Sensory characterization of the irritant properties of oleocanthal, a natural anti-inflammatory agent in extra virgin olive oils

Sara Cicerale1*, Paul A. S Breslin2, Gary K. Beauchamp2 & Russell S. J. Keast1

1School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Highway, Burwood, Victoria 3125, Australia
2 Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

Correspondence to be sent to: Sara Cicerale, School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Highway, Burwood, Victoria 3125 Australia. Phone: +61 3 9251 7286, Fax: +61 3 9244 6017. E-mail: cicerale@deakin.edu.au
Abstract

Oleocanthal is an olive oil phenolic possessing anti-inflammatory activity. Anecdotal evidence suggests that oleocanthal elicits a stinging sensation felt only at the back of the throat (oropharynx). Due to this compound possessing potentially health benefitting properties, investigation into the sensory aspects of oleocanthal is warranted to aid in future research. The important link between the perceptual aspects of oleocanthal and health benefits is the notion that variation in sensitivity to oleocanthal irritation may relate to potential differences in sensitivity to the pharmacologic action of this compound. The current study assessed the unique irritant attributes of oleocanthal including: its location of irritation, temporal profile, and individual differences in the perceived irritation. We show that the irritation elicited by oleocanthal was localized to the oropharynx ($p<0.001$) with little or no irritation in the anterior oral cavity. Peak irritation was perceived 15 secs post exposure and lasted over 180 secs. Oleocanthal irritation was more variable among individuals compared to the irritation elicited by CO$_2$ and the sweetness of sucrose. There was no correlation between intensity ratings of oleocanthal and CO$_2$ and oleocanthal and sucrose ($r=-0.15$, $n=50$, $p=0.92$ and $r=0.17$, $n=84$, $p=0.12$ respectively) suggesting independent mechanisms underlie the irritation of CO$_2$ and oleocanthal. The unusual spatial localization and independence of acid (CO$_2$) sensations suggest distinct nociceptors for oleocanthal are located in the oropharyngeal region of the oral cavity.

Key words: Oleocanthal; Irritation; Somatosensory system; Individual differences
**Introduction**

Newly pressed extra virgin olive oils (EVOOs) contain the olive oil phenolic, (-)-decarboxymethyl ligstroside aglycone, also known as oleocanthal (oleo- for olive, canth- for sting, and al- for aldehyde). Anecdotal evidence suggests that upon consumption of these EVOOs, oleocanthal elicits a concentration dependent irritation at the back of the throat (oropharynx) (Beauchamp *et al.*, 2005). Oleocanthal has been shown to mimic the pharmacology of ibuprofen, also an oropharyngeal irritant, in that oleocanthal has the capacity to inhibit the same cyclooxygenase (COX) enzymes in the inflammatory pathway as does ibuprofen, making oleocanthal a natural NSAID (Beauchamp *et al.*, 2005; Breslin *et al.*, 2001). The potential relationship between health benefits and the Mediterranean diet make oleocanthal a compound of interest, and an investigation into the apparent unique sensory aspects of oleocanthal is warranted to help direct future research. The important link between the perceptual aspects of oleocanthal and health benefits is the notion that variation in sensitivity to oleocanthal irritation may relate to potential differences in sensitivity to the pharmacologic action of this compound.

Mucous membranes in the oral, nasal, and pharyngeal regions are particularly sensitive to the effects of specific irritant chemicals. To stimulate the nociceptive and thermal neurons, chemicals must travel through the epithelia. Mucous membranes have shallow innervation, making them particularly sensitive to chemical stimuli. The need for the chemicals to penetrate the membrane in order to stimulate the nociceptors and thermal receptors is likely the reason chemesthetic sensations typically take longer than tastes and smells to develop and decline (Walker and Prescott, 2003; Green, 1996; Cain *et al.*, 2006; Keast, 2005).
Beauchamp et al. (Beauchamp et al., 2005) used a sensory-directed approach to isolate and identify oleocanthal. Although this approach has proved useful in the identification of oleocanthal, reproducibility of human subject ratings and individual variability in sensitivity to oleocanthal remains unexplored. Furthermore, the location of irritation has not been formally tested. Therefore, the aim of the current study was to further characterize the perceptual attributes of oleocanthal. This included the investigation of spatial and temporal patterning of irritation together with individual differences in perception of oleocanthal irritation. This information will help to elucidate the psychophysical properties of an unusual irritant and the very popular food ingredient that elicits it.

