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DEPARTMENT OF FOOD SCIENCE

PERCEPTUAL SORTING OF BITTER STIMULI

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A thesis
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ABSTRACT

People vary greatly in their sensitivities to bitter compounds. However, these compounds are all lumped under the umbrella term “bitter.” To further characterize bitter subattributes which may define these compounds, a protocol was developed by which thirteen bitter stimuli could be assessed using sorting, a semantic-free perceptual mapping technique. The clusters made by fifteen PROP nontasters and sixteen PROP tasters were compared, and the combined nontaster and taster data was analyzed. Similarity data were assessed using multidimensional scaling (MDS) to produce perceptual maps for the nontaster, taster, and combined nontaster and taster data. Overall, groupings were largely inconsistent and inconclusive, which pointed to shortcomings in the protocol or perhaps the lack of bitter subattributes. Limitations are discussed, and suggestions are provided for the optimization and future direction of characterizing bitter subqualities.

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Chapter 1

Introduction

Bitter taste:

Bitterness is one of the five prototypical taste qualities (in addition to sweet, sour, salty, and umami) that humans experience. Bitter perception and aversion to bitterness have served as important factors in food intake, used evolutionarily to detect toxicity in foods. Rejection of bitter compounds is innate from before birth, and serves as one of the key drivers of food rejection due to the negative affective response (Steiner 1974; Glendinning 1994). Common bitter foods and beverages include beer (due to hops), citrus peel, plants in the *Brassicaceae* family, coffee, unsweetened cocoa, escarole, and tonic water. Bitter sensations may stimulate a different response, due to a learned association, if the bitter taste is associated with a medicinal drug or reward, such as caffeine or tonic water (in a gin and tonic), compared to bitter tastes without anticipated positive consequences, such as a food that is unexpectedly bitter (Reed 2006). While bitterness may be a sign of toxicity, some naturally bitter-tasting are recognized for their health benefits; examples include the phenols present in tea, wine, and citrus fruits, and the organosulfur compounds found in cruciferous vegetables. Therefore, variability in bitter taste perception and sensitivity may influence food preferences, diet, and human health (Feeney et al. 2011; Tanaka 2007; Duffy 2007; Feeney 2011; Hayes et al. 2013).

Genetic variation and bitter variation:

Humans recognize thousands of diverse compounds that elicit bitter sensations (Meyerhof 2010). Bitter compounds are detected in the mouth by a subset of taste receptor cells through the expression of G-protein-coupled bitter taste receptors, *TAS2R* (*TASTE2 Receptors)*. In fact, 25 bitter taste receptor genes have been identified in humans thus far. These bitter receptors and respective coding genes contain high levels of allelic variation, which may also influence human response to bitter compounds in food products (reviewed by Hayes et al. 2013). Preliminary studies also suggest that variation in the perceived bitterness of regular foods and beverages, rather than pharmaceutical bitterants such as quinine or PROP, may be explained by common polymorphisms in the *TAS2R* bitter receptor genes. Due to the diversity of bitter taste receptors, individual studies that do not find a correlation between specific bitter taste indicators and liking or ingestion of particular foods do not necessarily invalidate the claim that chemosensory variation drives ingestive behaviors, and consequently, health and wellness (Hayes et al. 2011, 2013).

Bitterants include amines, amides, ureas, thioureas, amino acids, alkaloids, esters, azacycloalkanes, *N*-heterocyclic compounds, lactones, carbonyl compounds, phenols, crown ethers, terpenoids, secoiridoids, glycosides, flavonoids, steroids, acetylated sugars, isohumulones, fatty acids, peptides, phenols, carbamates, and ionic salts (Belitz et al. 1985; Spielman et al. 1992; DuBois et al. 2008). Many psychophysical studies on bitterness have been limited in the compounds or classes of compounds used. Notable individual differences exist in the bitterness and sensitivities of some bitter compounds. A wide range of responses to bitter compounds have been documented, with the most variation among individual perception of antithyroid compounds such as propylthiouracil (PROP) and phenylthiocarbamide (PTC) (Yokomukai et al. 1993; Fox 1932).

Individuals differ greatly in their sensitivities to phenylthiocarbamide (PTC), propylthiouracil (PROP), and other related compounds containing the functional group N – C = S (Lawless 1980) due largely to variation in the G-protein-coupled receptors hT2R38 (Binder 2009; Bufe et al. 2005). While genotype and the density of fungiform papillae contribute to PROP bitterness (Duffy et al. 2004), PTC and PROP perceived intensity may also be influenced by other receptors that may be involved in tasting high PROP concentrations (Hayes et al. 2008).

The variation in oral chemosensation is noted in terms of two related yet distinct phenotypes—the threshold (lowest detected concentration of a stimuli) and suprathreshold response (the magnitude of the perceived stimuli intensity at high concentrations) (Hayes and Keast 2011). The differences between tasters and nontasters may be observed in a bimodal distribution of detection thresholds. Individuals are classified as sensitive "tasters" of PTC/PROP if their detection threshold lies in the range of concentrations below the antimode of the population distribution. PTC/PROP "nontasters" are insensitive to the compounds, with a detection threshold above the antimode of the population distribution (Lawless 1980). However, participants for this sorting procedure were screened and chosen based on suprathreshold PROP intensity ratings made on the generalized Labeled Magnitude Scale (gLMS) in a previous study. The bitter groupings made in the sorting procedure will be compared between PROP/PTC "nontasters" and PROP/PTC "tasters," defined on the basis of suprathreshold ratings, not detection thresholds.

Behavioral evidence:

A rating and ranking procedure of various bitterants from different chemical classes by Delwiche and colleagues investigated the correlations of individual sensitivities in order to

determine the number and variety of bitterness transduction systems for the compounds. Two main clusters were observed, each containing subclusters of compounds more closely affiliated with one another, although the subclusters varied from one analysis to another. Notably, neither of the clusters—urea/phenylalanine/tryptophan/epicatechin, and quinine/caffeine/SOA/ denatonium benzoate/tetralone/magnesium sulfate—contained PROP, which formed a third isolated group (Delwiche et al. 2001). The different clusters of bitter compound sensitivities found in this study suggests that there may be variability in the qualitative aspects of these compounds which are all encompassed under the descriptor "bitter". However, there is just one term in the English language to describe bitterness. To help further define these sub-qualities, similarity profiles should be created using non-verbal techniques and Projective Mapping.

Sorting:

Projective Mapping is a class of methods that are used to build a multidimensional map of similarities between stimuli. These raw data are then analyzed using multivariate statistics like multidimensional scaling (MDS) to generate spatial maps which reflect the relationships between stimuli (e.g. Lim and Lawless 2005). Multidimensional Scaling is a group of multivariate statistical techniques used commonly to visually represent the similarity of items within a set of data. MDS can be used to create perceptual maps in which the spatial configuration allows for the observation of important differences in stimuli and any clusters or categories that may exist (Lawless et al. 1994). Multidimensional scaling of sorting data has been used to avoid semantic problems associated with descriptive analysis and psychophysical studies in which terms are preselected and generated by the experimenter (O'Mahony and Thompson 1997; Meilgaard et al. 1991; Lim and Lawless 2005). In other words, MDS allows for the exploration of attributes that

may be difficult to verbalize and does not require words to identify similarities and/or differences between stimuli (Nestrud and Lawless 2009).

A free sorting task consists of a single session in which participants examine a set of stimuli and group them according to similarity. All stimuli are presented simultaneously and are randomly presented to each participant. Participants are instructed to physically sort the stimuli into groups, in which two stimuli sorted into the same group are considered similar using criteria designated by the participant. Participants can use any criteria they wish to sort the stimuli, and are free to make as many groups as they want and place as many stimuli as they want into each group. When sorting is complete, participants may be asked to provide a word or term to characterize each group that they formed (Valentin et al.2012).

Sorting data is analyzed by counting how often each pair of stimuli is sorted into the same group. This allows for the calculation of a similarity matrix across the group with the stimuli as both the columns and rows of the matrix. The frequencies of co-sorting serve as data for multivariate analysis, and the matrix is submitted to MDS. The MDS may reveal several dimensions based upon the criteria participants use for their groupings, which are then used to construct the MDS spatial configuration (Nestrud and Lawless 2009). The distances between the stimuli on the spatial map are representative of their perceptual interrelationships and reflect the similarity/dissimilarity between stimuli in terms of their perceived characteristics (Lim and Lawless 2005). Stimuli that are close together on the MDS configuration are perceptually similar, while dissimilarity among stimuli is represented by distance. Sorting is a rapid, easy task to perform, and produces little fatigue and participant boredom (Lawless et al. 2004; Lawless 1989). The method has gained popularity as an alternative to classical descriptive analysis of food products (Valentin et al. 2012). For instance, the sorting task and MDS have been used on

commercial cheeses and cheese names (Lawless *et al.* 1995), divalent salts (Lim and Lawless 2005), grape jellies (Tang and Heymann 1999), and vanilla types (Heymann 1993).

Objectives:

Due to the numerous genes that encode different bitter receptors, and the allelic variation within them, it is hypothesized that individuals may perceive different types of bitterness based upon their genetics. By observing the phenotypic response of different bitter receptor genetic variants, this project is twofold. First, it will be determined whether individuals can sort bitter compounds into distinct groupings, and secondly, if the groups that people sort the bitterants into are based upon genetic differences.

The purpose of this study is to apply a semantic free sorting method to various bitterants; the spatial configuration of the sorting method may give insight into important differences and clusters of compounds, and can aid in the determination of the number of different groups of bitterants. Ultimately, the sorting procedure will aid in the determination of bitter subclusters under the global descriptor "bitter." Previous research has confirmed that bitter compounds that cluster together as a function of similar attributes share some form of common physiological mechanism (Delwiche et al. 2001). This study will focus on differentiating the subattributes that cause the bitterants to taste differently to participants. Better understanding of the qualitative aspects on bitterness will inform basic research on taste perception and may have practical applications for the food industry. The wide diversity of compounds that elicit bitter-taste sensations is vast, and this study will aid in the characterization of different bitter taste profiles.

Chapter 2

Materials and Methods

***Overview*:**

This study was performed in a single session on two separate groups of individuals previously phenotyped as tasters and nontasters of 3.2 mM PROP. All conditions, stimuli, and instructions were the same for each participant. All data were collected with the approval of the Penn State University Institutional Review Board and the informed consent of participants.

