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Masking Vegetable Bitterness to Improve Palatability Depends on Vegetable Type and Taste Phenotype

Mastaneh Sharafi,

Department of Allied Health Sciences, College of Agriculture and Natural Resources, University of Connecticut, 358 Mansfield Road, Unit 2101, Storrs 06269 CT, USA

John E. Hayes, and

Department of Food Science, College of Agricultural Sciences, The Pennsylvania State University, 220 Food Science Building, University Park 16802 PA, USA

Valerie B. Duffy

Department of Allied Health Sciences, College of Agriculture and Natural Resources, University of Connecticut, 358 Mansfield Road, Unit 2101, Storrs 06269 CT, USA

Abstract

Consumption of dark green vegetables falls short of recommendations, in part, because of unpleasant bitterness. A laboratory-based study of 37 adults was used to determine bitter and hedonic responses to vegetables (asparagus, Brussels sprouts, kale) with bitter masking agents (1.33 M sodium acetate, 10 and 32 mM sodium chloride, and 3.2 mM aspartame) and then characterized by taste phenotype and vegetable liking. In repeated-measures ANOVA, aspartame was most effective at suppressing bitterness and improving hedonic responses for all sampled vegetables. Among the sodium salts, 32 mM sodium chloride decreased bitterness for kale and sodium acetate reduced bitterness across all vegetables with a tendency to increase liking for Brussels sprouts, as release from mixture suppression increased perceived sweetness. Participants were nearly equally divided into three 6-*n*-propylthiouracil (PROP) phenotype groups. Those tasting the least PROP bitterness (non-tasters) reported least vegetable bitterness, and the additives produced little change in vegetable liking. Aspartame persisted as the most effective bitter blocker for the PROP tasters (medium, supertasters), improving vegetable liking for the medium tasters but too much sweetness for supertasters. The sodium salts showed some bitter blocking for PROP tasters, particularly sodium acetate, without significant gains in vegetable liking. Via a survey, adults characterized as low vegetable likers reported greater increase in vegetable liking with the maskers than did vegetable likers. These results suggest that bitter masking agents (mainly sweeteners) can suppress bitterness to increase acceptance if they are matched to perceived vegetable bitterness or to self-reported vegetable disliking.

Keywords

Bitter; Food preference; Genetics; Salt; Sweet; Taste; Vegetables

Introduction

Vegetable-rich diets have been shown to protect against chronic diseases and promote health (US Department of Agriculture and US Department of Health and Human Services 2010a). According to the 2010 Dietary Guidelines for Americans, daily consumption of 2.5 cups vegetables is advised for a 2,000-calorie diet (US Department of Agriculture and US Department of Health and Human Services 2010b). However, few individuals meet these recommendations. According to the US Behavioral Risk Factor Surveillance System, only one in four adults reported consuming at least three daily servings of vegetables when surveyed in 2000 and then again in 2009 (CDC 2010).

Vegetables provide diverse taste and textural and olfactory sensations, which join other factors (e.g., cost, availability/accessibility, safety, and quality) to determine whether or not vegetables are liked and consumed (Coulthard and Blissett 2009; Cox et al. 2012; Dammann and Smith 2009; Powell et al. 2009; Taylor et al. 2012). Strong bitterness deters vegetable acceptability, as it suppresses endogenous sweetness in vegetables (Dinehart et al. 2006) and may enhance disliked vegetable aromas (Duffy et al. 2007). An unfortunate relationship exists between the healthful components of green vegetables (e.g., phytonutrients) and the level of bitterness. Although beneficial to health (Drewnowski and Gomez-Carneros 2000), phytonutrients impart bitterness (Czepa and Hofmann 2004; Drewnowski and Gomez-Carneros 2000; Pasini et al. 2011; Roland et al. 2011) and other aversive oral sensations like astringency (Bajec and Pickering 2008; Kielhorn and Thorngate 1999) and irritation (Bennett et al. 2012; Cicerale et al. 2009). Since removing these compounds to improve taste eliminates their potential health benefit, suppressing vegetable bitterness may be an alternative to improve acceptability while preserving intake of these health-promoting compounds.

As recently reviewed (Bennett et al. 2012), bitter suppression occurs at the peripheral level through inhibition of taste receptors, the central cognitive level through perception of bitters mixed with other tastes or olfactory flavorings, or physically blocking the bitter stimulus from reaching receptors, as with fat or cyclodextrin. The sodium cation from sodium salts suppresses bitterness of aqueous pharmaceuticals (Keast and Breslin 2002; Mennella et al. 2003), even without adding a salty taste, which suggests peripheral inhibition at the receptor level (Keast and Breslin 2005). Sodium acetate (NaAc) has been shown to be effective at blocking the bitterness of aqueous pharmaceuticals (Keast and Breslin 2002). The bitter blocking of sodium salts and other compounds (e.g., β -cyclodextrin) varies with the bitter chemical structure and the mechanism of how the blocker impedes the bitter-receptor binding (Gaudette and Pickering 2012a). Adding a sweet-tasting compound suppresses bitterness via central integration of the mixture of taste qualities (i.e., mixture suppression) (Guadagni et al. 1974; Kroeze and Bartoshuk 1985; Lawless 1979). Through a conditioning paradigm, Capaldi and Privitera (2008) demonstrate simply adding sweetness to traditionally disliked vegetables (broccoli, cauliflower) can improve vegetable preference in college-aged adults and children.

