Chem. Senses 35: 229–238, 2010
doi:10.1093/chemse/bjq002
Advance Access publication January 25, 2010

Orthonasal and Retronasal but not Oral-Cavity-Only Discrimination of Vapor-Phase Fatty Acids

Bryson Bolton[1,4] and Bruce P. Halpern[2,3]

[1]Graduate Field of Food Science and Technology, Cornell University, Ithaca, NY 14853, USA, [2]Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853, USA and [3]Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

Correspondence to be sent to: Dr Bruce P. Halpern, Department of Psychology, Uris Hall, Cornell University, Ithaca, NY 14853-7601, USA. e-mail: bph1@cornell.edu

[4]Present address: Sensory Sciences—Research, Development and Quality, Kraft Foods, Inc., 801 Waukegan Road, Glenview, IL 60025, USA

Accepted December 30, 2009

Abstract

Discrimination of vapor-phase linoleic, oleic, and stearic fatty acids was studied using triangle tests. For each trial, 2 of the 3 modified odorant delivery containers (MODCs) had the same content and 1 was different. Contents were either mineral oil-diluted linoleic or oleic acids, with mineral oil in the other MODC (blanks) or undiluted stearic acid with NaCl in the other MODC (blanks). The task was to indicate which of the 3 MODC had the most different odor. Vapor-phase fatty acids and blanks were presented orthonasally, retronasally, or oral-cavity-only. It was found that all 3 fatty acids were discriminated from the blanks both orthonasally and retronasally, $P \leq 0.01$, one-tailed binomial tests. Orthonasally, 87% of 30 participants discriminated linoleic acid from blanks and 83% discriminated oleic and stearic acids. Retronasally, 93% discriminated linoleic acid from blanks, 57% discriminated oleic acid; 83% discriminated stearic acid. In contrast, with oral-cavity-only presentations, none of the fatty acids were discriminated from blanks, $P > 0.05$ (30% of 30 participants discriminated linoleic acid from blanks, $P = 0.71$; 47%, oleic and stearic acids, $P = 0.09$). These results demonstrate that human participants can discriminate linoleic, oleic, and stearic fatty acids both orthonasally and retronasally, confirming that humans can smell fatty acids.

Key words: fatty acids, human, oral cavity, orthonasal, retronasal, smell

Introduction

Fatty acids are common in foods, albeit often in ester form as a triglyceride (a.k.a. triacylglyceride). The 3 fatty acids of a triglyceride, which may all be the same fatty acid or may be 2 or 3 different fatty acids, are attached to a glycerol molecule (Potter and Hotchkiss 1998; Ophardt 2003; Lobb and Chow 2007). In addition to the triglyceride form, low concentrations of free fatty acids are also present in various foods (see Weiss 1983; McGee 2004; Chow 2007). The particular food-related fatty acids of present interest, linoleic, oleic, and stearic acids, are specifically described as long-chain, 18-carbon, unsaturated (linoeic and oleic) or saturated (stearic) carboxylic acids.

Notwithstanding the importance of fats and fatty acids in human nutrition and in food acceptance and flavor (McGee 2004; Stipanuk 2006), the types of sensory stimulation produced by fatty acids have been a matter of repeated disagreement, discussion, and investigation. Oral somatosensory stimulation resulting in perceived texture is generally ascribed to fats (e.g., Drewnowski 1987, 1997). However, the existence of chemosensory stimulation in humans by fats or fatty acids, that is, taste or smell responses, as distinguished from chemesthetic input (see Green 1996; Green et al. 2005; Simons and Carstens 2008), has received both positive and negative findings.

Evidence for human gustatory responses to oral emulsions containing fatty acids was provided by Chalè-Rush et al. 2007a and Mattes 2009b. In 2 subsequent series of experiments (Chalè-Rush et al. 2007b; Mattes 2009a), 3-alternative forced-choice detection thresholds were measured for both room temperature linoleic and oleic fatty acid emulsions and stearic fatty acid emulsions at 67–69 °C. The fatty acid emulsions contained, in addition to a fatty acid, gum acacia, ethylenediaminetetraacetic acid, water, and mineral oil. Detection thresholds were measured for orthonasal and

retronasal smell, for taste (in the taste condition, a nose clip was in place when the emulsion was in the oral cavity), and for a ''multimodal'' presentation condition (for the multimodal condition, an emulsion was in the oral cavity but no nose clip was used; the multimodal condition has sometimes been referred to as ''oral'' by other investigators, see Halpern 2004). In addition, an orthonasal ''olfactory irritation'' threshold (more commonly referred to as ''lateralization,'' e.g., Wysocki and Wise 2004; Cain et al. 2005, 2006; Frasnelli and Hummel 2005), in which participants indicated which nostril had been stimulated with the orthonasally presented fatty acid emulsion, was measured for linoleic acid (Chalè-Rush et al. 2007b).

Chalè-Rush et al. (2007b) and Mattes (2009a) reported that the measured smell and taste detection thresholds differed as a function of the presentation conditions and of the fatty acids. With regard to presentation conditions, retronasal detection thresholds were higher than all the other detection thresholds, and the taste detection thresholds were higher than the orthonasal detection thresholds. However, the multimodal (i.e., oral) thresholds were not significantly different from the orthonasal detection thresholds. With reference to detection threshold differences between the fatty acids, the orthonasal detection threshold for the oleic acid emulsion was the lowest and the retronasal detection threshold for the stearic acid emulsion was the highest. In addition, the ''orthonasal irritation'' (i.e., lateralization) threshold, which had been measured only for the linoleic acid emulsion, was numerically equal to the orthonasal linoleic acid emulsion detection threshold and did not differ significantly from the linoleic acid emulsion taste detection threshold (Chalè-Rush et al. 2007b). Despite these orthonasal and retronasal detection threshold findings for complex emulsions of linoleic, oleic, and stearic fatty acid, doubts have been expressed regarding the reality of human smelling of fatty acids (Mattes 2009a, 2009b).

Several issues regarding human detection of vapor-phase linoleic, oleic, and stearic fatty acids remain to be resolved. The multicomponent emulsions that were used by Chalè-Rush et al. (2007a, 2007b) in order to minimize somesthetic stimulation in the oral cavity make interpretation of the emulsions as sources of vapor-phase stimuli complex, whereas the high temperature of the stearic acid emulsion, chosen in order to solubilize the stearic acid, provided a pronounced temperature difference as well as, presumably, a higher vapor-phase concentration than could have been achieved at room temperature. Finally, the observation that the linoleic acid emulsion orthonasal irritation (i.e., lateralization) threshold was equal to the orthonasal detection threshold might suggest that this fatty acid, and perhaps the others, are very effective trigeminal stimuli (see Cain et al. 2006; Abraham et al. 2007).