Participants:

Participants were recruited based upon their participation in a previous study, GIANT-CS, Phase 2, conducted by the Sensory Evaluation Center at Penn State. Only PROP nontasters (intensity rating 0-16, poststandardization, on a gLMS) and PROP tasters (intensity rating 35-100, poststandardization, on a gLMS) were invited back to partake in this study. Participants for the GIANT-CS study were recruited from the Penn State campus and surrounding area in State College, Pennsylvania. To be eligible, individuals needed to be nonsmoking and Caucasian, between 21 and 45 years old, without any known defect in smell or taste and without tongue, lip, and cheek piercings. Additional exclusion criteria included pregnancy or nursing or having any history of the following: chronic pain conditions; choking or difficulty swallowing; and thyroid irregularities. For this substudy, participants were also screened for phenylketonuria

(PKU), as phenylalanine was included as a stimulus. Fifteen nontasters and sixteen tasters participated in the study.

Stimuli:

All samples were prepared in reverse osmosis (RO) water. Quinine hydrochloride (119 µM), sucrose octaacetate (SOA) (57 µM), urea (0.920 M), tetralone (10% v/v), t-phenylalanine (50.2 mM), L-tryptophan (26.9 mM), caffeine (10.9 mM), PROP (1 mM), PTC (3.17 mM), quinine sulfate (118.9 µM), and naringin (0.55 mM) were used as stimuli, with duplicates of both caffeine and tetralone to measure participant consistency when sorting. The stimuli concentrations for the QHCl, SOA, urea, tetralone, t-phenylalanine, L-tryptophan, and caffeine used in this experiment were previously matched in intensity and adapted from a design in literature (Delwiche et al. 2001). Concentrations for the other samples were also sourced from literature (Lawless 1980; Drewnowski et al. 1997; Bartoshuk et al. 1994; Hayes et al. 2008).The final concentrations for the present study were intensity matched in pilot testing by eleven individuals in the Hayes research group at Penn State.

Table 1: Concentrations and Applications of Bitter Stimuli

Stimuli	Concentration	Class of Compound	Application
Caffeine	10.9 mM	Alkaloid	Present in coffee, tea, and soft drinks
L-tryptophan	26.9 mM	Amino acid	Essential amino acid found in many plant and animal proteins
Naringin	0.55 mM	Flavonoid	Present in citrus fruit such as grapefruit, food additive
Propylthiouracil	1 mM	Antithyroid compound	Probe of phenotypic variation
Phenylthiocarbamide	3.17 mM	Carbamate	Probe of phenotypic variation
Quinine hydrochloride	119 µM	Alkaloid	Tonic water
Quinine sulfate	118.9	Alkaloid	Food safe bitterant
Sucrose Octaacetate	57 µM	Acetylated sugar	Food additive
Tetralone	10% v/v	Nonamino acid	Food grade hop extract from Kalsec used to provide bitterness in beer
t-phenylalanine	50.2 mM	Amino acid	Amino acid found in foods containing protein
Urea	0.920 M	Ureas	Stabilizer in medicine and pharmaceuticals; food additive in formulation and fermentation of yeast-raised baking goods, alcoholic beverages, and gelatin products; flavoring, humectant, and dehydrating agent

Procedure:

Ten milliliters of each of the stimuli were dispensed into a plastic medicine cup. Each cup was labeled with a random 3-digit blinding code. Sample order was randomized, and the medicine cups were arranged in sequential order on each tray. Each participant completed a training session prior to the sorting procedure. In the training session, participants were instructed to take the entire contents of each cup into their

mouths and to swish for five seconds (as if using mouthwash) before expectorating the sample. They then were told to rinse with RO water for the duration of the time break between each sample (90 seconds) or until no lingering sensations were perceived. All samples and rinse water were expectorated. Also during the training session, the participants learned about the sorting procedure they would use to group the samples.

After the training session, participants entered individual sensory booths in the Sensory Evaluation Center, where they received a tray containing all 13 stimuli. Participants were instructed to sort the cups into groups based on the similarity of the sensation elicited by each sample. Samples with similar sensations were to be put into the same group, and samples with different sensations were to be put into different groups. Each sample could only belong to one group. There was no limit to the number of samples per group, but there was not to be one large group, nor could each sample individually comprise its own group. The criteria on which these groupings were made were left up to the participants. Participants were given a blank piece of paper and pen to take notes with throughout the duration of the test. Additional samples could be requested for re-tasting at any point throughout the procedure.

After each participant tasted all of the samples, he or she was asked to make the final groupings. A comment box was used to record the number of groupings made, the registration codes of the samples within the group, and a descriptor word(s) which described each group. No list of descriptor words was provided to the participants. After the procedure in the booths was complete, the participants entered the same groupings into Websort (UXPunk, Chicago, IL), a web-based card-sorting software application.

Data Analysis:

Multidimensional scaling (MDS) on dissimilarity matrices was completed using The R Statistics Package (R Foundation for Statistical Computing). Data from the free sorting task was arranged in a binary matrix indicating whether or not two stimuli were grouped together or not. The similarity matrix was converted into a dissimilarity matrix and submitted to MDS. A regression function was applied to the dissimilarity data and a stress value that measured the quality of the fit between the input proximities and the output distances were generated.

In order to determine the number of dimensions that were to be used in the multivariate configurations, a Scree plot was used. The Scree plot displayed the stress values as a function of the dimension number. The objective was to find the best fit with the smallest number of possible dimensions. Ideally, the Scree plot should contain an "elbow" which indicates that the increasing dimensions no longer affect stress in a significant manner. Thus, the appropriate number of dimensions for each set of data was chosen (Jaworska and Chupetlovska-Anastasova 2009). Using these criteria, two dimensions were determined to be most appropriate for each the nontaster and taster, nontaster, and taster groups.

Agglomerative cluster analysis was then conducted on each of the matrices, and Ward's minimum variance method was used as the linkage criteria. Dendograms for each of the data sets were then constructed from the agglomerative hierarchal clustering of the sorting. In order to determine the appropriate number of clusters, a plot of the height of the amalgamation distance versus the Index was used. Large jumps in the amalgamation

height on this plot were indicative of stimuli that were joined in that step that were notably dissimilar from previously joined stimuli (Byrnes et al. under review). Two-dimensional MDS plots were then constructed and compared to the respective dendograms for determination of clusters

Chapter 3

Results

Nontaster Data:

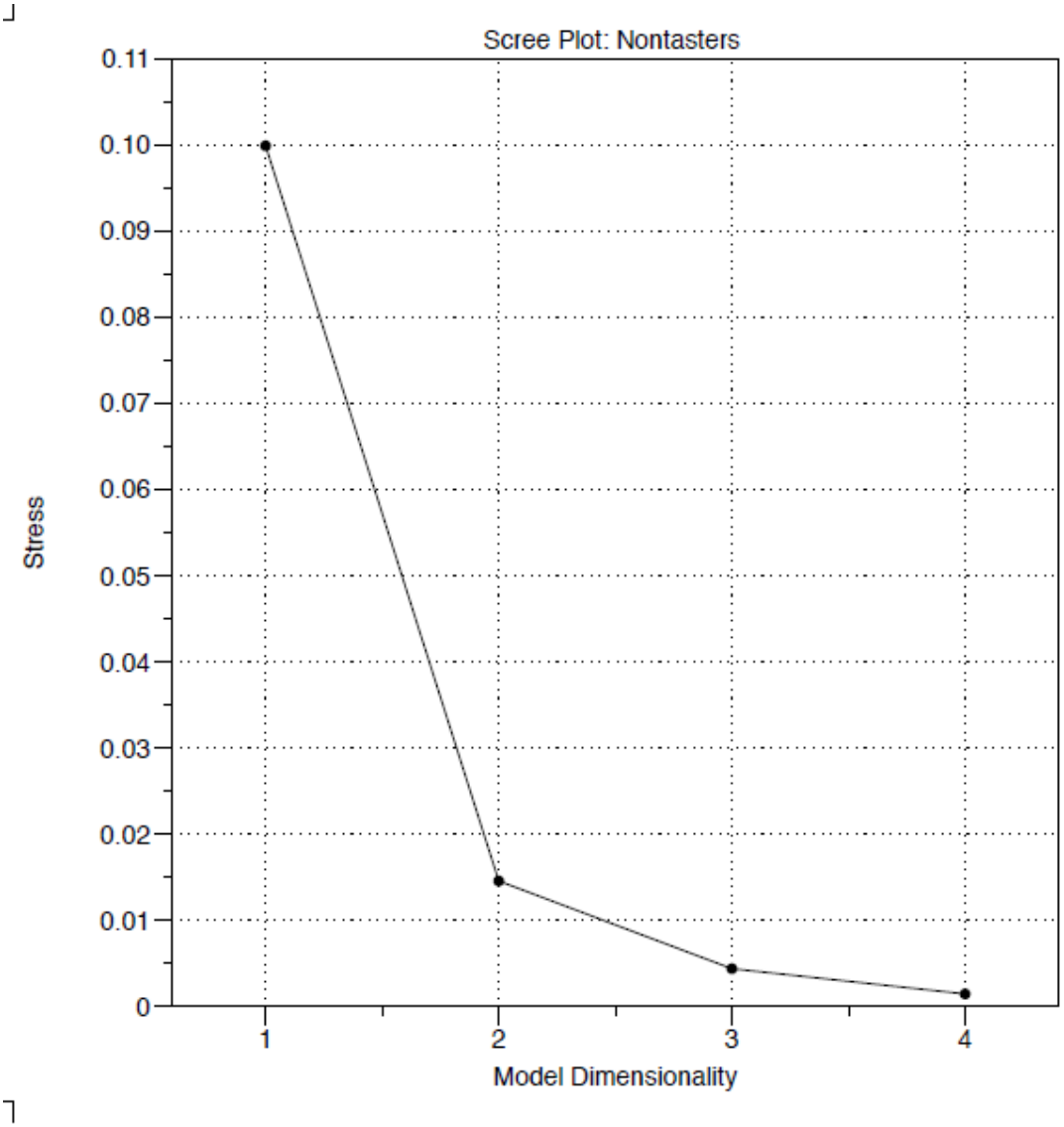

Figure 1: Scree Plot: Nontasters

Figure 1 shows the Scree plot created from the nontaster group data. Based upon the Scree plot, a two-dimensional solution was deemed appropriate for these data, as adding a third dimension did not significantly reduce the stress or aid in the interpretability of the data (2D stress=0.0146).

