

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

NFS Journal

journal homepage: <http://www.journals.elsevier.com/nfs-journal/>

Flavor misattribution: A novel approach to improving compliance and blinding in food-based clinical interventions

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ARTICLE INFO

Article history:

Received 29 May 2015

Received in revised form 29 July 2015

Accepted 29 July 2015

Available online 5 August 2015

Keywords:

Broccoli
Taste
Masking
Blinding
Sensory
Misattribution

ABSTRACT

Background: Clinical trials that test food-based interventions frequently suffer from ineffective blinding of study participants which can reduce the statistical power of reported outcomes, and can lead to poor compliance. This study used descriptive sensory analysis with highly trained evaluators, and well-validated statistical techniques to develop a protocol to mask the consumption of phytochemical-rich broccoli sprout extracts (BSEs³) for the use in clinical studies seeking to address a variety of conditions.

Methods: A trained sensory team identified foods and beverages that, when mixed with a BSE, showed promise in masking the extract's flavors. Established sensory evaluation techniques were then implemented by a group of seven trained descriptive sensory analysis panelists to deconstruct the sensory profile of each sample (BSE suspended in a delivery vehicle). The sensory characteristics were then clustered into dimensions based upon factor analysis and principal component analysis, followed by a test-retest protocol, to match complementary flavors from liquid-based food sources that would be readily available in the cultural context of our clinical test sites.

Results: Clustering of sensory attributes (dimensions) was identified and was both negatively and positively associated with the perception of glucoraphanin-rich and sulforaphane-rich BSE. Four dimensions were able to explain 73% of the sample set variability. Pineapple juice was identified as a complementary flavor that was most effective in masking broccoli complex attributes, and lime and ginger were effective in masking other "harsh" or objectionable flavor components of the BSE.

Conclusion: Effective beverages worked by invoking "flavor misattribution", wherein a food (broccoli extract) with an objectionable sensory characteristic was paired with a vector in which that characteristic was an acceptable component of the vector's flavor profile. Further development of this concept with an unlimited palate could be used to develop optimal carriers for food product development and/or to refine the approach for clinical trials based upon local taste preferences.

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1. Introduction

Food-based intervention trials often struggle to blind participants to their treatment condition because researchers are unable to mimic the sensory complexities of the food in question with an inert control [1]. For example, trials examining the antioxidant effects of long-term dark chocolate consumption have been studied frequently, yet effective

controls do not exist because the active polyphenolics lend a distinctive bitter taste that is not sufficiently replicated [2]. Trials testing unpalatable treatments may additionally suffer from low compliance and may not translate to large-scale studies, if successful, without improving palatability. However, manipulation of sensory characteristics has been used successfully to mask the flavor of anti-retroviral drugs and improve compliance in children who were unable to swallow pills [3]. Recent clinical studies have evaluated broccoli sprout extracts (BSEs) for a variety of indications [1,4–10], but their pungency, bitterness, and other sensory qualities greatly influence blinding and compliance [11]. Thus, in addition to sensory acceptability of food-based interventions, development of appropriate masking agents must also be a primary and necessary waypoint in the development of efficacious treatments.

Flavor masking has been utilized in an attempt to improve the commercial acceptability of functional food products that purport to offer

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³ BSEs: broccoli sprout extracts.

high antioxidants as well as for bioactive phytochemical supplements with objectionable sensory qualities. For clarification, masking in this publication refers to the concealment of objectionable flavors, whereas blinding refers to the inability of participants to determine their own experimental condition. Microencapsulation, for example, has been used to mask the flavor of DHA- and EPA-rich fish oils for non-traditional food applications such as orange juice and milk without noticeable flavor changes, and a variety of other approaches have been used for pharmaceutical taste masking [12,13]. While flavor masking is frequently employed in the commercialization of functional foods, it is seldom used to facilitate evaluation of their health claims during clinical trials. Were it more commonly used in a research setting, sensory evaluation and flavor masking might lead to a stronger body of functional food research [14]. Sensory testing has been employed for over a century; initially a simple system of food grading that has become increasingly sophisticated over the past 50 years [15,16]. More recently sensory scientists have formalized and codified methodologies, creating a discipline that enables scientists to conduct reproducible tests and provide data on which robust and defensible decisions can be made [17,18]. The use of highly trained evaluators as instruments, and the application of well-validated statistical analyses to the interpretation of results now permit very sensitive judgments to be made on the perceived sensory attributes of foods (e.g. appearance, odor, consistency, texture, and flavor).