To resolve these issues, suprathreshold discrimination experiments were designed utilizing vapor-phase presentations of linoleic and oleic fatty acids diluted with mineral oil, and vapor-phase presentation of undiluted stearic fatty acid, all at 21 ± 1 °C (Bolton 2009). Orthonasal and retronasal presentations were included to test the existence of vapor-phase fatty acid discrimination through the nostrils and from the oral cavity. Oral-cavity-only presentations were used to directly assess the possibility that stimulation of the oral cavity's trigeminal system with vapor-phase fatty acids would be sufficient to permit discrimination. Triangle tests (O'Mahony 1986; Lawless and Heymann 1998; Laska 2004; Stone and Sidel 2004; Meilgaard et al. 2007) were employed to determine whether vapor-phase fatty acids could be discriminated from blanks not containing fatty acids because triangle tests are basic forced-choice discrimination measures.

For the present experiments, only vapor-phase stimuli were available, thus eliminating nonvapor-phase sources of information for discrimination. All modified odorant deliver container (MODCs) held either an equal volume of liquid (linoleic or oleic fatty acid discrimination from mineral oil blank) or equal weights of solid (stearic fatty acid discrimination from NaCl blank). The task on each trial was to select the MODC with the most different odor. The hypotheses were 1) at suprathreshold concentrations, all 3 vapor-phase fatty acids would be discriminable from blanks that did not contain fatty acids both orthonasally and retronasally, 2) retronasal discrimination would be inferior to orthonasal discrimination, and 3) oral-cavity-only discrimination might be found for all 3 fatty acids.

Materials and methods

Participants

Participants were paid volunteers, receiving $6 for each session in which they participated. Ages ranged from 19 to 60 years across the 2 experiments. Specific numbers and ages will be given for each experiment. The participants were affiliated with Cornell University and were recruited using flyers that were posted around Cornell University's Ithaca NY campus. All participants were at least 18 years of age, could communicate in written and spoken American English, were nonsmokers, nonpregnant, and nonlactating. These were the only inclusion and exclusion criteria.

Participants were asked to not eat or drink anything for 1 h prior to the experiment. They were not screened for their ability to detect any odorants prior to the study nor were any other chemosensory data collected. The Cornell University Institutional Review Board (IRB) for Human Participants reviewed and approved the protocol. Each potential participant read the informed consent form, asked any questions they had, and, if they decided to participate in the study, signed the informed consent form that had been approved by the IRB. Participants were informed that the study would test their smelling ability.

Odorants

The fatty acids were linoleic acid (~60%, CAS no. 60-33-3), oleic acid (FCC, Kosher, FG, CAS no. 112-80-1), and

stearic acid (reagent grade, 95%, CAS no. 57-11-4), all from Sigma-Aldrich, Inc. The diluent for the linoleic and oleic acids, and the blank in their triangle tests (see below), was mineral oil, United States Pharmacopeia grade. The linoleic acid was diluted to 66.6% with mineral oil (volume/volume), where the undiluted linoleic acid was considered 100%, and the oleic acid was diluted to 40%. Dilutions were prepared on the day on which they were used. Stearic acid, a solid at 21 ± 1 °C, was used undiluted; the blank in the stearic acid triangle tests was NaCl (analytical reagent grade). The fatty acid concentrations were based upon a prior study (Tamburrino and Halpern 2007) and were verified for orthonasal detection by informal preliminary testing.

The undiluted linoleic and oleic acids were kept in the dark under Prepure Nitrogen at 4.5 °C. Stearic acid was stored in the dark at –18.5 °C. NaCl was stored in a dessicator in the presence of $CaCl_2$.

Odorant presentations

Odorant presentation containers

Vapor-phase fatty acid odorants and blanks for triangle tests (see below) were presented at 21 ± 1 °C in delivery containers modified from the odorant delivery container (ODC) design that had been used by Chen and Halpern (2008) and Parikh et al. (2009). The container was the same type of homopolymer polypropylene, black tapered elliptical container (Ellipso Portion Cups, Newspring Packaging) used by Chen and Halpern (2008) and Parikh et al. (2009). These containers were 118 mL in volume, 5.1 cm high, with an upper major axis of 7.8 cm and an upper minor axis of 4.9 cm. The lower major axis was 5.4 cm with a minor axis of 2.7 cm. The container had a tight-fitting, transparent homopolymer polypropylene elliptical lid. Modifications of the lid differed for the orthonasal versus the retronasal and oral-cavity-only experiments of the present study. These changes in the lid distinguished the MODCs of the present experiments from the ODC used by Chen and Halpern (2008) and Parikh et al. (2009). The MODC employed for the orthonasal triangle tests (orthonasal modified odorant delivery containers [O-MODC], see below) were first used in a preliminary experiment to ensure that they effectively delivered suprathreshold vapor-phase orthonasal odorants. Preliminary testing was not necessary for the MODC used for the retronasal and oral-cavity-only triangle tests (retronasal modified odorant delivery containers [R-MODC], see below) because they were functionally similar to those that had previously been used.

Odorant quantities

Five mL of mineral oil-diluted linoleic acid or oleic acid (including diluent), or of mineral oil, or 2 g of stearic acid (volume of 2.4 mL) or of NaCl (volume of 0.9 mL), were placed in an MODC, just covering the bottom.

Sequence

Participants received triangle discrimination tests (Lawless and Heymann 1998; Laska 2004; Meilgaard et al. 2007). On each trial of a triangle discrimination test, participants are presented with 3 samples. Two of the samples are the same and 1 is different. The triangle test is used to determine whether participants can differentiate between the 2 samples that are the same and the 1 that is different. To measure this, participants are asked to select the odd or most different of the 3 samples (Stone and Sidel 2004). A selection must be made on every trial. The probability of selecting the odd sample by chance is always 0.33 (Lawless and Heymann 1998).

In the present study, participants were presented with 3 MODC during each trial, with the 6 possible serving orders randomized across participants (Lawless and Heymann 1998). That is, if "F" represents a fatty acid and "B" represents its associated blank, the contents of the 3 MODC, randomized across participants for each fatty acid and associated blank, were BBF, BFB, FBB, FFB, FBF, and BFF. Thus on every trial for all fatty acids and presentation conditions (orthonasal, retronasal, and oral-cavity-only), participants received not only MODC with a fatty acid but also MODC holding an equal volume or an equal weight associated blank. For the mineral-oil-diluted linoleic and oleic fatty acids, the associated blank was an equal volume of mineral oil; for stearic fatty acid, which was used undiluted, the associated blank was an equal weight of NaCl. The 1 different MODC would contain either linoleic, oleic, or stearic fatty acid or a blank. Participants were asked to select the odd or most different of the 3 samples, with a selection of the most different MODC required on every trial. Each of the 3 MODC had unique 3-digit code numbers on them. The response was made by circling the code number of a MODC on a response sheet; a correct response was to select as most different the 1 different MODC. For each participant, the probability of a correct triangle test selection by chance on a trial, that is, selecting the 1 different MODC by chance on a trial, was 0.33.

