Variation in Thermally Induced Taste Response across Thermal Tasters

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Abstract

Thermal tasters (TTs) perceive thermally induced taste (thermal taste) sensations when the tongue is stimulated with temperature in the absence of gustatory stimuli, while thermal non tasters (TnTs) only perceive temperature. This is the first study to explore detailed differences in thermal taste responses across TTs. Using thermal taster status phenotyping, 37 TTs were recruited, and the temporal characteristics of thermal taste responses collected during repeat exposure to temperature stimulation. Phenotyping found sweet most frequently reported during warming stimulation, and bitter and sour when cooling, but a range of other sensations were stated. The taste quality, intensity, and number of tastes reported greatly varied. Furthermore, the temperature range when thermal taste was perceived differed across TTs and taste qualities, with some TTs perceiving a taste for a small temperature range, and others the whole trial. The onset of thermal sweet taste ranged between 22 and 38°C during temperature increase. This supports the hypothesis that TRPM5 may be involved in thermal sweet taste perception as TRPM5 is temperature activated between 15-35°C, and involved in sweet taste transduction. These findings also raised questions concerning the phenotyping protocol and classification currently used, thus indicating the need to review practices for future testing. This study has highlighted the hitherto unknown variation that exists in thermal taste response across TTs, provides some insights into possible mechanisms, and importantly emphasises the need for more research into this sensory phenomenon.

Key words: thermal taster; thermal taste; TRPM5; taste phenotype

1. Introduction

Multiple factors contribute to individual differences in orosensory perception, which in turn influence food choice, nutritional status, health and disease outcomes (Garcia-Bailo et al., 2009). Factors influencing variation in taste/orosensory perception are vast, and include taste phenotype, such as the well-evidenced 6-n-propylthiouracil (PROP) taster status (Bartoshuk et al., 2004) and the more recently discovered thermal taster status (Cruz and Green, 2000). Thermal tasters (TTs) perceive thermally induced taste sensations (thermal taste) when the tongue is temperature stimulated using a temperature thermode, in the absence of any gustatory stimuli, while those who only perceive temperature are termed thermal non-tasters (TnTs). The prevalence of TT has been reported to be between 20% (Bajec and Pickering, 2008) and 50% (Cruz and Green, 2000) of participants.

TTs are observed to report higher intensity ratings to chemical taste stimuli delivered at suprathreshold concentrations (Green and George, 2004, Green et al., 2005, Bajec and Pickering, 2008, Yang et al., 2014), as well as sucrose at detection threshold (Yang et al., 2014) and difference threshold for tartaric acid (Pickering and Kvas, 2016), when compared to TnTs. Observed intensity ratings for astringency, metallic (Bajec and Pickering, 2008) and temperature (Green and George, 2004, Bajec and Pickering, 2008, Hort et al., 2016) are higher for TTs than TnTs, whilst an advantage is not reported for capsaicin and menthol (Green et al., 2005, Yang et al., 2014). Evidence for altered responsiveness to olfactory stimulation is contradictory (Green and George, 2004, Yang et al., 2014). TTs perceptual advantage has been supported in a recent study showing increased cortical activation in multiple brain regions in response to gustatory-trigeminal stimuli in TTs compared to TnTs (Hort et al., 2016).

Some evidence suggests thermal taster status may also influence food preference (Pickering et al., 2016). However, the heightened oral responsiveness that TTs exhibit to attributes in alcohol and some food products does not always translate to a difference in overall preference (Pickering et al., 2010a, Pickering et al., 2010b, Pickering et al., 2016, Pickering and Klodnicki, 2016).

Little is understood about the mechanism responsible for thermal taste phenotype. One hypothesis is whether the variation in temperature sensitivity of gustatory neurons in the chorda tympani and glossopharyngeal nerves results in some individuals encoding a taste in response to thermal stimulation, thus resulting in a thermal taste response (Cruz and Green, 2000). A genetic mechanism is possible, and Transient Receptor Potential (TRP) cation channels involved in the transduction of chemical stimuli into taste, temperature, irritant and pungent sensations may be involved. The TRPM5 cation channel is a potential candidate for thermal taste as it is involved in the taste transduction of sweet, umami and bitter chemical tastes, and has been found to be temperature sensitive and activated between 15-35°C in the absence of gustatory stimuli (Talavera et al., 2005). Other cation channels associated with taste transduction may be involved in the perception of other thermal tastes (sour, salt, bitter) (Talavera et al., 2007) and oral sensations (metallic, spicy, mint).

An alternative theory is that TTs have a central nervous system gain mechanism which results in increased excitability in sensory integration areas where trigeminal, gustatory and olfactory inputs merge to produce a flavour perception (Green and George, 2004, Bajec and Pickering, 2008).

The most recent hypothesis is that there is variation in the physiology of fungiform papillae and co-innervation of the gustatory and trigeminal nerve fibres that innervate them, and cross wiring allows them to activate one another in TTs (Clark, 2011). This would explain the lack of difference in the perceived intensity of aroma across thermal taste phenotypes which was reported by Yang et al (2014).

Research to date has focussed on the differences in orosensory perception between TTs and TnTs, while little attention has been given to exploring individual differences in thermal taste responses between TTs alone. Variable sensations are perceived by TTs, with sweet, sour, salty, bitter (Cruz and Green, 2000), metallic, mint, (Hort et al., 2016) and spicy (Yang et al., 2014) having been reported. The number of tastes experienced, and the temperature at which a taste is elicited appears to vary. For example, sweet taste is more frequently reported when warming the tongue between 20-40°C, whilst cooling the tongue from 35-10°C evokes sourness, and saltiness as the temperature decreases from 10 to 5°C (Cruz and Green, 2000). However, the specific temperature range for which tastes are perceived has not been quantified, nor how this varies across TTs. The tongue area which is thermally stimulated has also been shown to influence taste perception, with sweet more frequently reported on the anterior tip, bitter at the posterior, and sour on the lateral edges of the tongue (Cruz and Green, 2000).

The overall aim of this study was to explore differences in thermally induced taste (thermal taste) responses across TTs. The first objective was to investigate the variability in taste qualities reported whilst warming/cooling the tongue tip using traditional thermal taster status phenotyping protocols, where a range of different thermal tastes were expected. As limited evidence details the temperature at which taste is perceived by TTs (Cruz and Green, 2000), the second objective was to explore the temporal thermal taste response to thermally stimulating the tongue, identify the taste quality, intensity, and temporal profile of perceived tastes within and across TTs, and identify the temperature at which taste was perceived. If the TRPM5 channel is the mechanism responsible for thermal sweet taste, it should be perceived between 15-35°C (Talavera et al., 2005).

2. Materials and Method

An initial phenotyping session was conducted to identify TTs. These individuals were then invited to attend two further study sessions. During session one (90 min), TTs were trained to use the general Labelled Magnitude Scale (gLMS), rated their temporal response to taste perceived in response to thermal stimulation, and identified the associated taste qualities. During session two (60 min), reproducibility of the temporal taste response to thermal stimulation was measured during 10 replicates of each temperature trial.

2.1. Participants

The study had ethical approval from the University of Nottingham Medical Ethics Committee. Participants gave written informed consent and an inconvenience allowance for participating was provided. Eighty five individuals were phenotyped for thermal taster status. All participants were healthy non-smokers, age 19 - 40 years, with no known taste or smell abnormalities or tongue piercings. Participants were instructed not to consume anything other than water for at least 1 h prior to all test sessions, which were individually conducted with each participant.