In previous clinical trials the biologically active phytochemical sulforaphane was shown to be more bioavailable when delivered in a sulforaphane-rich (SFR) BSE than in a BSE preparation rich in glucoraphanin (the biogenic precursor of sulforaphane; GRR), but participants noted a more objectionable flavor in the SFR samples as compared to the GRR [4,5]. A number of recent clinical trials have used mango juice as a vehicle for the BSE [4,6]; as have other trials still in progress — see www.clinicaltrials.gov [NCT00255775, NCT00994604, NCT00982319, NCT01008826, NCT01108003]. Acceptability of this beverage as both a masking and delivery agent is high amongst subjects in 5 separate trials in the USA, but in China it was not so well accepted. The sweetness of this juice was well tolerated in the USA, whereas Chinese subjects found it to be overly sweet and thus not as well accepted. An informal, blind survey showed a substantial preference for the GRR-BSE over the SFR-BSE beverage, indicating that masking was not entirely successful [1,4].

The investigation reported herein was therefore designed to develop beverage(s) that would better mask the flavor of BSE and facilitate its utilization in clinical trials in rural China and the USA without either interfering with its bioavailability or augmenting its chemoprotective activity. Two BSEs were evaluated, (GRR and SFR); the preparation of each has been previously described [1,4], and both were eventually used in a clinical trial in HeHe, Qidong, Jiangsu Province, PRC — a rural subsistence farming region of China near Shanghai [7]. GRR had been determined to be easier to mask and less pungent in flavor, therefore this study focused primarily on masking the SFR flavor [19]. In this novel, scientifically guided effort to develop culturally appropriate taste-masking carriers for BSE, a variety of vehicles (beverage and otherwise) were prepared and sampled by a panel of trained sensory evaluators. A preliminary screening for widespread acceptability focused upon the culinary preferences of the target trial population by using flavors and vehicles readily available in that region. The selected vehicles were then further tested using an array of standardized descriptors in order to identify sensory characteristics that masked the perception of the BSE.

2. Materials and methods

2.1. Broccoli sprout extract

Broccoli sprout extract (BSE) was produced at Johns Hopkins Medical School and supplied as one of two slightly different compositions: either a glucoraphanin-rich (GRR) BSE, or a sulforaphane-rich (SFR) BSE.

A “serving” of each consisted of enough powder to deliver 100 μmol of the phytochemical of interest (glucoraphanin or sulforaphane), consistent with what has been delivered in a number of clinical trials [4–10]. This amounted to 300 mg of GRR BSE and 500 mg of SFR BSE. These powders were prepared by boiling 3 day old broccoli sprouts and lyophilizing the aqueous extracts as previously described [1,4,6]. The resultant yellow/tan powders (BSE) are hygroscopic, completely water soluble, and have a strong odor and taste that has been described by untrained consumers as broccoli-like (GRR) and radish or daikon-like (SFR). All BSE powders were stored at −4 °C until mixed with test vehicles.

2.2. Preliminary screening

Culturally appropriate beverage flavors (or “vectors”) were identified by registered dietitians, based on their popularity in Asian food markets, since the initial use in a clinical trial was to be in China. These flavors included green tea, pineapple, mango, lime, grass jelly, tamarind, soy, soursop, melon, lychee, coconut, honey, and ginger (Table 1). In addition to beverages, the use of puddings, custards, and mochi was also explored. Two trained sensory panelists narrowed this preliminary group of flavors to include only those flavors with strong flavor-masking potential in two informal tasting sessions. 4 oz of each sample was mixed with a serving of BSE and evaluated for flavor-masking effectiveness. Mixtures were deemed ineffective if they were no better than water at masking the taste of BSE, based on the intensity of broccoli- or radish-related aromatics. Effective beverages successfully lowered these perceived aromatics whereas partially effective beverages elicited only a minor improvement compared to water. From this preliminary testing, ten beverages were chosen for formal sensory evaluation.

2.3. Masking beverage preparation

Both juices and flavored beverages were purchased from Asian Food Market (Piscataway, NJ) based on their anticipated availability in rural China (near Shanghai), and based upon the assessment of trained experts on both the cuisine and logistics at the trial site. Initially, eight experimental samples were created along with 2 control samples, one that

Table 1
Preliminary sensory analysis evaluation of masking and acceptability.