Across participants, the probability of selecting the 1 different MODC by chance on a triangle test trial can be calculated for various numbers of participants using the cumulative binomial probability distribution. These probabilities have been tabulated and published (Roessler et al. 1978; O'Mahony 1986; Lawless and Heymann 1998; Meilgaard et al. 2007). For the 30 participant sample size of the present study, selection by chance of the 1 different MODC on 1 trial by 15 participants would have a probability of 0.05. Correct selection of the 1 different MODC on 1 trial by 15 or more participants was taken as indicating statistically significant discrimination.

The order in which the 3 different odorants were presented across trials was randomized, with all participants receiving all 3 fatty acids. For each participant, odorant, and odorant presentation condition (orthonasal, retronasal, or oral-cavity-only), only 1 trial of a particular odorant and its associated blank was done. Thus, the data of these experiments consist of the number of participants who selected the MODC with content different from the other 2 as the one with the most different odor, that is, the number of correct selections indicating discrimination between a fatty acid and its associated blank.

During a trial the participant held a MODC without tilting it, removed the cap or caps from the tube(s) (see O-MODC and R-MODC, below) that extended above the lid, positioned the MODC so that vapor-phase inhalation could be carried out through the tubes that extended above the lid (O-MODC) or the straw that extended above the lid (R-MODC), and inhaled from a MODC up to 5 times, committing to memory the odor. After completing inhaling from 1 of the 3 MODC, the participant recapped its tube(s) and went on to inhale from another of the 3 MODC of that trial. Once the tube(s) were recapped that MODC was not used again. After all 3 MODC had been inhaled from and their odors committed to memory, the participant indicated which of the 3 was most different. A MODC was used for one odorant or its comparison stimulus, and for one participant, and then was discarded.

Orthonasal modified odorant delivery containers

For orthonasal odorant presentations, two 1 cm diameter openings were made along the long axis of the MODC lid, each 3 cm from an end. Into each opening an 8.2 cm length of a 5-mL Eppendorf ep disposable pipette tip (Eppendorf North America) was inserted perpendicular to the lid such that 4 cm projected below the lid when the disposable pipette tip was tightly fitted into the opening. These 2 disposable pipette tips, fixed in the lid but adjustable in angle, served as vapor-phase odorant delivery tubes during orthonasal triangle tests. To use an O-MODC, the participant held the O-MODC with its bottom approximately horizontal, tilted the 2 vapor-phase odorant delivery tubes so that they approximated the locations of that participant's external nares, and inhaled. When used with either stearic acid or NaCl in the container, to prevent possible particulate inhalation, 2.54×2.54 cm Kimwipe (low-lint, low-extractable scientific wipe; Kimberly-Clark) squares were taped around the ends of the tubes located inside the container. This lid, combined with the 118-mL elliptical container previously described, was the O-MODC used for orthonasal presentations. Aluminum foil was then secured/wrapped onto the lids of the O-MODC that contained stearic acid or NaCl to mask the identity of the sample. To further prevent visual identification by looking through the apical portion of the pipette tips, participants were asked to close their eyes during triangle test presentations of stearic acid and NaCl. Eye closure was monitored by an experimenter. These visual masking techniques were not used for linoleic and oleic acid triangle tests after benchtop testing revealed that identification did not occur. In order to close the odorant delivery tubes of the O-MODC except when in use by a participant, plastic caps cut from 5-ml sample vials could be placed on the ends of the tubes located outside the O-MODC (Figure 1).

Retronasal and oral-cavity-only MODC

Two openings were made in the lid along the long axis of the lid. One opening was 5 mm in diameter, 3.5 cm from one end of the lid; the other, 1.3 cm in diameter and 1.8 cm away from the 5 mm opening. Into the 5 mm opening a 6.5 cm length of 5 mm outer diameter homopolymer polypropylene straw (Jetware Unwrapped Plastic drinking straw, Jet Plastica Industries, Inc.) was inserted 3.25 cm, perpendicular to the lid. This straw fit tightly into the opening. When used with either stearic acid or NaCl in the container, to prevent possible particulate inhalation a 2.54×2.54 cm Kimwipe (low-lint, low-extractable scientific wipe; Kimberly-Clark) square was taped around the end of the straw located inside the container. Into the 1.3 cm opening that had been made in the lid a 4 cm length of 5-mL Eppendorf ep disposable pipette tip was inserted 2 cm, perpendicular to the lid. This lid, combined with the 118-ml elliptical container previously described, was the R-MODC used for retronasal and oral-cavity-only presentations. Aluminum foil was then secured/wrapped on to the lids of the R-MODC that contained stearic acid or NaCl to mask the identity of the sample. To further prevent visual identification by looking through the apical portion of the pipette tip, participants were asked to close their eyes during triangle test presentations of stearic

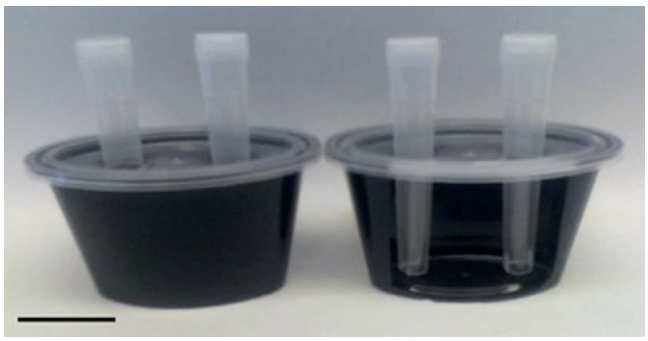

Figure 1 The O-MODC on the left shows an exterior view and the O-MODC on the right shows an interior view. The interior view was made possible by cutting away a portion of the wall of the O-MODC. The O-MODC shown in the interior view does not have Kimwipe squares taped around the interior ends of the tubes, as would be the case when used with presentations of stearic acid or its associated blank, NaCl. The plastic caps shown on the 2 tubes were removed by a participant before the participant angled the 2 tubes to approximate their nostrils, and inhaled. The horizontal calibration line represents 3 cm.

acid and NaCl. Eye closure was monitored by an experimenter. These visual masking techniques were not used for linoleic and oleic acid triangle tests after benchtop testing revealed that identification did not occur. To use a R-MODC, the participant held the R-MODC with its bottom approximately horizontal, placed their lips securely around the straw and inhaled. In order to minimize odorant flow from the R-MODC except when in use by a participant, a plastic cap cut from a 5-mL sample vial could be placed on the portion of the cut Eppendorf ep disposable pipette tip that was located outside the lid (Figure 2).

