Individual Differences in the Efficacy of Sodium Chloride and Sucrose as Bitterness Suppressors of Brassicaceae Vegetables

by

Lynn Melissa Wilkie

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Graduate Supervisory Committee:

Elizabeth D. Phillips, Chair
Carol Johnston
Federico Sanabria
Adam Cohen

ARIZONA STATE UNIVERSITY

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ABSTRACT

The unpleasant bitter taste found in many nutritious vegetables may deter their consumption. While bitterness suppression by prototypical tastants is well-studied in the chemical and pharmacological fields, mechanisms to reduce the bitterness of foods such as vegetables remain to be elucidated. Here tastants representing the taste primaries of salty and sweet were investigated as potential bitterness suppressors of three types of Brassicaceae vegetables. The secondary aim of these studies was to determine whether the bitter masking agents were differentially effective for bitter-sensitive and bitter-insensitive individuals.

In all experiments, participants rated vegetables plain and with the addition of tastants. In Experiments 1-3, sucrose and NNS suppressed the bitterness of broccoli, Brussels sprouts, and cauliflower, whereas NaCl did not. Varying concentrations of NaCl and sucrose were introduced in Experiment 4 to assess the dose-dependency of the effects. While sucrose was a robust bitterness suppressor, NaCl suppressed bitterness only for participants who perceived the plain Brussels sprouts as highly bitter. Experiment 5, through the implementation of a rigorous control condition, determined that some but not all of this effect can be accounted for by regression to the mean. Individual variability in taste perception as determined by sampling of aqueous bitter, salty, and sweet solutions did not influence the degree of suppression by NaCl or sucrose.

Consumption of vegetables is deterred by their bitter taste. Utilizing tastants to mask bitterness, a technique that preserves endogenous nutrients, can circumvent this issue. Sucrose is a robust bitter suppressor whereas the efficacy of NaCl is dependent upon bitterness perception of the plain vegetables.

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CHAPTER 1 – INTRODUCTION

Consumption of vegetables is critical to maintaining a healthy diet, lifestyle, and weight. Vegetables are an important source of phytonutrients (Murphy et al., 2012) and antioxidants. A recent review (Boeing et al., 2012) concluded there is sufficient evidence that consumption of vegetables and fruits reduces the risk of hypertension, coronary heart disease, stroke, and cancer (Birt, Hendrich, & Wang, 2001; van Duijnhoven et al., 2009). Despite these nutritional benefits, Americans consume only 41% of the 3-5 daily vegetable servings recommended by the 2010 Dietary Guidelines for Americans (USDA and USHSS, 2011).

One deterrent to vegetable consumption is their bitter taste (Dinehart et al., 2006), which may be caused by the phytonutrients (Roland et al., 2011) and other anti-carcinogenic compounds (Mithen et al., 2000). While taste is not the only factor considered in food decisions, it is often rated as most important (Glanz et al., 1998) and is the first way that foods are categorized (Connors et al., 2001). Interventions are needed to make vegetables taste more palatable and less bitter, and thereby increase their likelihood of consumption. Bitter suppression is also a desirable goal for other fields, such as the pharmaceutical industry, where the bitter taste of many drugs limits compliance of patients (Kumar et al., 2012; Ley, 2008; Mennella, Pepino, & Beauchamp, 2003).

Removing bitter tastants from vegetables via processing or selective breeding can be counterproductive because the nutrients are often eliminated concurrently (Reed & Knaapila, 2010). Instead, the addition of bitter masking agents would maintain endogenous health benefits while modulating taste and palatability. An additional advantage to the use of bitterness masking agents is that they may eventually become

unnecessary, as learning paradigms suggest that associating the flavor of the vegetables with a palatable taste will condition a preference for the plain vegetables over time (Holman, 1975; Yeomans, Gould, Mobini, & Prescott, 2008a; Zellner et al., 1983).

While the masking of bitter chemicals by prototypical tastants, particularly sodium salts and sweeteners, is well-studied (Kamen et al., 1961; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994; Breslin & Beauchamp, 1995; Prescott et al., 2001; Mennella et al., 2003; Keast et al., 2004b; Keast, 2008), there is less research applying these findings to healthy bitter foods (Sun-Waterhouse & Wadhwa, 2013). Here the goal is to determine the efficacy of these stimuli as bitterness suppressors of the complete food matrix of bitter vegetables. The secondary aim is to determine whether these tastants were differentially effective for bitter-sensitive and bitter-insensitive individuals. By administering nutritious taste stimuli and accounting for individual differences, these studies offer a personalized and practical extension of the chemical literature to improve the health profile of the diet.

Taste

Taste is the conscious experience produced when a ligand binds to its taste receptor cell (TRC). Taste is referred to as the 'nutritional gatekeeper' because the sensory input causes behavioral responses such as consumption or rejection that ultimately determine what is and is not allowed into the organism's body.

There has been debate about the exact nature of the sense of taste. Some argue that taste is a synthetic sense (Erickson, 1982; Erickson & Covey, 1980; Schiffman, McElroy, & Erickson, 1980) whereby stimuli synthesize into an experience that cannot be broken down into the sum of its parts. However, the prevailing view is that taste is an

analytic sense; a limited number of taste primaries combine to elicit multifaceted sensations (Bartoshuk, 1979; Breslin, Beauchap, & Pugh, 1996; Mattes, 2009). To be considered a taste primary, a sensation must be initiated through depolarization of specially-tuned TRCs and must not be able to be produced by a combination of other tastes (Mattes, 2009). Currently there are five accepted taste primaries: salty, sweet, sour, bitter, and umami. Taste perception of any food is based on the sensory integration of these five basic tastes, which are believed to each serve an evolutionary purpose of seeking nutrients or avoiding poisons (Chaudhari & Roper, 2010).

Foods are perceived as highly complex because the brain integrates input from other sensory modalities, resulting in *flavor* (Yarmolinsky, Zuker, & Ryba, 2009). Flavor perception and preference can be influenced by visual cues such as color (Spence et al., 2010), tactile cues such as viscosity (Kokini, 1987), somatosensory cues such as astringency (Negri, Morini, & Greco, 2011), olfactory cues such as aroma volatiles (Kader, 2008), and cognitive factors such as sensory expectations (Tuorila et al., 1998).

Taste Primaries.

Salt. A salty taste is elicited from sodium cations and anions, with common compounds including sodium chloride (NaCl), sodium acetate (NaAc), and sodium gluconate (Na-gluconate). Lithium salts such as zinc sulfate are also generally perceived as salty (Keast & Breslin, 2005).

Human infants cannot detect the taste of salt until around four months of age (Beauchamp, Cowart, & Moran, 1986). In adults, whether salt is perceived as palatable depends on several factors. Moderate salt is preferable to low or high concentrations (Flynn, Schulkin, & Havens, 1993; Stone & Pangborn, 1990), but which absolute

concentration is perceived as moderate varies between individuals (Hayes, Sullivan, & Duffy, 2010). Dietary history and current biological need are also influential. During short-term sodium depletion, preference for salty foods and preferred concentration rise (Beauchamp et al., 1990), as does the dopaminergic reward response to salt stimuli (Chang et al., 1988; McCaughey & Scott, 1998). However, five months on a low-salt diet reduced hedonic ratings of salty stimuli and preferred concentration of salt on crackers and in soup by over 80% (Bertino, Beauchamp, & Engelman, 1982).

As sodium is the primary cation in the extracellular fluid (Chandrashekar et al., 2010), it is believed that a positive hedonic response to mild concentrations of salt evolved to ensure a balanced distribution of water and osmotic pressure across the cellular membrane (McCaughey & Scott, 1998) and maintain blood circulation (Chaudhari & Roper, 2010).

Sweet. Sweetness naturally occurs in foods that are rich in simple carbohydrates, and compounds include sucrose, fructose, glucose, lactose, and maltose. Due to health concerns about excessive sucrose consumption there is a market for non-nutritive sweeteners (NNS), which provide a sweet taste with fewer calories. Examples include aspartame, saccharin, sucralose, acesulfame potassium, and neotame.

The taste of sweet is almost universally preferred, most likely because the sensory system adapted to detect and prefer sugars due to their energy content. A hedonic reward from the taste (Ventura & Mennella, 2011) would incentivize consumption of this fuel. Infants, even those born premature, exhibit a liking for sweet taste as measured either behaviorally by voluntary consumption (Desor, Maller, & Turner, 1973) or hedonically by a display of positive facial reactivity patterns (Steiner et al., 2001), demonstrating the

powerful and innate draw to sweetness. On average, children prefer a higher concentration of sucrose solution than do adults (Mennella et al., 2011). Ventura and Mennella (2011) speculate that this age-based preference reflects a greater need for consistent energy intake during the developmental period.

In adults, while sweet taste is generally regarded as palatable, there is wide individual variability in preferred concentration. The population has been categorized into three distinct groups based on the relationship of sucrose concentration to reported liking (Yeomans et al., 2007; Bartoshuk et al., 2006). One group, typically referred to as 'sweet likers,' exhibits a monotonic rise in hedonic ratings with increasing sweetness (Bartoshuk et al., 2006). In other words, the 'sweeter the better' function found in childhood persists into adulthood. A second group, which comprises the majority of adults, displays an inverted U-shape where liking increases with concentration until a certain point, above which the taste becomes too sweet and liking decreases (Yeomans et al., 2007; Bartoshuk et al., 2006). The apex of this function, however, is determined individually and can vary widely. Lastly, some participants show a monotonic decrease in hedonic ratings with increasing sweetener (Yeomans et al., 2007).

Sour. Sour taste reflects the acid content of a food (Chaudhari & Roper, 2010) and whether it is perceived as palatable depends highly on its concentration and context (Reed & Knaapila, 2010). Distaste for highly sour foods is very common and possibly evolved as a deterrent to the consumption of unripe fruits and a way to maintain a homeostatic acid-base balance (Chaudhari & Roper, 2010). Examples of sour-tasting compounds include citric acid, tartaric acid, and ascorbic acid.

Bitter. The taste of bitter can be elicited by the largest and most diverse group of chemical compounds of any of the taste primaries. Tastants include quinine hydrochloride (QHCl), phenylthiocarbamide (PTC), 6-*n*-propylthiouracil (PROP), urea, and denotonium benzoate.

Infants and non-human primates exhibit similar negative facial reactivity patterns (Berridge, 2000) to bitterness in proportion to their phylogenetic relation (Steiner et al., 2001). The rejection of bitterness is considered innate and even potentially a reflex due to its presence in anencephalic infants (Steiner, 1973) and decerebrate rats (Grill & Norgren, 1978). Evolutionarily, the tasting and rejecting of bitter foods serves to protect an organism from plant-based poisons, all of which have a bitter taste (Glendinning, 1994; Chaudhari & Roper, 2010).

However, distaste for bitterness can be modulated or even reversed for selected foods through experience or conditioning. Bitter foods have been shown to become palatable through an association with desirable pharmacological consequences (Beauchamp & Mennella, 2011), pairing with an already-liked taste (Fanselow & Birk, 1982; Johnston et al., 2011), or repeated exposure (Anzman-Frasca et al., 2012), demonstrating the adaptability of innate inclinations to the conditions of the environment.

Umami. Umami, from the Japanese word for *savory*, has only recently been accepted as a basic taste due to the discovery of receptors for its ligands (Nelson et al., 2002; Zhao et al., 2003). It is the taste of L-glutamate (Chaudhari & Roper, 2010) and other amino acids such as monosodium glutamate (MSG), inosine monophosphate (IMP), guanosine monophosphate (GMP), and adenosine monophosphate (AMP) (Fuke & Ueda, 1996). Some speculate that the taste of umami indicates protein content (Chaudhari &

Roper, 2010), although there is currently a lack of consensus as it is present in a diverse group of foods such as seafood, tomatoes, mushrooms, and cheese. Umami is generally unpalatable when tasted in isolation (Fuke & Ueda, 1996; McCabe & Rolls, 2007; Yamaguchi, 1987), yet added MSG increases hedonic ratings of many foods (Roininen et al., 1996; Kemp & Beauchamp, 1994) and has successfully been used as a palatable unconditioned stimulus to increase preference for a novel soup (Yeomans et al., 2008a).

Taste Perception and Physiology. There are approximately 2000-5000 taste buds in the oral cavity (Miller, 1995). Each contains around 100 heterogeneous taste receptor cells (TRCs) and thus responds to all stimuli (Chandrashekar et al., 2006). This finding is in contrast to the idea of the *tongue map*, or topographical segregation of receptors, which was a mistranslation of a German thesis (Hänig, 1901; as cited in Bartoshuk, 1989) and never scientifically evidenced (for an analysis of the controversy see Lindemann, 1999). Individual TRCs housed in the taste buds, in contrast, are specifically tuned to respond to only one of the taste primaries (Roper, 2013).

Localization of TRCs. Epithelial structures called *papillae* contain the taste buds (Yarmolinsky et al., 2009) and are further classified based on location. Fungiform papillae are on the anterior tongue, circumvallate papillae on the posterior, and foliate on the lateral folds (Roper, 2013; Bartoshuk et al., 2006; Yarmolinsky et al., 2009). Papillae have also recently been localized to the palate (Roper, 2013), throat (Bartoshuk et al., 2006), and duodenum (Jang et al., 2007)—in fact, throughout the gastrointestinal (GI) tract, or 'gut' (Negri et al., 2011). While the TRCs in the gut express similar G protein-coupled receptors (GPCRs) to those on the tongue, regulate comparable nutrient transporter expression, and activate the release of relevant hormones and

neurotransmitters (Depoortere, 2013), it is unclear whether the conscious perception of taste can be elicited by stimulation of these cells exclusively (Chaudhari & Roper, 2010).

Three Types of TRCs. TRCs are divided into three unique categories based on their structure and function. Type I cells behave similarly to glia in the nervous system in that their lamellar processes shroud other cells and limit the spread of transmitters (Dvoryanchikov et al., 2009; Pumplin, Yu, & Smith, 1997). Specifically, they degrade the adenosine triphosphate (ATP) released by other cells by synthesizing an ecto-ATPase (Bartel et al., 2006).

Type II (Receptor) cells express GPCRs and operate by metabotropic receptors (Roper, 2013). A ligand binding to a GPCR initiates a series of intracellular processes. G-protein subunits are released (Chandrashekar et al., 2006), which activate PLCβ2, an enzyme that digests plasma membrane phospholipids into IP3 (Roper, 2013). IP3 is a secondary messenger that diffuses to nearby intracellular stores of calcium and binds to its receptor IP3R3, opening its ion channels on the endoplasmic reticulum (Chaudhari & Roper, 2010). The net result is to mobilize and release Ca^{2+} into the cytosol (Simon et al., 2006; Roper, 2007). The release of Ca^{2+} allows the opening of ion channels (Yoshida et al., 2013) to let Na+ depolarize the membrane.

The communication of taste cells remained unidentified for some time, as the majority of TRCs (Yarmolinsky et al., 2009), including Type II cells (Clapp et al., 2004), do not possess synapses. It has recently been discovered that Type II cells secrete ATP onto afferent nerve fibers through pannexin1 hemichannels, the opening of which is triggered by their depolarization (Romanov et al., 2012).

Type III (Presynpatic) cells are unique in that they possess synapses (Yee et al., 2001), although their postsynaptic targets remain unidentified (Roper, 2013). As opposed to ATP, Type III cells release norepinephrine and two inhibitory neurotransmitters, serotonin and gamma-aminobutyric acid (GABA) (Roper, 2013).

Detection of the Taste Primaries. The mechanism for salty taste transduction is currently the least elucidated, but evidence points to the detection of sodium ligands by ionotropic receptors of Type I cells (Roper, 2013). It is hypothesized that Type I cells express epithelial sodium channels (ENaC) (Vandenbeuch, Clapp, & Kinnamon, 2008), which are essential for salt perception and palatability (Chandrashekar et al., 2010). If Type I cells expressed ENaC, Na+ ions could directly permeate the ion channels to depolarize the membrane (Chaudhari & Roper, 2010). Perception of a salty taste might also be elicited by the saturation of Na+ ions into taste buds' interstitial spaces (Rehnberg et al., 1993). Further, a recent study suggests that palatability of salt at low concentrations is mediated through ENaC, but excessively salty stimuli recruit the same pathways that cause the rejection response for sour and bitter tastes (Oka et al., 2013), explaining the inverted-U shaped relationship between concentration and hedonics (Flynn et al., 1993; Stone & Pangborn, 1990).

Type II cells detect sweet, bitter, and umami tastants. Sweet tastants bind to GPCR heterodimers of the receptors T1R2+T1R3 (Morini, Bassoli, & Temussi, 2005), whereas bitter tastants are ligands for a large number of T2R receptors (Chandrashekar et al., 2000). Umami tastants bind to T1R1+T1R3 (Chaudhari, Pereira, & Roper, 2009), although there is increasing evidence that umami taste is merely blunted, not eliminated, in T1R3 or T1R1 knockout (KO) mice (Damak et al., 2003; Kusuhara et al., 2013; Delay

et al., 2006). Kusuhara and colleagues (2013) also unexpectedly found diminished responsiveness to sucrose in T1R1 KO mice, suggesting more interrelatedness between sweet, bitter, and umami than previously thought.

To separate the effects of the receptors and their cells, Mueller and colleagues (2005) engineered mice to have bitter receptors in sweet cells, so that the sweet-sensing cells depolarized for bitter ligands. The mice showed comparable responses to a bitter-tasting liquid as control mice did to a sweet taste. These results indicate that perception of and behavioral responses to sweet and bitter appear to reflect solely the identity of the activated cells as opposed to the receptors or the tastants (Yarmolinsky et al., 2009; Chandrashekar et al., 2006).

Type III cells are directly stimulated by sour tastants (Huang et al., 2008) and activation of these cells is responsible for sour perception. However, Type III cells are also indirectly stimulated by sweet, bitter, and umami tastants *in situ* even though they do not possess receptors for these ligands (Tomchik et al., 2007). The proposed mechanism for this phenomenon is that the ATP secreted by adjacent Type II cells binds to the P2Y4 purinoceptors of Type III cells (Huang et al., 2007). This interaction highlights the importance of cell-cell communication (Roper, 2013).

Brain Pathways. Fungiform papillae are innervated by the chorda tympani (CT), lingual, and greater superficial petrosal nerves, while circumvallate and foliate papillae transmit signals primarily through the glossopharyngeal nerve (Roper, 2013; Bartoshuk et al., 2006; Yarmolinsky et al., 2009). The trigeminal nerve is also crucial to taste transduction, transmitting information on texture, viscosity, and oral burn and pain (Engelen & van der Bilt, 2008).

In primates, the taste-sensing nerves pass through the sensory ganglia and converge in the rostral section of the nucleus of the solitary tract (rNST) in the brainstem, where fibers project to the ventroposterior medial nucleus of the thalamus (VPMpc) (Carleton, Accolla, & Simon, 2010; Yarmolinsky et al., 2009). These fibers terminate in the primary gustatory cortex in the insula, where the neurons project to several areas, including the parabrachial nucleus (PBN), somatosensory cortex, and orbitofrontal cortex (Carleton et al., 2010). These three areas integrate taste inputs with those from other sensory modalities and determine final hedonic value and palatability.

Before such detailed physiological studies (Carleton et al., 2010; Yarmolinsky et al., 2009), the degree of specialization of receptors and nerves was unclear. The labeled line theory speculated that neurons and TRCs responding to each taste quality transmitted information to the brain via separate parallel pathways (Hellekant, Ninomiya, & Danilova, 1998). In contrast, combinatorial coding or across-fiber pattern theory argued that sensations were encoded by broadly-tuned receptors (Smith & Frank, 1993). Although there is behavioral evidence for both (Carleton et al., 2010), the physiological evidence now strongly favors the labeled line theory. There are specially-tuned TRCs (Yarmolinsky et al., 2009) and topographical segregation in the gustatory cortex (Chen et al., 2011) whereby distinctly separate neuronal clusters respond to one unique taste stimuli (Trivedi, 2012). However, the signal utilization of the same nerves and the convergence of these nerves in common brain areas suggest some degree of mild integration.

Bitterness Suppression

Bitterness suppression typically occurs in one of three ways. *Peripheral interactions* involve the tastants and the TRCs, occurring at the level of oral physiology (Keast & Breslin, 2002a; Kroeze & Bartoshuk, 1985; Sharafi et al., 2013). This can be tested with receptor agonists or antagonists and does not require conscious perception of the second tastant (Bennett, Zhou, & Hayes, 2012; Keast & Breslin, 2005). Therefore, it is suggested (Kroeze & Bartoshuk, 1985; Keast & Breslin, 2005) that peripheral interactions are caused by communication among taste receptors before nerve signals are transmitted to the brain. *Central cognitive interactions*, on the other hand, require perception of the tastants (Bennett et al., 2012), indicating involvement of the brain. This can also be referred to as mixture suppression and is typically tested by combining taste stimuli before administration (Keast & Breslin, 2005). Thirdly, bitter blocking compounds modulate the interactions of a subset of TAS2R38 receptors (Greene et al., 2011) by, for example, reducing the release of gustducin, a protein that codes for bitter taste (Gravina et al., 2003).

When two tastants are administered simultaneously, several outcomes are possible (reviewed by Breslin, 1996). Here, we are concerned primarily with whether the intensity of each tastant is increased or decreased from the addition of another. We will refer to these outcomes as *enhancement* and *suppression*, respectively. This is consistent with Keast & Breslin (2002a), who argue that it is difficult to determine whether an interaction is linear or non-linear (in which case, more specific terminology would be necessitated).

Sodium Chloride as a Bitterness Suppressor. The interaction of salty and bitter tastants administered via aqueous solution sampling by human participants is well-documented. When an effect is found, it is always bitterness or saltiness suppression (Kamen et al., 1961; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994; Breslin & Beauchamp, 1995; Prescott et al., 2001; Mennella et al., 2003; Keast et al., 2004; Keast, 2008). Although the finding that salt masks prototypical bitter tastants is relatively robust, the results do not necessarily translate to bitter foods such as vegetables. There are several factors influencing the effectiveness of sodium salts as bitterness suppressors.

First, the type of tastants chosen in the experimental design to represent both bitterness and saltiness create different perceptual reactions (Breslin, 1996; Keast & Breslin, 2002a). NaCl suppresses the bitterness of urea (Breslin & Beauchamp, 1997), potassium chloride (KCl) (Keast, Breslin, & Beauchamp, 2001), and caffeine (Mennella, Pepino, & Beauchamp, 2003), but not magnesium sulfate (MgSO4) (Breslin & Beauchamp, 1995; Keast et al., 2001), iso-α-acids (Yokomukai et al., 1994), or Tetralone (C10H10O) (Mennella et al., 2003). The variability by chemical is possibly due to the taste of bitterness having more transduction mechanisms (Breslin, 1996), receptors (Reed & Knaapila, 2010), molecule ligands (Ley, 2008), and individual variation among receptors (Feeney et al., 2011) than any of the other four basic tastes. Two other sodium salts, NaAc and Na-gluconate, are efficacious bitterness suppressors of urea, QHCl, caffeine, denatonium benzoate, ranitidine, and L-tryptophan (Breslin & Beauchamp, 1995; Mennella et al., 2003; Keast et al., 2004; Keast, 2008). Urea reciprocally suppressed the saltiness of both compounds, whereas QHCl did not (Breslin &

Beauchamp, 1995). Furthermore, NaAc is a more effective bitterness masking agent than NaCl on the same bitter stimuli (Sharafi et al., 2013), indicating the role of the salty tastant as well.