Vector	Masking of GR ^a	Masking of SF	Anticipated acceptability
Green tea	NE	NE	High
Grass jelly	E	NE	Low
Soursop	E	PE	Low
Mango drink	NE	PE	High
Tamarind	NE	PE	Low
Pineapple	E	PE	High
Soy milk	E	PE	Moderate
Calpico beverage	E	PE	Moderate
Mango pudding ^b	E		High
Melon mochi ^b	E		Low
Calpico lychee ^c		PE	Moderate
melon drink ^c		PE	Moderate
coconut juice ^c		NE	Moderate
honey/ginger ^c		PE	High
Calpico/soy ^c		E	Moderate
mango/pineapple ^c		PE	High
Calpico/pineapple ^c		E	High
pineapple/ginger/honey ^c		E	High
Calpico/ginger/honey ^c		E	High
pineapple/coconut/ginger/honey ^c		NE	High
Calpico/coconut ^c		NE	Moderate

^a NE — not effective, PE — partially effective, E — effective.

^b Vectors were not tested for SFR masking.

^c Vectors were not tested for GRR masking, for reasons described in the [Materials and methods](#) section.

Table 2
Formulation of masking beverages.

Beverage	Extract ^a	Symbol ^b	Ounces of juice/component added							
			Pineapple	Calpico	Silk	Lychee	Ginger	Lime	Mango	Water
Pineapple (P)	GRR	P-GR	12	–	–	–	–	–	–	–
Calpico (C)	GRR	C-GR	–	12	–	–	–	–	–	–
Calpico + Soy (CS)	SFR	CS-SF	–	6	6	–	–	–	–	–
Calpico + Pineapple (CP)	SFR	CP-SF	6	6	–	–	–	–	–	–
Pineapple + Ginger (PG)	SFR	PG-SF	–	8	–	–	4	–	–	–
Pineapple + Ginger + Lychee (PLG)	SFR	PLG-SF	4	–	–	4	4	–	–	–
Pineapple + Lime (PL)	SFR	PL-SF	5.64	–	–	–	–	0.72	–	5.64
Lychee + Ginger (LG)	–	LG-SF	–	–	–	8	4	–	–	–
Mango (M)	SFR	M-SF	–	–	–	–	–	–	12	–
Water (control; W)	–	W-SF	–	–	–	–	–	–	–	12

^a Either 300 or 500 mg of GRR or SFR BSE respectively were added to the components indicated.

^b Abbreviations given in “Symbol” column are also used in Figs. 1–2.

contained a sample beverage with no BSE and one that contained SFR BSE (the extract with the stronger aroma/flavor) suspended in water. Two additional experimental samples were added post-hoc to include the final masking formulation as well as a beverage used in previous research (Table 2) [1,19]. Permutations of 7 products were used in the experimental drink: pineapple juice (Dole Food Company, Westlake Village, CA), mango nectar (Jumex, Mexico City, Mexico), lime juice (Safeway, Pleasanton, CA), unsweetened soy milk (Silk; Whitewave Foods Co., Broomfield, CO), Calpico (Calpis Co., Tokyo, Japan), Calpico lychee (Calpis Co., Tokyo, Japan), and honey ginger instant tea (Xiamen Mingren Tea Industry Co., Xiamen, PRC). Both Calpico and Calpico lychee are non-carbonated dairy-containing soft drinks with citrus aromatics. Test beverages were stored at 4 °C until use. Each sample was prepared by whisking 3 servings of the respective BSE with 12 oz of the masking beverage(s) or, in the case of the control, water for 1 min. Exact formulations can be found in Table 2. Fewer experimental samples were run with GFR than with SFR BSE due to its less objectionable taste and the ease with which GFR can be masked. Beverages were tasted immediately after preparation.

2.4. Sensory evaluation

Beverages were evaluated in sequential monadic presentation using the Spectrum Method of Descriptive Analysis (SDA) [17,19]. Descriptive analysis is a method of sensory evaluation that generates quantitative, objective measurements by the detection and description of the sensory characteristics of a product. It is crucial to note that whereas this testing was conducted in a corporate, rather than an academic setting, it is primarily in such corporate settings that panels of highly trained sensory evaluators are available. All panelists had over 100 h of professional

