The olfactory perception of fat in foods

Shuo Mu

Propositions

1. Humans possess the ability to discriminate between foods differing in fat content through olfaction.
   (This thesis)

2. The perception of fatty acids does not qualify for basic taste perception.
   (This thesis)

3. AI technologies such as ChatGPT diminish scientific writing skills.

4. Industry is essential for the application of fundamental research results.

5. Satisfaction from efficient working is more addictive than satisfaction from hard working.

6. There is no junk food, only junk eating behavior.

Propositions belong to the thesis, entitled

The olfactory perception of fat in foods.

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Wageningen, 04 December 2023

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Content


1

Chapter 1

Introduction

Background

The basic senses of humans, including taste, smell, vision, hearing, and touch, play integral roles in perceiving food. Each sense provides unique sensory information that aids in perception of foods and discrimination between foods. Taste, also referred to as gustation, enables us to perceive fundamental taste qualities such as sweet, sour, salty, bitter, and umami. These taste qualities offer valuable information regarding food composition, for instance, sweet, and savory or umami tastes signal energy-rich carbohydrates and proteins, while bitterness is associated with potentially toxic substances in food [1-4]. Vision, through visual cues, provides crucial information about food appearance and assists in ingredient assessment, identification, and the qualitative evaluation of desirability and suitability of the food [5, 6]. The sense of hearing enables us to perceive and process auditory information, it can influence humans' perception of food through associated sounds, such as the crispness and staleness of chips [7]. The sense of touch, or tactile perception, predominantly contributes to the assessment of food through oral sensations, providing information about textural attributes such as tenderness, crispness, crunchiness, and smoothness [8, 9]. Olfaction, the sense of smell, contributes to the perception of food odors and flavors. Recent studies have also indicated that olfaction may contribute to the detection and identification of macronutrients in foods [10, 11].

From an evolutionary perspective, human perceive energy sources such as carbohydrates and proteins (4 kilocalorie/g) to be attractive (sweet taste for carbohydrates and umami taste for proteins), and thus motivate consumption of these macronutrients [12, 13]. Similarly, fat, which is a concentrated source of energy (9 kilocalorie/g) that provides more than twice the calories per gram compared to carbohydrates and proteins, should also have attractive sensory properties. The perception of fat is a complex multisensory percept, including gustatory, olfactory, and textural cues [14-16]. While gustatory and mouth textural perception of fat have been extensively researched [17-22], little is known about the olfactory perception of fat. Considering the excessive consumption of fat and it's negative contribution to human health [23-25], it could be beneficial to detect fat, and thus the energy content of foods from a distance, i.e., before it is put into the mouth. Therefore, this thesis aims to investigate the olfactory perception of fat. Sensory studies and chemical analyses were combined to examine the role of olfaction in fat perception and elucidate the chemical compounds involved in olfactory perception of fat.

Fat

Fat is an essential macronutrient that plays numerous crucial roles in the human body. It acts as a stored form of energy in the body and can be utilized during periods of increased energy demands or when other energy sources are limited [26]. Moreover, dietary fat is vital for the absorption of fat-soluble vitamins (such as vitamins A, D, E, and K). These nutrients rely on the presence of fat for proper absorption and utilization by the body. Fat also facilitates the absorption of fat-soluble antioxidants and supports the conversion of beta-carotene into vitamin A [27]. Furthermore, fat plays a significant role in hormone production. Certain hormones, including steroid hormones and sex hormones such as cortisol, testosterone, and estradiol, are derived from cholesterol, which is a type of fat. These hormones are indispensable for various physiological functions such as reproduction, growth, and metabolism [28]. Additionally, fat is an integral component of cell membranes, providing structural integrity and fluidity. This allows for proper cellular function, communication, and signaling [29]. Essential fatty acids, including omega-3 fatty acids and omega-6 fatty acids which cannot be synthesized by the body and must be obtained from the diet, play crucial roles in brain development, immune function, and the regulation of inflammation [30]. Certain fat-containing foods such as nuts, seeds, and fatty fish are rich sources of the essential nutrients, including vitamins, minerals, and antioxidants [31]. Consuming these foods as part of a balanced diet ensures a diverse array of nutrients that support overall health and well-being.

In addition to their physiological functions, fat contributes to the sensory perception of foods as it influences flavor and texture perception, and overall palatability. Fat can contribute to the flavor perception of food by providing a reservoir for hydrophobic flavor compounds and by interacting with odorants, intensifying the perception of odorants, and enhancing taste sensations such as sweetness [32, 33]. Moreover, fat influences the texture perception of foods. Fat provides desirable attributes like smoothness, creaminess, and lubrication, enhancing the mouthfeel of foods, specifically it contributes to the softness of baked goods, the tenderness of meats, and the creaminess of dairy products [34]. Thus, the perception of fat is a crucial factor that influences the intake of fat, a better understanding of perception of fat can aid in lowering fat content in food without losing hedonic appeal.

Recent research suggests that fat taste could be the sixth basic taste quality [14, 17, 19, 22]. Taste is the sensory experience associated with the detection and interpretation of various chemical substances, known as tastants, by specialized receptor cells on the tongue and other

parts of the oral cavity [35]. The traditional understanding of taste recognizes five primary taste qualities: sweet, sour, salty, bitter, and umami [36]. Sweet taste is typically associated with the perception of sugars, such as glucose, fructose, glycosides, and lactose[37]; Sour taste is triggered by acidic compounds, such as citric acid, acetic acid, lactic acid, tartaric acid, and malic acid [38]. Salty taste is associated with the perception of sodium ions (Na^+) and other salts [39]. Bitter taste is generally considered an aversive taste is often associated with substances such as caffeine, quinine, and certain vegetables like kale or Brussels sprouts [40]. Umami is a Japanese word that translates to "pleasant savory taste". It is characterized by the perception of amino acids, such as glutamate, which are commonly found in foods like meat, mushrooms, and aged cheeses [41].

Traditional tastants are suggested to have several characteristics in common [14]: 1) affective stimuli that can active receptors in mouth; 2) receptors specific for the class of stimuli on taste bud cells; 3) afferent fibers exist from taste bud cells to taste-processing regions of the brain; 4) downstream physiological effects that can be influenced by taste stimuli; 5) perception that independent from other sensory modalities. Previous studies already observed that the oral perception of fat does meet several of these characteristics. Fatty acids, which are the metabolic by -products of lipid breakdown, are suggested to be the affective stimuli of fat taste, as evidenced by psychophysical studies demonstrating the oral detectability of fatty acids by humans [42-44]. According to Animal studies, CD36 and G protein-coupled receptor 120 have been proposed as putative receptors for fatty acids. After their activation, a cascade of transduction takes place, leading to the release of neurotransmitters targeting afferent fibers, thus initiating the signaling process to the brain [45-47]. Furthermore, associations between fat taste and obesity were also observed. Consumers who were more sensitive to taste perception of fatty acids had lower energy intakes, consumed less total dietary fat, and were also better at detecting the fat content of food [48-50]. However, the perceptual quality of fatty acids is still open to debate. One difference between basic tastants and fatty acids is that tastants are supposed to be non-odorous and to be perceived by the gustatory system only, whereas fatty acids appear to be detectable through olfaction as well. More studies are needed to investigate the olfactory perception of tastants and fats/fatty acids.

Olfactory perception

Olfactory perception refers to the sense of smell, which is the ability to detect and perceive odors or smells in the surrounding environment. Olfaction is a complex sensory process that involves the detection, recognition, and interpretation of chemical compounds in the air through specialized sensory cells in the nose. When we encounter odorants, volatile chemical molecules are released from substances and enter the nasal cavity where olfactory sensory neurons are located. These sensory neurons contain specialized receptors known as olfactory receptors, which are responsible for detecting specific odorant molecules. When an odorant molecule binds to its corresponding olfactory receptor, it triggers a chemical signal that is transmitted to the brain, specifically to the olfactory bulb, which is the primary processing center for smell. From the olfactory bulb, the information is relayed to various brain regions involved in olfactory perception, memory, and emotional responses to smells. The brain interprets the information received from the olfactory receptors, enabling us to perceive and identify odors [51, 52].

Depending on the pathways, olfaction can be categorized as orthonasal and retronasal olfaction. Orthonasal olfaction refers to the route taken by odorants originating from the external environment, entering the nasal cavity via the nostrils. Retronasal olfaction refers to odorants entering the nasal cavity through the oral cavity and pharynx during food consumption [53]. Specifically, during oral processing, volatile molecules are released from food matrices. These odorants are transported from the oral cavity through the nasopharynx into the nasal cavity, where they are detected by olfactory receptors. Orthonasal olfaction is responsible for our ability to perceive and distinguish a wide range of odors in the surrounding world, and its' functions were identified as ingestion, avoiding environmental hazards, and social communication [54]. Retronasal olfaction is a unique mode of olfaction that especially occurs during eating and drinking, and thus contributes to flavor perception of food [11, 55-57].

Furthermore, the difference in anatomy and adsorption between ortho- and retronasal olfaction may cause disparity in odor perception [58, 59]. The odor quality and intensity perceived through retronasal and orthonasal olfaction might differ [60, 61]. Retronasal olfaction entails lower concentrations of odors in the nasal cavity compared to orthonasal olfaction leading to perception of lower odor intensities. The transfer of odors from the mouth to the nasal cavity involves interactions with saliva, food components, and other oral environments, potentially leading to dilution of odor concentrations [62]. Furthermore, the

contextual effects, subtle differences in nasal airflow, and differential trigeminal sensitivities of the respiratory epithelium may also contribute to the differences in perception through ortho- or retronasal olfaction [63]. Moreover, in contrast to orthonasal olfaction, which primarily pertains to odor perception in isolation from taste and other oral sensations, retronasal olfaction is intricately associated with taste and additional sensory inputs, including texture and temperature, thereby collectively contributing to the overall perception of flavor. From a physiological perspective, flavor perception may be a "supra-additive" response to olfactory, gustatory, and tactile stimuli [64]. Retronasal olfactory and gustatory inputs likely integrate into perception of flavor. Consequently, this raises one question: are tastants odorless, or is their taste actually a result of integrated flavor perception involving retronasal olfactory and gustatory inputs?

Olfactory perception of tastants

Although traditional tastants are typically considered non-volatile and odorless, there is limited evidence suggesting that humans may perceive tastants through olfaction. Mojet et al. [65] first hypothesized this by comparing the taste intensity perception of ten taste stimuli (NaCl, KCl, sucrose, aspartame, acetic, citric, caffeine, quinine, MSG, IMP) between elderly and young individuals. The young participants perceived the stimuli significantly more intense than the elderly participants, but this difference diminished when a nose clip was worn. Considering that olfactory sensitivity declines more rapidly with age than gustatory sensitivity [66], they proposed that retronasal smell of the tastant solutions was perceived by participants contributing to the taste intensity. To further verify whether tastants can be perceived through olfaction, they conducted additional research [67] and revealed that most tastants can be perceived through orthonasal olfaction and such perception may contribute to taste intensity ratings. Currently, only a limited number of studies have investigated olfactory perception of basic tastant solutions and the ability of humans to discriminate between tastant solutions and water based on smell. Furthermore, the underlying mechanisms of olfactory perception of tastants and the volatile compounds that might contribute to this discrimination capability remain unclear. However, more studies are needed to identify what exact volatile compounds contribute to the smell of tastant solution.

Olfactory perception of fatty acids and fat in foods

Several articles have explored the role of olfaction in perceiving fatty acids such as oleic, linoleic, and stearic acids. Humans were shown to be able to detect fatty acids through ortho- and retronasal olfaction [68], were able to discriminate odor of fatty acids solution at suprathreshold from blank through ortho- and retronasal olfaction [69], were able to discriminate retronasal odor of stearic acid from oleic and linoleic acids [70], and were able to linguistically identify the retronasal odor of three fatty acids at suprathreshold [71]. Overall, these studies indicate human olfaction plays a role in detection, discrimination, and identification of fatty acids.

Alongside the evidence that olfaction contributes to the perception of fat, there is an ongoing debate that the olfactory perception of fat may vary between individuals. While some research found demographics and fat consumption habits have no influence on the olfactory sensitivity in perceiving fat [10, 60, 72], one study observed positive relationships between intake of fat-rich nuts and sensitivity to fatty acids odor [73]. More studies are needed to explore the influence of individual difference on olfactory perception of fat.

Volatile compounds of fat odor

Dietary fats are triglycerides which are not volatile and thus may not be perceived by olfaction. Volatile compounds present in fat or metabolized from triglycerides rather than the triglycerides themselves have been suggested to act as the odor source facilitating detection of fat by smell in humans [15, 21, 74]. More than 40 volatile compounds, which were suggested to be metabolized from milk fat and might contribute to the perception of fat-related sensory attributes, were identified in the headspace of commercial milks [75-78]. Thermal processing of milk influences the volatile composition of milk. Higher concentrations of sulfide compounds, which contribute to off odors like cooked, stale, and sulfurous, were identified in UHT milks compared to raw and pasteurized milks [79]. The volatile compounds of raw meat are typically formed by lipid oxidation, lipid degradation, and microbial degradation whereas Maillard reactions, lipid oxidation, lipid thermal degradation, and lipid–Maillard reactions contribute to the volatile compound composition of roast meats [80, 81]. Although many studies already identified volatile compounds in real food matrices, their thresholds, and odor descriptors [82-84], it is still unknown whether or how these volatile compounds underpin humans' ability to discriminate fat content of food solely through olfaction. A better understanding of fat odor related compounds and their

contribution to fat content discrimination aids to the development of low-fat content food by reducing fat content without changing fat perception.

Thesis aim and outline

The role of olfaction in the perception of fat/fatty acids (and tastants) remains underexplored [67, 85]. Determining whether humans can perceive fatty acids and fat in foods through olfaction and can recognize these odors is crucial. It may offer the possibility of detecting energy content in foods from a distance. This thesis aimed to investigate how olfaction contributes to the perception of fat/fatty acids (and tastants) and to identify the difference in the volatile compound composition between foods differing in fat content. This thesis commences by investigating the olfactory perception of tastants and fatty acids. It subsequently delves into more complicated food matrices, exploring the olfactory perception of fat within real dairy and non-dairy foods. Within this thesis, several sub research questions are addressed (**Table 1**).

Table 1 Summary of the studies conducted in this thesis.

Chapter	Aim
2	Exploring olfactory perception of tastants and fatty acids, and profiling the volatile compounds that contribute to their olfactory perception
3	Reviewing the contribution of olfaction of dietary fat perception.
4	Investigating olfactory discrimination ability on fat content in milk matrices, and profiling the volatile compounds that contribute to olfactory discrimination.
5	Investigating olfactory discrimination ability on fat content in meat matrices and profiling the volatile compound composition that contribute to olfactory discrimination.

This thesis aims to answer the following three research questions: 1) Can humans perceive tastants and fatty acids through olfaction? 2) Can humans discriminate between real foods differing in fat content solely based on olfaction? 3) What volatile compounds facilitate the olfactory discrimination between foods differing in fat content? Olfactory triangle discrimination tests were conducted in **Chapter 2** to determine if humans can discriminate between fatty acid (and tastant) solutions and blank solutions by olfaction. Subsequently, a systematic scoping review was performed in **Chapter 3** to summarize research on olfactory perception of fat and identify potential knowledge gaps. In **Chapters 4** and **5**, olfactory triangle discrimination tests were performed to examine whether humans could discriminate fat content through olfaction in real food matrices such as milk and meat. In **Chapters 2, 4,** and **5**), the volatile compound compositions of the headspace of all stimuli were identified through GC-MS to explore potential volatile compounds that may influence the olfactory perception of tastant and fatty acid solutions and may facilitate the discrimination between foods differing in fat content. Finally, **Chapter 6** provides a general discussion, including

main findings of the thesis, implications of the results, methodological considerations, recommendations for future research, and the main conclusions.

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2

Chapter 2

Can humans smell tastants?

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Abstract:

Although general consensus suggests that tastants have no smell, there are limited indications that humans are able to perceive tastants via orthonasal olfaction. This study aims to (a) explore whether humans can discriminate between solutions of basic tastants and water through orthonasal and retronasal olfaction, (b) and if so, to examine what volatile odor compounds underlie the discrimination ability. Solutions of five basic tastants (sucrose, sodium chloride, citric acid, monosodium glutamate, quinine dissolved in water, at suprathreshold levels) and two fatty acids (oleic and linoleic acid dissolved in mineral oil, at suprathreshold levels) were prepared. Triangle discrimination tests were performed (n=41 in duplicate) and we found participants were able to distinguish all tastant solutions from blank through orthonasal olfaction ($p<0.05$ for quinine vs blank, $p<0.01$ for the other six comparisons). Only sucrose, sodium chloride, oleic acid, and linoleic acid were distinguished from blank by retronasal olfaction ($p<0.05$ for sucrose vs blank, $p<0.01$ for the other three comparisons). ITEX-GC-MS was applied to profile the headspace volatile composition of samples. Ethyl dichloroacetate, methylene chloride, and acetone were identified in the headspace of sucrose, MSG and quinine solutions but not in water (blank). Fat oxidation compounds such as alcohols and aldehydes were detected in the headspace of the oleic and linoleic acid solutions but not the mineral oil (blank). We conclude that tastant solutions can be discriminated from water and fatty acid solutions from mineral oil through orthonasal olfaction. Differences in the volatile headspace composition are likely to have facilitated the olfactory discrimination.

Introduction

Flavor is a multifaceted sensory experience that plays a crucial role in the perception and enjoyment of foods and beverages, its' perception guides food selection and promotes the ingestion of nutrients [1, 2]. Flavor encompasses the combination of gustatory, oral-somatosensory, and retronasal olfactory signals [3]. The gustatory system provides information about basic tastes – sweet, sour, bitter, salty, and umami - while ortho- and retronasal smell contribute to the perception of aromas and volatiles. Tastants are molecules that are dissolved in ingested foods and beverages that can bind to taste receptors on the human's tongue. They are supposed to be non-odorous and to be perceived by the gustatory system only. Although consensus view is that basic tastants have no smell, there are few indications that humans can discriminate tastant solutions from water solely by means of olfaction. Mojet, Köster, & Prinz [4]reported that participants discriminated between sucrose solutions and water by merely sniffing, and several participants consistently detected seven out of ten tastant solutions by olfaction. These results may suggest that some tastants in solution can be smelled. Similarly, Chen [5]observed that participants discriminated monosodium glutamate (MSG) and sucrose solutions from water through orthonasal, but not retronasal olfaction. At present, a very limited number of studies explored whether humans can olfactorily detect basic tastants and can discriminate between tastant solutions and water based on smell, and the mechanisms underlying the potential discrimination capability are unclear.

Fat taste has been suggested as a sixth basic taste. According to previous reviews [6, 7], there are specific receptors on the human tongue that respond to fatty acids, though others argue that the sensory experience of fat taste may actually be a combination of sensory modalities including taste, texture and aroma. Despite these controversies, and unlike for the other five basic tastes, there is consistent evidence showing that fatty acids can be smelled. Humans can discriminate fatty acids from mineral oil through ortho- and retronasal olfaction [8, 9], can discriminate oleic, linoleic, and stearic acids from each other through retronasal olfaction [10], and can describe the smell of these fatty acids [11]. Recent reviews provide overviews about olfactory fat perception [6, 12].

Evidence from animal studies suggests that tastants and fatty acids can be perceived via smell. Bell, Dennis, & Sly [13] found that olfactory bulbectomy in sheep decreased their aversion to sodium salt, while Rhinehart-Doty, Schumm, Smith, & Smith [14] demonstrated that rats can discriminate between sucrose solution concentrations by sensory cues other than

taste, possibly olfaction. The functional role of the olfactory sense in perceiving taste stimuli has been highlighted in animals, for example, played a role in the conditioned sucrose preference of mice. Zukerman, Touzani, Margolskee, & Sclafani [15] observed that mice displayed a decreased consumption of sucrose solution after olfactory bulbectomy. In addition to basic tastants, fatty acids can be detected through olfaction by animals. Mice lost their preference for fatty acid when they were anosmiated [16-18]. To summarize, olfaction may be involved in the detection of basic tastants and fatty acids in animals and potentially also in humans. However, the mechanisms underlying this ability remain unclear.

Taste receptors are not only distributed in the oral cavity, but also in other regions of the body such as the gut, large intestine, and the nasal cavity [19, 20]. Tastants typically have low volatility and are thus unlikely to be delivered to the nasal cavity. Previous studies hypothesized that olfactory discrimination between basic tastant solutions and water was facilitated by impurities in tastant solutions rather than the tastants themselves [4]. Others refuted this hypothesis as they observed that the purity grade of the tastants (i.e., sucrose in reagent grade, non-reagent grade, and food grade) did not influence olfactory discrimination ability, both in mice [15]and in humans [5]. However, none of these studies analyzed the headspace of the tastant solutions. Exploring the volatile compounds in the headspace of tastant solutions which may come from impurities may help to determine the odor-active compounds that facilitate discrimination between tastant solutions and water and may help to explain the mechanisms underlying the putative ability to detect or discriminate tastants via smell.

This study aims to (a) explore whether humans can discriminate between solutions of basic tastants and water through orthonasal and retronasal olfaction, (b) and if so, to examine what volatile odor compounds (VOCs) underlie this discrimination ability. Solutions of five basic tastants (sucrose, sodium chloride, citric acid, monosodium glutamate (MSG) and quinine dissolved in water) and two fatty acids (oleic and linoleic acid dissolved in mineral oil) were prepared and triangle discrimination tests were performed to assess whether the tastant solutions can be distinguished from the blanks (solvents) through ortho- and retronasal olfaction. The headspace composition of the volatile odor compounds was determined using In-Tube Extraction-Gas Chromatography-Mass Spectrometry (ITEX-GC-MS) and linked to the olfactory discrimination ability.

Materials and methods

Materials

Sucrose, sodium chloride, citric acid, monosodium glutamate (MSG), quinine (quinine monohydrochloride dihydrate), oleic acid, linoleic acid, and mineral oil were purchased from Merck KGaA (Darmstadt, Germany). Sucrose, sodium chloride (NaCl), citric acid, MSG, and quinine were stored (as recommended) at room temperature, oleic acid, linoleic acid, and mineral oil were stored (as recommended) at 4 ℃ before using. Milli-Q water (electrical resistivity 18.2 MΩ·cm at 25℃) produced using the Arium 611UF ultrapure water system (Sartorius Stedim Biotech GmbH, Göttingen, Germany) was used as solvent for five tastants and mineral oil was used as solvent for fatty acids. The concentrations of tastant solutions were chosen according to previous studies and their occurrence in foods and beverages [4, 11]. High concentrations of tastants and fatty acids were chosen that are easily perceivable by humans through taste, while still remaining within an ecological relevant tastant concentration range, so concentrations that are high but occur in common foods and beverages. The purity of solutes and final concentration of solutions are shown in **Table 1.**

Table 1. The purity of solutes, solvents, and concentration of tastant and fatty acid solutions.

Solutes	Solvents	Purity of solutes	Concentration (g/100g)
Sucrose	Milli-Q water	≥99.5%	25
Sodium chloride	Milli-Q water	≥99.0%	3
Citric acid	Milli-Q water	≥99.0%	5
MSG	Milli-Q water	≥98.0%	1
Quinine monohydrochloride dihydrate	Milli-Q water	≥90.0%	0.0083
Oleic acid	Mineral oil (neat)	≥90.0%	40
Linoleic acid	Mineral oil (neat)	58.0%-74.0%	40

Brown glass bottles (150 mL) were used for orthonasal testing, and specially designed cups (150 mL) were used for retronasal testing [21]. The setup and usage of special designed cups is shown in **Figure S1** in supplementary. Each brown bottle contained 60 g of tastant solutions or water or 50 g of fatty acid solutions or mineral oil, and each special designed cup contained 80g of water solutions or 60g of oil solutions. The amounts of tastant and fatty acid solutions were calculated based on their density to ensure a consistent volume in the bottles or cups. The solutions were prepared one day before testing and stored at 4 ℃. All samples were taken out the refrigerator one hour before testing to come to room temperature.

Participants

42 participants (mean age 24.0 ± 6.9 years; 8 males; mean BMI 21.6 ± 2.8 kg/m^2) took part in the study. All participants were non-smokers, not pregnant, not breast-feeding, nor currently on a calorie-restricted diet or have been in the past 2 months. All of them had a normal olfactory function according to the 16-item odor identification part of the Sniffing' Sticks test (score of ≥12; [22]). Participants were asked not to eat or drink anything other than water one hour prior to testing, nor wear any scented products on the day of testing. Their demographic information (age, gender, height, and weight) was collected through an online questionnaire. Written informed consents were provided by all participants prior to participation, financial reimbursements were transferred to participants when they completed all sessions. The study was exempt from review by the Medical Research Ethical Committee (number 2022-118-SBSEB-prc) according to the "Medical Research Involving Human Subjects Act" of The Netherlands (WMO in Dutch). The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013).

Study procedure

Sensory assessments were conducted in individual sensory booths at Wageningen University, the Netherlands. The sensory booths are well-ventilated to ensure an odor free environment. Participants attended four sessions of 30-40 minutes. The first session contained the Sniffing' Sticks test, and a training on how to use the special designed cups for retronasal olfactory testing (see **Figure S1** in supplementary material). The second and third sessions consisted of retronasal olfactory triangle discrimination tests. In each session, 7 sets of triangle comparisons were performed to compare each of the solutes (**Table 1**) to it solvent. The data obtained from the two sessions was treated as duplicate measures while different sample comparisons where performed (e.g., when triangle comparison ABB was performed in the second session, AAB performed in the third session was considered as its' duplicate; Presentation order within triangles (i.e., ABB, BAB, BBA) were randomized over participants in each session. The fourth session contained 14 sets of triangle comparisons (7 sets in duplicate) for orthonasal olfaction test, in random order. An example of the study design for all sessions is shown in **Table S1** in supplementary material.

The olfactory triangle discriminations were between tastant (or fatty acids) solution and blank (solvent respectively). In the test, participants were instructed to smell all three samples in the order samples were presented, and then select the different (odd) one out. Subsequently,

participants had to answer the following questions: “Did you distinguish the samples based on intensity of the odor, quality of odor, or did you just guess?” If participants answered ‘intensity’ they were then asked “Did you perceive the odd sample as more, or less intense compared to the other samples?”. Finally, they were asked “Which taste do you associate with the sample you smelled?”, with response options sweet, salty, bitter, sour, umami, sour, fat, other, or nothing. An inter-trial interval of approximately 1 minute was used between each triangle test. Participants were encouraged to smell their own skin between trials to prevent adaptation during the intervals.

Characterization of volatile compound composition

The characterizations of volatile compound composition were performed for all samples used in sensory test. The headspace volatile compound composition was determined by ITEX-GC-MS. 5 mL liquid sample was injected in a 20 mL vial. Vials were sealed and stored at 4℃ overnight and were removed from the refrigerator one hour before analysis. An auto-sampler (TriPlus, Thermo, USA) was employed for automatically loading and extracting samples. The vial was incubated at 60 ℃ for 10 mins before analyzing. The headspace of samples was extracted using a Tenax tube (GR 80/100, Buchem B.V., Minden, The Netherlands.) Extraction was set as 10 times with 1.3 mL each time. Injection was set as 1 mL headspace coupled with the desorption from Tenax tube.

A gas chromatograph system (Trace 1300, Thermo, USA) coupled with single quadrupole mass spectrometer (ISQ 7000, Thermo, USA) was employed to analyze the volatile composition of the headspace. Rxi-5SIL MS column (30 m x 0.25 mm, df = 1.0 µm, Restek GmbH, Schaberweg, Germany) was used in the analysis. The carrier gas was hydrogen, at 2.17 mL/min. The initial oven temperature was 40°C and was maintained for 2 min. The temperature was then increased to 250°C at 30°C/min and held for 2 mins. The mass spectrometry detection setting was full scan model, with mass range of 25 -250 *m/z*. All samples were measured in triplicate. Total ion chromatograms (TICs) were recorded and used for further analysis.

The chromatograms were recorded and analyzed using Thermo Scientific Dionex Chromeleon® 7.2 chromatography data system (CDS) software. Volatile compounds were identified by comparing their mass spectra and retention indices with the National Institute of Standards and Technology (NIST) database. Measurements were performed in triplicate for fatty acid solutions and mineral oil, and the compounds detected in all replicates were

recorded. As for tastant solutions and Milli Q water, only a few compounds were detected in the headspace based on our preliminary tests. To ensure measurement accuracy, two batches of samples were prepared, and each sample was measured in triplicate (yielding six measurements per sample). The compounds detected in at least five measurements were recorded. The mean values of the total ion currents (TICs) were calculated and used for further data analysis.

Statistical data analysis

Corresponding triplets of the triangle discrimination tests (e.g., AAB and ABB) were considered as duplicate measures, resulting in n=84 observation for retronasal test and n=82 observations for orthonasal test. The number of correct responses was summed up and the significance level (p) was calculated according to binominal tests. According to answers from the additional question of triangle discrimination tests, the proportion of responses to question 2 (discrimination based on odor intensity, quality, or guess) and question 3 (odor associate with sweet, salty, bitter, sour, umami, sour, fat, other, or nothing) were calculated based on the correct responses in triangle discrimination test. One-way ANOVA followed by Duncan test was performed to analyze differences in TIC of volatile compounds between samples using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL). A significance level of $p < 0.05$ was chosen for all analyses.

Results

Olfactory discrimination ability of tastant and fatty acids solutions

Figure 1 depicts the results of olfactory triangle tests conducted for orthonasal discrimination (I) and retronasal discrimination (II). Participants were able to discriminate between tastant or fatty acid solutions and the blank (water or mineral oil) through orthonasal olfaction (all p-values < 0.01, except for the comparison between quinine and the blank, p = 0.048). By means of retronasal olfaction, participants discriminated sucrose (p = 0.043), NaCl (p < 0.01), oleic acid (p < 0.01), and linoleic acid solutions (p < 0.01) from blank, but they were unable to discriminate citric acid (p = 0.213), MSG (p = 0.286), and quinine solutions (p = 0.850) from water.

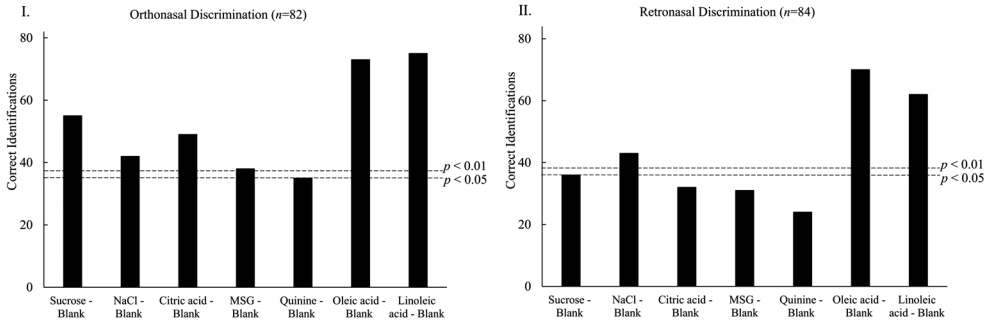

Figure 1. Number of correct identifications for triangle discrimination tests. I): orthonasal olfactory discrimination for prepared solutions and blanks (n = 82, 41 participants in duplicate). II): retronasal olfactory discrimination for prepared solutions and blanks (n = 84, 42 participants in duplicate). Dotted lines indicate the minimum number of correct identifications required at different significance levels. Milli Q water was used as blank for tastant solutions and mineral oil was used as blank for fatty acid solutions.

The proportions of responses (based on correct responses only) indicating whether participants based their judgment on odor intensity, quality, or guess are presented in **Figure 2**. Regarding orthonasal discrimination, more participants attributed their discrimination ability to odor quality (50-55%) as opposed to odor intensity (27-33%) for all comparisons except for quinine, oleic acid, and linoleic acid trials, where odor intensity (40-55%) and odor quality (40-53%) contributed equally to the discrimination. In terms of retronasal discrimination, more participants indicated that odor intensity (42-47%) contributed to the discrimination of sucrose, NaCl, MSG, and quinine, whereas both odor quality and odor intensity contributed equally to the discrimination of citric acid and oleic acid. Furthermore,

a higher proportion of participants made guesses in retronasal tests (22-46%) for tastant solutions compared to the corresponding orthonasal tests (14-21%).