A second factor mediating the relationship between salty and bitter taste stimuli is the concentration of the salt and bitter tastants. Sharafi and colleagues (2013) found that NaAc suppressed the bitterness of Brussels sprouts, kale, and asparagus, whereas NaCl masked bitterness only when presented at a high concentration on kale. In the chemical literature, NaCl suppresses the bitterness of caffeine at high (Breslin & Beauchamp, 1995) but not low (Kamen et al., 1961) concentrations, and amelioride and urea at moderate and high concentrations (Breslin & Beauchamp, 1995). Zinc lactate was found to suppress the bitterness of caffeine at moderate and high concentrations (Keast, 2008), although in general the effect of zinc salts on bitterness is less studied.

Lastly, individual variation in taste perception is an influential variable. Sharafi and colleagues (2013) found that vegetable dislikers (as identified by a survey) reported a greater increase in hedonic ratings when sodium salts were added to vegetables than did vegetable likers. Although the bitterness ratings of the vegetables by each of these groups is not reported, it is well-established that tasting vegetables as more bitter leads to decreased hedonic ratings and consumption (Dinehart et al., 2006; Drewnowski & Gomez-Carneros, 2000; Duffy et al., 2010). Therefore, it is reasonable to speculate that the vegetable dislikers in this study would have perceived high bitterness.

The evidence suggests that sodium salts mask bitterness peripherally. Kroeze and Bartoshuk (1985) employed a split-tongue procedure to measure the degree of suppression when QHCl and NaCl were presented spatially mixed versus spatially

separated by the tongue's midline. The authors found that the bitterness of QHCl was reduced by 66% when mixed with NaCl, but only by 20% when the chemicals were simultaneously applied to separate halves of the tongue (Kroeze & Bartoshuk, 1985). These results suggest that the suppression is primarily peripheral, although with a cognitive component as well. Further evidence for the peripheral suppression of bitterness by sodium salts is the finding that suppression occurs even when saltiness is barely perceived (Keast & Breslin, 2002a).

Keast and colleagues (2001) offer several compelling suggestions for why sodium salts are relatively robust bitter maskers. Sodium might alter affinity for bitter compounds at multiple stages of taste perception by forming a barrier between ligands and their receptors or interfering with intracellular processes (Keast et al., 2001). To these we add a theory that the ecto-ATPase released by Type I cells in response to salty stimuli degrades the ATP released by Type II cells in response to bitter stimuli, diminishing the conscious perception of bitterness by reducing transmission of the signal of bitterness through the nerve fibers. This theory helps explain why reciprocal saltiness suppression by bitter stimuli is also observed, although not why this is less common than bitterness suppression.

Sucrose as a Bitterness Suppressor. Combining sucrose with a bitter tastant consistently results in bitterness suppression (Kamen et al., 1961; Lawless, 1979; Kroeze & Bartoshuk, 1985; Breslin & Beauchamp, 1997, Prescott et al., 2001; Keast, 2008). There are occasions when no effect has been observed (Prescott et al., 2001; Keast, 2008), but these are when one or both of the tastants was given at a low concentration. The exact degree of suppression varies slightly by tastant and concentration, but overall

the relationship is remarkably robust. Additionally, reciprocal sweetness suppression typically occurs when both tastants are administered at relatively high concentrations (Prescott et al., 2001; Keast, 2008).

The blocking of bitter by sweet occurs centrally as opposed to peripherally because sucrose suppresses the bitterness of QHCl the same amount regardless of whether the tastants were mixed together before application or applied to separate halves of the tongue (Kroeze & Bartoshuk, 1985). Further evidence comes from Lawless (1979), who measured the bitterness of a QHCl+sucrose mixture after some participants rinsed with *Gymnema sylvestre*, an herb that exclusively blocks the perception of sweet taste. Participants who received the herb perceived the QHCl+sucrose mixture as equally bitter as did subjects receiving plain QHCl, indicating that the suppression was occurring at the neuronal and not molecular level.

Walters (1996) and Roy (1992) suggest that combining sweetness and bitterness decreases the perceived intensity of both because of a similar transduction mechanism, a concept known as *competitive inhibition*. Recent advances have illuminated the nature of this interaction. Detecting bitter and sweet tastes involves similar neurotransmitters, GPCRs (Margolskee, 2002), and signaling molecules (Zhang et al., 2003). Further, the final effect of both tastants is to mobilize intercellular Ca^{2+} and secrete ATP (Roper, 2013). There might be competition, such that bitter and sweet cannot be tasted at full intensity simultaneously. This theory is further supported by the reciprocal suppression of sweetness by bitter tastants (Lawless, 1979).

Quantifying Individual Differences

Individual Differences in Taste Sensitivity. Variation in the ability to taste the bitter chemical phenylthiocarbamide (PTC) was first discovered by Fox (1931) when a fortuitous accident resulted in some being released into the air. A colleague noted the bitter aftertaste of inhaling it, which Fox did not experience (Fox, 1931). Whether an individual perceives bitterness from PTC or its counterpart 6-*n*-propylthiouracil (PROP) was determined to be a heritable trait (Blakeslee, 1932), but the exact physiology is still not fully understood. Current research suggests a complex interplay of TAS2R38 genotype, bitter receptor expression, and number of fungiform papillae on the tongue (Hayes et al., 2008; Miller & Reedy, 1990; Duffy et al., 2010; Negri et al., 2012).

There is wide variation in sensitivity to PTC and PROP, leading to common measurement of this variable in recent taste perception studies (Khataan et al., 2009; Reed et al., 2010; Herz, 2011; O'Brien et al., 2010). Bitterness ratings of PTC-impregnated filter paper or aqueous solutions are continuous and bimodally distributed (Tepper, 1998). At first (Tepper, 1998) the population was divided into those who found PTC or PROP tasteless, named *nontasters*, and those who could taste any amount of bitterness, named *tasters*. Then, Bartoshuk (1991) recommended further division of the latter group into *moderate tasters* and *supertasters* due to the extreme variability in degree of perceived bitterness among tasters. Nontasters make up approximately 25% of the adult population, moderate tasters 50%, and supertasters 25% (Bartoshuk, 2000). Females are more often supertasters than are males and Caucasians have a lower percentage of supertasters than do Africans and Asians (Bartoshuk, 2000; Tepper & Nurse, 1996). However, there are numerous concerns about dividing participants into

such categories, such as the arbitrariness of the trichotomy (Tepper, 2008) now that it is understood that the trait does not follow a simple pattern of Mendelian inheritance (Drewnowski et al., 1997). While there is a clear distinction between nontasters and supertasters, there are no standard cutoffs for moderate tasters, in part due to the lack of consistency between rating scales (Tepper, 2008).

Development of Rating Scales. Taste is by definition a subjective experience, and the way that investigators quantify the intensity of a taste has evolved immensely. Bartoshuk and colleagues (2002; 2005) argue that assigning verbal descriptors to an experience is insufficient because the adjectives used to describe the intensity, such as 'weak' or 'strong,' are influenced by factors such as the noun to which they are referring, the respondent's personal experiences, and the context of the assessment.

Comparative taste psychology has benefited enormously from the development of numerical rating scales in the field of psychophysics. Hayes and Patterson (1921) developed the graphic rating scale, a continuous line with descriptive phrases printed underneath. Participants made a marking on the line that corresponded with the intensity of their sensory experience. Nine-point categorical Likert scales, with the range of 'none' to 'very strong,' also began to be used around this time and continue to be employed (e.g., Kaminski, Henderson, & Drewnowski, 2000). These scales had the advantage of quantifying the sensation. However, they still relied on adjectives for assessment and were ordinal—that is, a rating of 4 was larger, but not necessarily twice as large, as a rating of 2 (Bartoshuk et al., 2002).

Magnitude estimation (Stevens, 1956) asked participants to assign numbers to a sensation in a ratio way such that a number twice as big represented an experience twice

as intense. After normalizing the numbers, this method showed the rate at which the intensity of the sensations grew for each participant, but comparison of intensity across subjects was still not possible (Bartoshuk et al., 2002).

Aitken (1969) developed the Visual Analogue Scale (VAS) as a modification of the graphic rating scale meant to measure apprehension in fighter pilots. The endpoints of the scale were labeled with the minimum and maximum possible rating for the variable of interest—for Aitken (1969), 'maximal relaxation' to 'maximal panic.' Green and colleagues (1993) modified the VAS to develop the Labeled Magnitude Scale (LMS), which specifically measured oral sensations. The scale was anchored with 'strongest imaginable sensation,' although only referring to oral sensations due to the concern that the strongest imaginable sensation of any kind might be in another sensory modality and vary across participants (Bartoshuk et al., 2002). The 'imaginable' quantifier was meant to not limit the scale to the respondent's personal experience, but has recently been discovered to add noise to the data (Bartoshuk et al., 2012) and it is no longer recommended (Bartoshuk et al., 2005).

Since its development, many researchers have studied the appropriate distance on the LMS to place adjective descriptors (Borg, 1982; Moskowitz, 1977; Schutz & Cardello, 2001). Green and colleagues (1993) place the descriptors 'barely detectable,' 'weak,' 'moderate,' 'strong,' and 'very strong' in a roughly logarithmic fashion such that the final descriptor corresponds to the number 53 on a scale from 0 to 100. When used with the PTC strips, over half the participants rate the intensity of the strip as above 'very strong' (Bartoshuk, 2000), demonstrating why Likert scales of intensity where the final number corresponds to that phrase might obfuscate results. However, the very notion of

supertasters casts doubt on the assumption that the strongest imaginable oral sensation is the same for all participants. If two people are rating based on the most intense taste they have ever experienced or could imagine, the numbers are not comparable if one person is capable of tasting more intensely than the other (Bartoshuk et al., 2004; Bartoshuk et al., 2005).

To address these concerns Bartoshuk and colleagues (2002) introduced the generalized Labeled Magnitude Scale (gLMS). The scale is anchored with 'strongest sensation of any kind' so that participants must think about the taste in comparison to all sensory modalities. This is done for both the hedonic rating scale, with 'strongest liking/disliking of anything, not just food' as the endpoints, as well as for the intensities of saltiness, sweetness, sourness, and bitterness, with 'strongest sensation of anything, not just food' representing the highest rating. Although it can still be argued that the strongest sensation of any kind may not be the same for everyone, what is important is that the maximum does not differ systematically with PTC tasting ability (Bartoshuk et al., 2002). Typically studies using the gLMS (e.g., Cruickshanks et al., 2009) still use adjectives spaced according to the recommendation of Green and colleagues (1993). However, there are numerous cautions against the subjectivity of such descriptors and debate about whether they are necessary at all (Snyder, Fast, & Bartoshuk, 2004; Bartoshuk et al., 2005).

A separate but conceptually related technique (Stevens, 1959) involves asking participants to compare the intensity of what they taste to another sensory modality, usually a sound. This is termed *magnitude matching* and is meant to objectively capture the oral sensation by comparing the intensity to a non-oral sensation (Marks & Stevens,

1980). It does not need to be assumed that everybody hears exactly the same, only that hearing and taste are independent (Bartoshuk et al., 2005). Supertasters compare the bitterness of dark chocolate to a brighter light than do nontasters, even though most participants describe the taste as 'mildly bitter' (Fast, 2004; as cited in Bartoshuk et al., 2005). Fast's (2004) finding demonstrates the importance of using this technique and how easily the differentiation of taster types can be missed with improper measurement techniques such as adjective descriptors.

Current Studies

One practical application of discovering techniques to mask bitterness is modifying the taste of nutritious vegetables. In the studies to be reported here various concentrations of sodium chloride (NaCl), sucrose, and non-nutritive sweeteners (NNS) were added to broccoli, Brussels sprouts, and cauliflower as potential bitterness masking agents. Also of interest was whether these tastants were differentially effective for bitter-sensitive and bitter-insensitive individuals. The long-term goal of modulating the taste and palatability of these vegetables is to facilitate their consumption, thereby improving health.

In Experiment 1, sucrose suppressed the bitterness of broccoli and cauliflower whereas NaCl did not. In Experiment 2, Brussels sprouts replaced broccoli in order to investigate a more-bitter vegetable; the results were identical to that of Experiment 1. Three NNS—saccharin, aspartame, and sucralose—were used in Experiment 3 to determine whether the beneficial suppression effect of sucrose could be obtained without the addition of calories. The NNS all masked the bitter taste of the vegetables, suggesting them as an ideal strategy for temporarily improving palatability. In all three

experiments PTC taster phenotype was measured using filter papers and was treated as a continuous variable in analysis, and in no case could bitterness ratings predict the effect of NaCl or the sweeteners.

Several changes were made in Experiment 4. First, participants were instructed to record the strongest sensation, most liked sensation, and most disliked sensation they were using as references for the endpoints of the gLMS. Secondly, NaCl and sucrose were administered in various concentrations to assess the dose-dependency of their effects. Thirdly, PTC papers were replaced by aqueous QHCl, a bitter chemical that produces reliable bitterness ratings. The effect of NaCl and sucrose on QHCl was also assessed to determine similarities and differences to their effects on the full food matrix of vegetables. Fourthly, participants' sensitivities to NaCl and sucrose were measured with aqueous solutions. The results of Experiment 4 indicated that sucrose was indeed a robust bitterness masking agent at all concentrations. Hierarchical linear regression modeling revealed that NaCl suppressed bitterness only when the plain Brussels sprouts and cauliflower were perceived as highly bitter. This finding illuminated why NaCl was not found to suppress bitterness in Experiments 1-2 when all subjects were pooled for analysis.

The variable of QHCl sensitivity was not significantly predictive of the degree of bitterness suppression observed by NaCl or sucrose on the Brussels sprouts. Additionally, NaCl and sucrose both suppressed QHCl bitterness. Taken together, these findings suggest limitations to the use of QHCl as representative of bitter food stimuli.

Experiment 5 utilized a between-subjects design in order to determine the effect of repeated testing on variability in bitterness ratings. One group of participants tasted

Brussels sprouts plain and then with the addition of NaCl, whereas another group received two samples of the vegetable plain. Condition assignment was dummy-coded and linear regression models were employed to separate the conditions. The model was significant beyond the control condition for participants who received a relatively high but not low concentration of NaCl on their second sample of Brussels sprouts. These results indicate that some, but not all, of the effect in Experiment 4 can be explained by regression to the mean. There is a true phenomenon whereby NaCl masks bitterness most effectively for participants who taste vegetables as highly bitter.

CHAPTER 2 – SUCROSE AND NON-NUTRITIVE SWEETENERS, BUT NOT NACL, SUPPRESS THE BITTERNESS OF VEGETABLES

Experiment 1

Experiment 1 investigated the efficacy of NaCl and sucrose as bitter masking agents for broccoli and cauliflower. By using vegetables as the stimuli, this study builds on the work in the chemical and pharmacological fields (Ley, 2008; Sun-Waterhouse & Wadhwa, 2013) to determine whether prototypical tastants can make healthy foods more palatable. Additionally, variation in bitterness sensitivity was measured via suprathreshold ratings of PTC filter paper.

In order to investigate the effect of bitter masking agents on vegetables varying in bitterness, the stimuli were broccoli and cauliflower, which are both members of the Brassicaceae subfamily of the genus *Brassica*. Glucosinolates have been identified as the primary bittering agents of cruciferous vegetables such as these (Tepper, 2008; Dinehart et al., 2006). When plant tissue is broken down by chewing and digestion, glucosinolates are hydrolyzed and biologically active bitter substances are released (Mithen et al., 2000). Broccoli has approximately twice as dense a concentration of glucosinolates as does cauliflower (Carlson et al., 1987) and is primarily bittered by progoitrin (Drewnowski & Gomez-Carneros, 2000), whereas cauliflower contains neoglucobrassicin and sinigrin (Engel et al., 2002).

The hypotheses of Experiment 1 were: 1) NaCl and sucrose will suppress the bitterness of both broccoli and cauliflower, 2) the effectiveness of NaCl and sucrose as bitter masking agents will differ by PTC taster phenotype, and 3) NaCl and sucrose will mask bitterness and the most for participants who taste the plain vegetables as highly bitter.

Method

Subjects. Subjects were 111 Arizona State University undergraduate students participating for psychology course credit. Potential participants were turned away if they were under 18 years old, did not speak English as their primary language, or did not refrain from eating for two hours before the study as instructed. Nine students were excluded from analysis based on these criteria, leaving 102 subjects. Of these, there were 61 (60%) males and 41 (40%) females. Body mass index (BMI) ranged from 16.9 to 37.6, with an average of 22.8. In this between-subjects design, 50 (49%) were given broccoli with NaCl and cauliflower with sucrose, whereas 52 (51%) of participants were given the opposite.

Materials. Frozen broccoli and cauliflower (Bird's Eye Steamfresh) were cooked in a 900-watt microwave for five minutes and cut into 6 g pieces. For the sweetened version of the vegetables, 0.25 g raw granular sucrose was added to each piece after cooking. Pilot testing indicated that this concentration elicited moderate to strong (Green et al., 1993) gLMS sweetness ratings—an average of 43.6 and a range of 10 to 100.

For the salted piece of each vegetable, 0.125 g NaCl was added to each piece after cooking, which in a pilot test received an average saltiness rating of 49.5 with a range of 15 to 95. Experiment 1 builds on the work of Sharafi and colleagues (2013), who chose concentrations of NaCl eliciting a weakly salty taste when added to vegetables. The authors did not find significant differences in saltiness ratings between the vegetables plain and with NaCl, suggesting that an insufficient amount of NaCl was utilized. While NaCl is a peripheral bitter blocker and perception of the salty taste is not a requirement for its efficacy (Keast & Breslin, 2002a; Kroeze & Bartoshuk, 1985), the higher

concentration of NaCl suppressed bitterness while the lower one did not (Sharafi et al., 2013). Their findings indicate that a sufficiently salty taste should be perceived to best determine the influence of NaCl.

Bitterness sensitivity was assessed with PTC-impregnated filter paper (Neo/Sci Corporation). PTC papers were reliably used in other studies (Khataan et al., 2009; Reed et al., 2010; Herz, 2011; O'Brien et al., 2010) and suprathreshold bitterness ratings strongly correlate with TAS2R38 genotype (Khataan et al., 2009). Additionally, gLMS ratings of the papers are reproducible (Galindo-Cuspinera et al., 2009) and can predict longitudinal outcomes such as dentition (Pidamale et al., 2012). Filler questionnaires were distributed in order to ensure a delay between tasting of the PTC strip and tasting of the second sample of vegetables.

The scale used for both the taster strip and the food stimuli was a horizontal generalized Labeled Magnitude Scale (gLMS) adapted from Bartoshuk and colleagues (2002). The gLMS was updated for this study by removing the descriptive adjectives printed under the scales (e.g., 'weak, moderate, strong, very strong') in line with a recommendation from Bartoshuk and colleagues (2005). Although spaced to give the scale logarithmic properties (Moskowitz, 1977), adjectives are inherently subjective and their use in psychophysics has been denounced due to concerns that their meanings can be altered by context, experience, and the noun to which they are referring (Stevens, 1958; Bartoshuk et al., 2002; Snyder et al., 2004; Bartoshuk et al., 2005). For the hedonic scale, there were three labels: "-100 – strongest imaginable disliking of any kind" at the leftmost end, "0 – neutral" at the midpoint, and "100 – strongest imaginable liking of any kind" at the rightmost end. For the sensory scales, there were two labels: "0

– no sensation" at the leftmost end and "100 – strongest imaginable sensation" at the rightmost end.

Procedure. All procedures for all studies were approved by the Institutional Review Board (IRB). Participants were seated in individual cubicles to discourage communication and signed an informed consent document. Each taste stimulus had a corresponding paper with five horizontal gLMS lines: one hedonic scale for liking and four sensory scales for saltiness, sweetness, sourness, and bitterness. Participants were instructed to mark on each scale to indicate their liking and perception of each taste quality. When the scale was explained to participants, an emphasis was placed on the endpoints representing the strongest sensations they could imagine, which did not necessarily relate to taste or food. The same scale was used to assess all the taste stimuli in the experiment.

Participants first tasted and rated on this scale one piece of plain broccoli and one piece of plain cauliflower. After rating the samples participants filled out a demographic questionnaire assessing height, weight, ethnicity, dietary restrictions, and food allergies, which lasted approximately three minutes. Next, participants tasted and rated the PTC paper using the same scale, then completed a questionnaire lasting approximately eight minutes. Finally, participants were given one more piece of each vegetable with a bitterness masking agent. Whether they received broccoli with NaCl and cauliflower with sucrose or the opposite was randomized by day.

Statistical Analysis. A repeated-measures analysis of variance (ANOVA) was employed to determine the effect of NaCl and sucrose on the ratings of the vegetables. It was predicted that saltiness ratings would increase with the addition of NaCl and

sweetness ratings would increase with the addition of sucrose. The primary interest of the study was the effect of those tastants on bitterness and hedonic ratings.

To investigate the effect of individual differences in bitterness sensitivity, a change score was calculated for each participant as the difference in each taste quality between the baseline rating of the plain vegetable and the rating of the vegetable with the added bitter masking agent. Bitterness ratings of the plain vegetables and, separately, of the PTC-impregnated filter paper were treated as continuous variables and were used as independent variables in linear regression models to predict this change score. A Bonferroni correction was implemented to adjust for multiple comparisons, with a *p*-value for significance set at 0.004. Participants were not trichotomized into PTC taster groups due to concerns about the arbitrariness of such cut-off scores (Tepper, 2008) and the lack of clear boundaries between groups when the data were examined.

Lastly, split-plot ANOVAs were used to determine if vegetable type or gender of the participants interacted with the suppression effect. There was no significant interaction for either tastant of gender by tastant by vegetable when gender was added as a between-subjects variable to the repeated-measures ANOVA, nor an interaction of gender by sweetening or a main effect of gender, all F-values < 1. The same results were found for the hedonic ratings and saltiness or sweetness ratings, all F-values < 1. Therefore, the genders were pooled for successive analysis.

Results

The reported means for each taste quality can be seen in Table I. Because the *a priori* interest of this study was the effect of NaCl and sucrose separately, interactions of the tastants are not reported. Contrary to expectations, the plain broccoli and cauliflower

did not differ in bitterness, F (1, 101) = 1.92, p = 0.17, nor any other taste quality including hedonics, all F-values < 1.

Sodium Chloride.

Saltiness. The addition of NaCl increased saltiness ratings for both vegetables equally. While there was not a significant two-way interaction between salt and vegetable type on reported saltiness, F < 1, there was a main effect of the addition of salt increasing saltiness ratings, F (1, 111) = 93.79, p < 0.001.

Bitterness. NaCl did not affect bitterness ratings. There was neither an interaction between sample (plain or salted) and vegetable type nor a main effect of salt on bitterness, both F-values < 1.

Liking. Hedonic ratings did not change with the addition of NaCl. Both the interaction and main effect of salt on liking were non-significant, F-values < 1.