training in references and scaling as part of SDA and routinely conduct evaluations for the food industry which adds hundreds of hours of experience. Prior to training, panelists are screened based on sensory acuity and undergo an extensive training on flavor and taste intensity as well as qualitative flavor character references. The protocols and descriptive sensory parameters are highly standardized and widely used. The ballot was composed of 2 appearance, 14 aroma, and 14 flavor attributes as well as the 4 basic tastes and 4 chemical feeling factors (see Supplemental Tables S1–S3 for lists of attributes). Flavor intensity, chemical feeling factors and taste references are previously published and widely used in the sensory industry [17,21–23]. Qualitative aroma and flavor references or definitions are found in Table 3. Seven panelists were present during evaluation, and each was served approximately 45 g of each beverage in Table 2. Panelists first evaluated aroma and appearance, and then flavor and taste, expectorating completely. This study was approved by the Institutional Review Boards of the Johns Hopkins School of Medicine, and of Sensory Spectrum, Inc. Appearance, aroma, flavor, and chemical feeling factor attributes were evaluated for each beverage on a scale from 0 to 15, which includes tenths of a point, providing 150 points of differentiation (see Supplemental Tables S1–S3). Panelists rated each beverage during evaluation and one panelist recorded consensus attribute intensities. There was a wait time between samples where panelists were given spring water and unsalted saltine crackers to cleanse their palates. Experimental beverage presentation was random with the control beverage tasted last to reduce carry-over effects of the strong broccoli flavor. Thus, nine beverages were each scored by seven panelists.

2.5. Statistics

Data was analyzed using a multivariate technique called factor analysis (FA). Factor analysis is a method frequently used to summarize the variability present in a set of possibly correlated variables into a set of uncorrelated dimensions or factors that explain most of the variability in the original data. In this case and consistent with approaches frequently used with sensory data [24,25], factor analysis was run using all sensory variables. The initial extraction method was based on Principle Components Analysis (PCA) and performed on the correlation matrix [17]. The extraction was followed by an orthogonal varimax rotation, in order to conserve the orthogonality of the dimensions and to align the dimensions with the original variables more fully, allowing greater ease of interpretation of the new factors or dimensions. Number of dimensions was determined based on combined information from a scree-plot (flattening of the bend), eigenvalues associated with Principle Component prior to rotation (all eigenvalues greater than 2.0), and total variability explained by the factor solution after rotations (the solution accounts for 70% or more of the variability in the original variables) [17]. All analyses were performed using SAS, Version 9.2 (Cary, NC).

Table 3
Descriptive terms and references.

Term (aroma and flavor)	Qualitative reference
Broccoli complex	Raw or cooked broccoli
Citrus complex	General citrus category (e.g. lemon, lime, orange)
Cultured dairy	Yogurt or buttermilk
Dairy complex	All dairy (e.g. butter, milk, yogurt)
Floral	Flowers (e.g. rose, fruit blossoms)
Fruit complex	General category of fruit (e.g. berries, tropical, stone fruit)
Ginger	Raw or ground ginger
Green leafy	Raw green leafy vegetables (e.g. romaine lettuce)
Musty	Reminiscent of a dank basement or damp clothing
Radish complex	Red radish or daikon
Root vegetable	General root vegetable category (e.g. potatoes, carrots, taro)
Soy complex beany	Cooked soybeans (e.g. soy milk)
Sulfur	Elemental sulfur
Sweet aromatic	General category of sweet flavors (e.g. vanilla, caramelized)
Sweet green	Reminiscent of tender green stems (e.g. tulip stems)
Total impact	Total intensity of aroma or flavor impact

3. Results

3.1. Preliminary screening

Preliminary sensory screening (Table 1) was limited to simple evaluation of each food product's masking efficacy (effective, partially effective, and not effective) and anticipated consumer and study subject acceptability (low, moderate, high). Coconut juice, green tea, grass jelly, and mango were judged to be ineffective at masking one or both of the extract types. Testing of the pudding and mochi samples was discontinued due to perceived impracticality of using these formulations in the most immediate planned clinical trial (in a rural China setting). Soursop and tamarind were also excluded from further testing due to low anticipated acceptability outside of Asia, and have a low likelihood of being widely embraced in clinical trials. Pineapple juice, soy milk, honey ginger tea, Calpico lychee beverage, and Calpico beverage were identified as feasible vehicles for second stage testing. Based on the success of citrus flavors in Calpico, lime juice was included in the analysis as well, and formulations of these masking beverages are in Table 2. Results were compared against the efficacy of mango juice, a masking beverage previously used in clinical trials [1,4,6].

Table 4

Factor loadings of sensory attributes by dimension. Bolded data represent sensory attributes that load negatively (< -0.60) or positively (> 0.60) on a dimension. Total variability explained = 73%.