**General materials and methods**

Experiments were conducted at two independent sensory testing centers, in Melbourne, Australia and Philadelphia, U.S.A. The concentration of oleocanthal used in the studies varied due to natural variances in phenolic composition in EVOOs. Oleocanthal levels were quantified by high performance liquid chromatography (HPLC). See reference [1] for method details.

**Experiment 1- Location of oleocanthal irritation**

**Materials and methods**

Anecdotal evidence suggests that oleocanthal irritation is localized to the back of the throat. However, to date no studies have examined this. A within-subjects design was used to examine the location of oleocanthal irritation in the oral cavity. Twenty subjects (14 women, mean age 33.7±10.5 years) were recruited from Melbourne, Australia. Subjects gave their written informed consent prior to participation on an approved Institutional Review Board form (EC253-2006). All testing took place in the Sensory Laboratory at Deakin University.
Subjects were required to attend two training and three test sessions. Subjects were asked to refrain from consuming food and drink (except room temperature water) and use of chemesthetic agents (toothpaste, mouthwash and gum) 2 h prior to testing.

Subjects were trained in the use of the general labeled magnitude pseudo-logarithmic scale (gLMS) following the published standard procedures by Bartoshuk et al. (Bartoshuk et al., 2004; Green et al., 1996; Green et al., 1993). The gLMS is a labeled scale of sensation intensity that requires subjects to rate perceived intensity along a vertical axis containing the adjectives: barely detectable = 1.5, weak = 6, moderate = 17, strong = 35, very strong = 52 and strongest imaginable sensation of any kind = 100. The adjective placement was derived experimentally and yield data equivalent to magnitude estimation (Bartoshuk et al., 2004; Green et al., 1996; Green et al., 1993; Keast and Roper, 2007). Only the adjectives, and not their corresponding numbers, are only visible to the subjects. The experimenter receives numerical data from the scale (Keast and Roper, 2007).

After subjects were familiarized with the scale, they were given hypothetical stimuli and asked to rate their intensity on the scale. Feedback was given by the researchers as to where the general population rated those stimuli for intensity, helping the subjects to better understand how the scale should be used. After that the subjects were supplied with references for barely detectable (sweetness of a 50mM sucrose solution), weak (warmth of luke-warm water), and moderate (irritation of carbonated soda water) to sensory evaluate. For strong, very strong and strongest imaginable, subjects were given hypothetical examples. Subjects were trained to evaluate the irritation intensity of oleocanthal (54μg/g) in EVOO and CO₂ in soda water. Subjects were given as many samples as they required until the researchers felt they understood the instructions and were comfortable with the procedure.
Oleocanthal-containing EVOO was supplied by Redisland Australia (Australia) and soda water was supplied by Kirks Classics (Australia). All testing took place in a specialized sensory testing facility comprising seven individual booths. Each subject was isolated from other subjects by vertical dividers to eliminate interaction between subjects. Subjects also wore nose-clips to eliminate olfactory cues.

An aliquot of 5ml of EVOO and 15ml of soda water (for oropharyngeal testing) and 15ml of both EVOO and soda water (for anterior tongue and anterior mouth testing) were presented in 30ml polyethylene medicine cups (McFarlane Medical, Australia) in a randomized order. Subjects rinsed their mouths with filtered (fi) water (8 micron particulate filter with an activated charcoal filter, Dura®) at least three times over a 2 min period before commencement of testing. Each subject tested and rated (using the gLMS) EVOO for oleocanthal irritation and soda water for CO₂ irritation in the oropharynx, anterior tongue and anterior mouth (see Figure 1 for diagram of the oral cavity).