2.2. Phenotyping thermal taster status

Thermal taster status phenotyping was based on methods described by Bajec and Pickering (2008). A intra-oral ATS (Advanced Thermal Stimulator) peltier thermode (16 x 16 mm square surface) (Medoc, Israel) was used to deliver temperature stimulation on the tip of the tongue, as this has the highest fungiform papillae density (Shahbake et al., 2005) and has been shown to be most responsive to thermal taste (Cruz and Green, 2000, Yang, 2015). Before testing each participant the thermode was cleaned with 99% ethanol (Fischer Scientific, UK) and covered with a fresh piece of tasteless plastic wrap (Tesco, UK). The researcher instructed participants to position the thermode firmly in contact with the tongue (Green and George, 2004) prior to thermal stimulation. The warming trial started at 35°C, was reduced to 15°C, and then re-warmed to 40°C and held for 1 s (Fig. 1a). The cooling trial started at 35°C, was reduced to 5°C and held for 10 s (Fig. 1b). All temperature changes occurred at a rate of 1°C/s. Participants were instructed to 'attend' to the temperature increasing from 15 to 40°C during the warming trial, and to the whole of the cooling trial. At the end of each trial, the participant rated the intensity of the temperature when it reached its maximum on a gLMS. If a taste/s was perceived, a second gLMS was presented so each of the perceived taste qualities could be rated. Six categories of taste were listed for selection, the prototypical tastes (sweet, sour, salty, bitter, umami) and 'other (please state)' as other sensations (metallic, minty, spicy) have previously been associated with taste perception (Yang et al., 2014, Hort et al., 2016). Metallic has been proposed as a taste in the past (Bartoshuk, 1978), and some evidence indicates it may have a taste component (Epke et al., 2009, Lawless et al., 2004, Lawless et al., 2005, Skinner et al., 2017). Mint is typically considered to occur as a result of chemesthesis and aroma stimulation (Roper, 2014). However, sweetness is an important aspect of mintiness, and it is therefore possible that mintiness is reported due to combined perception of trigeminal temperature and sweet taste perceived (Hort et al., 2016). The general consensus is that spiciness occurs due to chemesthesis, however, the possible association with taste remains unclear (Roper, 2014). These attributes were included in order to explore the complete range of sensations reported in response to thermal stimulation, and to prevent attribute dumping onto the other attribute qualities. The gLMS consisted of a vertical line 230 mm high. Considering the line to be 100 units, unequal quasi-logarithmic spacing between word descriptors; 'no sensation', 'barely detectable', 'weak', 'moderate', 'strong', 'very strong' and 'strongest imaginable sensation of any kind', which were placed at 0, 1.4, 6, 17, 35, 53 and 100% of the scale respectively (Green et al., 1996). Two replicates of each temperature trial were delivered, and if the taste quality or presence of taste was inconsistent across replicates, a third trial was conducted to aid classification. A two-minute palate recovery break was given between replicates and warming/cooling trials. Warming trials preceded cooling trials to prevent possible adaptation from the intense, sustained cold stimulation of the cooling trial (Green and George, 2004). Participants were not made aware the purpose of the activity, and to reduce any bias of falsely reporting taste they were informed that taste is not always perceived. Verbal training on the basic 'taste' qualities was provided before the temperature trials were delivered; sweet as the sweetness experienced from sugar; salty as the sensation from table salt, sour as the sourness perceived from items such as lemon or vinegar, and bitterness like that perceived in coffee and tonic water, umami is a meaty savoury sensation associated with meat broth and mushrooms, and metallic like the sensation of metal or blood in the mouth. Participants were not trained on 'minty' and 'spicy' attributes. If reported, the researcher probed the nature of the perceived sensation, which was reported to be a sensation that occurred in addition to the perceived temperature.

Traditional thermal taste phenotyping classifies TTs as those individuals who report taste above weak in intensity, while those who report below weak are assigned to an uncategorised (*Uncat*) group. To explore the range of sensitivities reported, this study defined TTs as those individuals consistently reporting the same taste/s across two replicates of the warming and/or cooling trials at any intensity. Those only perceiving temperature were classified as TnTs, and those reporting taste inconsistently (taste quality or the presence of taste) across ≥ 2 replicates were characterised uncategorised (*Uncat)*. This resulted in 24 participants being identified as TTs. Thirteen participants who had previously been identified as TTs using the same temperature trials, were re-phenotyped and were again classified as TTs during the current study. The resulting 37 TTs, attended two subsequent sessions to further investigate the thermal taste phenomenon.

2.3. Modification of temperature trials

During preliminary testing, some individuals reported numbing of the tongue, and occasional pain when the traditional cooling trial was held at 5°C for 10 s, which is expected during this temperature range (Gardener and Johnson, 2013). A modified cooling trial was used for subsequent testing, which held at 5°C for 1 s instead of 10 s. To aid in palate recovery between replicates, both temperature trials were also extended to return to 35°C after reaching their destination of 40 or 5°C.

As the modified temperature trials contained both warming and cooling components, they are subsequently termed according to the temperature extremes reached during each trial; the ‘40°C trial’ (modified warming trial) lasting for 52 s (Fig. 1c), and the ‘5°C trial’ (modified cooling trial) lasting for 61 s (Fig. 1d). A specialised thermode holder-mouthpiece was used to standardise the positioning of the thermode on the tongue across both replicates and assessors (Fig. 1e). Traditional thermal taste phenotyping requires a response to be taken only during the ‘warming’ (15-40°C of the warming trial) or ‘cooling’ (35-5°C of the cooling trial) component of the temperature trial. Here, all subsequent responses were collected across the entirety of each modified temperature trial (35-35°C) to capture the complete temporal taste response to thermal stimulation.

2.4. Session 1

The aim of Session 1 was to familiarise participants with using the gLMS and study protocols, and record the nature of the taste/s they perceived. Participants were reminded that people do not always perceive taste to reduce any bias of falsely reporting taste.

2.4.1. Scale familiarisation

Participants were trained on the correct use of the gLMS (Bartoshuk et al., 2002). They were provided with a blank gLMS and instructed to add their strongest imaginable sensation at the top of the scale before rating the perceived intensity of 15 remembered or imagined sensations on the scale. This created each participants’ individualised reference gLMS which was presented during all subsequent testing to guide intensity ratings.

2.4.2. Temporal taste protocol familiarisation

Participants performed temporal response evaluations using an on screen gLMS (Presentation Software, Neurobehavioral System, San Francisco, US) and a rollerball to indicate either temperature or taste intensity perception in real time whilst the thermode was in contact with the tongue. Participants were familiarised with using the rollerball to rate the perceived temperature intensity of the thermode across each trial, by using the rollerball to rate on the gLMS in real time across the trial. Trials were then repeated during which participants rated only the intensity of any taste/s perceived on the gLMS, and not temperature. Here, they were clearly instructed that the rating should be at ‘no sensation’ when temperature alone was perceived, and only to rate if taste was perceived. If more than one taste was perceived they were instructed to rate the overall taste intensity.

2.4.3. Recording taste qualities associated with the temporal response

Preliminary testing (data not shown) revealed that some TTs reported more than one taste during a temperature trial. Consequently, temperature trials were undertaken to identify which taste/s were associated with which elements of the temporal taste response. A list of tastes (sweet, sour, salty, bitter, umami), metallic, and the option to report ‘other’ were presented to participants on a sheet. Two replicates of each temperature trial were delivered, during which the participant was instructed to point to the relevant word descriptors on the sheet to indicate; ‘no taste’, the taste quality, or ‘other’ sensation perceived across the trial in real time. If the ‘other’ option was selected, they were asked which sensation/s they had perceived once the trial finished. More than one sensation could be reported at any one time. The taste quality and temperature range at which taste/s were perceived was recorded. It should be acknowledged that attributes are more likely to be reported when presented as a list, as opposed to during free reporting (Lawless et al., 2005).

2.5. Session 2

The aim of Session 2 was to explore the variability in taste response across TTs, and its reproducibility within a TT across a large number of replicates. As before, participants were reminded that people do not always perceive taste to reduce any bias of falsely reporting taste.

2.5.1. Measuring the temporal taste response and reproducibility

Temperature trials were delivered using the modified protocols. A block of 10 repetitions of the 40°C trials was followed by a block of 10 repetitions of the 5°C trials. The inter-stimulus-interval (ISI) between replicates was reduced to 10 s as testing with a subset of the TTs revealed this duration to be long enough for the tongue to recover (data not shown). Participants were instructed to place their tongue back into their mouth during each ISI. The 40°C trial block preceded the 5°C trial block to prevent adaptation from the intense cold stimulation delivered during the 5°C trial. A 5 minute palate recovery break was given between the blocks. Participants were instructed to use the rollerball to rate the intensity of any perceived taste/s on the gLMS for all replicates of each trial, in the same manner indicated in section 2.4.2. At the end of each block of temperature trials participants verbally reported if any taste/s were perceived and these were recorded by the researcher.

2.6. Data analysis

2.6.1. Phenotyping thermal taster status

The percentage of individuals phenotyped as TT/TnT/*Uncat* was determined, and the frequency of taste sensations reported during the traditional warming and cooling trials identified. Chi-square tests were used to examine the relationship between the frequency of taste qualities perceived across warming and cooling trials. Analyses were performed using SPSS, version 21 (SPSS IBM, USA) with an α-risk of 0.05.

2.6.2. Taste qualities perceived during modified temperature trials

The taste qualities perceived by TTs were recorded from the taste identification temperature trials performed at the end of Session 1, and the tastes identified at the end of the replicate trials during Session 2. The mean maximum intensity (*Imax*) for each temporal taste reported across the 10 replicates for each participant was calculated using GraphPad Prism version 7.02 (GraphPad software, USA) using a threshold of 0.5 to ensure no spurious onsets were included. As gLMS data are typically log-distributed, all intensity ratings were log transformed prior to analysis resulting in values in the range of -1.4 to 2.

2.6.3. Reproducibility of temporal taste ratings

To measure reproducibility of the temporal taste ratings reported over the 10 replicates for an individual participant, a correlation analysis was performed between the temporal responses to each replicate (MATLAB R2015b), thus creating a correlation matrix between each pair of replicates for each temperature trial. The mean correlation coefficient (CC) from the correlation matrix was then computed for each temperature trial (5 and 40°C) for each participant. For each temperature trial, the 1st, 2nd, 3rd and 4th quartiles of the CC values were computed.

2.6.4. Categories of temporal taste responses

The average temporal response for each individual participant across the ten replicates was calculated for both the 40ºC and 5ºC temperature trial. To determine common temporal patterns of response across TTs, each individual average temporal response was included in a principal component analysis (PCA) for each temperature trial (MATLAB R2015b). The four principal components (PC) across the TT group and the variance explained by each component was determined and the resultant average time course for each PC computed.

In addition, for both the 40°C and 5°C temperature trial, for each individual participant, their replicates were included in a principal component analysis (PCA), and the first two PCs determined. From these, the time to the peak (TTP) of Principal Component 1 and Principal Component 2 was determined (MATLAB R2015b). These TTP values of the two PC components were then plotted against each other to group participants with separate categories of temporal responses.

