Thermal tasting: methodological considerations and implications for alcohol behaviour.

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Abstract

Thermal tasting is a phenomenon whereby some individuals perceive thermally-induced taste sensations when their tongue is warmed or cooled. These individuals, known as thermal tasters (TT), report a variety of thermally-induced tastes and the tastes reported can vary with temperature regime used and location on the tongue tested. TT are typically compared to thermal non-tasters (TnT), individuals who do not experience thermally-induced sensations. The literature suggests that TT give higher intensity ratings to orosensory stimuli than TnT; however, small sample sizes and differences in classification schemes between studies confound our understanding of TTS (thermal taste status). It is unknown whether the increased orosensory responsiveness of TT is universal or whether it is driven by a subgroup of TT. Furthermore, up to 50% of individuals are non-classifiable (NC). The largest database of individuals who have undergone TTS screening was compiled to address the literature gaps. Findings indicate that TT are more responsive than TnT to orosensory stimuli, regardless of the classification scheme used. The orosensory responsiveness of NC is not homogeneous, suggesting that NC are not a separate group but rather misclassified TT and TnT. Sweet TT are more likely than non-sweet TT to experience thermally-induced sensations during lingual warming. Similarly, sour TT are more likely than non-sour TT to report thermally-induced tastes during cooling. However, no differences in orosensory responsiveness based on these or other subgroups are identified, suggesting that the heightened orosensory responsiveness of TT is universal across this phenotype. The final study sought to characterize the binary interactions between ethanol and four orosensory stimuli (fructose, quinine, tartaric acid and alum sulphate) both overall and by comparing TT and TnT. In general, TT are more responsive

than TnT to all stimuli in the study. Few interactions between TTS and stimulus intensity exist suggesting that TT and TnT perceive the sensations elicited by alcoholic beverages similarly, albeit at different intensities. Together, the thesis helps inform best practices for TTS screening and classification, provides insights into TTS mechanisms and furthers our understanding of alcoholic beverage perception.

Key Words: ethanol, individual differences, orosensory responsiveness, alcohol consumption, taste interactions

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List of Abbreviations

Abbreviation	Definition
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ASIC	acid-sensing ion channels
BD, W, M, S, VS	anchor terms on the gLMS (barely detectable, weak, moderate, strong, very strong)
binary solutions/mixtures	aqueous solutions with two solutes
ENaC	amiloride-sensitive epithelial Na+ channels
FDR	false discovery rate
GCPR	G-protein coupled receptor
gDOL	generalized Degree of Liking Scale
gLMS	generalized Labeled Magnitude Scale
gVAS	generalized Visual Analogue Scale
I	Index of interaction
M	mean
NC or Uncats	non-classifiable participants
NC-AW	non-classifiable participants that provided at least one above "weak" response during thermal elicitation
NC-BWO	non-classifiable participants that did not provide any responses above "weak" during thermal elicitation
NC-NoREP	non-classifiable participants that did not report at least one reproducible thermally elicited sensation
NC-REP	non-classifiable participants that reported at least one reproducible thermally elicited sensation
NS	not significant
OR	odds ratio
orosensation	taste and chemesthetic sensations elicited in the oral cavity, excluding temperature-related sensations

Abbreviation	Definition
pMTs	PROP medium-tasters
pNTs	PROP non-tasters
PROP	6-n-propylthiouracil
pSTs	PROP super-tasters
PTC	phenylthiocarbamide
PTS	PROP taster status
SD	standard deviation
SE	standard error
single-factor subgroup	TT subgroup where only one criterion was used to classify participants
SNPs	single nucleotide polymorphisms
TAS1R	taste 1 receptor
TAS2R	taste 2 receptor
TCATA	temporal check-all-that-apply
TED or thermode	thermal elicitation device
TnTs or TnT	thermal non-tasters
TRP	transient receptor potential channels
TRPM	transient receptor potential melastatin channels
TRPV	transient receptor potential vanilloid channels
TTS	thermal taster status
TTs or TT	thermal taster
two-factor subgroup	TT subgroups where only two criteria were used to classify participants
unary solutions	aqueous solutions with one solute
v/v	volume per volume

Chapter 1: General Introduction

According to the *Global Status Report on Alcohol and Health* in 2016, 43% of individuals over the age of fifteen worldwide were current consumers of alcoholic beverages (World Health Organization, 2018). Understanding factors that impact alcohol consumption is important to reduce the harm associated with alcohol misuse, while also providing valuable consumer information to the alcoholic beverage industry.

Individual differences in the perception of oral taste and chemesthetic sensations exist and influence the development of food and alcohol preferences, intake, and health related outcomes (reviewed in Tepper, 2008; Garcia-Bailo et al., 2009; Hayes et al., 2013). As consumers often cite flavour as one of the most important factors in food choice (Glanz et al., 1998; Aggarwal et al., 2016; Kourouniotis et al., 2016; Small-Kelly, 2018), understanding how individual differences in orosensory perception influence food related choices is of interest to both the medical community and to food manufacturers.

Taste perception is partially under genetic control, and inter-individual differences can be understood by grouping people according to their taste genotype and phenotype (e.g. Talavera et al., 2005; Keskitalo et al., 2007; Tepper, 2008; Garcia-Bailo et al., 2009; Bering et al., 2013; Allen et al., 2014). One type of individual variation in taste perception is thermal taste status (TTS). Thermal taste was first reported by Cruz and Green (2000) who found that warming or cooling the tongue elicited taste sensations in some individuals. Subsequent studies focused on comparing these individuals, collectively referred to as thermal tasters (TT), with individuals who only perceived temperature changes during warming or cooling (thermal non-tasters; TnT). TT rate the intensity of suprathreshold basic tastes (sweet, sour, salty, bitter and umami) higher than TnT across multiple locations of the mouth (front/back of the tongue, soft palate) and when using a whole mouth sip and spit protocol (Green and George, 2004). Although not always significant, the increased responsiveness of TT to basic tastes compared to TnT has been confirmed in other studies (Green et al., 2005; Bajec and Pickering, 2008; Yang et al., 2014; Hort et al., 2016; Small-Kelly and Pickering, 2020).

For predominately chemesthetic stimuli, the results are less clear. Although TT are more responsive to alum sulphate, an astringent stimulus that also elicits sweetness (Bajec

and Pickering, 2008), there is no difference in responsiveness to burning (capsaicin; Green et al., 2005; Yang et al., 2014) or cooling stimuli (menthol; Green et al., 2005). Similarly, it is unclear whether TT have increased responsiveness over TnT in the perception of ortho- and retro-nasal aromas, as some studies have reported differences (Green et al., 2005) and others have not (Yang et al., 2014). TT appear to be more responsive to complex stimuli (ethanol and metallic salts), although it is unknown whether these differences can be attributed to the prototypical tastes, trigeminal sensations and/or retro-nasal aromas elicited by the stimuli (Bajec and Pickering, 2008; Small-Kelly and Pickering, 2020). Where findings across studies are equivocal, a significant challenge in reviewing and summarising the pertinent literature on thermal taste is assessing the significance of the methodological differences in data collection approaches and measures used across studies.

Although other taste phenotypes have been identified, such as 6-n-propylthiouracil (PROP) taster status and sweet-liking, they are independent of TTS (Pickering et al., 2010b; Bering et al., 2013; Yang et al., 2014, 2020). Thus, accurately screening participants for TTS can bring additional insights into food related behaviour. To this end, the current thesis focused on addressing knowledge gaps related to TTS screening and classification methods to help optimize future TTS protocols. The thesis also sought to extend our understanding of the differences between TT and TnT by investigating the perception of binary mixtures, as most studies have only tested for differences using unary solutions (Green and George, 2004; Green et al., 2005; Bajec and Pickering, 2008; Bajec et al., 2012; Yang et al., 2014, 2020; Nachtigal and Green, 2020; Small-Kelly and Pickering, 2020) or real food/beverages (Pickering et al., 2010b, 2010a, 2016; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016; Yang et al., 2018; Mitchell et al., 2019; Small-Kelly and Pickering, 2020). Studying how ethanol interacts with taste-relevant stimuli will provide insights into the flavour of alcoholic beverages. First, the overarching knowledge gaps at the time the thesis was conceived are introduced. This section is followed by a brief description of the thesis structure and chapters. Readers are referred to Chapter 2 for a general introduction to individual differences in taste perception and to their impact on alcohol related behaviour (preferences, consumption, alcohol use disorder risk).

1.1 Literature Gaps

1.1.1 Gap #1

Differences in taste responsiveness between TT and TnT are not always found, and such null results are often attributed to sample size.

The literature suggests that TT are more responsive to aqueous solutions of taste and chemesthetic stimuli compared to TnT. Nevertheless, in several studies the suggested differences in responsiveness are not always significant (Green et al., 2005; Bajec and Pickering, 2008; Bajec et al., 2012; Yang et al., 2014). Similarly, some non-significant instances of TT rating the taste and chemesthetic sensations elicited by foods and beverages have also been reported (Pickering et al., 2010a, 2010b, 2016; Pickering and Klodnicki, 2016). Several studies have suggested that small sample sizes may have contributed to the null results (Pickering et al., 2010a, 2016; Bajec et al., 2012; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016). It is unclear whether the studies were simply underpowered as they employed small sample sizes (typically 40-100), or instead if the reported differences between TT and TnT are spurious findings. Therefore, the relationship between supra-threshold orosensory responsiveness to aqueous solutions and TTS was re-examined using a much larger sample than previously employed (n = 708).

Additionally, scale use differences between TT and TnT have not been directly examined in the literature. Green and George (2004) propose that the equivalent ratings of fixed temperatures on non-gustatory sites given by TT and TnT suggest that differences in orosensory responsiveness between TT and TnT are not an artefact of scale use; rather, mechanistic differences likely underlie TTS. However, variation in scale use is widely acknowledged in the sensory and psychophysical literature, attributable to a range of cultural, psychological and biological factors, with the latter including taste phenotype (Bartoshuk et al., 2002). Therefore, differences in scale use between TT and TnT were tested directly.

1.1.2 Gap #2:

TT experience a wide range of taste sensations during thermal elicitation and the proportion of tastes reported can vary with the temperature regime and/or lingual

location tested. Based on these differences, more research is needed to determine whether TT are a homogeneous group or whether subgroups within TT exist.

By definition, TT are individuals who experience taste-related sensations when their tongue is warmed or cooled. However, the experiences of TT vary widely based on the orosensation reported (sweet, sour, bitter, salty, metallic, spicy, minty), the temperature regime (warming, cooling) and the location tested on the tongue (tip, 1-cm to the left, 1-cm to the right). For instance, thermal sweetness is more frequently elicited during warming than cooling (Cruz and Green, 2000; Green et al., 2005; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). Conversely, sourness and saltiness are more frequently elicited by cooling than warming (Cruz and Green, 2000; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). The proportion of participants reporting bitterness is typically higher during cooling than warming (Yang et al., 2014; Skinner et al., 2018), although it is roughly equivalent across both temperature regimes when three sites are tested on the edge of the tongue (Pickering and Kvas, 2016). Thermally-induced metallic tastes occur at similar proportions during both warming and cooling (Yang et al., 2014; Skinner et al., 2018). Further research is required to determine whether these results evidenced from small samples can be verified in a large sample.

Robust associations between the thermal taste experienced and the lingual location and/or temperature of thermal elicitation may provide insights into the mechanism(s) underlying the phenomenon. For example, TRPM5 is a heat-activated cation channel that is highly expressed in taste receptor cells and is involved in the perception of sweet, bitter and umami tastes (Talavera et al., 2005). Importantly, both TRPM5 and thermally-elicited sweetness can be activated in the same temperature range (Talavera et al., 2007; Skinner et al., 2018). Thus, by testing for an association between thermally-elicited sweetness and the warming cycle in a large sample, the results will help to support or refute the hypothesis that TRPM5 is involved in the perception of thermally-elicited sweetness.

Although on average, TT are more responsive than TnT to orosensory stimuli, orosensory responsiveness also varies considerably between TT. As such, it is possible that the heightened orosensory responses of TT are driven by a subset of individuals. For example, it has been hypothesized that TT who experience sweetness during thermal stimulation will rate sweet chemical stimuli (e.g., sucrose) higher than other TT (Pickering

and Klodnicki, 2016; Pickering and Kvas, 2016). Bajec et al. (2012) tested this hypothesis and found no differences in responsiveness between TT subgroups. Specifically, individuals who experienced thermally-elicited sweetness, sourness and bitterness, were not more responsive to aqueous solutions of sweet, sour and bitter chemical stimuli, respectively. However, those results are likely underpowered due to very small sample size for their TT subgroups (n=5-9) and more research is required to determine whether this hypothesis is supported in a large sample. Further, establishing the extent to which TT are a homogenous group will inform best practices when classifying TT in future studies.

1.1.3 Gap #3

Up to half of individuals are non-classifiable after TTS screening and it is not known whether they represent a third phenotypic group or whether are misclassified TT and TnT.

A third group of individuals, non-classifiable participants (NC), are typically excluded from studies as they cannot readily be classified as TT or TnT (Bajec and Pickering, 2008, 2010; Pickering et al., 2010a, 2010b, 2016; Bajec et al., 2012; Bering et al., 2013; Yang et al., 2014; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016). NC report purported thermally-induced sensations during thermal elicitation; however, these sensations are rated at low intensity or are not reproducible (Bajec and Pickering, 2008; Yang et al., 2014). This limitation makes it difficult to determine whether the thermally-elicited sensations reported by NC are valid or whether they simply reflect response bias common in self-report research. Thus, it is not known whether NC represent a distinct phenotypical sub-group of TT, or whether they are simply TT or TnT that have been misclassified. Furthermore, the taste and chemesthetic responsiveness of this group is not known. As NC may represent up 50% of the population, the responsiveness of NC as a whole to aqueous tastants was characterized. NC were also divided into subgroups based on the intensity or reproducibility of taste responses reported during thermal elicitation. Comparing the responsiveness to aqueous solutions of tastants for each NC subgroup to that of TT and TnT, will allow us to better assess their place within the TTS classification scheme. Together, the results will help to inform best practices for TTS classification in future studies.

1.1.4 Gap #4

TTS screening methods and classification schemes were developed when the phenomenon was newly discovered. Thus, a retrospective interrogation of existing data can provide insights into potential strategies to optimize methods.

Thermal taste was first described by Cruz and Green (2000) who identified the temperature regimes and locations mostly likely to elicit thermally-induced sensations. As the knowledge of thermal taste grew, TnT (Green and George, 2004) and NC (Bajec and Pickering, 2008) were added to the classification scheme and some studies reduced the number of locations tested on the tongue from three to one (Yang et al., 2014; Hort et al., 2016). The changes reflected a desire to optimize the TTS protocols based on best practices and emerging knowledge of TTS. Nevertheless, the impact of the changes to the TTS screening method and the TTS classification schemes have yet to be described in the literature.

Four primary methods and classification criteria have been identified in the literature are compared in this report to investigate the concordance across studies (See Table 3.1; Green and George, 2004; Bajec and Pickering, 2008; Yang et al., 2014; Hort et al., 2016). The most commonly used approach to screening and classifying participants was developed by Bajec and Pickering (2008). Under this approach, participants are screened for TTS using 12 trials, which are performed in two blocks. Each block consists of three warming cycles (one per location: tongue tip, 1-cm to the left, 1-cm to the right) followed by three cooling cycles (one per location; Bajec and Pickering, 2008). TT are defined as participants who reported the same, valid thermally-elicited taste sensation above weak on the gLMS (> 6 mm), during both replicates of the same location during the same temperature regime. Participants have to meet these requirements for one or more of the six combinations of location and temperature regime (warm/tip, warm/left, warm/right, cool/tip, cool/left and cool/right). TnT are defined as participants who reported no taste-related orosensation during thermal elicitation and all other participants were defined as NC. Two key differences between this approach and the other approaches in literature (Green and George, 2004; Yang et al., 2014; Hort et al., 2016) are highlighted below.

During TTS screening, most studies tested participants in 3 locations whereas others tested only one location (tip; Yang et al., 2014; Hort et al., 2016). Although the evidence is

limited, thermally-induced sweetness is rated highest at the tongue tip whereas thermal sourness is highest approximately 1-cm to the left and right of the tongue tip on the anterior edge (Cruz and Green, 2000). Thus, it is possible that not testing the tongue 1-cm to the right or left of the tip, could lead to TT being erroneously identified as TnT, as it may not capture all individuals who experience thermally-elicited sourness. Similarly, if the intensity of thermally-elicited sourness is lower on the tongue tip, sour TT may be classified as NC as the responses may not meet the minimum intensity requirement for classification as a TT when only the tongue tip is tested.

As the mechanism(s) underlying TTS have yet to be elucidated, the list of sensations considered valid thermally-elicited tastes when classifying participants varies. Although all studies accept the basic tastes (sweet, sour, bitter, salty, umami) as valid, most studies also include metallic as it is reported by a large proportion of individuals during TTS screening (Yang et al., 2014; Skinner et al., 2018). Some studies also include minty and/or spicy as valid thermally-elicited tastes (Yang et al., 2014; Hort et al., 2016; Skinner et al., 2018), which may or may not be proxies for cold or warm temperature. The defintion of TT was further modified by Yang et al (2014), who defined TT as individuals that report any thermally-elicited sensation during at least one warming and one cooling trial. Thus, depending on the list of thermally-elicited sensations considered valid, the proportion of TT, NC and TnT identified may vary. Differences in TTS screening methodology and classification schemes raise concern about the validity of comparing results across studies. To allow for direct comparison between the schemes, the raw TTS responses for each participant in a large dataset were used to populate TTS groups by applying previously reported criteria (Table 3.1). By testing for concordance between the four schemes, it can be determined whether the classification of individuals varies across studies, with implications for the extent to which findings can be dependably compared across studies that have employed different methods.

1.1.5 Gap #5

The increased responsiveness of TT compared to TnT has primarily been studied in simple aqueous solutions. Examining responses in binary mixtures will provide more nuanced insights into differences between the phenotypes, including suppression and enhancement effects.

Although the differences in responsiveness between TT and TnT in aqueous solutions are of interest, such solutions are not representative of normal food and beverages, with the latter examined in a few studies. TT also rate the dominant orosensations elicited by beer (Pickering et al., 2010a) and wine (Pickering et al., 2010b) higher than TnT, providing evidence that group differences in orosensory responsiveness between TT and TnT also extend to alcoholic beverages. Recently, Small-Kelly and Pickering (2020) provided further evidence by comparing the responsiveness of TT and TnT to ethanol (2-10%), a component of all alcoholic beverages.

Although bitterness intensity was similar for TT and TnT at 2% and 4% ethanol, TT rated the bitterness of 5%, 7% and 10% ethanol solutions higher than did TnT. The irritation/burning and sweetness of ethanol increased for both TT and TnT as the concentration of ethanol increased, but no group differences were identified. As only concentrations of ethanol below 11% have been examined to date (Small-Kelly, 2020), possible differences between TT and TnT in the sweetness and/or bitterness of ethanol at higher concentrations are yet to be determined. Importantly, the differences in intensity and dominance of the sensations elicited by ethanol likely drive the broad differences in the sensory properties of beer, wine and spirits (Nolden and Hayes, 2015). To this end, the current thesis examines three concentrations of ethanol, each representative of a major alcoholic beverage category: namely 5% (beer), 13% (wine) and 23% (diluted spirits).

When consumers drink alcoholic beverages, they make quick judgements about the flavour. Nevertheless, flavour perception is a complex phenomenon that involves integrating multi-modal sensory inputs including, taste, olfactory and chemesthetic responses (reviewed in Spence, 2015). By systematically varying the concentration of two compounds in binary mixtures, the interactions between individual compounds (enhancement or suppression) can start to be characterized (Keast and Breslin, 2002; Wilkie and Capaldi Phillips, 2014). This approach allows for a better understanding of how changing the composition of complex products, in this case alcoholic beverages, impacts their perception. Research is required to understand if/how TTS impacts the nature (i.e., no interaction, suppression or enhancement) or intensity of interactions in alcoholic experienced by TT and TnT. Importantly, if the nature of the interactions differs between TT and TnT, this finding will provide evidence that flavour perception differs between the

groups in more than just magnitude. To this end, the final chapter uses the optimised TTS methods developed in earlier chapters to examine the interactions between ethanol and four stimuli, each representing sensations commonly elicited by alcoholic beverages: namely fructose (sweet), quinine (bitter), tartaric acid (sour) and alum sulphate (astringency). Together, the results will provide important insights into the perception of alcoholic beverages in general. In addition, to the best of our knowledge, taste and chemesthetic interactions have not been investigated in TT and TnT providing novel insights into differences between the TTS groups.

1.2 Thesis structure

The following section provides an overview of each chapter of the thesis. Each chapter of the thesis was written as a manuscript for publication. As a result, some information is duplicated across chapters. An overview of the thesis structure is provided in Figure 1.1.

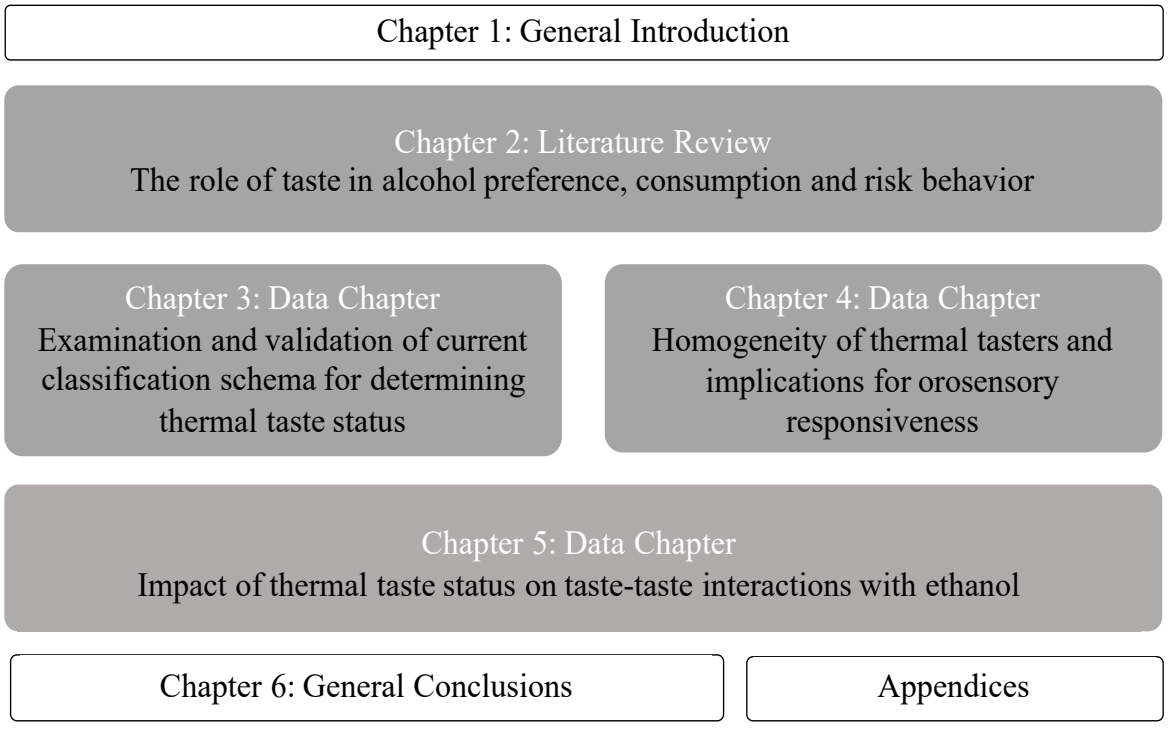

Figure 1.1: Overview of thesis structure and chapters.

1.2.1 Chapter 2

Chapter 2 is a comprehensive literature review of the role of taste in alcohol preference, consumption and risk behavior. After a brief introduction to taste perception, Chapter 2 outlines the sensations commonly elicited by ethanol and alcoholic beverages. Next, Chapter 2 summarizes the impact of individual differences in taste perception on alcohol related behaviour with a focus on three taste-related phenotypes (PTS, TTS and sweet-liking). Chapter 2 was published in full in *Critical Reviews in Food Science and Nutrition* (Thibodeau and Pickering, 2019; https://doi.org/10.1080/10408398.2017.1387759). The version of the manuscript include here has undergone minor updates to reflect newly available literature. This chapter is co-authored with Dr Gary Pickering.

1.2.2 Chapters 3 & 4

Chapter 3 and Chapter 4 are complementary as both investigate TTS classification schemes and methods in a large sample. Together, they provide a foundation for future TTS research as they provide insights into best practices for TTS screening.

Chapter 3 begins with a comparison of TT and TnT, to validate the findings reported in literature on smaller sample sizes. Next, Chapter 3 compares the concordance between TTS screening methods from different publications to determine if/how these results impact the proportion of TT, TnT and NC identified. Finally, Chapter 3 provides the first characterization of NC, in order to determine whether they are a unique phenotypic group or simply misclassified TT and TnT. Chapter 3 was published in full in *Chemosensory Perception* (Thibodeau et al., 2019; https://doi.org/10.1007/s12078-019-09264-w). This chapter is co-authored with Drs Gary Pickering, Anthony Saliba and Martha Bajec.

Chapter 4 examines whether TT subgroups exist and if/how their existence impacts orosensory responsiveness. Chapter 4 begins by characterizing the type of thermally elicited sensations experienced by TT during screening and the locations/temperature regimes where they are elicited. Furthermore, Chapter 4 tests whether any of these factors are associated and can provide insights into potential mechanisms underlying TTS. Next, TT are divided into subgroups based on the above criteria and tested for differences in orosensory responsiveness. Finally, Chapter 4 seeks to determine how training and scale availability impacts the types of orosensations reported during thermal elicitation. Chapter

4 was published in full in *Physiology & Behavior* (Thibodeau et al., 2020a; https://doi.org/10.1016/j.physbeh.2020.113160). Raw data, supplementary figures, and TT naming conventions from Chapter 4 were also published in *Data in Brief* and a full copy is included in Appendix I (Thibodeau et al., 2020b; https://doi.org/10.1016/j.dib.2020.106325). Both this chapter and Appendix I were co-authored with Drs Gary Pickering, Anthony Saliba and Martha Bajec.

1.2.3 Chapter 5

Chapter 5 investigates the perception of ethanol and four stimuli that represent commonly elicited sensations by alcoholic beverages: fructose (sweet), quinine (bitter), tartaric acid (sour) and alum sulphate (astringent). First, the perception of three concentrations of each stimuli is characterized and the responsiveness of TT and TnT is compared. Next, using a full factorial design, binary mixtures of ethanol and the other four stimuli are investigated to determine how TTS and stimulus concentration impact the perception of both dominant and non-dominant sensations. Where reliable dose-response functions were found, the Isobole method was used to test for interactions between ethanol and the alcohol-relevant stimuli. To better understand the perception of alcoholic beverages in general, the impact of ethanol and alcohol relevant stimuli concentrations on the orosensations elicited by the binary mixtures is discussed and compared to findings in model and alcoholic beverages. The impact of TTS is also discussed. Chapter 5 was accepted for publication in *Beverages* (Thibodeau and Pickering, 2021; https://doi.org/10.3390/beverages7020023). This chapter was co-authored with Dr Gary Pickering.

1.2.4 Chapter 6

The aim of Chapter 6 is to integrate and contextualize the findings from earlier chapters in the thesis and identify new literature gaps. To this end, Chapter 6 is divided into two parts. First, using the literature gaps identified in Chapter 1, the key findings from the thesis are summarized. Second, new research gaps are identified based on current literature and the findings from this thesis, and implications for practitioners and/or researchers in the field are highlighted.

Please note: Although the co-authors contributed to the published chapters, M.T. was ultimately responsible for developing research questions, designing the studies, executing the work and drafting the manuscripts.

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Chapter 2: The Role of Taste in Alcohol Preference, Consumption and Risk Behavior

2.1 Introduction

Alcohol consumption is often associated with negative health and personal consequences. For example, college students report missed classes, hangovers, and feelings of regret as a result of alcohol consumption (Park and Grant 2005). Physical consequences of high-risk drinking include liver damage, numbness, ulcers, reduced balance, vitamin deficiency/malnutrition, heart failure, memory loss, and the development of various cancers (Barbor et al., 2001). However, it is also positively associated with some measures of well-being, such as increased levels of relaxation, creativity, enjoyment of a meal and greater ability to express oneself (Park and Grant 2005). It has been argued that research in this area should only concern itself with the negative health consequences of alcohol; however, this ignores the equivocal nature of the literature on the healthiness of moderate consumption. Noteworthy, when individuals perceive wine as healthy, they do not consume more than individuals that consider wine unhealthy, and may in fact follow healthier consumption patterns (Saliba and Moran, 2010). A full discussion of the harm and benefit of alcohol is beyond the scope of this review, and the reader is referred to Walzem et al. (2008) for more information. Understanding the factors that affect alcohol intake is important for informing disease prevention and management interventions and policy. Conversely, the alcoholic beverage industry is interested in better understanding the drivers of alcohol preference and liking in non-clinical populations, as it can assist with market segmentation, and create opportunities for product development and optimisation.

Alcohol consumption is influenced by a diverse set of factors, including genetics, alcohol reactivity, social expectations and sanctions, gender, coping style, expectancy of alcohol related consequences, depression, self-esteem, sensation seeking, interpersonal relationships, and history of trauma (Nolen-Hoeksema, 2004). Taste also plays a role in mediating alcohol behaviour. For instance, in a study of 517 undergraduate students, alcoholic beverages represented a significant proportion of the food/beverage items to which participants reported taste aversions (the rejection of a substance due to

unpleasantness or illness; Logue et al., 1981). More recently, in a study of Japanese wine consumers, taste was rated as the most important factor influencing wine purchase decisions, behind (in descending order of importance), style, color, price, friend/family recommendation, variety of choice, back label info, wine magazine/critic recommendation, country of origin, sale item, vintage, front label design, brand, closures and alcohol content (Bruwer and Buller, 2012). Readers are referred to Betanchur et al. (2020) for a comprehensive review of factors that impact beer choice. While numerous, complex and interacting factors influence alcohol consumption and risk behaviour, the focus of this paper is on the role of taste.

2.1.1 Taste, Chemesthesis, Somesthesis and Orosensation

Formally, taste is the oral sensation produced when food or beverages are consumed, eliciting a response from chemoreceptors within the oral cavity. These sensations - referred to as the prototypical tastes - are sweet, sour, bitter, salty and umami (Bachmanov and Beauchamp, 2007). Two classes of prototypical tastes are recognized: the ion channel tastes (sour and salty) and g protein-coupled receptor (GPCR) tastes (sweet, bitter, and umami; Bachmanov and Beauchamp, 2007). Sourness and saltiness result from the depolarization of ions channels. Saltiness is elicited when amiloride-sensitive epithelial Na^+ channels (ENaC) are depolarized by Na^+, whereas sourness is elicited when acid-sensing ion channels (ASIC) are depolarized by free protons. Sweet, bitter, or umami are elicited when sapid compounds hydrogen bond to GPCRs on taste buds (Talavera et al., 2005). Taste 1 Receptor (TAS1R) genes encode for sweet and/or umami sensitive proteins, while Taste 2 Receptor (TAS2R) genes encode for proteins involved in bitterness transduction (Bachmanov and Beauchamp, 2007). In addition to the prototypical tastes, chemesthetic and somatosensory sensations are primarily elicited by stimulation of the trigeminal system, typically when transient receptor potential channels (TRP) are activated (Kolindorfer et al., 2015). Often these are also referred to as tactile sensations, and encompass percepts such as astringency, touch, heat, prickling, and burning. Collectively, prototypical taste, chemesthetic and somatosensory sensations are called orosensations.

The other key sensory modality involved in eating and drinking is retro-nasal olfaction, initiated by volatile compounds traveling from the mouth to olfactory receptor cells in the nasal cavity (Jackson, 2009). As orosensations and aroma are experienced

simultaneously during eating and drinking, they are often colloquially referred to simply as 'taste'. However, in this review the term 'flavour' will be used to describe the combined experience from orosensory and retro-nasal inputs, and 'taste' to indicate general orosensation.

2.1.2 Differences in Orosensory Perception

The perception of orosensations differs widely across individuals and varies with several biological and behavioral factors. These include gender (Wardwell et al., 2009), age (Fischer et al., 2013), and smoking status (Pepino and Mennella, 2007; Fischer et al., 2013), factors which may also directly associate with alcohol preference (Bajec, 2010), consumption (Duffy et al., 2004b; Pepino and Mennella, 2007; Bajec, 2010) and dependence (Kampov-Polevoy et al., 2004; Pepino and Mennella, 2007; Wronski et al., 2007). Orosensation is partially under genetic control, and inter-individual differences can also be understood by grouping people according to their taste genotype and phenotype (e.g., Allen et al., 2014; Bering et al., 2013; Garcia-Bailo at al., 2009; Keskitalo et al., 2007; Talavera et al., 2005; Tepper, 2008). Fox (1931) first reported that phenylthiocarbamide was tasteless to some individuals while eliciting a strong bitter sensation for others. This discovery sparked the development of research into taste phenotypes in an attempt to explain the striking differences observed between individuals (Wooding, 2006).

Phenotypes are traits under some genetic control such as eye colour or height, which can be observed and used to classify organisms into groups. For example, individuals can be divided by demographic characteristics (age or gender), by similar behaviors (smokers vs nonsmokers) or by similar responsiveness to tastants (taste phenotypes). Three important taste phenotypes from the literature that have been linked to alcohol behaviour are 6-n-propylthiouracil (PROP) taster status (PTS), sweet-liking, and thermal taster status (TTS). However, each phenotype is likely accounted for by different mechanisms. For instance, there is no (Bering et al., 2013; Pickering et al., 2010b) or very limited (Yang et al., 2014) association between PTS and TTS, and no association between PTS and sweet-liking has been found (Drewnowski et al., 1997).

Orosensations play a key role in food and beverage preferences and consumption. For example, in a 1998 nationwide study, 2967 American adults rated the importance of nutrition, taste, cost, convenience, and weight control in personal dietary choice (Glanz et

al., 1998). Taste followed by cost were the two most important factors in food choice reported in the study. Genetic variation in orosensory receptors contributes to differences in the perception of oral sensations across individuals (Figure 2.1). This in turn, influences the development of food preferences, food intake, and health related outcomes (reviewed in Garcia-Bailo et al., 2009; Hayes et al., 2013; Tepper, 2008). One way in which taste phenotypes might link with alcohol behaviour is through increased orosensory responsiveness in some phenotypic groups. For instance, higher perceived bitterness from ethanol might lead to an increase in its unpleasantness, and thus a decrease in total intake of alcoholic beverages (Tepper, 2008).

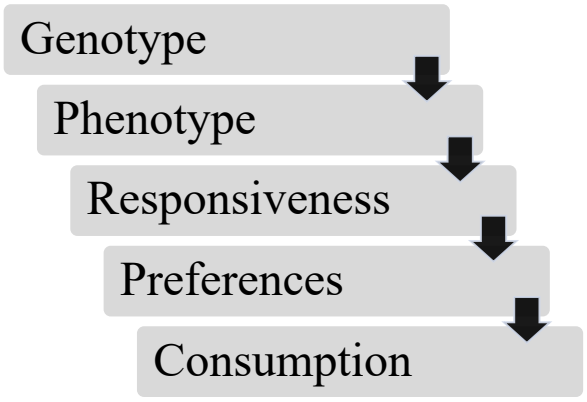

Figure 2.1: Model for the influence of taste genotype and phenotype on food related behaviour.

In this paper, the role of orosensation in alcohol consumption, preferences and the risk of an alcohol use disorder/alcoholism is critically reviewed. The paper begins with a description of the sensory characteristics associated with ethanol and their impact on alcohol behaviour. Subsequent sections describe the impact of individual differences in orosensory perception in general, and as operationalized by taste phenotypes. Genotypic differences that influence taste perception are also discussed and, where applicable, gender and age are considered. Next, the influence of orosensory responsiveness and taste phenotypes on alcohol related behaviour is examined. The paper concludes by arguing for the inclusion of orosensory responsiveness and/or taste phenotype data in future studies to build more comprehensive models of alcohol consumption and risk behaviors.

2.1.3 Methodological Challenges

A significant challenge in reviewing and summarising the pertinent literature is assessing the significance of the methodological differences in data collection approaches and measures used across studies, as best practices change and research foci can vary across groups. For example, alcohol use disorder risk can be assessed using several instruments, with for instance, six different screening tools suggested by the Australian government (Haber et al., 2009). Not only do these instruments differ in length and scale type, researchers must interpret the scores and choose appropriate cut-off points to define individuals with and without an alcohol use disorder. As a result, differences in the definition or criteria used in classification are common. Similarly, differences in the criteria used for classifying orosensory responsiveness and taste phenotypes are common, as are the measures of alcohol categories and intake. Among other factors, it is important to consider the choice of scales, standards/concentrations used for prototypical tastes, and co-variables examined in each study in assessing its contribution to the central thesis of this review. Importantly, when similar results are reported in studies that have used different methodological approaches, a degree of confidence can be assigned with respect to the robustness of the findings.

Most studies reviewed here use a relatively small sample size (n=30-200), as recruitment of participants can be challenging and the time required to administer tests limiting. A common theme in the discussion of null findings or those that only approach significance is that the limited sample size lacks the statistical power to establish significance, particularly when multiple variables are being evaluated simultaneously (e.g. Zhao et al., 2003). As a result, population based studies such as the Beaver Dam Offspring Study (n= 1500-2400; Cruickshanks et al., 2009; Fischer et al., 2014, 2013), the National Health and Nutrition Examination Survey (n = 4000, NHANES, Ng et al., 2019), the Italian Taste Project (n = 1225; Monteleone et al., 2017) or a recent report on over 1000 US wine consumers (Pickering et al., 2014) are valuable in confirming or refuting trends observed in smaller, more traditional lab-based studies. However, the methods used in population-based studies may be overly simplified to allow for large-scale testing outside the laboratory, and lack precision. For example, filter paper disks impregnated with tastants are commonly used to elicit orosensations rather than aqueous solutions, as they are easily

transported and can be prepared well in advance (e.g. Cruickshanks et al., 2009; Hayes and Pickering, 2012). However, the orosensations elicited by paper disks are typically localized to the area where the disk is applied, while aqueous solutions coat the entire mouth with sip and spit protocols. Similarly, some orosensory evaluation protocols involve self-administration of the stimuli by participants (e.g., Pickering et al., 2013), potentially introducing extra variability in the responses. Consequently, methodological differences should be considered when population- and laboratory-based studies report different findings.

The inclusion criteria used to select participants for a study may limit the generalizability of the results. For example, the evidence for a link between sweet-liking and alcoholism has been largely limited to male clinical populations. As a result, it is unclear if these results can be generalized to non-clinical populations or to females. Similarly, most research on thermal taste has been limited to convenience samples of college students, despite the fact that this cohort consume more alcohol and binge drink more frequently that their same-age peers who do not attend college, and typically reduce their alcohol consumption after graduation (Merrill and Carey, 2016). Thus, the drinking patterns of college student may not reflect lifetime drinking behaviour, and studies using college students may not be generalizable to other age cohorts.

2.2 Ethanol

2.2.1 Orosensory Characteristics

Ethanol (ethyl alcohol) is present in all alcoholic beverages and may play a key role in their sensory perception. Ethanol detection thresholds in aqueous solution are reported as between 0.87 to 1.43% v/v (Mattes and DiMeglio, 2001; Nolden et al., 2016), and are higher for males than females (Mattes and DiMeglio, 2001). Importantly, this range of threshold values is lower than the ethanol concentration found in most alcoholic beverages, confirming that ethanol likely contributes to the flavour of these products (Table 2.1). In aqueous solution it elicits sweetness, bitterness (Scinska et al., 2000; Mattes and DiMeglio, 2001; Allen et al., 2014; Nolden and Hayes, 2015; Nolden et al., 2016), sourness (Scinska et al., 2000; Mattes and DiMeglio, 2001), and saltiness (Mattes and DiMeglio, 2001). At and just above detection threshold in aqueous ethanol solutions, ethanol elicits bitterness

Table 2.1: Major alcoholic beverage categories and subtypes with typical ethanol concentrations, dominant tastants[1] and orosensations elicited.

Category and Ethanol Concentration (% v/v)	Major Subtypes	Dominant Tastant	Orosensations Elicited by Tastant	Literature Source
Beer (Typically: 3-7%, up to 16%)	All	Ethanol	Bitterness (Likely dominant), Sweetness, Burning/Tingling, Drying	Hardwick, et. al, 1995; Harwick, 1995; Parker, 2012
		Carbon Dioxide	Tingling, Prickling	
		Glycoproteins, Dextrin chains, Polypeptides & Gums	Fullness/Viscosity (By modifying the foam stability)	
		Polyphenols	Astringency, Bitterness	
		Organic Acids	Sourness	
		Sugars	Sweetness	
		Sulphates	Dryness	
		Chlorides	Fullness, Body	
	Hopped Styles	Hop Resins	Bitterness	
Wine (Typically: 11-13%, Full range 7.5%-20%; Fortified wines: 18-21%)	All	Ethanol	Bitterness (Likely dominant at lower concentrations), Burning/Tingling (Likely dominant at higher concentrations), Drying, Sweetness	Jackson, 2009; Jackson, 2012; Tredoux and Silva, 2012; Sowalsky and Noble, 1998.
		Organic Acids	Sourness, Astringency	
		Sugars	Sweetness, Viscosity	
		Glycerol	Sweetness (Minor contribution in dry wine)	
	White	Nonflavonoids (Tannins)	Bitterness, Astringency	
	Red	Flavonoids (Tannins)	Bitterness, Astringency	
	Sparkling	Carbon Dioxide	Tingling/Prickling	
	Desert	Glycerol	Viscosity	

[1] Tastant defined here as orosensory stimuli for ease of presentation

Table 2.1 (continued): Major alcoholic beverage categories and subtypes with typical ethanol concentrations, dominant tastants[1] and orosensations elicited.

Category and Ethanol Concentration (% v/v)	Major Subtypes	Dominant Tastant	Orosensations Elicited by Tastant	Literature Source
Spirits (Typically: 35%-45%, Full range: 30-86%)	All	Ethanol	Burning/Tingling (Likely dominant), Bitterness, Drying, Sweetness (Minor contribution from ethanol; typically major contribution from mixes/additives to unmixed spirits)	Aumatell, 2012; Bordeu, et al., 2012; Da Porto, 2012; Faria, 2012; Jack, 2012; Louw and Lambrechts, 2012; Lurton, et al., 2012; Villanueva-Rodriguez and Escalona-Buendia, 2012; Xu and Ji, 2012; Zabetakis, 2012
Other Sake: ~15%	Sake	Organic Acids	Sourness	Furrakawa, 2012; Hardwick et al., 1995
		Amino Acids, Peptides	Umami, Savoury	

[1] Tastant defined here as orosensory stimuli for ease of presentation

more strongly than other basic tastes (Mattes and DiMeglio, 2001). Recently, Nolden & Hayes (2015) have demonstrated that the dominant orosensation elicited by ethanol in aqueous solution changes with concentration. Bitterness dominates at 4% and 16% v/v ethanol, whereas burning/tingling is the dominant sensation at concentrations of 32% and 48% v/v. Mean pleasantness values for 0.3% to 10% (v/v) ethanol concentrations were negative on a line scale from -50 to 50 where 0 is assumed to represent a neutral pleasantness score (Scinska et al., 2000). Furthermore, orosensory intensity increased and pleasantness decreased with increasing ethanol concentration (Scinska et al., 2000), perhaps due to greater stinging, tingling, irritation and burning sensations (Green 1987, 1988; Allen et al., 2014; Nolden and Hayes, 2015; Nolden et al., 2016). Therefore, in

aqueous solution, ethanol is likely aversive regardless of the concentration, a finding with implications for alcoholic beverages discussed later.

Repeated exposure to ethanol can cause desensitization in the short term. Prescott & Swain-Campbell (2000) showed that when participants rate the intensity of ten sequential 47.5% ethanol solutions spaced at one-minute intervals, responsiveness decreases in latter samples. However, intensity ratings recover to near initial levels when an eleventh sample is tasted after a 10-minute break. If similar experiences occur with alcoholic beverages, this finding might predict that the aversive character of ethanol reduces across a drinking session, at least for some consumption patterns. Additionally, this suggests that the number of drinks consumed during a drinking occasion should be captured in studies examining inter-individual differences in orosensation, in addition to the traditional and simpler metric of total intake. However, the ecological validity and limits of the Prescott & Swain-Campbell (2000) finding remain to be determined. For example, taking several shots of spirits or slowly sipping a wine may not produce equal (or any) desensitization. Indeed, considerably more study is still needed to understand to what extent responses to ethanol aqueous solutions translates into perception of alcoholic beverages. The latter are much more complex, and this can affect orosensory ratings (Zamora et al., 2006). Responsiveness to ethanol aqueous solutions may not be a useful proxy for predicting responsiveness to more complex matrices; this may be especially true for ethanol consumed in solid or gel matrices, such as alcoholic ice cream, chocolates or gelatin desserts (e.g. Jell-o® shots).

2.2.2 Ethanol Perception and Alcohol Consumption

Only a limited number of studies have investigated this suggested association between ethanol responsiveness and alcoholic beverage behaviour. Nolden and Hayes (2015) recently reported that individual variation in the intensity of suprathreshold ethanol associates with the number of drinking occasions of beer, wine, straight spirits, and all alcoholic beverages when grouped together. In general, individuals who consume alcohol less frequently perceive greater bitterness and burning/tingling from ethanol, likely increasing its aversive character (Nolden and Hayes, 2015). The association between ethanol detection thresholds and alcohol consumption in some studies further supports this limited evidence that ethanol responsiveness and/or sensitivity mediates the number of drinking occasions. Moderate to heavy drinkers have a higher ethanol detection threshold

than individuals who abstain from alcohol or are light drinkers (Mattes, 1994). In contrast, no significant difference in ethanol detection thresholds was found when only beer consumption was examined (Mattes & DiMeglio, 2001). However, as these participants reported consuming other types of alcohol, beer consumption may not reflect trends in overall drinking.

Single nucleotide polymorphisms (SNPs) in three genes - TRPV1, TAS2R13, and TAS2R38 – have been shown to associate with differences in ethanol perception (Allen et al., 2014). These authors suggest that this variation may be predictive of alcohol consumption, as TRPV1 is a nocioreceptor associated with the perception of burning, and TAS2R13 and TAS2R38 are bitter taste receptors. Recently, Nolden et al. (2016) confirmed the link between the suprathreshold bitterness of ethanol and TAS2R38 genotypes; PAV/PAV individuals rate the bitterness of ethanol as significantly higher than AVI/AVI homozygotes or PAV/AVI heterozygotes. Dotson et al. (2012) reported that alcohol consumption varied by TAS2R13 and TAS2R38 genotype in patients with head and neck cancer. However, as head and neck cancer patients are typically undergoing radiation treatment and this is associated with taste abnormalities (Dotson et al., 2012), caution should be applied in generalising these results to healthy individuals. However, higher total alcohol consumption has been noted in healthy individuals with AVI/AVI genotypes compared to individuals with PAV/AVI and PAV/PAV genotypes (Duffy et al., 2004a; Hayes et al., 2011). With respect to alcohol use disorders, Wang et al., (2007) found no association between the maximum number of drinks consumed in a 24-hour period and TAS2R38 genotypes for Americans of European ancestry in a large study of families with a history of alcoholism. However, in African American females (n=105) but not males (n=114), the PA_ (PAV or PAI) haplotypes associated with a reduced number of drinks consumed in a 24-hour period compared to AA_ (AAV or AAI) or AVI haplotypes, although this finding did not extend to increased alcohol dependence.

Ethanol is not the only compound important in orosensation elicited by alcoholic beverages (Table 2.1). Sugars, organic acids, phenolics, ions, and carbon dioxide all contribute to the individual character of different alcoholic beverage categories (Piggott, 2012). The concentration and balance between these compounds are frequently manipulated during production to optimize flavour and define different beverage styles

(Blackman et al., 2010). For example, red and white wine differ in orosensory characteristics primarily due to the length of time the juice/must is in contact with the grape skins; the extra phenolics extracted from skins in red wine confer greater astringency and bitterness and help to differentiate the products (Brossaud et al., 2001; Yoo et al., 2012). Similarly, hard spirits consumed in the presence or absence of a mixer will differ in orosensory attributes, as the presence of a mix alters the characteristics of the final drink, including moderating the perception of ethanol (Lachemeir et al., 2014). Thus, the orosensory properties of specific alcoholic beverages may differ based on both ethanol concentration and other compositional differences.

2.3 Orosensory Responsiveness

2.3.1 Quinine Bitterness

Differences in suprathreshold responsiveness to prototypical tastants and irritants may partially predict alcohol consumption and preferences. This section begins by considering the link between these behaviors and perception of quinine, a bitterant that has been used extensively in psychophysical research on taste, both in its sulphate and hyrdochloride salt forms. As bitterness is generally perceived as unpleasant (Bredie et al., 2014), it has been hypothesized that increased bitter responsiveness associates with lower alcohol consumption. When quinine responsiveness was modelled in a large Mendelian randomization study (n = 438,870), increased quinine responsiveness was associated with a non-significant trend of increased frequency of overall alcohol consumption (Ong et al., 2018). However, the quinine responsiveness was not associated with the odds of being a heavy drinker (3+ occasions weekly; Ong et al., 2018). No difference in the real-world responsiveness to quinine and overall alcohol consumption was found by Fischer et al. (2013, 2014) in their large study of over 2,300 participants from the Beaver Dam Offspring Study. Ng et al. (2019) also failed to find any associations between overall alcohol consumption and quinine responsiveness using data from the NHANES study (n = 4990). However, these result fail to consider differences between alcoholic beverage types. For instance, intake of unmixed spirits might be expected to vary more with quinine responsiveness than other beverage categories, as they most closely resemble pure ethanol (Wisniewska et al., 2015), and increased responsiveness to ethanol has been previously

shown to associate with alcohol consumption. Surprisingly, however, consumption of unmixed spirits was not associated with suprathreshold differences in quinine bitterness in the recent report of Thibodeau, Bajec, and Pickering (2016). However, the bitterness of different compounds is typically associated only if they bind to the same TAS2R bitter receptor (Roura et al., 2015). Most previous studies have relied on quinine as a general proxy for bitterness, which elicits a response from 9 TAS2Rs (TAS2R4, TAS2R7, TAS2R10, TAS2R14, TAS2R39, TAS2R40, TAS2R43, TAS2R44, and TAS2R46; Meyerhof et al., 2010), whereas ethanol to date has only been shown to excite 2 bitter receptors; TAS2R38 and TAS2R13 (Allen et al., 2014). Thus, as ethanol and quinine do not appear to activate the same bitter taste receptors, it is possible that the expected effects on consumption from ethanol bitterness aversion are not fully captured when quinine is used, which may account for the null result for unmixed spirits intake in Thibodeau et al. (2017). These results suggest that when examining the relationship between orosensory responsiveness and alcohol response, preference or consumption, quinine is not an effective proxy for ethanol bitterness. Instead, an individual's response to or liking for ethanol should be measured directly or with a proxy that elicits the same TAS2R.

In contrast to unmixed spirits, quinine bitterness was associated with the monthly consumption of all alcoholic beverages (wine, beer, spirits and other combined) and all spirits combined (a combination of both mixed and unmixed spirits) in Thibodeau et al. (2017), with similar trends observed for beer intake and frequency. The pattern of results was non-linear, with individuals of intermediate quinine responsiveness consuming more alcohol than individuals with high or low responsiveness. Increased responsiveness to quinine bitterness was associated with an increased consumption of pale ale but was not associated with the consumption of lager (Higgins and Hayes, 2019; Higgins et al., 2020). It is possible that quinine responsiveness may be serving as a partial proxy for non-ethanol bitterants in alcoholic beverages that are present at optimal levels for the 'average' palate, particularly iso-α-acids (beer) and the phenolics malvidin-3-glucoside and (-) epicatechin (wine), all of which bind to TAS2Rs that overlap with quinine (Intelmann et al., 2009; Soares et al., 2013).

The impact of quinine bitterness on the liking of alcoholic beverages is not well described. In a study on sample beer, quinine bitterness was not associated with differences

in liking for the three styles tasted (imperial ale, session ale, lager; Higgins et al., 2020). More research is needed to determine if alcohol liking varies with quinine bitterness for other beverage styles.

Further evidence that intake may be associated with differences in the perception of compounds in alcoholic beverages other than ethanol comes from the study of Tanimura and Mattes (1993). These authors demonstrated that the detection threshold for iso-alpha-acids is approximately 4 times higher for heavy drinkers (> 8 beers weekly) than for slight consumers (<2 beers weekly), while that of moderate drinkers (3-7 beers weekly) was intermediate to but not statistically differentiated from the other two groups. However, sample sizes were very small (n=19, 5-8 per group), and thus some caution should be applied. It is possible that higher levels of beer intake lead to a greater tolerance for the bitterness of iso-alpha-acids, which in turn may facilitate a further increase in beer consumption. However, Higgins and Hayes (2019) reported that tetralone intensity (a hop extract) and pale ale consumption were positively correlated for pale ale consumers, but no correlation was reported for non-consumers of pale ales. This suggests that contrary to the earlier speculation, for a subset of alcohol consumers the bitterness elicited by hops may be desirable.

There is some, limited, evidence that quinine bitterness may also influence the risk of developing an alcohol use disorder. In-patient individuals undergoing treatment for alcoholism had higher quinine taste thresholds than a control group of non-alcoholics (Smith, 1972). However, there are several shortcomings in the information provided in the paper that make it difficult to fully evaluate the claims; specifically, what type of threshold was tested and what diagnostic criteria were used for alcoholism. A limited examination of individuals who abstain from all alcohol was conducted by Thibodeau et al. (2017), and they showed a tendency toward higher responsiveness than alcohol consumers to quinine bitterness, in addition to sweet, sour, and salty stimuli, perhaps suggesting that broadly-tuned orosensory responsiveness may be protective against alcohol use and misuse. Significantly more research is needed to expand on these initial findings and speculations.

2.3.2 PROP Bitterness

2.3.2.1 Classification & Orosensory Advantage

PTS (PROP taster status) measures an individual's responsiveness to the bitter compound, 6-n-propylthiouracil (PROP). As such, individuals are typically classified into three phenotypic groups; PROP non-tasters (pNTs) for whom PROP elicits little or no sensation, PROP medium-tasters (pMTs) for whom PROP elicits a mildly bitter sensation, and PROP super-tasters (pSTs) for whom PROP elicits a highly bitter sensation (Bartoshuk et al., 1994, 1999). The majority of studies examine the role of PROP responsiveness in perception and behavior by parsing individuals into one of these three groups, although several treat PROP responsiveness as a continuous variable; an approach that can be especially useful with modeling or correlation analysis (Lanier et al., 2005). The methods used to classify individuals into PTS groups have varied over the years and may contribute to some of the contrasting results on alcohol. Importantly, the early use of threshold methods to determine PTS has largely been replaced by suprathreshold methods as the later allows for the separation of pMTs and pSTs (Bartoshuk et al., 1994; Hayes and Keast, 2011; Tepper, 2008). A full discussion of the classification systems is beyond the scope of this review, and the reader is referred to Tepper (2008) for an in-depth consideration of the topic.

PROP bitterness is positively correlated with suprathreshold sweetness, bitterness (Bartoshuk et al., 1994; Bajec and Pickering, 2008; Fischer et al., 2014), saltiness, sourness, (Bajec and Pickering, 2008; Fischer et al., 2014), astringency, and metallic intensity (Bajec and Pickering, 2008) in aqueous solutions. As a result, it has been suggested that PTS may be a useful proxy for general orosensory responsiveness, should the findings in aqueous solutions extend to food and beverages (Bajec and Pickering, 2008). Several studies have established such an association between PROP responsiveness and perception of orosensations elicited by sampled foods and non-alcoholic beverages (Akella et al., 1997; Lanier et al., 2005; Bell and Tepper, 2006); below the extent to which this extends to ethanol and alcoholic beverages is reviewed, and the findings are summarized in Tables 2.2, 2.3 and 2.4.

Table 2.2: Summary of PROP/PTC-related studies on responsiveness to ethanol or sampled alcoholic beverages.

Author, (Date)	Stimulus (Concentration)	Method of PROP Operationalization	Participants (n)	Primary Findings
Bartoshuk et al., (1993)	Aqueous Solution (0.001 & 0.0032M)	Threshold & scaling (pNTs vs pMTs vs pSTs)	Not specified	pMTs & pSTs rated the bitterness of 10 – 50% ethanol higher than pNTs.
Prescott and Swain-Campbell, (2000)	Paper disk dipped in 0.3g/L PROP	Suprathreshold (pNTs vs pMTs vs pSTs)	College students/staff (61)	PROP tasters rated the intensity of ethanol higher than pNTs.
Mattes and DiMeglio, (2001)	Paper disk dipped in saturated PTC solution	Suprathreshold (PTC taster vs PTC non-taster)	Adults (50)	Familial history of alcoholism & responsiveness to 4.3 to 17% ethanol does not differ between PTC tasters & non-tasters.
Duffy at al., (2004)	Aqueous solutions (0.001 to 3.2 mM)	Threshold & scaling (pNTs vs pMTs vs pSTs)	Adults (83)	Increased PROP bitterness is associated with increased intensity from sampled ethanol & lower alcohol consumption.
Pickering et al., (2004)	Aqueous solution (0.32 mM)	Suprathreshold (pNTs vs pMTs vs pSTs)	College students/staff (25)	pNTs rated the bitterness, acidity & astringency of red wines lower than pMTs & pSTs.
Pickering and Robert, (2006)	Aqueous solution (Duplicate; 0.32 mM)	Suprathreshold (pNTs vs pSTs)	College students/staff (17)	pNTs rated the acidity, saltiness, heat/irritation, & astringency lower than pSTs in sampled red wines.
Pickering et al., (2010b)	Aqueous solution (Duplicate; 3.2 mM)	Suprathreshold (pNTs vs pMTs vs pSTs)	College students/staff (56)	No difference in orosensory responsiveness between PTS groups in sampled wines.
Carrai et al., (2017)	Taste disks (6 ranging from 0.1 to 10 mM)	Threshold (Staircase … method)	College students/staff or blood donors (528)	PROP sensitivity inversely associated with bitterness of a sampled red wine but no association for astringency or sourness.

Table 2.3: Summary of PROP/PTC-related studies on alcohol consumption and liking. The table is continued on the next page.

Author (Date)	Stimulus (Concentration)	Method of PROP Operationalization	Participants (n)	Primary Findings
Intranuovo and Powers, (1998)	Paper disk (Concentration not specified)	Suprathreshold (pNTs vs pMTs vs pSTs)	Adults (100)	pNTs consumed the most beer in their first year of drinking. pSTs reported higher bitterness lower liking from sampled beer.
Ullrich et al., (2004)	Aqueous solution (0.032, 0.32, 3.2 mM)	Threshold scaling (PROP tasters vs PROP non-tasters)	Adults (219)	Food adventurousness mediates alcohol liking in PROP tasters but not in pNTs.
Lanier et al., (2005)	Aqueous solution (3.2 mM)	Suprathreshold responsiveness (Continuous)	Adults (49)	PROP bitterness associated with the bitterness sweetness of scotch beer, which mediates alcohol consumption.
Pickering and Cullen, (2008)	Paper disk dipped in 50mM PROP	Suprathreshold (pNTs vs pMTs vs pSTs)	Alcohol Consumers (406)	pSTs like sparkling wine less than pNTs/ pMTs.
Bajec, (2010)	Aqueous solution (Duplicate; 0.32 mM)	Suprathreshold (pNTs vs pMTs vs pSTs)	College students/staff (132)	pMTs liked alcoholic beverages more than pNTs /pSTs. No differences in alcohol consumption across PTS groups.
Catanzaro et al., (2013)	Paper disk dipped in saturated PROP solution	Suprathreshold (pNTs vs pMTs vs pSTs)	College students (139)	PTS was not associated with difference in beer or red wine preferences.
Fischer et al., (2014)	Paper disk dipper in 1.0 M PROP	Suprathreshold responsiveness (Continuous)	Beaverdam Offspring Study Adults (2359)	PROP bitterness not associated with having consumed "any alcohol in the past year" or having "ever drank 4+ drinks/day".
Pickering et al., (2014)	Paper disk dipped in 50mM PROP	Suprathreshold (pNTs vs pMTs vs pSTs)	Wine Consumers (1101)	Increased PROP responsiveness associated with lower alcohol consumption, increased liking of sweet wine higher dislike of dry wine.

Table 2.3 (continued): Summary of PROP/PTC-related studies on alcohol consumption and liking.

Author (Date)	Stimulus (Concentration)	Method of PROP Operationalization	Participants (n)	Primary Findings
Pickering and Hayes, (2017)	Paper disk dipped in 50mM PROP	Suprathreshold (hypo-tasters vs hyper-tasters)	Alcohol Consumers (329)	PROP hyper-tasters liked sweet, dry fortified wine styles disliked red wine styles more than PROP hypo-tasters.
Beckett et al., (2017)	Aqueous solutions (6 ranging from 0.000017 to 0.0032 M) PROP	Threshold (PROP tasters vs PROP non-tasters)	Alcohol Consumers undergoing colonoscopy (180)	PROP tasters consumed less alcohol than PROP non-tasters.
Yang et al., (2018)	Aqueous solution (3.2 mM) PROP	Suprathreshold (pNTs vs pMTs vs pSTs)	Beer consumers (60)	pSTs and pMTs liked the sampled beers more than pNTs. pNTs were less content and excited but more bored that pMTs/pSTs.
Ong et al., (2018)	Aqueous solution (Duplicate;0.6 mM) PROP & Paper disk saturated (0.059 M) PROP	Suprathreshold responsiveness (continuous) in a Mendelian randomization study	To model PROP intensity by genotype: Brisbane Adolescent Twin Study participants (1757) To test for differences in alcohol consumption based on the modeled intensity: UK Biobank participants of white-British ancestry (438,870)	Higher modelled PROP responsiveness scores were associated with a decrease in the frequency of alcohol consumption. Heavy drinkers (3-4 events per week) tended to have lower modelled PROP responsiveness than non-heavy drinkers, but the trend was not statistically significant.
Fu et al., (2019)	PTC strip	Suprathreshold responsiveness (Continuous)	Wine consumers (519)	PTC responsiveness was not associated with differences in the frequency of alcohol consumption.
Concas et al., (2019)	Paper disk dipped in 50mM PROP	Suprathreshold responsiveness (Continuous)	Adults (3219)	PROP responsiveness was not correlated to the overall liking of alcoholic beverages.
Pierguidi et al., (2020)	Aqueous solution (Duplicate; 3.2 mM)	Suprathreshold responsiveness (Continuous)	Cocktail consumers (159)	Increased PROP responsiveness was associated with a decrease in the “shots of liquor” and “glasses of cocktails” consumed but did not impact the consumption of “cans of beer” or “glasses of wine”.

Table 2.4: Summary of PROP/PTC-related studies on alcohol use disorders or family history of alcoholism.

Author (Date)	Stimulus (Concentration)	Method of PROP Operationalization	Participants (n)	Primary Findings
Smith, (1972)	Aqueous solution (Duplicate; 0.057 mg/ml)	Suprathreshold (PTC tasters vs PTC non-tasters)	Inpatient alcoholics (27), drug (28) addicts & control group (unspecified)	Alcoholics are as likely as the control group to be PTC tasters or PTC non-tasters.
Swinson, (1973)	Aqueous solutions (14 ranging from 0.0009 to 7.64 mM)	Threshold (PTC taster vs PTC non-taster)	Inpatient Alcoholics & Control Group (411)	Alcoholics are as likely as the control group to be PTC tasters or PTC non-tasters.
Pelchat and Danowski, (1992)	Aqueous solutions (14 ranging from 0.000732 to 6.00 M)	Thresholds (PROP tasters vs PROP non-tasters)	College students/staff (55)	Children of alcoholics are more likely to be PROP non-tasters than children of non-alcoholics. No difference in the proportion of non-tasters between the children with & without alcoholism.
Kranzler et al., (1996)	Aqueous solutions (14 ranging from 0.000732 to 6.00 M)	Thresholds (PROP tasters vs PROP non-tasters)	Late adolescent & young adults (95)	No association between a paternal history of alcoholism & PTS.
Kranzler et al., (1998)	Aqueous solutions (14 ranging from 0.000732 to 6.00 M)	Thresholds (PROP tasters vs PROP non-tasters)	Outpatients being treated for AUD (90)	No association between a familial history of alcoholism & PTS (maternal, paternal or both).
DiCarlo and Powers (1998)	Paper disk dipped in saturated PROP solution	Suprathreshold (pNTs vs pMTs vs pSTs)	College aged students (100)	Individuals with a familial history of alcoholism are more like to be pNTs if they are not depressed or pSTs if they have symptoms of depression.

2.3.2.2 Genes associated with PROP Bitterness

PROP phenotypes are partially explained by genetic variation in the TAS2R38 gene for which two common haplotypes have been reported (Tepper, 2008). The recessive AVI "nontaster" allele is associated with reduced PROP responsiveness, while the dominant PAV "taster" allele is associated with higher PROP bitterness intensity (Duffy et al., 2004a; Calo et al., 2011). As a result, typically three diplotypes are studied; AVI/AVI homozygotes who rate PROP bitterness lowest (putatively pNTs), PAV/PAV homozygotes who rate PROP bitterness highest (putatively pSTs) and PAV/AVI heterozygotes (putatively pMTs) whom rate PROP bitterness higher than AVI/AVI at all concentrations and at high concentrations rate PROP bitterness lower than PAV/PAV (Duffy et al., 2004a; Calo et al., 2011; Fischer et al., 2014). The response of heterozygous individuals is variable, as higher within-group variation exists (Lipchock et al., 2013), possibly due to greater variation in gene expression (Lipchock et al., 2013). Three less common haplotypes also exist - PAI, AAV and AAI - with a prevalence of only 4% within the Caucasian population (Wang et al., 2007), and most studies do not report on them.

Polymorphism in the gustin gene (rs2274333; A/G), a trophic factor in taste bud development, is also associated with differences in PROP responsiveness (Calo et al., 2011). While the A allele may be required for supertasting, the gustin gene does not fully explain the variation in PROP responsiveness or PROP taster groups. However, when both TAS2R38 and gustin genotypes are accounted for, approximately 60% of the variation can be explained (Calo et al., 2011). New research determining how both genes might interact to modulate alcohol consumption, preferences and use disorder risk would be very appropriate.