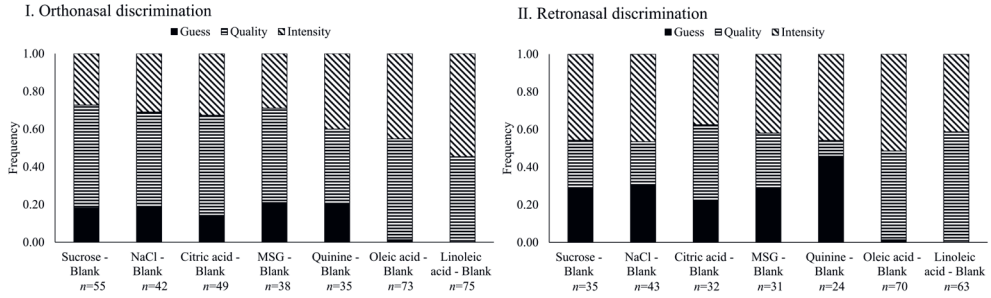

Figure 2. Reasons that participants provided for olfactory discrimination between solutions and blank. I): orthonasal discrimination. II): retronasal discrimination. All results were calculated based on correctly discriminated trials only; *n* indicates the number of correct responses for each sample comparison; Milli Q water was used as blank for tastant solutions and mineral oil was used as blank for fatty acid solutions.

In orthonasal and retronasal trials 45 and 46% of participants associated the odor of the sucrose solution with sweetness trial (**Figure 3**), respectively. For the other four tastants, only very few participants associated the odor of the tastant solution with the taste quality of the tastant trial (21 and 11% for NaCl, 7 and 10% for citric acid, 9 and 12% for MSG, 0 and 11% for quinine, in orthonasal and retronasal trials, respectively). For fatty acids, many participants associated the odor of oleic (68%) and linoleic acid solutions (56%) with sour taste in the orthonasal condition, whereas they associated the odor of oleic (54%) and linoleic acid (37%) solutions with fat taste in the retronasal condition.

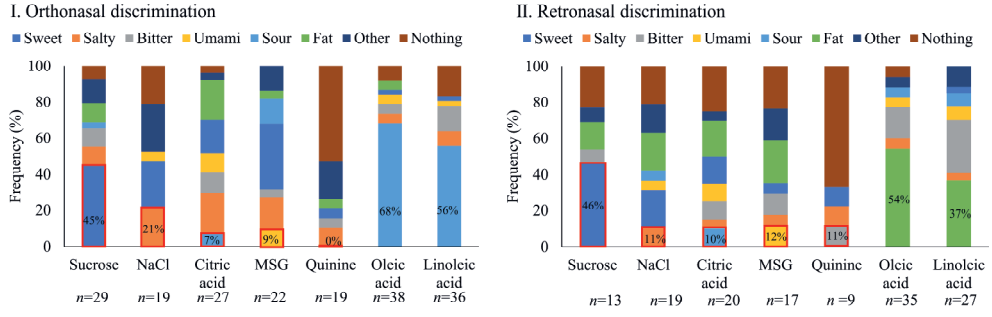

Figure 3. Frequency of the association of the odor of the tastant solution with the taste quality of the tastant. I): orthonasal discrimination. II): retronasal discrimination. Only correctly discriminated trials were included. The red boxes highlight the frequency when the odor of the tastant solutions matches the taste quality of the tastant.

Headspace volatile compound composition of tastant and fatty acid solutions

The compositions of volatile compounds in the headspace of tastant solutions and Milli Q water are presented in **Table 2**. Trichloromethane and diethyl azodicarboxylate were identified in the headspace of all five tastant solutions and Milli Q water. Trichloromethane was found to be significantly ($p < 0.05$) more abundant in the headspace of sucrose and quinine solutions compared to Milli Q water. No significant differences ($p > 0.05$) were observed in abundance of diethyl azodicarboxylate between tastant solutions and Milli Q water. Several compounds were identified only in the headspace of tastant solutions when compared to Milli Q water. Acetone and ethyl dichloroacetate were identified only in the headspace of the sucrose solution, while ethyl dichloroacetate and methylene chloride were detected only in the headspace of the quinine solution. Acetone was identified only in the headspace of the MSG solution. No compounds were identified in the headspace of the NaCl and citric acid solutions similar to Milli Q water.

Table 2. Peak area (total ion chromatogram, *10^6) of compounds detected in headspace of tastant solutions and Milli Q water. Results are expressed as mean ± SD (n = 5 or 6). Compounds that were detected in ≥5 measurements are reported. N: Not known. * Denotes significant differences in peak area of volatile compounds between a tastant solution and Milli Q water (independent samples T test, $p < 0.05$).

Compound	CAS Number	Sucrose	NaCl	Citric acid	MSG	Quinine	Milli Q Water	Odor quality
Trichloromethane	67-66-3	254.8±29.9*	37.3±12.2	36.1±8.9	49.1±9.0	398.5±53.3*	64.5±17.2	hay
Diethyl azodicarboxylate	1972-28-7	47.4±6.5	47.7±11.6	47.2±9.9	45.5±8.9	43.4±13.6	53.1±8.3	N
Acetone	67-64-1	21.8±4.6	-	-	64.3±4.9	-	-	chemical, ether, hay, nauseating, pungent, wood
Ethyl dichloroacetate	535-15-9	0.9±0.5	-	-	-	4.5±1.9	-	N
Methylene chloride	75-09-2	-	-	-	-	3.1±0.8	-	N

Table 3. Peak area (total ion chromatogram, *10^6) of compounds detected in the headspace of fatty acid solutions and mineral oil. Results are expressed as mean ± SD (n = 3). Compounds detected in all three measurements are reported. N: Not known. * Denotes significant differences in peak area between a fatty acid solution and minerals oil (independent samples T test, $p < 0.05$).

Compound Name	CAS Number	Oleic acid	Linoleic acid	Mineral oil	Odor quality
Acids					
Butyric acid	107-92-6	0.5±0.1	0.9±0.2	0.3±0.1	butter, cheese, must, rancid, sour, sweat
2-Methylbutanoic acid	116-53-0	-	0.4±0.1	-	butter, cheese, fermented, rancid, sour, sweat
Alcohols					
Ethanol	64-17-5	8.8±1.5	6.9±4.3	14.7±3.1	alcohol, floral, ripe apple, sweet
1-Propanol	71-23-8	9.9±0.4*	11.2±7.6*	1.7±1.3	alcohol, candy, must, plastic, pungent, ripe fruit, rum, sweet
trans-2-Octen-1-ol	18409-17-1	1.0±0.2	0.3±0.1	-	medicine, oil, plastic, soap
2-Penten-1-ol	20273-24-9	0.6±0.1	0.5±0.1	-	grass
3-Octen-2-ol	76649-14-4	0.2±0.1	1.1±0.1	-	N

Compound Name	CAS Number	Oleic acid	Linoleic acid	Mineral oil	Odor quality
1-Butanol	71-36-3	0.7±0.1	-	-	alcohol, fermented, fruit, medicine, phenol, putrid, solvent, sweat
Aldehydes					
2-Butenal	4170-30-3	2.9±1.1*	2.8±1.2	0.5±0.2	pungent
2,6-Nonadienal	557-48-2	0.3±0.1	0.2±0.1	0.2±0.1	cucumber, green, lettuce, melon, wax
2-Methyl-2-butenal	1115-11-3	0.8±0.4*	-	1.1±0.1	apple, fruit, grass, green, solvent
3-Methyl-butanal	590-86-3	1.5±0.5	1.5±0.2	-	acrid, almond, chocolate, cocoa, corn flakes, fermented, malt, pungent, sweat, sweet
Acetaldehyde	75-07-0	-	0.7±0.1	-	ether, floral, fruit, green apple, pungent, sweet
Hexanal	66-25-1	-	2.3±0.4	-	apple, cut grass, fresh, fruit, grass, green, oil
Esters					
Decanoic acid, ethyl ester	110-38-3	0.4±0.1*	0.2±0.0	0.2±0.0	brandy, burnt, fruit, geranium, grape, nut, pear, pleasant, soap
Isoamyl acetate	123-92-2	0.7±0.1	0.9±0.0	-	apple, banana, fruit, glue, pear, sweet, yeast
Ethyl 2-methylbutyrate	7452-79-1	0.2±0.1	1.3±0.1	-	anise, apple, bubble gum, fruit, kiwi, strawberry
Butanoic acid, methyl ester	623-42-7	1.3±0.1	-	-	apple, banana, cheese, ester, floral
Ketones					
Acetone	67-64-1	58.5±1.1*	23.5±12.3	23.4±6.2	chemical, ether, hay, nauseating, pungent, wood
Diacetyl	431-03-8	6.1±0.1*	10.2±1.8*	1.1±0.1	butter, caramel, cheese, cream, fruit, strawberry, sweet, yogurt
1-Octen-3-one	4312-99-6	0.9±0.8*	0.2±0.1	0.1±0.0	boiled mushroom, earth, green, metal, mushroom, sharp
2-Heptanone	110-43-0	4.0±0.5*	1.9±0.2	0.5±0.0	bell pepper, blue cheese, cinnamon, fruit, green, nut, sweet
2-Hexanone	591-78-6	51.3±0.3*	81.6±9.1*	1.4±0.3	ether

Compound Name	CAS Number	Oleic acid	Linoleic acid	Mineral oil	Odor quality
2-Pentanone	107-87-9	0.7±0.0	20.4±1.0*	0.9±0.2	burnt plastic, ether, fruit, kerosine, orange peel, pungent
2-Butanone	78-93-3	0.2±0.0	2.1±1.3	-	butterscotch, ether, fragant, fruit, pleasant, solvent, sweet
2,3-Pentanedione	600-14-6	-	1.1±0.2	-	bitter, butter, caramel, cream, fruit, strawberry, sweet, wine
Others					
2-Furfuryl-furan	1197-40-6	0.3±0.0	0.5±0.1	0.4±0.1	N
Dimethyl-Sulfide	75-18-3	18.8±7.7	22.2±12.4	24.2±6.3	cabbage, gasoline, organic, sulfur, wet earth
Methyl anisole	10568-38-4	0.4±0.1	1.1±0.3	-	N
3-Methyl-thiophene	616-44-4	0.8±0.0	1.6±0.0	-	astringent, burnt, plastic

The compositions of volatile compounds in the headspace of fatty acid solutions and mineral oil are presented in **Table 3**. The number of compounds detected in the headspace of oleic acid solution (26) was comparable to linoleic acid solution (27), and both were higher compared to mineral oil (15) demonstrating that fatty acid solutions had more abundant headspace volatile composition. Acids, alcohols, aldehydes, esters, and ketones were identified in all samples. *Trans*-2-octen-1-ol, 2-penten-1-ol, 3-octen-2-ol, isoamyl acetate, methyl anisole, 3-methyl-butanal, ethyl 2-methylbutyrate, 2-butanone, 3-methyl-thiophene, butanoic acid, methyl ester, 1-butanol, 2,3-pentanedione, acetaldehyde, 2-methyl-butanoic acid, and hexanal were identified in the headspace of both fatty acid solutions but were absent in the headspace of mineral oil. When comparing the headspace composition of oleic acid solution with mineral oil, 1-octen-3-one, acetone, 2-butenal, decanoic acid, ethyl ester, 2-heptanone, 1-butanol were more abundant and 2-methyl-2-butenal were less abundant in the headspace of oleic acid solutions. When comparing the headspace composition of linoleic acid with mineral oil, acetone, diacetyl, 2-hexanone, 2-pentanone, 1-propanol, ethyl 2-methylbutyrate, 2-butanone, 3-methyl-thiophene, and acetaldehyde were more abundant and 2-methyl-2-butenal was less abundant in the headspace of linoleic acid solution.

Discussion

This study aimed to (a) explore whether humans can discriminate between solutions of basic tastants and water through orthonasal and retronasal olfaction, (b) and if so, to examine what volatile odor compounds (VOCs) underlie the discrimination ability.

Humans can discriminate between tastants and fatty acid solutions from blanks through olfaction

The study demonstrated that participants distinguished all tastant solutions from blank (water) through orthonasal olfaction. Only sucrose, sodium chloride, oleic acid, and linoleic acid were distinguished from blank by retronasal olfaction. Previous studies investigated the olfactory discrimination ability of humans between tastant solutions at suprathreshold and blanks. Mojet et al. [4] investigated the orthonasal detection of tastant solutions and reported that "considerable number of subjects (21 out of 41) could regularly detect seven of the ten tastants by olfaction". However, they reported their result as percentages of odor detection after correction for chance guessing without describing how the correction was performed. In an unpublished study, Chen [5] explored the orthonasal and retronasal perception of tastant solutions by performing ortho- and retronasal olfactory triangle discrimination test between tastant solutions and water. Both studies indicated that only sucrose and MSG solutions were distinguishable from the blanks by orthonasal olfaction. Consistent with these findings, our study confirmed the discriminability of sucrose and MSG solutions at suprathreshold levels from water, orthonasally and retronasally. Furthermore, our study revealed that NaCl, citric acid, and quinine solutions could be discriminated from water through orthonasal olfaction (**Figure 1-I**) which is in contrast to the studies of Chen [5] and Mojet et al. [4]. Moreover, Chen reported that none of the five basic tastant solutions were discriminated from water through retronasal olfaction, while our results show that sucrose and NaCl solutions could be discriminated from water through retronasal olfaction. The disparity in findings may be attributed to differences in experimental design and data analysis. Chen [5] used 30 participants to perform the discrimination tests without replicates and without specifying the sample comparison design (AAB or ABB). Mojet et al. [4] recruited 41 participants and performed four alternative forced-choice tests without replicates. Our study recruited 41 (orthonasal) or 42 (retronasal) participants and performed measurements in duplicate (AAB and ABB are considered duplicates for one sample comparison in our study design), which resulted in 82 or 84 observations for each sample comparison. Furthermore, Chen [5]

compared discrimination response accuracy (%) with chance level (33% for triangle test) through one-sample t tests. We summed up the number of correct responses for each sample comparison and calculated the significance level (p) according to binominal tests as commonly done for triangle tests. Another factor that may influence the olfactory perception of tastant solution is the purity of blank water. Although Mojet et al. [4] indicated that the purity of water (such as demineralized, double-distilled, and Evian water) had no significant impact on the discriminability of most tastants at both individual and group levels, they observed that the use of double-distilled water enhanced the orthonasal discrimination ability of MSG solution, while the use of Evian water diminished it. Our study used Milli Q water, which is ultrapure water. The different waters used in these studies may have influenced the olfactory perception of tastant solutions, however, more chemical analyzes are necessary to further verify the difference in headspace volatile compound composition of water differing in purities as well as their olfactory perception.

Regarding fatty acids, our results (**Figure 1**) are consistent with previous findings that oleic and linoleic acid can be distinguished from blank mineral oil through both orthonasal and retronasal olfaction [8, 9]. Furthermore, our findings differ slightly from Bolton & Halpern [8], who observed that in the discrimination of oleic acid solution, more correct responses were obtained through orthonasal discrimination compared to retronasal discrimination. In our study, we observed this phenomenon for both oleic acid (73/82 correct responses for orthonasal discrimination, and 70/84 correct responses for retronasal discrimination) and linoleic acid (75/82 correct responses for orthonasal discrimination and 62/84 correct responses for retronasal discrimination). This difference could be attributed to the fact that retronasal olfactory thresholds for both oleic and linoleic acids were higher than orthonasal olfactory thresholds [9], making the perception of the odor of fatty acids easier through orthonasal olfaction compared to retronasal olfaction.

Participants indicated that both odor intensity and odor quality contributed to olfactory discrimination. A higher number of participants guessed during retronasal discrimination tests compared to orthonasal discrimination tests for the tastant solutions (**Figure 2**). This could be attributed to the in general lower sensitivity of retronasal olfaction compared to orthonasal olfaction [23]. A previous study [21] reported a lower odor intensity of milk through retronasal olfaction compared with orthonasal perception. It is possible that the odor of the tastant solutions was not strong enough to be reliably detected through retronasal olfaction, making retronasal discriminations more challenging. These findings align with our

discrimination results (**Figure 1**), where we observed more correct responses in orthonasal discrimination compared to relative retronasal discriminations.

To summarize, our study suggests that humans are capable of discriminating the headspace of tastant or fatty acid solutions from blank through orthonasal olfaction.

The perceived odor of tastant solutions is not associated with their taste quality

For all tastant solutions, olfactory discrimination was not associated with the specific taste quality of the tastant solution as less than 20% of particpants associated the odor of the tastant solution correctly with the taste quality of the tastant solution, with the exception of sucrose solutions, where 45-46% (ortho- and retronasal, respectively) of participants indicated that they perceived a sweet smell of sucrose solution. Previous animal studies have shown that mice are capable of sensing the odor of sucrose solutions. Rats can discriminate among sucrose solution concentrations by cues other than taste, possibly by olfaction [14]. Interestingly, the preference for sucrose solution still remained even when sweet taste receptor T1R3 was genetically knocked out, and such preference decreased when olfaction was blocked [15] . These findings suggest that sucrose solutions emit odors and those may influence the nutritional behavior of mice.

Regarding fatty acids, our study found that many participants associated the odor of oleic (68%) and linoleic acid (56%) solutions with sourness in the orthonasal condition, whereas they associated them with fat taste (54 and 37% for oleic and linoleic acid respectively) in the retronasal condition. This seems in line with the Volatile Compounds in Food Online database (https://www.vcf-online.nl), where oleic and linoleic acids have been reported to smell fatty and rancid, and Chukir et al[11], which describes The retronasal odor of oleic and linoleic acids as oily, olive oil, sunflower. In contrast to retronasal olfactory perception, the orthonasal odors of oleic and linoleic acids were more often associated with sourness. It is known that the same volatile molecules can be perceived differently at various concentrations [24], and between ortho- versus retronasal routes. Furthermore, the disparity in perceived odor quality between orthonasal and retronasal olfaction may be influenced by anatomical differences [25] and variations in the adsorption environment [23] along these two routes.

In conclusion, although our study shows that humans are able to distinguish between tastant solutions and blank by means of smell, they are not able to associate the odor of the tastant solution to its associated taste quality, except for fatty acid solutions.

Differences in volatile compound composition between headspaces of tastant and fatty acid solutions and blank might contribute to odor discrimination ability

Our study profiled volatile compound composition in the headspace of tastant solutions and Milli Q water to explore what volatile compounds contribute to olfactory discrimination. Acetone, which presents odor qualities such as ether, hay, and pungent, was identified only in the headspace of sucrose and MSG solutions. The presence of acetone might be related to the manufacturing process of sucrose as acetone might have been used as a (co-) solvent during sucrose purification (US4116712A - Solvent refining of sugar). The residual acetone may have contributed to odor discrimination between sucrose and MSG tastant solutions and Milli Q water. We observed that chlorinated compounds were present in all samples. Specifically, trichloromethane was identified in Milli Q water and all tastant solutions. Peak areas of trichloromethane were significantly higher in the headspace of sucrose and quinine solutions compared to water, whereas ethyl dichloroacetate and methylene chloride were only identified in the headspace of quinine solutions. Chlorinated compounds were previously identified in drinking [26] and public water supplies [27], their presence in water originates from water disinfection treatments [28, 29]. We do not have an explanation for observed differences in relative abundance of chlorinated compounds between tastant solution and water, but these differences might have contributed to olfactory discrimination. Diethyl azodicarboxylate was identified in all tastant solutions and water with similar abundances, so its presence probably did not influence olfactory discrimination. We acknowledged that NaCl and citric acid solutions were discirminated from water through olfaction, but we did not find any differences in volatile composition between their headspaces. We cannot link olfactory discrimination to headspace composition for these two (out of five) tastant solutions. The headspace analysis method (ITEX-GC-MS) used might have not been sufficiently senstive to detect all volatile compounds present in the headspace. More studies with different analysis and extraction methods are needed to further verify potential differences in volatile composition between headspace of tastant solutions and water.

Previous studies investigated the ortho- and retronasal olfactory perception of fatty acids and concluded that both oleic and linoleic acid have distinguishable odors compared to blank mineral oil [8, 9]. However, these studies did not specify the volatile compound composition in the headspace. In our study, we confirmed that humans can discriminate the odor of oleic

and linoleic acid from mineral oil using both ortho- and retronasal olfaction and extended this to we identify the volatile compounds that may facilitate this discrimination ability. Several alcohols and aldehydes, including trans-2-octen-1-ol, 2-penten-1-ol, 3-octen-2-ol, 1-butanol, 3-methyl-butanal, acetaldehyde, and hexanal, were only identified in oleic and linoleic fatty acid solutions but not the blank. Furthermore, 1-propanol, diacetyl, and 2-hexanone were identified with significantly larger peak areas in fatty acid solutions compared to mineral oil. These compounds were found to be odor active (Table 3), which likely facilitated olfactory discrimination between the fatty acid solutions and the mineral oil. The alcohols and aldehydes are well-known oxidation products of fatty acids [30, 31]. Cao et al. [32] suggested that several aldehydes, such as octanal, nonanal, decanal, and 2-decenal, could serve as oxidation indicators for oleic acid, while hexanal was closely associated with the oxidation of linoleic acid. We suggest that oxidation products of fatty acids contributed to the olfactory discrimination between fatty acids solutions and mineral oil.

We acknowledge that our study focused on qualitative rather than quantitative analysis of the volatile compound composition in the headspace of tastant and fatty acid solutions. It is important to note that volatile compounds contribute to odor perception only when their concentration exceeds the detection threshold. We can only speculate on whether the identified volatile compounds actually influenced olfactory perception, as concentrations of volatile compounds were not quantified. We were unable to obtain odor activity values, since area under the curve rather than absolute concentration of compounds was determined Future studies should determine odor activity values to validate our current findings.

Conclusions

Our study demonstrates that humans can discriminate between solutions of all five basic tastants and fatty acids from blanks through orthonasal olfaction, and can distinguish solutions of sucrose, NaCl, and two fatty acids from blank. The perceived odor qualities of tastant solutions are not associated with their taste quality whereas the perceived odor qualities of fatty acids are associated with fat. Difference in volatile compound composition between headspaces of solutions and blank contribute might have facilitated olfactory discrimination between tastant and fatty acid solutions and blanks. These findings warrant further investigations to explore how olfaction contributes to taste and fat perception.

CRediT authorship contribution statement

Shuo Mu: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Markus Stieger**: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration. **Sanne Boesveldt:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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Supplementary Material

Figure S1. The specially designed cups for retronasal olfactory testing. It contains a plastic cup, a silica gel lid with two holes, and a plastic straw plugged in one of the holes. The straw is positioned just above the liquid surface. When using the cup, participant first block their nose with a nose clip, then insert the straw in their mouth, inhale the air through the straw using their mouth, then remove the nose clip, and finally exhale via their nose.

Table S1. One example of detailed sample comparisons for each session. The italics in session 3 are considered as duplicate comparisons from session 2 for retronasal test. The italics in session 4 are considered as duplicate comparisons for orthonasal test.

Session 1 Screening and training.	Sniffin' Sticks test		
	Training on specially designed cups for retronasal olfactory testing		
	Pilot test		
Session 2 Retronasal triangle discrimination test	Sample comparison design		
	Sucrose	Sucrose	Milli Q water
	Sodium chloride	Milli Q water	Sodium chloride
	Milli Q water	Citric acid	Citric acid
	MSG	Milli Q water	MSG
	Quinine	Quinine	Milli Q water
	Mineral oil	Oleic acid	Oleic acid
	Linoleic acid	Linoleic acid	Mineral oil
Session 3 Retronasal triangle discrimination test	Sample comparison design		
	Milli Q water	Milli Q water	Sucrose
	Milli Q water	Milli Q water	Sodium chloride
	Citric acid	Milli Q water	Milli Q water
	Milli Q water	MSG	Milli Q water
	Milli Q water	Milli Q water	Quinine
	Oleic acid	Mineral oil	Mineral oil
	Mineral oil	Linoleic acid	Mineral oil
Session 4	Sample comparison design		

Orthonasal triangle discrimination test	Sucrose	Milli Q water	Sucrose
	Sodium chloride	Sodium chloride	Milli Q water
	Citric acid	Milli Q water	Citric acid
	Milli Q water	MSG	MSG
	Quinine	Quinine	Milli Q water
	Oleic acid	Mineral oil	Oleic acid
	Linoleic acid	Linoleic acid	Mineral oil
	Milli Q water	Milli Q water	Sucrose
	Milli Q water	Sodium chloride	Milli Q water
	Citric acid	Milli Q water	Milli Q water
	Milli Q water	Milli Q water	MSG
	Milli Q water	Milli Q water	Quinine
	Mineral oil	Oleic acid	Mineral oil
	Linoleic acid	Mineral oil	Mineral oil


3

Chapter 3

Smells like fat: A systematic scoping review on the contribution of olfaction to fat perception in humans and rodents.

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Abstract

Understanding how dietary fat is perceived by the senses is crucial in developing public health strategies aimed at curbing excessive fat intakes. Olfaction is one of several sensory modalities contributing to fat perception in foods, yet the nature and extent of its involvement is relatively unclear.

A systematic scoping literature review was conducted to identify and summarize relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents and highlight relevant knowledge gaps. The review was carried out in accordance with the PRISMA methodology, using combinations of olfaction-, fat- and perception-related search terms. Following searches in Scopus, Web of Science and PubMed databases, 42 articles were ultimately included.

Overall, findings are consistent with the notion that olfaction plays a role in the perception of dietary fat in rodents and humans. Rodents can perceive dietary fat via olfactory cues, and this ability may affect their preference for fat-containing feed. Humans can detect, discriminate, and identify fat and its constituents solely by olfaction, even when embedded within a complex food matrix. Food fat content can modulate the perception of various fat- and non-fat olfactory qualities, depending on the food matrix and odorant physio-chemical properties. On the other hand, the presence of fat-related odors can modify the perception of olfactory and non-olfactory sensory qualities (e.g., mouthfeel). Several knowledge gaps were identified, namely, the role of fat-related odors in eating behavior, the nature of chemical signals underlying olfactory fat perception and factors governing sensitivity to fat-related odors.

Introduction

Consumption of dietary fat is exceeding recommended daily intake requirements in many Western countries, including the Netherlands [1], in some accounting for up to 46% of the total daily energy intake [2]. Due to its high energy density and low effect on satiation, especially in obese individuals [3], fat is considered a major contributor to energy overconsumption and consequential development of obesity and related comorbidities [4-6]. Fat overconsumption is further exacerbated by its flavor, texture, and aroma-enhancing properties, all of which considerably contribute towards the pleasurable experience of eating [7-9]. The interaction of these factors has recently been illustrated by Teo et al. [10] who found that foods associated with fat-related flavors contributed most to higher energy intakes, independent of weight status.

Multiple sensory systems contribute to dietary fat perception [9, 11]. Fat is known to impart a range of mouthfeel sensations, such as thickness, creaminess, mouthcoating and smoothness [12-14], while the presence of free fatty acids can be detected in the oral cavity via taste receptors located on the human tongue [15-20]. In addition to mouthfeel and taste cues, the involvement of olfactory cues in fat perception has also been established. Flavor release studies identified various volatile compounds, belonging to different chemical classes as being associated with fat-related sensations [11, 21]. When released from foods or beverages, these volatiles bind to receptors located throughout the olfactory epithelium in the nasal cavity, which ultimately results in odor perception [22]. Orthonasal odors originate from the external environment and enter the nasal cavity via the nostrils. They are thought to be related to food source detection and the induction of appetite during the anticipatory phase of eating. Retronasal odors, on the other hand, enter the nasal cavity from the mouth during food consumption. They mainly contribute to flavor perception and may influence intake and satiation [22-25]. The two olfaction routes can yield distinct perceptions, even when odor intensities are matched [26]. In comparison to mouthfeel and taste, however, the involvement of olfaction in dietary fat perception seems to be relatively underexplored and much remains unclear about the nature and extent of its contribution.

Given the societal relevance of understanding sensory fat perception, and the lack of systematic literature reviews on this topic in academic literature, the current scoping review aimed at (1) systematically identifying and summarizing relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents, and (2) highlighting relevant knowledge gaps. The rationale behind focusing on broader literature, also involving

rodents, was to gain insight from mechanistic studies, which might not be feasible or ethical to conduct in human subjects.

Methods

Due to the broad nature of its aims, the current work is considered a systematic scoping review. It was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) methodology [27].

Search strategy

Three academic electronic databases (Scopus, PubMed and Web of Science) were searched for original articles published in English, without any publication date restrictions. Search strings included olfaction- (e.g., volatiles, orthonasal, aroma, odor) and fat-related words (e.g., fat, lipid, fatty acid, butter), combined with perception-related words or strings (e.g., flavor, discrimination, identification, chemosensory). Search strings for all three databases contained exclusion commands (excluding words such as cat, dog, insect, larvae from the search), to avoid articles beyond the scope of this review (e.g., insect studies). Detailed search strategies used in each database can be found in Supplementary Material A. Due to search algorithm differences, a specific search string was applied to each of the databases. It must be noted that the word "preference" in combination with fat-related words was excluded from the search string applied in the PubMed database. This was done to increase specificity, as inclusion of this combination mainly yielded articles deemed beyond the scope of this review. Early search results were evaluated to determine the relevance of obtained articles, and search term modifications were made prior to the formal search procedure. Reference lists of included articles were not searched for articles not captured by the searches. Manual searching was also not undertaken.

Article inclusion

Articles met eligibility criteria if they reported an investigation of olfactory exposure (ortho- or retronasal) to fat and its constituents, in isolation or via foods (real or model), beverages or emulsions in human or rodent subjects, utilizing sensory evaluation. Sensory evaluation was defined as a scientific approach utilizing a measure of perception, discrimination, identification, preference, acceptance and/or detection thresholds. Articles concerning the addition of fat-related aromas/flavorings to foods were included as well if their addition impacted relevant sensory attributes. Exclusion criteria involved fat perception not being the topic of research; lack of olfactory exposure to suitable fat sources (i.e. either no exposure to fat; or exposure to fat in combination with potentially confounding odor/flavor sources); lack

of reporting relevant outcomes resulting from olfactory exposure; articles focusing on volatile compounds without relevant sensory evaluation measures; reviews, meta-analyses, books, or book chapters; articles lacking an abstract; full-text unavailability; non-English publications; and non-peer reviewed publications.

Article Selection

Literature searches were performed up to April 2021 by three authors: PM, MS and FG. All identified items were exported to the reference software EndNote™ X9 (Clarivate Analytics) where they were organized, deduplicated and screened following the PRISMA guidelines [27]. Title and corresponding abstract screening were carried out by FG. Screening reliability was determined by calculating the Cohen's Kappa coefficient, after PM and FG screened a random sample of 116 titles and corresponding abstracts from the retrieved items (sample size was determined in accordance with the Cohen's Kappa methodology). The interrater reliability score amounted to 0.90, which indicated a strong agreement [28, 29]. Remaining potentially eligible items then underwent full-text screening, carried out by PM and MS. Any discordances regarding the ultimate inclusion of articles in the review were discussed by the reviewers until reaching a consensus. A list of citations excluded during the full-text screening process can be found in **Table S1**, Supplementary Material B.

Review Outcomes and Data Synthesis

Data from articles meeting all inclusion criteria were extracted. Extracted data included outcomes of interest relevant to our research question, study population characteristics (along with relevant population specifics, if applicable), stimuli (types used along with the applied manipulation, if applicable), route of olfactory exposure (orthonasal or retronasal), and relevant findings. Data were then evaluated and interpreted by all authors, tabulated per study, and listed by author name in an ascending alphabetical order. Rodent studies were distinguished from human ones and reported in a separate table. A narrative synthesis was ultimately conducted, meta-analysis was not performed due to the indirect nature of most of the identified work and lack of relevant and comparable data.