The perception of bitterness from vegetables varies, and some of this variability has a genetic basis. Best studied is variation in the bitterness of the thiourea (N-C=S) 6-*n*-propylthiouracil (PROP) for characterizing non-tasters, medium-tasters, and super-tasters (Bartoshuk et al. 1994). PROP tasters report greater bitterness from a broad range of compounds and foods/beverages including quinine hydrochloride (QHCl) (Hayes et al. 2008), naringin (Drewnowski et al. 1997), grapefruit juice (Drewnowski et al. 1997; Lanier et al. 2005), coffee (Lanier et al. 2005), green tea (Gayathri et al. 1997), alcoholic beverages (Intranuovo and Powers 1998; Lanier et al. 2005), and vegetables (Dinehart et al. 2006; Kaminski et al. 2000; Keller et al. 2002; Turnbull and Matisoo-Smith 2002). Those who

taste PROP as more bitter also report more sweetness from sucrose (Hayes and Duffy 2007) and aspartame (Duffy et al. 2006). Because oral sensations are important to dietary behaviors, and dietary behaviors to health, PROP bitterness could serve as biomarkers for diet-related chronic diseases (Duffy et al. 2007). Most evidence associates PROP phenotype or genotype with vegetable consumption, and this work was initially focused on vegetables high in compounds containing the N-C=S moiety, particularly Brassicaceae (Tepper 2008). Individuals with a greater propensity to experience bitterness have been shown to experience more negative sensations from vegetables, which could generalize to overall disliking for vegetables (Dinehart et al. 2006). Previous experiments from our laboratory found that supertaster adults reported greater bitterness and less natural sweetness in sampled, traditionally disliked green vegetables and reported lower consumption of all vegetables as assessed by food frequency survey (Dinehart et al. 2006). Similar results were found in an experimental study of preschool children, where PROP-sensitive children consumed less plain broccoli (Fisher et al. 2011). Interestingly, when ranch dressing was added to broccoli as a dip, a significant improvement was observed in broccoli consumption for the PROP-sensitive children. The dip likely provided peripheral suppression of broccoli bitterness by sodium ions as well as central suppression via added sweetness.

Although sodium-containing compounds and sweeteners have proven efficacy in suppressing the bitterness of pharmaceuticals and other bitters in model systems, the efficacy of these additives have not been sufficiently studied with regard to Brassicaceae vegetables like kale, and Brussels sprouts, or root vegetables like asparagus. We pilot-tested the procedures on 26 subjects to assure that the additives were matched to the level of bitterness across all three vegetable types and to obtain a range of sodium levels to test bitter blocking with limited saltiness. Zinc sulfate was pilot-tested as a bitter blocker but discontinued after testing ten participants as it reduced sweetness (Keast et al. 2004b), increased astringency, and diminished vegetable acceptability.

The first objective of the present study was to determine the impact of bitter suppressors on the bitterness and preference of green vegetables. We tested the high-intensity, non-nutritive sweetener aspartame, a centrally mediated bitter blocker, to deliver sweetness in a small volume without the added viscosity of an equivalently sweet carbohydrate sweetener. Aspartame is approved by the US Food and Drug Administration for safety as a general use sweetener and, according to a recently published evidence analysis (Fitch and Keim 2012), had a grade I=good level of evidence of not causing adverse effects in adults from peer-reviewed published human subjects. Based on our unpublished data from 75 adults, aqueous aspartame and sucrose, roughly matched for sweetness (between moderately and strongly sweet), did not produce significant differences in non-sweet qualities (sourness, bitterness, saltiness, irritation) or level of liking. The concentrations of sodium chloride (NaCl) were selected for their potential to enhance vegetable sweetness. Weak aqueous NaCl solutions can elicit sweet taste (Bartoshuk et al. 1978) and NaCl is commonly added to foods and in food preparation to enhance sweetness. NaAc was selected as it blocks bitterness in aqueous solution (Keast and Breslin 2002; Mennella et al. 2003), enhances sweetness in aqueous bitter/sweet mixtures (Breslin and Beauchamp 1997), and through pilot testing, produced between moderate and strong saltiness in aqueous solution yet only a weak salty taste when added to vegetables. The second objective was to examine the impact of PROP taster status on the suppression of bitterness and vegetable acceptability when bitter suppressors were added. We hypothesized peripheral and central bitter suppressors would both work to block bitterness and improve vegetable acceptability, but these effects would be more pronounced in those reporting greater PROP bitterness.

Methods

Subjects

Individuals were recruited through posters and word of mouth from the University community for this laboratory study. In a repeated-measures design, we tested the degree to which known bitter suppressors affected oral sensations (sweetness, sourness, saltiness, bitterness, and astringency) for three commonly disliked vegetables (asparagus, Brussels sprouts, and kale). Recruitment posters asked individuals to “participate in a study looking at why people eat what they do.” No specific emphasis was placed on the examination of vegetable preference, liking, or intake per se. The University of Connecticut Institutional Review Board approved all methods. Subjects provided informed, written consent and were paid for their time.

Thirty-seven reportedly healthy adults (21 females, ages 18 to 32 years), without severe dietary restrictions (e.g., food allergies or intolerance), participated in one session in a taste laboratory. This sample had nearly equal representation across the three PROP taster groups and was sufficient size to test the study objectives. Based on sample size calculations, a sample size of 27 was required to detect an effect size of 0.25 with power of 80% and $\alpha=0.05$ in a study design that include three groups (two within and one between group) and five measurements. All participants were prescreened via a web-based survey for study eligibility. One individual with high potential of pathology-related oral sensory alteration secondary to severe childhood history of otitis media requiring ear tube placement was excluded from participation. The screening also included the Revised Restraint Scale (Herman and Polivy 1980), and total restraint scores in our sample averaged less than published norms for college-aged students (Allison 1994). All but three participants were of European descent, and most were normal weight.