Other taste qualities. Sweetness ratings decreased with the addition of NaCl. There was no significant two-way interaction between salt and vegetable type on reported sweetness, F < 1, but there was a main effect of the addition of salt decreasing sweetness ratings, F (1, 110) = 4.65, p = 0.033. The addition of NaCl did not have a significant effect on reported liking of texture or sourness, both p-values < 0.30.

Individual Differences in Bitterness Sensitivity. Bitterness ratings of the plain vegetables had a significant negative relationship with the change in bitterness from the addition of NaCl for both broccoli, F (1, 82) = 42.24, p < 0.001, R^2 = 0.56, β = -0.719, and cauliflower, F (1, 27) = 26.89, p < 0.001, R^2 = 0.71, β = -0.723.

Bitterness ratings of the PTC paper could predict liking of the texture of plain broccoli, F (1, 85) = 5.81, p = 0.018, R^2 = 0.065, β = 0.244, but not those of cauliflower,

$F < 1$. Reported bitterness could not predict any of the other taste qualities of the vegetables and was not correlated with the bitterness rating of the plain vegetables, Pearsons' product-moment correlation coefficient (r) = 0.060, $p = 0.527$. In the linear regression analyses, bitterness rating could not predict the change in any taste qualities from the addition of NaCl, all F-values < 2.

Sucrose.

Sweetness. Sucrose increased the sweetness rating of the vegetables. While there was not a significant two-way interaction between sweetening and vegetable type on reported sweetness, $F < 1$, there was a main effect of sucrose on increasing ratings, F (1, 100) = 60.10, $p < 0.001$.

Bitterness. Perceived bitterness of both vegetables was decreased with the addition of sucrose. There was no interaction between sweetening and vegetable type, $F < 1$, but there was a main effect of sweetening on decreasing bitterness, F (1, 99) = 13.09, $p < 0.001$.

Liking. The addition of sucrose increased hedonic ratings for both vegetables equally. There was a main effect of sweetening on liking, F (1, 100) = 5.81, $p = 0.018$, and the interaction of sucrose and vegetable type was not significant, $F < 1$.

Other Taste Qualities. Sweetening did not have a significant effect on reported liking of taste, saltiness, or sourness, all F-values < 2.

Individual Differences in Bitterness Sensitivity. Bitterness ratings of plain broccoli had a negative relationship with the change in broccoli bitterness, F (1, 50) = 16.67, $p < 0.001$, $R^2 = 0.254$, $\beta = -0.330$. Bitterness ratings of plain cauliflower had a negative relationship with the change in cauliflower saltiness, F (1, 49) = 20.39, $p <$

0.001, $R^2 = 0.298$, $\beta = -0.683$, sourness, F (1, 49) = 37.91, $p < 0.001$, $R^2 = 0.441$, $\beta = -0.610$, and bitterness, F (1, 49) = 89.79, $p < 0.001$, $R^2 = 0.652$, $\beta = -0.832$.

Bitterness ratings of the PTC paper could not predict any taste quality of the plain vegetables, and was not correlated with the bitterness ratings of the plain vegetables, $r = -0.040$, $p = 0.691$. In the linear regression analyses, bitterness ratings could not predict the change in any taste qualities from the addition of sucrose, all F-values < 2. The lowest p-value was with the change in cauliflower sweetness as the dependent variable, F (1, 50) = 1.81, $p = 0.184$, $R^2 = 0.04$.

Discussion

Experiment 1 investigated the effect of NaCl and sucrose on bitterness and hedonic ratings of broccoli and cauliflower. Contrary to expectations, plain broccoli was not perceived by subjects as more bitter than plain cauliflower. This is likely due to glucosinolate concentration: although broccoli contains approximately twice as many glucosinolates per weight as cauliflower (Carlson et al., 1987), perhaps this difference is not extreme enough to produce differential perception of bitterness. Not surprisingly, there were no interactions between vegetable type and the effect of either of the tastants, and the results for both vegetables will be discussed together.

Adding sucrose significantly reduced perceived bitterness and increased liking and sweetness ratings for both vegetables. The bitterness suppression of sucrose is consistent with the theory that sweet and bitter tastes inhibit each other when tasted simultaneously (Walters, 1996; Roy, 1992; Lawless, 1982; Lawless, 1979). Sucrose also increased hedonic ratings compared to when the vegetables were served plain. Improved palatability is most likely a result of the increase in perceived sweetness and decrease in

perceived bitterness, as bitterness and liking are typically inversely related (Drewnowski & Gomez-Carneros, 2000). These findings suggest that sucrose can be used to reduce the bitterness of vegetables, thereby increasing acceptance and potentially facilitating consumption.

The addition of salt increased saltiness ratings for both vegetables. When all participants were pooled for analysis, NaCl was not an effective bitter masking agent. This result conflicts with chemical studies (Kamen et al., 1961; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994; Breslin & Beauchamp, 1995; Prescott et al., 2001; Mennella et al., 2003; Keast et al., 2004; Keast, 2008), indicating that experiments on prototypical chemical tastants might not generalize to the full food matrix of bitter vegetables. It is likely that this discrepancy represents differing physiological responses to the bitter ligands from PTC and glucosinolates, as the taste of bitter has multiple transduction mechanisms (Breslin, 1996; Reed & Knaapila, 2010; Ley, 2008; Feeney, O'Brien et al., 2011) that have not been fully elucidated.

When individual differences in bitterness sensitivity were taken into account, more specific results were illuminated. Bitterness rating of the plain vegetables broccoli and cauliflower had a negative relationship with the change in bitterness ratings from the addition of NaCl and sucrose, indicating that these tastants have especially effective bitterness suppressing properties for participants tasting plain vegetables as highly bitter. These results suggest that NaCl and sucrose will be effective bitterness suppressors for participants who taste vegetables as unpleasantly bitter, precisely the subpopulation for whom interventions to improve the taste of and heighten the consumption of vegetables is most crucial.

Individual differences in bitterness sensitivity as measured by PTC filter paper ratings, in contrast, were not predictive of either the taste qualities of the plain vegetables or the change from the addition of NaCl or sucrose. As sensitivity to vegetable bitterness is unable to be measured by PTC filter paper, perhaps this assessment is predictive of behavioral (Duffy et al., 2010; Drewnowski & Rock, 1995) but not hedonic outcomes. Alternatively, since both broccoli and cauliflower were given low bitterness ratings, it is possible that PTC taster phenotype is only associated with sensitivity to highly bitter foods. The promising implication of this finding is that sucrose, and to a lesser extent NaCl, will suppress bitterness to the same degree across people with large variability in PTC sensitivity.

Experiment 2

Broccoli and cauliflower were chosen in Experiment 1 to represent vegetables of varying degrees of bitterness, as one of our aims is to determine if there is an interaction between vegetable bitterness and sucrose masking efficacy. However, the two plain vegetables were not reported as significantly different in bitterness. Therefore, in Experiment 2 Brussels sprouts replaced broccoli, as their glucosinolates (Dinehart et al., 2006) cause them to be very bitter (Tepper, 1998), especially to supertasters (Kaminski et al., 2000). Specifically, Brussels sprouts contain three times the density of glucosinolates as broccoli and six times that of cauliflower (Carlson et al., 1987). A second update to the protocol was that only fresh vegetables were used for the remainder of the studies. While there has not been a conclusive and systematic investigation on the effect of freezing and packaging on glucosinolate levels (Mithen et al., 2000), Quinsac and colleagues (1994) suggest that different freezing techniques can affect the metabolism,

composition, and concentration of glucosinolates in vegetables. The hypotheses for this study were identical to those for Experiment 1.

Methods

Subjects. Subjects were 76 Arizona State University undergraduate students participating for course credit. Subjects were included for experimental analysis if they were 18 years of age and older, spoke English as their primary language, did not follow any dietary program, and did not have a history of or current ear infection, as otits media alters taste perception (Nelson et al., 2011). These criteria excluded seven participants, leaving 69 in the analyses. Of these, there were 43 (62%) males and 26 (38%) females. BMI ranged from 17.4 to 34.3, with an average of 22.8. In this between-subjects design, 33 (47%) of participants were given Brussels sprouts with NaCl and cauliflower with sucrose, while 36 (53%) were given the opposite.

Materials. Six whole fresh Brussels sprouts (144g) from a local grocery store were submerged in water and placed in a 900-watt microwave for four minutes, a method which maintains endogenous glucosinolates (Song & Thornalley, 2007). Separately, 40 g of fresh cauliflower was submerged in water and microwaved for five minutes. After cooking, the Brussels sprouts were cut into 6 g servings and the cauliflower was cut into 11 g servings. The concentrations of NaCl and sucrose were consistent with Experiment 1.

Procedure and Statistical Analysis. The procedure, rating scales, and data analyses were identical to that of Experiment 1.

Results

The reported means for each taste quality can be seen in Table I. Because the *a priori* interest of this study was the effect of NaCl and sucrose separately, interactions of the tastants are not reported. Brussels sprouts were rated as significantly more bitter than cauliflower, F (1, 67) = 4.67, p = 0.034, but the vegetables did not differ in any other taste quality including hedonics, all F-values < 2.

Sodium Chloride.

Saltiness. Saltiness ratings increased for both vegetables when NaCl was added. There was a significant interaction of salt by vegetable, F (1, 64) = 5.72, p = 0.020, and tests of simple effects revealed a significant increase in saltiness by 22 gLMS units for Brussels sprouts, F (1, 30) = 27.13, p < 0.001, and an increase by 39 gLMS units for cauliflower, F (1, 34) = 50.83, p < 0.001. The change in saltiness ratings could not be predicted by the change in sweetness, sourness, or bitterness ratings.

Bitterness. Salt did not change bitterness ratings for either vegetable, both F-values < 1. The change in bitterness ratings with the addition of salt could not be explained by the change in saltiness rating, F (1, 65) = 1.12, p = 0.297, R^2 = 0.017.

Liking. The addition of salt was not influential on ratings of liking. While there was a significant interaction of salt by vegetable, F (1, 64) = 6.19, p = 0.015, tests of simple effects revealed non-significant effects for both vegetables. The interaction resulted from a trend toward increasing liking for Brussels sprouts and decreasing liking for cauliflower, but neither reached significance. Using regression models, the change in liking from the addition of salt could not be explained by the change in perceived saltiness or sweetness. However, for Brussels sprouts only the change in liking could be

explained by the changes in perceived sourness, F (1, 30) = 14.66, p = 0.001, R^2 = 0.336, and bitterness, F (1, 30) = 4.16, p = 0.051, R^2 = 0.125. Liking decreased the most for participants for whom the addition of salt caused an increased perception of sourness or bitterness.

Other Taste Qualities. The addition of NaCl did not affect ratings of liking of texture, sweetness, or sourness, all F-values < 1.

Individual Differences in Bitterness Sensitivity. Bitterness ratings of the plain vegetables had a significant negative relationship with the change in bitterness from the addition of NaCl for both Brussels sprouts, F (1, 30) = 12.14, p = 0.002, R^2 = 0.29, β = -0.514, and cauliflower, F (1, 34) = 35.67, p < 0.001, R^2 = 0.72, β = -0.665.

Bitterness ratings of the PTC paper could not predict the liking, saltiness, sweetness, sourness, or bitterness of the plain vegetables, all F-values < 2. In the linear regression analyses, bitterness rating could not predict the change in any taste qualities from the addition of NaCl, all F-values < 7. The lowest p-value was with the change in cauliflower liking as the dependent variable, F (1, 33) = 6.15, p = 0.019, R^2 = 0.16, which did not reach significance due to a Bonferroni correction setting the criterion at p < 0.004.

Sucrose.

Sweetness. Sucrose increased the sweetness rating of the vegetables. There was a significant two-way interaction between sweetening and vegetable type on reported sweetness, F (1, 66) = 5.93, p = 0.02. Post-hoc tests revealed that sucrose increased sweetness ratings for both Brussels sprouts, F (1, 34) = 4.63, p = 0.039, and to a greater degree cauliflower, F (1, 32) = 22.08, p < 0.001.

Bitterness. Sucrose decreased the perceived bitterness of the vegetables. There was not a significant two-way interaction between sweetening and vegetable type on reported bitterness, $F < 1$, but there was a main effect of sucrose on decreasing bitterness, $F\ (1, 66) = 16.67$, $p < 0.001$.

Liking. Sucrose had no effect on liking. There was not a significant two-way interaction between sweetening and vegetable type, nor a main effect of sweetening, F-values < 1, on liking of the vegetables.

Other Taste Qualities. Sweetening did not increase vegetable saltiness or sourness, both F-values < 3.

Individual Differences in Bitterness Sensitivity. Bitterness ratings of the plain vegetables had a negative relationship with the change in bitterness with the addition of sucrose for both Brussels sprouts, $F\ (1, 34) = 8.88$, $p = 0.005$, $R^2 = 0.212$, $\beta = -0.414$, and cauliflower, $F\ (1, 32) = 605.84$, $p = < 0.001$, $R^2 = 0.951$, $\beta = -0.908$.

Bitterness rating of the PTC paper could not predict any taste quality of the plain vegetables. These ratings were correlated with the bitterness ratings of the plain Brussels sprouts, $r = 0.43$, $p = 0.009$, but not cauliflower, $r = 0.16$, $p = 0.372$. In the linear regression analyses, bitterness ratings of the PTC paper could not predict the change in any taste qualities from the addition of sucrose, all F-values < 2. The lowest p-value was with the change in Brussels sprouts sweetness as the dependent variable, $F\ (1, 34) = 1.20$, $p = 0.281$, $R^2 = 0.04$.

Gender. There was an interaction of gender and vegetable type on hedonic rating of the plain vegetables, $F\ (1, 67) = 4.13$, $p = 0.046$. Females gave higher hedonic ratings than males did for Brussels sprouts, $F\ (1, 34) = 5.81$, $p = 0.022$, but not cauliflower, $F <$

1. There was no interaction of gender by sweetening by vegetable when gender was added as a between-subjects variable to the repeated-measures ANOVA, nor an interaction of gender by sweetening or a main effect of gender, all *F*-values < 1.

There were no interactions of gender with the addition of the tastants, nor any predictive validity added to the regression models predicting the change scores.

Discussion

Brussels sprouts were rated as significantly more bitter than cauliflower, most likely due to their higher concentration of glucosinolates (Carlson et al., 1987). This allowed the data to reveal the effect of NaCl and sucrose on vegetables of varying bitterness. However, results did not differ by vegetable, indicating that the effects of NaCl and sucrose are consistent regardless of the bitterness of the vegetable onto which they are added.

Consistent with Experiment 1, NaCl did not have an effect on the bitterness of the two vegetables when all participants were pooled for analysis. Changes in liking for the Brussels sprouts with salt could be explained by increases in perceived sourness and bitterness, in contrast with the results of Experiment 1. The effect of salt might not be limited to salt perception, but can influence vegetable liking through changes in other taste qualities. Future studies might measure individual differences in perception of saltiness intensity, and to determine whether those who taste plain vegetables as highly bitter are the same as those who perceive added sourness from the addition of salt. This could be another subpopulation who do not benefit from NaCl as a bitter masking agent.

The addition of sucrose significantly decreased the reported bitterness of Brussels sprouts and cauliflower, consistent with Experiment 1. This result shows that sucrose can

be used even for highly bitter vegetables. Cauliflower, the less-bitter stimuli, with sucrose tasted sweeter than did Brussels sprouts with the same amount of sucrose, demonstrating the reciprocal suppression of sweetness by bitterness. This finding lends credence to the theory of competitive inhibition being responsible for the finding of bitterness suppression by sucrose (Walters, 1996; Roy, 1992).

Sucrose did not have an effect on liking for the vegetable stimuli in Experiment 2, despite suppressing their bitterness. This was unexpected, as generally tastes that are less bitter are more liked (Drewnowski & Gomez-Carneros, 2000). The lack of effect is possibly due to the finding that the Brussels sprouts with sucrose were perceived to be less sweet than the cauliflower with sucrose. Thus, the beneficial bitterness suppression of sucrose may be counteracted by the reciprocal sweetness suppression on especially bitter vegetables. Another reason could be individual variability in liking of sweet tastes (Yeomans et al., 2007; Bartoshuk et al., 2006), and perhaps this sample of subjects contained more sweet dislikers than did Experiment 1.

Similar to Experiment 1, bitterness rating of the plain vegetables had a negative relationship with the change in bitterness ratings from the addition of NaCl and sucrose, indicating that these tastants are especially effective bitterness suppressing properties for participants tasting plain vegetables as highly bitter. Bitterness ratings of the PTC taster strip could not predict the change score for any taste quality. Overall, these results suggest that sucrose is a robust bitter masking agent, whereas NaCl is more dependent upon individual differences in vegetable bitterness sensitivity.

Experiment 3

While Experiments 1 and 2 suggest that sucrose masks the bitter taste of vegetables, possibly facilitating consumption of important vitamins and minerals, there are many concerns about the caloric content and health risks, such as obesity (Jürgens et al., 2005), metabolic syndrome (Stanhope, 2012), and type 2 diabetes (Malik et al., 2010), associated with excess sucrose consumption. The purpose of Experiment 3 was to extend the finding that sucrose is a bitter suppresser to non-nutritive sweeteners (NNS), which are lower in calories. Three of the seven NNS determined to be safe and approved for use in the United States (Fitch & Keim, 2012) were investigated: saccharin, aspartame, and sucralose.

Saccharin (1,1-dioxo-1,2-benzothiazol-3-one) has a detection threshold 300 times lower than sucrose (Fitch & Keim, 2012). Despite its reputation, saccharin is not carcinogenic to humans and has been approved as a food additive (Touyz, 2011). One deterrent to the consumption of saccharin is it can have a bitter (Weihrauch & Diehl, 2004) or 'metallic' aftertaste, possibly due to the inhibition of carbonic anhydrases (Köhler et al., 2007) or the activation of the same taste receptor cells as metal salts such as copper and zinc (Riera et al., 2007). It has been speculated that individual differences in the perception of the bitterness of saccharin might be explained by PTC taster phenotype. There is no consensus in this area, with several studies (Bartoshuk, 1979; Gent & Bartoshuk, 1983) finding that bitterness perception of saccharin increased with PROP sensitivity but more recent studies (Kamerud & Delwiche, 2007; Rankin et al., 2004) reporting no relationship. If PTC supertasters perceived added bitterness from saccharin, the sweetener would not be effective masking agents for this population.

However, the results of Experiments 1 and 2 suggest that tasting PTC taster status is not related to tasting bitterness from other stimuli.

Aspartame (L-aspartyl-L-phenlalanine methyl ester) has a detection threshold 160-220 times lower than sucrose and is approved for general use (Fitch & Keim, 2012). It contains 4 kcal/g from added maltodextrin (Frank et al., 2008) and has the least risk for causing adverse effects in humans (Fitch & Keim, 2012).

Sucralose (trichlorogalactosucrose) is directly derived from sucrose and has a detection threshold 600 times lower (Frank et al., 2008). Frank and colleagues (2008) investigated whether sucrose and sucralose activated the same brain areas. Damak and colleagues suggest that sucrose is a ligand for more sweet receptors than is sucralose, as evidenced by T1R3 knockout (KO) mice showing a decreased preference for sucrose over water but absolutely no preference for sucralose. Frank and colleagues (2008) first determined concentrations of sucrose and sucralose for each individual human subject that were indistinguishable. Using an fMRI, the authors found that both sucrose and sucralose activated the frontal operculum and interior insula, but sucrose alone activated the left ventral striatum, anterior cingulate, and bilateral midbrain, among others. As these dopaminergic midbrain areas are involved in a subjective pleasantness response, the authors conclude that the brain distinguishes the sweeteners and the sucrose satisfies the reward system while sucralose does not (Frank et al., 2008). The physiological relevance of this modulation is that the use of NNS such as sucralose may consciously elicit the same experience of sweetness as a caloric sweetener such as sucrose, but ultimately the same reward system is not activated (Frank et al., 2008). This could potentially lead to the overconsumption of sweeteners (as well as the foods in which they are delivered) as

this reward is continuously sought and not achieved. However, as the food stimuli in the present experiments are nutritious vegetables, this is not a concern.

In relation to bitterness suppression, Ming and colleagues (1999) showed that several NNS inhibit the activation of the bitter receptor gustducin (Margolskee, 2002), which implies that they will suppress bitterness. Sharafi and colleagues (2013) recently found that aspartame suppresses the bitterness of kale, Brussels sprouts, and asparagus. Experiment 3 expands on this finding in several ways. Firstly, three different types of NNS are investigated. Secondly, a sucrose condition is included, permitting analysis to reveal not only the efficacy of NNS as bitter blockers but also how they compare to the effect of sucrose.

Due to the fact that NNS bind to the same receptor targets as sucrose, which also bind some bitter compounds (Ming et al., 1999), we predicted the same results as in Experiments 1 and 2. The hypotheses of Experiment 3 were: 1) NNS will decrease bitterness to the same degree as sucrose, 2) this suppression will not differ by PTC taster phenotype unless saccharin has a bitter aftertaste to supertasters, and 3) all sweeteners will be most effective for those participants tasting the Brussels sprouts as highly bitter.

Methods

Subjects. Subjects were 224 Arizona State University undergraduate students participating for psychology course credit. Based on the same exclusion criteria as Experiments 1 and 2, 25 participants were not included in the analysis, leaving 199. Of these, there were 109 (49%) males, 98 (49%) females, and 2 subjects who did not report gender. BMI ranged from 15.9 to 38.5 with an average of 22.9.

Materials. Brussels sprouts were prepared the same way as in Experiment 2. For the sweetened version, 0.25 g sucrose, sucralose, aspartame, or saccharin was added to each piece (Kroger). The NNS were approved for human consumption.

The sweeteners were matched for weight instead of perceived sweetness. The goal was to investigate the efficacy of bitter masking agents, and is possible that sweeteners interact with the bitterness of the vegetables in different ways—for example, some studies suggest that saccharin would add bitterness (Bartoshuk, 1979; Gent & Bartoshuk, 1983; Riera et al., 2007; Köhler et al., 2007). Bulking agents were not added.

Procedure. The rating scale for this experiment was modified based on a study by Bartoshuk and colleagues (2004) where subjects identified their strongest pain experience and instructed that the intensity of this situation was represented by the endpoint of the rating scale. Doing so was meant to prevent falsely inflated ratings of taste intensity by anchoring the top of the scale with a concrete sensation. Whereas in Experiments 1 and 2 participants were asked to think of the number 100 on the scale as describing their 'strongest imaginable sensation,' in Experiment 3 participants wrote down "the strongest physical sensation you can think of—a sight, sound, smell, taste, or touch" before the scale was explained. Although the sensations reported by the subjects differed in modality and objective intensity, the logic of the gLMS (Bartoshuk, 2000; Bartoshuk et al., 2002; Bartoshuk et al., 2005) only necessitates that the sensation is the strongest each individual participant could imagine. After recording this sensation, participants were instructed that a rating of 100 on any of the sensory scales meant that the sensation was as intense as the 'strongest imaginable sensation' they had identified. The hedonic portion of the gLMS remained the same as in Experiments 1 and 2.