Risk of Bias Assessment

To assess the quality of included studies, two authors (PM and MS) independently reviewed and evaluated each article in accordance with the Cochrane Association Risk of Bias

methodology [30]. Any discrepancies in risk of bias scores were discussed to reach agreements. Due to the nature of this review's topic, specific risk assessment domains were generated per study subject type. Risk evaluation domains for rodent studies included random group generation, researcher blinding, incomplete outcome reporting and selective reporting. Human studies were evaluated on stimulus randomization; isolation of olfaction from potentially confounding effects of taste, mouthfeel, and trigeminal sensations; participant blinding to sample identities; incomplete outcome reporting; and selective reporting. For each domain, the risk of bias was rated as "low risk", "some concern", "high risk" or "risk unclear", based on information reported in the included articles.

Findings

An overview of the search process and its results can be seen in the PRISMA flowchart in **Figure** 1. Database searches resulted in the identification of 2596 items from all sources, with 1703 of them remaining after deduplication. After title and abstract screening, 93 items remained and were assessed against our eligibility criteria. In total, 51 items were excluded: 4 were not about fat perception, 11 lacked olfactory exposure to suitable fat sources, 11 did not report relevant outcomes resulting from olfactory exposure, 4 focused on volatile chemical compounds without relevant sensory evaluation measures, 17 were either meta-analyses, reviews, books, or book chapters, and 4 were inaccessible. Full-text assessment ultimately resulted in 42 articles being included in the current review.

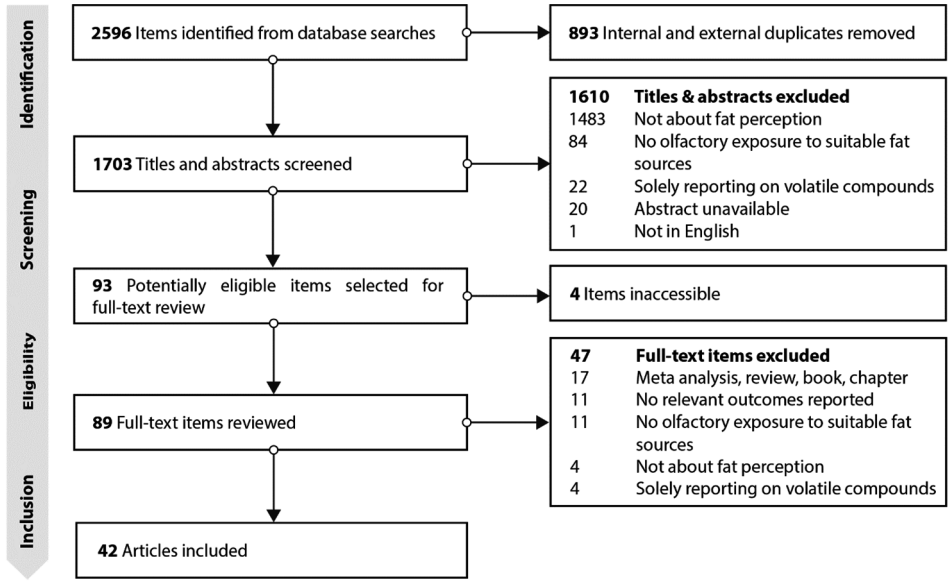

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the literature search to identify olfactory fat perception studies.

Rodent Studies

A summary of studies investigating olfactory fat perception in rodents is presented in **Table 1.** Six studies employed rodent subjects, namely mice [31-35] or rats [36]. In all cases wild-type controls were compared to either anosmiated [31-34, 36] or CD36 (cluster of differentiation 36) receptor-deficient specimens [35]. All rodent studies utilized preference paradigms in which animals were exposed to olfactory stimuli either via food varying in fat

content [31, 32, 36], scented paper [35], sucrose-based solutions [33], or corn oil and linoleic acid [34].

To summarize, rodents' preferences for fat-related odorants diminished when rodents were anosmiated [32, 34, 36] or lacked olfactory CD36 receptors [35]. Once their sense of smell was restored, preference for fat returned [32]. Moreover, following anosmiation, rodents lost their preference for aversion-inducing lipids [33]. Anosmiation, however, did not lead to a complete preference diminishment for fat in all cases. Despite anosmiation, Boone et al. [31] observed no preference alterations towards a high-fat diet, Ramirez [36] observed only a decrease in preference towards fat-containing mixtures, while Takeda et al. [34] observed a preference decrease only for corn oil containing higher fat levels.

Table 1. Summary of studies investigating olfactory fat perception in **rodents**.

Study	Outcome(s) of interest	Subjects	Stimuli	Relevant Findings	Interpretation
Boone et al. (2021) [31]	Changes in feeding patterns in response to varying access to different diets (standard or standard in combination with high fat).	A total of 96-120 (exact numbers per experiment n.s.) mixed-sex adult mice, either anosmiated via complete bilateral bulbectomy or sham-operated.	Standard diet (14% energy from fat) and high fat diet (60% energy from fat).	All mice, regardless of treatment (anosmiated or sham-operated) exhibited a preference for the high-fat diet.	Olfactory information is not relevant for the formation of high-fat food preferences.
Kinney and Antill (1996) [32]	Intake of food mixtures during a 2-h preference test.	36 male albino mice: 12 underwent bilateral olfactory nerve section, 12 underwent sham surgery (control), 12 untreated mice (control).	Corn oil-based high fat (3.42 kcal/g) and mineral oil-based low-fat (2.61 kcal/g) food mixes.	Pre-treatment, all mice preferred the high-fat food mixture; post-treatment, anosmic mice showed no preference for the high-fat mixture, preference for the high-fat mixture increased in the control groups. Preference for the high-fat mixture returned to anosmic mice after olfactory nerve recovery.	Olfactory information is relevant for the formation of high-fat food preferences.
Lee et al. (2015) [33]	Intake following two-bottle choice tests	8 – 12 week old mice (number and sex n.s.): Sham-operated (control) or anosmiated via olfactory nerve transection.	0.15 M sucrose solutions with 7.5 µM KOdiA-PC lipids (test) and without (control)	In contrast to normosmic controls, anosmiated mice exhibited preference for the solution containing the aversive KOdiA-PC lipid.	Olfaction is involved in the perception of lipids in mice.
Ramirez (1993) [36]	Preference scores following two-bottle preference tests.	20 female rats: 12 anosmiated via bulbectomy, 8 underwent sham surgery (control).	Carbohydrate- and fat-containing mixtures. Fat-containing mixtures included 0.5% corn oil, 1% corn oil, 0.5% triolein and 1% triolein.	Preference scores for fat-containing mixtures were lower in bulbectomized rats than in sham operated ones. Bulbectomized rats still exhibited preferences for fat-containing mixtures.	Preference for fat is mediated by olfactory and non-olfactory cues.

Study	Outcome(s) of interest	Subjects	Stimuli	Relevant Findings	Interpretation
Takeda et al. (2001) [34]	Voluntary intake of corn oil or linoleic acid; Place preference.	28 male mice: either sham-treated or anosmiated (via ZnSO4-induced olfactory blockade).	Corn oil (1, 3, 5 and 10%), linoleic acid and water.	Pre- treatment, mice preferred corn oil over the vehicle at all concentrations; post-treatment, sham-treated mice preferred corn oil over the vehicle at all concentrations, anosmiated mice preferred corn oil only at higher ones (5% and 10%), Place preference induced by corn oil was observed in both treatment conditions.	Multiple sensory modalities are involved in the perception of oil, olfactory stimuli might act as a signal for oil at low concentrations.
Xavier et al. (2016) [35]	Innate preference for scented filter paper inferred from Investigation time.	10 mice: 6 with CD36 receptor deficiency, 4 wild type (control).	Deodorized filter paper scented with PBS (control), amyl acetate (1 mM), or a lipid concentrate.	Contrary to wild type mice (control), CD36-deficient ones showed no preference for the lipid concentrate-scented filter paper.	Receptor CD36 is involved in the perception of fat-related odorants.

Human Studies

A summary of studies investigating olfactory fat perception in humans is presented in **Table 2.** Of the 36 studies employing human subjects, 8 presented olfactory stimuli orthonasally [37-44], 15 retronasally [13, 42, 45-58]and 13 through a combination of both olfaction routes [15, 43, 59-70]. Utilized sensory methodology included perceptual ratings [13, 37-40, 44, 48, 49, 51, 54, 55, 57, 58, 60-70]; discrimination testing [37, 50, 52, 53, 59]; detection [15, 56], difference [53, 56]and rejection [43] threshold testing; pairwise ranking [45]; time-intensity methods [46, 54, 65, 66, 69]; and identification testing [41, 47]. In addition to sensory methods, aroma volatile release or volatile compound composition analyses [39, 45, 46, 49, 50, 54, 55, 66, 69]and dietary intake assessments [37, 42] were carried out. Fatty acids were exclusively used as olfactory stimuli in six studies [15, 42, 47, 48, 52, 59], with subjects being exposed to either stearic, linoleic and oleic acid [15, 47, 52, 59]; taste strips containing varying levels of linoleic acid [48]; or oleic acid [42]. Food matrices served as olfactory stimuli in 31 human studies [13, 37-41, 43-46, 49-51, 53-58, 60-70]. The vast majority of food matrices were dairy product-based [13, 37, 38, 44-46, 50, 51, 53-58, 60, 61, 63-68, 70], others included meat products [40, 62, 69] margarine [39], oil and lard [41], chocolate [43]and agar gels [49]. Most studies utilizing foods added flavor/aroma volatiles to the matrices [45, 46, 49, 50, 53-55, 57, 60, 63-66, 68, 69], while some added free fatty acids [38, 43, 44].

Studies on the human ability to smell fatty acids found that 18-carbon fatty acids, namely linoleic, oleic and stearic, can be detected orthonasally [15, 42] and retronasally [15], with retronasal detection thresholds being higher than orthonasal ones [15]. Linoleic, oleic and stearic acids can also be discriminated from blanks ortho- and retronasally, with discrimination ability for oleic acid being lower for retronasal olfaction [59]; discriminated from each other retronasally [52]; and retronasally identified from blanks and each other, with their chemical structure (i.e., the number of double bonds) influencing identification [47]. Upon removing retronasal cues, the detection of linoleic acid on taste strips diminishes [48]. The addition of oleic and stearic acids to a corn starch solution had no effect on perception of creaminess odor [38], whereas adding short chain fatty acids, namely acetic, butanoic and hexanoic acid, to yogurt decreased yogurt-like odor intensity while simultaneously increasing intensities of off-flavors [44]. Chocolate containing linoleic fatty

acids was rejected at lower concentrations than chocolate containing oleic acid, whereas stearic acid had no effect on rejection thresholds [43].

Studies investigating olfactory fat perception ability in food matrices show that humans can orthonasally distinguish rapeseed oil, lard and oleic acid from non-fat controls [41] and discriminate fat content of dairy milks [37]. Moreover, the presence of retronasal cues can impact the ability to discriminate fat content in white sauces, milk, and yogurt, with the impact depending on the reference fat content, direction of comparison, and other factors such as added ingredients and the presence of sensory cues from other modalities [53]. The presence of retronasal cues enhances the perception of fattiness in dairy-based mixtures, while their elimination increases fat content detection and difference thresholds in cottage cheese [56], decreases the perception of creamy and fatty mouthfeel in vanilla custard and affects the perception of creaminess in sour cream [51]. In contrast, one study reported that elimination of retronasal cues does not affect fat content and creaminess perception in commercially available dairy products [13].

Fat content was reported to have differential effects on the release of flavor volatiles [39, 45, 46, 49, 50, 54, 55, 62, 65, 66, 69] and influenced the perception of various odors in diverse food matrices. Increases in fat content were found to decrease lemon flavor intensity, while increasing that of milk flavor in dairy desserts [45]; increase overall odor intensity in dairy milk [37]; decrease flavor intensities of 2-hexenyl acetate; anethole and terpinolene in yogurt [46]; increase creamy odor intensity in fresh cream and evaporated milk, with the increase being larger in evaporated milk, despite having a lower fat content than fresh cream [38]; increase butter and cheese odor in margarine, while decreasing that of cream [39]; increase blue cheese flavor in flavored agar gel [49]; decrease boiled odor in milk, while increasing creamy odor, flavor intensities and fattiness – a descriptor which was highly positively correlated with creamy aroma and flavor, and increased more in low-fat samples than in high-fat ones [63]; decrease strawberry flavor intensity in strawberry custard [50]; increase creaminess and butter note intensities in Gouda cheese [64]; decrease overall odor and flavor intensity and sharpness in strawberry ice cream [65]; decrease black pepper odor intensity in dry-ripened sausages [62]; decrease the odor intensity of linalool in dairy milk [66]; increase linalool odor intensity in strawberry-flavored milk while decreasing strawberry flavor intensity [54]; decrease intensities of various coffee-related (e.g., roast, coffee, burnt), but not milk-related (e.g., milky, butter, creamy) flavor qualities[67]; decrease flavor intensities of beta-damascenone, hexanal and ethyl butyrate in flavored dairy milk [55]; decrease

mushroom odor intensity, while increasing that of cocoa odor in mushroom and cocoa-flavored bologna sausages [69]; increase intensities of vanilla, caramel, milk odor and flavor, as well as cream and fat flavor in vanilla custards, while decreasing synthetic odor and chemical and sickly flavor [61]. Fat content was not found to affect cured ham odor intensity in cooked ham [40] and overall odor intensity in cheese [68].

Five studies investigated the perceptual consequences of adding fat-related odors to foods. In dairy milk, the addition of a cream aroma led to an increase in perceived fattiness [63], creaminess and thickness [60]; butter aroma added to cheese enhanced perceived creaminess and texture pleasantness [64] and fat content texture [68], while it enhanced fattiness when added to mashed potatoes [57]; fattiness was also enhanced after adding cream and onion aroma to potato chips [57]; the addition of a butter odor enhanced texture pleasantness in cheese [64].

Table 2. Summary of studies investigating olfactory fat perception in **human subjects**.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
STUDIES ON OLFACTORY PERCEPTION OF FAT IN ISOLATION						
Bolton and Halpern (2010)[59]	Discrimination ability between fatty acids and blanks.	Untrained: EXP 1(ortho- and retronasal session): n = 30 (13F); 26.6 ± 9.3 y EXP 2 (retronasal and oral-cavity-only session): n = 30 (16F); 26.0 ± 4.0 y	Orthonasal Retronasal (I)	Linoleic and oleic acids compared to mineral oil (control); Undiluted stearic acid compared to NaCl (control).	All fatty acids were discriminated from control, ortho- and retronasally: Orthonasally, 87% of subjects discriminated linoleic acid from blanks and 83% discriminated oleic and stearic acids; Retronasally, 93% discriminated linoleic acid from control, 57% discriminated oleic acid and 83% discriminated stearic acid. Discrimination ability did not differ between the routes for linoleic and stearic acids, it was lower for oleic acid in the retronasal condition.	Humans can ortho- and retronasally distinguish fatty acids from non-fatty acid-containing controls.
Chale-Rush et al. (2007)[15]	Orthonasal, retronasal and multimodal detection thresholds of different fatty acids	Untrained; 6-n-Propylthiouracil tasters; n = 22 (7F); 21.2 ± 0.6 y; BMI 23.6 ± 0.4; body fat 18.3 ± 1.3 %	Orthonasal Retronasal (I, C)	Linoleic, oxidized linoleic, oleic, and stearic acids varying in concentration.	Retronasal detection thresholds were higher than those of other exposure routes for all fatty acids. Detection thresholds for linoleic acid were lowest for orthonasal olfaction compared to other exposure routes. For oxidized linoleic and oleic acids, orthonasal thresholds did not differ from those of multimodal exposure but were lower than those of taste. Stearic acid detection thresholds did not differ between orthonasal, taste and multimodal exposure. No correlations between the different thresholds were observed.	Humans can smell 18-carbon fatty acids. Olfaction contributes independently to the perception of fatty acids. Retronasal olfaction is less sensitive to the presence of fatty acids than other chemosensory systems.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Chukir et al. (2013)[47]	Linguistic identification derived from Check-All-That-Apply methodology following retronasal inhalation.	Untrained; n = 36 (24F); 18 – 71 y (median 21 y)	Retronasal (I)	Fatty acids: linoleic, oleic, and stearic; non-fatty acid stimuli (controls): geraniol and phenylethyl alcohol.	Fatty acid-containing samples received identifications that consistently differed from those ascribed to controls. Stearic acid was identified differently from linoleic and oleic acids by approximately one-third of assessors. Linoleic and oleic acids mostly received the same, partly food-related identifications: Linoleic acid identifications included: new plastic, rubbery, sunflower, peanut oil, olive oil and oily. Oleic acid identifications included: new plastic, rubbery, sunflower, peanut oil, margarine, olive oil and oily. Stearic acid identifications included: new plastic, rubbery, sunflower, and oily - the proportion of rubbery for stearic acid was about twice that for linoleic and oleic acids. Identifications of the three fatty acids were consistently different from those of non-fatty acid stimuli.	18-carbon fatty acids can be identified retronasally. Linoleic and oleic fatty acids may contribute to flavor perception.
Ebba et al. (2012)[48]	Perceptual ratings of fat-related taste quality intensities.	Untrained; n = 88 (51F); 18 – 74 y (mean 25.1 y)	Retronasal (C)	Taste strips containing mineral oil (control), linoleic acid in amounts of 1.1, 1.3, 1.5, and 1.7 μmol.	The perceived taste intensity of linoleic acid decreased by 40% when retronasal olfaction was eliminated via nose clips.	Olfaction is involved in the perception of fatty acids and can enhance fat-related taste qualities.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Kallas and Halpern (2011)[52]	Discrimination ability between fatty acids.	Untrained; n = 40 (30F); 18 – 36 y	Retronasal (I)	Linoleic (40.5%), oleic (40.0%) and stearic acids, all at suprathreshold levels; Linoleic acid (0.005% – subthreshold level concentration) compared to mineral oil (control).	Vapor phase stearic acids were discriminated from vapor-phase linoleic or oleic fatty acids: 70% of subjects discriminated between stearic and linoleic acids; 65% discriminated between stearic and oleic acids. Oleic and linoleic fatty acids were discriminated by 38% of subjects. No discrimination occurred in "negative control" trials.	Humans can discriminate 18-carbon fatty acids using solely retronasal olfaction.
Kindleysides et al. (2017)[42]	Fatty acid olfactory detection thresholds. Dietary intake of key food groups.	Untrained; n = 50F; 18 – 45 y (median 26 y); median BMI 24 (31 normal-weight, 11 overweight, 8 obese)	Orthonasal	Oleic acid (combined with mineral oil), varying in concentration (6, 12, 24, 48, 95, 190, and 380 mM).	Olfactory detection curves increased with higher concentration of oleic acid. Oleic acid taste and olfactory detection abilities were positively correlated. Oleic acid olfactory sensitivity was not related to body composition. Dietary intakes of nuts, nut spreads, and seeds were positively correlated with high olfactory sensitivity to oleic acid.	Oleic fatty acid can be detected orthonasally. While olfactory sensitivity to oleic fatty acid is independent of body composition, it is related to the habitual consumption of fat-containing foods and gustatory sensitivity to oleic acid.
STUDIES ON OLFACTORY PERCEPTION OF FAT EMBEDDED WITHIN FOOD MATRICES						
Arancibia et al. (2015)[45]	Relative intensities of lemon and milk flavors assessed	Sensory session: trained; n = 28 (16F); 23 – 55 y Aroma release session: n = 8	Retronasal (C)	Lemon-flavored (added linalool and cis-3-hexen-1-ol) dairy desserts with added thickeners	Lemon flavor intensity was higher in dairy desserts with a lower fat content, while milk flavor intensity was higher in desserts with a higher fat content.	Fat content influences *in vivo* release of certain flavor compounds, which affects their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	via pairwise ranking. Aroma release parameters following nose-space sampling.			and varying in fat content: 0.14% and 3.5% fat.	Linalool release was lower in desserts with a higher fat content.	
Boesveldt and Lundstrom (2014)[37]	Orthonasal discrimination ability between fat levels in dairy milk. Perceptual ratings of intensity, pleasantness. Habitual fat intake.	EXP 1: untrained; n = 30 (16F), 27.3 ± 4.2 y, BMI 23.1 ± 3.1 EXP 2: untrained; n = 18 (12F), 22.1 ± 1.2 y, BMI 22.7 ± 3.1 EXP 3: Normal-weight – untrained; n = 30 (15F), 25.0 ± 3.7 y, BMI 22.5 ± 1.8; Overweight – untrained; n = 30 (18F), 30.6 ± 7.2 y, BMI 35.6 ± 8.4	Orthonasal	Manipulated milk samples varying in fat content (skimmed, semi-skimmed, whole):	Skimmed milk samples were discriminated from whole milk ones in all experiments. In EXP 1 and EXP 2, skimmed milk was not discriminated from whole milk; in EXP 3 skimmed milk was not discriminated from semi-skimmed milk. There was no difference between normal-weight and overweight subjects discrimination performance. In EXP 1 and EXP 2, perceived intensity increased with increasing fat content, while pleasantness decreased. In EXP 2, perceived pleasantness did not differ between the samples. In EXP 3, perceived intensity, but not pleasantness was lower in the overweight group. Discrimination ability was not correlated to BMI or habitual dairy fat consumption parameters in any of the experiments.	Humans can smell differences between dairy milks differing in fat level, using solely orthonasal olfaction. This ability seems independent from habitual dairy fat consumption and BMI.
Brauss et al. (1999)[46]	Time-intensity parameters related to the	Trained; n = 10	Retronasal (C)	Flavored yogurts varying in fat	Flavor compound volatility and perceived flavor intensities decreased with increasing fat content.	Fat content diminishes the volatility of certain flavor

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	perception of flavorings (2-hexenyl acetate, anethole and terpinolene) Aroma release parameters following nose space sampling.			content (0.2, 3.5 and 10%).		compounds, which affects their perception.
Bult et al. (2007)[60]	Perceptual ratings of overall flavor intensity, thickness, and creaminess (taken whilst milk-like foods were present in the mouth and a cream odor was presented retro- or orthonasally).	Untrained; n = 11 (3F); 41 ± 11 y	Orthonasal Retronasal (I, C)	Fresh skim milk (0.075% fat content) with an added cream aroma.	The odor stimulus increased intensities of thickness and creaminess, but only when the odor was presented retronasally. This was most pronounced when odors coincided with swallowing.	Fat-related retronasal odors can enhance fat-related mouthfeel sensations via cross-modal interactions.
Chen and Eaton (2012)[38]	Perceptual ratings of creaminess following orthonasal, taste, taste and	Untrained; dairy-consumers, familiar with creamy foods; n = 16 (14F); 21 – 25 y	Orthonasal	Fresh single cream (19.1% fat), evaporated milk (9.0% fat), corn starch solution and corn starch solution	Orthonasal creaminess ratings were higher for fat-containing samples than non-fat ones. Despite having a higher fat content, single cream was rated as being less creamy than evaporated milk.	Olfaction is involved in the perception of creaminess. Fat content influences the intensity of creamy odor.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	mouthfeel, and multimodal exposure.			with added oleic and stearic fatty acids (0.1%).	The presence of fatty acids had no influence on creaminess aroma ratings.	Oleic and stearic fatty acids do not elicit a creamy aroma.
Dadalı and Elmacı (2019)[39]	Perceptual ratings of butter, creamy, cheesy, animal-like, margarine and oxidized aroma. Relative amounts of volatiles in the headspace following fat and emulsifier content manipulation.	Trained; n = 10 (8F); 23 – 54 y	Orthonasal	Model margarines varying in fat content (60, 70 and 80%).	The release of 2,3-butanedione and butanoic acid was higher in model margarines with 70% and 80% fat content. The release of 2-heptanone, 2-nonanone, 2-undeca-none, hexanoic acid, and delta-decalactone was higher in margarines with a lower fat ratio. Fattier margarines were rated higher in terms of butter and cheese aroma. Cream aroma was rated as being more intense in lower-fat margarines.	Fat content influences the volatility of certain flavor compounds, which affects their perception.
Fernandez et al. (2000)[40]	Perceptual ratings of smell intensity.	Trained; n = 12	Orthonasal	Cooked ham slices varying in fat content (≤ 2%; 2-3%; 3-4%; > 4%).	Smell intensity of cured ham (pork) was not affected by fat content.	Fat content alterations do not necessarily modify smell intensity.
Frank et al. (2015)[49]	Perceptual ratings of blue cheese flavor and overall flavor intensities. Aroma release parameters	Trained; n = 10	Retronasal (C)	Agar gels varying in fat content (0%, 10%) and aromatized with blue cheese-related volatiles.	Fat-containing agar gels were rated as more intense in terms of blue cheese flavor. Fat content had differential effects on the release of several volatiles, depending on their solubility and lipophilicity.	Fat content influences the volatility of certain flavor compounds, which affected their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	following headspace sampling.					
Frøst et al. (2001)[63]	Perceptual ratings of creamy aroma, cream flavor, and total fattiness (meta descriptor).	Trained; n = 7	Orthonasal Retronasal (C)	Commercially available dairy milk varying in fat content (0.1, 1.3 and 3.5%) with added cream aroma (0 or 0.75 g/L), thickener (0 or 1 g/L) and whitener (0 or 1 g/L).	With increasing fat content, Intensities of creamy odor and flavor increased, while boiled milk odor decreased. The magnitude of perceived difference in fattiness was much larger between 0.1 and 1.3% fat samples than between 1.3 and 3.5% ones. Samples with added cream aroma scored higher in terms of total fattiness. Total fattiness was highly positively correlated with creamy odor and flavor.	The addition of fat-related odors to milk enhanced the perception of milk fat content.
Glumac and Chen (2020)[41]	Proportion of correct answers to the question: "Is this perceived as oil/fat?", posed following exposure via various sensory modalities.	Untrained; n = 30 (15F); 27.3 ± 2.0 y; BMI 18.5 – 25.9	Orthonasal	Commercial rapeseed oil, commercial lard, plant-sourced oleic acid, food grade silicone oil, food-grade glycerol, and food-grade xanthan gum solution.	Using only orthonasal cues, subjects correctly identified rapeseed oil, lard and oleic acid as oil/fat-containing, while silicon, glycerol and xanthan gum solution were correctly identified as non-oil/fat. Aroma seemed to be the most informative sensory modality for oil/fat recognition, followed by tactile and taste sensations.	Humans can identify fat-containing food samples using solely orthonasal cues. For fat recognition, orthonasal olfactory cues are more informative than tactile and taste-related ones.
González-Tomás et al. (2007)[50]	Relative intensity of strawberry flavor assessed	Aroma release: n = 10	Retronasal (C)	Model, strawberry-flavored custards varying in fat content (0.14% and	Fat content influenced strawberry flavor intensity and release: Strawberry flavor of 0.14% fat samples was more intense than that of 3.5% ones.	Increases in fat content may diminish the volatility of certain flavor compounds, in turn

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	via pairwise comparison. Aroma release parameters following nose-space sampling.	Sensory evaluation: Trained; n = 39		3.5%), starch and emulsifier concentrations.	Volatile release was higher in 0.14% milk fat samples than in 3.5% ones.	modulating their perceived intensity.
Han et al. (2019)[64]	Perceptual ratings of cheese creaminess, butter note, overall flavor and cheese texture pleasantness following consumption of cheese cubes in the presence of either ortho- or retronasal butter odor delivered at various points of the oral processing cycle.	Untrained; n = 20 (8F); 25 – 29 y	Orthonasal Retronasal (C)	Butter odor at two concentrations: low (just above the detection threshold) and high (well above the detection threshold) Gouda cheese varying in fat content (20, 30 and 40%).	Creaminess, butter note intensity and texture pleasantness were enhanced by the addition of a butter odor – effects were more pronounced when a low odor concentration was presented and varied with the timing of odor presentation and cheese fat content: Perceived creaminess increased when butter odor was presented retronasally at the start of chewing. Perceived butter note intensity peaked when the odor was delivered retronasally during chewing in regardless of the butter odor concentration. Perceived texture pleasantness was enhanced when butter odor was delivered orthonasally before chewing. Perceived creaminess and butter note intensities increased with increasing fat content.	Fat content affects the olfactory perception of creaminess. Fat-related odors can enhance the perception of cheese-related attributes via cross-modal interactions. These enhancements are more pronounced at lower odor concentrations.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Hyvönen et al. (2003)[65]	Time-intensity parameters related to the perception of strawberry flavor release and melting. 6 perceptual ratings, including fattiness and creaminess.	Untrained: Time-intensity panel: n = 15 (9F); 28 y (SD n.s.) Descriptive panel: n = 35 (23F); 31 y (SD n.s.)	Orthonasal Retronasal (C)	Strawberry-flavored ice cream varying in fat content (0, 5, 9, 14 and 18%), prepared using dairy and vegetable fat.	Flavor release from vegetable fat-based ice cream samples was slightly faster than from dairy fat-based ones. Intensity and sharpness of ice cream aroma and flavor were higher in fat-free ice cream samples than fat-containing ones No differences in the intensities of aroma and flavor attributes were observed in samples containing 5% of fat or more.	Fat type may influence the volatility of certain odor/flavor compounds, without affecting their perception. Fat content influences the volatility of certain odor/flavor compounds, which may affect their perception.
Jervis et al. (2014)[51]	Perceptual creaminess ratings of sour cream in various conditions: Normal consumption (control); visual exposure only; visual exposure while stirring; stirring while blindfolded; tasting while blindfolded; tasting while	Untrained: Control session: n = 274 Test sessions: n = 100 – 111	Retronasal (C)	12 samples representing the sensory space of commercial sour creams, with fat content ranging between 0 and 33%.	When the retronasal pathway was inhibited using a nose clip, creaminess perception was different from control (where all sensory modalities were used) – perceived creaminess decreased in most cases. Inhibition of retronasal olfaction had the greatest impact on creaminess perception compared to other modalities.	Retronasal olfaction is involved in the perception of creaminess.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	blindfolded and wearing a nose clip; tasting while only wearing a nose clip.					
Le Calvé et al. (2015)[53]	Discrimination ability and fat difference thresholds between various food matrices varying in fat.	Trained; n = 35 - 50	Retronasal (C)	Different food matrices varying in fat, sugar and flavoring content: White sauces containing 7.5 - 32.5% fat; dairy milks containing 0 - 3.8% fat; yogurts containing 0 – 3.5% fat.	White Sauces: The addition of olfactory cues during tasting of flavored and unflavored samples could modulate discrimination ability, depending on the reference fat content and the direction of comparison. Milk: Fat content discrimination was possible only when olfactory and/or vision cues were involved. In sucrose-enriched samples, the involvement of olfaction reduced discrimination ability. The addition of flavors had no effect on fat discrimination. Yoghurt: Fat discrimination was not possible in the absence of olfactory and/or visual cues. The same results were observed in sucrose-enriched samples. In samples with added flavor and/or fruit preparation fat discrimination was possible, but the ability was reduced.	Retronasal fat discrimination ability depends on product type and reference fat content. Retronasal olfaction, along with other sensory systems, is involved in food fat content discrimination.
Fonseca et al. (2015) [62]	Perceptual ratings of flavor intensity and	Trained; n = 10	Orthonasal Retronasal (C)	Sausages varying in fat content: 10, 20 and 30%.	Whereas fat content had no effect on flavor intensity, perceived black pepper odor	Increases in fat content may diminish the volatility of certain

