IMPACT OF SODIUM CHLORIDE ON LIKING OF CRUCIFEROUS VEGETABLES

THESIS

Presented in Partial Fulfillment of the Requirements for the degree of Master of Science
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By
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ABSTRACT

In developed countries, a universal recommendation to improve overall nutritional status is greater consumption of vegetables. Bitterness of nutritionally beneficial cruciferous vegetables is a major reason for rejection. While sodium chloride can suppress bitterness, nutritional recommendations caution against over-consumption of salt. This study determined the minimum amount of salt required to increase palatability and suppress bitterness for three distinct age groups. “College-aged panelists” (Chapter 2) and “Older panelists” (Chapter 3) rated overall liking, saltiness, and bitterness of broccoli samples with varying levels of salt. They also indicated their perceptual ideals of saltiness and bitterness for broccoli. “Younger panelists” (Chapter 4) were asked to rank their preference for broccoli samples with varying levels of salt. The results indicate that liking of cruciferous vegetables for all panels peaks somewhere between 150 and 450 mg of salt per serving. Highest overall liking, with adequate saltiness and suppressed bitterness, occurs around 350 mg.
Dedicated to my supportive parents, Jim and Dawn Balitsis,
and my encouraging fiancé Aaron Koop.
ACKNOWLEDGMENTS

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Major Field: Food Science and Nutrition
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CHAPTER 1

INTRODUCTION

Vegetables from the genus *Brassica* have widely been regarded as possessing cancer preventive effects (Beecher, 1994). Broccoli, Brussels sprouts, and cabbage (Table 1) are members of the genus *Brassica* and are commonly known as cruciferous vegetables (Murillo and Mehta, 2001).

<table>
<thead>
<tr>
<th>Common Members of the Cruciferous Vegetable Group (genus <em>Brassica</em>)</th>
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<tr>
<td>Arugula</td>
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<td>Beet greens</td>
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<td>Broccoli</td>
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<td>Daikon</td>
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<td>Garden cress</td>
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Epidemiological studies have consistently shown that regular consumption of fruits and vegetables, including cruciferous vegetables, is strongly associated with reduced risk of developing chronic diseases such as cancer, cardiovascular disease, hypertension, and stroke (Duyn and Pivan, 2000). These findings are attributed to
phytochemicals, which are bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic disease (American Institute for Cancer Research, 1996). Such epidemiological studies have also suggested that the additive and synergistic effects of complex mixtures of phytochemicals found in whole fruits and vegetables are responsible for potent antioxidant and anticancer activities (Liu, 2004). Cruciferous vegetables contain many phytochemicals which contribute to human health including the following: dietary fiber, vitamins, minerals, terpenes, carotenoids, flavonoids, glucosinolates, isothiocyanates, phenols, tannins, anthoxanthins, and dithiolthiones (Duyn and Pivonka, 2000).

In the United States as well as many other countries, a common recommendation to the public is to increase vegetable consumption in order to reduce disease risks (Duyn and Pivonka, 2000). Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection (Dinehart, et al., 2006). The bitterness of *Brassica* vegetables was found to highly correlate (0.90) to the content of the phytochemical glucosinolate (Rouseff, 1990). Ironically, the same vegetables that are the highest in phytonutrients (and thus the most beneficial to health) are often the vegetables that are highest in bitterness (Moreno, et al., 2006). Thus, there is incentive to decrease the bitterness of these vegetables, in turn increasing their palatability and hopefully their consumption.

Bellisle, et al. (2000) investigated the influence of palatability on eating behavior. 564 participants maintained food diaries for 7 days in which they recorded their intake along with a rating of the palatability of each individual item eaten and of the entire meal on a 7-point scale. Bellisle et al. (2000) found that most meals that are self-selected are palatable and that only 9.3% are rated as unpalatable. Meals that were highest in
palatability were 44% larger than meals that were low in palatability. In addition, meals with the highest palatability rating are on the average 44.2% larger than the average meals with the lowest ratings (Bellisle, et al., 2000). These findings suggest that one strategy for increasing vegetable consumption, especially the consumption of the highly nutritious bitter-tasting vegetables such as broccoli, is to increase their palatability.

Sodium chloride has been demonstrated to be an effective bitter suppressant (Keast, et al., 2004). However, high sodium intake has been shown to sometimes negatively impact health. Although it is difficult to determine the actual impact of salt consumption due to the influence of other confounding variables like socioeconomic status, obesity, level of physical exercise, genetics, and intake of alcohol, fruits, vegetables, or dairy products (Taubes, 1998), there is evidence that excessive salt intake increases blood pressure and leads to the resulting risk of hypertension (Vollmer, et al., 2001). Other studies have also linked high sodium intake to other adverse health effects like heart disease, stroke, and kidney disease due to the additional retention of sodium and water necessary for the kidney to excrete excessive amounts of salt (Evilly, 2002).

Vollmer et al. (2001) examined the effects of “Dietary Approaches to Stop Hypertension” (the DASH diet) on blood pressure. The DASH diet, which is promoted by the National Heart, Lung, and Blood Institute, limits sodium intake and cholesterol, is high in fiber, potassium, calcium, and magnesium, and is moderately high in protein (Liebman, 1997). Vollmer et al (2001) concluded that the DASH diet (with its reduced sodium intake) produced mean blood pressure reductions of 6.6 mm Hg for systolic blood pressure (SBP) and 3.2 mm Hg for diastolic blood pressure (DBP). A similar study showed that the DASH diet, exercise, and weight loss had even greater effects on blood
pressure with mean reductions of 9.5 mm Hg for SBP and 5.3 mm Hg for DBP in overweight persons with medication-treated hypertension (Miller, et al., 2002). Since these studies both increased vegetable intake and reduced salt intake, it is difficult to determine which factor was more important in the reduction of high blood pressure.

Despite the common recommendation to restrict sodium intake (Mahan and Escott-Stump, 2004, US Department of Health and Human Services, 2005), both sodium and chloride (the components of salt) are necessary for the survival of humans. Sodium, one of the primary electrolytes in the body, is involved in regulating the water content of the body and maintaining the electrolyte balance inside and outside of cells (Gibson, et al., 2000, Laszlo, 2001). Salt has been used for thousands of years in practically every culture of the world not only to enhance the taste of foods, but to support life. In 640 B.C., Romans even used salt as a form of payment for soldiers, which was the origin of the word salary (Kurlansky, 2002). In modern settled civilizations people have come to take salt for granted because it is so plentiful. However, it is no coincidence that archaeologists have pinpointed the locations of the first human settlements to be along seashores, where extraction of the sea’s salt can be accomplished by simple evaporation in the sun (Laszlo, 2001). Inland migrations came later as a result of coastal dwellers specializing in the production of salt to be sent along trails to those living inland (Laszlo, 2001). Salt is so important to life that contrary to today’s lifestyle, early civilizations planned their lives around maintaining an adequate supply of this mysterious chemical.

While the blood pressure of some individuals does fall with a reduction in salt intake, the blood pressure of others do not, suggesting that individuals vary in their salt sensitivity (Obarzanek, et al., 2003). In addition, several genetic loci that play a role in
salt absorption and blood pressure regulation have been identified (Evilly, 2002), which suggests heritability does play an important role in the effect of salt on blood pressure regulation. It has been estimated that about 30-50% of hypertensives and 15-25% of normotensives are salt sensitive (Mahan and Escott-Stump, 2004). The importance of dietary salt restrictions thus depends upon several factors, and individuals should consider other risk factors for salt-sensitive hypertension, such as race (e.g., Blacks are more prone to hypertension than Caucasians), obesity, advanced age, diabetes, renal dysfunction, and use of the drug cyclosporine (Mahan and Escott-Stump, 2004), before resorting to strictly restricting salt intake. Despite this, many food companies have attempted to reduce sodium concentrations in their products or in low-sodium versions of their products for many years because of sodium’s perceived threat (Clifcorn, 1953). Currently, ingredient developers and food manufacturers continue to create reformulated food products in response to health concerns and consumer requests for more low-sodium food choices (Nachay, 2008).

Not only does salt often enhance the flavor of foods, salt also has many other sensory and processing effects on foods. Salt affects the water-holding capacity, fat-binding, texture, stability, and shelf life of foods (Gibson, et al., 2000). Accordingly, the removal of sodium leads to many processing and product development challenges. Phelps et al. (2006) have discussed several potential approaches to reducing salt in food products including stepwise reduction (assuming that consumers will adapt to a less salty taste if changes are made incrementally over time), use of salt replacers, use of salt enhancers,
and modification of the physical form of salt. They showed that to maintain high palatability while reducing sodium requires different approaches for different food types (Phelps, et al., 2006).

Specifically, Phelps et al. (2006) found that although modifying the crystal size and shape of salt can increase the perception of salty taste of a given concentration (due to enhancement of specific surface area and rapid dissolution rate), such alterations can support only modest sodium decreases if consumer acceptance is to be maintained. Similarly, despite the fact that potassium chloride is the most common salt substitute, Desmond (2006) found that blends which contain more potassium than a 50/50 sodium chloride/potassium chloride blend result in a significant increase in bitterness and a significant loss of observed saltiness. Other flavor enhancing and masking compounds also can be utilized to improve food products and limit sodium content, such as monosodium glutamate, nucleotides, amino acids, lactates, and yeast extracts. These flavor enhancers have been shown to successfully deliver up to 50% reductions in salt without producing unacceptable off flavors. Unfortunately, Phelps et al. (2006) found that salt replacers and enhancers can not deliver the clean salty taste of sodium chloride, especially when used in high concentrations. Frequently, bitter and sour notes result when sodium levels are reduced and replacers and enhancers are used. The loss of consumer acceptance that occurs when sodium levels are lowered despite the use of the aforementioned strategies indicates just how important sodium chloride is to food palatability. At least for certain foods, salt is one of the primary determinants of acceptability.
Adams, et al. (1995) assessed the magnitude of sodium reduction that could be made without significantly changing the perception of saltiness and acceptability of many common food entrees. 190 subjects rated the perceived saltiness and overall acceptability of “regular” and “low-sodium” entrees. For six of the eight entrees tested (meatballs, beef stew, pork with barbeque sauce, tuna with noodles, spaghetti with meat sauce, and chicken with rice), consumers perceived a significant difference in saltiness between the low-sodium and regular food entrees. However, for two of them, chicken stew and chicken a la king, no significant difference was found, despite the fact that all low sodium entrees contained approximately the same levels of sodium. These findings suggest that the concentration of sodium alone does not determine the perception of saltiness in a complex food system. Instead, sodium interacts with other sensory components such as spices, fats, and perhaps even the physical structure of the food.

Furthermore, acceptance, like perceived intensity of saltiness, was not solely related to salt concentrations. Four of the eight entrees (chicken stew, chicken a la king, spaghetti with meat sauce, and chicken with rice) reduced sodium by 52-89% and were not found to be significantly different in acceptability from the regular entrees. However, four others (meatballs, beef stew, pork with barbeque sauce, and tuna with noodles) were reduced in sodium by similar amounts (60-91%) and were significantly lower in acceptability than the regular entrees. This suggests perception of salt intensity and acceptability are product specific and thus salt reductions need to be adjusted individually by food processors and preparers to ensure commercial success (Adams, et al., 1995).

In a second experiment, Adams, et al. (1995) had 380 subjects rate both the perception of saltiness and the acceptability of a variety of prepared foods and
Panelists rated food items that were systematically reduced in sodium content. Commercially prepared foods (breads, snacks, and vegetable juices) were used to achieve these ratings. The levels of sodium for these foods ranged from 0.01-0.60%. The perception of saltiness increased as the concentration of sodium increased across each of the commercially prepared food types. The lowest sodium level for both vegetable and tomato juice (0.03 and 0.01 respectively) were found to be significantly less accepted than the other three sodium concentrations of these juices. However, no significant differences in acceptance were found over concentration levels for the bread (whole wheat and oatmeal) and snack food items (potato and tortilla chips) (Adams, et al., 1995).

Prepared foods (meatloaf, creole sauce, meat loaf with creole sauce, and mashed potatoes) were used to compare perceptions of both simple and complex recipes. The levels of sodium for these foods ranged from 0.03-0.63%. The perception of saltiness increased as the concentration of sodium increased for all food items except for meatloaf. Acceptability was highest for mashed potatoes at 0.14% sodium, for meatloaf at 0.31% sodium, for creole sauce at 0.60% sodium, and for meatloaf and creole sauce at 0.60% sodium. However, meatloaf and meatloaf with creole sauce were equally acceptable at all concentrations. Subjects rated the highest sodium concentration for creole sauce and the second lowest sodium concentration for mashed potatoes most acceptable due to the differences in compositions and complexities. The liking of the different foods was not consistently for low or high salt formulations across different foods. Furthermore, the perceived intensity of saltiness at a given concentration of sodium differs according to the
Finally, the more simple the food item (fewer ingredients), the greater the perceived saltiness (Adams, et al., 1995).

Salt’s important role in food preservation, improving food taste, and balance of water in cells may be well understood now, but this chemical’s complex interactions with other chemicals and the source of its bitter-suppressive ability is still largely unknown. Paradoxically, salt enhances some flavors in foods while suppressing others (Breslin and Beauchamp, 1997, Keast, et al., 2001). To better understand this inconsistency of action, researchers tested the hypothesis that salts selectively filter flavors by suppressing unpleasant tastes like bitterness and increasing more palatable tastes like sweetness. Breslin and Beauchamp (1997) had 21 participants assess aqueous mixtures of urea (bitter), sucrose (sweet), and sodium acetate (a salt selected for its mild taste and decreased potential of causing rating interference due to high saltiness). Participants rated the bitterness, sweetness, and ‘otherness’ of all possible combinations of three concentrations of urea (0.0, 0.5, 1.0 M), four of sucrose (0.0, 0.1, 0.3, 0.5 M) and three of sodium acetate (0.0, 0.1, 0.3 M) using the method of magnitude estimation. There was indeed a selective suppression of the taste components by sodium acetate, with the bitterness of urea being suppressed much more than the sweetness of sucrose. The sucrose–urea mixtures with added salt were relatively less bitter and more sweet than when sodium acetate was not added. Since the addition of sodium acetate to sucrose in the absence of urea did not enhance sweetness, it appears the sweet enhancement is due to bitter suppression (which prevents the bitterness of urea from suppressing the
sweetness of the sucrose). These results imply that in order to develop a salt substitute for bitter foods, attention must be paid to replacing sodium chloride’s dual role as both flavor enhancer and bitterness inhibitor.

