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The nose and tongue as chemical detectors

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Humans have evolved sensory systems that respond to a variety of environmental stimuli. For example, we have receptors in our eyes that are activated by visible light (400-700nM wavelength) within the electromagnetic spectrum (sight), or ears that house auditory cilia that respond to pressure changes in air (sound), and pressure sensitive receptors beneath the skin that respond to mechanical stimulation (touch/somatosensation). We also evolved sensory systems to detect chemicals in the environment: taste, smell, and chemical irritation (somatosensory system). The chemical senses, with receptor mechanisms located in the oro-nasal region, are the gatekeepers of our digestive system and therefore have a role in determining the food and beverages we consume. The chemical senses must be robust and broadly tuned to detect tens of thousands of volatile and non-volatile compounds, including novel compounds that we may never encounter. The aim of this paper is to give an overview of human chemical sensory systems and how these systems are used as detectors to help scientific research. What follows is a description of the physiology of the chemical senses, and an example of chemical separation combined with human sensory perception to isolate and identify a natural non-steroidal anti-inflammatory compound in olive oil.

The science of smell

The immense diversity of flavours we associate with foods are primarily derived via the volatile compounds that are released in the oral cavity when foods or liquids are chewed and swallowed. A simple experiment demonstrates the influence the sense of smell has on flavour perception; if you taste a grated apple and onion with your nose plugged (index finger and thumb pinching your nostrils closed) it is near impossible to distinguish between the two, yet with your nose unplugged the task is very easy. When the nose is plugged, there is no airflow over the olfactory epithelium effectively removing aroma from the overall flavour and we must distinguish between the two samples by taste attributes alone. During eating, the sense of smell is activated when volatile compounds flow from the mouth via the naso-pharynx passage and access receptors in the nasal cavity (retronasal). The sense of smell is also activated via active sniffing of air where volatile compounds travel to the olfactory receptors through the nostrils (orthonasal).

Peripheral Olfactory Pathways

Volatile compounds access small, thin sheet of cells high up in the nasal cavity called the olfactory epithelium. The olfactory epithelium houses the olfactory receptors on olfactory neuronal cilia within the mucus layer [1]. Quantitatively we have millions of olfactory receptors housed in the olfactory epithelium that are comprised of approximately 360 qualitatively different receptors. Odorants dissolved in the mucus can access the olfactory receptors, but only if the volatile compound has the chemical key for the receptor will the transduction process begin and the individual perceive a quality (e.g., rose, caramel, cut grass). The system is broadly tuned as an olfactory receptor may be activated by multiple compounds and a single compound may activate multiple olfactory receptors. An electrical signal is sent to

the olfactory bulb where the neuronal activity pattern responsible for the quality is established.

Central Olfactory Pathways

Olfactory information is modified by inhibitory and excitatory interactions within and between the olfactory bulbs, and by modulation from systems of axons descending from higher areas of the brain. The olfactory signals have an unusually direct and widespread influence on the parts of the forebrain that have roles in emotion, motivation, and certain kinds of memory.

The science of somatosensations

Chemical irritant sensations can be experienced all over the body due to activation of the chemically sensitive neurons of the somatosensory system. Our mouth and nose (and other mucus membrane regions) are particularly sensitive to certain chemical irritants such as carbon dioxide, menthol, and capsaicin due to a porous epithelium structure allowing chemicals to diffuse through the protective barrier to the nerve endings beneath. In contrast to the mucus membranes, hairy and glabrous skin has increased stratum corneum that forms a protective barrier to the penetration of chemicals. Chemical activation of the nerve endings in the mucus membrane of the oro-nasal region produces a number of diverse sensations that are an essential component of flavour. There are a number of adjectives to describe the sensations; the burn of chili pepper, the warmth of ethanol, the tingle of CO_2, the pungency of wasabi. However, there is not the qualitative breath of adjectives as we have for the sense of smell.

Peripheral chemesthesis pathways

Free endings of individual nerve fibers innervate both the oral and nasal mucosa and have specialized sensory receptors that respond to both heat [2, 3], cold [4] (both of which evoke thermal and pain sensations) and innocuous stimuli that do not contribute to pain [5]. These oro-nasal sensations are conveyed to the central processing areas of the brain via polymodal nociceptors of the trigeminal nerve (5th cranial nerve), the vagus (10th cranial nerve) and glossopharyngeal (9th cranial nerve) nerve, and the chorda tympani nerve (7th cranial nerve). The detection mechanisms of the irritation system are required to identify a diverse range of chemical structures including salts, acids, alcohols, and alkaloids.