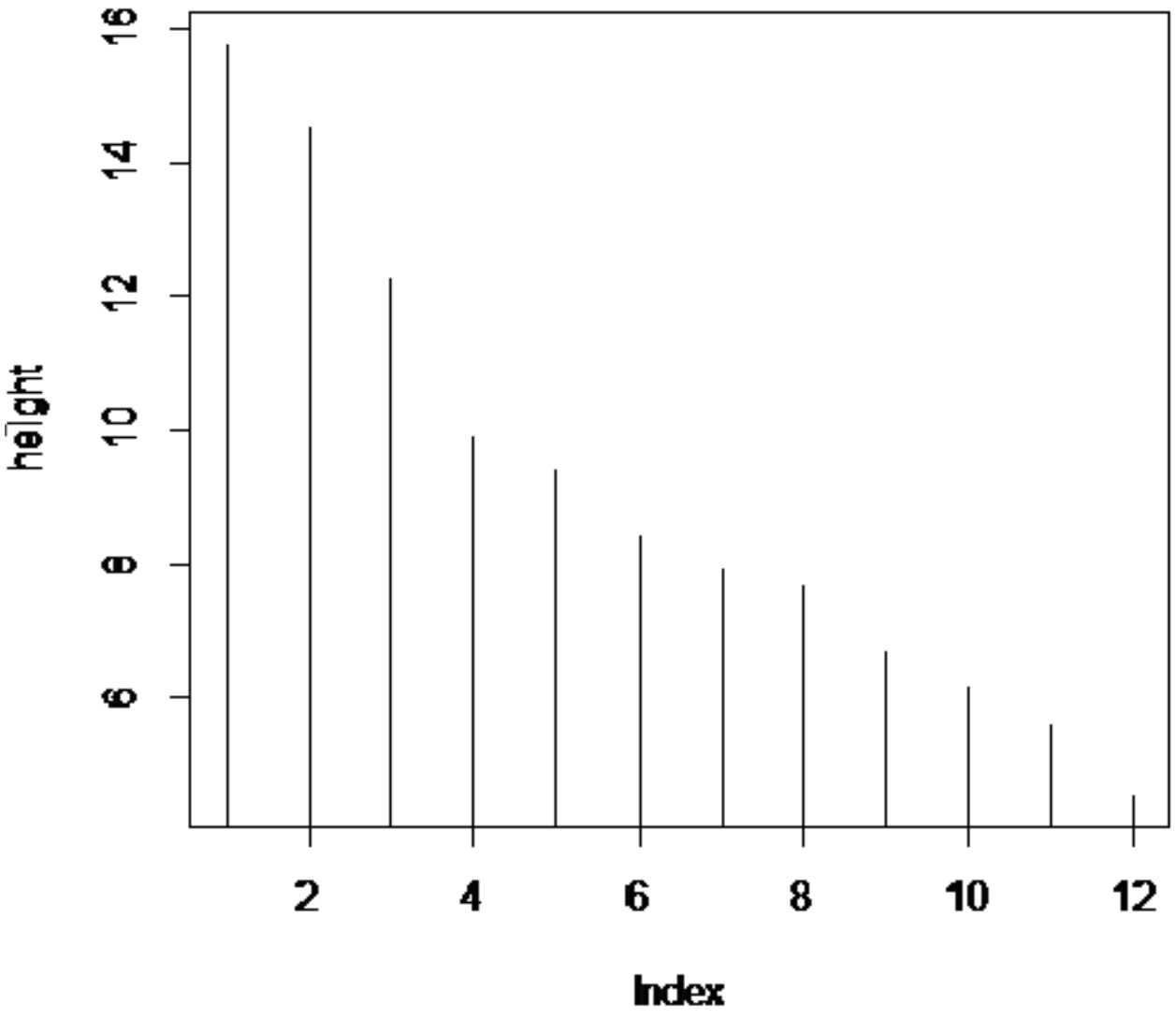

Figure 2: Joining Distance Plot: Nontasters

Figure 2 was used to determine the appropriate number of clusters for the nontaster group. On this joining distance plot, a large jump in amalgamation height was observed around a height of 10, indicating that four clusters of bitterants may be found in this data set.