2.6.5. Temperature range of taste responses

To explore variation in the temperature range at which tastes were perceived, Graphpad Prism software was used to identify the onset and offset temperature at which taste/s were reported by each TT during each replicate of their temporal response from Session 2, and the means (± 1 stdev) were calculated. In some cases two temporal taste peaks were reported during a single temperature trial, but the taste intensity rating did not return to zero between the peaks. In these cases the onset of the second taste was identified to be the time at which an increase in taste intensity rating was reported in the waveform.

3. Results

3.1. Phenotyping thermal taster status

Of the 85 participants attending the phenotyping session, 28% were TTs, 51% TnTs, and 21% *Uncat*. Notably seven participants classified as TTs would have been classified as *Uncat* if using the traditional phenotyping methodology administering only 2 rather than 3 replicates of each temperature trial. The current protocol permitted TTs to report taste on only 2 of the 3 replicates administered. Of the total 37 TTs, data from one participant was removed due to contradictions in temporal taste ratings and what was reported verbally, leaving 36 (13 male/23 female) participants for analysis. When phenotyping, the tastes most frequently reported during the traditional warming trial were sweet (42%), metallic (13%) and spicy (13%) (Fig. 2a), and during the traditional cooling trial were sour (25%), bitter (25%) and metallic (17%) (Fig. 2b). Chi-square analysis indicated that the tastes reported were significantly associated with the temperature trial ($p=0.001$), where sweet was reported more frequently during the warming trial, and bitter and sour more frequently during the cooling trial.

3.2. Variation in temporal taste responses

Variation across TTs was observed in terms of the taste quality, intensity, and number of tastes perceived, the shape of the temporal taste response, and the temperature range at which taste was perceived.

3.2.1. Taste qualities perceived during modified temperature trials

A range of different taste qualities were perceived by TTs during the modified temperature trials (Table 1 and 2). Only 4 TTs reported 'no taste' across one of the two temperature trials, and the number of perceived tastes ranged from 0-4 during one temperature trial. The reported intensity also varied, with *Imax* ranging from 0.17 (below barely detectable) to 1.94 (above very strong) on the gLMS. Two TTs reported taste intensity below weak on the gLMS, and ordinarily would have been classified as *Uncat* if using traditional phenotyping protocols. In most cases (69%) one individual taste was reported alongside one temporal response. However, in 31% of responses, multiple tastes (2-4) were associated with a single temporal response, or taste was reported at an inconsistent temperature range across replicates.

3.2.2. Reproducibility of temporal taste ratings

Table 1 and 2 provide the mean correlation coefficients (CC) from the correlation matrix for each individual for the 40°C and 5°C temperature trials respectively. A higher mean correlation was found for the 5°C temperature trial (median CC of 0.76) compared to the 40°C temperature trial (median CC of 0.67). Figure 3 shows correlation matrices for the 10 replicates of the a) 40°C trial, and b) 5°C trial, with an example correlation matrix for an individual participant within the i) first, ii) second, iii) third, and iv) fourth quartiles. Correlation coefficients identified consistent temporal taste responses were rated across the 10 replicates of the temperature trials by most TTs, whilst a small number reported inconsistently across replicates by either perceiving taste on <10 replicates of a temperature trial, and/or by reporting taste at inconsistent temperature ranges across replicates (Table 1 and 2).

3.2.3. Categories of temporal taste responses

PCA analysis performed on the average temporal response across TTs indicated that for the 40°C trial, 4 principal components accounted for 85% of the variation in the data. The temporal responses associated with each PC are shown in Figure 4a reflecting 4 different patterns of response relating to number and onset of temporal taste intensity peaks. PC1 reflected trials where participants perceived taste during the cooling stage, which increased in intensity to a second peak at the end of the warming stage. PC2 represented those trials with two peaks, where the first peak was initiated during the cooling stage and peaked when the temperature reached 15°C. A second, less intense, peak was then observed during the warming stage. PC3 reflects those trials with one peak during the warming period which peaked at the end of the trial (the early bumps observed in the cooling element relate to a couple of erroneous replicates). Finally, PC4 reflected responses with two peaks, similar to PC2, but with an earlier first peak. For the 5°C temperature trial, the 4 principal components accounted for a higher, 92%, of the variance, and Figure 5a shows the temporal responses associated with each component which again differed in relation to number of peaks and time of onset. PC1 revealed trials where participants reported only one taste peak which began during the cooling period and peaked at the lowest temperature before fading. PC2 showed a much later onset and peak of taste intensity perception which started in the middle of the warming phase of the trial. PC3 highlighted responses with two peaks in taste intensity perception, one began during the cooling element of the trial which faded before a second peak occurred in the middle of the warming element, and continued to rise until the end of the trial. PC4 also reflected responses with 2 peaks, but with onsets arising earlier during both the cooling and warming elements.

The results of the PCA on individual participant replicates are shown in Figure 4b and 5b. These plot the time to peak of PC1 versus PC2 for each individual participant for the 40°C temperature trial (Figure 4b) and the 5°C temperature trial (Figure 5b). For each temperature trial, four subgroups of TTs can be observed, which relate to the groups of temporal responses identified in Figure 4a and Figure 5a according to the timing of the peaks of taste intensities.

3.2.4. Temperature range of taste responses

Tastes (Table 1 and 2) were reported at variable temperature ranges during the 40°C (Fig 6) and 5°C (Fig 7) trials. In line with the phenotyping results, sweet was most frequently reported when warming the tongue, and bitter when cooling. Interestingly sweet was reported alone during 28% of total responses, and always when the temperature was increasing with the onset ranging between 22 and 38°C. Bitter was reported alone during 17% of total responses. Although the onset predominantly occurred when the temperature was decreasing (between 32 and 18°C), onset did occur as temperature increased on three trials (between 19 and 25°C). Other tastes were not reported alone with a temporal response at a high enough frequency to report the temperature range of perception. Other thermal sensations (salt, umami, metallic and spicy) were not generally reported alone, therefore the temperature range of each was not isolated or discussed. Tastes were associated with a brief temperature range for some TTs (as small as 3.3°C), whilst others perceived taste/s across a wider range spanning most of the trial (as much as 58°C, which includes a warming and cooling spell), showing variation in the taste/temperature specificities across TTs. It is also noteworthy that some tastes elicited during cooling of the tongue persisted as the temperature increased during the subsequent warming component of the trial.

4. Discussion

4.1. Thermal taster status phenotyping

Twenty eight percent of participants phenotyped in this study were TTs, which is within the 20% (Bajec and Pickering, 2008) - 50% (Cruz and Green, 2000) range previously reported. Fifty one percent of participants were classified as TnTs, within the range previously identified 29% (Yang et al., 2014) to 77% (Hort et al., 2016), but higher than the typical 35-40% reported in most studies (Bajec and Pickering, 2008, Bajec and Pickering, 2010, Pickering et al., 2010a, Pickering et al., 2010b, Pickering et al., 2016). Twenty one percent of participants were *Uncat*, lower than previous findings which range from 23% (Pickering et al., 2016) – 42% (Yang et al., 2014), and considerably lower than the 33-42% typically reported (Bajec and Pickering, 2008, Bajec and Pickering, 2010, Bajec et al., 2012, Yang et al., 2014). The variation across studies is likely due to differences in the classification methods used, indicating the need for a more standardised approach.

Traditional phenotyping requires taste intensity to be reported above weak intensity on the gLMS. Apart from the initial paper reporting the thermal taste phenomenon (Cruz and Green, 2000), this is the first study to classify individuals reporting taste below weak intensity as TTs (n=2). These individuals continue to report taste, which would not be experienced by TnTs. Classifying them as *Uncat*, as traditional methods stipulate, results in the TT group containing only those with high intensity thermal taste responses. Therefore, prevalence estimates are likely skewed to show a lower percentage of TTs than is representative of those perceiving tastes. Additionally, further distinction between TTs and *Uncat* individuals can be made by administering a third replicate of a temperature trial when taste is reported inconsistently across the first 2 replicates. Using this method in the current study resulted in 7 participants who traditionally would have been *Uncat* to be assigned to the TT group. Other considerations that need to be addressed include whether an individual should be classified as a TT if they perceive only prototypical tastes or 'other' sensations, and the number of tongue locations tested. Improving phenotyping practices to reduce the number of individuals assigned to the *Uncat* group would increase those included within a study population, improving understanding of this taste phenotype over a wider percentage of the population when exploring impact on oral responsiveness, and food preference and behaviours. Alternatively, as this group make up a significant proportion of the population, the *Uncat* group should be included as a unique category within the thermal taste phenotype, and included in all analysis and group comparisons.

Phenotyping using the traditional temperature trials found sweet, metallic and spicy most frequently reported during the warming trial, and sour and bitter during the cooling trial. Sweet was perceived significantly more frequently during the warming trial, and bitter and sour during the cooling trial. Early literature on TTs failed to report which taste qualities were perceived, and more recently some researchers have grouped tastes perceived across both trials together (Pickering et al., 2016, Pickering and Klodnicki, 2016). When tastes have been identified across separate trials, sweet, metallic and bitter are frequently perceived when warming the tongue, and sour, bitter, metallic and salt when cooling (Cruz and Green, 2000, Yang et al., 2014, Hort et al., 2016, Pickering and Kvas, 2016), as found in the current study.