**INSERT FIGURE 1**

For evaluation of oleocanthal irritation in the oropharynx, the method of sensory evaluation was adapted from Beauchamp and colleagues (Beauchamp et al., 2005). Subjects were required to place the oil in their mouths and tilt their head back to allow the oil to drizzle down the back of their throat. Subjects were asked to keep the oil at the back of the throat for ~5 secs then swallow the sample in two stages and rate the peak intensity of irritation after 20 secs. Swallowing the sample in two stages meant that the subject swallowed the oil and then immediately swallowed again ensuring the oropharynx was coated with the oil. For evaluation
of CO₂ irritation in the oropharynx, subjects were asked to gargle the sample at the back of their throats for 5 secs, swallow the sample, and then rate peak intensity of irritation after 20 secs. For the anterior tongue, a tongue dip method was used for both stimuli, where subjects were asked to place their tongue in the sample for 5 secs, take their tongue out of sample and rate the peak intensity of irritation after 20 secs (Keast and Breslin, 2002). For the anterior mouth, subjects were asked to rinse both stimuli in their mouth for 5 secs, spit and then rate the peak intensity of irritation after 20 secs. All evaluations were performed in duplicate.

Data analysis

Data were analyzed using SPSS for Windows, version 14.0. One-way repeated measures ANOVA with Bonferroni correction was used to determine if a difference in perceived oleocanthal irritation existed between the oropharynx, anterior tongue and anterior mouth.

Results

Oleocanthal irritation was greater in the oropharynx compared to the anterior tongue or anterior mouth (Figure 2) [Wilks’ Lambda=0.07, F(2, 38)=265.70, p<0.05]. No significant difference in irritation was observed between the anterior tongue and anterior mouth (p=1.00). CO₂ irritation was perceived equally at all three sites of the oral cavity [Wilks’ Lambda=0.07, F(2, 38)=2.08, p=0.14].

INSERT FIGURE 2
Experiment 2- Test-retest reliability

Materials and methods

Test-retest reliability of oleocanthal irritation ratings was conducted to determine the reproducibility and thus reliability of such ratings. Materials and methods are equivalent to those in experiment except as otherwise stated. A within-subjects design was used to examine the test-retest reliability of oleocanthal irritation intensity ratings. Thirteen subjects (10 women, mean age 32.7±10.4 years) were recruited from Melbourne, Australia. Subjects were required to attend two training and six test sessions.

Subjects were trained to evaluate the irritation intensity of oleocanthal in EVOO, irritation intensity of CO₂ in soda water and intensity of sweetness of sucrose. EVOO containing 54µg/g of oleocanthal, soda water and a 200mM sucrose solution were used in the experiment. The soda water and sucrose solutions were included in the experiment as control stimuli. Sucrose was supplied by pure Australian white sugar resources and Fi water was used to make the sucrose solution.

An aliquot of 5ml of oil and 15ml of soda water and sucrose solution were presented in 30ml polyethylene medicine cups. In any one session, one sample of EVOO, soda water and sucrose solution were evaluated. Each stimulus was evaluated on six separate occasions. For the evaluation of CO₂ irritation in the anterior mouth and sweetness of sucrose, subjects were asked to rinse both stimuli in their mouth for 5 secs, spit and then rate the peak intensity of irritation (for CO₂) and sweetness (for sucrose) after 20 secs.
Data analysis

A Pearson’s product-moment coefficients correlation was conducted between the averaged values of the first and last three ratings of each of the stimuli to determine oleocanthal, CO₂ and sucrose test-retest reliability. A paired-samples t-test was also conducted to establish if there was a statistically significant difference between the averaged values of the first and last three ratings of each of the stimuli.