2.3.2.3 PROP Bitterness and Responsiveness to Ethanol and Alcoholic beverages

PTS may be associated with suprathreshold responsiveness to ethanol. When a probe with 50% v/v alcohol was placed on the tongue, individuals who rated PROP bitterness lower also rated less burning sensation and greater liking of the stimulus (Duffy et al., 2004b). Similarly, PROP tasters rated the intensity of a 47.5% w/v ethanol aqueous solution higher than non-tasters (Prescott and Swain-Campbell, 2000), and pSTs rated the bitterness and irritation of an ethanol solution (concentration not stated) significantly higher

than pMTs and pNTs (Bartoshuk et al., 1994). These differences have not been replicated to date with phenylthiocarbamide (PTC) – a PROP analogue used primarily in the earlier literature; no difference was found for suprathreshold ethanol responsiveness across the concentration range examined of 4.3% to 17% v/v between PTC tasters and PTC non-tasters (Mattes and DiMeglio, 2001). However, given that PTC and PROP activate the same TAS2R receptors and typically elicit similar orosensory responsiveness and behavioural correlates (Roura et al., 2015), this null result may be due to the PTC categorisation method used. That is, grouping individuals with intermediate PTC responsiveness with those of high responsiveness (analogous to combining pMTs and pSTs) may not allow for differences in ethanol perception to be fully captured. Given that the intensity and predominance of both bitterness and burning elicited by ethanol change with concentration (Nolden and Hayes, 2015), further research should examine the responsiveness of all PTS and PTC groups across the range of ethanol levels typically found in alcoholic beverages (3-45 % v/v).

The perception of alcoholic beverages differs across PTS groups. In a small study of untrained panellists (n=25), pNTs reported lower bitterness, astringency, and sourness from red wine than pMTs and pSTs (Pickering et al., 2004). In a subsequent study, a panel of individuals trained in descriptive analysis (n=16) largely confirmed these results, with sourness, saltiness, heat/irritation, and overall astringency (although not bitterness) rated less intense by pNTs than pSTs across sixteen commercial red wines (Pickering and Robert, 2006). With beer, pSTs rated the bitterness of Urquell pilsner but not Budweiser as significantly higher than pMTs and pNTs (Intranuovo and Powers, 1998). In contrast, no differences in orosensory responsiveness were found across PTS groups by Pickering et al., (2010b). As speculated by Bajec et al. (2012), this may be attributable to the use of pectin as an inter-stimulus rinse in the study; pectin is capable of binding proteins and may have interacted with gustin to reduce its contribution to perceptual differences between the PTS groups. Alternatively, Pickering et al. (2010b) used a higher concentration of PROP to classify individuals than the earlier studies (3.2 mM vs 0.32 mM), which may explain the contradictory findings. On balance, the existent literature suggests that PROP responsiveness mediates the perception of orosensations in some alcoholic beverages, with pSTs being more responsive than pNTs.

2.3.2.4 PROP Bitterness and Alcoholic Beverage Preferences and Consumption

Despite these differences in orosensation, the literature is somewhat equivocal on whether PROP responsiveness predicts or mediates preference and consumption behaviours. Daily consumption of alcoholic beverages was lower for PROP tasters (pMTs and pSTs combined) than for pNTs in a convenience cohort of Australians drinkers (Beckett et al., 2017). When PROP responsiveness was modelled in a Mendelian randomization study, increased PROP responsiveness was associated with a decrease in the frequency of overall alcohol consumption (Ong et al., 2018). Increased PROP responsiveness was also associated with a decrease in the consumption of "shots of liquor" and "glasses of cocktail" per week but not "cans of beer" or "glasses of wine" (Pierguidi et al., 2020). In contrast, no association was found between PROP bitterness and alcohol consumption in a study of 329 Canadian alcohol consumers (Pickering and Hayes, 2017) or PTC bitterness and alcohol consumption in a study of 519 wine consumers (Fu et al., 2019). Similarly, Fischer et al. (2014) reported no significant association between PROP bitterness ratings and alcohol consumption. However, while their population-based study had a large sample size (n=2359), the questions asked likely did not capture alcohol consumption fully. Alcohol intake was measured by asking two dichotomous questions; whether participants had consumed "any alcohol in the past year" and whether they had "ever drank 4+ drinks/day", with yes/no response options for both. Thus, their data did not discriminate finer aspects of the consumption patterns of drinkers, and a continuous measure of alcohol intake may be more illuminating. Indeed, when alcohol consumption is more precisely measured (e.g. monthly frequency and total intake), some variation with PROP responsiveness has been reported. For instance, Pickering et al. (2014) found that total wine intake decreased as PROP bitterness increased in a study of over 1000 US wine consumers.

Australians with the TAS2R38 (rs713598) tasting genotype (PAV/PAV and PAV/AVI combined) reported lower daily consumption of alcoholic beverages than participants with the non-tasting genotype (AVI/AVI; Beckett et al., 2017), while no association was found in a study of Korean adults (Choi et al., 2017). However, Koreans with PAV/AVI or AVI/AVI diplotypes were more likely to have never consumed alcohol than individuals with the PAV/PAV diplotype (Choi et al., 2017). Surprisingly, wine

consumers with the tasting genotype for TAS2R38, consumed alcohol more frequently than those with the non-tasting genotype (Fu et al., 2019). As wine experts are more likely to be pSTs than pNTs (Hayes and Pickering, 2012; Pickering et al., 2013) and the wine consumers were recruited at tasting events, it is possible the consumers had a high degree of involvement with wine, which impacted alcohol consumption differently than would be expected in the general public.

Liking and preferences may also vary with PTS and are of particular interest to alcoholic beverage producers and marketers. pSTs report lower liking scores than other PTS groups for dry table wine, fortified wine (Pickering et al., 2014) and sparkling wine (Pickering and Cullen, 2008; Pickering et al., 2014); all wine styles that are potentially more unpleasant due to higher sourness, irritation, and/or bitterness. pSTs preferred sweet wines and wine-based beverages (Pickering et al., 2014), consistent with the higher liking scores for all five sweet wine styles of the most PROP responsive individuals in Pickering and Hayes (2017). However, in contrast with Pickering et al. (2014), the latter study of 320 participants showed that the most PROP responsive individuals also gave higher liking scores for wines that elicit the predominantly aversive orosensations of dryness, carbonation, and heat. The authors speculate that the discrepancy in findings may largely be attributed to differences between the cohorts, as non-drinkers were excluded from their study, and one third of participants were wine professionals, indicating a higher than normal involvement with wine. Bajec (2010) found that pMTs preferred sweet table and desert/ice wines more than pNTs, with pSTs indicating an intermediate liking score. A general trend of higher liking from pMTs was reported across all alcoholic beverage types in the latter study. Intranuovo and Powers (1998) showed that male pNTs reported higher liking of sampled beer than male pSTs, a finding which may have contributed to pNTs reporting higher beer consumption than pSTs (genders combined) during their first year of drinking. However, Yang et al. (2018) found the opposite, that pSTs and pMTs liked sampled beers that varied in carbonation level and serving temperature, more than pNTs.

Some studies have failed to find a relationship (Catanzaro et al., 2013; Pickering et al., 2010b; Concas et al., 2019). Overall liking of alcoholic beverages was not correlated with PROP responsiveness in a large study of Italians (n = 3219; Concas et al., 2019). The liking scores of American college students (n=139) for beer and red wine did not differ

with PTS in the report of Catanzaro et al. (2013), although the scale used (1 = "I hate it" to 5 = "I love it") was likely more restrictive than those employed by Bajec (2010; 7-point hedonic) and Pickering et al. (2014; gDOL). Further, the latter two studies allowed participants to indicate that they were unfamiliar with an alcohol beverage, ensuring that liking ratings were only obtained from individuals who reported familiarity with each beverage type.

2.3.2.5 PROP and Alcohol Behaviour - Mediators

It has been suggested that food neophobia - the fear of trying new foods resulting in food avoidance (Pliner and Hobden, 1992) - mediates the effect of PTS on food liking. Ullrich et al. (2004) reported that food adventurous PROP tasters liked more foods than their non-adventurous counterparts, while adventurousness had minimum effect in non-tasters. The possibility that the neophobia trait might also mediate the relationship between PROP responsiveness and alcohol behaviour, however, has not been thoroughly investigated. While preferences/liking of alcoholic beverages have been associated with both food (Logue and Smith, 1986; Ullrich et al., 2004) and alcoholic beverage (Pickering et al., 2014) neophobia, PTS did not mediate this relationship in the study on wine liking of Pickering et al. (2014). In contrast, Ullrich et al. (2004) reported that PROP tasters who were more food adventurous liked strong alcohol more than tasters who were less food adventurous, providing some preliminary evidence that food/alcoholic beverage adventurousness may mediate the association between PROP responsiveness and alcohol liking. However, this finding should be replicated, and consideration given to incorporating a more discriminating hedonic scale than their dichotomous 'like' or 'dislike' measure, as well as assessing intake behavior.

Few studies have directly assessed and modeled the role of sensory factors as predictors or mediators of alcohol liking and behavior by examining responses to sampled beverages. The work of Lanier et al. (2005) is the noteworthy exception, in which the relationship between the bitterness and sweetness elicited by sampled scotch and beer were investigated in relation to PROP responsiveness, alcohol preference and alcohol intake in college students using linear regression analysis (Figure 2.2). Lower PROP responsiveness predicted higher sweetness and lower bitterness ratings in scotch, which in turn contributed to higher alcohol consumption. Similarly, lower PROP responsiveness predicted lower

bitterness in beer, and both bitterness and sweetness ratings independently predicted higher preference for the beer which in turn contributed to higher total alcohol consumption. Interestingly, while PROP responsiveness associated with the bitterness of both sampled beverages, it did not directly mediate alcohol intake, suggesting the need to collect broader orosensory data when investigating taste/alcohol behaviour relationships. In particular, responsiveness to product components presented at ecologically-valid concentrations are important, given that the dominant orosensations elicited by ethanol are highly dependent on concentration (Nolden and Hayes, 2015). The use of partial structural equation modelling in the Lanier et al. (2005) study allows for the relative contribution of each measure to be examined in relation to the other variables, which can facilitate deeper insights into the complexities of alcohol behavior. Further research using their general approach would be valuable and should be extended to include consideration of the full range of orosensations elicited by alcohol and a wider range of alcoholic beverage types.

2.3.2.6 PROP Bitterness and Alcoholism

In two early studies using phenylthiocarbamide, the proportion of PTC tasters and non-tasters did not differ between alcoholics undergoing treatment and a control group (Smith, 1972; Swinson, 1973). However, little detail is provided about the control groups in these reports, including their size, which makes it challenging to evaluate these null results. Subsequent studies have shown conflicting findings. Pelchat and Danowski (1992) reported that children of alcoholics were significantly more likely to be pNTs than children of non-alcoholics, as defined by the Michigan Alcohol Screening test (MAST). Conversely, family history of alcoholism did not differ with PTC (Mattes and DiMeglio, 2001) or PROP (Kranzler et al., 1996, 1998; Robb and Pickering, 2019) responsiveness in subsequent reports, with Robb and Pickering (2019) also failing to show a relationship between PTS and Alcohol Use Disorders Identification Test (AUDIT) classification. One possible explanation for the null results of these latter studies is that relevant co-variables, including depression, were not assessed.

DiCarlo and Powers (1998) reported that college students with a family history of alcoholism (either parent) are more likely to be pNTs if there is no family history of depression, or more likely to be pSTs if there is family history of depression, hinting that

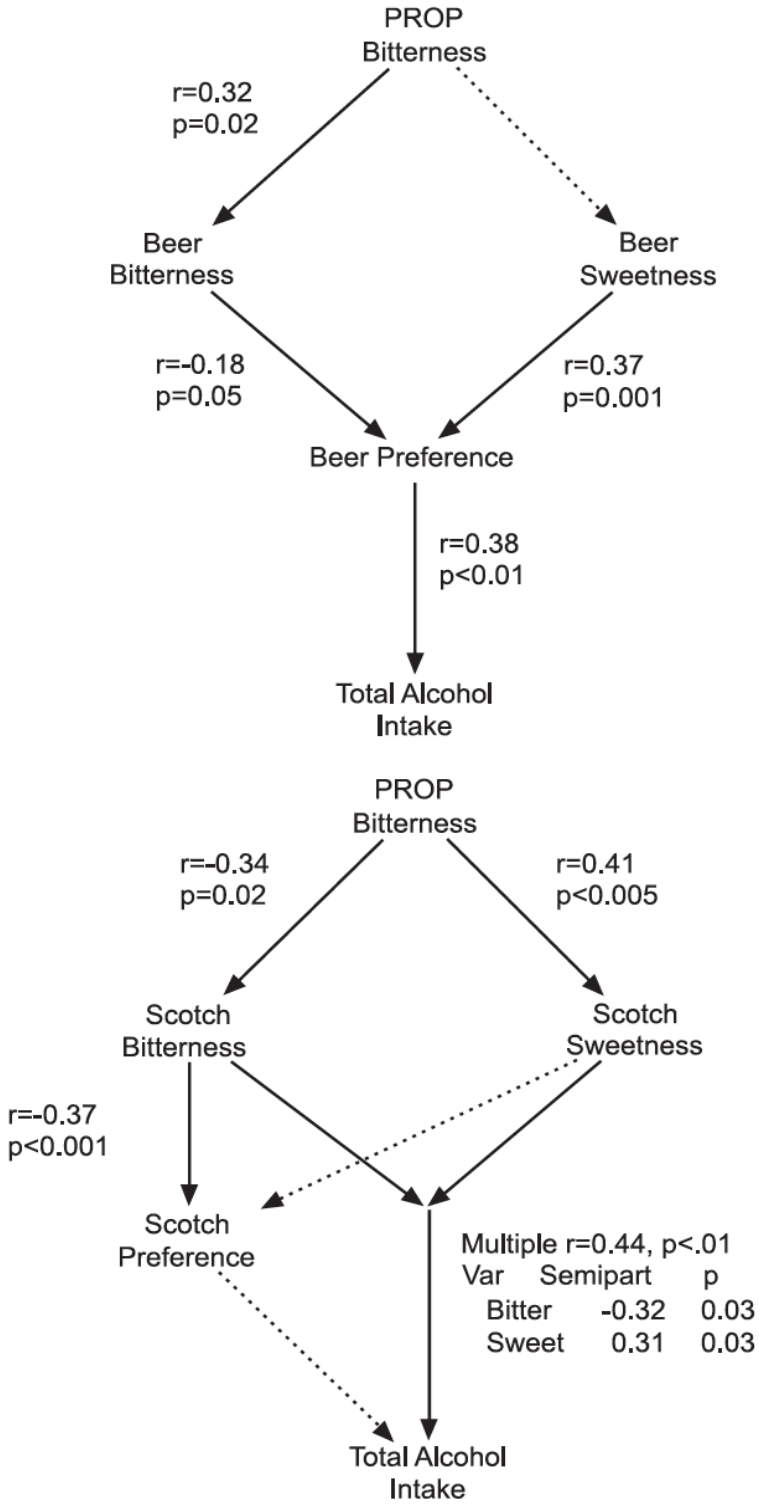

Figure 2.2:Model of the relationship between PROP bitterness, sweetness, bitterness, preference and alcohol consumption for beer and scotch. Significant relationships are shown with solid lines while dotted lines are not significant. From Lanier et al. (2005).

PROP responsiveness may be differentially associated with the two different types of alcoholism. Type 1 alcoholism, known as 'milieu-limited', is associated with depression, late onset (after age 25), genetic predisposition (family history) and environmental factors (family home with high levels of alcohol consumption; DiCarlo and Powers, 1998). In contrast, Type 2 alcoholism, 'male-limited', is typically experienced by males (onset before age 25) with alcoholic fathers regardless of their upbringing (DiCarlo and Powers, 1998). The age range of the cohort used in DiCarlo and Powers (1998) study should be expanded in future work beyond college students, as the onset of Type 1 alcoholism typically occurs after the age of 25.

One theory for the association of PTS with alcoholism is that pNTs experience the aversive orosensory qualities of alcohol less strongly, leading them to consume alcohol at a younger age, which may put them at increased risk of developing alcoholism. However, no difference in TAS2R38 genotype was found between age of first intoxication or age of commencing regular drinking in a study of high-risk families (Wang et al., 2007). The balance of the existent literature does not support an association between PROP/PTC responsiveness and alcoholism, although as indicated above, family history of depression amongst other possible mediating factors should be considered in future studies.

2.3.3 Other Bitter Taste Receptors and Genes

Two SNPs in TAS2R16 have been associated with increased alcohol consumption. Homozygous AA (rs846672) individuals reported consuming alcoholic beverages more frequently than AC or CC subjects, and CG and GG individuals trended toward higher total intake and frequency of consumption than CC homozygotes (Hayes et al., 2011). As noted by the authors, a larger sample should be examined (current n=96) as the two SNPs are not in linkage disequilibrium. It is currently unknown if these SNPs make independent contributions to alcohol consumption patterns. When examined in larger study of wine consumers (n = 519), TAS2R16 (rs846664, rs846672) was not associated with differences in the frequency of alcohol consumption (Fu et al., 2019). However, alcohol frequency was measured as a categorical variable ("less than two drinks per week", "2-7 drinks per week", "greater than 7 drinks per week"), thus further study in a large sample where alcohol consumption is treated as a continuous variable may confirm the findings of Hayes et al. (2011). However, the K172N (rs846664) allele is associated with an increased risk of

developing alcohol dependence (Hinrichs et al., 2006) and an increased maximum number of drinks consumed in a 24-hour period (Wang et al., 2007), suggesting it may play an important role in alcohol consumption. However, this may not extend to sampled scotch whisky, as TAS2R16 genotypes were not predictive of the intensity of taste sensations (sweet, sour, bitter or salty) or liking elicited by this product (Hayes et al., 2011). The frequency of alcohol consumption is not associated with CA6 (gustin; rs227433), GNAT3 (rs1524600), TAS2R19 (rs10772420), TAS2R20 (rs12226920), TAS2R43 (rs1443637), TAS2R46 (rs2708377), TAS2R50 (rs10772397), TAS2R60 (rs4595035), TAS2R8 (rs1548803) or TRPA1 (rs11988795; Fu et al., 2019).

2.3.4 Sweet-liking

2.3.4.1 Introduction

Sweet-liking is a measure of an individual's hedonic response (liking or preference) to sweetness and is typically measured by determining preference for a series of sucrose solutions of increasing concentration. While no standard method of classification has been established, individuals are often defined as sweet-likers if they most prefer high sucrose concentrations (0.4-0.8M) and sweet-dislikers if they most prefer lower sucrose concentrations (Kampov-Polevoy et al., 1997, 2001, 2003a, 2014; Kranzler et al., 2001; Wronski et al., 2007; Tremblay et al., 2009; Lange et al., 2010). However, other methods have been employed (e.g. Looy et al., 1992; Looy and Weingarten, 1992; Asao et al., 2015), and indeed, contradictory results in the sweet-liking and alcohol use disorder literature are often attributed to differences in the classification of sweet-likers and sweet-dislikers (see Iatridi et al., 2019 for a review). Results with other sugars suggest that sweet-liking is a robust phenomenon which may be generalizable to complex sweet substances (Looy et al., 1992).

Preference for liking of sweet solutions is partially heritable; approximately 50% of the variation in liking of a 20% w/v sucrose solution was associated with genetic factors in a study of 663 female twin pairs (Keskitalo et al., 2007). As the molecular mechanisms underlying sweet preferences are largely unknown, candidate genes and allele studies are not yet possible to further current understanding of the phenomenon (Hayes et al., 2013).

As a result, the studies reviewed below have all measured sweet liking at the phenotypic level.

2.3.4.2 Sweet-liking and Alcohol

Sweet-likers may consume more alcohol and be at greater risk of developing an alcohol use disorder. As previously discussed, ethanol elicits sweetness and has been shown to activate sweetener responsive neural fibres in gustatory nerves (reviewed in Bachmanov et al., 2011). In addition, ethanol and sweet solutions activate overlapping central mechanisms, namely the opioidergic, serotonergic and dopaminergic systems, potentially making the reward associated with alcohol consumption consistent with that of sugar consumption (Levine et al., 2003; Bachmanov et al., 2011). In a preliminary imaging study, sucrose solutions were shown to activate the bilateral orbitofrontal cortex and the right ventral striatum, both areas associated with reward (Kareken et al., 2013). Interestingly, the number of drinks consumed on a day when drinking was positively correlated with left orbitofrontal cortex response when consuming a sucrose solution (Kareken et al., 2013). As a result, it has been theorized that individuals at risk of alcoholism may experience an enhanced reward to alcohol if they also experience greater preference for sweetness (Kampov-Polevoy et al., 2001). In individuals with an abnormally low basal level of endogenous reward, stronger stimulation, such as a sweeter tasting food or more alcohol, may be required to elicit equivalent responses to individuals with high basal levels of endogenous reward (Kareken et al., 2013).

Individuals with the CT genotype for TAS1R3 rs307355 are more likely to be heavy drinkers (>30 g alcohol/day) than light drinkers (<30 g/alcohol/day) compared to individuals with the CC genotype (Choi et al., 2017). These results suggest a genetic mechanism that may impact sweetness perception and further research is warranted to determine if TAS1R3 is associated with sweet-liking.

The link between sweet-liking and alcohol was first reported in a study of male alcoholics with a diagnosis of alcohol dependence based on the DSM-III-R and males who had never received a diagnosis of alcoholism. Significantly more alcoholic men preferred the highest sucrose solution (0.83M, 65% vs 16%) and were classified as sweet-likers compared to the nonalcoholic group (Kampov-Polevoy et al., 1997). This finding was subsequently confirmed when the sample size of the above study was expanded (Kampov-

Polevoy et al., 1998) and in a report comparing hospitalized men with and without alcoholism (Kampov-Polevoy et al., 2001). Within alcoholics, greater consumption of alcoholic beverages was associated with a higher detection threshold for sucrose (Silva et al., 2016). Additionally, alcoholics had a higher detection threshold for sucrose than non-alcoholics who were matched for age, gender and income (3.78 vs 1.39 g/L in water; Silva et al., 2016).

In contrast, no differences in sucrose solution preferences or sweet-liking phenotypes were found when abstinent alcoholic men and men without a history of alcohol use disorder were compared (Bogucka-Bonikowska et al., 2001; Wronski et al., 2007). Additionally, Tremblay et al. (2009) found no difference between alcoholics and a control group for liking of a 0.83M sucrose solution, but reported that alcoholics did prefer 0.05M sucrose more than the controls. However, when sex, age, education, smoking status, number of drinking days and number of standard drinks during the 30 days preceding testing were included, the effect was no longer significant, suggesting that the differences between groups were due to factors other than a diagnosis of alcoholism.

Interestingly, alcohol dependent individuals were more likely to be sweet-likers than control individuals when newly sober (4-30 days), but no difference was found at 6 months (Krahn et al., 2006). Furthermore, sweet-liking alcohol dependent individuals were less likely to maintain their sobriety during the 6 month period (Krahn et al., 2006). In contrast, no difference in sweet-liking was found between alcohol dependent individuals and control groups when tested twice within 30 days of beginning a treatment program (Kampov-Polevoy et al., 2001, 2003b). Thus, it remains unclear if alcoholism leads to a preference for sweeter solutions, or if a preference for sweeter solutions predisposes individuals to alcohol use disorders (Kampov-Polevoy et al., 1997).

A paternal history of alcoholism has been associated with greater preference for sweet solutions (sweet-liking) in hospitalized alcoholics when compared to non-alcoholic men (Kampov-Polevoy et al., 2001), in individuals without a lifetime history of alcohol or drug abuse (Kampov-Polevoy et al., 2003a) and in residential patients with a history of alcoholism, drug dependence or psychiatric conditions (Kampov-Polevoy et al., 2003b, 2004). Furthermore, greater preference for sweet-solutions was reported in females with a first or second degree familial history of alcoholism (Pepino and Mennella, 2007), and male

alcoholics with a first degree maternal or paternal history of alcoholism are more likely to be sweet-likers and rate 0.83 M sucrose higher more intensely than male alcoholics without such familial history (Wronski et al., 2007). Sweet-likers were 2.7 times more likely to have a family history of alcoholism (Lange et al., 2010). However, no difference in the proportion of sweet-likers and sweet-dislikers was found when non-alcoholic men with or without a paternal history of alcoholism were compared (Kranzler et al., 2001), or when sons of male alcoholics and males without a first/second degree family history of alcoholism were compared (Scinska et al., 2001). Further, neither sweet-liking nor responsiveness was associated with familial history of alcoholism in a non-clinical samples (Robb and Pickering, 2019; Eiler et al., 2017).

Recently, Bouhlal et al. (2018) studied the impact of sweet-liker status in a sample where most participants met the diagnostic criteria for current alcohol dependence (54 out of 55; DSM-IV). They found that sweet-liking was not associated with differences in the age of first drink, the recent number of heavy drinking days or the average number of drinks per day when drinking. However, alcohol dependent sweet-likers had higher cravings for alcohol than alcohol dependent sweet-dislikers (Bouhlal et al., 2018). Alcohol dependent sweet-likers also preferred spirits to non-spirits while the opposite was true for sweet-dislikers (Bouhlal et al., 2018).

Sweet-liking may be more predictive of alcohol related behaviour in males than females. Men but not women with alcohol-related problems (individuals who meet at least one criterion of the DSM-III-R without meeting the requirements for a DSM-III-R diagnosis of an alcohol use disorder) are more likely to be sweet-likers (Lange et al., 2010). Similarly, male sweet-likers have also been shown to consume more alcohol on average per month than male sweet-dislikers (Robb and Pickering, 2019). Therefore, while sweet-liking may be associated with a familial history of alcoholism and alcohol consumption, the nature of the relationship may differ between the sexes.

Personality traits mediate the association between sweet-liking and alcohol behaviour. While sweet-liking is associated with increased risk of alcohol-related problems, that risk is increased in sweet-likers with high novelty seeking traits (Lange et al., 2010; Kampov-Polevoy et al., 2014). Additionally, the finding by Mennella et al. (2010) that a family history of alcoholism was associated with an increased preference for sucrose

solutions in 5-12 year old children was largely driven by the children who were also classified as depressed, as measured by the 23-item Pictoral Depression scale (Mennella et al., 2010). Individuals with a preference for white wine with added fructose (20 g/L) reported higher levels of impulsiveness and lower levels of openness than individual that preferred the same wine with no fructose addition (Saliba et al., 2009). Thus, it would seem prudent to include measures of openness, depression and novelty seeking (including impulsiveness) as potential co-variables in future studies on sweet-liking and alcohol behaviour.

2.3.5 Other Orosensations and Alcohol Behaviour

One consistent trend emerges across all orosensations when the association between responsiveness and alcohol consumption is examined; individuals who are the most responsive to orosensations typically consume lower quantities of alcohol (Fischer et al., 2013; Thibodeau et al., 2017). In fact, individuals who consumed more than four drinks per day were more likely to exhibit taste impairment compared to non-drinkers (Liu et al., 2016). Furthermore, individuals who avoid alcohol are significantly more responsive than alcohol consumers to sourness, with a similar trend reported for bitterness and sweetness (Thibodeau et al., 2017). This may suggest that individuals with increased responsiveness to orosensations experience the aversive sensory characteristics of alcoholic beverages more strongly than individuals with lower responsiveness, leading to lower consumption or avoidance. In contrast, the highest rates of alcohol consumption have been reported in individuals with low or moderate responsiveness, depending on the specific orosensation under consideration (Fischer et al., 2013; Thibodeau et al., 2017).

In the Beaver Dam Offspring Study (n > 2000), alcohol consumption was not linearly related to suprathreshold responsiveness to saltiness or sourness (Fischer et al., 2013). Moderate alcohol consumers (15-74g ethanol/week) had significantly lower responsiveness to saltiness but not sourness, when compared to non-drinkers or heavy drinkers (>140g/week; Fischer et al., 2013). Furthermore, the consumption of an alcoholic beverage in the past year was associated with significantly lower ratings of suprathreshold sourness and saltiness (Fischer et al., 2013). In contrast, Ng et al. (2019) found only limited associations between suprathreshold saltiness after testing salty responsiveness under three conditions (1 M NaCl on tongue tip, 0.32 and 1 M NaCl whole mouth rinse) and dividing

the participants into four groups (men 40-59, men 60+, women 40-59, women 60+). Current drinkers (only women, aged 60+) were more responsive to 0.32 M sodium chloride than non-drinkers. Ng et al. (2019) also considered the type of alcoholic beverages consumed (wine only, distilled spirits only, cordial/liqueur/cocktail only, 2+ beverage types). Wine consumers rated the saltiness of 1 M NaCl (tongue) lower than non-drinkers but no significant associations were found for any of the other combinations of conditions or the type of alcoholic beverage consumed. Sour responsiveness was associated with both beer and wine consumption, and metallic responsiveness with dry wine intake, in the study of Thibodeau et al. (2017). As sour or metallic responsiveness increased, alcohol consumption decreased, suggesting that these sensations may be largely aversive for some consumers when elicited by these alcoholic beverages (Thibodeau et al., 2017).

Astringency responsiveness also appears to associate with wine consumption, but the nature of the relationship varies between red and white wine. Individuals with intermediate astringency responsiveness consumed more red wine than those with high or low responsiveness, whereas white wine intake decreased with increasing astringency responsiveness (Thibodeau et al., 2017). These results suggest that when astringency is expected in wine, as when elicited by the ubiquitous phenolic constituents of red wine, its level is optimized for the average consumer's palate. However, as astringency is not typically expected in white wine, it is perceived as aversive, with a corresponding effect on intake.

Perception of sourness and saltiness may also be linked to risk of developing an alcohol use disorder. Family history of alcoholism is associated with decreased liking of and increased responsiveness to sourness (Sandstrom et al., 2003), and decreased liking of saltiness (Scinska et al., 2001; Sandstrom et al., 2003). However, Sandstrom et al. (2003) found no association between sourness or saltiness and alcohol consumption, although their intake measure may have been oversimplified with only two groups (low and high consumption) used in the analysis. While saltiness and sourness have been reported as sensations elicited by ethanol (Scinska et al., 2000; Mattes and DiMeglio, 2001), bitterness and heat/irritation are the dominant sensations from ethanol at the concentrations found in most alcoholic beverages (Nolden and Hayes, 2015). Consequently, the increased risk of

developing an alcohol use disorder noted above in Sandstrom et al. (2003) and Scinska et al. (2001) may simply be reflecting more generalised orosensory responsiveness.

2.3.6 Thermal Tasting

2.3.6.1 Introduction

Thermal tasting represents another taste phenotype whereby orosensory responsiveness may associate with alcohol consumption behaviour. Thermal taster status (TTS) is determined when the tip of the tongue is cooled or heated, producing a phantom taste in thermal tasters (TTs) and no taste sensations in thermal non-tasters (TnTs; Green & George, 2004). Individuals who fail to meet these classification criteria (uncategorized; Uncats) are also identified, but typically excluded from studies as part of the initial screening process (Bajec and Pickering, 2008; Yang et al., 2014). TTs tend to rate aqueous solutions of sour, bitter, sweet, salty, umami, metallic and astringent stimuli higher than TnTs (Green and George, 2004; Bajec and Pickering, 2008; Hort et al., 2016; Yang et al., 2014) as well as cold and warm stimuli (Bajec and Pickering, 2008; Yang et al., 2014), and are more discriminating of CO_2 levels (Hort et al., 2016). TTs also have lower detection thresholds for sweetness than TnTs (Yang et al., 2014), and a trend toward lower difference thresholds for sweetness, sourness, and bitterness (Pickering and Kvas, 2016).

These differences in orosensations associate with self-reported liking of a large range of food items (Bajec and Pickering, 2010), particularly significant given that reported liking may be a more accurate proxy for consumption than many traditional dietary intake measures (Duffy, 2007). However, only limited associations were found between TTS and sampled foods and non-alcoholic beverages in the more recent reports of Pickering and Klodnicki (2016) and Pickering et al. (2016), possibly attributable to small samples sizes. It has been speculated that TTS may link with alcohol consumption; specifically, that TTs consume less as they experience the orosensations more intensely than TnTs, and ethanol elicits primarily aversive sensations (Thibodeau, 2015).

2.3.6.2 Thermal Tasting and Alcoholic Beverages

In contrast with most sampled foods, TTs appear more responsive than TnTs to the orosensations elicited by alcoholic beverages. When five aqueous solutions of ethanol (2-10% v/v) were tasted, TTs tended to rate the bitterness and burning but not the sweetness

of the higher ethanol concentrations higher than TnTs, although the difference was not significant (Small-Kelly and Pickering, 2020). In beer, TTs rated bitterness (Pickering et al., 2010a, Small-Kelly and Pickering, 2020), sourness (Pickering et al., 2010a, Small-Kelly and Pickering, 2020), sweetness (Pickering et al., 2010a), and overall intensity (Small-Kelly and Pickering, 2020) higher than did TnTs. In addition, when evaluating dealcoholized beer using TCATA (Temporal Check-All-That-Apply), TTs were more likely than TnTs to describe the aftertaste of the beer as astringent, bitter, sour (Mitchell et al., 2019). In wine, the bitterness, sweetness, sourness, astringency, and overall flavour intensity were rated higher by TTs for white and red wine (Pickering et al., 2010b). The sourness and astringency/drying was rated higher by TTs than TnTs in cider (Small-Kelly and Pickering, 2020). Noteworthy, these attributes represent the dominant orosensations typically elicited by these products. Further, TTs tend toward lower difference thresholds for sweetness, sourness, and bitterness in neutral white wine; a trend that reached statistical significance for sourness (Pickering and Kvas, 2016). As such, TTs may be able to better discriminate smaller differences in the orosensory properties of alcoholic beverages than TnTs.

These differences in orosensory responsiveness and discrimination may contribute to the increase liking of alcoholic beverages reported by TnTs. TnTs reported significantly higher liking of bourbon, brandy, vodka, mixed tequila, and dry red wine compared to TTs (Bajec, 2010). Furthermore, a trend of higher liking of beer, spirits (overall, mixed and unmixed) and wine (overall, sweet and dry) by TnTs was observed (Bajec, 2010). In contrast, when sampling beers that varied in temperature and/or carbonation level, no differences in liking between TTs and TnTs were found (Yang et al., 2018). However, TTs rated several emotions elicited by the beers (tame/safe, curious, underwhelmed, shocked, bored, disgusted) higher than TnTs (Yang et al., 2018). These results partially support the hypothesis that greater liking is associated with lower responsiveness to the aversive sensations elicited by alcoholic beverages.

2.3.6.3 Thermal Tasting - Other Considerations

To date, all reports on thermal tasting have treated TTs as a homogenous group. However, TTs may experience sweet, salty, sour, bitter (Green and George, 2004; Yang et al., 2014; Hort et al., 2016; Pickering and Kvas, 2016), savoury (Yang et al., 2014), minty

(Hort et al., 2016), or metallic (Yang et al., 2014; Hort et al., 2016; Pickering and Kvas, 2016) sensations on lingual thermal stimulation, and up to a third report more than one phantom taste sensation during testing (Green and George, 2004). This heterogeneity of experience amongst TTs led Bajec and Pickering (2008) to speculate that differences in orosensory responsiveness and related behavioural differences between TTs and TnTs may be due to only a subset of TTs. For example, it is possible that the greater bitter responsiveness of TTs overall is only due to higher ratings from those TTs who experience bitterness during the lingual thermal stimulation procedure used to determine TTS. If this is true, the association between alcohol consumption and/or preference and TTS may be masked by a classification scheme that is too general. For instance, bitter TTs may consume less alcohol than other TTs, as they may experience the aversive bitterness elicited by ethanol more strongly. Conversely, sweet TTs may consume more alcohol than other TTs, as their higher responsiveness to sweetness may help mask ethanol bitterness. Therefore, additional research is required to establish if TT sub-groups based on the orosensation(s) elicited during lingual thermal stimulation represent ecologically valid phenotypes, and how they may vary in their alcohol behaviour.

Up to 40% of individuals in thermal taste studies are not classified as either TTs or TnTs (Uncat). Uncats report experiencing phantom taste sensations during lingual heating and/or cooling; however, these sensations are either rated below the minimum intensity threshold necessary or are not reproducible (Bajec and Pickering, 2008; Yang et al., 2014). Therefore, it is not known if Uncats represent a distinct phenotypical sub-group, or if they are simply TTs or TnTs that have been misclassified. As Uncats have yet to be characterized with respect to orosensory responsiveness and appear to represent a large proportion of the population, further research is warranted, which should also include consideration of TTS and alcoholism/alcohol use disorders.

2.4 Conclusion

The orosensations elicited by alcoholic beverages are not experienced uniformly across individuals and these differences impact alcohol preferences, consumption and risk of developing an alcohol use disorder. In general, individuals who are more responsive to nominally aversive orosensations (bitterness, irritation, sourness and astringency), report

lower preference for and consumption of alcoholic beverages. Furthermore, an increased preference for sweetness may be associated with a familial risk of developing an alcohol use disorder. However, contradictory findings are numerous in the literature, which while in part are attributable to methodological differences, also indicate that the drivers underlying alcohol behaviours are highly complex and cannot be predicted by a single taste-related factor, including genotype, orosensory responsiveness, ethanol responsiveness, PROP taster status, thermal taster status or sweet-liking. Future research would benefit from taking a wider, multi-factorial approach to studying the key orosensory drivers of alcohol intake and incorporating statistical techniques such as partial regression modeling that allow for clearer elucidation of the interaction between multiple variables. Finally, as ethanol elicits chemesthetic and prototypical taste sensations, both should be considered when assessing orosensory responsiveness and its association with alcoholic beverage behaviours.

2.5 Link to Published Version

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2.6 References

Note: Articles marked with "*" were not in the original published version and have been added to provide an update literature review.

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Chapter 3: Examination and validation of classification schema for determining thermal taste status

3.1 Introduction

Individual differences in the perception of oral sensations exist and influence the development of food preferences, food intake, and health related outcomes (reviewed in Tepper 2008; Garcia-Bailo et al., 2009; Hayes et al., 2013). Thermal taster status (TTS), a taste phenotype reported in literature and source of individual variation, is determined when the tongue is cooled or warmed (Cruz and Green 2000). This produces a thermally-induced taste sensation in thermal tasters (TT) but no taste response in thermal non-tasters (TnT; Green and George 2004). A third group of individuals (NC; non-classifiable or uncategorizable) cannot readily be classified as TT or TnT and have been excluded from most previous studies.

While TTS responses are reproducible across multiple trials (Skinner et al., 2018), the mechanism(s) underlying TTS have yet to be fully elucidated. It is currently unknown if the thermally-induced tastes experienced by TT are due to a central gain and/or peripheral mechanism. TRPM5 has been suggested as a possible peripheral mediator of thermal sweetness. TRPM5 is a heat-activated cation channel that is highly expressed in taste receptor cells (Talavera et al., 2005). Importantly, the gustatory nerve response of *Trpm5* knockout mice is reduced compared to that of wild-type mice (Talavera et al., 2005). In addition, sweetness has been reported by TT during warming to temperature that can also activate TRPM5 (Skinner et al., 2018). Using fMRI, elevated cortical activation of taste brain regions of humans was found in TT compared to TnT when tasting sweet solutions with varying concentration of carbon dioxide (CO_2). As CO_2 increased, cortical activation of taste, somatosensory and reward areas of the brain increased in TnT but only the somatosensory regions increased for TT (Hort et al., 2016). Differences in the activation of the taste regions of the brain in both TT and TnT suggest a cross-wiring of receptors in TT at the periphery (Hort et al., 2016).

Green and George (2004) propose that TTS is not mediated by innervation density as three different cranial nerves were tested for orosensory responsiveness, yielding similar

results. No difference in salivary flow rate and fungiform papillae density were found between TT and TnT, suggesting that neither is involved in TT (Bajec and Pickering 2008).

3.1.1 Taste Advantage

Interestingly, TT rate the intensity of suprathreshold tastants (sweet, sour, salty, bitter and umami) elicited in aqueous solution higher than TnT across multiple locations of the mouth (front/back of the tongue, soft palate) using a whole mouth sip and spit protocol (Green and George 2004). While not always significant, the trend of increased responsiveness, defined here as higher intensity ratings, of TT to basic tastes compared to TnT has been confirmed in other studies (Green et al., 2005; Bajec and Pickering 2008; Yang et al., 2014; Hort et al., 2016). The relationship between TTS and taste thresholds is less clear, as TT had a significantly lower detection threshold for sucrose but not NaCl or caffeine (Yang et al., 2014).

Generally, TT are more responsive than TnT to complex stimuli intensity including capsaicin (Yang et al., 2014), ethanol (Small-Kelly and Pickering 2019), alum – an astringent (Bajec and Pickering 2008) and iron (II) sulphate – a metallic stimulus (Bajec and Pickering 2008). TT were also better able to discriminate CO_2 levels in sucrose solutions than TnT when the CO_2 level was high (Hort et al., 2016). In contrast, no significant differences were found between phenotypes for the intensity of taste and chemesthetic attributes elicited by capsaicin and menthol when presented on either the tongue or the vermillion border of the lip (Green et al., 2005). Also, detection thresholds for both N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (WS3) – a trigeminal stimulant - and capsaicin did not differ with TTS (Yang et al., 2014).

While the differences in responsiveness between TT and TnT in aqueous solutions are of interest, such solutions are not representative of normal food and beverages; these have been examined in further studies. TT rated the individual orosensations (taste & chemesthetic) and overall intensities elicited by beer (Pickering et al., 2010a; Small-Kelly & Pickering, 2019) and wine (Pickering et al., 2010b) higher than did TnT. TT also report experiencing taste sensations more frequently over time when consuming de-alcoholised beer (Mitchell et al., 2018). Additionally, TT have a lower difference threshold for tartaric acid in a neutral white wine than TnT, with a similar non-significant trend observed for sucrose but not quinine (Pickering and Kvas 2016). The findings for sampled foods are

more equivocal. Pickering and Klodnicki (2016) found no differences in intensity scores for the dominant orosensations elicited by a range of 15 solid food items, while TT tended to give higher intensity ratings to sampled foods than TnT in Pickering et al. (2016), although this only reached significance for bitter items. The weaker relationship between TTS and the perception of sampled foods compared to beverages may be the result of small sample sizes, although Pickering et al. (2016) have speculated that liquids may be better able to activate the thermal taster 'advantage' by recruiting more receptors within the oral cavity.

Texture and bitterness may account for differences in food preference between TT and TnT (Bajec and Pickering 2010; Pickering and Klodnicki 2016). Self-reported liking of soft and bitter foods was higher for TnT than TT in Bajec & Pickering (2010), a trend that is mirrored for sampled food liking (Pickering and Klodnicki 2016). In contrast, wine and beer preferences were not associated with TTS (Pickering et al., 2010a,b; Yang et al., 2018). Small sample sizes may have contributed to null results, masking an association between TTS and food preferences. The inclusion of power statistics in future research would allow for greater confidence in interpreting the results. In addition to more traditional hedonic ratings, food products can also be discriminated by measuring emotional responses (Meiselman 2015). After consuming commercial beer samples, TT rated six of out ten emotional categories higher than TnT (Yang et al., 2018). Further research is required to determine if this trend extends to other food and beverage products.

Overall, the literature suggests that TT may have an advantage in the perception of orosensations compared to TnT, and importantly there are no reports of TnT rating orosensations in aqueous solutions, beverages or food as more intense than TT. However, as many of the results are not significant, it is unclear if the studies were simply underpowered due to small sample sizes (typically 40-100), or instead the reported differences between TT and TnT are spurious findings.

3.1.2 Temperature and Scale Use

The intensity of warming the tongue from 15°C to 35-40°C is rated higher by TT than TnT (Bajec and Pickering 2008; Hort et al., 2016). Contrasting results for cooling the tongue from 35°C to 5°C are reported with TT being more (Bajec and Pickering 2008) or equally (Hort et al., 2016) responsive to cooling than TnT. TT also rated warm and cold

aqueous stimuli higher than TnT when applied to the tongue with a cotton swab (Yang et al., 2014). Together, this suggests TT may be more responsive to both changing and fixed temperatures compared to TnT within the oral cavity. However, no difference in the perception of static temperatures applied to the palm or vermillion border of the lip has been reported (Green and George 2004).

Scale use differences between TT and TnT have not been directly examined in the literature. Green and George (2004) propose that the equivalent ratings of fixed temperatures on non-gustatory sites given by TT and TnT suggest that differences in orosensory responsiveness between TT and TnT are not an artefact of scale use; rather, mechanistic differences likely underlie the thermal tasting phenomenon. However, variation in scale use is widely acknowledged in the sensory and psychophysical literature, attributable to a range of cultural, psychological and biological factors, with the latter including taste phenotype (Bartoshuk et al., 2002). Therefore, the lack of difference in scale use between TT and TnT should be tested directly.

3.1.3 Methodological Differences

Methodological differences exist across labs and studies in how TTS is determined (Table 3.1). Full details of the TTS elicitation procedures are included in the materials and methods section. Key differences in approaches are the number of locations tested on the tongue, the size of the probe and the number of trials performed. Most studies test for a thermal response in three locations on the tongue (tip, 1-cm to the left, 1-cm to the right). However, four studies only tested one location, the tip of the tongue (Yang et al., 2014, 2018; Hort et al., 2016; Skinner et al., 2018). Three different probe sizes were used to elicit temperature changes ranging from 28.26 mm^2 (Yang et al., 2014; Hort et al., 2016) to 256 mm^2 (Skinner et al., 2018; Yang et al., 2018) with all other studies using a 64 mm^2 probe. Two replicates, where one replicate is defined as one trial for each combination of temperature regime and location tested, were performed in most studies. Skinner et al. (2018) modified the protocol by adding a third replicate in order to re-test when trials from the first two replicates where inconsistent. In contrast, the first two studies on TTS used one full replicate and, if necessary, a second replicate to confirm the presence of a thermally induced taste (Green and George 2004; Green et al., 2005).

Table 3.1: Methodological and classification approaches used to determine thermal taster status (TT = thermal taster, TnT = thermal non-taster, NC = non-classifiable). Table continues on next page.

Scheme Letter & Article	A Green and George (2004)	B Bajec and Pickering (2008)	C Yang et al. (2014)	D Hort et al. (2016)	E Skinner et al. (2018)
Subsequent articles using the same scheme	Green et al. (2005)[a]	Bajec and Pickering (2010); Bajec et al. (2012); Bering et al. (2013); Pickering et al. (2010a, 2010b, 2016); Pickering and Klodnicki (2016); Pickering and Kvas (2016), Mitchell et al. (2018); Small-Kelly and Pickering (2019).	None	Yang et al. (2018)[c]	None
Methodological Differences					
Location	3: tip, 1-cm to left of midline, 1-cm to right of the midline	3: tip, 1-cm to left of midline, 1-cm to right of the midline	1: tip	1: tip	1:tip
Temperatures	Warming: 15°C to 35°C Cooling: 35°C to 15°C	Warming: 15°C to 40°C Cooling: 35°C to 5°C	Warming: 15°C to 40°C Cooling: 35°C to 5°C	Warming: 15°C to 40°C Cooling: 35°C to 5°C	Warming: 15°C to 40°C Cooling: 35°C to 5°C
Repetitions	2: 1 full, 1 as needed	2	2	2	3: 2 full, 1 as needed
Probe Size	64 mm^2 (Shape not reported)	64 mm^2 (Square)	28.26 mm^2 (Truncated Cone)	28.26 mm^2 (Truncated Cone)[c]	256 mm^2 (Square)[c]

[a]Participants recruited by Green et al. (2005) were tested using the same methodology as Green and George (2004) but only participants reporting sweet thermal taste during warming were retained.

[b] Participants were classified as "TnT" if they reported experiencing an "other" taste sensation that when described was heat related (e.g. spicy, hot peppers), cold related (e.g., minty, menthol), or a mouthfeel (e.g. drying, tingling).

[c]Participants recruited by Yang et al. (2018) were tested using the same methodology as Hort et al. (2016) except that a larger probe was used (256 mm^2 square).

Table 3.1 (continued): Methodological and classification approaches used to determine thermal taster status (TT = thermal taster, TnT = thermal non-taster, NC = non-classifiable).

Scheme Letter & Article	A Green and George (2004)	B Bajec and Pickering (2008)	C Yang et al. (2014)	D Hort et al. (2016)	E Skinner et al. (2018)
TTS Classification – Definitions					
TT	Reports a taste sensation above 'weak' on first rep that can be confirmed when the same location and temperature regime is re-tested.	Reports the same taste sensation, at the same location, during the same temperature cycle above "weak".	Reports any taste sensations above 'weak' during all trials. Taste sensations do not need to be the same across trials.	Reports any taste sensations above "weak" both warming and/or both cooling trials. Taste sensations do not need to be the same across trials.	Reports the same taste sensation in two or more replicates of the same warming and/or cooling trial regardless of the intensity.
TnT	Any participants not classified as a TT.	Reports no taste sensations (including "other") during all trials.[b]	Reports no taste sensations during all trials.	Reports no taste sensations during all trials.	Reports no taste sensations during all trials.
NC	Not included in this scheme.	Any participants not classified as a TT or TnT.	Any participants not classified as a TT or TnT.	None reported under this scheme.	Any participants not classified as a TT or TnT.
Valid thermally-induced tastes used for classification	Sweet[a], sour, salty and bitter.	Sweet, sour, salty, bitter, umami (savoury) and metallic[b].	Sweet, sour, salty, bitter, savoury, metallic and other.	Sweet, sour, salty, bitter, umami, other (minty).	Sweet, sour, salty, bitter, umami and other (spicy, metallic, minty).
TTS Classification – Percentages (Ranges for all studies using this scheme)					
TT	46-54%	20-38%	27%	23%	28%
TnT	46-54%	24-40%	30%	77%	51%
NC	0%	24-50%	43%	0%	21%
TTS Classification – Percentages (Data from current study)					
TT	31%	31%	9%	30%	N/A
TnT	69%	26%	31%	30%	N/A
NC	0%	43%	60%	40%	N/A

[a]Participants recruited by Green et al. (2005) were tested using the same methodology as Green and George (2004) but only participants reporting sweet thermal taste during warming were retained.

[b] Participants were classified as "TnT" if they reported experiencing an "other" taste sensation that when described was heat related (e.g. spicy, hot peppers), cold related (e.g., minty, menthol), or a mouthfeel (e.g. drying, tingling).

[c]Participants recruited by Yang et al. (2018) were tested using the same methodology as Hort et al. (2016) except that a larger probe was used (256 mm^2 square).

Green and George (2004) were the first to classify participants into TT and TnT. Participants were classified as TT if they reported a taste sensation above "weak" on the gLMS that could be confirmed after re-testing under the same conditions. All other participants were classified as TnT. Bajec and Pickering (2008) modified the criteria by classifying participants as TT if they reported the same taste above "weak" at the same location and using the same temperature regime in duplicate assessments. Participants who did not report any taste sensations across all twelve runs were classified as TnT, while all other participants were excluded from testing and were considered a new group; non-classifiables (NC). All subsequent studies adopted Bajec et al. (2008) definitions of TnT and NC (if reported), while the definitions of TT was modified as follows in four studies. Skinner et al. (2018) modified the definition of TT in two ways; no minimum intensity was enforced for the thermally induced tastes and the same taste needed to be reported in only 2 or 3 of the warming and/or cooling trials. Hort et al. (2016) and Yang et al. (2018) classified participants as TT if they reported any combination of tastes above "weak" during both warming and/or both cooling trials. Using a more conservative approach, Yang et al. (2014) classified participants as TT if they reported any taste sensation above "weak" during all four trials. Differences in methodology and classification criteria raise concern about the validity of comparing results across studies.

The classification schemes also differed in the thermally induced tastes that were considered valid. Early studies (Green and George 2004; Green et al., 2005) considered reports of *sweetness, sourness/acidity, saltiness* and/or *bitterness* valid, and used these measurements when determining TTS. Subsequent studies expanded the list of valid thermally elicited tastes/orosensations to include *umami/savoury* (all), *metallic* (all), *spicy* (Yang et al., 2014; Skinner et al., 2018)*, tingly* (Yang et al., 2014) and/or *minty* (Hort et al., 2016; Skinner et al., 2018). More research is required to determine the significance of differences in the thermally elicited tastes reported by TT but is beyond the scope of this manuscript.

3.1.4 Non-Classifiable Participants

NC have always been excluded from studies as part of the initial screening process and have yet to be characterized. NC report purported thermally-induced taste sensations during thermal elicitation; however, these sensations are rated at low intensity or are not reproducible (Bajec and Pickering 2008; Yang et al., 2014). Thus, it is not known if NC represent a distinct phenotypical sub-group of TT, or if they are simply TT or TnT that have been misclassified. Furthermore, the orosensory and temperature responsiveness of this group is not known despite the fact that they may represent up to 50% of the population (Pickering and Klodnicki 2016).

3.1.5 Other Considerations

Another important taste phenotype is PROP taster status, which measures an individual's response to the bitter compound, 6-*n*-propylthiouracil (PROP). Individuals are classified into three groups; PROP non-tasters (pNTs) for whom PROP elicits little or no sensation, PROP medium-tasters (pMTs) for whom PROP elicits a mildly bitter sensation and PROP super-tasters (pSTs) for whom PROP elicits a highly bitter sensation (Bartoshuk et al., 1999). PROP taste intensity has been found in several studies to be a useful proxy for general orosensory responsiveness (e.g. Bartoshuk et al., 1994; Prescott et al., 2001; Fischer et al., 2014), much like TTS, raising the question of whether the phenotypes are linked. Indeed, Yang et al. (2014) reported that within pMTs, TT rated taste, trigeminal and aroma stimuli intensity higher than TnT, with the opposite trend observed for pSTs. Noteworthy however are the low cell numbers in some of these analyses, with, for instance, only 9 pSTs TnT. Significant interactions were also found in the emotional responses of participants to sampled beer. Within TnT, individuals classified as pST felt more *content, tame/safe* and *curious* than individuals classified as pNT. However, within TT no corresponding difference were found (Yang et al., 2018). In contrast, evidence for the independence of the phenotypes is suggested by the absence of interaction between PTS and TTS for intensity scores for orosensations elicited by both aqueous solutions (Bajec and Pickering 2008) and wine (Pickering et al., 2010b). Re-examining the relationship between TTS and PTS for orosensory responsiveness in a much larger sample should provide additional clarity on this question and is a secondary objective of the current study.

3.1.6 Study Aims

The primary aim of this study is to more fully investigate TTS and its classification in a large sample (n=708). The study and corresponding aims are structured into three parts:

1. To compare responsiveness (orosensory, temperature, PROP), scale use, and demographic characteristics of TT and TnT.
2. To determine the concordance between previously published TTS classification methods and implications for orosensory responsiveness patterns.
3. To characterize the orosensory and temperature responsiveness of NC to inform how they should be treated in future research.

3.2 Materials and Methods

3.2.1 Participants

A convenience sample of 815 participants was recruited from Brock University and the surrounding community in eleven recruitment drives ("cohorts"). Incentive for participation was offered in the form of entry into a monetary/gift card draw or participation credit towards select courses. Informed consent was obtained from all individual participants and all procedures were cleared by the Brock Research Ethics Board. 107 participants were excluded for failing to appropriately use scales during training (see "scales" section for further details).

A final sample size of 708 participants was retained with a mean age of 25.5 years +/- 9.6 SD (range: 17-75). The sample consisted of 223 males, 484 females and 1 individual of undisclosed gender. Ethnicity was assessed according to the method in Bajec & Pickering (2008); 550 participants identified as Caucasian, 155 identified as Non-Caucasian (29 Chinese, 19 South Asian, 10 South East Asian, 26 Black, 5 Filipino, 1 Japanese, 12 Latin American, 7 Arab, 5 Aboriginal and 41 Other/Mixed Race) and 3 participants did not disclose an ethnicity. A summary of the demographic information by cohort is included in (Table S3.1).

As the sample was obtained from multiple recruitment drives performed over several years, minor differences in the methods used exist across the cohorts. These differences reflect changes in best practices, as informed by the developing sensory and

thermal tasting literature and differences in study aims across the cohorts. Differences between the methods and materials used for each cohort are summarized in the subsequent sections and in Table 3.2 and Supplementary Table 3.2.

3.2.2 Scales

Two intensity scales, the generalized Visual Analogue Scale (gVAS) and the generalized Labeled Magnitude Scale (gLMS) were used for data collection (Bartoshuk et al., 2002, 2004). All scale data was collected on paper. The gVAS is a vertical scale anchored with "NS – No Sensation" at the bottom (0 mm) and with "SE – Strongest sensation of any kind that you have ever Experienced" at the top (100 mm). In addition, the scale is divided into 4 equal segments by three marks (25 mm, 50 mm and 75 mm). The gLMS is a vertical scale anchored with "No Sensation" at the base (0 mm) and "Strongest Imaginable" at the top (100 mm). Other terms on the scale include "Barely Detectable" (1.5 mm), "Weak" (6 mm), "Moderate" (17 mm), "Strong" (35 mm) and "Very Strong" (53 mm). Participants were asked to use the anchor terms as they would in their daily life and to rate sensations on both scales by marking a single horizontal line where appropriate.

Prior to the collection of psychophysical data participants were provided with both oral and written instruction on use of the scales. All participants were trained on scale use by rating five to fifteen remembered sensations (Bajec and Pickering 2008). As no psychophysical data was collected using the gVAS for Cohort 6, no training for the gVAS was provided to them. In order to screen for appropriate scale use participants were required to rate "the pain of biting your tongue" more intensely than the "touch sensation of a pill on your tongue" on both the gLMS and gVAS. This approach assumes that the sensations are not perceptually equivalent despite being from different modalities, and follows similar approaches used with sound-related remembered sensations (Cruickshanks et al., 2009) and solutions of different concentrations (Galindo-Cuspinera et al., 2009). 107 participants from Cohorts 1-5 and 7-11 did not use the scales appropriately and were excluded. Participants from Cohort 6 did not rate the "touch sensation of a pill on your tongue" so they could not be screened for appropriate scale use. All Cohort 6 participants were included in the study despite this limitation.

Table 3.2: Summary of tastants used by each cohort.

Cohort(s)	1	2	3	4	5, 7 & 10	6	8 & 9	11
Sweet	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[b]	250 mM Sucrose[c]	250 mM Sucrose[b]	250 mM Sucrose[b]
Salty				180 mM NaCl[f]	180 mM NaCl[d]	180 mM NaCl[e]	180 mM NaCl[f]	180 mM NaCl[d]
Sour	3.25 mM Citric Acid[g]	3.33 mM Citric Acid[g]	3.25 mM Citric Acid[g]	3.33 mM Citric Acid[g]	3.25 mM Citric Acid[g]	4.47 mM Tartaric Acid[h]	3.25 mM Citric Acid[g]	3.25 mM Citric Acid[g]
Bitter	0.0275 mM Quinine monohydrochloride[a]	0.022 g/L Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]	0.022 g/L Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]	0.0255 mM Quinine Sulphate[j]	0.0275 mM Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]
Umami	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]		125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]
Metallic					1.0 mM Cupric sulfate[b]	0.3 & 3 m Iron (III) Sulfate[k]		1.0 mM Cupric sulfate[b]
Astringent	0.877 mM Aluminum Sulfate[a]	0.877 mM Aluminum Sulfate[a]	0.877 mM Aluminum Sulfate[a]			0.73 & 14.6 mM Aluminum Sulfate[a]		0.0877 mM Aluminum Sulphate[a]

[a]Sigma-Aldrich, MO, USA
[b]BioShop, ON, Canada
[c]Lantic Sugar Ltd., QC, Canada
[d]ACP Chemicals Inc., QC, Canada
[e]Windsor, QC, Canada
[f]Caledon Laboratories, ON, Canada
[g]Fisher Scientific, NY, USA
[h]Carl Roth KG, distributed by Atomergic Chemetals Corp., NY, USA
[i]SAFC Supply Solutions, MO, USA
[j]Novopharm, ON, Canada
[k]J.T. Baker, NJ, USA

3.2.3 Orosensory Responsiveness

The primary purpose of collecting orosensory responsiveness data was to familiarize participants with prototypical tastants for later identification during TTS elicitation. Table 3.2 provides a full description of the tastants and concentrations used for each cohort. All cohorts were familiarized with sweet, sour and bitter (n=708). Other oral sensations included salty (n=592, Cohorts 4-11), umami (n=580, Cohorts 1-5 & 7-11), metallic (n=362, Cohorts 5-7, 10 & 11) and astringent (n=349, Cohorts 1-3, 6 & 11). All solutions were prepared volumetrically in pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA) or distilled water and refrigerated when not in use. Solutions were discarded within 7 days (sweet, sour, salty) or 48 hours (bitter, umami). Metallic and astringent solutions were prepared within 3 hours of testing. All solutions were presented in a randomized order and at room temperature.

Initially, participants were presented with 20 ml of each solution in medicine cups or clear wine glasses labelled with the identity of the solution and asked to swish each solution on their palate for 5 s before expectorating. Participants waited a further 10 s before rating the maximum intensity of the elicited sensation on a gLMS (Cohort 6) or gVAS (Cohorts 1-5,7-11; Bajec and Pickering 2008). Participants were asked to follow the above instructions, but the exact timings were not formally monitored. Each solution was tasted in a randomized sequence and participants rinsed with filtered water (Brita, ON, Canada) prior to and after each solution but no specific interstimulus interval was enforced. In order to minimize potential carry over effects, soda crackers (Cohorts 5, 7, 10 & 11) or a 5g/L pectin solution (Cohorts 1-3, 6) were provided as palate cleansers, but no additional palate cleanser was provided to Cohort 4. Participants then repeated the above procedure using blind-coded samples presented in re-randomized order. In addition to rating the maximum intensity of the sensation elicited for each sample, they were asked to identify the sensation. If participants were unable to successfully identify the blind-coded solutions the entire procedure was repeated (Cohorts 5, 7, 10 & 11). Cohorts 1, 8 and 9 only repeated the second half of the procedure with the blind-coded samples. Cohorts 2-4 did not repeat the procedure in the event of a failure to correctly identify the orosensations. As this study used a large sample, incorrect identification of the blind-coded solutions was not used as an exclusion criterion. Differences in the protocols used to obtain orosensory

responsiveness scores necessitated the conversion of raw scores to z-scores (see "Data Treatment"). As a result, quantification of the magnitude of any differences between cohorts is not possible. However, the relative differences in orosensory responsiveness between TTS groups, especially in light of the large sample size, are nonetheless informative.

3.2.4 Thermal Taste Status Determination

Thermal stimulation was performed using a 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode). Prior to use, the thermode was rinsed with 70% ethanol (Commercial Alcohols, ON, Canada) and wrapped in a fresh piece of plastic wrap (Compliments, ON, Canada). The thermal taste eliciting procedures of Bajec and Pickering (2008) were employed. Two different cycles were used: a warming cycle and a cooling cycle. Warming cycles started at 35°C, then cooled to 15°C before final re-warming to 40°C and holding for 1 s. Since only the warming portion of the cycle was of interest, participants were asked to rate the maximum intensity of sensations during the re-warming phase of the cycle (from 15°C to 40°C). For convenience, a beep signalled the beginning of this period. Cooling cycles started at 35°C, subsequently cooling to 5°C and holding for 10 s. Since no warming occurs during this cycle, participants were asked to rate the maximum intensity of sensations through the entire cycle. For both cycles, all temperature transitions occurred at approximately 1°C/s.

Prior to collection of TTS responses, participants underwent training runs to become familiar with the temperature cycles and the thermode. Cohorts 1-5 and 7-11 rated the maximum intensity of both warming and cooling trials on gLMS when the thermode was applied to the palm and vermillion border of the bottom lip. Cohorts 1-3 performed this task in duplicate, with all other cohorts completing the task only once. Cohort 6 was familiarized with the thermode by rating the temperature and any taste sensations elicited by the thermode when applied at 37°C for 10s on the tongue tip.

Three locations on the edge of the tongue were tested for each participant: the very tip of tongue along the midline, 1 cm to the left from the midline and 1 cm to the right from the midline. A total of 12 runs was performed for each participant in two blocks. Each block consisted of 3 warming cycles (one per location) followed by 3 cooling cycles (one

per location) with no minimum interstimulus breaks between runs. A minimum 3-min break was taken between blocks. After each run, participants were provided with a new ballot with individual gLMSs labelled “heat” or “cold”, “sweet”, “salty”, “sour”, “bitter”, and “other”. Participants then rated the maximum intensity of any sensations perceived using the corresponding scales. The ballot used by participants in Cohorts 5, 7, 10 and 11 included two additional gLMSs (“umami” and “metallic”). In order to mitigate response bias, prior to testing participants were told that not all individuals will experience taste sensations, that the sensations experienced may or may not vary across runs and that the researcher was equally interested in those who do and do not perceive taste sensations (Green and George 2004; Green et al., 2005).

3.2.5 PROP Determination

Most participants (Cohorts 1-3, 5, 7-8 & 10-11) rinsed with a 10 ml aqueous solution of 3.2 mM 6-n-propylthiouracil (PROP) for 5 s prior to expectoration. After a 10 s wait, participants rated the maximum intensity of the sensation on a gLMS. PROP responsiveness was always measured at the end of a session to reduce possible carryover effects. Using the same sip and spit protocol, Cohorts 4 and 9 rinsed with 20 ml of 3.2 mM PROP and Cohort 6 rinsed with 20 ml of 0.32 mM PROP (Table S3.2).

3.2.6 Data Treatment

Normality and equality of variance (Levene’s test, $p > 0.05$) was tested for all continuous variables (data not shown). For variables where $n > 300$, Fisher’s skewness values of +/- 2 and Fisher’s kurtosis values of +/- 7 indicated that the variable was normally distributed (Kim 2013). For variables with 50 to 300 participants, a z-score of less than 3.29 for Fisher’s skewness and Fisher’s kurtosis indicated that the variable was normally distributed. Unless otherwise noted, all variables met the above assumptions. Age was right-skewed as disproportionate number of undergraduate students were recruited. Therefore, a log transformation was performed on the raw age scores. However, log(age) failed to meet the assumption of homogeneity of regression slopes required to include it as a covariate in ANCOVA. As a result, age (yrs) was binned into 4 similarly sized groups “17-19”, “20-23”, “24-30” and “31+” to allow application of parametric statistics. Unless otherwise noted, all analyses were carried out using these binned age groups.

Direct comparison of orosensory responsiveness scores was not possible due to differences in scale, tastants, stimulus concentrations, and/or the number of exposures. For all tastants, mean responsiveness scores were calculated for each participant from all replicates (labelled and blind-coded). Next, the mean scores from each cohort were converted to z-scores separately. Lastly, the z-scores for each cohort were combined for final analysis. Temperature responsiveness was averaged across all trials for each combination of location (tongue tip, tongue left, tongue right, lip and palm) and temperature cycle (warming and cooling). Temperature ratings did not meet the assumption of homogeneity of variance (data not shown); as a result, all analyses on these variables were conducted using non-parametric methods.

