test demonstrate statistically significant $p < 0.05$ evidence of which of canned tomato juice is the best accepted by these panelists. Future research goal for this product should move forward with canned tomato juice and find an ideal packaging for the broccoli sprout powder that could be made into a ready-to-drink type of product. According to this data, the most appropriate next step would be a non-blinded test to assess the acceptability of the panelists seeing the juice in a can versus a plastic bottled tomato juice. In past studies consumers complained about off metallic taste in canned tomato juice used in sensory panels (Riddle et al., 2012), which is why packaging was tested in the present work, but this did not seem to be an issue addressed by any of the panelists, even more so they consistently rated higher the canned tomato juice than the plastic bottled tomato juice.

5.6 Colorimeter

As an indicator of color stability, the L, a, and b values were recorded for 8 weeks for each of the BSP samples including raw, sous vide and steamed powders. The values reported correspond to the average of the triplicate color measurement taken on the corresponding week. For the raw broccoli sprout powder, the 'a' and 'b' parameters demonstrated that it has gone through a change, specifically in its green and yellow color respectively. Because the BSP powders were stored at 4℃ in as low humidity refrigerator this change it has undergone is a process commonly known as rotting (Pogson and Morris et al. 1997).

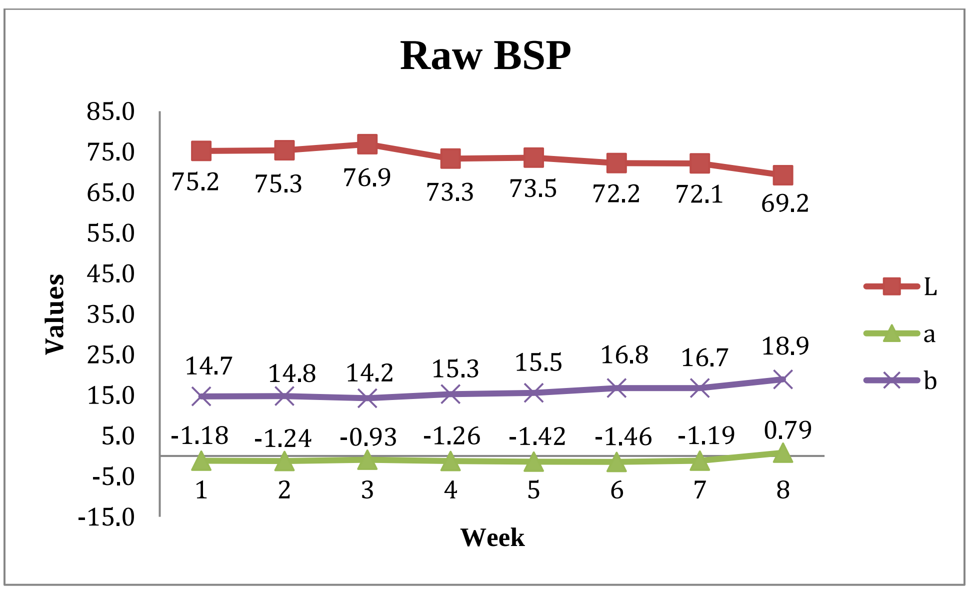


Figure 20 Raw Broccoli Sprout Powder 8-week Color Study

In Figure 20, it is easily observed there was a slow but steady change in all 3 parameters L, a, and b. For the parameter ‘L’, it is indicating a slow but steady decrease of its perceived darkness, from a value of 75.2 to 69.2. On week 8 it is thought that the powder might have been exposed to water, or contaminated since the values are much more different than the average values for all other samples and for the redness or greenness indicator ‘a’, where its positive value indicates that it is redder than green, which could be a potential indicator of spoilage. It has also been suggested that this difference could be due to endogenous ethylene (Hyodo et al., 1994). Therefore, a package that could minimize ethylene in the storage conditions of the BSP can be effective in extending the shelf life storage of the BSP. Broccoli is in general, known to go from a green to a yellow color, because of senescence; but according to the parameter ‘b’, the values always indicate a yellow color, until the last week when it is increased from 14.7 to 18.9, a significant augmentation in the observed yellow pigmentation. The average values for the colorimeter measurements for the raw BSP during this 8-week study were 73.5, -1.0, and 15.9 for L, a, and b respectively.

In the next Figure 21, the 8-week color measurements for the sous vide BSP are shown. While the numbers differ from that of the raw BSP, the trend is very similar.