Other than these modifications to the rating scale and the exclusive utilization of Brussels sprouts, the procedure was identical to that of Experiments 1 and 2. In this between-subjects design, 58 (29%) subjects received sucrose as their sweetener, 44 (22%) saccharin, 35 (17%) aspartame, and 65 (33%) sucralose.

Statistical Analysis. The statistical analyses were identical to that of Experiments 1 and 2.

Results

The reported means for each taste quality can be seen in Table I.

Sweetness. There was a significant interaction between sweetening and type of sweetener on reported sweetness, F (3, 189) = 6.68, $p < 0.001$. Tests of simple effects revealed that sweetness ratings of the unsweetened Brussels sprouts did not differ between the sucrose concentration conditions, $F < 1$, but did for the sweetened vegetables, F (3, 199) = 4.29, $p = 0.006$. Post-hoc tests revealed that both vegetables sweetened with sucrose were perceived to be less sweet than those sweetened with NNS, all p-values < 0.019, the three conditions of which did not differ from each other, all p-values > 0.207.

Bitterness. All sweeteners decreased bitterness to the same degree. There was no interaction between sweetening and type of sweetener on reported bitterness, $F < 1$, but there was a main effect of sweetening on decreasing bitterness, F (1, 187) = 29.90, $p < 0.001$.

Liking. All sweeteners increased liking to the same degree. There was no interaction between sweetening and type of sweetener on liking, $F < 2$, but there was a main effect of sweetening on increasing liking, F (1, 187) = 8.41, $p = 0.004$.

Other Taste Qualities. Sweetening did not influence vegetable saltiness or sourness, both *F*-values < 3.

Individual Differences in Bitterness Sensitivity. Since sweetener condition was not influential on bitterness ratings of the plain Brussels sprouts or the change score, the sweetener conditions were pooled for the linear regression analysis. Bitterness ratings of the plain Brussels sprouts had a negative relationship with the change in bitterness with the addition of a sweetener, F (1, 188) = 150.85, $p < 0.001$, $R^2 = 0.447$, $\beta = -0.606$.

Bitterness ratings of the PTC-impregnated paper predicted bitterness ratings of the unsweetened Brussels sprouts, F (1, 195) = 7.58, $p = 0.006$, $R^2 = 0.04$, but not the liking or sweetness ratings, both *F*-values < 1. In the linear regression analyses, bitterness rating could not predict the change in any taste qualities from the addition of the sweeteners, all *F*-values < 2. The lowest *p*-value was with the change in Brussels sprouts sweetness as the dependent variable, F (1, 34) = 1.20, $p = 0.281$, $R^2 = 0.04$.

Gender. In a univariate ANOVA there was no significant effect of gender on rated bitterness, liking, or sweetness ratings of the plain Brussels sprouts, all *F*-values < 3. When gender was added as a between-subjects factor to the repeated-measures ANOVA, there were no significant interactions for bitterness and sweetness ratings. For hedonic ratings there was no gender by sweetener by sweetening interaction, but there was a gender by sweetening interaction, F (1, 183) = 4.09, $p = 0.044$. Collapsing across sweetener condition, post-hoc tests revealed that sweetening significantly increased hedonic rating for men, F (1, 94) = 20.89, $p < 0.001$, but had no effect for women, F (1, 95) = 0.54, $p = 0.463$.

Strongest Imaginable Sensation. For the 'strongest imaginable sensation' reported by subjects, 15% reported a sight, 15% a sound, 9% a taste, 8% an odor, 45% a touch (including physical pain), and 7% a sensation that could not be quantified as physical, such as an emotion (see Table IV). Only 15% of participants gave an extremely high (>80) bitterness rating to the PTC paper in this study, as compared to 43% in Experiment 1 and 37% in Experiment 2, suggesting that the modification to the scale was an effective way to conceptualize the endpoints.

Discussion

Saccharin, aspartame, and sucralose were investigated as bitterness masking agents to determine if they were as efficacious as sucrose. All three NNS suppressed bitterness to the same degree as sucrose when matched by weight. Because they contain fewer calories than sucrose, these results suggest that NNS are ideal to reducing the objectionable bitter taste of vegetables. The sweeteners also increased reported liking of the Brussels sprouts, especially for men. Together, these results suggest that the addition of NNS to bitter vegetables will increase sweetness, suppress bitterness, and improve palatability, making them ideal low-calorie bitter masking agents to promote healthy eating.

Consistent with Experiments 1 and 2, individual variability in bitterness sensitivity to the plain vegetable stimuli but not the PTC taster strips affected the change in bitterness ratings from the addition of the sweeteners. Specifically, the sweeteners were especially effective bitterness masking agents for participants who tasted the plain Brussels sprouts as highly bitter. In contrast, PTC taster phenotype was not predictive of the change in bitterness ratings with the addition of sucrose. However, bitterness ratings

of the PTC strip were positively correlated with perceived bitterness of the plain vegetables. These results indicate that while the filter paper provides a useful measure of bitterness sensitivity, changes due to the addition of sucrose cannot be predicted from this variable.

Some studies (Bartoshuk, 1979; Gent & Bartoshuk, 1983; Weihrauch & Diehl, 2004) suggest that saccharin would only add bitterness for participants who perceived the PTC strip as highly bitter, yet this was not substantiated by our data, consistent with more recent literature (Kamerud & Delwiche, 2007; Rankin et al., 2004). Thus, any NNS are effective and will not increase bitterness even for those who are genetically sensitive to bitterness.

While the addition of sweeteners decreased bitterness and increased sweetness to the same degree for both men and women, there was a gender effect on hedonic ratings. For men the addition of any of the sweeteners significantly increased hedonic ratings, whereas sweetening had no effect on hedonic ratings for women. These results suggest that the bitterness of vegetables contributes more to the unpleasant taste for men than it does for women, as men's hedonic rating increased when the bitterness was masked. The differential contribution of bitterness to palatability of vegetables by gender should be explored in further studies. Sweetening, particularly by low-calorie NNS, might therefore be an especially effective strategy to increase vegetable consumption for men.

The amount of sucrose and NNS added to the Brussels sprouts and cauliflower was matched by weight. While we did find that both vegetables sweetened with NNS were sweeter than those sweetened with sucrose, the effect was not as robust as previous studies (Frank et al., 2008; Fitch & Keim, 2012) would suggest: the vegetables sweetened

with NNS were on average 1.5 times as sweet as those with sucrose. Future studies will benefit from pilot tests conducted with the sweeteners—served on vegetables as opposed to in aqueous solutions—to determine the proper amounts of each sweetener to elicit the same average sweetness rating.

The finding that three different NNS masked vegetable bitterness just as effectively as sucrose has several implications. First, it can be assumed that the masking effect is due to the taste of sweet, as opposed to any other attribute of sucrose. This fits with the theory of competitive inhibition (Walters, 1996; Roy, 1992) and should be further tested with varying nutritive and non-caloric sweeteners. Second, these findings indicate that the intervention of using sweetness to decrease bitterness can be done without the addition of calories.

CHAPTER 3 – NACL SUPPRESSES VEGETABLE BITTERNESS ONLY WHEN PLAIN VEGETABLES ARE PERCEIVED AS HIGHLY BITTER

In Experiments 1-3 sucrose and NNS were robust bitter masking agents, whereas NaCl was only effective for participants who perceived the plain vegetables as highly bitter. Experiment 4 investigated multiple amounts of NaCl and sucrose, as the concentration of NaCl relative to other tastants can be influential in taste mixture studies (Breslin, 1996; Breslin & Beauchamp, 1995). Additionally, potential dose-dependent effects were considered. The ideal outcome would be that even a low amount of sucrose suppresses vegetable bitterness.

As there are individual differences in the perception of saltiness and sweetness in addition to bitterness (Hayes et al., 2010; Miller & Reedy, 1990; Bartoshuk et al., 2006; Gent & Bartoshuk, 1983), aqueous solutions of NaCl and sucrose were added to the experimental procedure to obtain data on salt and sweet taste sensitivity. Furthermore, the PTC taster strips were replaced with QHCl as a prototypical bitter tastant. QHCl is commonly used in biological and psychophysical assays to measure bitterness response (Breslin, 1996; Chandrashekar et al., 2010; Loney et al., 2012). Taste sensitivity strongly correlates with PTC taster status (Hayes et al., 2008), so the results will be comparable with those of Experiments 1-3.

In addition to using QHCl to measure bitterness sensitivity, solutions of QHCl mixed with NaCl (QHCl+NaCl) and sucrose (QHCl+sucrose) were administered. The aim was to determine whether NaCl and sucrose differentially affect the bitterness of the vegetable stimuli and QHCl. If this were the case, the generalizability of taste compound studies to whole foods would be called into question.

Experiment 4

Methods

Subjects. Subjects were 306 Arizona State University undergraduate students participating for psychology course credit. In addition to meeting the exclusion criteria from Experiments 1-3, participants must have provided an answer to all three 'strongest sensations,' (see below), rated plain water as below 50 on intensity, and not rated one of the quinine solutions as 20 or more gLMS bitterness units lower than the previous concentration. These criteria indicate a lack of understanding and proper utilization of the gLMS. These exclusions left 266 participants for analysis: 161 (61%) males and 105 (39%) females, with an average age of 19.4 and an average BMI of 22.7.

In this mixed design, all participants received plain Brussels sprouts and plain cauliflower, then one piece of each vegetable with NaCl and one piece of each with sucrose. For Brussels sprouts, 109 (41%) received the low concentration of NaCl, 82 (31%) moderate, and 75 (28%) high; 84 (32%) received the low concentration of sucrose, 99 (37%) moderate, and 83 (31%) high. For cauliflower, 83 (31%) received the low concentration of NaCl, 85 (32%) moderate, and 98 (37%) high; 83 (31%) received the low concentration of sucrose, 84 (32%) moderate, and 99 (37%) high.

Materials. The vegetables were cooked for 10 min in a vegetable steamer, a method that maintains endogenous glucosinolate levels (Song & Thornalley, 2007). For the salted and sweetened versions, the low concentration condition received 0.125 g, the moderate concentration 0.25 g, and the high concentration 0.5 g per piece of either NaCl or sucrose. A pilot test revealed these to taste an average of 25 gLMS saltiness and sweetness units different from each other, respectively.

To assess bitter tasting ability, a series of three QHCl solutions were administered. The low concentration was 0.03 mM (Miller & Reedy, 1990), the moderate concentration was 0.32mM (Duffy et al., 2010), and the high concentration was 1.0 mM (Kroeze & Bartoshuk, 1985). The QHCl+NaCl mixture contained 1.0 mM QHCl and 0.16 mM NaCl. A pilot test solution containing 1.0 mM QHCl and 0.32 M NaCl (Kroeze & Bartoshuk, 1985) was perceived by participants as excessively salty (gLMS saltiness ratings on average >70), so the concentration of NaCl was adjusted to 0.16 mM. A plain salt solution of the same concentration was included to assess sensitivity to the taste of salt. The QHCl+sucrose mixture contained 1.0 mM QHCl and 0.64 M sucrose, whereas the plain sucrose solution contained 0.64 M sucrose. The same pilot test revealed these solutions to taste moderately sweet (average gLMS sweetness ratings of 43, with a range of 0 to 90).

In line with NIH guidelines and previous research (Keast & Breslin, 2002a; Yeomans et al., 2007) the solutions were made each week, kept in non-transparent bottles in a refrigerator overnight, and brought to room temperature at least 2 h before the first session. They were presented as 10 mL servings (Yeomans et al., 2007) in 20 mL cups and identified to the participants with numbers.

Procedures. Participants were greeted and seated. After signing an informed consent document, they were instructed to record the strongest sensation of any kind they had personally experienced, as well as the sensations they experienced that they liked the most and disliked the most. The experimenters emphasized that the sensation did not necessarily have to relate to food or eating. Experienced rather than imaginable sensations were requested because the 'imaginable' qualifier of the gLMS adds noise to

the data (Bartoshuk et al., 2012; Bartoshuk et al., 2005). Participants were instructed that a rating of 100 on any of the sensory scales meant that the sensation was as intense as the sensation they had identified. They were similarly instructed that a rating of -100 or 100 on the hedonic scale meant that the sensations were as disliked or liked as the sensations they had recorded, respectively. The first stimulus was plain water, which gives participants practice and allows for the exclusion of participants who did not understand the scale or did not use it correctly.

Next, participants rated all three quinine solutions in ascending order on liking, intensity (to capture overall perception), saltiness, sweetness, sourness, and bitterness. They were instructed to take the solution into their mouth, swirl it while they counted to three, expectorate it, and then rate it on the scale—a whole mouth sip-and-spit technique (Keast & Breslin, 2002a) that stimulates 90% of taste buds (Miller, 1991). No subject swallowed the solution. Twenty seconds was taken between each tasting, during which time the subjects took a drink of their water (Yeomans et al., 2007).

After this, participants completed a filler questionnaire lasting approximately 4 min. Then, they were given four vegetables: one piece each of Brussels sprouts and cauliflower plain, and one piece with the addition of one concentration of NaCl or sucrose. The concentrations given were semi-randomized such that each participant did not receive the same concentration of both tastants. For example, Brussels sprouts with 0.125 g NaCl could be accompanied by cauliflower with 0.25 g or 0.5 g sucrose but not 0.125 g. The samples were identified to the subjects by numbers. The plain samples were always eaten first, but vegetable type was randomized by day. Participants were

instructed to eat the stimuli at their own pace and rate them on the scale provided, taking a sip of water between each sample.

Participants then completed another filler questionnaire lasting approximately six minutes and were then given the NaCl and sucrose solutions. They were given the same instructions to sip-and-spit the solutions. After another filler questionnaire lasting approximately five minutes, subjects were given the QHCl+NaCl and QHCl+sucrose solutions. Finally, participants were thanked and debriefed.

Statistical Analysis. Univariate ANOVAs were employed to determine whether concentration assignment determined the taste quality ratings of each vegetable with NaCl and sucrose. A Bonferroni correction was implemented to adjust for multiple comparisons, with a *p*-value for significance set at 0.004. Post-hoc tests of simple effects were employed when the relationship was significant. Repeated-measures ANOVAs were used to determine whether there was an interaction of tastant concentration and change from baseline.

Additionally, for each participant a change score was computed for how their rated attributes of the second samples differed from their baseline ratings of the plain vegetables. A series of hierarchical linear regression models were performed to determine the factors influencing this change score, using the concentration of salt as a covariate. Predictors were determined theoretically. *A priori* Pearson's r tests to assess multicollinearity of predictors were performed and each model is composed of predictors that are not significantly correlated at the $p < 0.05$ level. In all models the variance inflation factor (VIF) < 5, suggesting that the estimated βs are well-established.

Results

Descriptive statistics for each taste quality for the vegetables and aqueous solutions can be seen in Table I.

Sodium Chloride.

Saltiness. Saltiness ratings of the salted Brussels sprouts different significantly by NaCl concentration assignment, F (2, 265) = 34.55, $p < 0.001$, and cauliflower, F (2, 265) = 18.09, $p < 0.001$. Post-hoc tests revealed significant differences between all conditions, p-values < 0.001, with higher ratings given to the vegetables with higher concentrations of salt. The only exception was that there was no significant difference in saltiness ratings between the 0.125 g and 0.25 g conditions of cauliflower, $p = 0.157$.

Bitterness. Bitterness ratings of the salted Brussels sprouts or cauliflower were not affected by salt concentration, both F-values < 1. There was not an interaction of sample (plain or salted) and salt concentration in predicting bitterness ratings for Brussels sprouts or cauliflower, both F-values < 4.

Liking. Hedonic ratings of the salted Brussels sprouts were the same between all groups, $F < 3$, but differed for cauliflower, F (2, 264) = 6.76, $p = 0.001$. There was a significant interaction of sample and concentration on hedonic ratings for Brussels sprouts, F (2, 263) = 24.66, $p < 0.001$, with post-hoc tests revealing significant differences between each condition except 0.125 g and 0.25 g. For cauliflower, the interaction was also significant, F (2, 262) = 14.07, $p < 0.001$, but none of the post-hoc comparisons reached significance as determined by our Bonferroni correction.

Other Taste Qualities. Salt concentration could not determine the sourness ratings of the salted Brussels sprouts or cauliflower, both F-values < 3. There was not a

significant interaction of sample and concentration on sourness ratings for Brussels sprouts or cauliflower, both *F*-values < 2.

Intensity ratings of the salted Brussels sprouts and cauliflower did vary by condition assignment, both *p*-values < 0.001. There was a significant interaction of sample and concentration on intensity ratings for Brussels sprouts, F (2, 262) = 20.91, p < 0.001, and cauliflower, F (2, 263) = 8.80, p < 0.001.

Hierarchical Linear Regression Models. Results of all hierarchical linear regression models can be seen in Table II. Relevant results are discussed here.

There was a negative relationship between bitterness rating of the plain Brussels sprouts and the change in bitterness when salt was added, indicating that salt decreased bitterness for participants who tasted the plain Brussels sprouts as highly bitter. Adding the predictors of bitterness and saltiness of the plain Brussels sprouts resulted in a significant ΔR^2 over the step one model, which consisted of concentration of salt alone. Only the bitterness of the plain Brussels sprouts independently contributed significantly to the model (in the negative direction). When the analysis was repeated with this variable as the only predictor the model was again significant over the step one model, F (1, 265) = 35.02, p < 0.001, R^2 = 0.211, β = -0.513. Identical results were found for cauliflower when sensitivity to the taste of salt was measured from the aqueous NaCl solution. These findings indicate that salt increased bitterness when the plain vegetables were not perceived as bitter and decreased bitterness when the plain vegetables were perceived as bitter, regardless of individual differences in salt intensity perception.

There was a negative relationship between hedonic rating of the plain Brussels sprouts and the change in liking when salt was added. Hedonic rating and saltiness rating

of the salt solution were added to the step one model of concentration, and only the former significantly contributed. When liking of plain Brussels sprouts was the only predictor, the model was also significant, F (1, 265) = 42.32, $p < 0.001$, $R^2 = 0.243$, $\beta = -0.419$. This analysis was repeated for cauliflower and the results were identical. These results indicate that salt increased liking when the plain vegetables were disliked, and decreased liking when the plain vegetables were liked, regardless of salt intensity perception.

Sensitivity to the taste of salt was positively correlated with the change in saltiness ratings with the addition of NaCl. When liking of plain Brussels sprouts and the saltiness of the salt solution were step two predictors, only ratings of the solution independently contributed significantly to the model. The results were repeated for cauliflower with saltiness of plain cauliflower and saltiness of the aqueous solution as the predictors, and the same result was obtained. These findings indicate that salt intensity perception as measured by an aqueous solution is predictive of the increase in saltiness from baseline when NaCl is added to a taste stimulus.

Sucrose.

Sweetness. Sweetness ratings of the Brussels sprouts with sugar differed significantly by sucrose concentration, F (2, 265) = 11.30, $p < 0.001$, and cauliflower, F (2, 263) = 9.86, $p < 0.001$. There was an interaction of sample (plain or sweetened) and sucrose concentration on sweetness ratings of the sweetened Brussels sprouts and cauliflower, both p-values < 0.001. Post-hoc tests revealed a significant difference between the 0.125 g and 0.50 g conditions, $p < 0.001$, for both Brussels sprouts and cauliflower.

Bitterness. Bitterness ratings of the Brussels sprouts or cauliflower with sugar were not affected by sucrose condition assignment, both F-values < 1. There was an interaction between sample and concentration on bitterness ratings for Brussels sprouts, F (2, 263) = 4.27, p = 0.015. Post-hoc tests revealed no significant differences between any of the conditions with our Bonferroni correction. This interaction was not significant for cauliflower, F (2, 260) = 0.43, p = 0.653. While bitterness ratings are suppressed from baseline, low concentrations of sucrose are equally efficacious as higher concentrations.

Liking. Sucrose concentration could not determine hedonic ratings of the Brussels sprouts or cauliflower with sugar, both F-values < 4. There was an interaction of sample and sucrose concentration on hedonic ratings of the sweetened Brussels sprouts, F (2, 263) = 6.72, p = 0.001. However, post-hoc tests determined no significant differences between any of the conditions. The interaction was not significant for cauliflower, F < 1.

Other Taste Qualities. Saltiness ratings of the Brussels sprouts or cauliflower were not affected by condition, both F-values < 2. There was not an interaction between sample and concentration on saltiness ratings, both F-values < 2.

Sourness ratings of the Brussels sprouts or cauliflower did not vary by sucrose condition, both F-values < 1. There was not an interaction between sample and concentration on sourness ratings, both F-values < 3.

Intensity ratings of the vegetables with sucrose were affected by sucrose concentration, both p-values < 0.005. Post-hoc tests revealed significant differences between the 0.125 g and 0.50 conditions, both p-values < 0.004. There was not a significant interaction of sample and sucrose concentration on intensity ratings for

Brussels sprouts, F (2, 262) = 2.30, p = 0.102, but there was for cauliflower, F (2, 263) = 11.24, $p < 0.001$. Post-hoc tests revealed no significant differences between the conditions for cauliflower.

Hierarchical Linear Regression Models. Results of all hierarchical linear regression models can be seen in Table II. Relevant results are discussed here.

The variables of interest for the change in Brussels sprouts bitterness with the addition of sucrose were the bitterness rating of the plain Brussels sprouts and the sweetness of the sucrose solution. However, because these predictors were correlated they could not be analyzed in the same model. When investigated separately they were both significantly negatively correlated with the outcome variable, both p-values < 0.001. Liking of plain Brussels sprouts and of the sucrose solution were did not add predictive validity to the model. For cauliflower, only the bitterness of plain cauliflower was associated with the change score. Thus, sucrose was most effective at masking bitterness for participants who have heightened taste sensitivity to both sweetness and bitterness (as determined by ratings of vegetable stimuli, not PTC filter paper).

For the change in liking of Brussels sprouts with the addition of sucrose, both the liking of plain Brussels sprouts and of the sugar solution independently contributed to the model above the step one of concentration. Liking of plain Brussels sprouts was negatively associated with the change score, whereas liking of the sucrose solution had a positive β-weight. For cauliflower, liking of the sugar solution contributed significantly independently to the model but sweetness rating of the cauliflower with sucrose did not. These results indicate that sucrose is most effective at increasing palatability for participants who dislike bitter vegetables and strongly like the taste of sweet.

The change in Brussels sprouts and cauliflower sweetness with added sucrose could be positively predicted by the rated sweetness of the sucrose solution, but not by the liking of the vegetables plain. Sweetness sensitivity as measured by an aqueous solution is predictive of the increase in sweetness from baseline when sucrose is added to a taste stimulus.

Quinine Solutions.

The addition of salt to the strong quinine solution decreased reported bitterness, F (1, 262) = 48.62, $p < 0.001$, as did the addition of sucrose, F (1, 263) = 30.54, $p < 0.001$.