Attributes ^a	Dimension 1	Dimension 2	Dimension 3	Dimension 4
	(Broccoli)	(Ginger)	(Soy/bean)	(Citrus)
% variability explained	25%	20%	16%	12%
Radish complex flav ^b	0.92	0.04	0.12	0.04
amt vis particles ^c	0.90	-0.07	0.04	0.10
Broccoli complex flav	0.86	-0.33	0.19	-0.23
Broccoli complex	0.83	-0.27	0.31	-0.21
Total aroma	0.83	0.44	0.08	0.04
Bitter	0.72	0.28	-0.35	0.43
Musty	0.68	-0.30	0.05	-0.26
Total impact	0.66	0.52	-0.14	0.09
Green leafy flav	0.65	0.00	-0.08	-0.38
Sulfur	0.57	-0.32	0.43	0.00
Green leafy	0.56	0.03	0.13	-0.41
Radish complex	0.53	0.06	0.47	0.17
Sweet	-0.60	0.31	0.03	-0.05
Ginger	-0.06	0.89	-0.29	0.09
Burn	0.38	0.81	0.01	0.32
Heat	0.17	0.77	-0.32	-0.06
Floral	-0.27	0.72	-0.04	0.03
Floral flav	-0.16	0.70	-0.11	0.09
Nasal pungency	-0.15	0.65	0.16	-0.05
Sweet aromatic flav	-0.37	0.65	-0.01	0.01
Cultured dairy	-0.54	-0.55	-0.11	0.28
Sweet green	-0.54	-0.55	-0.11	0.28
Dairy complex	-0.54	-0.55	-0.11	0.28
Sweet aromatic	-0.07	0.00	0.96	-0.02
Soy complex beany flav	-0.07	0.00	0.96	-0.02
Soy complex beany	-0.07	0.00	0.96	-0.02
Root vegetable	0.33	-0.18	0.76	-0.23
Sulfur flav	0.24	-0.13	0.54	0.02
Sour	-0.20	-0.29	-0.54	0.48
Astringency	-0.12	0.24	0.01	0.90
Citrus complex	-0.30	-0.51	-0.28	0.70
Fruit complex	-0.11	0.38	-0.49	0.69
Citrus	-0.37	-0.57	-0.19	0.63
Fruit complex flav	-0.33	-0.04	-0.24	-0.44
Ginger flav	-0.40	0.53	-0.36	-0.58
Salt	0.00	0.00	0.00	0.00

^a Abbreviations used in this column appear also on Figs. 1 and 2. These are the descriptive terms used by sensory panelists.

^b flav' – flavor.

^c amt vis particles' – amount of visible particles (turbidity).

3.2. Sensory analysis results

The consensus data for each sample's aroma, flavor/taste, and chemical feeling factor attributes are found in Supplementary Tables S1, S2, and S3, respectively. These data were analyzed by factor analysis (based upon Principal Component Analysis). A desirable factor analysis solution will usually explain at least 70% of the original variability in the data set [26]. Based on examination of the scree plot of eigenvalues (data not shown), a four-factor solution was selected that accounted for 73% of the sample set variability. Accordingly, Dimensions 1, 2, 3, and 4 account for 25%, 20%, 16%, and 12% of overall variability respectively (Table 4). Along with total percent of the variability explained, Table 4 highlights factor loadings for each of the original variable along each of the new dimensions. Factor loadings represent a measure of the degree to which an attribute is associated with a specific dimension. They are statistically determined numerical values between -1.0 and 1.0 (very much like a correlation coefficient). An attribute is referred to as "loading" on a dimension if it has a score >0.6 (positively loaded) or <-0.6 (negatively loaded). Dimension 1 has high positive loading of the attributes broccoli flavor, green leafy flavor, musty and sulfur aroma, and bitter taste and strongly negative loading of sweet taste and will be subsequently referred to as the "broccoli dimension". Dimension 2 is characterized by ginger aroma, heat, burn, and nasal pungency and will be subsequently referred to as the "ginger dimension". Dimension 3 is characterized by root vegetable aroma soy/bean flavor and aroma, and sweet aroma and will be subsequently referred to as the "soy/root dimension". Dimension 4 is characterized by citrus and fruit (tropical) flavors and will be subsequently referred to as the "citrus dimension".

Two selected maps, resulting from the factor analysis, demonstrate the quality of the associations of the broccoli and ginger dimensions (Fig. 1) and of the broccoli and citrus dimensions (Fig. 2). Vectors (primarily beverages) high in the ginger dimension were positively associated with some broccoli attributes and negatively associated with others (Fig. 1 and Table 5), however panelists noted that ginger-containing SFR samples had less noticeable daikon or radish flavor. Vectors with attributes highest in citrus dimension are mid-range to low in the broccoli dimension (Fig. 2). Samples with high overall soy/root attributes also tend to have higher factor loadings for broccoli attributes.

