Title: Investigating the oronasal contributions to metallic perception.

Running title: Oronasal contributions to metallic perception.

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Abstract

Metallic taints elicited when consuming food can be unpleasant for the consumer, and are therefore problematic to food manufacturers. Although metallic has been proposed as a taste in the past, evidence remains inconclusive. This study investigates the oral and nasal contributions to metallic perception using sensory evaluation and headspace analysis using gas chromatography mass spectrometry (GC-MS). When sniffing the headspace over divalent salt solutions some were discriminated from water. GC-MS did not detect volatiles in the sample headspace, one hypothesis being that sample volatiles react with phospholipids in the nasal cavity and it is lipid oxidation products which are perceived. Copper sulphate was reported as metallic when tasted with the nose occluded to eliminate retronasal perception, suggesting a gustatory or trigeminal mechanism may be involved. This work indicates orthonasal stimulation is involved in metallic perception, and contributes to the ongoing debate over metallic being a taste, trigeminal or flavour response.

Key words ‘divalent salts’ ‘metallic’ ‘orthonasal’ ‘retronasal’ ‘taste’ ‘trigeminal’

Introduction

Metallic taints experienced when consuming food have negative implications for consumer acceptability, and therefore for food manufacturers. Such taints can arise from artificial sweeteners (Schiffman *et al*., 1979), when fortifying foods with compounds such as ferrous sulphate ($FeSO_4$) (Hurrell, 2002), and when consuming food from metal serving utensils (Piqueras-Fiszman *et al*., 2012). This problematic sensation extends beyond food and is associated with some medications (Gould *et al*., 1988), can be reported as a phantom sensation by cancer patients (Ravasco, 2005), those suffering from taste distortion (Nordin *et al*., 2004) and during burning mouth syndrome (Grushka, 1987). Developing strategies to mask this metallic sensation is therefore important, but to do this a better understanding of the mechanisms involved in its perception is needed. There are currently five widely recognised and accepted tastes (sweet, sour, salty, bitter and umami), and while metallic has been proposed as an additional taste quality (Bartoshuk, 1978), this is controversial and evidence remains inconclusive.

Divalent salts, electrical currents (Lawless *et al*., 2005), and solid metal (Laughlin *et al*., 2011) have been found to stimulate a metallic sensation when placed on the tongue. Volatiles can stimulate the olfactory pathway via the orthonasal (nose) and retronasal (nasopharynx) routes (Visschers *et al*., 2006). Using a nose clip to occlude the nose is a well-recognised technique for blocking the retronasal pathway to isolate the taste and oral trigeminal components of a stimuli from the retronasal aspects (Murphy and Cain, 1980). Occluding the nose significantly reduces the frequency (Hettinger, 1990) and intensity (Lawless *et al*., 2004) at which metallic is reported after oral exposure to $FeSO_4$, indicating retronasal stimulation is involved. This retronasal metallic sensation is commonly perceived to originate in the mouth and can inaccurately be identified as a taste, a process termed oral referral (Lim and Johnson, 2012). The predominant hypothesis relating to metallic perception states that lipid oxidation of the phospholipid bilayer in the oral cavity occurs after contact with divalent salts, releasing aldehydes and ketones which stimulate the retronasal pathway and elicit metallic perception (Omur-Ozbek *et al*., 2012). However, a reduction in metallic perception with nasal occlusion is not reported for $CuSO_4$, suggesting a taste or trigeminal mechanism is also involved (Epke *et al*., 2009). It is unknown whether volatiles released from the sample itself could also elicit lipid oxidation when coming into contact with the tissue in the nasal cavity via the orthonasal route, and to our knowledge the orthonasal sensations related to divalent salts have rarely been investigated.

This study had several objectives. The first was to identify if divalent salts can be detected orthonasally when smelling the sample headspace, and the second to identify the sensory qualities perceived when tasting the salts. The next objective was to assess the impact of retronasal stimulation on sample perception by evaluating the samples with the nose both open and occluded. An additional aim was to establish whether perceptual differences were observed across the different anions of ferrous salts. Finally headspace analysis was used to determine if any volatiles could be detected in the sample headspace.

Materials and Methods

Subjects

Subjects included staff and students at the University of Nottingham (23 females and 6 males), 29 were recruited in line with the ISO Standards for conducting a triangle test (BS: ISO 4120, 2004). All were non-smokers, aged 18-45 years old, reported being healthy, and having no known taste or smell abnormalities. The study had ethical approval from the University of Nottingham medical Ethics Committee (Q13112014 SoB Sensory Sci). Subjects gave written informed consent and an inconvenience allowance was provided. Subjects were instructed not to consume anything but water for at least one hour before testing.