Kemp and Beauchamp (1994) examined the flavor modifying effects of sodium chloride. Fifteen subjects assessed the effects of varying concentrations of sodium chloride upon the modification of taste, flavor, and flavor with a somatosensory (i.e., mouthfeel) component. Participants rated the effects of sodium chloride on intensity of taste compounds (sucrose, citric acid, quinine sulfate, and sodium chloride), and the flavor and odor intensity of different flavor substances (imitation butter flavor, celery extract, lemon extract, mint extract, imitation pistachio flavor, menthol, and citral). Sodium chloride increased or decreased perceived intensities, depending upon the stimulus assessed (Kemp and Beauchamp, 1994). Sodium chloride significantly decreased the perceived intensities for sweetness, bitterness, and sourness of the compounds which corresponds to each basic taste. High concentrations of sodium chloride significantly lowered citral, lemon, menthol, and mint flavors, but not the other flavors (Kemp and Beauchamp, 1994).

The food industry is increasingly pressured by consumers and health authorities to provide healthier foods with optimal palatability. Often times improving health benefits requires adding bitter compounds such as antioxidants, flavonoids, and calcium, and phytochemicals like glucosinolates. Breslin and Beauchamp (1995) conducted a series of twelve studies to gain more insight into the complex relationship that salts and bitter compounds play in the perception of overall bitterness. Depending upon the panel, 12-27 subjects rated the perceived intensity of the bitterness and saltiness of all possible
combinations of multiple concentration levels of bitter compounds (quinine hydrochloride, caffeine, magnesium sulfate, amiloride, potassium chloride, and urea) with multiple concentration levels of salts (sodium chloride, sodium acetate, sodium gluconate, potassium chloride, lithium chloride, and L-arginine-L-aspartame) using magnitude estimation. They found that all bitter compounds were suppressed by sodium chloride at some combination of concentrations. However, the extent of suppression differed for each bitter compound, despite the fact that they were matched for intensity. In most mixtures, saltiness was affected less than bitterness, i.e. sodium salts suppressed bitterness of the bitter compounds more than the bitter compounds suppressed the saltiness of the sodium salts.

Since urea was the bitter compound most effectively suppressed by sodium chloride, it was used to determine the bitter suppressing roles of salt anions and cations, i.e., whether suppression of the bitter taste is attributed to the sodium ion, the chloride ion, or both (Breslin and Beauchamp, 1995). Sodium chloride, sodium acetate, and sodium gluconate were used for anion manipulation, while potassium chloride, lithium chloride, and arginine-aspartame were used for cation manipulation. All three sodium salts and lithium chloride had a suppressive effect on the bitterness of urea, and the bitterness suppression was independent of perceived saltiness. However, potassium chloride and arginine-aspartame increased the bitterness of the solution when mixed with urea. Thus, only sodium and lithium ions appear to be effective in suppressing bitterness, and the authors suggest that their suppressive effects act at the periphery, as opposed to their taste properties acting centrally (Breslin and Beauchamp, 1995). The distinction between the two mechanisms is discussed further below.
Keast and Breslin (2002) further examined bitterness suppression by sodium chloride and sodium gluconate of aqueous solutions of L-tryptophan, L-phenylalanine, urea, quinine HCl, and magnesium sulfate of matched bitterness intensity. Thirteen subjects rated bitter-salt solutions for sweetness, sourness, saltiness, bitterness, and savoriness using the Labeled Magnitude Scale. Once again, the sodium salts were shown to significantly reduce bitterness and the amount of suppression varied for the different bitter compounds. Sodium chloride and sodium gluconate decreased the bitterness of the solutions similarly, with both significantly suppressing the bitterness of tryptophan, phenylalanine, and quinine, and neither significantly suppressing the bitterness of magnesium sulfate. However, sodium chloride significantly inhibited the bitterness of urea, while sodium gluconate only suppressed urea marginally.

There are two main theories to explain the taste suppression in mixtures containing qualitatively different tastes. The first is via activity at the periphery, and assumes suppression takes place at the receptor level. The second is via a central mechanism, somewhere in the processing of taste. Kroeze and Bartoshuk (1985) utilized split-tongue taste stimulation to investigate this question. Ten subjects used magnitude estimation to rate the perceived bitterness intensity of quinine hydrochloride, sucrose, sodium chloride, and deionized water, alone and in combination. Stimulation occurred through a tongue box that consisted of a barrier that split the tongue surface into two anterior halves and allowed a different solution to be presented to each side of the tongue simultaneously. Both sucrose and sodium chloride were able to significantly suppress the bitterness of quinine. However, the quinine-sodium chloride mixture produced significantly more bitterness suppression with the spatially mixed than with the spatially
separated stimulus components. This suggests bitterness suppression in mixtures with sucrose occurs centrally and bitterness suppression in mixtures with sodium chloride occurs both peripherally and centrally. In contrast, the findings of Breslin and Beauchamp (1995) suggested sodium ions suppress bitterness only at the periphery. Regardless, both studies indicate that sodium chloride suppresses the perception of bitterness (Breslin and Beauchamp, 1995, Kroeze and Bartoshuk, 1985).

Compounds known as glucosinolates are present in a variety of vegetables, especially cruciferous vegetables, and are responsible for their bitter taste (Schonhof, et al., 2004). Not only can sodium chloride enhance the palatability of these vegetables by its own taste, but also potentially by suppressing the bitterness of these compounds. Furthermore, the main behavioral interventions which have been proven to reduce blood pressure are exercise, weight loss, and a diet (like the DASH diet) that promotes a decreased intake of total and saturated fat and an increased intake of low fat dairy products, fruits, vegetables, and whole grains (Bacon, et al., 2004). Such a multi-faceted intervention has been shown to have greater overall effects in lowering blood pressure that does salt reduction alone. It is possible that a moderate use of dietary salt will increase vegetable consumption and that the benefits from the increased vegetable consumption may outweigh any costs associated with a minor increase in salt consumption.
In order to encourage a greater consumption of vital foods like cruciferous vegetables, this study attempts to determine the lowest amount of sodium chloride that still results in high palatability of broccoli, a representative cruciferous vegetable. Many processed foods, including vegetables, have high levels of added salt which may be in excess of this optimal amount.

The following chapters of this thesis explore the measurement of sodium chloride required to suppress bitterness for specific age groups. In chapter 2, the results of bitterness suppression by sodium chloride for young and middle aged adults in a college campus population are discussed. This chapter also discusses the methods of measuring actual sodium chloride absorption by the broccoli. In chapter 3, the taste perception and results of bitterness suppression by sodium chloride for an elderly age group are explored. In chapter 4, the taste perception and results of bitterness suppression by sodium chloride for young school aged children are investigated. Chapter 5 discusses this entire body of work as a whole.
2.1 Introduction

The most readily accessible group of subjects for investigation was individuals recruited from the university campus; these volunteers were predominately young to middle aged adults, although a few older individuals were also included. This group was the most extensively tested and the results from this group were used to direct the investigations reported in subsequent chapters conducted on populations of older individuals (Chapter 3) and young children (Chapter 4). Dose-response curves were constructed for liking, bitterness, and saltiness. Instrumental analysis was also conducted to determine how much of the added salt doses were actually incorporated into the broccoli samples.

2.2 Materials & Methods

*Materials*

Broccoli was prepared by filling each of three containers (Gladware 64 oz. Deep Dish, Oakland, CA) with 85g (one standard serving) of frozen broccoli florets (Birds Eye Baby Broccoli Florets, Rochester, NY) and cooking the broccoli for 3 minutes in a
microwave oven (Sharp Carousel, 1200 watts). The three containers were shaken and their positions rotated every minute of the three-minute cooking interval. After cooking, a pre-weighed portion (0, 150, 350, 450, 550, or 750 mg), depending upon desired dosage level) of sodium chloride, (Fisher Scientific, Fair Lawn, NJ) was evenly distributed across the florets by gentle shaking for ten seconds. The sample with no added sodium chloride was also shaken for consistency of preparation across stimuli.

For the sensory assessments, after thorough distribution of the sodium chloride dose, three broccoli florets of each level were placed into separate 1-ounce plastic cups (Solo Plastic Soufflés, Solo Cup Company) and labeled with neutral random three-digit codes. For instrumental assessments, once the broccoli was removed from the cooking containers, 50 mL of deionized water was poured into each container and shaken vigorously for ten seconds. Any sodium chloride not absorbed by the broccoli and thus left in the container was dissolved into the water. The resulting solution was then assessed using a chloride analyzer (M-926, Nelson-Jameson, Inc., Marshfield, WI) to determine its sodium chloride content.

Subjects

Volunteer participants were recruited from the Ohio State University. The panel consisted of 75 volunteers (42 female and 33 male) who ranged from 18-65+ years of age. The majority of panelists were younger than 36 years of age, with only two panelists aged 56 and older. The remaining panelists included adults distributed in the following age groups: forty-six aged 18-25 years, eighteen aged 26-35 years, and nine aged 36-55 years. All subjects gave informed consent, in accordance with the policies of The Ohio State University Office of Responsible Research Practices.
Procedures

All materials and procedures were approved by the OSU Office of Responsible Research Practices (and assigned protocol number 2007E0103) before experimentation began. Panelist assessments were made in ten individual sensory testing booths, each of which was equipped with a computer monitor, keyboard, and mouse. Data were collected using Compusense® five version 4.6 software (Compusense Inc, Guelph, Ontario, Canada). Broccoli samples were presented in a restricted random order such that sample order was counterbalanced across all subjects. Each panelist also received a room temperature (roughly 20-25 °C) cup of water (Ice Mountain Drinking Water, Greenwich, CT) for rinsing.

Before beginning their assessments, panelists were told that they would be making three assessments from each portion and to refrain from eating the entire broccoli serving when making their first assessment. They began their assessments by rinsing three times and then rating all six broccoli samples for overall liking on the 9-point hedonic scale (Figure 1), rinsing twice between each sample. Next, they rinsed three more times and rated the same six samples of broccoli for saltiness on a 10-point category scale (ranging from “1 = not salty” to “10 = very salty,” as shown in Figure 2), rinsing twice between each sample. In addition, each panelist indicated his/her ideal level of saltiness on this same intensity scale. Finally, after rinsing three more times, panelists rated bitterness of the same six samples on a 10-point category scale (ranging from “1 = not bitter” to “10 = very bitter,” as shown in Figure 3), still rinsing twice between each sample. As with saltiness, each panelist indicated his/her ideal level of bitterness on the same intensity scale.
Please rate your OVERALL LIKING for each sample on the following scale. Please rinse your mouth 2 times between each sample.

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<td>Like Slightly</td>
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<td>Dislike Moderately</td>
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<td>Dislike Extremely</td>
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*Figure 1. Nine-point hedonic scale used to rate overall liking of broccoli samples.*

From left to right, please rate the SALTINESS for each sample and your 'perceptual IDEAL' on the following scale. Please rinse your mouth 2 times between each sample. *(Ideal = IDE)*

Not Salty | Very Salty

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*Figure 2. Ten-point category scale used to rate saltiness of broccoli samples.*
Figure 3. Ten-point category scale used to rate bitterness of broccoli samples.

For an instrumental assessment of incorporated sodium chloride, after creating a sodium chloride solution in the broccoli cooking container, the solution was poured through a course filter (Mr. Coffee type) to remove any broccoli particles. The chloride level of three batches of broccoli were assessed for each level of added sodium chloride in quadruplicate using a chloride analyzer (M-926, Nelson-Jameson, Inc., Marshfield, WI), resulting in twelve assessments for each sodium chloride level.

Statistical Analysis

Ratings of overall liking (all six salt levels of broccoli), saltiness (all six salt levels and the ideal), and bitterness (all six salt levels and the ideal) were analyzed using three separate repeated measures ANOVAs (Statistica 7, Statsoft Inc., Tulsa, OK) and Fisher’s LSD post-hoc analysis. In addition, a simple linear regression between added salt and each of the sensory attributes was conducted and Pearson’s Product Moment Correlation Coefficient was calculated for each.
Instrumental measures of sodium chloride were analyzed by calculating the averages of the replicates for each level tested. By subtracting these averages of sodium chloride not absorbed from the amount of sodium chloride added, the average amount of sodium chloride absorbed for each dose level were determined. The average percentage of sodium chloride absorption also was calculated for each level.

2.3 Results

*Sodium Chloride Absorption* – The average absorption of sodium chloride as compared to the amount actually added was 94.3% with very little variation; both the highest and lowest percentages had less than 1% difference from the average. As the amount of added sodium chloride increased, so too did the amount of sodium chloride not incorporated into the broccoli. The amount of salt not absorbed (Figure 4), and correspondingly the amount absorbed (Figure 5), increased in a linear fashion.
Figure 4. Mean sodium chloride not absorbed by broccoli for each level of added sodium chloride (n=12).

Figure 5. Mean sodium chloride absorbed by broccoli for each level of added sodium chloride (n=12).
Overall Liking - Broccoli with 350 mg of added salt received the highest average overall liking rating. All broccoli samples with added salt were liked significantly more than broccoli without any added salt ($p<0.05$) (Table 2 & Figure 6). In addition, broccoli with 350 mg of added salt was liked significantly more than broccoli with 150 mg of added salt ($p<0.05$).

Table 2. Mean ratings of each category question for each sodium chloride level (n=75). Samples with the same superscripts are not significantly different from each other (Fisher’s LSD, $\alpha = 0.05$).

* Theoretically the ideal level of overall liking will be the maximum value on the scale and thus was not measured.

Figure 6. Mean ratings for overall liking of each sodium chloride level (n=75)
Saltiness- Broccoli with 350 and 450 mg of added salt were the only samples whose average saltiness ratings were not significantly different from the panelists’ average ideal level of saltiness \( (p>0.05) \) (Table 2 & Figure 7). In addition, the saltiness of broccoli with no and 150 mg of added salt were not perceived to be significantly different from each other \( (p>0.05) \).

![Figure 7. Mean ratings for saltiness of each sodium chloride level (n=75) (The large unfilled circle represents the mean rating of saltiness for the perceptual ideal)](image)

\[
\text{Saltiness} = 0.0071x + 2.4 \\
R^2 = 0.97 \\
p < 0.0005
\]
**Bitterness** - The perceived bitterness of levels 0, 550, and 750 mg were all significantly different than the perceptual ideal for bitterness \((p<0.05)\) (Table 2 & Figure 8). However, 150, 350, and 450 mg were not significantly different than the perceptual ideal for bitterness \((p>0.05)\). The lowest perceived bitterness occurred at 350 mg of sodium chloride. Also, there was no significant difference between the bitterness ratings of 0 and 550 mg of sodium chloride \((p>0.05)\).

