

The sense of smell: contributions of orthonasal and retronasal perception applied to metallic flavor of drinking water

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ABSTRACT

The human senses are important assessment tools that assist the water industry with effective water quality and water security monitoring. Flavor perception is the combination of taste and odor; odor perception is a combination of orthonasal and retronasal perception. Orthonasal perception is associated with “the sniff” and describes the entry of odor molecules through the nostrils to the nasal cavity followed by interaction with odor receptor neurons. Retronasal perception describes the mechanism in which odor molecules present in the mouth are transported to the back of the nasopharynx and then to the odor receptor neurons. Although both pathways bring the odor molecules to the olfactory epithelium where they are perceived and processed by the brain, the retronasal route is more important for humans in terms of detecting ingested odors. For copper (II), the metallic flavour is a combination of a weak bitter taste and a strong metallic odor that is produced as a biochemical reaction in the mouth. Copper (II) in the presence of oxygen causes lipid oxidation of arachidonic, linoleic, and oleic acids in the mouth. A more thorough understanding of the sense of smell will greatly aid the water industry in its quest to produce a palatable product.

Key words | odor, olfactory, orthonasal, retronasal, smell, taste

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INTRODUCTION: WHAT IS FLAVOR IN WATER?

Water is a refreshing beverage that is enjoyed throughout the world. Although water utilities and treatment personnel have always been concerned with drinking water palatability, increasing consumer demand for water free of unwanted tastes and odors has led to a greater focus on the sensory quality of drinking water (Devesa *et al.* 2004; Dietrich 2006; Bae *et al.* 2007; Burlingame & Mackey 2007).

The senses of taste and smell are referred to as the “chemical senses” because they detect chemicals. Chemicals that can be tasted are called “tastants” and chemicals that can be smelled are called “odorants”. These two senses are well integrated and it is therefore often difficult for humans to distinguish between taste and smell when eating foods and drinking beverages. Human perception of flavor provides both the enjoyment of eating and protection

from spoiled or toxic foods and beverages. Human survival instincts and personal experiences contribute to an individual’s ability to discriminate foods and beverages that do not have the “right” flavor.

Consumers generally rate the flavor, or combined taste and odor of drinking water, but frequently refer to this only as “taste”. Water treatment personnel are concerned with the tastes, odors, and flavor of their product. As such, utilities will employ flavor profile analysis (Standard Method 2170; APHA 2006) to independently rate the water’s taste and odor qualities. There are only five tastes: sour, sweet, salty, bitter and umami (or “savory” which was added in the 1990s) (Burlingame *et al.* 2007). Each of these five tastes is associated with one of five receptor types in the mouth, or oral cavity. In contrast to the five tastes, there are

an estimated 10,000–100,000 odors which can be detected and perceived by the nearly 350 types of human odor receptors (Sullivan *et al.* 1995; Buck 2004). The human sense of smell detects volatile odorants that provide us with the complex flavors we perceive. Beverages like tea and coffee would just be perceived as bitter tasting if not for the multifaceted aromas generated by odorants in these products. When we ingest a beverage, flavor is actually the combination of feeling in the mouth; the five tastants interacting with taste buds and signalling the brain about sweet, salty, sour, bitter and umami; and the many aromas that we smell through our nostrils and re-smell because the odor molecules volatilize in our mouth and also travel through the back of the throat to the nose.

The intricacies of the human sense of smell are due not only to the myriad of olfactory receptors and the multitude of odorants that these can detect, but also to the two pathways to the nose: the orthonasal and retronasal passages. Orthonasal odors are those that enter the nose from the outside environment through the nostrils. Retronasal, which means “behind the nose”, represents odors that travel to the nose from the mouth or inside the body (Figure 1). Retronasal perception is the major pathway for giving “flavor” to foods and beverages (Burdach & Doty 1987; Shepherd 2006).

Understanding human physiology and the sense of smell is critical to the water industry’s ability to produce water without taste-and-odor problems. Utilities rely on customer feedback as a component of their water quality program and also as indicators of potential water security breaches. It is necessary for utilities to understand the ability of humans to detect water quality problems. The human chemical senses are instruments that act as drinking water quality sensors both in the laboratory and in the home. Like any instrument or sensor, it is necessary to understand the operating principles including mechanisms of reaction, detection limits, sensitivities, and interferences. The purpose of this article is to provide insight into the principles of operations of the human sense of smell, specifically: 1) inform water industry personnel and researchers about the current state of knowledge of the olfactory system; 2) demonstrate its value to assessing the flavor of water and also evaluating odor problems in drinking water with metallic flavour as an example.