Procedure

Data were collected in one session in the following order: orientation to the scaling procedure; liking and taste quality ratings of prototypical tastants, including 0.32 and 1 M sucrose and 0.32 mM and 1 mM quinine; surveyed liking/disliking of 28 foods and 11 non-food items, including three vegetables (green beans, raw carrot, broccoli); a seven-item standard dietary screener to measure total fruit and vegetable consumption (Havas et al. 1995); liking and taste quality ratings of sampled vegetables lightly misted with water (control) or bitter blocker; and PROP phenotype.

For oral sampling, the experimenter provided each stimulus, one at a time, encouraging participants to sample fully (aqueous solutions for 5 s, vegetable samples masticated), to rinse thoroughly with deionized water after each, and to re-rinse if there was a lingering taste. The intensity and affective ratings were collected using a general Labeled Magnitude Scale (gLMS) (Bartoshuk et al. 2004), a modification of the LMS (Green et al. 1996; Green et al. 1993) that changes the top of the scale to ‘strongest imaginable sensation of any kind.’ For the intensity and hedonic ratings, the gLMS was oriented vertically, ranging from ‘no sensation’ (0) to ‘strongest imaginable sensation of any kind’ at the top (100), with adjectives of ‘barely detectable’ (1.4), ‘weak’ (6), ‘moderate’ (17), ‘strong’ (35), and ‘very strong’ (53). For orientation to the gLMS scale, subjects rated intensities of 16 remembered sensations including brightness, loudness, tastes, and oral irritation/burn. All of the participants were able to order correctly the intensities of loudness (whisper<conversation<loudest sound) and brightness (dimly lit<well-lit<brightest light) sensations. For the surveyed liking/disliking ratings, the gLMS was oriented horizontally, where the extreme left of the scale was labeled as ‘strongest imaginable liking’ (+100) and the extreme right side labeled as ‘strongest imaginable disliking’ (−100).

Sampled Vegetables—Following a published procedure (Dinehart et al. 2006), participants orally sampled asparagus, Brussels sprouts (Bird's Eye®, Rochester, NY), and kale (Big Y Foods, Inc, Springfield, MA), purchased from the freezer section of a grocery store, and portioned into individual sealed plastic bags (one spear head of asparagus, 1/2 Brussels sprout, 4 g kale). Vegetables for testing were held in sealed plastic bags in the refrigerator no more than 24 h. On the day of testing, vegetables were taken from the refrigerator and served at room temperature.

Stimuli—Participants tasted 15 vegetable stimuli—three vegetable types, each plain and with four different additives, presented in randomized order. For each, participants first rated the intensity of sweet, sour, salty, bitter, burn/astringency, and then the level of liking/disliking. All vegetables were misted in a standardized protocol with 0.5 mL distilled water (control), 0.5 mL of 3.2 mM aspartame (NutraSweet), and 0.5 mL of sodium salts—10 mM NaCl, 32 mM NaCl (Kosher Salt, Diamond Crystal, Minneapolis, MN), and 1.33 M trihydrate sodium acetate (NaAc, Fisher USP, Fair Lawn, NJ). When added as 0.5 mL per vegetable sample, the salts ranged in added sodium (0.1 mg Na for 10 mM NaCl, 0.4 mg Na for 32 mM NaCl, 15 mg Na for 1.33 M NaAc) to assess bitter blocking and potential enhancement of natural vegetable sweetness. The aspartame level was selected to be perceived as roughly equivalent to the sweetness from 1 M sucrose (DuBois et al. 1991). Vegetables were misted with the additive solution within 30 min of testing. Although participants were free to swallow or expectorate the vegetable sample and then rinsed their mouth with deionized (>15 M Ω) water after each food sample, they were asked to report the intensity rating before swallowing or expectorating the vegetable samples.

PROP Taste Phenotype—At the conclusion of the test session, participants rated the intensities of a series of aqueous NaCl (0.5 log steps from 0.01 to 1 M), tones (1,000 Hz, 50 to 98 dB), and aqueous PROP (3.2, 1, 0.32, 0.1, and 0.032 mM, Sigma, St. Louis, MO), with PROP presented last and randomized within the series. The ratings of NaCl and tones provided a standard for comparing the bitterness of PROP across individuals (Bartoshuk et al. 1998). The participants were instructed to swish for 5 s, spit, rate the intensities using the gLMS, and rinse with deionized (>15 M Ω) water after each solution.

Statistical Analysis

Initial reproducibility analysis was performed to determine participants' consistency in rating prototypical taste qualities (Gaudette and Pickering 2012b). Coefficient of variation was calculated between sucrose (0.32 and 1 M) for perceived sweetness, duplicate measures of 32 mM NaCl for perceived saltiness, quinine (0.32 and 1 mM), as well as PROP (1 and 3.2 mM) for perceived bitterness. The inclusion criterion was a coefficient variation of <100 % for at least three of the four perceived tastes; all of the participants met this criterion. The effect of additive on taste and preference ratings was tested with repeated-measures ANOVA, using age, sex, and 74 dB tones as covariates, and with *t* tests as planned comparisons (Keppel 1991). Associations between individual variables were tested with the non-parametric Spearman's rank order correlation. Standard linear regression analysis was used to assess the relationship between composite vegetable taste and hedonic rating variables as well as the composite hedonic rating and vegetable intake variables (Tabachnick and Fidell 2001), reporting the correlation coefficient, semipartial correlation coefficient (*sr*—unique variance explained by each independent variable), or percent variance (*r*²). The criterion for significance was *p* 0.05.