Sensory Stimuli

Divalent salts were dissolved in deionised water from a reverse osmosis unit at supra-threshold concentrations (Table 1). Pharmaceutical or food grade compounds were used where possible: $FeSO_4$, $CuSO_4$, and $CaCl_2$. Otherwise reagent grade was used: $FeCl_2$ and FeGlu.

Pilot studies with researchers at the Sensory Science Centre at the University of Nottingham showed the samples to be equi-intense when assessed orally. Samples were made fresh every three hours to minimize oxidation effects (Lim and Lawless, 2005). A deionised water control sample was evaluated so that any sensations elicited from the water itself could be decoupled from that of the divalent salts. Samples (5 ml) were presented according to a randomised balanced design in odourless plastic medicine cups at room temperature, and were labelled with random three digit codes. Deionised water was provided for palate cleansing before and after all samples were consumed.

Table 1. Sample, formula, source and concentration of the 5 divalent salts sourced from Sigma Aldrich, Missouri, USA or Spectrum Chemicals, Northamptonshire, UK.

Stimulus	Formula	Source	Concentration (M)
Calcium chloride dehydrate	$CaCl_2 \cdot 2H_2O$	Sigma Aldrich	0.015
Iron II chloride tetrahydrate	$FeCl_2 \cdot 4H_2O$	Sigma Aldrich	0.002
Iron II D gluconate dihydrate	$FeC_{12}H_{22}O_{14} \cdot 2H_2O$	Spectrum Chemicals	0.001
Iron II sulphate heptahydrate	$FeSO_4 \cdot 7H_2O$	Sigma Aldrich	0.003
Copper II sulphate pentahydrate	$CuO_4S \cdot 5H_2O$	Sigma Aldrich	0.015

Sensory Methods

All data were collected on FIZZ software (Biosystems, Cergy-Pontoise, France). Tests were conducted in an air conditioned room (20±1°C) in individual booths designed to ISO Standards (BS: ISO 8589, 1988). The experimental procedure was divided into two parts.

Experiment 1

In the first session 5 triangle tests (one for each divalent salt) were conducted to determine if they could be differentiated from the water control. Order of presentation was randomised and balanced across subjects following British Standards (2005) protocols. Red lighting was used in the test area to disguise any potential visual cues. Samples were presented in lidded medicine cups and the lid removed when assessing the sample. Subjects were instructed to smell the headspace above the three samples and identify the odd one out.

Experiment 2

Subjects attended 2 further sessions. Before completing testing the different attribute qualities were described to them: sweet as the sweetness experienced from sugar; salty as the sensation from table salt; bitterness as found in coffee and tonic water; astringent as the ‘drying or puckering’ mouthfeel sensation experienced from red wine, green banana or strong tea; tingling as the mouthfeel sensation elicited by carbonated beverages; and metallic being like the taste of blood or metal. Reference samples to represent the attributes tested were not delivered so as to avoid restricting the qualities reported to the constraints of that specific reference sample. This is particularly important when evaluating metallic, as the metallic quality is reported to differ across divalent salts (Schiffman, 2000). To ensure the full range of oral receptors were coated, subjects were instructed to ingest the whole sample, hold it in the mouth, and lift the tongue to the palate 3 times before swallowing. They were asked to rate (on a 10-point line scale) their perceived maximum intensity for sweet, salty, bitter, metallic, astringent, and tingling, as these attributes are commonly reported to be associated with divalent salts during preliminary testing or in previous literature. A scale labelled from ‘none’ to ‘very intense’ was provided for each attribute. The option to report ‘other’ sensations was also given to reduce the occurrence of attribute dumping (Clark and Lawless, 1994). A 1 min inter-stimulus interval including palate cleansing with deionised water was compulsory. Samples were assessed under two conditions: (a) with the nose open, and (b) with the nose occluded using a swimming nose clip (Slazenger, Shirebrook, UK). Two repetitions were collected for each sample under each condition. During each session 50% of the subjects tested samples with the nose open, and 50% with the nose occluded, with the condition being reversed during the second session. Data was collected under Northern Hemisphere daylight lighting.

Data Analysis

To determine if the divalent salts could be detected orthonasally during the triangle test, the number of correct identifications was tested for significance using binomial statistics (α= 0.05) (BS ISO 6658: 2005). A three factor (sample, nose condition, replicate) analysis of variance (ANOVA) with interaction (sample*nose condition) and Tukey’s Honestly Significant Difference (HSD) post hoc test were undertaken to identify where any differences existed across sample intensity ratings. SPSS, version 21 (SPSS IBM, USA) was used for all analyses (α=0.05).