4.2. Variation in taste response across TTs

This is the first study to evidence detailed differences in the taste response across TTs. It has been demonstrated that TTs not only perceive different taste qualities, but the number of tastes perceived, their intensity, the reproducibility of the response, and the temperature range at which they are detected also varies.

4.2.1. **Taste qualities perceived during modified temperature trials**

A number of different taste qualities were perceived during the modified temperature trials (Table 1 and 2). Participants perceived between 0 and 4 tastes across a trial, however, only four TTs reported no taste on one of the temperature trials. Notably this questions the need to use two separate temperature trials when phenotyping for, or investigating, thermal taste. Sweet was the taste most frequently reported alone, followed by bitter. However, as many as three tastes were reported within one temporal peak by some TTs, indicating they may arise together or merge from one to another. Another possibility is that participants may have struggled to articulate the taste perceived, or that the plastic mouthpiece which has not been used in previous studies had an effect on the perceived responses. Reported taste intensity varied considerably from 0.19 (< barely detectable) to 1.94 (> very strong) on the gLMS, showing a diverse spectrum of responsiveness to temperature induced taste perception, as seen with chemical tastants (Garcia-Bailo et al., 2009). This full range of perceived taste intensities are not usually considered as current phenotyping practices categorise individuals reporting taste intensity below weak to the *uncat* group, highlighting the need to revise phenotyping methods.

4.2.2. Reproducibility of temporal taste responses

Mean CC values identified temporal taste ratings were more consistent across the 10 replicates of the 5°C trial (Table 2) compared to the 40°C trial (Table 1). This is likely due to the complexity of the temperature changes during the 40°C trial, which first cools the tongue from 35-15°C, before warming to 40°C, before returning to 35°C. Again, this highlights the need to explore and understand the impact of delivering thermal stimulation that varies in both the range of temperatures delivered, and degree of temperature change on the perceived thermal taste response. This should aim to optimise both the frequency and range of sensations reported, and their reproducibility. Interestingly, low CC values were associated with different types of inconsistent reporting (Table 1 and 2). The first type was those with taste being reported on less than 10 of the replicates, which could indicate lower sensitivity in the mechanism responsible for eliciting thermal taste, resulting in a taste not always being perceived by some TTs. One hypothesis being that there is a 'spectrum' of thermal taste responsiveness, resulting in not all individuals perceiving taste on all replicates. This effect may be more prevalent when delivering large numbers of replicates, as conducted in the current study. The second type of inconsistent reporting occurs when taste is reported at a variable temperature range across replicates. In contrast, other TTs reported taste highly reproducibly across all 10 replicates with mean CC values as high as 0.925. One hypothesis is that the mechanism responsible for thermal taste in some TTs is highly specific and results in taste being perceived at a specific and reproducible temperature within the trial during every replicate, whereas for others the mechanism, or mechanisms, elicit taste/s at variable temperature ranges resulting in inconsistent reporting across replicates. These latter responses were frequently associated with multiple (2-4) tastes (Table 1 and 2), where participants reported taste arising interchangeably across the trial, and/or that more than one taste may occur at one time. This indicates more than one mechanism may be involved in eliciting the different taste qualities, which occur in parallel for some TTs. It should also be noted that by combining both a cooling and a warming element in the modified trial, the reporting of more than one taste, and hence within-trial taste response variability, is not surprising as some TTs do report taste on both modes of stimulation.

4.2.3. **Categories of temporal taste responses**

PCA on the averaged taste intensity responses across all TTs identified categories of responses associated with the four principal components for the 40°C (Fig 4a) and 5°C (Fig 5a) temperature trials, which accounted for 85 and 92% of the variance respectively. PCA on the individual participant replicates allowed grouping of the TTs according to their time to peak for PC1 and PC2 for each temperature trial (Fig 4b and 5b), which was associated with the different categories of temporal responses identified. For the first time, this quantifies the complexity of the temporal taste responses reported within and across TTs. Sometimes, a single taste peak was perceived (Fig 4a PC3 and Fig 5a PC2). These responses frequently occurred over a short temperature range, which could indicate specificity in the temperature sensitivity of the mechanism involved. In other cases, TTs detected a taste on each of the warming and cooling elements of the temperature trials, leading to two peaks, but with variable onsets, durations, and intensities (Fig 4a PC2 and PC4, Fig 5a PC3 and PC4). In these cases, the intensity of the first taste associated with cooling was always more intense than that of the second taste associated with warming, which may be due to an interaction with the perceived temperature delivered, as cooling to 5 or 15°C reaches a greater variation from body temperature than warming to 40°C. Another common response was when taste was reported across most of the temperature trial (Fig 4a PC1), but where one peak was reported to be associated with the cooling component of the trial, and then rose in intensity to identify a second peak. This associates with verbal reporting that tastes sometimes merged from one to another with no ‘off’ period between. Finally, a common response during the 5°C trial was reporting of an intense taste peak during the cooling component of the trial, which declined as the temperature increased, and started to rise again before the trial finished (Fig 5a PC1). This indicates individuals who perceived a taste associated with cooling the tongue, and the beginning of a second taste associated with warming the tongue, which would continue to develop if the trial continued for longer. These findings highlight the need to explore a more diverse range of thermal stimulation paradigms in order to understand the occurrence, persistence, intensity of taste, and interaction between tastes when delivering temperature at greater temperature extremes (for example >40°C), temperature at different rates of temperature change (°C/s), and delivery of continuous temperatures for prolonged periods. It may be that alternative temperature trials optimise the range of sensations reported, and better differentiate between those experienced when cooling the tongue compared to those associated with warming it. Understanding these elements could contribute towards developing alternative phenotyping practices that do not require expensive thermal stimulation devices, and can be adopted by a wider range of individuals in both research, clinical and health profession environments to forward understanding of this unique and fascinating phenotype.

4.2.4. Temperature range of the taste responses

Sweet taste was frequently reported alone, which allowed an associated temperature range to be identified. The TRPM5 channel is a possible mechanism for thermal sweet taste as it is temperature sensitive and activated by temperature between 15-35°C in the absence of gustatory stimuli, and also modulates sensitivity to sweet taste (Talavera et al., 2005). It is therefore possible that temperature stimulation activates gustatory nerve fibres via the TRPM5 to elicit 'thermal' sweetness. However, this does not explain the selectivity for sweet when the TRPM5 is also involved in the transduction of bitter and umami tastes. Here, the onset of sweet taste ranged between 22 to 38°C as the temperature increased, thus supporting the hypothesis of the TRPM5 being involved as it is temperature activated between 15-35°C. The sweet onset only occurred at a temperature > 35°C on one occasion, this may be due to a latency effect in responding to the stimulus when using the rollerball.

Bitterness was also frequently reported alone, with the taste onset predominantly when the tongue was cooled, (ranging between 32 to 18°C), which is in agreement with bitter being frequently reported during the traditional cooling trial (Cruz and Green, 2000, Yang et al., 2014, Pickering and Kvas, 2016). However, on three trials the onset of bitterness occurred when warming the tongue (between 19 and 25°C). Interestingly, bitter has is also reported during the traditional warming trial (Pickering and Kvas, 2016, Hort et al., 2016). It is worth noting that traditional phenotyping specifies participants 'attend' to only part of the warming trial, as the temperature increases (15-40°C). Here, responses were collected across the entirety of both modified temperature trials (35-35°C). Figure 6 and 7 show tastes elicited during cooling of the tongue often persisted as the temperature increased during the 'warming' component of the trials. Some tastes reported during the warming component of the traditional warming trial when phenotyping may therefore be associated with the pre-cooling temperatures. This could, at least in part, explain why some tastes typically associated with cooling the tongue are reported during the warming trial (such as bitter, sour and salty). This study demonstrates sweet is most frequently associated with true warming of the tongue, after the pre-cool taste has diminished. Bitter was occasionally reported when warming of the tongue, but this response was infrequent.

In the past, some researchers have classified TTs as those reporting only prototypical taste qualities (Cruz and Green, 2000, Bajec et al., 2012), whilst others, including the current study, have permitted 'other' attributes (minty, metallic, spicy) (Yang et al., 2014, Hort et al., 2016, Pickering and Klodnicki, 2016, Pickering and Kvas, 2016, Pickering et al., 2016). Although controversial, it is important to understand how these sensations relate to the thermal taste phenomenon, and to characterise the complete range of sensation reported in addition to the perceived temperature across TTs. Here TTs reporting mint did so during the cooling element of the trail which calls into question the hypothesis that it relates to an association with a thermally induced sweet taste as the latter is more associated with warming of the tongue. Future work should focus on better understanding the nature of these responses. It would be interesting to provide participants with prototypical chemical reference stimuli (ferrous sulphate, menthol and capsaicin) and identify the similarities/differences in the response to both thermal and chemical sensations. Another approach could be to utilise functional Magnetic Resonance Imaging to compare the cortical response to the thermal sensations with that of the equivalent chemical sensations. TTs could also be categorised into a group perceiving only minty or spicy sensations, and a second group perceiving prototypical tastes. Thermally stimulating the tongue to perceive these sensations whilst imaging the brain could also identify similarities or differences in the responses to aid in understanding the nature of the sensations.