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	black pepper odor.				intensity decreased with increasing fat content.	odor/flavor compounds, in turn modulating their perceived intensity.
Mela (1988)[13]	Perceptual ratings of fat content and creaminess.	Untrained: EXP 1: n = 20 (12F); 27 y (SD n.s.) EXP 2: n = 20 (12F); 23 y (SD n.s.)	Retronasal (C)	Commercially available skim milk (0.5%), whole milk (3.3%), light cream (11.6%), a mixture of light and heavy cream (24%) and heavy cream (36%).	Elimination of olfactory cues had no effect on fat content perception in both experiments.	Fat perception is not driven by olfactory cues. Inhibiting olfactory cues might influence hedonic perception.
Miettinen et al. (2003)[66]	Perceptual intensity ratings of diacetyl and linalool aromas. Time-intensity parameters related to the perception of diacetyl and linalool aromas. Aroma release parameters following headspace sampling.	Trained; n = 12; 28 y (SD n.s.)	Orthonasal Retronasal (C)	Commercial non-fat milk with added rapeseed oil at levels of 0%, 1%, 5%, and 10% (v/v) and flavored with either diacetyl or linalool.	With increasing fat content, linalool was retained in the matrix, while the release of diacetyl was not affected. The addition of 1% of fat to the matrix sufficed to reduce the headspace linalool concentration and orthonasal, but not retronasal, intensity. The perception of linalool aroma in the sample containing most fat lasted a shorter time than in samples containing less fat.	Increases in fat content may diminish the volatility of certain odor/flavor compounds, in turn modulating their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Miettinen et al. (2004)[54]	Perceptual ratings of first impression and after taste-related attribute intensities (free choice profiling). Time-intensity parameters related to the perception of strawberry and linalool flavor. Aroma release parameters following nose space sampling.	Trained; n = 12 (9F); 29.5 y (SD n.s.)	Retronasal (C)	Strawberry-linalool-flavored milks varying in fat content: 0%, 0.5%, and 5%.	With increasing fat content, the maximum perceived intensity of linalool reduced, while the maximum perceived intensity of strawberry flavor increased. Linalool was retained in the matrix as fat content increased. Strawberry aroma of the fattiest sample lingered the longest, but no temporal differences were found in the release of linalool.	Increases in fat content may diminish the volatility of certain flavor compounds, in turn modulating their perception.
Parat-Wilhelms et al. (2005)[67]	Perceptual ratings of coffee-related odor and taste/retronasal odor attributes: buttery, milky, creamy, sour, caramel, aromatic,	Trained; n = 15	Orthonasal Retronasal (C)	Coffee beverages with or without added milk, varying in fat content (0, 3.5 and 7.0%).	An increase in the amount of fat in the milk samples (from 3.5% to 7.0%) led to a decrease in the perceived intensity of coffee-related descriptors. No differences in the milk-related descriptors were found between the two milk samples.	Increases in fat content may diminish the perception of certain flavors.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
	roasted, coffee, butter, burnt.					
Roberts, Pollien, Antille, et al. (2003)[55]	Flavor compound intensities Aroma release parameters following headspace and nose space sampling.	Trained; n = 5	Retronasal (C)	Food matrices varying in fat content with added aroma compounds: Water, skim (0.033% fat), semi-skim (2.7% fat) and whole milk (3.8% fat) containing either beta-damascenone, hexanal, ethyl butyrate, benzaldehyde and 2,3-butanedione.	Fat content influenced perceived intensities of the different compounds in all conditions. Volatility and Intensities of the most lipophilic compounds (beta-damascenone, hexanal and ethyl butyrate) decreased with increasing fat content.	Increases in fat content may diminish the volatility of certain flavor compounds, in turn reducing their perceived intensity.
Running et al. (2017)[43]	Orthonasal (linoleic, oleic) and flavor (stearic) rejection thresholds.	Untrained: Linoleic acid test: n = 75 (49F); 31.1 y (SD n.s.) Oleic acid test: n = 69 (48F); 34.3 y (SD n.s.) Stearic acid test: n = 80 (21F); 32.1 y (SD n.s.)	Orthonasal	Dark chocolate containing different concentrations (0.04 – 2.5%; w/w) of free fatty acids: linoleic, oleic, stearic.	Chocolate containing the polyunsaturated fatty acid (linoleic) was rejected at lower concentrations than the one containing monounsaturated fatty acid (oleic) in both orthonasal and taste conditions. Stearic acid-containing chocolate was not rejected at any concentration.	The addition of fatty acids to a food matrix may unfavorably alter its odor-related qualities. The degree of fatty acid unsaturation influences rejection following orthonasal exposure (the more unsaturated, at

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
						lower concentrations it gets rejected). Saturated fatty acids do not seem to contribute to flavor preference.
Rychlik et al. (2006)[44]	Perceptual ratings of sour, cheese-like and yoghurt-like odor intensities.	Trained; n = 8	Orthonasal	Yogurt (3.5% fat) without (control) or with added short-chain fatty acids: acetic, butanoic, hexanoic, octanoic, decanoic and dodecanoic.	The addition of free fatty acids to fresh yogurt diminished yogurt-like odor intensity, while enhancing intensities of off-flavor-related cheese-like and sour odors.	The addition of fatty acids to a food matrix may unfavorably alter its odor-related qualities.
Schoumacker et al. (2017)[56]	Fat content detection and difference thresholds.	Untrained; n = 40 (18F); 25 – 76 y (mean 55 y); BMI 18.1 – 36.7 (mean 24.2 kg/m^2)	Retronasal (C)	Cottage cheese mixtures containing 1, 2, 3, 4, 5, 6, 7, 7.8, 9, 10 and 11% fat.	Eliminating olfaction using nose clips resulted in higher detection and difference thresholds.	Retronasal olfaction contributes to the perception of fat in food.
Syarifuddin et al. (2016)[68]	Perceptual ratings of several odor, taste and texture attribute intensities, including perceived fat content.	Untrained; n = 31 (21F); 10 – 61 y	Orthonasal Retronasal (C)	Model cheeses varying in fat content (20%, 40%), added aroma (none, sardine, butter), salt (0.5%, 1.5%) and pH at renneting (5.0, 6.2).	Perceptual ratings of fat content texture increased after the addition of a butter aroma. Fat content had no effect on overall odor intensity, regardless of the added aroma.	The addition of fat-related odors can enhance the perception of fat-related texture sensations.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Ventanas et al. (2010)[69]	Perceptual ratings of mushroom and cocoa odor and flavor. Time-intensity parameters related to the perception of mushroom flavor. Aroma release parameters following headspace sampling.	Trained; n = 8 (5F); 25 – 59 y	Orthonasal Retronasal (C)	Mushroom and cocoa-flavored cooked bologna sausages varying in NaCl and fat content (from 4.4 to 22.5% fat).	With increasing fat content, mushroom odor intensity decreased, while that of cocoa odor increased. With increasing fat content, mushroom and cocoa flavor intensities decreased. Duration of mushroom flavor perception decreased with increasing fat content. Fat content influenced the volatility of mushroom but not cocoa flavor-related volatiles.	Increases in fat content may diminish the volatility of certain odor/flavor compounds, in turn modulating their perception.
Weenen et al. (2005)[70]	Perceptual ratings of 60 odor, taste/flavor, mouthfeel, and aftertaste-related attributes.	Trained; n = n.s.	Orthonasal Retronasal (C)	10 commercially available vanilla custards (fat content between <0.5 and 3.5%); Mayonnaises (fat content between 0 and 80%); Warm sauces (mainly starch-based).	The use of nose clips decreased the perception of creamy and fatty mouthfeel in vanilla custards.	Retronasal, cues contribute to the perception of fat-related mouthfeel sensations.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
de Wijk et al. (2003)[61]	Perceptual ratings of 66 descriptive attributes, including 6 odor and 11 flavor-related sensations.	Trained; n = 9 (7F) (22 – 49 y)	Orthonasal Retronasal (C)	16 vanilla-flavored model custards varying in fat content (0 and 4.5%), carrageenan and starch.	Compared to 0% fat custard samples, 4.5% fat ones were rated as more intense in terms of vanilla, caramel and milk odor, and less intense in terms of synthetic odor. 4.5% custard samples were also rated as more intense in terms of vanilla, caramel, milk, cream and fat flavor and less intense in terms of chemical and sickly flavor.	Increases in fat content may alter the perception of certain odor and flavor-related sensory qualities.
Yackinous and Guinard (2000)[57]	Perceptual ratings of fattiness, intensity and liking.	Untrained; n = 106 (66F); 19.3 ± 1.6 y; BMI 21.9 ± 2.7	Retronasal (C)	4 foods varying in fat and flavor concentration: Butter-flavored mashed potatoes: (0.5% fat + 0.08% flavor; 15% fat + 3.75% flavor); Dairy-flavored vanilla pudding (4% fat + 0.05% flavor; 28% fat + 1.75% flavor); Sour cream and onion-flavored potato chips (1% fat + 0.05% flavor; 5% fat + 1.00% flavor);	The use of nose clips reduced the perception of fattiness across all investigated foods. A product-specific effect of flavor concentration on fattiness ratings was observed: The addition of high levels of fatty-type flavors enhanced the perception of fattiness in mashed potatoes and potato chips.	Olfaction contributes to the perception of fat in food. Adding fat-related flavors to foods can enhance the perception of their fattiness.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
				White chocolate-flavored chocolate drink (5.29% fat + 4.50% flavor; 15.87% fat + 7.00% flavor).		
Zhou et al. (2016)[58]	Perceptual ratings of fattiness intensity following exposure to mixtures differing in fat content via various combinations of sensory modalities (taste, taste + odor, taste + mouthfeel, all modalities).	EXP 1: n = 46 (21F); 19 – 53 y; BMI 16.5 – 43.5 EXP 2: n = 51 (35F); 18 – 55 y; BMI 17.0 – 39.3	Retronasal (C)	EXP 1: Five mixtures differing in fat content (0, 7.5, 10, 15 and 20%) produced from non-fat skimmed milk, single cream (19.1% fat) and double cream (50.5% fat). EXP 2: Two more mixtures differing in fat content were added (2.5 and 5.5%).	Perceived fattiness intensity rated from taste + odor (without nose clips) was higher than that from just taste (with nose clips) or all modalities. Perceived fattiness intensity rated from all modalities was higher than just from taste + mouthfeel.	Retronasal olfaction contributes to the perception of fat.

EXP, experiment; **n.s.**, not specified; **n**, sample size (**F**, female); **y**, years of age (mean ± SD / range); **BMI**, body mass index, expressed in kg/m2 as mean ± SD or range); **I**, isolated from taste and mouthfeel (e.g., inhalation); **C**, combined with taste and mouthfeel (e.g., during ingestion);

Risk of Bias Assessment

Risk of bias evaluations of included rodent studies are presented in **Figures S1** and **S2** in Supplementary Material C. No information reported in rodent studies indicated a high bias risk or concerns in any of the evaluated domains. Overall, there was a considerable amount of unclear risk of bias due to lack of explicit reporting, particularly not stating whether the researchers were blinded to treatments.

Risk of bias evaluations of included human studies are presented in **Figures S3** and **S4** in Supplementary Material C. In human studies, there was a moderate amount of unclear risk of bias due to lack of explicit reporting on stimulus presentation orders and participant blinding. Moreover, incomplete outcome reporting (i.e., attrition bias) could not be assessed in several studies due to lack of clarity regarding the inclusion of all participants in the final outcome reports. Not isolating olfaction from effects of potentially confounding sensory modalities, namely taste, mouthfeel and trigeminal sensations was identified as a common source of high bias risk or concerns. Most of the "some concerns" judgements in this domain were given when mouthfeel and taste effects were clearly eliminated, but potential involvement of the trigeminal system could not be ruled out completely, or when orthonasal exposure was combined with non-isolated retronasal exposure.

Discussion

This systematic scoping review aimed at (1) identifying and summarizing relevant evidence on the contribution of olfaction to dietary fat perception and (2) highlighting relevant knowledge gaps. It yields consistent evidence supporting the notion that olfaction is involved in the perception of dietary fat in rodents and humans. Olfaction alone is sufficient for detecting fat and its components (i.e. fatty acids), whether they are present on their own or as part of a complex food matrix. Food fat content plays a considerable role in modulating the perception of various fat- and non-fat-related olfactory qualities, depending on the food matrix and odorant properties. Furthermore, the perception of fat in food can be influenced by the addition of fat-related odors, which may enhance olfactory, as well as non-olfactory fat-related attributes, such as mouthfeel.

Albeit limited, evidence from rodent studies supports the involvement of olfaction in fat perception. With the exception of Boone et al. [31], all studies demonstrated that olfactory cues contribute to the formation of preferences towards fat-related odorants [32-36]. Anosmiation having no effect on preference in the case of Boone et al. [31], and preference partially diminishing following anosmiation in the case of Ramirez [36] and Takeda et al. [34], suggests that preference for fat in rodents is mediated by olfactory, as well as non-olfactory cues. Moreover, anosmiation eliminating preference only for low-fat stimuli, as shown by Takeda et al. [34], points towards olfaction in rodents acting as a signaling mechanism for fat at lower concentrations. Lastly, as suggested by Xavier et al [35], receptor CD36 seems to play a role in detecting fat-related stimuli in rodents.

Findings of human studies utilizing free fatty acids as olfactory stimuli are aligned in suggesting that humans possess the ability of perceiving fatty acids via the olfactory system [15, 42-44, 47, 48, 52, 59]. The interpretation of some findings, however, requires caution. It must be acknowledged that although most studies [15, 42, 47, 52, 59], attempted to isolate olfactory inputs from potentially confounding effects of non-olfactory systems (e.g., vision, gustation, somatosensation), only Bolton and Halpern [59] verified the absence of trigeminal system involvement. They did so by demonstrating that the presentation of fatty acids to the oral cavity resulted in no discrimination from blanks. As the oral cavity is innervated by trigeminal but not olfactory nerve branches [71], this shows that the discrimination observed by Bolton and Halpern [59] was indeed olfaction-based and provides the most convincing evidence of 18-carbon fatty acids being effective olfactory stimuli. The involvement of olfaction in fatty acid perception is further corroborated by the fact that elimination of

retronasal cues considerably decreases the perceived taste intensity of linoleic acid presented to the oral cavity [48].

Clearly, sensations elicited via olfactory exposure to fat in its isolated form (i.e., fatty acids) are sufficient to evoke perception. However, since fat-related odorants are usually perceived in conjunction with a multitude of other stimuli present in a particular food matrix, the more relevant question is whether fat can be smelled when embedded within a food matrix, and if so, how does that influence perception. Various studies on the matter demonstrated that, even when dietary fat is embedded within a food matrix, olfactory cues enable or facilitate its perception. Using solely olfaction, humans are able to distinguish natural oils and oleic acid from non-fat controls [41] and discriminate between fat content differences in dairy milk [37]. The latter has been replicated by our own experiments as well (not included in this review as they were unpublished at the time of search), where we observed that ortho- or retronasal cues in isolation are sufficient to allow for dairy fat content discrimination [72], and identified headspace composition differences underlying the ability [73]. The involvement of olfaction in detecting food fat content differences seems to be particularly relevant in certain food products, as demonstrated by [53], who observed that fat content discrimination in milk and yoghurt was possible only after retronasal cues were added to those of other sensory modalities. They also showed that, despite olfaction not being crucial for discriminating fat content in white sauces, retronasal cues can modulate fat content discrimination, depending on the fat content levels being compared and added sweeteners or flavors. Similarly, elimination of retronasal cues via the use of nose clips has been reported to hinder food fat content discrimination [56] and affect the perception of fat-related qualities [51, 58]. The role of olfaction in perceiving fat embedded within food is further underscored by findings that the addition of fatty acids to a food matrix unfavorably alters odor-related qualities by producing off-odors [44], which may lead to rejection, depending on fatty acid type [43]. All in all, although relatively limited, evidence suggests that olfactory cues are integral for the perception of fat in food [51, 53, 56, 58]. They not only signal its presence [41, 44], but may also provide information about its quantity [37, 72, 73] or type [43]. These findings, in combination with those from studies on fatty acids, indicate that humans possess a functional olfaction-based system for detecting dietary fat in isolation or when part of a food matrix.

Studies investigating the effects of fat content on odor perception found that fat content impacts (i.e. accentuates or diminishes) intensities of various fat and non-fat olfaction-related qualities, in a range of diverse food matrices [37-39, 45, 46, 49, 50, 54, 61-67, 69]. Some

qualities, such as creaminess, seem to be positively related to fat content [38, 39, 63, 64], yet the relationship is not always linear [38, 39]. It has to be acknowledged that fat content alterations do not always modulate olfaction-related qualities, as was the case in Fernandez et al. [40] and Syarifuddin et al. [68]. Olfaction-related quality or intensity shifts following fat content alteration, likely arise from changes in the volatility of odorous compounds contained the food matrix. Various factors, such as lipophilicity and solubility [11, 21], modulate their release, which influences subsequent perception, as demonstrated by several studies included in the current review [39, 45, 46, 49, 50, 54, 55, 62, 65, 66, 69]. In most instances, increases in fat content seem to accentuate the perception of fat-related flavor volatiles, while diminishing that of non-fat-related ones. There are, however, exceptions. For example, as demonstrated by Dadali & Elmaci [39], the release of Hexanoic acid, a fat-related odorant responsible for eliciting fatty, waxy or cheesy qualities, decreased despite an increase in fat content. Further discussion about the intricacies behind factors that influence fat-related volatile release are beyond the scope of the current review - for further information on the matter, see the review on flavor compound and food ingredient interactions and their influence on flavor perception by Guichard [21]. In summary, fat content clearly has an influence on the perception of food-related odors and/or flavors. Olfaction-related perceptual consequences of fat content alteration depend on the food matrix and physio-chemical properties of the odorants in question [21].

Conversely, the perception of fat content-related attributes can be modified by the presence of odors associated with fat. All studies exploring perceptual effects of adding fat-related odors to foods observed an enhancement of fat-related qualities [57, 60, 63, 64, 68]. The enhancement, however, is not limited solely to olfaction-related attributes, but may also affect non-olfactory ones, such as thickness [60], fat-related mouthfeel [68], and texture pleasantness [64]. The enhancing effects of odors on other sensory modalities have also been demonstrated by Ebba et al. [48], observing that the removal of retronasal cues diminished taste intensity of linoleic acid, and Weenen et al. [70], where their absence diminished creamy and fatty mouthfeel. These findings underscore the multi- and cross-modal nature of fat perception [11], wherein the presence of fat-related odors can enhance fat-related mouthfeel and even taste sensations. For additional information on the taste-enhancing potential of odors, see the reviews by Ai and Han [74] and Spence [75]. For insights on fat-related odor-mouthfeel interactions, see the review by Guichard et al. [11].

All human studies included in this review, with the exception of Mela[13], demonstrated that olfaction is involved in the perception of fat or fat-related odors to some degree. Several even found that dietary fat can be perceived using solely olfactory cues [37, 41, 47, 52, 53, 59]. We speculate that the low sample serving temperature (4°C) in the study of Mela et al (11) might have reduced the volatility of fat-related odorants, thus hindering the perception of sensory differences between the fat content of their samples. Since fat perception is multi-modal, the exact contribution of olfaction to the overall flavor percept is difficult to approximate. Not only because of the inherent difficulty in disentangling olfactory inputs from non-olfactory ones, but also due to complex cross-modal interactions occurring between olfaction and other modalities, as discussed above. Nevertheless, findings of the current review clearly show that olfaction has a relevant, even independent, role to play in the perception of dietary fat in humans.

Another relevant point that requires discussion is on the differential role the two olfactory routes might play in fat perception, given that they seem to serve distinct purposes in the context of eating [23, 25]. Few studies included in the current review aimed specifically at comparing the two routes. Nevertheless, some observations can be highlighted. Although free fatty acids can be perceived by either route, retronasal olfaction seems to be less sensitive to their presence [15]. The two routes, however, are relatively comparable in discriminating between specific fatty acid types [59]. As demonstrated by our recent work on the topic [72] the routes are also comparable in discriminating fat content of dairy milk. When it comes to perception of fat-related odors in the context of food, Han et al. [64] compared the two routes and observed differential effects on perception of butter aroma delivered during consumption of cheese, depending on the route of delivery. Specifically, when delivered retronasally, butter aroma enhanced creaminess and butter note intensity, while orthonasally it enhanced texture pleasantness. In contrast, Bult et al. [60] reported enhancements to creaminess and thickness in dairy milk following retronasal, but not orthonasal exposure to cream aroma. In summary, there seem to be differences in fat perception between the olfactory routes. However, to reach reliable conclusions, more research focusing specifically on the distinctions between the two is needed. For an overview of distinctions between ortho- and retronasal olfaction in the context of flavor perception in general, see the review by Goldberg et al. [25].

The current work has identified several other relevant knowledge gaps that require attention in order to further our comprehension of the topic. One of the more relevant blind spots is

the potential impact of olfactory fat perception on subsequent eating behavior. Apart from six studies, whose findings on fat odor-related hedonics [37, 43, 51, 57, 64, 68] merely hint at possible behavioral implications without experimentally determining them, no other study included in this review aimed at investigating the potential behavioral consequences of fat-related odors. It must be acknowledged that much is still unclear about how, and under what circumstances, food odors impact eating behavior. Although it has been established that orthonasal food odors can induce appetite specific for the cued product during the anticipatory phase of eating, findings on their effects on food choice and intake are limited and conflicting [23]. The effect of retronasal exposure to food odors on eating behavior has received even less attention. While there is some evidence of their influence on appetite [76], which does not seem to translate into actual food intake [23], reports on their potential role in food choice are practically non-existent, even more so when it comes to behavioral consequences of fat-related odors. Future studies should therefore aim to fill this important knowledge gap by investigating potential effects of exposure to various ambient and retronasal fat-related odors on appetite, food choice and intake. One of the key prerequisites to this approach is the elucidation of the exact nature of fat-related olfactory chemical signals. Although fatty acids seem to be effective olfactory stimuli on their own [15, 47, 52, 59], most fat-related odors largely originate from volatile compounds bound to dietary fats – which are known to act as volatile compound reservoirs [77-80]. Future research should thus aim to identify effective fat-related olfactory stimuli; extend the knowledge on headspace compositions of different fat-based food matrices, varying in fat content and type; and establish which volatiles underly specific fat-related olfactory qualities (e.g., using gas chromatography-olfactometry or proton transfer reaction-mass spectrometry). Efforts should also be focused towards identifying fat-related olfactory receptors and elucidating their role. Examining the exact role of receptor CD36, which was suggested to be involved in the perception of fat-related odorants in rodents [35], appears a reasonable initial step. Lastly, and similar to previous work for fat taste [81], additional work is required to illuminate factors governing olfactory sensitivity to fat-related odorants. Sensitivity to fat-related odors seems independent of body composition [37, 42, 72], and has been found to be related with gustatory sensitivity to oleic acid [42]. Moreover, our own findings show that olfactory fat content discrimination ability is independent of habitual consumption [72, 73]. However, the evidence base is limited, which warrants further investigation. Future studies should thus aim to replicate initial findings on the topic and seek other potential influences (e.g., genetics).

Lastly, expanding the knowledge on mouthfeel and taste-enhancing qualities of specific fat-related odors might also prove worthwhile, especially for commercial applications. Specifically, the addition of fat-related odors to foods as fat substitutes seems a potentially viable approach for reducing food fat content in various food products, without compromising on their appealing fat-related sensory characteristics and negatively impacting food choice and intake. Considering that fat flavor-related foods seem to contribute most to energy intakes [10], the development of such sensory optimized foods might help maintain existing dietary flavor patterns, while moderating dietary energy density, as suggested by Teo et al. [10] and Forde & de Graaf [82]. Findings on the interactions between olfaction and other sensory modalities involved in fat perception could thus prove instrumental in developing strategies aimed at curbing excess dietary fat intakes.

The current review is the first to summarize findings specific to olfactory fat perception. It yields consistent evidence supportive of olfaction's contribution to the perception of fat, yet conclusions are inherently influenced by the studies selected for inclusion. Our choices of search strings, literature eligibility criteria and their appraisal, and the decision to forgo manual literature searching and sifting through reference lists of included articles are likely to have resulted in the omission of other relevant studies. Publication bias remains a possibility as well. Furthermore, potential bias sources should be considered when interpreting reported findings, particularly those that arise from interactions between olfaction and potentially confounding sensory modalities (see **Figures 3** and **5**), namely taste, mouthfeel and trigeminal sensations. The risks of cross-modal interactions are, however, generally difficult to avoid, mainly due to the inherent complexity in separating retronasal olfaction from other sensations, particularly when it comes to flavor release studies. Even when olfaction is completely isolated from mouthfeel and taste, prying it apart from trigeminal sensations is virtually impossible. Since most odorants can activate the trigeminal system [25], we decided to take a conservative approach when scoring this domain, to raise caution when interpreting results. This resulted in multiple studies receiving "some concerns" bias risk scores. Nevertheless, we deem the methodological quality and validity of findings reported in this review as high. Especially considering that findings from the vast majority of included studies are aligned. Furthermore, the main conclusions of this review were drawn from studies where the bias risk due to potentially confounding effects of other sensory modalities was minimized. Future work on olfactory fat perception should consider

employing control conditions, where possible, wherein the potential involvement of the trigeminal system can be established (as demonstrated by Bolton and Halpern [59]).

Conclusion

Our findings support the notion that olfaction contributes to the perception of dietary fat in rodents and humans. The identified evidence base, although relatively heterogenous and limited in some areas, is consistent in showing that olfaction is involved in detecting, discriminating, and identifying fat and its constituents, when either isolated or embedded within a complex food matrix. When embedded within complex food matrices, fat content and type can modulate the perception of various fat- and non-fat related olfactory qualities, likely by influencing the volatility of odorous compounds. Furthermore, the addition of fat-related odorants to a food matrix may modulate not only its olfactory, but also non-olfactory sensory characteristics, such as mouthfeel. This demonstrates that, although olfaction can act as an independent fat-sensing modality, it also interacts with other sensory systems. Several knowledge gaps have been identified by the current review, including the role of fat-related odors in the choice and intake of various foods; the nature of chemical signals underlying olfactory fat perception; and factors governing olfactory sensitivity to fat-related odors. Replication of included studies and examination of suggested knowledge gaps are warranted given the public health and commercial relevance of this topic. Potentially, the cross-modal nature of olfactory cues in fat perception could be exploited in product reformulation. Specifically, fat-related odorants could be used as dietary fat substitutes, to enhance palatability in various low-fat or reduced-fat food products. The current systematic scoping review is the first of its kind focusing specifically on the olfactory component of fat perception. It provides an extensive overview of the topic, which has the potential of facilitating future research and providing useful information to the food industry.

Authorship contribution statement

Mu Shuo: Conceptualization, Methodology, Investigation, Data curation, Writing – Review & Editing, Project Administration; **Pirc Matjaž:** Conceptualization, Methodology, Investigation, Data curation, Writing – Original Draft, Writing – Review & Editing, Project Administration; **Frissen Gino:** Methodology, Investigation, Data curation; **Stieger Markus:** Conceptualization, Supervision, Writing – Review & Editing, Project Administration; **Boesveldt Sanne:** Conceptualization, Supervision, Writing – Review & Editing, Project Administration

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Supplementary Material A

The following search strings (per online database) were applied to perform the literature search.

Scopus: (((TITLE-ABS-KEY (volatile OR volatiles OR orthonasal OR orthonasally OR retronasal OR retronasally OR aroma OR aromas OR olfaction OR olfactory OR smell OR smells OR smelling OR odorous OR odorant OR odorants OR odorant OR odorants OR odor OR odors OR odor OR odors)) AND (TITLE-ABS-KEY (fat OR fats OR lipid OR lipids OR "fatty acid" OR "fatty acids" OR fatty OR fattiness OR creamy OR creaminess OR greasiness OR greasy OR oiliness OR oily OR butter OR buttery OR butteriness OR rancid OR rancidness OR rancidity))) AND ((TITLE-ABS-KEY ((flavor) AND (creaminess OR creamy))) OR (TITLE-ABS-KEY ("fat flavor")) OR (TITLE (perception OR discriminat* OR preference)) OR (TITLE-ABS-KEY ((discrimination OR absolute OR difference OR flavor OR olfactory OR odor OR smell OR identification OR sensory OR detection) PRE/3 (threshold))) OR (TITLE-ABS-KEY ((preference) W/10 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((perception) W/3 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((identification) W/3 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY (chemosensation OR chemosensory OR chemosensitivity)) OR (TITLE-ABS-KEY ((sense) W/15 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((sensation) W/15 (fat OR lipid OR "fatty acid"))))) AND NOT (cat OR dog OR "honey bee" OR bioelectronic OR electronic OR "bank voles" OR larvae OR larval OR insect OR beetle OR mosquito) AND (LIMIT-TO (LANGUAGE , "English"))

Web of Science: (((TS=(volatile OR volatiles OR orthonasal OR orthonasally OR retronasal OR retronasally OR aroma OR aromas OR olfaction OR olfactory OR smell OR smells OR smelling OR odorous OR odorant OR odorants OR odorant OR odorants OR odor OR odors OR odor OR odors)) AND (TS=(fat OR fats OR lipid OR lipids OR "fatty acid" OR "fatty acids" OR fatty OR fattiness OR creamy OR creaminess OR greasiness OR greasy OR oiliness OR oily OR butter OR buttery OR butteriness OR rancid OR rancidness OR rancidity))) AND ((TS=((flavor OR flavors OR flavor OR flavors) and (creaminess OR creamy))) OR (TS=("fat flavor" OR "fat flavor")) OR (TI=(perception)) OR (TI=(discriminat*)) OR (TI=(preference)) OR (TS=((discrimination OR absolute OR difference OR flavor OR flavors OR flavor OR flavors OR olfactory OR odor OR odors OR odor OR odors OR smell OR identification OR sensory OR detection) NEAR/3 (threshold$)))

OR (TS=((preference$) NEAR/10 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((perception$) NEAR/3 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((identification$) NEAR/3 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=(chemosensation OR chemosensory OR chemosensitivity)) OR (TS=((sense) NEAR/15 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((sensation$) NEAR/15 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids")))) NOT(ALL=(cat OR dog OR "honey bee" OR bioelectronic OR electronic OR "bank voles" OR larvae OR larval OR insect OR beetle OR mosquito))) AND LANGUAGE: (English)
Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Pubmed: ((volatile[Title/Abstract] OR volatiles[Title/Abstract] OR orthonasal[Title/Abstract] OR orthonasally[Title/Abstract] OR retronasal[Title/Abstract] OR retronasally[Title/Abstract] OR aroma[Title/Abstract] OR aromas[Title/Abstract] OR olfaction[Title/Abstract] OR olfactory[Title/Abstract] OR smell[Title/Abstract] OR smells[Title/Abstract] OR smelling[Title/Abstract] OR odorous[Title/Abstract] OR odorant[Title/Abstract] OR odorants[Title/Abstract] OR odorant[Title/Abstract] OR odorants[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract] OR fatty[Title/Abstract] OR fattiness[Title/Abstract] OR creamy[Title/Abstract] OR creaminess[Title/Abstract] OR greasiness[Title/Abstract] OR greasy[Title/Abstract] OR oiliness[Title/Abstract] OR oily[Title/Abstract] OR butter[Title/Abstract] OR buttery[Title/Abstract] OR butteriness[Title/Abstract] OR rancid[Title/Abstract] OR rancidness[Title/Abstract] OR rancidity[Title/Abstract])) AND (((flavor[Title/Abstract] OR flavors[Title/Abstract] OR flavor[Title/Abstract] OR flavors[Title/Abstract]) AND (creaminess[Title/Abstract] OR creamy[Title/Abstract])) OR (“fat flavor”[Title/Abstract] OR “fat flavor”[Title/Abstract]) OR (perception[Title]) OR (discriminat*[Title]) OR (preference[Title]) OR ((discrimination[Title/Abstract] OR absolute[Title/Abstract] OR difference[Title/Abstract] OR flavor[Title/Abstract] OR flavors[Title/Abstract] OR flavor[Title/Abstract] OR flavors[Title/Abstract] OR olfactory[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract] OR smell[Title/Abstract] OR identification[Title/Abstract] OR sensory[Title/Abstract] OR

detection[Title/Abstract]) AND (threshold[Title/Abstract] OR thresholds[Title/Abstract])) OR ((perception[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((identification[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((chemosensation[Title/Abstract] OR chemosensory[Title/Abstract] OR chemosensitivity[Title/Abstract])) OR ((sense[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((sensation[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract]))) NOT (cat[All] OR dog[All] OR “honey bee”[All] OR bioelectronic[All] OR electronic[All] OR “bank voles”[All] OR larvae[All] OR larval[All] OR insect[All] OR beetle[All] OR mosquito[All])

Filter: English language

Supplementary Material B

Reference	Title	Exclusion Reason
Bisulco and Slotnick, 2003	Olfactory discrimination of short chain fatty acids in rats with large bilateral lesions of the olfactory bulbs	Lack of olfactory exposure to suitable fat sources.
Borg and Seubert, 2017	Lipids in Eating and Appetite Regulation – A Neuro-Cognitive Perspective	Review, meta-analysis, book, chapter
Burseg et al., 2009	Flavor perception in biscuits; Correlating sensory properties with composition, aroma release, and texture	No relevant outcomes resulting from olfactory exposure reported.
Calkins and Hodgen, 2007	A fresh look at meat flavor	Review, meta-analysis, book, chapter
de Roos, 1997	How lipids influence food flavor	Unavailable
De Roos, 2005	How lipids influence flavor perception	Review, meta-analysis, book, chapter
Delahunty, 1996	Comparison of dynamic flavor release from hard cheeses and analysis of headspace volatiles from the mouth with flavor perception during consumption	Lack of olfactory exposure to suitable fat sources.
Drake et al., 2010	Impact of fat reduction on flavor and flavor chemistry of Cheddar cheeses	Lack of olfactory exposure to suitable fat sources.
Drake et al., 2010	Influence of fat on flavor and flavor development in cheddar cheese	Review, meta-analysis, book, chapter
Drewnowski, 1997	Why do we like fat?	Review, meta-analysis, book, chapter
Feyzi et al., 2020	A study on aroma release and perception of saffron ice cream using in-vitro and in-vivo approaches	Lack of olfactory exposure to suitable fat sources.
Folkenberg and Martens, 2003	Sensory properties of low fat yoghurts. Part A: Effect of fat content, fermentation culture and addition of non-fat dry milk on the sensory properties of plain yoghurts	Unavailable
Francis and Eldeghaidy, 2015	Imaging methodologies and applications for nutrition research: what can functional MRI offer?	Review, meta-analysis, book, chapter
Frank et al., 2011	Proton transfer reaction mass spectrometry and time intensity perceptual measurement of flavor release from lipid emulsions using trained human subjects	Focusing on volatile compounds without relevant sensory evaluation measures
Fuentes et al., 2013	Effect of intramuscular fat content and serving temperature on temporal sensory perception of sliced and vacuum packaged dry-cured ham	Lack of olfactory exposure to suitable fat sources.