Bitterness rating of the strong quinine solution could predict the liking of plain Brussels sprouts, F (1, 263) = 6.82, $p = 0.010$, $R^2 = 0.025$, $\beta = 0.160$; the change in saltiness ratings of Brussels sprouts with the addition of salt, F (1, 262) = 11.66, $p = 0.001$, $R^2 = 0.043$, $\beta = 0.203$; the change in saltiness ratings of cauliflower with the addition of salt, F (1, 262) = 27.16, $p < 0.001$, $R^2 = 0.094$, $\beta = 0.291$; the change in sweetness ratings of the Brussels sprouts with sugar, F (1, 263) = 8.37, $p = 0.004$, $R^2 = 0.031$, $\beta = 0.137$; the change in sweetness ratings of the cauliflower with sugar, F (1, 262) = 24.54, $p < 0.001$, $R^2 = 0.086$, $\beta = 0.221$ and the change in bitterness ratings of the cauliflower with sugar, F (1, 260) = 4.58, $p = 0.033$, $R^2 = 0.017$, $\beta = 0.046$.

Gender.

In no case was there an interaction of gender and vegetable type on the ratings of the plain vegetables. Men gave higher hedonic ratings than women did, F (1, 262) = 6.87, $p = 0.009$, but there were no gender differences on perceived taste qualities, F-values < 1.

Vegetables with Sodium Chloride. For the rated saltiness of the vegetables there was an interaction of concentration by gender, F (3, 866.2) = 5.77, p = 0.001. Tests of simple effects revealed a significant increase in saltiness ratings by increasing concentration of salt for both males, F (3, 642) = 258.82, $p < 0.001$, and females, F (3, 418) = 339.62, $p < 0.001$. Females reported more saltiness than males did at the highest concentration of salt.

For the rated sourness of the vegetables there was an interaction of concentration by gender, F (3, 858.3) = 3.06, p = 0.028. Tests of simple effects revealed a significant increase in sourness ratings by increasing concentration of salt for both males, F (3, 642) = 6.59, $p < 0.001$, and females, F (3, 418) = 9.92, $p < 0.001$. Males reported more sourness than females did at the moderate concentration of salt.

There were no interactions of salt concentration by gender, F-values from 0.020 to 0.990, p-values > 0.397, nor main effects of gender, F-values from 0.079 to 0.761, p-values > 0.443, on ratings of sweetness, sourness, bitterness, intensity, or liking of the vegetables.

Vegetables With Sucrose. There were no interactions of sucrose concentration by gender, F-values from 0.231 to 0.882, p-values > 0.221, nor main effects of gender, F-values from 0.001 to 0.528, p-values > 0.684, on ratings of any taste qualities of the vegetables.

Aqueous Solutions. For the aqueous solutions, the following effects were significant, all p-values < 0.030 according to a Bonferroni correction: men rated water higher on intensity than women did; women disliked all three QHCl solutions more than men did, and rated them as more intense and bitter; women disliked the plain NaCl

solution more than men did, and rated it as more intense; women disliked the QHCl+NaCl mixture more than men did, and rated it as more intense; men liked the plain sucrose solution more than women did; women disliked the QHCl+sucrose mixture more than men did, and rated it as more intense and more bitter.

Discussion

The primary purpose of this study was to illuminate a potential dose-dependency of the effects found in Experiments 1-3. To that end, Brussels sprouts and cauliflower were administered with one of three concentrations of NaCl and sucrose. Participants also sampled aqueous solutions of QHCl, NaCl, sucrose, and their combinations.

For sucrose, all concentrations suppressed bitterness compared to baseline ratings. Even the lowest amount, which elicited relatively weak (Green et al., 1993) gLMS sweetness ratings, reduced bitterness ratings compared to baseline. These results indicate that sucrose is a robust bitterness suppressor even when a sweet taste is not strongly perceived. Future studies should administer even lower amounts of sucrose in order to determine at what concentration bitterness is not suppressed, and whether this concentration varies by individual taste sensitivity to sweetness and bitterness.

Sweetness ratings of the sucrose aqueous solution predicted the change in bitterness ratings from the addition of sucrose. Sucrose was most effective at masking bitterness for participants who have heightened intensity perception of both bitterness and sweetness. This finding is consistent with the theory of competitive inhibition, as increased perception of each of these tastes would compete with the other. Not surprisingly, the change in liking from the addition of sucrose was highest for participants who gave high hedonic ratings to the aqueous sucrose solution. Therefore, adding

sucrose to vegetables might be most effective for 'sweet likers,' (Yeomans et al., 2007; Bartoshuk et al., 2006), and generally speaks to the importance of personalizing dietary strategies.

None of the concentrations of NaCl suppressed bitterness when all participants were pooled for analysis, even though the concentrations ranged from eliciting low to extremely high (Green et al., 1993) gLMS saltiness ratings. Consistent with Experiments 1 and 2, bitterness ratings of the plain vegetables could predict the change in bitterness ratings when salt was added, in a model where concentration condition was factored out. Because no suppression of bitterness by NaCl was observed even at the highest concentration, this result cannot be accounted for by a heightened perception of saltiness by participants who also perceive high bitterness from the vegetable stimuli.

The suppression of bitterness by salt is complex and dependent on bitterness sensitivity. This relationship is not, however, influenced by individual differences in saltiness perception—saltiness ratings of the aqueous NaCl solution were not predictive of the change score in bitterness rating from the addition of salt. This finding suggests that salt perception is not critical in determining the effect of NaCl on bitterness perception, supporting the idea that salt suppresses bitterness peripherally (Kroeze & Bartoshuk, 1985; Keast, 2003).

Administering aqueous NaCl and sucrose showed a wide range of saltiness and sweetness ratings, allowing identification of individual differences in sensitivity. Ratings of both solutions were positively correlated with the change in saltiness or sweetness (respectively) ratings from baseline, suggesting accuracy to the method of administering aqueous solutions to assess taste sensitivity.

Both NaCl and sucrose suppressed the bitterness of QHCl when all subjects were pooled. This finding is consistent with the chemical literature (Kamen et al., 1961; Lawless, 1979; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994; Breslin & Beauchamp, 1995; Breslin & Beauchamp, 1997, Prescott et al., 2001; Prescott et al., 2001; Keast et al., 2004; Keast, 2008) and our hypotheses. Replicating the bitter suppression effect of NaCl on QHCl in the same group of subjects where no effect was observed for vegetables gives validity to the unexpected findings and suggests a true phenomenon whereby the effect of NaCl is different on vegetables than it is in a prototypical bitter tastant. Perhaps this is because the NaCl is interacting with some other taste quality in the vegetables. Alternatively, because the taste of bitter is more complex and varied (Breslin, 1996; Reed & Knaapila, 2010; Ley, 2008; Feeney et al., 2011) than the other basic tastes, it is possible that NaCl interacts with the bitter ligands of QHCl and glucosinolates differently. Further physiological studies are needed to elucidate the mechanism behind the transduction of these differing compounds.

The finding that NaCl interacts with the bitterness of QHCl differently than the bitterness of Brussels sprouts and cauliflower limits the generalizability of QHCl as a bitter tastant representing bitter foods. However, there were several instances in which bitterness ratings of QHCl were predictive of other important variables, such as liking of the plain Brussels sprouts and the change in bitterness ratings when sucrose was added to cauliflower. QHCl ratings were therefore more informative than the PTC ratings of Experiments 1-3, even though typically bitterness ratings of QHCl and PTC are highly correlated (Hayes et al., 2008). QHCl is recommended as a metric of bitterness

sensitivity, but the interaction of QHCl with NaCl is not representative of the interaction of NaCl and glucosinolates.

Experiment 5

The linear regression model results obtained in Experiment 4 could have been the result of regression to the mean or variability due to repeated testing. Bitterness ratings might not have high test-retest reliability (Guttman, 1945), and participants who gave high bitterness ratings for the plain vegetables might have decreased their bitterness ratings of the second sample due to an experimental artifact of multiple samplings. The design of Experiment 4 does not allow for the separation of change due to repeated testing and that due to our manipulation. Thus, Experiment 5 added a stricter control group. Some of the participants received two samples of plain Brussels sprouts, while the rest received the first sample plain and the second sample with the addition of NaCl in one of two concentrations. If bitterness ratings of the second plain sample were consistent with the first sample in the control group and the linear regression model was non-significant, the data would suggest that the changes from the addition of salt were a reflection of a true phenomenon.

Methods

Subjects. Subjects were 377 Arizona State University undergraduate students participating for psychology course credit. After exclusions were applied based on the same criteria as Experiment 4, 341 participants were included in analysis: 194 (57%) males and 148 (43%) females, with an average age of 19.3 and an average BMI of 23.2.

All participants received two samples of Brussels sprouts and two samples of aqueous QHCl. The first sample of each stimulus was plain for both conditions. The

second sample, depending on condition, was either plain or with the addition of NaCl. Some participants (Control Group) received two plain samples of Brussels sprouts, one plain sample of QHCl, and one sample of QHCl+NaCl (97 participants; 28%). Another group received one plain sample of Brussels sprouts, one sample of Brussels sprouts with NaCl, and two plain samples of aqueous QHCl. The amount of NaCl served on the Brussels varied. In total, 94 (27%) received 0.09g and 152 (44%) received 0.30 g.

Materials. The Brussels sprouts were cooked identically to those in Experiment 4. For the salted version, the low concentration condition received 0.09 g and the high concentration 0.30 g of NaCl per piece. Concentrations of aqueous solutions were based on the results of Experiment 4. The plain QHCl solution was 0.01 mM, and the QHCl+NaCl solution contained an additional 0.004 mM NaCl. The solutions were mixed, stored, and administered to participants in the same way as in Experiment 4.

Procedures. The informed consent procedure and rating scales were identical to that of Experiment 4. The gLMS contained scales for liking of taste, liking of texture, saltiness, sweetness, sourness, and bitterness. Participants sipped water and filled out a questionnaire lasting approximately four minutes between each taste stimuli to reduce carryover effects.

Subjects first tasted and rated the plain QHCl using the whole mouth sip-and-spit technique, then consumed one piece of plain Brussels sprouts. Next, they tasted and rated a second aqueous solution and vegetable. Whether these second samples were plain or with the addition of NaCl depended on the condition.

Statistical Analysis. The variables of interest were: 1) how the change in bitterness ratings of the salted samples compared to the change from repeated testing, 2)

how the change in bitterness ratings of QHCl compared to Brussels sprouts, and 3) how the change in bitterness ratings compared between bitter-sensitive and bitter-insensitive participants. Repeated-measures ANOVAs were employed as in Experiments 1-4 to determine changes from baseline ratings, and here the assigned condition of the nature of the second sample was added as a between-subjects factor to assess interactions. Additionally, univariate ANOVAs were performed for each quality on only ratings of the second sample, as this method accounts for experimental artifacts, such as sensory-specific satiety from tasting the same stimulus twice (Rolls, 1986), that could be affecting ratings of the second sample.

A multiple regression model was conducted to determine whether the change in bitterness ratings from baseline could be predicted by the rating of the plain Brussels sprouts, as was suggested by the results of Experiment 4. Condition was dummy coded to compare the regression coefficients. If the model is significant over and above the Control Group, it will be suggested that the relationship cannot be purely accounted for by regression to the mean.

Results

Descriptive statistics for each taste quality for the vegetables and aqueous solutions can be seen in Table I.

Sodium Chloride.

Saltiness. There was a significant interaction of salt condition and change in saltiness ratings, F (2, 339) = 237.33, $p < 0.001$. Post-hoc tests revealed that each condition differed from the others, all p-values < 0.001, with saltiness ratings increasing the most in the 0.30 g NaCl condition.

NaCl concentration assignment had a significant effect on the saltiness ratings of the second sample of Brussels sprouts, F (2, 342) = 228.02, $p < 0.001$, with post-hoc tests revealing a significant rise in saltiness between each condition. As expected, group assignment could not predict the saltiness ratings of the first sample.

Bitterness. There was a significant interaction of salt condition and change in bitterness ratings in a repeated-measures ANOVA, F (2, 339) = 3.29, $p = 0.038$. However, post-hoc tests revealed that none of the conditions were significantly different from each other. The lowest p-value was 0.093 between the plain condition and 0.09 g NaCl condition. Bitterness ratings of the second sample did not differ between the conditions when all subjects were pooled, F (2, 341) = 1.78, $p = 0.170$.

Liking. Liking of taste could be significantly negatively predicted by bitterness intensity and sourness ratings, and positively predicted by saltiness and sweetness ratings.

There was a significant interaction of salt condition on hedonic ratings in a repeated-measures ANOVA, F (2, 340) = 3.13, $p = 0.045$. Post-hoc tests revealed a difference between the 0.09 g NaCl condition and the two others, both p-values < 0.009. Hedonic ratings increased from baseline in the 0.09 g NaCl condition, whereas those ratings decreased in the plain and 0.30 g NaCl condition.

For liking of taste of the second sample of Brussels sprouts, there was a significant effect of NaCl on ratings, F (2, 342) = 7.03, $p = 0.001$. Post-hoc tests revealed that the plain and 0.30 g salt groups were both significantly lower in liking than the 0.09 g salt group, both p-values < 0.001, but not from each other, $p = 0.784$. Condition also significantly predicted ratings of liking of texture, F (2, 342) = 6.28, $p = 0.002$, with post-

hoc tests revealing that both salt concentrations significantly increased liking of texture ratings over the group who received the second sample of Brussels sprouts plain.

Other Taste Qualities. NaCl condition increased rated intensity of the Brussels sprouts, F (2, 341) = 19.72, $p < 0.001$. Post-hoc tests revealed a significant increase in intensity ratings between each condition with a heightened amount of salt. Salt concentration of the second sample of Brussels sprouts did not significantly affect sweetness, sourness, or fattiness ratings.

Linear Regression Models. A linear regression model predicting the change in bitterness ratings from the baseline bitterness ratings and two dummy-coded condition variables was significant, F (3, 341) = 45.23, $p < 0.001$. The variable of baseline bitterness was highly significant, as predicted, t (341) = -11.25, $p < 0.001$. The 0.09g NaCl condition was not predictive over and above the Control Group, t (341) = -1.36, p = 0.174, suggesting that decreases in bitterness ratings from this group were artifacts of repeated testing. The 0.30 NaCl condition, on the other hand, was significantly predictive, t (341) = -2.29, p = 0.022. These results indicate that strong, but not subtle, NaCl is an efficacious bitterness suppressor for participants who taste vegetables as highly bitter, over and above the variance in ratings that can be explained by regression to the mean or repeated testing. The intercept of this model was not significant, t (341) = 1.04, p = 0.256.

Each condition was then analyzed separately. When Brussels sprouts were tasted plain twice, the bitterness of the first sample was a marginally significant predictor for the change score of the bitterness between the two samples, F (1, 96) = 4.07, p = 0.046, R^2 = 0.041, β = -0.124. The strength of the effect increased for the group receiving

Brussels sprouts with 0.09 g NaCl, F (1, 93) = 32.49, $p < 0.001$, $R^2 = 0.261$, $\beta = -0.451$, and again for the group receiving 0.30 g NaCl, F (1, 150) = 110.07, $p < 0.001$, $R^2 = 0.425$, $\beta = -0.697$, indicating a dose-dependency of the effect.

Quinine Solutions. Consistent with Experiment 4, bitterness of the quinine solution could not predict the bitterness ratings of the plain Brussels sprouts, F (33, 341) = 1.41, $p = 0.071$. The addition of salt to the quinine solution did not affect bitterness ratings, F (1, 92) = 1.19, $p = 0.279$. As expected, bitterness ratings also did not change for participants who sampled the same plain solution twice, F (1, 96) = 0.67, $p = 0.414$.

Gender. There were no gender differences on the hedonic or sensory attributes of the plain Brussels sprouts, F-values < 1. Men disliked the plain QHCl solution marginally more than women did, F (1, 341) = 3.98, $p = 0.047$, and there were no differences in ratings of the sensory attributes of the solution, F-values < 1.

Habitual Brussels Sprouts Consumption. The 9-point Likert scale of Brussels sprouts consumption completed by subjects was converted into a yearly score, ranging from 0 (never) to 365 (daily). More frequent consumption was associated with a higher liking of taste rating for the plain Brussels sprouts, F (1, 342) = 7.18, $p = 0.008$, $R^2 = 0.021$, $\beta = 0.178$, but not any of the sensory attributes. Habitual eating of Brussels sprouts could not predict bitterness ratings of the plain QHCl, F (1, 341) = 1.78, $p = 0.183$.

For participants who received the second sample of Brussels sprouts with salt, more frequent consumption was associated with a higher liking for the taste, intensity ratings, and saltiness ratings, p-values < 0.05, but not any of the change scores from baseline ratings of the plain vegetables, F-values < 1.

Discussion

Experiment 5 aimed to determine whether the linear regression models employed in Experiment 4 were significant due to an artifact of regression to the mean. A condition was instituted where participants tasted the plain Brussels sprouts stimuli twice to discover if ratings would change between the two samples. High consistency in ratings was found for this Control Group, yet the model of predicting the bitterness change score from the bitterness ratings of the plain vegetables reached significance for these participants. The model was significant over and above this condition for participants who received the higher, but not the lower, amount of salt on their second sample of Brussels sprouts. Therefore, some of the effect was due to regression to the mean, particularly when the salt concentration was low, but there is still a true phenomenon.

In contrast to Experiment 4, the addition of NaCl to QHCl did not change bitterness ratings. This is most likely due to the fact that that the concentration of NaCl added to the QHCl was much lower than that of Experiment 4. Due to the lower saltiness ratings, it can be concluded that an insufficient amount of NaCl was used to elicit a perceptibly salty taste.

In Experiment 5 the variable of habitual Brussels sprouts consumption was investigated to determine whether it influenced either ratings of the plain Brussels sprouts or the change scores. Higher habitual consumption was positively associated with liking of Brussels sprouts, but had no relationship with bitterness ratings or any of the change scores. These findings are consistent with the literature that exposure contributes to liking (Anzman-Frasca et al., 2012; Owens, Capaldi, & Sheffer, 1993; Wardle et al., 2003).

CHAPTER 4 – GENERAL DISCUSSION

These studies investigated the influence of sodium chloride (NaCl), sucrose, and non-nutritive sweeteners (NNS) on perceived taste qualities of the Brassicaceae vegetables broccoli, cauliflower, and Brussels sprouts. Secondary variables of interest were vegetable bitterness, the effect of the tastants on bitter quinine hydrochloride (QHCl), and whether individual differences in bitterness sensitivity influenced the relationships.

In Experiment 1, sucrose suppressed the bitterness of broccoli and cauliflower whereas NaCl did not. Because the two vegetables were not perceived as differing in bitterness, Brussels sprouts replaced broccoli in Experiment 2 and the results were replicated. Saccharin, aspartame, and sucralose were investigated in Experiment 3 and were found to suppress the bitterness of Brussels sprouts identically to sucrose. In all three studies, phenylthiocarbamide (PTC) taster phenotype was not predictive of the bitterness ratings of the vegetables or the change scores from the addition of the tastants.

Experiment 4 examined three concentrations of NaCl and sucrose on Brussels sprouts and cauliflower in addition to those tastants in aqueous quinine hydrochloride (QHCl). None of the concentrations of NaCl suppressed bitterness when all subjects were pooled, but hierarchical linear regression modeling revealed that bitterness was suppressed only for participants who perceived the vegetables as highly bitter. Sucrose suppressed vegetable bitterness equally at all three concentrations, and both tastants significantly masked the bitterness of QHCl.

Experiment 5 investigated the possibility that the results of the regression models in Experiment 4 were due to participants who rated the plain vegetables as highly bitter giving lower bitterness ratings of the second samples due to regression to the mean. A

control condition was instituted in which participants tasted plain Brussels sprouts twice, and the same analyses were run. Bitterness rating of the plain vegetables could not significantly predict the change in bitterness with the addition of NaCl over and above the control group for participants tasting a low amount of NaCl on Brussels sprouts, but it was significant for those served a higher amount. These results indicate that some, but not all, of the effect could be explained by regression to the mean.

Overall, sucrose and NNS were found to be wide-ranging bitterness masking agents for all participants for both vegetables and bitter compounds. The effect of NaCl on bitterness was dependent on individual differences in vegetable bitterness sensitivity and the nature of the bitter stimulus. Participants who tasted the vegetable stimuli as highly bitter reported the greatest bitterness suppression effect from NaCl, while NaCl decreased the bitterness of QHCl for all participants.

Sodium Chloride

Effect of NaCl on Brassicaceae Vegetable Bitterness and Liking Depends on Taste Perception. In all experiments it was found that the addition of NaCl did not influence the bitterness or hedonic ratings of broccoli, Brussels sprouts, and cauliflower compared to baseline. This null effect was consistent for these three vegetables although they varied in bitterness. Furthermore, in Experiment 4 the differing concentrations of NaCl elicited average saltiness ratings ranging from weak to strong (Green et al., 1993), and bitterness ratings were not reduced in any of the three conditions compared to the first plain sample of Brussels sprouts and cauliflower.

Further investigation illuminated the influence of perception of the plain vegetables. The reported bitterness of the vegetable stimuli with NaCl decreased

compared to the plain rating for participants who reported the most bitterness from the plain vegetables. Additionally, liking of the three vegetables with NaCl increased the most over the plain stimuli for participants reporting a low liking of plain vegetables. Experiment 5 confirmed that only a part of this relationship was due to regression to the mean from participants who gave high bitterness ratings to the first sample adjusting downwards. A large proportion of the effect, especially with a higher concentration of salt, reflects a true phenomenon whereby participants respond differently to the addition of NaCl based on how they perceived the plain Brussels sprouts.

The finding that salt differentially affects individual perception based on bitterness sensitivity is consistent with that of Sharafi and colleagues (2013), where participants categorized as vegetable dislikers via a survey reported a greater increase in hedonic ratings with the addition of NaCl and NaAc than those categorized as vegetable likers. The present experiments extended their findings by having participants taste the vegetables instead of using a survey and treating hedonic ratings as a continuous variable in a regression model.

Even though there was not an overall trend of NaCl decreasing bitterness and increasing liking for Brussels sprouts and cauliflower, there is a subset of people for whom salting vegetables will be beneficial. Crucially, NaCl reduces bitterness and improves palatability for people who taste plain vegetables as bitter and unpleasant, precisely the population for whom taste enhancement is most imperative. As bitterness and liking are inversely related (Drewnowski & Gomez-Carneros, 2000) and a pleasant taste is crucial for consumption (Glanz et al., 1998; Connors et al., 2001), targeted

interventions should account for individual differences in taste perception and encourage the addition of salt for consumers who dislike vegetables and taste them as highly bitter.

Effect of NaCl on Bitterness Depends on the Type of Tastant. In contrast to the vegetable stimuli, NaCl decreased the bitterness of QHCl solutions across all participants. The differential effect of the same sodium salt on various bitter tastants is supported by previous research (Breslin, 1996; Breslin & Beauchamp, 1995; Keast & Breslin, 2002a, 2002b; Mennella et al., 2003; Sharafi et al., 2013) and is most likely due to the complexity of bitter taste transduction (Breslin, 1996; Reed & Knaapila, 2010; Ley, 2008; Feeney et al., 2011).