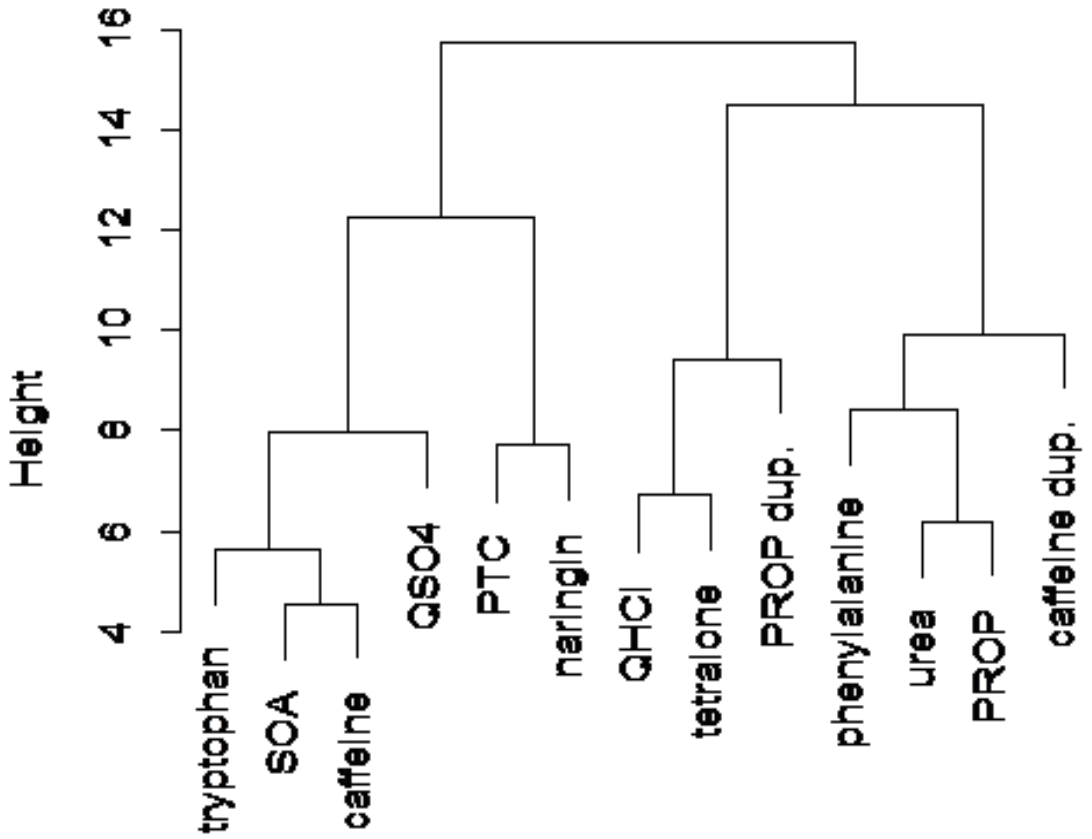

Figure 3: Dendogram of 2D Nontaster Configuration

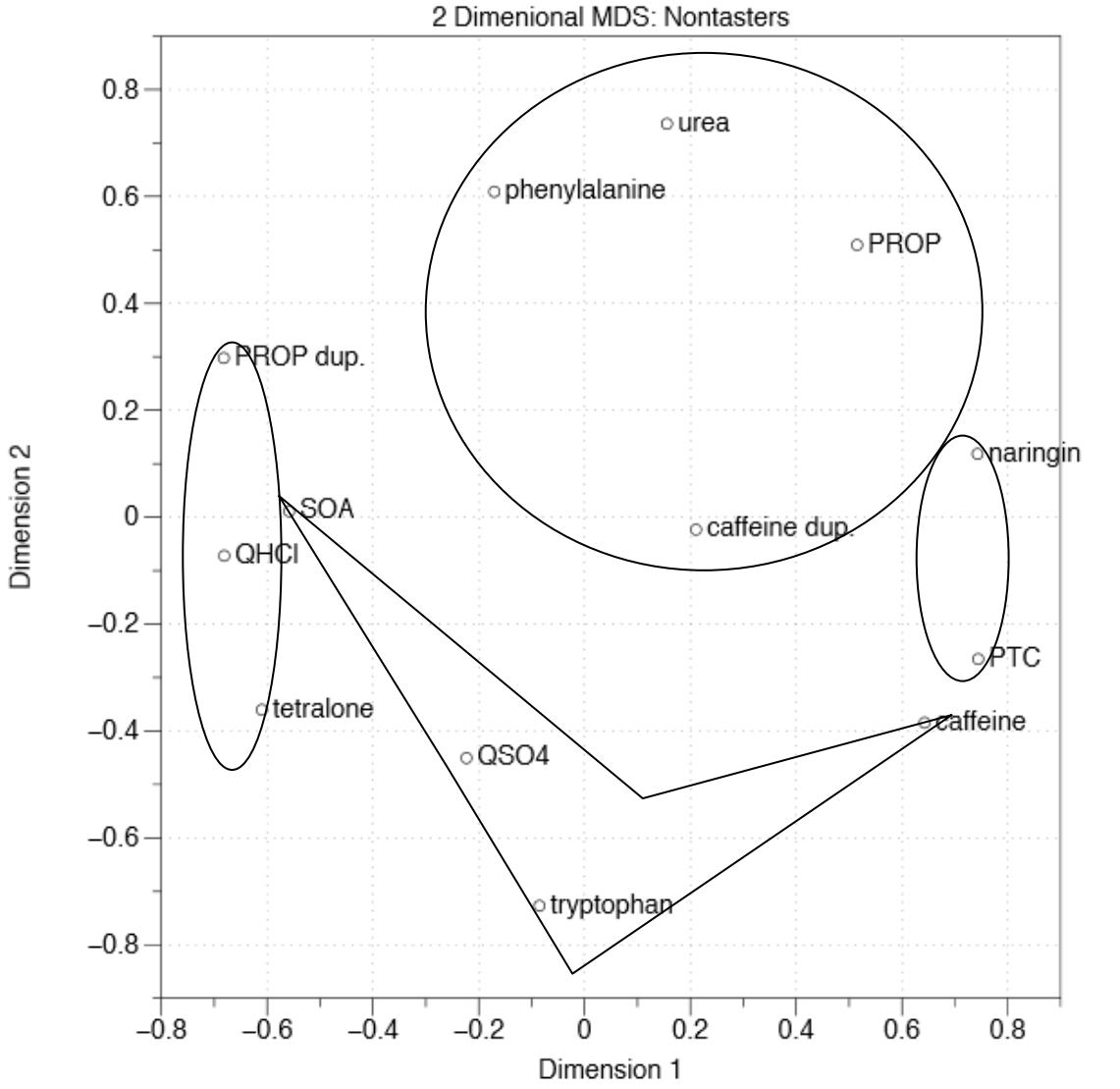

Figure 4: Two-dimensional MDS Plot: Nontasters

As found in Figure 2, four clusters were deemed appropriate for the nontaster sorting data. Therefore, on the dendogram (Figure 3), the four clusters are determined by using the agglomerative height from Figure 2. The four clusters obtained are: tryptophan-SOA-caffeine-quinine sulfate; PTC-naringin; quinine hydrochloride-tetralone-PROP duplicate; and urea-PROP-caffeine duplicate. The agglomerative coefficient for this configuration is 0.55, indicating that the clustering structure is not very strong.

When the aforementioned four clusters from Figure 3 were observed on the 2-Dimensional MDS plot (Figure 4), the stimuli that grouped together on the dendogram were not closely spatially oriented. The rough groupings made on the plot are the four clusters from the dendogram

***Taster Data*:**

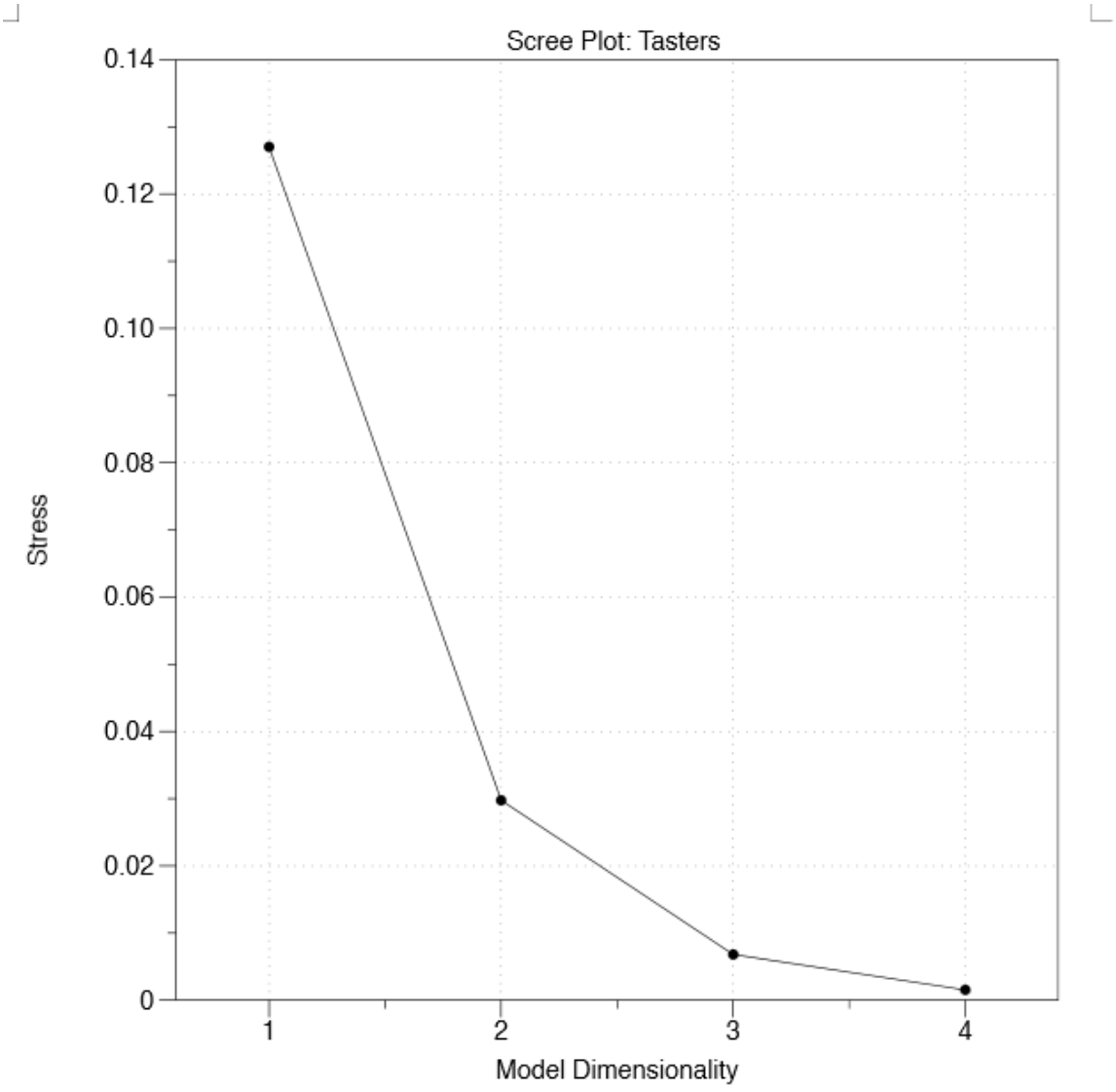

Figure 5: Scree Plot: Tasters

Figure 5 shows the Scree plot from the taster group data. Based upon this plot, a two-dimensional solution was again deemed appropriate for these data. The 2D stress value for this set of data is 0.0298

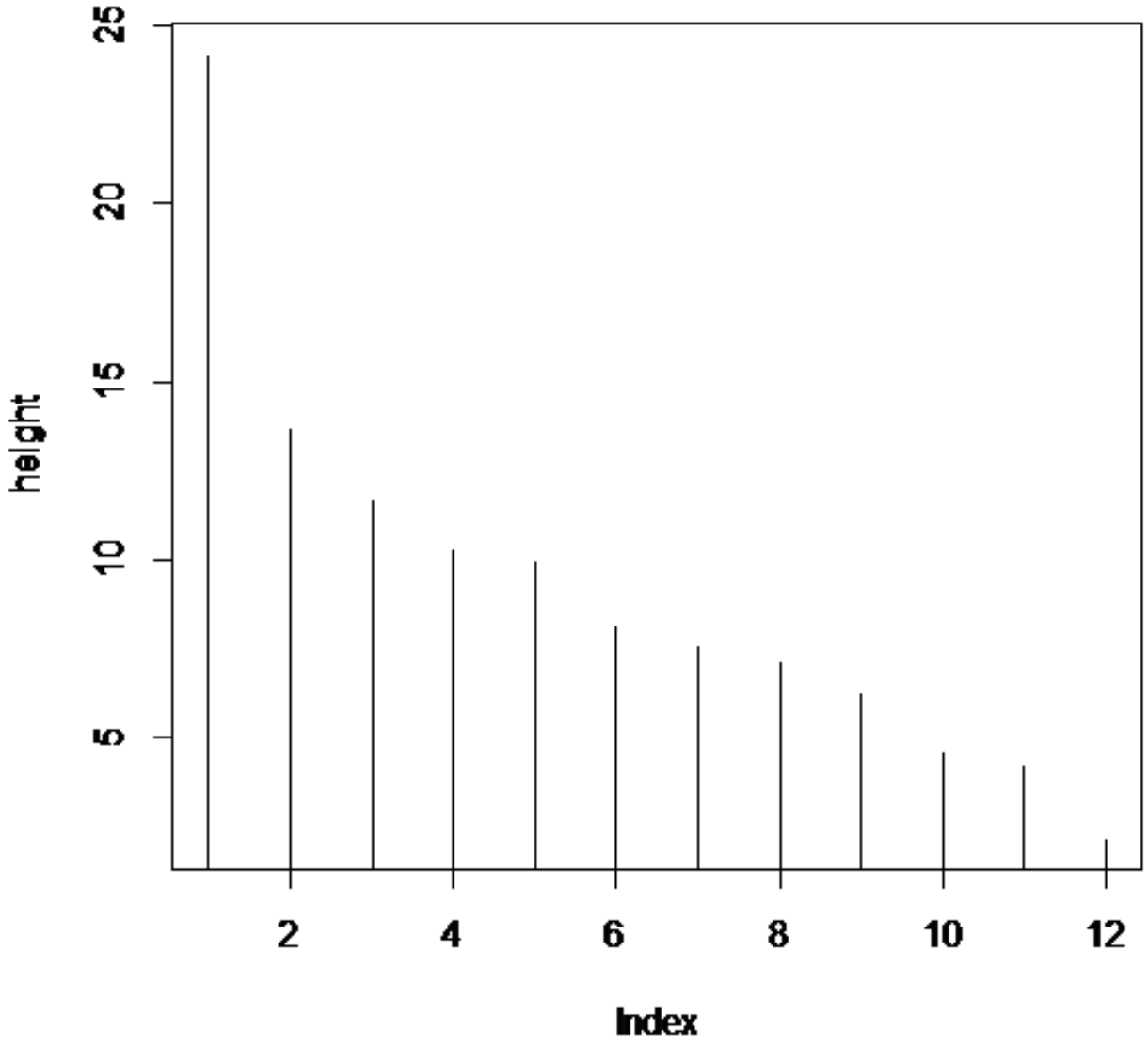

Figure 6: Joining Distance Plot: Tasters

Figure 6 was used to determine the appropriate number of clusters for the taster group. On this plot, three large jumps in amalgamation height were observed, indicating that either ten, six, or three clusters of bitterants may be found in this data set.