Reference	Title	Exclusion Reason
Garvey et al., 2019	Factors influencing the sensory perception of reformulated baked confectionary products	Review, meta-analysis, book, chapter
Guichard, 2001	Interactions between flavor compounds and food ingredients and their influence on flavor perception	Review, meta-analysis, book, chapter
Guichard and Relkin, 2008	Flavor release from food emulsions varying in their composition in fat and proteins and its effect on flavor perception	Unavailable
Guichard et al., 2013	Flavor release and sensory perception in cheeses	Review, meta-analysis, book, chapter
Guichard et al., 2018	Physiological mechanisms explaining human differences in fat perception and liking in food spreads-a review	Review, meta-analysis, book, chapter
Hatakeyama et al., 2014	Optimising aroma quality in curry sauce products using in vivo aroma release measurements	Focusing on volatile compounds without relevant sensory evaluation measures
Hatchwell, 1996	Implications of Fat on Flavor	Review, meta-analysis, book, chapter
Henneberry et al., 2015	Sensory quality of unheated and heated Mozzarella-style cheeses with different fat, salt and calcium levels	Lack of olfactory exposure to suitable fat sources.
Kanta et al., 2019	Eliciting the Sensory Modalities of Fat Reformulated Yoghurt Ice Cream Using Oligosaccharides	Lack of olfactory exposure to suitable fat sources.
Larue, 1978	Oral cues involved in the rat's selective intake of fats	Unavailable
Le Calvé et al., 2019	Capturing key sensory moments during biscuit consumption: Using TDS to evaluate several concurrent sensory modalities	No relevant outcomes resulting from olfactory exposure reported.
Lim et al., 2010	Effect of flaxseed oil towards physicochemical and sensory characteristic of reduced fat ice creams and its stability in ice creams upon storage	No relevant outcomes resulting from olfactory exposure reported.
McDaniel et al., 1969	Influence of Free Fatty Acids on Sweet Cream Butter Flavor	No relevant outcomes resulting from olfactory exposure reported.
Morquecho-Campos et al., 2020	Smelling our appetite? The influence of food odors on congruent appetite, food preferences and intake	Review, meta-analysis, book, chapter
Neugebauer et al., 2020	Characterization of the Key Odorants in High-Quality Extra Virgin Olive Oils and Certified Off-Flavor Oils to Elucidate Aroma Compounds Causing a Rancid Off-Flavor	Focusing on volatile compounds without relevant sensory evaluation measures

Reference	Title	Exclusion Reason
Nishimura and Saiga, 2019	Umami compounds and fats involved in koku attribute of pork sausages	Review, meta-analysis, book, chapter
Overington et al., 2010	Flavor release and perception in cheese bases	No relevant outcomes resulting from olfactory exposure reported.
Pepino and Mennella, 2014	Cigarette smoking and obesity are associated with decreased fat perception in women	No relevant outcomes resulting from olfactory exposure reported.
Postma et al., 2020	Food preferences and intake in a population of Dutch individuals with self-reported smell loss: An online survey	Not about fat peception
Schlutt et al., 2007	Sensory-directed identification of creaminess-enhancing volatiles and semivolatiles in full-fat cream	Focusing on volatile compounds without relevant sensory evaluation measures
Shepard et al., 2013	Relating sensory and chemical properties of sour cream to consumer acceptance	Not about fat perception
Shiota et al., 2011	Model studies on volatile release from different semisolid fat blends correlated with changes in sensory perception	Not about fat peception
Shojaei et al., 2006	Measurement and manipulation of aroma delivery allows control of perceived fruit flavor in low- And regular-fat milks	Lack of olfactory exposure to suitable fat sources.
Stevenson et al., 2016	Chemosensory Abilities in Consumers of a Western-Style Diet	Lack of olfactory exposure to suitable fat sources.
Strugnell, 1995	Consumer acceptance of fat substitutes	Review, meta-analysis, book, chapter
Summo et al., 2020	Effectiveness of Oat-Hull-Based Ingredient as Fat Replacer to Produce Low Fat Burger with High Beta-Glucans Content	Lack of olfactory exposure to suitable fat sources.
Tamsma et al., 1969	Contribution of Milk Fat to the Flavor of Milk	No relevant outcomes resulting from olfactory exposure reported.
Tepper and Kuang, 1996	Perception of fat in a milk model system using multidimensional scaling	No relevant outcomes resulting from olfactory exposure reported.
Tomaschunas et al., 2013	Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fiber as fat replacers	Lack of olfactory exposure to suitable fat sources.
Tsuruta et al., 1999	The orosensory recognition of long-chain fatty acids in rats	No relevant outcomes resulting from olfactory exposure reported.

Reference	Title	Exclusion Reason
Tucker et al., 2012	Olfactory ability and object memory in three mouse models of varying body weight, metabolic hormones, and adiposity	Not about fat perception
Ulla et al., 2016	Genetic basis of flavor sensitivity and food preferences	Review, meta-analysis, book, chapter
Upadhyay et al., 2020	Perception of creaminess in foods	Review, meta-analysis, book, chapter
Van den Oever, 2006	Fat reduction in foods: Microstructure control of oral texture, taste, and aroma in reduced oil systems	Review, meta-analysis, book, chapter
Yackinous and Guinard, 2001	Relation between PROP taster status and fat perception, touch, and olfaction	No relevant outcomes resulting from olfactory exposure reported.
Yackinous et al., 1999	Internal preference mapping of hedonic ratings for Ranch salad dressings varying in fat and garlic flavor	No relevant outcomes resulting from olfactory exposure reported.

Table S1: Studies excluded during full-text screening

Supplementary Material C

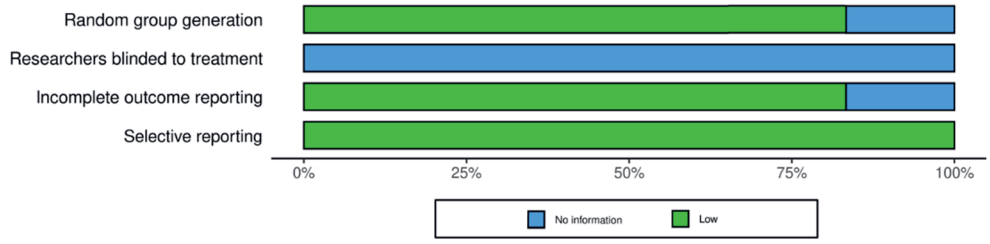

Figure S1. Risk of bias assessment graph for rodent studies.

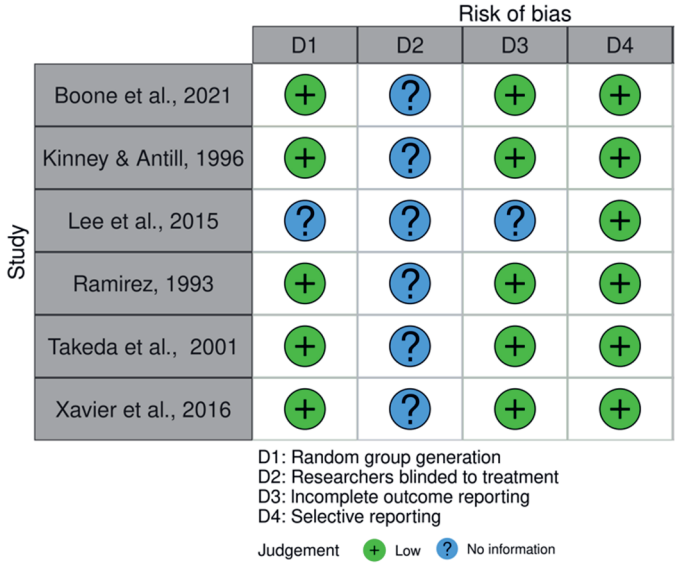

Figure S2. Risk of bias assessment summary table for rodent studies.

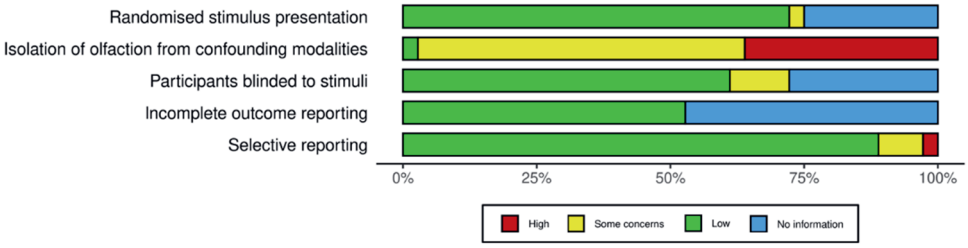

Figure S3. Risk of bias assessment graph for human studies.

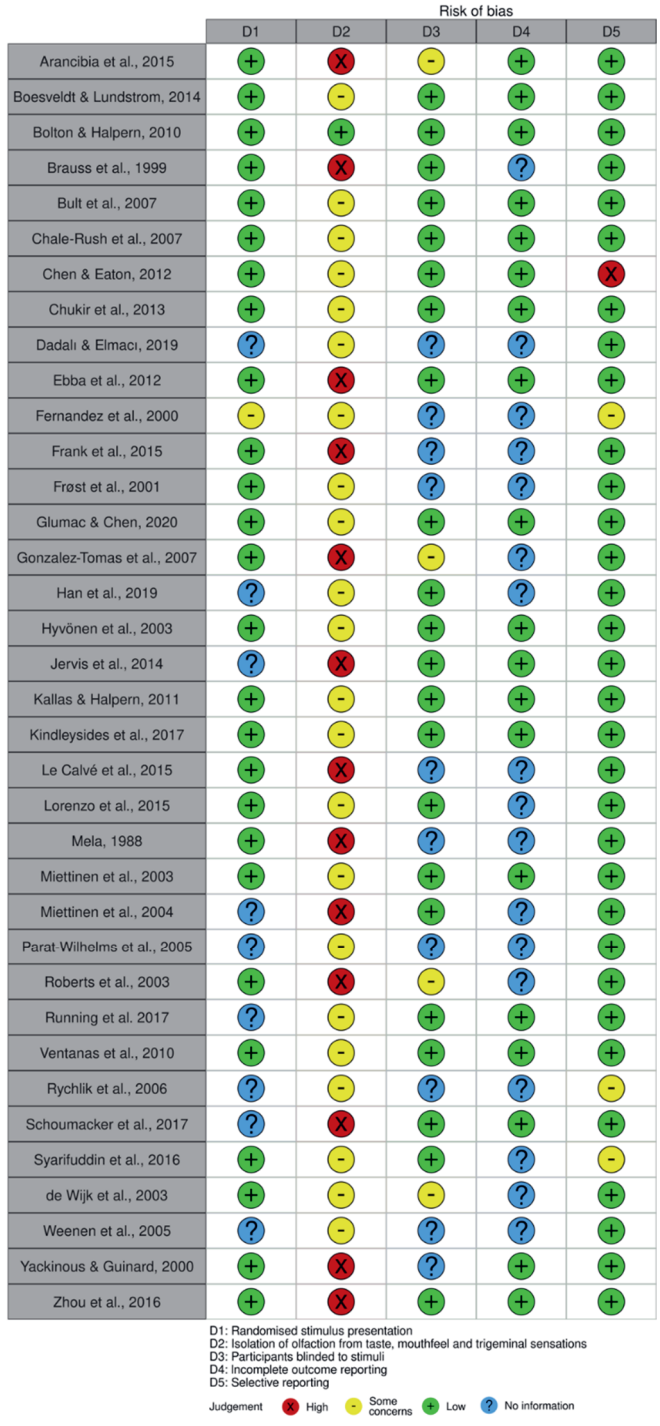

Figure S4. Risk of bias assessment summary table for human studies.


4

Chapter 4

Olfactory discrimination of fat content in milks is facilitated by differences in volatile compound composition rather than odor intensity.

Shuo Mu, Markus Stieger, Sanne Boesveldt.

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Abstract

The mechanisms underlying the ability to olfactorily discriminate fat content in milks remain unknown. In this study, discrimination triangle tests and HS-SPME-GC-MS analyses were performed to (a) compare olfactory fat discrimination capability of pasteurized and UHT milks differing in fat content and (b) to explore how volatile odor compound (VOC) composition of milks influences olfactory fat discrimination capability. We found Differences in fat content can be discriminated through olfaction in pasteurized milks but not in UHT milks. Different VOC compositions were observed in pasteurized milks varying in fat content while similar VOC composition were observed in UHT milks. Principal component analysis of the sensory and chemical data suggests that unique VOCs only found in skim pasteurized milks contribute to its' olfactory fat distinguishability. High concentrations of acetoin and 2-heptanone in all UHT milks may mask odor difference and may lead to the UHT milks being undistinguishable by olfaction.

Introduction

Fat is an important part of our daily diet because of its' high energy density and highly palatable flavor. Many studies demonstrated that multiple senses, including taste, smell, somatosensory and in-mouth tactile sensations are involved in fat perception [1-3]. Fat also impacts odor perception as it can act as a reservoir retaining lipophilic volatile odor compounds (VOC) and repel hydrophilic VOCs. In contrast to numerous studies exploring gustatory perception of fat and fatty acids [2, 4, 5], little is known about the olfactory perception of fats and fatty acids.

Both animals and humans are able to smell fat. Anosmic mice, modeled by deficient CD36 [6], surgery to remove olfactory bulbs [7], ZnSO4 treatment [8] or sectioning olfactory nerve [9], lost their preference for high fat content feeds. Humans can detect fatty acids (linoleic, oleic, and stearic acid), both orthonasally and retronasally [10]. Humans can retronasally detect the presence of fat in milks [11] and discriminate between different concentrations of fat in milk (0, 1.5 and 3.5%) based on orthonasal smell [12]. Descriptive sensory analysis of margarines showed that with increasing fat content, butter and cheese odor intensity increased while creamy odor intensity decreased [13]. These results suggest that smell contributes to fat perception in dairy foods in addition to mouthfeel and taste. However, the mechanisms underlying the ability to detect or discriminate fat content in dairy foods through olfaction are still unknown.

Dietary fats are triglycerides which are not volatile and thus cannot be perceived by olfaction. VOCs present in fat or metabolized from triglycerides rather than the triglycerides themselves have been suggested to act as the odor source facilitating detection of fat by smell in humans. More than 40 different VOCs were identified in the headspace of commercial pasteurized and UHT milks, including hexanal, heptanal, octanal, nonanal and 2-heptanone [14-16]. These VOCs were reported to be metabolized from milk fat and were suggested to contribute to perception of fat-related sensory attributes [17]. It is still unknown whether or how these compounds or other VOCs present in milk underpin humans' ability to discriminate fat content of milks by smell. Thermal processing (pasteurization, high-temperature short time processing, ultra-high temperature processing, etc.) influences VOC composition and odor perception of milks. Many VOCs generated during the thermal processing of milk have been associated with cooked, stale, and sulfurous notes and are considered off-flavors [18]. Dimethyl sulfide, 2-hexanone, 2-heptanone, 2-nonanone, 2-undecanone, 2-methylpropanal, 3-methylbutanal, heptanal, and decanal concentrations were

higher in UHT milks than in raw and pasteurized milks. These VOCs could therefore be important contributors to the off flavor of UHT milk [19]. To summarize, the volatile composition of milks is influenced by the processing conditions, and it is unknown whether olfactory fat perception and discrimination ability of milks is influenced by thermal processing.

The aims of this study were (a) to investigate olfactory fat discrimination capability of pasteurized and UHT milks differing in fat content (0.5, 1.5, 3.5% fat) and (b) to explore how VOC composition of milks influences olfactory fat discrimination capability. We hypothesize that the ability to discriminate fat content of milks by smell is influenced by the VOC composition. Our findings may contribute to a better understanding of the mechanisms underlying the capability of humans to detect and discriminate fat content in foods.

Materials and methods

Three commercially available pasteurized milks (De Zaanse Hoeve, 0.5, 1.5 and 3.5% fat) and three commercially available UHT milks (AH Houdbare, 0.5, 1.5 and 3.5% fat) were purchased from a local supermarket (Albert Heijn, Wageningen, The Netherlands). Pasteurized and UHT milks with low (0.5%), medium (1.5%) and full fat content (3.5%) were labeled as P0.5, P1.5, P3.5, U0.5, U1.5 and U3.5. Ethyl pentanoate (CAS-No. 539-82-2) as internal standard was purchased from Merck KGaA (Darmstadt, Germany).

Participants

N=33 participants (mean age 23.1±4.4 years; 11 men; body mass index (BMI) between 18.5 and 27.5 kg/m^2) recruited from the Wageningen area participated in the study. All participants were non-smokers, not lactose intolerant, not pregnant, not breast-feeding, not currently on a calorie-restricted diet or have been in the past 2 months, and had a normal functioning sense of smell (a score of >12 as determined by the 16-item odor identification part of the Sniffing' Sticks [20]. Participants were asked not to eat or drink anything other than water one hour prior to testing, nor wear any scented products on the day of testing. Demographic information (age, gender and BMI were collected through an online questionnaire. All participants provided written informed consent prior to participation and were paid €25 after finishing all sessions. The Medical Ethics Review Committee of Wageningen University (METC-WU) approved the study (NL51747.081.14; ABR 51747).

Study procedure.

All sensory assessments were conducted in individual sensory booths at Wageningen University and Research, The Netherlands. The sensory booths were and well-ventilated to ensure an odorless environment. Participants attended three sessions of 30-50 min. In the first session a Dairy Food Frequency Questionnaire was filled in and perceptual rating tests were performed. In the second and third session, triangle discrimination tests were performed. In the second session, triangle discrimination tests between pasteurized milks or UHT milks with different fat content were performed. In the third session, triangle discrimination tests between pasteurized milks and UHT milks with same fat content were performed.

Perceptual rating test. Participants (N=33) rated the perceived intensity and pleasantness of each milk sample on a 100-unit visual analog scale. The intensity scale ranged from "not perceivable at all" on the left to "extremely intense" on the right. The pleasantness scale

ranged from “not pleasant at all” to “extremely pleasant”. Milks (30 g) were served in 50 mL amber glass vials with lid and labeled with three random digit codes. Participants were asked to remove the lid, smell the sample and rate the perceived odor intensity and odor pleasantness. The presentation order of samples was randomized. Each trial consisted of one sample and the interval between trials was at least 1 minute.

Triangle discrimination test. Participants (N=33) were presented with a series of odor triangle discrimination tests. Each trial consisted of three vials labeled with three random numbers, two vials contained the same milk sample, and one contained a different one. Participants were asked to smell each vial once following the presentation order and choose the odd one out. Presentation order of the sample triplets was randomized in each session. All samples were presented in 50 ml amber glass vials, containing a total of 30 g of milk. Participants duplicated the assessment by performing sample triplets AAB and ABB, so that N=66 observations were obtained per triangle discrimination test. In total, 18 discrimination tests were performed by each participant during the two sessions (12 in session 2 and 6 in session 3), using an inter-trial interval of approximately 1 min between each triplet.

Headspace Solid Phase Micro Extraction Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS)

The headspace of milks was extracted using a SPME fiber (50/30 μm, CAR/PDMS, Supelco, Bellefonte, USA). Ethyl pentanoate (internal standard) was dissolved in distilled water to prepare the internal standard solution at a concentration of 87 μg/mL. 50 μL internal standard solution together with 5 mL milk were added to a sample glass vial. The extraction mode was automatic. The vial was placed in the incubator for 30 min at room temperature. The SPME fiber was then automatically inserted into the headspace of the vial for 30 min at room temperature to adsorb volatiles. After extraction, the loaded SPME fiber was immediately injected into the injection port of the GC-MS to desorb for 5 min at 230 °C.

An GC-MS instrument (Thermo Fisher Scientific) equipped with EI source was used. Samples were analyzed on a Stabilwax DA capillary column (30 m × 0.25 mm ID × 0.25 μm). Helium (99.999% purity) was used as carrier gas, and the column flow rate was set at 1.20 mL/min (29.32 cm/s) in splitless injection mode. The injector temperature was 230 °C. The initial oven temperature was 40 °C and was maintained for 2 min. The temperature was then increased to 180 °C at 3 °C/min, held for 2 min, increased to 220 °C at 5 °C/min, and finally steadied for 3 min. The mass spectrometry detection conditions were as follows: mass detector temperature 150 °C; electron impact mode 70 eV; ion source temperature 240 °C;

transmission line temperature 250 °C; and mass range m/z 40–450 in full scan mode. All samples were measured in triplicate.

The chromatograms were recorded and analyzed using Thermo Scientific Dionex Chromeleon® 7.2 chromatography data system (CDS) software. Volatile odor compounds (VOC) were identified by comparing their mass spectra and retention indices with the National Institute of Standards and Technology (NIST) database. A semi-quantitative method was used in this study. The concentration of each VOC was calculated by comparing its peak area with the internal standard. Each sample was measured in triplicate, and the mean value was applied for further analysis. Odor thresholds and odor quality of all VOCs were obtained from VCF Volatile Compounds in Food online database (https://www.vcf-online.nl).

Statistical data analysis

SPSS 25.0 (SPSS Inc., Chicago, IL) was used to perform statistical data analysis. A significance level of p = 0.05 was chosen. Corresponding triplets of the triangle discrimination tests (e.g., AAB and ABB) were considered duplicate measures resulting in six comparisons, each with 66 assessments (N=33 participants assessing triangles in duplicate). The number of correct trials was summed up and the significance level (*p*) was calculated using binominal tests. To explore whether demographic characteristics (age, gender, BMI) or dairy consumption habits (DCF, DI, DFI) influence the ability to discriminate fat content through orthonasal olfaction, binary logistic regression was applied to each sample comparison. The number of summed up trials of each sample comparison was set as dependent factor, while gender, age, BMI, DCF, DI and DFI were set as covariates (gender was categorized as indicator). One-way ANOVA followed by Duncan test was used to analyze differences in perceptual rating scores. Principal component analysis (PCA) and partial least-squares regression (PLSR) were performed using XLSTAT 2019 (Addinsoft, New York, NY). To investigate the similarity of VOC compositions between samples, PCA was performed based on the relative concentrations of all VOCs in samples. To determine whether concentration of VOCs were higher than thresholds, Odor Activity Values (OAV)were calculated as the ratio between relative concentration of the compound and its' detection threshold. OAVs of VOCs were calculated based on Table 1 and detailed OAV data is shown in **Table S2** in the supplementary data. Only the VOCs of which concentrations were higher than their thresholds were included in the following analysis. To explore the

key VOC responsible for perceived intensity and pleasantness of milks, PLSR was performed based on the OAV data and perceptual rating scores, where x variables were the OAV in samples and y variables were rating scores for perceived intensity and pleasantness. To investigate VOCs that influence the olfactory judgement of fat content, a PCA was performed on OAV data and triangle test results. The OAV difference in each sample comparison (e.g., OAV difference of acetoin between sample A and sample B is the absolute value of acetoin OAV in sample B minus that in sample A) and the number of correct responses of that sample comparison was used in PCA.

Result

Olfactory discrimination ability of pasteurized and UHT milks differing in fat content

Figure 1 shows the results of the triangle tests for pasteurized and UHT milk. Pasteurized milks differing in fat content can be distinguished based on orthonasal smell ($p = 0.0270$ for 1.5-3.5% and $p = 0.0037$ for 0.5-1.5% and 0.5-3.5% comparisons). In contrast, participants are not able to discriminate the smell between UHT milks differing in fat content ($p = 0.1797$ for 0.5-1.5% and $p = 0.1209$ for 1.5-3.5% and 0.5-3.5% comparisons). Participants are able to orthonasally smell the difference between pasteurized and UHT milks at all three fat levels ($p < 0.001$ for comparisons between UHT and pasteurized milks at 0.5, 1.5 and 3.5% fat content).

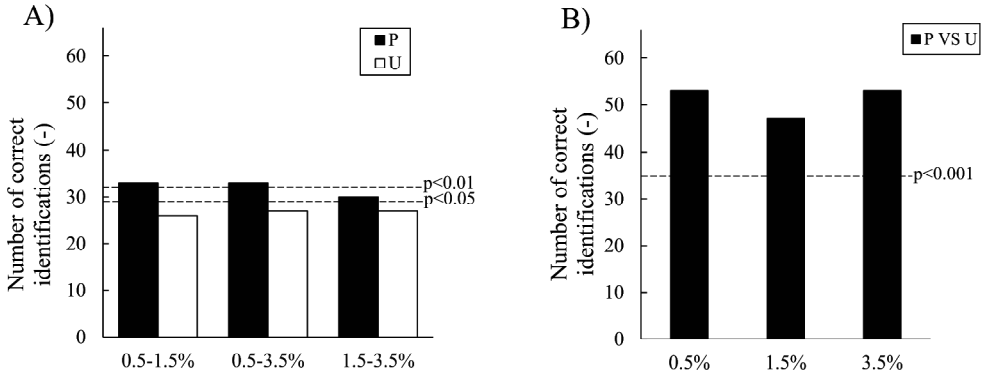

Figure 1 Total number of correct answers for each triangle discrimination test. A) olfactory discrimination between 0.5 vs 1.5%, 0.5 vs 3.5%, and, 1.5 vs 3.5% fat content in pasteurized and UHT milks. B) olfactory discrimination between pasteurized and UHT milk with 0.5%, 1.5%, and 3.5% fat content. Dotted lines indicate the minimum number of correct identifications required at different significance levels (N = 66, 33 participants in duplicate). P stands for pasteurized milk; U stands for UHT milks.

Odor intensity and odor pleasantness of pasteurized and UHT milks differing in fat content

Figure 2 shows the perceived odor intensity and odor pleasantness ratings for all milks. No significant difference ($p > 0.05$) in odor intensity is observed between UHT or pasteurized differing in fat content. Significant higher ($p < 0.05$) ratings of odor intensity are found for UHT milks compared with pasteurized milks at each fat content. Neither fat content nor thermal processing (pasteurized vs UHT) have a significant impact on perceived odor pleasantness for all milk samples ($p > 0.05$). The result of binary logistic regressions (**Table**

S1, in supplementary data) indicates that gender, age, BMI, Dairy Consumption Frequency (DCF), Dairy Intake (DI), and Dairy Fat Intake (DFI) did not significantly influence the olfactory discrimination ability of milks differing in fat content.

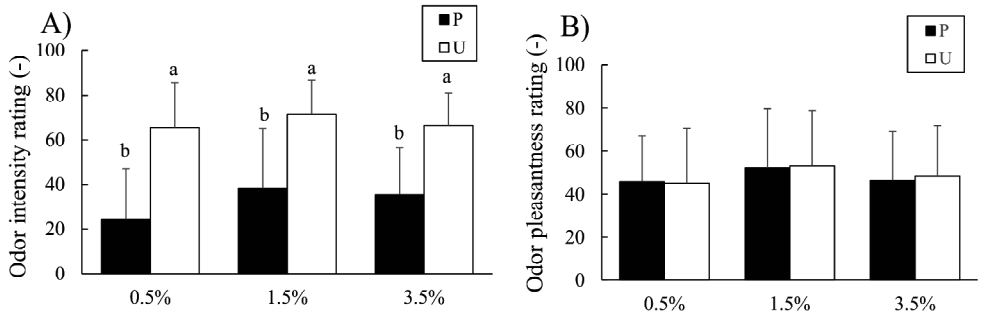

Figure 2 Odor intensity (A) and odor pleasantness (B) ratings of milk samples differing in fat content measured on 100mm VAS. P denotes pasteurized milk; U denotes UHT milks. Different letters above bars denote significant differences between means ($p < 0.05$). Error bars denote standard deviation.

Compositions of VOCs in pasteurized and UHT milks differing in fat content

The VOCs detected in the pasteurized and UHT milks differing in fat content are shown in **Table 1**. Ethyl butyrate, 2-undecanone, 1-decanol, 2-methyl-1-butanol, 2-methyl-propanal, 2-methyl-butanal were only found in P0.5 whereas butanal and hexanal were only found in P3.5.

PCA was performed on the HS-SPME- GC-MS data and the results are shown in **Figure 3**. The first and second principal component explain 67.34% (34.11% for F1 and 33.23 for F2) of the total variance in the HS-SPME- GC-MS data. The three UHT milks (U0.5, U1.5, and U3.5) are positioned close to each other in the lower left quadrant with overlapping confidence ellipses, indicating similar VOC compositions of UHT milks differing in fat content. Their VOC composition is characterized mainly by hexanal, butanal, dimethyl sulfone and 2-butanone. In contrast, the three pasteurized milks differing in fat content (P0.5, P1.5, and P3.5) are separately positioned across the other three quadrants. The distance in the PCA between the three pasteurized milks is considerably larger than the distance between the three UHT milks. The confidence ellipse of the pasteurized milk P0.5 does not overlap with P1.5 and P3.5 and is located far away from P1.5 and P3.5, indicating that the VOC composition of P0.5 differs strongly from the VOC composition of P1.5 and P3.5. The confidence ellipses of P1.5 and P3.5 overlap only slightly suggesting that the composition differs significantly between P1.5 and P3.5 for several VOCs (2-pentanone, 2-butanone, 2-

nonanone, 2-heptanone, butanoic acid, 1-hexanol, hexanoic acid, octanoic acid, and 3-methyl-butanoic acid; see Table 1) and for only few VOCs (acetoin, 1-pentanol, and ethanol; see **Table 1**) no significant difference are observed. Overall, P0.5 is characterized by ethyl butyrate, 2-methy-butanal, 1-decanol, 2-methy-1-butanol, and 2-undecanone; P1.5 is characterized by higher concentration of 2-butanone and 1-hexanol; and P3.5 is characterized by higher concentration of 2-pentanone, 2-nonanone, 2-heptanone, butanoic acid, hexanoic acid, 1-octanol, octanoic acid, 3-methyl-butanoic acid.