Nose clip

All retronasal and oral-cavity-only trial sequences began with the participant wearing a nose clip (Spirometrics Nose Clip no. 2104, Spirometrics). The nose clip was removed after inhalation but before exhalation for retronasal triangle test discrimination trials but remained in place during oral-cavity-only triangle test discriminations trials. Each nose clip was used for one participant and one odorant presentation method (retronasal or oral-cavity-only) and then discarded.

Orthonasal and retronasal fatty acid smelling tests

The participants were 30 paid volunteers, 17 males and 13 females. Age range = 19–60 years, mean = 26.6 years, standard deviation (SD) = 9.3 years. For purposes of this study, orthonasal smelling was described as inhaling through the nose. Retronasal smelling was described as smelling from inside of the mouth while exhaling out the nose. Both procedures were demonstrated by an experimenter to each participant. After seeing the demonstration, a participant was asked to demonstrate the orthonasal and retronasal procedures before beginning the triangle tests. Each participant did both orthonasal and retronasal triangle tests for linoleic, oleic, and stearic fatty acids and their associated blanks. The odd numbered participants received the orthonasal tests first, and the even numbered participants received the retronasal tests first. There was a 2- to 3-min time interval between orthonasal and retronasal odorant testing.

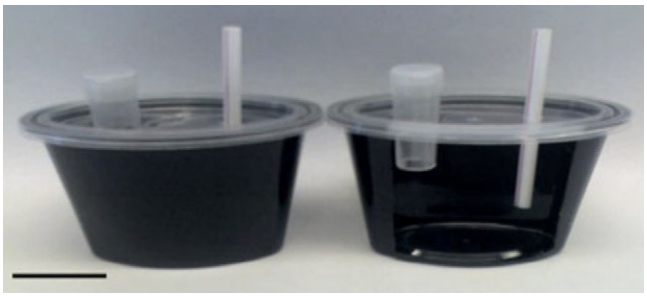

Figure 2 Retronasal and oral-cavity-only MODCs. The R-MODC on the left shows an exterior view and the R-MODC on the right shows an interior view. The interior view was made possible by cutting away a portion of the wall of the R-MODC. The R-MODC shown in the interior view does not have a Kimwipe square taped around the interior end of the straw, as would be the case when used with presentations of stearic acid or its associated blank, NaCl. The plastic cap shown on the left-hand tube was removed by a participant before the participant placed their lips securely around the straw (the right-hand, uncapped tube), and inhaled. The horizontal calibration line represents 3 cm.

Orthonasal smelling: An orthonasal trial began with the participant picking up one O-MODC, holding it horizontally, and removing the 2 caps from the 2 plastic pipette tips. The participant next angled the pipette tips of that O-MODC so that the pipette tips corresponded to their nostrils, with the outer edges of the tubes gently grazing the outer rims of their nostrils. Then the participants inhaled moderately one time, removed the tubes from their nostrils, and exhaled through their nostrils. The O-MODC placement/inhalation/exhalation orthonasal procedure could be repeated up to 5 times for that O-MODC.

Retronasal smelling: A retronasal trial began with the participant putting on their nose clip so that breathing through the nose was not possible. Then they picked up one R-MODC and removed the cap from the pipette tip. They placed their lips around the straw and inhaled moderately once. The participant then removed the straw from their mouth area, keeping their lips closed. Then they removed their nose clip and exhaled through their nose while keeping their mouth closed. After 2–3 s, the nose clip/inhalation/exhalation retronasal procedure could be repeated up to 5 times for that R-MODC.

Oral-cavity-only fatty acid smelling tests

The participants were 30 paid volunteers, 14 males and 16 females. Age range = 20–42 years, mean = 26 years, SD = 4 years. Nine of the 30 participants were from the orthonasal and retronasal experiment. For the purposes of this study, oral-cavity-only fatty acid smelling was described as inhaling and exhaling through the mouth while keeping a nose clip secured onto the nose. The procedure was the same as that for retronasal smelling (see above), except that the nose clip remained in place and exhalation as well as inhalation were through the mouth.

Statistical analyses

The data of these triangle test discrimination experiments consisted of the number of participants who selected the 1 MODC that differed in odor from the other 2 MODC of a trial as the one with the most different odor, that is, the number of correct responses for each odorant in each of the 3 presentation conditions. Because there were 30 participants for each presentation condition, the number of correct responses for each odorant and presentation condition could range between 0 and 30. For each participant, odorant, and odorant presentation condition (orthonasal, retronasal, or oral-cavity-only), only 1 trial of a particular odorant and its associated blank (a total of 3 MODC) was done by each participant. On every trial each participant was forced to

make a decision. The relevant data are the number of correct selections, that is, the proportion correct for a specified sample size.

The triangle discrimination test is considered a one-tailed test. That is the case because there is only one correct answer, and performance below chance is not of interest (Amerine et al. 1965; Roessler et al. 1978; O'Mahony 1986; Lawless and Heymann 1998; MacRae 1995; Meilgaard et al. 2007). The probability of a correct selection by chance on 1 trial is 0.33. Tabulated cumulative binomial distribution values (Roessler et al. 1978; O'Mahony 1986; Lawless and Heymann 1998; Meilgaard et al. 2007) indicate that for a triangle discrimination test with 30 participants, 15 correct selections would have a chance probability of 0.05; 17 correct selections of 0.01; and 19 correct selections of 0.001 (Roessler et al. 1978; O'Mahony 1986; Meilgaard et al. 2007). Consequently, the probability of chance selection of the 1 MODC with contents different from the 2 other MODC of a trial by ≥15 of the 30 participants was ≤0.05, and a statistically significant discrimination was noted.

Differences in number of correct discriminations between male and female participants, and between the 3 fatty acids, were evaluated using likelihood ratio contingency analyses (Williams 1976).