In cases where scale use differed across TTS, standardized orosensory and temperature responsiveness scores were used (Part 3). Individual standardization factors (gVAS and gLMS) were generated for each participant by dividing their "brightness of the sun when looking directly at it" rating by the mean rating of their cohort (Bartoshuk et al., 2002; Bajec and Pickering 2008). Raw orosensory and temperature responses were subsequently divided by the appropriate standardization factor and extreme outliers removed (any value 3x greater than the interquartile range above the 3rd quartile; (Kamerud and Delwiche 2007; Bajec and Pickering 2008). Generally, the participants who were removed scored the brightness of the sun lower than average resulting in a correspondingly low standardization factor. In turn, this disproportionately inflated their standardized taste and temperature responsiveness scores (many scores were 3+ times greater than the total scale length) which would have significantly skewed the results. Overall, for each variable 2.9-4.6% of the responses were considered outliers and removed from the data set to prevent skewing the data. Finally, as with the unstandardized data, orosensory responsiveness scores were converted to z-scores by cohort.

3.2.7 TTS and PROP Classification

TTS classification schemes differ across the literature. To allow for a comparison between the schemes, the raw TTS responses for each participant were used to populate TTS groups by applying previously reported criteria (Table 3.1). Specifically, TTS groups were derived using the classification schemes and methods of Green and George (2004), Bajec and Pickering (2008), Yang et al. (2014) and Hort et al. (2016) and will be referred

to in order of publication as Schemes A, B, C and D, respectively. As participants in Schemes C and D were only tested on the tongue tip, responses on the left or right of the tongue tip were ignored when classifying participants under these two schemes. In addition, it was assumed that the responses elicited by the 64mm^2 probe in this study are consistent with those elicited by the smaller 28.26 mm^2 probe used in Schemes C and D (Table 3.1). Scheme E, the scheme employed by Skinner et al. (2018) was not included in the analysis as it relies on three thermal elicitation replicates but only two were available in the data set.

Due to concentration and volume differences across cohorts, raw PROP responsiveness scores were not used (Table S3.2). Instead, each cohort was divided into tertiles based on mean responsiveness scores. Percentiles were calculated based on the closest observations to the 33rd and 66th percentiles and were used to separate the groups. From the least responsive to most responsive tertile, participants were categorized as hypo-tasters, medium-tasters and hyper-tasters using the approach of Pickering and Hayes (2017). Tertiles from different cohort were subsequently combined for final analysis.

3.2.8 Data Analysis

All data analysis was performed using XLSTAT Version 19.02 (Addinsoft, NY, USA) and Microsoft® Excel® for Mac 2011 (Microsoft ®, ON, Canada). The significance criterion (α) for all analyses was set at $p = 0.05$.

3.2.8.1 Part 1 – Comparison of TT and TnT

The aim of Part 1 was to verify trends in orosensory responsiveness reported in prior literature using a much larger sample size (n=708). As all previous studies have excluded NC, they were also excluded from Part 1. All analyses were conducted using TTS classification Scheme B because it is the method most frequently used in the literature. In addition, the methodology used for thermal-elicitation corresponds to Scheme B.

To test for associations with TTS, Chi-squared contingency tests were performed for categorical age, gender, ethnicity and PTS with Fisher's exact test as a post-hoc test. A Student's t-test was also performed to confirm the age difference between TT and TnT using log(age). In order to determine if scale use differed between TT and TnT, Student's t-tests were conducted on the remembered sensation "the brightness of the sun when looking directly at it" as rated on both the gVAS and gLMS.

Differences in orosensory responsiveness were examined using Student's t-test with z-scores as the dependent variables and TTS as the independent variable. In order to account for possible confounding variables, four-way ANOVAs including all two-way interactions were performed on orosensory z-scores, using TTS, categorical age, ethnicity and gender as independent variables. In order to improve the power of the model, non-significant interaction terms were excluded and the analyses re-run. Means in all ANOVAs were examined using Tukey's HSD. To assess the relative effect of age, gender, ethnicity and PTS compared to TTS on orosensory responsiveness, Eta-squared values were derived from a five-way ANOVA with all two-way interactions included. The effect size is considered small, medium or large, when Eta-squared values exceed 0.01, 0.06 or 0.140, respectively (Lakens 2013). Differences in temperature responsiveness between TT and TnT were tested using Mann-Whitney U tests with critical values of 13775 and 19947 for non-lingual (palm/lip) ratings and tongue ratings (middle, left, right), respectively.

3.2.8.2 Part 2 – Comparison of Classification Schemes

The aim of Part 2 was to determine if differences in orosensory responsiveness between TT and TnT are similar regardless of the classification scheme employed. In order to compare TTS classification schemes, inter-judge concordance in the assignment of participants as TT, TnT and NC was assessed using Fleiss' kappa (κ). The analysis was repeated using only Schemes B, C & D to determine if concordance improved for these schemes in which NC were allowed. Finally, pairwise comparisons of all schemes using Cohen's kappa (κ) were completed to assess the concordance more closely. The quality of the agreement was deemed poor ($\kappa < 0.200$), fair ($\kappa = 0.201 – 0.400$), moderate ($\kappa = 0.401 – 0.600$), good ($\kappa = 0.601 – 0.800$) or very good ($\kappa = 0.801 – 1.0$ after Kwiecien et al. (2011). As participants' TTS could vary with classification scheme, mean orosensory responsiveness values were calculated for each scheme.

3.2.8.3 Part 3 – Characterization of NC

The aim of Part 3 was to characterize NC and to establish best practices for their classification in future studies. Scheme B was selected for TTS classification as it was the only scheme to test all three lingual locations and include a provision for the classification of NC (Table 3.1). In addition, the highest percentage of NCs is reported in Scheme B

(Pickering and Klodnicki 2016). Differences in gVAS and gLMS use between TT, TnT and NC were examined using Kruskal-Wallis for the remembered sensation "the brightness of the sun when looking directly at it. The Steel-Dwass-Critchlow-Fligner procedure was used as a post-hoc test. As scale use differed between NC and TT, standardized orosensory and temperature responsiveness data was used for this part. Differences in orosensory responsiveness between TT, TnT and NC were tested with 1-way ANOVAs with Tukey's HSD as the means separation test. Similarly, differences in temperature responsiveness were assessed using Kruskal-Wallis.

In order to further elucidate the question of whether NC are a standalone TTS group or misclassified TT or TnT, concordance between the first and second replicates of thermal elicitation was determined using Fleiss' Kappa. As concordance was only moderate for NC, the possibility that NC could be grouped into sub-categories based on their responses to thermal elicitation was examined. First, NC were divided into participants that provided at least one above "weak" taste response to thermal elicitation (NC – AW) and those with taste responses below "weak" only (NC – BWO). Second, NC were divided based on the reproducibility of a taste response during thermal elicitation. Participants who reported the same taste, at the same location regardless of intensity were defined as reproducible (NC – REP) and all others were defined as non-reproducible (NC – NoREP). Differences in orosensory and temperature responsiveness based on the intensity (TT, TnT, NC – AW, NC – BWO) and reproducibility (TT, TnT, NC – REP, NC – NoREP) of thermal responses were tested with ANOVA and Kruskal-Wallis, as described in the previous paragraph.

3.3 Results

3.3.1 Part 1 - Comparison of TT and TnT

Classification of participants using Scheme B resulted in 218 TT, 183 TnT and 307 NC (Table 3.3). With access to a large data set (n=708), the first aim was to re-examine the findings previously reported with respect to the general characteristics and orosensory responsiveness of TT and TnT. As NC have always been excluded in previous literature, only TT and TnT were tested.

Table 3.3: Summary of demographic information by thermal taster status. (AW: Non-classifiable participants (NC) with one or more above "weak" taste responses; BWO: NC with only "weak" or below taste responses; REP: NC with one or more reproducible taste responses; NoREP: NC with no reproducible taste responses.)

Thermal Taste Status	Thermal Tasters	Thermal Non-Tasters	Non-Classifiables				
			All	AW	BWO	REP	NoREP
Number	218	183	307	172	135	149	158
Age							
Mean	24.8	27.0	25.2	25.0	25.3	25.4	24.9
Standard Deviation	9.2	10.7	9.1	8.9	9.4	9.4	8.9
Minimum	18	17	18	18	18	18	18
Maximum	68	75	67	67	60	62	67
Gender							
Female	158	122	204	123	81	95	109
Male	60	61	102	48	54	54	48
Not Reported	0	0	1	1	0	0	1
Ethnicity							
Caucasian	177	153	220	123	97	115	105
Non-Caucasian	40	30	85	47	38	34	51
Not Reported	1	0	2	2	0	0	2

3.3.1.1 Age, Gender, Ethnicity and PTS

Log(Age) differed significantly between TT and TnT when tested with a Student's t-test (n=394, p[t =2.46] = 0.015). While the range of ages (years) was similar for both TT (18-68) and TnT (17-75), mean and median ages were lower for TT ($\bar{x}$ = 24.8 +/- 9.2, med = 21) than TnT ($\bar{x}$ = 27.0 +/- 10.7, med = 23). Categorical age and TTS were also significantly associated based on Chi-squared contingency analysis (n = 394, p[χ^2 = 8.54] = 0.036). Post hoc tests revealed there were significantly more TT and less TnT than expected by chance in the 17- 19 year old group, while there were fewer TT amongst 24 to 30 year olds than expected. An overall trend of TT being overrepresented and TnT underrepresented in 17-23 year olds was also observed, with the opposite trend apparent in those ≥24 years old. As the trends for categorical age and log(age) were consistent, the categorical age bins were deemed suitable for use in subsequent analyses.

Chi-square contingency tests showed no association between gender (n = 401, p[χ^2 = 1.59] = 0.207), ethnicity (n = 400, p[χ^2 = 0.29] = 0.593) or PTS (n = 401, p[χ^2 = 3.86] = 0.145) and TTS. Student's-t tests indicated that ratings of "the brightness of the sun when looking directly at it" did not differ between TT and TnT on the gVAS (n=335, p[t =

2.29]=0.389) or gLMS (n=400, p[1.62]=0.499). As a result, temperature and orosensory responsiveness were not standardized prior to further analyses.

3.3.1.2 Orosensory Responsiveness

Student's t-test showed that orosensory responsiveness differed significantly between TT and TnT for sweet (n=401, p[t = 2.45] = 0.015), sour (n=401, p[t = 2.38] = 0.018), bitter (n=401, p[t = 2.07] = 0.039), salty (n=321, p[t = 2.08] = 0.038), metallic (n=158, p[t = 2.95] = 0.004) and astringency (n=191, p[t = 1.98] = 0.049). In all cases, TT were more responsive than TnT (Figure 3.1), a trend that was mirrored for umami responsiveness (n=335, p[t = 1.75] = 0.081).

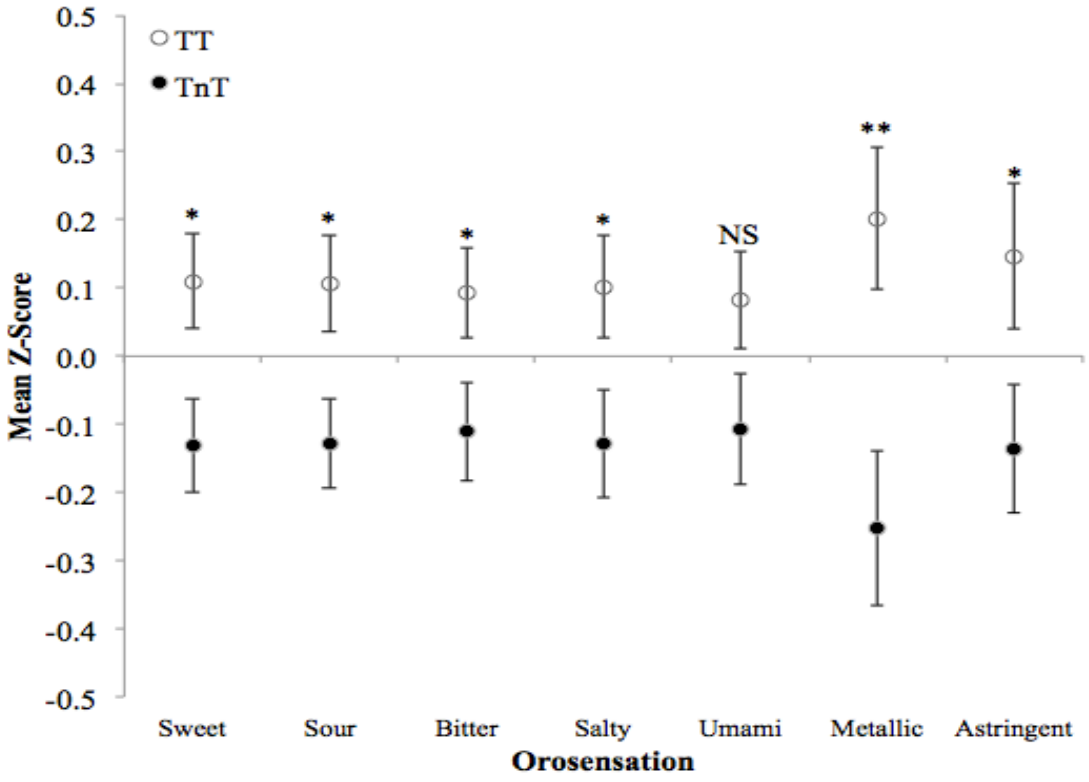


Figure 3.1: Mean orosensory responsiveness (z-score +/- standard error of the mean) of thermal tasters (TT) and thermal non-tasters (TnT) to aqueous solutions of tastants. Differences tested using Student's t test (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

The impact of age, gender, ethnicity and TTS on orosensory responsiveness was evaluated using a four-way ANOVA. TT were significantly more responsive than TnT to

sweet (n=393, p[F = 5.43] = 0.020), sour (n=393, p[F = 4.65] = 0.028) and metallic (n=153, p[F = 5.57] = 0.011) stimuli. Females were more responsive to sour than males (n=393, p[F = 4.31] = 0.035). No other main effects for age, gender, ethnicity or TTS were found. There were no significant 2-way interactions except for an age*gender effect for metallic responsiveness (n=153, p[F = 14.72] = 0.001), which was higher for males than females aged 17-19, 24-30 and 31+, but lower for males than females aged 20-23.

Eta-squared values for age, gender, ethnicity, TTS and PTS are summarized in Table 3.4. Effect sizes were greatest for PTS with medium effects calculated for *salty* and *astringent*, and small effects for other orosensations. In addition, small effect sizes were found for TTS (*sweet, sour,* and *metallic*), age (*salty, umami, metallic* and *astringent*), gender (*sour)* and ethnicity (*metallic*). The relative order of effect sizes for TTS, age, gender and ethnicity varied with orosensation.

Table 3.4: Eta-squared values showing the relative effect of age, gender, ethnicity, thermal taster status (thermal tasters vs. thermal non-tasters; TTS) and PROP taster status (PTS) derived from ANOVA.

Factor	Eta-Squared (η^2)						
	Sweet	Sour	Bitter	Salty	Umami	Metallic	Astringent
Age	0.003	0.009	0.005	0.014	0.017	0.051	0.026
Gender	0.005	0.010	0.000	0.001	0.000	0.000	0.009
Ethnicity	0.001	0.004	0.002	0.001	0.002	0.024	0.002
TTS	0.011	0.010	0.007	0.004	0.004	0.019	0.008
PTS	0.034	0.012	0.017	0.088	0.044	0.042	0.061

3.3.1.3 Temperature Responsiveness

Mann-Whitney U tests showed that non-lingual temperature responsiveness differed significantly between TT and TnT when the palm was warmed (n=335, p[U = 15502.5] = 0.049) and cooled (n=335, p[U = 16460.0] = 0.002) and when the lip was cooled (n=335, p[U = 17076.0] = 0.0002). In all cases, TT were more responsive than TnT (Figure 3.2), a trend that was also observed with warming of the lip. Similarly, TT were more responsive for thermal stimulation of the tongue) when the tip (n=401, p[U = 16210.5] = 0.001), the right (n=401, p[U = 15888.5] = 0.0004), and the left was warmed (n=401, p[U = 16425.5] = 0.002), and when the tip (n=401, p[U = 16805.5] = 0.007), the right (n=401, p[U = 16034.0] = 0.001) and the left was cooled (n=401, p[U = 16743.5] = 0.006) (Figure 3.2).

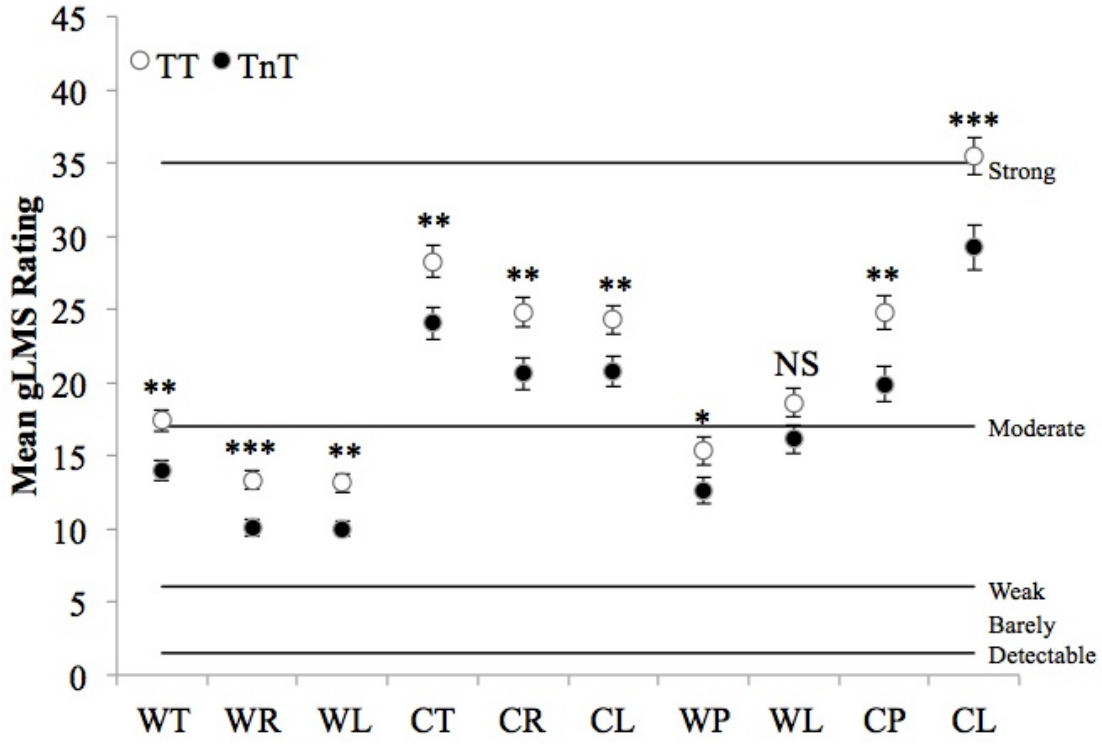


Figure 3.2: Mean temperature responsiveness (+/- standard error of the mean) of thermal tasters (TT) and thermal non-tasters (TnT) after warming (W) or cooling (C) cycles on the palm (P), lip (L) and tongue (T=tip, R=right, L=left). Vertical lines indicate the position of anchor terms on the gLMS. Differences tested using Mann-Whitney U test (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

3.3.2 Part 2 – Comparison of Classification Schemes

The proportion of participants classified as TT, TnT and NC differed between the classification schemes (Figure 3.3). Overall concordance was moderate, ($\kappa = 0.406$) with a total of 219 participants (31%) being consistently classified as TT (8%), TnT (23%) or NC (0%) in all classification schemes. Agreement between the schemes was higher for TT ($\kappa = 0.554$) and TnT ($\kappa = 0.456$) than for NC ($\kappa = 0.234$). The lower concordance for NC is attributable at least in part to the absence of a NC grouping in Scheme A, leading us to assess the concordance between the other schemes. 454 participants (64%) were consistently classified under Schemes B, C and D, resulting in good overall concordance ($\kappa = 0.618$). Concordance was improved for TnT and NC but was reduced for TT. Participants whose TTS was not consistent under Schemes B, C and D were represented as TT/NC (26%), TnT/NC (7%), TT/TnT (2%) and TT/NC/TnT (1%). Pairwise comparisons

showed that agreement between schemes ranged from poor (A & C), to fair (A & B; A & D), to moderate (B & C) to good (B & D; C & D), with full details given in Table 3.5. As the level of concordance differed across the schemes, mean orosensory responsiveness was calculated (Figure 3.4). Mean z-scores were positive for TT and negative for TnT for all orosensations under all schemes; regardless of scheme or orosensation, TT were more responsive than TnT.

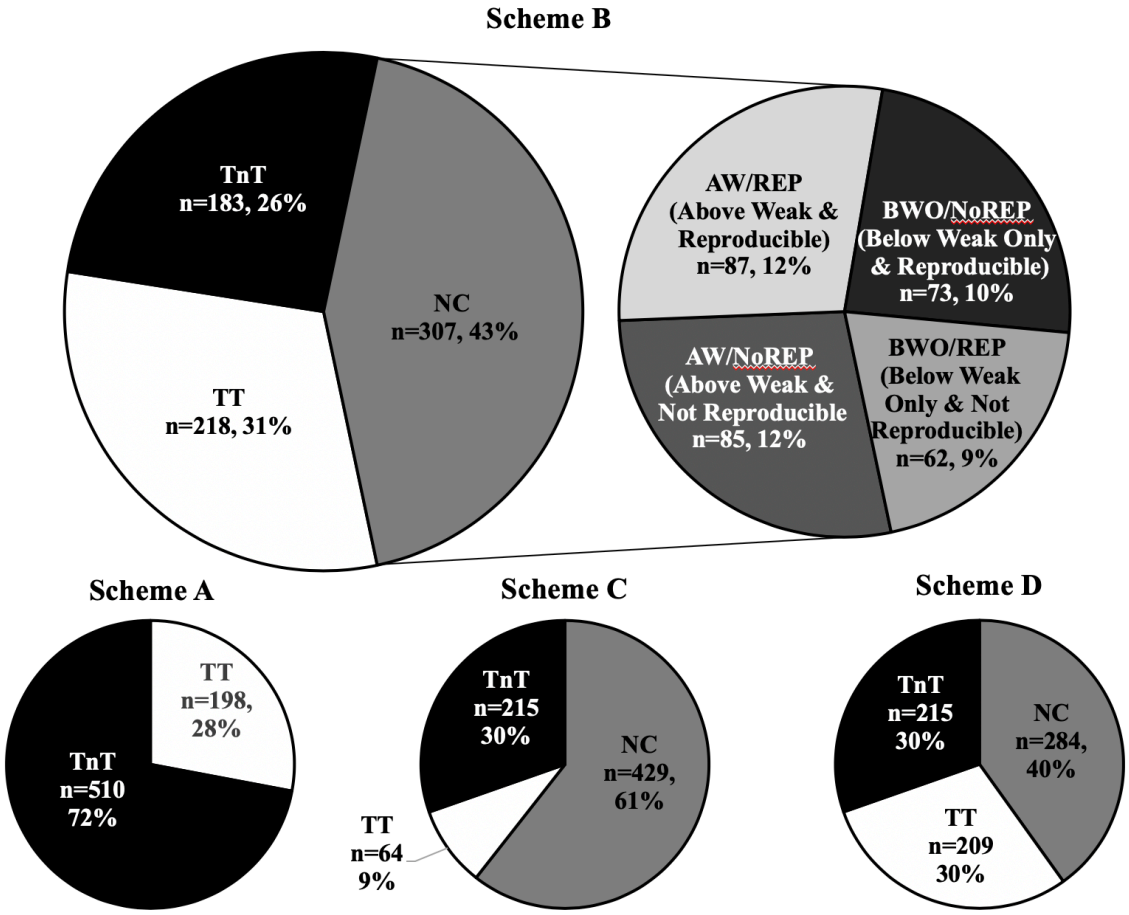


Figure 3.3: Thermal taster status abundances for all classification Schemes (A = Green and George, 2004; B = Bajec and Pickering, 2008; C = Yang et al., 2014; and D = Hort et al., 2016). Groups include thermal tasters (TT), thermal non-tasters (TnT) and non-classifiable participants (NC). NC were divided into four subgroups; AW/REP – NC with at least one above "weak" and one reproducible taste response during thermal stimulation, AW/NoREP – NC with at least one above "weak" taste response but no reproducible taste response during thermal stimulation, BWO/REP – NC with no above "weak" taste response but at least one reproducible taste response during thermal stimulation, and BWO/NoREP – NC with no above "weak" or reproducible taste response during thermal stimulation.

Table 3.5: Concordance between classification Schemes (A = Green and George, 2004, B = Bajec and Pickering, 2008, C = Yang et al., 2014; and D = Hort et al., 2016) and level of agreement (VG = very good, good, Mod = moderate, fair, poor). TT = Thermal tasters, TnT = Thermal non-tasters, NC = Non-classifiables.

Schemes	Overall Percent Match*	Kappa (κ)				Level of Agreement			
		Overall	TT	TnT	NC	Overall	TT	TnT	NC
A & B	54%	0.365	0.932	0.238	0.000	Fair	VG	Fair	Poor
A & C	36%	0.156	0.319	0.227	0.000	Poor	Fair	Fair	Poor
A & D	49%	0.274	0.610	0.227	0.000	Fair	Good	Fair	Poor
B & C	69%	0.512	0.324	0.763	0.439	Mod	Fair	Good	Mod
B & D	79%	0.677	0.661	0.763	0.620	Good	Good	Good	Good
C & D	80%	0.679	0.384	1.000	0.607	Good	Fair	VG	Good
B, C & D	64%	0.618	0.457	0.844	0.547	Good	Mod	VG	Mod
A, B, C, D	30%	0.406	0.554	0.456	0.234	Mod	Mod	Mod	Fair

*Percentage of participants classified in the same group across the tested classification schemes.

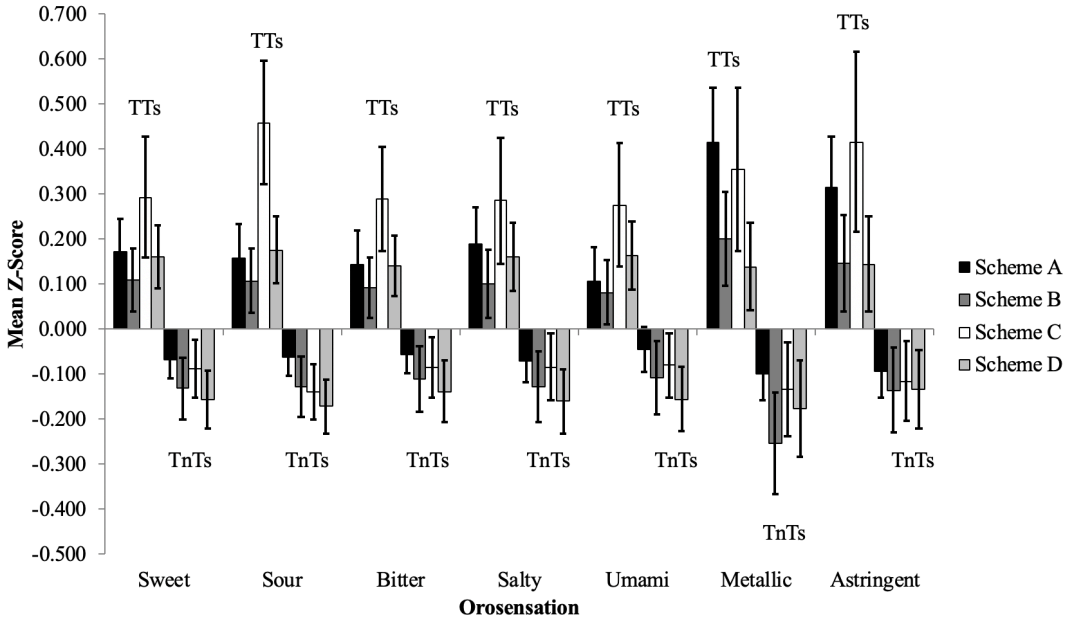


Figure 3.4: Mean orosensory responsiveness (z-score +/- standard error of the mean) of thermal tasters (TT) and thermal non-tasters (TnT) for all classification schemes (A = Green and George, 2004, B = Bajec and Pickering, 2008, C = Yang et al., 2014; and D = Hort et al., 2016).

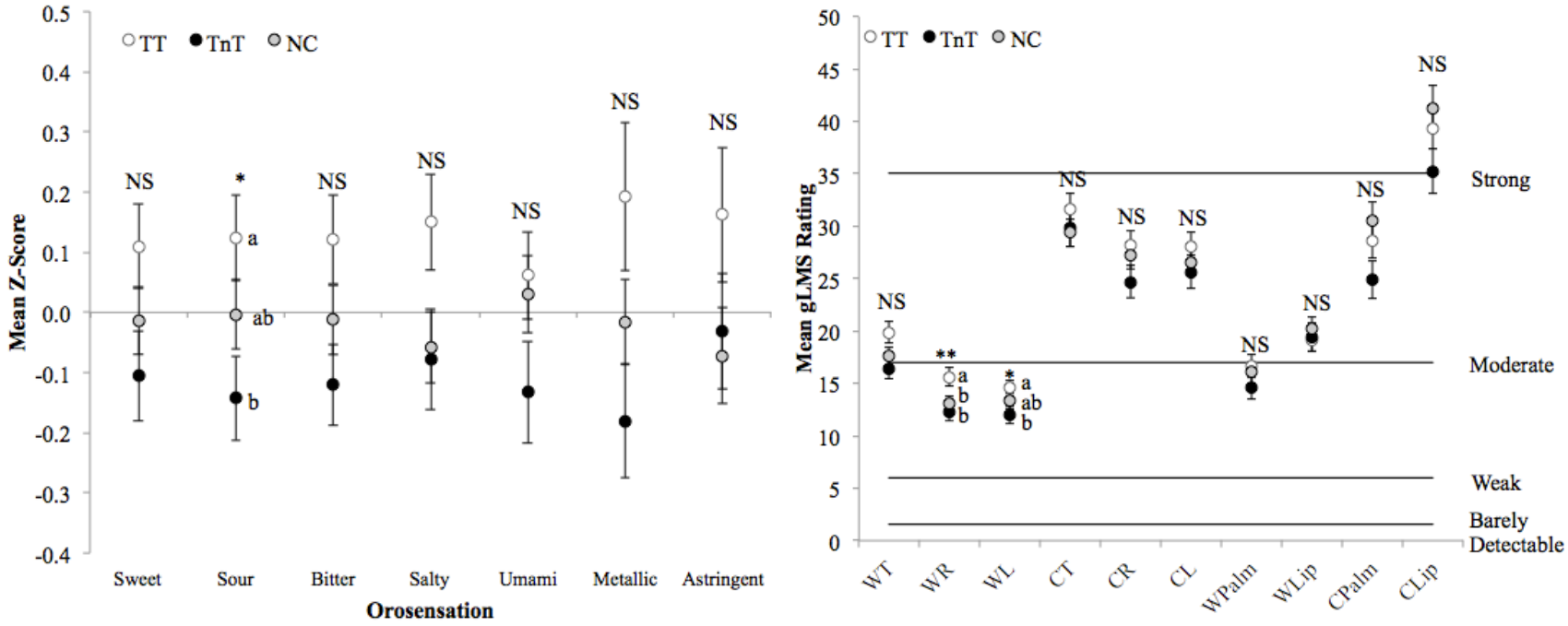
3.3.3 Part 3 – Characterization of NC

Demographic characterization of NC using Scheme B showed similar proportions of males (n=102) and females (n=204) to that observed for TT and TnT (Table 3.3). In contrast, the proportion of Non-Caucasians was higher for NC (28%) than TT (18%) and TnT (16%). While the age range of NC (18-67yrs) was similar to TT and TnT, the mean and median ages of NC ($\tilde{x}$ = 25.2 +/- 9.1, med = 22) was intermediate to TT and TnT. ANOVA indicated that ratings of "the brightness of the sun when looking directly at it" differed significantly by TTS for the gVAS (n=580, p[F = 8.18]=0.017) and approached significance for the gLMS (n=707, p[F = 5.84]=0.054). Post-hoc test tests indicated NC scale use was lower than TnT on the gVAS and lower than TT on the gLMS. In addition, even when not significant, the mean rank and mean ratings of NCs was lower than TT and TnT.

The mean orosensory responsiveness of NC was between that of TT and TnT for all orosensations except astringency where NC had the lowest responsiveness (Figure 3.5a). TT ratings for sour were significantly higher than those of TnT (n=686, p[F=3.51]=0.030), while sweet (n=687, p[F=2.35]=0.096), bitter (n=684, p[F=2.89]=0.056), salty (n=570, p[F=2.87]=0.058), and metallic (n=353, p[F =2.76]=0.064) approached significance.

The relative order of temperature responsiveness varied between TT, TnT and NC (Figure 3.5b). In all cases except the warming of the lip, TT were more responsive than TnT. Kruskal-Wallis demonstrated that significant differences were found for warming of the right (n=691, p[K=11.34]=0.003) and left (n=686, p[K=7.71]=0.021) of the tongue. Post hoc tests showed TT were significantly more responsive than TnT for both locations but TT were only more responsive than NC on the right side. Differences in temperature responsiveness approached significance for the warming of the tongue tip (n=678, p[K=5.38]=0.068) and the cooling of the right side of the tongue ((n=686, p[K=5.31]=0.070).

When participants are tested for thermal taste, two replicates of the thermal elicitation procedure are required. Each replicate was treated as a separate trial to determine the concordance. Overall concordance was good ($\kappa = 0.468$) with a total of 550 participants (78%) being consistently classified as TT (n=275, 39%), TnT (n=183, 26%) or NC (n=92, 13%). Agreement between the replicates was higher for TT ($\kappa = 0.674$) and TnT ($\kappa = 0.765$)



(a) (b)

Figure 3.5: (a) Mean orosensory responsiveness (z-score +/- standard error of the mean) of thermal tasters (TT), thermal non-tasters (TnT) and non-classifiables (NC) to aqueous solutions of tastants. Differences tested by ANOVA. (b) Mean temperature responsiveness (+/- standard error of the mean) of TT, TnT and NC after warming (W) or cooling (C) cycles on the palm, lip and tongue (T=tip, R=right, L=left). Vertical lines indicate the position of anchor terms on the gLMS. Differences tested using Kruskal-Wallis. Means with different letters differ (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

than for NC ($\kappa = 0.468$). The remaining participants were classified as TT/NC (n=87, 12%), TnT/NC (n=43, 6%) or TT/TnT (n=28, 4%).

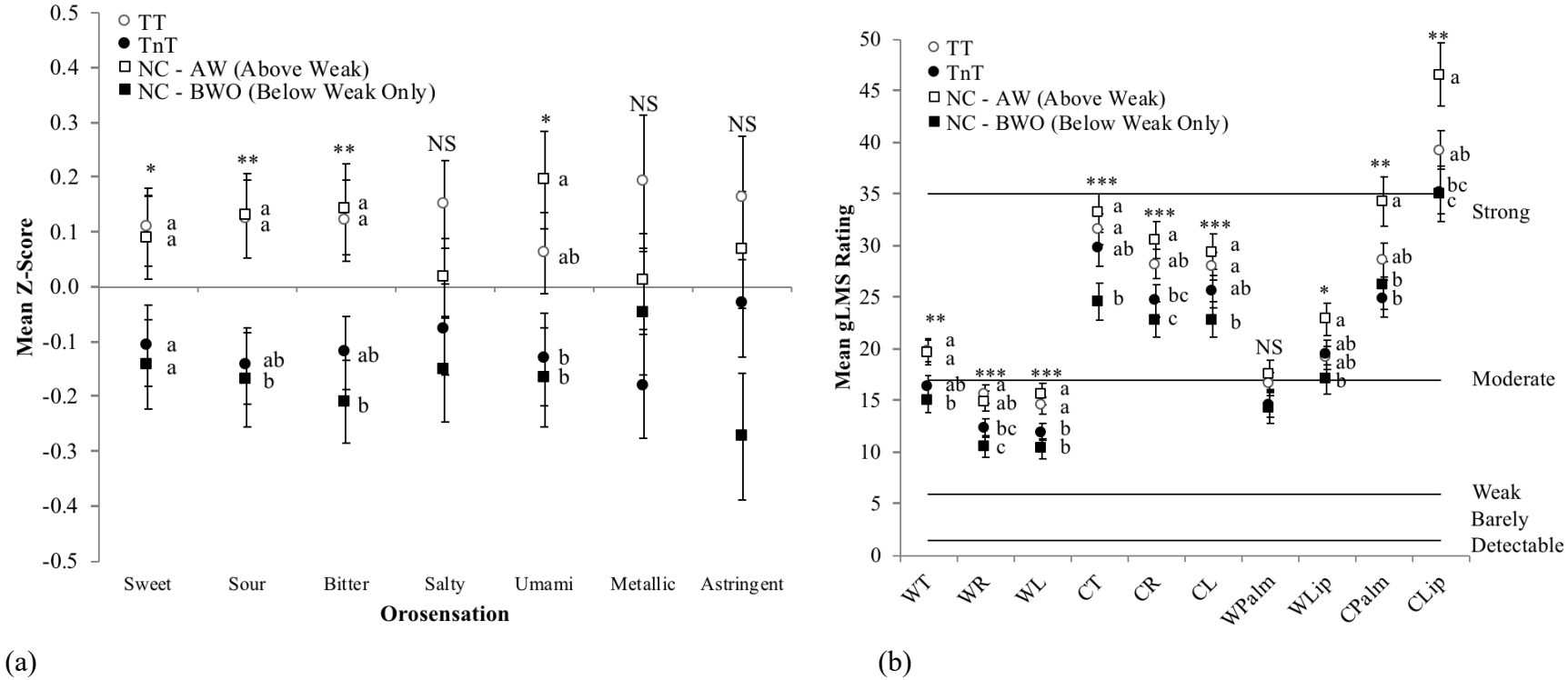
3.3.3.1 Importance of Intensity in Thermal Taste Responses

The relevance of the intensity rating of taste(s) elicited during thermal stimulation on orosensory and temperature responsiveness in NC was assessed by dividing them into two groups; NC-AW (n=172) and NC-BWO (n=135). NC-AW were participants that had provided a minimum of one above "weak" thermal taste response during either replicate while NC-BWO provided ratings of below "weak" for both replicates. Two response scenarios qualified NC for a NC-AW categorization. They either gave an above "weak" response during one of the replicates (n=115), or above "weak" responses during both replicates but for different orosensations or locations (n=57). Demographic details of NC-AW and NC-BWO are provided in Table 3.3.

In general, the orosensory responsiveness of NC-AW mirrored that of TT and that of NC-BWO mirrored that of TnT (Figure 3.6a). ANOVA showed that orosensory responsiveness between the four groups differed significantly for sweet (n=677, p[F=2.92]=0.033), sour (n=676 p[F=4.62]=0.003), bitter (n=684, p[F=5.07]=0.002) and umami (n=560, p[F=3.78]=0.010), while salty (n=570, p[F=2.52]=0.057) and astringent (n=339, p[F =2.56]=0.055) approached significance. Similar to orosensory responsiveness, a trend of NC-AW rating temperature as more intense than NC-BWO was observed (Figure 3.6b). Kruskal-Wallis demonstrated significant differences for warming of the lip (n=558, p[K=8.89]=0.031), tongue tip (n=678, p[K=14.1]=0.003), right side of the tongue (n=691, p[K=27.70]<0.001) and left side of the tongue (n=686, p[K=27.63]<0.001), as well as the cooling of the palm (n=554, p[K=13.93]=0.003), lip (n=560, p[K=16.22]=0.001), tongue tip (n=684, p[K=18.657]<0.001), right side of the tongue (n=686, p[K=19.58]<0.001) and left side of the tongue (n=686, p[K=17.87]<0.001).

3.3.3.2 Importance of Reproducibility in Thermal Taste Responses

In addition to intensity, in most studies the taste reported during thermal stimulation must be reproducible in order for an individual to be classified as a TT. Therefore, the impact of requiring an individual to report a reproducible sensation during thermal elicitation was tested using the temperature and orosensory responsiveness of NC,



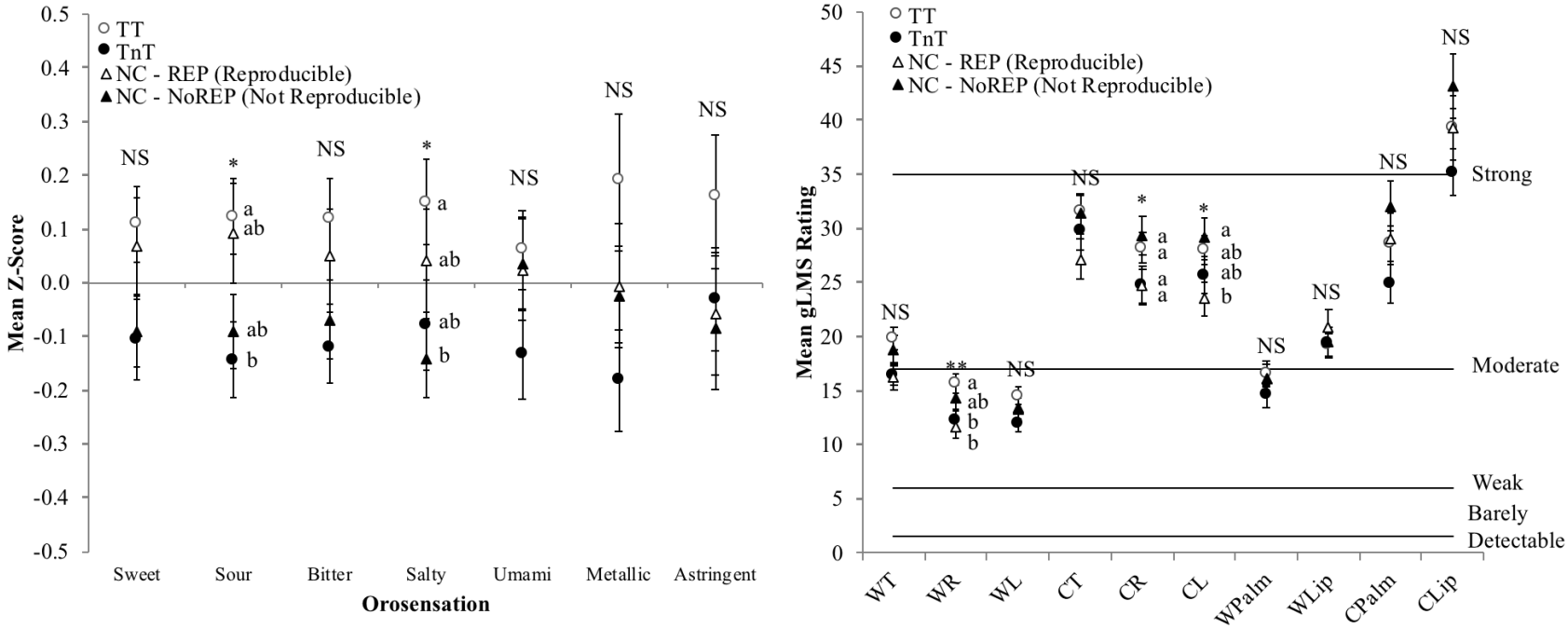
(a) (b)

Figure 3.6: Mean orosensory responsiveness (z-score +/- standard error of the mean) to aqueous solutions of tastants of thermal tasters (TT), thermal non-tasters (TnT), non-classifiable participants with one or more taste responses above "weak" during thermal stimulation (NC-AW) and non-classifiable participants with no taste responses above "weak" during thermal stimulation (NC-BWO). Differences tested by ANOVA. (b) Mean temperature responsiveness (+/- standard error of the mean) of TT, TnT, NC-AW and NC-BWO after warming (W) or cooling (C) cycles on the palm, lip and tongue (T=tip, R=right, L=left). Vertical lines indicate the position of anchor terms on the gLMS. Differences tested using Kruskal-Wallis. Means with different letters differ (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Differences tested using Kruskal-Wallis. Means with different letters differ (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

regardless of the intensity of sensation experienced. NC were divided into those who reported the same taste quality at the same location during both thermal elicitation sessions (NC-REP; n=149), and all other NC (NC-NoREP; n=158). Three response scenarios qualified NC for a NC-REP categorization. Participants could report reproducible taste sensations that were below 'weak' on the gLMS for both replicates (n=78), the intensity of the replicates could vary with one replicate above 'weak' and one below 'weak' (n=30) or a combination of both (n=41). Demographic details of NC-REP and NC-NoREP are provided in Table 3.3.

In general, for familiar orosensations (sweet, sour, bitter and salty) the responsiveness of TT and NC-REP, and of TnT and NC-NoREP were similar (Figure 3.7a). For the more unfamiliar orosensations (umami, metallic and astringent) the responsiveness of NC-REP and NC-NoREP was similar to each other. ANOVA showed that orosensory responsiveness between the four groups differed significantly for sour (n=686 p[F=3.20]=0.023) and salty (n=570, p[F=2.65]=0.048). While no significant difference was found for umami, metallic or astringency, sweet (n=687, p[F=2.20]=0.087) and bitter (n=684, p[F=2.28]=0.078) approached significance. In contrast to orosensory responsiveness, a trend of TT and NC-NoREP, and TnT and NC-REP rating temperature responses similarly was found (Figure 3.7b). Significant differences exist between the four groups using Kruskal-Wallis for warming (n=691, p[K=13.18]=0.004) and cooling of the right side of the tongue (n=686, p[K=9.76]=0.021) and for cooling of the left side of the tongue (n=686, p[K=9.88]=0.020). No differences were found for the warming and cooling of the palm, and the cooling of the lip.

When reproducibility and intensity are used together to classify NC, similar sample sizes are obtained: NC-AW/REP (n=87), NC-AW/NoREP (n=85), NC-BWO/REP (n=62) and NC-BWO/NoREP (n=73, Figure 3.3).



(a) (b)

Figure 3.7: (a) Mean orosensory responsiveness (z-score +/- standard error of the mean) to aqueous solutions of tastants of thermal tasters (TT), thermal non-tasters (TnT), non-classifiable participants with one or more reproducible taste responses during thermal stimulation (NC-REP) and non-classifiable participants with no reproducible taste responses during thermal stimulation (NC-NoREP). Differences tested by ANOVA. (b) Mean temperature responsiveness (+/- standard error of the mean) of TT, TnT, NC-REP and NC-NoREP after warming (W) or cooling (C) cycles on the palm, lip and tongue (T=tip, R=right, L=left). Vertical lines indicate the position of anchor terms on the gLMS. Differences tested using Kruskal-Wallis. Means with different letters differ (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

3.4 Discussion

3.4.1 Part 1 – Comparison of TT and TnT

3.4.1.1 Age, Gender, Ethnicity, and PTS

TT were on average 2.2 years younger than TnT. To our knowledge, this is the first report of an age difference; previous studies that have examined the relationship between TTS and age found no effect (Bajec and Pickering 2008; Pickering and Kvas 2016). As these previously published results were based on data from Cohorts 6 and 8, it is likely that the small but significant difference in age was not found in those studies due to lack of power in the analyses. As only 19% of the sample was above the age of 30, targeted recruitment of participants over 30 years in future studies would aid in determining the extent and ecological validity of this result. In the interim, this finding suggests that age should be controlled for in analysis of TTS data, by for instance, including it as a co-variate. This may be particularly salient when examining relationships between TTS and other factors known to vary with age, such as sensory sensitivity and responsiveness (Mojet et al., 2001).

No differences in gender were found between TT and TnT, consistent with several other reports (Bajec and Pickering 2008; Pickering et al., 2010b; Pickering and Kvas 2016), including one study from outside the authors' lab using independently obtained data (Yang et al., 2014). This finding suggests that the mechanisms underpinning thermal tasting may be independent of gender. No difference in the proportion of TT and TnT was found between Caucasian and non-Caucasians. While on the surface this suggests that ethnicity and TTS are not associated, it is possible that by grouping of all non-Caucasian participants into a single category may be masking potential relationships.

No association between PTS and TTS was found, in agreement with the trends reported in other studies from the authors' lab (Bajec and Pickering 2008, 2010; Pickering et al., 2010b; Bering et al., 2013), and independent data (Yang et al., 2014; Hort et al., 2016). Additionally, this null result is consistent with genetic data, with Bering et al. (2013) finding no relationship between TTS and the TAS2R38 and Gustin genotypes that are associated with PROP responsiveness (Duffy et al., 2004; Calo et al., 2011). In contrast

when ratings are treated as continuous, PROP was rated as more intense by TT than TnT (Green and George 2004). The inconsistency may be due to TTS classification differences between studies. While Green & George (2004) combined NC and TnT, most other studies have excluded NC. It is possible that the reduced responsiveness to PROP by TnT may be driven by NC, a hypothesis that should be investigated in future studies.

3.4.1.2 Scale Use and Orosensory Responsiveness

The finding that TT and TnT use scales in a similar way agrees with Green and George (2004), confirming that scale use differences do not account for the variation in orosensory responsiveness between these phenotypes. TT were more responsive to supra-threshold orosensations than TnT, consistent with published results for Cohort 6 (Bajec and Pickering 2008) and independently obtained data (Green and George 2004; Green et al., 2005; Yang et al., 2014; Hort et al., 2016). Therefore, previous findings that have trended in this direction but failed to reach significance (Green et al., 2005; Yang et al., 2014; Hort et al., 2016) have likely simply been underpowered. The lack of a significant difference between TT and TnT for umami responsiveness is unexpected. The lack of a TTS effect cannot be explained by low stimulus intensity as all tastants were approximately equi-intense (data not shown). However, as the means +/- SE do not overlap, TT have a positive z-score and TnT have a negative z-score, this finding does not contradict the overall trend. These findings provide strong support for the hypothesis that TT have an orosensory advantage over TnT for suprathreshold orosensory stimuli. Despite the fact that variation in orosensory perception influences our relationship with food (e.g. preferences, intake, emotional responses) and diet-related outcomes (reviewed in Garcia-Bailo et al., 2009; Hayes, et al., 2013; Tepper, 2008), our understanding of the impact of TTS remains limited.

In the 4-way ANOVAs, the lack of significant interactions between TTS and gender, ethnicity or PTS further support the independence of each variable from TTS. It is possible that the TTS*Gender interaction in the perception of salty and TTS*Ethnicity interaction of astringency in the perception reported by Bajec & Pickering (2008) were Type I errors.

Eta-squared values are useful because they calculate the proportion of variation within a data set than can be explained by group membership for each factor (ie. when η^2=0.10, 10% of the variation is explained by the corresponding factor; Lakens 2013). In this study, PTS has a larger effect on orosensory responsiveness than TTS, confirming the

trend previously reported for Cohort 6 (Bajec and Pickering 2008). It is also noteworthy that no large effects were reported, and that total variation for each orosensation explained by all variables ranged from 3% - 11%. Together, these findings suggest that additional factors are needed to fully account for variation in orosensory responsiveness, as well as highlighting the complexity of orosensory perception.

3.4.1.3 Temperature Responsiveness

The greater responsiveness of TT to lingual temperature changes is consistent with previous results from Cohort 6 (Bajec and Pickering 2008) and independent data (Hort et al., 2016). In contrast with Green and George (2004), TT were also more responsive to warming and cooling at two non-lingual sites. The conflicting results can likely be attributed to differences in the definition of TnT or sample size. If the difference in temperature responsiveness is generalizable to other parts of the body, it may provide indirect evidence for the role of TRPM5 in mediating thermally-induced sweetness. Additionally, these finding may be explored further in a wider examination of how temperature-sensing mechanisms and associated genes not connected with taste perception may vary with TTS. Given that the warming and cooling cycles used in these protocols may also elicit pain (Green 2004), the potentially confounding influence of pain pathways should also be considered in interpreting these results.

The coolness elicited by cold/frozen food when taken into the mouth and the re-warming of the tongue after it is swallowed may be similar to the temperature changes experienced during the thermal elicitation procedures. This raises the possibility that TT may experience thermally-induced tastes during the consumption of cold or frozen products. This speculation should be investigated further, including the possibility that thermal taste(s) elicited by cold foods and beverages may associate with liking and consumption. Similarly, TT may experience thermally-induced tastes when eating warm or hot products. The maximum temperature used in thermal elicitation protocols is close to body temperature (40°C) and below the serving temperature for many warm or hot products. Therefore, more research is required to determine if TT and TnT react similarly to these products. It remains to be determined what effect the advantage experienced by TT from warming and cooling areas outside the oral cavity has under more ecologically valid contexts outside of eating and drinking.

3.4.2 Part 2 – Comparison of Classification Schemes

As expected, concordance is highest between schemes that use similar classification approaches. Scheme A is most concordant with Scheme B as both tested for a thermal elicitation response at three lingual locations and required the same thermally induced taste to be reported in duplicate trials. Similarly, Schemes C and D, which only tested one location but did not require the same thermally-induced taste sensation to be reported, were most concordant. Testing three locations led to the identification of more TT (198-218 vs 64-209) but fewer TnT (183 vs 215). Thus, testing three locations on the tongue in future studies should reduce the risk of a TT being classified as a NC or TnT, and reduce the false identification of TT or NC as TnT. However, the current study did not take into account the size of the probe used for thermal stimulation. Most studies, including this one, tested participants in 3 locations using a 64 mm^2 probe, while others tested one location (tip) using a 28.26 mm^2 probe (Yang et al., 2014; Hort et al., 2016). It is possible that the larger 256 mm^2 probe used by Skinner et al. (2018) and Yang et al. (2018) simultaneously stimulated more than one of the three lingual sites traditionally examined separately during testing. Further study is required to determine if the same level of discrimination between TTS groups can be achieved with the larger probe.

It is currently unclear if the mechanism underlying thermal taste during warming and cooling cycles is the same. TRPM5 may be associated with sweet thermal tasting during warming (Talavera et al., 2005), suggesting that the mechanism may be specific to individual tastes and/or temperature regimes. Until the mechanism(s) underlying thermal tasting is well understood, requiring participants to report a thermal taste during *both* warming and cooling cycles in order to qualify as a TT (e.g. Scheme C) is not recommended. The inclusion of NC in classification procedures (rather than dumping them in with TnT) greatly increased the concordance between schemes and is also recommended in future studies. As TT were more responsive to orosensory stimuli under all schemes, the requirement for TT to report the same taste sensation in replicate trials may not be necessary. Further research is required to determine if TT are a homogenous group but is beyond the scope of this paper. Orosensory responsiveness patterns for TT and TnT were similar across Schemes A, B, C and D, giving confidence that the main findings from studies on orosensation and TTS that appear in the literature to date can be compared.

3.4.3 Part 3 – Characterization of NC

To the best of our knowledge, this is the first paper to characterize NC because it is difficult to interpret their data. Early publications based on Scheme A did not include NC, instead all participants who were not classified as TT were classified as TnT. This definition of TnT is problematic because a subset of individuals do in fact report experiencing thermally induced tastes that are low in intensity or not reproducible. As such, these individuals were re-classified as NC in subsequent studies and excluded, in order to produce TnT groups with homogenous response patterns (Bajec and Pickering 2008). NC should be included in future studies as they represented up to 60% of individuals within this study. Also, including NC may assist in understanding the mechanisms underlying thermal taste.

A higher proportion of NC were non-Caucasian than compared to TT and TnT. As the non-Caucasian group was highly diverse, the implications of this result remain unclear and further study is required. The trend of NC being intermediate in age, orosensory responsiveness and temperature responsiveness to TT and TnT may suggest that NC represent a distinct phenotypical group. More likely, the trend may suggest that NC are misclassified TT and TnT for whom averaged data yields intermediate responses.

Interestingly, NC rated the "brightness of the sun" significantly lower than TT on the gLMS, despite the fact that the scale is generalized. More research is required to determine how standardizing the thermally elicited responses, which are collected on the gLMS, would impact the proportions of individuals classified at TT and NC.

During thermal elicitation, two replicates are used to classify participants. In order to determine if one replicate is sufficient to determine TTS, each replicate was treated as independent. Overall concordance was good ($\kappa = 0.468$) but 22% of participants were classified into different groups across the two replicates. In addition, 4% of participants were classified as TT during both replicates when considered separately but would be considered NC when the replicates are combined under classification schemes where the same taste sensation needs to be reported across trials. In order to avoid the misclassification of participants, the use of a minimum of two replicates to determine TTS is recommended.

3.4.3.1 Importance of Intensity & Reproducibility in Thermal Taste Responses

In most classification schemes, a valid thermal taste response is obtained when participants rate the intensity of a taste above "weak" on the gLMS. When included as a requirement for TT, the minimum intensity offers greater confidence that the taste response reported is real rather than a response bias by the participant. However, Skinner et al. (2018) argued that the choice of "weak" as a cut-off point was arbitrary as it was not based on empirical evidence. Furthermore, when this requirement was removed by Skinner et al. (2018), 5% of TT identified would otherwise have been NC. To examine the effect of how this minimum score criterion ('threshold') influences TTS classification, NC were divided into participants that experience an above threshold response (NC-AW) and those that do not (NC-BWO), regardless of the reproducibility of their scores. Interestingly for both temperature and orosensory responsiveness, NC-AW were more responsive than NC-BWO, suggesting that NC-AW and NC-BWO may be misclassified TT and TnT, respectively.

Two replicates of thermal taste elicitation are performed during testing. While TT and TnT are consistently classified in both trials, NC may or may not be consistent in both trials. Under Schemes A, B & E, a reproducible thermal taste occurs when the same orosensation is reported at the same location during the same temperature regime. This is a requirement for TT as reproducibility offers greater confidence that the thermal taste reported is real. Following this logic, NC were divided into NC-REP and NC-NoREP, regardless of the intensity of the sensation. While NC-REP were more responsive than NC-NoREP to orosensations, the opposite trend was observed for temperature responsiveness, response patterns not typical of miscategorised TT and TnT.

Together, the differences in orosensory and temperature responsiveness of the four NC subgroups confirm that NC cannot be considered a homogenous group. While some NC subgroups had similar responsiveness patterns to TT and TnT (NC-AW & NC-BWO), other did not (NC-REP & NC-NoREP). The contrasting results likely suggest that some NCs are misclassified. Further analysis and testing would be required to validate any proposed NC subgroups and would be greatly aided by a better understanding of the mechanism(s) underlying thermal taste. Ideally, four groups of NC would be maintained in future research; NC-AW/REP, NC-AW/NoREP, NC-BWO/REP, and NC-BWO/NoREP.

As NC are heterogeneous in their orosensory responsiveness, the homogeneity of TT should also be investigated, but is beyond the scope of this study.

Based on these findings, it appears acceptable to increase sample sizes in future studies by combining NC-AW with TT and NC-BWO with TnT, but this should be limited to investigations aimed at understanding orosensory or temperature responsiveness. Ideally, data would be analyzed using all four groups first, and only combined if the NC-AW and NC-BWO response patterns are similar to those of TT and TnT, respectively. Caution should be applied when using this approach to examine other associates of TTS until analysis of the responses of the NC sub-groups are completed, and the mechanism(s) underlying thermal tasting are more fully elucidated.

3.5 Conclusion

A large sample size allowed for confirmation of trends reported in the literature on thermal taste status. Importantly, it was shown that TT report higher responsiveness to a wide range of orosensory stimuli compared to TnT regardless of the TTS classification scheme used. After comparing the main classification schemes used in the field, it can be concluded that all three locations on the tongue (tip, left, right) should be tested during thermal elicitation procedures. However, further research is required to determine if the three locations can be stimulated simultaneously using a larger probe. In addition, two or more replications of each location/temperature regime should be performed. NC are an important group with heterogeneic orosensory responsiveness and represent up to 60% of the population. Future research should consider incorporating NC subgroups in analyses in order to significantly boost sample size, although the approaches used for re-classifying NC should be informed by the aims of the study and the guidelines outlined in this paper.

3.6 Link to published version

https://doi.org/10.1007/s12078-019-09264-w

3.7 References

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3.8 Supplementary Tables

Table S3.1: Summary of demographic information and publications by cohort (ND = Not Disclosed).

Cohort	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**
Number	64	31	21	76	94	128	17	77	74	21	105	708
Age												
17-19	5	9	1	20	37	20	11	19	44	4	25	27.5%
20-23	14	7	6	24	35	19	3	30	14	12	55	30.9%
24-30	21	8	8	24	11	34	1	14	8	3	13	20.5%
31+	24	7	6	7	4	54	0	14	5	1	12	18.9%
ND	0	0	0	1	7	1	2	0	3	1	0	2.1%
Gender												
Female	39	18	9	46	73	85	12	47	67	16	72	68.4%
Male	25	13	12	30	21	43	5	30	6	5	33	31.5%
ND	0	0	0	0	0	0	0	0	1	0	0	0.1%
Ethnicity												
Caucasian	55	31	20	60	60	104	12	61	58	19	70	77.7%
Non-Caucasian	9	0	1	15	34	23	5	16	15	2	35	21.9%
ND	0	0	0	1	0	1	0	0	1	0	0	0.4%
Data Used in Earlier Publications	Yes [a, e]	Yes [b, e]	Yes [c, e]	Yes [d, e]	Yes [e]	Yes [e, f]	No	Yes [g]	Yes [h]	No	No	No

[a]Bajec et al., (2012)
[b]Pickering et al., Bajec, (2010a)
[c]Pickering et al., (2010b)
[d]Pickering et al., (2016)
[e]Thibodeau et al., (2017)
[f]Bajec and Pickering, (2008); Bajec and Pickering, (2010)
[g]Pickering and Kvas, (2016)
[h]Pickering and Klodninki, (2016)

Table S3.2: Summary of methodological differences between cohorts.

Cohort	**1**	**2, 3**	**4**	**5, 7, 10 & 11**	**6**	**8**	**9**
Remembered Sensations							
Scales Rated	gVAS and gLMS	gVAS and gLMS	gVAS and gLMS	gVAS and gLMS	gLMS Only	gVAS and gLMS	gVAS and gLMS
"pain from biting your tongue", "brightness of the sun when you are looking directly at it", "burning sensation of eating a whole hot pepper"	Yes	Yes	Yes	Yes	Yes	Yes	Yes
"touch sensation of a pill on your tongue", "sweetness of cotton candy"	Yes	Yes	Yes	Yes		Yes	Yes
"coolness of an ice-cold beverage", "sourness of a lemon"	Yes	Yes			Yes		
"sweetness of a banana", "heat of drinking hot tea", "burn of cinnamon gum", "coolness of a peppermint candy", "warmth of sipping lukewarm water", "bitterness of black coffee", "saltiness of ocean water", "tingling from a carbonated drink"	Yes	Yes					
Orosensory Responsiveness							
Scale Used	gVAS	gVAS	gVAS	gVAS	gLMS	gVAS	gVAS
Palate Cleansers in Addition to Filtered Water	5 g/L Pectin	5 g/L Pectin	None	Soda Crackers	5 g/L Pectin	None	None
Number of Blind Presentations	1 or 2	1	1	1 or 2	1	1 or 2	1 or 2
Number of Labelled Presentations	1	1	1	1 or 2	1	1	1
Total Number of Presentations	2 or 3	2	2	2 or 4	2	2 or 3	2 or 3
Thermal Taste							
Training Runs (Palm & Lip, Warming & Cooling Cycles)	2	2	1	1		1	1
Training Runs (Tongue 10s at 37C)					2		
Temperature, Sweet, Salty, Sour, Bitter & Other Scales	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Umami & Metallic Scales				Yes			
PROP							
Concentration	3.2 mM	3.2 mM	3.2 mM	3.2 mM	0.32 mM	3.2 mM	3.2 mM
Volume	10 ml	10 ml	20 ml	10 ml	20 ml	10 ml	20 ml

Chapter 4: Homogeneity of thermal tasters and implications for mechanisms and classification

4.1 Introduction

Diet is an important determinant of overall health (Sofi et al., 2008; Milajerdi et al., 2018) and consumers often cite flavour as one of the most important factors in food choice (Glanz et al., 1998; Aggarwal et al., 2016; Kourouniotis et al., 2016). Therefore, understanding how individual differences in orosensory perception influence the development of food preferences, food intake, and health related outcomes is of interest (reviewed in Tepper, 2008; Garcia-Bailo et al., 2009; Hayes et al., 2013b). One such type of individual variation in taste perception is thermal taste status (TTS). Thermal taste was first reported by Cruz and Green (2000) who found that warming or cooling the tongue elicited orosensations in some individuals. Further research has identified individuals who experience one or more orosensations during thermal stimulation, including sweet, sour, salty, bitter, umami/savoury, metallic, minty and spicy (Table 4.1; Cruz and Green, 2000; Bajec et al., 2012; Yang et al., 2014; Hort et al., 2016; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016; Pickering et al., 2016; Karunanayaka et al., 2018; Skinner et al., 2018) in addition to the change in temperature. Subsequent studies focused on comparing these individuals, collectively referred to as thermal tasters (TT), with individuals who only perceived temperature changes during warming or cooling (thermal non-tasters; TnT). For simplicity, the term "orosensation" will be used to describe taste and chemesthetic sensations elicited in the oral cavity, but not temperature.

A third group of individuals, non-classifiable participants (NC), are typically excluded as they cannot readily be classified as TT or TnT (Bajec and Pickering, 2008; Yang et al., 2014). Like TT, NC report orosensations during warming and/or cooling. However, unlike TT the sensations are not reproducible or are reported at low intensity, making it difficult to determine if they are valid or if they simply reflect response bias common in self-report research. Importantly, there is no evidence that NC constitute a third distinct thermal taste phenotype, but instead are most likely misclassified TT or TnT (Thibodeau et al., 2019, Chapter 3). Fatty/oily, chemical and electric orosensations have

Table 4.1: Orosensations (%) reported by thermal tasters during thermal elicitation using the warming cycle, the cooling cycle or across both the warming and cooling cycle (overall).

Paper	Pickering et al., (2016)	Pickering and Klodnicki, (2016)	Pickering and Kvas, (2016)		Yang et al., (2014)		Skinner et al., (2018)		Current Study		
Sample Size	25	23	21		56		37		267*		
Temperature Regime	Overall	Overall	Warming	Cooling	Warming	Cooling	Warming	Cooling	Overall	Warming	Cooling
Sweet	20%	18%	27%	7%	22%	8%	42%	7%	19%	28%	8%
Salty	22%	12%	6%	27%	3%	6%	5%	5%	12%	11%	14%
Sour	23%	24%	17%	33%	10%	18%	8%	25%	21%	10%	31%
Bitter	33%	34%	33%	30%	19%	26%	11%	25%	26%	25%	27%
Metallic					30%	27%	13%	17%	13%	12%	14%
Other	2%	12%	17%	3%	13%	14%					
Savoury/Umami					3%	1%			3%	4%	2%
Minty							8%	13%	1%	0%	1%
Spicy							13%	8%	6%	10%	2%

*The sample includes the 254 TT identified when *sweet, salty, sour, bitter* and *metallic* were considered valid tastes in combination with those identified when *minty* (n=2) and s*picy* (n=11) were also considered valid. The total number of taste qualities reported is 416 (228 warming, 248 cooling) and are greater than the number of participants as 35% of participants reported more than one taste. Taste qualities were only counted once in the overall percentages if they occurred during both heating and cooling.

also been reported after thermal stimulation (Karunanayaka et al., 2018) but it is unknown if they would meet the requirements to qualify an individual as a TT. Readers are referred to Thibodeau et al. (2019, Chapter 3) for a full discussion of NC and how they differ from TT and TnT.