QHCl has commonly been used in experiments studying bitterness suppression (Kamen et al., 1961; Lawless, 1979; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994; Breslin & Beauchamp, 1995; Prescott et al., 2001; Keast et al., 2004). The results of the present study indicate that NaCl interacts with QHCl fundamentally differently than it does with the full food matrix of fresh vegetables, and perhaps this chemical is not representative of bitterness in general. These results as well as the discrepancies of chemical studies (Breslin, 1996; Breslin & Beauchamp, 1995; Keast & Breslin, 2002a, 2002b; Mennella et al., 2003; Sharafi et al., 2013) suggest the lack of generalizeability of any one bitter compound. Future studies should employ even more types of bitter vegetables to further elucidate the relationship between these tastes.

Although not studied here, previous literature suggests that the type of salty tastant is influential in the combination of taste primaries. Sharafi and colleagues (2013) found that NaAc was a more effective bitter masking agent than NaCl on the stimuli of

Brussels sprouts, kale, and asparagus, and various salty tastants differentially affect sweeteners (Keast et al., 2004). The effects of $MgSO_4$ (Keast et al., 2004), NaAc (Breslin & Beauchamp, 1995; Sharafi et al., 2013), sodium gluconate (Breslin & Beauchamp, 1995), and various zinc salts (Keast, 2003; Keast, 2008) on vegetables should be investigated.

Sensitivity to the Taste of NaCl. Saltiness ratings of the vegetables with NaCl increased the most for participants who reported the most saltiness from the NaCl solution. These results suggest that individual variability in taste perception influences the perception of saltiness. Presumably there is an optimal saltiness level for vegetables to improve palatability, but it is likely that this is individually determined based on NaCl taste sensitivity and hedonic response (Hayes et al., 2010), bitter taste sensitivity, and environmental factors such as saltiness of diet (Bertino et al., 1982). Future studies would benefit from administering multiple concentrations of aqueous NaCl in order to obtain a dose-dependent concentration curve for each participant (Hayes et al., 2010; Stone & Pangborn, 1990).

Furthermore, individual variability in perception of the same amount of NaCl may be confounding results. Some studies (Keast & Breslin, 2002a, 2002b) aimed to circumvent the issue of individual differences in taste intensity perception by pre-testing and selecting concentrations that were matched for relative intensity to subjects, not absolute concentration. This method would more accurately capture the interaction of perceived salty and bitter *tastes* as opposed to that of salty and bitter tastants.

Speculative Mechanism. Keast and colleagues (2001) offer several compelling suggestions for why sodium salts are bitter maskers. Sodium might alter affinity for

bitter compounds at multiple stages of taste perception by forming a barrier between ligands and their receptors or by interfering with intracellular processes (Keast et al., 2001). To these we add a speculative theory for why NaCl would only be an efficacious bitterness masker for participants who perceived the plain vegetables as highly bitter. It is possible that NaCl suppresses bitterness because salt-sensing Type I cells restrict the spread of ATP secreted by Type II cells, diminishing the conscious perception of bitterness. If this were the case, in order for the ecto-ATPase to have a bitterness suppression effect, ATP would need to be released as a result of bitter taste perception. As such, NaCl masks bitterness in proportion to how much bitterness is tasted from the plain vegetables. However, this mechanism does not explain why the addition of NaCl increased bitterness ratings for patients not reporting any bitterness from the plain vegetables in Experiment 5.

Sucrose

Sucrose as a Bitterness Suppressor. In Experiments 1-4, sweetening three Brassicaceae vegetables and aqueous bitter solutions suppressed bitterness, consistent with the chemical literature (Kamen et al., 1961; Lawless, 1979; Kroeze & Bartoshuk, 1985; Breslin & Beauchamp, 1997, Prescott et al., 2001; Keast et al., 2004; Keast, 2008). This effect persisted for vegetables of varying degrees of bitterness and in two studies also improved reported liking of the vegetables. The benefit of sweetening is twofold: more nutrients will be consumed if perceived bitterness is reduced (Drewnowski & Gomez-Carneros, 2000) and the taste buds are conditioned to associate the sweetness with the vegetable flavor.

Importantly, the suppression of bitterness by sweetness is not limited to sucrose but was found from three common NNS as well. This finding is consistent with Sharafi and colleagues (2013), who found bitterness suppression by aspartame for Brussels sprouts, kale, and asparagus. The efficacy of NNS suggests a method to decrease the bitterness of vegetables, a complaint that often deters their consumption (Dinehart et al., 2006), without adding calories or removing endogenous nutritious bitter compounds (Roland et al., 2011; Reed & Knaapila, 2010) from the foods.

Palatability. In Experiments 1 and 3, but not Experiment 2, it was found that the addition of a sweetener increased reported liking of the vegetables. Increased hedonic ratings are most likely a direct result of the suppression of bitterness, as in general tastes that are less bitter are more preferred (Drewnowski & Gomez-Carneros, 2000).

The change in palatability with the addition of a sweetener may be explained by observed wide variability in liking of different concentrations of sweetness (Yeomans et al., 2007; Bartoshuk et al., 2006). Such individual differences can be due to early exposure to sugar (Pepino & Mennella, 2005), experienced gastrointestinal feedback from caloric sweet foods (Capaldi et al., 1987), and perception of non-sweet taste attributes from sucrose (Looy & Weingarten, 1992).

PTC taste sensitivity is also correlated with being a sweet disliker in adults (Yeomans et al., 2007), so it is likely that the greater proportion of supertasters in Experiment 2 contributed to the lack of increase of palatability from the addition of sucrose. Interestingly, the correlation between taster phenotype and sweet liking cannot be explained by the enhanced perceived sweetness intensity by supertasters (Yeomans et al., 2007). Therefore, although PTC taster phenotype could not predict the change in

liking with the addition of sweeteners in these three studies, sweet liker status may be a secondary variable affecting this relationship and should be quantified in future studies.

Gender might also influence the change in palatability by the addition of a sweetener. In Experiment 3 sweeteners only increased the hedonic ratings of Brussels sprouts for men, even though men and women tasted the same decrease in bitterness and increase in sweetness from the addition of sweeteners. This finding illustrates a distinction between taste perception and palatability and suggests that bitter taste is a primary deterrent of vegetable liking for men but not for women.

Non-Nutritive Sweeteners. In Experiment 3 the common NNS saccharin, aspartame, and sucralose had the same effect as sucrose in reducing bitterness and increasing liking, and have an even more pronounced effect on increasing sweetness. NNS are therefore ideal bitterness masking agents because they are very low in calories.

NNS and naturally occurring sugars are digested and utilized by the human body in functionally identical ways (Fitch & Keim, 2012). However, NNS might not be received identically to sucrose in the brain. Although several NNS bind to the same receptor targets as sucrose (Ming et al., 1999) and activate common taste pathways (Frank et al., 2008), sucralose fails to activate dopaminergic midbrain regions involved in the subjective pleasantness response and does not obtain the same brain reward as sucrose (Frank et al., 2008). Frank and colleagues (2008) suspect that this mechanism might lead to overconsumption, but this is not a concern here due to the utilization of nutritious vegetables as stimuli.

Speculative Mechanism. Although not measured directly, the data are consistent with the theory that the chemical compounds of the sweeteners are acting in competition

with the bitter glucosinolates in the three vegetables to produce inhibition of both tastes. The finding of sweetness suppressing bitterness aligns with previous research (Kroeze & Bartoshuk, 1985; Lawless, 1982; Margolskee, 2002; Zhang et al., 1983) and expands findings in the chemical and pharmaceutical fields (Ley, 2008; Sun-Waterhouse & Wadhwa, 2013) to perception of a full food matrix.

One speculative explanation for these data is that because bitter and sweet tastants both result in the secretion of ATP from Type II cells onto afferent nerve fibers and adjacent Type III cells (Roper, 2013), the maximum resulting subjective taste intensity must be split between the two tastes. Therefore, when experienced in conjunction with sweet, the intensity of bitterness will not be as strong as when tasted alone, and vice versa. Evidence for this theory comes from the mutual suppression of sweetness by bitterness in Experiment 2, where the sweetened Brussels sprouts were reported as less sweet than the sweetened cauliflower. The reciprocal suppression of sweetness by bitterness is consistent with chemical studies when both sucrose and QHCl were administered at relatively high concentrations (Prescott et al., 2001; Keast, 2008).

By the same logic whereby sweet suppresses bitter, sweet and umami should exhibit reciprocal suppression by competing for the pathways of Type II cells. There have been no human perceptual studies on the interaction of sweet and umami tastants, but one rodent physiological study by Sako and colleagues (2003) helps illuminate the relationship. The CT nerve responses of rats were measured for solutions of sweeteners (sucrose, glucose, fructose, and maltose), MSG, and a combination of each sweetener with MSG. A heightened CT response was recorded for the combinations. The authors suggest that the colocalization of sweet and umami receptors in the same TRCs might be

responsible for the synergistic response of blending these stimuli (Sako et al., 2003). Given that these results suggest sweet and umami enhancement instead of suppression, either sweet and bitter tastants recruit similar pathways while umami is separate or the theory of competitive inhibition is incorrect in explaining why sweet suppresses bitter. More physiological studies are needed to determine the nerve pathways recruited and explain whether competitive inhibition occurs and, if so, why sweet and umami are an exception. Alternatively, the results of Sako and colleagues (2003) might be confounded because of the presence of Na+ ions in MSG (Barylko-Pikielna & Kostyra, 2007), since salt enhances sweetness at low concentrations (Keast & Breslin, 2002; Breslin, 1996).

Quinine Hydrochloride

QHCl solutions were administered to assess individual variability in bitterness sensitivity and to determine whether NaCl and sucrose suppressed the bitterness of a bitter stimulus besides vegetables. QHCl was a more valid metric of individual differences than PTC as bitterness ratings of aqueous QHCl were able to predict several other variables in Experiment 4, including liking of the plain Brussels sprouts. However, bitterness ratings of the plain Brussels sprouts were not significantly associated with bitterness ratings of the QHCl in Experiments 4 and 5, contrary to expectations. This finding suggests that extreme sensitivity to the bitter tastes of glucosinolates and QHCl exist in separate populations, possibly indicating the existence of 'vegetable supertasters' previously unidentified by tastant sampling.

In Experiment 4, NaCl and sucrose suppressed the bitterness of QHCl. This finding is consistent with the chemical literature (Kamen et al., 1961; Lawless, 1979; Kroeze & Bartoshuk, 1985; Schifferstein & Frijters, 1992; Frijters & Schifferstein, 1994;

Breslin & Beauchamp, 1995; Breslin & Beauchamp, 1997, Prescott et al., 2001; Prescott et al., 2001; Keast et al., 2004; Keast, 2008) and our original hypotheses. Sucrose is therefore a robust bitterness suppressor for multiple types of bitter tastants, whereas the effect of NaCl is more complex and depends on the bitter tastant.

The finding that NaCl suppressed the bitterness of QHCl for the same group of participants where this effect was not observed for Brussels sprouts gives credibility to the unexpected results and suggests that a true phenomenon was observed in Experiment 4. Possibly, NaCl is interacting with some other taste quality in the vegetables besides bitterness, or NaCl responds differently to the bitter ligands of QHCl and glucosinolates. The generalizability of QHCl as a tastant which can represent bitter foods is called into question by these findings.

PTC Sensitivity

In no study were bitterness ratings of the PTC filter paper predictive of sensory or hedonic ratings of the plain vegetables, indicating that sensitivity to vegetable bitterness is unable to be measured by this metric. It is likely that this discrepancy represents differing physiological responses to the bitter ligands from PTC and glucosinolates, as the taste of bitterness has a higher number of transduction mechanisms (Breslin, 1996), receptors (Reed & Knaapila, 2010), potential molecule ligands (Ley, 2008), and individual variation (Feeney et al., 2011) than the other taste primaries. PTC taster phenotype is therefore not recommended as a measure of sensitivity to the bitterness of vegetable stimuli.

In Experiments 1-3 bitterness rating of the PTC-impregnated filter paper was unrelated to the degree of bitterness suppression by NaCl or sucrose, in agreement with

Sharafi and colleagues (2013). In contrast to Sharafi and colleagues (2013), who found that aspartame only increased hedonic ratings for medium tasters, here PTC taster phenotype was also unrelated to the hedonic ratings of the sweetened vegetables. This incongruence is most likely mediated by the perceived sweetness of the vegetables. Here sweetness increased comparably regardless of PTC tasting ability, whereas Sharafi and colleagues (2013) found that supertasters perceived an unpalatably high amount of sweetness from the vegetables with aspartame.

Psychophysical Scaling

Fewer participants gave an extremely high bitterness rating to the PTC paper in Experiment 3 than in Experiments 1 and 2. This is most likely due to the improvements to the scale whereby participants reported their 'strongest sensations' to anchor the scale. Participants were less likely to give an inflated rating when forced to compare the intensity of what they tasted to their specific 'strongest sensations' as opposed to the concept in abstract. It is recommended that future studies utilize this modification in order to facilitate participant understanding of the scope of the scale.

Only 9% of participants identified a taste sensation as their 'strongest imaginable sensation.' Since the logic of the gLMS breaks down if the scale is anchored with the sensation of interest (Bartoshuk et al., 2004; Bartoshuk, 2000; Bartoshuk et al., 2002), it would be prudent for future studies to explicitly instruct participants to not use a taste sensation or exclude those that do from data analysis.

It was hypothesized that participants who reported higher bitterness ratings of the PTC filter paper would be more likely to report a taste exemplar as their 'strongest sensation,' in line with research that these participants have a greater possible maximum

taste intensity (Bartoshuk, 2000). However, this was not the case. The majority of participants reported a sensation of touch or pain, consistent with Bartoshuk (2000). Thus, although supertasters may perceive a higher intensity from taste than do nontasters, physical pain is more intense than taste for both groups.

Central and Peripheral Suppression

Salt suppresses the bitterness of aqueous compounds peripherally whereas sucrose does so centrally (Kroeze & Bartoshuk, 1985). The current studies did not contain any experimental manipulations to differentiate the concepts, but future studies should investigate whether suppression occurs in a similar manner for aqueous solutions and vegetables. One common technique is the split-tongue procedure employed by Kroeze and Bartoshuk (1985), but this would be methodologically difficult as solid foods require chewing, which distributes particles throughout the oral cavity.

A more practical approach to evidence these theories would be to utilize a concentration of NaCl and sucrose that is not perceptible to the participant. It would be expected that participants who perceive a suppression effect from the addition of NaCl would show a similar reaction regardless of whether the stimulus tasted salty or not, whereas sucrose suppression would only occur when sweetness was perceptible.

The emerging field of volatiles (Tieman et al., 2012) could also help elucidate whether sweetness suppresses bitterness centrally or peripherally. Sweet aroma volatiles cause the perception of sweetness without adding sucrose or NNS (Tieman et al., 2012). If these volatiles were able to successfully mask the bitterness of vegetables, it could be concluded that the suppression was occurring centrally as opposed to peripherally. Such

a discovery would also propose a way to suppress the bitter taste of vegetables and potentially condition preference without the addition of a single calorie.

Habitual Brussels Sprouts Consumption

In Experiment 5, participants indicated how frequently they consumed a variety of vegetables including the target, Brussels sprouts. More frequent consumption was associated with a greater liking of plain Brussels sprouts, but not any sensory attributes of the vegetables or aqueous QHCl. These findings are consistent with the literature that exposure to a taste stimulus increases its palatability (Anzman-Frasca et al., 2012; Owens et al., 1993; Wardle et al., 2003) but does not affect how the stimulus tastes.

Implications

The most important extension of the finding that sucrose and NNS suppress bitterness is determining the impact on consumption. There are many ways to increase the palatability and consumption of disliked tastes, such as mere exposure (Pliner, 1982; Hausner, Olsen, & Møller, 2012; Anzman-Frasca et al., 2012), flavor-flavor learning (Holman, 1975; Yeomans et al., 2008a; Ackroff & Sclafani, 2011) and flavor-nutrient learning (Zellner et al., 1983; Yeomans et al., 2008b; Yeomans, 2012). The bitterness suppression of sucrose and NNS found here might facilitate these processes by improving the palatability of the initial exposure, thereby encouraging voluntary second and third exposures that may eventually lead to a permanent shift in preference. Flavor-nutrient learning in particular relies on consuming sufficient calories to receive gastrointestinal feedback (Yeomans 2012), so the use of sucrose to increase consumption of vegetables would facilitate this process.

A focus should be given to implementing the use of sucrose and NNS for children, as preferences from childhood are long-lasting (Nicklaus et al., 2004) and children may be more willing to try a new food if it is sweetened. Although parents may be hesitant to serve their children extra sucrose, flavor-flavor and flavor-nutrient learning ensure that a preference will emerge for the *unsweetened* vegetables if the sucrose is used for a sufficient number of trials. According to these learning principles, the sweetener will not become a crutch.

It is clear from the results of Experiments 4 and 5 that NaCl should not be utilized as a bitter masking agent without first assessing individual bitterness sensitivity to vegetables. NaCl should be administered for people who find the taste of it on vegetables pleasant or who perceive plain vegetables as objectionably bitter. In general, the results speak to the importance of personalizing dietary strategies.

Conclusions

The bitter taste of vegetables can hinder their acceptance, but this is not an insurmountable problem. Sucrose and non-nutritive sweeteners were found to decrease the bitterness of broccoli, cauliflower, and Brussels sprouts. The addition of sweeteners to vegetables is recommended improve the initial palatability of nutritious foods and allow for the multiple exposures necessary for flavor-flavor and flavor-nutrient learning to take place.

Sodium chloride (NaCl) has been found to suppress the bitter taste of chemical tastants, and this was replicated here with quinine hydrochloride (QHCl). Yet research on the effect of NaCl on the taste of vegetables is limited. Here it was found that NaCl had no effect on the bitter taste of Brussels sprouts and cauliflower across all participants,

calling into question the generalizeability of bitter chemicals to the complex matrix of real foods.

Bitterness perception of the plain vegetables proved to be an influential variable in determining the nature of the interaction of salt with vegetable bitterness. When the plain vegetables were rated as highly bitter, NaCl suppressed bitterness; similarly, when the plain vegetables were disliked, NaCl improved hedonic ratings. Adding salt to vegetables is a beneficial taste modification strategy for consumers who dislike vegetables and perceive their taste as highly bitter.

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Table I.

Means and Standard Errors of the Mean (SEM) for the gLMS Ratings of All Taste Qualities for All Taste Stimuli, Experiments 1-5.

	Liking of taste	Liking of texture	Intensity	Saltiness	Sweetness	Sourness	Bitterness
Experiment 1							
Broccoli							
Plain	25.1 (3.2)	19.8 (3.2)		14.9 (1.8)	17.9 (2.1)	4.4 (0.9)	14.3 (1.7)
Second sample							
NaCl, 0.125g	23.2 (5.1)	33.9 (12.1)		50.6 (3.4) ***	12.8 (1.9) *	10.9 (2.1) **	12.9 (2.2)
Sucrose, 0.25g	33.7 (6.1) *	25.9 (5.4) *		15.0 (3.0)	41.3 (4.1) ***	5.4 (1.8)	9.5 (2.6) **
Cauliflower							
Plain	14.3 (4.0)	19.5 (3.8)		10.6 (2.3)	16.6 (2.8)	6.4 (2.2)	11.7 (2.1)
Second sample							
NaCl, 0.125g	24.1 (6.8)	22.9 (6.5)		41.3 (5.8) ***	18.0 (5.3)	6.6 (2.6)	9.8 (2.9)
Sucrose, 0.25g	17.8 (5.7)	22.9 (4.5)		6.2 (2.1)	33.2 (3.8) ***	2.9 (1.2)	4.3 (1.6) *
Experiment 2							
Brussels sprouts							
Plain	11.3 (4.4)	14.5 (4.9)		12.6 (2.2)	10.8 (1.8)	5.9 (1.8)	22.8 (3.1)
Second sample							
NaCl, 0.125g	18.1 (6.7)	15.3 (5.6)		38.3 (5.1) ***	15.6 (3.8) *	9.5 (3.4)	14.9 (4.3)
Sucrose, 0.25g	14.3 (7.7)	21.5 (6.5)		16.3 (3.8) *	17.1 (3.6)	4.7 (1.9)	17.6 (4.0) *

Cauliflower						
Plain	16.9 (4.1)	11.3 (5.1)	5.9 (1.5)	8.8 (1.6)	2.8 (1.1)	13.7 (2.5)
Second sample						
NaCl, 0.125g	8.3 (8.3)	19.2 (6.6)	45.9 (5.3) ***	10.3 (3.2)	6.8 (2.6)	9.3 (2.7)
Sucrose, 0.25g	19.8 (6.3)	14.8 (6.0) *	7.1 (2.1)	27.7 (4.9) ***	1.9 (0.9)	2.1 (0.8) ***

Experiment 3

Brussels sprouts						
Plain	6.3 (3.0)	1.7 (3.1)	10.3 (1.2)	12.9 (1.4)	5.7 (0.9)	25.2 (1.9)
Second sample						
Sucrose, 0.25g	16.1 (5.2) **	4.1 (5.2)	10 (2.3)	25.7 (3.5) **	2.0 (0.6)	20.5 (2.9) **
Saccharin, 0.25g	9.5 (6.6)	0.5 (5.3) **	8.9 (2.0)	38.8 (4.1) ***	1.7 (0.7)	13.8 (3.4)
Aspartame, 0.25g	22.5 (8.9)	22.4 (8.0)	8.2 (3.1)	45.6 (5.1) ***	5.9 (2.9)	11.0 (2.6) **
Sucralose, 0.25g	12.7 (5.6) **	12.0 (5.4) *	10.9 (2.3)	37.9 (3.5) ***	5.5 (1.5)	12.7 (2.4) ***

Experiment 4

Brussels sprouts						
Plain	8.5 (1.9)	16.5 (1.1)	3.5 (0.6)	5.7 (0.6)	2.5 (0.5)	11.4 (1.0)
Second sample NaCl						
0.125g	10.1 (3.8) *	32.7 (2.2) ***	41.1 (2.7) ***	5.8 (1.2)	4.2 (0.9)	10.7 (1.9)
0.25g	7.2 (4.7)	37.4 (2.4) ***	55.2 (3.0) ***	5.8 (1.2)	6.8 (1.9) *	9.6 (2.1)
0.5g	-23.75 (5.1) ***	53.5 (3.1) ***	74.2 (2.6) ***	3.5 (1.5)	8.4 (1.9) ***	9.9 (2.3)
Second sample sucrose						
0.125g	11.9 (3.7)	18.6 (1.8) *	4.8 (1.3) *	24.7 (2.5) ***	1.8 (0.9)	4.6 (1.2)
0.25g	25.2 (3.2) ***	24.3 (1.9) ***	8.9 (1.6) ***	32.5 (2.3) ***	0.6 (0.2) *	5.6 (1.0) ***
0.5g	14.3 (4.5)	27.6 (2.5) ***	5.9 (1.9)	43.3 (3.2) ***	2.8 (1.4)	5.5 (1.6) ***