Headspace Analysis

Headspace solid phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS) were used to explore whether any volatiles were present in the sample headspace.

Samples

Samples were prepared using the chemicals indicated in Table 1 (0.0, 0.003, 0.3M). Samples consisted of 8 ml of solution placed in 20 ml amber glass headspace vial that was commercially clean, used as supplied, and capped with Teflon-lined silicone crimp caps.

GC-MS Analysis

Samples were tested in a Thermo Scientific Gas Chromatography Mass Spectrometer (Thermo Scientific, Hemel Hempstead, UK). A Supelco solid phase microextraction (SPME) sampling unit was used, with a 50/30 nanometer DVB/CAR/PDMS Stableflex fibre which was exposed to the headspace of the vial for 10 minutes to extract the volatiles using an out of tray method. Fibre was desorbed in the injection port at 230 °C, for 5 minutes and in splitless mode. A Trace GC Ultra was used to run GC analysis using a ZB-wax GC column (Phenomenex), which was 30 metres in length, 0.25 ID mm, 1.00 film thickness and using a helium flow rate constant pressure at 18 PSI. The temperature programme was 40 °C for 1 minute, then heated to 250 °C at 8 °C/min and held for 1 minute. Mass spectrometry Dual Stage Quadrupole (DSQ) was run with a full scan for mass range of *m/z* 15-200 and an ion source temperature of 200 °C, and mass scan starting at 0.5 minutes. Each sample was run in triplicate, and sample presentation order was randomised to eliminate order effects.

Data Analysis

The National Institute of Standards and Technology library was used to identify compounds that were likely present in the samples. Background subtraction was undertaken to identify compounds present in the sample headspace that were not in the water control. The same method was used to compare differences across divalent salts, as well as the low and high concentration samples. A specific search for the selected mass fragments of 1-octen-3-one (mwt 126g/mol) and 1-nonen-3-one (mwt 140g/mol) was undertaken, as they have previously been reported in the headspace of divalent salts (Lubran *et al*., 2005). Differences across the 3 replicates for each sample were compared for consistency.

Results

Sensory Characterisation

Experiment 1

Table 2 lists the number of correct identifications and related probability values for the triangle tests investigating orthonasal stimulation. $FeCl_2$ and $FeSO_4$ were the only samples discriminated from the water control.

Table 2. Frequency of correct identifications and associated p values in triangle tests.

	$CaCl_2$	$FeCl_2$	FeGlu	$FeSO_4$	$CuSO_4$
Correct response	10	22	7	15	11
p value	0.55	<0.001	0.90	0.03	0.36

Experiment 2

ANOVA showed that global intensity ratings differed across replicates for metallic ($p < 0.001$) and astringency ($p = 0.019$) only, where replicate 1 was rated higher than 2. However, Tukey results showed this difference across replicates was not significant ($p > 0.05$) when analysing intensity ratings for these attributes at the individual sample level. The only significant interaction between sample and nose condition occurred with the metallic attribute, which was due to a magnitude effect where the ferrous salts were rated significantly more intense ($p < 0.001$) than all other samples under the nose open condition. ANOVA showed that nose condition had an effect on global attribute intensity rating as all attributes except for tingling ($p = 0.254$) were rated higher ($p < 0.05$) with the nose open compared to occluded.

Fig. 1. shows metallic ratings for all ferrous salts were significantly higher than the water control sample with the nose open ($p < 0.05$), but not with the nose occluded ($p > 0.05$). $CuSO_4$ was perceived significantly more metallic than water with the nose open ($p < 0.001$) and occluded ($p = 0.038$). $CaCl_2$ was not rated more metallic ($p > 0.05$) than water under either nose condition.