![Diagram showing bitterness ratings](image)

*Figure 8. Mean ratings for bitterness of each sodium chloride level \((n=75)\)*
2.4 Discussion

The data suggests a sodium chloride level near or at 350 mg is the optimum level of sodium chloride for assessments of liking, appropriate saltiness, and adequate bitterness suppression. The level of salt in canned vegetables is typically more than twice that at around 750 mg of added sodium chloride (as determined by inspection of sodium levels on can labels). The level of sodium in the canned vegetables is far above that which is needed for adequate bitterness suppression and palatability. For the predominantly young to middle aged adults of the test population, broccoli with 350 mg of added salt received the highest average overall liking rating. For bitterness suppression, a range of added salt levels from 150-450 mg was sufficient to achieve the panelist’s ideal level. In addition, 350-450 mg of added salt was sufficient to achieve the panelist’s ideal level of saltiness for taste preference. This suggests that the high levels of added sodium in canned vegetables are unnecessary and that more moderate use of salt could potentially increase vegetable consumption.

The results of this study are consistent with the findings of Adams et al. (1995), discussed previously in Chapter 1. Adams et al (1995) concluded that the more simple the food item (i.e., the fewer ingredients a food contained), the greater the perceived saltiness (Adams, et al., 1995). Similarly, the very simple food item (broccoli) and preparation (microwaving) used in this investigation required a very low sodium percentage (0.002%) to achieve maximum palatability, suggesting salt is readily perceived in this simple, two-ingredient food.

Previous researchers have shown that sodium suppresses the bitterness of a wide variety of compounds (Breslin and Beauchamp, 1995, Breslin and Beauchamp, 1997,
Keast and Breslin, 2002, Keast, et al., 2001, Kemp and Beauchamp, 1994, Kroeze and Bartoshuk, 1985). Results of the current investigation expand upon this body of work by showing that sodium chloride suppresses the bitterness of broccoli (due predominantly to glucosinolates). However, for broccoli there appears to be a small range of values at which suppression is effective. Only 150, 350, and 450 mg of added sodium chloride were able to significantly decrease the perception of bitterness for broccoli. When sodium chloride was added at even higher levels, bitterness ratings returned to a value similar to when no salt was added. However, these findings must be interpreted with caution given that untrained panelists were used in the current investigation. It is possible that rather than rating bitterness per se, they were rating their “dislike” of the samples. Further investigation of the phenomenon with a trained panel is warranted before time is invested in the development of complicated hypotheses to explain this failure to suppress bitterness in broccoli at the higher levels of sodium chloride.

The 2005 Dietary Guidelines for Americans published by the U.S. Dept. of Health and Human Services and the U.S. Dept. of Agriculture recommends consuming less than 2,300 mg (approximately 1 tsp of salt) of sodium per day (US Department of Health and Human Services, 2005). This would be the equivalent sodium content contained in 5,846 mg of sodium chloride. According to this document the range of sodium content of frozen vegetables (all types) is 2-160 mg. This would be the equivalent sodium content contained in 5-407 mg of sodium chloride. Therefore, one serving of broccoli (with 350 mg of sodium chloride) shown to be highly palatable would fall within the range of sodium content of typical frozen vegetables and would expend about 6% of the daily
recommended sodium intake. Other references recommend consuming no more than 2,400 mg of sodium (or 6,000 mg of sodium chloride) for hypertension prevention which would also make the proposed broccoli preparation with 350 mg of sodium chloride expend just under 6% of the daily recommended sodium intake (Mahan and Escott-Stump, 2004). This small percentage of daily sodium expenditure seems well worth the health benefits that accompany the consumption of broccoli and other vegetables.

2.5 Conclusions

It was determined that a level of roughly 350 mg of added salt was sufficient to achieve desirable saltiness, preference, and bitterness suppression of broccoli in the college population assessed. This sodium level is within the range of sodium contained in frozen vegetables, but far less than the typical sodium contained in canned vegetables. Since medical researchers caution against over consumption of sodium, it is important for consumers to spend their daily sodium allotment wisely. If 350 mg of sodium chloride equates to only 6% of daily recommended intake, it is sodium “well spent” in increasing the desirability (and chances of consumption) of cruciferous vegetables. The seemingly low sodium level that this broccoli needs could be attributed to its simple preparation and lack of additional ingredients. However, as noted previously, perceptions of saltiness and acceptability are product specific; this optimal sodium level can not reasonably be assumed to be appropriate for other vegetables or food products.
CHAPTER 3

PREFERRED SALT LEVEL OF AN OLDER POPULATION FOR BROCCOLI

3.1 Introduction

In the year 2011, the oldest of the Baby Boomers (those born between 1946 and 1964) will start turning 65. Between 2010 and 2030 the number of older people in the U.S. will increase dramatically from 35 million in 2000 to a projected 71.5 million in 2030, more than doubling (Federal Interagency Forum on Aging-Related Statistics, 2008). Prior research indicates that the sense of smell diminishes with increasing age and that certain areas of the brain associated with olfactory processing typically show neuropathological changes with age (Finkelstein and Schiffman, 1999). Losses in taste perception in the elderly can be attributed to alterations in the normal renewal of taste buds, slowing from the typical turnover rate of 10.5 days in healthy adults (Schiffman, 1993). In addition, while taste loss is generally modest in healthy aging individuals, when daily medications become necessary they often have significant negative effects on taste perception (Finkelstein and Schiffman, 1999).

Schiffman (1997) reported that both detection and recognition thresholds are elevated in older individuals for sweet, salty, sour, and bitter tastes. Compared with young subjects, the average detection thresholds for elderly individuals with 1 or more medical conditions and on an average of 3.4 medications were 11.6 times higher for
sodium salts and 7.0 times higher for bitter compounds. In addition, the literature suggests elderly people perceive suprathreshold solutions of sweet, salty, sour, and bitter tasting compounds as less intense than do young people (Schiffman, 1997).

Mojet (2001) determined thresholds for a variety of compounds (including NaCl, caffeine, and quinine HCl, among others) of 21 older (60-75 years of age) and 22 young (19-33 years of age) subjects using a staircase and two-alternative forced choice procedure. It was found that the older group experienced a general decrease in taste perception, with a significant (p<0.007) increase in thresholds for NaCl, although there was no significant increase (p > 0.05) in thresholds for caffeine or quinine HCl (Mojet, et al., 2001).

In contrast, Murphy and Gilmore (1989) found the greatest age related losses in suprathreshold taste were associated with bitterness. They had elderly (65-83 years of age) and young (18-31 years of age) subjects assess the perceived intensity of various concentrations of single and mixed solutions of sucrose, caffeine, sodium chloride, and citric acid using magnitude estimation. For all mixed and unmixed solutions containing bitter compounds, the elderly subjects’ intensity judgments for bitterness were significantly lower than those of the young subjects. In contrast, citric acid, sucrose, and NaCl showed smaller difference between age groups (Murphy and Gilmore, 1989). Taken together, this suggests that with increasing age, there are complex changes in taste perception that depend upon the taste quality. Specifically, it suggests that bitter thresholds are unaltered while suprathreshold intensities are suppressed, while in contrast NaCl thresholds are elevated while suprathreshold intensities remain largely unaltered.
In a separate study, Gilmore and Murphy (1989) also assessed suprathreshold taste discrimination ability in elderly (67-77 years of age) and young (18-25 years of age) subjects by determining the Weber ratio (the minimum noticeable difference to the stimulus intensity) for sweet and bitter stimuli. While the just-noticeable differences for the elderly subjects were significantly larger than those for the young subjects for the bitter stimuli (caffeine), no significant difference was found between the age groups for the just-noticeable differences for sucrose (Gilmore and Murphy, 1989). Combining this finding with those of Mojet (2001) and Murphy and Gilmore (1989), this suggests that although bitter thresholds are unaltered, suprathreshold intensities of bitter compounds are suppressed and the ability to discriminate suprathreshold levels of bitter compounds is reduced.

Using a forced-choice procedure, Nordin, et al. (2003) measured the Weber ratio for salt solutions of 30 elderly (65-78 years old) and 30 young (18-25 years old) subjects. Subjects were asked to compare three aqueous salt solutions (100, 200, and 400 mM of NaCl) with comparison stimuli which were 70%, 82%, 94%, 106%, 118%, and 130% of each standard. Weber ratios were significantly larger for elderly subjects than for young adult subjects for NaCl, suggesting that elderly subjects have a decreased ability to discriminate between taste intensities of sodium chloride (Nordin, et al., 2003). Combining this finding with those of Mojet (2001) and Murphy and Gilmore (1989), this suggests that although NaCl suprathreshold intensities are unaltered, NaCl thresholds are elevated and the ability to discriminate suprathreshold levels of NaCl is reduced.

Stevens and Cain (1993) examined sensitivity to flavor changes of different age groups by comparing young (18-32 years), middle-aged (35-56 years), and elderly (66-89 years) subjects. They found that the sensitivity to flavor changes decreased with age, with elderly subjects showing the least sensitivity.
years) subjects. Each subject performed triangle tests, in which the subject assessed tomato soup with and without added salt, and carrot soup with and without added marjoram (spice). For both soups, the young were better able to discriminate than the middle-aged, who were in turn better able to discriminate than the elderly (Stevens and Cain, 1993). These results suggest that there is a gradual loss in taste or flavor acuity with aging, in agreement with the findings of Gilmore and Murphy (1989) and Nordin, et al. (2003).

Drewnowski, et al. (1996) examined the link between salt taste sensitivity and both preferences and sodium intake in 24 younger (aged 20-30 years) and 24 healthy older (aged 60-75 years) subjects. Hedonic preferences and intensity ratings on salt in aqueous solutions (ranging from 0.04 to 0.48 M) and in broth (ranging from 0.04 to 0.64 M) were determined and each subject performed dietary intake measures including one 24-hour food recall interview followed by 14 consecutive days of diet records. Ratings of hedonic preference and the desire to consume salted soups showed that older subjects preferred less salty soups than did younger people. In addition, estimated sodium intakes appeared to be independent of taste preference profiles for salty soups. Age-related deficits in salt taste perception did not result in increased sodium consumption. Subjects categorized as salt likers and salt dislikers by their hedonic responses had identical sodium intakes (Drewnowski, et al., 1996).

Thus the relationship between age-related changes in taste sensitivity, preferences, and intake are complex. The findings of Murphy and Gilmore (1989) indicate that the suprathreshold levels of NaCl will be equally intense across all age groups, suggesting that for a given level of perceived bitterness, suppression would be
equal across age groups. However, the findings of Murphy and Gilmore (1989) also suggest that suprathreshold levels of bitter compounds are less bitter to older subjects than to younger subjects, suggesting that for the older subjects, broccoli had less inherent bitterness to suppress. In addition, the findings of Drewnowski, et al. (1996) suggest that the elderly prefer less salty tastes. As a result of this complex relationship, it is unclear how the preferred salt level on broccoli would be impacted by aging. Thus, the salt level preference of an older population for broccoli was determined.

3.2 Materials & Methods

Materials

All materials used were identical to those used in Experiment 2. However, only three added levels of sodium chloride per serving of broccoli (85 g) were used (0 mg, 150 mg, and 350 mg). As before, each sample was labeled with neutral random three-digit codes.

Subjects

Volunteer participants were recruited from the Westminster-Thurber Community (a retirement home and assisted living community) and Gillie Senior 50+ Center (a senior recreation center), both located in Columbus, Ohio. The panel consisted of 50 volunteers (40 female, 10 male) who were 60+ years of age. The panelists included older adults distributed in the following age groups: two aged 60-69 years, eleven aged 70-74, seven aged 75-79, eleven aged 80-84, and nineteen aged 85 and older. All subjects gave informed consent, in accordance with the policies of The Ohio State University Office of Responsible Research Practices.
Procedures

All materials and procedures were approved by the OSU Office of Responsible Research Practices (and assigned protocol number 2007E0479) before experimentation began. The evaluation procedure was largely the same as that employed in chapter two, differing only in that the number of samples assessed was reduced from six to three and that assessments were made on paper ballots rather than on computer. Each panelist participated in a private area and made their assessments with the aid of a trained helper. Broccoli samples were presented in a restricted random order such that sample order was counterbalanced across all subjects. Each panelist also received a room temperature (roughly 20-25 °C) cup of water (Ice Mountain Drinking Water, Greenwich, CT) for rinsing.

Before beginning their assessments, panelists were told that they would be making three assessments for each portion and to refrain from eating the entire broccoli serving when making their first assessment. They began their evaluations by rinsing three times and then rating all three broccoli samples for overall liking on the 9-point hedonic scale (Figure 9), rinsing twice between each sample. Subsequently, they rinsed three more times and rated the same three samples of broccoli for saltiness on a 10-point category scale (ranging from “1 = not salty” to “10 = very salty”, as shown in Figure 10), rinsing twice between each sample. In addition, each panelist indicated his/her ideal level of saltiness on this same scale. Finally, after rinsing three more times, panelists rated bitterness of the same three samples on a 10-point category scale (ranging from “1 = not
bitter” to “10 = very bitter,” as shown in Figure 11), still rinsing twice between each sample. As with saltiness, each panelist indicated his/her ideal level of bitterness on the same scale.

Figure 9. 9-point hedonic scale used to rate overall liking of broccoli samples
Please rinse your mouth 3 times with water.
Now you will TASTE the same samples from left to right and answer the following questions...

Please rate the **SALTINESS** of each sample and your 'perceptual IDEAL' on the following scale. Please rinse your mouth 2 times between each sample.

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**Figure 10. 10-point category scale used to rate saltiness of broccoli samples**

Please rinse your mouth 3 times with water.
Now you will TASTE the same samples from left to right and answer the following questions ...

Please rate the **BITTERNESS** of each sample and your 'perceptual IDEAL' on the following scale. Please rinse your mouth 2 times between each sample.

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**Figure 11. 10-point category scale used to rate bitterness of broccoli samples**
Statistical Analysis

Ratings of overall liking (all three salt levels of broccoli), saltiness (all three salt levels and the ideal), and bitterness (all three salt levels and the ideal) were analyzed using three separate repeated measures ANOVAs (Statistica 7, Statsoft Inc., Tulsa, OK) and Fisher’s LSD post-hoc analysis, when appropriate. In addition, simple linear regression between added salt and each of the sensory attributes was conducted and Pearson’s Product Moment Correlation Coefficient was calculated for each dataset.
3.3 Results

*Overall Liking*- Broccoli with 350 mg of added salt received the highest average overall liking rating and was liked significantly more than broccoli with no and 150 mg of added salt (LSD, $p<0.05$). There was no significant difference in liking of broccoli with no and 150 mg of added salt (LSD, $p>0.05$) (see Table 3 and Figure 12).

![Figure 12. Mean ratings for overall liking of each sodium chloride level (n=50).](image)

$Liking = 0.0023x + 6.66$

$R^2 = 0.98$

$p > 0.05$
Saltiness- Broccoli with 350 mg of added salt was significantly saltier (LSD, \( p<0.05 \)) than the samples with 0 and 150 mg of added salt. However, no significant difference in saltiness was found between broccoli with 0 and 150 mg of added salt (LSD, \( p>0.05 \)). The ideal level of saltiness was significantly higher than both 0 and 150 mg of added salt (LSD, \( p<0.05 \)) and was significantly less than 350 mg of added salt (LSD, \( p<0.05 \)) (see Table 3 or Figure 13).