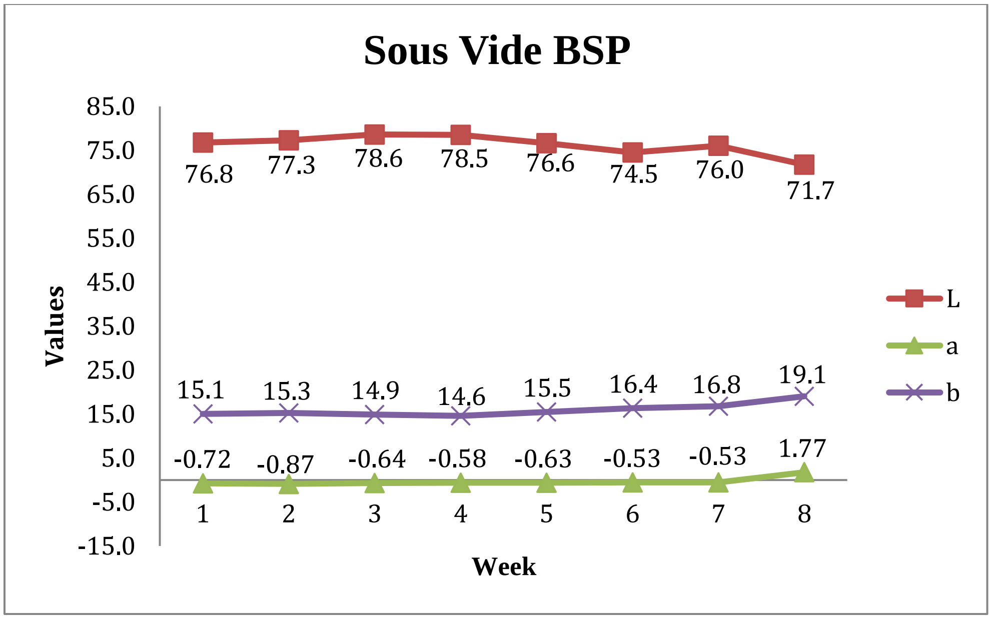


Figure 21 Sous Vide Broccoli Sprout Powder 8-week Colorimeter Measurements

For the first parameter 'L', indicator of dark- lightness, the values also seem to go in a decreasing pattern from a value of 76.8 to 71.7. The green to red parameter also seems to increase significantly observed in the 'a' values beginning at -0.72 to a value of 1.77 which is indicative of a red (not green) color; as reported by the raw BSP sample. The parameter indicating its yellowness was also significantly increased from a value of 15.1 to 19.1. This is also indicative of the putrefaction (Pogson and Morris et al. 1997) of the powder with 8 weeks of storage, in these storage conditions of 4℃ in the corresponding resealable plastic bag.

For the steamed BSP results on Figure 22, the same trends are observed for all three-color parameters. The decrease in the 'L' value from 75.2 to a value of 69.2 indicates a change in the perceived darkness which was reduced after 8 weeks.

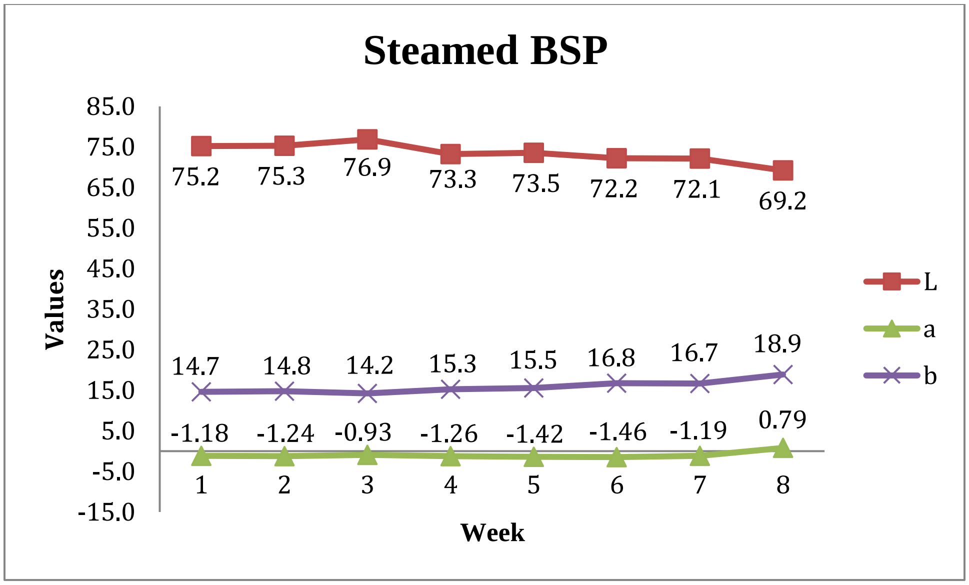


Figure 22 Steamed Broccoli Sprout Powder 8-week Colorimeter Measurements

For the redness/greenness indicator ‘a’ the values changed from -1.18 which is indicative of a green color to a positive value of 0.79 indicative of a red color. This is the same pattern observed for the raw and sous vide BSP as well. This could be indicating a need for microbiological future work to determine if there was any bacterial contamination that caused this redness to appear in the powders. For the parameter ‘b’, a significant increase in the yellowness was also observed from week 1 at 14.7 to week 8 at 18.9. These values are not significantly different than those observed from the raw and sous vide powders and exhibit the same patterns for all the color parameter indicators. Future work could be focused on microbiological spoilage due to contamination or growth of bacteria or mold in the powders or examine for chemical spoilage due to endogenous ethylene causing spoilage in the powders.