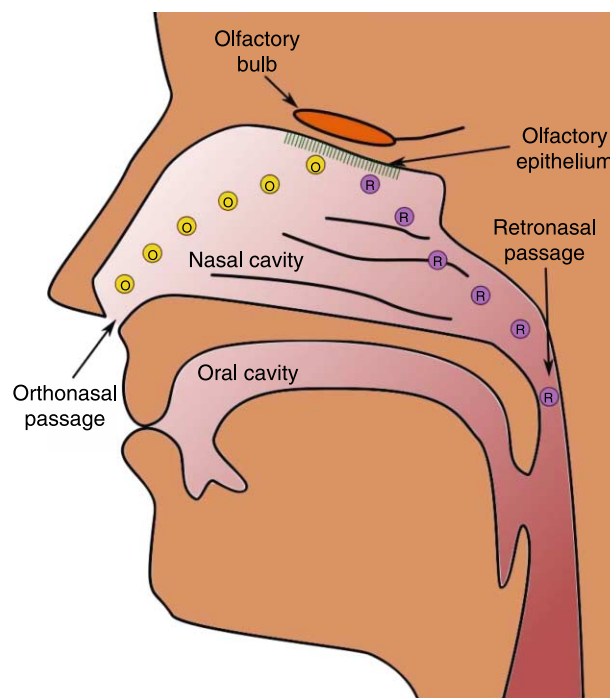


Figure 1 | Schematic of the human olfactory system. The odorants labelled “O” travel to the olfactory epithelium through the nostrils, or orthonasal pathway; the odorants labelled “R” travel through the nasopharynx, or retronasal passage, to the olfactory epithelium. Odorant molecules interact with the olfactory epithelium to generate a signal that is transmitted to the brain via the olfactory bulb. The three horizontal lines represent the turbinates which provide turbulence and mixing to air in the nose.

PHYSIOLOGY OF THE NOSE AND OLFACTORY SYSTEM

The nose connects humans to their gaseous environment. Its functions are respiration (the inhalation of oxygen and exhalation of carbon dioxide) and smelling. The term “olfactory” is used to describe those components of the nose associated with the sense of smell. The olfactory system is designed to detect volatile components and determine if they are odorous (Figure 1). The exterior portion of the olfactory system is the nose, which possesses a nasal septum that divides it into two chambers, each served by a nostril, or anterior nares. The olfactory epithelium is designed to capture odorants and translate them into signals that are sent to the olfactory bulb and then the brain.

Orthonasal odor: via the nostrils

When odorants are inhaled from the exterior environment through the nostrils, orthonasal nasal odors are perceived.

A non-turbulent airstream, with or without odorants, enters the nostrils and is restricted and accelerated into the main nasal cavity where it becomes turbulent. As inhaled air expands into the nasal chamber, it is directed upward to the olfactory epithelium by the turbinates or nasal conchae. The turbinates are three horizontal outgrowths on the sides of the nasal cavity. Air and odorants move quickly through the nose with typical flows of 250 mL/sec. During a sniff, the odor intensity increases due to airspeed, resulting in mixing and causing more odorant molecules to reach the epithelium (Cowart & Rawson 2000).

Interestingly, air and odorants are humidified and warmed or cooled to within one degree of body temperature (37°C) as they pass through the nasal cavity. The warming of odorants by the nose lends credence to the use of warmer temperatures for odor analysis as in flavor profile analysis (Standard Method 2170; APHA 2006) or hexanal as an odor standard (Ömür-Özbek & Dietrich 2008).

Retronasal odor: via the mouth

The retronasal passage, which is also called the nasopharynx, connects the mouth and nose. Retronasal perception is the main reason humans experience flavors (Cowart & Rawson 2000). It is also the reason people shoot water out their noses if they swallow and laugh at the same time. Retronasal stimulation occurs during eating because volatile molecules are released in the oral cavity and then pumped by mouth movements from the back of the oral cavity up through the nasopharynx and to the nasal cavity olfactory epithelium. Retronasal odors are activated when humans breathe out during mastication or swallowing (Shepherd 2006). Concentration of odorants and mastication are known to affect retronasal perception (Burdach & Doty 1987) and ingesting foods and beverages causes the concentrations from retronasal exposure to be higher than those from the orthonasal route (Pierce & Halpern 1996).

Organization of the olfactory epithelium

Odorants and air entering the nasal cavity via either the orthonasal or retronasal routes are directed to the olfactory epithelium, which is a mucus membrane lining the nasal cavity. The approximately 2 cm by 5 cm olfactory epithelium

is located on the roof of the nasal cavity, about 7 cm behind and above the nostrils (Cowart & Rawson 2000). While the nose and the retronasal passage move odorants to the olfactory epithelium, it is the olfactory epithelium and olfactory bulb that detect and process the odorants into recognizable odors like cherry, musty, medicinal, or lemon. It is a complex task to sort out odorous compounds, and scientists are just beginning to unravel the olfactory system.