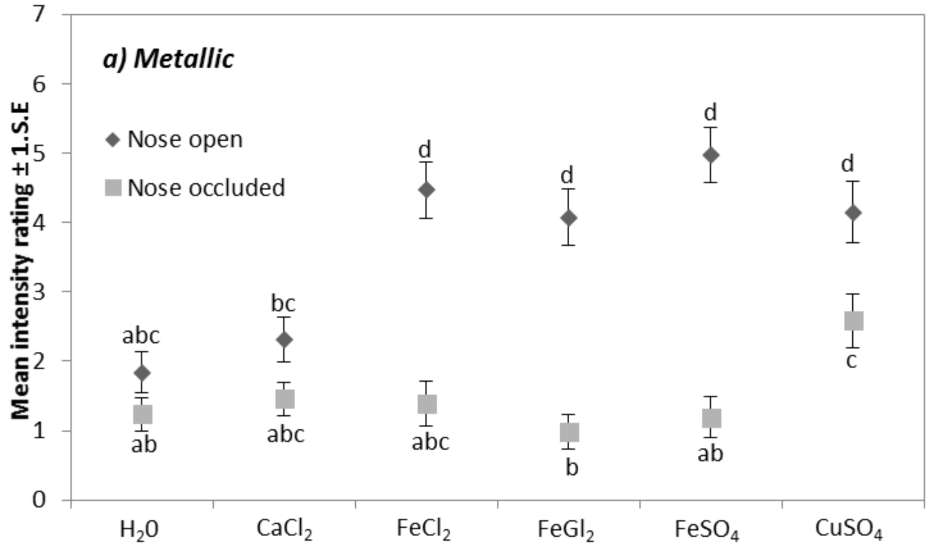
Tukey results for divalent salt attribute qualities significantly higher ($p < 0.05$) than that of the water control are shown in Table 3. From these findings $CaCl_2$ was reported to be bitter, astringent and salty, ferrous salts metallic and sometimes sweet, while $CuSO_4$ was the most complex sample for which all attributes excluding sweet were reported.

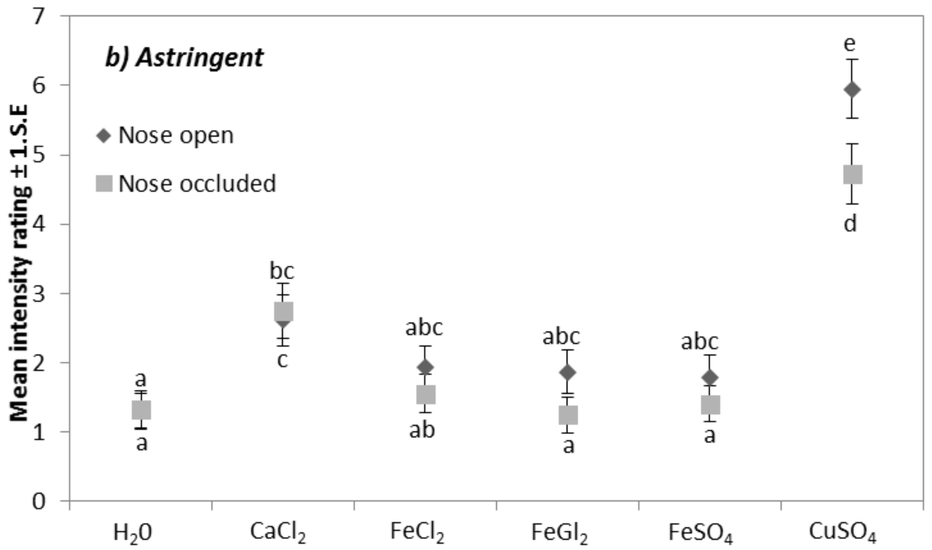
Table 3. Tukey results showing sample attribute intensity ratings compared to the water control under the nose open (NO) and nose closed conditions (NC) with significance level indicated; < 0.05*, < 0.01**, < 0.001***.

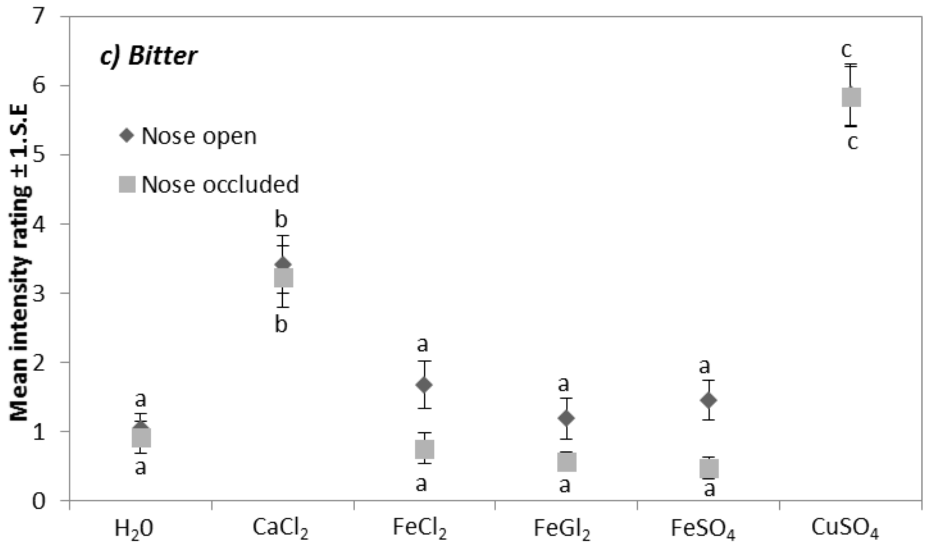
Divalent salt	**$CaCl_2$**	**$CaCl_2$**	**$FeCl_2$**	**$FeCl_2$**	**FeGlu**	**FeGlu**	**$FeSO_4$**	**$FeSO_4$**	**$CuSO_4$**	**$CuSO_4$**
Nose condition	**NO**	**NC**	**NO**	**NC**	**NO**	**NC**	**NO**	**NC**	**NO**	**NC**
Metallic	2.32	1.46	4.47***	1.39	4.08***	0.99	4.97***	1.19	4.14***	2.58*
Astringent	2.61*	2.75**	1.94	1.55	1.87	1.24	1.79	1.41	5.95***	4.72***
Bitter	3.41***	3.24***	1.67	0.76	1.19	0.57	1.45	0.47	5.87***	5.84***
Tingling	0.52	0.57	0.46	0.33	0.64	0.42	0.32	0.33	1.12**	0.94
Sweet	0.57	0.45	2.19***	1.3	1.43	0.94	2.08***	1.88***	1	0.51
Salty	2.43***	2.11***	1.27	0.48	1.06	0.41	1.05	0.6	1.3	1.63**