4.1.1 Differences between Thermal Tasters and Thermal Non-Tasters

Despite variation in the strict definitions of TT and TnT (Thibodeau et al., 2019, Chapter 3), on balance the literature strongly supports the finding that TT rate the intensity of suprathreshold aqueous solutions of prototypical tastants higher than TnT (Green and George, 2004; Green et al., 2005; Bajec and Pickering, 2008; Bajec et al., 2012; Yang et al., 2014; Hort et al., 2016; Thibodeau et al., 2019, Chapter 3; Small-Kelly and Pickering, 2020). The increased orosensory responsiveness of TT occurs at multiple gustatory sites (tongue tip, circumvallate papillae and soft palate) and also when using whole-mouth sip-and-spit protocols (Green and George, 2004). TT are also more responsive to the orosensations elicited by sampled beverages (Pickering et al., 2010a, b, 2016; Pickering and Kvas, 2016; Mitchell et al., 2019; Small-Kelly and Pickering, 2020), but only limited differences in responsiveness to solid foods have yet been shown (Pickering and Klodnicki, 2016; Pickering et al., 2016).

For predominately trigeminal stimuli, the results are less clear. While thermal tasters are more responsive to alum sulphate, an astringent stimuli that also elicits sweetness (Bajec and Pickering, 2008; Thibodeau et al., 2019, Chapter 3), there is no difference in responsiveness to burning (capsaicin; Green et al., 2005; Yang et al., 2014) or cooling stimuli (menthol; Green et al., 2005). Similarly, it is unclear if TT have increased responsiveness over TnT in the perception of ortho- and retro-nasal aromas as some studies have reported differences (Green et al., 2005) and others have not (Yang et al., 2014). TT are more responsive to complex stimuli (ethanol and metallic salts), although it is unknown if these differences are caused by the prototypical tastes, trigeminal sensations and/or retro-nasal aromas elicited by the stimuli (Bajec and Pickering, 2008; Thibodeau et al., 2019, Chapter 3; Small-Kelly and Pickering, 2020).

4.1.2 Differences within Thermal Tasters

By definition, TT are individuals who experience orosensations when their tongue is warmed or cooled. However, the experiences of TT vary widely based on the orosensation reported, the temperature regime and the location tested on the tongue. Sweet and sour thermally-elicited tastes can be elicited across the entire anterior edge of the tongue (Cruz and Green, 2000). The rating of thermally-induced sweetness has been reported as highest at the tongue tip while thermal sourness is highest approximately 1-cm to the left and right of the tongue tip on the anterior edge (Cruz and Green, 2000). Cooling the posterior region of the tongue, in the circumvallate papillae region, produces thermally-elicited sourness and bitterness (Cruz and Green, 2000). More research is required to determine if the proportion of thermally-elicited orosensations varies by location tested on the tongue.

Thermal sweetness is more frequently elicited during warming than cooling (Cruz and Green, 2000; Green et al., 2005; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). Conversely, sourness and saltiness are more frequently elicited by cooling than warming (Cruz and Green, 2000; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). The proportion of participants reporting bitterness is typically higher during cooling than warming (Yang et al., 2014; Skinner et al., 2018), although it is roughly equivalent across both temperature regimes when three sites are tested on the edge of the tongue (Pickering and Kvas, 2016). Metallic thermally-induced tastes occur at similar proportions during both warming and cooling (Yang et al., 2014; Skinner et al., 2018). Further research is required to determine if these trends evidenced from small samples can be verified in a large sample. Importantly, the interaction between the thermal taste experienced and the lingual location and/or temperature of thermal elicitation may provide insights into the mechanism(s) underlying the phenomenon.

The identification of subgroups within TT is further complicated as Skinner et al. (2018) reported that 31% of TT experienced multiple thermally-elicited orosensations within the same trial. Isolating the individual orosensations is also difficult as salty, umami, metallic and spicy thermally-elicited sensations often overlapped when studied temporally. Between-trial variation also exists as Green et al. (2005) reported that one third of TT who

experienced sweetness during warming also reported saltiness and/or sourness during cooling (Green et al., 2005).

While on average, TT are more responsive than TnT to orosensory stimuli, the orosensory responsiveness of TT also varies considerably between TT (Thibodeau et al., 2019, Chapter 3). As such, it is possible that the heightened orosensory responses of TT are driven by a subset of individuals. For example, it has been hypothesized that TT who experience sweetness during thermal stimulation will rate sweet chemical stimuli (e.g. sucrose) higher than other TT (Pickering and Klodnicki, 2016; Pickering and Kvas, 2016). Bajec et al. (2012) tested this hypothesis and found no differences in responsiveness between TT subgroups. Specifically, individuals who experienced thermally-elicited sweetness, sourness and bitterness, were not more responsive to aqueous solutions of sweet, sour and bitter chemical stimuli, respectively. However, those results are likely underpowered due to a small sample size (n=5-9) and more research is required to determine if this hypothesis is supported in a large sample. Further, establishing the extent to which TT are a homogenous group will inform best practices when classifying TT in future studies.

4.1.3 Thermal Taste Mechanism(s)

The wide variety of responses to thermal stimulation support the hypothesis that multiple mechanisms underlie the thermal taste phenomenon (Skinner et al., 2018). ENaC (epithelial amiloride-sensitive sodium channel) and TRPM5 (transient receptor potential M5) have been suggested as possible peripheral mediators of thermally-induced saltiness and sweetness, respectively.

ENaCs are expressed in taste receptor cells, are involved in the perception of saltiness (Bachmanov and Beauchamp, 2007), and can be activated by cooling from 30°C to 15°C (Askwith et al., 2001; Chraibi and Horisberger, 2003). As the activation range of ENaC overlaps the temperature range used in the cooling cycle of TT studies (35 to 5°C) and thermally-elicited saltiness is more common during cooling than warming, ENaC may play a role in thermally-elicited saltiness (Cruz and Green, 2000; Talavera et al., 2007). Unfortunately, a clear activation range for thermally-elicited saltiness could not be

established to test this hypothesis (Skinner et al., 2018). If true, then temperature changes may act directly on taste receptors to induce thermally-elicited tastes.

TRPM5 is a heat-activated cation channel that is highly expressed in taste receptor cells and is involved in the perception of sweet, bitter and umami tastes (Talavera et al., 2005). The gustatory nerve response of *Trpm5* knockout mice is reduced compared to that of wild-type mice (Talavera et al., 2005). Importantly, both TRPM5 and thermally-elicited sweetness can be activated in the same temperature range (Talavera et al., 2007; Skinner et al., 2018). In contrast, the onset of thermally-elicited bitterness is predominantly during cooling and no temperature range has been established for thermally-elicited umami (Skinner et al., 2018). Therefore, evidence for the role of TRPM5 in tastes other than sweet is limited. This suggests that changes in temperature may act directly on taste transduction pathways to produce thermally-elicited sensations.

Differences in the activation of the taste regions of the brain in both TT and TnT support the hypothesis of a cross-wiring of receptors in TT at the periphery (Hort et al., 2016). As the CO_2 concentration increased in a cold sweet solution, cortical activation of both the primary and secondary somatosensory cortices and taste regions of the brain increased in TnT, but only the secondary somatosensory region increased for TT. Hort et al. (2016) suggest that TT only displayed limited increases in cortical activation as gustatory and trigeminal nerves were already highly stimulated by the cold temperature and sweet stimuli in the sample. This suggests that taste and temperature-related pathways may be cross-wired in TT or a subset thereof such that activation of one pathway (e.g. temperature) can activate the other (e.g. taste).

During TTS screening, participants are typically required to report the same taste sensations across matching replicates. If the mechanism(s) underlying thermal taste originate with taste and/or temperature receptors, then it would be anticipated that participants would experience the same taste reproducibly. However, if thermal tastes originate further upstream or centrally, participants may experience a range of thermally-elicited orosensations. Interestingly, some participants report interchangeable or multiple sensations across replicates, and at times the sensations merge together (Skinner et al., 2018). Further support for a centrally-mediated mechanism underlying TTS comes from the reports of increased responsiveness of TT to stimuli outside the oral cavity, including

some ortho-nasal aromas (Green et al., 2005) and temperature stimuli (Thibodeau et al., 2019, Chapter 3). TT also showed elevated cortical activation of brain taste regions compared to TnT when tasting sweet solutions (Hort et al., 2016). It may be too simplistic to assume that the mechanism(s) underlying TTS are either peripherally- or centrally-mediated. Rather, some individuals may experience thermally-elicited orosensations that originate peripherally, others centrally and some both.

4.1.4 Study Aims

As much remains unknown about the mechanism(s) underlying TTS, this study sought to better characterize the breadth of experiences reported by TT during both thermal stimulation and in response to aqueous chemical stimuli. To this end, data was combined from twelve TTS study cohorts to create the largest single sample of TT reported to date (n=254). The large sample size allowed us to investigate the following aims which guided the analyses conducted:

(1) To more fully characterize the experiences reported by TT during thermal elicitation and to identify potential subgroups based on:
    a. The type of thermally-elicited orosensation experienced (e.g. sweet TT).
    b. The location on the tongue at which the orosensation is experienced (e.g. tip TT).
    c. The temperature regime during which the orosensation is experienced (e.g. warm TT).

(2) To identify potential TT subgroups based on any combination of two factors from (1a) to (1c) by determining if TT are more likely to be members of both subgroups.

(3) To determine if the increased orosensory responsiveness of TT over TnT is universal or if it is confined to one or more of the subgroups identified above.

(4) To examine the practical implications of if/how methodological differences impact the type of thermally-elicited orosensation reported.

4.2 Materials and Methods

4.2.1 Participants

975 participants were recruited during 12 mutually-exclusive recruitments drives ('cohorts') from Brock University and the surrounding community, of which 905 completed the study in full. Compensation was offered as an incentive to participants in the form of entry into a monetary/gift card draw or participation credit towards select courses. Informed consent was obtained for all participants and all procedures were cleared by the Brock Research and Ethics Board (REB-05-258, 08-006, 08-065, 08-216, 10-193, 12-116, 12-181,14-119, 14-120, 15-018, 15-176, 17-031). A total of 70 participants did not complete the TTS screening procedure and were excluded from the study. In addition, failure to appropriately use the scales during training led to the exclusion of 124 participants (see Section 4.2.2.1 for further details). The final sample consisted of 781 participants with a mean age of 25.1 years +/- 9.4 SD of which 556 were female, 223 were male and two did not disclose gender.

4.2.2 Thermal Taste Status Screening

The thermal taste status of participants was determined based on the protocol of Bajec and Pickering (2008). However, there were minor difference in the methods used across the cohorts. These differences reflect changes in best practices, as informed by the developing sensory and thermal tasting literature and differences in study aims across cohorts. The following section briefly describes the methods used to screen for TTS, highlights key methodological differences across cohorts and when applicable explains how the changes optimized the protocol. Full details of the procedures are provided in Thibodeau et al. (2019, Chapter 3) for Cohorts 1-11 and Mitchell et al. (2019) for Cohort 12, while Supplementary Table 4.1 summarises methodological differences. Please note, cohorts are not numbered chronologically and the order of data collection was as follows: 6, 1-4, 8-9, 5, 7, 10-12.

4.2.2.1 Scales

Two intensity scales, the generalized Visual Analogue Scale (gVAS) and the generalized Labeled Magnitude Scale (gLMS) were used for data collection (Bartoshuk et al., 2002, 2004). All participants were trained on scale use by rating five to fifteen

remembered sensations (Bajec and Pickering, 2008). Two procedures were implemented to screen for appropriate scale use by the participants. For Cohort 12, the most recent cohort, participants were required to rate the "the brightness of the sun when staring directly at it" more intensely than "the brightness of a dimly lit room". Cohorts 1-5, 7-11 were required to rate "the pain of biting your tongue" more intensely than the "touch sensation of a pill on your tongue". This approach assumes that the sensations are not perceptually equivalent and that participants who failed to rate the sensations appropriately did not fully understand the scales. Similar approaches to screening for scale use have been used previously (Cruickshanks et al., 2009; Galindo-Cuspinera et al., 2009; Thibodeau et al., 2019, Chapter 3). As participants from Cohort 6, the first cohort, did not rate the "touch sensation of a pill on your tongue" or "the brightness of a dimly lit room", no screening of these participants was performed.

4.2.2.2 Orosensory Responsiveness

Participants tasted aqueous solutions eliciting common orosensations primarily to aid with the later identification of thermally-elicited sensations (Table 4.2). All cohorts were presented with exemplars of *sweet*, *sour* and *bitter*. Additional oral sensations included in training were *salty* (Cohorts 4-12, n=215), *umami* (Cohorts 1-5 & 7-12, n=226), *metallic* (Cohorts 5-7, 10-12, n=125) and *astringent* (Cohorts 1-3, 6 & 11, n=92). All solutions were prepared volumetrically in pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA) or distilled water and refrigerated when not in use. Solutions were discarded within 7 days (sweet, sour, salty) or 48 hours (bitter, umami). Metallic and astringent solutions were prepared within 3 hours of testing. All solutions were presented in a randomized order and at room temperature. In early cohorts, the choice of oral sensations used in training was based on the specific aim of the individual study. However, training has been optimized in the most recent cohorts (5, 7, 10-12). Participants are now always familiarized with exemplars of common thermally-elicited orosensations (*sweet, sour, salty, bitter, umami* and *metallic*) and TTS ballots include scales for each of these orosensations later in TTS screening.

The number of replicates and the type of replicate varied across cohorts. All cohorts started with a labelled replicate, where the identity of each orosensation was indicated on the sample. Participants were presented with 20 ml of each solution in medicine cups or

Table 4.2: Summary of tastants used by each cohort.

Cohort(s)	1	2	3	4	5, 7, 10 & 12	6	8 & 9	11
Sweet	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[a]	250 mM Sucrose[b]	250 mM Sucrose[c]	250 mM Sucrose[b]	250 mM Sucrose[b]
Salty				180 mM NaCl[f]	180 mM NaCl[d]	180 mM NaCl[e]	180 mM NaCl[f]	180 mM NaCl[d]
Sour	3.25 mM Citric Acid[g]	3.33 mM Citric Acid[g]	3.25 mM Citric Acid[g]	3.33 mM Citric Acid[g]	3.25 mM Citric Acid[g]	4.47 mM Tartaric Acid[h]	3.25 mM Citric Acid[g]	3.25 mM Citric Acid[g]
Bitter	0.0275 mM Quinine monohydrochloride[a]	0.022 g/L Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]	0.022 g/L Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]	0.0255 mM Quinine Sulphate[j]	0.0275 mM Quinine monohydrochloride[i]	0.0275 mM Quinine monohydrochloride[i]
Umami	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]		125 mM L-glutamic acid monosodium salt hydrate[a]	125 mM L-glutamic acid monosodium salt hydrate[a]
Metallic					1.0 mM Copper (II) sulfate[b]	0.3 & 3 m Iron (II) Sulfate[k]		1.0 mM Cupric sulfate[b]
Astringent	0.877 mM Aluminum Sulfate[a]	0.877 mM Aluminum Sulfate[a]	0.877 mM Aluminum Sulfate[a]			0.73 & 14.6 mM Aluminum Sulfate[a]		0.0877 mM Aluminum Sulphate[a]

[a]Sigma-Aldrich, MO, USA
[b]BioShop, ON, Canada
[c]Lantic Sugar Ltd., QC, Canada
[d]ACP Chemicals Inc., QC, Canada
[e]Windsor, QC, Canada
[f]Caledon Laboratories, ON, Canada
[g]Fisher Scientific, NY, USA
[h]Carl Roth KG, distributed by Atomergic Chemetals Corp., NY, USA
[i]SAFC Supply Solutions, MO, USA
[j]Novopharm, ON, Canada
[k]J.T. Baker, NJ, USA

clear wine glasses and asked to swish each solution on their palate for 5 seconds before expectorating. Participants waited a further 10 seconds before rating the maximum intensity of the elicited sensation on a gLMS (Cohorts 6 & 12) or gVAS (Cohorts 1-5,7-11; Bajec and Pickering, 2008). Each solution was tasted in the presented sequence and participants rinsed with filtered water (Brita, ON, Canada) prior to and after each solution. In order to minimize possible carry over effects of the metallic and astringent stimuli, unsalted soda crackers (Cohorts 5, 7, 10-12) or a 5g/L pectin solution (Cohorts 1-3, 6) were provided as palate cleansers. The last exercise consisted of evaluating blind-coded replicate samples, which were identical to labelled replicates except that the labels were replaced with 3-digit codes and in addition to rating the maximum intensity of the sensation elicited, participants were asked to identify the oral sensation elicited. Cohort 12 completed a single labelled replicate, and Cohorts 1-11 completed up to three additional replicates, of which at least one was blind-coded.

4.2.2.3 Thermal Taste Status Determination and Classification

Thermal stimulation was performed using a 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode). Prior to use, the thermode was rinsed with 70% ethanol (Commercial Alcohols, ON, Canada) and wrapped in a fresh piece of plastic wrap (Compliments, ON, Canada). Two different cycles were used: a warming cycle and a cooling cycle. Warming cycles started at 35°C, then cooled to 15°C before final re-warming to 40°C and holding for 1 second. Since only the warming portion of the cycle was of interest, participants were asked to rate the maximum intensity of sensations during the re-warming phase of the cycle (from 15°C to 40°C). For convenience, a beep signalled the beginning of this period. Cooling cycles started at 35°C, subsequently cooling to 5°C and holding for 10 seconds. Since no warming occurs during this cycle, participants were asked to rate the maximum intensity of sensations through the entire cycle. For both cycles, all temperature transitions occurred at approximately 1°C/second.

Prior to collection of TTS responses, participants underwent training runs to become familiar with the thermode. The earliest cohort (Cohort 6) was familiarized with the thermode by rating the temperature and any taste sensations elicited by the thermode when applied at 37°C for 10s on the tongue tip. While this familiarized participants with

the touch sensation of the thermode, participants were not exposed to the range or speed of temperature changes characteristic of the warming and cooling trials prior to data collection. To address this, all subsequent participants (Cohorts 1-5 and 7-12) rated the maximum intensity of both warming and cooling trials on gLMS when the thermode was applied to the palm and vermillion border of the bottom lip. Cohorts 1-3 performed this task in duplicate while all other cohorts completed this task only once.

Three locations on the edge of the tongue were tested for each participant: the very tip of tongue along the midline, 1 cm to the left from the midline and 1 cm to the right from the midline. A total of 12 runs were performed for each participant in two blocks. Each block consisted of three warming cycles (one per location) followed by three cooling cycles (one per location). A minimum 3-minute break was taken between blocks. All participants rated any sensations (*heat*, *cold*, *sweet*, *salty*, *sour*, *bitter*, and *other*) elicited using a paper ballot with individual gLMS scales for each. For the most recent cohorts (Cohort 5, 7, 10-12), the paper ballot was modified by adding gLMS scales for *umami* and *metallic*. These change reflected the widespread acceptance of *umami* as a fifth basic taste and large number of participants reporting *metallic* tastes using the *other* scale in earlier cohorts.

TTS classification was determined using the methods of Bajec and Pickering (2008). TT were defined as participants who reported the same, valid thermally-elicited taste sensation above weak on the gLMS (> 6 mm), during both replicates of the same location during the same temperature regime. Participants had to meet these requirements for one or more of the six combinations of location and temperature regime (warm/tip, warm/left, warm/right, cool/tip, cool/left and cool/right). Valid thermally-elicited tastes included *sweet, salty, sour, bitter, umami* and *metallic.* TnT were defined as participants who reported no taste-related orosensation during thermal elicitation. TnT could report temperature (e.g. *heat, cold*), texture (e.g. *astringency, drying),* pressure or pain related sensations during thermal elicitation. All other participants were defined as non-classifiable.

4.2.3 TT Subgroups

TT were then divided into subgroups based on the orosensation(s) reported, the temperature regime(s) or the location of the thermally-elicited orosensation(s). For example, all TT who experienced *sweet* thermally-elicited sensations, regardless of the location or temperature regime, were classified as *sweet* TT. These TT subgroups are referred to as

single-factor subgroups because only one criterion was used to classify participants. At minimum, each participant belongs to three single-factor subgroups; one each based on orosensation, location and temperature regime. However, TT may belong to additional subgroups if they experience multiple thermally-elicited orosensations or if the sensation(s) is experienced at more than one location or during both temperature regimes. In order to assess the importance of membership to two subgroups by the same participant, TT were also divided into subgroups based on two criteria which are referred to as two-factor subgroups. Two-factor subgroups were named by joining the two single-factor group names with an "&" to indicate that was a member of both groups at the same time. For example, TT who experience thermally-elicited *sweetness* during *warming* were classified as both *sweet&warm* TT. Similarly, TT who experience thermally *sweetness* and *sourness* at the same location and during the same temperature regime were classified as *sweet&sour* TT. When a broader definition was required, as when testing for associations between the subgroups, the "/" was used to indicate the participant was a member of both groups regardless of if it occurred at the same time. As such, all "&" subgroups are "/" subgroups but the reverse is not always true. For example, a TT that experienced thermally-elicited *sweetness* during *cooling* and thermally-elicited *bitterness* during *warming* was classified as a *sweet/warm* TT but not a *sweet&warm* TT. In contrast, a TT that experienced thermally-elicited *sweetness* during *warming* was classified as a *sweet/warm* TT and a *sweet&warm* TT.

Prototypical tastes are broadly divided into two classes based on mechanism. G-protein-coupled receptors (GCPR) are responsible for the perception of sweetness, bitterness and umami while ion channels are responsible for the perception of saltiness and sourness (Bachmanov and Beauchamp, 2007). To assess the importance of mechanism, *sweet* TT, *bitter* TT and *umami* TT are collapsed into a single group called *GCPR* TT. Similarly, *salty* TT and *sour* TT were defined as *Ion* TT. TT are required to report the same taste sensation across replicate trials in most but not all (Yang et al., 2014, 2018; Hort et al., 2016) studies. To investigate this, in the "Unmatched TT" section, *matched* TT, and *unmatched* TT were compared. Participants previously identified as TT are referred to as *matched TT* in this section as they report the same thermally-elicited orosensation during

replicate trials. *Unmatched* TT are defined as other participants who report any combination of thermally-elicited orosensations above 'weak' on the gLMS during replicate trials.

During TTS screening participants are provided with an "other" scale to prevent attribute dumping. When participants use this scale, they are asked to provide a description of the sensation in one or two words. Most often, the terms used to describe the "other" sensation fall with two categories, *spicy* (e.g. "spice", "spicy" "burning", "cinnamon", "peppery", "chili flakes", "hot pepper", "pepper" or *minty* (e.g. "menthol", "mint", "minty", "medicinal", "peppermint"). Some studies have included *minty* and/or *spicy* as valid thermally-elicited tastes (Hort et al., 2016; Skinner et al., 2018; Yang et al., 2018). As *minty* and *spicy* may be proxies for *cold* and *heat*, these responses were first treated as temperature-related sensations and ignored when classifying participants in the current study. As a result, participants that experienced a thermally-elicited *spicy* or *minty* sensation could be classified as TnT if they did not experience any other thermally-induced orosensations. Similarly, participants were only classified as TT if they reported a different thermally-induced orosensation (e.g. *sweet*). Instead, in the "Spicy & Minty" section, *minty* and *spicy* were added to the list of valid thermally-elicited tastes for classification and analysis purposes. Readers are referred to Table 4.3 for a summary of the naming conventions followed for the TT subgroups, with fuller descriptions in the *Data in Brief* article than corresponds with this work (Thibodeau et al., 2020, Chapter 7).

4.2.4 Data Analysis

All data analysis was performed using XLSTAT Version 19.02 (Addinsoft, NY, USA) and Microsoft® Excel® for Mac 2011 (Microsoft ®**,** ON, Canada). Due to the exploratory nature of this study, high numbers of statistical tests were performed. To correct for multiple comparisons, the false-discovery rate control method of Benjamini and Hochberg was applied to each group of statistical tests (Benjamini and Hochberg, 1995; Glickman et al., 2014). In brief, all p-values within a group of statistical tests are sorted from the lowest value to the highest value, where "n" is the total number of statistical tests and "i" is the rank order of the p-values. The adjusted significance level for each p-value is calculated using the formula d x i/n where "d" is the maximum false-discovery rate. If the actual p-value of a statistical test is lower than the adjusted p-value, the null hypothesis is

Table 4.3: Summary of the definitions and naming conventions for thermal taster (TT) subgroups.

One-factor TT Subgroups		
Based on the orosensation experienced	Sweet TT	Experiences *sweet* thermally-elicited sensations. Similarly for: Sour TT, Salty TT, Bitter TT, Umami TT, Metallic TT, Minty TT & Spicy TT.
	GCPR TT	Experiences *sweet, bitter* and/or *umami* thermally-elicited sensations.
	Ion TT	Experiences *sour* and/or *salty* thermally-elicited sensations.
	Matched TT	Experiences the same thermally-elicited orosensation reproducibly.
	Unmatched TT	Experiences different thermally-elicited orosensations during each trial of the same location during the same temperature regime.
Based on the temperature regime during which orosensations are experienced	Warm TT	Experiences thermally-elicited sensations when the tongue is warmed. Similarly for Cool TT
	Onlywarm TT	A warm TT who is not a cool TT. Similarly for Onlycool TT
	Warmandcool TT	A warm TT who is also a cool TT.
Based on the location at which orosensations are experienced	Tip TT	Experiences any orosensation when the tip of the tongue is warmed and/or cooled. Similarly for Left TT & Right TT.
Two-factor TT Subgroups (The same conventions apply to all two-factors groups. Two factor subgroups can be formed from any two one-factor subgroups. Examples are provided for sweet TT and a second group.)		
Member of both subgroups simultaneously (Group1&Group2 TT)	Sweet&warm TT	Experiences sweet thermally-elicited sensations during warming trials.
	Sweet&tip TT	Experiences sweet thermally-elicited sensations at the tip of the tongue.
	Sweet&sour TT	Experiences sweet and sour thermally-elicited sensations at the same location and during the same temperature regime.
Member of both subgroups Group1/Group2 TT)	Sweet/warm TT	Experiences thermally-elicited sweetness during warming and/or cooling and experiences any thermally-elicited orosensation during warming. By definition all sweet&warm TT are sweet/warm TT but not the reverse.

rejected, and the test is deemed statistically significant. A false-discovery rate of 0.05 was used for all calculations.

Normality and equality of variance (Levene's test, $p > 0.05$) were tested for all continuous variables (data not shown). For variables with less than 50 participants, z-scores of less than 1.96 for Fisher's skewness and Fisher's kurtosis indicated that the variable was normally distributed (Kim, 2013). Similarly, for variables with 50 to 300 participants, z-scores below 3.29 indicated the variable was normally distributed. Unless otherwise noted, all variables met the above assumptions. For Mann-Whitney U tests, the p-value were approximated due to large sample sizes by standardizing raw U (z-scores). Therefore, prior to correction for multiple comparisons, Mann-Whitney U tests were significant if the $U_{standardized}$ was greater than 1.96.

4.2.4.1 Orosensory Responsiveness, Unmatched, Spicy and Minty

TT were divided into subgroups based on the groups described (Section 4.2.3). Direct comparison of orosensory responsiveness scores was not possible due to differences in scale, tastants, stimulus concentrations, and/or the number of exposures. For all tastants, mean responsiveness scores were calculated for each participant from all replicates (labelled and blind-coded). Next, the mean scores from each cohort were converted to z-scores separately. Lastly, the z-scores for each cohort were combined for final analysis. Student's t tests were performed to determine if the mean z-score orosensory responsiveness (*sweet, salty, sour, bitter, umami, metallic* and *astringent*) of a TT subgroup differed from all other TT who were not part of that subgroup (e.g. *sweet* TT vs not *sweet* TT). Results from all single-factor TT subgroups, including those based on thermally-elicited orosensation, location, temperature, *GCPR* TT, *Ion* TT, *Unmatched* TT and *Spicy* TT were combined when correcting for multiple comparisons. If orosensory responsiveness failed to meet the assumption of normality, a Mann-Whitney U test was substituted.

4.2.4.2 Two-Factor TTS Subgroups

In order to determine if some orosensations, locations or temperature regimes were associated, 2X2 Fisher's exact tests were performed. When testing for an association between *sweet* TT (group 1) and *warm* TT (group 2), the four groups were as follows: *sweet/warm* TT (in both groups), *sweet* TT who are not *warm* TT (in group 1 only), *warm*

TT who are not *sweet* TT (in group 2 only) and participants who were not *sweet/warm* TT, *sweet* TT or *warm* TT (not in groups 1 and/or 2). After correction for multiple comparisons, odds-ratios (OR) were calculated for any significant pairs as a measure of effect size.

For each significant pair, participants were re-classified into TT subgroups. Pairs with an odds ratio above 1 were more likely to occur together than chance. Thus, participants were divided into two groups; *factor1&factor2* TT or other TT. Pairs with an odds ratio < 1 were less likely to occur together so participants were divided into not *factor1&factor2* TT and other TT. Mann-Whitney U was used to compare the mean z-score orosensory responsiveness of each newly identified TT subgroup. Results from all two-factor TT subgroups were combined when correcting for multiple comparisons. A similar analysis of three-factor subgroups (e.g. *sweet/warm/tip* TT) was not possible due to small sample sizes.

4.2.4.3 Temperature Responsiveness

TT rate the warmth of the warming and the cold of the cooling cycle higher than TnT (Thibodeau et al., 2019, Chapter 3). Much like the orosensory responsiveness of TT, it is unknown if all TT are more responsive to temperature changes or if this is driven by a subset of TT. Mean temperature responsiveness scores for each participant at both lingual (*tip*, *left* and *right*) and non-lingual (*palm*, *lip*) locations were log transformed as ratings on the gLMS are often log-normal (Hayes et al., 2013a). Zeros were replaced with 0.5 (or 0.5 mm), the smallest measurement possible when measuring the paper ballots by hand. Despite the log transformation, the variance was not equal across groups so non-parametric statistic were employed. Kruskal-Wallis was used to compare the temperature responsiveness of *onlywarm* TT, *onlycool* TT and *warmandcool* TT. Mann-Whitney U was used to compare the temperature responsiveness of *warm&sweet* TT to all other TT. Similarly, the temperature responsiveness of *warm&bitter* TT, *sour&cool* TT and *bitter&cool* TT was tested. As these were the only subgroups identified where the temperature regime at which thermally-elicited orosensation was experienced was used to include or exclude participants in the subgroup, the analysis was limited to these subgroups.

Significant differences in temperature responsiveness were found between *onlywarm* TT, *onlycool* TT and *warmandcool* TT. Kruskal-Wallis was used to determine if scale use differences between these groups may have confounded these findings. Scale

use was tested by comparing the ratings of the remembered sensation "the brightness of the sun when looking directly at it" on both the gVAS and gLMS. Finally, ANOVA was used to determine if orosensory responsiveness also differed across these mutually exclusive groups.

4.2.4.4 Training: Scales & Aqueous Stimuli

During thermal taste determination, participants are asked to identify any thermally-elicited orosensation they experience. The effect of two methodological choices on the types of TT identified was tested, namely, the type of aqueous stimuli used during training and the type of scales provided on the thermal-elicitation response ballot. To test the importance of training with a *salty* stimulus during training, participants were divided into two groups: those who received a *salty* stimulus and those who did not. Separate Fisher's exact tests (2X2) were performed to determine if the number of *sweet* TT, *salty* TT, *sour* TT, *bitter* TT, *umami* TT or *metallic* TT differed across the two groups. Similarly, participants were split based on training with *umami, metallic* and *astringent* stimuli and tested for differences in the number of TT per subgroup identified. As all participants trained with *sweet, sour* and *bitter* stimuli, the effect of their inclusion/exclusion in protocols could not be determined.

When participants underwent thermal taste determination, all participants used response ballots with scales labelled *sweet, salty, sour, bitter* and *other*. However, some participants also had scales labelled *umami* and *metallic*. To test for the impact of this difference, separate Fisher's exact tests were used to determine if the number of *sweet* TT, *salty* TT, *sour* TT, *bitter* TT, *umami* TT or *metallic* TT identified differed between the two groups. Results from this section were combined when correcting for multiple comparisons and odds ratios were calculated for any significant findings.

4.3 Results

The primary focus of this study was on characterizing the experiences of TT. Nevertheless, TTS classification resulted in the identification of 254 TT, 207 TnT and 323 NC. The following analyses focused only on differences within TT. Readers are referred to Thibodeau (2019, Chapter 3) for information on differences between TT, TnT and NC.

The orosensations reported by TT during thermal elicitation varied widely across participants. First, possible TT subgroups using one factor were characterized, namely by the type of thermally-elicited orosensation, the temperature regime or location at which the orosensation was elicited. A total of 107 *bitter* TT, 89 *sour* TT, 77 *sweet* TT, 51 *salty* TT, 53 *metallic* TT, and 11 *umami* TT were identified (Figure 4.1). The total number of TT by orosensation exceeded the total number of TT as some participants were TT for two (n=54), three (n=24), four (n=8) or five (n=2) orosensations (Figure 4.2). The percentage of taste qualities reported overall, during warming only and during cooling only can be found in Table 4.1. TT can also be divided based on the temperature regime(s) under which they experienced reproducible thermally-elicited tastes. A total of 157 *warm* TT and 179 *cool* TT were identified and of these 80 participants were *warmandcool* TT (Figure 4.2). TT can also be classified based on the location at which thermally elicited orosensations are experienced. A total of 157 *tip* TT, 140 *right* TT and 142 *left* TT were identified. Overlap

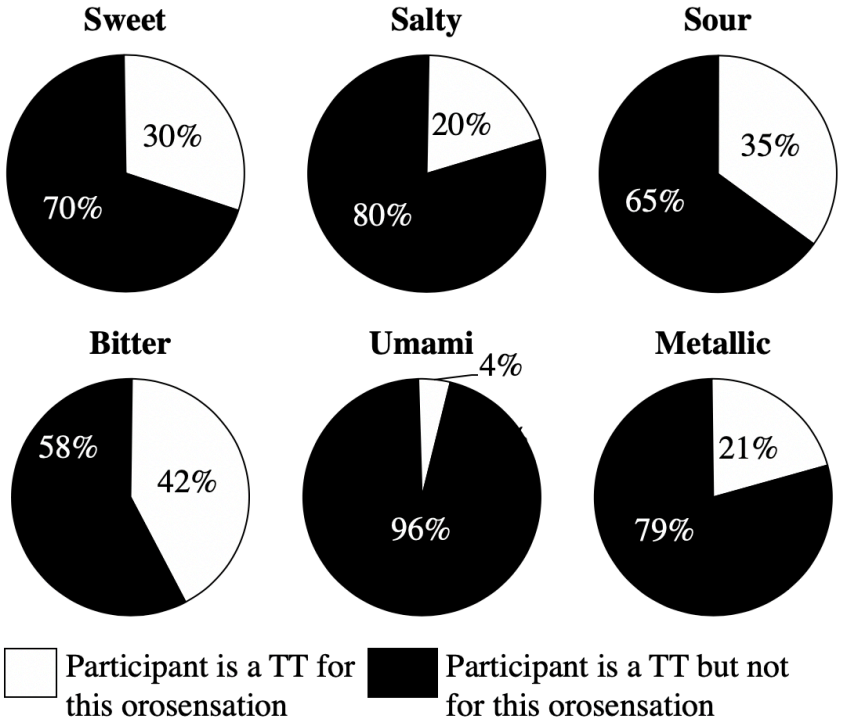

Figure 4.1: Overall percentage of thermal tasters experiencing sweet, salty, sour, bitter, umami and metallic thermally-induced orosensations (n=254). Note: When the percentage of TT experiencing each of the orosensations is summed, the total does not add to 100% as thermal tasters were able to report more than one orosensation.

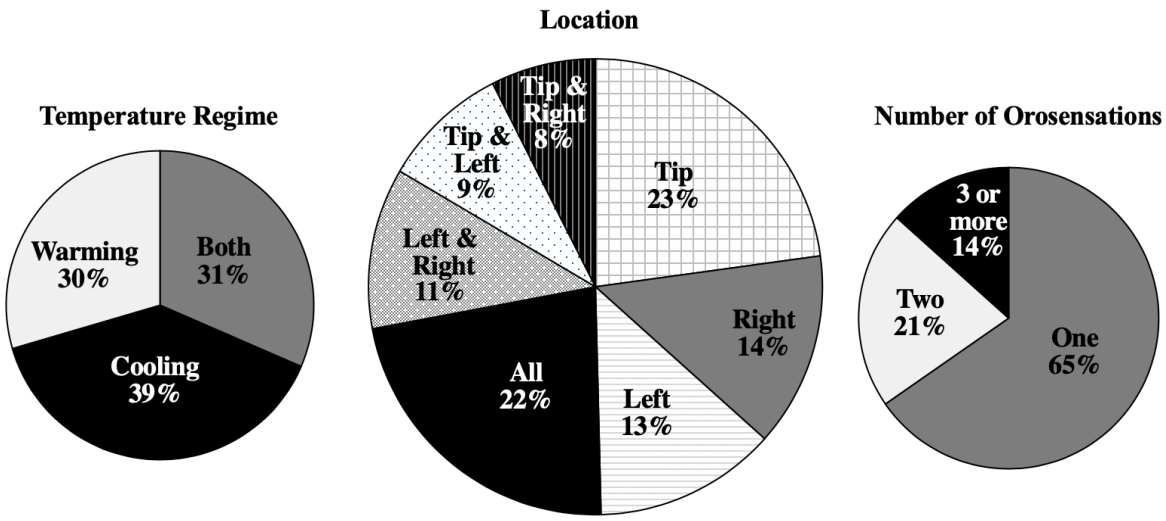

Figure 4.2: Overall percentage of thermal tasters experiencing reproducible and above threshold thermally-induced orosensations based on temperature regime, location and number of orosensations reported (n=254).

between the groups was considerable as 128 participants were TT for more than one location; 29 *left/right* TT, 19 *tip/right* TT, 23 *tip/left* TT and 57 *tip/left/right* TT were found (Figure 4.2).

4.3.1 Orosensory Responsiveness

When TT are treated as a single group, they are more responsive to orosensory stimuli than TnT. However, it is unknown if this difference is universal or if the heightened responsiveness is due to one or more subgroups. *Umami* TT (n=11) appeared to be more responsive to aqueous *sweet* stimuli than participants who were not *umami* TT (n=243; t=2.78, p=0.006). However, this was not significant after correction for multiple comparisons and no differences were found for the other orosensations. Similarly, no differences in orosensory responsiveness were found for *sweet* TT, *salty* TT, *sour* TT, *bitter* TT, *metallic* TT, *warm* TT, *cool* TT, *tip* TT, *left* TT, *warmandcool* TT, *right* TT, *GCPR* TT or *Ion* TT (Supplementary Tables 4.2-4.4). In addition, no trends were apparent when mean orosensory responsiveness was plotted for each TT subgroup, providing strong evidence that the heightened orosensory responsiveness of TT is universal (see Thibodeau et al., 2020, Chapter 7). Furthermore, these findings contradict the speculation that the taste

experienced during thermal elicitation predicts a corresponding specificity of taste gain in the 'real world'; that is (for example), only *sweet* TT are more responsive to sweet foods.

4.3.2 Unmatched TT

A key requirement of most TTS classification schemes is that TT report the same orosensation in replicate trials. To test the importance of this requirement, *unmatched* TT (n=32) were identified from the NC participants who had previously been excluded. No significant differences in the mean orosensory responsiveness of *unmatched* TT and *matched* TT for aqueous solutions of tastants were found (p >. 0.12, Supplementary Tables 4.2-4.4, see Thibodeau et al., (2020), Chapter 7 for figures).

4.3.3 Spicy and Minty

In order to determine if *spicy* or *minty* should be considered thermally-elicited tastes, abundances for each category were calculated. When *spicy* and *minty* were considered valid thermal tastes, 25 *spicy* TT were identified who had been classified as TT (n=14), non-classifiable (n=10) or TnT (n=1) under Bajec and Pickering (2008). Similarly, 3 *menthol/minty* TT were identified who had formerly been classified as non-classifiable (n=2) or TT (n=1). In addition, 13 TnT were reclassified as non-classifiable, when *spicy* (n=6) and *menthol/minty* (n=7) were included as valid thermally-elicited tastes. The TTS of 95.7% participants remained unchanged (239 TT, 311 NC, 193 TnT). No significant differences in the orosensory responsiveness of *spicy* TT and *non-spicy* TT were found (Supplementary Tables 4.2-4.4, see Thibodeau et al., (2020), Chapter 7 for figures). Due to low sample size, the orosensory responsiveness of *minty* TT (n=3) was not compared to that of *non-minty* TT.

4.3.4 Two-Factor TT Subgroups

Next, the association between any combination of two factors related to thermal elicitation was tested. Eleven pairs of TT subgroups were found to be positively associated using Fisher's exact test (see Table 4.4 for p-values). The two strongest association were found between *sweet* TT and *warm* TT, where *sweet* TT were 9.13 times more like to also be *warm* TT (OR), and *sour* TT were 8.21 times more likely to be *cool* TT. For the remaining nine pairs the odds ratio was between 2.0-3.0 and are indicated above the solid lines in Figure 4.3. Four pairs of TT subgroups were negatively associated with odds ratios

Table 4.4: Fisher's exact test results (p-values) and false-discovery rate corrected critical values for the association between any two single-factor TT subgroups. Critical values are calculated using the formula d x i/n where d = 0.05, n = 54 and i = rank order of the p-value. *Significant findings after correcting for multiple comparisons using the false-discovery rate method.

Type of Thermal Taster	p-value (False-discovery rate corrected critical values)									
	Tip	Right	Left	Cool	Warm	Sweet	Salty	Sour	Bitter	Umami
Right	**0.007*** (0.012)									
Left	**0.051** (0.022)	**0.057** (0.025)								
Cool	**0.023** (0.018)	**0.002*** (0.004)	**0.018** (0.016)							
Warm	**0.008*** (0.013)	**0.520** (0.041)	**0.002*** (0.005)	N/A						
Sweet	**0.024** (0.019)	**0.683** (0.044)	**0.336** (0.036)	**0.0005*** (0.0028)	**< 0.0001*** (0.0019)					
Salty	**0.016** (0.015)	**0.271** (0.034)	**0.114** (0.028)	**0.040** (0.020)	**0.261** (0.033)	**0.496** (0.040)				
Sour	**0.343** (0.037)	**0.005*** (0.007)	**0.004*** (0.006)	**< 0.0001*** (0.0009)	**0.031** (0.019)	**0.010*** (0.014)	**0.413** (0.038)			
Bitter	**0.006*** (0.009)	**0.022** (0.017)	**0.005*** (0.008)	**0.003*** (0.006)	**0.006*** (0.010)	**0.053** (0.023)	**0.433** (0.039)	**0.594** (0.043)		
Umami	**0.540** (0.042)	**0.759** (0.047)	**0.119** (0.029)	**1.000** (0.049)	**0.055** (0.024)	**0.739** (0.045)	**0.699** (0.044)	**0.752** (0.046)	**0.765** (0.048)	
Metallic	**0.113** (0.027)	**0.278** (0.035)	**0.120** (0.030)	**0.130** (0.031)	**0.156** (0.031)	**0.045** (0.021)	**0.084** (0.026)	**0.006*** (0.011)	**1.000** (0.050)	**0.248** (0.032)

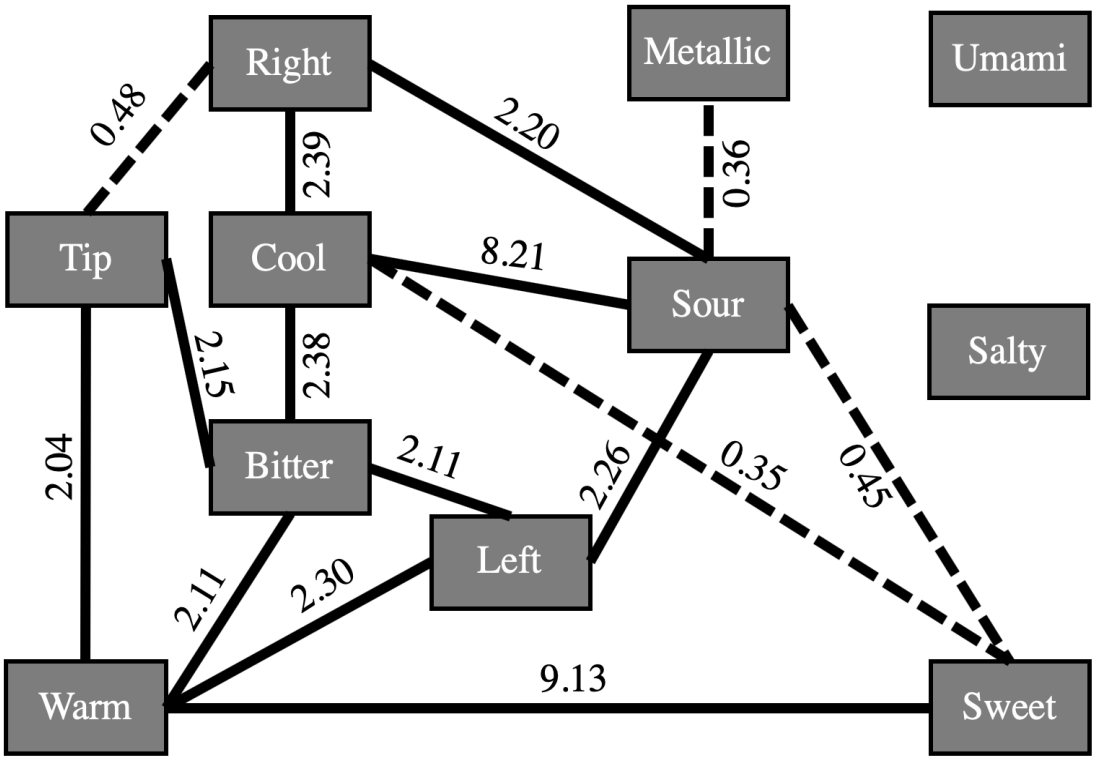

Figure 4.3: Summary of Fisher's exact test results measuring the association between the type of thermal taste (*sweet, sour, salty, bitter, umami* or *metallic*), location (*tip, left* or *right)* and temperature regime *(warming* or *cooling)* during thermal elicitation. Attributes linked with a solid line are more likely to occur together (e.g. S*weet* thermal tastes are more likely to be reported during *warming).* Attributes linked with a dashed line are less likely to be reported at the same time. Attributes that are not connected by a line are not associated. Odds ratio for each significant pair shown on lines.

under 0.5 and are shown as dashed lines in Figure 4.3. Notably, *sweet* TT were less likely to be *sour* TT (OR=0.45) or *cool* TT (OR=0.35). Mann-Whitney U was used to test for significant differences in orosensory responsiveness between the newly-identified two-factor TT subgroups and other TT. After correction for multiple comparisons, no significant differences in orosensory responsiveness based on any two-factor TT subgroups were found (Supplementary Tables 4.5-4.8). No trends were observed when mean orosensory responsiveness was graphed for each TT subgroup (see Thibodeau et al., 2020, Chapter 7).

4.3.5 Temperature Responsiveness

Warmandcool TT rated the heat elicited by the heating cycle significantly higher on the *left* (K=14.1, p=0.001) and *right* (K=13.8, p=0.001) of the tongue than *onlywarm* and *onlycool* TT (Figure 4.4). Similar trends for the intensity of warming of the *lip* (K=7.0. p=0.031) and cooling of the *tip* (K=10.9, p=0.004), the *left* (K=7.4, p=0.025) and the *right* (K=7.25, p=0.027) of the tongue were observed, but were not significant after correction for multiple comparisons. There were no differences in scale use observed between the groups on the gLMS (K=2.8, p = 0.243) or gVAS (K=1.8, p=0.400). In addition, no significant differences in orosensory responsiveness were found between the three groups for *sweet* (F=0.82, p=0.440), *salty* (K=0.62, p=0.735), s*our* (F=0.24, p=0.788), *bitter* (F=0.20, p=0.817), *umami* (F=0.16, p=0.848), *metallic* (F=0.14, p=0.866) or *astringent* (F=0.50, p=0.609). No differences in temperature responsiveness were found for any of the two-factor TT subgroups tested (Supplementary Table 4.9).

4.3.6 Training: Scales & Aqueous Stimuli

The impact of methodological differences on the prevalence of different subgroups of TT identified was investigated. When an aqueous *metallic* stimulus was included during training prior to thermal elicitation, more *metallic* TT were identified than in the absence of training with a *metallic* stimulus ($p<0.0001$, OR=20.1). The inclusion of *umami* and *metallic* scales to the thermal elicitation ballot increased the number of *umami* TT (p=0.003, OR= 7.9) and *metallic* TT ($p<0.0001$, OR=13.7) identified (Figure 4.5). None of the methodological differences impacted the number of *sweet* TT, *salty* TT, *sour* TT or *bitter* TT identified (Supplementary Table 4.10).

4.4 Discussion

The findings in this study represent the first comprehensive analysis of the experiences reported by TT during TTS screening in a large sample (n=254). The large proportion of TT experiencing *sweet*, *salty*, *sour, bitter* and *metallic* (21-42%) thermally-elicited sensations is consistent with previous literature (Yang et al., 2014; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016; Pickering et al., 2016; Skinner et al., 2018) and suggests their importance in the TT phenomenon. Conversely, thermally-elicited

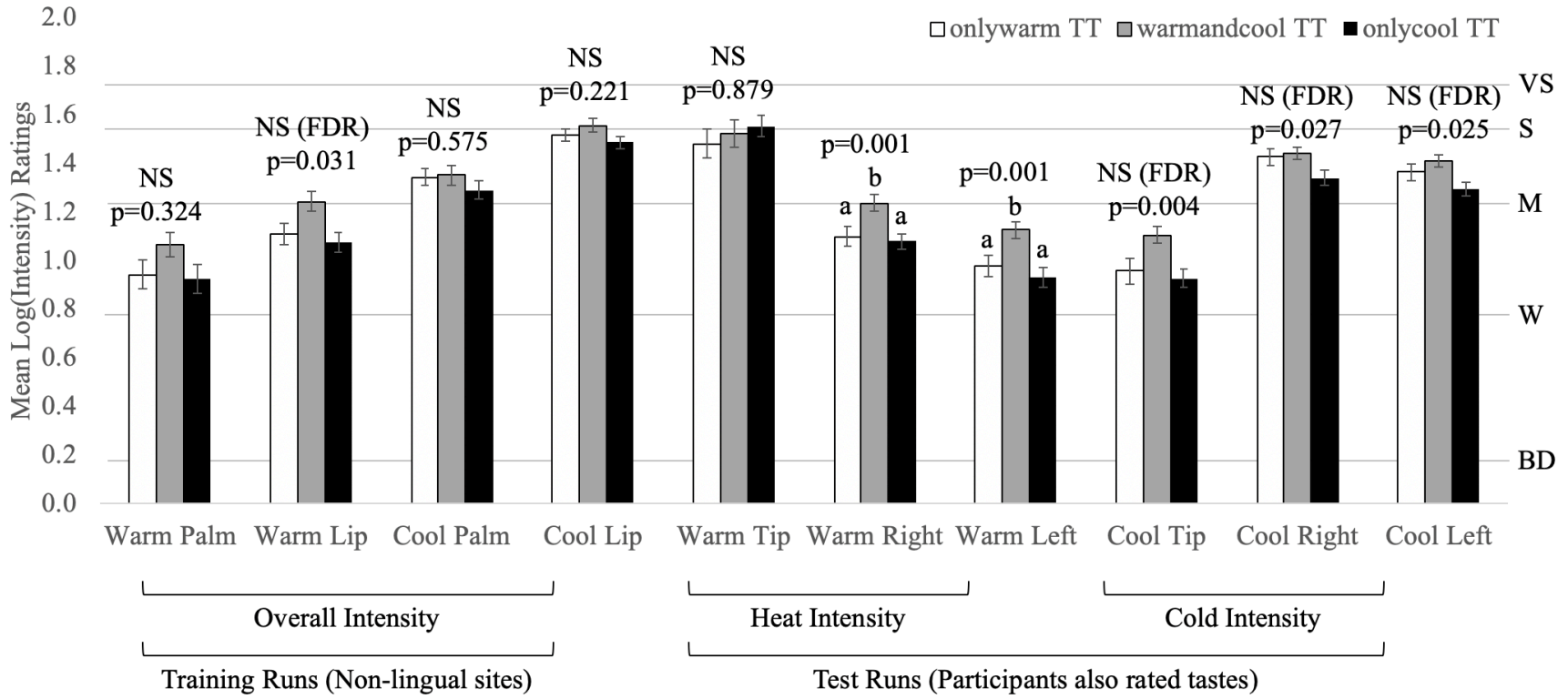

Figure 4.4: Mean temperature responsiveness (+/- SE) of *onlywarm* TT, *onlycool* TT and *warmandcool* TT to warming and cooling of the palm, lip and tongue (tip, 1-cm to the left, 1-cm to the right). Differences tested using Kruskal-Wallis. Means with different letters differ (NS = non-significant, NS(FDR) = non-significant after false discovery rate correction). Vertical lines indicate the position of anchor terms on the gLMS (BD = barely detectable, W = weak, M = moderate, S= strong, VS = very strong).

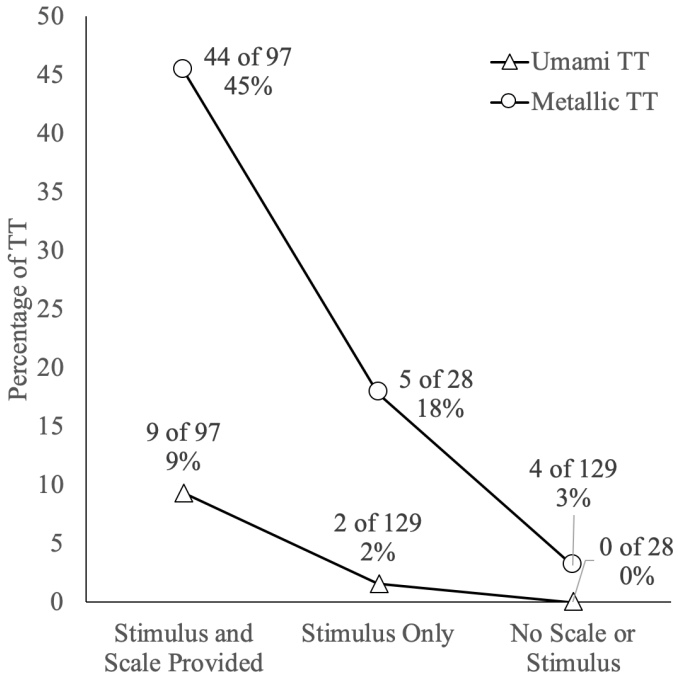

Figure 4.5: Percentage of *umami* (triangles) and *metallic* (circles) TT identified based on the inclusion/exclusion of matching aqueous orosensory stimuli during training and including *umami* and *metallic* scales on the ballot used to report thermal tastes.

umami is relatively rare as only 11 participants (4%) were *umami* TT, of which only four individuals were *umami* TT only. Consistent with Skinner et al. (2018), thirty-five percent of participants belong to two or more taste-related TT subgroups.

The proportion of TT who experience thermally-elicited orosensations during warming only, during cooling only, and during both warming and cooling is roughly equivalent in the current study (Figure 4.2). Interestingly, Skinner et al. (2018) showed that thermally-elicited tastes with onsets during the cooling period could persist beyond the end of cooling and into the warming phase of a trial. As 31% of participants are both *warm* TT and *cool* TT, it is unlikely that thermally-elicited sensations carried over from cooling to warming account for all these observations. Rather, is it likely that most participants experience distinct thermally-elicited sensations across both temperature regimes. However, more research is required to determine the proportion of TT for which this is true and if/how this impacts the number/type of thermally-elicited sensations reported during the warming trials. The proportion of individuals that experienced thermally-induced

orosensations was roughly equivalent across all three lingual locations (*tip*, *left*, *right*) with 50% reporting sensations in two or three locations. The equal proportions may result from the fact that all three locations are located on the anterior two-thirds of the tongue and served by the same nerves (chorda tympani and trigeminal (Martin, 2013).

It is important to note that the percentages reported here and throughout the paper only include thermally-elicited tastes that were both above threshold (>weak, 6 mm on gLMS) and reproducible, unless otherwise specified. These criteria reduced the risk of including data that were due to response bias, which may be accentuated by the lengthy TTS screening procedure, but also possibly reduced the overall number of TT identified both overall and within subgroups.

4.4.1 Insights into Thermal Taste Mechanism(s)

Sweet TT were more likely to also be *warm* TT and less likely to be *cool* TT, providing further support for the role of TRPM5 in thermally-elicited *sweet* orosensations. It also confirms the observations of previous work in a larger sample size (Cruz and Green, 2000; Green et al., 2005; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). *Sour* TT were more likely to also be *cool* TT, *right* TT and *left* TT, also consistent with previous literature (Cruz and Green, 2000; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018). Interestingly, *sour* TT were also less likely to be *sweet* TT or *metallic* TT, suggesting that different peripheral mechanism(s) may be involved. The odds ratios were also highest for *sweet/warm* TT and *sour/cool* TT making them good starting points for research investigating peripheral mechanism(s), and such work is encouraged.

Consistent with previous studies where three locations were tested, *bitter* TT were more likely to also be *warm* TT, *cool* TT, *left* TT and *right* TT (Pickering and Kvas, 2016). This likely reflects the fact that thermally-elicited *bitterness* is the most common taste-related orosensation observed in the study, thus it is more likely to occur universally, and that its elicitation is independent of temperature regime and location along the edge of the tongue. *Metallic* TT and *salty* TT were equally likely to be TT for both temperature regimes and all three locations. As expected based on previous literature (Cruz and Green, 2000; Yang et al., 2014; Pickering and Kvas, 2016; Skinner et al., 2018), a trend of *salty* TT being more likely to also be *cool* TT was observed. Much like thermally-induced *bitterness,* thermally-induced *salty* and *metallic* orosensations are likely independent of temperature

regime and location. *Umami* TT were not significantly associated with any other single-factor TT subgroup, likely a result of the low number of *umami* TT in the sample.

No significant differences in orosensory responsiveness were found for any of the TT subgroups tested based on thermally-elicited orosensation, temperature regime or location. No difference in responsiveness was found based on mechanism similarities in taste perception (*GCPR* TT and *ion* TT) or when broader definitions of TT were used (*Unmatched* TT and *Spicy* TT). Furthermore, none of the pairs of TT subgroups that were significantly associated (e.g. *sweet&warm* TT) differed in orosensory responsiveness. Contrary to earlier speculation (Pickering and Kvas, 2016; Pickering et al., 2016), *sweet* TT were not more responsive to *sweet* chemical stimuli or less responsive to non-*sweet* stimuli. Similarly, no differences in responsiveness were found for *salty, sour, bitter, umami* or *metallic.* Taken together, this provides the first comprehensive evidence that the heightened orosensory responsiveness of TT is universal and confirms the findings of a preliminary analysis by Bajec et al. (2012). This result suggests that TT can be treated as a homogeneous group in studies where differences in orosensory response rather than the mechanism(s) underlying TTS are of primary interest. Furthermore, if desired the definition of TT can be broadened to include *unmatched* TT and *spicy* TT as their orosensory responsiveness is consistent with the TT who experience thermally-elicited tastes. Importantly, this will reduce the recruitment burden for future studies and allow for more rapid contributions to the TTS field.

It is logical to assume that the same mechanism drives the heightened orosensory responsiveness of TT and TT's experience of thermally-elicited orosensations, as it is the most parsimonious theory. If true, the lack of differences supports a centrally-mediated mechanism of increased responsiveness to oral stimuli in TT. Alternatively, gustatory and trigeminal pathways must be cross-wired far enough along the transduction pathway allowing the gains to extend to all orosensations, not just those reported during thermal elicitation. More research is required to determine if structural differences in the central nervous system of TT and TnT exist. TT are more responsive to the *warmth* of the warming cycle and the *cold* of the cooling cycle than TnT (Thibodeau et al., 2019, Chapter 3). Therefore, it was speculated that differences in temperature responsiveness may also exist between TT subgroups. While the analysis was limited, it was found that *warmandcool* TT

were more responsive to most temperature changes than *warmonly* TT and *coolonly* TT (Figure 4.4). However, the three groups did not differ in orosensory responsiveness. Importantly, scale use did not differ between these groups nor did it differ when Thibodeau et al. (2019, Chapter 3) compared the scale use of TT and TnT. More research is required to confirm if temperature and orosensory responsiveness are correlated and if/how this can be exploited to understand the TTS mechanism(s).

Our findings provide some insights into possible peripherally- and centrally-mediated mechanism(s) for TTS and supports continued research into both. It is possible that the two postulated mechanism(s) are not mutually exclusive, and that some TT benefit from both.

4.4.2 Insights into Thermal Taste Classification and Methods

4.4.2.1 Training: Scales and Aqueous Stimuli

Differences in familiarity with *salty, umami* and *metallic* sensations may explain why methodological differences in TTS screening impacted the proportion of TT subgroups identified. The proportion of *salty* TT did not vary if training using *salty* stimulus was included or excluded. The widespread availability of salty foods likely meant that participants were already sufficiently familiarized with saltiness so that additional training did not impact the rate of *salty* TT identification.

The inclusion or exclusion of an *umami* stimulus prior to thermal elicitation did not impact the proportion of *umami* TT identified. It is possible that the limited training provided (1-4 exposures) was not sufficient to overcome the low levels of familiarity with *umami* (Singh et al., 2015; Cecchini et al., 2019). However, more *umami* TT were identified when an *umami* scale was included on the response ballot. As over 60 classes of terms have been used to describe the *umami*-eliciting stimulus MSG (L-glutamic acid monosodium salt hydrate) in a European cohort (Cecchini et al., 2019), the prompt provided by the labelled scale may have been helpful in identifying *umami* sensations during thermal stimulation. The findings are further complicated by the fact that MSG, the stimulus used for training on *umami,* also elicits a salty taste. Importantly, providing training with an *umami* stimulus did not impact the proportion of *salty* TT identified despite the fact that MSG also tastes *salty.* As a result, it is unlikely that *salty* TT are incorrectly identified as

umami TT or vis versa. While the number of *umami* TT identified is small, this provides evidence that thermally-elicited *umami* sensations are real.

During thermal elicitation, the proportion of *metallic* TT increased significantly when training with a metallic stimulus was provided before testing and/or a *metallic* scale was included on the response ballot. This finding is consistent with Lawless et al. (2005) who found that ferrous solutions were more likely to be described as *metallic* when the descriptor was embedded in a list of options. Importantly, if the proportion of individuals who had a *metallic* scale and were trained with a *metallic* stimulus prior to thermal elicitation is correct, the number of *metallic* TT would more than double. Conservatively, this suggests that up to 5% of NC and/or TnT are misclassified *metallic* TT in the absence of scales and training. The inclusion of oleogustus (a fatty taste) as a sixth basic taste is still debated (reviewed in Besnard et al., 2016; Running and Mattes, 2016). When *fatty/oily* was embedded in the list of possible sensations it was reported by 41% of participants during warming (Karunanayaka et al., 2018). There is no evidence in the present study for a thermally-elicited *oleogustus* sensation as it was not reported by any individual (TT or NC). Similarly to *umami* and *metallic*, to conclusively determine if thermally-elicited *oleogustus* is real, participants well familiarized with the sensation should be tested for TTS.

4.4.2.2 Spicy & Minty

The percentage of *spicy* TT in the current study is lower than previously reported for both heating and cooling (Skinner et al., 2018). Furthermore, results from the current study do not support the inclusion of *minty* as a valid thermal taste as it was only reported by three participants.

Spicy and *minty* thermally-elicited orosensations are considered valid thermal taste responses in some studies (Yang et al., 2014, 2018; Hort et al., 2016; Skinner et al., 2018). When excluded in TTS studies it is argued that these terms are simply proxies for temperature, namely for warmth and cold, respectively (Thibodeau et al., 2019, Chapter 3). Some receptors can be activated by both temperature and chemical stimuli including TRPV1 (≥42°C & capsaicin), TRPA1 (≤17°C & menthol) and TRPM8 (≤25°C and cinnamaldehyde; Dhaka et al., 2006). As the activation ranges of TRPM8 and TRPA1 fall within the temperatures elicited by the cooling cycle, it is possible that they account for the reported *spicy* and *minty* sensations, respectively. The threshold for activation of TRPV1

by heat is 2°C higher than the maximum temperature of the warming trial. However, as individual differences exist in temperature perception in humans (Manrique and Zald, 2006; Green and Akirav, 2007), it is plausible that thermally-elicited *spiciness* results from the activation of TRPV1. Nevertheless, even after asking participants to clarify any *spicy* or *minty* thermally-elicited tastes, Skinner et al. (2018) found that between 8-13% reported these sensations as distinct from any changes in temperature experienced. Participants reporting *spiciness* and/or *mintiness* as distinct from temperature may be using the terms as proxies for the mild pain elicited by the *warming* and *cooling* cycle. More research into the neural responses of *spicy* and *minty* TT may help resolve the true nature of these sensations during TTS screening.

An additional consideration for the identification of *spicy* and *minty* TT is in the terminology used when instructing participants on how to complete TTS ballots. If participants are asked to report "any taste" sensations they experience as a result of thermal stimulation, they may only report *sweet, salty, sour* and *bitter* tastes due to the demand characteristics of the instructions (Orne, 1962; McCambridge et al., 2012). That is, participants may dismiss any other sensations they experience because they believe the correct answer is one of the four basic tastes commonly taught in childhood. As many studies allow participants to concurrently rate the temperature experienced during warming or cooling, they may attribute any *spicy* or *minty* orosensation to the temperature scales even if they are perceptually distinct from the temperature felt. Therefore, the instructions to participants should be "any sensation" elicited from thermal elicitation.

4.4.3 Other Considerations

Metallic is considered a valid thermally-elicited taste due to the high proportion of TT who report it during thermal stimulation in both the current study and previous literature (Yang et al., 2014; Skinner et al., 2018). It remains unclear if *metallic* stimuli elicit a distinct prototypical *metallic* taste in addition to retro-nasal aromas (Lawless et al., 2005; Epke et al., 2009; Skinner et al., 2017; Wang et al., 2019). Therefore, identifying the mechanism(s) underlying thermally-elicited *metallic* sensations may help our understanding of *metallic* perception more broadly.