The mucus coating on the olfactory epithelium serves to capture and dissolve odorants, which are also called chemical stimuli. A typical adult produces 1–2 L of mucus per day. The olfactory epithelium contains millions of olfactory receptor neurons, which are frequently referred to as just “odor receptors” (Cowart & Rawson 2000). These neurons, or nerves, are the only ones in the human body that are in direct contact with the outside environment, which may explain why they are the only type of neuron that is regularly replaced; replacement occurs at 4–8 week intervals (Menco & Morrison 2003). At one end of the olfactory receptor neuron there are odor-binding proteins, called receptors that bind the odorants dissolved in the mucus of the olfactory epithelium. Each neuron contains only one type of odor receptor, which means that each neuron can interact with only certain types of odor molecules (Buck 2004). Once an odorant binds to the olfactory receptor, a series of biochemical reactions occur that lead to an electrical signal being sent to the brain. The receptor is at one end of a continuous sensory neuron that passes through the bone behind the nose and connects to a glomerulus in the olfactory bulb. The glomeruli in the olfactory bulb serve to “organize” the odorant signals and then send messages to the brain.

DETECTING AND RECOGNIZING DIFFERENT ODORANTS—IT IS ALL ABOUT CODES AND COMBINATIONS

The 2004 Nobel Prize in Medicine was awarded to Drs Linda Buck and Richard Axel for their research explaining the sense of smell through understanding the genetics of odor receptors. Their research revealed that a large multigene family controls the odor receptor neurons in the olfactory epithelium (Buck & Axel 1991; Buck 2004;

NobelPrize.org 2008). Approximately 350 different odor receptors were identified in humans; interestingly, mice are reported to have about 1000 different odor receptors. Next they unravelled how these different receptors detect thousands of odors because it was clear that one receptor type did not code for one odor. Instead, the olfactory epithelium is organized into spatial zones that contain nonoverlapping sets of odor receptors/neurons that are expressed by select genes. Each zone contains groups of several different types of odor receptors, but one type of odor receptor is only scattered throughout one zone. Odor molecules interact with certain receptors in select zones. This means that a specific odorant activates some zones, but not others, allowing a few zones the ability to detect a lot of odors because different combinations represent different odorants.

As stated by Dr. Buck: “Studies revealed that one odor receptor can recognize multiple odorants and that a single odorant is detected by multiple odor receptors, but, importantly, different odorants are recognized by different combinations of odor receptors” (Buck 2004). Different combinations of which odor receptors are activated by odorants allow tens of thousands of recognizable odors from just 350 different types of odor receptors.

A subtle change in the chemical structure of an odorant will alter its odor receptor code and thus the perceived odor. Short chain aliphatic alcohols have pleasant odors while alkanes with the same carbon-chain length are odorless and carboxylic acids with the same carbon-chain length are acrid. Interestingly, changing the odorant concentration can also change its receptor code and this is why the same chemical compound can have very different odor descriptors at different concentrations (Buck 2004). For example, linolenic acid smells watermelon-like at low concentrations, then melon-like and finally waxy as the concentration increases from $\mu\text{g/L}$ to mg/L (Rashash *et al.* 1997).

How are signals from the odor receptor neurons converted to recognition of an odor by the brain? Odors are currently thought to be represented by complex spatial patterns that are more akin to images, like faces, than to facts, like hair colors (Sullivan *et al.* 1995; Shepherd 2006). The millions of individual odor receptor neurons in the olfactory epithelium, which represent approximately 350

different types of odor receptors, converge upon 2,000–7,800 glomerulii in the adult olfactory bulb (Coward & Rawson 2000). Although odor receptor neurons for a specific type of odorant are scattered throughout a zone of the olfactory epithelium, similar odor receptor neurons all converge to the same type of glomerulus in the olfactory bulb. Each glomerulus receives signals from only one type of odor receptor. In this manner, the odor signals are organized spatially before they are sent to the brain for detection and recognition. The nose and the olfactory epithelium first broadly organize odor information, and then the olfactory bulb creates highly organized spatial patterns that humans call “odors”. Interestingly, odor images appear to be learned which is why different cultures have different cuisines. Taste is more innate; bitter typically evokes disgust and sweet universally evokes pleasure for people across the world.

DIFFERENCES IN ORTHONASAL AND RETRONASAL PERCEPTION

Orthonasal odor perception is associated with the nasal “sniff” leading odorants to the olfactory epithelium. Retronasal odor perception describes the mechanism in which odorants are transported from the mouth to the nasopharynx and then to the olfactory epithelium. These combined routes to the olfactory epithelium are called the “duality of the sense of smell” (Rozin 1982). Does the ability to detect odors vary with the pathway, with one being more sensitive than another?