Central chemesthesis pathways

Very similar to the pathways of taste, see below.

The science of taste

The role of the taste system is to identify and distinguish between nutrients and toxins. The human gustatory system is capable of identifying five major taste qualities: sweet, sour, bitter, salty, and umami, and perhaps several sub-qualities. This is a relatively small number of qualities given the vast number and structural diversity of chemical compounds that elicit taste.

Peripheral Taste Pathways

The taste system is stimulated when non-volatile compounds stimulate specialized epithelial taste receptor cells on the tongue as well as the soft palate, pharynx, and epiglottis. Humans have on average 300,000 taste receptor cells selected in groups of about 50 into 6000 taste buds. About 2/3rds of the buds are housed in small projections on the tongue called papillae, which are shaped like ridges

(foliate papillae), pimples (vallate papillae), or mushrooms (fungiform papillae). The chemically sensitive part of a taste receptor cell is its small membrane region, called the apical end, near the surface of the tongue. The apical ends have thin extensions called microvilli that project into the taste pore, a small opening on the surface of the tongue that is exposed to the contents of the mouth.

Taste transduction involves different processes, and each basic taste may have one or more mechanisms. Salty and sour tastes presumably perceived after activation of ion channels on taste cells, while sweet, bitter and umami tastes are initiated via G-protein coupled receptors.

Central Taste Pathways

The chemical code that was recognised by a receptor mechanism is converted to electrical signal that is transferred to processing regions of the brain. The taste signal is sent from taste buds to the nucleus of the solitary tract, which is the engine of taste; to the parabrachial nucleus; to the thalamic area which responds to touch and temperature as well as taste; to the primary taste cortex which is probably responsible for determining taste quality; to the secondary taste cortex where visual, somatosensory, and olfactory input may converge with taste [6]; to the amygdala where cells are sensitive to physiological condition (e.g., satiety); to the hypothalamus which is involved with hedonic judgements of stimuli [7].

Individual chemical sensory systems as analytic detectors

Gas chromatography-olfaction (GC-O) is a common form of a sensory process interfaced with analytic separation [8]. The nose detects a wide variety of volatile chemicals, and assuming the 'sniffer' has adequate training the intensity and quality of smell can be quantified yielding valuable information for the researcher.

Far less common is the use of high performance liquid chromatography-gustation (HPLC-G) where analytic separation of non-volatile compounds is linked with the tongue. As taste is a proximal sense which requires compounds to be solublized, preferably in water, the steps involved in preparing a sample for human evaluation are more tedious than GC-O. HPLC-G is an emerging scientific technique, and a German research group developed a taste dilution analysis (TDA) to evaluate the flavour influence of non-volatile compounds in a number of natural compounds, in particular those generated via Maillard reactions [9-11]. However, TDA investigates threshold effects of compounds, and these effects do not necessarily translate to suprathreshold intensity we experience in foods, therefore TDA has limited practical utility.

What follows is an overview of sensory directed approach to isolate and identify a non-volatile throat irritating compound in virgin olive oils using HPLC-G [12].

Isolation and identification of oleocanthal, an irritating compound in virgin olive oil

The major component of olive oil is lipid, primarily triacylglycerol and some free fatty acids. In addition, virgin olive oils have a variety of non-saponifiable phenolic compounds (1-2%) whose structural properties have been well characterized [13]. The different phenolic compounds in olive fruits such as phenyl acids, flavonoids and secoiridoids [14-18], are reported to have health benefits [19] and contribute to the oxidative stability and flavour of the oil. However, while the polyphenols are assumed to impart astringent, bitter, and pungent sensations associated with olive oil,

very little is known about the influence the individual compounds contribute to the organoleptic properties.

During a tasting of freshly pressed olive oil in Sicily, Prof Gary Beauchamp noticed the pharyngeal irritation associated with the freshly pressed oil remarkably similar to the pharyngeal irritation associated with ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) [20]. The perceptual similarity lead to a collaborative project aimed at discovering, identifying, and synthesizing the irritating compound in olive oil, followed by assessing its pharmacologic similarity with ibuprofen.

Ten olive oils from different geographical regions (Italy, Greece, USA) were chosen for the experiment. The pharyngeal irritation in each of the olive oils was assessed and the oil with the highest irritant sensation was chosen for the sensory directed extraction procedure. The method for isolation of the phenolic portion of virgin olive oil was modified from the research of Montedoro and colleagues [21, 22].