Karunanayaka et al. (2018) found that 48% of participants reported a *metallic* taste when a silver probe was applied to the tongue and the temperature of the probe was held at

a static 25°C. The researchers believed that the silver plate applied to the tongue was inert, but this finding suggests that the silver plate itself in the absence of a temperature change can elicit a metallic taste. This challenges the validity of considering thermally-elicited *metallic* sensations as real. It is possible that the appearance of a shiny metallic-looking probe may cue participants to report a metallic taste even if it is not experienced. It is difficult to assess the importance of these findings due to limitations in the methodology. By applying the probe to the tongue (~35°C) at a starting temperature of 25°C, rapid cooling of the tongue would occur at the start of all trials, including the warming, cooling and negative control trials where the probe was held at 25°C. Importantly, in all studies except Karunanayaka et al. (2018), the probe is wrapped in a piece of clear plastic wrap, creating a barrier between the probe and the tongue which should prevent chemically-induced *metallic* sensations. Further research is needed to confirm that the *metallic* orosensations reported during thermal elicitation are real.

4.5 Conclusions

Our study is the first to characterize the thermally-elicited sensations reported by TT as well as the temperature regimes and locations at which they were elicited in a large sample. Thermally-elicited *sweetness* and *sourness* were elicited significantly more frequently during the *warming* cycle and the *cooling* cycle, respectively. Despite the identification of several TT subgroups, no differences in orosensory responsiveness for any subgroups were identified. Taken together, this suggests that multiple mechanism(s) underlie TTS and that they may be peripherally- and/or centrally-mediated. Practically, the findings support the continued treatment of TT as a single homogenous group in studies where the primary aim is not to investigate the TTS mechanism(s). In addition, the orosensory responsiveness of *spicy* TT and *unmatched* TT does not differ from other TT. Therefore, the definition of TT can be broadened to include *spicy* TT and *unmatched* TT if desired.

4.6 Link to Published Version

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4.7 References

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4.8 Supplementary Materials

4.8.1 Supplementary Tables

Table S4.1: Summary of methodological differences between cohorts.

Cohort	**1**	**2, 3**	**4**	**5, 7, 10 & 11**	**6**	**8**	**9**	**12**
Remembered Sensations								
Scales Rated	gVAS and gLMS	gVAS and gLMS	gVAS and gLMS	gVAS and gLMS	gLMS only	gVAS and gLMS	gVAS and gLMS	gLMS only
"pain from biting your tongue", "brightness of the sun when you are looking directly at it", "burning sensation of eating a whole hot pepper"	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
"sweetness of cotton candy"	Yes	Yes	Yes	Yes		Yes	Yes	Yes
"touch sensation of a pill on your tongue"	Yes	Yes	Yes	Yes		Yes	Yes	
“the brightness of a dimly lit room”								Yes
"coolness of an ice-cold beverage", "sourness of a lemon"	Yes	Yes			Yes			
"sweetness of a banana", "heat of drinking hot tea", "burn of cinnamon gum", "coolness of a peppermint candy", "warmth of sipping lukewarm water", "bitterness of black coffee", "saltiness of ocean water", "tingling from a carbonated drink"	Yes	Yes						
Orosensory Responsiveness								
Scale Used	gVAS	gVAS	gVAS	gVAS	gLMS	gVAS	gVAS	gLMS
Palate Cleansers in Addition to Filtered Water	5 g/L Pectin	5 g/L Pectin	None	Soda Crackers	5 g/L Pectin	None	None	None
Number of Blind Orosensory Stimuli Replicates	1 or 2	1	1	1 or 2	1	1 or 2	1 or 2	0
Number of Labelled Orosensory Stimuli Replicates	1	1	1	1 or 2	1	1	1	1
Total Number of Orosensory Stimuli Replicates	2 or 3	2	2	2 or 4	2	2 or 3	2 or 3	1
Thermal Taste								
Training Runs (Palm & Lip, Warming & Cooling Cycles)	2	2	1	1		1	1	1
Training Runs (Tongue 10s at 37°C)					2			
Temperature, Sweet, Salty, Sour, Bitter & Other Scales	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Umami & Metallic Scales				Yes				Yes

Table S4.2: T-test results comparing the responsiveness of thermal taste subgroups to aqueous solutions (*sweet, salty* and *sour)*. After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli (Critical t-value)		Sweet (t crit = 1.97)				Salty (t crit = 1.97)				Sour (t crit = 1.97)			
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	Obs. t	p-value	Group 1 Number	Group 2 Number	Obs. t	p-value	Group 1 Number	Group 2 Number	Obs.t	p-value
Taste (s) experienced during thermal elicitation													
Sweet	*Not Sweet*	77	177	1.31	0.191	64	151	0.71	0.477	77	177	0.05	0.961
Salty	*Not Salty*	51	203	0.68	0.500	41	174	0.91	0.362	51	203	0.12[b]	0.907[a]
Sour	*Not Sour*	89	165	0.14	0.889	73	142	1.01	0.313	89	165	0.70	0.486
Bitter	*Not Bitter*	107	147	0.49	0.627	91	124	1.54	0.124	107	147	0.72	0.471
Umami	*Not Umami*	11	243	2.78	0.006	10	205	0.78	0.434	11	243	0.56[b]	0.574[a]
Metallic	*Not Metallic*	53	201	0.56	0.574	50	165	0.92	0.357	53	201	1.34	0.183
GCPR	*Not GCPR*	164	90	1.00	0.320	137	78	1.81	0.072	164	90	0.79	0.432
Ion	*Not Ion*	125	129	0.33	0.739	102	113	1.44	0.152	125	129	0.37	0.715
Unmatched	*Matched*	32	254	0.27	0.785	30	215	0.96	0.340	32	254	1.19[b]	0.235[a]
Spicy	*Not Spicy*	25	240	0.12	0.906	23	202	1.29	0.200	25	240	0.77	0.443
Location at which the thermally-elicited taste was experienced during thermal elicitation													
Tip	*Not Tip*	157	97	1.00	0.319	130	85	0.65	0.514	157	97	0.24	0.810
Right	*Not Right*	140	114	0.05	0.958	117	98	0.48	0.634	140	114	0.51	0.609
Left	*Not Left*	142	112	0.38	0.705	118	97	0.94	0.350	142	112	0.12[b]	0.901[a]
Temperature during which the thermally-elicited taste was experienced during thermal elicitation													
Cool	*Not Cool*	179	75	0.02	0.987	150	65	0.42[b]	0.674[a]	179	75	0.69	0.491
Warm	*Not Warm*	155	99	1.11	0.269	130	85	0.78[b]	0.433[a]	155	99	0.41	0.682
Warmandcool	*Not Warmandcool*	80	174	1.15	0.252	65	150	0.10	0.921	80	174	0.25	0.806

[a] Failed to meet the assumptions of a t-test. P-value obtained from Mann-Whitney U Test where U_{crit}=1.96. [b]$U_{standardized.}$

Table S4.3: T-test results comparing the responsiveness of thermal taste subgroups to aqueous solutions (*bitter, umami,* and *metallic)*. After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli (Critical t-value)		Bitter (t crit = 1.97)				Umami (t-crit = 1.97)				Metallic (t-crit = 1.98)			
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	Obs. t	p-value	Group 1 Number	Group 2 Number	Obs. t	p-value	Group 1 Number	Group 2 Number	Obs. t	p-value
Taste (s) experienced during thermal elicitation													
Sweet	*Not Sweet*	77	177	0.32	0.753	66	160	1.27	0.207	41	84	0.32	0.748
Salty	*Not Salty*	51	203	0.23	0.816	44	182	0.78	0.434	22	103	0.09	0.925
Sour	*Not Sour*	89	165	0.52	0.601	78	148	1.09	0.279	41	84	0.18[b]	0.856[a]
Bitter	*Not Bitter*	107	147	0.22	0.823	97	129	0.71	0.477	43	82	1.18	0.241
Umami	*Not Umami*	11	243	0.13	0.900	11	215	1.52	0.131	9	116	0.01	0.990
Metallic	*Not Metallic*	53	201	0.75[b]	0.456[a]	48	178	0.75	0.452	49	76	1.40	0.165
GCPR	*Not GCPR*	164	90	0.09	0.931	147	79	0.85	0.399	73	52	1.14	0.212
Ion	*Not Ion*	125	129	0.30	0.764	109	117	0.87	0.384	54	71	1.23	0.222
Unmatched	*Matched*	32	254	0.14	0.890	26	226	0.86	0.391	20	125	1.55	0.122
Spicy	*Not Spicy*	25	240	1.06	0.292	20	215	1.00	0.32	14	116	0.67	0.506
Location at which the thermally-elicited taste was experienced during thermal elicitation													
Tip	*Not Tip*	157	97	0.05	0.961	137	89	1.24	0.217	83	42	1.08	0.281
Right	*Not Right*	140	114	0.55	0.584	121	105	0.52	0.607	75	50	0.64	0.524
Left	*Not Left*	142	112	0.82	0.416	132	94	0.55	0.584	69	56	0.73	0.470
Temperature during which the thermally-elicited taste was experienced during thermal elicitation													
Cool	*Not Cool*	179	75	0.44	0.658	158	68	0.03	0.977	92	33	0.44	0.660
Warm	*Not Warm*	155	99	0.16	0.872	137	89	0.47	0.636	75	50	0.07	0.942
Warmandcool	*Not WarmandCool*	80	174	0.61	0.546	69	157	0.53	0.596	42	83	0.10	0.921

[a] Failed to meet the assumptions of a t-test. P-value obtained from Mann-Whitney U Test where U_{crit}=1.96. [b]$U_{standardized.}$

Table S4.4: T-test results comparing the responsiveness of thermal taste subgroups to aqueous solutions (*astringent)*. After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli (Critical t-value)			Astringent (1.98)		
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	Obs. t	p-value
Taste (s) experienced during thermal elicitation					
Sweet	*Not Sweet*	29	63	0.73	0.466
Salty	*Not Salty*	21	71	0.95	0.343
Sour	*Not Sour*	35	57	0.91[b]	0.366[a]
Bitter	*Not Bitter*	35	57	1.08	0.285
Umami	*Not Umami*	3	89	0.37[b]	0.709[a]
Metallic	*Not Metallic*	21	71	0.81	0.419
GCPR	*Not GCPR*	58	34	1.82	0.720
Ion	*Not Ion Channel*	49	43	1.97[b]	0.049[a]
Unmatched[b]	*Matched*	13	92	1.53	0.129
Spicy	*Not Spicy*	9	87	1.09	0.278
Location at which the thermally-elicited taste was experienced during thermal elicitation					
Tip	*Not Tip*	65	27	0.50	0.622
Right	*Not Right*	57	35	0.73	0.470
Left	*Not Left*	49	43	0.76	0.449
Temperature during which the thermally-elicited taste was experienced during thermal elicitation					
Cool	*Not Cool*	69	23	0.45	0.652
Warm	*Not Warm*	55	37	1.15[b]	0.252[a]
Warmandcool	*Not Warmandcool*	32	60	0.61	0.541

[a] Failed to meet the assumptions of a t-test. P-value obtained from Mann-Whitney U Test where U_{crit}=1.96. [b]$U_{standardized.}$

Table S4.5: Mann-Whitney U results comparing the responsiveness of two-factor thermal taste subgroups to aqueous solutions (*sweet* and *salty)*. After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli		Sweet					Salty				
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value
Bitter & Cool	*All Other*	67	187	6051.5	0.41	0.680	58	157	4046.0	1.25	0.211
Bitter & Left	*All Other*	52	202	5387.5	0.29	0.775	42	173	3301.0	0.92	0.359
Bitter & Tip	*All Other*	53	201	5875.5	1.15	0.249	42	173	3797.5	0.45	0.650
Left & Sour	*All Other*	49	205	5193.0	0.37	0.713	39	176	3193.0	0.68	0.497
Left & Warm	*All Other*	75	179	6945.0	0.43	0.664	60	155	4091.5	1.36	0.173
Right & Cool	*All Other*	95	159	7511.0	0.07	0.942	82	133	5353.0	0.23	0.822
Right & Sour	*All Other*	46	208	4880.5	0.21	0.831	39	176	3317.5	0.32	0.746
Sour & Cool	*All Other*	77	177	6369.0	0.83	0.408	61	154	4186.5	1.24	0.215
Warm & Bitter	*All Other*	58	196	5726.5	0.09	0.932	48	167	4166.5	0.28	0.776
Warm & Sweet	*All Other*	64	190	5568.5	1.01	0.315	51	164	3835.0	0.89	0.372
Warm & Tip	*All Other*	88	166	6703.0	1.08	0.281	71	144	4399.0	1.66	0.097
Not Sour & Sweet	*All Other*	106	148	6919.5	1.60	0.110	93	122	4996.0	1.50	0.134
Not Sweet & Cool	*All Other*	40	214	3824.0	1.07	0.286	35	180	3260.0	0.33	0.745
Not Tip & Right	*All Other*	33	221	3870.0	0.57	0.571	28	187	2401.5	0.70	0.482
Not Metallic & Sour	*All Other*	122	132	7975.5	0.13	0.897	102	113	5974.0	0.46	0.644

Table S4.6: Mann-Whitney U results comparing the responsiveness of two-factor thermal taste subgroups to aqueous solutions (*sour* and *bitter)*. After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli		Sour					Bitter				
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value
Bitter & Cool	*All Other*	67	187	6051.5	1.13	0.258	67	187	6483.0	0.42	0.673
Bitter & Left	*All Other*	52	202	5954.5	1.49	0.137	52	202	6036.0	1.66	0.097
Bitter & Tip	*All Other*	53	201	6568.0	2.61	0.009	53	201	5284.5	0.09	0.930
Left & Sour	*All Other*	49	205	4822.5	0.43	0.666	49	205	4103.0	1.99	0.047
Left & Warm	*All Other*	75	179	6349.5	0.68	0.497	75	179	6128.5	1.09	0.275
Right & Cool	*All Other*	95	159	6089.0	2.58	0.010	95	159	7754.0	0.36	0.723
Right & Sour	*All Other*	46	208	3760.0	2.27	0.023	46	208	5254.0	1.04	0.298
Sour & Cool	*All Other*	77	177	6120.5	1.29	0.198	77	177	7060.5	0.46	0.648
Warm & Bitter	*All Other*	58	196	5329.0	0.72	0.471	58	196	5595.5	0.18	0.858
Warm & Sweet	*All Other*	64	190	6178.0	0.19	0.848	64	190	5913.5	0.33	0.744
Warm & Tip	*All Other*	88	166	6360.0	1.69	0.090	88	166	7549.5	0.44	0.660
Not Sour & Sweet	*All Other*	106	148	7645.0	0.34	0.731	106	148	8333.5	0.85	0.397
Not Sweet & Cool	*All Other*	40	214	3870.0	0.96	0.337	40	214	4163.0	0.27	0.785
Not Tip & Right	*All Other*	33	221	3655.0	0.02	0.984	33	221	4143.0	1.26	0.208
Not Metallic & Sour	*All Other*	122	132	7755.5	0.51	0.613	122	132	8107.5	0.094	0.925

Table S4.7: Mann-Whitney U results comparing the responsiveness of thermal taste two-factor subgroups to aqueous solutions (*umami,* and *metallic).* After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli		Umami					Metallic				
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value	Group 1 Number	Group 2 Number	$U_{observed}$	$U_{standardized}$	p-value
Bitter & Cool	*All Other*	58	168	4778.0	0.22	0.828	29	96	1467.0	0.44	0.663
Bitter & Left	*All Other*	48	178	4250.0	0.05	0.957	17	108	1041.0	0.88	0.378
Bitter & Tip	*All Other*	48	178	4670.0	0.99	0.323	19	106	1187.5	1.24	0.216
Left & Sour	*All Other*	42	184	4167.0	0.79	0.429	25	100	1003.5	1.52	0.129
Left & Warm	*All Other*	67	159	5420.5	0.21	0.835	32	93	1322.0	0.94	0.349
Right & Cool	*All Other*	81	145	5652.5	0.47	0.641	51	74	1824.0	0.31	0.754
Right & Sour	*All Other*	39	187	3588.0	0.16	0.876	23	103	1132.0	0.26	0.796
Sour & Cool	*All Other*	69	157	4933.5	1.07	0.286	34	91	1509.0	0.21	0.835
Warm & Bitter	*All Other*	53	173	4739.5	0.37	0.711	24	101	1015.0	1.23	0.218
Warm & Sweet	*All Other*	56	170	3963.0	1.88	0.061	31	94	1494.5	0.21	0.832
Warm & Tip	*All Other*	76	150	5003.5	1.50	0.134	48	77	1818.0	0.15	0.881
Not Sour & Sweet	*All Other*	97	129	5648.5	1.25	0.212	50	75	1956.0	0.41	0.685
Not Sweet & Cool	*All Other*	38	188	3015.0	1.51	0.130	15	110	905.5	0.61	0.543
Not Tip & Right	*All Other*	32	194	3176.5	0.21	0.834	11	114	712.0	0.74	0.461
Not Metallic & Sour	*All Other*	109	117	6373.0	0.10	0.006	45	80	1593.5	1.06	0.289

Table S4.8: Mann-Whitney U results comparing the responsiveness of two-factor thermal taste subgroups to aqueous solutions (*astringent).* After correction for multiple comparisons using false-discovery rate, no significant differences were found.

Orosensory Stimuli		Astringent				
Type of Thermal Taster (Group 1)	Type of Thermal Taster (Group 2)	Group 1 Number	Group 2 Number	$U_{critical}$	$U_{observed}$	p-value
Bitter & Cool	*All Other*	25	67	902.5	0.57	0.571
Bitter & Left	*All Other*	20	72	733.5	0.12	0.902
Bitter & Tip	*All Other*	19	73	756.5	0.60	0.547
Left & Sour	*All Other*	21	71	619.5	1.17	0.243
Left & Warm	*All Other*	28	64	831.5	0.54	0.587
Right & Cool	*All Other*	39	53	1107.0	0.58	0.564
Right & Sour	*All Other*	72	136	859.0	1.31	0.190
Sour & Cool	*All Other*	31	61	1057.5	0.92	0.357
Warm & Bitter	*All Other*	19	73	706.0	0.12	0.908
Warm & Sweet	*All Other*	25	67	695.5	1.24	0.214
Warm & Tip	*All Other*	38	54	999.0	0.21	0.834
Not Sour & Sweet	*All Other*	34	58	908.0	0.63	0.531
Not Sweet & Cool	*All Other*	11	81	347.5	1.17	0.241
Not Tip & Right	*All Other*	7	85	327.5	0.43	0.664
Not Metallic & Sour	*All Other*	82	80	918.5	0.95	0.341

Table S4.9: Kruskal-Wallis (three groups, K_{crit}=5.99) and Mann-Whitney U (two groups, U_{crit}=1.96) p-values for differences in temperature responsiveness to warming and cooling of the palm, lip and three locations on the tongue. Only p-values marked with "*" were significant after correction for multiple comparison using the false discovery rate method.

	onlywarm TT vs *onlycool* TT vs *warmandcool* TT	*bitter&cool* TT vs *other* TT	*sour&cool* TT vs *other* TT	*warm&bitter* TT vs *other* TT	*warm&sweet* TT vs *other* TT
Non-Lingual Sites					
n (in order of the groups)	68, 89, 69	58, 168	69, 157	53, 173	56, 170
Warming Cycle					
Palm	0.324	0.209	0.989	0.649	0.961
	K= 2.25	$U_{standard}$=1.26	$U_{standard}$=0.01	$U_{standard}$=0.46	$U_{standard}$=0.05
Lip	0.031	0.507	0.334	0.583	0.162
	K=6.97	$U_{standard}$=0.66	$U_{standard}$=0.97	$U_{standard}$=0.55	$U_{standard}$=1.40
Cooling Cycle					
Palm	0.575	0.479	0.648	0.442	0.691
	K=1.11	$U_{standard}$=0.71	$U_{standard}$=0.46	$U_{standard}$=0.44	$U_{standard}$=0.40
Lip	0.221	0.949	0.268	0.801	0.718
	K=3.02	$U_{standard}$=0.06	$U_{standard}$=1.11	$U_{standard}$=0.25	$U_{standard}$=0.36
Lingual Sites n (in order of the groups)	75, 99, 80	67, 187	77, 177	58, 196	64, 190
Warming Cycle					
Tip	0.879	0.995	0.352	0.612	0.409
	K=0.26	$U_{standard}$=0.01	$U_{standard}$=0.93	$U_{standard}$=0.51	$U_{standard}$=0.83
Right	**0.001***	0.251	0.975	0.218	0.277
	K=13.86	$U_{standard}$=1.15	$U_{standard}$=0.03	$U_{standard}$=1.23	$U_{standard}$=1.09
Left	**0.001***	0.338	0.82	0.555	0.096
	K=14.12	$U_{standard}$=0.96	$U_{standard}$=0.23	$U_{standard}$=0.59	$U_{standard}$=1.67
Cooling Cycle					
Tip	0.004	0.246	0.433	0.758	0.080
	K=10.85	$U_{standard}$=1.16	$U_{standard}$=0.78	$U_{standard}$=0.31	$U_{standard}$=1.75
Right	0.027	0.941	0.063	0.032	0.438
	K=7.25	$U_{standard}$=0.07	$U_{standard}$=1.86	$U_{standard}$=2.14	$U_{standard}$=0.78
Left	0.025	0.771	0.332	0.084	0.452
	K=7.41	$U_{standard}$=0.29	$U_{standard}$=0.97	$U_{standard}$=1.73	$U_{standard}$=0.75

Table S4.10: Fisher's exact test results (p-values) evaluating the effect of methodological differences. Differences are the inclusion/exclusion of (i) *metallic*, *umami*, *salty*, and *astringent* stimuli when familiarizing participants with orosensations before thermal taste elicitation, and (ii) *umami* and *metallic* scales on the ballot used to report thermal tastes during thermal elicitation. *Significant findings after correcting for multiple comparisons using the false discovery rate method (d=0.05).

	Aqueous orosensations that were absent or present during training				Thermal taste ballot with (n=97) or without (n=157) *umami* and *metallic* scales
	Salty (n_{absent}=39, $n_{present}$=215)	Umami (n_{absent}=28, $n_{present}$=226)	Metallic (n_{absent}=129, $n_{present}$=125)	Astringent (n_{absent}=92, $n_{present}$=162)	
Sweet TT	0.706	0.282	0.415	0.777	0.889
Salty TT	0.385	0.462	0.351	0.419	0.197
Sour TT	0.466	0.676	0.511	0.495	0.343
Bitter TT	1.000	0.546	0.016	0.356	0.050
Umami TT	1.000	0.616	0.032	0.751	0.003*
Metallic TT	0.031	0.808	<0.0001*	0.630	<0.0001*

Chapter 5: Impact of Thermal Taste Status on Taste-Taste Interactions with Ethanol

5.1 Introduction

According to the *Global Status Report on Alcohol and Health* in 2016, 43% of individuals over the age of fifteen worldwide were current consumers of alcoholic beverages (World Health Organization, 2018). Alcohol misuse is associated with several negative health and social effects including increasing the risk of cancer, neuropsychiatric disorders, cardiovascular disease, digestive diseases and accidental injury/death (Barbor et al., 2001; World Health Organization, 2018). In contrast, moderate consumption of alcoholic beverages may also be associated with increased well-being including relaxation, creativity or the ability to express oneself (Park and Grant, 2005), and reduced adverse cardiovascular events (Krenz and Korthuis, 2012). As a result, understanding factors that impact alcohol consumption is important to reduce the harm associated with alcohol misuse, while also providing valuable consumer information to the alcoholic beverage industry.

Alcohol consumption is influenced by several factors including gender, individual differences, genetics, social expectations and sanctions, interpersonal relationships, personality, demographics and socioeconomic status (Nolen-Hoeksema, 2004; Tepper, 2008; Chartier et al., 2017; Fu et al., 2019). Consumers also identify flavour as one of the most important factors when purchasing alcoholic beverages (Bruwer and Buller, 2012; Small-Kelly, 2018). Individuals who are more responsive to both taste and chemesthetic sensations tend to report lower liking and consumption of alcoholic beverages than those less responsive. It is possible that this reduction in liking may be due to increased responsiveness to the nominally aversive sensations (bitterness, irritation, sourness and astringency) that are commonly elicited by alcoholic beverages (reviewed in Thibodeau and Pickering, 2019, Chapter 2). As alcoholic beverages are complex matrices that vary considerably in flavour and composition, the study sought to better understand how ethanol impacts the perception of prototypical stimuli that elicit sensations common in alcoholic beverages. Furthermore, if/how these results were impacted by individual differences in taste perception (the thermal taste phenotype) was tested. Although olfactory stimuli also

contribute to the flavour of alcoholic beverages, the scope of the current research was limited to focus on taste (sweet, sour, bitter) and chemesthetic (astringent, burning/tingling) sensations.

5.1.1 Ethanol

The defining characteristic of alcoholic beverages is the presence of ethanol (ethyl alcohol), the primary product of fermentation. Ethanol concentrations vary with beverage style and are typically 3-7% (vol/vol) in beer, 11-16% (vol/vol) in wine and 35-45% (vol/vol) in spirits (Thibodeau and Pickering, 2019, Chapter 2). For simplicity and unless otherwise noted, ethanol concentrations are reported as % (vol/vol) throughout the manuscript. In aqueous solutions, ethanol elicits sweetness (Berg et al., 1955; Wilson et al., 1973; Scinska et al., 2000; Mattes and DiMeglio, 2001; Allen et al., 2014; Nolden and Hayes, 2015; Nolden et al., 2016; Small-Kelly and Pickering, 2020), bitterness (Wilson et al., 1973; Scinska et al., 2000; Mattes and DiMeglio, 2001; Allen et al., 2014; Nolden and Hayes, 2015; Nolden et al., 2016; Small-Kelly and Pickering, 2020), astringency (Nolden and Hayes, 2015) and irritation/burning (Wilson et al., 1973; Green, 1987, 1988; Allen et al., 2014; Nolden and Hayes, 2015; Small-Kelly and Pickering, 2020). Sourness is also reported is some studies but usually at low intensity or by only a small proportion of participants (Scinska et al., 2000; Mattes and DiMeglio, 2001; Nolden et al., 2016). In real and model alcoholic beverages, ethanol concentration has also been shown to impact the perception of perceived viscosity, density and body (Pickering et al., 1998; Nurgel and Pickering, 2005; Gawel et al., 2007). Taken together, the literature strongly supports that ethanol is a complex stimulus capable of eliciting multiple taste and chemesthetic sensations.

The intensity and relative dominance of the sensations elicited by ethanol vary with concentration. Nolden and Hayes (2015) asked participants to rate the intensity of five ethanol concentrations (4%, 8%, 16%, 32% and 48%) on generalized Labelled Magnitude Scales (gLMS). The bitterness, burning/tingling, drying and sweetness elicited by ethanol was roughly equivalent at 4% and was rated between “barely detectable” and “weak”. Sweetness increased slightly as ethanol concentration increased but remained at or below “weak”. Bitterness, drying and burning/tingling increased from around “weak” at 8% ethanol to above “moderate” at 48% ethanol. Bitterness was rated as the most intense

sensation at 8%, whereas burning/tingling was the most intense at higher ethanol concentrations (32% and 48%). The differences in intensity and dominance of the sensations elicited by ethanol likely drive the broad differences in the sensory properties of beer, wine and spirits.

5.1.2 Orosensory interactions

The composition of alcoholic beverages varies widely across styles (beer, wine, spirits) and production practices can be used to optimize the flavour profile. Broadly speaking, other compounds that contribute to the taste and chemesthetic sensations elicited by alcoholic beverages include but are not limited to organic acids (sourness), hop resins (bitterness), sugars (sweetness), carbon dioxide (tingling/prickling), and tannins (astringency, bitterness; Thibodeau and Pickering, 2019, Chapter 2).

When consumers drink alcoholic beverages, they make quick judgements about the flavour. Nevertheless, flavour perception is complex phenomenon that involves integrating multi-modal sensory inputs including, taste, olfactory and chemesthetic responses (reviewed in: Spence, 2015). Psychophysical curves can be used to characterise the nature of the interaction between two compounds as additive, suppressive or synergistic (Keast and Breslin, 2002). If the combined intensity of two compounds can be predicted from the psychophysical curves of each individual compound, the combined intensity of the two compounds is said to be additive. Roughly, additivity (no interaction) occurs when the intensity of the binary mixture is equal to the summed intensity of unary solutions of both components in the mixture (AB = A + B). If the combined intensity of two compounds is lower than predicted (AB < A + B), the interaction is suppressive (Keast and Breslin, 2002). For example, bitterness is typically supressed by the addition of a sweet stimuli (Keast and Breslin, 2002; Wilkie and Capaldi Phillips, 2014). Conversely, if the combined perceived intensity of two compounds is higher than predicted (AB > A + B), the interaction is synergistic (Keast and Breslin, 2002). As true synergy is difficult to measure, the more general term ‘enhancement’ is used to describe when the intensity of two compounds is greater than the intensity of each compound individually (Keast and Breslin, 2002). For example, bitterness tends to be enhanced by the addition of a sour stimuli (Keast and Breslin, 2002; Wilkie and Capaldi Phillips, 2014). Importantly, the nature of the interaction between

two stimuli can vary based on concentration (Keast and Breslin, 2002; Wilkie and Capaldi Phillips, 2014).

5.1.3 Ethanol and Taste/Chemesthetic Stimuli

To better understand how the compounds in alcoholic beverages interact with ethanol to elicit the flavour of alcoholic beverages, several studies have investigated the interactions that occur in binary mixtures of ethanol and spiked aqueous solutions. Although less ecologically valid than using real or model alcoholic beverages, these studies provide insights into how ethanol concentration may modify the perception of specific stimuli in alcoholic beverages.

Three studies have investigated the interaction between organic acids (citric acid, tartaric acid) and ethanol. In general, increased ethanol concentration leads to a decrease in sourness (Martin and Pangborn, 1970; Zamora et al., 2006; Guirao et al., 2013). However, this trend is typically observed when pH and organic acid concentration are higher (Zamora et al., 2006; Guirao et al., 2013), and at lower organic acid concentrations, it is possible for ethanol to enhance the sourness (Guirao et al., 2013). However, astringency was not rated in any of these studies despite being elicited by both ethanol and organic acids (Sowalsky and Noble, 1998), and thus is a potential confounding variable not yet accounted for in the literature.

The interaction between ethanol and sweet stimuli is concentration-dependent and likely impacted by the choice of sweet compound (Martin and Pangborn, 1970; Hoopman et al., 1993; Calviño, 1998). At higher concentrations (> 12%), ethanol tends to suppress the sweetness of sugars. In contrast, at lower concentrations (< 12%), ethanol can enhance or have no effect on the perceived intensity of sweet stimuli. However, the impacts of sweeteners on the sensations elicited by ethanol are less well understood, suggesting that further research into the interactions between sweet stimuli and ethanol is warranted.

The nature of the interactions between ethanol and other stimuli that elicit bitterness and/or astringency are largely uncharacterized. An aqueous tannin extract solution (0.4%) was described as less bitter and more astringent than when 5% ethanol was added (Lea and Arnold, 1978). Martin and Pangborn (1970), found that adding ethanol to quinine solutions did not impact bitterness. However, although four concentrations of quinine (0.001% to 0.004%) and four concentrations of ethanol (4 to 16%) were included in the study, a full

factorial design was not used, limiting the ability to draw wider conclusions from the results. Overall, more research is required to more fully characterize the interactions between ethanol and prototypical taste and chemesthetic stimuli.

5.1.4 Other Considerations: Thermal Taste

Although the perception of alcoholic beverages can vary based on their composition, individual differences in taste and chemesthetic perception also exist (Hayes and Keast, 2011). For example, thermal tasters (TT) are individuals that reliably experience taste sensations when their tongue is warmed and/or cooled, whereas thermal non-tasters (TnT) do not (Green and George, 2004; Bajec and Pickering, 2008; Yang et al., 2014; Thibodeau et al., 2019, Chapter 3). TT also rate the intensity of suprathreshold aqueous prototypical tastants and some trigeminal stimuli higher than thermal non-tasters (Green and George, 2004; Green et al., 2005; Bajec and Pickering, 2008; Bajec et al., 2012; Yang et al., 2014; Hort et al., 2016; Thibodeau et al., 2019, Chapter 3; Small-Kelly and Pickering, 2020). TT also rate the dominant orosensations elicited by beer (Pickering et al., 2010a) and wine (Pickering et al., 2010b) higher than TnT. Recently, Small-Kelly and Pickering (2020) compared the responsiveness of TT and TnT to ethanol ranging from 2-10%. Although bitterness intensity was similar for TT and TnT at 2% and 4% ethanol, TT rated the bitterness of 5%, 7% and 10% ethanol solutions higher than TnT. The irritation/burning and sweetness of ethanol increased for both TT and TnT as the concentration of ethanol increased, but no group differences were identified. As only concentrations of ethanol below 11% have been examined to date, possible differences between TT and TnT in the sweetness and/or bitterness of ethanol at higher concentrations are yet to be determined. More research is required to understand how the differences in orosensory perception between TT and TnT impact their perception of alcoholic beverages. In addition, to the best of our knowledge, taste and chemesthetic interactions have not been investigated in TT and TnT. To address these gaps in the literature, all participation were screened for thermal taste status before data collection.

5.1.5 Study Aims

Although interactions between ethanol and stimuli that elicit key orosensations in alcoholic beverages have been previously investigated, more research is needed to fully

characterize the relationships. Here, the interactions between ethanol and four stimuli (fructose, quinine, aluminium sulphate and tartaric acid) are investigated, which elicit taste and/or chemesthetic sensations that are common in alcoholic beverages. For each combination, a full-factorial design was used consisting of four concentrations of ethanol approximately representative of major beverage categories (0% - dealcoholized, 5% - beer, 13% - wine and 23% - spirits) and four concentrations of each stimulus (absent, low, medium and high). Trained participants rated six orosensations (sweet, sour, bitter, burning/tingling, astringency and other) when evaluating the samples using the gLMS. This strategy allowed for the interactions of both dominant and non-dominant sensations to be captured. In addition, the descriptive anchor terms on the gLMS allow for the ecological validity of the observed differences in intensity ratings to be characterized. Further, it was determined whether the increased orosensory responsiveness of TT compared to TnT, extends to binary mixtures, and whether the nature of the inter-actions differ based on thermal taste status. Taken together, the findings provide a more comprehensive understanding of the interactions between ethanol and taste/chemesthetic stimuli.

5.2 Materials & Methods

The study was divided into six 1-hour sessions. First, participants underwent thermal taste status screening (Session 1) followed by orosensory training (Session 2). Next, during the data collection phase, the order of Sessions 3A, 3B, 3C, 3D was randomized across participants. Although participants were encouraged to complete the full study, this randomization allowed for data from participants who completed a minimum of three sessions to be included, allowing for an increased sample size. Full details of the sessions are given below and an overview is provided in Figure 5.1.

Initially, a convenience sample of 142 participants was recruited from Brock University and the surrounding community to Session 1. Participants were eligible for the study if they were 19 to 40 years old, self-reported non-smokers, were free of tongue damage or abnormalities and did not have severe food allergies. Gender differences in taste perception exist (Michon et al., 2009; Thibodeau et al., 2019, Chapter 3), so to reduce their potential confounding effects, given the relatively small sample size, only female participants were eligible for the study. At the start of Session 1, participants were oriented

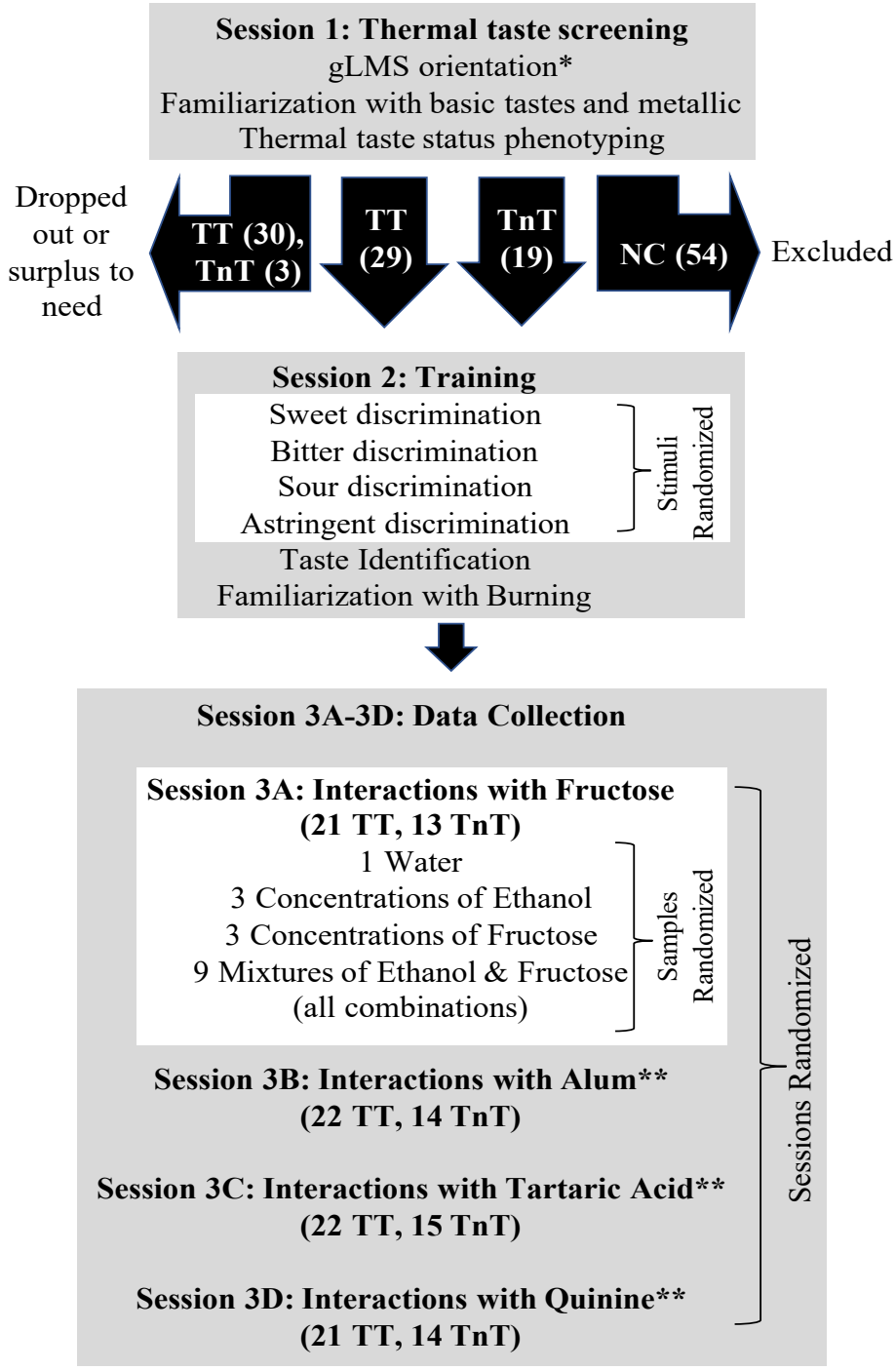

Figure 5.1: Overview of experimental design. (TT = thermal taster, TnT = thermal non-taster, NC = non-classifiable, *Note: Seven participants did not successfully complete the gLMS orientation task. As a result, of the 142 eligible participants recruited to the study, only 135 completed Session 1. ** = Design per Session 3A except for unique stimulus compound.)

to the gLMS and practiced using the scale by rating five remembered sensations. Participants that incorrectly rated the *brightness of a dimly lit restaurant* higher than the *brightness of the sun when staring directly at it* were also excluded from the study (n = 7). All data were collected in individual sensory booths at Brock University. To improve retention, participants were paid a modest honorarium for their participation or were provided credit towards select courses. Written informed consent was obtained from all participants. All procedures were cleared by the Brock University Bioethics Research Board (17-168) and were in accordance with the Declaration of Helsinki.

48 participants (29 TT & 19 TnT) completed Session 2. Others were excluded because they were non-classifiable (54), they dropped out of the study (10 TT, 3 TnT) or they were identified as TT (20) after the recruitment target for TT had been met. The number of participants per session varied slightly as follows; Session 3A (21 TT and 13 TnT), Session 3B (22 TT and 14 TnT), Session 3C (22 TT and 15 TnT) and Session 3D (21 TT and 14 TnT). Overall, 18 TT and 13 TnT completed all six sessions.

5.2.1 Thermal Taste Screening

Thermal taste screening was performed using the methods of Mitchell et al. (2019), which are an adapted version of the methods first used by Bajec and Pickering (2008). Readers are referred to Section 5.5 (Appendix A) for full details of the TTS screening protocol. Of the 135 individuals that completed Session 1, 59 TT, 22 TnT, and 54 non-classifiable participants were identified.

5.2.2 Training (Session 2) and Data Collection (Sessions 3A-3D)

5.2.2.1 Orosensory Stimuli

To investigate the interactions in alcoholic beverages, four stimuli were selected to represent commonly elicited sensations; sweet (D-Fructose UltraPure Grade; Burlington, BioShop, Canada), bitter (Quinine monohydrochloride; SAFC Supply Solutions, St. Louis, MO, USA), sour (L-(+)-Tartaric acid ; SAFC Supply Solutions, St. Louis, MO, USA) and astringent (Aluminium sulphate; Sigma-Aldrich, St. Louis, MO, USA). A literature search was conducted to identify potential concentrations for each stimuli (Settle et al., 1986; Schiffman et al., 1995; Keast and Roper, 2007; Bajec and Pickering, 2008; Low et al., 2017). Two rounds of bench testing followed (data not shown), leading to the identification

of three concentrations of each stimuli (low, medium and high; Table 5.1). The aim was to select concentrations for the stimuli that were perceptually different, miscible with all ethanol levels, and tolerated by participants.

Table 5.1: Orosensory stimuli and concentrations used in taste-taste interactions.

Stimulus	Orosensation(s) Elicited	Units	Concentration		
			Low	Medium	High
Fructose	Sweet	mM	140	280	960
Quinine	Biter	mM	0.025	0.040	0.100
Tartaric acid	Sour (primary), Astringent	mM	2.75	6.91	17.4
Alum	Astringent (primary), Sour	mM	0.73	2.05	5.43
Ethanol	Sweet, Bitter, Astringent, Burning* (varies based on concentration)	% (vol/vol)	5	13	23

* Relative intensity varies with concentration

Three ethanol (Beverage grade, Ethyl Alcohol 95% Kosher, Storechem Alcohols Ltd. Burlington, ON, Canada) concentrations were chosen to represent different beverages types; 5% (vol/vol) for beer, 13% (vol/vol) for wine and 23% (vol/vol) for distilled spirits (Thibodeau and Pickering, 2019, Chapter 2). Although most distilled spirits are typically 35-45% ethanol, 23% ethanol was chosen to ensure that the total volume of pure ethanol each participant was exposed to during each session was below one standard drink. This choice increased the participants' tolerance of the samples and reduced the risk of inebriation, allowing for all samples for each taste stimulus and ethanol combination to be evaluated during the same session. Distilled spirits are often diluted to this concentration before sensory evaluation in industry (Ickes and Cadwallader, 2017). Furthermore, 23% is below the upper discrimination taste threshold for ethanol, which Lachenmier et al. (2014) estimate is approximately 40%. Samples prepared from mixing two stimuli (ethanol and one other) will be referred to as binary solutions. In contrast, samples with only one stimulus (ethanol or one other) will be referred to as unary solutions.

A concentrated stock solution of each stimulus was prepared volumetrically with pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA). Stock solutions were well mixed and then further combined/diluted with pure water order to obtain the desired unary and binary solutions (see below for full details). Tartaric acid and fructose solutions

(stock and samples) were discarded within 7 days of preparing the stock solution, regardless of when the final samples were prepared. Similarly, quinine solutions and alum solutions were discarded within 36 hours and 12 hours respectively. Solutions were stored in the fridge when not in use and sample solutions were brought to room temperature on the day of testing.

During Sessions 2 and 3A-3D, 10 ml blind-coded (3-digit) samples were presented to participants in 2 oz portion cups with lids to prevent ethanol evaporation. Unless otherwise noted, all samples were evaluated using a sip-and-spit protocol. Participants were instructed to take the entire sample, swirl for 5 seconds, expectorate and then rate the maximum intensity of the sensation on a gLMS 10 seconds after expectorating. Participants were required to rinse with filtered water between samples, and soda crackers were available ad libitum. All intensity ratings were collected using individual gLMS (Bartoshuk et al., 2004).

5.2.2.2 Session 2

As participants were recruited from the community and did not have any formal sensory evaluation training, a brief orosensory training session was held before data collection. Each participant was required to complete three tasks (Figure 5.1), which were administered using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada).

First as part of the ranking task, participants were asked to familiarize themselves with the sensation elicited by unary solutions representing sweet (fructose), astringency (aluminium sulphate), sour (tartaric acid) and bitter (quinine). One sensation at a time, participants were presented with a set of three samples, one each of the low, medium and high intensity concentrations. For each set of solutions, participants were told what the primary orosensation elicited was and asked to familiarize themselves with it (Table 5.1; for example, sweet for fructose). To ensure that participants were actively engaged in the familiarization task, they were asked to rank the three samples in order of intensity. Both the order of sample sets and the order of samples within a set were randomized. One-minute breaks were enforced between sample sets.

Second, as part of the identification task, participants were presented with a flight of four samples: one each of the medium intensity fructose, aluminium sulphate, tartaric acid and quinine. Participants were asked to taste each sample one at a time and identify

the primary sensation elicited from six options (sweet, bitter, sour, astringent, no sensation or other). As the aim of this task was to help train participants, after each sample feedback was automatically provided for correct ("Great job! Sample (3-digit code) is (correct orosensation)") or incorrect responses ("Sample (3-digit code) typically tastes (correct orosensation)"). Samples were randomized and one-minute breaks were enforced between sample sets.

Third, to familiarize participants with burning/tingling, they were presented with a ~5 ml sample of aqueous Capsaicin (Sigma-Aldrich, St. Louis, MO, USA). Participants were asked to extend their tongue and briefly dip the tip into the solution. The capsaicin solution was prepared in two steps. First a saturated stock solution was prepared by adding 30.5 mg/L of capsaicin to water and stirring gently. Second, 1.965 ml of the supernatant was further dissolved in water, yielding a maximum capsaicin concentration of 1.2 mg/L. Bench testing showed that it could reliably elicit a mild burning/tingling sensation, which was well tolerated by all participants.

5.2.2.3 Session 3A-3D

Data collection was performed across four sessions where each session was used to investigate the interaction between ethanol and one stimulus: 3A (fructose), 3B (aluminium sulphate), 3C (tartaric acid) and 3D (quinine). Although the samples varied between sessions based on the stimulus of interest, the same method was used in each session. To illustrate the method, a detailed description of Session 3A is provided below and can be used as a model for Sessions 3B-3D.

In Session 3A, participants were presented with sixteen 10 ml samples consisting of 1 pure water, 3 unary solutions of ethanol (5%, 13%, 23%), 3 unary solutions of fructose (low, medium, high) and 9 binary solutions of ethanol and fructose. Binary solutions were prepared using a 3X3 design so that one of each combination of ethanol (5%, 13%, 23%) and fructose (low, medium, high) was included. Samples were presented in randomized order. Using the sip-and-spit protocol from Session 2, participants tasted each of the samples and rated the maximum intensity of the sweet, sour, bitter, astringency, burning/tingling and other on a separate gLMS for each sensation. To reduce the potential effects of ethanol desensitization on intensity ratings (Prescott and Swain-Campbell, 2000), minimum 2-minute breaks were enforced between samples, the maximum ethanol

concentration of any one sample was 23% and participants were instructed to rinse with water at least once between samples. Water and soda crackers were also available *ad libitum* if participants desired further palate cleansing between samples. As nasal irritation thresholds for ethanol are up to 1000 times lower than ethanol taste thresholds, participants wore nose clips during all tastings (Mattes and DiMeglio, 2001).

5.2.3 Data Analysis

All data analysis was performed using XLSTAT Version 2020.3.1 (Addinsoft, NY, USA) and Microsoft® Excel® for Mac Version 16.43 (Microsoft ®). Significance for all analyses was set at $P = 0.05$. All graphics were generated using in RStudio Version 1.1.463 (RStudio, Inc.) using ggplot2 Version 3.2.1 (Wickham, 2016) and gridExtra Version 2.3 (Auguie, 2017).

5.2.3.1 Data Treatment

Maximum intensity ratings (sweet, sour, bitter, astringent, burning/tingling) were log transformed using the formula ($\log_{10}$ (intensity rating +1)) for all gLMS responses to improve normality (Green et al., 2005; Bajec and Pickering, 2008). Although log transformations do not always improve the normality of data collected using the gLMS (Hayes et al., 2013), a visual comparison of histograms showed that log transformation improved the normality of the data (data not shown). The non-normality of the log transformed data is likely attributable to the large number of absent or low intensity responses for the non-dominant orosensations elicited by the stimuli.

Unary solutions of ethanol (5%, 13%, 23%) and water were tasted in all four data collection sessions (3A-3D). Data for participants that did not complete all data collection sessions were excluded to eliminate context effects due to differences in the binary solutions presented across the sessions. In addition, the mean of log transformed intensity ratings were calculated by averaging responses for all four sessions. As the other unary solutions (fructose, aluminium sulphate, tartaric acid, quinine) and all binary solutions were only tasted once, no means were calculated and data from all participants that completed the session were included.

5.2.3.2 Orosensory Training

Results from orosensory training were examined to briefly assess the discrimination and identification ability of participants (29 TT, 19 TnT). The discriminatory ability of TT and TnT was assessed by counting the number of times each participant correctly ranked the low and high intensity sample of each stimuli (fructose, aluminium sulphate, tartaric acid and quinine) during the ranking task. The ability of participants to identify orosensations was assess by counting the number of stimuli correctly identified by each participant during the identification task. To assess whether TT and TnT performed equally, Mann-Whitney U was used to compare scores for both tasks as data was not normally distributed (Shapiro Wilks, $P < 0.001$).

5.2.3.3 Unary Solutions

Data for the unary solutions from Sessions 3A-3D were analyzed to better characterize the perception of ethanol. Boxplots were generated for each orosensation and 2-way ANOVA with interactions was used to investigate the impact of thermal taste status (TT and TnT) and ethanol concentration (5%, 13%, 23%) on mean orosensory ratings. Effect size was calculated for all main effects and interactions to assess the relative importance of each. Effect sizes were considered small, medium or large, when η^2_p values exceeded 0.01, 0.06, or 0.140 respectively (Lakens, 2013). Although the data were not normally distributed (data not shown), ANOVA is largely robust to deviations from normality. A stimulus concentration*TTS interaction has been reported for saccharine but not sucrose or sodium chloride (Green et al., 2005). Thus, as most studies on orosensory responsiveness and TTS included only a single concentration of a tastant or did not test for interactions (Green et al., 2005; Bajec and Pickering, 2008; Yang et al., 2018; Thibodeau et al., 2019, Chapter 3), the decision to employ ANOVA despite this limitation was made. Importantly, ANOVA allowed for the interaction between stimulus concentration and thermal taste status to be tested, which is not possible to the best of our knowledge using the non-parametric alternative Kruskal-Wallis. As a precaution, non-parametric statistics (Kruskal-Wallis) were also applied using six groups (TT-low, TT-medium, TT-high, TnT-low, TnT-medium, TnT-high) and confirmed that similar results were observed. Furthermore, all data was log transformed (see Section 2.3.1) as transformation improved normality.

Participants also tasted low, medium and high intensity solutions of fructose (Session 3A), aluminium sulphate (Session 3B), tartaric acid (Session 3C) and quinine (Session 3D). Intensity scores for the unary solutions of each stimulus (low, medium, high) were extracted from the respective sessions and the same data analysis approach used to investigate ethanol perception was used. Boxplots were generated to visualize the data and used to select the attributes for further analysis. Two-way ANOVA comparing intensity ratings by concentration (low, medium, high) and thermal taste status (TT, TnT) were completed for the sweetness of fructose, the astringency and sourness of aluminium sulphate, the sourness and astringency of tartaric acid, and the bitterness of quinine.

5.2.3.4 Binary Mixtures

Data for the binary solutions from Sessions 3A-3D were analyzed to better characterize interactions between ethanol and four stimuli (fructose, aluminium sulphate, tartaric acid, quinine). Data from each session were assessed separately and the approach described below for ethanol and fructose (Session 3A) was applied to the other sessions. Separate three-way ANOVAs were performed to compare the intensity of the sweetness, bitterness, sourness, astringency and burning/tingling elicited for the nine binary solutions of fructose and ethanol. Factors included in the model were thermal taste status (TT, TnT), ethanol concentration (5%, 13%, 23%), fructose concentration (low, medium, high) and all two-way interactions.

Binary interactions between two stimuli can be modelled using the isobole method to better determine whether true enhancement or suppression has occurred (Sühnel, 1993; Fleming et al., 2016; Wang et al., 2018). Importantly, good dose-response models are required for unary solutions of both components of the binary mixture as they are used to generate the values used in the interaction calculations (Sühnel, 1993). To identify good candidates for modelling using the isobole method, simple linear regression was performed to determine whether stimulus concentration (log transformed) could be used to significantly predict intensity ratings for each sensation (sweet, sour, bitter, astringent or burning/tingling) for each set of unary solutions (ethanol, fructose, tartaric acid, aluminium sulphate quinine). Two candidates were identified for modelling: the astringency of aluminium sulphate/ethanol binary solutions and the bitterness of quinine/ethanol binary solutions. In both cases, linear models for both stimuli predicted the intensity of the

orosensation of interest. The index of interaction (I) was calculated for each pair using the formula $(c_A/C_A)+ (c_B/C_B)$, where A and B are the two compounds in the binary mixture and "c_A" and "c_B" are the actual concentration of the compounds A and B. "C_A" and "C_B" are the concentrations of compounds A and B needed to achieve the same intensity as in the binary mixture, as predicted from the linear models of the unary solutions. The compounds in the binary mixture supressed, enhanced or had no effect on the perception of orosensations when "I" was above 1.1, below 0.9 or between 0.9-1.1, respectively (Wang et al., 2018).

5.2.3.5 Other Considerations

Ethanol is a complex stimulus that elicits multiple orosensations and the number of sensations elicited varies between participants (Scinska et al., 2000). To determine if/how these patterns are impacted by thermal taste status, the number of scales used by TT and TnT was compared. Similarly, to intensity scores, only the data of participants that completed all data collection sessions were included. Scale use was calculated in two steps. For each session (3A-3D), the number of scales used was determined by counting the number of scales with ratings above "no sensation" (0 on gLMS) for each concentration of ethanol (5%, 13%, 23%) and water. As participants were provided with six scales (sweet, bitter, sour, astringent, burning/tingling, other*)*, scores ranged for 0 to 6, (0 = no scales, 6 = all scales). Second, the mean number of scales used for each participant was calculated by averaging the number of scales used for each sample in Sessions 3A-3D. As the data was not normally distributed, Mann-Whitney U (TT vs TnT) and kernel density estimates were generated for TT and TnT to compare the distribution of scores. Furthermore, as Mann-Whitney U compares group medians, any differences in scale use found will not be driven by outliers. The same approach was also used to compare the number of scales used by TT and TnT in response to the other unary (fructose, aluminium sulphate, tartaric acid, quinine) and all binary mixtures. However, unlike ethanol and water, these samples were only tasted by participants once, so raw scores were used instead of means.

5.3 Results

5.3.1 Orosensory Training

Although the training provided in Session 2 was brief, the participants' ability to discriminate the samples and correctly identify the sensations was considered sufficient. During the ranking task, 82% (24 TT, 15 TnT) of participants who completed Session 2 (29 TT; 19 TnT), were able to discriminate the low intensity and high intensity samples for all stimuli by ranking each set in the correct order. The remaining participants were also largely successful as they ranked three (4 TT, 4 TnT) or two (1 TT) of low and high intensity samples in the correct order. The number of stimuli for which low and high concentrations were correctly discriminated did not differ between TT (M = 3.8, SD = 0.5) and TnT (M = 3.8, SD = 0.4), ($U_{standardized} > 0.001$, P = 0.976). During the identification task, 28 participants (58%; 16 TT, 12 TnT) correctly identified all four stimuli. Of the remaining participants, 11 correctly identified three stimuli (7 TT, 4 TnT), seven correctly identified two stimuli (4 TT, 3 TnT) and two correctly identified two stimuli (2 TT). The number of stimuli correctly identified did not differ between TT (M = 3.3, SD = 1.0) and TnT (M = 3.5, SD = 0.8), ($U_{standardized} > 0.001$, P = .555). As TT and TnT had sufficient and equivalent abilities to both identify and discriminate the key orosensations, no participants were excluded based on these results.

5.3.2 Unary Solutions

Overall, ethanol elicited sweetness, bitterness, astringency and burning/tingling but not sourness, with intensity varying with concentration (Supplementary Figure 5.1). To better characterize the sensations elicited, 2-way ANOVAs were performed for each sensation with thermal taste status (TT and TnT) and concentration (5, 13, 23 %) as the independent variables (Figure 5.2, Supplementary Table 5.1). Increasing ethanol concentration led to an increase in bitterness (F(2,86) = 10.2, P < 0.001) and burning/tingling (F(2,86) = 95.9, P < 0.001). Similar non-significant results were found for astringency (F(2,86) = 2.7, P = 0.070). The sweetness of ethanol did not vary with ethanol concentration (F(2,86) = 1.2, P = 0.294). TT were significantly more responsive to sweetness (F(1,86) = 17.4, P < 0.001) and astringency (F(1,86) = 23.0, P < 0.001), while the similar results for bitterness (F(1,86) = 3.6, P = 0.059) and burning/tingling (F(1,86) =

3.1, $P = 0.083$) were not significant. When effect sizes were compared (Table 5.2), large effects were found based on ethanol concentration for the dominant sensations (bitterness, burning/tingling), and for thermal taste status for the non-dominant intensity sensations (sweetness, astringency). No significant interactions were found, suggesting the response patterns of TT and TnT do not vary based on ethanol concentration.

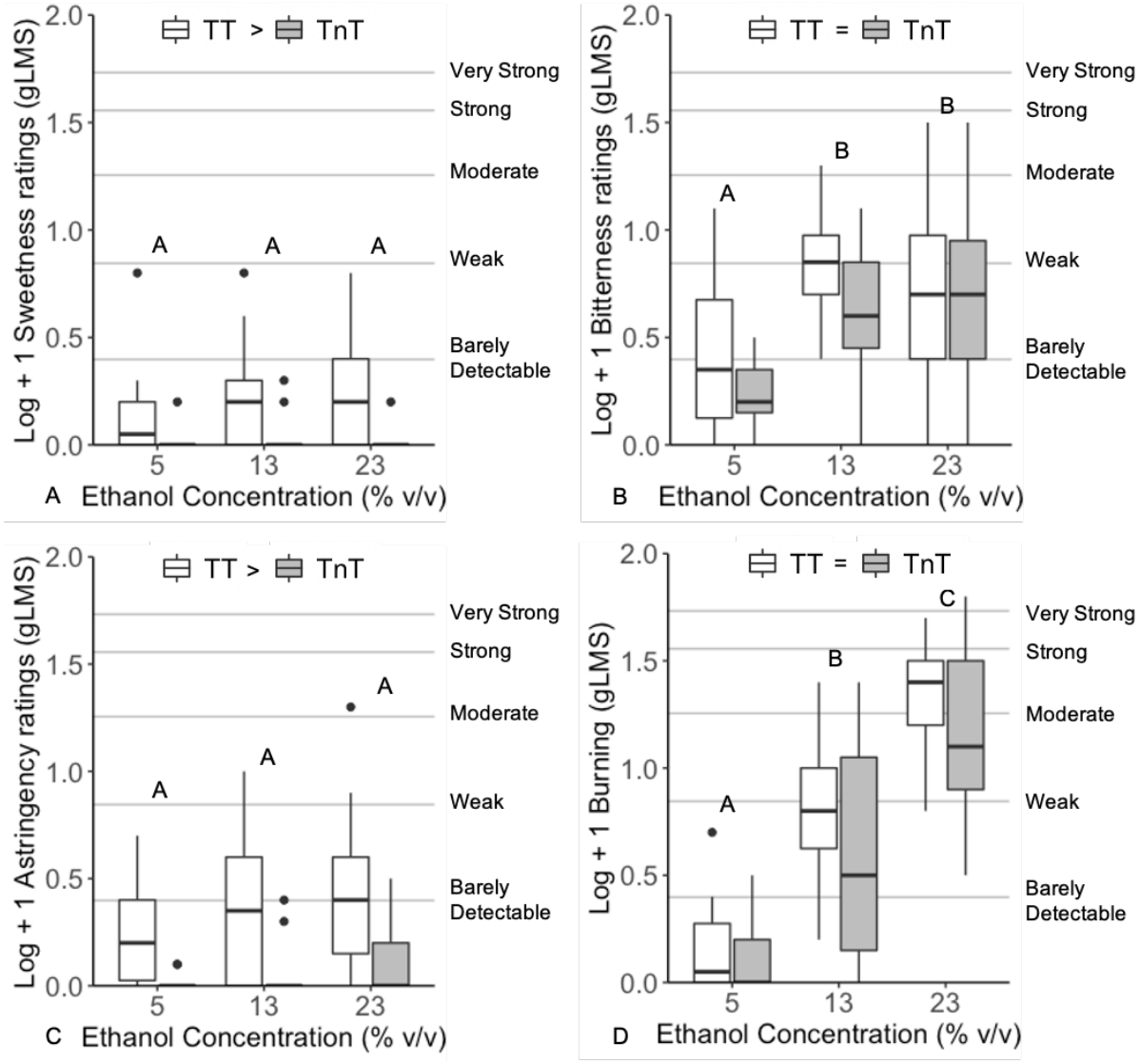

Figure 5.2: Boxplots of mean intensity elicited by unary solutions of ethanol by concentration (5, 13, 23% vol/vol) and thermal taste status (18 TT, 11 TnT) for sweet (a), bitter (b), astringent (c) and burning/tingling (d). Significant differences between concentrations are shown with different letters above the boxplots. Significant differences between TT and TnT are indicated by the mathematical symbols in the legend ("=" no difference; ">" TT rate the sensation higher than TnT).

Table 5.2: Summary of effect sizes for two-way ANOVAs comparing intensity ratings by thermal taste status (TT and TnT) and stimuli concentration (low, medium, high) to orosensations elicited by unary solutions of ethanol, fructose, quinine, tartaric acid and aluminium sulphate. Note: The effect size is considered small, medium, or large, when η^2_p values exceed 0.01 (light grey), 0.06 (dark grey), or 0.140 (black), respectively (Lakens, 2013). Levels of significance in the corresponding ANOVAs are denoted by "*" and "#", when $P < 0.05$ or $P < 0.10$, respectively.

Effect size (η^2_p)										
Stimuli	Ethanol				Fructose	Quinine	Tartaric acid		Alum sulphate	
Orosensation / Factor in ANOVA	Bitter	Burning/tingling	Sweet	Astringent	Sweet	Bitter	Sour	Astringent	Astringent	Sour
Thermal taste status (TTS)	0.04#	0.04#	0.18*	0.22*	0.14*	< 0.01	.02	0.01	0.01	0.12*
Stimulus concentration (Conc)	0.20*	0.70*	0.03	0.06#	0.55*	0.07*	.26*	0.04	0.24*	0.19*
TTS*Conc	< 0.01	0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< .01	< 0.01	0.08*

Unary solutions of fructose and quinine each elicited one primary sensation (Supplementary Figures 5.2-5.3), sweetness and bitterness, respectively. In contrast and as expected (Peleg et al., 1998; Sowalsky and Noble, 1998), tartaric acid and aluminium sulphate each elicited two orosensations (sourness and astringency), although the dominant sensation differed between the two (Supplementary Figures 5.4-5.5). For these sensations/stimulus pairs (Figure 5.3; Supplementary Table 5.1), 2-way ANOVAs were used to compare the intensity ratings based on thermal taste status (TT and TnT) and concentration (low, medium, high). As expected, increased concentration also led to increased intensity for the dominant sensations elicited by fructose (sweetness; $F(2,101) = 58.0$, $P < 0.001$), quinine (bitterness*;* $F(2,104) = 3.6$, $P = 0.030$), tartaric acid (sourness*;*

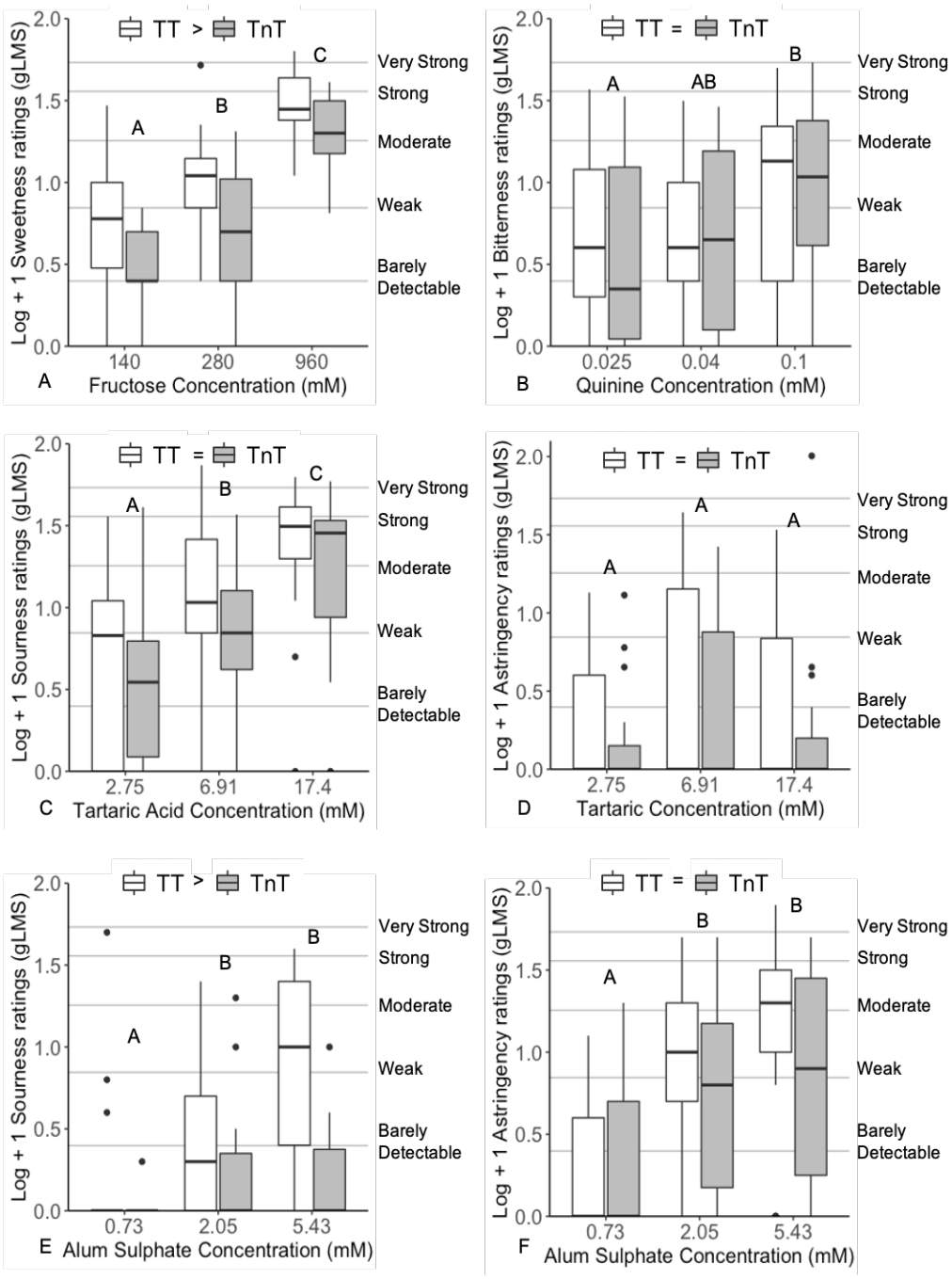

Figure 5.3: Unary solution boxplots of the mean sweetness of fructose (A), bitterness of quinine (B), sourness of tartaric acid (C), astringency of tartaric acid (D), sourness of alum sulphate (E) and astringency of alum sulphate (F) by concentration (low, medium, high) and thermal taste status (TT, TnT). Significant differences between mean concentrations are shown with different letters above the boxplots. Significant differences between thermal tasters (TT, n = 21-22) and thermal non-tasters (TnT, n = 13-15) are indicated by the mathematical symbols in the legend ("=" no difference; ">" TT rate the sensation higher than TnT).