The differences in sensitivity and recognition between orthonasal and retronasal perception is an area of active research. Little empirical evidence exists for a substantial qualitative difference between orthonasal and retronasal perception within the human population, although select individuals are known to exhibit significant differences in orthonasal and retronasal perception.

Saliva, temperature and mastication in the mouth alter a food or beverage and these alterations influence the partitioning of odorants into the air phase, thus affecting the volatile odorants that reach the oral cavity and nasal cavity from the oral cavity. Differences in airflow patterns, the direction of odorant movement across the olfactory

epithelium, and inhalation/exhalation frequencies are all known to produce different patterns of airflow and possibly odor sensitivity and perception (Burdach & Doty 1987; Cowart & Rawson 2000). The direction of air flow from the ortho- or retronasal passages to the olfactory epithelium were implicated in the efficiency with which individuals could identify an odorant delivered to the olfactory epithelium via these two routes (Pierce & Halpern 1996). An increase of about 20% in odorant identification was observed for odorants entering via the orthonasal route, but the explanation for this slight increase is not known.

Recent evidence suggests that neural processing, and hence perception, depends on whether an odorant travels the orthonasal or retronasal pathway to the nasal cavity, although the differences were less than a factor of two (Sun & Halpern 2005). Hummel (2008) performed experiments that demonstrated human subjects perceived lower thresholds for chocolate and lavender when the odorant was given via the orthonasal route. The author postulated that differences in airflow between the two routes of exposure affected the absorption of the odorants in the olfactory epithelium. Additionally, during Functional Magnetic Resonance Imaging, different regions of the brain were activated depending on whether a stimulant was presented through the ortho- or retronasal pathway. The brain activation may be due to the odorant or to the psychology as to whether the odor is anticipated as a reward for a substance in the mouth.

DISTINGUISHING TASTE VS. ODOR PERCEPTION FOR COPPER IN DRINKING WATER

How are these two chemical senses distinguished? It is easy: use a nose clip to prevent volatile odorants from travelling through the orthonasal and retronasal passages to the nasal cavity. For example, the widely reported “metallic”

taste of copper, which is problematic in drinking water, is really a combination of a slight taste and a significant retronasal odor (Lawless *et al.* 2004; Epke & Lawless 2007; Ömür-Özbek & Dietrich 2008). Retronasal perception related to ingested metals was first documented by Hettinger *et al.* (1990) who found a decrease in taste of ferrous sulfate when nose was occluded. As shown in Table 1, the flavor thresholds of copper salts are 2–3 times higher when the nose is closed and the retronasal odors cannot reach the olfactory epithelium.

Our research group performed extensive sensory testing with copper (II) ions under various water quality regimens. When the nose was occluded with a nose clip, the human subject could barely perceive a copper sensation. Human subjects wearing “speedo” brand nose clips were placed in sensory testing booths and sequentially provided 3 ounce solo cups with 10 ml of copper (II) sulfate solution from 0.1 to 5 mg/l. The subjects sipped and swished the solution in their mouths, then record if they detected a “copper” sensation. After tasting the final solution of 5 mg/l CuSO₄ only a few subjects reported perceiving copper as bitter with nose clips on, but all six subjects were amazed that they detected a strong and disgusting “metallic” and/or “bloody” sensation upon removing their nose clips.

Cuppert *et al.* (2006) demonstrated that speciation of copper affected perception. The research demonstrated that soluble free copper (II) ion or copper (II) complexes were both detected by the chemical senses but that particulate copper (II) was poorly detected. Flavor testing by 36 human subjects with their noses open compared copper (II) dissolved in pH 5.5 distilled water, and pH 6.5 and pH 7.4 reference tap water containing 110 mg/L total dissolved solids and 34 mg/l HCO₃⁻; no other transition metals or organic carbon was added. Because the flavor of copper was shown to linger and/or cause adaptation, subjects only tested one concentration per day. The group flavor thresholds were similar in

Table 1 | Thresholds of iron and copper measured for human subjects with their noses open or their noses closed with a nose clip (Epke & Lawless 2007). The nose open thresholds allow for orthonasal and retronasal odors

Metal salt	Human detection threshold		Nose open mg/l	Nose closed mg/l	Ratio of thresholds closed/open
	Nose open μ M	Nose closed μ M			
CuSO ₄	7.8	24.6	0.50	1.6	3.2
CuCl ₂	8.2	15.6	0.52	1.0	1.9

the distilled water and the reference tap water indicating that copper complexes did not alter perception (Figure 2). Flavor thresholds were calculated using both geometric mean group thresholds and by logistic regression (ASTM 1432-91 1991). The factor of 2 difference between these methods is not substantial given the different criteria for calculating the group thresholds. The logistic regression group threshold is based on when 50% of the 36 human subjects could taste copper; the geometric mean group threshold is based on calculating a geometric mean based on 36 individual thresholds. The copper flavor thresholds were all below the maximum solubility of copper in these waters: 8 mg/l Cu in pH 5.5 distilled water; 4 mg/l Cu in pH 6.5 reference tap water; and 1.3 mg/l Cu in pH 7.4 reference tap water. When the pH was raised to 8.5 for a 5 mg/L total copper solution with only 0.3 mg/l soluble Cu, the copper flavor was difficult to perceive even in the presence of 4.7 mg/l particulate copper.