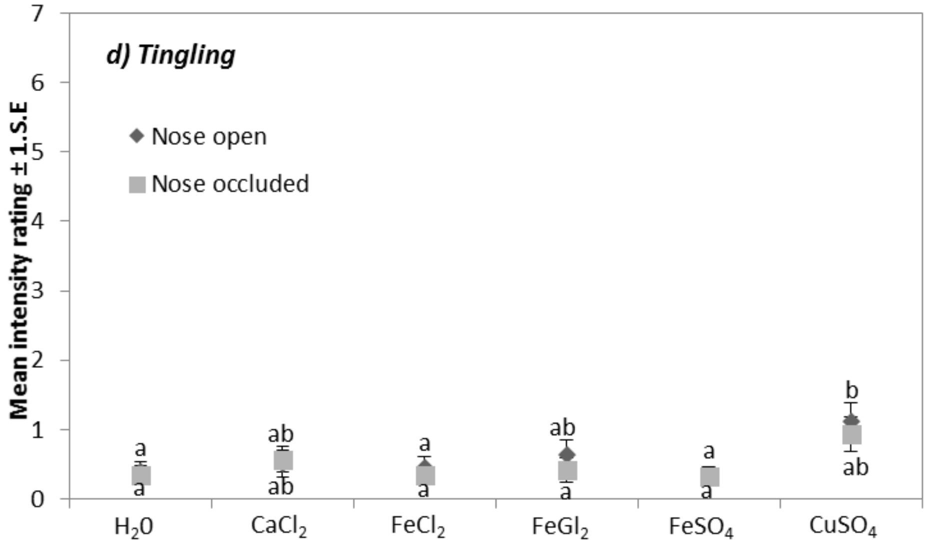
Headspace Analysis

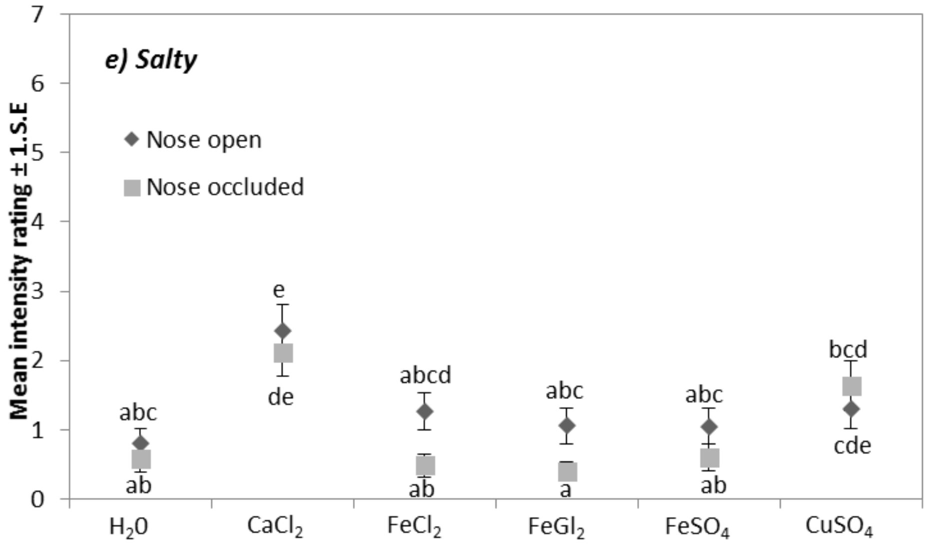
Results across sample replicates were consistent, with the exception of $FeCl_2$ where ethyl ether, ethyl chloride, ethyl acetate, ethanol and ethyl chloroacetate were found in replicate 1, but not replicate 2 or 3. No compounds were identified in any other sample.