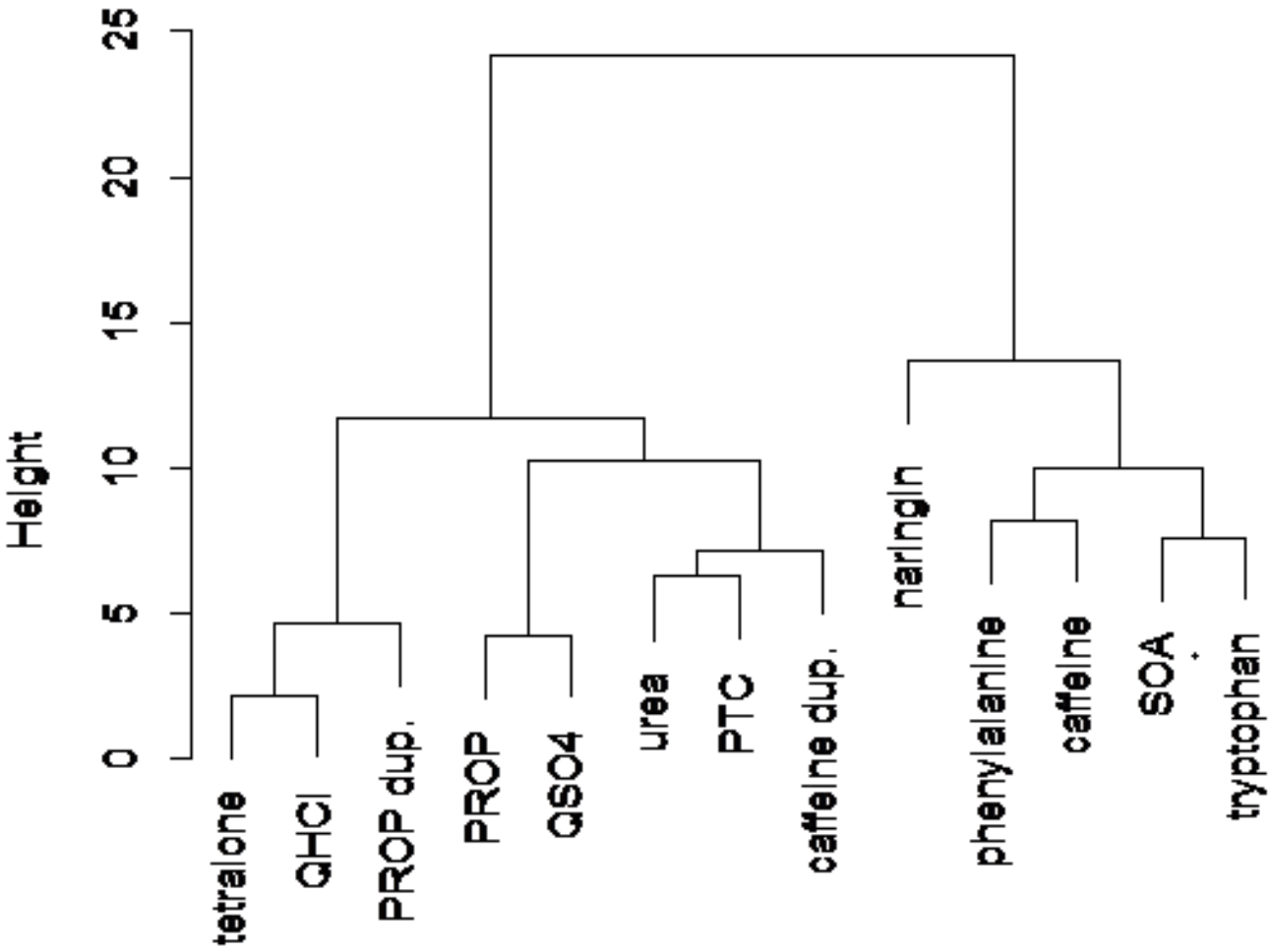

Figure 7: Dendogram of 2D Taster Configuration

There was no clear indication of the number of clusters from the plot of amalgamation distance versus joining order. Therefore, the groupings from Figure 7 and Figure 8 were compared in order to determine the most appropriate number of clusters for the taster data set. From the dendogram, with three clusters, the groupings would be: tetralone-quinine hydrochloride-PROP duplicate-PROP-quinine sulfate-urea-PTC-caffeine duplicate; naringin; and phenylalanine-caffeine-SOA-tryptophan. With six clusters, the groupings are: tetralone-quinine

hydrochloride-PROP duplicate; PROP-quinine sulfate; urea-PTC-caffeine duplicate; naringin; phenylalanine-caffeine; and SOA-tryptophan. Finally, the ten clusters would be: tetralone-quinine-hydrochloride-PROP duplicate; PROP-quinine sulfate; urea; PTC; caffeine duplicate; naringin; phenylalanine; caffeine; SOA; and tryptophan. The agglomerative coefficient for this configuration is 0.74, indicating that the clustering structure is somewhat strong

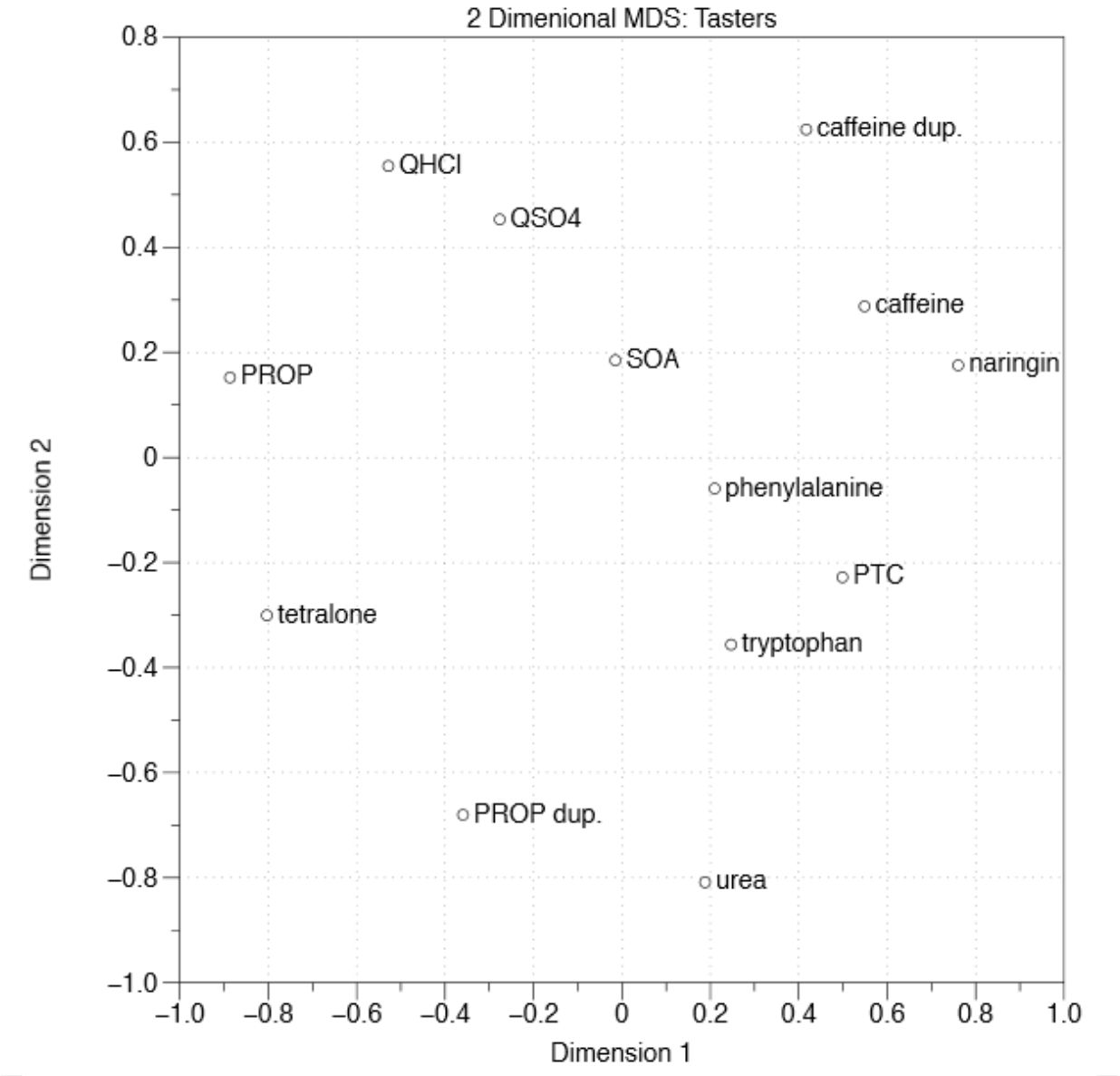

Figure 8: Two-dimensional MDS Plot: Tasters

When applying these groups to the two-dimensional MDS plot, the stimuli that clustered together on the dendogram were not spatially oriented closely with either three, six, or ten groups. To avoid clutter on the plot, groupings were not drawn in on Figure 8.