An examination of individual copper-flavor thresholds with respect to copper solubility, sensory thresholds and regulatory levels is highlighted in Figure 3. Based on data in Figure 3, between 70–80% of consumers would be able to detect copper (II) in their water if the concentration was at or below the USEPA SMCL of 1 mg/l (USEPA 1997), the USEPA action level of 1.3 mg/l Cu (USEPA 1991), or the WHO (1998) health based guideline of 2 mg/l. Interestingly, these sensory and health based levels and the ability of consumers to detect copper correspond to the region where copper is present in its soluble form, which is up to 1.3 mg/l at pH 7.4. Both the solubility and cumulative taste threshold curves level out at about 2 mg/l which is below the 4 mg/l Cu that is likely to cause gastrointestinal problems. When the total copper concentration increases above 1.3 mg/l all

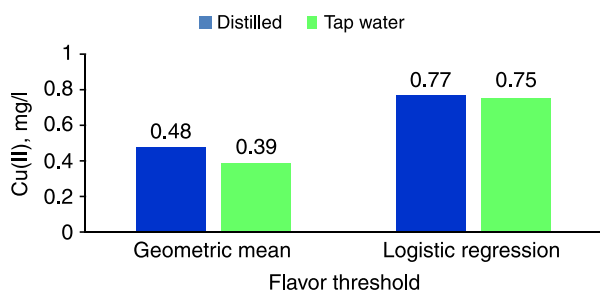


Figure 2 | Comparison of copper (II) flavor thresholds in pH 5.5 distilled water or pH 7.4 reference tap water.

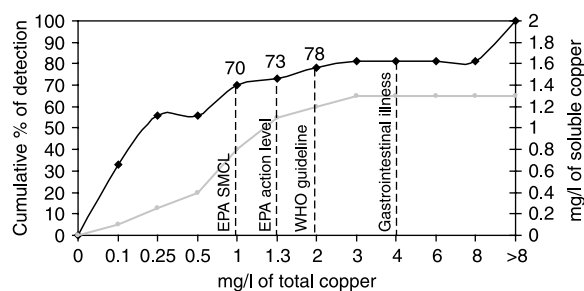


Figure 3 | Cumulative percent of human subjects' abilities to detect copper in pH 7.4 reference tap water and the corresponding total and dissolved copper concentrations. Sensory and concentrations of regulatory or health concern are indicated. Copper concentration ≥ 4 mg/l are likely to cause gastrointestinal upset (Pizzaro *et al.* 1999). Source of sensory and copper concentration data: Cuppett *et al.* 2006.

additional copper added would be particulate at pH 7.4. Based on the data presented in Figures 3 and 4, it appears that if a person cannot detect copper when the copper is soluble, then it is very unlikely they would taste a higher concentration with particulate copper. This is of concern, because concentrations above 4 mg/l are likely to both contain copper precipitates and cause gastrointestinal upset. The results of this study only directly pertain to a pH 7.4 water, because that is the pH where the maximum soluble copper is 1.3 mg/l. Waters at lower pH values would possess higher soluble copper concentrations and potentially $> 80\%$ of the population could detect copper. Conversely, drinking waters with higher pH values would have less soluble copper potentially resulting in $< 80\%$ of the population being able to detect its flavor.

Copper (II) is clearly detect by both taste and odor in the human mouth. A question arises, what is the source of the metallic odor? The metallic odor is a biochemical reaction that happens in the mouth. The source is metal-induced oxidation of unsaturated lipids in oral tissues. Arachadonic, linoleic, and oleic acids are the major unsaturated fatty acids in human epithelial tissues including skin and the mouth (Lekholm & Svennerholm 1977). The double bonds in unsaturated fatty acids are known to be very prone to oxidation and form volatile aldehydes and ketones that are responsible for the undesirable off-flavors in foods (Schulz 1985). The same biochemical reactions that happen in foods happen in the human mouth.