An original objective of this study was to isolate the temperature range associated with each temporal rating and its associated taste quality as this may elucidate or eliminate temperature sensitive mechanisms such as TRPs that have been proposed as possible mechanisms. However, this was not possible with the more complex responses where multiple tastes were sometimes reported with one temporal rating (Table 1 and 2, Fig 6 and 7) indicating they arose together and/or interchangeably. In other instances (participant 32 and 33 during the 40°C trial), up to four tastes were perceived during a temperature trial, and were associated with inconsistent temporal ratings across replicates of the temperature trial. Better characterisation of these complex responses would aid in further determining the temperature range of perception across the wider range of thermal taste responses than was achieved in the current study, and would contribute to elucidating the mechanism/s, such as the TRP channels, that may be involved in the response. Adopting a Temporal Check All That Apply (TCATA) approach could effectively capture the temperature range of each individual taste perceived, and may aid in better characterising the more complex responses exhibited by some TTs, or a time intensity approach that measures the temporal response to each reported taste individually. This could also influence characterisation of groups of TTs exhibiting certain responses. For example sub categorisation of TTs reporting sweet compared to those reporting bitter, has been proposed as a way to explore differences across TTs (Bajec and Pickering, 2010). However, as only one paper reports such sub categorisation (Bajec et al., 2012) this deserves further investigation in order to better understand the wider impact of the variance in taste responses observed across TTs.

It cannot be ruled out that the experimental approach adopted to investigate TTs in more depth may itself have contributed to some of the variation in taste responses observed across TTs, which would not have influenced findings from previous studies adopting traditional thermal taste phenotyping protocols. These factors include collecting 'overall temporal taste intensity', as opposed to collecting a temporal taste intensity rating for each individual taste quality across separate replicates of the temperature trials, asking participants to report the perceived taste quality at the end of the 10 replicates of the temperature trials, as opposed to collecting a response after each individual replicate, and the decision not to deliver reference taste solutions when training participants on the taste qualities.

TTs are frequently observed to rate the intensity of gustatory and some trigeminal stimuli more intensely than TnTs (Green and George, 2004, Green et al., 2005, Bajec and Pickering, 2008, Yang et al., 2014), as well as some attributes in complex foods and beverages (Pickering et al., 2010a, Pickering et al., 2010b, Pickering et al., 2016, Pickering and Klodnicki, 2016) which may be associated with food preference (Pickering et al., 2016). It is unknown whether thermal sensations are also elicited when consuming food and beverage at warm and/or cool temperatures. If so, this may also have implications for food preference. For example this could explain why some individuals report metallic taints in cold beer that others do not perceive. Understanding the temperature range at which thermal tastes are perceived in the laboratory setting, such as that performed in the current study, aids in indicating the temperature range at which the sensations may also be perceived when consuming food and beverage.

4. Conclusion

This is the first study to report detailed variation in the thermal taste response within TTs. The taste quality, intensity, and number of tastes perceived was highly variable across participants. A number of different categories of temporal taste responses were identified when delivering thermal stimulation, and the temperature range at which taste was elicited differed across taste qualities and TTs. The onset of sweet taste was frequently reported as the temperature increased between 22-35°C, supporting the hypothesis that the TRPM5 may be involved in sweet perception. The findings of this study also raise questions over the phenotyping classification currently used, and highlights the need to review these protocols. This includes implementing methods to reduce the number of individuals uncategorised due to inconsistent reporting across replicates of temperature trials, or for reporting taste at a low intensity. These findings highlight the vast perceptual differences in taste perception across TTs in response to thermal stimulation of the tongue, and may suggest different mechanisms including the involvement of TRPs, variation in fungiform papillae anatomy and temperature sensitive gustatory neurons are involved. Understanding variation within and across TTs, and sub-categorising the different types of responses, may contribute to informing the impact that this may have on the perception of food and beverage during everyday consumption

5. Funding

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Figure and table legends

Figure.1. Thermode temperature across traditional warming (a), and cooling (b) trials, and modified 40°C (c) and 5°C (d) trials. Arrows (< --- >) indicate when participants were instructed to 'attend' to the test. e) mouthpiece used to guide the positioning of thermode on the tongue.

Figure. 2. Taste qualities (%) reported by TTs when phenotyping to classify TT status during the traditional warming (a) and cooling (b) trials.

Figure. 3. Correlation matrix showing example reproducibility in temporal taste ratings across 10 replicates for one participant of the a) 40°C trial, and b) 5°C trial for the i) first, ii) second, iii) third, and iv) fourth quartile, where the overall correlation coefficient (CC) for each example is indicated on individual design matrix.

Figure 4. PCA results associated with the 40°C trial. a) PCA analysis performed on the average temporal taste response across TTs identified four principal components which accounted for 85% of the variation in the data, the associated temporal responses are shown. b) PCA analysis performed on individual participant temporal taste responses identified four subgroups when plotting the time to peak of PC1 against PC2, these groups relate to the temporal responses identified in Figure 4a.

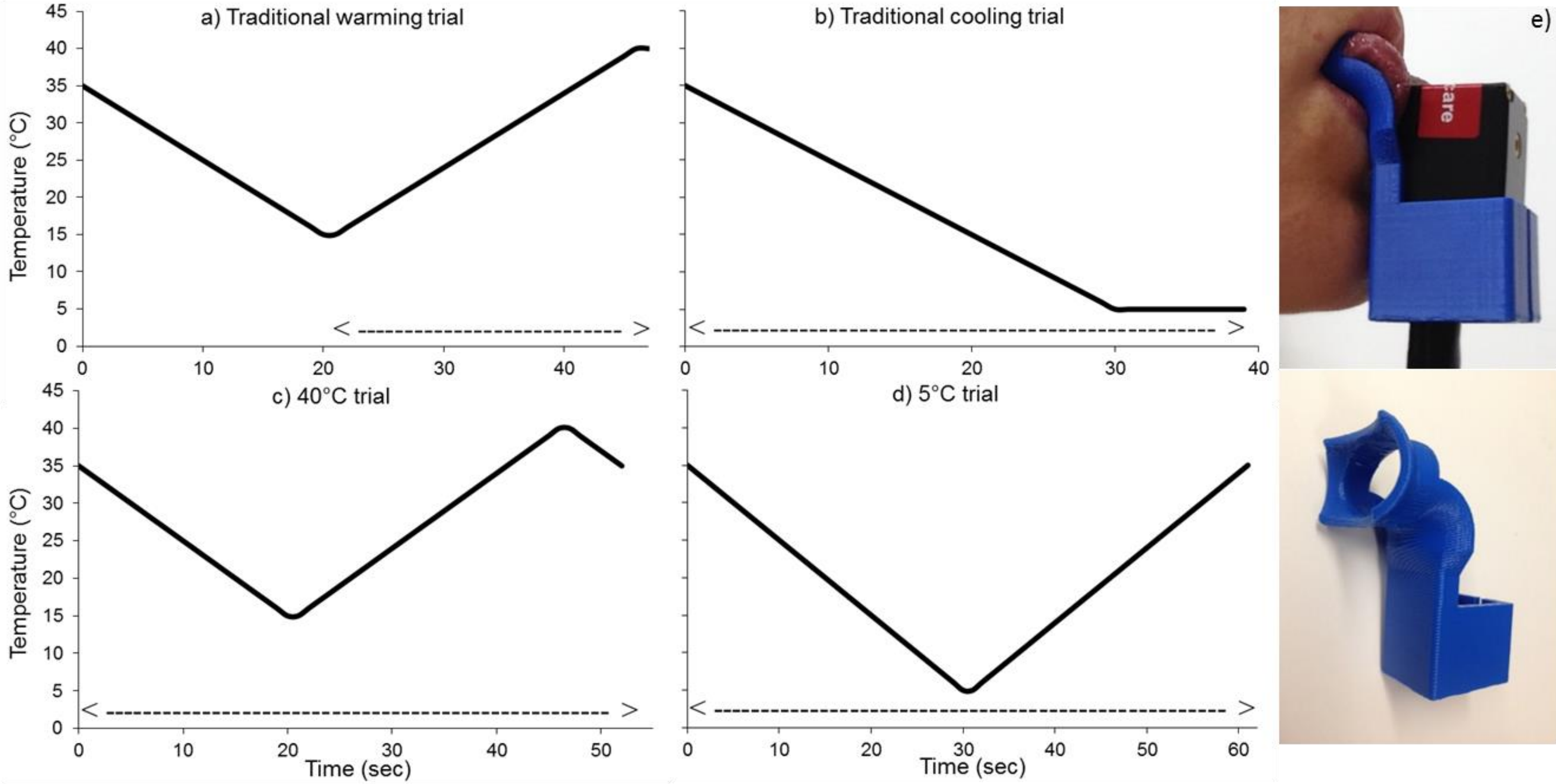
Figure 5 PCA results associated with the 5°C trial. a) PCA analysis performed on the average temporal taste response across TTs identified four principal components which accounted for 92% of the variation in the data, the associated temporal responses are shown. b) PCA analysis performed on individual participant temporal taste responses identified four subgroups when plotting the time to peak of PC1 against PC2, these groups relate to the temporal responses identified in Figure 5a.

Figure. 6. Mean temperature range over which the temporal taste response was reported by each participant (P) during the 40°C trial. Error bars show ± 1 S.D of the mean onset and offset of taste. White boxes indicate when the temperature of the thermode was warming (↑) or cooling (↓) the tongue (± 1°C/s).