Results

Preliminary study on O-MODC

Twenty-nine of 30 participants selected the O-MODC containing orange extract and the O-MODC containing strawberry extract as the ones with the most different odor; 30 selected the O-MODC containing peppermint extract (sunflower oil diluent and blanks; extracts from Frontier Natural Flavors Co-op). For all 3 preliminary study triangle test outcomes, the probability of selecting the natural extract–containing O-MODC by chance the observed number of times (≥29 correct selections for 30 participants) was <0.001 (cumulative binomial probability distribution, Roessler et al. 1978; Meilgaard et al. 2007). This outcome indicated that the O-MODC was effective in orthonasal triangle tests with vapor-phase odorants. Prior studies had found that an ODC similar to the R-MODC was effective for retronasal and oral-cavity-only vapor-phase presentations (Chen and Halpern 2008; Parikh et al. 2009; Stephenson and Halpern 2009). Consequently, no preliminary testing of the R-MODC was done.

Tests of fatty acid smelling

The triangle test discrimination experiments had been designed to measure to what extent, if any, vapor-phase linoleic, oleic, and stearic fatty acids could be discriminated from blanks not containing a fatty acid and whether there were differences between these fatty acids in the degree of discrimination.

Test of orthonasal fatty acid smelling

Using the orthonasal procedure (inhalation and exhalation through the nostrils), 26 of 30 participants selected the 1 different O-MODC when linoleic fatty acid was present and 25 selected the 1 different O-MODC when oleic or stearic fatty acids were present (Figure 3). For all 3 triangle test orthonasal fatty acid discrimination outcomes, the probability of selecting the 1 different O-MODC by chance the observed number of times was <0.001 (≥25 correct selections for 30 participants, cumulative binomial probability distribution, Roessler et al. 1978; Meilgaard et al. 2007).

Test of retronasal fatty acid smelling

Using the retronasal procedure (inhalation through the mouth, exhalation through the nostrils after the nose clip was removed), 28 of 30 participants selected the 1 different R-MODC when linoleic fatty acid was present; 17 selected the 1 different R-MODC when oleic fatty acid was present; 25 selected the 1 different R-MODC when stearic fatty acid was present (Figure 3). For all 3 retronasal fatty acid discrimination triangle tests, the probability of selecting the 1 different R-MODC by chance the observed number of times was ≤0.01 (≥17 correct selections for 30 participants, cumulative binomial probability distribution, Roessler et al. 1978; Meilgaard et al. 2007). For linoleic and stearic fatty acids, the probability of selecting the 1 different R-MODC by chance

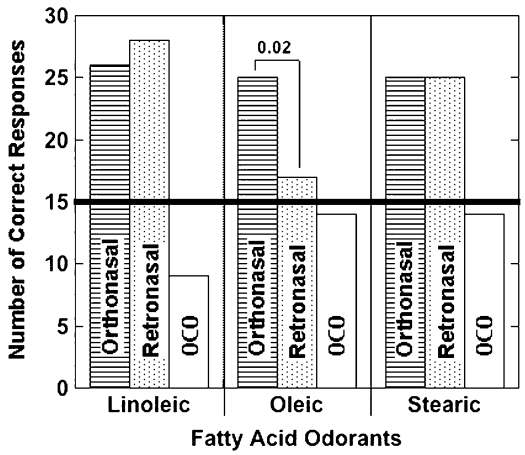

Figure 3 The number of correct responses for 30 participants for orthonasal (bars with horizontal lines), retronasal (gray bars), and oral-cavity-only (OCO, clear bars) triangle discrimination tests using MODC with each of 3 fatty acids. Linoleic, vapor-phase linoleic acid versus mineral oil; Oleic, vapor-phase oleic acid versus mineral oil; Stearic, vapor-phase stearic acid (solid) versus NaCl. Number of correct responses, total number of correct responses for each fatty acid triangle test across the 30 participants. The horizontal black bar at 15 correct responses represents the minimum number of correct judgments needed for $P = 0.05$ for a triangle test when $n = 30$. The rectangular bracket spanning the orthonasal and retronasal oleic acid correct responses indicates that the numbers of these 2 correct responses were significantly different at $P = 0.02$ (likelihood ratio). There were no other statistically significant differences between orthonasal and retronasal triangle tests, $P \geq 0.39$.

the observed number of times was <0.001 (≥25 correct selections for 30 participants, cumulative binomial probability distribution, Roessler et al. 1978; Meilgaard et al. 2007).

Gender effects for orthonasal versus retronasal smelling

Likelihood ratio measures indicated that, across orthonasal and retronasal oleic acid triangle tests, female participants were significantly more likely than male participants to make correct responses, $P = 0.03$ (likelihood ratio, Williams 1976). The corresponding contingency analysis for oleic acid showed female total response percentage of ~80% correct; males, ~65%. There were no significant differences between male and female participants for number of correct responses for linoleic or stearic acid for either orthonasal or retronasal tests, $P \geq 0.6$ (likelihood ratio, Williams 1976). For linoleic and stearic acids, males and females were both ≥75% correct.

Comparisons between orthonasal and retronasal smelling test outcomes

Likelihood ratio measures indicated that, between orthonasal and retronasal oleic acid triangle tests, participants were significantly more likely to make correct responses for orthonasal than for retronasal smelling, $P = 0.02$ (likelihood ratio, Williams 1976; Figure 3). The corresponding contingency analysis for oleic acid showed orthonasal total response percentage of 83% correct, and for retronasal smelling, 57% correct. For linoleic and stearic fatty acids, there were no statistically significant differences between numbers of orthonasal and retronasal correct triangle test responses, $P \geq 0.39$ (likelihood ratio, Williams 1976). The corresponding contingency analyses for linoleic and stearic acids showed orthonasal and retronasal total correct response percentage of ≥83%.

Test of oral-cavity-only fatty acid smelling

Using the oral-cavity-only procedure (nose clip not removed, inhalation and exhalation through the mouth), 9 of 30 participants selected the 1 different R-MODC when linoleic fatty acid was present and 14 selected the 1 different R-MODC when either oleic or stearic fatty acids were present (Figure 3). For all 3 triangle test oral-cavity-only fatty acid discrimination outcomes, the probability of selecting oral-cavity-only the correct R-MODC by chance the observed number of times was >0.05 (cumulative binomial probability distribution, Roessler et al. 1978; Meilgaard et al. 2007). For linoleic acid, the probability of selecting the correct oral-cavity-only R-MODC by chance the observed number of times (9 correct selections by 30 participants) was 0.714. For oleic and stearic fatty acids, the probability of selecting the correct oral-cavity-only R-MODC by chance the observed number of times (14 correct selections by 30 participants) was 0.09 (cumulative binomial probability distribution, Roessler et al. 1978).