Results

Sensory and Hedonic Ratings of Vegetables

Among the three sampled vegetables, bitterness averaged as the most pronounced yet most variable taste quality; mean ratings ranged from no sensation to very strong (Table 1). A significant negative association between bitterness and endogenous sweetness of plain Brussels sprouts was found; those who rated Brussels sprouts as more bitter perceived them as less sweet ($\rho = -0.34, p < 0.05$). This association was not significant for asparagus or kale. Hedonic ratings for the individual vegetables averaged between weakly disliked to neutral and were highly variable, ranging from greater than very strong disliking to above moderate liking. Bitterness was the primary driver of hedonic ratings for the sampled vegetables; there was also a trend for a separate contribution of sweetness, consistent with prior work (Dinehart et al. 2006). Averaged across all vegetables, 28 % of the variance ($p = 0.005$) in the mean hedonic rating was explained by a multiple regression model containing bitterness ($\beta = -0.48, p < 0.01$) and sweetness ($\beta = 0.25, p = 0.10$). Affective ratings for the three vegetables obtained by survey averaged from strongly disliked to strongly liked. Although surveyed liking was correlated significantly with hedonic ratings for the sampled vegetables ($r = 0.59, p = 0.001$), only surveyed vegetable liking was significantly correlated with vegetable consumption ($r = 0.44, p < 0.05$).

Oral Sensations from Vegetables with Bitter Masking Agents

Modeling bitterness in a two-way repeated-measures ANOVA revealed a significant interaction ($F(8,280) = 5.03, p < 0.0001$) between vegetable (asparagus, Brussels sprouts, kale) and additive (water, four additives). In a similar ANOVA model for the liking of sampled vegetables, only a significant main effect of additive ($F(4, 140) = 3.64, p < 0.01$) was observed; the additive by vegetable interaction was marginal ($F(8, 280) = 1.82, p = 0.07$).

Aspartame significantly suppressed bitterness for all three sampled vegetables (Fig. 1). Vegetables with added aspartame averaged just above moderately sweet, compared with strongly sweet for the aqueous aspartame. Aspartame also significantly improved the hedonic rating, shifting the ratings from disliking to liking for Brussels sprouts ($p = 0.01$) and kale ($p = 0.01$) as well as enhancing liking for asparagus ($p < 0.05$).

As illustrated in Fig. 1, although 32 mM NaCl decreased bitterness for kale ($p < 0.05$), NaAc was the most effective sodium salt to reduce bitterness for Brussels sprouts ($p < 0.05$), kale ($p < 0.05$), and asparagus ($p < 0.0001$). The bitter suppression occurred with minimal, although significant (all p 's < 0.001) increase in vegetable saltiness (vegetables plus NaAc ranged in saltiness from weak to moderately intense, compared with that between moderate and strong saltiness for the aqueous NaAc). The perceived saltiness of the vegetables with added NaCl salts was not different (all p 's > 0.25) from the saltiness of the plain vegetables. (For comparison, saltiness for aqueous 10 and 32 mM NaCl averaged just above moderate and just above strong, respectively). Although adding NaAc and 32 mM NaCl increased the perceived vegetable sweetness significantly ($p < 0.05$), averaging still below weakly sweet, none of the added sodium salts produced significant change in the hedonic rating for the vegetables. However, NaAc tended to increase the preference for Brussels sprouts ($p = 0.07$). In a sub-analysis, the shift in hedonic rating with added NaAc was related to change in sweetness. Of those who found Brussels sprouts less pleasant with added NaAc, 14 of 15 reported less or no change in sweetness, and of those who found Brussels sprouts more pleasant with added NaAc, 13 of 22 reported an increase in sweetness (two-tailed Fisher exact probability test < 0.01). In summary, the 10 and 32 mM NaCl failed to increase sweetness enough to shift hedonic value of the sampled vegetables. NaAc appeared to

provide sufficient bitter blocking but only increased liking if there was a concurrent increase in perceived sweetness.

Individual Variation—PROP Effects

Participants showed diversity in PROP bitterness ratings, with 12 non-tasters (3.2 mM PROP below 24, which is just above moderate), 12 medium tasters (between 24 and 51, which is very strong), and 13 supertasters (51+; above very strong). In this cohort, there was no significant effect of age or sex on PROP bitterness (p 's>0.2). Similar to our previous finding (Dinehart et al. 2006), PROP bitterness showed a closer association with the bitterness of vegetables than either vegetable hedonic rating or reported vegetable intake. As a continuous variable, PROP bitterness was significantly associated with average bitterness across all of the sampled vegetables ($r=0.37$, $p<0.05$). Tested as a categorical variable by Fisher's exact two-tailed test, PROP non-tasters were more likely to report the average bitterness as less than weak–moderately bitter ($p<0.05$), whereas PROP supertasters were more likely to report the vegetables as greater than this bitterness level. PROP bitterness did not correlate significantly with reported vegetable liking from sampled or surveyed vegetables. However, the variance in hedonic ratings for the sampled vegetables was significantly greater in the supertasters and medium tasters than in the non-tasters ($p<0.001$ and $p<0.05$, respectively). For example, the range of hedonic ratings in the supertasters ranged from strongly disliked to moderate liking and, in the non-tasters, from weakly disliked to weakly liked.