Of the samples tested, the two GRR samples had lower loading scores on Dimension 1, the "broccoli dimension", than any of the SFR samples (Pineapple, -0.68; Calpico -0.90). Amongst the SFR samples the lychee-ginger (LG-SF) and calpico-soy (CS-SF) had the lowest loading scores along the broccoli dimension, -0.01 and -0.12 respectively. The pineapple-lime sample (PL-SF) had a loading score of 0.04 as compared to the mango (0.46) and water (1.00). Pineapple juice alone did the best job in masking attributes associated with broccoli complex (P-GR) (Fig. 1 and Table 5), however it was ineffective at masking the SFR and was dropped after the preliminary screening. On the other hand, juices containing ginger (PG, PLG, CG, LG) were all effective in masking the radish components and its heat/burn association (Fig. 1 and Table 5). The fact that attributes which load well on the citrus/dairy dimension do not load well on the broccoli dimension, and that the converse is true, is illustrated in Fig. 2 and Table 5.

4. Discussion

Rudimentary flavor masking uses desirable flavors like chocolate, vanilla, and strawberry to "drown" or overwhelm undesirable sensory characteristics by creating an exaggeratedly high awareness of the masking agent. However, this technique is, by-and-large unsuccessful since olfactory physiology permits the human sensory apparatus to detect the presence of many aromatics with exquisite sensitivity [20], even in the presence of much stronger odors [17]. One of the most promising recent technologies in food science has focused on microencapsulation or binding as a way of eliminating these "undesirable" aromatics. Such

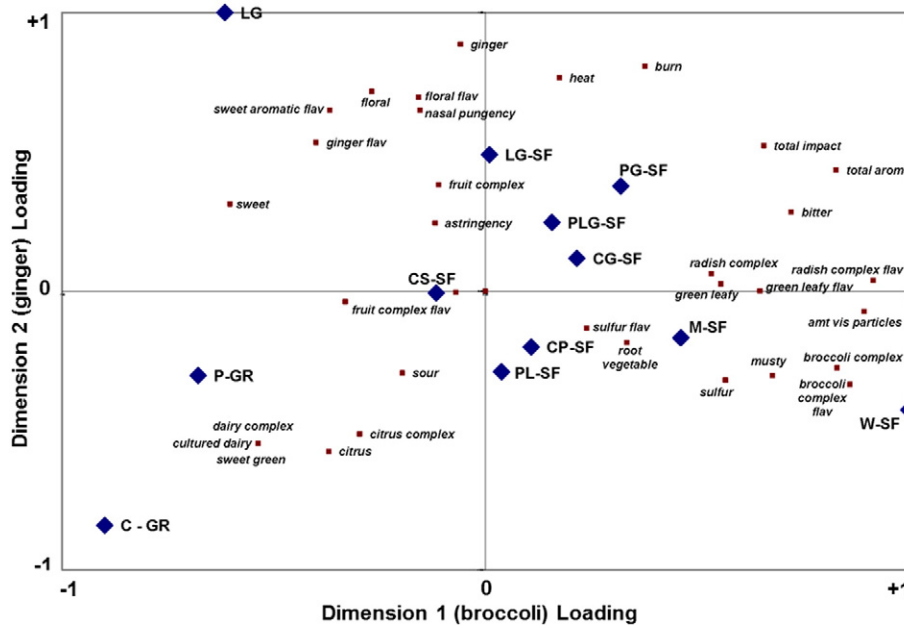


Fig. 1. Location of test beverages (♦) in a grid plotting sensory attributes (■) of broccoli (Dimension 1) vs. ginger (Dimension 2). Symbols (see also Table 1) are: P – pineapple, C – calpico, CS – calpico + soy, CP – calpico + pineapple, PG – pineapple + ginger, PLG – pineapple + ginger/lychee, PL – pineapple + lime, LG – lychee + ginger, M – mango, W – water; addition of either glucoraphanin-rich BSE (GR) or sulforaphane-rich BSE (SF) to beverages is indicated.

solutions are not appropriate for clinical trials though, due to their unknown potential to attenuate target bioavailability and efficacy. The initial decision to use mango juice as a carrier and masking agent for BSE was based on the perception that the flavor was popular in the target populations for a number of clinical trials, however pairing of mango and broccoli flavors was not adequately accounted for. Proper flavor masking must pair broccoli with desirable complementary flavors. Often in sensory science, particularly descriptive analysis, many of the attributes that are measured can be correlated with one another. Factor analysis uncovers a new set of uncorrelated latent variables, called “factors”, based on the original set attributes. It is commonly used in sensory

science and social sciences to simplify larger data sets from questionnaires or ballots.