Gender effects for oral-cavity-only smelling

Likelihood ratio measures indicated that for oral-cavity-only fatty acid triangle tests, there were no statistically significant differences between female and male participants in number of correct responses, $P \geq 0.52$ (likelihood ratio, Williams 1976). The corresponding contingency analysis for linoleic acid showed ~25% correct responses for females and 30% correct for males. For oleic and stearic acids, ~40% of the responses were correct for females and ~50% were correct for males.

Discussion

The 3 hypotheses

1. At suprathreshold concentrations, all 3 vapor-phase fatty acids would be discriminable from blanks not containing fatty acids both orthonasally and retronasally.

The results of the present study were strongly positive. Not only were statistically significant orthonasal and retronasal discrimination of the 3 fatty acids observed but also more than 80% of the 30 participants showed orthonasal selection of the 1 different MODC, substantially exceeding the minimum number of correct selections required for statistical significance. There were comparable or higher percentage retronasal correct selections with linoleic and stearic fatty acids. These outcomes supported the first hypothesis.

2. Retronasal discrimination would be inferior to orthonasal discrimination.

In the present study, a difference in discrimination between orthonasal and retronasal smelling was found only for oleic acid, which was discriminated significantly less retronasally than orthonasally. In contrast, discrimination of stearic acid did not differ between orthonasal and retronasal tests, with equal percentages of participants selecting the 1 different MODC when stearic acid was present during orthonasal and retronasal presentations. In addition, the percentage of participants correctly selecting the 1 different MODC when linoleic acid was present was greater retronasally than orthonasally. Thus, in 2 of 3 instances the second hypothesis, that orthonasal discrimination would be different from and superior to retronasal discrimination, was not confirmed. The one statistically significant difference for fatty acids between orthonasal and retronasal discrimination of the present study, less retronasal than orthonasal discrimination for oleic acid, is the converse of a prediction that might have been made from a prior detection threshold study (Chalè-Rush et al. 2007b). In that investigation, the numerical values of the detection thresholds for the oleic acid emulsion were lower than those for linoleic and stearic acid emulsions,

suggesting that, at threshold, the oleic acid emulsion had been a more effective stimulus. In other studies it had previously been observed that retronasal odorant identification tended to be inferior to orthonasal identification if relatively low concentrations were employed but that the differences disappeared with higher concentrations (Halpern 2004). Perhaps the linoleic and stearic acid concentrations of the present study were high enough to preclude reduced retronasal discrimination compared with orthonasal?

3. Oral-cavity-only discrimination might be found for all 3 fatty acids.

Because the oral cavity is innervated by trigeminal nerve branches but has no olfactory nerve innervation (see Bereiter et al. 2008; Sessle 2008), responses from the oral cavity to vapor-phase fatty acids would necessarily involve trigeminal input. A priori, oral-cavity-only discrimination of fatty acids was considered a possibility for several trigeminal-related reasons. One reason was the previous observation of nasal cavity lateralization of linoleic acid (Chalè-Rush et al. 2007b), suggesting effective trigeminal stimulation of nasal cavity trigeminal branches. Another reason for hypothesizing that oral-cavity-only discrimination of fatty acids might occur were reports that some odorants, such as peppermint extract and DL-menthol, could be identified under some circumstances with only oral cavity stimulation, whereas orange and strawberry extracts, although not identifiable, could be discriminated from their solvents (Dragich and Halpern 2008; Parikh et al. 2009). Nonetheless, under the conditions of the present study, neither linoleic, oleic nor stearic fatty acid, when presented only to the oral cavity, could be discriminated from blanks that did not contain fatty acids. It had previously been observed that retronasally identifiable vapor-phase trigeminal stimuli such as eugenol, heptyl alcohol, nonanal, 1-octanal, and valeric acid could not be identified when restricted to the oral cavity (Parikh et al. 2009), suggesting that the trigeminal receptor populations in the oral and nasal cavities may have different characteristics.

It might be proposed that the sample size or the odorant concentrations of the present study may have been limitations or that a triangle test may not be sufficiently sensitive to detect limited oral-cavity-only discrimination of fatty acids (see Lawless and Heymann 1998, for triangle test sensitivity). The present sample size could perhaps be considered a limiting factor because 14 of the 30 participants selected the oral-cavity-only R-MODC containing stearic and oleic fatty acids from their associated blanks ($P = 0.09$), whereas for an sample size of 30, one more correct triangle test selection, that is, correct triangle test selections by 15 participants, would have been required to reach $P = 0.05$ (Roessler et al. 1978; Meilgaard et al. 2007, Figure 3). This could raise the possible suggestion that a larger sample size might result in significant oral-cavity-only discrimination of stearic and oleic fatty acids. However, the required number of correct responses for $P = 0.05$ increases with sample size, reaching 19 for a sample size of 40 and 23 for a sample size of 50 (Roessler et al. 1978; Meilgaard et al. 2007), perhaps countering speculation that a larger sample size would necessarily yield a statistically significant oral-cavity-only outcome. For linoleic acid, with only 9 of 30 oral-cavity-only participants selecting the 1 different R-MODC ($P = 0.71$), statistically significant discrimination in the oral cavity seems quite unlikely if the only change in procedure were a larger sample size.

Perhaps a stronger case can be made for concentration as a limiting factor? Both the linoleic and the oleic acids of the present study were diluted with mineral oil. Although the concentrations that were employed in the present study were clearly suprathreshold for orthonasal and retronasal discriminations, one might imagine that oral-cavity-only discrimination of vapor-phase linoleic or oleic fatty acid might occur if higher concentrations were employed. It should be noted that stearic acid concentration is not relevant in this context because it was used undiluted, and consequently its vapor-phase concentration at the presented temperature, 21 $\pm$ 1 $^{\circ}$C, could not be increased. It would be appropriate to test oral cavity discrimination of higher concentrations of vapor-phase linoleic and oleic acid than were used in the present study, although the present absence of discrimination for undiluted stearic acid may suggest that no oral-cavity-only discrimination of undiluted linoleic and oleic acids would occur.

The triangle discrimination tests employed in the present experiments are considered less sensitive that other discrimination measures under some circumstances (e.g., Francois and Sauvageot 1987; Lawless and Heymann 1998; Rousseau et al. 1999, 2002). Triangle tests were clearly sufficient to document orthonasal and retronasal discrimination of vapor-phase fatty acids. However, perhaps a more sensitive discrimination measure such as duo-trio tests (Lawless and Heymann 1998) might reveal oral-cavity-only discrimination of vapor-phase linoleic or oleic fatty acids?