a) Metallic
Mean intensity rating ±1.S.E
Nose open
Nose occluded
abc
bc
d
d
d
d
ab
abc
abc
b
ab
c
H20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$


b) Astringent
Mean intensity rating ±1.S.E
Nose open
Nose occluded
e
d
bc
c
abc
abc
abc
a
a
ab
a
a
H20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$


c) Bitter
Mean intensity rating ±1.S.E
Nose open
Nose occluded
c
c
b
b
a
a
a
a
a
a
a
a
H20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$


d) Tingling
Nose open
Nose occluded
Mean intensity rating ±1.S.E
7
6
5
4
3
2
1
0
a
a
ab
ab
a
a
ab
a
a
a
b
ab
H_20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$


e) Salty
Nose open
Nose occluded
Mean intensity rating ±1.S.E
7
6
5
4
3
2
1
0
abc
ab
e
de
abcd
ab
abc
a
abc
ab
bcd
cde
H_20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$

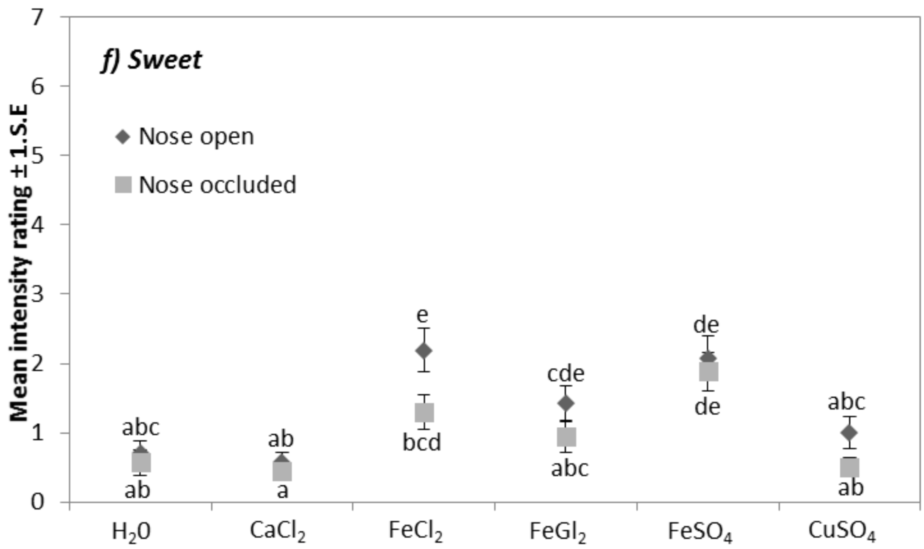

f) Sweet
Nose open
Nose occluded
Mean intensity rating ±1.S.E
7
6
5
4
3
2
1
0
abc
ab
ab
a
e
bcd
cde
abc
de
de
abc
ab
H_20
$CaCl_2$
$FeCl_2$
$FeGl_2$
$FeSO_4$
$CuSO_4$

Figure 1. Attribute intensity ratings with the nose open and occluded. Mean intensity rating ± 1 standard error, for a) metallic, b) astringent, c) bitter, d) tingling, e) salty and f) sweet. Data points with different letters abcde show significant differences ($p < 0.05$) across samples and nose conditions according to the Tukey post hoc test.

Discussion

Orthonasal Perception

Orthonasal stimulation by divalent salts has not been well researched, therefore its possible contribution to metallic perception is poorly understood. $FeSO_4$ solutions are thought to produce little (Lubran *et al*., 2005) or no (Lawless *et al*., 2004) aroma as they are not typically considered volatile. In contrast to Lawless *et al.* (2004), the current study found $FeSO_4$ was discriminated from water, indicating orthonasal stimulation is occurring, Table 2. This variance across studies could be due to the different sample concentrations, or discrimination tests used (Ennis *et al*., 2014).