This study shows that pineapple juice alone effectively masks a large portion of the broccoli flavor characteristics, as illustrated graphically by the very negative scoring (low loading) in Dimension 1 of P-GR as shown in Fig. 1. However, pineapple juice is not as successful in masking the radish complex which is more dominant in the SFR samples, and more problematic from a clinical trial standpoint. In Dimension 2, samples with ginger have high factor loadings, suggesting that when adding ginger the radish flavor and chemical feeling factors may be attributed to the ginger, instead of the SFR BSE (Fig. 1 and Table 4). No samples

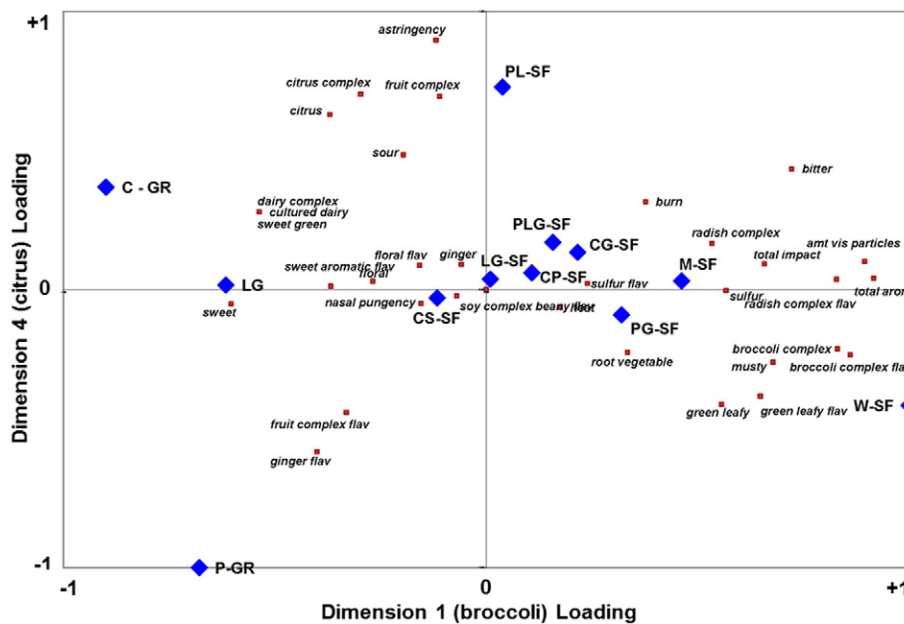


Fig. 2. Location of test beverages (♦) in a grid plotting sensory attributes (■) of broccoli (Dimension 1) vs. citrus/dairy (Dimension 4). Symbols (see also Table 1) are: P – pineapple, C – calpico, CS – calpico + soy, CP – calpico + pineapple, PG – pineapple + ginger, PLG – pineapple + ginger/lychee, PL – pineapple + lime, LG – lychee + ginger, M – mango, W – water; addition of either glucoraphanin-rich BSE (GR) or sulforaphane-rich BSE (SF) to beverages is indicated.

Table 5

Factor scores for the four dimensions accounting for greatest overall variability (73%), based upon scree plot of eigenvalues.

Sample ^a	Dimension 1	Dimension 2	Dimension 3	Dimension 4
P-GR	-0.68	-0.30	-0.18	-1.00
C-GR	-0.90	-0.84	-0.12	0.37
CS-SF	-0.12	-0.01	1.00	-0.03
CP-SF	0.11	-0.20	-0.07	0.06
PG-SF	0.32	0.38	-0.26	-0.09
CG-SF	0.22	0.12	-0.14	0.13
PLG-SF	0.16	0.25	-0.15	0.17
LG-SF	0.01	0.49	-0.05	0.04
LG	-0.62	1.00	-0.02	0.02
W-SF	1.00	-0.43	-0.03	-0.42
M-SF	0.46	-0.17	0.13	0.03
PL-SF	0.04	-0.29	-0.13	0.73

^a Sample designations: P – pineapple, C – calpico, CS – calpico + soy, CP – calpico + pineapple, PG – pineapple + ginger, PLG – pineapple + ginger/lychee, PL – pineapple + lime, LG – lychee + ginger, M – mango, W – water; addition of either glucoraphanin-rich BSE (GR) or sulforaphane-rich BSE (SF) to beverages is indicated.

were perceived to be high in both citrus and broccoli/radish flavors, raising interest in further investigation of the utility of other citrus flavors in such a misattribution strategy. These flavors, as one would expect with successful misattribution, might be mutually exclusive so that consumers would either perceive the flavors as “broccoli” or completely attribute them to “citrus”.