Figure. 7. Mean temperature range over which the temporal taste response was reported by each participant (P) during the 5°C trial. Error bars show ± 1 S.D of the mean onset and offset of taste. White boxes indicate when the temperature of the thermode was warming (↑) or cooling (↓) the tongue (± 1°C/s).

Table. 1. Taste/s and mean intensity (stdev) reported during 40°C trial. *Inconsistent reporting across replicates prevented the mean taste intensity being calculated for some participants. Correlation coefficient (CC) indicates consistency of rating across 10 replicates. Final column indicates nature of inconsistency where possible.

Table. 2. Taste/s and mean intensity (stdev) reported during 5°C trial. *Inconsistent reporting across replicates prevented the mean taste intensity being calculated for some participants. Correlation coefficient (CC) indicates consistency of rating across 10 replicates.



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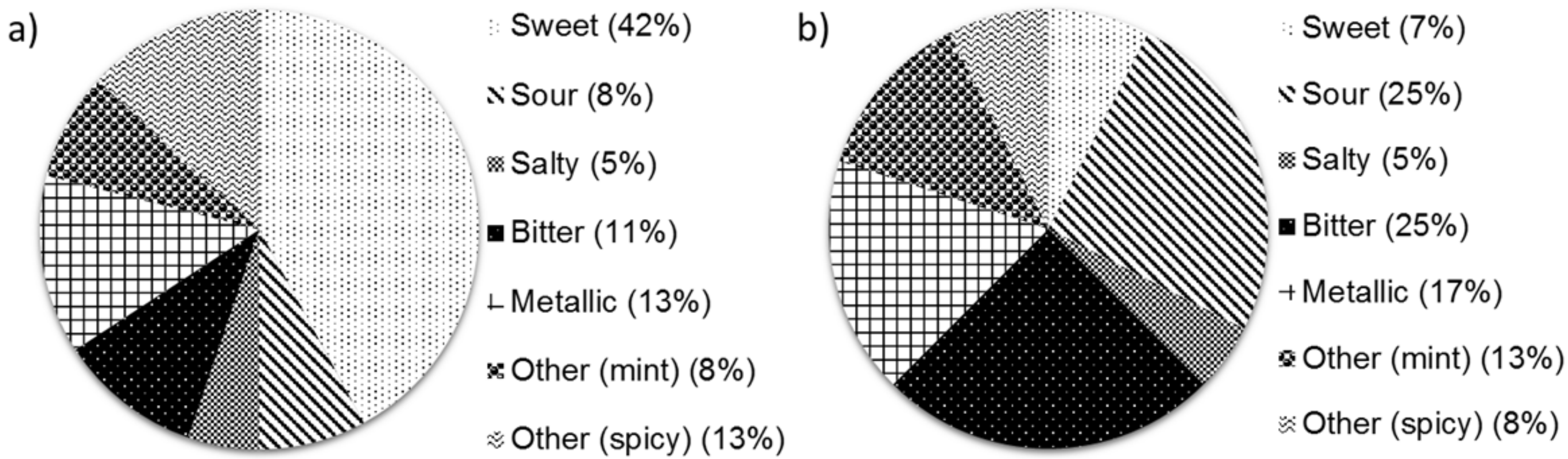

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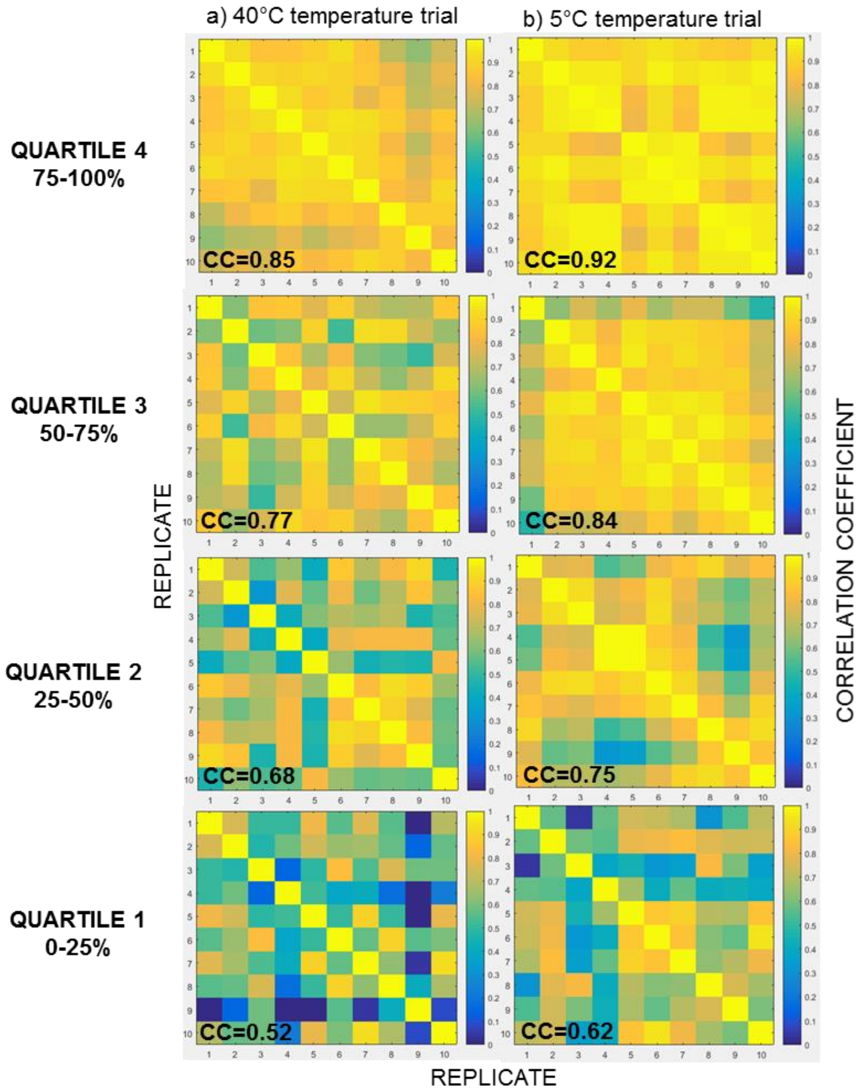

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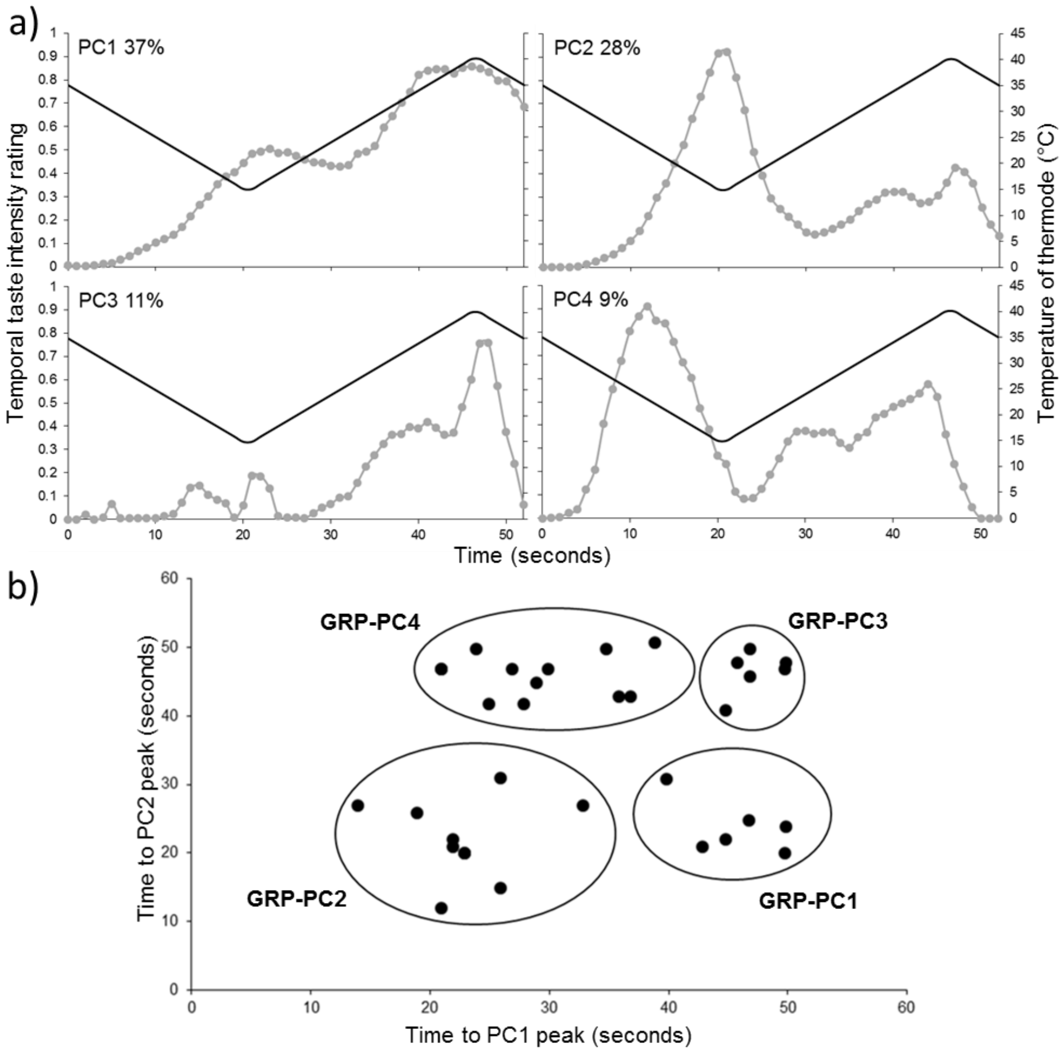