Results

Correlation analysis revealed highly reproducible CO₂ \( (r=0.94, n=13, p<0.05) \) and sucrose \( (r=0.98, n=13, p<0.05) \) ratings for all subjects. Test-retest reliability of oleocanthal ratings revealed a slightly weaker correlation \( (r=0.61, n=13, p<0.05) \) than that for CO₂ and sucrose. Refer to Table 1 for subject gLMS rating (mean±SE) for each stimulus. Furthermore, there was no statistical significant difference between the averaged values of the first and last three ratings of each of the stimuli \( (p>0.05) \).

INSERT TABLE 1

Experiment 3- Time-intensity profile of oleocanthal oropharyngeal irritation

Materials and methods

The time-intensity profile of oleocanthal oropharyngeal irritation was examined to determine the time at which irritation is most intense. This information will aid with the establishment of methods for future studies that utilize the sensory directed-approach for the determination of oleocanthal concentration in extra virgin olive oils. Materials and methods are equivalent to those in experiment one except as otherwise stated.
A within-subjects design was used to examine the time-intensity profile of oropharyngeal irritation of oleocanthal. Thirteen subjects (10 women, mean age 23.0±4.0 years) were recruited from Philadelphia, U.S.A. Subjects gave their written informed consent prior to participation on an approved Institutional Review Board form (SETBAPP5005). Subjects were required to attend 10 training and nine test sessions. The 10 training sessions were used to familiarise subjects with the stimulus and rating oleocanthal irritation over a period of time. They were also used to obtain consistency in ratings. In each of the nine test sessions, one olive oil was presented and rated over a period of time.

Oleocanthal-containing EVOO (43μg/g) was supplied by Lucini Italia (Italy) and corn oil was purchased from Wholefoods Supermarket (U.S.A). Corn oil was used as a diluent to reduce the level of oleocanthal in the EVOO. Three levels of dilution were used: 100% EVOO, 0% corn oil; 75% EVOO, 25% corn oil; 50% EVOO, 50% corn oil.

An aliquot of 3.5ml of oil was presented in 30ml polyethylene medicine cups. Subjects were asked to rate the intensity of irritation at the oropharynx elicited by the EVOO, EVOO-corn oil, and corn oil on the gLMS. In each session, only one oil was evaluated. Subjects were given an unidentified sample of oil, required to swallow the sample in two stages, then rate the intensity of throat irritation at nine time points over a three minute period (0, 5, 15, 30, 45, 60, 90, 120, and 180 secs). All evaluations were performed in triplicate and presentation order of oils was randomized.

**Data analysis**

A two-way ANOVA was used to determine if there was a significant main effect of time and concentration on oleocanthal irritation.
**Results**

The temporal pattern of oropharyngeal irritation from oleocanthal is shown in Figure 3. Results from a nine by three (time x concentration) two-way ANOVA of EVOO revealed there was a significant main effect of time \([F(8,18)=9.5, p<0.001]\) and of concentration \([F(2,24)= 6.9, p<0.05]\) at 15 secs post exposure. There was no interaction between time and concentration \([F(16,10)= 0.8, p=0.6]\). Post hoc pair wise tests demonstrated that the intensity of irritation from all three concentrations of oleocanthal at 180 secs was significantly more than at time zero \((p<0.05)\), indicating that duration of sensation exceeded 180 secs. Peak irritation was perceived at 15 secs post exposure and lasted over 180 secs.

There were significant differences in irritation intensity between the highest \((43\mu g/g)\) and lowest \((18\mu g/g)\) oleocanthal concentration time intensity curves \((p<0.05)\). The \(32\mu g/g\) oleocanthal time intensity curve was not significantly different from \(43\mu g/g\) \((p=0.6)\) and \(18\mu g/g\) \((p=0.9)\) time intensity curves.