Flavor attribution, the process of mentally assigning perceived aromatics to a specific ingredient source, can be as important as true flavor masking [21]. As discussed previously, it may be impossible to ever truly hide the sensory characteristics of an unpalatable additive. This may work in one's favor, however, if one can successfully reassign the distasteful characteristic to an acceptable/positive- rather than a negative source (perceived as off-note). For example, masking the pungency of radish flavor using ginger may cause subjects to mentally reassign the pungency from the radish (the negatively perceived source) to that of the ginger (an acceptable and positive source). This phenomenon, termed ‘flavor misattribution’, can be used to mask the *source* of a flavor, rather than the flavor itself. Mango has very little aromatic similarity to broccoli and thus adding the broccoli flavor (BSE), to mango juice may create either an exaggerated awareness of the broccoli flavor, or a perception of spoilage or off-flavors in the mango. Pineapple and citrus (an aromatic in the Calpico and lychee beverages) are two flavors in which the bitter or leafy green notes of broccoli might be expected and accepted. In fact, others have noted the utility of furaneol, a component of the pineapple flavor, in masking grass-like and earthy aromatics such as those found in broccoli [22]. Ginger and daikon share the chemical feeling factors of nasal pungency and heat/burn, thus the daikon flavor may be adequately masked if its pungency can be misattributed to the presence of ginger in a beverage.

There is a citrus component to the ginger flavor, and since lime also has green (stemmy/leafy) and citrus flavor components, it is a natural flavor fit in a misattribution masking strategy. Ginger and lime both share geraniol which imparts the citrus-like floral note [23]. In the case of the SFR samples, addition of lime paired with the heat/burn combines for an association reminiscent of ginger versus radish. The attraction of this approach is in finding flavors that are compatible or complimentary rather than drowning offending flavors with sugar or pungent aromatics. Based on the prevalence of lactose-intolerance amongst Asian populations, dairy-based beverages were judged not to be appropriate for the clinical trials that this study was initially designed to serve. Due to their low loading scores in the broccoli dimension, citrus flavors have great potential for use in future trials. Due to considerations outside the scope of this paper, ginger was rejected as an additive for the studies for which BSE was designated (it was the only one of the agents tested, which substantially induced the chemoprotective enzymes that were to be studied in the trial this study was initially designed to serve). However, ginger is a highly effective masking agent.

The combination of pineapple and lime juice (as described in Table 2) was thus taken to rural China where a 12 week, daily intervention was carried out in a cohort of 300 free-living individuals [7]. Since the juices are shelf-stable canned products, there were no issues with product stability; broccoli sprout powders were admixed with them at the study site, and daily doses could be frozen, or consumed directly with no change in perceived flavor-masking ability over the duration of the study. Analyses of broccoli sprout glucoraphanin and sulforaphane stability in juice samples (doses) performed many months later revealed no degradation of active ingredients.

5. Conclusions

A limited sensory sampling of several beverages has identified beverages that effectively mask the sensory attributes of BSE. Care must be taken when pairing ingredients for Western palates vs. East Asian palates since such pairings have been shown to be very region-specific [18]. This analysis may also prove useful to larger interventions and epidemiological studies in which compliance with a prescribed diet (e.g. broccoli) cannot be strictly controlled. Other future benefits of such a novel flavor-misattribution approach could include the design of more palatable food products for disease prevention and increased health-span.

Abbreviations used

BSE	broccoli sprout extract
FA	factor analysis
PCA	principal component analysis
GRR	glucoraphanin-rich
SFR	sulforaphane-rich

Conflicts of interest

None.

Acknowledgements

We are grateful for funding provided by The Lewis B. and Dorothy Cullman Foundation; USPHS grants P01 ES0906052, R01 CA39416, R01CA93780; and an MHS/RD International Health Internship provided to JEB by Sensory Spectrum, LLC. Assistance was also provided by AR Krogmann, TW Kensler, PA Egner, KH Kensler, Y Koelliker, KK Stephenson, and KL Wade.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.nfs.2015.07.001>.

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