***Taster and Nontaster Data*:**

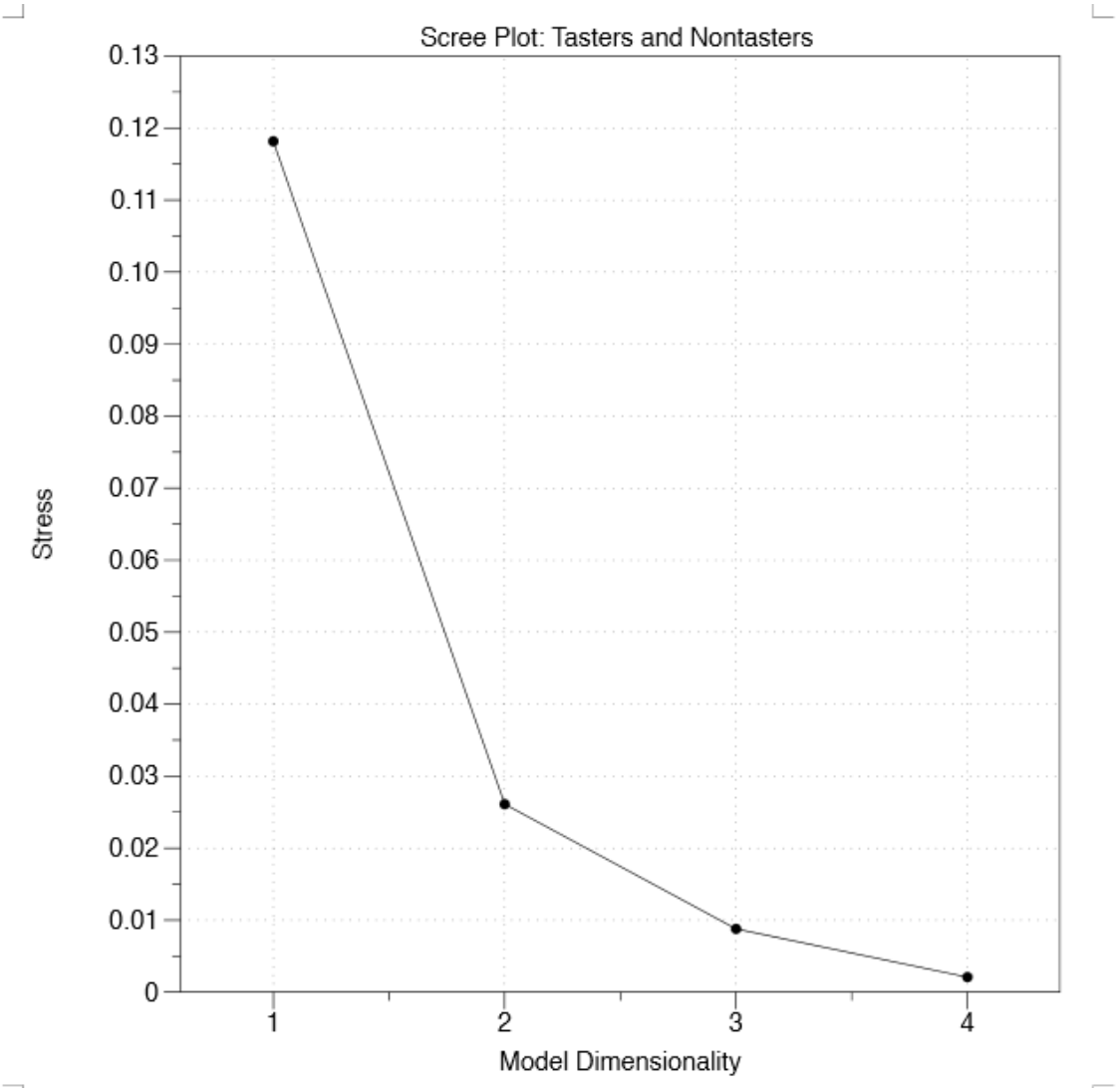

Figure 9: Scree Plot: Nontasters and Tasters

Figure 9 shows the Scree plot from combined taster and nontaster group data. Based upon this Scree plot, the elbow indicated that a two-dimensional solution was again deemed appropriate. The 2D stress value for this set of data is 0.0261.

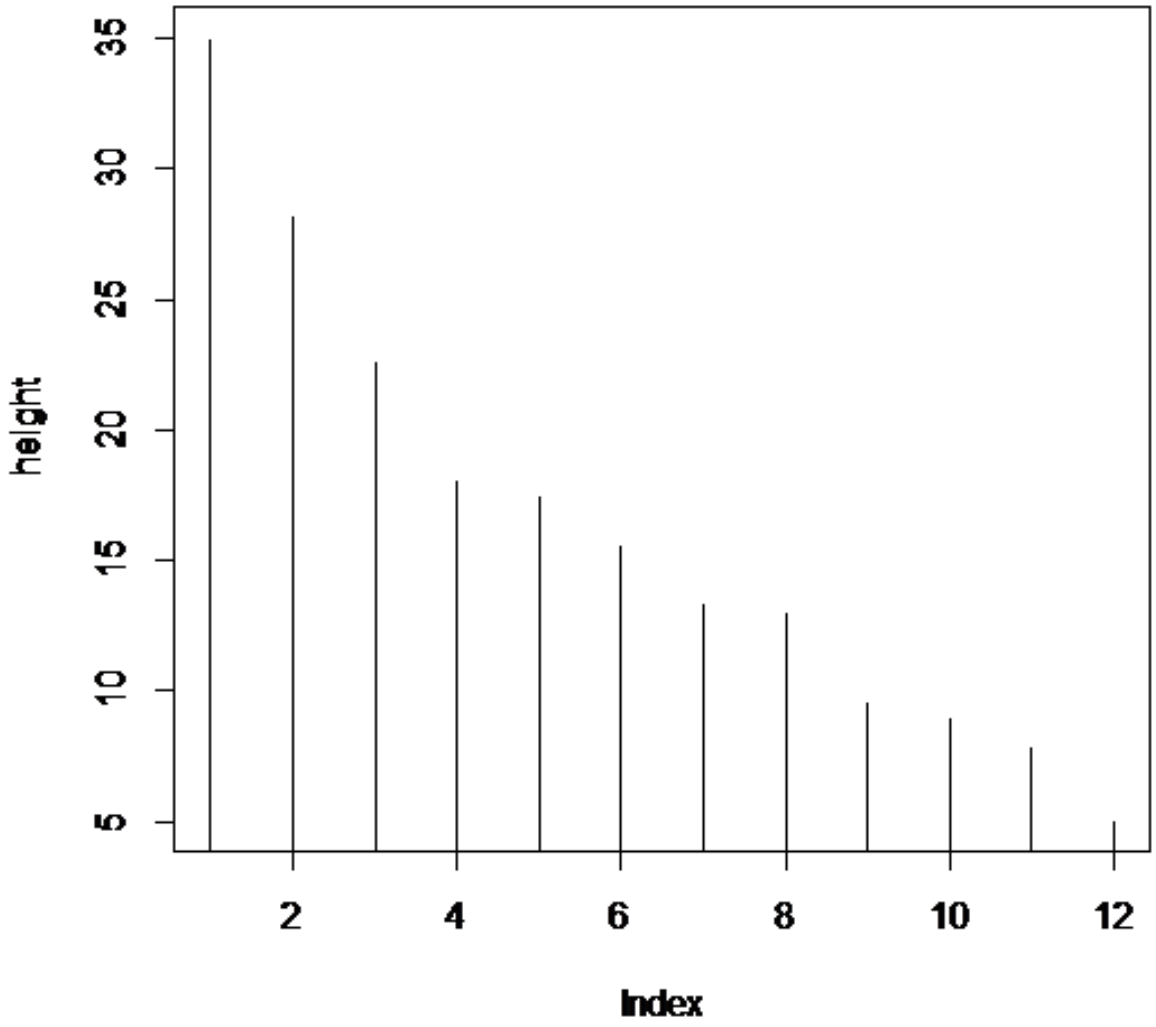

Figure 10: Joining Distance Plot: Taster and Nontaster

The joining distance plot (Figure 10) was used to determine the appropriate number of clusters for the combined taster and nontaster group. This time, large jumps in amalgamation height suggested that four, three, or two clusters of bitterants may be appropriate for this group.

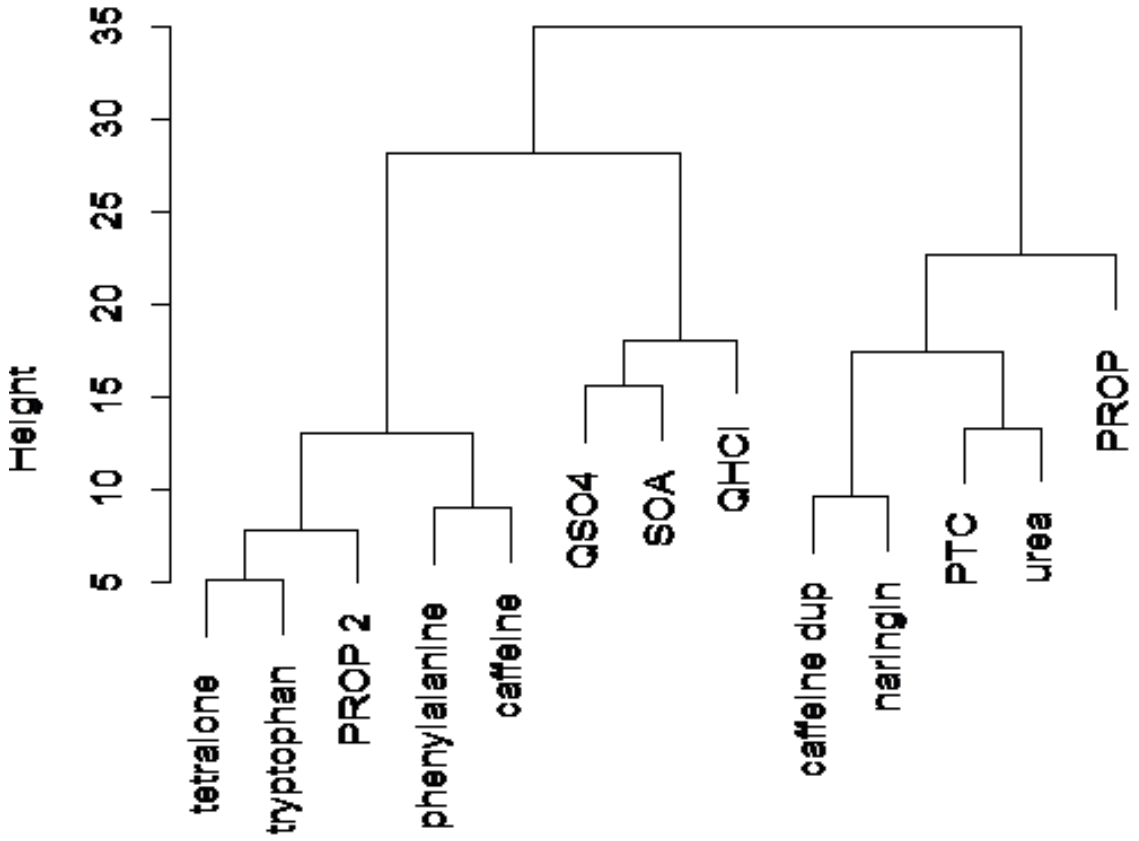

Figure 11: Dendogram of 2D Nontaster and Taster Configuration

There was again no clear indication of the number of clusters from the joining distance plot. Therefore, the dendogram (Figure 11) and 2-dimendional MDS plot (Figure 12) were compared in order to determine which stimuli clustered together. From the dendogram, four clusters would include the groupings: tetralone-tryptophan-PROP duplicate-phenylalanine-caffeine; quinine sulfate-SOA-quinine hydrochloride; caffeine duplicate-naringin-PTC-urea; and PROP. If three clusters were appropriate, the groupings would be: tetralone-tryptophan-PROP duplicate-phenylalanine-caffeine-quinine sulfate-SOA-quinine hydrochloride; caffeine duplicate-naringin-PTC-urea; and PROP. Finally, with two clusters, the bitterants would sort into: tetralone-tryptophan-PROP duplicate-phenylalanine-caffeine-quinine sulfate-SOA-quinine hydrochloride; and caffeine duplicate-naringin-PTC-urea-PROP. The agglomerative coefficient

for the taster and nontaster data combined is 0.66, which indicates that this clustering structure was less well defined than the nontaster clusters, but more defined than the taster groupings.