F(2, 110) = 18.5, *P* < 0.001) and aluminium sulphate (astringency*; F*(2,104) = 15.5, *P* < 0.001). The intensity of non-dominant sensations also increased significantly for aluminium sulphate (sourness; F(2,104) = 11.9, P < 0.001) but not for tartaric acid (astringency; F(2, 110) = 1.9, P = 0.149). TT were more responsive than TnT to the sweetness of fructose (F(1,101) = 15.0, P < 0.001) and the sourness of aluminium sulphate (F(1,104) = 13.2, P < 0.001). A significant interaction between thermal taste status and aluminium sulphate concentration was found for the perception of sourness (Figure 5.3E). Whereas TT rated the sourness of the high concentration of aluminium sulphate as more intense than the low concentration, TnT ratings did not differ for the same samples (F(2,104) = 4.2, P = 0.018). No other main effects nor interactions were found (Supplementary Table 5.1).

5.3.3 Binary Mixtures

To better understand how differences in the composition of alcoholic beverage impact their perception, binary mixtures of ethanol and four stimuli (fructose, aluminium sulphate, tartaric and quinine) were examined (Figures 5.4-5.7). Results of 3-Way ANOVAs comparing the impacts of ethanol concentration (5%, 13%, 23%), changes in the concentration of the other stimuli (low, medium, high) and thermal taste status (TT, TnT) are provided in Supplementary Table 5.2 and results are described below.

Ethanol concentration significantly impacted the perception of bitterness and burning/tingling and similar results were observed across all binary mixture types. In binary mixtures of ethanol with fructose, tartaric acid or aluminium sulphate, samples with 5% ethanol were less bitter than those with 13% or 23% ethanol. In contrast, bitterness did not vary significantly with ethanol concentration in quinine/ethanol mixtures. For all binary solution types, as ethanol concentration increased, ratings of burning/tingling also increased significantly. Regardless of the type of stimulus used in the binary mixture with ethanol, the burning/tingling of 5%, 13% and 23% had a similar intensity.

Ethanol concentration impacted the perception of sweetness, sourness and astringency (Figures 5.4-5.7), although effects varied based on the binary mixture type.

Sweetness decreased as ethanol concentration increased in mixtures with fructose, but the opposite was true in mixtures with quinine. The sweetness did not vary with alcohol concentration in mixtures with aluminium sulphate or tartaric acid. Sourness decreased

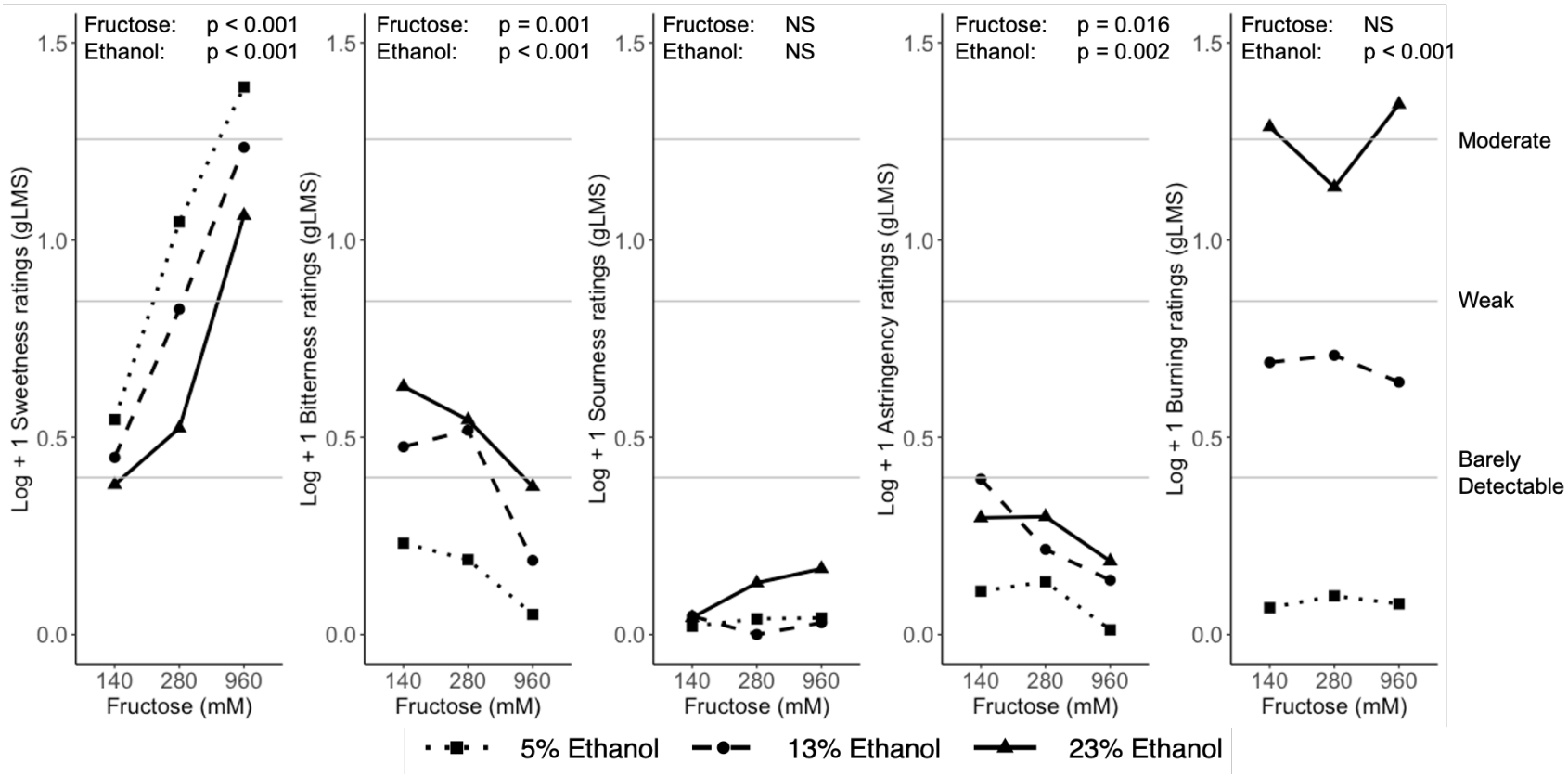

Figure 5.4: Mean ratings of sweetness, bitterness, sourness, astringency and burning/tingling in the binary solutions of ethanol (5%, 13%, 23% vol/vol) and fructose (140 mM, 280 mM, 960 mM). Significant differences are indicated from the p-values above each graph (NS = not significant). A full summary of the model including the effect of thermal taste status and 2-way interactions is included in Supplementary Table 5.2.

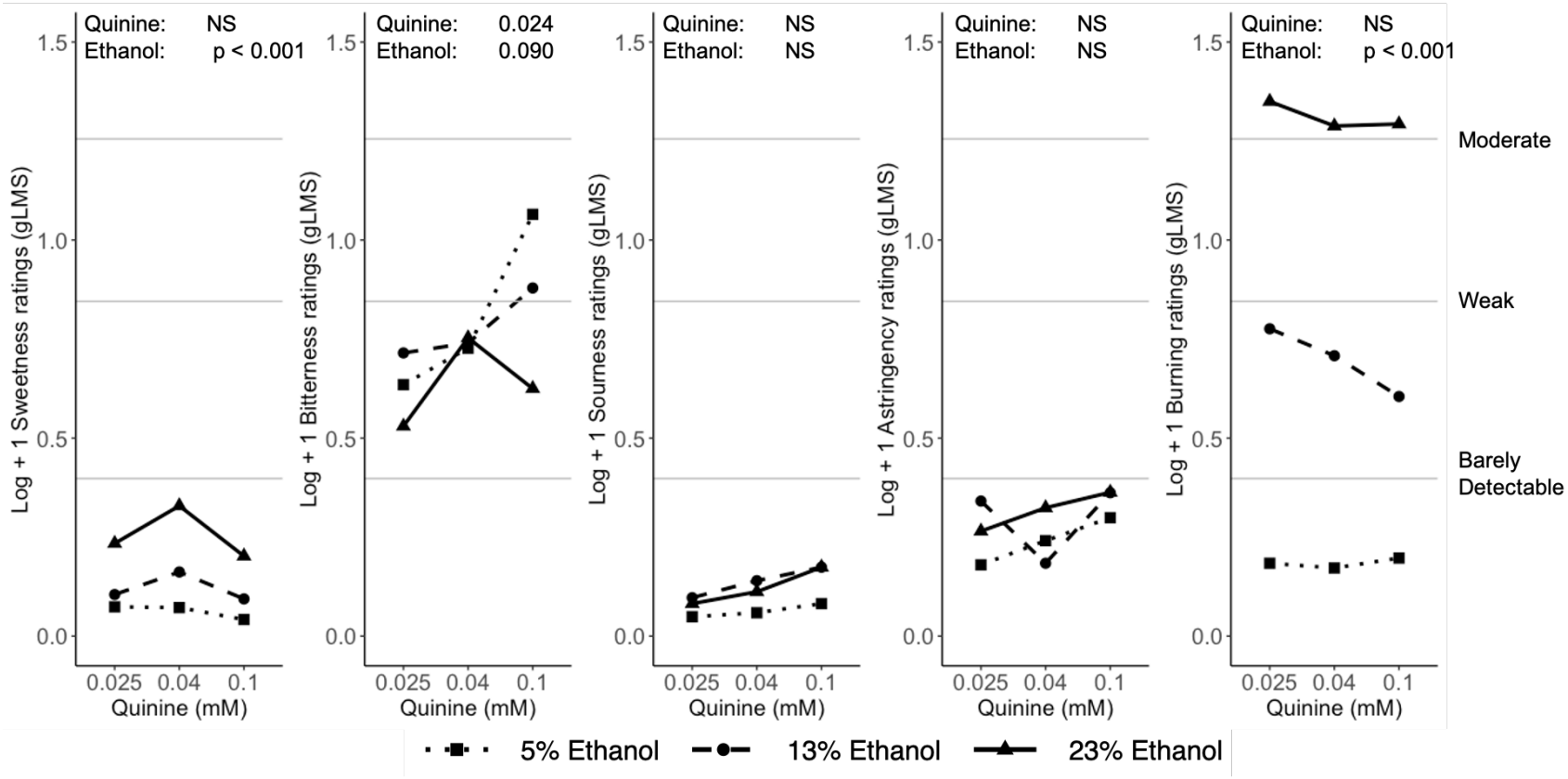

Figure 5.5: Mean ratings of sweetness, bitterness, sourness, astringency and burning/tingling in the binary solutions of ethanol (5%, 13%, 23% vol/vol) and quinine (0.025 mM, 0.040 mM, 0.100 mM). Significant differences are indicated from the p-values above each graph (NS = not significant). A full summary of the model including the effect of thermal taste status and 2-way interactions is included in Supplementary Table 5.2.

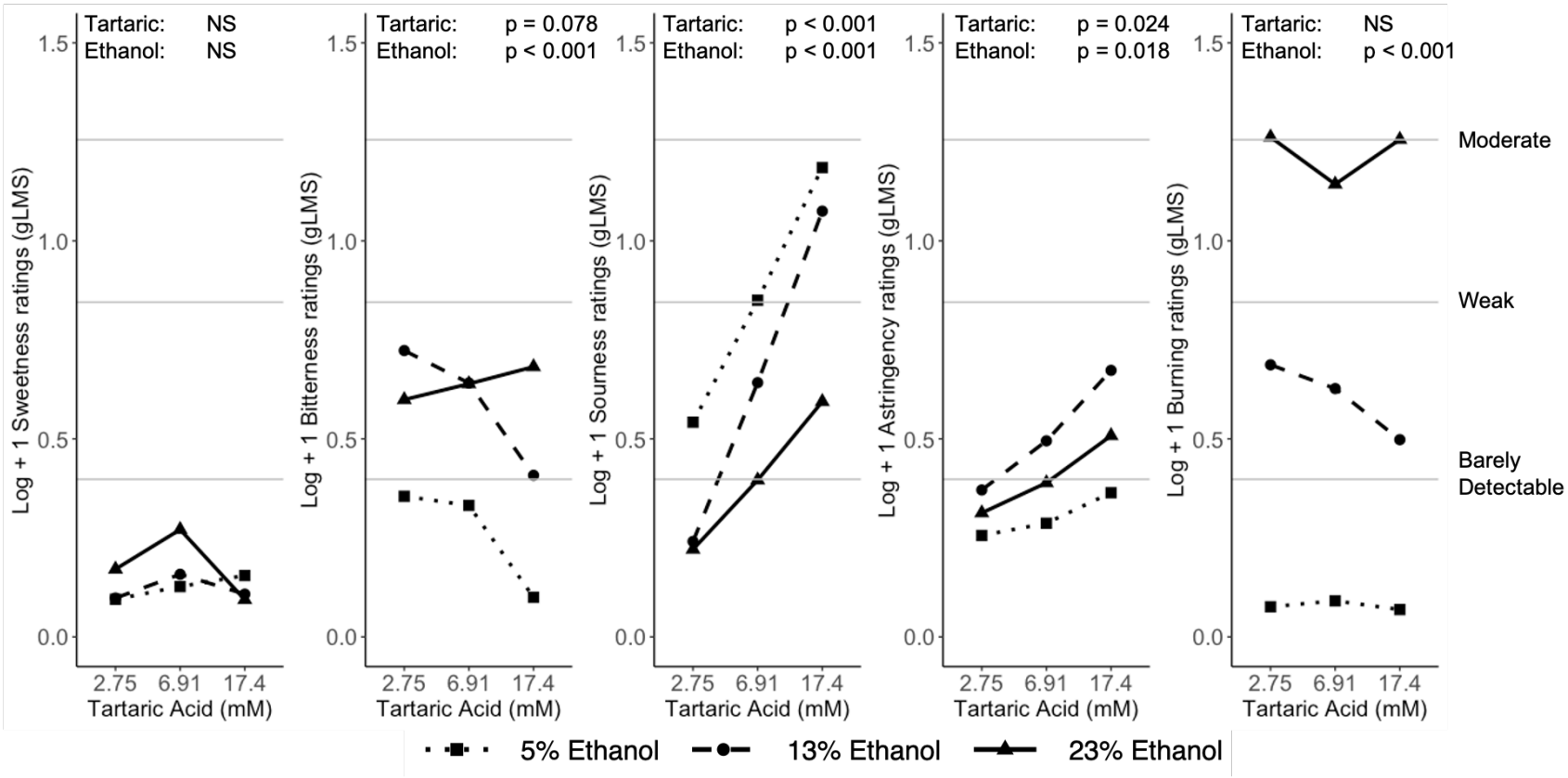

Figure 5.6: Mean ratings of sweetness, bitterness, sourness, astringency and burning/tingling in the binary solutions of ethanol (5%, 13%, 23% vol/vol) and tartaric acid (2.75 mM, 6.91 mM, 17.4 mM). Significant differences are indicated from the p-values above each graph (NS = not significant). A full summary of the model including the effect of thermal taste status and 2-way interactions is included in Supplementary Table 5.2.

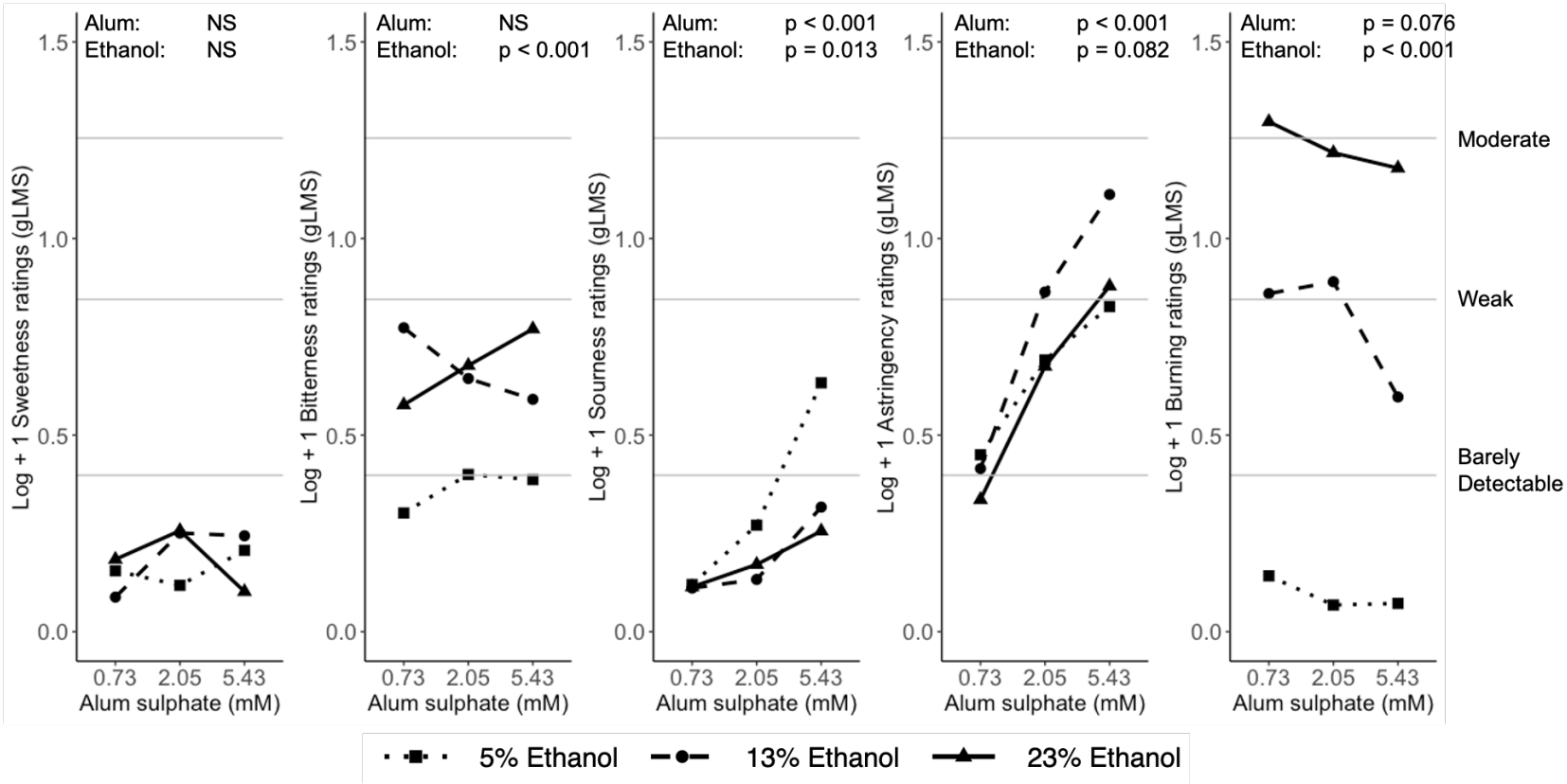

Figure 5.7: Mean ratings of sweetness, bitterness, sourness, astringency and burning/tingling in the binary solutions of ethanol (5%, 13%, 23% vol/vol) and alum sulphate (0.73 mM, 2.05 mM, 5.43 mM). Significant differences are indicated from the p-values above each graph (NS = not significant). A full summary of the model including the effect of thermal taste status and 2-way interactions is included in Supplementary Table 5.2.

significantly as ethanol concentration increased in binary mixtures with tartaric acid and aluminium sulphate. No difference in sourness based on ethanol concentration was found in mixtures with fructose or quinine. In binary mixtures with tartaric acid (Figure 5.6), 13% ethanol was the most astringent and 5% ethanol was the least astringent. However, neither 5% nor 13% ethanol differed significantly from the 23% ethanol mixture. In contrast, the astringency in binary mixtures of ethanol and fructose increased between 5% to 13% ethanol but did not differ between 13% and 23% ethanol (Figure 5.4). However, a significant interaction between ethanol concentration and thermal taste status showed that this result was only true for TT. Astringency was not impacted by ethanol concentration in binary mixtures with quinine or aluminium sulphate.

The fructose, quinine, tartaric acid and aluminium sulphate concentrations also impacted the perception of their respective binary mixtures (Figures 5.4-5.7, Supplementary Table 5.2). Quinine, aluminium sulphate and tartaric acid concentration predominantly impacted only the orosensations commonly elicited by their respective unary solutions. Increasing the concentration of quinine in binary mixtures with ethanol increased the bitterness (Figure 5.5). In binary solutions of ethanol and tartaric acid (Figure 5.6) or aluminium sulphate (Figure 5.7), both sourness and astringency increased significantly as the concentration of tartaric acid or aluminium sulphate increased. Similarly, increasing the concentration of fructose in binary mixtures with ethanol also increased the sweetness. However, increasing the fructose concentration also resulted in lower intensity of bitterness and astringency (Figure 5.4).

Thermal tasters had higher mean orosensory ratings than TnT for binary mixtures of ethanol and fructose (sweetness, astringency, burning/tingling), quinine (sweet), tartaric acid (bitter, sour) and aluminium sulphate (sweet, astringent; $P < 0.05$; Supplementary Table 5.2). Significant differences in the burning/tingling of aluminium sulphate were also found based on thermal taste status, although TT and TnT could not be separated by the means separation test (Supplementary Table 5.2). Regardless of significance, TT rated all the orosensations elicited by each of the binary mixtures higher than did TnT. An interaction between thermal taste status and ethanol concentration for burning/tingling in binary mixtures of ethanol and quinine showed TT more responsive to 23% ethanol, but

not 5% or 13% ethanol. Overall, the results strongly support the hypothesis that TT are more responsive than TnT.

5.3.3.1 Index of Interaction

The isobole method was used to better characterize interactions in the binary mixtures. First, intensity ratings were modelled based on the concentration of stimuli in the unary solutions (ethanol, fructose, quinine, tartaric acid, aluminium sulphate). Simple linear regression was performed to determine which sensations (sweet, sour, bitter, astringency, burning/tingling*)* could be used to predict intensity ratings based on unary solution concentrations (Supplementary Table 5.3). The intensity of bitterness ($F(1,86) = 14.2$, $P < 0.001$, $R^2 = 0.14$), astringency ($F(1,86) = 4.5$, $P = 0.038$, $R^2 = 0.05$) and burning/tingling ($F(1,86) = 182.6$, $P < 0.001$, $R^2 = 0.69$) could be predicted by ethanol concentration. Similarly, sensations elicited by unary solutions of fructose (sweetness), aluminium sulphate (sour, astringent, bitter), tartaric acid (sourness) and quinine (bitterness) could be predicted from their concentration (Supplementary Table 5.3). As expected, the slopes in the linear regressions were consistent with the boxplots for the unary solutions (Supplementary Figures 5.1-5.5). Based on the regression results, the bitterness of quinine/ethanol mixtures and the astringency of aluminium sulphate/ethanol mixtures were selected for analysis using the isobole method (see Material and Methods – Binary mixtures). In binary mixtures, the bitterness was suppressed for all combinations of ethanol and quinine (Table 5.3). Similarly, the astringency was suppressed for eight of the nine combinations of ethanol and aluminium sulphate (Table 5.4). The only exception was 13% ethanol and 2.05 mM aluminium sulphate, where astringency was additive.

5.3.4 Other Considerations

As TT are more responsive than TnT to the orosensations elicited by both the unary solutions and binary mixtures, the number of scales used to describe the samples by TT and TnT was investigated. Kernel-density estimates were generated for all the samples (Figure 5.8, Supplementary Figures 5.6-5.10) and Mann-Whitney U was used to compare the median number of scales used by TT and TnT (Supplementary Tables 5.4-5.8). TT used significantly more scales than TnT to describe water ($U = 169.5$, $P = 0.001$), 5% ethanol ($U = 143.0$, $P = 0.009$), 13% ethanol ($U = 153.0$, $P = 0.012$), 140 mM fructose (low

Table 5.3: Isobole calculations for bitterness interactions in binary mixtures of ethanol and quinine (n = 35).

Ethanol % (vol/vol)	Quinine (mM)	Mean log(bitter) in binary mixture	Actual log(ethanol) concentration (c_{EtOH})	log(ethanol) to achieve the same log(bitterness) as in mixture (C_{EtOH})	Actual log(1+ quinine) concentration ($c_{Quinine}$)	Concentration of log(1 + quinine) to achieve the same bitterness as in mixture ($C_{Quinine}$)	Index of interaction (I)	Nature of interaction
5	0.025	0.635	0.699	1.141	0.011	0.014	1.41	Suppression
5	0.040	0.727	0.699	1.310	0.017	0.022	1.31	Suppression
5	0.100	1.065	0.699	1.934	0.041	0.053	1.14	Suppression
13	0.025	0.715	1.114	1.289	0.011	0.021	1.38	Suppression
13	0.040	0.740	1.114	1.334	0.017	0.023	1.57	Suppression
13	0.100	0.879	1.114	1.590	0.041	0.036	1.84	Suppression
23	0.025	0.530	1.362	0.947	0.011	0.004	4.24	Suppression
23	0.040	0.753	1.362	1.357	0.017	0.024	1.70	Suppression
23	0.100	0.625	1.362	1.122	0.041	0.013	4.50	Suppression

Table 5.4: Isobole calculations for astringency interactions in binary mixtures of ethanol and alum sulphate (n = 36).

Ethanol % (vol/vol)	Alum sulphate (mM)	Mean log(astringency) in binary mixture	Actual log(ethanol) (c_{EtOH})	log(ethanol) to obtain the mean log(astringency) in binary mixture (C_{EtOH})	Actual log(alum sulphate) (c_{alum})	log(alum sulphate) to obtain the mean log(astringency) in binary mixture (C_{Alum})	Index of interaction (I)	Nature of interaction
5	0.73	0.450	0.699	1.920	-0.137	-0.042	3.65	Suppression
5	2.05	0.691	0.699	2.907	0.312	0.265	1.42	Suppression
5	5.43	0.827	0.699	3.463	0.735	0.437	1.88	Suppression
13	0.73	0.415	1.114	1.774	-0.137	-0.087	2.20	Suppression
13	2.05	0.864	1.114	3.614	0.312	0.484	0.95	Additive
13	5.43	1.112	1.114	4.631	0.735	0.799	1.16	Suppression
23	0.73	0.336	1.362	1.450	-0.137	-0.187	1.67	Suppression
23	2.05	0.675	1.362	2.842	0.312	0.245	1.75	Suppression
23	5.43	0.879	1.362	3.675	0.735	0.503	1.83	Suppression

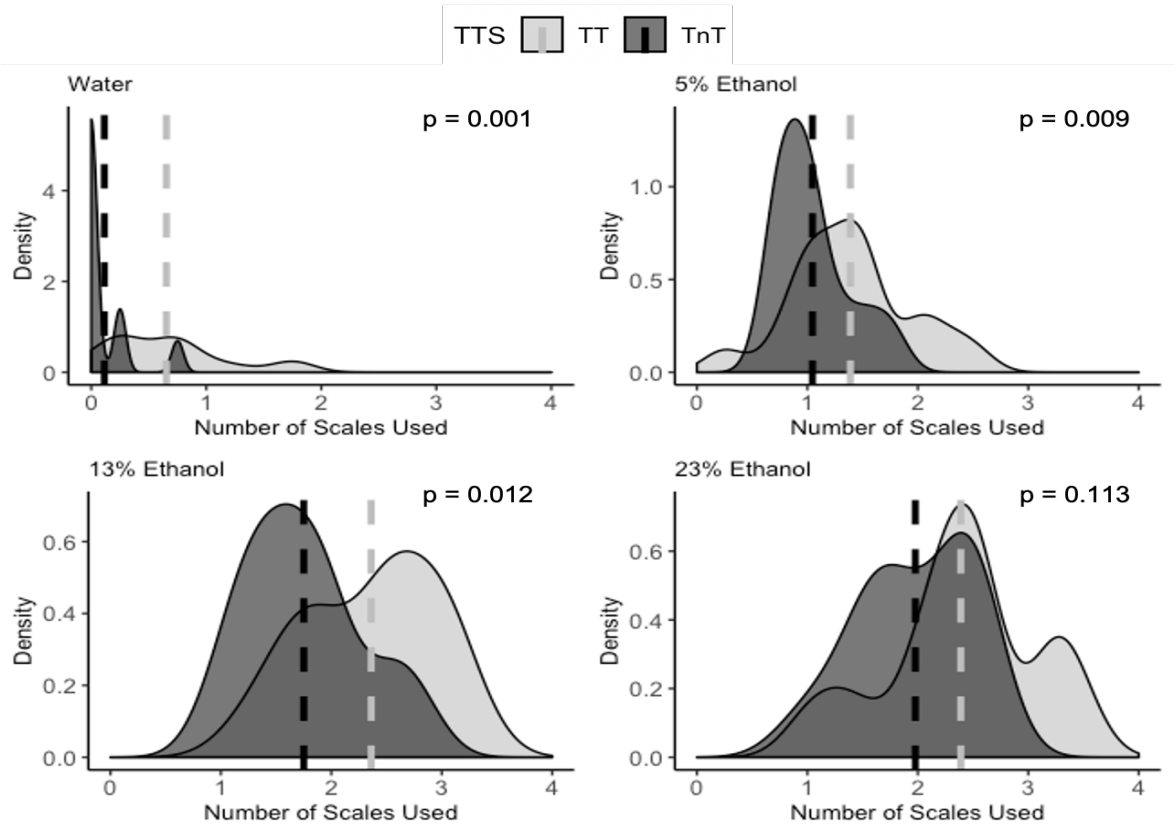

Figure 5.8: Kernel density estimates of the mean number of scales used by thermal tasters (TT, $n = 18$) and thermal non-tasters (TnT, $n = 11$) when rating unary aqueous solutions of ethanol (0%, 5%, 13% and 23% vol/vol). Dashed lines indicate the median values and p-values indicate if the medians differ significantly (NS = not significant).

intensity; $U = 182.5$, $P = 0.032$), 2.05 mM aluminium sulphate (medium intensity; $U = 213.5$, $p = 0.021$) and 5.43 mM aluminium sulphate (high intensity; $U = 217.0$, $P = 0.015$). Similarly, TT used mores scales to describe some binary solutions; 5% ethanol/960 mM fructose ($U = 191$, $P < 0.001$), 13% ethanol/0.73 mM aluminium sulphate ($U = 220.5$, $P = 0.007$), 5% ethanol/5.43 mM aluminium sulphate ($U = 205.0$, $P = 0.040$) and 23% ethanol/6.91 mM tartaric acid ($U = 215$, $P = 0.027$).

5.4 Discussion

5.4.1 Unary Solutions

Consistent with prior literature, ethanol elicited sweetness, bitterness, astringency and burning/tingling (Berg et al., 1955; Wilson et al., 1973; Green, 1987, 1988; Scinska et

al., 2000; Mattes and DiMeglio, 2001; Allen et al., 2014; Nolden and Hayes, 2015; Nolden et al., 2016; Small-Kelly and Pickering, 2020). The three ethanol concentrations (5%, 13%, 23%) differed significantly based on bitterness and burning/tingling, but no differences were found for sweetness or astringency. When ethanol from the concentrations typical in beer (5%) and wine (13%) were compared, both the bitterness and the burning/tingling sensations increased with ethanol concentration. However, when ethanol concentrations typical in wine (13%) and diluted spirits (23%) were compared, only burning/tingling increased with ethanol concentration. Although simple dilution may be an appropriate strategy to reduce the burning of an alcoholic beverage, it may not be sufficient to reduce the aversive sensations of bitterness or astringency for all starting concentrations of ethanol. More research is required to better characterize the implication of these findings in actual alcoholic beverages, especially in cases where producers are seeking to develop low or reduced ethanol products with flavour profiles similar to their full-strength counterparts.

Mean ratings of the orosensations elicited by ethanol were lower than those previously reported (Nolden and Hayes, 2015; Small-Kelly and Pickering, 2020). Despite the use of similar protocols (choice of scale, volume, whole mouth rinse), methodological differences may explain the lower ratings in the current study. Burning/tingling ratings were likely reduced as participants in the current study were required to wear nose clips to prevent ethanol from eliciting these sensations in the nasal cavity (Mattes and DiMeglio, 2001). More research is needed to determine whether nasal occlusion disrupts cross-modal interactions between nasal burning/tingling and the other sensations rated, reducing their intensity. It is also possible that as both unary and binary solutions were presented in the same session, the inclusion of the higher intensity binary mixtures reduced the overall intensity ratings of the unary ethanol solutions due to a context effect (Schifferstein and Frijters, 1992; Ferris et al., 2003). Importantly, the relative dominance of sensations and relative intensity of each ethanol concentration was maintained.

5.4.2 Binary Mixtures

5.4.2.1 Sweet

The impact of ethanol concentration on the sweetness of the binary solutions varied based on the non-ethanol stimuli included in the mixture. In binary mixtures with quinine

and ethanol, sweetness increased as ethanol concentration increased (Figure 5.5). In contrast, in binary mixtures with fructose (Figure 5.4), higher ethanol concentration was associated with reduced sweetness. As fructose elicits higher levels of sweetness than ethanol, it is likely that the other and more dominant sensations elicited by ethanol (bitterness, burning/tingling*)* suppressed the sweetness of fructose. The results are consistent with Hoopman et al. (1993) who found that the overall intensity of aqueous solutions of sweet stimuli is reduced as the concentration of ethanol increases from ~12% to 35% (vol/vol; reported as 10 – 30% wt/wt) ethanol. However, when binary mixtures of 0%, 5% and 12% ethanol and the same sugars were compared the overall intensity does not vary or may even increase as ethanol concentration increases (Hoopman et al., 1993). Although Hoopman et al. (1993) showed that the effect of ethanol on sweetness varied with concentration, the results should be interpreted with caution as only overall intensity was measured. That is, it is unclear if/how overall intensity was a useful proxy for the sweetness of the samples. Mixed results were also reported by Martin and Pangborn (1970) who found that aqueous sucrose solutions were sweeter when ethanol was added during a forced choice exercise, but when the samples were rated no differences in sweetness intensity were found. Meanwhile, Calviño et al. (1998) found that in mixtures with aspartame, sweetness did not vary with ethanol concentration (0 – 8%).

Ethanol concentration also impacts the intensity of sweetness of alcoholic beverages at low concentrations. In model beer (0 – 4.5%), sweetness increases with ethanol concentration (Clark et al., 2011). In contrast, in wine and model wine solutions, sweetness intensity does not vary with ethanol concentration over the range 7-14% (Nurgel and Pickering, 2005; Gawel et al., 2007; Jones et al., 2008; Cretin et al., 2018). Taken together, the current study and prior literature demonstrate that the impact of ethanol on the perception of sweetness depends on the concentration of both ethanol and the other stimuli in the mixture.

5.4.2.2 Bitter

Bitterness did not vary with ethanol concentration in binary mixtures with quinine. This replicates the findings of Martin and Pangborn (1970) but is not consistent with the results from the other binary mixture types or with studies in alcoholic beverages. In binary mixtures of ethanol and stimuli that did not elicit bitterness (fructose, tartaric acid, alum

sulphate), bitterness was significantly lower at 5% ethanol than at 13% or 23%, matching the trend observed in the unary solution of ethanol. In real and model alcoholic beverages (cider, beer, wine) increased ethanol concentration is associated with higher bitterness in most (Lea and Arnold, 1978; Fischer and Noble, 1994; Vidal et al., 2004; Nurgel and Pickering, 2005; Fontoin et al., 2008; Jones et al., 2008; Clark et al., 2011; Villamor et al., 2013; Gawel et al., 2013; Cretin et al., 2018; Poveromo and Hopfer, 2019; Harwood et al., 2020) but not all (Frost et al., 2017) studies. It is possible that the bitterness elicited by quinine, which increased with quinine concentration, may have masked any effects of ethanol bitterness. Nevertheless, the isobole method results showed that bitterness was suppressed in binary mixtures of ethanol and quinine. More research is required to determine if/how bitterness varies in binary mixtures of ethanol and other bitter compounds.

In binary mixtures of ethanol and fructose, increasing fructose concentration reduced bitterness. This finding is not unexpected as adding sweet stimuli to model alcoholic beverages also decreases bitterness in most (Lea and Arnold, 1978; Nurgel and Pickering, 2006; Jones et al., 2008; Clark et al., 2011) but not all (Villamor et al., 2013) studies. Increasing organic acid concentration decreases the bitterness in model wine (Fontoin et al., 2008) but the opposite has been found in cider (Lea and Arnold, 1978). The current study found that tartaric acid or alum sulphate concentration did not impact bitterness in binary mixture with ethanol. Thus, the impact of sour/astringent stimuli in binary mixtures with ethanol may be matrix dependent. Overall, the current study and prior literature demonstrate that the bitterness of ethanol and alcoholic beverages can be manipulated by changing their composition.

5.4.2.3 Sour

When ethanol was mixed with tartaric acid or aluminium sulphate, the impact of ethanol concentration on sourness followed the same pattern. In both binary mixture types, as the concentration of ethanol increased the sourness decreased, suggesting a robust effect. The findings are consistent with previous studies on organic acid and ethanol mixtures (Martin and Pangborn, 1970; Zamora et al., 2006). Also, consistent with the current study, Guirao et al. (2013) found that as ethanol concentration increases, sourness decreases, although this observation only held when both the ethanol and citric acid concentrations were high. The impact of ethanol concentration on the sourness of alcoholic beverages is

less clear. When dealcoholized model or real red wines (0%) were compared to wines with ethanol (6-16%; Demiglio and Pickering, 2008; Villamor et al., 2013), sourness was lower in the wines with ethanol. However, sourness does not vary with different concentrations of ethanol in model and real wines in most (Demiglio and Pickering, 2008; Jones et al., 2008; Villamor et al., 2013) but not all studies (Fischer and Noble, 1994). More research is needed to fully characterize the interactions between ethanol and organic acids in aqueous solutions and alcoholic beverages. In particular, studying a wider range of organic acid concentrations while simultaneously measuring pH is recommended.

5.4.2.4 Astringency

As expected, the astringency of aluminium sulphate and ethanol mixtures increased as aluminium sulphate concentration increased. This finding is consistent with observations in unary solution of aluminium sulphate (Figure 5.3F, Supplementary Figure 5.5) and in studies where astringent stimuli (phenolics) are added to model or real wine (Fontoin et al., 2008; Gawel et al., 2013; Villamor et al., 2013). Ethanol did not impact the astringency of binary ethanol and aluminium sulphate mixtures. However, in binary mixtures with both tartaric acid and fructose, 5% ethanol was less astringent than 13 % ethanol. The impact of ethanol concentration on astringency in real and model alcoholic beverages varies across studies (Lea and Arnold, 1978; Vidal et al., 2004; Demiglio and Pickering, 2008; Fontoin et al., 2008; Jones et al., 2008; Gawel et al., 2013; Villamor et al., 2013), thus the conflicting findings are not unexpected. The isobole method showed that the astringency in the binary mixtures of ethanol and aluminium sulphate was suppressed for most mixtures (Table 5.4). Together, the results suggest that simply mixing any concentration of ethanol with aluminium sulphate reduced the astringency similarly.

In binary solutions of ethanol and tartaric acid, both compounds impacted the perception of astringency. As tartaric acid increased so did the astringency of binary mixtures with ethanol. This result is not consistent with astringency perception in the unary solutions (Figure 5.3D, Supplementary Figure 5.4) nor studies in model wine (Fontoin et al., 2008). However, some studies have shown that astringency is increased in wine when pH is decreased (Demiglio and Pickering, 2008; Fontoin et al., 2008). Together, the results suggest that changes in astringency associated with organic acids are likely driven by changes in pH rather than actual concentration. As the binary mixtures in the current study

were simple and therefore not highly buffered, it is possible that the change in tartaric acid concentration also led to changes in pH.

In binary mixtures of fructose and ethanol, samples with 5% ethanol were less astringent than samples with 13% or 23%. Previous research showed that adding fructose or glycerol did not impact the astringency of wine (Jones et al., 2008; Villamor et al., 2013). However, the concentrations used were much lower (fructose, 1- 11mM; glycerol, 100 mM). As fructose itself does not elicit astringency (Supplementary Figure 5.2), it is likely that the fructose suppressed the astringency from ethanol.

5.4.2.5 Burning/tingling

Regardless of the stimuli mixed with ethanol, burning/tingling always increased as ethanol concentration increased (Figures 5.4-5.7). This result is consistent with most (Nurgel and Pickering, 2005; Gawel et al., 2007, 2013; Jones et al., 2008; Clark et al., 2011; Villamor et al., 2013; Harwood et al., 2020) but not all (Frost et al., 2017) previous research in wine and beer (model or real). The relative intensity of the burning/tingling was the same for all four binary mixture types and in unary solutions (Figure 5.4-5.7). Burning/tingling ratings were well differentiated between ethanol concentrations typically found in beer (5%; below barely detectable), wine (13%; between barely detectable and weak) and in dilute spirits (23%; moderate). Together, the results suggest that burning/tingling is a key sensory characteristic of alcoholic beverages and is likely a key differentiator of styles.

The concentration of non-ethanol stimuli in the binary mixture did not impact the burning/tingling ratings. This finding was unexpected as previous research showed that adding sweet compounds to model solutions, beer or wine led to decreased ratings of burning/tingling-like sensations in most (Calviño, 1998; Nurgel and Pickering, 2006; Gawel et al., 2007; Jones et al., 2008; Clark et al., 2011) but not all (Villamor et al., 2013) studies. In addition, adding phenolics to white wine increased burning/tingling at lower ethanol concentrations (> 12.5%) but had no effect on red wine (Gawel et al., 2013). It is possible that the current study failed to capture the impacts of non-ethanol stimuli on burning/tingling as participants were required to wear nose clips. This choice limited the burning/tingling to the oral cavity, eliminating the impacts of nasal irritation from ethanol (Mattes and DiMeglio, 2001). In addition, the use of a wider range of ethanol

concentrations than most studies and the choice of label (burning/tingling vs heat, irritation, pungency, warming, hotness) may have limited the ability to detect small but significant changes in burning/tingling. More research is required to determine, if/how the burning/tingling of ethanol is impacted by non-ethanol stimuli. Furthermore, collecting information using scales with descriptive anchor terms, such as the gLMS, would allow researchers to determine whether differences found are ecologically-valid or likely too small for a consumer to detect.

5.4.3 Thermal Taste Status

As expected, TT were more responsive than TnT to many of the sensations elicited by the unary solutions (Green and George, 2004; Bajec and Pickering, 2008; Yang et al., 2014; Thibodeau et al., 2019, Chapter 3). Importantly, the current study also demonstrated that TT are also more responsive to both dominant and non-dominant sensations in binary mixtures. Although not all sample intensities varied with thermal taste status, such as the bitterness of quinine, no instances of TnT being more responsive than TT were found. Despite differences in responsiveness, relatively few interactions were reported between thermal taste status and stimuli concentration in the binary solutions. This observation suggests that despite the increased responsiveness of TT compared to TnT, the relative intensity of sensations elicited in binary mixtures is the same for both phenotypes. If true, changing the composition of alcoholic beverages to optimize flavour will lead to similar changes in the taste and chemesthetic profile of the product for both TT and TnT, albeit at different absolute intensities. Further research is encouraged to determine whether this finding is generalizable to different combinations of binary compounds in more complex samples or in solid food products.

Nolden and Hayes (2015) found that individuals who were more responsive to ethanol also tended to consume alcoholic beverages less frequently. Variation in ethanol responsiveness between TT and TnT reported here and in the literature (Small-Kelly and Pickering, 2020) suggest that differences in alcoholic beverage consumption may be partially attributable to thermal taste status. As the dominant sensations elicited by ethanol are nominally aversive, it is possible that the increased responsiveness of TT compared to TnT may also lead to lower alcohol consumption. However, to date only limited differences between TT and TnT in monthly alcohol consumption have been reported (Thibodeau,

2015). Thibodeau et al. (2017) found that alcohol consumption was not always linearly associated with orosensory responsiveness. Individuals with intermediate responsiveness to bitterness and astringency, tended to drink more alcohol than low or high responders (Thibodeau et al., 2017). The authors attribute this observation to the fact that the flavour of alcoholic beverages is likely be optimized by producers for the 'average' consumer. Importantly, alcoholic beverages are one of a growing number of products for which a wide variety of styles and flavours are available. Thus, research into the impact of TTS or other taste-related phenotypes is needed to determine if, rather than reducing their consumption of alcoholic beverages, consumers instead shift their consumption towards alcoholic beverages that are optimized for their palate. All other factors being equal (e.g., price, availability, social context), each consumer likely selects alcoholic beverages that best balance the taste sensations, chemesthetic sensations and aromas they find appetitive with the ones they aversive find aversive. By considering the volume and the proportion of alcoholic beverages consumed across categories (e.g., beer vs wine), types (e.g., red wine vs white wine) or styles (e.g., dry white wine vs sweet white wine), a more nuanced picture of alcohol consumption can be obtained. Furthermore, empirical research where consumers create their optimal alcoholic beverage (e.g., mix your own cocktail), may also provide insights into how taste impacts the consumption of alcoholic beverages at the individual level. Importantly, empirical research would allow for more control over the many intrinsic and extrinsic factors that also impact alcohol consumption (Betancur et al., 2020).

For unary solutions of ethanol, effects sizes were higher for the non-dominant attributes (sweetness and astringency) than the dominant attributes (bitterness and burning/tingling). These findings likely resulted from the increased number of scales used by TT compared to TnT when describing ethanol (Figure 5.8) and aluminium sulphate (Supplementary Figure 5.6). The simplest explanation for this finding is that TT have lower detection thresholds than TnT, and thus experience a wider range of low intensity sensations. However, suprathreshold intensity ratings and detections thresholds are not always associated (Mojet et al., 2005; Keast and Roper, 2007; Yang et al., 2014). Additionally, only detection thresholds for sucrose have been shown to differ between TT and TnT when taste (sucrose, sodium chloride, caffeine), trigeminal (capsaicin, N-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide) and aroma (ethyl butyrate, isoamyl acetate)

were examined (Yang et al., 2014). Thus, differences in detection thresholds may not explain the differences in scale use between TT and TnT.

TT and TnT did not differ in their ability to identify the primary orosensation elicited by a stimulus after a familiarization task, nor to discriminate different concentrations of the same stimulus. In both cases, TT and TnT performed equally, suggesting that the increased responsiveness of TT compared to TnT did not impact these tasks. However, as the data was collected during a training session, the results should be interpreted with caution. That is, during the identification task participants were provided with feedback after each sample, replicate samples were not included to re-test their abilities, and the ability to discriminate samples was limited to comparing low and high intensity stimuli. Thus, TT and TnT may differ in their ability to discriminate stimuli closer in intensity, supported by the lower discrimination thresholds of TT for tartaric acid in white wine reported by Pickering and Kvas (2016). Further research is encouraged to determine whether these preliminary results apply to a wider range of stimuli and in broader contexts.

5.4.4 Limitations and Other Considerations

A key limitation of the current study was the number of sensations rated as absent (0 on the gLMS) resulting in zero-inflated data. Despite log transformation the data remained right-skewed, which was largely attributed to the zeros in the data set. Although zero-inflated data is common in psychological research (Yang et al., 2017), it limited the treatment of stimulus concentration as a continuous variable. Instead, concentrations were treated as a categorical variable in the ANOVA, which is more robust to deviations from normality than ANCOVA (Field, 2013). Although more extensive analysis of interactions using the isobole method was planned, it was not possible and only a limited regression analysis performed. Readers are advised to interpret the results of the regression analysis (Supplementary Table 5.3) with caution as R^2 values are low, likely due to the right-skew of the data. Similarly, interactions results for the isobole analyses (Tables 5.4 & 5.5) should be treated as preliminary due to the limitations of the underlying regressions (Sühnel, 1993). Nevertheless, the isobole results demonstrate that in binary mixtures of ethanol and quinine or aluminium sulphate, bitterness and astringency are (respectively) largely suppressed. These results may be due to mixture suppression, which is common when solution

complexity is increased (Keast and Breslin, 2002). Where appropriate the index of interaction was calculated, determining whether enhancement or suppression has truly occurred and complementing the ANOVA, where potential interactions can be inferred but not tested.

Although other studies have investigated the interactions between ethanol and alcohol-related taste and chemesthetic stimuli, the current study was designed to address important gaps in the literature. With the exception of Martin and Pangborn (1970), previous studies on binary mixtures of ethanol with taste/chemesthetic stimuli only investigated a single stimulus or a group of stimuli that elicited the same orosensation. By examining stimuli that elicit four different orosensations, if/how changes in ethanol concentration impacted each of the binary mixture types was determined. For example, burning/tingling increased as ethanol concentration increased in all four binary mixture types and was not impacted by the concentration of other stimuli. In contrast, adding ethanol decreased the sweetness in binary mixtures with fructose but the opposite was true in binary mixtures with quinine. Furthermore, providing participants with six scales when rating the binary solutions reduced the risk of attribute dumping, allowing for a more complete understanding of the interactions between the stimuli. For example, previous studies on the interactions between organic acid and ethanol (Martin and Pangborn, 1970; Zamora et al., 2006; Guirao et al., 2013), did not measure the astringency elicited in the samples despite the fact that organic acid elicit both sensations (Sowalsky and Noble, 1998). As participants in the current study rated both the sourness and astringency of the binary mixtures of ethanol and tartaric acid, it was demonstrated that increasing the ethanol concentration reduced the sourness while simultaneously increasing the astringency of the binary mixtures.

Finally, by screening participants for thermal taste status, the impacts of individual taste differences on the perception of binary mixtures was also investigated. Importantly, few interactions were found between thermal taste status and the concentrations of ethanol and/or the other stimuli in the binary mixtures. These results suggest that despite differences in the magnitude of the sensations elicited, the nature of interactions (enhancement and/or suppression) was the same in both groups. Sex is not associated with differences in TTS classification (Bajec and Pickering, 2008; Yang et al., 2014; Thibodeau

et al., 2019, Chapter 3). Nevertheless, as the study only included female participants, more research with males is encouraged to determine whether sex-related differences exist. Additionally, as the sample size is relatively small, such expansion would allow for an examination of the findings with a larger sample. More work is also encouraged to determine whether trends exist for other taste-related phenotypes where differences in the perception of ethanol have been reported (e.g. 6-n-propylthiouracil (PROP) taster status (Bartoshuk et al., 1993; Prescott and Swain-Campbell, 2000; Duffy et al., 2004). Together, the results of the current study provide insights into how the taste and chemesthetic profile of alcoholic beverages can be manipulated by changing their composition. More research is encouraged to determine if/how the trends reported here apply in more complex mixtures and in real alcoholic beverages, especially in beer and spirits, as most published research uses model or real wines.

5.5 Appendix A – Thermal Taste Screening (Session 1)

Before training (Session 2) and data collection (Session 3A-3D), participants underwent TTS screening. First, participants were trained on the gLMS to ensure that ratings were generalized across all possible sensations. To this end, participants were asked to write down the strongest imaginable sensation they could think of, painful or otherwise, at the top of a blank gLMS (Hort et al., 2016). Participants were then verbally oriented to the gLMS and asked to rate the intensity of five remembered stimuli. Next, labelled 20 mL aqueous solutions were presented to participants to familiarize them with sensations that might be elicited during thermal stimulation later in the session, as it can increase the number of TT identified (Thibodeau et al., 2019, Chapter 3). These samples were prepared with pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA) and were exemplars of sweet (sucrose 85.58 g/L; Burlington, BioShop, Canada), sour (citric acid 0.62 g/L; Fisher Scientific, Fair Lawn, NJ, USA), bitter (quinine monohydrochloride dehydrate 0.011 g/L; SAFC Supply Solutions, St. Louis, MO, USA), metallic (cupric sulphate 0.25 g/L; Sigma-Aldrich, St. Louis, MO, USA), salty (sodium chloride 10.5 g/L; Sigma-Aldrich, St. Louis, MO, USA), and umami (L-glutamic acid monosodium salt hydrate 21.14 g/L; Sigma-Aldrich, St. Louis, MO, USA), the most common sensations

reported by TT (Thibodeau et al., 2020, Chapter 4). Participants tasted each sample using a sip-and-spit protocol and rated the maximum intensity of each on a gLMS.

Thermal stimulation was performed using a 64 mm2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode). Warming cycles started at 35°C, then cooled to 15°C before final re-warming to 40°C and holding for 1 second. Because only the warming portion of the cycle was of interest, participants were asked to rate the maximum intensity of sensations during the re-warming phase of the cycle (from 15°C to 40°C). Cooling cycles started at 35°C, subsequently cooling to 5°C and holding for 10 seconds. Because no warming occurs during this cycle, participants were asked to rate the maximum intensity of sensations through the entire cycle (Bajec and Pickering, 2008).

Before collection of thermal taste responses, participants underwent four training runs to become familiar with the temperature cycles and the thermode. Participants rated the maximum intensity of the temperature elicited when the thermode was applied to the palm and the vermillion border of the lip during both warming and cooling trials. Next, the experimenter applied the thermode to each participant's extended tongue. Three locations on the edge of the tongue (the most anterior tip of the tongue, ~1 cm to the right of the midline and ~1 cm to the left of the midline) were tested in randomized order. 12 runs were performed for each participant in two blocks. Each block consisted of three warming cycles (one per location) followed by three cooling cycles (one per location). After each trial, participants were instructed to rate the intensity on the gLMS of any oral sensations perceived, including temperature, on eight individual scales titled heat or cold, sweet, salty, sour, bitter, umami, metallic and other. Participants were tested using all combination of two temperature regimes and at three locations on the tongue, as testing under all six conditions leads to increased identification of TT (Thibodeau et al., 2020, Chapter 4).

Thermal taste status classification was determined using the methods of Bajec et al. (2008) as this scheme has been successfully used for previous data collected from the available thermode, it has been validated in a large data set and it has good concordance with most of the schemes (Thibodeau et al., 2019, Chapter 3). TT were defined as participants who reported the same, thermally-elicited taste sensation above weak on the gLMS (> 6 mm) during both replicates of the same location during the same temperature

regime. TnT were defined as participants who reported no taste-related orosensation during thermal elicitation.

5.6 Link to Published Version

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5.7 References

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5.8 Supplementary Materials

5.8.1 Supplementary Tables

Table S5.1: Two-way ANOVA and Kruskal-Wallis analyses comparing intensity ratings by thermal taste status (TT and TnT) and stimulus concentration (low, medium, high) for orosensations elicited by unary solutions of ethanol, fructose, quinine, tartaric acid and alum sulphate.

Stimulus	Orosensation rated	Two-way ANOVA						Kruskal-Wallis	
		Thermal taste status (TTS)		Stimuli Concentration (Conc)		TTS*Conc		*K*	*P*
		F(df)	*P*	*F*(df)	*P*	*F*(df)	*P*		
Ethanol	Sweet	17.4 (1, 86)	< 0.001	1.2 (2, 86)	0.294	0.8 (2, 86)	0.435	21.5	0.001
	Bitter	3.6 (1,86)	0.059	10.2 (2, 86)	< 0.001	0.3 (2, 86)	0.745	22.1	0.001
	Astringent	23.0 (1, 86)	< 0.001	2.7 (2, 86)	0.070	0.4 (2, 86)	0.675	23.9	0.002
	Burning/tingling	3.1 (1, 86)	0.083	95.9 (2, 86)	< 0.001	0.6 (2, 86)	0.572	61.7	< 0.001
Fructose	Sweet	15.0 (1, 101)	< 0.001	58.0 (2, 101)	< 0.001	0.2 (2, 101)	0.794	62.2	< 0.001
Quinine	Bitter	0.01 (1, 104)	0.933	3.6 (2, 104)	0.030	0.2 (2, 104)	0.806	7.2	0.206
Tartaric acid	Sour	2.2 (1, 110)	0.140	18.5 (2, 110)	< 0.001	0.1 (2, 110)	0.885	33.5	< 0.001
	Astringent	1.1 (1, 100)	0.318	1.9 (2, 110)	0.149	0.1 (2, 110)	0.891	3.9	< 0.001
Alum sulphate	Astringent	1.3 (1, 104)	0.252	15.5 (2, 104)	< 0.001	0.5 (2, 104)	0.614	26.5	< 0.001
	Sour	13.2 (1, 104)	< 0.001	11.9 (2, 104)	< 0.001	4.2 (2, 104)	0.018	30.1	0.560

Table S5.2: Three-way ANOVA comparing the intensity of orosensations elicited by binary mixtures of ethanol and one stimuli (fructose, quinine, tartaric acid, alum sulphate). Factors in each model include thermal taste status (TTS; thermal taster, thermal non-taster), ethanol concentration (5%, 13%, 23% vol/vol) and stimuli concentration (low, medium, high).

Binary Mixture and Factors in Model	*Df*	Sweet		Bitter		Sour		Astringent		Burning/ tingling	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Fructose & Ethanol											
Overall Model	13, 305	16.2	< 0.001	3.4	< 0.001	1.2	0.259	4.1	< 0.001	31.3	< 0.001
TTS	1, 305	40.8	< 0.001	0.5	0.472			21.8	< 0.001	26.6	< 0.001
Ethanol	2, 305	13.1	< 0.001	12.9	< 0.001			6.5	0.002	185.2	< 0.001
Fructose	2, 305	68.0	< 0.001	6.8	0.001			4.2	0.016	0.3	0.773
TTS*Ethanol	2, 305	0.3	0.734	0.9	0.388			3.1	0.046	2.6	0.074
TTS*Fructose	2, 305	0.7	0.514	0.5	0.633			0.6	0.543	0.1	0.892
Ethanol*Fructose	4, 305	1.3	0.287	0.4	0.822			0.8	0.549	1.1	0.378
Quinine & Ethanol											
Overall Model	13, 314	3.9	< 0.001	1.6	0.087	0.5	0.895	0.6	0.856	19.6	< 0.001
TTS	1, 314	23.9	< 0.001	0.6	0.437					2.5	0.112
Ethanol	2, 314	9.0	< 0.001	2.4	0.090					120.8	< 0.001
Quinine	2, 314	1.3	0.264	3.8	0.024					0.5	0.604
TTS*Ethanol	2, 314	2.7	0.067	0.1	0.900					3.9	0.021
TTS*Quinine	2, 314	0.0	0.995	0.7	0.504					0.6	0.574
Ethanol*Quinine	4, 314	0.3	0.909	1.5	0.195					0.3	0.879

Table S5.2 (continued): Three-way ANOVA comparing the intensity of orosensations elicited by binary mixtures of ethanol and one stimuli (fructose, quinine, tartaric acid, alum sulphate). Factors in each model include thermal taste status (TTS; TT, TnT), ethanol concentration (5%, 13%, 23% vol/vol) and stimuli concentration (low, medium, high).

Binary Mixture and Factors in Model	*Df*	Sweet		Bitter		Sour		Astringent		Burning/ tingling	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Tartaric Acid & Ethanol											
Overall Model	13, 332	0.9	0.573	4.5	< 0.001	9.8	< 0.001	1.6	0.074	21.4	< 0.001
TTS	1, 332			15.9	< 0.001	4.3	0.039	1.7	0.195	3.0	0.083
Ethanol	2, 332			14.0	< 0.001	19.9	< 0.001	4.1	0.018	134.6	< 0.001
Tartaric Acid	2, 332			2.6	0.078	36.5	< 0.001	3.8	0.024	0.5	0.590
TTS*Ethanol	2, 332			0.4	0.693	0.9	0.393	0.3	0.715	0.8	0.464
TTS*Tartaric Acid	2, 332			1.3	0.284	0.6	0.537	1.0	0.357	0.4	0.675
Ethanol*Tartaric Acid	4, 332			1.4	0.238	1.7	0.147	0.3	0.887	0.7	0.596
Alum & Ethanol											
Overall Model	13, 314	2.7	0.001	2.3	0.007	3.3	< 0.001	7.1	< 0.001	22.8	< 0.001
TTS	1, 314	23.2	< 0.001	2.8	0.093	0.7	0.389	30.2	< 0.001	3.9	0.049
Ethanol	2, 314	0.2	0.792	9.1	< 0.001	4.4	0.013	2.5	0.082	140.9	< 0.001
Alum	2, 314	0.9	0.422	0.1	0.962	11.7	< 0.001	23.3	< 0.001	2.6	0.076
TTS*Ethanol	2, 314	0.1	0.873	1.7	0.185	1.2	0.311	3.0	0.051	0.6	0.530
TTS*Alum	2, 314	1.3	0.267	0.6	0.535	0.3	0.772	0.6	0.570	0.1	0.938
Ethanol*Alum	4, 314	1.7	0.153	1.0	0.428	1.9	0.115	0.7	0.599	0.9	0.441

Table S5.3: Simple linear regressions used to predict the intensity of orosensations based on the concentration of unary solutions of stimuli (ethanol, fructose, alum sulphate, tartaric acid and quinine). Terms of the regression equation are provided for significant models only (NA = All participants rated the burning/tingling of fructose unary solutions as "no sensation" or 0 on gLMS, thus, regression was not possible as there was no variance).

Stimulus (concentration)	Orosensation	Linear regression parameters			Regression equation terms Intensity = m (concentration) + b	
		R^2	F	p	Intercept (b)	Slope (m)
Ethanol log(% vol/vol)	Sweet	0.02	2.1	0.152		
	Bitter	0.14	14.2	< 0.001	0.016	0.543
	Sour	0.01	0.4	0.508		
	Astringent	0.05	4.5	0.038	-0.018	0.244
	Burning/ tingling	0.69	182.6	< 0.001	-1.062	1.685
Fructose log(mM)	Sweet	0.51	104.0	< 0.001	-1.441	0.957
	Bitter	0.01	1.2	0.270		
	Sour	0.01	0.7	0.391		
	Astringent	0.04	3.7	0.057		
	Burning/ tingling	NA	NA	NA		
Quinine log(1+mM)	Sweet	< 0.01	0.02	0.865		
	Bitter	0.07	7.5	0.007	0.489	10.819
	Sour	0.02	1.9	0.168		
	Astringent	< 0.01	0.1	0.716		
	Burning/ tingling	< 0.01	0.05	0.822		
Tartaric acid log(mM)	Sweet	< 0.01	< 0.01	0.947		
	Bitter	0.01	1.0	0.314		
	Sour	0.26	37.4	< .001	0.007	1.032
	Astringent	0.01	0.6	0.433		
	Burning/ tingling	0.01	1.4	0.232		
Alum sulphate log(mM)	Sweet	0.01	0.9	0.358		
	Bitter	0.06	6.6	0.012	0.063	0.234
	Sour	0.17	20.3	< 0.001	0.167	0.582
	Astringent	0.21	28.1	< 0.001	0.483	0.786
	Burning/ tingling	0.02	2.1	0.154		

Table S5.4: Summary of Mann-Whitney U results comparing the mean number of scales used by TT and TnT when rating water and unary solutions of ethanol, fructose, quinine, tartaric acid and alum sulphate. (# = interpret with caution as the variance of TnT scores is 0).

Stimuli	Concentration	TT		TnT		*U*	*p*
		n	*M*	*n*	*M*		
Water	N/A	18	0.7	11	0.1	169.5	0.001
Ethanol	5% vol/vol	18	1.4	11	1.0	143.0	0.009
	13% vol/vol	18	2.4	11	1.8	153.0	0.012
	23% vol/vol	18	2.4	11	2.0	134.0	0.113
Fructose	140 mM	21	1.3	13	0.9	182.5	0.032
	280 mM	21	1.1	13	1.0	148.5	0.798
	960 mM	21	1.1	13	1.0	156.0	< .001#
Quinine	0.025 mM	21	1.4	14	1.2	165.5	0.571
	0.040 mM	21	1.4	14	1.1	180.5	0.251
	0.100 mM	21	1.4	14	1.1	183.0	0.210
Tartaric acid	2.75 mM	22	1.5	15	1.2	206.0	0.200
	6.91 mM	22	1.4	15	1.5	160.5	0.208
	17.4 mM	22	1.7	15	1.3	207.5	0.178
Alum sulphate	0.73 mM	21	1.0	14	0.7	188.0	0.161
	2.05 mM	21	2.0	14	1.4	213.5	0.021
	5.43 mM	21	2.4	14	1.6	217.0	0.015

Table S5.5: Summary of Mann-Whitney U results comparing the mean number of scales used by TT (n = 21) and TnT (n = 13) when rating binary solutions of fructose and ethanol.

Fructose Concentration	Ethanol Concentration (vol/vol)	TT		TnT		*U*	*p*
		M	*SE*	*M*	*SE*		
140 mM	5%	1.7	0.2	1.4	0.2	155.5	0.569
	13%	2.3	0.2	1.9	0.3	168.5	0.251
	23%	2.4	0.2	2.2	0.2	156	0.496
280 mM	5%	1.8	0.2	1.5	0.2	164	0.334
	13%	2.4	0.2	2.2	0.2	160	0.417
	23%	2.4	0.2	2.4	0.3	131	0.823
960 mM	5%	1.4	0.1	1.1	0.1	191	< 0.001
	13%	2.1	0.1	1.9	0.2	160.5	0.418
	23%	2.8	0.2	2.4	0.2	170.5	0.216

Table S5.6: Summary of Mann-Whitney U results comparing the mean number of scales used by TT (n = 21) and TnT (n = 14) when rating binary solutions of quinine and ethanol.