![Figure 13. Mean ratings for saltiness of each sodium chloride level (n=50). The large unfilled circle represents the mean rating of saltiness for the perceptual ideal.](image-url)
**Bitterness**- The bitterness of all three levels and the ideal were not perceived to be significantly different from each other (ANOVA, \(p > 0.05\)) (see Table 3 or Figure 14). This suggests that for this subject population, sodium did not reduce the bitterness of the broccoli samples. However, not only did the bitterness ratings of the broccoli samples not differ from one another, they also did not differ from the ideal.

Figure 14. Mean ratings for bitterness of each sodium chloride level (n=50). The large unfilled circle represents the mean rating of bitterness for the perceptual ideal.
Table 3. Mean ratings of each category question for each sodium chloride level (n=50). Within a row, samples with the same superscript are not significantly different from one another.
* Theoretically the ideal level of overall liking will be the maximum value on the scale and thus was not measured.

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<td>Overall Liking</td>
<td>6.7 a</td>
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<td>7.5 b</td>
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<td>&lt;0.001</td>
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<tr>
<td>Saltiness</td>
<td>2.3 a</td>
<td>2.9 a</td>
<td>4.7 c</td>
<td>3.8 b</td>
<td>&lt;0.001</td>
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<td>Bitterness</td>
<td>2.6 a</td>
<td>2.2 a</td>
<td>2.6 a</td>
<td>2.1 a</td>
<td>0.130</td>
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3.4 Discussion

Unlike in the previous chapter, in this investigation no evidence was found to indicate that sodium chloride suppressed the bitterness of broccoli. However, it is known that perceived bitterness declines with age (Murphy and Gilmore, 1989). Combined with the fact that the bitterness of all the broccoli samples and the ideal level of bitterness were not significantly different implies that these older subjects did not perceive much bitterness in these samples. In addition, the findings of Gilmore and Murphy (1989) indicate that the ability to distinguish between different levels of bitterness decreases with age, which may further explain why no difference was found between broccoli samples and the ideal. Thus, although the lack of bitterness suppression reported in this chapter contradicts the findings of the previous chapter, the findings of the current investigation do fit well into the entire body of relevant findings to date.

In contradiction with Nordin, et al. (2003) who found that elderly subjects have a decreased ability to discriminate between taste intensities of sodium chloride, no such evidence of a decline in ability to distinguish salt levels with age was found. In this chapter, the older group found broccoli with 350 mg NaCl to be significantly saltier than broccoli with both 0 and 150 mg NaCl, but did not distinguish the saltiness of broccoli
with 0 mg NaCl from that with 150 mg NaCl. These results match those of the previous chapter with the college campus population. However, the stimuli used by Nordin, et al. (2003) were quite different from those used in these studies. In addition, the current investigations used much larger differences in NaCl between levels than did Nordin, et al. (2003). These differences between studies seem likely to account for the apparent discrepancy.

In Chapter 2, the average ideal level of saltiness for younger adults was 5.6. The linear regression of perceived saltiness and mg of added NaCl was $y = 0.0071x + 2.4$, where $y$ is the perceived saltiness and $x$ is the mg of added sodium chloride. This equation can be rewritten as $x = 141y – 338$. Thus, it can be estimated that the ideal level of saltiness for the college campus population would arise from 452 mg of added NaCl. In contrast, the average ideal level of saltiness for the older adults assessed in Chapter 3 was 3.8 and the linear regression equation was $y = 0.0071x + 2.10$, or $x = 141y – 296$. Thus the ideal level of saltiness for the older population would arise from 240 mg of added NaCl. These results indicate that older adults prefer their broccoli to be less salty than do younger adults, in agreement with the findings of Drewnowski, et al. (1996). However, for both groups tested 350 mg of added NaCl was the best liked of all samples presented. It seems likely that 350 mg of added NaCl would appeal to both groups despite the fact that it is somewhat low for the younger population and somewhat high for the older population.
3.5 Conclusions

In this population, 350 mg of added salt was preferred more than 0 and 150 mg of added salt, with the ideal level of saltiness being significantly more salty than both 0 and 150 mg of added salt and significantly less salty than 350 mg of added salt, making their ideal level somewhat lower than the group tested in Chapter 2. Panelist discrimination of saltiness was similar across both groups, but the older group showed less discrimination of the bitterness than the younger group. For both groups, the sample with 350 mg of added NaCl was the best liked. Lifestyle modifications for hypertension management includes a salt restriction of 2,400 mg sodium (or 6,000 mg sodium chloride) daily (Mahan and Escott-Stump, 2004 and US Department of Health and Human Services, et al., 2003). The preferred broccoli preparation with 350 mg of sodium chloride would expend just under 6% of the daily recommended sodium intake, making this salt level also acceptable for hypertensive individuals. Individuals with more severe hypertension and have been placed on a more strict sodium diet should follow instructions set by their doctor and/or dietician.
CHAPTER 4

PREFERRED SALT LEVEL OF CHILDREN FOR BROCCOLI

4.1 Introduction

The American Institute for Cancer Research (AICR), in their publication “Guidelines for Cancer Prevention,” has recommended consuming at least five servings of fruits and vegetables per day throughout life for defense against cancer and chronic diseases like heart disease, stroke, and diabetes (The American Institute for Cancer Research, 1997). The protective effect of cruciferous vegetables is strongest for cancers of the mouth, pharynx, larynx, esophagus, and stomach. Although it is beneficial to improve dietary habits at any stage of life, the AICR stresses the effectiveness of maintaining abundant vegetable consumption throughout life. Therefore, it is especially important to encourage young children to consume a diet high in vegetables like broccoli in order to impart good habits towards disease prevention.

Mennella, et al. (2003) investigated the bitterness suppression of several bitter tasting compounds (including urea, caffeine, and tetralone) by sodium gluconate for both children and adults. 41 mother-children pairs, with the children ranging in age from 7 to 10 years old, were tested. A forced-choice procedure was used wherein each subject was presented with all possible pairs of the four solutions (bitter, salt, bitter + salt, and water), one pair at a time in duplicate, and was asked to choose which solution of the pair tasted
more bitter during one test session and which tasted better during another test session. Finally at the end of the sessions, subjects were presented with each of the four solutions and asked to rank them from most to least preferred. Sodium gluconate suppressed the bitterness and enhanced the acceptance of urea and, to a lesser extent, caffeine, in both children and adults. In contrast, sodium gluconate enhanced the bitterness of tetralone in children and decreased the liking of tetralone in both children and adults. Overall, children preferred salt more than adults, as indicated by the preference rankings.

Oram et al. (2001) investigated the differences between the ability of adults and children to perceive tastes in binary mixtures. 50 children (aged 8-9 years) and 30 adults (aged 18-39 years) indicated whether sweet, salty, and sour tastes were present in aqueous solutions containing sucrose, sodium chloride, citric acid, and all possible binary mixtures of these compounds. While adults were able to correctly recognize the two tastes in all binary mixtures, children recognized only one. In contrast, for both age groups suppression of one or both components was recorded with most mixtures. However, while children reported suppression of sourness for the sodium chloride-citric acid mixture, adults reported suppression of saltiness. Furthermore, children did not report any suppression of sweetness or saltiness for mixtures containing citric acid, while adults did. These results suggest that children experience taste mixtures differently than do adults, which may alter their preferred sodium level for broccoli. Thus, their preference for sodium chloride on broccoli was measured.
4.2 Materials & Methods

Materials

All materials and sample preparation used were identical to those used in Experiment 1, although only three salt levels were used on the broccoli samples: 0 mg, 150 mg, and 350 mg. As before, each sample was labeled with random three-digit codes.

Subjects

Volunteer participants were recruited from grade school children attending Scarlet and Gray Agricultural Day at the Ohio State University on May 18, 2007. The panel consisted of 88 volunteers (42 female, 46 male) who ranged from 9-12 years of age. All subjects gave informed consent, in accordance with the policies of The Ohio State University Office of Responsible Research Practices. Permission slips were sent home and signed by parents and children prior to the event and the children gave verbal permission the day of the event.

Procedures

All materials and procedures were approved by the OSU Office of Responsible Research Practices (and assigned protocol number 2007E0306) before experimentation began. Each panelist participated in sensory testing booths equipped with computer monitors, keyboards and a mouse at each of ten stations. Data were collected using Compusense® five version 4.6 software (Compusense Inc, Guelph, Ontario, Canada). Broccoli samples were presented in a restricted random order such that sample order was counterbalanced across all subjects. Each panelist also received a room temperature (roughly 20-25 °C) cup of water (Ice Mountain Drinking Water, Greenwich, CT) for rinsing.
Panelists were instructed to first rinse the mouth three times with water. Then, they tasted each broccoli sample, rinsing twice between each sample, and ranked the samples for preference. They were instructed to first select the sample they like the most (Figure 15), and then next select the sample they like second most (Figure 16). By default, the remaining sample was the least liked.

Please taste the samples from left to right. 1st: decide which sample you like the MOST and click on its number. 2nd: click on the number of the sample you like second most. The sample you like LEAST will automatically be selected 3rd.

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<th>Samples</th>
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<td>862</td>
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<td>574</td>
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<tr>
<td>358</td>
</tr>
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</table>

Select Sample to Rank
First

Figure 15. 1st step of ranking exercise used to rate overall liking of broccoli samples
Please taste the samples from left to right. 1st: decide which sample you like the MOST and click on its number. 2nd: click on the number of the sample you like second most. The sample you like LEAST will automatically be selected 3rd.

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<th>Samples</th>
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<tbody>
<tr>
<td>862</td>
<td>358</td>
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</table>

| Ranked Samples | 574 |

Select Sample to Rank
Second

Clear Selections and Start Again

Reverse Last Action

Figure 16. 2\textsuperscript{nd} step of ranking exercise used to rate overall liking of broccoli samples

**Statistical Analysis**

Mean rank scores across the panelists were computed for each broccoli sample using Friedman Analysis of Rank procedure and Basker’s tables (Basker, 1988) for critical values of difference. In addition, Spearman’s rho for the regression of the liking ranks and the rank-order of added sodium chloride levels was calculated. For plotting, to make comparison to previous chapter results more straight-forward, rank values were inverted from those used by the panelists such that the best liked sample was given the highest rank of 3 and the least liked sample was given the lowest rank of 1.
4.3 Results

For the children 350 mg of sodium chloride was most frequently rated as the most liked level, 150 mg of sodium chloride was most frequently rated as the second most liked level, and 0 mg of sodium chloride was most frequently rated as the least liked level. However, there was no significant difference (Friedman, $p>0.05$) between the liking of broccoli with 150 and 350 mg of sodium chloride (Figure 17), while broccoli with no added sodium was liked significantly less (Friedman, $p<0.05$) than both.

Figure 17. Mean rank for each sodium chloride level ($n=88$)  
($1 = \text{least preferred}; 3 = \text{most preferred}$)
4.4 Discussion

The findings of the current study are consistent with those of Mennella, et al. (2003). In both instances, sodium salts were found to enhance the acceptance of bitter compounds presumably by suppression of the bitter taste in both children and adults. This experiment shows that children prefer the same (or perhaps slightly less) salt than do the adults in Chapter 2. It is unclear if in fact they prefer less saltiness than do the older adults in Chapter 3, as it is possible that the optimum salt level for children lies somewhere in between 150 and 350 mg added sodium chloride, as it does for the older adults. Additional research would be necessary to further refine the optimum level and make this determination. In order to further define an optimal salt level for children, a subsequent study should use similar procedures but test salt levels of 150, 250, and 350 mg of added sodium chloride. It is important to mention that any additional studies should continue with the use of small sample sets to avoid overwhelming children.

It is interesting to note that once again the broccoli with 350 mg of added sodium chloride was the best liked sample of all those tested. Although this level may be higher than the optimum level for the young group (as discussed above), and it is both higher than the ideal level of the older adults and lower than the ideal of the college campus population, all three groups rated this level as their most preferred among all levels tested. Thus, for all groups, this moderate addition of sodium chloride would increase palatability of broccoli and encourage its consumption.
4.5 Conclusions

The results of this experiment showed that children preferred broccoli with added salt more than broccoli with no salt. In addition, it was shown that overall they liked the two levels of added salt (150 and 350 mg) equally. This suggests that a moderate addition of sodium to broccoli will increase its palatability and thus encourage its increased consumption. It seems certain that the benefits of this increased consumption of broccoli would outweigh any increased risk due to the associated minor increase in sodium consumption.
CONCLUSIONS

Glucosinolates are the main phytochemicals present in a variety of vegetables, especially cruciferous vegetables; glucosinolates have a bitter taste (Schonhof, et al., 2004). Sodium chloride enhances the palatability of these vegetables by adding saltiness as well as by suppressing bitterness. In order to encourage a greater consumption of vital foods like cruciferous vegetables, this study has estimated the lowest amount of sodium chloride that still results in high palatability of broccoli, a representative cruciferous vegetable. This thesis quantitatively measured the amount of sodium chloride required to suppress bitterness for specific age groups of the population.

In chapter 2, the bitterness suppression by sodium chloride for young and middle aged adults in a college campus population was determined. This chapter also determined via measures of chloride absorption that roughly 94.3% of the sodium chloride added to the broccoli was absorbed into the sample. In this study, it was determined that a level of roughly 350 mg of added salt was sufficient to achieve desirable saltiness, preference, and bitterness suppression of broccoli in the college population assessed. This sodium level is within the range of sodium contained in frozen vegetables, but far less than the typical sodium contained in canned vegetables. Since many medical experts caution against over consumption of sodium, it is important that consumers spend their daily
sodium allotment wisely. Since 350 mg of salt equates to only 6% of daily recommended intake, it should be considered sodium “well spent” in increasing the desirability (and chances of consumption) of cruciferous vegetables.

In chapter 3, the taste perception and bitterness suppression by sodium chloride for an elderly age group was established. In this population, 350 mg of added salt was preferred more than 0 and 150 mg of added salt, with the ideal level of saltiness being significantly more salty than both 0 and 150 mg of added salt and significantly less salty than 350 mg of added salt, making their ideal level somewhat lower than the adults from a college campus population. Panelist discrimination of saltiness was similar across both groups, but the older group showed less discrimination of the bitterness than the younger group. For both groups, the sample with 350 mg of added salt was the best liked.