Effects of Masking Agents on Vegetable Bitterness and Liking by PROP

Status—The masking agents showed differential effects on vegetable bitterness and hedonic rating based on PROP taster status; they were most effective for the medium tasters (Fig. 2). In three-way repeated-measures ANOVA (PROP taster group by additive by vegetable), PROP ($F(2,30)=5.32$, $p=0.01$) and additive ($F(4, 132)=8.72$, $p<0.0001$) showed separated main effects on vegetable bitterness; there was neither significant main effect of vegetable type nor any interactions except for the vegetable type by additive interactions described previously. For the medium taster group, aspartame suppressed bitterness across all vegetables (Fig. 2). Among the sodium salts (Fig. 3), NaAc was as effective as aspartame for blocking bitterness in medium tasters, whereas the 32 mM NaCl suppressed bitterness only for kale. For PROP supertasters, only aspartame suppressed bitterness across all vegetables concurrent with significant increase in sweetness, averaging just below strong sweetness (Fig. 2). The sodium salts had less consistent effects for supertasters; NaAc (Fig. 3) and 10 mM NaCl ($p=0.01$) decreased bitterness of asparagus (but not kale and Brussels sprouts). For non-tasters, there was less bitterness suppression, which would be expected due to a floor effect. Through analysis with a sign test, at least 10 of 12 non-tasters reported less bitterness for the vegetable plus additive ($p<0.05$) for aspartame and NaAc but not for NaCl.

For vegetable hedonic rating, there was a trend for three-way interaction of additive by vegetable type by PROP status ($F(16, 264)=1.52$, $p<0.1$). As shown in Fig. 2, only medium tasters had significant improvement in hedonic rating with aspartame added to Brussels sprouts and kale as well as 10 mM NaCl added to Brussels sprouts ($p<0.05$). For supertasters, in spite of significant bitter reduction by aspartame, no significant changes in hedonic rating were observed. Further analysis showed that, after addition of aspartame, the perceived sweetness of sampled vegetables was significantly higher among supertasters compared with medium tasters ($p<0.05$) and non-tasters ($p<0.01$), without significant difference in other taste qualities including sourness and irritation. Sodium salts also did not have a significant influence on vegetable hedonic rating for the supertaster group; the response was more variable than either the medium or non-taster groups. Consistent with

bitterness, non-tasters failed to show significant average difference in hedonic ratings from plain versus additive vegetables; the sign test only showed a trend with 9 of 12 non-tasters reported more liking for vegetables plus aspartame and NaAc ($p=0.07$).

Do Additives Improve Liking for Vegetable Dislikers Assessed by Survey?

Using the survey reported liking across three vegetables, participants were divided into high vegetable likers ($n=9$, averaging strongly like), moderate vegetable likers ($n=16$, averaging moderately like), and vegetable dislikers ($n=9$, averaging moderately dislike) for a three-way repeated-measures ANOVA (vegetable liking group by additive by vegetable) on vegetable hedonic ratings. We observed significant main effects of liking group ($F(2, 27)=4.19$, $p<0.05$) as well as a significant interaction between liking group and additive ($F(8,120)=3.21$, $p<0.01$) and vegetable type ($F(4,60)=3.56$, $p=0.01$). The three-way interaction was not significant. Vegetable dislikers reported improvements in liking when aspartame was added to Brussels sprouts ($p=0.01$), asparagus ($p=0.01$), and kale ($p=0.08$). Moderate vegetable likers showed some improvement in hedonic rating of sampled vegetables with added aspartame (kale, $p<0.001$; Brussels sprouts, $p=0.07$). For individuals who reported high liking for vegetables on the survey, none of the additives significantly increased liking ratings for the sampled vegetables.

Discussion

General Findings

Vegetable consumption, particularly green vegetables, falls short of public health recommendations. Although vegetables contain health-promoting phytonutrients, they taste bitter (Czepa and Hofmann 2004; Drewnowski and Gomez-Carneros 2000; Pasini et al. 2011; Roland et al. 2011). The present study examined the taste and hedonic responses to predominantly bitter vegetables that are frequently disliked, when bitter masking agents (sweetener, sodium salts) were added. Our general finding was that adding a small amount of a non-nutritive sweetener (equivalent in sweetening power to 0.75 teaspoon of sugar to 0.5 cup of vegetables) decreased the bitterness significantly and shifted the hedonic rating for all three vegetables from disliked to liked. The ability of the sodium salts to block bitterness of the vegetables was dependent on the level of added sodium—NaAc blocked bitterness across all vegetables (approximately 160 mg Na to ½ cup of vegetables) while the 32 mM NaCl (approximately 4 mg Na to ½ cup of vegetables) blocked bitterness of only one vegetable. Averaged across all three sampled vegetables, aspartame, NaAc, and 32 mM NaCl decreased perceived bitterness by 71 %, 42 %, and 22 %, respectively. With peripheral reduction of bitterness at the receptor by sodium ions, we might expect release of mixture suppression, which should increase the endogenous sweetness of the vegetables. However, NaAc only improved the hedonic value of one vegetable, Brussels sprouts, by concurrently increasing the perceived sweetness. Otherwise, the added sodium salts did not have a consistent effect on sweetness to improve vegetable liking. The masking agents were most effective for individuals who had a greater propensity to experience bitterness and for individuals who self-reported prior disliking for vegetables via survey.

Overall Effects of Additives to Modify Vegetable Bitterness and Enhance Vegetable Preference

Across all of the participants, aspartame was most effective at reducing bitterness and increasing vegetable hedonic rating. The aspartame-vegetable mixture was about 41 % less sweet than aspartame alone and 71 % less bitter than the vegetable alone. The average shift across the study sample from disliking of sampled vegetables to liking could have resulted from central cognitive suppression of bitterness, as shown previously (Lawless 1979) and was most effective when bitterness was balanced with sweetness. Exposing adults and

children to broccoli or grapefruit juice with increased sweetness conditioned a preference for these foods, with only 3 days of exposure for adults, and the preference lasted even in response to the unsweetened version (Capaldi and Privitera 2008). In unpublished pilot data with children, we found that two of three children preferred cooked vegetables misted with light sweetness to vegetables misted only with water (Napoleone et al. 2007). Recent research suggests that, through food technology and food chemistry, the level of olfactory volatiles that are sweet in quality can be manipulated to increase the perceived sweetness of a vegetable, such as tomatoes, without need of additives (Tieman et al. 2012).