Insight to reactions in the human mouth can be gained from the research of Glindemann *et al.* (2006) who investigated the source of the "metallic" odor from contact

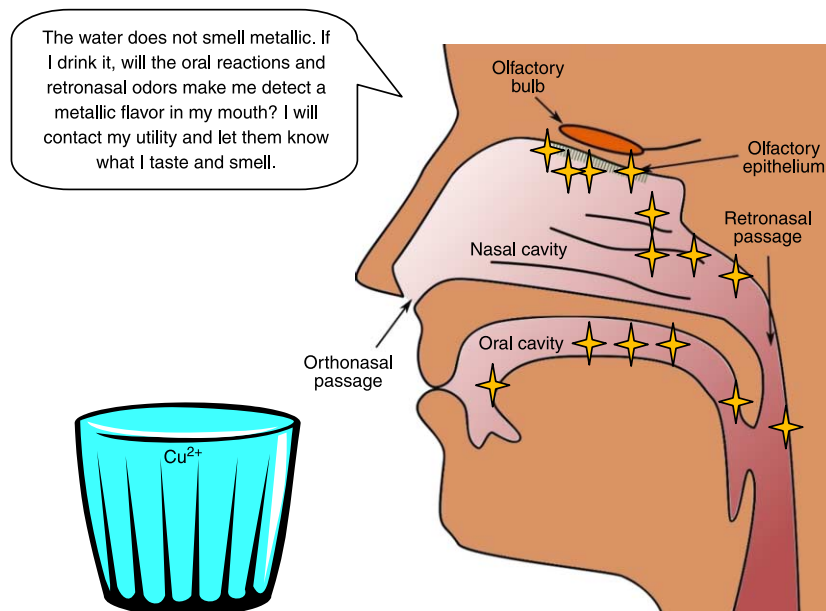


Figure 4 | Schematic of a human as an informed water quality sensor that provides customer feedback about metallic flavour to their drinking water provider. ✦ Represent odorant molecules.

of ferrous ion, iron metal, ferric ion, cupric ion, cuprous ion, and copper metal on the skin of the palm for human subjects. About 110 nmole metal ion/dm² skin was applied. Research with copper (II) demonstrated that both copper solutions and copper metal on sweaty palms also produced the “metallic” odor. The mechanism is lipid oxidation of skin fatty acids (including oleic, arachidonic and linoleic acids) by metals in the presence of oxygen to produce abundant C₆ to C₁₀ n-aldehydes and at least 5 more minor peaks of unsaturated aldehydes and ketones. The musty-mushroom-metallic odorant 1-octen-3-one was determined to be the most important “metallic flavor” component as it has an air-odor threshold of 50 ng/m³ (0.05 ppm–V/V in air) and contributed about one-third of the odor intensity. The same series of carbonyls was detected for copper (II, I, metal) as for ferrous/Fe^o, including 1-octen-3-one. The metallic odor and carbonyl production of copper (II) was not as intense as for an equivalent molar concentrations of iron (II), which was the most reactive ion. Iron (III) did not produce the metallic odor in contact with skin.

Ömür-Özbek *et al.* (2008) demonstrated that copper (II) caused lipid oxidation in the mouth. Immediately after ingesting 10 mg/l (180 µM) copper, 13 human subjects reported developing a strong “metallic” flavour. Lipid oxidation products were measured in their saliva using the

colorimetric thiobarbituric acid reactive substances (TBARs) method which is widely used in the food and medical industries to measure malondialdehyde, a universal lipid oxidation product (Yagi 1998; Hodossy & Celec 2005). Like human skin, oleic, linoleic and arachidonic acids are major unsaturated fatty acids in the human oral epithelium (Lekholm & Svennerholm 1977) and these readily react with copper (II) in the presence of oxygen to form lipid peroxides that further react to form aldehydes and ketones (Aust & Svingen 1982).

Besides copper-induced lipid oxidation in the oral cavity, copper (II) also interacts with salivary proteins and volatile odorants in the mouth to alter sensory perception (Hong *et al.* 2006, 2008). Copper (II) interacted with the salivary proteins mucin and α-amylase at pH 7.5 but not pH 7 or 6.5 when added at 2.5 mg/l Cu to an artificial saliva consisting of salts and proteins. The pH of saliva is typically 6.5–7.0. When four odorants were added to the artificial saliva, an increase in the headspace concentrations of hexanal, butyl acetate, 2-heptanone, and ethyl hexanoate was observed at pH 6.5 when compared to the saliva solution without copper. No change in volatility of these odorants was observed at pH 7.0 and at 7.5 there was a slight decrease in volatility. At pH 7.5, presence of copper in the artificial saliva system containing mucin and α-amylase decreased headspace volatile concentration. The effect of

copper on volatiles at pH 6.5 was thought to be due to increased solubility of copper at lower pH while salivary proteins seem to interact with copper at pH 7.5. Ultrafiltration studies demonstrated that particulate copper was present in saliva at pH 7 but not at pH 5.5 and that copper (II) bound to proteins at either pH. The copper interaction may change configuration of protein binding sites for volatile odorants and this can be a source of altered sensory perception in foods/beverages or even the retronasal odor of carbonyls produced by lipid oxidation.