In chapter 4, the taste perception of bitterness suppression by sodium chloride for young school aged children was determined. The results of this experiment showed that children preferred broccoli with added salt more than broccoli with no salt. In addition, it was shown that overall they liked the two levels of added salt (150 and 350 mg) equally. This suggests that a moderate addition of sodium to broccoli will increase its palatability and thus encourage its increased consumption. It seems likely that the benefits of this increased consumption of broccoli would outweigh any increased risk due to the associated minor increase in sodium consumption.
Finer tuning of the level of sodium chloride required for bitterness suppression and maximum palatability could be achieved with additional testing. The results from this study and similar investigations can be utilized in large scale food service operations such as school lunch programs, retirement home kitchens, restaurants, and food manufacturing to reduce total sodium levels consistent with recommendations from the medical community.
BIBLIOGRAPHY


APPENDIX A

IRB APPLICATION, BALLOT, AND EXPERIMENTAL DESIGN

PREFERRED SALT LEVEL OF A COLLEGE CAMPUS POPULATION FOR BROCCOLI
APPLICATION FOR EXEMPTION
FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

All research activities involving the use of human beings as research subjects must be reviewed and approved by an Ohio State University Institutional Review Board (IRB), unless the Office of Responsible Research Practices (ORRP) determines that the research falls into one or more of the categories of exemption established by federal regulation.

Exempt research is generally short term in nature. It must be performed “as written,” i.e. the investigators do not make changes in the research design, the selection of subjects, the informed consent process, or the instrumentation during the course of the study. If changes are necessary, re-application is required.

A determination that research is exempt does not absolve the investigators from ensuring that the welfare of human subjects participating in research activities is protected, and that methods used and information provided to gain subject consent are appropriate to the activity. Investigators may not solicit subject participation or begin data collection until they have received approval from the appropriate Institutional Review Board OR written concurrence that research has been determined to be exempt.

All OSU Investigators who participate in human subjects research must be appropriately trained in human subjects protection. See http://orrp.osu.edu/humansubjects/training.cfm for more details.

There is no deadline or timeline for submitting exempt applications for review. Applications are processed as received. Each application must include a research proposal. The proposal must include (at a minimum) the following items: the background literature review, the research question, a description of the research methods including sample size and data collection procedures, and a data analysis plan.

Please allow up to three weeks for processing.

If you have questions regarding the application process or the review of exempt protocols, please contact Janet Schulte, Office of Responsible Research Practices.

Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu

A COMPLETE APPLICATION PACKET INCLUDES THE FOLLOWING MATERIALS:

☐ Title page (attached). Identifies the investigators. Lists the protocol title and the source of funding.
☐ Screening questions (attached). Identifies the categories of exemption and solicits responses to screening questions.
☐ Description of the proposed research (questions #1 through #9, attached). Includes responses to questions about the objective(s) of the research, the methodology that will be used to gain informed consent from the subjects, and the measures taken to protect the confidentiality of information obtained in research.

☐ Research proposal (see question #1).
☐ Grant proposal. Must be included when externally-sponsored funding is being sought.
☐ Letter(s) of support (see question #4).
☐ Copies of surveys, instruments, questionnaires, interview questions, focus group topics, and/or data collection sheets (see question #5).
☐ Recruitment letter (see question #8).
☐ Consent form (see question #9).

SEND ONE COPY OF YOUR APPLICATION TO:
Office of Responsible Research Practices
300 Research Foundation Building
1960 Kenny Road
Columbus OH 43210-1063
Fax (614) 688-0366

TITLE PAGE - APPLICATION FOR EXEMPTION FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

Principal Investigator

Name: Jeannine F. Delwiche

Department or College: Food Science & Technology

Campus Address (room, building, street address):
110 Parker Food Science Building
2015 Fyffe Road
Columbus, OH 43210

Signature: Date:

Phone: 614-247-6756
E-mail: Delwiche.1@osu.edu

Fax: 614-292-0218
<table>
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<tr>
<th>Co-Investigator</th>
<th>Name: Jennifer K. Balitsis</th>
<th>Phone: 614-247-6831</th>
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<tr>
<td>University Status:</td>
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<td>Campus Address (room, building, street address) or Mailing Address: 110 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210</td>
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<td></td>
<td>Staff</td>
<td>E-mail: <a href="mailto:Balitsis.1@osu.edu">Balitsis.1@osu.edu</a></td>
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<td>Undergraduate Student</td>
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<td>Student</td>
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<td>Research has been determined to be exempt under these categories: ____________________. Research may begin as of the date of determination listed below.</td>
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<tr>
<td>Disapproved.</td>
<td>The proposed research does not fall within the categories of exemption. Submit an application to the appropriate Institutional Review Board for review.</td>
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<td>Office of Responsible Research Practices</td>
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The purpose of the Application for Exemption is two-fold: (a) to determine whether the proposed research qualifies for exemption from review and continuing oversight by an Institutional Review Board; and, if so, (b) to ensure that the informed consent process protects the rights and welfare of human subjects in research. Please respond to the following questions and provide the requested documentation.

Have all investigators completed the required web-based course in the protection of human research subjects? ☒Yes ☐No

If No, see [http://orrp.osu.edu/humansubjects/citi.cfm](http://orrp.osu.edu/humansubjects/citi.cfm) for more information.

EDUCATIONAL REQUIREMENTS MUST BE SATISFIED PRIOR TO SUBMITTING THE APPLICATION FOR IRB REVIEW.
Please check the categories of exemption for which you are applying. The list of categories is located at the end of this application. You may check more than one box.

EXEMPT CATEGORY:  1  2  3  4  5  6

SCREENING QUESTIONS: If you check YES to any of the questions below, your research is not exempt. Do not complete the exempt application. Submit an application to the appropriate Institutional Review Board for review.

Does any part of the research require that subjects be deceived?  □ Yes  □ No

Will research expose human subjects to discomfort or harassment beyond levels encountered in daily life?  □ Yes  □ No

Could disclosure of the subjects’ responses outside the research reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects’ financial standing, employability, or reputation?  □ Yes  □ No

Will fetuses, pregnant women, human in vitro fertilization, or individuals involuntarily confined or detained in penal institutions be subjects of the study?  □ Yes  □ No

For research proposed under category 2, will research involve surveys, interview procedures, or observation of public behavior with individuals under the age of 18?  □ Yes  □ No

For research proposed under category 4, will any of the data, documents, records, pathological specimens, or diagnostic specimens be collected or come into existence after the date you apply for exemption?  □ Yes  □ No

For research proposed under category 4, will any of the information obtained from data, documents, records, pathological specimens, or diagnostic specimens that come from private sources be recorded by the investigator in such a manner that subjects can be identified directly or through identifiers linked to the subjects?  □ Yes  □ No

IF YOU CHECKED YES TO ANY OF THE QUESTIONS ABOVE, YOUR RESEARCH IS NOT EXEMPT.

IF YOU HAVE CHECKED NO TO ALL OF THE QUESTIONS ABOVE, YOUR RESEARCH MAY BE EXEMPT. PLEASE CONTINUE WITH THE EXEMPT APPLICATION.
If you have questions about the application or review process, please contact Janet Schulte, Office of Responsible Research Practices. Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu

For purposes of this application, “research” includes the recruitment of human subjects as well as data collection and analysis. None of these research activities may begin until the investigator has received a protocol number AND has received written concurrence that the proposed research is exempt. The “date of determination” on page one of this application is assigned by the Office of Responsible Research Practices; it indicates the date when research may begin.

Please describe your study clearly and completely, using a style of language that can easily be understood by someone who is not familiar with your research.

**GENERAL QUESTIONS REGARDING THE PROPOSED RESEARCH**

1. **Describe the purpose of the research activity to be undertaken. Describe how it involves human subjects. Respond in the space provided here, or attach a research proposal and/or grant proposal containing the requested information.**

   **Description:** A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phytonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will develop an experimental model to quantitatively measure the amount of salt required to suppress bitterness in vegetables and increase palatability by building a dose-response curve. From this curve, it will be possible to extract the optimum level of sodium chloride to encourage higher consumption of vegetables without leading to the over consumption of sodium chloride.

2. **Provide a brief description of the subjects you plan to recruit and the criteria used in the selection process. Indicate whether subjects are 18 years of age or older.**

   **Description:** Subjects will be recruited from volunteers that are 18 years of age or older who are not pregnant and not nursing. They will be recruited based on availability and willingness to participate.
3. Describe how the proposed research meets the criteria for exemption from IRB review and oversight. (Refer to the criteria on the last page of this application that correspond to the category or categories you checked on the screening sheet.)

**Description:** This research meets the criteria for exemption under category 6. Subjects will taste wholesome foods and respond to questions about liking and attributes of the foods. Foods tasted will include cooked broccoli with varying levels of sodium chloride.

4. Will your subjects be recruited through schools, employers, and/or community agencies or organizations, and/or are you required to obtain permission to access data that is not publicly available? If the answer is yes, provide a letter of support from the person authorized to give you access to the subjects or to the data in question. More than one letter may be required.

- [x] Does not apply.
- [ ] Letter(s) attached.

**Comments:** Subjects will be recruited from the faculty, staff, and students proximate to the Parker FST building and neighboring areas.

5. Describe the means you will use to obtain data. Check all boxes that apply.

- [x] Surveys or questionnaires distributed by mail or in person. I am attaching a copy of the instrument(s).
- [ ] Surveys distributed through the Internet, through listservs, or through E-mail. I am attaching a copy of the instrument(s). Provide the Internet address:
- [ ] Interviews. I am attaching a copy of the interview questions.
- [ ] Focus groups. I am attaching a copy of the questions that will shape the discussion.
- [ ] Observation of public behavior.
- [ ] Observation of activities in school classrooms.
- [ ] Audiotapes. I will obtain consent from the subjects to tape their responses.
- [ ] Videotapes. I will obtain consent from the subjects to tape their activities or responses.
- [ ] Review of existing records, including databases, medical records, school records, etc. I am attaching a copy of the data collection sheet. I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All of the information in the records to be reviewed exists as of the date of submission of this application.
- [ ] Tissue specimens. All of the specimens have already been collected and are “on the shelf.” I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.
6. Indicate the date when you plan to begin research, and the date when you anticipate that data analysis will be complete.

Begin date: 2/26/2007  
End date: 2/26/2008

CONFIDENTIALITY

- Investigators are required to protect the confidentiality of the information obtained during research, unless the subjects (a) explicitly agree to be identified or quoted, and/or (b) explicitly agree to the release of material captured on audiotapes or videotapes for use in presentations or conferences.

7. Provide a brief description of the measures you will take to protect confidentiality. Please describe how you will protect the identity of the subjects, their responses, and any data that you obtain from private records or capture on audiotape or videotape. Describe the disposition of the data and/or the tapes once the study has been completed.

Description: No identifying information will be collected.

INFORMED CONSENT

- In most cases, investigators are required to obtain informed consent from their subjects before collecting data. Respond to questions #8 and #9 to indicate how you will inform your subjects about the research and how you will obtain and document their consent.
- Subjects must be told what they will be asked to do if they agree to participate in research, how long it will take, and how you will protect the confidentiality of the information they provide.
- Subjects must be told that their participation is voluntary, they can refuse to answer questions that they do not wish to answer, and they can refuse to participate or they can withdraw at any time without penalty or repercussion.
- With few exceptions, written consent of the child’s parent(s) or guardian(s) is required if subjects are under the age of 18. In addition, children 14 years of age or older should be asked to give written assent (agreement) to participate. Children younger than 13 years of age should be asked to give verbal assent (agreement) to participate.
- Provide a means for subjects to contact the investigator(s) if they have questions or concerns about the research. Make it clear to the subjects that you are affiliated with The Ohio State University.
8. What information do you plan to give to your subjects before you ask for their consent? Use a style of language that simply and clearly explains the research to your subjects. Respond in the space provided here, or attach a copy of the information you plan to provide to your subjects and/or their parents or guardians. (Note: if you use more than one method of recruitment, you may check more than one box)

☐ Letter(s) attached. I will give each of the subjects a copy of this letter.
☐ I will be contacting subjects by phone or in person. I am attaching a script that contains the information I will give them.
☐ Does not apply. My data analysis is limited to existing records or tissue specimens.
☒ Response: Information about the study will be presented on the first screen of the Compusense® data collection software (see attached questionnaire).

9. How do you plan to document informed consent? Read all of the options before checking the appropriate boxes. (A sample consent form is attached to this application.)

☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to sign a written consent form. I am attaching a copy of the consent form.
☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to give verbal consent to participate in this research study.
☐ The subjects are 18 years of age or older. I am distributing a survey or questionnaire to the subjects. They can choose whether or not they want to respond. I am requesting a waiver of written consent.
☐ The subjects are under the age of 18. I am attaching a copy of the consent form that I will use to obtain consent from their parents or guardians and assent (agreement) from subjects who are 14 years of age or older.
☐ Some of the subjects are 18 years of age or older, and some are younger than 18. I have checked more than one box above to reflect the methods I will use to document informed consent.
☐ Does not apply. My data analysis is limited to existing records or tissue specimens.
☒ Other. Please explain and provide justification for your request. Data will be collected via Compusense® software where subjects will indicate their ratings of the samples. Prior to making these ratings, a screen will describe the study and ask panelists to indicate informed consent by clicking “Yes, I consent to participate.”

☐ Comments:
Recruitment Letter – for distribution by email and flyer

Department of Food Science and Technology
Sensory Science Group
110 Parker Food Science and Technology Building
2015 Fyffe Road
Columbus, OH 43210

Phone 614-247-6864
FAX 614-292-0218

PARTICIPANTS NEEDED
Jeannine Delwiche, Principal Investigator

Date: TBA
Time: TBA
Place: Parker 122 (Sensory Booths)

This study is designed to look at consumer perception of cooked broccoli. In this study, you will be presented with several samples on three small trays and you will be asked to assess them. At the end, you will be asked a few demographic questions such as gender and age.

Your answers will be entered directly into the computer using the mouse and/or keyboard. This has been estimated to take less than 15 minutes but you may take as long as you need. Your participation is voluntary, and you can refuse to answer questions that you do not wish to answer. Also, you can withdraw at any time without penalty or repercussions. Your responses will in no way be linked to your identity and you will be compensated with candy.