The ability of the sodium salts to suppress vegetable bitterness is consistent with research on the role of sodium to suppress bitterness in model systems (Breslin and Beauchamp 1995; Keast and Breslin 2002; Kroeze and Bartoshuk 1985). For example, the bitterness of individual aqueous pharmaceutical agents is reduced when NaAc and NaCl are added (Keast and Breslin 2002). Averaged across all three sampled vegetables, we found that NaAc decreased perceived bitterness by 42 %, which is close to the 55 % bitterness suppression seen in pharmaceuticals (Keast and Breslin 2002). The greater ability for NaAc to suppress bitterness would be expected. Based on the molecular weight, NaAc provided about 40 times the amount of sodium than the top concentration of NaCl (32 mM). In an unpublished laboratory experiment (Balitsis 2008), five different levels of NaCl (150, 350, 450, 550, and 750 mg) were added to 85 g of broccoli; 350 mg produced highest reduction in bitterness, concurrent increases in saltiness, and significant improvements to liking. The present study found a greater increase in hedonic ratings with a NaCl level equivalent to 420 mg added to 85 g of vegetables, with less increase in saltiness and an equivalent reduction in bitterness. We did not observe a general enhancement of vegetable sweetness with added salts. The ability of NaAc to block bitterness and enhance sweetness, as observed in aqueous mixtures with urea (Breslin and Beauchamp 1997), appears to be vegetable-specific and not observed in all individuals. Nonetheless, it appears that the aspartame additive exceeded the effect of the sodium salts on vegetable bitterness and hedonic rating.

PROP Taster Status Effects on Vegetables' Oral Sensation

Genetic diversity exists in the multiple chemosensory mechanisms and receptors that respond to a variety of chemicals and produce differential perception of bitterness and preference for foods, such as vegetables. Vegetables contain multiple bitter ligands that may reinforce other negative oral sensations (e.g., strong retronasal sulfur flavor) and suppress positive oral sensations (e.g., natural sweetness). Although there are increasing numbers of ways to characterize variation in bitter taste perception (Hayes and Keast 2011), differential response to the bitterness of concentrated PROP has been the most studied and linked to heightened overall oral sensation related to greater numbers of taste receptors (e.g., fungiform papillae) and increased cortical processing (Bembich et al. 2010). In the present study, PROP supertasters reported more bitterness from traditionally disliked green vegetables and consistent with previous findings (Dinehart et al. 2006), this elevated bitterness was associated with lower affinity for vegetables, which in turn, was associated with lower vegetable consumption. Of note, however, the present study was not powered to confirm a path of association between PROP bitterness and vegetable consumption, as our primary goal was to determine the efficacy of bitter blockers on vegetable hedonics.

The additive levels used in the present study appeared most optimal to suppress bitterness and enhance hedonic rating for PROP medium tasters (Fig. 2). The added aspartame produced a perceivable increase in sweetness that reduced, yet balanced, with bitterness to improve liking for Brussels sprouts and kale (asparagus showed the same pattern, but the change in liking was not significant). The non-taster and supertaster groups showed an imbalance in bitterness and sweetness of the vegetable plus added aspartame. PROP non-tasters reported less bitterness from vegetables to suppress the sweetness of the added

aspartame. PROP supertasters reported highest sweetness from the added aspartame, consistent with prior work (Duffy et al. 2006), which exceeded the natural level of vegetable bitterness. Collectively, these findings suggest that the level of sweetness added to vegetables needs to be individualized and titrated to the perceived bitterness of the vegetables.

Of the sodium salts, NaAc was more effective at blocking bitterness, and this effect was most apparent in the medium tasters. However, added NaAc did not produce a consistent increase in hedonic ratings. Added salt may need to improve sweetness to change vegetable preference as suggested by the hedonic ratings of plain vegetables, the survey data, and the sub-analysis with Brussels sprouts. Low levels of sodium salts, including NaCl, are known to enhance sweetness (Barisas et al. 2006) but failed to work as well as the added sweetener to enhance liking. The addition of low levels of sodium with sweet may be needed to enhance liking as suggested in research with single bitter compounds (Breslin and Beauchamp 1997; Gaudette and Pickering 2012b; Keast and Breslin 2005). The application to vegetable bitterness and preference is unknown. A recent study has found that preschoolers who were PROP tasters showed improvements in liking for broccoli when served with a dip that was salty and flavorful (Fisher et al. 2011). Our study failed to show the increase in PROP supertaster's hedonic ratings, when sodium salts or aspartame were added to the vegetables. Supertasters may need a broader change in oral sensations from vegetables, and not just saltiness (Hayes et al. 2010) or sweetness, to improve hedonic rating. In addition, supertasters showed higher variability in responses to plain vegetables and additives, which suggests that there might be some other unmeasured moderators in the relationship between supertasting and vegetable liking. Importantly too, PROP bitterness likely does not capture all of the variability in unpleasant sensations from vegetables (e.g., sulfurous odors, textures, etc.) and may not fully differentiate individual preference for vegetables.