Using SPME to collect volatiles in the sample headspace, and the human nose as a sensitive and selective detector of the odour active compounds using gas chromatography olfactometry (GCO), Lubran *et al.* (2005) identified the odorants 1-nonen-3-one and 1-octen-3-one, which were described as 'metallic', in $FeSO_4$ sample headspace. These volatiles were not detected in the current study, which may be due to differing sample temperature and purge times used across studies. Here GC-MS headspace analysis did not identify any volatiles present in the $FeSO_4$ or $FeCl_2$ sample that were not present in the water control, which could be because the GC-MS equipment is not sensitive enough to detect the compounds perceived by the human nose. Sample detection during the triangle test could arise from the release of low concentration volatiles from the sample itself, or another hypothesis being that volatiles released from the sample could cause lipid oxidation upon contact with tissue in the nasal cavity, and it is the by products that are detected, as found in the oral cavity (*Omur-Ozbek et al*., 2012). When smelled orthonasally FeSO4 has been described as a 'tingling irritation' (Lubran *et al*., 2005), and so another question that arises is whether the reported sensation is due to an aroma and/or trigeminal response. Compounds which were not present in the water control were detected in the headspace of replicate 1 of the $FeCl_2$ sample. As they were not present in replicate 2 or 3 and are not typically associated with $FeCl_2$, this is likely due to some form of contamination.

Results from the orthonasal sensory testing indicate that volatiles released from ferrous salts could impact metallic perception more than once thought, and thus highlights the need for more research investigating this quality. A description of the attribute quality detected when orthonasally sniffing the sample headspace was not collected, but further exploration is recommended.

Retronasal and Oral Metallic Perception

In line with previous research (Lim and Lawless, 2006) Fig. 1a shows that occluding the nose significantly reduced (p < 0.05) the intensity of the metallic sensation reported for ferrous salts, supporting the hypothesis that retronasal stimulation is the key driver of metallic for these salts. When $FeSO_4$ comes into contact with skin on the hand (Glinderman *et al*., 2006) and oral cavity (Omur-Ozbek *et al*., 2012) lipid oxidation occurs, causing the formation of formaldehyde, acetaldehyde, proprionaldehyde and increased protein-carbonyls, which are thought to stimulate the retronasal pathway, eliciting a metallic sensation. ANOVA showed the global intensity rating for metallic was higher (p < 0.001) on replicate 1, although this was not seen with the Tukey analysis at the individual sample level. This global effect could be due to a reduced rate of lipid oxidation and subsequent metallic perception on replicate 2, therefore future testing could benefit from increased palate cleansing time between samples, or a reduction in the number of samples tested per session. Increased 'metallic' smelling volatiles were found in the headspace of $FeSO_4$ samples at 37 °C but not 22 °C during GCO (Lubran *et al*., 2005), suggesting the temperature inside the mouth may stimulate the release of volatiles that are not associated with lipid oxidation, but may be detected retronasally and contribute to metallic perception. $FeSO_4$ (≥ 5mM) can be discriminated from water with the nose occluded (Lim and Lawless, 2005), and when asked to describe the sensation assessors reported bitter, sour, sweet, astringent, metallic and electric. When applying the same sample to a non-gustatory part of the lip the solution could not be discriminated, suggesting there may be a gustatory component to metallic perception. The current study reports a different response for $CuSO_4$; while occluding the nose reduced the metallic rating (p = 0.006), it remained higher (p = 0.038) than that of the water control. $CuSO_4$ also induces lipid oxidation and the subsequent volatile release (Omur-Ozbeck *et al*., 2012), which explains the difference observed across nose conditions. However, this does not explain the metallic quality reported under the nose occluded condition both here and in previous studies (Epke *et al*., 2009; Lawless *et al*., 2004). The same result is also seen for both solid metal stimuli and electrical stimulation of the tongue (Lawless *et al*., 2005), which has led to the hypothesis that different mechanisms may be involved in metallic perception reported across stimuli. Transient receptor potentials (TRP) are a family of cation channels involved in the transduction of chemical stimuli into taste, olfaction and trigeminal sensations, and have been associated with the perception of divalent salts (Riera *et al*., 2007). One possible mechanism is the involvement of TRPV1, TRPM5 and T1R3. When expressed in cultured cells in vitro, the TRPV1 was activated not only by artificial sweeteners which have been found to evoke a metallic quality, but also by solutions of FeSO4, $CuSO_4$ and zinc salts, thus suggesting it may be involved in metallic perception (Riera *et al*., 2007). Comparing the behavioural response to divalent salts in wild type (WT), TRPV1 knockout (KO), TRPM5 KO and T1R3 KO mice, Riera *et al.* (2009) found these channels likely to influence perception as measured preference for divalent salts differed across the mice. However, divalent salts have multiple sensory attributes making it difficult to pinpoint which of these are affecting sample perception (Spence *et al*., 2015) and the hedonic differences observed.