The HPLC chromatogram of the phenolic fraction of olive oil was viewed and separated in fractions corresponding to peak detection (Figure 1). The HPLC eluant was collected in glass vials according to the predetermined fraction elution times. Baseline eluant where no peaks were detected were also collected and combined into one fraction giving a total of 16 fractions. The corresponding fractions from 10 HPLC runs were collected, combined, then evaporated under vacuum (kPa, 30°C), and freeze-dried to remove the solvents. The residues of the fractions were dissolved in water, filtered through a 0.45μm filter, and evaluated for taste and oral irritation properties. Fraction seven was the only fraction to elicit pharyngeal irritation (Figure 1). The fractions were further purified by C18 cartridge and the sample was dissolved in $CDCl_3$ for detailed 1D and 2D NMR analysis. The compound was positively identified as 2-(4-hydroxyphenyl) ethyl, 4-formyl-3-(2-oxoethyl)-4-hexenoic acid

ester (Figure 2), the deacetoxy-dialdehydic ligstroside aglycone that had previously been reported as a secoiridoid derivative in virgin olive oil [21]. Both enantiomers of deacetoxy-dialdehydic ligstroside aglycone were synthesized (13 steps), but in nature only (-)- exists and the optical rotation of the natural enantiomer was found to be levorotary. We named the compound oleocanthal (oleo-olive, canth-sting, al-aldehyde). The concentration of oleocanthal in the ten olive oils was determined by HPLC and there was a strong correlation between intensity of pharyngeal irritation the concentration of oleocanthal in virgin olive oils (r=0.9) (Figure 3). Moreover, there was a strong correlation between increasing concentration of synthetic (-)oleocanthal and pharyngeal irritation.

Cyclooxygenase and lipoxygenase assays conducted with synthetic (-)-oleocanthal demonstrated that, as predicted, it is a natural NSAID with an anti-inflammatory profile and potency strikingly similar to that of ibuprofen. We concluded that oleocanthal may play a significant role in the health benefits of a Mediterranean diet. The finding provides proof that similar perceptual properties between novel compounds and pharmaceuticals may predict pharmacologic activity in vivo. The research was published in Nature [12].

Conclusion

Humans have highly sensitive biological detection systems that respond to thousands of diverse chemicals, and the utility of human noses and tongues as analytic chemical detectors is still in its infancy. The systematic sensory directed research investigating olive oil irritation is an example of the wealth of valuable information that can be obtained when we understand and use human sensory systems as an analytic tool.

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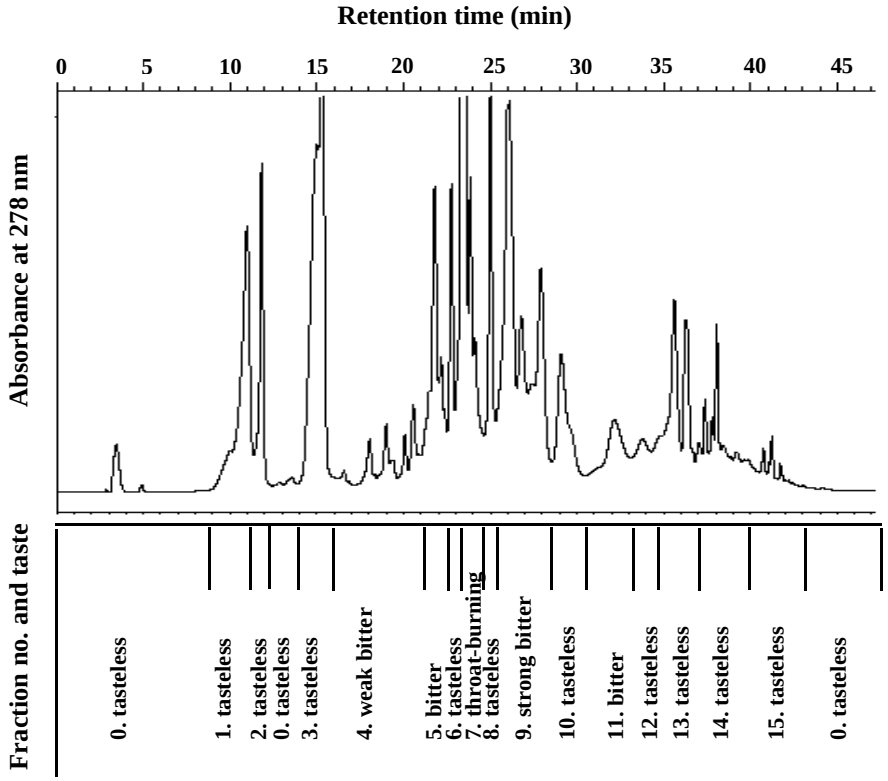
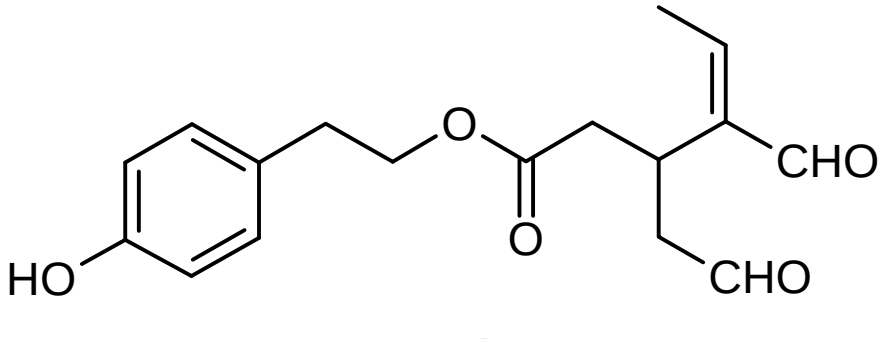
Figures