Additives Improve Vegetable Preference for Low Vegetable Likers Identified by Survey

Vegetable taste testing may not be required to identify individuals who respond to modifying vegetable tastes to improve vegetable consumption. Surveyed liking for common vegetables correlated significantly with the hedonic rating for sampled vegetables, and most of participants who reported disliking vegetables also consumed vegetables less frequently. These findings suggest that survey responses to vegetable liking/disliking can be a proxy for negative vegetable tastes as well as for vegetable intake (Dinehart et al. 2006; Drewnowski and Hann 1999; Duffy et al. 2007, 2009; Larson et al. 2008; Wind et al. 2006). Our results showed that a simple food liking survey can identify adults who have low affinity towards vegetables, and critically, these individuals responded best to additives intended to improve the acceptability of vegetables. Although low preference for vegetables develops from a complex interplay of taste genetics, exposure, availability, and personality traits, we have found that adults with a genetic predisposition to taste vegetables as more bitter and less sweet generalize this sensory hindrance and report consuming less of all types of vegetables (Dinehart et al. 2006; Duffy et al. 2010). Ideally, vegetable dislikers would be identified early in life so that strategies could be implemented to identify genetic taste vulnerabilities to vegetable disliking (Fisher et al. 2011) or novel interventions to condition a preference (Capaldi and Privitera 2008).

Strengths, Limitations, and Conclusions

This study examined how bitter suppressors affect bitterness of three traditionally disliked green vegetables, while considering how the individual variability in taste and oral sensations influenced the bitter suppression and vegetable hedonic rating. This paper extends research beyond bitter suppression in model systems (single pharmaceuticals) to

whole foods that are the basis for healthy diets. However, a number of limitations should be acknowledged. The sample size was small and relatively homogenous to increase internal validity, and all testing occurred in a laboratory setting. Thus, the findings may not generalize to samples with diversity in age, health status, and ancestry as well as testing in the field. The protocol only tested three vegetables in a form that may not be typical to avoid experimental bias. Vegetables are diverse in bitter compounds and oral sensory characteristics, particularly as they are processed from farm to plate. The additives may not produce the same results on inherently sweeter vegetables or those where overcooking produces strong unpleasant odors and flavors. The study also did not assess potential effects of monosodium glutamate, which also has been shown to suppress bitterness in aqueous solution (Keast et al. 2004a), with less certain effects in complex foods such as vegetables (Essed et al. 2009). Finally, a non-nutritive sweetener, aspartame, was used in small volume to avoid further sensory changes. Additives may not be required with cooking techniques to enhance natural vegetable sweetness (e.g., caramelization via roasting), as well as with vegetables that have optimal freshness or are rich in sweet-quality flavor volatiles (Tieman et al. 2012). The concentration of NaCl used here may not have been high enough to produce effective bitter suppression and impart a salty taste, which may be preferred (Barisas et al. 2006), particularly for those predisposed to perceive bitter in vegetables as more intense and have greater affinity for saltiness in bitter foods (Hayes et al. 2010). Serving a bitter vegetable with a dip may be more effective to increase preference for the genetically vulnerable individuals (Fisher et al. 2011). Finally, the present study did not test a 'combination-therapy' approach, which uses sodium ions and a sweetener simultaneously to exploit peripheral and central bitter suppression to improve vegetable liking (Keast and Breslin 2005; Gaudette and Pickering 2012b).

In summary, due to the under consumption of healthful vegetables, the need exists for new strategies to improve vegetable acceptability and hence vegetable intake. Previous studies have focused on repeated exposure, conditional learning, and school-based education to increase preference and hence vegetable consumption (Anzman-Frasca et al. 2012; Birch and Marlin 1982; Capaldi and Privitera 2008; Heim et al. 2009; Remington et al. 2012), yet a recent systematic meta-analysis shows minimal impacts ability to improve vegetable intake (Evans et al. 2012). Failing to establish a preference for vegetables may explain a decay of effects of a behavioral intervention to increase vegetable consumption (Hoffman et al. 2011). Evaluation of the combined impact of behavioral-based interventions with taste modification, horticultural practices to improve vegetable sensory characteristics, and preparation techniques on vegetable preference and intake is needed. The present research demonstrated that addition of small amounts of a sweetener to vegetables could decrease bitterness and increase hedonic rating, especially for those with a predisposition to taste elevated bitterness, or those who report low affinity for vegetables. A simple liking survey may serve as a proxy to identify individuals who are responsive to sensory-based interventions to improve vegetable sensations and preference.

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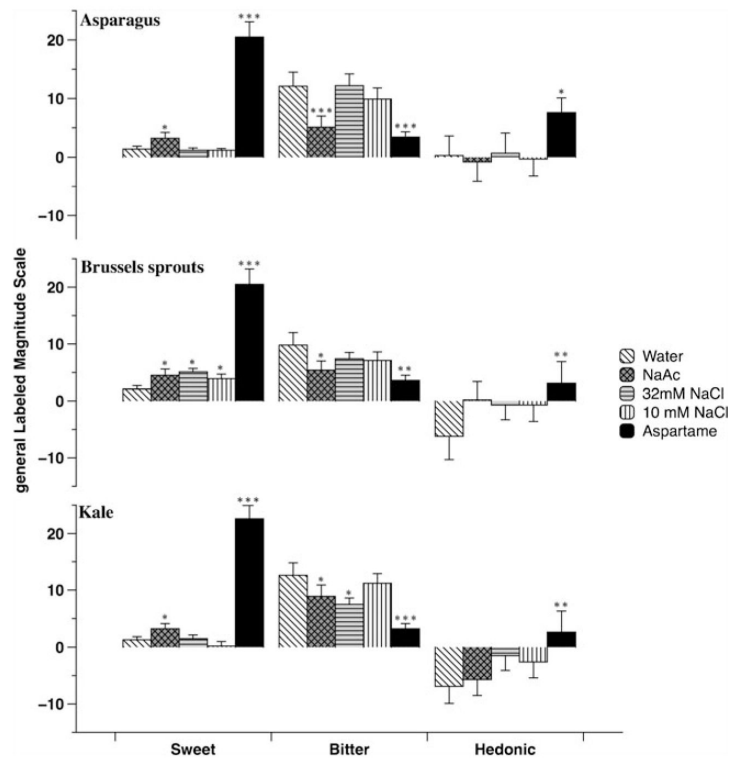