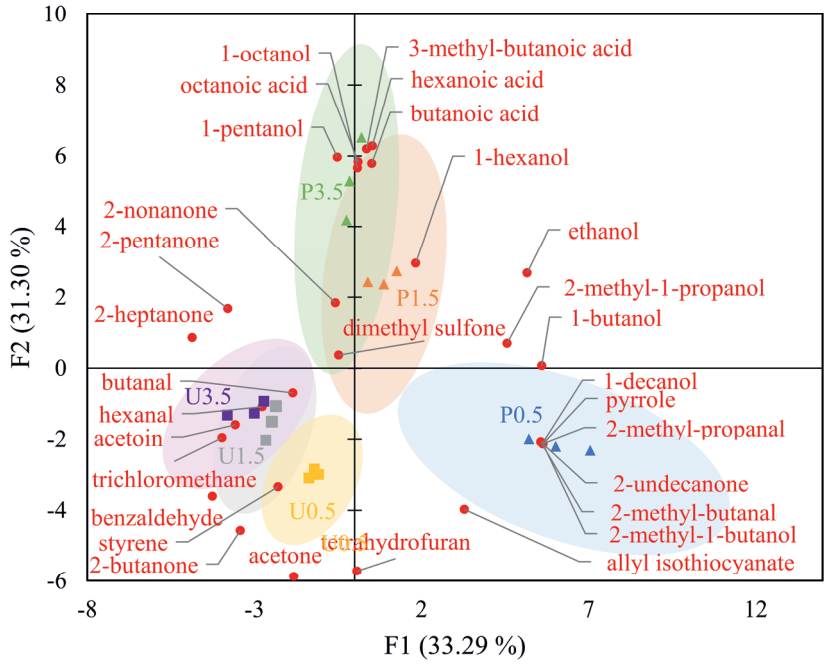

Figure 3 PCA of VOCs in pasteurized and UHT milks differing in fat content. P0.5 denotes pasteurized milk with 0.5% fat content; P1.5 denotes pasteurized milk with 1.5% fat content, P3.5 denotes pasteurized milk with 3.5% fat content; U0.5 denotes UHT milk with 0.5% fat content; U1.5 denotes UHT milk with 1.5% fat content; U3.5 denotes UHT milk with 3.5% fat content. The confidence ellipses show 95% confidence intervals.

Table 1 Relative abundance, odor threshold and odor quality of VOCs in pasteurized (P) and UHT (U) milks differing in fat content (0.5; 1.5 and 3.5%).

Compound	Concentration (mg/L)						Threshold (mg/L)	Odor quality
	P0.5	P1.5	P3.5	U0.5	U1.5	U3.5		
tetrahydrofuran	9.398 ± 1.354[a]	2.520 ± 0.857[c]	1.469 ± 0.685[c]	12.997 ± 2.354[a]	7.770 ± 1.895[b]	7.977 ± 2.548[b]	OW, d: 10.200	Ether-like
2-pentanone	1.733 ± 0.687[c]	2.540 ± 0.897[c]	7.247 ± 1.875[a]	2.205 ± 0.895[c]	8.317 ± 2.547[a]	5.335 ± 1.547[b]	OW, d: 0.350	burnt plastic, ether, fruit, kerosine, pungent
acetoin	0.184 ± 0.058[c]	0.250 ± 0.011[c]	0.202 ± 0.059[c]	0.375± 0.112[c]	0.888 ± 0.124[b]	3.592 ± 0.657[a]	OW, d: 0.014	butter, cream, green pepper, rancid, sweat
2-butanone	7.651 ± 1.356[d]	13.320 ± 2.548[c]	0.757 ± 0.157e	34.436 ± 5.658[a]	27.391 ± 5.223[ab]	20.401 ± 3.875[b]	OW, d: 5.800 OA, r: 16.000	ether, fragant, fruit, pleasant, sweet
2-nonanone	0.514 ± 0.054[b]	0.167 ± 0.034[c]	0.695 ± 0.085[a]	0.200 ± 0.036[c]	0.645 ± 0.087[a]	0.673 ±0.105[a]	OA, d: 0.032	fragant, fruit, hot milk, pleasant
dimethyl sulfone	0.193 ± 0.023[b]	0.107 ± 0.018[c]	0.113 ± 0.068[c]	-	0.253 ± 0.025[a]	0.243 ± 0.008[a]	N	burnt, sulfur/flavorless
1-pentanol	0.150 ± 0.008[b]	0.409 ± 0.096[a]	0.558 ± 0.186[a]	-	0.202 ± 0.013[c]	0.320 ± 0.045[b]	OW, d: 0.153	balsamic, fruit, green, medicine, yeast
acetone	16.015 ± 2.548[c]	-	-	26.979 ± 3.548[a]	22.506 ± 4.574[ab]	20.991 ± 3.578[b]	OW, d: 48.000 OW, r: 78.000	chemical, ether, nauseating, pungent
2-heptanone	-	0.289 ± 0.015[b]	5.960 ± 1.589[a]	3.658 ± 0.897[b]	4.845 ± 0.875[ab]	6.802 ± 1.857[a]	OW, d: 0.023	blue cheese, cinnamon, green, nut

Compound	Concentration (mg/L)						Threshold (mg/L)	Odor quality
	P0.5	P1.5	P3.5	U0.5	U1.5	U3.5		
benzaldehyde	-	-	-	0.399 ± 0.045[a]	0.403 ± 0.035[a]	0.261 ± 0.109[a]	OA, r: 4.100	bitter almond, burnt sugar, cherry, malt, roasted pepper
butanoic acid	0.030 ± 0.007[c]	0.081 ± 0.015[b]	0.440 ± 0.099[a]	-	-	-	OW, d: 0.00094 OW, r: 0.0160	butter, cheese, rancid, sour, sweat
1-hexanol	0.135 ± 0.006[b]	0.793 ± 0.048[a]	0.110 ± 0.004[b]	-	-	-	OW, d: 0.040 OW, r: 0.380	flower, fruit, green, herb, wood
hexanoic acid	-	3.407 ± 0.987[b]	4.452 ± 0.753[a]	-	-	-	OW, d: 0.048	acid, cheese, goat, pungent, rancid
ethanol	4.101± 1.834[a]	2.881 ± 1.984[a]	3.184 ± 1.745[a]	-	-	-	OW, d: 0.620 OW, r: 0.665	alcohol, floral, ripe apple, sweet
allyl isothiocyanate	0.184 ± 0.015[a]	-	-	0.201 ± 0.036[a]	-	-	OW, d: 0.375	garlic, mustard, pungent, sulfur
styrene	-	-	-	0.180 ± 0.094[a]	0.114 ± 0.067[a]	-	OA, d: 26.400	balsamic, gasoline, plastic, rubber, solvent
trichloromethane	-	-	-	-	1.018 ± 0.057[b]	1.690 ± 0.82[a]	OW, d: 3700	misleadingly-pleasant ethereal odor, leading to olfactory fatigue
1-octanol	-	0.376 ± 0.035[b]	2.921 ± 0.985[a]	-	-	-	OW, d: 0.023 OW, r: 0.500	detergent, fat, jasmine, lemon, metal

Compound	Concentration (mg/L)						Threshold (mg/L)	Odor quality
	P0.5	P1.5	P3.5	U0.5	U1.5	U3.5		
2-methyl-1-propanol	1.001 ± 0.187[a]	1.308 ± 0.298[a]	-	-	-	-	OA, d: 2.000 OA, r: 5.400	apple, cocoa, fusel, malt
1-butanol	0.628 ± 0.095[a]	-	0.521 ± 0.185[a]	-	-	-	OA, r: 45.000	alcohol, fruit, medicine, phenol, solvent
octanoic acid	-	0.776 ± 0.047[b]	4.105 ± 0.982[a]	-	-	-	OA, d: 0.0051	acid, cheese, fat, rancid, sweat
3-methyl-butanoic acid	-	0.113 ± 0.045[b]	0.255 ± 0.087[a]	-	-	-	OA: 0.001-0.002	cheese, fecal, putrid fruit, rancid, sweat
butanal	-	-	-	-	-	0.129 ± 0.032	OW, d: 0.100	banana, green, pungent
hexanal	-	-	-	-	-	0.127 ± 0.084	OW, d: 0.230	fresh, fruit, grass, green, oil
pyrrole	0.255 ± 0.009	-	-	-	-	-	OW, d: 20.000	nut, sweet
2-undecanone	0.232 ± 0.008	-	-	-	-	-	OW, d: 0.0055	fresh, green, orange, pineapple, rose
1-decanol	0.320 ± 0.085	-	-	-	-	-	OW, r: 19.400	fat, oil, plastic
2-methyl-1-butanol	0.257 ± 0.007	-	-	-	-	-	OW, d: 0.140 OW, r: 0.830	banana, fusel oil, green, malt, medicine
2-methyl-propanal	0.454 ± 0.015	-	-	-	-	-	OW, d: 0.140 OW, r: 0.410	caramel, cocoa, green, malt, nut

Compound	Concentration (mg/L)						Threshold (mg/L)	Odor quality
	P0.5	P1.5	P3.5	U0.5	U1.5	U3.5		
2-methyl-butanal	0.028 ± 0.009	-	-	-	-	-	OW, d: 0.0015 OW, r: 0.0044	almond, cocoa, fermented, hazelnut, malt

Results are expressed as mean ± SD (n = 3). a-d: Mean values in the same row with different superscripts differ significantly ($p < 0.05$) per compound; OA: Odor threshold in air (mg/m^3); OW: Odor threshold in water (mg/L); d: detection threshold; r: recognition threshold; -: compound was not detected. Bold font means the measured concentration is higher than its' detected threshold. The odor detection threshold and odor quality were obtained from references listed in VCF online (www.vcf-online.nl).

VOC compositions responsible for olfactory discrimination ability of pasteurized and UHT milks differing in fat content

To explore volatile compound compositions responsible for the olfactory discrimination ability of pasteurized and UHT milks differing in fat content, PLS-DA was performed among olfactory discrimination ability of each sample comparison and absolute difference of OAVs of each sample comparison. The results are shown in Fig. 4. Dimension 1 explains 52.5% of the predictor (volatile compounds) and 87.0% of the response (olfactory discrimination ability), while dimension 2 explains 14.3% of predictor variables and 10.4% of response variance. Olfactory "distinguishable" milks are located on the negative side of dimension 1 whereas olfactory "indistinguishable" milks are located on the positive side of dimension 1. The VIPs of 1-hexanol, 3-methyl-butanoic acid, hexanoic acid, ethanol, octanoic acid, acetoin, 1-octanol, butanoic acid, 2-undecanone, 2-methyl-1-butanol, 2-methyl-propanal, 2-methyl-butanal, and 2-heptanone were > 1 (Table S5 in supplementary material), indicating that these volatile compounds contribute to the olfactory discrimination of milks differing in fat content. All UHT milk comparisons are distributed together on the positive side of dimension 1, indicating that UHT milks have similar absolute differences of OAVs. However, the pasteurized milks are diffusely distributed on the negative side of dimension 1, indicating that different volatile compound compositions contribute to the olfactory discrimination of these milks. Specifically, 2-undecanone, 2-methyl-1-butanol, 2-methyl-propanal, 2-methyl-butanal, hexanoic acid, butanoic acid, 1-octanol, octanoic acid and ethanol contribute to the ability to discriminate between P0.5 and the other two pasteurized milks (P1.5 and P3.5). 1-hexanol specifically contributes to the ability to discriminate between P1.5 and P3.5. Acetoin and 2-heptanone are observed to contribute to the indistinguishable odor of UHT milk comparisons.

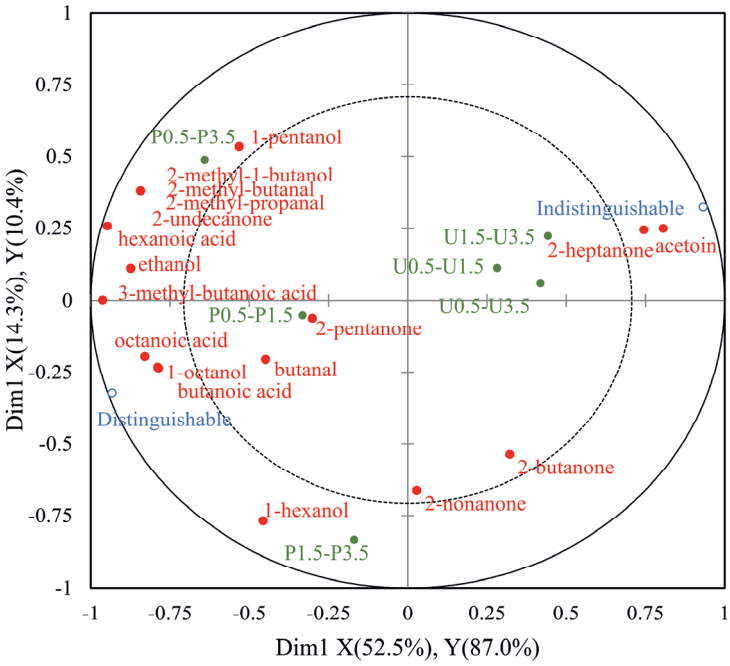

Figure 4 PLS-DA of VOC compositions and triangle test results. The PLS-DA was performed among olfactory distinguishability of each sample comparison and absolute difference of volatile compound content in each sample comparison. P0.5 denotes pasteurized milk with 0.5% fat content; P1.5 denotes pasteurized milk with 1.5% fat content, P3.5 denotes pasteurized milk with 3.5% fat content; U0.5 denotes UHT milk with 0.5% fat content; U1.5 denotes UHT milk with 1.5% fat content; U3.5 denotes UHT milk with 3.5% fat content. Blue dots represent olfactory distinguishability, green dots represent sample comparisons, and red dots represent volatile compounds.

VOC compositions responsible for odor intensity and pleasantness rating of pasteurized and UHT milks differing in fat content

Figure 5 shows the result of PLSR correlating the VOC composition with odor intensity and odor pleasantness ratings. Dimension 1 explains 52.7% of the predictor (key odor compounds) and 32.2% of the response (sensory perception), while dimension 2 explains 16.6% of predictor variables and 26.5% of response variance. Intensity ratings load strongly positive on dimension 1. All pasteurized milks are located on the negative side of dimension 1 while all UHT milks and odor intensity are located on the positive side of dimension 1. This indicates that odor intensity is more strongly associated with UHT milks compared with pasteurized milks, in line with our result that UHT milks have higher odor intensity ratings (**Figure 2B**).

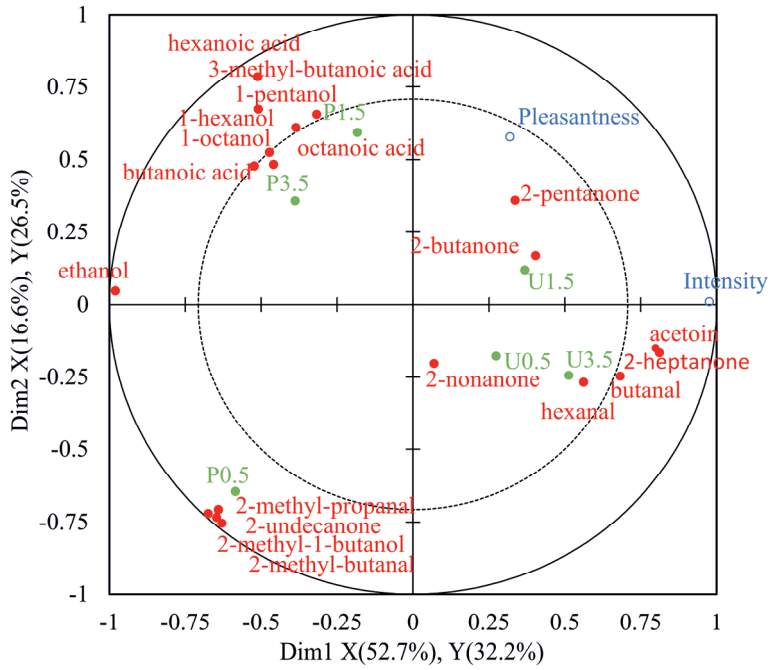

Figure 5. PLRS correlation matrix of OAV results and perceptual rating scores of pasteurized and UHT milks differing in fat content. P denotes pasteurized milk, U denotes UHT milk and numbers indicate fat content (0.5, 1.5 and 3.5%). Blue dots represent sensory attributes, green dots represent milk samples, and red dots represent VOCs.

Discussion

The study aimed (a) to explore olfactory fat discrimination capability of pasteurized and UHT milks differing in fat content (0.5, 1.5, and 3.5% fat content) and (b) to explore how volatile odor compound (VOC) composition of milks influences olfactory fat discrimination capability. Olfactory discrimination tests, odor intensity and pleasantness ratings and HS-SPME-GC-MS analysis were combined to achieve these goals. We found that participants were able to discriminate the smell of all fat levels for pasteurized but not for UHT milks, and that this ability was not related to any demographic characteristics or dairy consumption frequency. Different VOC compositions were observed among pasteurized milks with various fat content, while those of UHT milks with various fat content were similar. PCA suggests that olfactory discrimination between pasteurized milks is facilitated by differences in VOC compositions and not by perceived odor intensity.

Olfactory fat discrimination capability of pasteurized and UHT milks

To the best of our knowledge, this is the first study that compared olfactory fat discrimination in pasteurized and UHT milks. Boesveldt and Lundstrom [12] previously reported that humans can orthonasally detect difference in fat content of reconstituted milks. Pirc et al. [21] confirmed these findings in manipulated milks differing in fat content (milks prepared with different mixing ratios of either milk powder and water or cream and skim milk) and reported that humans are also capable of discriminating fat content of milks solely based on retronasal olfaction. Our study extended these findings towards commercially available milks differing in fat content. We found that the thermal processing of milks affects the olfactory discrimination ability as it may affect VOC compositions. Pasteurized milks cover 70% of the global milk market share and more than 90% of the countries in the world mainly consume pasteurized milk. Thus, our findings suggest that consumers can, based on smell only, discriminate the milks that are consumed globally the most. We observed that the ability to olfactorily discriminate between milks differing in fat content was not affected by demographics nor dairy consumption habits, which is in line with previous studies [12, 21]. However, for non-dairy foods, Kindleysides et al. [3] observed that consumers who have higher intake of seeds, nuts and nut spreads are more sensitive to detect the smell of oleic acid. Thus, the type of fat and type of food should be taken into consideration when exploring effects of dietary habits on olfactory fat discrimination. Looking into the consumption of

overall fat rather than certain types of fat or foods could help to reveal potential relationships between dietary habits and fat discrimination capability by smell.

Why pasteurized milks with different fat content can be distinguished through smell but UHT milks not

We explored the underlying reason why pasteurized milks are olfactory distinguishable depending on fat content while UHT milks are not. About 98% of the fatty acids in milk are in triacylglycerol form and not volatile, thus in order to contribute to the odor, they must be transformed into volatile compounds, either by lipase present in raw milks or by (thermal) processing of the milks. Many of the key aromas typically found in milk are generated from short- and medium-chain fatty acids present in milk fat. The unsaturated fatty acids are transformed into aldehydes, acids, and alcohols, whereas the free fatty acids are transformed into esters [22]. The majority of VOCs detected in pasteurized and UHT milks in our study have been previously reported [23-25]. Our results showed that the concentration of VOCs in pasteurized and UHT milks did not proportionally increase with increasing fat content: for instance, for 2-butanone, the highest concentration was found in the UHT milk with the lowest fat content (U0.5). This is in line with other studies [14, 16] and can be explained by the fact that the partition of volatile compounds between vapor and continuous phase is not only governed by the vapor pressure but also by the activity coefficient. Volatiles may be found in higher concentration in the headspace over low-fat matrices than in the headspace over full-fat matrices [26].

In pasteurized milks with low fat content (P0.5) we found several unique VOCs that were absent in pasteurized milk with higher fat content (P1.5 and P3.5), including 2-undecanone, 2-methyl-1-butanol, 2-methyl-propanal, and 2-methyl-butanal. The log P values of these VOCs are higher than 0, which means these compounds are lipophilic. The absence of these VOCs in P1.5 and P 3.5, at least partially, might be due to a protective effect of the milk fat against the release of volatile compounds from the milk during headspace sampling [27]. Similar results were reported previously [28], in which lipophilic VOCs 2-decanone and 2-undecanone, were only found in skim pasteurized milks but not full fat pasteurized milks. In our study, these four compounds can have contributed to the odor of P0.5 as their concentrations were above their detection threshold values. Furthermore, we observed that the absence of 2-heptanone and hexanoic acid in P0.5 also contributed to the olfactory discrimination between P0.5 and the other two milks (P1.5 and P3.5). To summarize, P0.5

had a unique VOC composition which may have contributed to its' distinguishable odor. When comparing the VOCs between P1.5 and P3.5, the concentration as well as OAV of each VOC in the two milks were different. Higher OAVs of butanoic acid, 1-octanol, octanoic acid, and 2-nonanone were found in P3.5 whereas higher OAV of 2-butanone was found in P1.5. These differences in the VOCs composition may have contributed to the olfactory discrimination between P1.5 and P3.5.

For UHT milks, unlike pasteurized milks, the least abundance of VOCs was observed in U0.5. Dimethyl sulfone,1-pentanol, and 2-heptanone were not found in U0.5 but were present in U1.5 and U3.5. 1-pentanol is usually derived from short-chain unsaturated fatty acids in dairy products; 2-heptanone can be formed by β-oxidation of fatty acids, followed by decarboxylation [16]. The concentration of these dairy fat related VOCs could have been reduced when the fat content of the milks was reduced during the skimming process. Similar results were reported by another study demonstrating that less or even no 1-pentanol and 2-heptanone were found in skim UHT milks compared to full fat UHT milks [16]. 2-pentanone, 2-heptanone 2-nonanone, 2-butanone, and acetoin were found in all three UHT milks in our study. Ketones were reported to be prevalent and important aroma compounds in UHT milks [16, 22, 29]. All these ketones can be generated from fatty acids, formed by β-ketoacid decarboxylation [30] while acetoin was reported to be generated from citrate, which can be generated from triglycerides in dairy products [31]. We also observed that the different OAVs between UHT milks are mainly contributed to acetoin, butanal, and hexanal. Although the VOC compositions and OAVs are different between the three UHT milks, participants could not olfactorily discriminate between the three UHT milks differing in fat content. We speculate that the high abundance of 2-heptanone and acetoin, both being milky/buttery/creamy [13, 32], may have masked odor difference between U0.5, U1.5 and U3.5. The OAVs of 2-heptanone and acetoin observed in our study were much higher than those of other VOCs in UHT milks. Furthermore, our PLSR result showed that 2-heptanone and acetoin seem to contribute to the higher perceived odor intensity of UHT milks. Hence, we speculate that the high abundance of 2-heptanone and acetoin may have led to strong perceptions of cream related odor sensations in all UHT milks and may have masked other odor differences leading to high odor intensity scores but indistinguishable odor qualities of UHT milks.

Overall, the unique VOC composition of P0.5 contributes to its' olfactory discrimination capability while olfactory discrimination between P1.5 and P3.5 is contributed by

concentration differences of several VOCs. Higher odor intensity of UHT milks differing in fat content may have masked odor differences and may have disabled their olfactory discrimination.

Why UHT milks can be distinguished from pasteurized milks with same fat content through smell

Our results showed that participants can detect the differences in smell between pasteurized and UHT milks at the same fat content. This is likely due to the thermal processing applied to the milks that creates different VOC compositions, which in turn leads to different odor perceptions. Many studies indicated that thermal processing has a major impact on the VOC composition of milks, and that the key changes in VOC composition of milks during thermal treatment have been associated with Maillard reactions [33]. Such changes in VOC composition lead to stronger flavor of UHT milks compared to pasteurized milks [34], in line with our results hat UHT milks have higher odor intensity ratings than pasteurized milks with same fat content. Furthermore, we also explored the VOCs that are responsible for perceived odor intensity by performing PLSR among OAV data (**Table S2**, Supplementary Data) and perceptual odor intensity rating results. Our results show that 2-heptanone and acetoin, which milky/buttery/creamy odor, have seem to contribute to the higher perceived odor intensity of UHT milks. We speculate that the odor perception triggered by these compounds is the main reason why UHT milks can be distinguished from pasteurized milks with same fat content though olfaction.

Limitations and future recommendations

In order to investigate the VOCs composition underlying olfactory fat discrimination, when sampling the headspace of the milks, we aimed to mimic what participants sniff during the sensory test. We thus selected HS-SPME to extract the VOCs from the milks. Although this is a frequently used method to quantify VOCs in foods [15, 19, 35], limited absorption space in the SPME fiber may have led to competitive adsorption among VOCs, which can decrease quantification precision. OAVs were included in our study to estimate olfactory contribution of each VOC to the odor, but this approach has limitations as the correlations between OAV and perceived intensity are non-linear. Furthermore, perceptual interactions between VOCs are ignored by this approach as the presence of one VOC can influence the odor perception of other VOCs. While it has been shown by several studies including ours that milks differing

in fat content can be discriminated based on smell, it is unknown whether humans can discriminate between other, non-dairy foods differing in fat content based on smell. Future studies should explore whether these findings of olfactory discrimination capability are generalizable from dairy foods towards non-dairy foods such as meats. Finally, future studies should explore how the ability to discriminate fat content of foods affects eating behavior and food choice.

Conclusions

Our study demonstrates that humans are able to discriminate between varying fat contents in pasteurized milks, but not in UHT milks, based on smell. The special VOC composition in pasteurized milks with low fat content contributes to its' distinguishable odor, whereas strong odor intensity of UHT milks may mask odor difference between UHT milks differing in fat content leading to UHT milks being undistinguishable by smell. The demonstration of different olfactory fat content detection in milks and the explanation of potential mechanism may aid to the production of low-fat food by modifying VOC composition to stimulus sensory perception of full-fat product, and thus aids in the reduction of fat intake.

Authorship contribution statement

Shuo Mu: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Markus Stieger**: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration. **Sanne Boesveldt:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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Supplementary Material

Table S1 Binary logistic regression result of each triangle test.

pasteurized milk
fat content 0.5% vs 1.5%

		Score	df	Sig.
Variables	gender	.030	1	.862
	age	.441	1	.507
	BMI	.000	1	.986
	DCF	.009	1	.926
	DI	.098	1	.754
	DFI	.848	1	.357
Overall Statistics		2.134	6	.907

0.5% fat content
pasteurized milk vs UHT milk

		Score	df	Sig.
Variables	gender	2.347	1	.126
	age	.639	1	.424
	BMI	.800	1	.371
	DCF	2.512	1	.113
	DI	3.318	1	.069
	DFI	.417	1	.519
Overall Statistics		7.887	6	.247

pasteurized milk
fat content 0.5% vs 3.5%

		Score	df	Sig.
Variables	gender	.273	1	.602
	age	.052	1	.819
	BMI	.446	1	.504
	DCF	.911	1	.340
	DI	.014	1	.907
	DFI	1.394	1	.238
Overall Statistics		5.498	6	.482

1.5% fat content
pasteurized milk vs UHT milk

		Score	df	Sig.
Variables	gender	.037	1	.848
	age	.223	1	.637
	BMI	.554	1	.457
	DCF	.534	1	.465
	DI	.864	1	.352
	DFI	.028	1	.868
Overall Statistics		1.694	6	.946

pasteurized milk
fat content1.5% vs 3.5%

		Score	df	Sig.
Variables	gender	2.475	1	.116
	age	.342	1	.559
	BMI	1.732	1	.188
	DCF	.001	1	.970
	DI	.504	1	.478
	DFI	.001	1	.973
Overall Statistics		8.525	6	.202

3.5% fat content
pasteurized milk vs UHT milk

		Score	df	Sig.
Variables	gender	.048	1	.827
	age	1.303	1	.254
	BMI	2.514	1	.113
	DCF	.497	1	.481
	DI	.000	1	.987
	DFI	.436	1	.509
Overall Statistics		5.079	6	.534

Table S2 Calculated OAV of VOCs in different milk samples.

VOC	P0.5	P1.5	P3.5	U0.5	U1.5	U3.5
2-Pentanone	4.951±1.962^{c}	7.257±2.562^{c}	20.705±5.357^{a}	6.300±2.557^{c}	23.762±7.277^{a}	15.242±4.420^{b}
Acetoin	13.142±4.143^{e}	17.857±0.786^{d}	14.428±4.214^{e}	26.785±8.000^{c}	63.428±8.857^{b}	256.571± 46.929^{a}
2-Butanone	1.3191±0.234^{c}	2.296±0.439^{bc}	0.131±0.027^{d}	5.937±0.976^{a}	4.722±0.901^{ab}	3.517±0.668^{b}
2-Nonanone	16.062±1.688^{b}	5.218±1.063^{c}	21.718±2.656^{a}	6.250±1.125^{c}	20.156±2.719^{a}	21.031±3.281^{a}
1-Pentanol	0.980±0.052^{b}	2.673±0.627^{a}	3.647±1.216^{a}	-	1.320±0.085^{b}	2.091±0.294^{a}
2-Heptanone	-	12.565±0.652^{c}	259.130±69.087^{a}	159.043±39^{b}	210.652±38.043^{a}	295.739±80.739^{a}
Butanoic acid	31.914±7.44^{c}	86.170±10.723^{b}	468.085±65.543^{a}	-	-	-
1-Hexanol	3.375±0.150^{b}	19.825±1.200^{a}	2.750±0.100^{b}	-	-	-
Hexanoic acid	-	70.979±20.563^{b}	92.750±15.688^{a}	-	-	-
Ethanol	6.614±2.958^{a}	4.646±3.200^{a}	5.135±2.815^{a}	-	-	-
1-Octanol	-	16.347±1.522^{b}	127.000±42.826^{a}	-	-	-
Octanoic acid	-	15.215±1.92^{b}	80.4902±19.254^{a}	-	-	-
Butanoic acid, 3-methyl-	-	56.500±2.250^{b}	127.500±43.200^{a}	-	-	-
Butanal	-	-	-	-	-	1.290±0.320
Hexanal	-	-	-	-	-	1.856±0.365
2-Undecanone	42.181±1.455	-	-	-	-	-
1-Butanol, 2-methyl-	1.835±0.050	-	-	-	-	-
Propanal, 2-methyl-	3.242±0.107	-	-	-	-	-
Butanal, 2-methyl-	18.666±6.000	-	-	-	-	-

Results are expressed as mean ± SD (n = 3). a-e: Mean values in the same row with different superscripts differ ($p < 0.05$) for OAV of compounds.

Table S3 Variable Importance in the Projection (VIP) for PlS-DA

Variable	VIP 1 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP 2 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)
1-hexanol	1.089	0.667	-0.625	2.804	1.364	0.593	-0.159	2.888
3-methyl-butanoic acid	1.336	0.174	0.889	1.783	1.262	0.135	0.916	1.609
hexanoic acid	1.240	0.207	0.708	1.772	1.184	0.201	0.668	1.700
ethanol	1.249	0.314	0.442	2.056	1.181	0.329	0.335	2.028
octanoic acid	1.208	0.344	0.324	2.092	1.146	0.304	0.364	1.927
acetoin	1.176	0.391	0.170	2.181	1.115	0.393	0.105	2.125
1-octanol	1.162	0.378	0.191	2.133	1.105	0.340	0.232	1.978
butanoic acid	1.159	0.380	0.182	2.136	1.102	0.342	0.222	1.981
2-undecanone	1.060	0.336	0.196	1.925	1.029	0.302	0.253	1.806
2-methyl-1-butanol	1.060	0.336	0.196	1.925	1.029	0.302	0.253	1.806
2-methyl-propanal	1.060	0.336	0.196	1.925	1.029	0.302	0.253	1.806
2-methyl-butanal	1.060	0.336	0.196	1.925	1.029	0.302	0.253	1.806
butanal	1.060	0.438	-0.065	2.185	1.002	0.439	-0.125	2.130
1-pentanol	0.441	0.594	-1.085	1.968	0.730	0.339	-0.140	1.601
2-heptanone	0.656	0.566	-0.799	2.110	0.622	0.497	-0.655	1.899
2-butanone	0.230	0.432	-0.881	1.341	0.488	0.145	0.116	0.861
2-nonanone	0.168	0.317	-0.647	0.983	0.443	0.386	-0.550	1.435
2-pentanone	0.260	0.581	-1.233	1.753	0.402	0.380	-0.574	1.378

Table S4 Pearson correlation analysis between individual volatiles and the fat contents of milk.