Quinine Concentration	Ethanol Concentration (vol/vol)	TT		TnT		*U*	*p*
		M	*SE*	*M*	*SE*		
0.025 mM	5%	1.6	0.2	1.4	0.1	167	0.540
	13%	2.0	0.2	1.6	0.2	175	0.364
	23%	2.4	0.2	1.9	0.3	183	0.238
0.040 mM	5%	1.5	0.2	1.4	0.1	162.5	0.967
	13%	2.1	0.2	1.9	0.3	173	0.364
	23%	2.6	0.3	2.1	0.2	175	0.355
0.100 mM	5%	1.8	0.2	1.3	0.1	192	0.110
	13%	2.3	0.2	1.8	0.3	192.5	0.118
	23%	2.3	0.2	1.9	0.2	182.5	0.222

Table S5.7: Summary of Mann-Whitney U results comparing the mean number of scales used by TT (n = 22) and TnT (n = 15) when rating binary solutions of tartaric acid and ethanol.

Tartaric Acid Concentration	Ethanol Concentration (vol/vol)	TT		TnT		*U*	*p*
		M	*SE*	*M*	*SE*		
2.75 mM	5%	1.9	0.2	1.4	0.2	212	0.136
	13%	2.3	0.2	2.1	0.2	183	0.599
	23%	2.3	0.2	1.9	0.2	209.5	0.182
6.91 mM	5%	1.8	0.1	1.7	0.2	189	0.468
	13%	2.5	0.2	2.5	0.3	166.5	0.983
	23%	2.8	0.2	2.0	0.2	235	0.027
17.4 mM	5%	1.8	0.2	1.3	0.1	215	0.086
	13%	2.6	0.2	2.3	0.3	197.5	0.311
	23%	2.6	0.3	2.4	0.3	186	0.482

Table S5.8: Summary of Mann-Whitney U results comparing the mean number of scales used by TT (n = 21) and TnT (n = 14) when rating binary solutions of alum sulphate and ethanol.

Alum Sulphate Concentration	Ethanol Concentration (vol/vol)	TT		TnT		*U*	*p*
		M	*SE*	*M*	*SE*		
0.73 mM	5%	1.7	0.2	1.4	0.2	175.5	0.355
	13%	2.5	0.2	1.7	0.2	220.5	0.007
	23%	2.4	0.2	1.9	0.2	184	0.210
2.05 mM	5%	2.1	0.2	1.4	0.1	203	0.050
	13%	2.7	0.2	2.2	0.3	186	0.166
	23%	2.7	0.2	2.2	0.3	181	0.267
5.43 mM	5%	2.3	0.2	1.7	0.2	205	0.040
	13%	2.8	0.2	2.1	0.3	199	0.075
	23%	2.6	0.3	2.4	0.3	166	0.568

5.8.2 Supplementary Figures

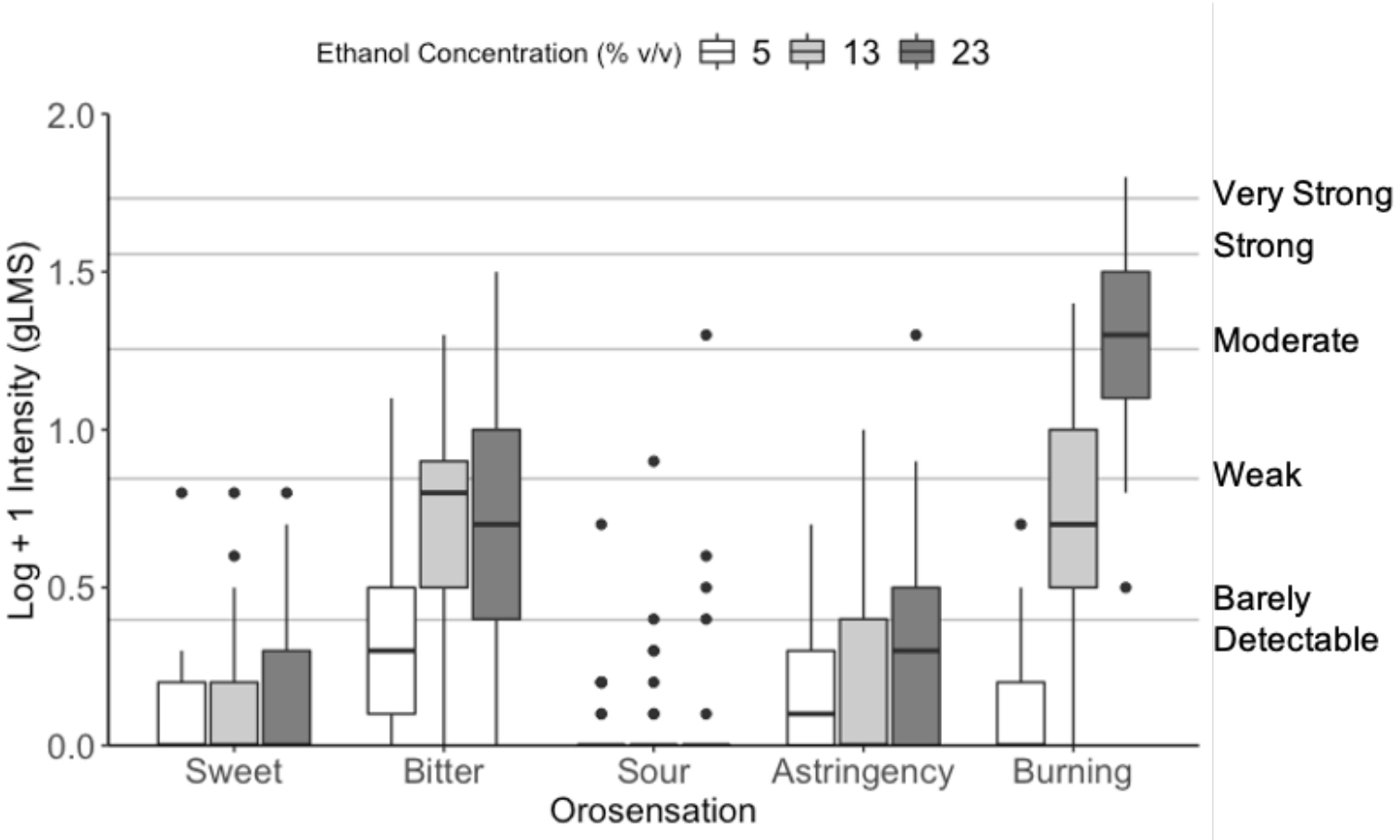

Figure S5.1: Boxplots of mean responsiveness to orosensations elicited by unary solutions of ethanol. Data is for participants that completed all sessions (3A-3D; $n = 29$).

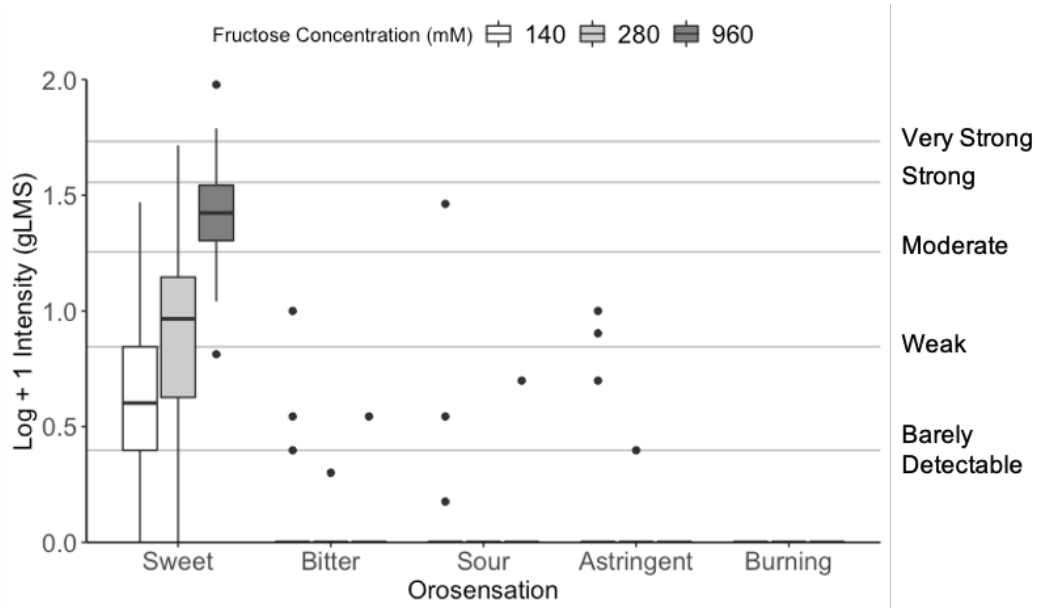

Figure S5.2: Boxplots of mean responsiveness to orosensations elicited by unary solutions of fructose. Data is for participants that completed all Session 3A (n = 34).

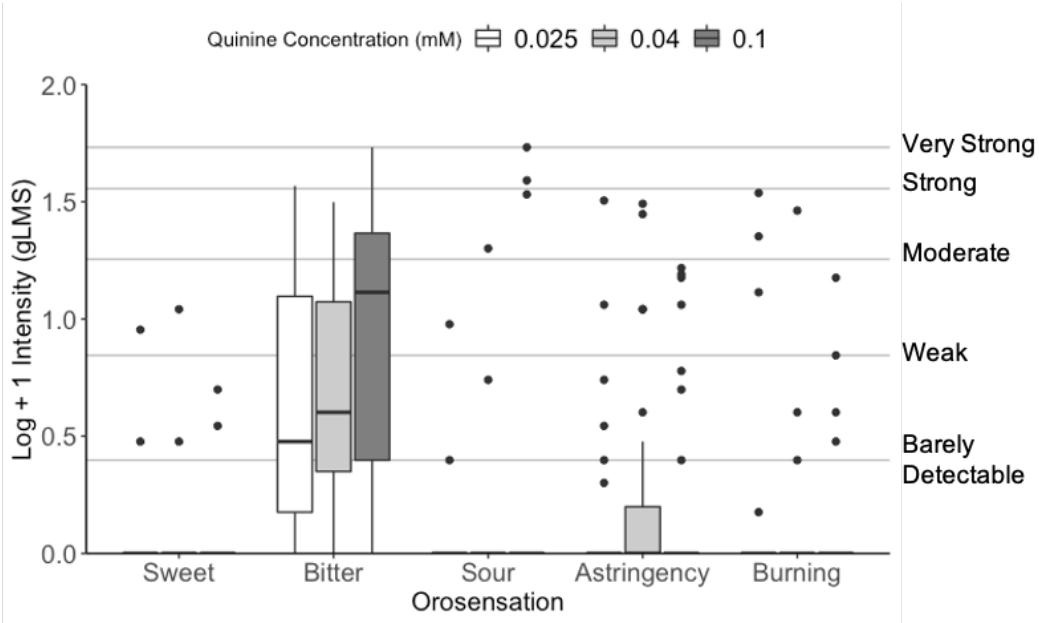

Figure S5.3: Boxplots of mean responsiveness to orosensations elicited by unary solutions of quinine. Data is for participants that completed all Session 3D (n = 35).

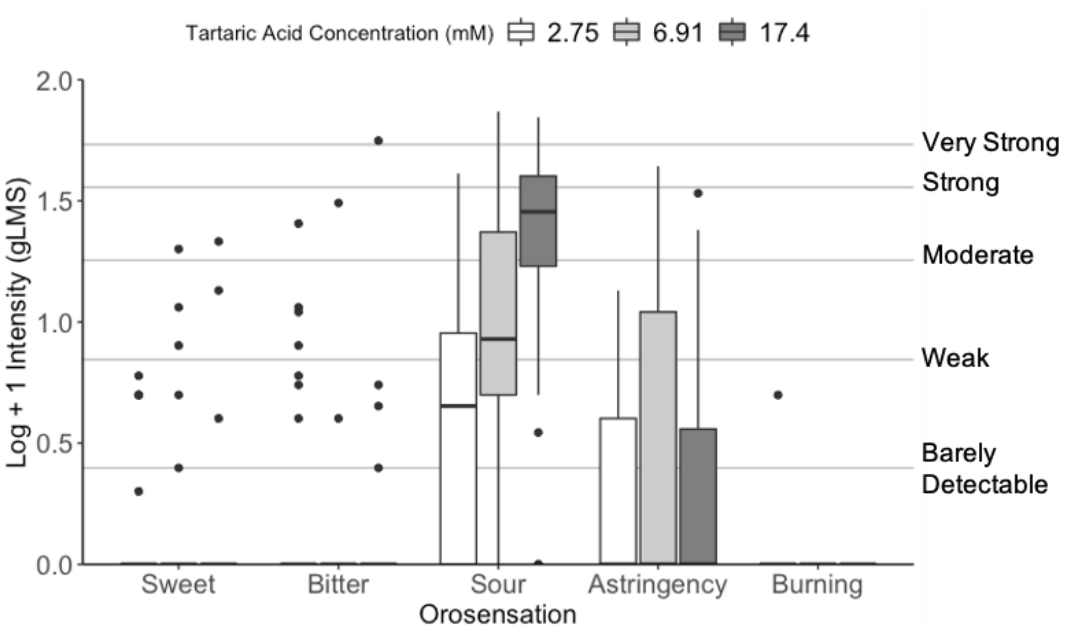

Figure S5.4: Boxplots of mean responsiveness to orosensations elicited by unary solutions of tartaric acid. Data is for participants that completed all Session 3C (n = 37).

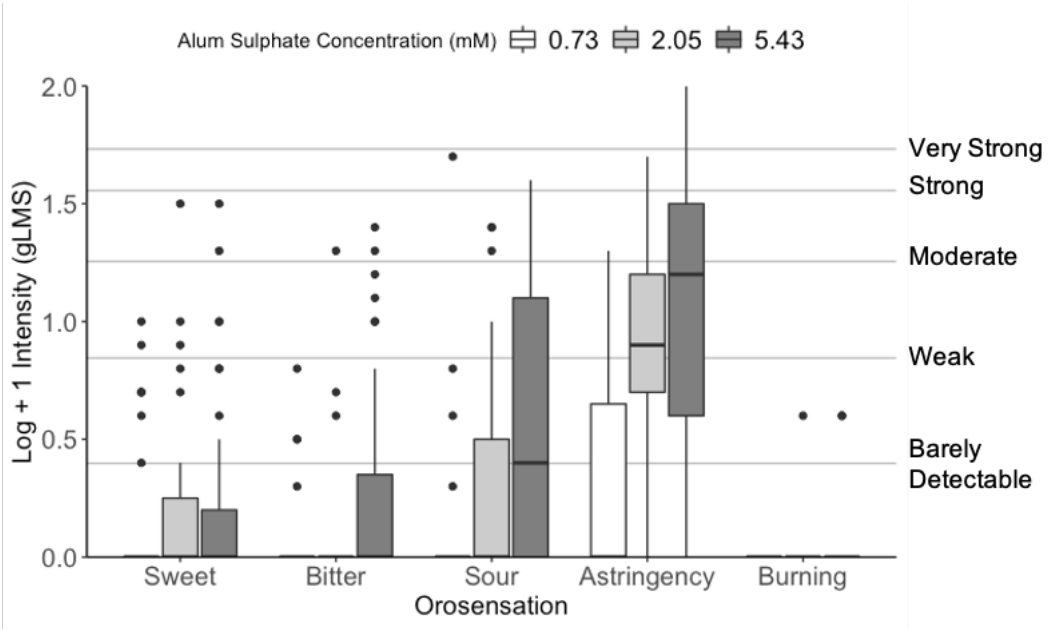

Figure S5.5: Boxplots of mean responsiveness to orosensations elicited by unary solutions of alum sulphate. Data is for participants that completed all Session 3A (n = 36).

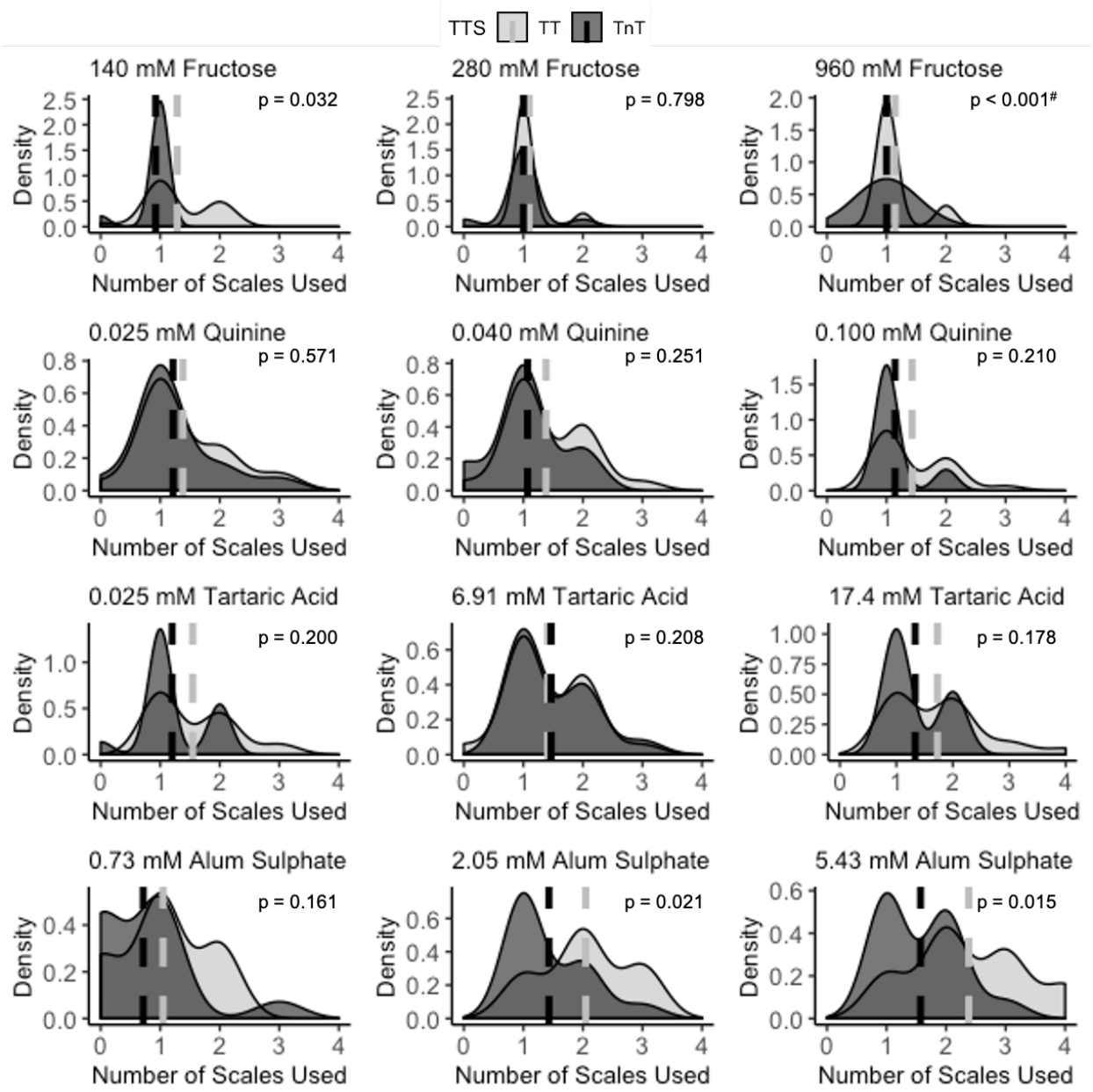

Figure S5.6: Kernel density estimates of the mean number of scales used by thermal tasters (TT, n = 21-22) and thermal non-tasters (TnT, n = 13-15) when rating unary aqueous solutions of fructose, alum sulphate, tartaric acid and quinine. Dashed lines indicate the mean values and p-values indicate if the medians differ significantly (NS = not significant, # = interpret with caution as the variance of TnT scores is 0).

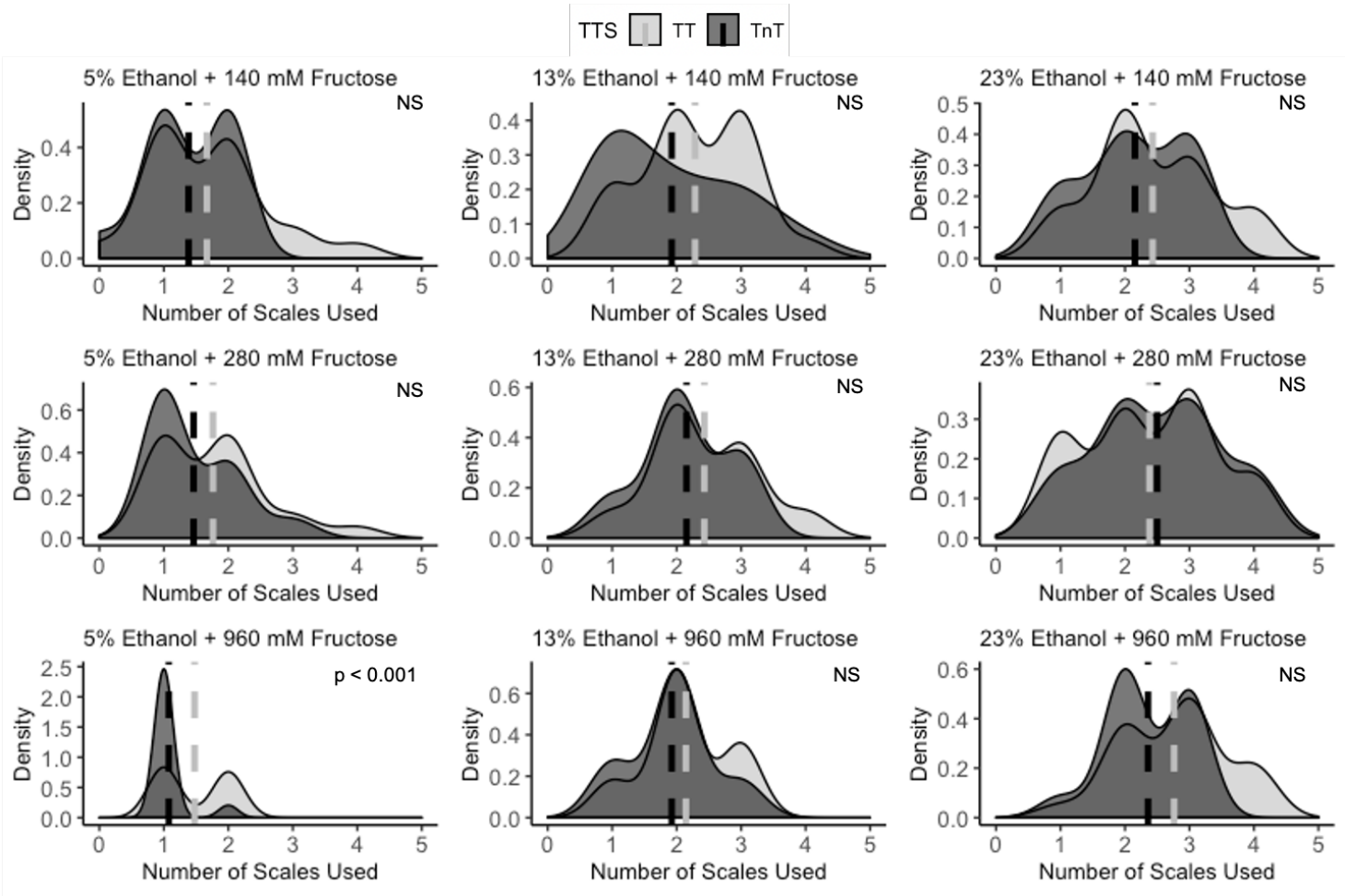

Figure S5.7: Kernel density estimates of the mean number of scales used by thermal tasters (TT, $n = 21$) and thermal non-tasters (TnT, $n = 13$) when rating binary solutions of fructose and ethanol. Dashed lines indicate the mean values and p-values indicate if the medians differ significantly (NS = not significant).

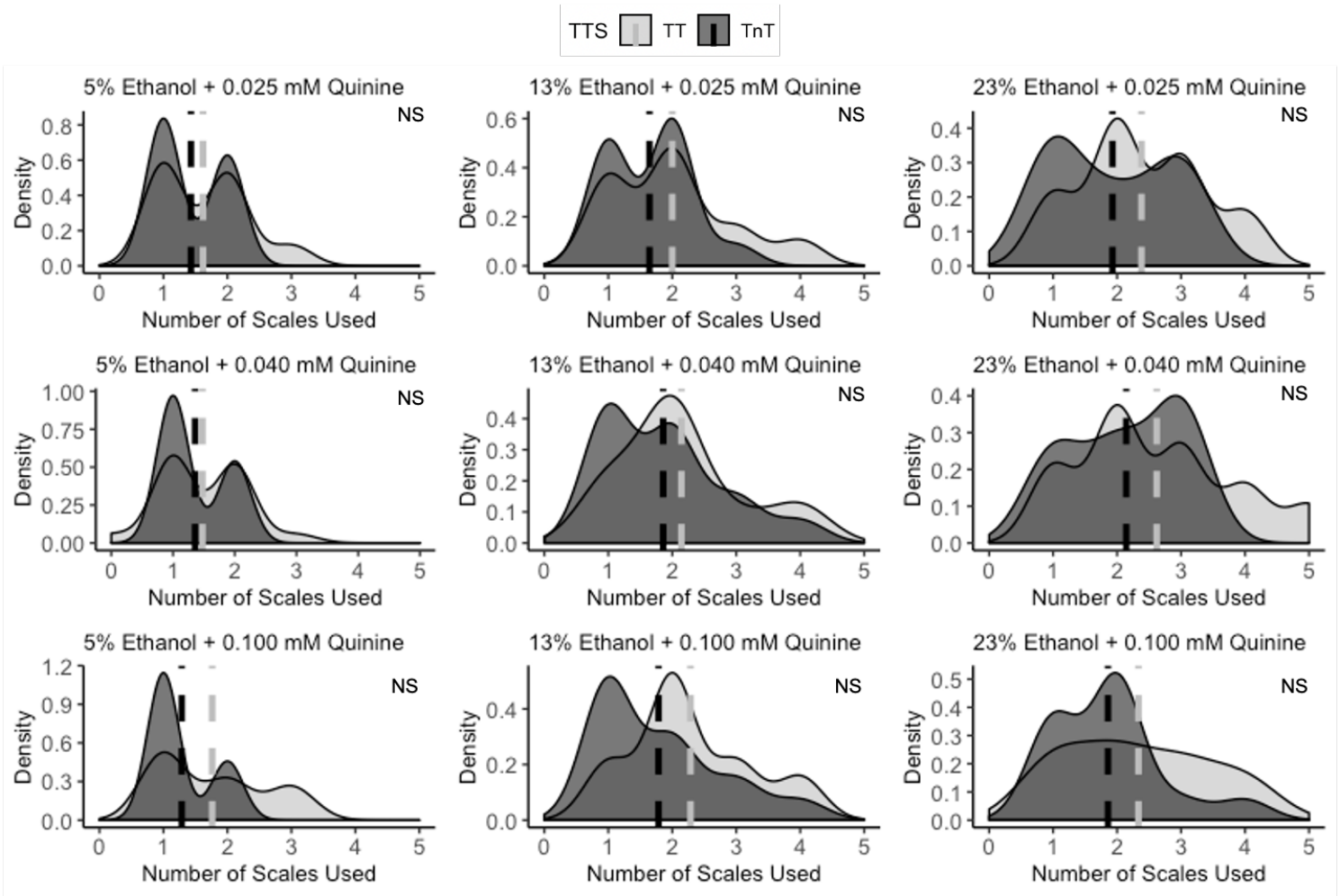

Figure S5.8: Kernel density estimates of the mean number of scales used by thermal tasters (TT, $n = 21$) and thermal non-tasters (TnT, $n = 14$) when rating binary solutions of quinine and ethanol. Dashed lines indicate the mean values and p-values indicate if the medians differ significantly (NS = not significant).

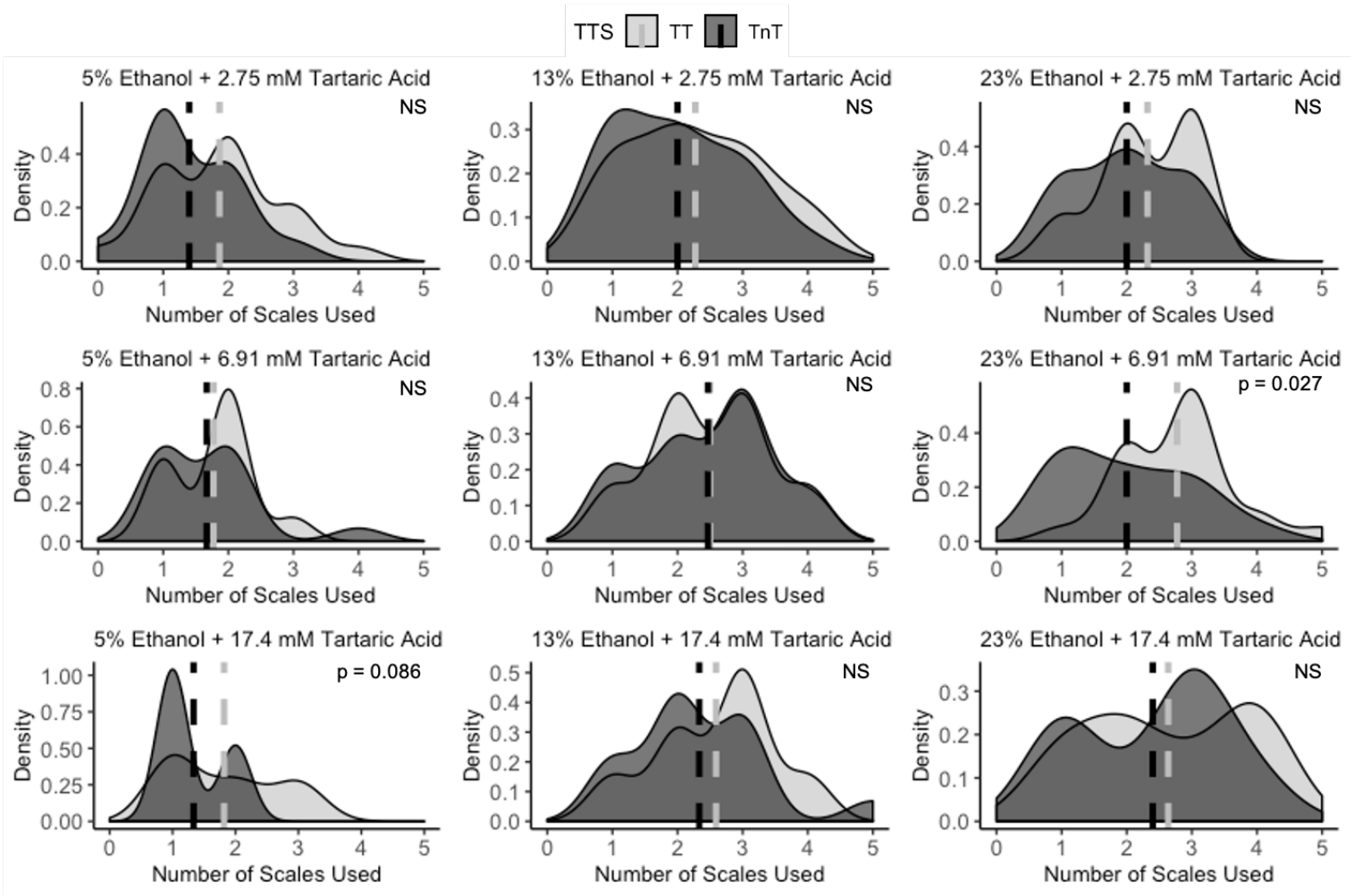

Figure S5.9: Kernel density estimates of the mean number of scales used by thermal tasters (TT, n = 22) and thermal non-tasters (TnT, n = 15) when rating binary solutions of tartaric acid and ethanol. Dashed lines indicate the mean values and p-values indicate if the medians differ significantly (NS = not significant).

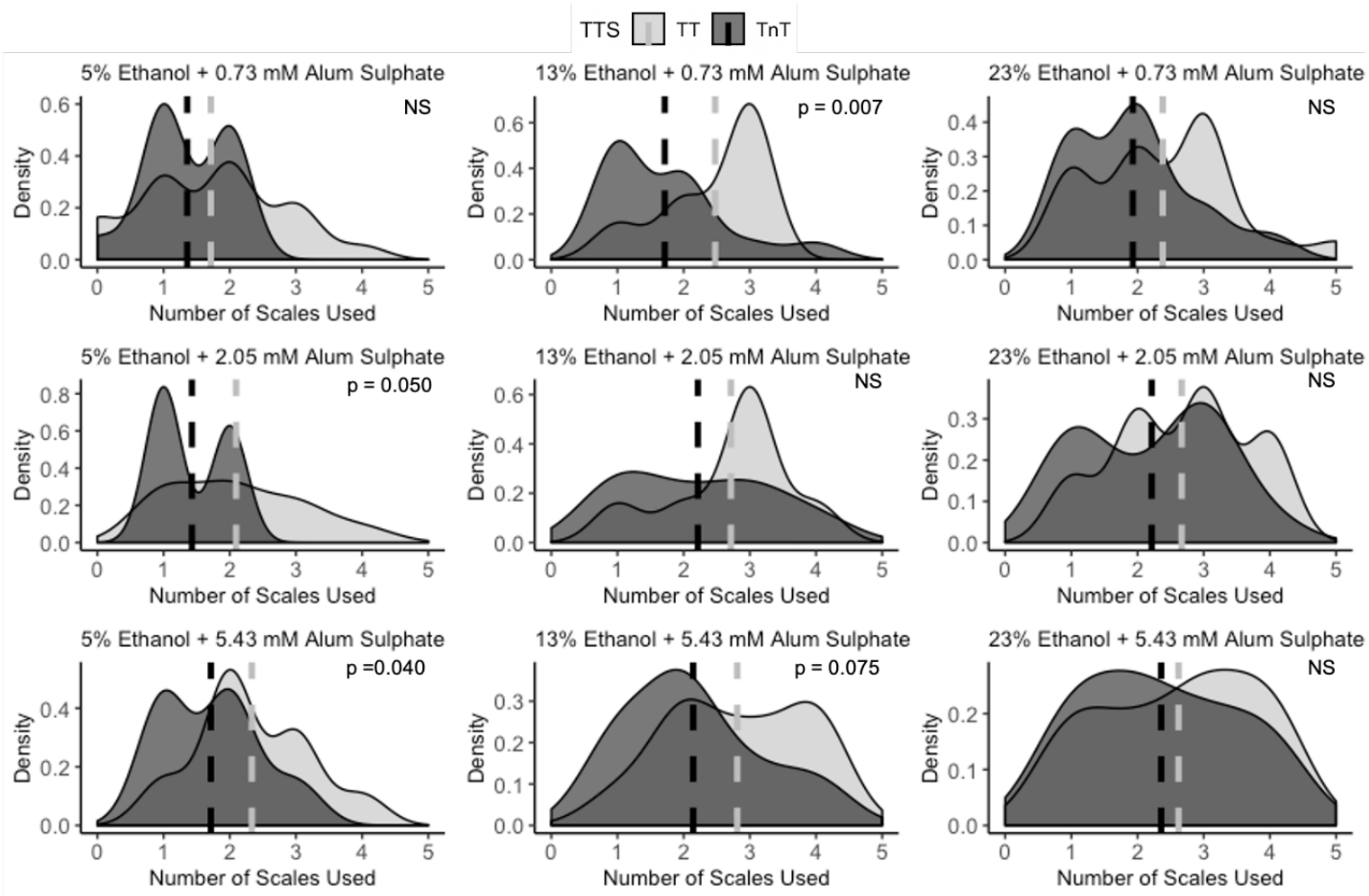

Figure S5.10: Kernel density estimates of the mean number of scales used by thermal tasters (TT, n = 21) and thermal non-tasters (n = 14) when rating binary solutions of alum sulphate and ethanol. Dashed lines indicate the mean values and p-values indicate if the medians differ significantly (NS = not significant.)

Chapter 6: General Conclusions

Together, the chapters in this thesis provide good evidence to support the existence of thermal taster status (TTS), provide new insights into the differences both between and within TTS phenotypes (thermal tasters – TT; thermal non-tasters – TnT; non-classifiable NC) and inform best practices in the field moving forward. Furthermore, insights into the perception of alcoholic beverages were obtained by studying binary mixtures of ethanol and stimuli that represent common orosensations elicited in alcoholic beverages. The current chapter is divided into two parts. First, using the literature gaps identified in Chapter 1, the key findings from the thesis are summarized. Second, new research gaps are identified based on current literature and the findings from this thesis. Readers are referred to earlier chapters for a full consideration of each study's limitations in-situ.

6.1 Summary of findings and implications

6.1.1 Gap #1

Differences in taste responsiveness between TT and TnT are not always found, and such null results are often attributed to sample size.

Results in Chapter 3 confirm that TT are more responsive to orosensory stimuli than TnT, regardless of the classification scheme employed. TT also rate temperature changes at both lingual and non-lingual sites higher than TnT. Although neither of these findings are new in literature, they are important as they are consistent with the findings reported in other studies, where significant differences were not always found (Green and George, 2004; Green et al., 2005; Bajec and Pickering, 2008; Yang et al., 2014; Hort et al., 2016). Furthermore, as the first study to directly test for scale use differences between TT and TnT, it was confirmed that scale use differences do not account for the variation in orosensory responsiveness between these phenotypes. Together, with the increased orosensory responsiveness of TT compared to TnT to the unary solutions in Chapter 5, the Chapter 3 results and literature strongly supports that TTS is an important source of individual differences in orosensory perception.

6.1.2 Gap #2

TT experience a wide range of taste sensations during thermal elicitation and the proportion of tastes reported can vary with the temperature regime and/or lingual location tested. Based on these differences, more research is needed to determine whether TT are a homogeneous group or whether subgroups within TT exist.

The findings in Chapter 4 represent the first comprehensive analysis of the experiences reported by TT during TTS screening in a large sample (n=254). Consistent with previous literature, it was found that a large proportion of TT experienced sweet, salty, sour, bitter and metallic thermally-elicited sensations (Yang et al., 2014; Pickering and Klodnicki, 2016; Pickering and Kvas, 2016; Pickering et al., 2016; Skinner et al., 2018) and approximately one third of participants experience more than one type of thermally-elicited sensation (Skinner et al., 2018). Fifteen combinations of thermally-elicited orosensations, locations and temperature regimes were significantly associated, providing potential hints towards the mechanism(s) underlying TTS. Notably, sweet TT were nine times more likely to also be warm TT, and sour TT were eight times more likely to be cool TT. Contrary to earlier speculation (Pickering and Kvas, 2016; Pickering et al., 2016), no differences in orosensory responsiveness were found for any of the TT subgroups tested based on thermally-elicited orosensation, temperature regime or location. It is logical to assume that the same mechanism drives the heightened orosensory responsiveness of TT and TT's experience of thermally-elicited orosensations, as it is the most parsimonious theory. If true, the lack of differences support a centrally-mediated mechanism of increased responsiveness to oral stimuli in TT. Alternatively, gustatory and trigeminal pathways must be cross-wired far enough along the transduction pathway allowing the gains to extend to all orosensations, not just those reported during thermal elicitation. Taken together, these results provide the first comprehensive evidence that the heightened orosensory responsiveness of TT is universal and confirms the findings of a preliminary analysis by Bajec et al. (2012). This result suggests that TT can be treated as a homogeneous group in studies where differences in orosensory response rather than the mechanism(s) underlying TTS are of primary interest.

6.1.3 Gap #3

Up to half of individuals are non-classifiable after TTS screening and it is not known whether they represent a third phenotypic group or whether are misclassified TT and TnT.

Chapter 3 is the first published paper to characterize NC individuals. Rather than a distinct phenotypic group, the pattern of NC being intermediate in age, orosensory responsiveness and temperature responsiveness to TT and TnT suggests that NC may be misclassified TT and TnT. This hypothesis was further supported when NC were divided into subgroups based on the two criteria used to define TT, namely the reproducibility and intensity of the thermally-elicited sensations reported. NC that met at least one of these criteria (see Chapter 3 for full details) tended to have higher orosensory than those that did not. Thus, NC that reported reproducible or higher intensity thermally-elicited sensations, tended to behave more like TT whereas those that did not tended to behave more like TnT. Together, the differences in orosensory and temperature responsiveness of the four NC subgroups confirm that NC cannot be considered a homogenous group. As NC represent up to 50% of participants screened for TTS, the decision to combine subgroups with TT and TnT to increase sample size may reduce the recruitment burden associated with TTS research but should be done with careful consideration of the study's objectives.

6.1.4 Gap #4

TTS screening methods and classification schemes were developed when the phenomenon was newly discovered. Thus, a retrospective interrogation of existing data can provide insights into potential strategies to optimize methods.

Results for Chapter 3 and Chapter 4 helped to inform the TTS screening protocols used in Chapter 5 and best practices for future research on TTS. TTS screening should employ at least two replicates for all locations (tip, left, right) and temperature regimes (warming, cooling), while also providing participants with training and scales for all anticipated thermally-elicited sensations. These practices are associated with an increased identification of TT and a decreased identification of TnT. Although reduced TnT identification can be a limitation, it reduces the odds that a TnT is misclassified as a TT, which would strongly distort the group differences. In addition, maintaining the ability to classify individuals as NC can increase confidence that individuals classified as TT or TnT

are accurately classified. However, NC should not be treated as a separate group from that of TT and TnT. In fact, for larger population-based studies, the definitions of TT and TnT can likely be relaxed. This strategy would allow for more NC to be re-classified as TT or TnT, reduce recruitment burdens and allow for testing to ensure individuals previously classified as NC behaved similarly to the group into which they were added. Smaller studies on basic psychophysics or on mechanisms underlying TTS may prefer to maintain the narrower definitions of TT and TnT.

6.1.5 Gap #5

The increased responsiveness of TT compared to TnT has primarily been studied in simple aqueous solutions. Examining responses in binary mixtures will provide more nuanced insights into differences between the phenotypes, including suppression and enhancement effects.

Chapter 5 demonstrated that TT are generally more responsive to the orosensations elicited in binary mixtures of ethanol and four stimuli that are representative of common orosensations (sweet – fructose, bitter – quinine, sour – tartaric acid, astringent – aluminium sulphate). Although not all sample intensities varied with TTS, no instances of TnT being more responsive than TT were found. Together, the results in Chapter 5 provide the first evidence that the increased orosensory responsiveness of TT compared to TnT to unary solutions, also extends to binary mixtures. Importantly, few interactions were found between TTS and the concentrations of ethanol and/or the other stimuli in the binary mixtures. These findings suggest that despite differences in the magnitude of the sensations elicited, the nature of interactions (enhancement and/or suppression) was the same in both groups.

Chapter 5 also demonstrated how ethanol interacts with the four stimuli providing insights into the general perception of alcoholic beverages. Burning/tingling increased as ethanol concentration increased in all four binary mixture types and was not impacted by the concentration of other stimuli. In contrast, adding ethanol decreased the sweetness in binary mixtures with fructose but the opposite was true in binary mixtures with quinine. Furthermore, providing participants with six scales when rating the binary solutions reduced the risk of attribute dumping, allowing for a more complete understanding of the interactions between ethanol and stimuli that elicit both astringency and sourness (tartaric

acid, alum sulphate). In particular, it was demonstrated that increasing ethanol concentration reduced the sourness while simultaneously increasing the astringency of the binary mixtures of ethanol and tartaric acid. While increasing the alcohol concentration also increased the sourness of binary mixtures with alum sulphate, astringency was not impacted. As taste impacts alcohol consumption and preferences (Chapter 2), the insights into binary mixtures provide clues into the complex interactions that ultimately produce the flavour of alcoholic beverages.

6.2 Future research

As summarized above, the current thesis provided several novel insights into TTS and its impact on alcohol perception. Nevertheless, several literature gaps remain, a selection of which, are discussed below with an aim to highlighting potential directions for future research.

6.2.1 TTS Methodology and Classification

Chapter 3 and 4 included the first investigations into the impact of methodological changes on TTS since the protocols were established. Nevertheless, as these observations were all made looking at data retrospectively, empirical studies where these factors are manipulated are necessary to confirm the findings and to establish causation. Several other aspects of TTS screening could be optimized if empirical studies were designed to test the protocols.

Recently, Sollai et al. (2017) developed a silver probe that makes electrophysiological recordings of the tongue surface. The probe allows for objective and non-invasive measurements of gustatory system activation, free from the inherent bias common in self-report research. The probe is circular and has an opening so that a taste stimulus can be delivered to the surface of the tongue. Using this technology, Sollai et al. (2017) demonstrated that electrophysiological responses varied across PROP (6-n-propylthiouracil) taster status groups and related genotypes. Thus, more research is encouraged to determine whether similar differences exist between TT, TnT and NC in response to basic taste stimuli or real foods/beverages. In addition, if the probe can be adapted so that a thermode can be applied in the central opening, differences between TT, TnT and NC during thermal stimulation may also be tested. Importantly, if clear

differences in activation can be identified between TT and TnT, activation patterns may provide insights into the mechanism(s) underlying TTS. In addition, activation patterns could be used to validate the NC subgroups from Chapter 3 and support or refute the current hypothesis that they are misclassified TT and TnT.

Chapter 3 provides a comprehensive investigation into group differences between TT, TnT and NC but more research is needed to fully characterize the phenotypic groups. In particular, the requirement that TT report a thermally-elicited taste as "above weak" (6 > on gLMS), essentially treats the ratings as dichotomous despite the fact that continuous data has been collected. Further research is encouraged to determine if "above weak" is the appropriate cut-off for the minimum intensity of thermally-elicited sensations. As the scale use of TT and NC differs, further research is needed to determine if/how standardizing the intensity of thermally-elicited sensations impacts the proportion of TT and NC identified. Finally, more research is required to determine whether the intensity of thermally-elicited sensations is correlated with the intensity of aqueous solutions of chemical stimuli. It is possible that if TT subgroups were created based on the intensity of thermally-elicited sensations rather than based on the type of thermally-elicited sensation, temperature regime or location, that differences in orosensory responsiveness between the groups would be found. If true, this theory could be akin to PROP taster status, where both PROP medium-tasters and PROP super-tasters, experience bitterness when exposed to PROP but at different intensities. Differences have been reported between PROP medium-tasters and super-tasters to food related behaviour (e.g., liking and consumption of alcoholic beverages; Chapter 2). Thus, determining whether the intensity of thermally-elicited sensations is associated with differences in general orosensory responsiveness, is a logical first step in assessing whether TT subgroups not considered in this thesis exist and their potential impact on food related behaviour.

TT are more responsive to the temperature changes used during TTS screening than TnT (Green and George, 2004; Bajec and Pickering, 2008; Yang et al., 2014; Hort et al., 2016; Chapter 3). Thus, sensitivity to temperature change may be a key factor underlying the TTS mechanism. Yet, as individual differences exist in temperature perception in humans (Manrique and Zald, 2006; Green and Akirav, 2007), it is plausible that the temperature regimes employed during thermal elicitation do not represent the full range of

temperature regimes capable of producing thermally-elicited sensations. In particular, no studies warm the tongue above 40°C despite the fact that hot beverages, such as coffee, are often served at much higher temperatures (60-80°C; Brown and Diller, 2008). Furthermore, as hot beverages typically cool rapidly once they enter the oral cavity, testing for thermally elicited sensations using slower or faster rates of temperature changes than employed in current studies (1.0-1.5°C/s), may also impact the type and/or number of thermally-elicited sensations reported. If TT experience thermally-elicited tastes concurrently with chemically-induced tastes during normal eating a drinking, characterizing the magnitude and speed of temperature changes in the oral cavity (e.g., Lee et al., 2003; Choi et al., 2016) may provide insights into more ecologically valid temperature regimes for TTS screening and studies. The development of a temperature probe that is not damaged during the consumption of solid foods is encouraged as it could provide more general insights into how temperature impacts the perception of food during ecologically valid eating contexts. Furthermore, the link between thermally-elicited sensations and food temperature should be investigated, including the possibility that thermal taste(s) elicited by hot or cold foods and beverages may associate with liking and consumption.

6.2.2 TTS Mechanism(s)

Chapter 4 provides some insights into possible peripherally-mediated mechanism(s) for TTS. Although the work was largely exploratory in nature, it helps to inform future studies that could directly test for mechanistic difference between TT and TnT. The finding in Chapter 4 that sweet TT were nine times more likely to experience thermally-elicited sensation during warming, adds to the body of evidence suggesting an association between thermally-elicited sweetness and TRPM5. Both TRPM5 and thermally-elicited sweetness are activated during warming across similar temperature ranges (Talavera et al., 2007; Skinner et al., 2018). Findings are also consistent with a recent study by Nachtigal and Green (2020) who demonstrated that lactisol, an inverse agonist of TAS1R2/TAS1R3, inhibits thermally elicited sweetness in individuals known to be sweet TT. The authors suggest that heating from a cold temperature, as in the warming cycle, may cause a conformational change in the TAS1R2/TAS1R3 dimer but more research is needed to identify how this change generates thermally-elicited sweetness. Although the potential

role of TRPM5 and the TAS1R2/TAS1R3 dimer in thermally-elicited sweetness is of interest, only 25% of TT experienced thermally-elicited sweetness during warming, which is just over 8% of individuals that successfully completed TTS screening in Chapter 4. Thus, research into peripheral mechanism(s) for the other thermally-elicited sensations is also warranted. In particular, identifying a potential candidate gene/protein for thermally-elicited sourness, which is associated with the cooling cycle, is of interest. More research is also encouraged to determine if/how the candidate genes/proteins account for individual differences and importantly, how these candidates elicit thermally-elicited sensations in TT but not in TnT.

Veldhuizen et al. (2020) recently identified an amygdala-thalamic circuit, which may explain centrally-mediated difference in orosensory responsiveness. Individuals that were more responsive to three suprathreshold tastes (sweet, sour, salty) also had increased activation of the left amygdala and decreased activation of the bilateral cuneus regions of the brain. Although Hort et al. (2016) did not report any differences between TT and TnT in the activation of the amygdala to a sweet stimulus, more research is encouraged to determine whether brain regions associated with the amygdala-thalamic circuit vary between TT, NC subgroups and TnT. If centrally-mediated effects are involved, then TTS phenotypic differences may extend beyond differences in orosensory responsiveness, something that was demonstrated with differences in temperature perception in Chapter 3. As a result, further comparing the groups may provide a unique opportunity to better understands how differences in central gain manifest into phenotypic differences across a wide range of fields (e.g., emotional responses, personality differences, diet-related disease risk).

Despite being the most frequently reported sensation, the existence of a metallic thermally-elicited taste is sometimes attributed to the use of a metallic or metallic-looking probe (Nachtigal and Green, 2020; Chapter 4). Thus, a study using a non-metallic probe for TTS screening could be used test this hypothesis. In addition, it remains unclear whether chemical metallic stimuli elicit a distinct prototypical metallic taste in addition to retro-nasal aromas (Lawless et al., 2005; Epke et al., 2009; Skinner et al., 2017; Wang et al., 2019). As the aromas associated with metallic stimuli are at least partially attributed to the lipid oxidation within the oral cavity (Ömür-Özbek et al., 2012), screening participants

with and without nose clips would determine whether retro-nasal aroma also plays a role in thermally-elicited metallic. Furthermore, if the mechanim(s) underlying thermally-induced metallic sensations can be identified, it may provide further insights into other metallic sensations. A persistent metallic taste is a common side effect of chemotherapy and has been described as a form of phantageusia (reviewed in IJpma et al., 2015; Reith and Spence, 2020), a "taste in the mouth for which no external stimulus can be found" (Merriam-Webster, n.d.). One hypothesis for the metallic tastes in chemotherapy patients is that it may be caused by localized taste damage (Ijpma et al., 2015). Although highly speculative, it is possible that the sustained change in temperature during warming and cooling, as evidenced by the mild discomfort reported by some participants during TTS screening, may cause mild but reversible taste damage/distortion, akin to what has been theorized for chemotherapy-induced metallic. As such, it is worth assessing whether thermally- and chemotherapy-elicited metallic tastes share a common mechanism(s) so that potential connections between the phenomenon can be exploited to further our understanding of both.

6.2.3 TTS and Food/Alcohol

As confirmed in Chapter 3, the greater taste acuity of TT compared to TnT is a robust phenomenon. This finding is similar to the increased taste acuity observed in PROP super-tasters compared to PROP non-tasters (Bartoshuk et al., 1994, 1999; Bajec and Pickering, 2008; Yang et al., 2014). Wine experts are more likely to be PROP super-tasters than PROP non-tasters (Hayes and Pickering, 2012; Pickering et al., 2013), which may represent an active gene/environment interaction. The authors hypothesize those with greater acuity (PROP super-tasters) are more likely to select professions where greater acuity will be a net benefit (e.g., sommelier, winemaker, wine vendor). As a result, it is possible that experts and the average consumer are not tasting wine similarly, leading to a disconnect between expert recommendations and the consumer experience. Similar research has yet to be conducted in the thermal taste context, but it is possible that due to their increased responsiveness to aqueous solutions (Chapters 3 & 5) and alcoholic beverages (Pickering et al., 2010b, 2010a; Small-Kelly and Pickering, 2020), that TT are more likely than TnT to also be wine experts. However, as only the magnitude and not the relative intensity of the sensations elicited in the binary mixtures differed between TT and

TnT, it is likely that the disconnect between consumer and expert perception of alcoholic beverages may be less consequential. More research is encouraged to determine if/how active gene/environment interactions impact the behavior of TTS subgroups and other taste-related phenotypes.

Two studies on TTS have investigated the perception of solid food products, each only identifying limited differences between TT and TnT (Pickering and Klodnicki, 2016; Pickering et al., 2016). These results contrast with the findings from Chapters 3 and 5 where aqueous solutions where rated higher by TT than TnT, a pattern mirrored in studies using real beverages (Pickering et al., 2010b, 2010a; Small-Kelly and Pickering, 2020). As solid foods form a bolus during chewing whereas liquids do not, solid foods are likely to stimulate a lower proportion of oral receptors at any given time compared to aqueous solutions or beverages. The authors speculate the surface area in the oral cavity stimulated by solid foods compared to aqueous solutions/beverages may not be sufficient to evoke the increased responsiveness of TT. Future research should consider whether changing the proportion of the oral cavity exposed to food or the length of exposure, impacts the orosensory responsiveness of TT and TnT. In addition, aqueous solutions or beverages may also elicit faster and shorter temperature changes within the oral cavity. Thus, as noted above, characterizing intra-oral temperature during food and beverage consumption is encouraged.

The authors also suggest that the relatively small sample sizes employed when testing solid foods (~25 TT, ~25 TnT) may not have been large enough to detect differences attributable to TTS phenotypes (Pickering and Klodnicki, 2016; Pickering et al., 2016). This hypothesis is supported by eta-squared values from Chapter 3 and 5, showing that the effect of TTS on orosensory responsiveness for the dominant orosensations elicited by a stimulus are generally characterized as low (Lakens, 2013). As the power of a study can be increased by increasing the sample size (Sullivan and Feinn, 2012), research using larger sample sizes are necessary to confirm whether the increased orosensory responsiveness of TT compared to TnT, also extends to food. Alternatively, when recruiting larger sample sizes is time/cost prohibitive, the power of a study can also be increased by limiting measurement error (Sullivan and Feinn, 2012). Thus, using narrow inclusion criteria to reduce other sources of individual differences in taste perception (e.g., age, gender, obesity,

smoking; Tepper et al., 2017), can allow for significant differences to be found as demonstrated in Chapter 5.

Variation in ethanol responsiveness between TT and TnT reported in Chapter 5 and in the literature (Small-Kelly and Pickering, 2020) suggest that that differences in alcoholic beverage consumption may be partially attributable to TTS. As the dominant sensations elicited by ethanol are nominally aversive, it is possible that the increased responsiveness of TT compared to TnT may also lead to lower alcohol consumption. However, to date only limited differences between TT and TnT in monthly alcohol consumption have been reported (Thibodeau, 2015). Thibodeau et al. (2017) found that alcohol consumption was not always linearly associated with orosensory responsiveness. Individuals with intermediate responsiveness to bitterness and astringency, tended to drink more alcohol than low or high responders (Thibodeau et al., 2017). The authors attribute this observation to the fact that the flavour of alcoholic beverages is likely be optimized by producers for the 'average' consumer. Importantly, alcoholic beverages are one of a growing number of products for which a wide variety of styles and flavours are available. Thus, research into the impact of TTS or other taste-related phenotypes is needed to determine if, rather than reducing their consumption of alcoholic beverages, consumers instead shift their consumption towards alcoholic beverages that are optimized for their palate. All other factors being equal (e.g., price, availability, social context), each consumer likely selects alcoholic beverages that best balance the taste sensations, chemesthetic sensations and aromas they find appetitive with the ones they aversive find aversive. By considering the volume and the proportion of alcoholic beverages consumed across categories (e.g., beer vs wine), types (e.g., red wine vs white wine) or styles (e.g., dry white wine vs sweet white wine), a more nuanced picture of alcohol consumption can be obtained. Furthermore, empirical research where consumers create their optimal alcoholic beverage (e.g., mix your own cocktail), may also provide insights into how taste impacts the consumption of alcoholic beverages at the individual level. Importantly, empirical research would allow for more control over the many intrinsic and extrinsic factors that also impact alcohol consumption (Betancur et al., 2020).

6.2.4 Conclusion

The work in this thesis addressed several important gaps in the thermal taste literature and included several novel findings. It demonstrated that NC are likely misclassified TT and TnT, that TT can be treated as a homogeneous group in studies unrelated to mechanism and provide insights into the optimization of screening protocols. Together, these findings provide the key information that will allow for the development of faster TTS screening protocols, which in turn will facilitate larger population based studies. Furthermore, the work in this thesis extended our understanding of the interactions of orosensory stimuli in alcoholic beverages and provided further evidence that TT and TnT differ in alcoholic beverage perception. Significant gaps in the literature on TTS remain and this thesis informs important areas for further investigation. Concurrent research into several aspects of TTS is encouraged to advance the field as a whole and to better characterize the bigger picture contribution of this phenotype to food/beverage consumption, liking and diet-related health risks.

6.3 References

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Chapter 7: Appendices

7.1 Appendix I: Thermal Taster Subgroups and Orosensory Responsiveness Dataset

7.1.1 Specifications Table

Subject	Sensory Systems
Specific subject area	Individual differences in taste, chemesthetic and thermal perception in the oral cavity.
Type of data	Figure
How data were acquired	Thermally-elicited responses were obtained using a thermal elicitation device (TED). The TED is a computer-controlled 64 mm^2 Peltier device with thermocouple feedback attached to a water-circulated heat sink (Brock University, Machine Shop). See Thibodeau et al. (2020) for full details.
Data format	Thermal taste classification – Raw: Data is provided as a binary (yes/no), indicating if each participant belongs to specific subgroups. Orosensory Stimuli Intensity – Z-scores: Data converted to z-scores by cohort and combined is available in the attached data set. Figures are also provided showing mean scores by TT subgroup.
Parameters for data collection	A large dataset of TTS screening data was obtained by combining the results of 12 recruitment drives ('cohorts'). All cohorts were composed of convenience samples recruited from the Brock University student population and surrounding community. As the goal of this data set is to investigate differences within thermal tasters (TT), only data for participants that could be classified as TT was retained for analysis.
Description of data collection	Thermal taste status data was acquired by applying the TED to the edge of participants' tongues and asking them to rate any sensations elicited on generalized Labelled Magnitude Scales. In a separate task, participants also provided intensity ratings to aqueous solutions of orosensory stimuli (sweet, sour, salty, bitter and umami, astringency, metallic) by rinsing with the sample using a sip-and-spit protocol.
Data source location	Institution: Brock University, City/Town/Region: St. Catharines, Ontario Country: Canada
Data accessibility	With the article

Related research article	M. Thibodeau, M.Bajec, A. Saliba & G. Pickering, Homogeneity of thermal tasters and implications for mechanisms and classification, Physiology & Behavior. (2020) 227: 113160. (See Chapter 4 for full text)

7.1.2 Abstract

Thermal taste is a phenomenon whereby some individuals, known as thermal tasters (TT) experience taste sensations when their tongue is warmed or cooled. It was first reported in 2000 by Cruz and Green (2000) and since then, most research has focused on comparing TT to thermal non-tasters (TnT; individuals who do not experience thermally-elicited sensations). As TT rate the intensity of taste stimuli higher than TnT, understanding the nature of this difference may help inform how individual differences in taste perception impact consumer liking and consumption of food and beverages. However, as the mechanism(s) underlying thermal tasting are yet to be fully elucidated, it is unclear if TT should be considered a homogeneous group or if subgroups exist. A dataset was created to help determine if the orosensory advantage is universal across all TT, or if it is mainly attributable to one or more subgroups of TT. To this end, the thermal taste screening data of 297 TT from 12 previous recruitment drives ('cohorts') was combined. This created the largest dataset of TT reported to date in a single study, allowing for an in-depth analysis of the differences between TT. After training on appropriate scale use, participants were familiarized with common taste and chemesthetic stimuli (sweet, sour, salty, bitter, umami, astringent and metallic). Using a sip-and-spit protocol, participants rinsed with the stimuli and rated the maximum intensity each stimulus elicited on a generalized Visual Analogue Scale (gVAS) or a generalized Labeled Magnitude Scale (gLMS). To account for minor methodological differences between the cohorts, ratings from each cohort were first converted to z-scores before being combined into the overall dataset. Next, participants underwent a series of 12 trials that assessed response to a thermal elicitation device during which each combination of two temperature regimes (warming and cooling) and three lingual sites (tongue tip, 1 cm to left, 1 cm to the right) were examined in duplicate. Participants were asked to rate the maximum intensity of any sensations experienced during each trial. TT were classified into subgroups based on the type of thermally-elicited taste

reported (typically sweet, sour, salty, bitter, metallic), the temperature regime during which the sensation was elicited (warming or cooling) and the location on the tongue tested at which the sensation was experienced. Figures are provided that show the mean intensity ratings of aqueous solutions of chemical stimuli and corresponding standard errors for each of the TT subgroups. In addition, the TT Subgroup Naming Conventions provided should allow for a consistent and clear use of terminology across future thermal taste research. Readers are referred to *Homogeneity of thermal tasters and implications for mechanisms and classification* (Thibodeau et al., 2020, Chapter 4) for a full discussion of how these findings inform our understanding of the mechanism(s) underlying thermal taste and the practical implications of methodological differences in determining thermal taste status.

7.1.3 Value of the Data

- Thermal tasters (TT) are individuals that experience taste sensations when their tongue is heated or cooled while thermal non-tasters (TnT) do not (Bajec and Pickering, 2008; Yang at al., 2014; Green and George, 2004; Thibodeau et al., 2019). Patterns in the types of TT responses based on the temperature regime, location or the type of orosensation provide insights into potential TT subgroups. Identifying these subgroups provide insights into the mechanisms underlying thermal taste. The attached dataset includes extensive TT subtype classification details for a large sample of TT (n=299).
- These data will benefit researchers interested in understanding variation in human orosensation. TT rate the intensity of taste stimuli higher than TnT (Bajec and Pickering, 2008; Yang at al., 2014; Green and George, 2004; Thibodeau et al., 2019). However, it is unknown if the orosensory advantage of TT is universal or if it is driven by a subset of TT. For example, it has been hypothesized that sweet TT rate sweet stimuli (e.g. sucrose) higher than other TT (Pickering and Klodnicki, 2016; Pickering and Kvas, 2016). Figures are provided comparing the orosensory responsiveness of several TT subgroups identified by Thibodeau et al., (2020), to investigate this hypothesis.
- Researchers interested in the thermal taste status phenomenon will have access to a dataset consisting of a large sample of TTs and their responses both to thermal taste

elicitation and to aqueous orosensory stimuli. Together, these results can inform future TTS screening protocols, recruitment targets and classification methods.

- TRPM5, a heat-activated cation channel expressed in taste receptor cells, may be involved in the thermally-elicited sweetness experienced by some thermal tasters (Talavera et al., 2005; Talavera et al., 2007). However, only 30% of participants report thermally elicited sweetness suggesting that additional mechanism(s) may underlie the phenomenon. By providing the raw data, researchers may gain insights into additional genes and/or pathways worthy of study.
- TT rate the intensity of alcoholic beverages and the emotions they elicit higher than TnT (Pickering et al., 2010a; Pickering et al., 2010b; Yang et al., 2018). Improving our understanding of how TT should be categorised in future studies (e.g. as a homogeneous group or as discrete subgroups) allows for further insights into the basis of individual differences in food/beverage preferences, intake and diet-related health outcomes.

7.1.4 Data Description

The primary aim of this manuscript is to visually display the relationship between orosensory responsiveness and TT subgroups. Each figure shows the mean orosensory responsiveness and corresponding standard error to aqueous solutions of sweet, salty, sour, bitter, umami, metallic & astringent stimuli of a TT subgroup compared to TT who are not a part of the subgroup. Readers are referred to the "TT Subgroup Naming Conventions" for full details of the inclusion criteria for each subgroup.

The raw data associated with each of the figures can be found in the accompanying file "Thermal Taster Subgroups and Orosensory Responsiveness Dataset.xlxs". The dataset includes information on each participants cohort (Column A), a unique identifier for each participant (ID, Column B) and z-scores of mean orosensory responsiveness for six common orosensations (Columns C-I). The remaining columns provided the TT subgroup status of each participant using a binary (Yes/No) system of coding. "Yes" is used to indicate that a participant is part of the TT subgroup listed in the heading, while "No" indicates that they are not. For example, the participant with ID code 14 is a both a Sweet TT and a Sour TT but is not a Salty TT, a Bitter TT, an Umami TT or a Metallic TT.

Readers should consult the "TT Subgroup Naming Conventions" for full details of the inclusion criteria of each TT subgroup.

The data is divided across five tabs as follows:

(1) One-factor (n=254): Patterns in the types of TT responses to thermal elicitation may provide insights into potential TT subgroups. This tab includes the data for TT subgroups based on the orosensation experienced when the tongue was heated or cooled (see Figure 7.1, Figure 7.2A, Figure 7.2B; Columns O-V),the location at which orosensations were experienced (see Figure 7.3; Columns J-L) and based on the temperature regime during which temperature regimes were experienced (see Figures 7.4-7 5; Columns M-N).