Fig 1. Oral sensations changed when tastant modifiers (water, aspartame, sodium acetate, and NaCl, 32 and 10 mM) were added to sampled vegetables of asparagus, Brussels sprouts, and kale (significantly different from vegetable+water, * p 0.05, ** p 0.01, and *** p 0.001)

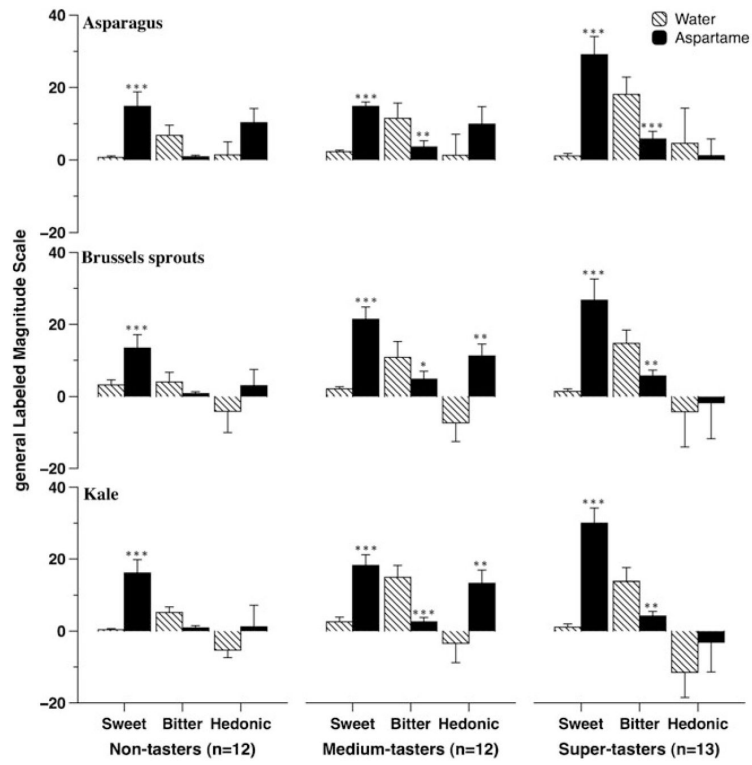


Fig. 2. Aspartame interacted with PROP taster status to influence taste and hedonic ratings of sampled vegetables (significantly different from vegetable+water, * p 0.05, ** p 0.01, and *** p 0.001)

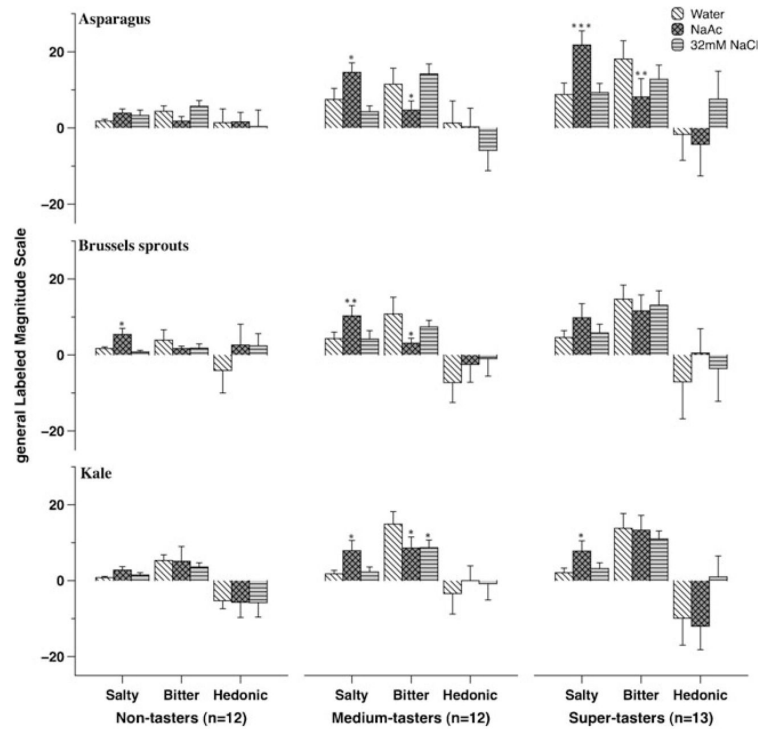


Fig. 3. Sodium salts interacted with PROP taster status to influence taste and hedonic ratings of sampled vegetables (significantly different from vegetable+water, * p 0.05, ** p 0.01, and *** p 0.001)

Table 1

Individual taste qualities from plain sampled vegetables reported on the gLMS (Bartoshuk et al. 2004), where 1,4=barely detectable, 6=weak, 17=moderate, 35=strong, and 53=very strong

	Mean	Standard error	Range
Asparagus			
Bitterness	11.0	2.2	0 to 53
Sweetness	1.4	0.5	0 to 14
Sourness	2.4	0.8	0 to 20
Saltiness	6.4	1.6	0 to 30
Hedonic	-0.4	3.4	-66 to 35
Brussels sprouts			
Bitterness	9.1	2.4	0 to 50
Sweetness	2.2	0.6	0 to 17
Sourness	2.4	1.1	0 to 26
Saltiness	3.7	0.9	0 to 20
Hedonic	-7.4	4.2	-95 to 39
Kale			
Bitterness	11.2	2.0	0 to 50
Sweetness	1.4	0.5	0 to 14
Sourness	1.6	0.7	0 to 19
Saltiness	1.6	0.5	0 to 11
Hedonic	-5.7	2.3	-34 to 34