Overall, the present data provide no evidence of oral-cavity-only discrimination of vapor-phase oleic, linoleic, or stearic fatty acids, and offer no indication that the oral cavity trigeminal sensory system is responsive to vapor-phase long-chain, 18 carbon carboxylic fatty acids.

Oral responses to fats in foods

If retronasal responses to vapor-phase fatty acids are relevant to judgments of foods, one would expect that manipulation of the fat content of foods would produce differences in judgment when taste stimulation (solution in the oral cavity, nose clip in place) is compared with oral stimulation (solution in the oral cavity but no nose clip). Fat-related judgments comparing taste with oral stimulation conditions have been examined in several studies.

In one such study, Yackinous and Guinard (2000) manipulated the fat levels of mashed potatoes, vanilla pudding, potato chips, and chocolate drink by varying the vegetable oil

content. They observed that judgments of the fattiness of the foods presented as taste stimuli (in the oral cavity with a nose clip in place) significantly increased when the nose clip was removed from the participants. Removing the nose clip permitted oral stimulation (see Halpern 2004), in which access to the nasal cavities is available in addition to all the forms of stimulation made possible by having substances in, and directly in contact with, the tissues of the oral cavity. The authors stated: ''The effect of the nose clips demonstrates the important role of flavor, or the olfactory component of fat-containing foods, in fat perception''.

In contrast, Mela and Christensen (1987) reported that judgments of the oiliness of a dry, corn-based food that was coated with a range of concentrations of hydrogenated vegetable oils did not change when participants wore nose clips. There were a number of differences between the Yackinous and Guinard (2000) and the Mela and Christensen (1987) studies that may have been factors in finding an effect of nose clip removal, and hence of smelling fats, in the former but not in the latter. These differences included the nature and diversity of the foods, the sample sizes (106 participants for Yackinous and Guinard 2000, vs. 17 participants for Mela and Christensen 1987), the specific attribute that was judged (fattiness vs. oiliness) and the psychophysical methods (category scale vs. magnitude estimation).

Another study (Schiffman et al. 1998) reported that the presence or absence of a nose clip generally did not alter detection thresholds of oil emulsions placed in the oral cavity. The 3 oils were bleached and deodorized soybean oil, medium chain length triglycerides, and mineral oil. The emulsifiers were acacia gum, Emplex (sodium stearcyl-2-lactylate), Tween-80 (polysorbate 80), and Na-caseinate. Only for medium chain length triglycerides emulsified with Emplex did the mean detection threshold concentration for the 12 participants decrease appreciably (from 6.68 ± 1.6% to 3.93 ± 0.72%, means and SDs) when the nose clip was removed. The latter condition is perhaps closest to that in Chalè-Rush et al. (2007b) in that detection thresholds of oral triglycerides in an emulsion were measured. It is interesting that under these circumstances Schiffman et al. (1998) observed a sizeable effect of nose clip removal. However, with the other emulsifiers, the Schiffman et al. (1998) mean detection thresholds for medium chain length triglycerides changed less than one SD with nose clip removal.

Comparisons among these investigations, and between them and the present study, are difficult because the stimuli do not correspond (foods or emulsions containing fats vs. the diluted or undiluted single fatty acids of the present study), unknown potential concentrations of any fatty acids versus the specified concentrations of single fatty acids of the present study, the presentations were oral rather than the vapor-phase method employed in the present study, and, in most cases, judgments of oiliness or fattiness were measured. Nonetheless, the Yackinous and Guinard (2000) results suggest that vapor-phase fatty acids would be detected retronasally, whereas the Mela and Christensen (1987) and Schiffman et al. (1998) reports imply that vapor-phase fatty acids would not be detected. It should be noted that an extensive literature that is not discussed in the present report exists on responses in the oral cavity to fats (e.g., Gilbertson 1998; de Araujo and Rolls 2004; Fushiki and Kawai 2005; Mizushige et al. 2007).

In conclusion, in the present study both orthonasal and retronasal discrimination of vapor-phase, mineral oil-diluted linoleic and fatty acids, and undiluted stearic acid, measured with triangle tests, were observed. These findings support those of Chalè-Rush et al. (2007b) and provide sufficient data to state that humans can smell fatty acids. In contrast, no oral-cavity-only discrimination of the fatty acids was found, providing no evidence for oral cavity trigeminal responses to vapor-phase fatty acids. Whether orthonasally or retronasally presented vapor-phase fatty acids can be discriminated from each other, or evoke sensory input that is sufficient to permit consistent use of linguistic labels, that is, identification, remain to be determined.

Funding

This work was supported by the PepsiCo Foundation; the Henry and Ruth Herzog Graduate Research Fund; and a Susan Linn Sage Professorship.

Acknowledgements

We thank Thomas A. Cleland, Harry T. Lawless, Robert E. Johnston, Christiane Linster, and the anonymous reviewers for comments on earlier versions of this manuscript.

References

Abraham MH, Sánchez-Moreno RS, Cometto-Muñiz JE, Cain WS. 2007. Quantitative structure–activity analysis on the relative sensitivity of the olfactory and the nasal trigeminal chemosensory systems. Chem Senses. 32:711–719.

Amerine MA, Pangborn RM, Roessler EB. 1965. Principles of sensory evaluation of food. New York: Academic Press 602 p.

Bereiter DA, Hargreaves KM, Hu JW. 2008. Trigeminal mechanisms of nociception: peripheral and brainstem organization. In: Bushnell MC, Basbaum AI, editors. The senses: a comprehensive reference. Volume 5, Pain. On-line, Elsevier. p. 435–460.

Bolton B. 2009. Orthonasal, retronasal, and oral-cavity-only discrimination of vapor phase fatty acids [Internet]. [MSc thesis]. [Ithaca (NY)]: Cornell University Available from: http://hdl.handle.net/1813/13787 [cited 2009 Dec 27].

Cain WS, Lee N-S, Wise PM, Roland Schmidt R, Ahn B-H, Cometto-Muñiz JE. 2006. Chemesthesis from volatile organic compounds: psychophysical and neural responses. Physiol Behav. 88:317–324.

Cain WS, Wijk RA, Jalowayski AA, Pilla Caminha G, Schmidt R. 2005. Odor and chemesthesis from brief exposures to TXIB. Indoor Air. 15:445–457.

Chalè-Rush A, Burgess JR, Mattes RD. 2007a. Evidence for human orosensory (taste?) sensitivity to free fatty acids. Chem Senses. 32:423–431.

Chalè-Rush A, Burgess JR, Mattes RD. 2007b. Multiple routes of chemosensitivity to free fatty acids in humans. Am J Physiol Gastrointestinal Liver Physiol. 292:G1206–G1212.