If you have any questions or concerns please contact Jeannine Delwiche at Delwiche.1@osu.edu, 614-247-6756 or Jennifer Balitsis at Balitsis.1@osu.edu, 614-247-6831. Both are affiliated with The Ohio State University.
PROPOSAL COVER SHEET 2007
Dose-response curve of sodium chloride on vegetable palatability

PRINCIPAL INVESTIGATOR
Name: Jeannine Delwiche
Institution: Ohio State University
Address: Department of Food Science & Technology
2015 Fyffe Road
Columbus, OH 43210
Phone: 614-247-6756 Fax: 614-292-0218
Email: delwiche.1@osu.edu

PROPOSAL INFORMATION

Title: Dose-response curve of sodium chloride on vegetable palatability

Summary Statement

A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phytonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will develop an experimental model to quantitatively measure the amount of salt required to suppress bitterness in vegetables and increase palatability by building a dose-response curve. From this curve, it will be possible to extract the optimum level of sodium chloride to encourage higher consumption of vegetables without leading to the over consumption of sodium chloride.
Dose-response curve of sodium chloride on vegetable palatability

Submitted by: The Ohio State University and the Salt Institute

Background: In an effort to encourage lower sodium consumption, UK policy makers have removed salt shakers from school lunchrooms. As a result, students are not only eating less sodium but also, unfortunately, fewer vegetables. It is well established that most children need to increase their vegetable consumption, thus the approach of complete sodium elimination seems misguided. The use of a limited amount of sodium to create vegetables that taste good will do far more to promote an optimal diet than does eliminating the likely small amount of sodium it would require to make vegetables palatable. Therefore a more pragmatic solution is to find the optimal level of salt at which people will be more likely to eat their vegetables. Knowing the small amount of salt required to make vegetables palatable to will encourage US policy makers to consider the inclusive benefit/risk package before repeating the actions of their UK counterparts. The establishment of such a dose-response curve also has the potential to serve as a practical guide in the formulation of restaurant and processed foods.

Objective: The objective of this study is to determine the optimal level of salt use in order to increase the likelihood that children will eat their vegetables in school lunches.

Approach: Consumer liking assessments will be made of frozen broccoli, cooked and prepared with salt levels varying from no added salt to 350 mg sodium/serving (the typical level in canned vegetables). Assessments of 5 levels of sodium will be made by 75-100 individuals at the Ohio State University on–site location. From this preliminary testing, the candidate level of optimum sodium level will be refined and subsequent testing of preferred levels will be conducted with students at a grade school, as well as individuals in a retirement community.

Deliverables to the Salt Institute: Determination of the appropriate level of salt to use in order to increase vegetable palatability for three age-groups.

Budget:

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<thead>
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<th>Cost</th>
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<tr>
<td>Labor:</td>
<td>$22,000</td>
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<tr>
<td>(Technician and graduate student salary/benefits/tuition)</td>
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<td>Supplies:</td>
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<td>Total:</td>
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Questionnaire:

Welcome to Sensory Testing!
Jeannine Delwiche, Principal Investigator

This study is designed to look at consumer perception of cooked broccoli. In this study, you will be presented with several samples on three small trays and you will be asked to assess them. At the end, you will be asked a few demographic questions such as gender and age.

Your answers will be entered directly into the computer using the mouse and/or keyboard. This has been estimated to take less than 15 minutes but you may take as long as you need. Your responses will in no way be linked to your identity and you will be compensated with candy at the end. If you have any questions, please feel free to ask the attendant at any time.

If you wish to participate, please read the following statement and indicate your consent to participate.

INFORMED CONSENT STATEMENT

I understand the purpose, procedures and time requirements of this study. All questions have been answered to my satisfaction. I may withdraw at any time without penalty. I am 18 years of age or older. I am not pregnant and not nursing. I freely and voluntarily give my consent to participate by circling 'YES' below.

YES  no

<present a tray containing 6 samples of different broccoli preparations for flavor evaluation>

Please rinse your mouth 3 times with water.
Now you will TASTE the samples from left to right and answer the following questions…

Please rate your OVERALL LIKING of each sample on the following scale.
Please rinse your mouth 2 times between each sample.

<table>
<thead>
<tr>
<th>436</th>
<th>972</th>
<th>891</th>
<th>647</th>
<th>513</th>
<th>263</th>
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</thead>
</table>
| ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | ![ ] | Like very much
<present a new tray containing the same 6 samples of different broccoli preparations for flavor evaluation>

Please rinse your mouth 3 times with water.
Now you will TASTE the samples from left to right and answer the following questions…

Please rate the SALTINESS of each sample and your ‘perceptual IDEAL’ on the following scale.
Please rinse your mouth 2 times between each sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Not salty</th>
<th>Very salty</th>
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</thead>
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<tr>
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<tr>
<td>Sample 263</td>
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<tr>
<td>IDEAL</td>
<td>□ □ □ □ □ □ □ □ □ □</td>
<td>□ □ □ □ □ □ □ □ □ □</td>
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</tbody>
</table>

<present another new tray containing the same 6 samples of different broccoli preparations for flavor evaluation>

Please rinse your mouth 3 times with water.
Now you will TASTE the samples from left to right and answer the following questions…

Please rate the BITTERNESS of each sample and your ‘perceptual IDEAL’ on the following scale. Please rinse your mouth 2 times between each sample.

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<th>Sample</th>
<th>Not bitter</th>
<th>Very bitter</th>
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</tbody>
</table>
Please indicate your gender.

- Male
- Female

Please indicate your age category.

- 18-20 years
- 21-25 years
- 26-35 years
- 36-45 years
- 46-55 years
- 56-65 years
- over 65 years
Project: Preferred Salt Level in Broccoli for College Campus Population Design

Plan:

Description: Williams Design (6 treatments)
Type: Quantitative Descriptive
Samples: 6
Presented: 6
Blocks: 6 [Base Block]  
\[ \times 17 \text{ [Factor]} \]
\[ = 102 \text{ [Entire Block]} \]

Options:

Blinding Codes: Constant
Sample Randomization: No
Block Randomization: No
Registration: Panelists Must Register
Sample Set Distribution: Bind Sample Sets to Panelists

Sessions:

Number of Sessions: 1

Samples:

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<td>1</td>
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<tr>
<td>2</td>
<td>2</td>
<td>150 mg</td>
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<tr>
<td>3</td>
<td>3</td>
<td>350 mg</td>
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<td>4</td>
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<td>550 mg</td>
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<tr>
<td>6</td>
<td>6</td>
<td>750 mg</td>
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Blinding Codes for Session 1

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<th>Product Code</th>
<th>Product Name</th>
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<tr>
<td>6</td>
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## Layout for Session 1

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APPENDIX B

IRB APPLICATION, BALLOT, AND EXPERIMENTAL DESIGN

PREFERRED SALT LEVEL OF AN OLDER POPULATION FOR BROCCOLI
APPLICATION FOR EXEMPTION
FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

All research activities involving the use of human beings as research subjects must be reviewed and approved by an Ohio State University Institutional Review Board (IRB), unless the Office of Responsible Research Practices (ORRP) determines that the research falls into one or more of the categories of exemption established by federal regulation.

Exempt research is generally short term in nature. It must be performed “as written,” i.e. the investigators do not make changes in the research design, the selection of subjects, the informed consent process, or the instrumentation during the course of the study. If changes are necessary, re-application is required.

A determination that research is exempt does not absolve the investigators from ensuring that the welfare of human subjects participating in research activities is protected, and that methods used and information provided to gain subject consent are appropriate to the activity. Investigators may not solicit subject participation or begin data collection until they have received approval from the appropriate Institutional Review Board OR written concurrence that research has been determined to be exempt.

All OSU Investigators who participate in human subjects research must be appropriately trained in human subjects protection. See http://orrp.osu.edu/humansubjects/training.cfm for more details.

There is no deadline or timeline for submitting exempt applications for review. Applications are processed as received. Each application must include a research proposal. The proposal must include (at a minimum) the following items: the background literature review, the research question, a description of the research methods including sample size and data collection procedures, and a data analysis plan.

Please allow up to three weeks for processing.

If you have questions regarding the application process or the review of exempt protocols, please contact Janet Schulte, Office of Responsible Research Practices.
Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu

A COMPLETE APPLICATION PACKET INCLUDES THE FOLLOWING MATERIALS:

- Title page (attached). Identifies the investigators. Lists the protocol title and the source of funding.
- Screening questions (attached). Identifies the categories of exemption and solicits responses to screening questions.
- Description of the proposed research (questions #1 through #9, attached). Includes responses to questions about the objective(s) of the research, the methodology that will be used to gain informed consent from the subjects, and the measures taken to protect the confidentiality of information obtained in research.
- Research proposal (see question #1).
- Grant proposal. Must be included when externally-sponsored funding is being sought.
☐ Letter(s) of support (see question #4).
☐ Copies of surveys, instruments, questionnaires, interview questions, focus group topics, and/or data collection sheets (see question #5).
☐ Recruitment letter (see question #8).
☐ Consent form (see question #9).

SEND ONE COPY OF YOUR APPLICATION TO:
Office of Responsible Research Practices
300 Research Foundation Building
1960 Kenny Road
Columbus OH 43210-1063
Fax (614) 688-0366

### TITLE PAGE - APPLICATION FOR EXEMPTION FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Name: Ken Lee</th>
<th>Phone: 614-292-7797</th>
</tr>
</thead>
<tbody>
<tr>
<td>University Title:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☑ Professor</td>
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<tr>
<td>☑ Associate Professor</td>
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<td>☑ Assistant Professor</td>
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<tr>
<td>☑ Instructor</td>
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<tr>
<td>☑ Other. Please specify. (May require prior approval.)</td>
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<td></td>
</tr>
<tr>
<td>Department or College:</td>
<td>Food Science &amp; Technology</td>
<td></td>
</tr>
<tr>
<td>Campus Address (room, building, street address):</td>
<td>110 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210</td>
<td></td>
</tr>
<tr>
<td>Signature:</td>
<td></td>
<td>Fax: 614-292-0218</td>
</tr>
<tr>
<td>Date:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-mail: <a href="mailto:Lee.133@osu.edu">Lee.133@osu.edu</a></td>
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<th>Co-Investigator</th>
<th>Name: Jeannine F. Delwiche</th>
<th>Phone: 609-580-4301</th>
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<tr>
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<tr>
<td>Campus Address (room, building, street address) or Mailing Address:</td>
<td>Firmenich Incorporated P.O. Box 5880 Princeton, NJ 08543</td>
<td></td>
</tr>
<tr>
<td>Signature:</td>
<td></td>
<td>Fax: 609-452-9486</td>
</tr>
<tr>
<td>Date:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-mail: <a href="mailto:Jeannine.Delwiche@Firmenich.com">Jeannine.Delwiche@Firmenich.com</a></td>
<td></td>
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<tr>
<td>Phone:</td>
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<tr>
<td>Co-Investigator</td>
<td>Name: Jennifer K. Balitsis</td>
<td>Phone: 614-247-6831</td>
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</tr>
<tr>
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<td>E-mail: <a href="mailto:Balitsis.1@osu.edu">Balitsis.1@osu.edu</a></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Staff</td>
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<td></td>
</tr>
<tr>
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<tr>
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<td>Signature:</td>
<td>Fax: 614-292-0218</td>
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<table>
<thead>
<tr>
<th>Protocol Title</th>
<th>Dose-response curve of sodium chloride on vegetable palatability</th>
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</thead>
</table>

<table>
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<tr>
<th>Source of Funding</th>
<th>CIFT Grant</th>
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For Office Use Only

- Approval: Research has been determined to be exempt under these categories: ____________________. Research may begin as of the date of determination listed below.
- Disapproval: The proposed research does not fall within the categories of exemption. Submit an application to the appropriate Institutional Review Board for review.

<table>
<thead>
<tr>
<th>Date of determination:</th>
<th>Signature:</th>
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<tr>
<td></td>
<td>Office of Responsible Research Practices</td>
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</table>

The purpose of the Application for Exemption is two-fold: (a) to determine whether the proposed research qualifies for exemption from review and continuing oversight by an Institutional Review Board; and, if so, (b) to ensure that the informed consent process protects the rights and welfare of human subjects in research. Please respond to the following questions and provide the requested documentation.

Have all investigators completed the required web-based course in the protection of human research subjects? ☑Yes ☐No

If No, see [http://orrp.osu.edu/humansubjects/citi.cfm](http://orrp.osu.edu/humansubjects/citi.cfm) for more information. EDUCATIONAL REQUIREMENTS MUST BE SATISFIED PRIOR TO SUBMITTING THE APPLICATION FOR IRB REVIEW.
Please check the categories of exemption for which you are applying. The list of categories is located at the end of this application. You may check more than one box.

**EXEMPT CATEGORY:**

1  2  3  4  5  6

**SCREENING QUESTIONS:** If you check YES to any of the questions below, your research is not exempt. Do not complete the exempt application. Submit an application to the appropriate Institutional Review Board for review.

- Does any part of the research require that subjects be deceived? [ ] Yes [x] No
- Will research expose human subjects to discomfort or harassment beyond levels encountered in daily life? [ ] Yes [x] No
- Could disclosure of the subjects’ responses outside the research reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects’ financial standing, employability, or reputation? [ ] Yes [x] No
- Will fetuses, pregnant women, human in vitro fertilization, or individuals involuntarily confined or detained in penal institutions be subjects of the study? [ ] Yes [x] No
- For research proposed under category 2, will research involve surveys, interview procedures, or observation of public behavior with individuals under the age of 18? [ ] Yes [x] No
- For research proposed under category 4, will any of the data, documents, records, pathological specimens, or diagnostic specimens be collected or come into existence after the date you apply for exemption? [ ] Yes [x] No
- For research proposed under category 4, will any of the information obtained from data, documents, records, pathological specimens, or diagnostic specimens that come from private sources be recorded by the investigator in such a manner that subjects can be identified directly or through identifiers linked to the subjects? [ ] Yes [x] No

If you checked YES to any of the questions above, your research is not exempt.

If you have checked NO to all of the questions above, your research may be exempt. Please continue with the exempt application.

If you have questions about the application or review process, please contact Janet Schulte, Office of Responsible Research Practices. Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu
For purposes of this application, “research” includes the recruitment of human subjects as well as data collection and analysis. None of these research activities may begin until the investigator has received a protocol number AND has received written concurrence that the proposed research is exempt. The “date of determination” on page one of this application is assigned by the Office of Responsible Research Practices; it indicates the date when research may begin.

Please describe your study clearly and completely, using a style of language that can easily be understood by someone who is not familiar with your research.

**GENERAL QUESTIONS REGARDING THE PROPOSED RESEARCH**

1. **Describe the purpose of the research activity to be undertaken. Describe how it involves human subjects. Respond in the space provided here, or attach a research proposal and/or grant proposal containing the requested information.**

   **Description:** A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phytonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will extract the optimum level of sodium chloride to encourage higher consumption of vegetables without leading to the over-consumption of sodium chloride.

2. **Provide a brief description of the subjects you plan to recruit and the criteria used in the selection process. Indicate whether subjects are 18 years of age or older.**

   **Description:** Subjects will be recruited from volunteers that are 55 years of age or older who are not pregnant and not nursing. They will be recruited from the Westminster-Thurber retirement Community based on availability and willingness to participate.