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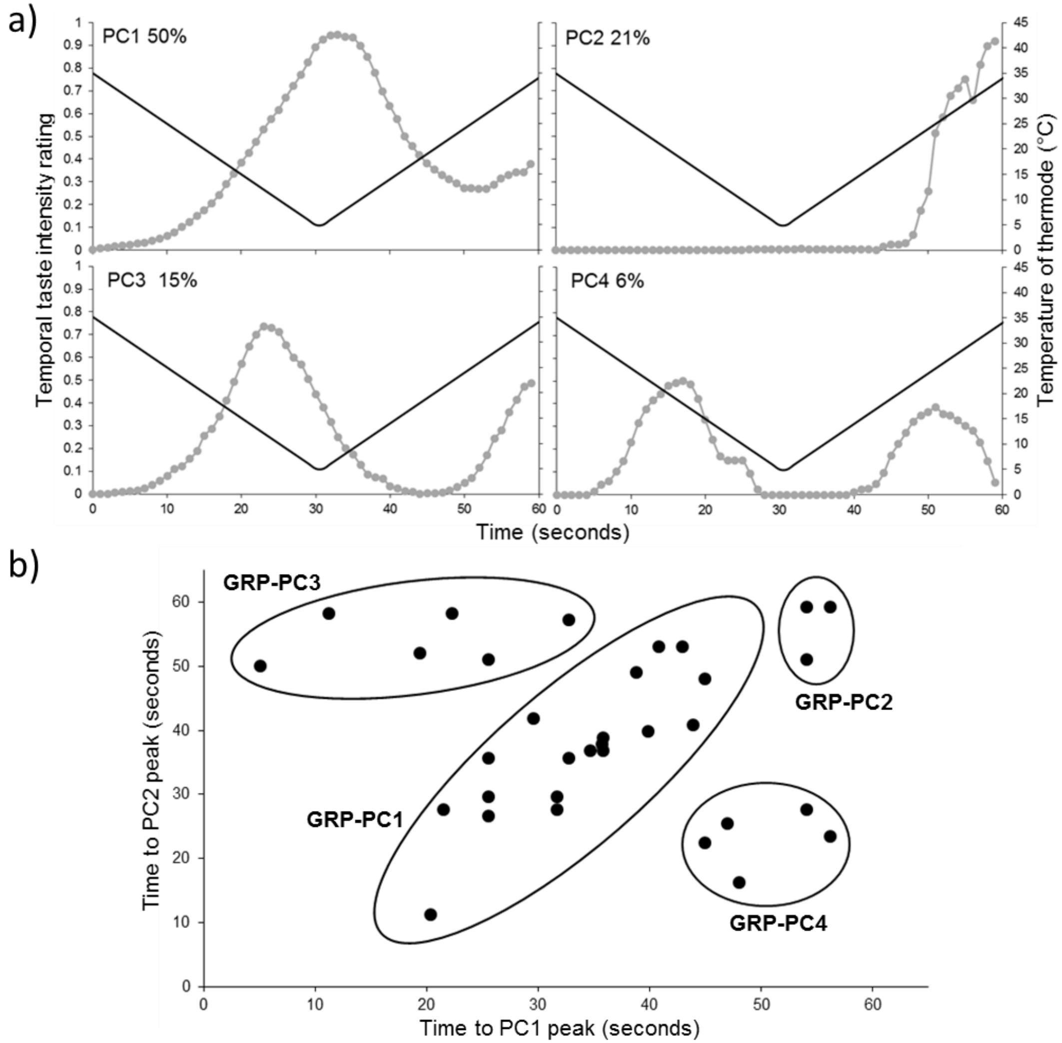
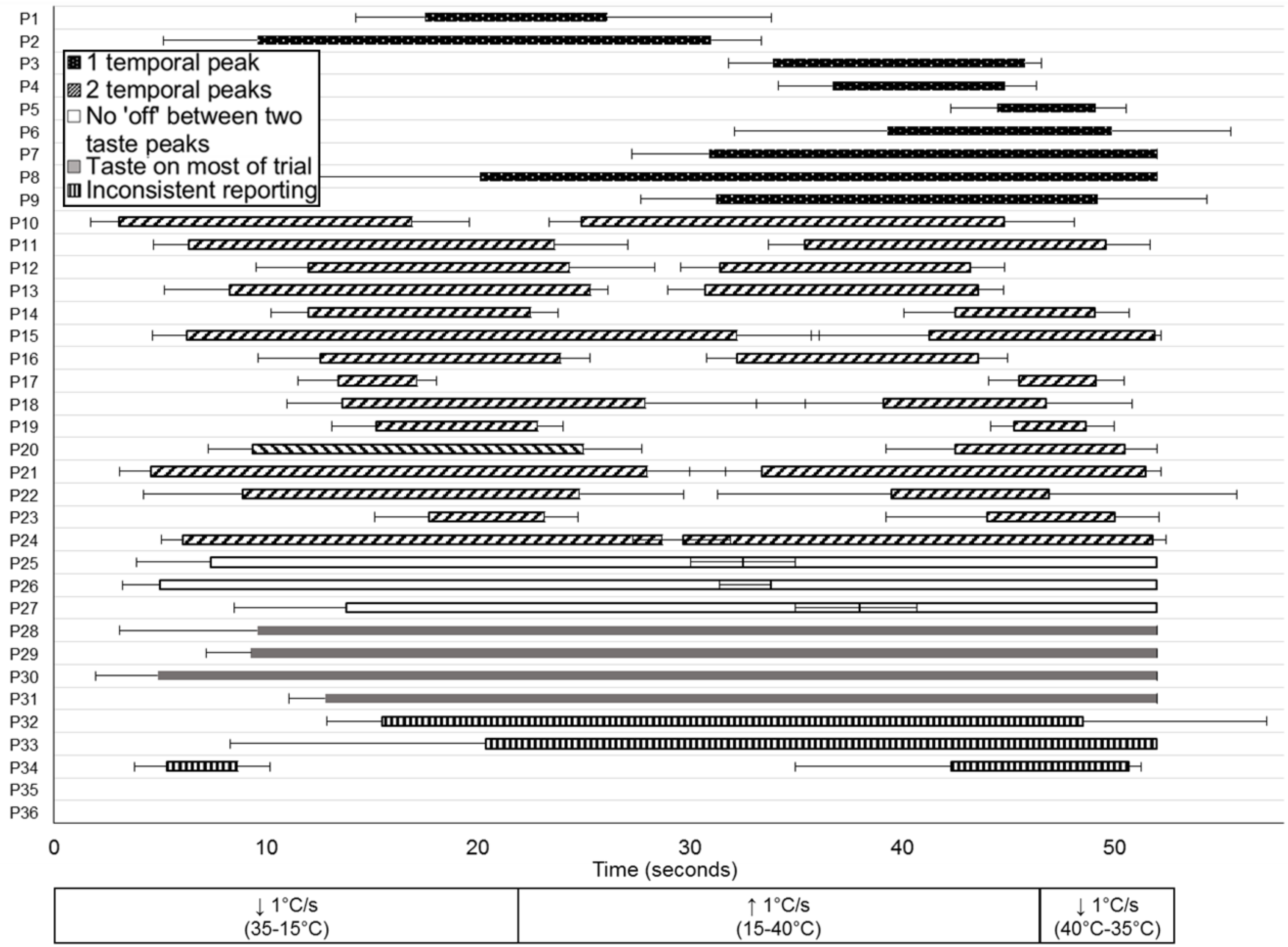


Figure 5. PCA results associated with the 5°C trial. a) PCA analysis performed on the average temporal taste response across TTs identified four principal components which accounted for 92% of the variation in the data, the associated temporal responses are shown. b) PCA analysis performed on individual participant temporal taste responses identified four subgroups when plotting the time to peak of PC1 against PC2, these groups relate to the temporal responses identified in Figure 5a.