This research with copper leads to several suggestions for other researchers to consider when designing taste and odor studies. These are: 1) testing with and without nose clips at the beginning of the study would be valuable and provide insight into retronasal odor as a potential mechanism; 2) because metal flavor may be due to the biochemical reaction of oxidation of lipids in oral tissues, several hours should be allowed between taste/flavor testing of samples with the metal so that the lipids can “renew” themselves; 3) presence of the metal as soluble or particulate form should be considered as a function of both pH and ionic content; 4) although solid phase micro-extraction was successful at measuring carbonyls formed on the skin it was not successful in the mouth for studies with copper(II)—this was thought to be due to a lower Henry’s Law constant for volatilization of carbonyls from saliva and also the loss of carbonyls due to exhalation; 5) metals in the mouth may interact with taste receptors, produce odors, and cause changes in saliva and protein composition, all or which may contribute to “flavor”.

SUMMARY

The chemical senses of taste and smell serve to both provide pleasure and to protect humans from ingesting toxic foods/beverages. Humans are designed to perceive flavor, which combines taste and odor into one overall experience. In their role as water quality monitors, humans use their senses to evaluate the quality of the water and provide useful feedback to utilities. The role of orthonasal odor is to allow humans to evaluate water before they ingest it, while the retronasal odor process allows a person to evaluate odors in drinking water after it is ingested. For copper (II), the metallic flavour is a combination of a weak bitter taste and a stronger metallic odor that is produced as

a biochemical reaction in the mouth. Copper (II) in the presence of oxygen causes lipid oxidation of arachidonic, linoleic, and oleic acids, which are lipid in the mouth, to produce odorous volatiles including carbonyls. The odorous carbonyls are perceived mostly by the retronasal mechanism. Thus, metallic flavour perception in drinking water is due to a series of complex reactions initiated by ingesting copper (II).

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REFERENCES

- American Public Health Association (APHA) 2006 Flavor profile analysis. section 2170. *Standard Methods for the Examination of Water and Wastewater*, 20th edition. APHA, Washington, DC.
- American Standards for Testing Materials (ASTM) 1991 Standard Practice for Defining and Calculating Individual and Group Sensory Thresholds from Forced-Choice Data Sets of Intermediate Size. E-1432-91. In *Annual Book of Standards*, Vol. 15.07 American Society for Testing of Materials, Philadelphia, PA, pp. 67–74.
- Aust, S. D. & Svingen, B. A. 1982 The role of iron in enzymatic lipid peroxidation. In: Pryor, W. A. (ed.) *Free Radicals in Biology*. Academic Press, New York, NY, pp. 1–18.
- Bae, B. U., Shin, H. S. & Choi, J. J. 2007 Taste and odour issues in South Korea’s drinking water industry. *Water Sci. Technol.* **55**(5), 203–208.
- Buck, L. 2004 Olfactory receptors and odor coding in mammals. *Nutr. Rev.* **62**(11), S184–S188.
- Buck, L. & Axel, L. 1991 A novel multigene family may encode odor receptors: a molecular basis for odor recognition. *Cell* **65**, 175–187.
- Burdach, K. & Doty, R. 1987 The effects of mouth movement, swallowing and spitting on retronasal perception. *Physiol. Behav.* **41**, 353–356.
- Burlingame, G. A. & Mackey, E. D. 2007 Philadelphia obtains useful information from its customers about taste and odour quality. *Water Sci. Technol.* **55**(5), 257–263.