Chen V, Halpern BP. 2008. Retronasal but not oral-cavity-only identification of "purely olfactory" odorants. Chem Senses. 33:107–18.

Chow CK. 2007. Fatty acids in foods and their health implications. 3rd ed. Boca Raton (FL): CRC Press 1281 p.

de Araujo IE, Rolls ET. 2004. Representation in the human brain of food texture and oral fat. J Neurosci. 24:3086–3093.

Dragich AM, Halpern BP. 2008. An oral cavity component in retronasal smelling of natural extracts. Physiol Behav. 93:521–528.

Drewnowski A. 1987. Fats and food texture: sensory and hedonic evaluations. In: Moskowitz HR, editor. Food texture. New York: Marcel Dekker. p. 217–250.

Drewnowski A. 1997. Why do we like fat? J Am Diet Assoc. 97:S58–S62.

Francois P, Sauvageot F. 1987. Comparison of the efficiency of pair, duo-trio, and triangle tests. J Sens Stud. 3:81–94.

Frasnelli J, Hummel T. 2005. Intranasal trigeminal thresholds in healthy subjects. Environ Toxicol Pharmacol. 19:575–580.

Fushiki T, Kawai T. 2005. Chemical reception of fats in the oral cavity and the mechanism of addiction to dietary fats. Chem Senses. 30(Suppl 1):184–185.

Gilbertson TA. 1998. Gustatory mechanisms for the detection of fat. Curr Opinion Neurobiol. 8:447–452.

Green BG. 1996. Chemesthesis: pungency as a component of flavor. Trends Food Sci Tech. 7:415–420.

Green BG, Alvarez-Reeves M, George P, Akirav C. 2005. Chemesthesis and taste: evidence of independent processing of sensation intensity. Physiol Behav. 86:526–537.

Halpern BP. 2004. When are oral cavity odorants available for retronasal olfaction? In: Deibler KD, Delwiche J, editors. Handbook of flavor characterization: sensory analysis, chemistry, and physiology. New York: Marcel Dekker. p. 51–63.

Laska M. 2004. Olfactory discrimination ability of human subjects for enantiomers with an isopropenyl group at the chiral center. Chem Senses. 29:143–152.

Lawless HT, Heymann H. 1998. Sensory evaluation of food: principles and practices. New York: Chapman & Hall 819 p.

Lobb K, Chow CC. 2007. Fatty acid classification and nomenclature. In: Chow CK, editor. Fatty acids in foods and their health implications. 3rd ed. Boca Raton (FL): CRC Press. p. 1–16.

MacRae AW. 1995. Confidence intervals for the triangle test can give reassurance that products are similar. Food Qual Prefer. 6:61–67.

Mattes RD. 2009a. Is there a fatty acid taste? Ann Rev Nutr. 29:305–327.

Mattes RD. 2009b. Oral detection of short-, medium-, and long-chain free fatty acids in humans. Chem Senses. 34:145–150.

McGee H. 2004. On food and cooking. New York: Scribner 884 p.

Meilgaard M, Civille GV, Carr BT. 2007. Sensory evaluation techniques. 4th ed. Boca Raton (FL): Taylor & Francis 448 p.

Mela DS, Christensen DM. 1987. Sensory assessment of oiliness in a low moisture food. J Sens Stud. 2:273–281.

Mizushige T, Inoue K, Fushiki T. 2007. Why is fat so tasty? Chemical reception of fatty acid on the tongue. J Nutr Sci Vitaminol. 53:1–4.

O'Mahony M. 1986. Sensory evaluation of food: statistical methods and procedures. New York: M. Dekker 487 p.

Ophardt CE. 2003. Triglycerides. Virtual chembook [Internet]. Elmhurst College Available from: http://www.elmhurst.edu/~chm/vchembook/552triglycerides.html [cited 2009 Dec 27].

Parikh V, Lee-Lim AP, Halpern BP. 2009. Retronasal and oral-cavity-only identification of air-phase trigeminal stimuli. Chemos Percep. 2:9–24.

Potter NN, Hotchkiss JH. 1998. Food science. 5th edition. New York: Springer 608 p.

Roessler EB, Pangborn RM, Sidel JL, Stone H. 1978. Expanded statistical tables for estimating significance in paired—preference, paired–difference, duo–trio and triangle tests. J Food Sci. 43:940–943.

Rousseau B, Stroh S, O'Mahony M. 2002. Investigating more powerful discrimination tests with consumers: effects of memory and response bias. Food Qual Pref. 13:39–45.

Rousseau R, Rogeaux M, O'Mahony M. 1999. Mustard discrimination by same- different and triangle tests: aspects of irritation, memory and criteria. Food Qual Pref. 10:173–184.

Schiffman SS, Graham BG, Sattely-Miller EA, Warwick ZS. 1998. Orosensory perception of dietary fat. Curr Dir Psychol Sci. 7:136–143.

Sessle BJ. 2008. Sensation from the face. In: Squire LR, editor. Sensory systems. Somatosensation. Haptics/touch. Encyclopedia of neuroscience. Amsterdam (The Netherlands): Elsevier Online. p. 575–582.

Simons CT, Carstens E. 2008. Oral chemesthesis and taste. In: Firestein S, Beauchamp GK, editors. The senses: a comprehensive reference. Volume 4. Olfaction and taste. On-line, Elsevier. p. 345–369.

Stephenson D, Halpern BP. 2009. No oral-cavity-only discrimination of purely olfactory odorants. Chem Senses. 34:121–126.

Stipanuk MH. 2006. Biochemical, physiological, & molecular aspects of human nutrition. 2nd ed. St. Louis (MO): Saunders Elsevier 1212 p.

Stone H, Sidel JL. 2004. Sensory evaluation practices. 3rd ed. Amsterdam (The Netherlands): Elsevier Academic Press 377 p.

Tamburrino R, Halpern BP. 2007. Identification of air-phase fatty acids: both retronasal and orthonasal failure [abstract]. Chem Senses. 32:A15.

Weiss TJ. 1983. Food oils and their uses. 2nd ed. Westport (CT): AVI Publishing \.

Williams DA. 1976. Improved likelihood ratio tests for complete contingency tables. Biometrika. 63:33–37.

Wysocki CJ, Wise P. 2004. Methods, approaches, and caveats for functionally evaluating olfaction and chemesthesis. In: Deibler KD, Delwiche J, editors. Handbook of flavor characterization. Sensory analysis, chemistry, and physiology. New York: Marcel Dekker. p. 1–40.

Yackinous C, Guinard J-X. 2000. Flavor manipulation can enhance the impression of fat in some foods. J Food Sci. 65:909–914.