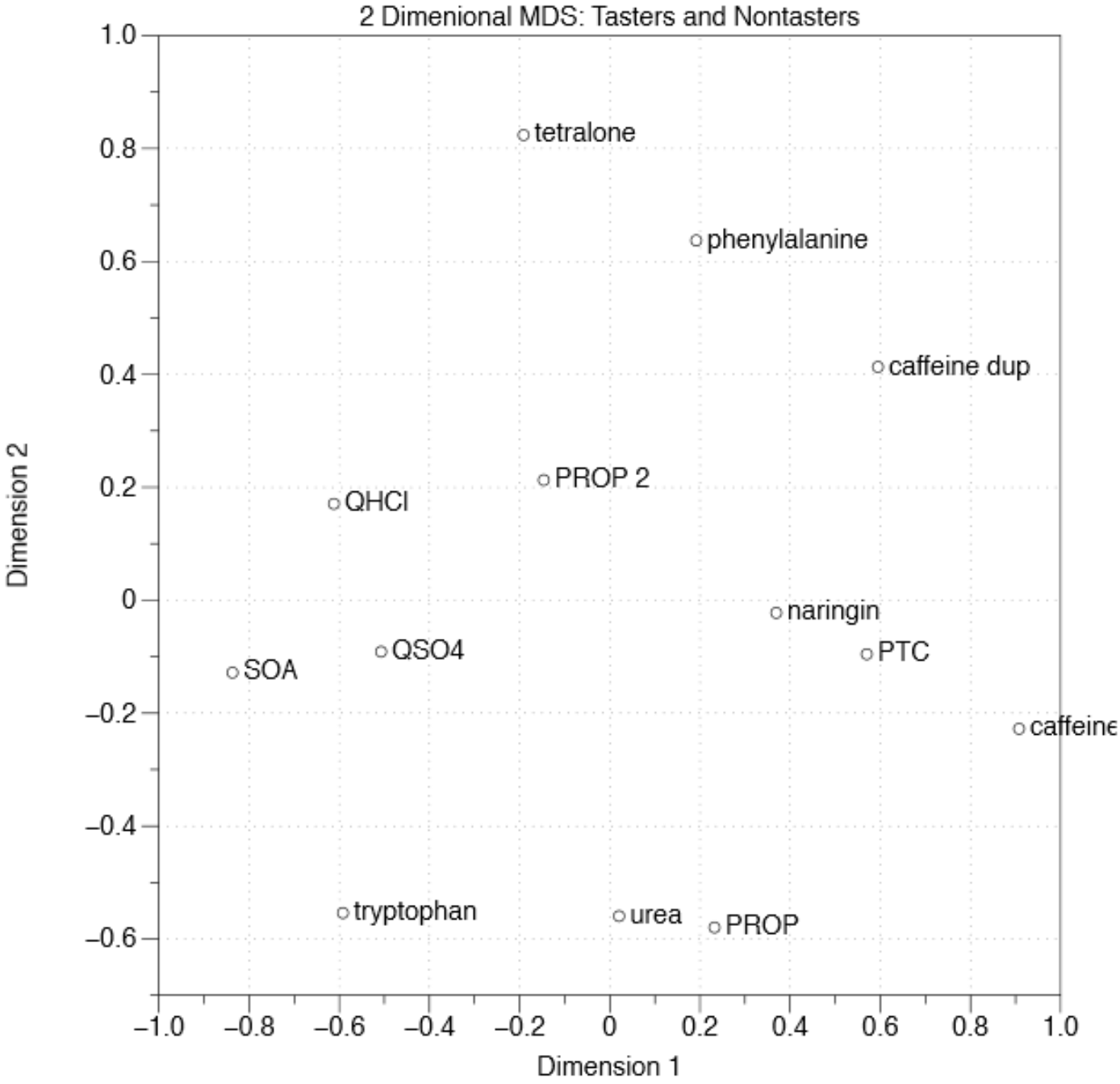

Figure 12:Two-dimensional MDS Plot: Tasters and Nontasters

When looking at the groups from Figure 11 on the two-dimensional MDS plot, the bitterants that grouped together on the dendogram were again not close on the spatial plot with four, three, or two clusters. Again, to avoid confusion, the clusters were not drawn in on Figure 12.

Chapter 4

Discussion

Limitations:

Bitter stimuli are difficult samples to work with due to the highly fatiguing nature of tasting multiple samples in a row. This procedure, which included tasting thirteen bitter stimuli, may have been overwhelming for participants. Because bitter compounds are often associated with toxicity and are often rejected, this procedure may have been difficult for participants to complete due to a negative affective response. The participants were asked to sort the stimuli into groups based on the similarity of the sensation elicited by each sample. If a participant had an adverse reaction to a bitterant, then the participant may not have sorted the sample based upon certain subattribute, but rather on his/her degree of disliking for the sample. Adaption may have also occurred due to the high number of bitter stimuli tasted by each participant.

The actual sorting task may have also been challenging for participants to complete. Because there is only one word in the English language for "bitter," participants may have had trouble determining a basis by which to sort the stimuli. Additionally, because this was a free sorting task, no list of descriptor terms was given to participants. Instead, participants had to generate their own descriptors to define each of the groups that they created. While the free sort procedure may have eliminated bias in term generation, providing a predefined list of descriptors may produce different results. The descriptor terms that were generated are displayed in the Appendix. Due to a lack of consistent, significant attributes, dimensions were unable to be defined.

This procedure was completed in isolated sensory booths without any supervision from a researcher due to time limitations. Therefore, it is unknown whether or not participants took the entire contents of each cup into their mouth and swished the sample around in their mouths for the indicated time (5 s) before expectorating, as prompted by the instructions. Furthermore, the participants may not have rinsed thoroughly between samples. Inadequate rinsing could cause carry over effects between stimuli and therefore lead to inaccurate sorting. If participants were in a rush to complete the procedure because of any unpleasant sensations, improper tasting techniques may have been employed to finish the test as quickly as possible. Any disagreeable sensations elicited by a sample may have prevented participants from retasting samples. While participants had the option of retasting any sample as many times as necessary in order to be sure of their groupings, few participants chose to so. The lack of additional tastings may have led to hasty groupings. Furthermore, it is highly unlikely that participants were able to remember the sensation elicited by each stimulus throughout the duration of the test, especially if samples were not retasted. Instead of holding a single session for the whole study, one-on-one data collection may be more accurate, produce clearer results, and avoid many of the above-mentioned problems.

Here, a minimum interstimulus interval of 90 seconds was set with rinsing ad libitum, even with retasting. The total stimuli number (13) was chosen to allow participants to complete the entire task within a one-hour session. Many previous psychophysical studies on bitterness have been limited to only a few compounds. Such a limitation makes it difficult to find relationships across many compounds. Thus, compounds from multiple classes of bitter-tasting compounds (see Table 1) were chosen for this study, further validating the high stimulus number. The stimuli were previously intensity matched in order to ensure that concentrations were high enough to evoke an appropriate sensation, yet low enough to ensure that the sensation dissipated after 90 seconds and was not overtly unpleasant to the participants. The tasting order was

randomized and counterbalanced to avoid any systematic bias due to carryover effects. Thus, while care was taken to design an appropriate protocol, it may not be possible, under these conditions, to conduct a sorting procedure with the challenges presented by using a large number of bitter stimuli.

Sorting procedures have been shown to stabilize with approximately 25-30 participants (Faye et al. 2006). The taster and nontaster group contained 31 participants; however, the taster group by itself only was comprised of 16 people, and the nontaster group only contained 15 people. Therefore, the sample size may be too small to draw definitive conclusions for the taster and nontaster groups separately. Due to a lack of genetic data available (PROP tasters vs. nontasters), the sample size for this study was lower than planned.

Discussion of Results:

On each of the dendograms and two-dimensional MDS plots for the nontaster, taster, and nontaster and taster groups, it can be noted that the duplicate samples (caffeine and PROP) were not sorted consistently. Figure 11 shows that caffeine and PROP were not in the same cluster as their duplicate sample even when only two groups were suggested. The duplicate samples were part of the procedure to probe for consistent sorting. Due to the inconsistencies observed in the duplicate samples, the quality and accuracy of the sorting data in each the nontaster, taster, and nontaster and taster groups are in question.

Moreover, the discrepancies between the groups of bitterants observed on the dendograms and the clusters noted on the perceptual maps also undermine the validity of the data. When the four groups of bitterants from the dendogram of the nontaster data (Figure 3) were

observed on the MDS plot (Figure 4), the samples within these four groups were not always in the same regions on the plot. Furthermore, while the agreement between the dendogram and MDS plot clusters appeared to be the clearest in the nontaster data set, the low agglomerative coefficient (0.55) suggests that these clusters are only weakly defined.

A look at the taster data proves even less conclusive. When the exact number of groups on the dendogram (Figure 7) was unclear from the joining distance plot (Figure 6), observation of the perceptual map (Figure 8) was hoped to elucidate both the number of clusters and the bitterants within these clusters. This was not the case, however. With three groupings, perversely large regions of space were occupied by a single group, indicating that stimuli that clustered together on the dendogram were not similarly spatially oriented. With six or ten groups, as proposed by Figure 6, many stimuli were in an individual group, and those stimuli that were grouped with other stimuli did not occupy the same region of the map. While the agglomerative coefficient (0.74) suggested that these clusters were more strongly defined than the nontaster clusters, visually, the perceptual map proves otherwise. Surprisingly, these PROP tasters were not able to sort even the duplicate PROP samples into the same groupings. It was hypothesized that PROP tasters and nontasters may sort bitter stimuli differently based upon differences in their genetics. The groupings observed on both the nontaster and taster perceptual maps are blatantly different. However, due largely to the small sample size, whether or not the genetic differences between PROP nontasters and tasters is responsible for the different clusters on the MDS plots cannot be concluded.