3. **Describe how the proposed research meets the criteria for exemption from IRB review and oversight. (Refer to the criteria on the last page of this application that correspond to the category or categories you checked on the screening sheet.)**

   **Description:** This research meets the criteria for exemption under category 6. Subjects will taste wholesome foods and respond to questions about liking and attributes of the foods. Foods tasted will include cooked broccoli with three varying levels of sodium chloride, with the lowest being no added salt and the highest being well below levels typically found in canned vegetables.
4. Will your subjects be recruited through schools, employers, and/or community agencies or organizations, and/or are you required to obtain permission to access data that is not publicly available? If the answer is yes, provide a letter of support from the person authorized to give you access to the subjects or to the data in question. More than one letter may be required.

☐ Does not apply.
☒ Letter(s) attached.
☒ Comments: Subjects will be recruited from the residents of the Westminster-Thurber Community, a continuing care retirement community. A letter of support from the authorized person at Westminster Thurber Community has been attached.

5. Describe the means you will use to obtain data. Check all boxes that apply.

☒ Surveys or questionnaires distributed by mail or in person. I am attaching a copy of the instrument(s).
☐ Surveys distributed through the Internet, through listservs, or through E-mail. I am attaching a copy of the instrument(s). Provide the Internet address:
☐ Interviews. I am attaching a copy of the interview questions.
☐ Focus groups. I am attaching a copy of the questions that will shape the discussion.
☐ Observation of public behavior.
☐ Observation of activities in school classrooms.
☐ Audiotapes. I will obtain consent from the subjects to tape their responses.
☐ Videotapes. I will obtain consent from the subjects to tape their activities or responses.
☐ Review of existing records, including databases, medical records, school records, etc. I am attaching a copy of the data collection sheet. I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All of the information in the records to be reviewed exists as of the date of submission of this application.
☐ Tissue specimens. All of the specimens have already been collected and are “on the shelf.” I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.

6. Indicate the date when you plan to begin research, and the date when you anticipate that data analysis will be complete.

Begin date: 7/1/2007  End date: 7/1/2008
CONFIDENTIALITY

- Investigators are required to protect the confidentiality of the information obtained during research, unless the subjects (a) explicitly agree to be identified or quoted, and/or (b) explicitly agree to the release of material captured on audiotapes or videotapes for use in presentations or conferences.

7. Provide a brief description of the measures you will take to protect confidentiality. Please describe how you will protect the identity of the subjects, their responses, and any data that you obtain from private records or capture on audiotape or videotape. Describe the disposition of the data and/or the tapes once the study has been completed.

Description: No identifying information will be collected.

INFORMED CONSENT

- In most cases, investigators are required to obtain informed consent from their subjects before collecting data. Respond to questions #8 and #9 to indicate how you will inform your subjects about the research and how you will obtain and document their consent.
- Subjects must be told what they will be asked to do if they agree to participate in research, how long it will take, and how you will protect the confidentiality of the information they provide.
- Subjects must be told that their participation is voluntary, they can refuse to answer questions that they do not wish to answer, and they can refuse to participate or they can withdraw at any time without penalty or repercussion.
- With few exceptions, written consent of the child’s parent(s) or guardian(s) is required if subjects are under the age of 18. In addition, children 14 years of age or older should be asked to give written assent (agreement) to participate. Children younger than 13 years of age should be asked to give verbal assent (agreement) to participate.
- Provide a means for subjects to contact the investigator(s) if they have questions or concerns about the research. Make it clear to the subjects that you are affiliated with The Ohio State University.
8. What information do you plan to give to your subjects before you ask for their consent? Use a style of language that simply and clearly explains the research to your subjects. Respond in the space provided here, or attach a copy of the information you plan to provide to your subjects and/or their parents or guardians. (Note: if you use more than one method of recruitment, you may check more than one box)

☐ Letter(s) attached. I will give each of the subjects a copy of this letter.
☐ I will be contacting subjects by phone or in person. I am attaching a script that contains the information I will give them.
☐ Does not apply. My data analysis is limited to existing records or tissue specimens.
☒ Response: Information about the study will be presented on the first page of the questionnaire (see attached questionnaire).

9. How do you plan to document informed consent? Read all of the options before checking the appropriate boxes. (A sample consent form is attached to this application.)

☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to sign a written consent form. I am attaching a copy of the consent form.
☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to give verbal consent to participate in this research study.
☐ The subjects are 18 years of age or older. I am distributing a survey or questionnaire to the subjects. They can choose whether or not they want to respond. I am requesting a waiver of written consent.
☐ The subjects are under the age of 18. I am attaching a copy of the consent form that I will use to obtain consent from their parents or guardians and assent (agreement) from subjects who are 14 years of age or older.
☐ Some of the subjects are 18 years of age or older, and some are younger than 18. I have checked more than one box above to reflect the methods I will use to document informed consent.
☐ Does not apply. My data analysis is limited to existing records or tissue specimens.
☒ Other. Please explain and provide justification for your request. Data will be collected via a paper questionnaire where subjects will indicate their ratings of the samples. Prior to making these ratings, the first page will describe the study and ask panelists to indicate informed consent by checking “Yes, I consent to participate.”
☐ Comments:
Recruitment Letter – for distribution by flyer

PARTICIPANTS NEEDED
Jeannine Delwiche, Principal Investigator

Date: TBA
Time: TBA
Place: Westminster-Thurber Community

This study is designed to look at consumer perception of cooked broccoli. In this study, you will be presented with several samples on a small tray and you will be asked to assess them. At the end, you will be asked a few demographic questions such as gender and age.

Your answers will be indicated directly on the paper questionnaire provided. This has been estimated to take less than 10 minutes but you may take as long as you need. Your participation is voluntary, and you can refuse to answer questions that you do not wish to answer. Also, you can withdraw at any time without penalty or repercussions. Your responses will in no way be linked to your identity and you will be compensated with candy.

If you have any questions or concerns please contact Jennifer Balitsis at Balitsis.1@osu.edu, 614-247-6831. Graduate Research Assistant with The Ohio State University.
PROPOSAL INFORMATION

Title: Dose-response curve of sodium chloride on vegetable palatability

Summary Statement

A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phytonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will develop an experimental model to quantitatively measure the amount of salt required to suppress bitterness in vegetables and increase palatability by building a dose-response curve. From this curve, it will be possible to extract the optimum level of sodium chloride to encourage higher consumption of vegetables without leading to the over consumption of sodium chloride.
Dose-response curve of sodium chloride on vegetable palatability

Submitted by: The Ohio State University and the Salt Institute

Background: In an effort to encourage lower sodium consumption, UK policy makers have removed salt shakers from school lunchrooms. As a result, students are not only eating less sodium but also, unfortunately, fewer vegetables. It is well established that most children need to increase their vegetable consumption, thus the approach of complete sodium elimination seems misguided. The use of a limited amount of sodium to create vegetables that taste good will do far more to promote an optimal diet than does eliminating the likely small amount of sodium it would require to make vegetables palatable. Therefore a more pragmatic solution is to find the optimal level of salt at which people will be more likely to eat their vegetables. Knowing the small amount of salt required to make vegetables palatable to will encourage US policy makers to consider the inclusive benefit/risk package before repeating the actions of their UK counterparts. The establishment of such a dose-response curve also has the potential to serve as a practical guide in the formulation of restaurant and processed foods.

Objective: The objective of this study is to determine the optimal level of salt use in order to increase the likelihood that children will eat their vegetables in school lunches.

Approach: Consumer liking assessments will be made of frozen broccoli, cooked and prepared with salt levels varying from no added salt to 350 mg sodium/serving (the typical level in canned vegetables). Assessments of 5 levels of sodium will be made by 75-100 individuals at the Ohio State University on-site location. From this preliminary testing, the candidate level of optimum sodium level will be refined and subsequent testing of preferred levels will be conducted with students at a grade school, as well as individuals in a retirement community.

Deliverables to the Salt Institute: Determination of the appropriate level of salt to use in order to increase vegetable palatability for three age-groups.

Budget:

Labor: $22,000
    (Technician and graduate student salary/benefits/tuition)

Supplies: $  3,000

Total: $25,000
This study is designed to look at consumer perception of cooked broccoli. In this study, you will be presented with several samples on a small tray and you will be asked to assess them. At the end, you will be asked a few demographic questions such as gender and age.

You will mark your answers directly on your paper questionnaire. This has been estimated to take less than 10 minutes but you may take as long as you need. Your responses will in no way be linked to your identity and you will be compensated with candy at the end. If you have any questions, please feel free to ask the attendant at any time.

If you wish to participate, please read the following statement and indicate your consent to participate.

<table>
<thead>
<tr>
<th>INFORMED CONSENT STATEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I understand the purpose, procedures and time requirements of this study. All questions have been answered to my satisfaction. I may withdraw at any time without penalty. I am 18 years of age or older. I am not pregnant and not nursing. I freely and voluntarily give my consent to participate by circling 'YES' below.</td>
</tr>
<tr>
<td>YES no</td>
</tr>
</tbody>
</table>

<a tray should now be placed in front of you containing 3 samples of different broccoli preparations for flavor evaluation, if not, please inform the attendant that you need your samples>

Please rinse your mouth 3 times with water. Now you will TASTE the samples from left to right and answer the following questions… (Please don’t eat your entire broccoli at once as you will need to answer three questions about each sample)

Please rate your OVERALL LIKING of each sample on the following scale. Please rinse your mouth 2 times between each sample.
Please rinse your mouth 3 times with water.
Now you will TASTE the same samples from left to right and answer the following questions...

Please rate the SALTINESS of each sample and your ‘perceptual IDEAL’ on the following scale.
Please rinse your mouth 2 times between each sample.

<table>
<thead>
<tr>
<th>Sample 574</th>
<th>dislike extremely much</th>
<th>dislike much</th>
<th>dislike moderately much</th>
<th>dislike slightly</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like much</th>
<th>like extremely</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 358</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please rinse your mouth 3 times with water.
Now you will TASTE the same samples from left to right and answer the following questions...

Please rate the BITTERNESS of each sample and your ‘perceptual IDEAL’ on the following scale.
Please rinse your mouth 2 times between each sample.

<table>
<thead>
<tr>
<th>Sample 574</th>
<th>Not salty</th>
<th>Very salty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 562</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 358</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDEAL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Please indicate your gender.

- Male
- Female

Please indicate your age category.

- 55-59 years
- 60-64 years
- 65-69 years
- 70-74 years
- 75-79 years
- 80-84 years
- 85 years and over
Project: Preferred Salt Level in Broccoli for an Older Population Design

Plan:

Description: Copy of All Possible Permutations
Type: Quantitative Descriptive
Samples: 3
Presented: 3
Blocks: 100 [Base Block]
  \[1 \times 1 = 100\] [Entire Block]

Options:

Blinding Codes: Constant
Sample Randomization: Yes
Block Randomization: No

Registration: Panelists Will NOT Register
Sample Set Distribution: Panelist Will Indicate Sample Set Received

Sessions:

Number of Sessions: 1

Samples:

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Blinding Codes for Session 1

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## Layout for Session 1

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</table>
APPENDIX C

IRB APPLICATION, BALLOT, AND EXPERIMENTAL DESIGN

PREFERRED SALT LEVEL OF CHILDREN FOR BROCCOLI
APPLICATION FOR EXEMPTION
FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

All research activities involving the use of human beings as research subjects must be reviewed and approved by an Ohio State University Institutional Review Board (IRB), unless the Office of Responsible Research Practices (ORRP) determines that the research falls into one or more of the categories of exemption established by federal regulation.

Exempt research is generally short term in nature. It must be performed “as written,” i.e. the investigators do not make changes in the research design, the selection of subjects, the informed consent process, or the instrumentation during the course of the study. If changes are necessary, re-application is required.

A determination that research is exempt does not absolve the investigators from ensuring that the welfare of human subjects participating in research activities is protected, and that methods used and information provided to gain subject consent are appropriate to the activity. Investigators may not solicit subject participation or begin data collection until they have received approval from the appropriate Institutional Review Board OR written concurrence that research has been determined to be exempt.

All OSU Investigators who participate in human subjects research must be appropriately trained in human subjects protection. See http://orrp.osu.edu/humansubjects/training.cfm for more details.

There is no deadline or timeline for submitting exempt applications for review. Applications are processed as received. Each application must include a research proposal. The proposal must include (at a minimum) the following items: the background literature review, the research question, a description of the research methods including sample size and data collection procedures, and a data analysis plan.

Please allow up to three weeks for processing.

If you have questions regarding the application process or the review of exempt protocols, please contact Janet Schulte, Office of Responsible Research Practices.
Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu

A COMPLETE APPLICATION PACKET INCLUDES THE FOLLOWING MATERIALS:
☐ Title page (attached). Identifies the investigators. Lists the protocol title and the source of funding.
☐ Screening questions (attached). Identifies the categories of exemption and solicits responses to screening questions.
Description of the proposed research (questions #1 through #9, attached). Includes responses to questions about the objective(s) of the research, the methodology that will be used to gain informed consent from the subjects, and the measures taken to protect the confidentiality of information obtained in research.

Research proposal (see question #1).
Grant proposal. Must be included when externally-sponsored funding is being sought.
Letter(s) of support (see question #4).
Copies of surveys, instruments, questionnaires, interview questions, focus group topics, and/or data collection sheets (see question #5).
Recruitment letter (see question #8).
Consent form (see question #9).

SEND ONE COPY OF YOUR APPLICATION TO:
Office of Responsible Research Practices
300 Research Foundation Building
1960 Kenny Road
Columbus OH 43210-1063
Fax (614) 688-0366

TITLE PAGE - APPLICATION FOR EXEMPTION FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

Principal Investigator
University Title:
☐ Professor
☒ Associate Professor
☐ Assistant Professor
☐ Instructor
☐ Other. Please specify. (May require prior approval.)

Name: Ken Lee
Department or College: Food Science & Technology
Campus Address (room, building, street address):
110 Parker Food Science Building
2015 Fyffe Road
Columbus, OH 43210

Signature:
Date:
Phone:
614-292-7797
E-mail:
Lee.133@osu.edu
Fax:
614-292-0218
<table>
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<tr>
<th>Co-Investigator</th>
<th>Name: Jeannine F. Delwiche</th>
<th>Phone: 609-580-4301</th>
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<td>Campus Address (room, building, street address) or Mailing Address: Firmenich Incorporated P.O. Box 5880 Princeton, NJ 08543</td>
</tr>
<tr>
<td>Staff</td>
<td>Graduate Student</td>
<td>Firmenich Incorporated P.O. Box 5880 Princeton, NJ 08543</td>
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<tr>
<td>Undergraduate</td>
<td>Student</td>
<td>E-mail: <a href="mailto:Jeannine.Delwiche@Firmenich.com">Jeannine.Delwiche@Firmenich.com</a></td>
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<td></td>
<td>Signature:</td>
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<tr>
<td></td>
<td>Date:</td>
<td>Fax: 609-452-9486</td>
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<th>Co-Investigator</th>
<th>Name: Jennifer Balitsis</th>
<th>Phone: 614-247-6831</th>
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<tr>
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<td>Campus Address (room, building, street address) or Mailing Address: 110 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210</td>
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| Protocol Title | Effects of sodium chloride on children’s liking of cruciferous vegetables |

| Source of Funding | CIFT Grant |

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<tr>
<td>□ Approved.</td>
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<tr>
<td>□ Disapproved.</td>
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<tr>
<td>Date of determination:</td>
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Office of Responsible Research Practices
The purpose of the Application for Exemption is two-fold: (a) to determine whether the proposed research qualifies for exemption from review and continuing oversight by an Institutional Review Board; and, if so, (b) to ensure that the informed consent process protects the rights and welfare of human subjects in research. Please respond to the following questions and provide the requested documentation.