Figure 1 HPLC-Gustation chromatogram. A typical HPLC chromatogram of the phenolic fraction of virgin olive oil combined with fraction isolation corresponding to major peaks and taste quality associated with each fraction

Figure 2 Chemical structure of oleocanthal, the throat-burning deacetoxy-dialdehydic ligstroside aglycone identified in virgin olive oils.

Figure 3 Correlation between pharyngeal irritation and concentration of oleocanthal in ten olive oils. X-axis represents the concentration of oleocanthal in olive oils (μg/l). Y-axis represents pharyngeal irritation intensity using the general labeled magnitude scale [23]. The dashed line represents 2nd order best fit plot.


Retention time (min)
0
5
10
15
20
25
30
35
40
45
Absorbance at 278 nm
Fraction no. and taste
0. tasteless
1. tasteless
2. tasteless
0. tasteless
3. tasteless
4. weak bitter
5. bitter
6. tasteless
7. throat-burning
8. tasteless
9. strong bitter
10. tasteless
11. bitter
12. tasteless
13. tasteless
14. tasteless
15. tasteless
0. tasteless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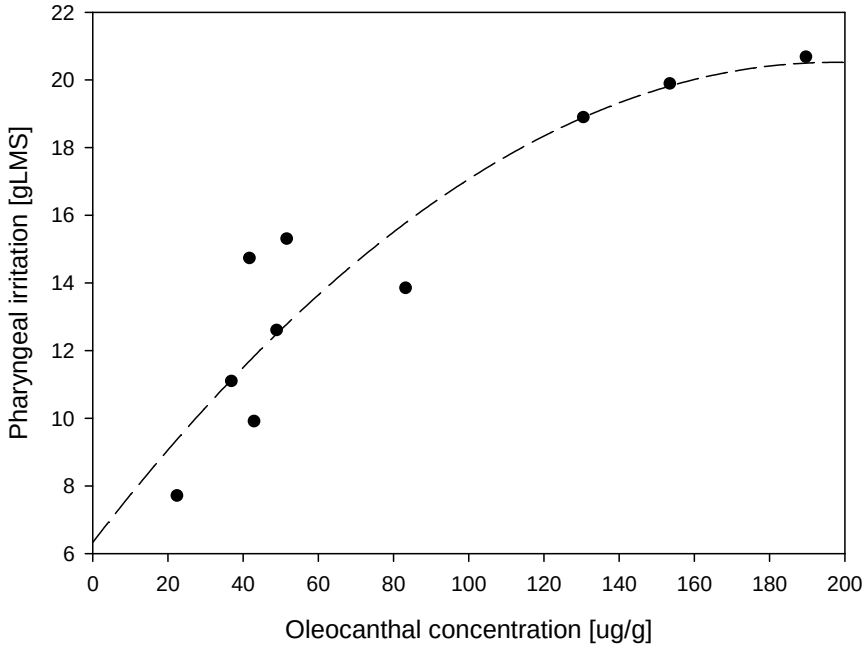
Pharyngeal irritation [gLMS]
22
20
18
16
14
12
10
8
6
0
20
40
60
80
100
120
140
160
180
200
Oleocanthal concentration [ug/g]