Chapter 6: Conclusion

As part of this product development project, we could synthesize a functional ITC- enriched tomato juice using only broccoli sprouts as the source of bioactive ITCs. The main goal was successfully achieved by using a sensory panel to determine if the public accepted the product with better acceptability than past attempts. We were also able to characterize some of the physicochemical parameters of the juice and study how the addition of the broccoli sprout powder affects the properties of the 100% tomato juice. Because of its potential health benefits for BC prevention, the combination of tomato juice with broccoli sprout powder was meant to be a target for this BC cohort, potentially to be used in future BC prevention clinical trials. With this goal in mind, our aim was to have a product high in bioactive ITC phytochemical content while retaining high organoleptic properties in order to have successful clinical adherence and for long term human consumption. As future work, packaging strategies should be attempted to be able to administer a ready to drink juice with a serving of the broccoli sprout powder to be added immediately before consumption. Upon completion of this work, canned tomato juice with the sous vide broccoli sprout powder seems to be like the ideal combination for future endeavors. Furthermore, the distribution, bioavailability, and metabolism kinetics of the ITC-rich tomato juice should also be further tested and understood.

References

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Appendix A: Approval Letters of IRB for the Sensory Evaluation Study

THE OHIO STATE UNIVERSITY

Office of Responsible Research Practices
300 Research Administration building
1960 Kenny Road
Columbus, OH 43210-1063
Phone (614) 688-8457
Fax (614) 688-0366
orrp.osu.edu

01/22/2018

Study Number: 2018E0038

Study Title: Sensory Evaluation of a Tomato Juice with added Broccoli Sprout Powder Copy
Principal investigator: Yael Vodovotz
Date of determination: 01/22/2018
Qualifying exempt category: #6

Dear Yael Vodovotz,
The Office of Responsible Research Practices has determined the above referenced project exempt from IRB review.

Please note the following about this determination:

- Retain a copy of this correspondence for your records.
- Only the Ohio State staff and students named on the application are approved as Ohio State investigators and/or key personnel for this study.
- Simple changes to personnel that do not require changes to materials can be submitted for review and approval through Buck- IRB.
- No other changes may be made to exempt research (e.g., to recruitment procedures, advertisements, instruments, protocol, etc.). If changes are needed, a new application for exemption must be submitted for review and approval prior to implementing the changes.
- Records relating to the research (including signed consent forms) must be retained and available for audit for at least 5 years after the study is closed. For more information, see university policies, Institutional Data and Research Data.
- It is the responsibility of the investigators to promptly report events that may represent unanticipated problems involving risks to subjects or others.

This determination is issued under The Ohio State University's OHRP Federalwide Assurance #00006378. Human research protection program policies, procedures, and guidance can be found on the ORRP website.
Please feel free to contact the Office of Responsible Research Practices with any questions or concerns.
Jacob Stoddard
stoddard.13@osu.edu
(614) 292-0526

Appendix B: Ballot for the Sensory Evaluation

Individual samples will be presented, please evaluate each one at a time and answer all questions for each sample before proceeding on to the next. Please be sure that the sample number corresponds to the sample number on the evaluation sheets. Tests for this section of the study each will include:

Tasting section (section I)

1. Acceptability tests: ask for your level of liking of the tomato-based juices. You will rate overall liking, appearance, aroma, flavor and viscosity.

2. Just-About Right is an important test to measure how we can make the product better for commercialization. We would like you to test the expected aroma, thickness, flavor (saltiness/bitterness).

3. As part of the evaluation for the juice mostly preferred by panelist based only on taste. The goal of this test is to know which of the two juices you prefer.

*Please rinse your mouth 3 times with water before starting a new sample and have a bite of the provided cracker.

*Please taste the samples carefully and according to sample number on each page. You may not go back and re-taste the samples once you completed all the questions for that sample.