Variables in Pasteurized milks	fat content	tetrahydrofuran	2-pentanone	acetoin	2-butanone	2-nonanone	dimethyl sulfone	1-pentanol	butanoic acid	1-hexanol	ethanol
fat content	**1**	**-0.806**	**0.921**	-0.102	**-0.674**	0.108	-0.002	**0.847**	**0.952**	-0.220	-0.279
tetrahydrofuran	**-0.806**	**1**	-0.652	-0.210	0.168	-0.273	0.458	**-0.801**	-0.626	-0.345	0.600
2-pentanone	**0.921**	-0.652	**1**	-0.325	**-0.797**	0.189	0.026	0.664	**0.880**	-0.393	-0.275
acetoin	-0.102	-0.210	-0.325	**1**	0.465	0.045	-0.580	0.256	-0.079	0.420	-0.205
2-butanone	**-0.674**	0.168	**-0.797**	0.465	**1**	-0.138	-0.511	-0.350	**-0.782**	**0.838**	0.008
2-nonanone	0.108	-0.273	0.189	0.045	-0.138	**1**	-0.317	0.138	0.055	0.078	-0.478
dimethyl sulfone	-0.002	0.458	0.026	-0.580	-0.511	-0.317	**1**	-0.324	0.127	**-0.721**	0.360
1-pentanol	**0.847**	**-0.801**	0.664	0.256	-0.350	0.138	-0.324	**1**	**0.838**	0.111	-0.097
butanoic acid	**0.952**	-0.626	**0.880**	-0.079	**-0.782**	0.055	0.127	**0.838**	**1**	-0.413	-0.070
1-hexanol	-0.220	-0.345	-0.393	0.420	**0.838**	0.078	**-0.721**	0.111	-0.413	**1**	-0.212
ethanol	-0.279	0.600	-0.275	-0.205	0.008	-0.478	0.360	-0.097	-0.070	-0.212	**1**

Values in bold are different from 0 with a significance level alpha = 0.05

Variables in UHT milks	fat content	tetrahydrofuran	2-pentanone	acetoin	2-butanone	2-nonanone	acetone	2-heptanone	benzaldehyde
fat content	**1**	-0.587	0.553	**0.962**	**-0.799**	**0.783**	-0.567	**0.865**	-0.107
tetrahydrofuran	-0.587	**1**	**-0.735**	-0.567	0.398	**-0.832**	0.575	-0.656	-0.126
2-pentanone	0.553	**-0.735**	**1**	0.390	**-0.695**	**0.677**	**-0.814**	0.344	0.063
acetoin	**0.962**	-0.567	0.390	**1**	**-0.686**	**0.709**	-0.453	**0.905**	-0.144
2-butanone	**-0.799**	0.398	**-0.695**	**-0.686**	**1**	-0.543	0.655	-0.422	-0.044

Variables in UHT milks	fat content	tetrahydrofuran	2-pentanone	acetoin	2-butanone	2-nonanone	acetone	2-heptanone	benzaldehyde
2-nonanone	0.783	-0.832	0.677	0.709	-0.543	**1**	-0.389	**0.836**	0.279
acetone	-0.567	0.575	**-0.814**	-0.453	0.655	-0.389	**1**	-0.279	0.422
2-heptanone	**0.865**	-0.656	0.344	**0.905**	-0.422	**0.836**	-0.279	**1**	-0.042
benzaldehyde	-0.107	-0.126	0.063	-0.144	-0.044	0.279	0.422	-0.042	**1**

Values in bold are different from 0 with a significance level alpha = 0.05

Questionnaire dairy consumption

This questionnaire is about dairy products and will be used to make an estimation of the dairy products you consumed the last month. The following remarks are important and should be considered during the answering of the questionnaire.

- Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.
- The questions cover the last month, this means the last 4 weeks. This includes both weekdays and weekends
- The products you drank or ate at birthdays, weddings, receptions, etc. should be taken into account as well.
- If you had a completely different diet than normal for example due to illness or holiday for the last month, consider the month before the last month to fill in the questions.
- If you don't use a product at all, fill in 'Not this month'. Always give an answer

1a. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? *(Please do not include chocolate milk, hot chocolate and flavored milk or* yogurt*)*

- o Not this month (go to question 2)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

1b. Each day you drank **milk as a beverage**, how much did you usually drink?

- o Less than 1 cup (8 ounces)
- o 1-1.5 cups (8 to 12 ounces)
- o More than 1.5 cups (12 ounces)

1c. How often was the milk **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

2a. How often did you drink chocolate milk as a beverage (including hot chocolate)?

- o Not this month (go to question 3)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

2b. Each day you drank **chocolate milk as a beverage**, how much did you usually drink?

- o Less than 1 cup (8 ounces)
- o 1-1.5 cups (8 to 12 ounces)
- o More than 1.5 cups (12 ounces)

2c. How often was the chocolate milk **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

3a. How often did you drink **flavored milks as a beverage**?

- o Not this month (go to question 4)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

3b. Each day you drank **flavored milks**, how much did you usually drink?

- o Less than 1 cup (8 ounces)
- o 1-1.5 cups (8 to 12 ounces)
- o More than 1.5 cups (12 ounces)

3c. How often was the flavored milk **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

4a. How often did you drink **yogurt as a beverage?**

- o Not this month (go to question 5)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week

- 6-7 days per week

4b. Each of the days you drank **drink yogurt**, how much did you usually drink?

- Less than 1 cup (8 ounces)
- 1-1.5 cups (8 to 12 ounces)
- More than 1.5 cups (12 ounces)

4c. How often was the yoghurt **reduced-fat or fat-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

5a. Do you consume **milk with cereals**?

- Not this month (go to question 6)
- 1 day per month or less
- 2-3 days per month
- 1 day per week
- 2-3 days per week
- 4-5 days per week

5b. Each time **milk was added to your cold cereal,** how much was usually added?

- Less than 1 cup (8 ounces)
- 1-1.5 cups (8 to 12 ounces)
- More than 1.5 cups (12 ounces)

5c. How often was the milk **reduced-fat or fat-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

6a. How often did you eat **yogurt**?

- o Not this month (go to question 7)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

6b. Each time you ate **yogurt**, how much did you usually eat?

- o Less than ½ cup or less than 1 container
- o 0 ½ to 1 cup or 1 container
- o 0 More than 1 cup or more than 1 container

6c. How often was the yogurt you ate **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

7a. How often did you eat **cottage cheese**?

- o Not this month (go to question 8)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

7b. Each time you ate **cottage cheese**, how much did you usually eat?

- o Less than 1 cup (8 ounces)
- o 1-1.5 cups (8 to 12 ounces)
- o More than 1.5 cups (12 ounces)

7c. How often was the cottage cheese you ate **reduced-fat or fat-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

8a. How often did you eat **pudding or custard**?

- Not this month (go to question 9)
- 1 day per month or less
- 2-3 days per month
- 1 day per week
- 2-3 days per week
- 4-5 days per week
- 6-7 days per week

8b. Each time you ate **pudding or custard,** how much did you usually eat?

- Less than 1 cup (8 ounces)
- 1-1.5 cups (8 to 12 ounces)
- More than 1.5 cups (12 ounces)

8c. How often was the pudding or custard you ate **reduced-fat or fat-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

9a. How often did you eat **sour cream**?

- Not this month (go to question 10)
- 1 day per month or less
- 2-3 days per month
- 1 day per week
- 2-3 days per week
- 4-5 days per week
- 6-7 days per week

9b. Each time you ate **sour cream,** how much did you usually eat?

- o Less than 1 tablespoon
- o 1 to 3 tablespoon
- o More than 3 tablespoons

9c. How often was the sour-cream you ate **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

10a. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

- o Not this month (go to question 11)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week
- o 6-7 days per week

10b. Each time you ate **cheese**, how much did you usually eat?

- o Less than ½ ounce or less than 1 slice
- o ½ to 1½ ounces or 1 slice
- o More than 1½ ounces or more than 1 slice

10c. How often was the cheese you ate **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

11a. How often did you eat **cream cheese?**

- o Not this month (go to question 12)

- 1 day per month or less
- 2-3 days per month
- 1 day per week
- 2-3 days per week
- 4-5 days per week
- 6-7 days per week

11b. Each time you ate **cream cheese**, how much did you usually eat?

- Less than 1 tablespoon
- 1 to 3 tablespoon
- More than 3 tablespoons

11c. How often was the cream cheese you ate **reduced-fat or fat-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

12a. How often did you eat **whipped cream?**

- Not this month (go to question 13)
- 1 day per month or less
- 2-3 days per month
- 1 day per week
- 2-3 days per week
- 4-5 days per week
- 6-7 days per week

12b. Each time you ate **whipped cream**, how much did you usually eat?

- Less than 1 tablespoon
- 1 to 3 tablespoon
- More than 3 tablespoons

12c. How often was the whipped cream you ate **reduced-fat or fat-free**?

- Almost never or never

- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

13a. How often do you drink **coffee or tea with milk**?

- o Not this month (last question, thank you for completing this questionnaire)
- o 1 day per month or less
- o 2-3 days per month
- o 1 day per week
- o 2-3 days per week
- o 4-5 days per week

13b. Each time **milk was added to your coffee or tea**, how much was usually added?

- o Less than 1 tablespoon
- o 1 to 3 tablespoon
- o More than 3 tablespoons

13 c. How often was the milk **reduced-fat or fat-free**?

- o Almost never or never
- o About ¼ of the time
- o About ½ of the time
- o About ¾ of the time
- o Almost always or always

This is the last question, thank you very much for completing this questionnaire

Based on the Diet History Questionnaire, Version 2.0. National Institutes of Health, Applied Research Program, National Cancer Institute,2010. & the Dutch Dairy Food Frequency Questionnaire, Wageningen University, Department Human Nutrition, 2005


5

Chapter 5

How volatile composition facilitates olfactory discrimination of fat content in beef and pork.

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Submitted for publication.

Abstract:

Foods differing in fat content can be distinguished through olfaction alone. The mechanisms underlying the ability of humans to discriminate between foods differing in fat content through olfaction are underexplored. In this study, beef and pork samples were prepared (raw and roasted) with low (muscle tissue; raw: 2-5%; roasted: 5%), medium (muscle tissue with lard; raw: 25-30%; roasted: 36-44%), and high (lard; raw: 40-42%; roasted: 69-70%) fat content. Olfactory triangle discrimination tests and ranking tests were performed to explore whether humans can discriminate and rank fat content of the samples through orthonasal olfaction. GC-MS was used to characterize the volatile compound compositions of the headspace of samples differing in fat content. Partial least-squares regression and partial least squares-discriminant analysis were performed to determine the volatile compounds that were responsible for olfactory fat content discrimination. We found that fat content in both raw and roasted samples can be distinguished through orthonasal olfaction. Perceived odor differences did not always contribute to olfactory identification of fat content. Roasted samples with higher fat content had more abundant fatty acids, aldehydes, and ketones. Phthalic acid, isobutyl 2-ropylpentyl ester, and carbon disulfide facilitated the olfactory discrimination of fat content in raw pork and beef samples. 2-Methyl-propanal, benzaldehyde, 1-hydroxy-2-propanone, 2,3-pentanedione, 2,5-octanedione, and 2-butanone contributed to odor differences of roasted beef samples differing in fat content. We conclude that meat samples differing in fat content differ in volatile compound composition of the headspace, and these differences facilitate discrimination between samples differing in fat content based on olfaction alone.

Introduction

Overconsumption of dietary fat can contribute to the development of overweight, obesity and non-communicable diseases [1]. A better understanding of how humans perceive fat (content) in foods may potentially help to guide consumers to reduce their dietary fat intake. Fat or energy content of foods may be assessed through olfactory cues before foods are put in the mouth. Many studies showed that dietary fat can be perceived through the sense of smell. Several rodent studies demonstrated that blocking olfaction decreased their preference for high fat feeds [2-4]. These findings indicate that animals can smell the odor associated with fat content an ability that might help them find high caloric feed. Human studies showed that 18-carbon fatty acids, including linoleic, oleic, and stearic fatty acids, can be detected by orthonasal and retronasal olfaction [5, 6] and can be distinguished from each other through olfaction [7]. These studies suggest that humans can smell fatty acids as well.

Several studies explored the olfactory perception of fat in foods. Boesveldt and Lundstrom [8] found that odors of reconstituted milks differing in fat content can be distinguished through orthonasal olfaction. Le Calvé et al. [9] further reported that retronasal olfaction is involved in discriminating fat content of milks and yogurts. Pirc et al. [10] recently confirmed that humans can discriminate between milks differing in fat content through orthonasal and retronasal olfaction. Moreover, we [11] previously observed that humans can discriminate between commercial pasteurized milks differing in fat content based on olfaction, but not between commercial ultra-high temperature (UHT) treated milks. The unique volatile compound compositions of pasteurized milks differing in fat contributed to the olfactory discrimination of pasteurized milks. In contrast, the strong odor intensity of UHT milks may mask odor differences between UHT milks differing in fat content leading to UHT milks being undistinguishable by smell. To summarize, these studies showed that humans can discriminate between dairy foods differing in fat content based on olfaction. However, it is unknown whether this capability is limited to dairy foods or can be generalized across food categories to, for example, meats. Fernandez et al. [12] demonstrated that intramuscular fat content ranging from 1.4% to 4.7% did not influence smell intensity of cured pork ham.

The volatile compounds of raw meat are typically formed by lipid oxidation, lipid degradation, and microbial degradation [13]. As for roasted meats, Maillard reactions, lipid oxidation, lipid thermal degradation, and lipid–Maillard reactions contribute to their volatile compound composition, the volatile compounds found in meats, their thresholds and odor descriptors have been reviewed before [14]. Fat participates in all these reactions as a

precursor which influences the volatile compound composition of roasted meats [15]. Furthermore, the presence of fatty acids in meats also influences the odor of meat. For example, linoleic fatty acids in meat auto-oxidize and form 2-nonenal, 2,4-decadienal, 1-octen-3-one, 2,4-nonadienal, these compounds contributing to a meaty odor. The oxidation of arachidonic acid forms *trans*-4,5-epoxy-(E)-2-decenal, 1-octen-3-one, 2,4-decadienal, 2,4,7-tridecatrienal, and hexanal, all these compounds have a distinct aroma and thereby contribute to the olfactory percept of meat [16]. Based on these studies, we hypothesize that humans can discriminate between meats differing in fat content based on smell only, and that this ability is influenced by the volatile compound composition of meats.

This study aims to 1) determine whether humans can discriminate between meat samples (pork and beef; raw and roasted) differing in fat content (muscle tissue; muscle tissue with lard; lard) through olfaction and 2) explore the volatile compound composition that facilitates olfactory discrimination of meat samples differing in fat content. Our findings may contribute to a better understanding of the mechanisms underlying the olfactory perception of fat in foods.

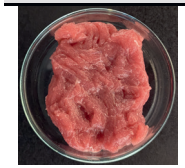
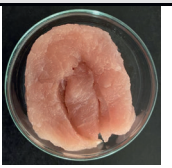
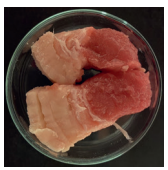
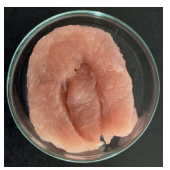
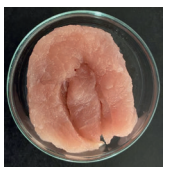
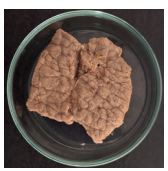
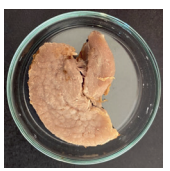
Materials and methods

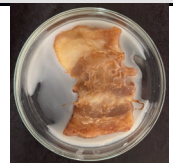
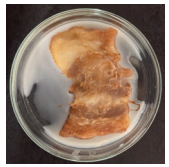
Materials

Raw and roasted beef and pork meats with low-, medium-, and high-fat content were used. Beef sirloin steak (No.7, sliced from the thin loin, Albert Heijn Excellent Entrecote, The Netherlands) and pork bacon (AH Speklap à la minute naturel, The Netherlands) were purchased from a local supermarket (Albert Heijn, Wageningen, The Netherlands). The nutritional composition of the meats is shown in **Table S1** in supplementary materials. Meats were bought every test day and freshly prepared each test day. Replicated measurements were performed on different test day. We purchased meats in the supermarket since we aimed for high ecological validity. We consequently have no control on variations caused by diet and breed between individual animals as they occur in real life.

As **Table 1** shows, meat samples differing in fat content were established using different meat components. low fat refers to muscle tissue of beef and pork from which tallow/lard has been removed manually using a knife. Medium fat refers to beef and pork with muscle tissue and tallow/lard as purchased from the supermarket. High fat refers to the tallow/lard of beef/pork from which the muscle tissue has been removed manually using a knife. Meat samples were assessed raw and roasted, as 10g samples. Raw samples were used as purchased without further processing. Roasted samples were prepared in an oven. Meat samples were wrapped in aluminum foil and roasted at 180°C for 8 min. The raw and roasted samples were placed into odorless glass Petri dishes with lids (60 x15 mm). As shown in **Table 1**, a total of 12 samples (2 types of meat (Beef (B), Pork (P)) x 3 fat levels (Low (L), Medium (M), High (H)) x 2 preparation conditions (Raw (R), rOasted (O))) were prepared. All samples were prepared one day before sensory testing and were stored overnight in the fridge at 4°C. Samples were taken out of the fridge one hour before testing to reach room temperature with the lid closed.

Table 1. Sample codes together with sample descriptions, and fat content of all beef and pork samples. Pictures show 10 g of sample in glass petri dishes as used for the sensory evaluation. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content (muscle tissue); M = Medium fat content (muscle tissue with lard); H = High fat content (lard). Fat content was determined using the Folch method.

Beef	Sample code	Description	Fat content (%, w/w)	Pork	Sample code	Description	Fat content (%, w/w)
	RBL	Raw beef with low fat content	4.5 ± 0.2		RPL	Raw pork with low fat content	2.2 ± 0.1
	RBM	Raw beef with medium fat content	24.9 ± 1.9		RPM	Raw pork with medium fat content	29.7 ± 1.4
	RBH	Raw beef with high fat content	41.8 ± 0.6		RPH	Raw pork with high fat content	40.2 ± 0.7
	OBL	Roasted beef with low fat content	5.2 ± 0.4		OPL	Roasted pork with low fat content	5.1 ± 0.2

Beef	Sample code	Description	Fat content (%, w/w)	Pork	Sample code	Description	Fat content (%, w/w)
	OBM	Roasted beef with medium fat content	36.2 ± 3.1		OPM	Roasted pork with medium fat content	43.9 ± 3.3
	OBH	Roasted beef with high fat content	70.1 ± 1.6		OPH	Roasted pork with high fat content	69.3 ± 0.6

Participants

43 participants (mean age 25.0 ± 4.9 years; 5 males; mean Body Mass Index (BMI) 21.4 ± 2.0 kg/m^2) recruited from the Wageningen area participated in the study. All participants were non-smokers, not pregnant, not breast-feeding, non-vegetarian/vegan, not currently on a calorie-restricted diet or have been in the past 2 months, had a normal functioning sense of smell (tested by the 16-item odor identification part of the Sniffing' Sticks [17] using a score of ≥12 as cutoff for normal olfactory function). Participants were asked not to eat or drink anything other than water one hour prior to testing, nor wear any scented products on the day of testing. Demographic information (age, gender, height, and weight) was collected through an online questionnaire. All participants provided written informed consent prior to participation and were paid a financial reimbursement after completion of all sessions. The study was exempt from review by the Medical Research Ethical Committee according to the "Medical Research Involving Human Subjects Act" of The Netherlands (WMO in Dutch). The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki.

Study procedure

Sensory assessments were conducted in individual sensory booths at Wageningen University, the Netherlands. The sensory booths were well-ventilated to ensure an odorless environment. Participants attended three sessions of 30-50 min. Sniffing' Sticks test and olfactory triangle discrimination tests were performed in the first session, while the second and third sessions consisted of olfactory triangle discrimination tests and ranking tests. Pork samples were assessed in the first session, beef and pork samples were assessed in the second and third session. Sample comparisons were performed in duplicate in random order (e.g. for triangle discrimination test, comparison ABB was performed in the first or second session, and duplicate comparison BAA was performed in the second or third session, different comparison orders ABB, BAA, and ABA were randomly arranged in each session; for the ranking test, comparison ABC was performed in the second session, and duplicate comparison was performed in the third session, where different comparison orders ABC, ACB, BAC, BCA, CBA, and CAB were randomly arranged in each session). An example of the study design for sample comparisons for all sessions is shown in **Table S2** in

supplementary material. Participants were blindfolded during all sensory tests to eliminate visual cues when sniffing the headspace of the samples.

Olfactory triangle discrimination test

Participants were presented with a series of olfactory triangle discrimination tests. Each trial consisted of three petri dishes, two dishes containing the same sample, and one containing a different sample (though from the same type of meat). Beef samples were only compared with beef samples with different fat content, and pork samples were only compared with pork samples differing in fat content. Raw samples were only compared with raw samples and roasted samples only with roasted samples. Blindfolded participants were assisted by researchers to smell each sample and asked to choose the odd one out, so the sample that smells different from the other two. Subsequently, participants had to (orally) answer the following question, "Did you distinguish the samples based on differences in intensity of the smell, quality of smell, other reasons, or unknown reasons?". Participants (n=43) performed each comparison in duplicate by assessing sample triplets AAB and ABB, so that n=86 observations were obtained for each sample comparison. In total, 24 discrimination tests were performed by each participant during the three sessions (6 in the first session, 9 in the second and third session), using an inter-trial interval of approximately 1 minute between each triplet. Participants were encouraged to smell their own skin between trials to prevent adaptation during the intervals.

Ranking test

In the second and third session, participants were presented with a series of olfactory ranking tests. Each trial contained three samples differing in fat content (of the same meat, so beef or pork, prepared either raw or roasted). Blindfolded participants (n=43) smelled the samples and ranked them in order of perceived fat content from lowest to highest. Participants could re-smell samples after the first round of three samples. Presentation order between sample triplets was randomized. Participants duplicated the ranking tests during the second and third sessions with different presentation order, resulting in n=86 observations per ranking test. The interval between trials was approximately 1 minute. Participants were encouraged to smell their own skin to restore smell function and prevent adaptation or olfactory fatigue during the intervals.

Fat content determination

Samples were prepared in the same way as for sensory testing. Before determination of fat content, samples were freeze-dried (Alpha 2-4LDplus freeze dryer, Martin Christ, Germany) for 48 hours and milled into a powder in liquid nitrogen using a Freezer Mill (6875D, SPEX Europe, UK). Powdered samples were stored at -18℃ and thawed at -4℃ overnight before fat content determination. Fat content of all samples was determined using the Folch method, based on the partitioning of lipids in a biphasic mixture of chloroform and methanol. The detailed description of the Folch method is provided in **Method S1** in supplemental material. Fat content was determined in triplicate for all samples.

Characterization of volatile compound composition

The headspace volatile compound composition was determined by Headspace-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS). The headspace of samples was extracted using a SPME fiber (50/30 μm, DVB/CAR/PDMS, Supelco, Bellefonte, USA). One gram of sample was put in a 10 mL vial. Vials were sealed and stored at 4℃ overnight before analysis. An auto-sampler (TriPlus, Thermo, USA) was employed for automatically loading and extracting samples. The vial was placed in the incubator for 1 hour at room temperature. The SPME fiber was then automatically inserted into the headspace of the vial for 30 min to adsorb volatile compounds. After extraction, the loaded SPME fiber was immediately injected into the injection port of the GC-MS for 5 min at 230 °C for desorption.

A gas chromatograph system (Trace GC Ultra, Thermo, USA) coupled with mass spectrometer (DSQ II, Thermo, USA) was employed to explore the volatile composition of the headspace. Samples were analyzed on a Stabil wax DA capillary column (30 m × 0.25 mm × 0.25 μm). Helium (99.999% purity) was used as carrier gas, and the column flow rate was set at 1.20 mL/min in spitless injection mode. The initial oven temperature was 40°C and was maintained for 2 min. The temperature was then increased to 90°C at 3°C/min, held for 5 min, then increased to 200°C at 5°C/min, and finally increased to 230°C at 15°C/min, hold for 10 min. The mass spectrometry detection conditions were as follows: electron impact mode 70 eV; ion source temperature 225°C; and mass range *m/z* 40–450 in full scan mode. Total ion chromatograms (TICs) were recorded and used for further analysis.

The chromatograms were recorded and analyzed using Thermo Scientific Dionex Chromeleon® 7.2 chromatography data system (CDS) software. Volatile compounds were

identified by comparing their mass spectra and retention indices with the National Institute of Standards and Technology (NIST) database. All samples were measured in triplicate. The compounds detected in all three measurements were recorded, the observed mean values of TIC were determined (referred to as relative abundance) and used for further data analysis. Odor descriptors of all volatile compounds were obtained from the online Volatile Compounds in Food database (VFC) (https://www.vcf-online.nl. Van Dongen & Donders, n.d.).

Statistical data analysis

Corresponding triplets of the triangle discrimination tests (e.g., AAB and ABB) were considered as duplicate measures, resulting in 12 comparisons: for both raw and roasted, beef and pork, low vs medium vs high fat content. The number of correct trials was summed up and the significance level (p) was calculated using binominal tests. Furthermore, according to answers from the additional question of triangle tests, the proportion of responses (discrimination based on odor intensity, quality, or unknown reasons) based on the correct discrimination responses were calculated. A significance level of $p < 0.05$ was chosen for all analyses. Ranking data was analyzed by Friedman testing followed by Nemenyi post-hoc tests to explore significant differences among mean sample ranks using Real Statistics Resource Pack software (Release 7.6, Charles Zaiontz). One-way ANOVA followed by Duncan test was performed to analyze differences in peak area of volatile compounds between samples using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL).

To investigate differences of volatile compound compositions between samples, principal component analysis (PCA) was performed based on the peak area of all volatiles of all samples using XLSTAT 2019 (Addinsoft, New York, NY). Pearson correlation analysis (the data was considered normal based on Shapiro–Wilk testing) was performed among volatiles found in pork and beef samples differing in fat content to explore (linear) correlations between fat content and headspace volatile composition, raw pork, raw beef, roasted pork, and roasted beef were analyzed independently. To investigate volatile compounds that are related to olfactory discrimination of the samples differing in fat content, a partial least squares discrimination analysis (PLS-DA) was performed on peak area and triangle test result for pork samples using XLSTAT 2019. The peak area difference in each sample comparison (e.g., peak area difference of acetoin between sample A and sample B is the absolute value of peak of acetoin in sample B minus that in sample A) was set as explanatory variable, and

the olfactory discrimination ability (discriminable/not discriminable, based on group level analysis) of that sample comparison was set as dependent variable. Since all beef comparisons were olfactory discriminable, PLS-DA was not applicable. Therefore, a partial least squares regression (PLSR) was performed to investigate the relationships between olfactory discrimination ability and volatile compound composition for beef samples. The peak area difference in each sample comparison was set as explanatory variable and the number of correct responses of that sample comparison was set as dependent variable. Variable importance in the projection (VIP) of variables were calculated.

Results

Olfactory discrimination ability of fat content of raw and roasted meat samples

Figure 1 shows the results of the olfactory triangle tests for raw and roasted beef (I) and pork (II) differing in fat content. Both raw and roasted beef differing in fat content were olfactorily distinguished from each other [p = 0.038 for RBL-RBM (difference in fat content (wt%) between samples Δ[F] = 20.4%) and RBM-RBH (Δ[F] = 16.9%), $p < 0.001$ for RBL-RBH (Δ[F] = 37.3%), OBL-OBM (Δ[F] = 31.0%), OBL-OBH (Δ[F] = 64.9%), and OBM-OBH (Δ[F] = 33.9%)). However, such olfactory discrimination ability was not observed for pork samples. For raw pork, participants were only able to distinguish between low and high fat content [p = 0.023 for RPL-RPH (Δ[F] = 38.0%)] through olfaction. Roasted pork with low fat content could be olfactorily distinguished from medium and high fat content [p < 0.001 for OPL-OPM (Δ[F] = 38.8%) and OPL-OPH (Δ[F] = 64.2%)], but the comparison of medium vs high fat content was olfactorily undistinguishable (Δ[F] = 25.4%). Moreover, the number of correct identifications for roasted pork comparisons were always higher than those for the corresponding raw pork comparisons (except for the PM-PH comparison).

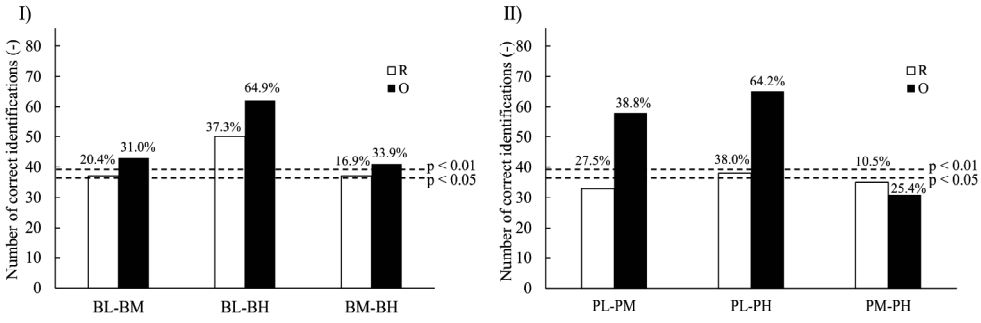


Figure 1. Total number of correct identifications for each triangle discrimination test. I): olfactory discrimination for raw and roasted beef differing in fat content. II): olfactory discrimination for raw and roasted pork differing in fat content. Dotted lines indicate the minimum number of correct identifications required at different significance levels (N = 86, 43 participants in duplicate). B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The numbers above each bar indicate the average difference in fat content (*n*=3) between samples that were compared.

The result of proportion of responses (discrimination based on odor intensity, quality, or unknown reasons) is shown in **Figure S1** in supplementary material. Participants attributed their discrimination ability mostly to odor intensity (> 50%) for the raw samples differing in

fat content except for RBM-RBH (41%). For roasted beef and pork, odor intensity (32-51% for beef comparisons, 31-55% for pork comparisons) and odor quality (44-53% for beef comparisons, 31-58% for pork comparisons), more or less equally, contributed to the perceived odor difference.

Olfactory ranking of perceived fat content of raw and roasted meat samples.

Participants were able to rank samples according to their fat content based on smell for most beef and pork samples, except for raw pork (**Figure 2**). RBL was correctly ranked as sample with the lowest fat content, but RBM and RBH were ranked equally according to their fat content. For roasted sample, both OBH and OPH were correctly ranked as the highest fat content, but OBL (or OPL) and OBM (or OPM) were ranked equally according to their fat content.