**INSERT FIGURE 3**

**Experiment 4- Individual variation in oleocanthal oropharyngeal irritation**

**Materials and methods**

This experiment was conducted to determine the extent of variation in perceived intensity of oleocanthal irritation among the general population. Materials and methods are equivalent to those in experiment one except as otherwise stated. A between-subjects design was used to examine individual variation in oleocanthal oropharyngeal irritation intensity. The experiment
was carried out on two separate occasions with two different population groups. Both groups of subjects were required to attend one session which consisted of training and testing.

Group A- Subjects (n=50, 40 women, mean age 23.0±5.0 years) were recruited from Philadelphia, U.S.A. Subjects were asked to rate the irritation intensity of oleocanthal-containing EVOO (154μg/g) (Laudemio, Italy) on the gLMS. As a control irritant, subjects were asked to rate the intensity of mouth irritation elicited by soda water. Group B- Subjects (n=84, 76 women, mean age 20.7±3.7 years) were recruited from Melbourne, Australia. Subjects were asked to rate the irritation intensity of oleocanthal-containing EVOO (70μg/g) on the gLMS. As a control stimulus, subjects were asked to rate the intensity of sweetness elicited by a 200mM sucrose solution.

Group A subjects were given 3.5ml of oleocanthal-containing EVOO and 10ml of soda water in 30ml polyethylene medicine cups. Subjects were instructed to rate the peak intensity of irritation in the oropharynx for oil and anterior mouth for soda water. All evaluations were made in triplicate. For group B, subjects were given 5ml of oleocanthal-containing EVOO and 15ml of a 200mM sucrose solution in 30ml polyethylene medicine cups. Subjects were instructed to rate the peak intensity of oropharyngeal irritation for oil and intensity of sweetness elicited by sucrose.

Data analysis

Pearson product-moment coefficients correlation was also conducted to analyze the relationship between oleocanthal irritation intensity, CO₂ irritation intensity and the sweetness of sucrose intensity. Mean, range, and variance values were used to determine variability in
perceived oleocanthal irritation among individuals. \( P \) values <0.05 were considered significant.

**Results**

Group A results demonstrated a greater mean, range, and variance in perceived intensity of oropharyngeal irritation from oleocanthal-containing EVOO (\( n=50 \), mean \( gLMS \) 25.2, range \( gLMS \) 10-49, variance 79.1) compared with anterior oral irritation from \( CO_2 \) (\( n=50 \), mean \( gLMS \) 10.3, range \( gLMS \) 5-14, variance 3.6). Figure 4 shows respective histograms with an overlay of a normal distribution. There was no correlation between perceived intensity of oleocanthal irritation and soda water irritation (\( r=-0.15, n=50, p=0.92 \)) indicating a lack of a shared mechanism between the stimuli.

**INSERT FIGURE 4**

Group B results also demonstrated a greater mean, range, and variance in perceived intensity of oropharyngeal irritation from EVOO (\( n=84 \), mean \( gLMS \) 24.0, range \( gLMS \) 1-81, variance 243.3) compared with the intensity of sucrose sweetness (\( n=84 \), mean \( gLMS \) 9.8, range \( gLMS \) 0-34, variance 45.0). There was no correlation between oleocanthal oropharyngeal irritation and sucrose sweetness intensity ratings (\( r=0.17, n=84, p=0.12 \)) indicating that irritation was likely to be independent of an individual’s idiosyncratic use of the \( gLMS \) or is a result of an overall effect like supertasting or a central gain in absence. Figure 5 shows respective histograms with an overlay of a normal distribution.

**INSERT FIGURE 5**

**General discussion**
This study demonstrates that oleocanthal irritation is greatest in the oropharyngeal region of the oral cavity and the irritation produced is not correlated with that of CO₂ irritation and therefore does not elicit irritation via a generalized acid sensing mechanism. These findings may be are result of the existence of chemesthetic receptors located in the oropharyngeal region that respond specifically to the natural non-steroidal anti-inflammatory compound, oleocanthal. Alternatively, it remains possible that oleocanthal activates TRPV1 or other such receptors in the throat.