(2) Two-factor (n=254): Each TT belongs to a minimum of three single-factor TT subgroup, suggesting that subgroups based on two factors (e.g. the location and temperature regime) may also be of interest. To this end, Thibodeau et al. (2020) tested 54 combinations ('pairs') of two TT subgroups to determine if a participant that belongs to TT subgroup A was significantly more or less likely to also belong TT subgroup B. Eleven pairs of TT subgroups were positively associated (e.g. Sweet TT were 9 times more likely to also be warm TT) and four pairs were negatively associated (e.g. Sweet TT were 2.2 times less likely to also be Sour TT). For pairs that were positively associated, participants that were members of both subgroups simultaneously (e.g. sweet&warm TT: the participant experienced thermally-elicited sweetness during warming) were compared to the remaining TT (Figures 7.6-7.8; Columns J-T). For pairs that were negatively associated, participants that were not members of either subgroup in the pairs (e.g., not sweet TT and/or sour TT) were compared to TT who were members of one or both of the subgroups for the pair (see Figure 7.9; Columns U-X).

(3) Spicy (n=265): Includes an expanded dataset with 25 participants who can be classified as Spicy TT if it is included in the list of valid thermally-elicited sensations (Figure 7.2C; Column J). Eleven participants IDs (255-265) would not have met the criteria for classification as TT if Spicy was not considered valid (Column K).

(4) Unmatched (n=286): Includes an expanded dataset with 32 participants (IDs 266-297) who can be classified as Unmatched TT if participants are not required to report the same thermally-elicited sensations in corresponding trials (see Figure 7.2D; Column J).

(5) Sample Sizes: As this study is retrospective in nature, not all cohorts were exposed to each of the orosensory stimuli. For convenience, this tab summarizes the sample sizes for each of the TT subgroups.

Please note: As z-scores are calculated using sample means and standard deviations, small differences in the z-scores of some participants exist (1-254) as the number of participants included in the calculations varied. Each participant is assigned the same unique identifier if they are included in multiple tabs of the spreadsheet, for comparison purposes.

7.1.5 Experimental Design, Materials and Methods

The primary aim of this manuscript was to create a large data set of TT responses. To this end, data from the TTS screening procedures of 12 recruitments drives ('cohorts') was combined. 975 participants were recruited from Brock University and the surrounding community, of which 905 completed the study in full. Failure to appropriately use the scales during training led to the exclusion of an additional 124 participants. The final data set includes only the responses for the 297 participants who could be classified as thermal tasters. A description of the experimental design, materials and method is provided next and readers are referred to Thibodeau et al. (2020) for a comprehensive description.

7.1.5.1 Data Collection

The thermal taste status of all participants was determined based on the protocol of Bajec and Pickering (2008), with minor difference in the methods used across the cohorts. These differences reflect changes in best practices, as informed by the developing sensory and thermal tasting literature and differences in study aims across cohorts. The following section briefly describes the methods used to screen for TTS.

After providing informed consent and basic demographic information, participants were training on the appropriate use of two intensity scales, the generalized Visual Analogue Scale (gVAS) and the generalized Labeled Magnitude Scale (gLMS). After, a verbal description of the scale from the researcher, participants were asked to rate the

maximum intensity of a series of remembered sensations on each of the scales (Bajec and Pickering 2008). Two procedures were implemented to screen for appropriate scale use by the participants. For Cohort 12, the most recent cohort, participants were required to rate the "the brightness of the sun when staring directly at it" more intensely than "the brightness of a dimly lit room". Cohorts 1-5, 7-11 were required to rate "the pain of biting your tongue" more intensely than the "touch sensation of a pill on your tongue". As participants from Cohort 6, the first cohort, did not rate the "touch sensation of a pill on your tongue" or "the brightness of a dimly lit room", no screening for scale use of these participants was performed.

Using a sip-and-spit protocol, participants rinsed with aqueous solutions eliciting common orosensations primarily to aid with the later identification of thermally-elicited sensations (see Table 2 from Thibodeau et al. (2020) for full details). All cohorts were presented with exemplars of sweet, sour and bitter. Additional oral sensations included in training were salty (Cohorts 4-12), umami (Cohorts 1-5 & 7-12), metallic (Cohorts 5-7, 10-12) and astringent (Cohorts 1-3, 6 & 11). Readers are referred to the data file for a full summary of the sample sizes. All solutions were presented in a randomized order and at room temperature. For each stimulus, participants were presented with 20 ml of each solution in medicine cups or clear wine glasses and asked to swish each solution on their palate for five seconds before expectorating. Participants waited a further 10 seconds before rating the maximum intensity of the elicited sensation on a gLMS (Cohorts 6 & 12) or gVAS (Cohorts 1-5,7-11; Bajec and Pickering, 2008). Each solution was tasted in the presented sequence and participants rinsed with filtered water (Brita, ON, Canada) prior to and after each solution. In order to minimize possible carry-over effects of the metallic and astringent stimuli, unsalted soda crackers (Cohorts 5, 7, 10-12) or a 5g/L pectin solution (Cohorts 1-3, 6) were provided as palate cleansers. Direct comparison of orosensory responsiveness scores was not possible due to differences in scale, tastants, stimulus concentrations, and/or the number of exposures across cohorts. For all tastants, mean responsiveness scores were calculated for each participant from all replicates. Next, the mean scores from each cohort were converted to z-scores separately. Lastly, the z-scores for each cohort were combined for final analysis.

Thermal stimulation was performed using a 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode). Two different cycles were used: a warming cycle and a cooling cycle. Warming cycles started at 35°C, then cooled to 15°C before final re-warming to 40°C and holding for 1 second. Participants were only asked to rate the maximum intensity of sensations during the warming phase of the cycle. Cooling cycles started at 35°C, with subsequent cooling to 5°C and holding for 10 seconds. Participants were asked to report any sensations regardless of when they occurred during the cooling cycle.

Three locations on the edge of the tongue were tested for each participant: the very tip of tongue along the midline, 1 cm to the left from the midline and 1 cm to the right from the midline. A total of 12 runs were performed for each participant in two blocks. Each block consisted of three warming cycles (one per location) followed by three cooling cycles (one per location). A minimum 3-minute break was taken between blocks. All participants rated any sensations (heat, cold, sweet, salty, sour, bitter, and other) elicited using a paper ballot with individual gLMS scales for each. For the most recent cohorts (Cohort 5, 7, 10-12), the paper ballot was modified by adding gLMS scales for umami and metallic.

TTS classification was determined using the methods of Bajec and Pickering (2008). TT were defined as participants who reported the same, valid thermally-elicited taste sensation above weak on the gLMS (> 6 mm) during both replicates of the same location during the same temperature regime (n=254). Valid thermally-elicited tastes were sweet, salty, sour, bitter, umami and metallic. All other participants (thermal non-tasters and non-classifiable), were excluded from the dataset.

7.1.5.2 TT Subgroup Naming Conventions

TT were divided into subgroups based on the orosensation(s) reported, the temperature regime(s) and the location of the thermally-elicited orosensation(s). The following conventions were followed in classifying and naming the groups:

(1) Participants who experienced sweetness above 'weak' on the gLMS during both replicates for at least one temperature regime and location combination are referred to as sweet TT. Similarly, participants that report a different orosensation are defined as salty TT, sour TT, bitter TT, umami TT or metallic TT based on the orosensation reported.

(2) Participants who reported the same orosensations (sweet, salty, sour, bitter, umami or metallic) above 'weak' during both warming replicates for at least one location are referred to as warm TT. Similarly, participants who experience thermally-induced orosensations during cooling are cool TT.

(3) Participants who experienced the same orosensations (sweet, salty, sour, bitter, umami or metallic) above 'weak' during both warming and/or cooling replicates at the tongue tip are tip TT. Similarly, participants who experience thermally-induced orosensations on the left or right side of the tongue are defined as left TT or right TT.

(4) At minimum, each participant belongs to three subgroups; one from each of (1) – (3) above. However, TT may belong to more subgroups if they experience multiple thermally-elicited orosensations above 'weak' or if the sensation(s) is experience at more than one location or during both temperature regimes. Membership of more than one group is designated by an "&" or "/". The use of each symbol is demonstrated below using the example of a participant who is both a sweet TT and warm TT.

    a. The "&" symbol indicates that the participant is a member of both groups simultaneously. Thus, a sweet&warm TT reports thermally-induced sweetness during both warming replicates at a minimum of one location.

    b. A sweet/warm TT could be either a sweet&warm TT or a TT that does not report thermally-induced sweetness during both warming replicates at a minimum of one location. E.g. they could report saltiness during warming and sweetness during cooling.

    c. Please note: It is not possible to be a warm&cold TT as the warming and cooling regimes were tested during separate trials. Similarly, it is not possible to be a left&right TT, left&tip TT or right&tip TT. However, as multiple orosensations can be elicited in a single trial, it is possible to use the "&" symbol for two thermally-elicited orosensations. For example, a TT that experiences both thermally-elicited sweetness and sourness at the same location and during the same temperature regime is a sweet&sour TT.

(5) TT subgroups defined in (1)-(3) are referred to as single-factor subgroups because only one criterion was used to classify participants. TT subgroups from (4) are referred to as two-factor subgroups.

7.1.5.3 Figure Generation

The mean orosensory responsiveness z-score (sweet, salty, sour, bitter, umami, metallic and astringent) was plotted for each TT subgroup comparing it to all other TT who were not part of that subgroup (e.g. sweet TT vs not sweet TT). Subgroups examined include single-factor subgroups based on the naming conventions (1; see Figure 7.1), (2; see Figure 7.4) and (3; see Figure 7.3).

Additional TT subgroups were all developed and plotted based on the following criteria:

- Prototypical tastes are broadly divided into two classes based on mechanism. G-protein-coupled receptors (GCPR) are responsible for the perception of sweetness, bitterness and umami while ion channels are responsible for the perception of saltiness and sourness (Bachmanov and Beauchamp, 2007). To assess the importance of mechanism, sweet TT, bitter TT and umami TT are collapsed into a single group called GCPR TT (Figure 7.2A). Similarly, salty TT and sour TT were defined as Ion TT (Figure 7.2B).
- Three mutually exclusive subgroups based on temperature regime are also recognized. Individuals that experience thermally-elicited tastes during only warming, only cooling or both warming and cooling, are referred to as onlywarm TT, onlycool TT and warmandcool TT, respectively (Figure 7.5).
- Two-factor TT subgroups, as outlined in naming convention (4), allow for more precision when defining TT subgroups. This may be important in understanding the association between TTS screening responses and the orosensory advantage of TT. For example, 77 participants are sweet TT but only 64 experience thermally-elicited sweetness during warming. Therefore, by excluding the 13 TT who experience thermally-elicited sweetness during cooling, noise in the dataset may be reduced. As 54 TT subgroups can be established, based on naming convention (4a) alone, a more targeted approach was necessary when selecting two-factor TT subgroups to examine. Two-factor TT subgroups were selected based on the findings of Thibodeau et al. (2020) who used Fisher's exact tests to test for association between pairs of single-factor TT subgroups. Pairs that occurred together significantly more often than by chance (n=11) were examined by

classifying individuals as factor1&factor2 TT or not factor1&factor2 TT (Figures 7.6-7.8). Pairs that occurred together significantly less often than by chance (n=4) were used as the basis for TT subgroups by comparing participants that were not factor1/factor2 TT, to those that were (Figure 7.9).

While the naming conventions provide a clear definition of a TT, other studies have further expanded their definition of TT. This is possible as the mechanism(s) underlying thermal taste are not well elucidated. The orosensory responsiveness of two additional TT subgroups was investigated by re-classifying participants as follows:

- Consistent with some studies (Yang et al., 2014; Yang et al., 2018; Hort et al., 2016; Skinner et al., 2018), the list of valid thermally-elicited orosensations was expanded to include spicy. Similarly to naming convention (1), participants who reported spicy above 'weak' on the gLMS during both replicates for at least one temperature regime and location combination were classified as spicy TT. This was possible as participants were able to rate the intensity of spicy orosensations using the "other" scale provided.
- TT are required to report the same taste sensation across replicate trials in most but not all (Yang et al., 2014; Yang et al., 2018; Hort et al., 2016) studies. The TT dataset was expanded to included Unmatched TT; participants who reported two different thermally-elicited sensations above 'weak' on the gLMS during replicate trials and who had previously been classified as thermal non-tasters or non-classifiable.

7.1.6 Figures

7.1.6.1 Single-Factor TT Subgroups

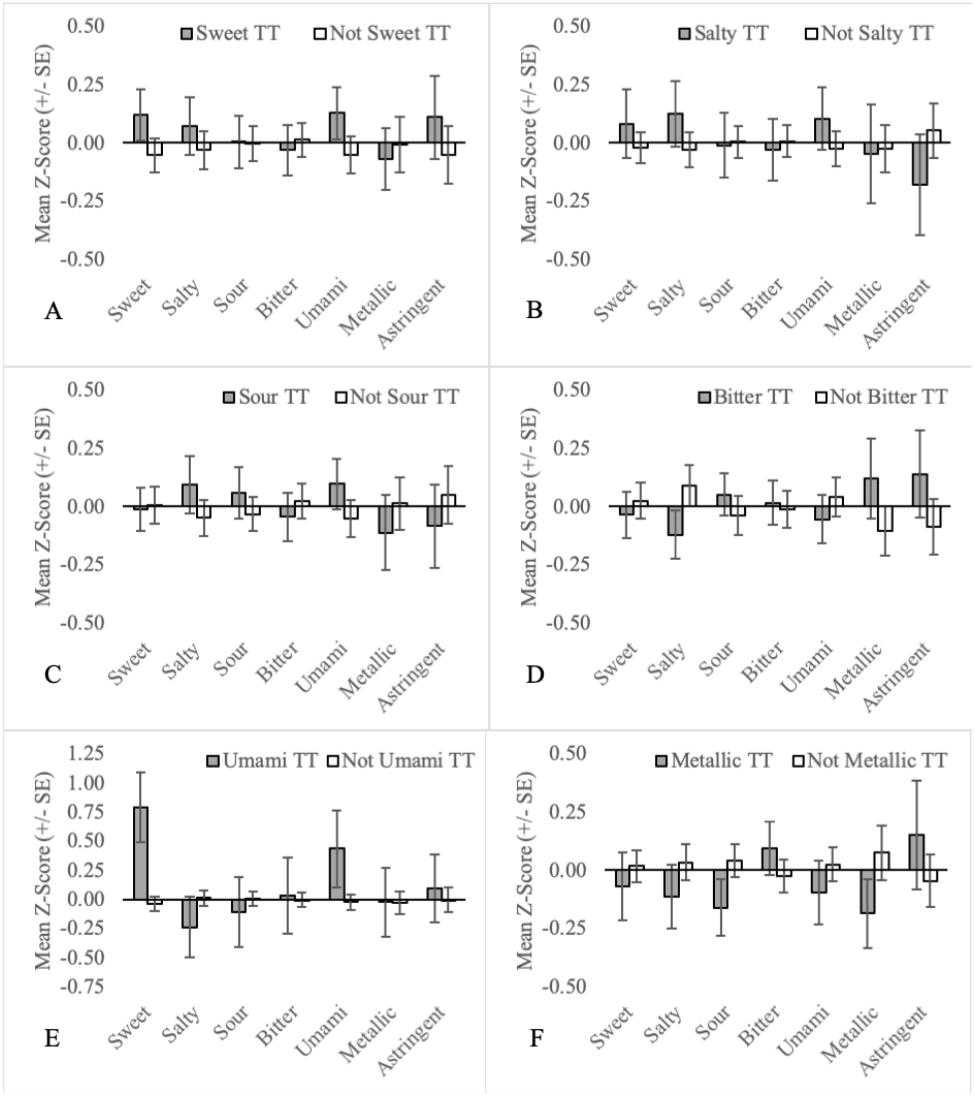

Figure 7.1: Mean orosensory responsiveness (+/- SE) of sweet TT (A), salty TT (B), sour TT (C), bitter TT (D), umami TT (E) and metallic TT (F) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

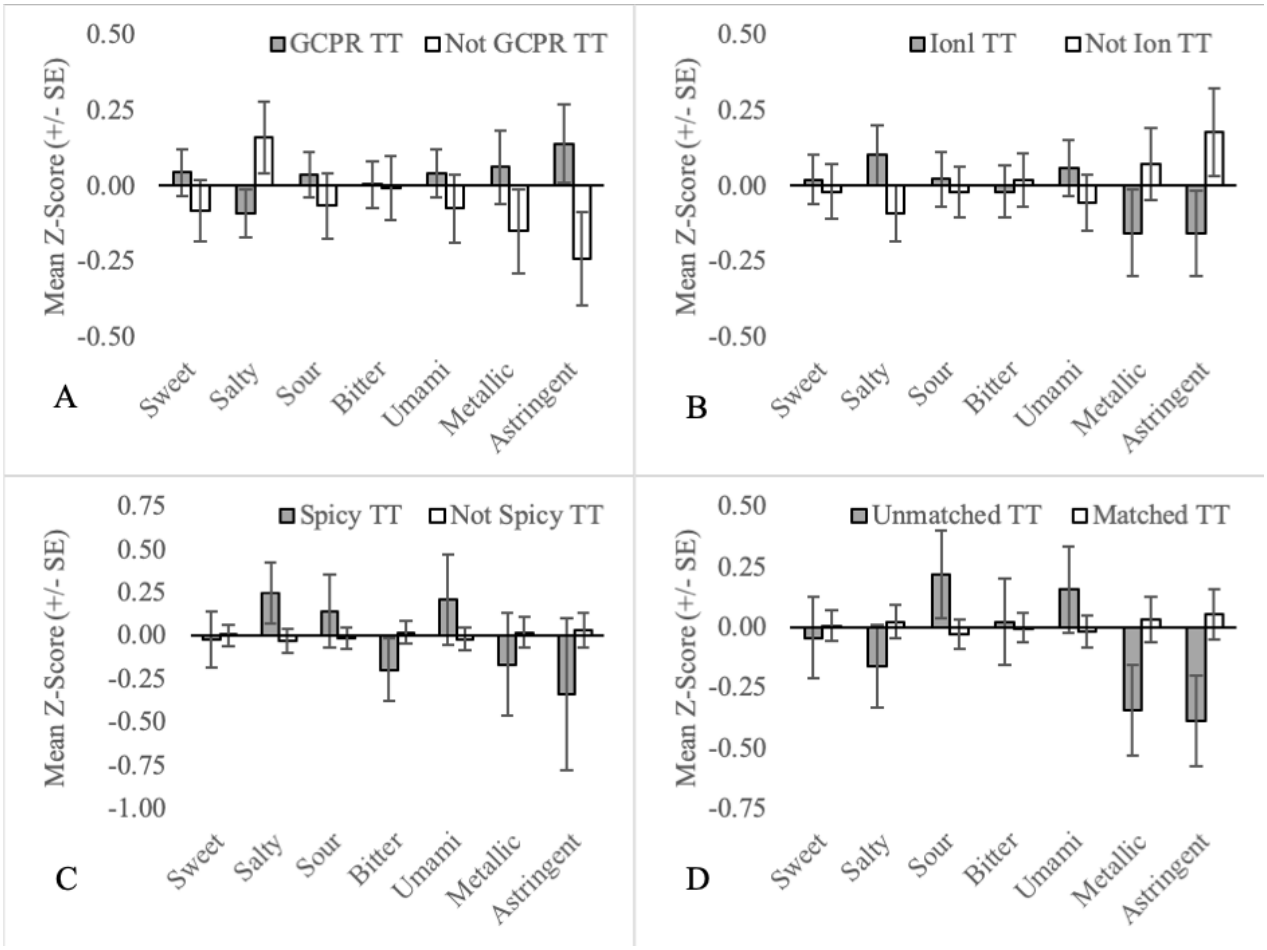

Figure 7.2: Mean orosensory responsiveness (+/- SE) of GCPR TT (A), Ion TT (B), Spicy TT (C) and Unmatched TT (D) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

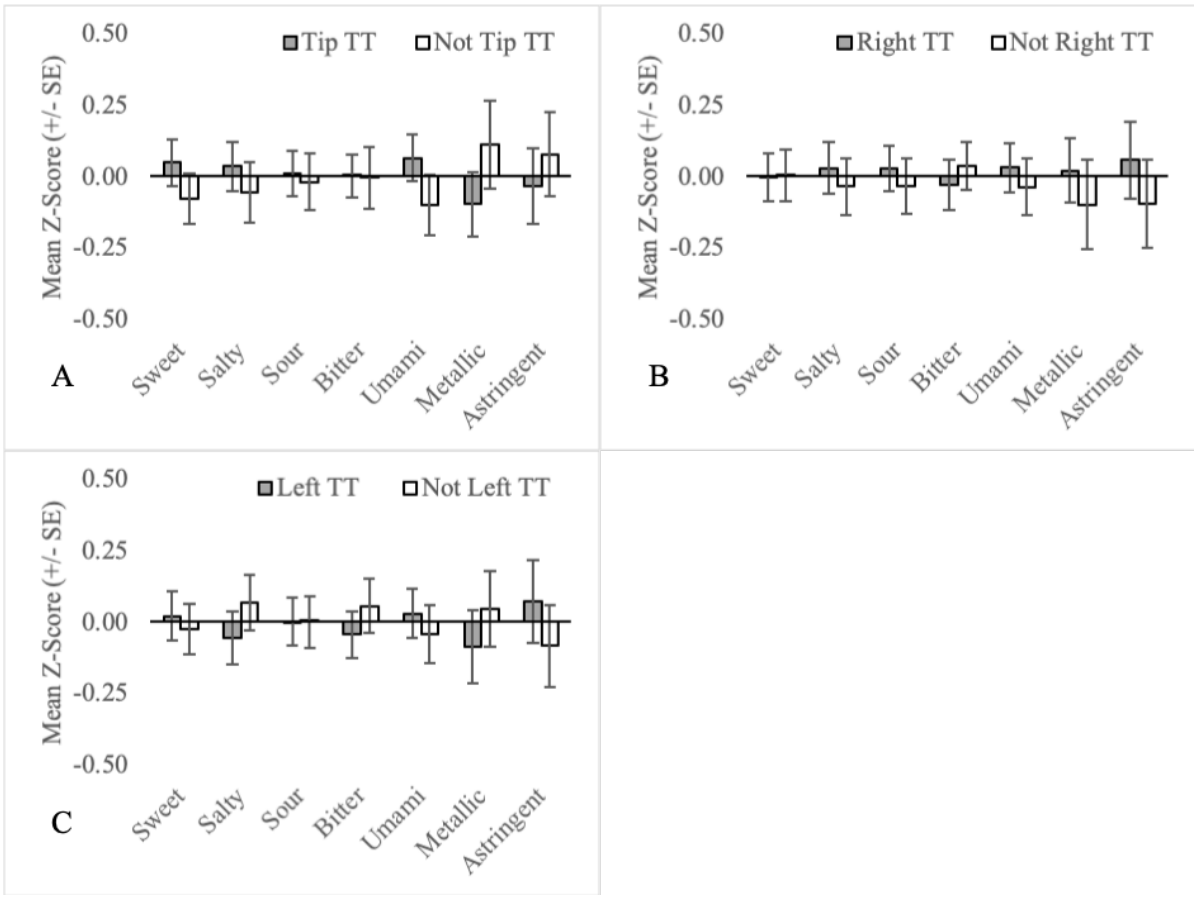

Figure 7.3: Mean orosensory responsiveness (+/- SE) of tip TT (A), right TT (B) and left TT (C) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

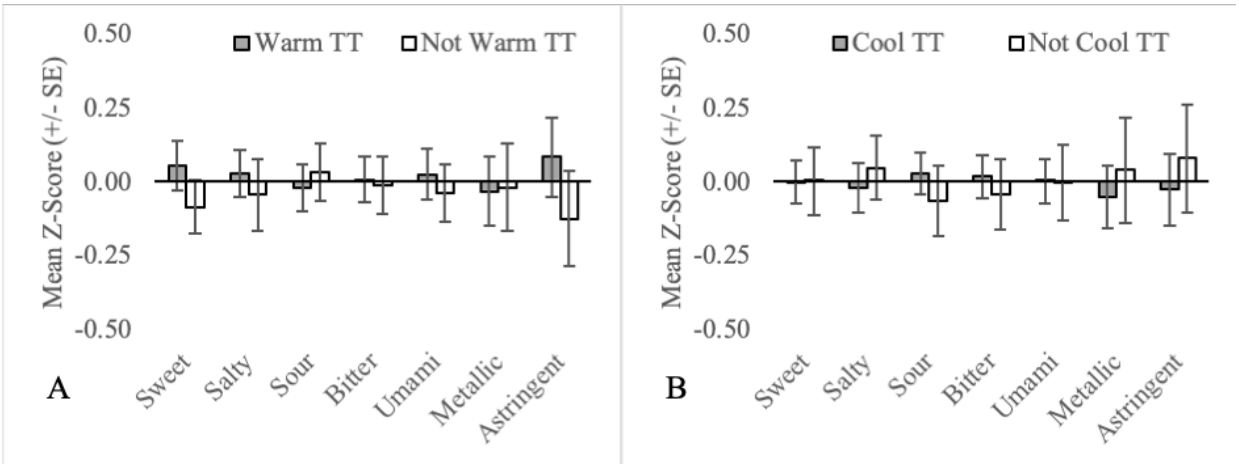

Figure 7.4: Mean orosensory responsiveness (+/- SE) of warm TT (A) and cool TT (B) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

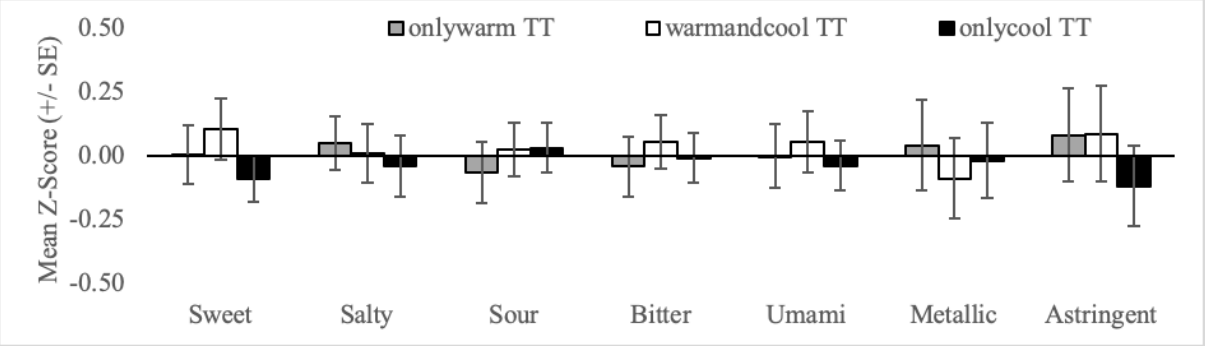

Figure 7.5: Mean orosensory responsiveness (+/- SE) of onlywarm TT, warmandcool TT and onlycool TT to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

7.1.6.2 Two-factor TT Subgroups

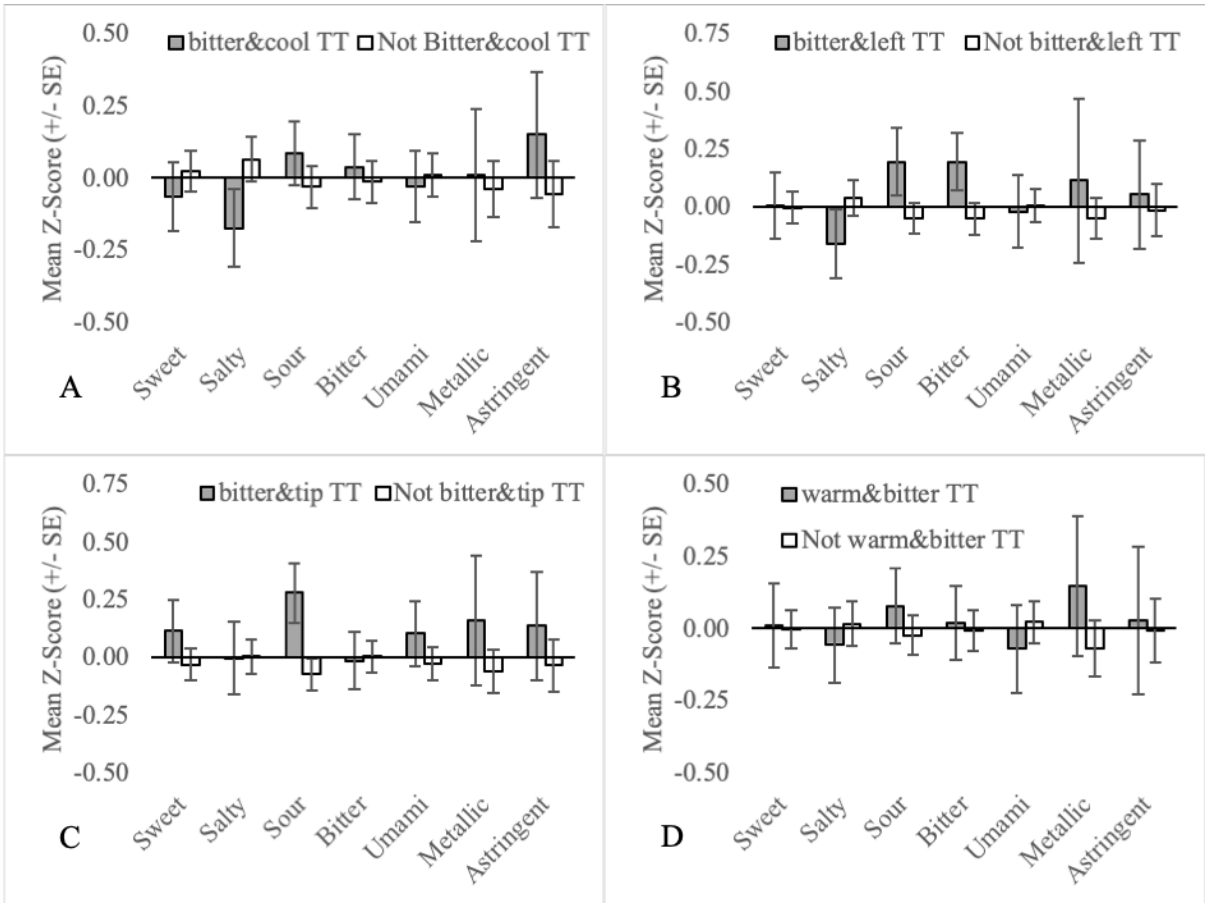

Figure 7.6: Mean orosensory responsiveness (+/- SE) of bitter&cool TT (A), bitter&left TT (B), bitter&tip TT (C) and warm&bitter TT (D) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

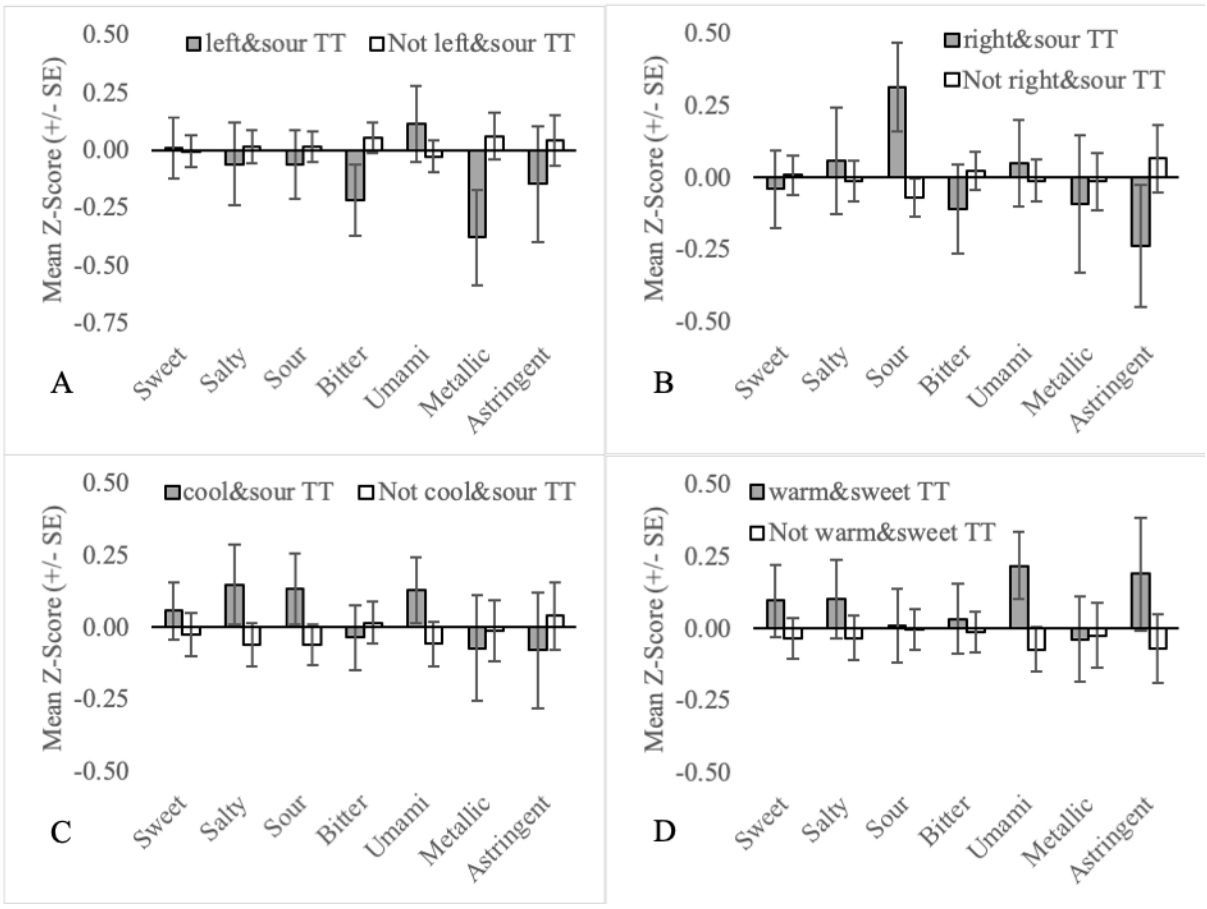

Figure 7.7: Mean orosensory responsiveness (+/- SE) of left&sour TT (A), right&sour TT (B), cool&sour TT (C) and warm&sweet TT (D) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

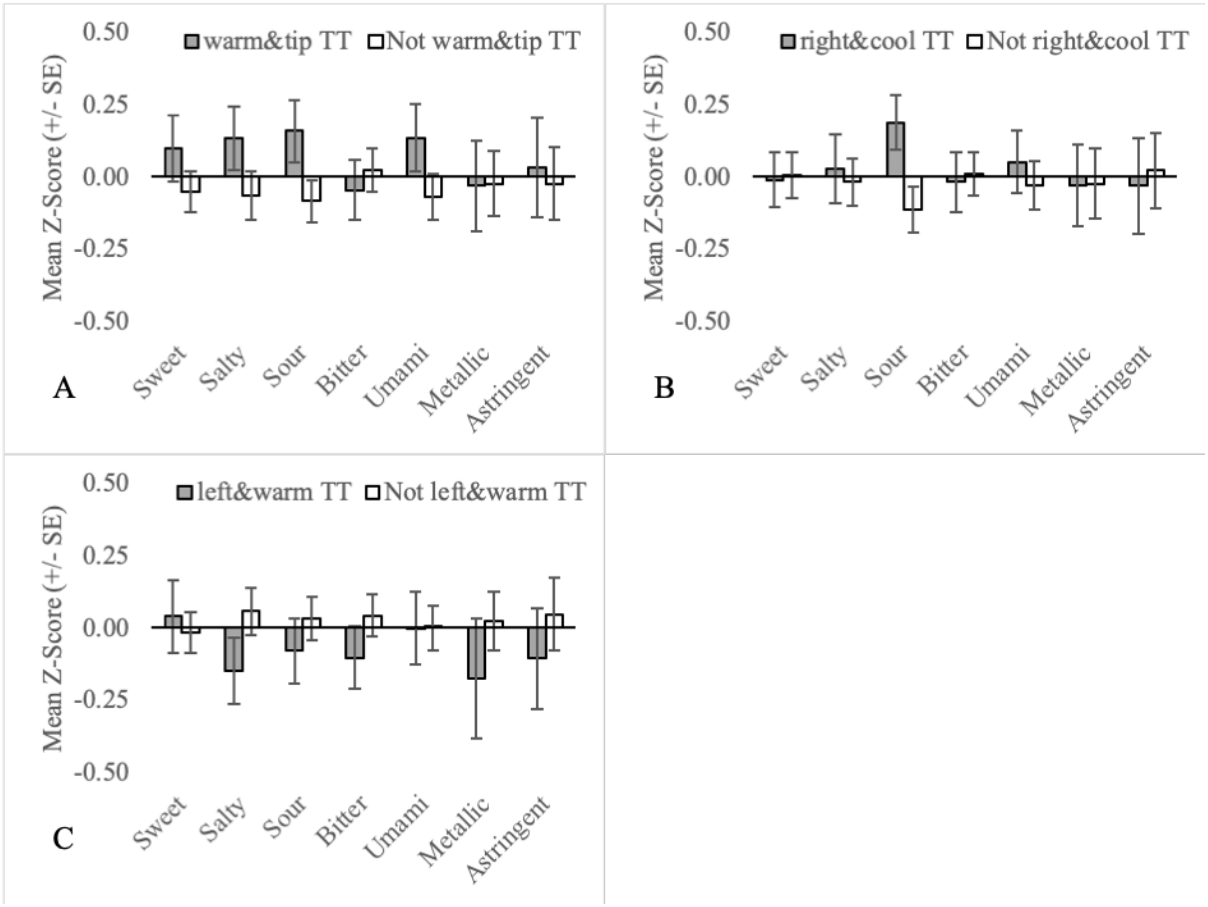

Figure 7.8: Mean orosensory responsiveness (+/- SE) of warm&tip TT (A), right&cool TT (B) and left&warm TT (C) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

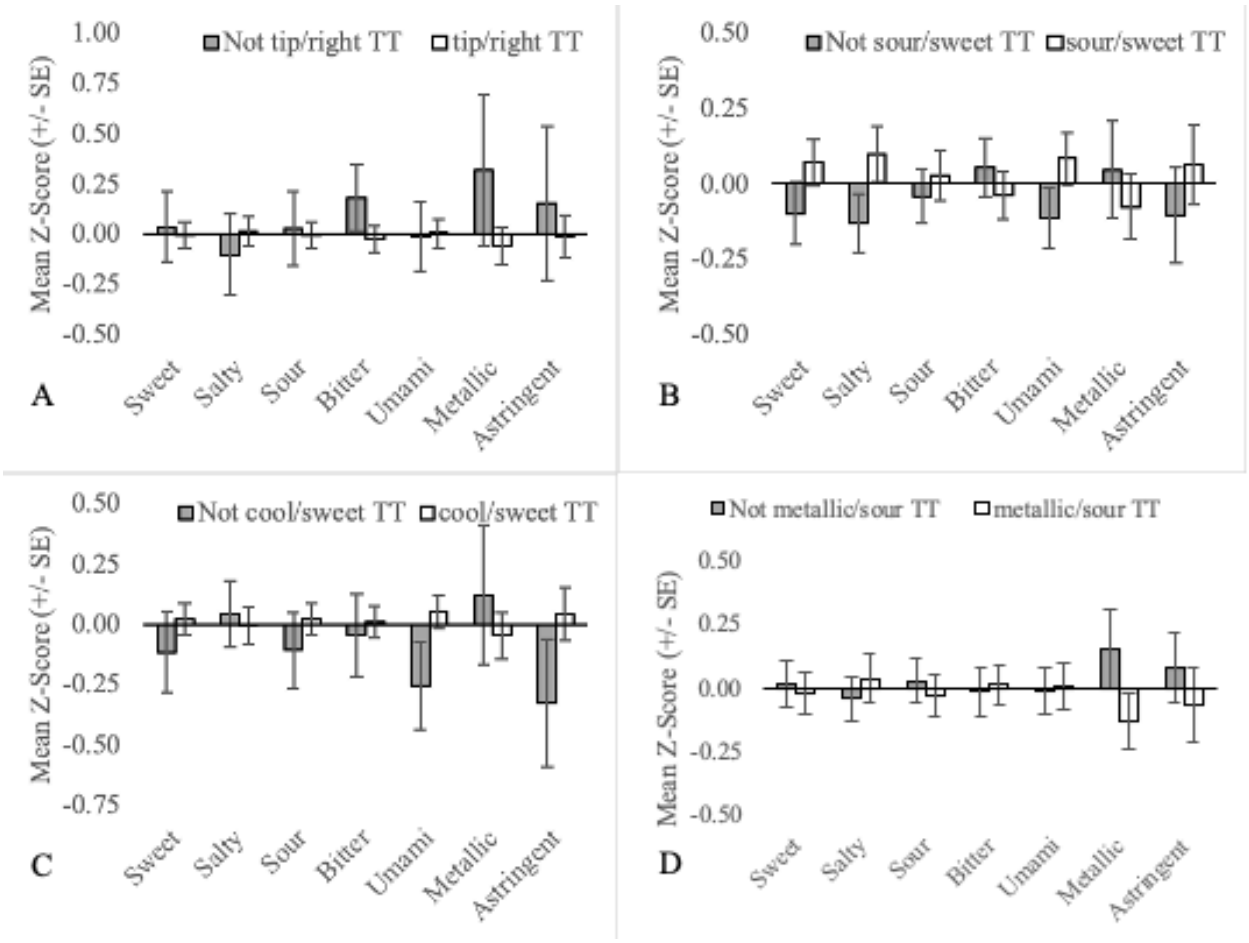

Figure 7.9: Mean orosensory responsiveness (+/- SE) of not tip/right TT (A), not sour/sweet TT (B), not cool/sweet TT (C) and not metallic/sour TT (D) to aqueous solutions (sweet, salty, sour, bitter, umami, metallic & astringent).

7.1.7 Link to Published Version

https://doi.org/10.1016/j.dib.2020.106325

7.1.8 References

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7.2 Appendix II: Chapter 4 Materials

7.2.1 Information Letter, Consent Form, Compensation Form

Information Letter

Date: January 2018
Project Title: Impact of Thermal Taste Status on Taste-Taste Interactions with Ethanol

Principal Investigator/Faculty Supervisor: Dr. Gary Pickering, Professor, Department of Biological Sciences, Brock University, (905) 688-5550 Ext. 4715
gpickering@brocku.ca

Student Principal Investigator: Margaret Thibodeau, PhD Candidate, Biological Sciences

Co-investigator: Dr Ping Liang, Professor, Department of Biological Sciences

INVITATION

You are invited to participate in a PhD study that involves research on thermal tasting and the perception of ethanol. Thermal tasting is the ability to perceive phantom taste sensations as a result of heating or cooling of the tongue. The main objective of this study is to examine differences between different thermal taste groups in their perception of different taste sensations and ethanol. Similarly, a secondary question in this project seek to determine if an individual's sensitivity to the bitter compound 6-n-propylthouracil (PROP) or preference for sweet taste also impact ethanol perception. Finally, the genetic origin of thermal tasting will be investigated. Participation in this study requires you to attend 1-6 sessions, each of which will take approximately 1 hour of your time, resulting in up to 6 hours.

COMPENSATION

You will receive a $10 honorarium per session of participation up to a maximum of $60. All honorariums will be paid in the form of Brock University Campus Store gift cards. Participants that withdraw from the study or fail to complete the study, will be compensated using a pro-rated approach. They will receive a $10 honorarium for each session they completed.

Participants who are enrolled in PSYC courses that attend all sessions may select to receive a combination of research credit and an honorarium. As each session lasts one hour, for each hour claimed as research credit, the value of the honorarium will be reduced by $10. If a participant withdraws prior to completing all sessions, they will only receive research credit for any sessions completed.

Some participants will be screened out of the study after the first or second session. If you are screened out after the first session, you will receive a $10 honorarium or 1 hour

of research credit. If you are screened out after the second session, you will receive a $20 honorarium, 2 hours of research credit or $10 honorarium and 1 hour of research credit.

Some participants have participated in previous studies on thermal taste in the Pickering lab. Considerable overlap exists between the first session of this study and the first session of other thermal taste studies. Therefore, these participants may consent to skip the first session. However, these participants will be required to consent to have the previously collected data added to this study. Furthermore, as these participants will only complete 5 sessions, compensation will be capped at a $50 honorarium or 5 hours of research credit. Alternatively, participants that have previously participated in a thermal taste study may choose to complete the study in its entirety.

WHAT'S INVOLVED

In session 1, you will provide demographic information and your thermal taster status will be determined by heating and cooling small areas of your palm, lip and tongue. In session 2, you will be trained to identify four taste sensations and a photo of your tongue will be taken to measure the density of your taste buds. In sessions 3-6 you will taste a series of solutions with ethanol and different taste sensations. Full training will be provided. You will also be asked to provide a spit sample from which DNA may later be extracted in Dr Liang's lab to help determine which genes are responsible for thermal tasting. Participation in the DNA collection is not required in order to complete all other portions of the study and will not impact compensation. Additionally, you will be asked to complete a set of short questionnaires during breaks in the sessions that are concerned with emotional aspects of food and eating behaviour, taste preferences and behavioural motivation. Some of the participants that are screened out after the first session, will be invited to complete the series of short questionnaires described above, in a modified version of the second session. The modified version will also take one hour and can be completed in the lab or at home as you will be provided with a confidential link to the surveys.

Please note that no consumption of alcohol is allowed or will be tolerated in this study. You must spit out all solutions when asked to taste them. Participants that fail to follow these instructions will be asked to leave and will not be allowed to continue in the study.

RESPONSIBILITIES

To participate in this study, participants need to schedule six, 1-hour blocks of time to come to the CCOVI Sensory Lab (H301) or the research lab (MCH315) located in Brock University. Time slots will be available during normal working hours, after working hours, and on weekends, as agreed upon by the participant and the Principal Student Investigator. Additionally, participants may be asked to complete a survey(s) presented during breaks.

POTENTIAL BENEFITS AND RISKS

Possible benefits of participation include the opportunity to gain a greater awareness of your palate and the ability to discriminate between different taste sensations. Individuals will also develop knowledge of taste phenotypes and how they can influence individual

taste variations, including their own. Further, participants gain the opportunity to be one of only a few hundred individuals to have their thermal taster status determined. Other benefits include contributing to the scientific community and adding to the existing knowledge of thermal tasting research.

The anticipated risks associated with participation in this study are no greater than those encountered in normal daily food and beverage consumption. Specifically, there is a risk of adverse reaction or allergy when ingesting substances. The following page lists the ingredients used in this study. Please review it and inform the Student Principal Investigator if you have any known allergies. In case of an allergic reaction, the lab is equipped with an Epi-pen to reduce the potential risk. All substances to be tasted are safe, are of food-quality grade and are presented at room temperature. However, some participants may find the solutions bitter, astringent or sour which may be unpleasant. There is also a risk of intoxication as you will be asked to taste solutions which include ethanol. In order to mitigate this risk all samples must be spit out and you must rinse with water after each sample. Participants that fail to follow the rules will be asked to leave immediately. Furthermore, participants that arrive at a session intoxicated, will not be allowed to participate.

The study also requires that a toothbrush-sized probe be place on your palm, lip and the tip of your tongue. The probe will be heated or cooled to induce a temperature change which may cause very mild discomfort. To reduce this risk, the probe is calibrated to stay with 5°C and 40°C which is well within the normal range for eating and drinking. As the probe is not disposable, it will be rinsed with 70% ethanol between uses and covered with a fresh piece of saran wrap that can be disposed.

There is also a risk that you may feel obligated or coerced to participate in this study. Please note that participation is entirely voluntary (details below). Furthermore, participation in the DNA collection is not required in order to complete all other portions of the study and will not impact compensation. This study requires participants to answer questions related to alcohol consumption, alcohol related behaviour and your emotions. If you become upset about any issues that have been raised in this study, counseling services are available:
For Brock students and staff: 905-688-5550 x4750
For others: 905-984-3003

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you wish, you may decline to answer any questions or participate in any component of the study. Further, you may decide to withdraw from this study at any time and may do so without any penalty or loss of benefits to which you are entitled. To withdraw from the study, please contact Gary Pickering (gpickering@brocku.ca) or Margaret Thibodeau (mt10xw@brocku.ca) and every effort will be made to destroy your data. Once the study is complete and the data has been de-identified, it will only be possible to destroy your data if you remember your three-digit code.

CONFIDENTIALITY

All information and data you provide is considered confidential; your name will not be disclosed to anyone outside of the researchers listed above and the Research Assistant(s). Each participant will be assigned a 3-digit code at the beginning of the study, and your data will be stored using your 3-digit code and not your name. The DNA samples will be stored in secured -20 and -80^{o}C freezers in Dr Liang's lab. Access to samples will be limited to Dr Liang and approved researcher(s) working under his direct supervision. Samples will be destroyed on completion of the project. Furthermore, because our interest is in the average responses of the entire group of participants, you will not be identified individually in any way in written reports of this research. Data collected during this study will be stored in a secure filing cabinet located in a locked office. Unless you consent to be contacted for future studies, your name will be deleted from the records once data collection is complete. Unless you consent to be contacted for future studies, your name and emergency contact information will be deleted from the records once data collection is complete. All other data will be kept indefinitely.

PUBLICATION OF RESULTS

Results of this study may be published in professional journals and presented at conferences. Feedback about this study will be available to you by contacting Margaret Thibodeau at mt10xw@brocku.ca. The results from this study will become available in late 2018.

CONTACT INFORMATION AND ETHICS CLEARANCE

If you have any questions about this study or require further information, please contact the Principal Investigator/Faculty Supervisor or the Student Principal Investigator contact information provided above. This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University 17-168. If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.Thank you for your assistance in this project. Please keep a copy of this form for your records.

INGREDIENT LIST

Sucrose (Table sugar)
Citric acid (Sour compound common in citrus fruit)
Quinine monohydrochloride dehydrate (Bitter compound in tonic water)
L-glutamic acid monosodium salt hydrate (MSG)
Sodium chloride (Table salt)
Cupric sulphate
6-n-propylthiouracil (PROP)
Water
SCOPE^{TM} Classic Mouthwash
Ethanol (Alcohol)
Alum Sulphate
Tartaric acid (Sour compound common in grapes)
Fructose (Type of sugar)
Unsalted crackers (gluten-free available upon request)
Capsaicin
Various candies or chocolates (optional)

CONSENT FORM

I agree to participate in this study described above. I have made this decision based on the information I have read in the Information-Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may continue to ask questions throughout the study and in the future. I understand that I may withdraw this consent at any time and I will not be subjected to any penalty or discriminatory treatment.

Name: _____

Signature: _____ Date: _____

Please check YES or NO to saliva collection for the purpose of determining the genetic basis of thermal tasting. Note: This is not required for participation in the study.

☐YES ☐NO

Please check YES, NO or NOT APPLICABLE if you consent to use data collected from previous thermal taste studies in the Pickering lab in lieu of completing session 1.

☐YES ☐NO ☐NOT APPLICABLE

Please provide an emergency contact that we can call in case of an emergency

Contact: _____
Phone Number: _____
Relation: _____

Please check the box and fill out the contact information below if you **ARE** interested in being contacted to participate in future studies conducted by the Pickering Lab. Please note that many of our lab's studies have considerable overlap. As a result, if you consent to being contacted, we will retain your name and 3-digit code so that you do not have to repeat the same tasks. Please contact us if you change your mind at any time and your name will be removed from the list.

☐
Email Address: _____
Phone Number: _____

COMPENSATION FORM

Impact of Thermal Taste Status on Taste-Taste Interactions with Ethanol

Name: _____ Student Number: _____

As a thank you for your participation in this study, you may choose from three compensation options. Please select your choice below, by checking the appropriate boxes, and fill in the appropriate information after completing each session.

Session	Type of Compensation (Check One)			Course Code for Credit	Date	Participant's Signature
	$10 Honorarium	1 Hour of Research Credit	Not Applicable			
1						
2						
3						
4						
5						
6						

To be completed at end of study:

I acknowledge that I have received compensation for participation in the study.

Gift Card Number(s): _____

Participant's Signature: _____ Date: _____

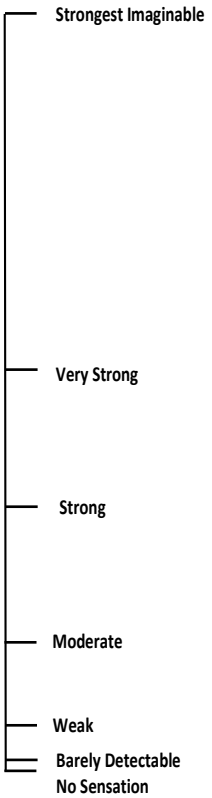
7.2.2 Session 1 Data Collection Ballots

Ballot: gLMS Strongest Imaginable

You are being asked to brainstorm the strongest imaginable sensation across all types of sensations. It should represent the most intense—including painful—sensation that you can ever imagine experiencing. Once you have a sensation in mind, please write that sensation beside the "Strongest Imaginable" line on the scale.

The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing by indicating where it lies on a scale of all possible sensations.

In the following tasks, when you are asked to use the scale, you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.



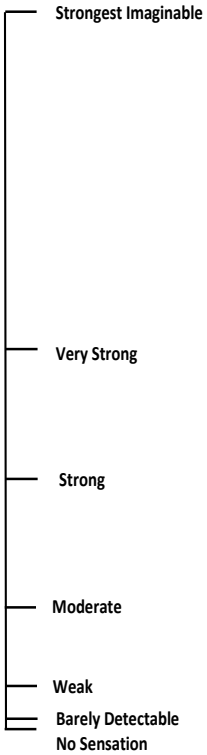
Ballot: gLMS Remembered Sensations

You are being asked to rate the intensity of a remembered sensation, namely, **the brightness of the sun** when staring directly at it, by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing. When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

Please mark the scale with a horizontal line only.

brightness of the sun when staring directly at

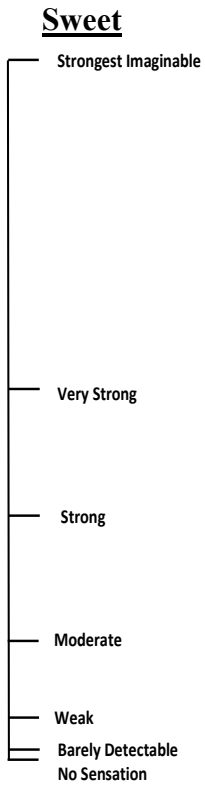


Alternate versions: "sweetness of cotton candy", "touch sensation of a pill on your tongue", "burning sensation of eating a whole chili pepper"

Ballot: gLMS Taste Solutions

You are being asked to rate **the intensity of sweet,** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing. Please take the entire volume of the sample provided, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit out). After you have expectorated, wait approximately ten (10) seconds and then rate the maximum intensity that you perceived in the preceding fifteen (15) seconds. Please keep in mind that you are rating the **maximum intensity** for sweetness, whenever it may occur. When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*



Alternate versions: "bitter", "sour", "salty", "umami", "metallic"

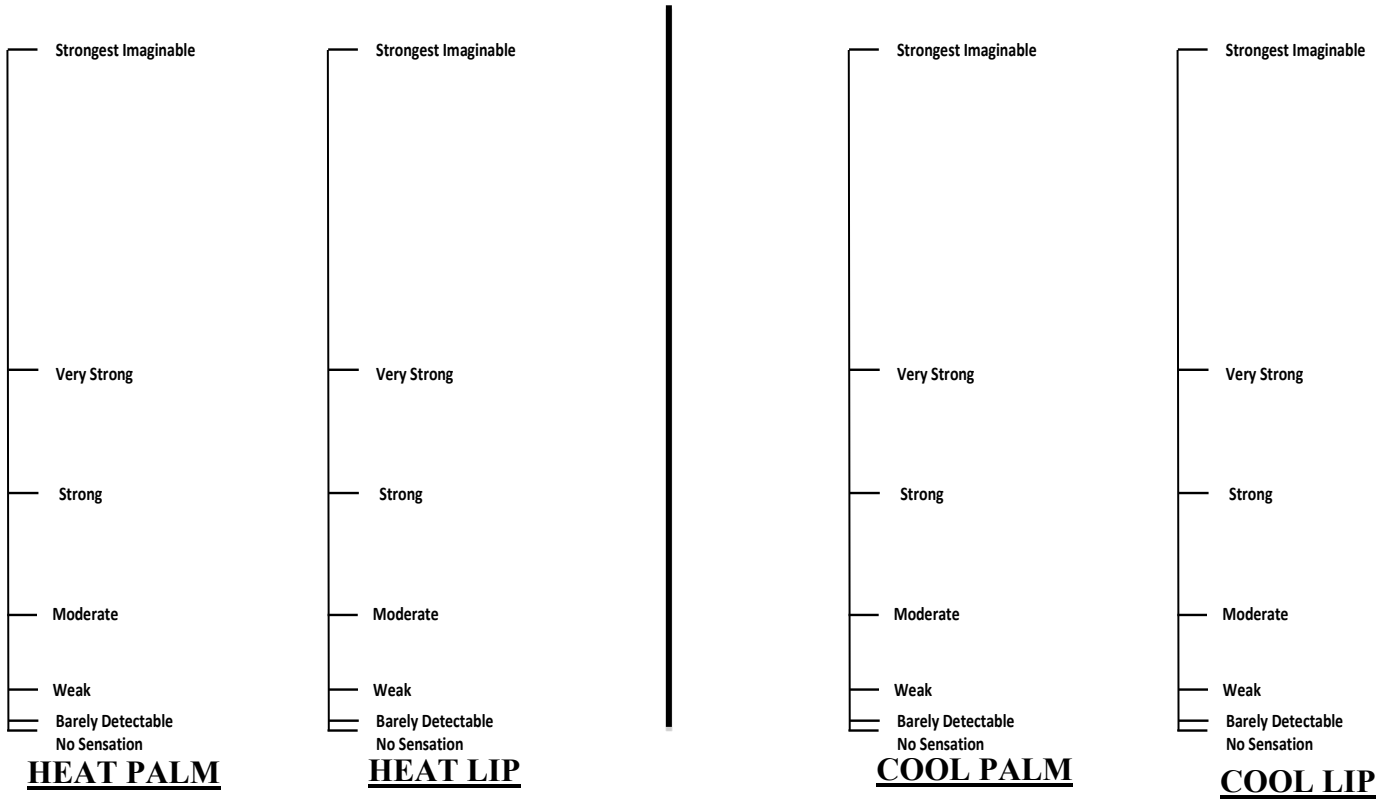
Ballot: gLMS Palm & Lip

You are being asked to rate the intensity of the **temperature applied to your lip and the temperature applied to the palm of your hand** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

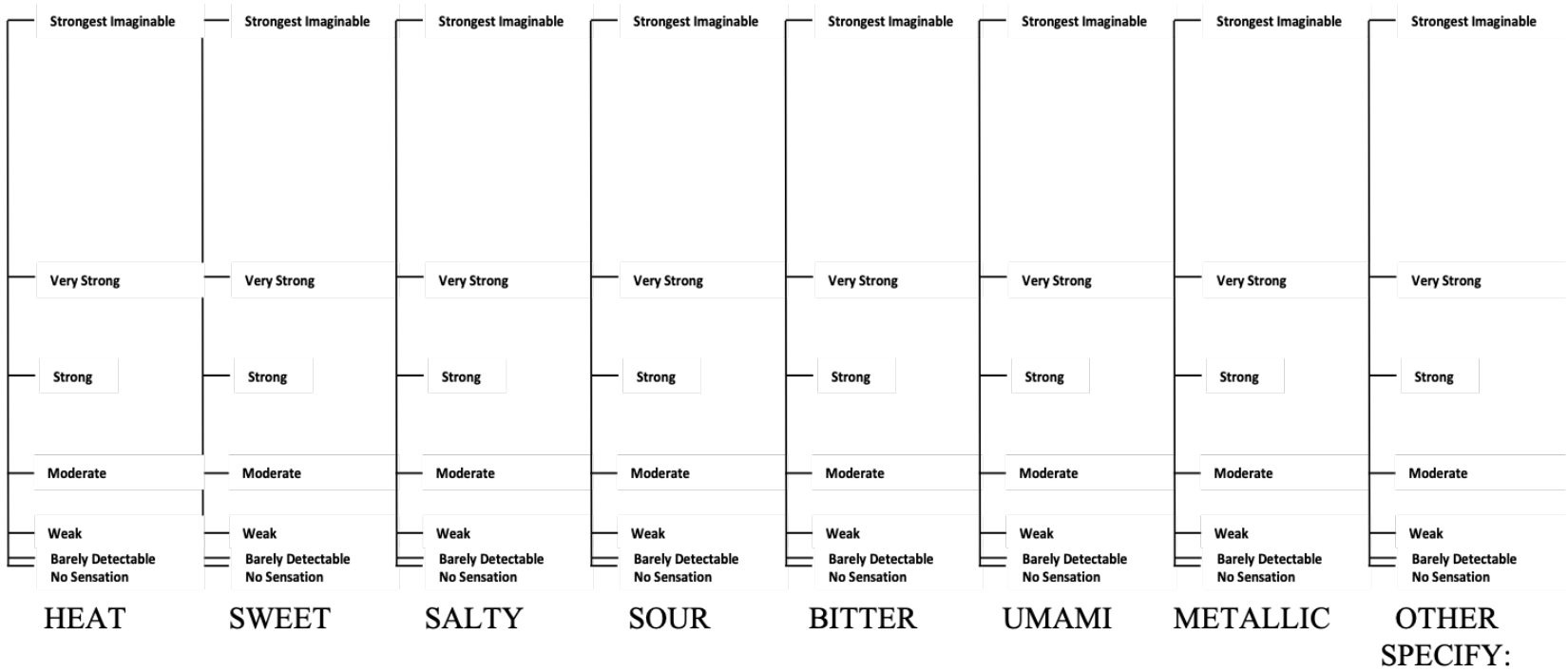
Please mark the scale with a horizontal line only.



Ballot: gLMS Warming Cycle

You are being asked to rate the intensity of the sensations you experience upon **heating of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing. When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate. It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

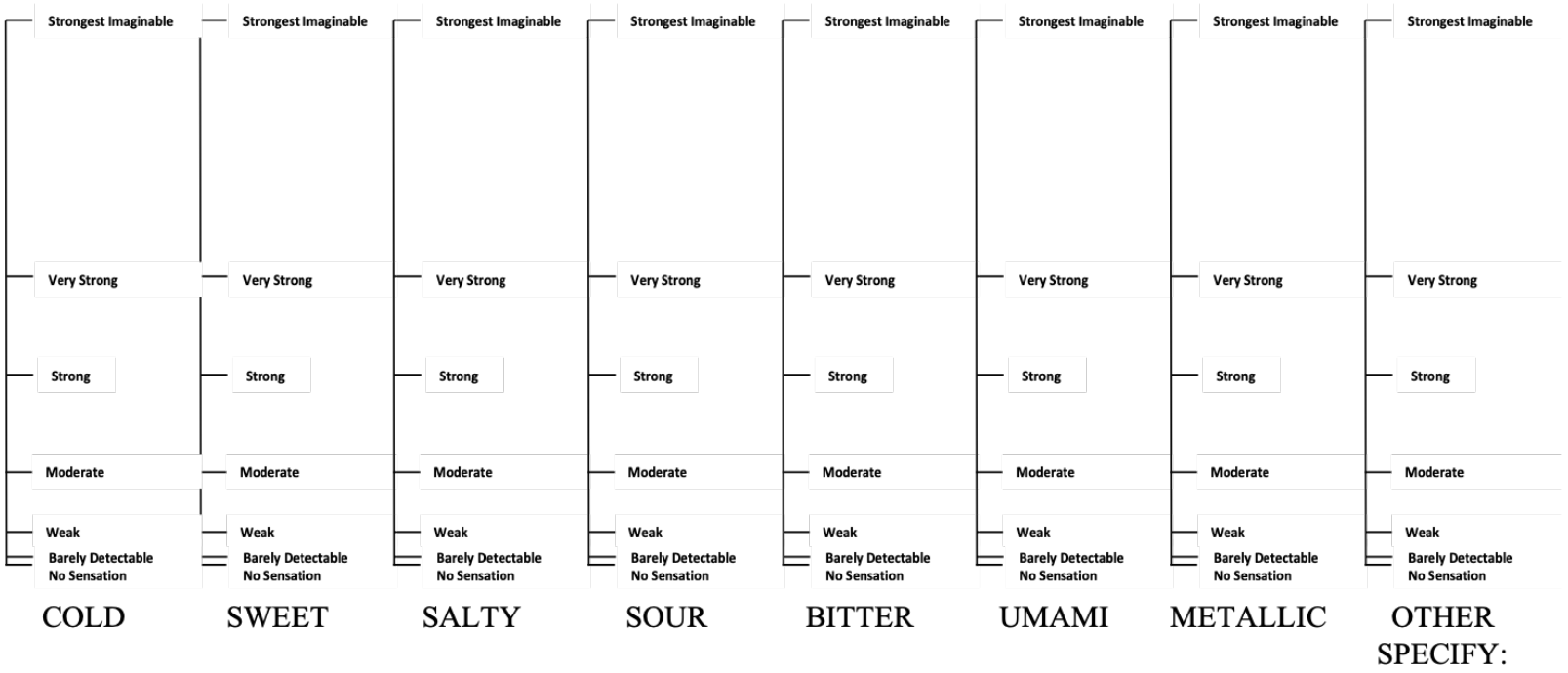
Please mark the scale with a horizontal line only



Ballot: gLMS Cooling Cycle

You are being asked to rate the intensity of the sensations you experience upon **cooling of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing. When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate. It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

Please mark the scale with a horizontal line only



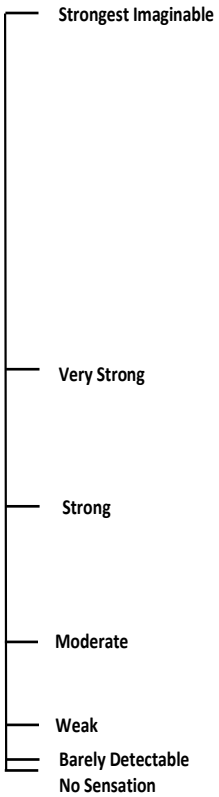
Ballot: gLMS PROP

Please take the entire volume of the sample provided **(6-n-propylthiouracil or PROP)**, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit it out). After you have expectorated, wait approximately ten (10) seconds and then rate the intensity that you perceived in the preceding fifteen (15) seconds, by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But, do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

Please mark the scale with a horizontal line only.



7.2.3 Session 3A-3D Data Collection Ballots

You are being asked to rate the intensity of the sensations you experience when tasting **sample (3-digit code)** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing. When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation. Then, fine tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate. It is important to note that the top of the scale is "strongest imaginable", which represents the most intense—including painful—sensation that you can ever imagine experiencing.

Please mark the scale with a horizontal line only