- Burlingame, G. A., Dietrich, A. M. & Whelton, A. J. 2007 The taste of tap water. *JAWWA* **99**(5), 100–112.
- Cowart, B. & Rawson, N. 2000 Olfaction. In: Bruce Goldstein, E. (ed.) *Blackwell Handbook of Perception*. Blackwell Publishing, pp. 567–595.
- Cuppett, J., Duncan, S. & Dietrich, A. M. 2006 Evaluation of copper speciation and water quality factors that affect aqueous copper tasting sensitivity. *Chem. Senses* **36**, 689–697.
- Devesa, R., Fabrellas, C., Cardeñoso, R., Matia, L., Ventura, F. & Salvatella, N. 2004 The panel of Aigues de Barcelona: 15 years of history. *Water Sci. Technol.* **49**(9), 145–151.
- Dietrich, A. M. 2006 Aesthetic issues for drinking water. *J. Water Health* **4**, 1–11.
- Epke, M. & Lawless, H. T. 2007 Retronasal smell and detection thresholds of iron and copper. *Physiol. Behav.* **92**, 487–491.
- Hettinger, T. P., Myers, W. E. & Frank, M. E. 1990 Role of olfaction in perception of non-traditional 'taste' stimuli. *Chem. Senses* **15**, 755–760.
- Hodosy, J. & Celec, P. 2005 Daytime of sampling, tooth-brushing and ascorbic acid influence on salivary thiobarbituric acid reactive substances—a potential clinical marker of gingival status. *Dis. Mark.* **21**, 203–207.
- Hong, J. H., Duncan, S. E., Dietrich, A. M. & O'Keefe, S. 2006 Effect of copper on the volatility of aroma compounds in a model mouth system. *J. Agric. Food Chem.* **54**, 9168–9175.
- Hong, J. H., Duncan, S. E., O'Keefe, S. & Dietrich, A. M. 2008 Ultrafiltration as a tool to study binding of copper to salivary proteins. *Food Chem.* **113**(1), 180–184.
- Hummel, T. 2008 Retronasal perception of odors. *Chem. Biodivers.* **5**(6), 853–861.
- Glindemann, D., Dietrich, A. M., Staerk, H.-J. & Kuschik, P. 2006 The two odors of iron when touched or pickled: (skin) carbonyl compounds and organophosphines. *Angew. Chem. Int. Ed.* **45**(42), 7006–7009.
- Lawless, H., Shlake, S., Smythe, J., Lim, J., Yang, H., Chapman, K. & Bolton, B. 2004 Metallic taste and retronasal smell. *Chem. Senses* **29**(1), 25–31.
- Lekholm, U. & Svennerholm, L. 1977 Lipid pattern and fatty acid composition of human palatal oral epithelium. *Scand. J. Dent. Res.* **85**, 279–290.
- Menco, B. & Morrison, E. 2003 Morphology of the mammalian olfactory epithelium: form, fine structure, function, and pathology. In: Doty, R. (ed.) *Handbook of Olfaction and Gustation*, 2nd edition, pp. 32–97.
- Nobel Prize 2008 *Linda Buck and Richard Axel, 2004 Nobel Prize in Medicine*. http://nobelprize.org/nobel_prizes/medicine/laureates/2004/ (accessed August 2008).
- Ömür-Özbek, P. & Dietrich, A. M. 2008 Developing hexanal as an odor reference standard for sensory analysis of drinking water. *Water Res.* **42**, 2598–2604.
- Ömür-Özbek, P., Dietrich, A. M., Duncan, S. E. & Lee, Y.-W. 2008 Metallic flavor of iron and copper in drinking water, presented at the *IWA 8th International Symposium on Off-Flavours in the Aquatic Environment, October 2008 Seoul, Korea*.
- Pierce, J. & Halpern, B. P. 1996 Orthonasal and retronasal odorant identification based upon vapor phase input from common substances. *Chem. Senses* **21**, 529–543.
- Pizarro, F., Olivares, M., Uauy, R., Contreras, P., Rebelo, A. & Gidi, V. 1999 Acute gastrointestinal effects of graded levels of copper in drinking water. *Environ. Health Perspect.* **107**, 117–121.
- Rashash, D. M., Dietrich, A. M. & Hoehn, R. C. 1997 FPA of selected odorous compounds. *JAWWA* **89**(2), 131–142.
- Rozin, P. 1982 Taste-smell confusion and the duality of the olfactory smell. *Percept. Psychophys.* **31**, 397–401.
- Schulz, H. 1985 Oxidation of fatty acids. In: Vance, D. E. & Vance, J. E. (eds) *Biochemistry of Lipids and Membranes*. The Benjamin/Cummings Publishing Company, Inc, Menlo Park, CA, p. 117.
- Shepherd, G. 2006 Smell images and the flavor system in the human brain. *Nature* **444**(16), 316–321.
- Sullivan, S., Ressler, K. & Buck, L. 1995 Spatial patterning and information coding in the olfactory system. *Curr. Opin. Genet. Dev.* **5**(4), 516–523.
- Sun, B. & Halpern, B. 2005 Identification of orthonasal and retronasal odorant pairs. *Chem. Senses* **30**(9), 393–706.
- USEPA 1991 Maximum contaminant level goals and national primary drinking water for lead and copper. final rule. *Fed. Regist.* **56**, 26460–26564.
- USEPA 1997 National secondary drinking water regulations final rule. *Fed. Regist.* **44**(140), 42195.
- World Health Organization (WHO) 1998 *Guidelines for Drinking Water Quality*. 2nd. Addendum to Vol. 2. Health Criteria and Other Supporting Information. Geneva Switzerland.
- Yagi, K. 1998 Simple assay for the level of total lipid peroxides in serum or plasma. *Meth. Mol. Biol.* **108**, 101–106.

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