While the sample size for the combined nontaster and taster data is sufficiently large enough for a sorting procedure to draw conclusions, the indeterminate clusters again observed on the spatial plot (Figure 12) does not allow for such. When agglomerative hierarchical clustering

suggested two, three, or four groups for the perceptual space, the resulting clusters encompassed large regions of space. These crude clusters, in addition to the agglomerative coefficient value of 0.66, may infer the participants' inability to readily identify a basis by which to sort a bitter taste or sensation. The close association found between phenylalanine, tryptophan, and urea as well as the association between caffeine, SOA, and quinine in previous studies is only partially supported by the sorting data in this procedure (Delwiche et al. 2001; Yokomukai et al. 1993; Lawless 1979; McBurney et al. 1972). With four clusters, phenylalanine and tryptophan cluster together, as do both of the quinine samples and SOA. Phenylalanine and tryptophan were expected to group together since both bitterants are amino acids, as were the two alkaloids, quinine hydrochloride and quinine sulfate. While one PROP sample is in an individual cluster, similarly noted in the Delwiche and Yokomukai studies, the PROP duplicate is grouped with phenylalanine and tryptophan (Delwiche et al. 2001; Yokomukai et al. 1993). Instead of clustering with SOA and the quinine samples, caffeine was found in two different groups. Furthermore, urea was not sorted with the amino acids, as predicted by Delwiche. Therefore, for the combined nontaster and taster data, definitive conclusions cannot be drawn, due in part to the inconsistent duplicate sample sorting in conjunction with many nonsensical groupings observed on the MDS plot.

Future Directions:

As noted previously, the study limitations largely impeded conclusive evidence towards determining whether or not bitter subattributes exist and whether or not the genetic differences between PROP nontasters and tasters lead to differences in bitter stimuli sorting. However, altering the procedure may lead to more robust, definite results.

To start, increasing the sample size to include at least 25-30 of both nontasters and tasters will allow for conclusions to be made regarding any noted differences in bitterant sorting. Changing the data collection to one-on-one testing sessions may aid in eliminating many procedural shortcomings that may occur when participants are unsupervised by a researcher, such as improper stimuli intake or inadequate rinsing. If thirteen stimuli are deemed too fatiguing, perhaps the number of stimuli should be reduced to only include one bitterant from each class of compounds (i.e. only including one of the amino acids). Increasing the interstimulis interval may also be necessary to ensure that carryover effects do not influence stimuli sorting. Results may vary if PROP were not included in the sorting procedure, since it may introduce a priori contextual effects for the participants who could not taste it and for those who were very sensitive to it (Delwiche et al. 2001).

Results may be different if the groupings were made by genetic supertasters instead of tasters and then compared to the groupings made by PROP nontasters (Hayes and Keast 2011). Additionally, it would be interesting to note differences in bitter stimuli sorting if participants wore a nose clip for the duration of the procedure. The nose clip would help determine if any ortho- or retronasal odors influence the participants when sorting stimuli. Moreover, providing the participants with a predefined list of terms in another sorting procedure may help to define the axes on the perceptual maps and distinguish which attributes are significant when sorting bitterants.

Chapter 5

Conclusion

As seen above, the nontaster, taster, and combined nontaster and taster groups were unable to group bitter stimuli based primarily upon varying subattributes. While the basis of the groupings could not be determined, many descriptors were generated to describe the bitter groupings formed. Descriptor terms to characterize each of the participants' groupings were not provided to participants in this procedure because of the uncertainty of words besides "bitter" to appropriately describe the stimuli. Now that participants have provided an idea of their bases for sorting the stimuli, the next phases of this study may involve using these descriptors as part of a predetermined list of terms used by the next participants to describe clusters of bitterants.

The groupings made by the nontasters and tasters varied in the number of groups, the stimuli within each group, and the placement of the clusters on the spatial plots. Due largely to procedural limitations and a small sample size, conclusive evidence was not found regarding the differences in sorting observed between PROP nontasters and tasters. This is not to suggest, however, that subattributes do not exist or that there is not a difference between the clusters of bitterants formed by tasters and nontasters. Improvements to the sorting design and sample may allow for the formation of clearer clusters and hopefully a better understanding of any differences that arise in nontaster versus taster bitter clusters.

Overall, this study suggests that without the aforementioned revisions to the procedure, that the sorting task may only be utilized to determine, at minimum, rudimentary differences

between bitter stimuli. The high degree of fatigue that arises with using a large number of bitter stimuli coupled with the possibility of adaptation poses problematic for accurate data collection. Additionally, the inconsistency in sorting observed with the duplicate samples of caffeine and PROP calls into question whether or not naïve participants can consistently differentiate between the perceptual qualities of different bitterants. Due to success with sorting chemesthetic compounds (Byrnes et al. under review), which are also difficult stimuli to work with, there is hope that this sorting procedure may be utilized to begin understanding the qualitative aspects of the broad term "bitter."

Appendix A

Supplemental Material

Group Descriptors: Terms Generated by Participants and the Number of Stimuli in Each Group

Group Descriptor	Number of Stimuli in Group
"Sweet/flavor" sensation	2
Ale bitter	2
Alkaline with after burn	3
Also watery mouthfeel but with a slight citrus-like sourness/bitterness	4
Astringent Bitter	3
Astringent	2
Astringent bitter	4
Astringent, Bitter	1
Bad alkaline with some aftertaste	3
Barely any taste at all	2
Barely bitter sensation	4
Barely Noticeable/Slightly Bitter	5
Bitter	2
Bitter	5
Bitter	4
Bitter Aftertaste Only	2
Bitter, astringent, and Stingy	1
Bitter, But Less Strong	3
Bitter, but not as bitter as group 1, but more bitter than group 2	4
Bitter, Coating Mouth Feel	1
Bitter/Burn	4
Bitter/Sour?	1
Bland/watery taste	3
Blank/No Sensation	3
Burning bitter	2
Chemical-bitter	3
Coating Mouth Feel, But No Other Strong Sensation	1
Dark bitter	3
Different kind of bitter	3
Dry and bitter	2
Drying sensation	2

Extremely bitter	2
Extreme bitter	1
Extremely Bitter	5
Extremely Bitter	2
Extremely Bitter	3
Fairly Bitter	4
Fresh, plain bitter	1
Grapefruit-sour-bitter, pleasant	3
Green-tea bitter	1
Slightly Bitter	4
Alkaline	4
Moderately Bitter	6
Quite Bitter	2
Very Bitter	1
Harsh Bitter	1
High-end medium bitter, slightly sour	3
Hint of bitter	3
Hops-like, plant bitterness	3
Horrid aftertaste- bitter and astringent	6
Initial aftertaste- bitter	7
Intense bitter flavor, horrible aftertaste	2
Light bitter	1
Like bitter veggies - gross	2
Like licking a stamp	4
Like vomit	1
Lite Bitter	5
Little Bitter Taste	4
Low-end medium bitter flavor, slightly lemony	2
Medium Bitter	4
Medium Bitter Flavor	4
Medium Bitter with something else, not sweet/sour/salt	1
Medium Bitter/ Mild Sweet	2
Medium Bitterness	3
Mild alkaline taste with minimal aftertaste	3
Mild Bitter	3
Mild bitter	5
Mild Bitter Flavor	5
Mild Bitter/ Mild Sweet	1
Mild bitterness	1
Mild bitterness with a lingering taste	6
Mild with a strong after taste (medium bitterness)	3

Mildly bitter	5
Mildly bitter, including after taste	3
Mildly bitter, slightly drying	2
Moderate + cacao	1
Moderate + cacao+ burning sensation	1
Moderate + earthy	1
Moderate + other undescriptive flavor	1
Moderate bitterness	3
Moderate, metallic	1
Moderately Bitter	3
Moderately bitter	3
Moderately Bitter	2
More sour/acidic taste	1
Neutral	6
Neutral Astringent	1
No Real Sensation	1
No Sensation	4
No sensation	4
No Strong Sensation	2
No taste, mild or moderate bitter after taste	4
Not too shabby	2
Nothing, tastes like water	2
Perfumey/Aromatic	2
Persistent, moderate bitter sensation through and after tasting	5
Pungent bitter sensation at first taste	2
Rubbing Alcohol, astringent	1
Salty Bitter	3
Severe Bitter Flavor	4
Sharp Metallic Bitter	2
Slightly Bitter	3
Slightly bitter	2
Slightly bitter aftertaste	2
Slightly Bitter/Neutral	4
Soapy, not pleasant	2
Sour	1
Sour Bitter	1
Sour Bitter	1
Sour/Bitter	1
Spice/Cinnamon	2
Spicy bitter	1
Strong alkaline/sour taste with aftertaste	4

Strong Bitter	7
Strong Bitter	1
Strong bitter	2
Strong bitter flavor	1
Strong bitter sensation	4
Strong bitter start, little after taste	3
Strong bitter start, strong after taste	1
Strong Bitter, Slightly Metallic	6
Strong bitterness	5
Strong bitterness+ burning sensation	2
Strong, unpleasant grapefruit	2
Stronger Alkaline	4
Stronger bitter	1
Stronger bitter than stronger bitter	2
Strongly Bitter	6
Sulfuric Bitter	1
Sweet Bitter	1
Terrible	2
Very Bitter	2
Very bitter	2
Very bitter	3
Very bitter	2
Very Bitter	4
Very Mild Bitter	2
Very mild bitter flavor	2
Very mild, almost no taste	1
Very Sour	1
Very strong bitter	1
Very Strong Bitter	2
Very strong bitter flavor	4
Very weak flavor	1
Watery - No Significant Taste/Bitterness	4
Watery, dry mouthfeel, and bitter/plastic-like aftertaste	1
Watery, slight bitterness, created dry mouthfeel	3
Weakest bitter flavor	1
Weakly Bitter	4
Weakly Bitter, Aspirin	4

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