The localized irritation of oleocanthal is surprising in light of the fact that nociceptive neurons typically respond to most irritants (Green, 2004). For instance, chemical irritants such as capsaicin and menthol evoke irritant sensations throughout the oral cavity and other mucus membrane areas of the body. Chemical irritation of a restricted area (as in the case of oleocanthal) is rare (Green, 2004). Similarly, ibuprofen solely irritates the oropharynx suggesting that this mucosal region possesses sensory receptors specific to compounds structurally related to oleocanthal and ibuprofen (Breslin et al., 2001).

All subjects gave reproducible ratings for both CO₂ mouth irritation and sucrose sweetness. Oleocanthal oropharyngeal irritation ratings were less reliable than for CO₂ and sucrose but are never the less somewhat reliable r=0.61. The cause(s) of this reduced reproducibility for oleocanthal oropharyngeal irritation ratings is not entirely clear. The difference in reproducibility between CO₂ and oleocanthal irritation ratings may be due to differences in diffusion through the epithelium. Furthermore, differences in salivary composition from test to test (Breslin et al., 2001) and thickness of the mucus layer present at the back of the throat at time of testing might affect oleocanthal ratings. Further research is required to explore the
factors that affect the variability in perceived oleocanthal oropharyngeal irritation. Nevertheless, the reliability was sufficiently high to yield meaningful data.

There was variability among subjects in perceived irritation from oleocanthal. Such individual variation in perception of oleocanthal may be related directly to the specific form and quantity of receptors in the oral cavity, as has been reported with other oral stimuli such as 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC) bitterness (Green, 2004; Breslin and Spector, 2008). There were non-significant correlations between ratings of oleocanthal irritation and the irritation of CO₂ or the sweetness of sucrose. Thus, the large variability in perceived intensity should not be attributed to an individual’s idiosyncratic use of the gLMS and indicates irritation elicited by oleocanthal and the irritation elicited by CO₂ access somewhat different physiological mechanisms. Similar to the findings of Breslin et al (Breslin et al., 2001) regarding ibuprofen, the large inter-individual variation and non-correlation with CO₂ intensity suggest oleocanthal irritation may be due to specific receptors in the oropharynx that differ from person to person in their density or their ability to bind and respond to oleocanthal.

A limitation of the study to consider was that the majority of participants were women and therefore the results obtained may not generalize to men. Future studies could include a greater proportion of men to investigate if there are gender differences regarding the perceptual attributes of oleocanthal.

In summary, oleocanthal irritation was localized to the oropharyngeal region of the oral cavity and is highly variable among individuals. Taken together our findings suggest that chemical-
specific receptors are located in the oropharyngeal region of the oral cavity that responds to oleocanthal.

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References


Tables

Table 1: Subject gLMS rating (mean±SE) for EVOO, soda water and sucrose.

Figures

Fig. 1: The oropharynx, anterior tongue and anterior mouth.

Fig. 2: Bar graph depicting gLMS ratings (mean±SE) for intensity of oleocanthal and CO₂ irritation on the oropharynx, anterior tongue and anterior mouth.

Fig. 3: Temporal profile of oleocanthal irritation.

Fig. 4: Histograms of rating frequency (gLMS) of irritant intensity of oleocanthal and CO₂. The x-axis represents the average irritation on the gLMS by an individual subject. The Y-axis represents the number of subjects. Each bar represents the number of people who rated the irritation at the specified intensity range.

Fig. 5: Histograms of rating frequency (gLMS) of irritant intensity of oleocanthal and sweetness of sucrose. The x-axis represents the average irritation on the gLMS by an individual subject. The Y-axis represents the number of subjects. Each bar represents the number of people who rated the irritation at the specified intensity range.
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<th>Subject number</th>
<th>EVOO containing 54µg/g oleocanthal (mean±SE)</th>
<th>Soda water (mean±SE)</th>
<th>200mM sucrose (mean±SE)</th>
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Anterior mouth
Oropharynx
Anterior tongue