Section I - Acceptability Test

Check the box that best describes your opinion of juice _______ Subject ID:

Please rate your **OVERALL LIKING**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike Like	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Please rate the **APPEARANCE**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike Like	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Please rate the **AROMA (SMELL)**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike Like	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Please rate the **FLAVOR**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike Like	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Please rate the **VISCOSITY (FLOW)**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike Like	Like Slightly	Like Moderately	Like Very Much	Like Extremely

Just About Right Test

Tomato and Tomato Broccoli Juice Trial Subject ID:

CHECK THE CORRESPONDING BOX THAT BEST DESCRIBES YOUR IMPRESSION OF JUICE SAMPLE # ___

AROMA

Much Too Little	Too Little	Just About Right	Too Much	Much Too Much

THICKNESS

Much Too Little	Too Little	Just About Right	Too Much	Much Too Much

FLAVOR

Saltiness

Much Too Little	Too Little	Just About Right	Too Much	Much Too Much

Bitterness

Much Too Little	Too Little	Just About Right	Too Much	Much Too Much

From the characteristic mentioned above which contributed to your acceptance of the juices?

____

From the characteristic mentioned above which contributed to your dislike of the juices?

____

If the juice was marketed as having health benefits and potential cancer preventative effects, would that contribute to your acceptance of the juices?

____

Preference Test

Tomato Broccoli Juice **Subject ID:**

Taste the two coded samples in the following order: ________ ________

Which of these two samples do you prefer? ________

Would you be willing to drink a 6 oz of tomato juice with the same ratio of juice to broccoli sprout powder once a day? If so, would you be willing to drink once a day for 30 days?

____

Additional Comments:

Appendix C: Consent form for Sensory Trial

The Ohio State University Consent to Participate in Research

Study Title:	**Sensory Evaluation of Tomato Juice with Broccoli Sprout Powder**
Researcher:	**Yael Vodovotz, PhD**
Sponsor:	**None**

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate.

Your participation is voluntary.

Please consider the information carefully. Feel free to ask questions before making your decision whether or not to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form.

Purpose: The objective of this study is to evaluate the consumer acceptance of two 100% tomato juice samples with and without broccoli sprout powder to determine their palatability and acceptability among tomato juice drinkers. The aim is studying consumer acceptance of these juices is that the juice's appearance, flavor and viscosity the characteristics that influence consumers' acceptability and willingness to consume.

Procedures/Tasks: You will be asked to drink the various juice samples. After drinking each sample provided for each test you will complete a series of three of tests. The three tests are as follows:

1. Acceptability tests are used to obtain the likability or palatability of 4 tomato juice samples. The participant will be blinded to which juice is from which package, 1 will be from a metal can the other from a plastic bottle, participants will be asked to taste with and without the broccoli sprout powder, for a total of 4 samples. A 9-point Hedonic scale will be used to describe overall acceptability, appearance, aroma, flavor and viscosity (1-dislike extremely, 9-like extremely) of each.
2. A 5-point horizontal Just About Right scale (JAR) is used to optimize the formulation of the juice for enhanced customer acceptability. The attributes examined are as follows: aroma, thickness (viscosity) and flavor (saltiness and bitterness) (1-Much too little, 5-Much too much).
3. A preference test is used to see which of the two juices consumers prefer. The participant will be served 2 juices, one from the can source the other from the plastic bottle both with broccoli sprout powder incorporated. Comparing the juices using a

horizontal line scale ranging from 1 to 10 (1 being the lowest and 10 being the highest intensity).

Duration: The study will last approximately 20-25 minutes.

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

Risks and Benefits: The risks of this study are extremely low unless you are allergic or intolerant to tomato, tomato juice, citric acid, broccoli, crucifers, as well as wheat or gluten-free crackers. All of the products prepared for human consumption in this project contain wholesome food ingredients including freeze-dried broccoli sprout powder in 100% tomato juice. The broccoli sprouts to be used in this study are certified GRAS by the FDA at the levels added to the juices. No chemical/environmental contaminant will be added in these products. Juices will be provided in 3 oz servings with 0.5 grams of broccoli sprout powder and will not present any risk to recruited healthy subjects. This study will be conducted in a reasonable eating behavior by the subjects.

Confidentiality:

All information will be stored in a secure computerized database. At the onset of the experimental session, you will be asked to provide general demographic information including age, gender and ethnicity. In some cases additional information regarding eating and dietary habits may be obtained. These data will be collected using secured computerized data acquisition software.

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor, if any, or agency (including the Food and Drug Administration for FDA-regulated research) supporting the study.

Incentives: When you complete the tests you will receive a $10. If the questionnaires are not complete because you decided to discontinue the study you will still receive the $10.

Participant Rights:

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

This study has been determined Exempt from IRB Review.

For questions, concerns, or complaints about the study you may contact Carla J. Rodriguez, the study coordinator, at rodriguez.766@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research tea m, you m ay contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251 or by e-mail at hsconcerns@osu.edu.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Yael Vodovotz at vodovotz.1@osu.edu.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form**.**

Printed name of subject	**Signature of subject**
	Date and time AM/PM
Printed name of person authorized to consent for subject (when applicable)	**Signature of person authorized to consent for subject (when applicable)**
Relationship to the subject	**Date and time** AM/PM

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent	**Signature of person obtaining consent**
	Date and time AM/PM