Cauliflower						
Plain	19.7 (1.8)	12.6 (0.9)	2.6 (0.4)	5.7 (0.6)	0.9 (0.2)	5.9 (0.8)
Second sample NaCl						
0.125g	20.4 (4.2)	24.7 (2.4) ***	33.9 (3.1) ***	4.8 (1.1)	3.5 (1.2) *	6.7 (1.6)
0.25g	3.3 (4.5) ***	40.3 (2.8) ***	52.8 (3.3) ***	4.2 (1.4)	6.2 (1.7) **	9.4 (2.0) *
0.5g	-0.5 (4.0) ***	40.8 (2.5) ***	58.8 (2.7) ***	3.6 (1.1) *	6.1 (1.7) **	7.6 (1.8) *
Second sample sucrose						
0.125g	25.2 (3.8)	17.6 (1.8) ***	4.1 (0.9)	26.3 (2.8) ***	1.2 (0.6)	4.2 (1.0)
0.25g	19.6 (3.2)	20.0 (1.7) ***	2.7 (0.8)	30.7 (2.6) ***	0.7 (0.4)	3.3 (1.1)
0.5g	17.5 (3.1)	26.6 (1.9) ***	2.7 (1.1)	42.0 (2.6) ***	0.3 (0.1)	2.3 (0.6) ***
Quinine						
0.03mM	-20.9 (2.1)	18.1 (1.5)	3.1 (0.6)	0.5 (0.2)	2.9 (0.7)	28.4 (1.9)
0.32mM	-40.2 (2.2)	34.4 (1.7)	6.1 (0.9)	0.3 (0.1)	5.3 (0.9)	48.5 (1.9)
1.0mM	-54.2 (2.3)	48.7 (1.9)	8.6 (1.2)	0.3 (0.2)	6.9 (1.1)	63.9 (1.8)
Second sample 1.0mM						
NaCl, 0.32mM	-52.7 (2.2)	55.3 (1.7) ***	43.2 (1.8) ***	1.2 (0.4)	12.4 (1.4) **	48.1 (1.9) ***
Sucrose, 0.64M	-44.9 (2.3) ***	53.8 (1.7) ***	7.9 (0.9)	21.9 (1.6) ***	15.8 (1.5) ***	52.8 (1.8) ***
Water						
Plain	24.9 (2.0)	2.5 (0.3)	1.7 (0.4)	0.5 (0.2)	0.1 (0.1)	1.5 (0.6)
NaCl, 0.16mM	-11.4 (1.9) ***	31.0 (1.5) ***	45.2 (1.8) ***	1.5 (0.4) *	4.9 (0.9) ***	8.8 (1.3) ***
Sucrose, 0.32M	28.4 (2.2)	30.2 (1.4) ***	1.5 (0.4)	53.3 (1.7) ***	2.9 (1.1) **	1.5 (0.5) *

Experiment 5

Brussels sprouts

Plain	-0.2 (2.3)	-1.0 (2.2)	26.7 (1.1)	9.4 (0.7)	3.7 (0.4)	3.7 (0.6)	20.0 (1.3)
Second sample							
Plain	-7.4 (4.5) *	-10.5 (4.0) **	27.9 (2.2)	10.6 (1.3)	3.3 (0.9)	5.4 (1.3)	22.8 (2.6)
NaCl, 0.09g	14.5 (5.2) *	9.3 (4.4) *	36.7 (2.3) ***	41.5 (2.6) ***	4.6 (0.9)	6.9 (1.6) **	17.1 (1.9)
NaCl, 0.30g	-5.8 (3.6)	0.7 (2.9)	45.9 (1.8) ***	67.6 (1.8) ***	4.2 (0.9)	10.2 (1.5) ***	18.6 (1.8)
Quinine							
0.005mM	-8.0 (1.5)	12.7 (1.6)	11.9 (0.8)	3.5 (0.6)	1.9 (0.3)	3.5 (0.5)	15.9 (1.2)
Second sample							
0.005mM	-13.0 (1.7) ***	9.8 (1.6)	13.1 (1.1) **	3.9 (0.6)	1.6 (0.4)	5.2 (0.7) ***	17.4 (1.3) *
NaCl, 0.004mM	-30.3 (2.8) ***	2.4 (3.8) ***	27.1 (2.1) ***	36.7 (2.7) ***	1.5 (0.5)	7.3 (1.6)	20.4 (2.0)
Water							
Plain	20.6 (1.5)	27.4 (1.7)	6.2 (0.6)	0.6 (0.2)	0.9 (0.2)	0.3 (0.1)	0.5 (0.1)

* significantly different from the first plain sample, $p < 0.05$
** significantly different from the first plain sample, $p < 0.01$
*** significantly different from the first plain sample, $p < 0.001$

Table II.

Results of Hierarchical Linear Modeling Analyses, Experiment 4.

Outcome variable	**Measures**	**Overall model R^2**	**Overall model ΔF**	**Sig**	**Overall model *df***	**Predictor β**	**Sig**
Change in bitterness with added NaCl for Brussels sprouts	*Step One* Concentration of NaCl on Brussels sprouts	0.006	1.47		1, 263	1.82	
	Step Two Bitterness of plain Brussels sprouts	0.212	34.16	***	2, 261	-0.517	***
	Saltiness of Brussels sprouts with NaCl					0.023	
Change in bitterness with added NaCl for cauliflower	*Step One* Concentration of NaCl on cauliflower	0.015	3.99	*	1, 263	2.53	*
	Step Two Bitterness of plain cauliflower	0.123	16.01	***	2, 261	-0.469	***
	Saltiness of NaCl solution					-0.001	
Change in liking with added NaCl for Brussels sprouts	*Step One* Concentration of NaCl on Brussels sprouts	0.151	46.88	***	1, 264	-20.62	***
	Step Two	0.247	16.66	***	2, 262		

	Liking of plain Brussels sprouts					-0.419	***
	Saltiness of NaCl solution					0.085	
Change in liking with added NaCl for cauliflower	*Step One*	0.054	14.95	***	1, 263	-11.51	***
	Concentration of NaCl on cauliflower						
	Step Two	0.185	20.97	***	2, 261		
	Liking of plain cauliflower					-0.508	***
	Saltiness of NaCl solution					0.071	
Change in saltiness with added NaCl for Brussels sprouts	*Step One*	0.197	64.59	***	1, 263	16.84	***
	Concentration of NaCl on Brussels sprouts						
	Step Two	0.26	11.03	***	2, 261		
	Liking of plain Brussels sprouts					0.038	
	Saltiness of NaCl solution					0.269	***
Change in saltiness with added NaCl for cauliflower	*Step One*	0.114	33.77	***	1, 263	12.37	***
	Concentration of NaCl on cauliflower						
	Step Two	0.188	11.91	***	2, 261		
	Saltiness of plain cauliflower					-0.485	
	Saltiness of NaCl solution					0.277	***

Change in bitterness with added sucrose for Brussels sprouts	*Step One* Concentration of sucrose on Brussels sprouts	0.018	4.81	*	1, 263	-2.66	*
	Step Two Liking of plain Brussels sprouts	0.074	7.91	***	2, 261	0.046	
	Sweetness rating of sucrose solution					-0.125	***
Change in bitterness with added sucrose for Brussels sprouts	*Step One* Concentration of sucrose on Brussels sprouts	0.018	4.91	*	1, 264	-2.67	*
	Step Two Bitterness of plain Brussels sprouts	0.563	163.43	***	2, 262	-0.659	***
	Liking of sucrose solution					-0.009	
Change in bitterness with added sucrose for cauliflower	*Step One* Concentration of sucrose on cauliflower	0.007	1.71		1, 259	-1.09	
	Step Two Bitterness of plain cauliflower	0.51	131.83	***	2, 257	-0.668	***
	Bitterness of highest concentration of quinine					0.033	*
Change in	*Step One*	0.005	1.24		1, 264	2.79	

liking with added sucrose for Brussels sprouts	Concentration of sucrose on Brussels sprouts						
	Step Two	0.191	30.2	***	2, 262		
	Liking of plain Brussels sprouts					-0.39	***
	Liking of sucrose solution					0.19	***
Change in liking with added sucrose for cauliflower	*Step One*	0.002	0.47		1, 264	-1.42	
	Concentration of sucrose on cauliflower						
	Step Two	0.107	15.47	***	2, 262		
	Liking of sucrose solution					0.251	***
	Sweetness rating of cauliflower with sucrose					0.034	
Change in sweetness with added sucrose for Brussels sprouts	*Step One*	0.096	27.84	***	1, 263		
	Concentration of sucrose on Brussels sprouts						
	Step Two	0.273	31.77	***	2, 261		
	Liking of plain Brussels sprouts					-0.55	
	Sweetness of sucrose solution					0.37	***
Change in	*Step One*	0.085	24.22	***	1, 262	8.397	***

sweetness with added sucrose for cauliflower	Concentration of sucrose on cauliflower							
	Step Two Sweetness of sucrose solution	0.303	81.93	***	1, 261		0.394	***

* = p< 0.05
** = p< 0.01
*** = p < 0.001

Note. Betas reported are those from the step at which the variable was entered into the equation.

Table III.

Results of Linear Modeling Analyses, Experiment 5.

Outcome variable	**Measures**	**Overall model *R square***	**Overall model *ΔF***	**Sig**	**Overall model *df***	**Predictor β**	**Sig**
Change in Brussels sprouts bitterness with added salt	Concentration of salt on Brussels sprouts	0.018	6.19	*	1, 341	-2.69	*
		0.286	67.94	***	2, 341		
	Bitterness of plain Brussels sprouts					-11.29	***
Change in Brussels sprouts liking with added salt	Concentration of salt on Brussels sprouts	0.001	0.267		1, 342	0.304	
		0.121	23.40	***	2, 342		
	Liking of plain Brussels sprouts					-6.82	***

* = p< 0.05
** = p< 0.01
*** = p < 0.001

Note. Betas reported are those from the step at which the variable was entered into the equation.

Table IV.

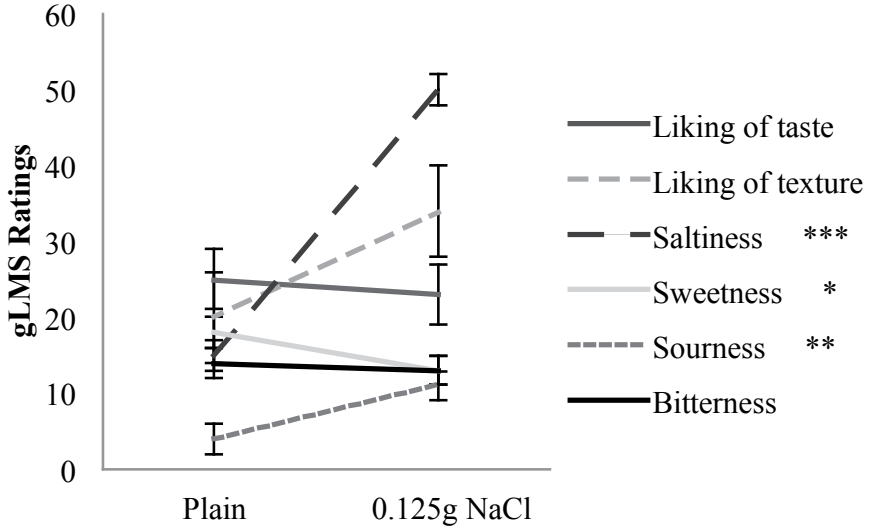
Strongest Imaginable and Experienced Sensations Reported by Participants, Coded, Experiments 3-5.

	Strongest sensation	Most liked sensation	Most disliked sensation
Experiment 3			
Strongest imaginable physical sensation			
Sight	14.2%		
Sound	15.2%		
Smell	8.5%		
Taste	7.6%		
Touch	44.1%		
Other	6.6%		
Experiment 4			
Strongest experienced sensation			
Sight	0.0%	0.0%	0.6%
Sound	0.0%	0.6%	0.6%
Smell	0.6%	1.2%	1.8%
Taste	14.2%	17.2%	14.8%
Touch	30.2%	9.5%	36.1%
Emotion	13.6%	29.0%	27.2%
Sexual	6.5%	21.3%	0.0%
Adrenaline	22.5%	12.4%	4.7%
Burn	2.4%	0.0%	1.2%
Absence of sensation	0.6%	0.6%	1.8%
Internal state	4.1%	4.7%	5.9%
Other	5.3%	2.4%	5.3%
Experiment 5			
Strongest experienced physical sensation			
Sight	7.3%	10.2%	2.0%
Sound	9.6%	7.9%	9.9%
Smell	15.5%	13.7%	18.7%
Taste	11.1%	26.5%	13.4%

Touch	44.3%	28.9%	45.8%
Other	12.2%	12.8%	10.2%

Figure 1.

A) Change in Sensory gLMS Ratings When Broccoli Was Served Plain and with 0.125 g NaCl, Experiment 1.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with 0.125 g NaCl, Experiment 1.

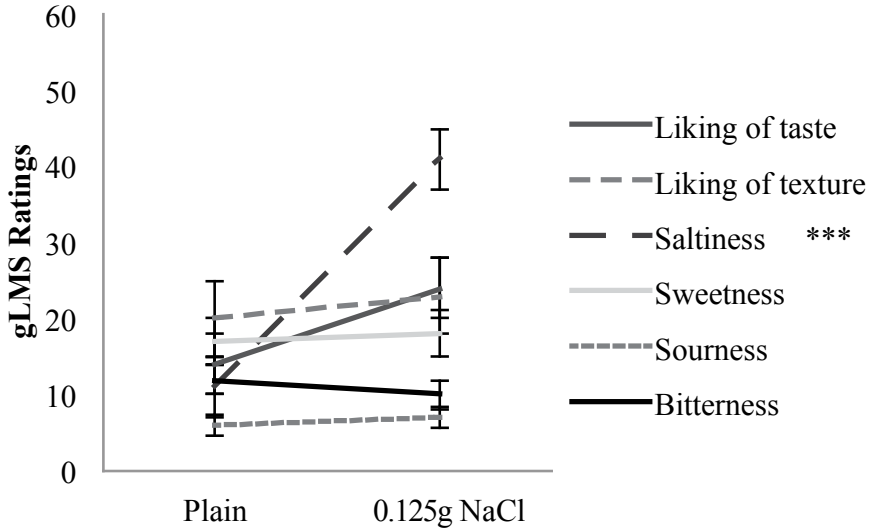
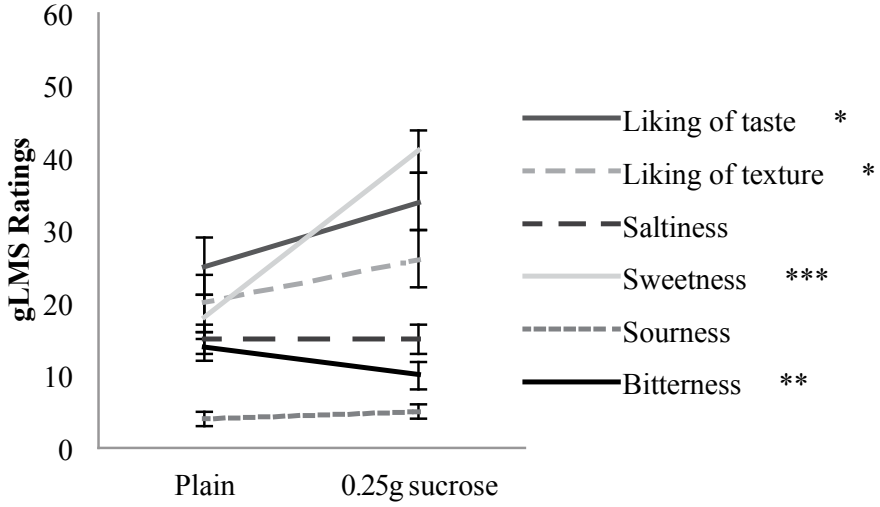


Figure 2.

A) Change in Sensory gLMS Ratings when Broccoli was Served Plain and with 0.25 g Sucrose, Experiment 1.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with 0.25 g Sucrose, Experiment 1.

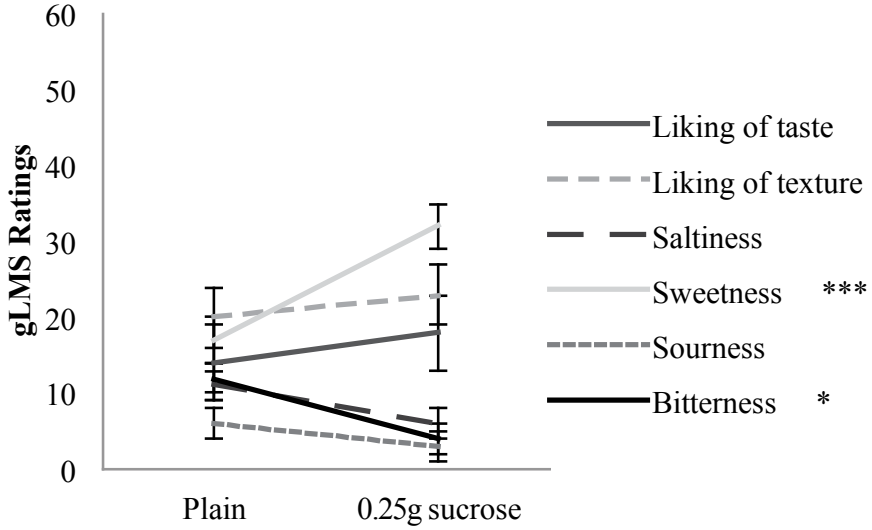
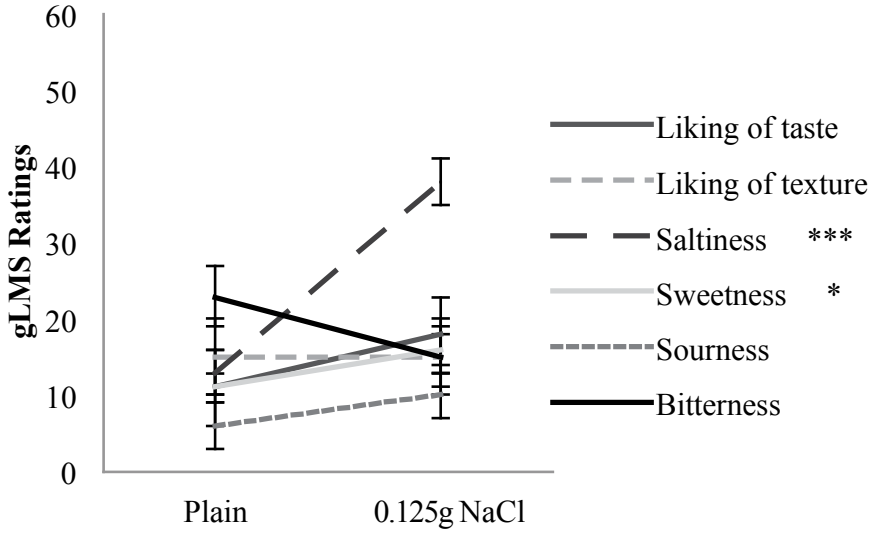


Figure 3.

A) Change in Sensory gLMS Ratings When Brussels Sprouts Were Served Plain and with 0.125 g NaCl, Experiment 2.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with 0.125 g NaCl, Experiment 2.

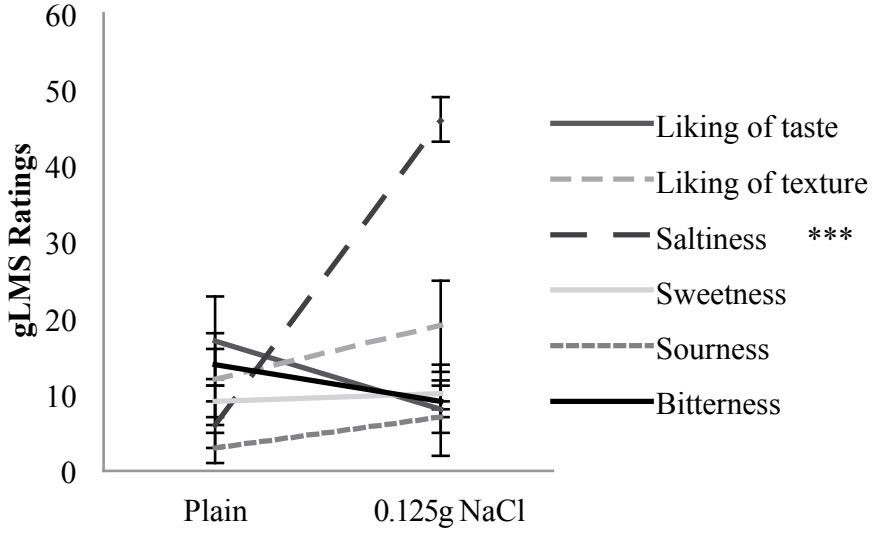
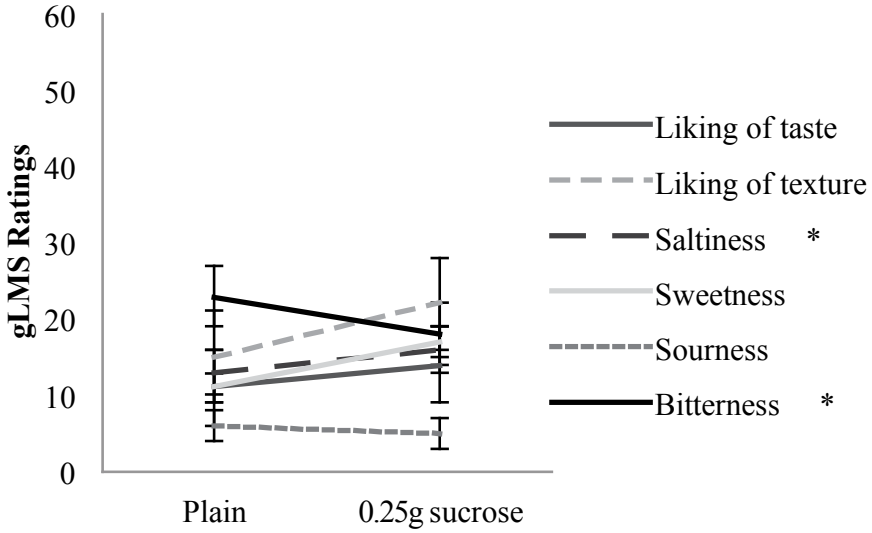


Figure 4.

A) Change in Sensory gLMS Ratings When Brussels Sprouts were Served Plain and with 0.25 g Sucrose, Experiment 2.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with 0.25 g Sucrose, Experiment 2.

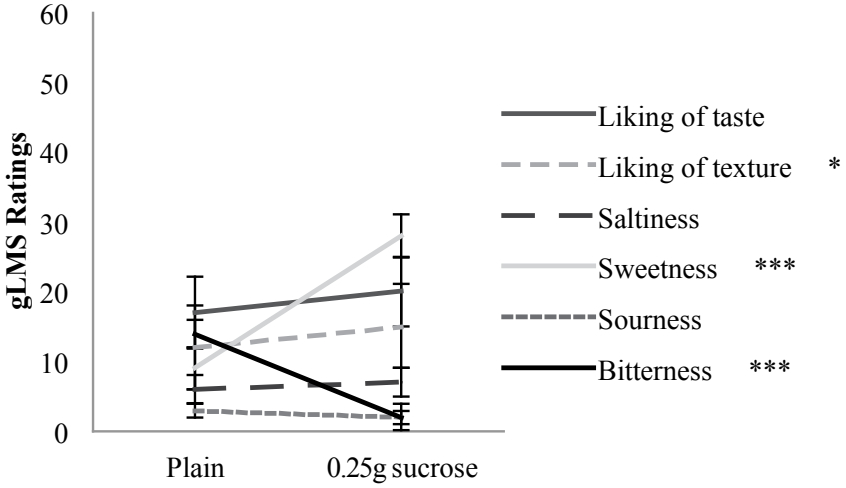


Figure 5.