**Have all investigators completed the required web-based course in the protection of human research subjects?**

- [ ] Yes
- [x] No

If No, see [http://orrp.osu.edu/humansubjects/citi.cfm](http://orrp.osu.edu/humansubjects/citi.cfm) for more information.

EDUCATIONAL REQUIREMENTS MUST BE SATISFIED PRIOR TO SUBMITTING THE APPLICATION FOR IRB REVIEW.

Please check the categories of exemption for which you are applying. The list of categories is located at the end of this application. You may check more than one box.

<table>
<thead>
<tr>
<th>EXEMPT CATEGORY:</th>
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**SCREENING QUESTIONS:** If you check **YES** to any of the questions below, your research is not exempt. Do not complete the exempt application. Submit an application to the appropriate Institutional Review Board for review.

<table>
<thead>
<tr>
<th>Question</th>
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<th>No</th>
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<tbody>
<tr>
<td>Does any part of the research require that subjects be deceived?</td>
<td></td>
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<tr>
<td>Will research expose human subjects to discomfort or harassment beyond levels encountered in daily life?</td>
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<tr>
<td>Could disclosure of the subjects’ responses outside the research reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects’ financial standing, employability, or reputation?</td>
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<tr>
<td>Will fetuses, pregnant women, human <em>in vitro</em> fertilization, or individuals involuntarily confined or detained in penal institutions be subjects of the study?</td>
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<tr>
<td>For research proposed under category 2, will research involve surveys, interview procedures, or observation of public behavior with individuals under the age of 18?</td>
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<tr>
<td>For research proposed under category 4, will any of the data, documents, records, pathological specimens, or diagnostic specimens be collected or come into existence after the date you apply for exemption?</td>
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</table>
For research proposed under category 4, will any of the information obtained from data, documents, records, pathological specimens, or diagnostic specimens that come from private sources be recorded by the investigator in such a manner that subjects can be identified directly or through identifiers linked to the subjects?

☐ Yes  ☒ No

IF YOU CHECKED YES TO ANY OF THE QUESTIONS ABOVE, YOUR RESEARCH IS NOT EXEMPT.

IF YOU HAVE CHECKED NO TO ALL OF THE QUESTIONS ABOVE, YOUR RESEARCH MAY BE EXEMPT. PLEASE CONTINUE WITH THE EXEMPT APPLICATION.

If you have questions about the application or review process, please contact Janet Schulte, Office of Responsible Research Practices. Phone: 688-0389 / Fax: 688-0366 / E-mail: schulte.58@osu.edu

For purposes of this application, “research” includes the recruitment of human subjects as well as data collection and analysis. None of these research activities may begin until the investigator has received a protocol number AND has received written concurrence that the proposed research is exempt. The “date of determination” on page one of this application is assigned by the Office of Responsible Research Practices; it indicates the date when research may begin.

Please describe your study clearly and completely, using a style of language that can easily be understood by someone who is not familiar with your research.

---

**GENERAL QUESTIONS REGARDING THE PROPOSED RESEARCH**

1. **Describe the purpose of the research activity to be undertaken. Describe how it involves human subjects. Respond in the space provided here, or attach a research proposal and/or grant proposal containing the requested information.**

**Description:** A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phyttonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will examine the impact of 2 low salt concentrations versus no salt on liking of broccoli as an indicator of consumption likelihood.
2. **Provide a brief description of the subjects you plan to recruit and the criteria used in the selection process. Indicate whether subjects are 18 years of age or older.**

**Description:** Subjects will be recruited from volunteers attending Scarlet & Gray Agriculture Day that are under 18 years of age and have consent forms signed by themselves and their parents. They will be recruited based on scheduling of this event and willingness to participate.

3. **Describe how the proposed research meets the criteria for exemption from IRB review and oversight. (Refer to the criteria on the last page of this application that correspond to the category or categories you checked on the screening sheet.)**

**Description:** This research meets the criteria for exemption under category 6. Subjects will taste wholesome foods and respond to questions about liking or preference of the foods. Foods tasted will include cooked broccoli with varying levels of sodium chloride (maximum 350 mg of salt per serving, far less than the amount found in canned vegetables).

4. **Will your subjects be recruited through schools, employers, and/or community agencies or organizations, and/or are you required to obtain permission to access data that is not publicly available? If the answer is yes, provide a letter of support from the person authorized to give you access to the subjects or to the data in question. More than one letter may be required.**

- [x] Does not apply.
- [ ] Letter(s) attached.

**Comments:** Subjects will be recruited from volunteers attending Scarlet & Gray Agriculture Day that are under 18 years of age (ages 8-12 are being targeted) and who have consent forms signed by both themselves and their parents. They will be recruited based on scheduling of this event and willingness to participate.
5. Describe the means you will use to obtain data. Check all boxes that apply.

☒ Surveys or questionnaires distributed by mail or in person. I am attaching a copy of the instrument(s).
☐ Surveys distributed through the Internet, through listservs, or through E-mail. I am attaching a copy of the instrument(s). Provide the Internet address:
☐ Interviews. I am attaching a copy of the interview questions.
☐ Focus groups. I am attaching a copy of the questions that will shape the discussion.
☒ Observation of public behavior.
☐ Observation of activities in school classrooms.
☐ Audiotapes. I will obtain consent from the subjects to tape their responses.
☐ Videotapes. I will obtain consent from the subjects to tape their activities or responses.
☐ Review of existing records, including databases, medical records, school records, etc. I am attaching a copy of the data collection sheet. I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All of the information in the records to be reviewed exists as of the date of submission of this application.
☐ Tissue specimens. All of the specimens have already been collected and are “on the shelf.” I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.

6. Indicate the date when you plan to begin research, and the date when you anticipate that data analysis will be complete.

Begin date: 5/18/2007 End date: 5/18/2008
CONFIDENTIALITY

- Investigators are required to protect the confidentiality of the information obtained during research, unless the subjects (a) explicitly agree to be identified or quoted, and/or (b) explicitly agree to the release of material captured on audiotapes or videotapes for use in presentations or conferences.

7. Provide a brief description of the measures you will take to protect confidentiality. Please describe how you will protect the identity of the subjects, their responses, and any data that you obtain from private records or capture on audiotape or videotape. Describe the disposition of the data and/or the tapes once the study has been completed.

   Description: No identifying information will be collected.

INFORMED CONSENT

- In most cases, investigators are required to obtain informed consent from their subjects before collecting data. Respond to questions #8 and #9 to indicate how you will inform your subjects about the research and how you will obtain and document their consent.
- Subjects must be told what they will be asked to do if they agree to participate in research, how long it will take, and how you will protect the confidentiality of the information they provide.
- Subjects must be told that their participation is voluntary, they can refuse to answer questions that they do not wish to answer, and they can refuse to participate or they can withdraw at any time without penalty or repercussion.
- With few exceptions, written consent of the child’s parent(s) or guardian(s) is required if subjects are under the age of 18. In addition, children 14 years of age or older should be asked to give written assent (agreement) to participate. Children younger than 13 years of age should be asked to give verbal assent (agreement) to participate.
- Provide a means for subjects to contact the investigator(s) if they have questions or concerns about the research. Make it clear to the subjects that you are affiliated with The Ohio State University.

8. What information do you plan to give to your subjects before you ask for their consent? Use a style of language that simply and clearly explains the research to your subjects. Respond in the space provided here, or attach a copy of the information you plan to provide to your subjects and/or their parents or guardians. (Note: if you use more than one method of recruitment, you may check more than one box)

   ✗ Letter(s) attached. I will give each of the subjects a copy of this letter: letters will go home for parents before the event and they will be asked to indicate consent by signing.
I will be contacting subjects by phone or in person. I am attaching a script that contains the information I will give them: The procedure will be explained to the children the day of the taste panel. Since they will be under 13 years old, verbal assent will be required before participating.

☐ Does not apply. My data analysis is limited to existing records or tissue specimens.

☒ Response: Information about the study will be presented on the first screen of the Compusense® data collection software (see attached questionnaire).

9. How do you plan to document informed consent? Read all of the options before checking the appropriate boxes. (A sample consent form is attached to this application.)

☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to sign a written consent form. I am attaching a copy of the consent form.

☐ The subjects are 18 years of age or older. Before collecting data, I will ask them to give verbal consent to participate in this research study.

☐ The subjects are 18 years of age or older. I am distributing a survey or questionnaire to the subjects. They can choose whether or not they want to respond. I am requesting a waiver of written consent.

☒ The subjects are under the age of 18. I am attaching a copy of the consent form that I will use to obtain consent from their parents or guardians and assent (agreement) from subjects who are 14 years of age or older.

☐ Some of the subjects are 18 years of age or older, and some are younger than 18. I have checked more than one box above to reflect the methods I will use to document informed consent.

☐ Does not apply. My data analysis is limited to existing records or tissue specimens.

☒ Other. Please explain and provide justification for your request. Data will be collected via Compusense® software where subjects will indicate their ratings of the samples. Prior to making these ratings, a screen will describe the study and ask panelists to indicate informed consent by clicking “Yes, I consent to participate.”

☐ Comments:
SCARLET & GRAY AGRICULTURE DAY: SENSORY SCIENCE PANEL

On May 18th, your child will have the opportunity to participate in and learn about the importance of scientific research at the University level. The objective of this study is to determine the optimal preparation, with the use of salt, in order to increase the likelihood that children will eat their vegetables in school lunches. A universal recommendation to improve the overall nutritional status of all individuals is the greater consumption of vegetables. Bitterness is a major determinant of a vegetable’s palatability and a major reason for rejection. Ironically, the same vegetables that are the highest in phytonutrients and thus the best for us are often the vegetables that are highest in bitterness. While sodium chloride has been demonstrated to be an effective bitter suppressant, nutritional recommendations caution against the over-consumption of sodium chloride. This study will develop an experimental model to quantitatively measure the amount of salt required to suppress bitterness in vegetables and increase palatability. The information derived from this study will help to extract the optimum level of sodium chloride to encourage higher consumption of vegetables without leading to the over consumption of sodium chloride.

Your child will be asked to taste three broccoli samples that have been prepared differently (maximum of 350 mg of salt per serving, far less than the level of salt in most canned vegetables). They will then be asked to choose which broccoli sample they like the most and which broccoli sample they like the least. They will then be asked their gender and age. Their answers will be entered directly into a computer using a mouse and/or keyboard. Their personal information will be kept confidential at all times. Your child may choose to quit the study at any point in time if they feel uncomfortable. This should take approximately 5 minutes but they may take as much time as they need. At the conclusion of the study, the child will be allowed to take one of the serving cups of broccoli (his/her choice). If you or your child has any questions, please feel free to ask at any time, you may contact the researchers at lee.133@osu.edu, delwiche.1@osu.edu, or balitsis.1@osu.edu.

CONSENT FOR PARTICIPATION IN RESEARCH
I consent to participating in (or my child's participation in) research entitled: Effects of Sodium Chloride on Children’s Liking of Cruciferous Vegetables. The purpose of the study, the procedures to be followed, and the expected duration of my (my child’s) participation have been described for me. Possible benefits of the study have also been described above.
I acknowledge that I have had the opportunity to obtain additional information regarding the study through the contact information provided above and that any questions I have raised have been answered to my full satisfaction. Furthermore, I understand that I am (my child is) free to withdraw consent at any time and to discontinue participation in the study without prejudice to me (my child).

Finally, I acknowledge that I have read and fully understand the consent form. I sign it freely and voluntarily.

Parent print name_________________________________ Parent Sign name_________________________________

Child print name_________________________________ Child sign name_________________________________
Questionnaire:

Welcome to Sensory Testing!
Ken Lee & Jeannine Delwiche, Principal Investigators

In this study, you will be presented with three samples on a small tray and you will be asked to choose the one you like the most, and the one you like the least. At the end, you will be asked your gender and age.

Your answers will be entered directly into the computer using the mouse and/or keyboard. This should take less than 5 minutes, but you may take as long as you need. Your responses will in no way be linked to your identity and you will be compensated with candy at the end. If you have any questions, please feel free to ask the attendant at any time.

If you wish to participate, please read the following statement and indicate your consent to participate.

INFORMED CONSENT STATEMENT

I understand the purpose, procedures and time requirements of this study. All questions have been answered to my satisfaction. I may withdraw at any time without penalty. I freely and voluntarily give my consent to participate by circling 'YES' below.

YES  no

<present a tray containing 3 samples of different broccoli preparations for evaluation>

Please rinse your mouth 3 times with water.
Now you will TASTE the samples from left to right and answer the following question…

Please choose, by clicking with your mouse, the broccoli sample that you like the MOST. Please rinse your mouth 2 times between each cup of broccoli.

647  513  263
□  □  □  My FAVORITE

Please choose, by clicking with your mouse, the broccoli sample that you like the LEAST. Please rinse your mouth 2 times between each cup of broccoli.

647  513  263
□  □  □  My LEAST favorite
Please indicate your gender.

- Male
- Female

Please indicate your age category.

- 8 years
- 9 years
- 10 years
- 11 years
- 12 years
- 13 years

Thank You for Participating! Please feel free to take ONE (but ONLY ONE) of the serving cups with you and let a researcher know if you would like your cup refilled. (Choice made by subject will be recorded)
Project: Preferred Salt Level in Broccoli for Children Design

Plan:

Description: All Possible Combinations  
Type: Quantitative Descriptive  
Samples: 3  
Presented: 3  
Blocks: 100 [Base Block]  
\[ \times 1 \text{ [Factor]} \]  
\[ = 100 \text{ [Entire Block]} \]

Options:

Blinding Codes: Constant  
Sample Randomization: Yes  
Block Randomization: No  
Registration: Panelists Must Register  
Sample Set Distribution: Bind Sample Sets to Panelists

Sessions:

Number of Sessions: 1

Samples:

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Blinding Codes for Session 1

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