No difference in metallic rating for $CaCl_2$ was reported when compared to water with the nose open or occluded ($p < 0.005$). Although it has previously been reported as metallic, the rating has not always been compared to a water control (Lawless *et al*., 2003; Yang and Lawless, 2005) and the metallic perception could, at least in part, be attributed to the metallic quality reported for deionised water (Dalton *et al*., 2000). However, Lawless *et al.* (2004) found that metallic intensity varied across different calcium anions, indicating a metallic component that was not observed in the current study.

Oronasal Qualities of Divalent Salts

An additional objective was to determine the non-metallic qualities reported for the samples, and differences across ferrous salts. Attributes discussed in this section were identified as those reported significantly higher ($p < 0.05$) than the water control sample, Table 3. Occluding the nose did not affect bitter and astringency ratings, which is typically expected for gustatory and trigeminal stimuli (Lim and Lawless, 2006). A reduction in sweetness was reported with nasal occlusion for the

$FeCl_2$ sample (p = 0.028), but not for $FeSO_4$ (p = 1.00). One hypothesis being that the perceived sweetness for $FeCl_2$ is a result of sweet 'smelling' volatiles that are perceived as taste due to gustatory referral (Lim and Johnson, 2012). Alternatively, volatiles detected from this sample enhanced sweet perception when the nose was open, as is often seen with taste aroma interactions (Noble, 1996), including enhancement of sweetness (Pfeiffer *et al*., 2005). Saltiness was only reported for $CuSO_4$ under the nose occluded condition, perhaps because the intense metallic sensation dominates perception under the nose open condition. Tingling was reported for $CuSO_4$ with the nose open but not occluded, indicating the sensation originates from nasal stimulation as a result of pungency produced by volatiles that are detected retronasally (Cometto-Muniz and Hernandez, 1990). Volatiles could be released from lipid oxidation, or directly from the sample due to the temperature increase in the oral cavity, which would explain why they were not detected orthonasally.

Sweet was reported for the ferrous salts, while attributes reported in prior literature are bitter, astringent, sweet, sour, salty (Lim and Lawless, 2006), soapy and sulphurous (Hettinger *et al*., 1990). $CuSO_4$ was found to elicit bitter, astringent, salty and tingling. Prior research has found copper salts to be astringent, bitter (Lawless *et al*., 2004) and sour (Epke *et al*., 2009). $CaCl_2$ was found to be bitter, astringent and salty, while previously reported attributes are bitter, salty, sour, umami and astringent (Lawless *et al*., 2003; Yang and Lawless 2005). Potential reasons for the limited attribute qualities reported in this study compared to those evidenced elsewhere are multifactorial; here naïve assessors were used, in comparison to a trained panel that has previously been used to provide detailed descriptive profiles (Yang and Lawless, 2005; Epke *et al*., 2009). The attributes which subjects were asked to rate were limited to 6 to avoid overwhelming the naïve assessors. Although subjects were given the option to report 'other' perceived attributes, this restricted list may have reduced the qualities reported as attributes are more likely to be rated when listed as opposed to free choice profiling (Lawless *et al*., 2005). Another consideration being that the sample concentration affected the qualities perceived across studies (Murphy and Cain, 1980). Divalent salt attribute qualities change over time (Yang and Lawless, 2006) and so the point at which the intensity rating is taken (immediate or aftertaste) may have contributed to the variability.

The anion can affect the sensory qualities exhibited by divalent salts. Here differences across ferrous anions were observed; unlike FeGlu, $FeCl_2$ and $FeSO_4$ had a perceivable orthonasal aroma and were sweet. Similar anion effects for ferrous salts have been evidenced by a number of researchers (Lawless *et al*., 2003; Lim and Lawless, 2006; Yang and Lawless, 2005; Yang and Lawless, 2006) and would be interesting to further explore, with attention focussed on differences in the headspace volatiles.

Conclusion

Discrimination testing found orthonasal detection of ferrous salts may contribute to their perception more than previously thought. This could either be due to the detection of volatiles coming from the sample itself, from lipid oxidation by products when the sample volatiles come into contact with tissue in the nasal cavity. Headspace analysis of the samples did not find volatiles which could explain the sample discrimination. Occluding the nose when tasting samples reduces the metallic perception for ferrous salts, indicating retronasal stimulation is important for these samples. However, metallic is still perceived for $CuSO_4$ when the nose is occluded, suggesting a second gustatory or trigeminal mechanism is involved for this sample. This work contributes to the ongoing debate over metallic being a taste, trigeminal or flavour response. Although metallic may have a gustatory component, particularly for $CuSO_4$, it is thought that defining it as a taste could be a misnomer, particularly when referring to the sensation which arises from $FeSO_4$, therefore metallic 'sensation' may be a more accurate description.

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