Change in Sensory gLMS Bitterness Ratings When Brussels Sprouts Were Served Plain and with 0.25 g Sucrose, Saccharin, Aspartame, or Sucralose, Experiment 3. All Sweeteners Significantly Reduced Reported Bitterness.

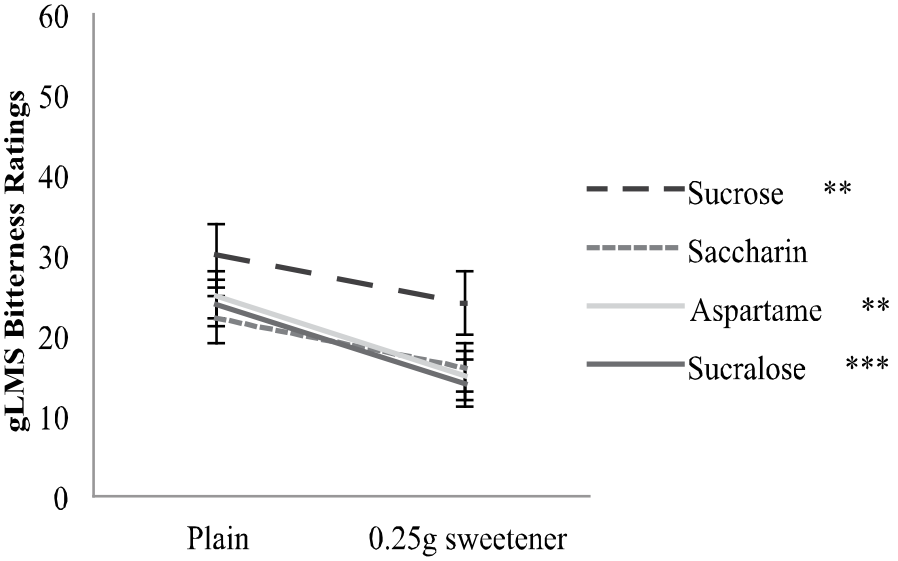
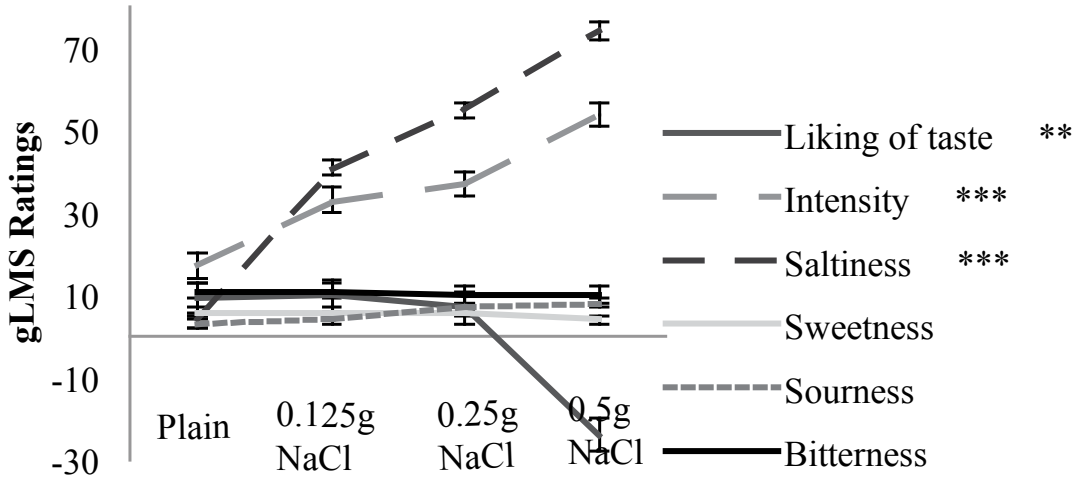


Figure 6.

A) Change in Sensory gLMS Ratings When Brussels Sprouts Were Served Plain and with NaCl, Experiment 4.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with NaCl, Experiment 4.

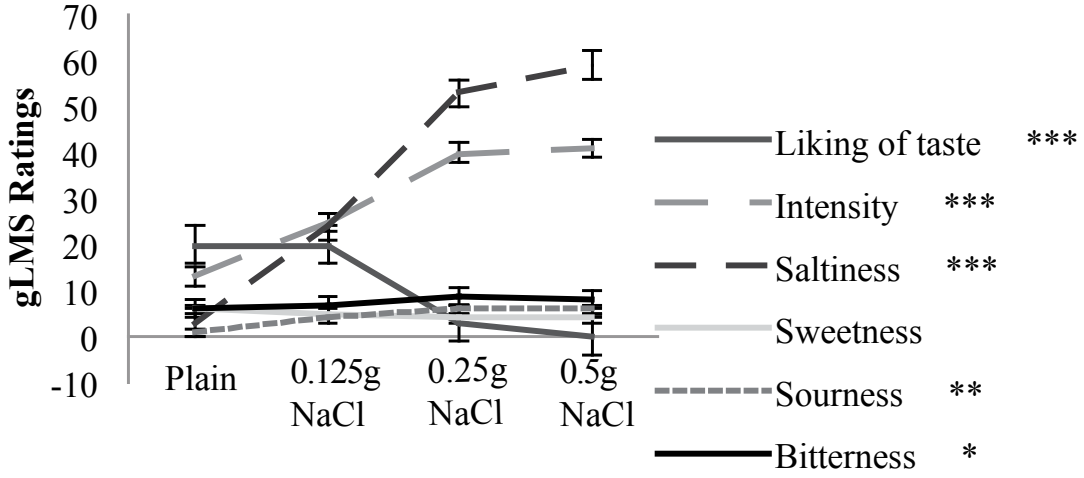
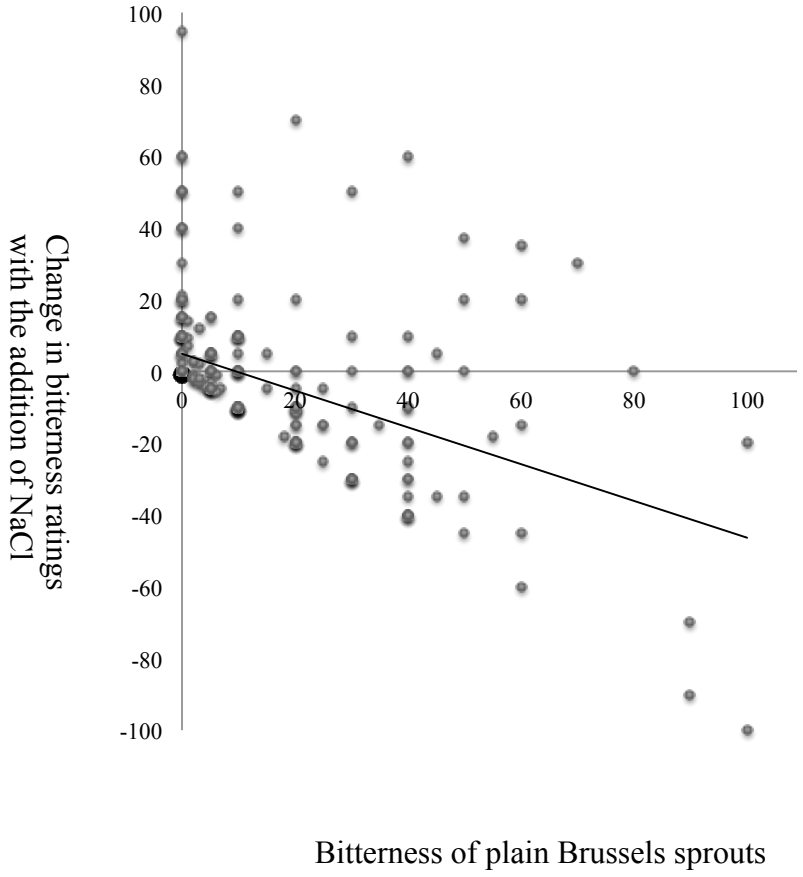


Figure 7.

A) Bitterness Rating of Plain Brussels Sprouts Significantly Negatively Predicts the Change in Bitterness When NaCl was Added, $\beta = -0.513$, Experiment 4. A Negative Change Score Indicates Bitterness Perception was Reduced.



B) Bitterness Rating of Plain Cauliflower Significantly Negatively Predicts the Change in Bitterness When NaCl was Added, β = -0.469, Experimnt 4. A Negative Change Score Indicates Bitterness Perception was Reduced.

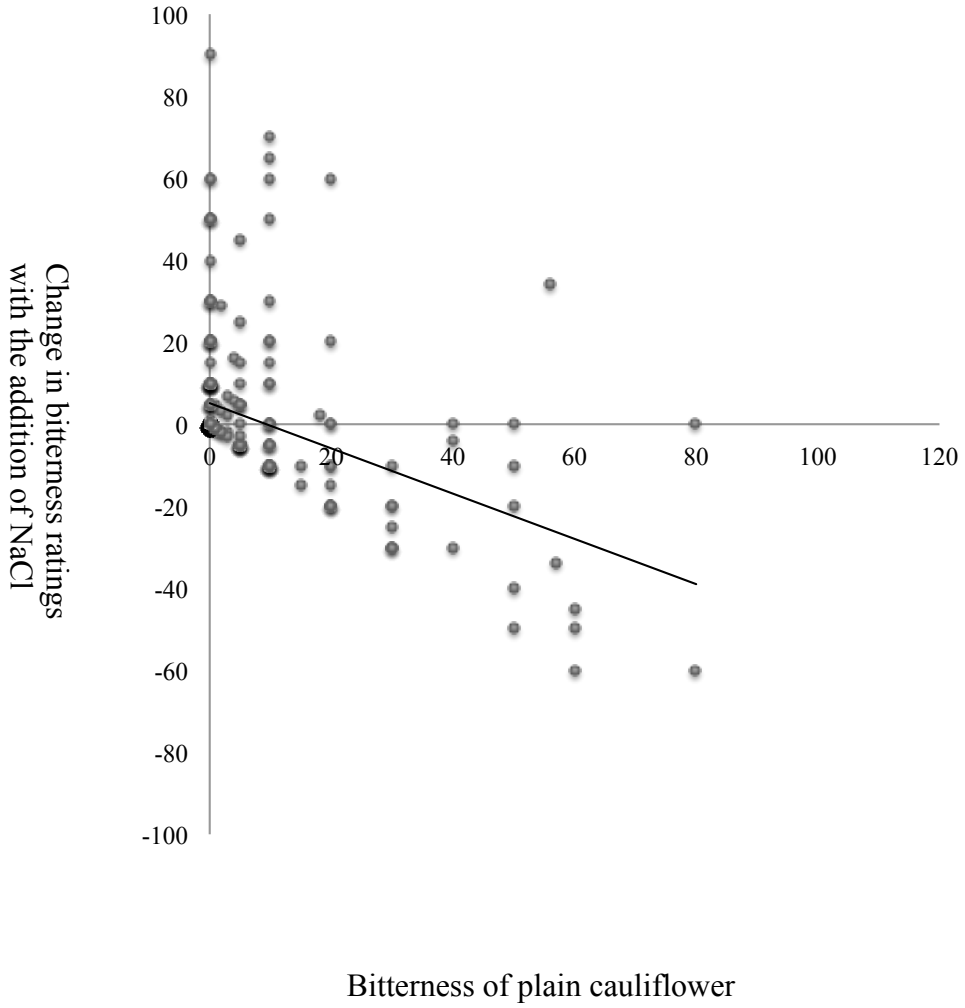
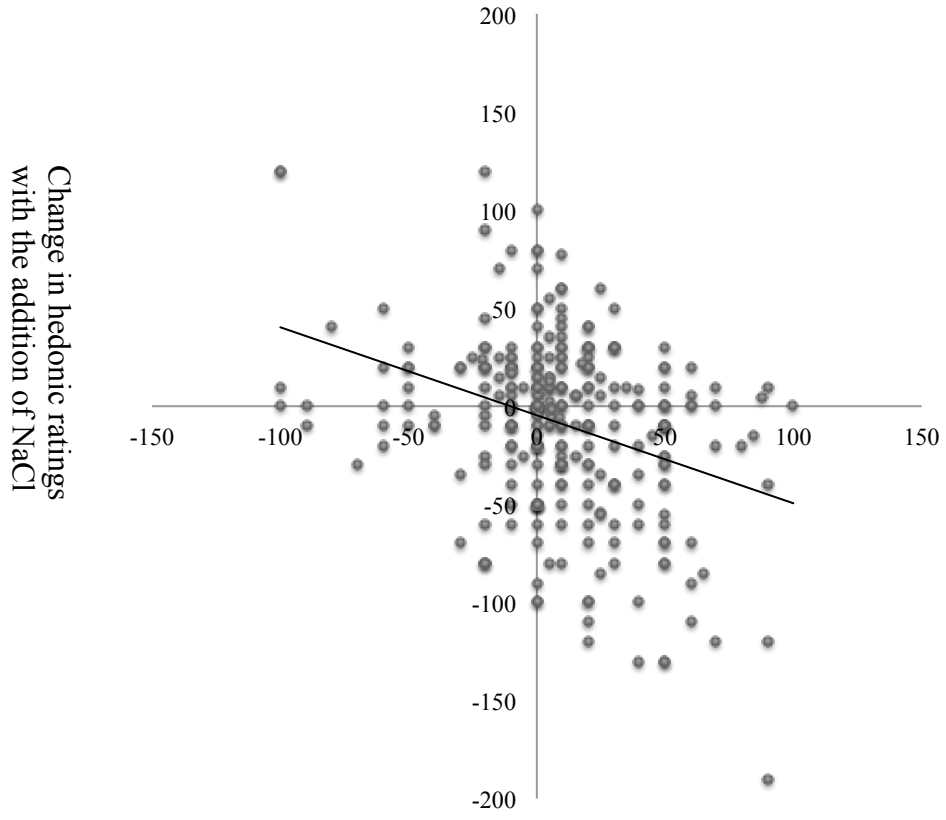


Figure 8.

A) Liking of Plain Brussels Sprouts Significantly Negatively Predicts the Change in Liking when NaCl was Added, β = -.419, Experiment 4. A Positive Change Score Indicates Liking Increased.



B) Liking of Plain Cauliflower Significantly Negatively Predicts the Change in Liking When NaCl was Added, β = -.502, Experiment 4. A Positive Change Score Indicates Liking Increased.

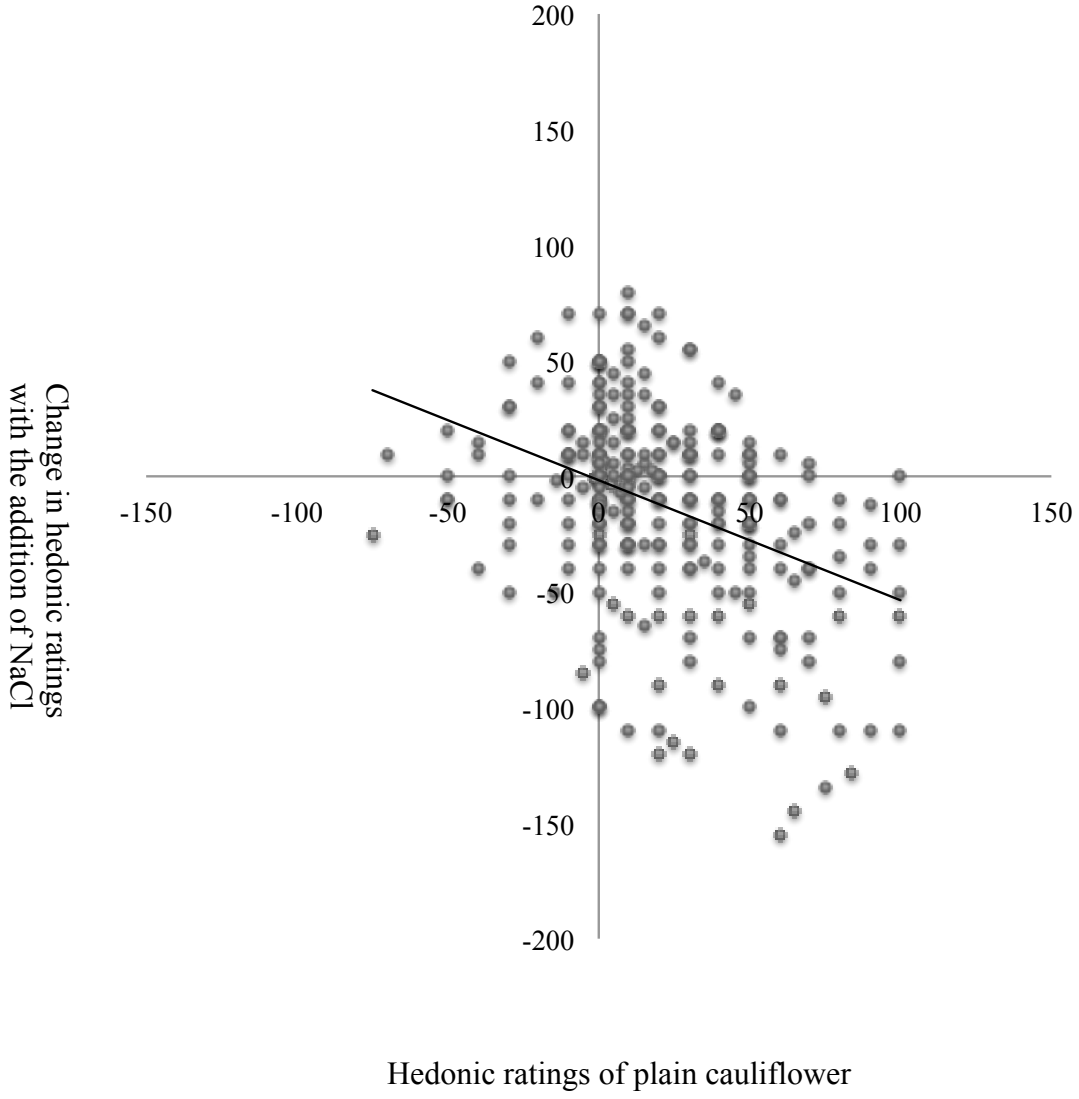
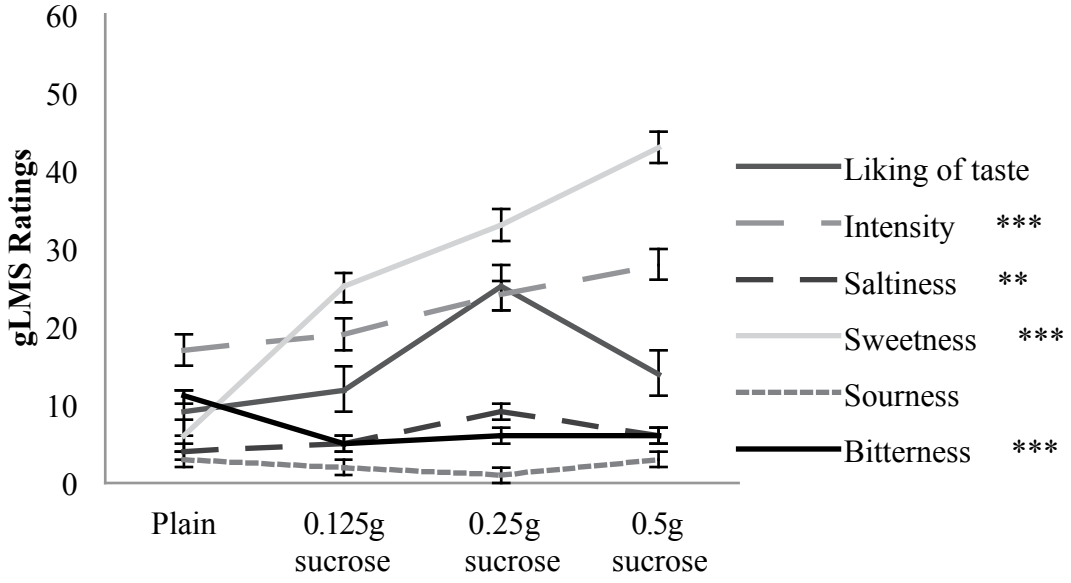


Figure 9.

A) Change in Sensory gLMS Ratings When Brussels Sprouts Were Served Plain and with Sucrose, Experiment 4.



B) Change in Sensory gLMS Ratings When Cauliflower was Served Plain and with Sucrose, Experiment 4.

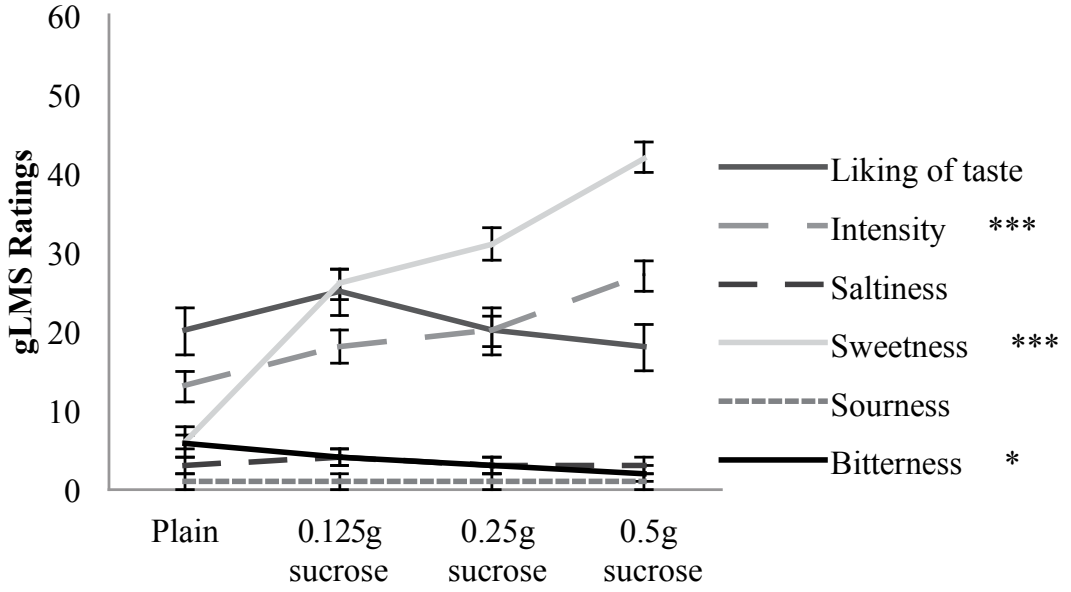


Figure 10.

Bitterness Rating of the First Sample of Plain Brussels Sprouts Significantly Negatively Predicts the Change in Bitterness Ratings Between the First and Second Samples, Experiment 5. Baseline Bitterness Ratings Predict the Change Score Over and Above the Control Group for the 0.30 g NaCl Group Only.