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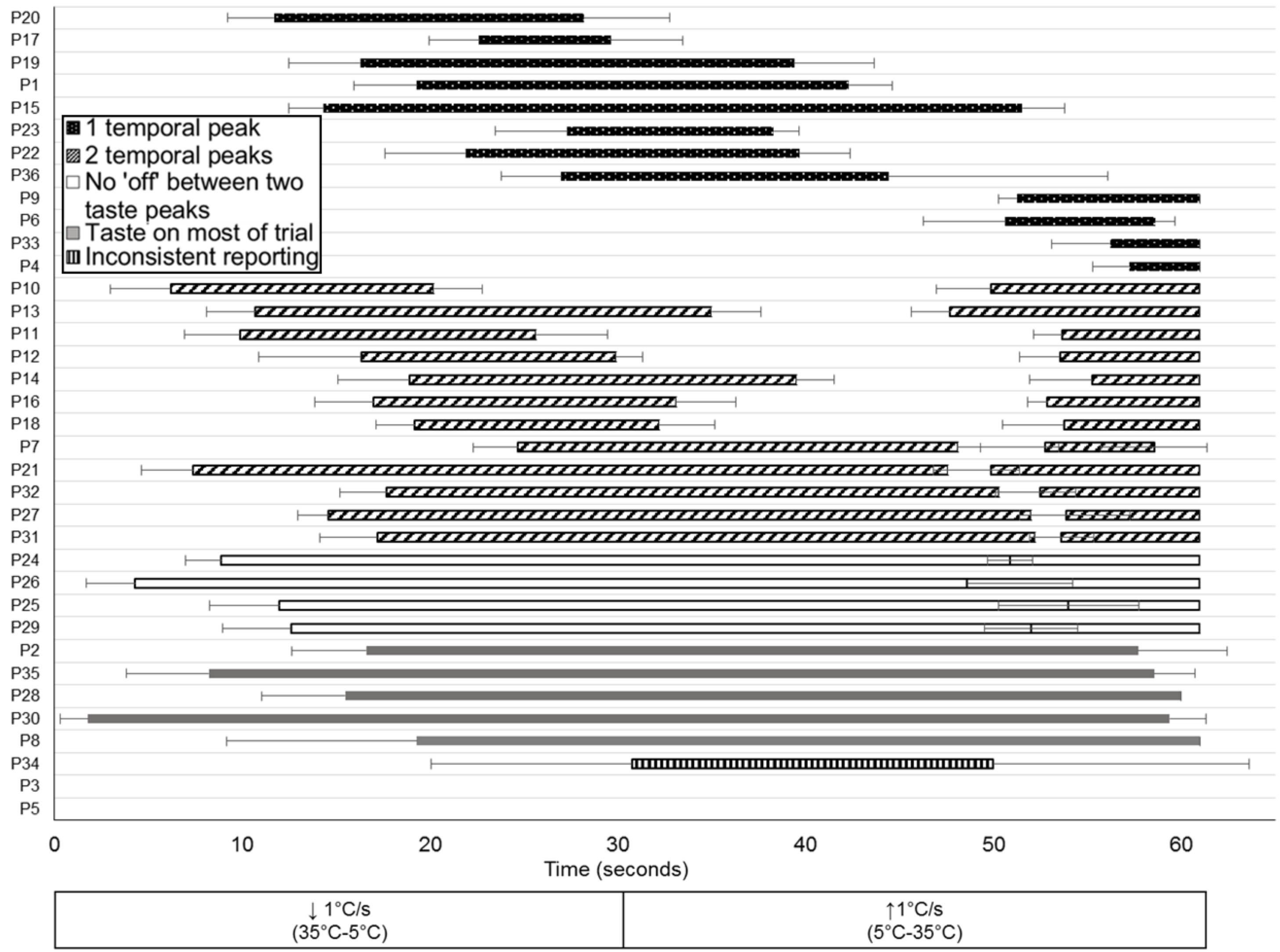

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Participant	First Taste/s	Mean intensity	Second taste/s	Mean intensity	CC	Rationale for low CC
1	Spicy	0.92 (0.41)			0.557	
2	Bitter	1.35 (0.68)			0.755	
3	Sweet	1.08 (0.02)			0.565	
4	Sweet	0.71 (0.56)			0.659	Rating on <10 replicates
5	Sweet	0.72 (0.51)			0.404	Rating on <10 replicates
6	Sweet	1.41 (0.25)			0.541	
7	Salty, sweet	1.20 (0.62)			0.686	
8	Salty, sweet	1.64 (0.93)			0.846	
9	Bitter, salty, umami	0.79 (0.60)			0.617	Rating on <10 replicates
10	Bitter	1.60 (0.50)	Bitter	1.54 (0.72)	0.659	
11	Bitter	1.29 (0.50)	Sweet	1.26 (0.39)	0.749	
12	Mint	1.56 (1.07)	Sweet	1.18 (0.73)	0.517	
13	Sour	1.65 (0.76)	Sweet	1.49 (0.60)	0.767	
14	Mint	1.33 (0.64)	Sweet	1.50 (0.83)	0.617	
15	Bitter	1.53 (0.62)	Salty	1.19 (0.81)	0.763	
16	Sour	1.22 (0.64)	Sweet	1.11 (0.56)	0.765	
17	Mint	0.23 (0.27)	Spicy	0.36 (0.03)	0.270	Rating on <10 replicates
18	Minty	1.31 (0.78)	Sweet	1.12 (0.76)	0.470	Taste perceived across similar temperature range but non-overlapping onsets/offsets
19	Metallic	0.94 (0.44)	Spicy	0.66 (0.04)	0.632	
20	Bitter	1.09 (0.30)	Spicy	1.00 (0.36)	0.662	
21	Mint, sweet	1.37 (0.53)	Spicy, sweet	1.42 (0.64)	0.628	
22	Minty	1.13 (0.72)	Bitter, spicy	0.96 (0.61)	0.602	
23	Metallic, bitter	0.67 (0.59)	Metallic, bitter	0.47 (0.59)	0.298	Rating on <10 replicates
24	Bitter, sour	1.77 (0.98)	Sweet	1.72 (0.42)	0.814	
25	Bitter	1.43 (0.78)	Sweet	1.44 (0.68)	0.824	
26	Mint, bitter	1.76 (1.10)	Sweet	1.72 (0.94)	0.822	
27	Mint	1.18 (0.83)	Sweet	0.99 (0.43)	0.560	
28	Salty, sweet	1.31 (0.86)			0.667	
29	Bitter, sweet	1.50 (0.73)			0.699	
30	Metallic, sweet	1.37 (0.78)			0.765	
31	Sour, bitter, sweet	1.43 (0.73)			0.828	
32	Sour, salt, sweet, spicy	*			0.428	Inconsistent across replicates
33	Bitter, sour, sweet	*			0.459	Inconsistent across replicates
34	Bitter	*	Sweet	*	0.008	Rating on <10 replicates and inconsistent across replicates
35	No taste				N/A	
36	No taste				N/A	

Table. 2. Taste/s and mean intensity (stdev) reported during 5°C trial. *Inconsistent reporting across replicates prevented the mean taste intensity being calculated for some participants. Correlation coefficient (CC) indicates consistency of rating across 10 replicates. Final column indicates nature of inconsistency where possible.

Participant	First Taste/s	Mean intensity	Second taste/s	Mean intensity	CC	Rationale for low CC
1	Spicy	0.91 (0.98)			0.844	
2	Bitter	1.35 (0.62)			0.889	
3	No taste				N/A	
4	Sweet	0.81 (0.50)			0.699	Rating on <10 replicates
5	No taste				N/A	
6	Bitter, sweet	0.97 (0.90)			0.686	Rating on <10 replicates
7	Salty	1.62 (0.65)	Sweet	1.08 (0.49)	0.801	
8	Bitter, salt, sweet, umami	*			0.556	
9	Bitter	0.29 (0.31)			0.865	
10	Bitter	1.56 (1.89)	Bitter	1.48 (0.50)	0.597	
11	Bitter	1.16 (0.49)	Sweet	1.15 (0.68)	0.579	
12	Mint, salt	1.20 (0.88)	Sweet	0.98 (0.47)	0.435	Taste perceived across similar temperature range but non-overlapping onsets/offsets
13	Sour	1.67 (0.56)	Sweet	1.41 (0.80)	0.810	
14	Minty	1.14 (0.67)	Sweet	0.69 (0.30)	0.754	
15	Bitter	1.66 (0.50)			0.924	
16	Sour	1.42 (0.82)	Sweet	1.13 (0.57)	0.823	
17	Metallic, mint	0.09 (0.05)			0.266	Rating <10 replicates
18	Minty	1.21 (0.65)	Sweet	1.05 (0.52)	0.794	
19	Metallic, sour, bitter	1.18 (0.84)			0.692	
20	Bitter	1.12 (0.50)			0.617	
21	Sweet, mint, salt	1.48 (0.36)	Sweet	1.24 (0.67)	0.754	
22	Minty	1.24 (0.73)			0.742	
23	Metallic	1.14 (0.81)			0.666	
24	Sour, bitter	1.87 (0.88)	Sweet	1.43 (0.61)	0.925	
25	Bitter	1.59 (0.52)	Sweet	1.35 (0.85)	0.905	
26	Minty, bitter, sour	1.78 (0.89)	Sweet	1.76 (0.86)	0.799	
27	Minty	1.52 (0.89)	Sweet	1.15 (0.65)	0.761	
28	Salty, sweet	1.32 (0.68)			0.794	
29	Bitter	1.51 (0.54)	Sweet	1.13 (0.55)	0.840	
30	Metallic	1.46 (0.62)			0.823	
31	Sour	1.49 (0.41)	Bitter, sweet	1.24 (0.74)	0.823	
32	Sour	1.65 (0.60)	Sweet	1.11 (0.45)	0.845	
33	Bitter, sour, sweet	1.23 (1.01)			0.474	Rating on <10 replicates
34	Bitter, sweet	*			0.002	Rating on <10 replicates and inconsistent across replicates
35	Sour	1.94 (0.27)			0.900	
36	Sour, spicy	1.02 (0.98)			0.587	Rating on <10 replicates

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