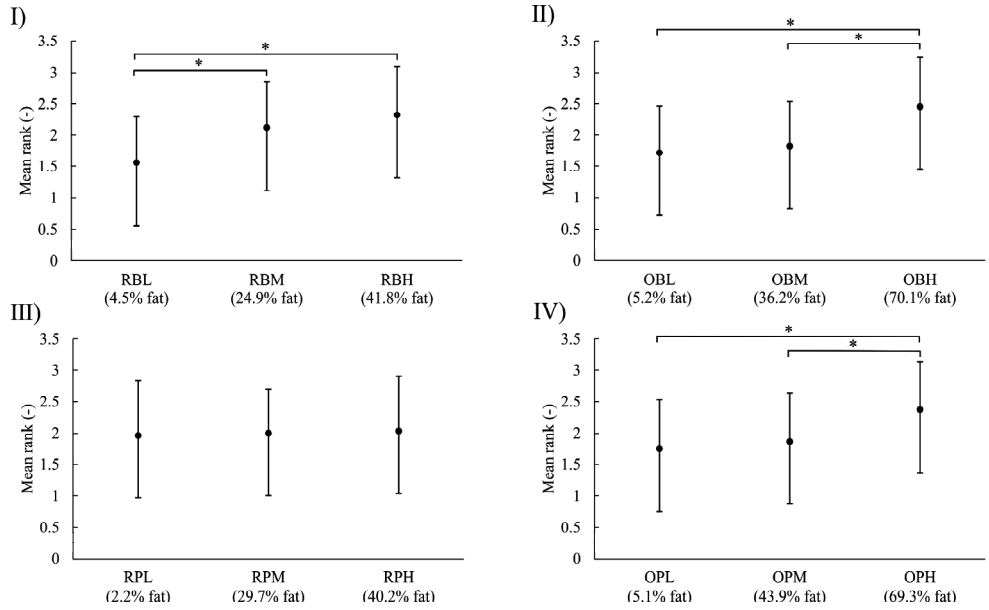


Figure 2. Results of ranking test based on perceived fat content by smell (n=86, 43 participants in duplicate), mean ranks and standard error of the means are shown. I): Raw beef with low, medium, and high fat content; II): Roasted beef with low, medium, and high fat content; III): Raw pork with low, medium, and high fat content; IV): Roasted pork with low, medium, and high fat content. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The numbers below the sample codes indicate the average fat content (n=3) of meats. * Indicates a significant difference between two mean ranks ($p < 0.05$).

Headspace volatile compound composition of beef and pork samples differing in fat content

The headspace volatile compound compositions of the raw and roasted beef and pork samples differing in fat content are shown in **Table 2**. In total 36 volatile compounds were identified in raw beef and 50 volatile compounds in roasted beef. For pork, in total 16 volatile compounds were identified in raw pork and 52 volatile compounds in roasted pork. Acids, alcohols, aldehydes, alkanes, esters, ketones, nitrogen, and sulfur compounds were identified in both beef and pork samples. Several acids and aldehydes, including acetic acid, butanoic acid, hexanoic acid, nonanoic acid, heptanal, hexanal, nonanal, octanal, and pentanal, were identified in beef and pork. More sulfur compounds were identified in pork than in beef samples. Only one sulfur compound, carbon disulfide, was identified in beef samples. Pearson correlation analyses (**Tables S3 – S6** in supplementary material) indicated that several volatile compounds were positively linear correlated with fat content. Specifically, acetic acid, butanoic acid, and 2,3-butanedione in raw beef; 2-Propanol, 1-methoxy-, 2-methyl-butanal, 3-methyl-butanal, toluene, and acetonitrile in roasted beef; Acetoin in raw pork; Carbon disulfide, dimethyl sulfone, 1-methoxy-2-propanol, 3-methylbutanal, 2-methylbutanal, acetoin, acetonitrile, and pentane in roasted pork positively correlated with fat content.

Table 2. Relative abundance (TIC, $*10^6$) and odor quality of volatile compounds of raw and roasted beef and pork samples. The compounds detected in all three measurements were recorded. Results are expressed as observed means ± standard error of the mean ($n = 3$). B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. a-e: Mean values in the same row with different superscript letters differ significantly ($p < 0.05$) in peak area of the compound; -: compound was not detected; NK: not known. Bold fonts indicate volatile compounds that were identified both in beef and pork. The odor descriptor was obtained from references listed in VCF online (www.vcf-online.nl).

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
Acids								
Acetic acid	64-19-7	34.67±1.74^{bc}	89.94±12.99^{b}	204.74±44.6^{a}	-	51.42±6.21^{bc}	206.78±34.31^{a}	acid, fruit, pungent, sour, vinegar
Butanoic acid	107-92-6	6.76±0.91^{b}	18.87±8.8^{ab}	30.12±3.55^{a}	-	-	14.79±3.74^{b}	butter, cheese, must, rancid, sour, sweat
Hexanoic acid	142-62-1	-	13.62±1.9^{a}	12.64±2.12^{a}	-	-	6.42±1.04^{b}	acid, cheese, goat, pungent, rancid
Mercaptoacetic acid, 2tms derivative	-	6.39±0.9^{a}	6.99±0.15^{a}	7.01±0.51^{a}	7.08±0.14^{a}	6.77±0.27^{a}	7.77±1.72	
n-Hexadecanoic acid	57-10-3	-	-	-	-	119.63±14.83	-	rancid, wax
Nonanoic acid	112-05-0	-	-	-	11.82±1.42^{a}	7.55±1.54^{b}	5.74±0.34^{b}	fat, green, sour
Phthalic acid, 5-methylhex-2-yl isobutyl ester	-	6.57±0.2^{a}	5.27±0.32^{b}	-	-	-	-	NK
Phthalic acid, hex-3-yl isobutyl ester	-	-	-	-	5.38±0.31	-	-	NK
Phthalic acid, isobutyl 2-propylpentyl ester	-	-	6.33±0.82	-	-	-	-	NK
Alcohols								
1-Hexanol	111-27-3	-	-	-	5.91±1.05	-	-	bread, flower, fruit, green, herb, wood

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
1-Octen-3-ol	3391-86-4	-	-	-	12.8±2.99^{a}	10.54±1.82^{a}	3.33±0.36^{b}	earth, fat, floral, green, herb, mold, mushroom
1-Pentanol	71-41-0	15.07±1.79^{bc}	15.31±0.33^{bc}	12.48±0.68^{c}	38.49±6.02^{a}	25.43±4.81^{b}	17.38±2.34^{bc}	balsamic, fruit, green, medicine, yeast
1-Penten-3-ol	616-25-1	4.83±0.11^{c}	6.45±0.36^{bc}	-	16.52±3.74^{a}	12.59±3.28^{ab}	2.95±0.24^{c}	burnt, fish, grass, green, meat, wet earth
1-Undecanol	112-42-5	5.28±0.46	-	-	-	-	-	citrus, mandarin
2-Propanol, 1-methoxy-	107-98-2	-	-	-	11.05±1.97^{c}	26.69±3.95^{b}	58.33±10.33^{a}	pleasant, ethereal odor
2-Propen-1-ol	107-18-6	-	-	10.15±0.24	-	-	-	pungent, mustard-like odor
Ethanol	64-17-5	-	-	5.66±0.41	-	-	-	fragrant, vinous odor
Aldehydes								
2-Methyl-butanal	96-17-3	-	-	-	28.62±5.08^{c}	104.44±14.5^{b}	163.71±26.52^{a}	almond, chocolate, cocoa, fermented, hazelnut, malt, nut
2-Methyl-propanal	78-84-2	-	-	-	-	39.71±4.65^{b}	117.02±13.2^{a}	caramel, cocoa, floral, fresh, green, malt, nut
3-Methyl-butanal	590-86-3	-	-	10.36±0.55^{bc}	54.52±4.28^{bc}	202.58±124.59	697.42±66.7^{ab}	acrid, almond, chocolate, cocoa, malt, pungent
Acetaldehyde	75-07-0	-	-	-	74.64±9.69^{a}	73.46±7.21^{a}	64.47±7.87^{a}	pungent, fruity odor
Benzaldehyde	100-52-7	-	-	-	18.02±5.28^{a}	7.74±0.49^{b}	-	almond, bitter almond, burnt sugar, cherry, malt, roasted pepper, sweet

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
Benzeneacetaldehyde	122-78-1	-	-	-	-	-	33.13±3.75	berry, geranium, honey, nut, pungent
Heptanal	111-71-7	82.03±15.15[a]	33.03±8.36[b]	34.3±4.25[b]	-	-	-	citrus, dry fish, fat, green, nut, soap, sweet
Hexanal	66-25-1	-	-	-	1736.6±255.54[a]	376.92±30.29[b]	51.11±5.45[c]	cut grass, fresh, fruit, grass, green, oil
Nonanal	124-19-6	-	-	-	47.33±9.53[a]	-	5.9±1.87[b]	citrus, fat, floral, green, paint, pungent, sweet
Octanal	124-13-0	-	-	-	37.38±2.59	-	-	citrus, fat, green, nut, pungent
Pentanal	110-62-3	-	-	-	164.93±17.04[a]	154.32±9.19[a]	84.56±13.8[b]	almond, chemical, green, malt, oil, pungent
Alkanes								
2,2,6-Trimethyl-octane	62016-28-8	22.4±9.55[a]	8.66±1.03[b]	-	-	-	-	NK
2,2,7,7-Tetramethyloctane	1071-31-4	-	-	-	5.47±1.09	-	-	NK
2,3,4-Trimethylhexane	921-47-1	681.49±36.72	-	-	-	-	-	NK
2,3,4-Trimethyl-hexane	921-47-1	-	295.51±41.33[a]	250.92±35.24[a]	281.44±28.69[a]	125.5±18.82[b]	79.23±15.93[bc]	NK
2,5,6-Trimethyl-octane	62016-14-2	-	-	-	26.5±2.97	-	-	NK
3-Methyl-hexane	589-34-4	-	341.78±22.47[a]	205.32±38.81[b]	319.47±28.43[a]	114.19±14.04[c]	112.25±9.03[c]	NK
5-Ethyl-2,2,3-trimethyl-heptane	62199-06-8	8.06±0.97[b]	-	19.19±2.31[a]	-	-	-	NK
Decane	124-18-5	7.48±0.72[b]	-	-	-	21.02±4.27[a]	-	gasoline-like

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
Decane, 2,5,6-trimethyl-	62108-23-0	7.91±0.18	-	-	-	-	-	NK
n-Hexane	110-54-3	323.6±16.92^{b}	246.82±21.37^{b}	-	237.28±35.22^{b}	-	560.7±59.88^{a}	gasoline-like
Nonane	111-84-2	-	-	51.98±5.48	-	-	-	alkane
Octane	111-65-9	410.82±18.04^{a}	220.95±35.53^{b}	223.98±36.28^{b}	342.02±56.46^{a}	157.91±23.34^{bc}	83.73±13^{c}	alkane, fat, flower, oil, sweet
Alkenes								
2-Octene	111-67-1	187.7±17.76^{a}	84±12.91^{b}	61.62±8.7^{b}	227.83±38.21^{a}	65.42±7.24^{b}	47.14±8.06^{b}	NK
Trans-2-octene	13389-42-9	137.25±2.04	-	-	-	-	-	NK
Esters								
1-Propen-2-ol, acetate	108-22-5	-	-	-	-	87.7±10.22	-	fruity
Acetic acid ethenyl ester	108-05-4	-	-	48.19±8.37^{a}	-	7.42±1.66^{b}	-	alcohol, ester, fruit, wine
Dibutyl phthalate	84-74-2	-	-	5.7±0.22^{b}	-	-	6.66±0.27^{a}	NK
Diethyl phthalate	84-66-2	4.76±0.11^{a}	5.05±0.82^{a}	4.45±0.25^{a}	4.32±0.28^{a}	5.21±0.72^{a}	4.82±0.5^{a}	NK
Glycerol 1,2-diacetate	102-62-5	-	-	-	-	7.71±1.16^{b}	15.88±0.8^{a}	slight, fatty odor
Ketones								
1-Hydroxy-2-propanone	116-09-6	-	-	-	-	14.84±5.08^{b}	50.5±3.62^{a}	butter, herb, malt, pungent
2,3-Butanedione	431-03-8	283.49±14.47^{b}	520.66±28.59^{a}	529.12±23.42^{a}	-	-	-	buttery odor
2,3-Pentanedione	600-14-6	-	-	-	16.55±4.77^{a}	13.98±5.31^{a}	12.55±2.04^{a}	sweet, fermented dairy and creamy, popcorn buttery
2,5-Octanedione	3214-41-3	-	-	-	38.28±5.74^{a}	18.71±4.8^{b}	-	NK

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
2-Butanone	78-93-3	-	-	-	87.32±4.29[a]	38.08±3.38[b]	25.89±2.72[c]	sweet, pungent, fragrant, mint-like odor
Acetoin	513-86-0	3016.37±269.06[b]	4160.78±228.38[a]	3670.84±155.36[a]	216.04±14.85[c]	195.55±4.02[c]	167.56±14.29[c]	buttery, bland, woody, yogurt odor
Monoaromatics								
Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	49.35±3.66[a]	6.09±1.72[b]	-	10.63±0.2[b]	-	-	NK
Benzene, 1,3-dimethyl-	108-38-3	-	-	-	-	12.51±3.14	-	sweet odor
Ethylbenzene	100-41-4	-	-	-	-	18.46±2.28[b]	31.82±3.71[a]	sweet, gasoline-like
p-Xylene	106-42-3	-	-	-	-	33.08±4.59[a]	30.79±6.8[a]	sweet, aromatic odor
Toluene	108-88-3	-	-	-	20.81±3.47[c]	116.15±9.23[a]	93.04±5.53[b]	sweet, pungent, benzene-like
Nitrogen Compounds								
1-Methyldodecylamine	7311-30-0	105.98±13.23[a]	-	13.33±0.09[b]	-	-	-	NK
2,5-Dimethyl-pyrazine	123-32-0	-	-	-	-	6.59±0.76[b]	16.8±3.03[a]	burnt, burnt plastic, cocoa, medicine, roasted, roast beef, roasted nut
2-Methyl-pyrimidine	5053-43-0	-	-	-	-	-	6.74±0.96	NK
2-Octanamine	693-16-3	79.44±3.27	-	-	-	-	-	NK
4-Amino-1-pentanol	927-55-9	-	-	77.79±6.77	-	-	-	NK
Acetonitrile	75-05-8	-	-	-	27.46±3.28[c]	55.19±4.79[b]	81.5±6.74[a]	sweet, ethereal odor
Methylpent-4-enylamine	5831-72-1	94.19±5.29[a]	-	44.26±4.08[b]	-	-	-	NK
Sulfur compounds								

Volatiles in beef	Cas Number	RBL	RBM	RBH	OBL	OBM	OBH	Odor descriptor
Carbon disulfide	75-15-0	567.65±45.02[a]	49.97±6.76[c]	53.18±4.73[c]	131.24±14.39[b]	49.73±6.48[c]	48.03±5.88[c]	vegetable sulfide
Others								
2-Ethyl-oxetane	4737-47-7	-	-	213.34±8.18	-	-	-	NK
Trichloromethane	67-66-3	21.72±8.2[c]	-	-	726.62±55.38[a]	369.9±36.64[b]	271.58±36.03[b]	Hay

Volatiles in pork	Cas Number	RPL	RPM	RPH	OPL	OPM	OPH	Odor descriptor
Acids								
Acetic acid	64-19-7	-	-	-	-	176.39±17.71[b]	264.1±18.58[a]	acid, fruit, pungent, sour, vinegar
Butanoic acid	107-92-6	-	-	-	-	11.75±1.1[b]	17.25±0.92[a]	butter, cheese, must, rancid, sour, sweat
Cis-13-octadecenoic acid	13126-39-1	-	-	-	-	-	21.96±2.82	NK
Hexanoic acid	142-62-1	-	-	-	-	-	8.25±0.85	acid, cheese, goat, pungent, rancid
n-Hexadecanoic acid	57-10-3	-	-	-	-	21.03±2.09[a]	11.63±1.01[b]	
Nonanoic acid	112-05-0	-	-	-	-	-	4.32±0.28	fat, green, sour
Trans-13-octadecenoic acid	693-71-0	-	-	-	-	-	72.3±3.99	NK
Alcohols								
1-Methoxy-2-propanol	107-98-2	-	-	-	8.82±0.54[c]	49.95±4.01[a]	42.97±2.81[b]	mild, ethereal
1-Pentanol	71-41-0	11.6±2.88[b]	4.83±0.31[c]	4.28±0.31[c]	37.66±3.62[a]	18.48±4.13[b]	12.43±1.47[b]	balsamic, fruit, green, medicine, yeast

Volatiles in pork	Cas Number	RPL	RPM	RPH	OPL	OPM	OPH	Odor descriptor
1-Penten-3-ol	67928-92-1	-	-	-	5.81±0.9[a]	3.74±0.61[b]	-	burnt, fish, grass, green, meat
Ethanol	64-17-5	-	-	6.41±0.46	-	-	-	alcohol, floral, ripe apple
Isopropyl alcohol	67-63-0	15.58±0.73[a]	12.74±1.19[b]	15.55±0.88[a]	-	-	-	rubbing alcohol
Oct-1-en-3-ol	3391-86-4	-	-	-	22.63±2.45	-	-	sweet earthy
Aldehydes								
2-Methylbutanal	96-17-3	-	-	27.37±3.58[c]	24.38±1.41[c]	70.88±7.75[b]	439±22.19[a]	almond, chocolate, cocoa, fermented, malt, nut
2-Methylpropanal	78-84-2	-	-	-	-	56.03±7.4[b]	472.75±39.21[a]	caramel, cocoa, floral, fresh, green, malt, nut
3-Methylbutanal	590-86-3	166.21±10.83[b]	10.88±2.03[c]	18.35±4.09[c]	25.41±1.29[c]	314.76±27.26[b]	1305.46±207.91[a]	acrid, almond, chocolate, cocoa, malt, pungent
Benzaldehyde	100-52-7	-	-	-	14.28±3.32	-	-	almond, burnt, cherry, malt, roasted pepper
Ethanal	64-17-5	-	-	-	65.4±2.21	-	-	pungent choking
Heptanal	111-71-7	-	-	-	35.13±3.61	-	-	citrus, dry fish, fat, green, nut, soap
Hexanal	66-25-1	-	-	-	2995.39±332.63[a]	398.9±31.73[b]	87.38±4.76[b]	cut grass, fresh, fruit, grass, green, oil
Nonanal	124-19-6	-	-	-	31.45±3.26	-	-	citrus, fat, floral, green, paint, pungent
Octanal	124-13-0	-	-	-	15.37±2.05[a]	-	5.73±0.42[b]	citrus, fat, green, nut, pungent
Pentanal	110-62-3	-	-	-	148.81±11.59	-	-	almond, chemical, green, malt, oil, pungent
Alkanes								
Dodecane	112-40-3	11.44±0.7[a]	-	12.49±1.64[a]	-	-	-	alkane, undesirable

Volatiles in pork	Cas Number	RPL	RPM	RPH	OPL	OPM	OPH	Odor descriptor
Heptane	142-82-5	-	-	-	-	76.31±4.73^{b}	188.95±19.27^{a}	alkane, burnt matches, floral, plastic
n-Hexane	110-54-3	-	-	14.45±0.93^{c}	255.69±33.09^{a}	305.45±14.45^{a}	149.26±13.85^{b}	alkane
Octane	111-65-9	-	-	-	47.5±4.01^{a}	34.52±2.53^{b}	42.47±1.74^{a}	alkane
Pentane	109-66-0	-	-	-	99.81±4.69^{c}	189.23±19.72^{b}	257.84±5.7^{a}	a petroleum-like odor
Tetradecane	629-59-4	1.84±0.05^{a}	-	1.95±0.16^{a}	-	-	-	alkane, hydrocarbon
Esters								
1,2,3-Propanetriol, diacetate	101364-64-1	-	-	-	-	-	8.41±1.58	slight fatty odor
Acetic acid ethenyl ester	108-05-4	-	-	-	13.61±1.84	-	-	alcohol, ester, fruit, wine
Butyrolactone	96-48-0	-	-	-	-	-	28.87±4.91	pleasant, faint
Diethyl phthalate	84-66-2	7.26±1.5^{abc}	10.63±0.96^{a}	9.18±1.78^{ab}	5.08±0.20^{c}	5.69±0.53^{bc}	5.4±0.22^{c}	no odor
Isopropenyl acetate	108-22-5	-	-	-	-	88.21±1.92	-	NK
Nitrogen Compounds								
2,5-Dimethylpyrazine	123-32-0	-	-	-	-	10.31±2.63^{b}	47.74±2.92^{a}	burnt, cocoa, roasted, roasted beef, roasted nut
2-Ethyl-6-methylpyrazine	13925-03-6	-	-	-	-	-	14.9±1.17	grass, green, nut, roasted
3-Ethyl-2,5-dimethylpyrazine	13360-65-1	-	-	-	-	-	8.38±1.18	broth, chocolate, earth, potato, roast
Acetonitrile	75-05-8	-	-	2.89±0.29^{c}	31.44±2.86^{b}	77.15±5.93^{a}	73.21±2.57^{a}	sweet, ethereal
Cyclohexen-1-carbonitrile	1855-63-6	19.53±2.84	-	-	-	-	-	NK
Methylpyrazine	109-08-0	-	-	-	-	-	21.08±1.39	burnt, cocoa, hazelnut, nut, popcorn, roasted

Volatiles in pork	Cas Number	RPL	RPM	RPH	OPL	OPM	OPH	Odor descriptor
Pyrrole	109-97-7	-	-	-	-	-	8.31±0.51	nut, sweet
Trimethylpyrazine	14667-55-1	-	-	-	-	-	18.52±4.29	burnt, cocoa, earth, must, potato, roast
Ketones								
2,3-Butanedione	431-03-8	-	-	17.92±1.88	-	-	-	butter, caramel, cheese, cream, fruit, yogurt
2,3-Pentanedione	600-14-6	-	-	-	-	-	36.19±4.13	bitter, butter, caramel, cream, fruit, wine
2-Butanone	78-93-3	-	-	-	104.03±5.6^{a}	40.24±2.94^{b}	-	butterscotch, ether, fragrant, fruit, pleasant
2-Methyl-3-octanone	923-28-4	-	-	-	54.3±6.65	-	-	NK
Acetoin	513-86-0	11.53±0.78^{c}	29.57±3.23^{b}	96.48±10.77^{a}	13.11±0.23^{c}	22.69±2.26^{bc}	37.87±4.77^{b}	buttery
Acetone	67-64-1	23.78±1.94^{b}	11.46±1.04^{c}	23.6±2.27^{b}	107.8±5.77^{a}	-	-	chemical, ether, hay, pungent, wood
Hydroxyacetone	116-09-6	-	-	-	-	46.46±4.08^{b}	85.67±11.86^{a}	butter, herb, irritant, malt, pungent
Sulfur compounds								
Carbon disulfide	75-15-0	642.66±190.31^{a}	222.75±63.07^{b}	83.14±21.72^{b}	143.56±19.99^{b}	185.72±39.62^{b}	240.29±21.7^{b}	vegetable sulfide
Dimethyl disulfide	624-92-0	-	-	-	5.5±1.09	-	-	cabbage, garlic, meat, onion, putrid, sulfur
Dimethyl sulfone	67-71-0	-	-	-	3.4±0.85^{c}	11.33±3.55^{bc}	27.41±5.89^{a}	burnt, sulfur
Methanethiol	74-93-1	-	-	-	82.3±5.45^{a}	-	74.48±11.08^{a}	cabbage, garlic, gasoline, putrid, sulfur
Others								
2-Methyltetrahydrofuran-3-one	159551-39-0	-	-	-	-	-	9.91±1.18	NK

Volatiles in pork	Cas Number	RPL	RPM	RPH	OPL	OPM	OPH	Odor descriptor
2-Pentylfuran	3777-69-3	-	-	-	15.03±2.18^{a}	4.98±0.65^{b}	5.12±0.8^{b}	butter, floral, fruit, green, green bean
Carbon dioxide	124-38-9	226.11±12.79^{c}	320.54±15.87^{b}	430.77±9.88^{a}	-	47.03±4.22^{d}	51.35±5.02^{d}	no odor
Chloroform	67-66-3	250.71±13.93^{c}	24.36±1.59^{d}	25.97±3.55^{d}	887.56±93.1^{a}	494.38±14.32^{b}	253.55±34.48^{c}	pleasant, etheric,
Vinyl isopropyl ether	926-65-8	-	-	-	-	-	49.59±3.92	NK

PCA was performed on the HS-SPME-GC-MS data of beef and pork separately (**Figure 3**). The first and second principal component explain 59.9 (34.9% for F1 and 25.0% for F2) of the total variance for beef and 79.05% of the total variance (44.8% for F1 and 34.2% for F2) for pork. The results clearly show that, as expected, raw and roasted beef and pork samples displayed very different volatile compound compositions. Acids and nitrogen compounds were mostly identified in raw beef whereas various aldehydes were found in roasted beef. Raw pork was characterized by carbon disulfide, dodecane, tetradecane, 2-propanol, ethanol, and diethyl phthalate, whereas roasted pork was characterized by more acids, alcohols, aldehydes, and ketones.

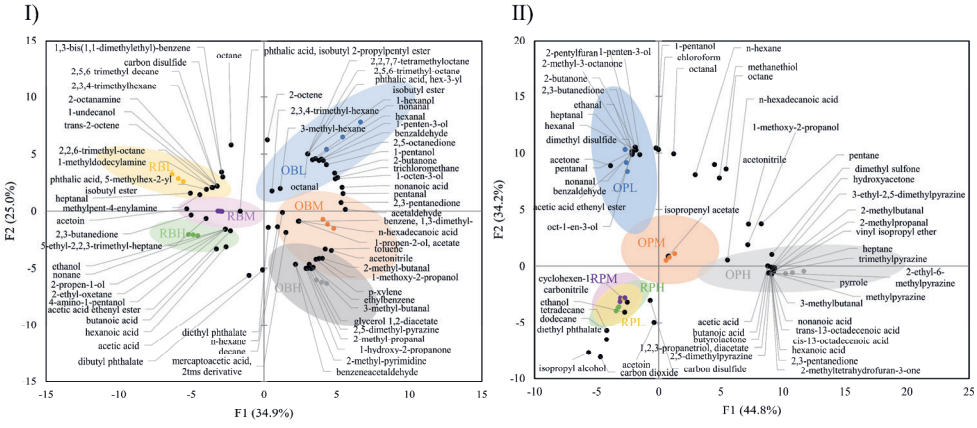


Figure 3. PCA of headspace volatile compound composition of raw and roasted beef and pork meats differing in fat content. I): PCA of beef meats. II): PCA of pork meats. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The confidence ellipses show 95% confidence intervals.

The confidence ellipses of RBL, RBM, and RBH were located separately on the negative side of F1, indicating that all raw beef samples differing in fat content had different volatile compound composition. However, different results were obtained for pork: the confidence ellipses of RPL, RPM, and RPH overlapped completely, indicating raw pork samples differing in fat content were similar in volatile composition. For roasted samples, both beef and pork samples displayed similar trends: OBL (or OPL), OBM (or OPM), and OBH (or OPH) were positioned separately in the PCA, and their confidence ellipses only partly overlapped. These results indicate that the volatile compound compositions partly differed between beef (or pork) differing in fat content.

Relationships between volatile compound composition and olfactory discrimination ability of beef and pork samples

For beef, in total 69.0% (52.6% from Dim1, 16.4% from Dim2) of explanatory variables and 96.5% (58.2% from Dim1,38.3% from Dim2) of dependent variables are explained by the result of PLSR (**Figure 4-I**). For pork, in total 70.0% (37.6% from Dim1; 32.4% from Dim2) of explanatory variables and 81.5% (58.9% from Dim1; 22.6% from Dim2) dependent variables are explained by the result of PLS-DA (**Figure 4-II**).

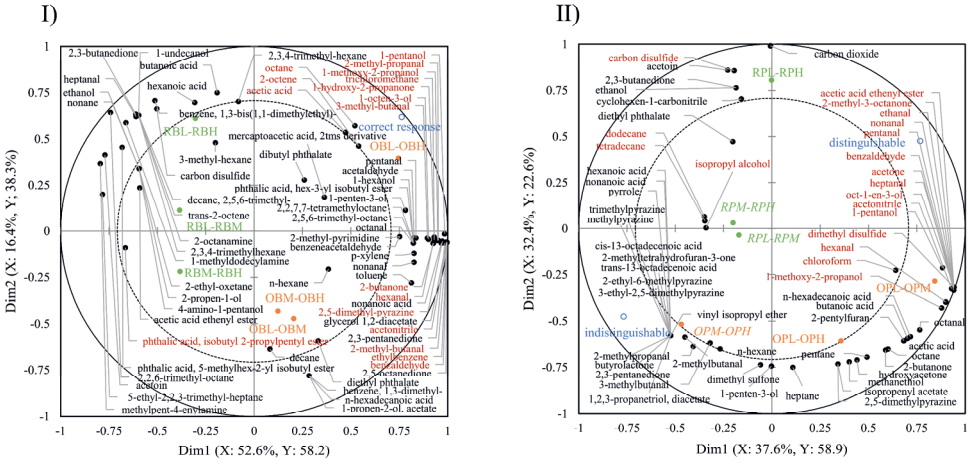


Figure 4. I) PLSR of volatile compound compositions and triangle test results of beef meats. The PLSR was performed among number of correct identifications of each sample comparison and absolute difference in peak area of volatile compound in each sample comparison. II): PLS-DA of volatile compound compositions and triangle test results of pork meats. The PLS-DA was performed among absolute difference in peak area of volatile compound in each sample comparison and olfactory distinguishability of that sample comparison. The sample comparisons in italic indicate they are olfactory indistinguishable comparisons. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The volatile compounds in red indicate that VIPs were > 1 both in Comp1 and Comp2.

For beef samples, the comparisons of raw samples were positioned separately on the negative side of Dim1, indicating differences in volatile compound composition between raw beef comparisons. The VIP of isobutyl 2-propylpentyl ester, which only identified RBM, is > 1 both in Comp1 and Comp2 (**Table S7** in supplementary material). This indicates isobutyl 2-propylpentyl ester may be correlated with correct responses of triangle discrimination tests for raw beef comparisons. Differences in volatile compounds including 1-pentanol, 2-methyl-propanal, 1-methoxy-2-propanol, trichloromethane, 1-hydroxy-2-propanone, 1-octen-3-ol, 3-methyl-butanal, 2-butanone, hexanal, 2,5-dimethyl-pyrazine, acetonitrile, 2-

methyl-butanal, ethylbenzene, and benzaldehyde correlated with correct responses for the comparisons of OBL-OBM and OBM-OBH. Octane, 2-octene, and acetic acid correlated with correct responses for the comparison of OBL-OBH.

For pork samples, all olfactory indistinguishable sample comparisons were positioned on the negative side of Dim1 while all olfactory distinguishable sample comparisons except RPL-RPH were positioned on the positive side of Dim1. This separation indicates that different volatile compounds contributed to their olfactory distinguishability. Specifically, carbon disulfide influenced the olfactory discrimination between RPL-RPH. Dodecane, tetradecane, isopropyl alcohol, acetic acid ethenyl ester, 2-methyl-3-octanone, ethanal, nonanal, pentanal, benzaldehyde, acetone, heptanal, oct-1-en-3-ol, acetonitrile, 1-pentanol, dimethyl disulfide, hexanal, chloroform, and 1-methoxy-2-propanol may have influenced the olfactory discrimination between OPL-OPM.

Discussion

This study aimed to 1) determine whether humans can discriminate between various meat samples, differing in fat content through olfaction and 2) preliminarily explore the volatile compound composition that facilitates olfactory discrimination of meat samples differing in fat content.

Odor differences of meat samples are not always identified as difference in fat content

Participants were able to discriminate between all raw and roasted beef samples, while they could only distinguish low from medium (roasted, 38.8% fat content difference) or high (raw and roasted, 38.0% and 64.2% fat content difference, respectively) fat pork samples. We also observed that the fat content difference in olfactory indistinguishable comparisons (27.5% for raw pork in low vs high fat content; 10.5% for raw pork in medium vs high fat content; 25.4% for roasted pork in medium vs high fat content) were smaller than those of olfactory distinguishable comparisons. These results are in line with Pirc et al [10] who reported that olfactory discrimination between no-fat-containing versus high-fat-containing milks is easier than discrimination between varying levels of fat-containing milks, and that the (absolute) difference between fat content needs to be larger for fat-containing samples in order to be discriminated. Overall, our results showed that humans can discriminate between meat samples differing in fat content (muscle tissue, muscle tissue with lard, lard) based on smell only, which confirms our hypothesis that the olfactory discrimination between foods differing in fat content is not limited to dairy foods but can be extended to meats.

We observed that all raw beef sample comparisons were olfactory distinguishable whereas for raw pork, only the comparison of low vs high fat content was olfactory distinguishable. The volatile compound composition may explain this. All beef samples differing in fat content have different volatile compound compositions whereas raw pork samples have similar volatile compound compositions. Acetic acid, phthalic acid, isobutyl 2-propylpentyl ester, 1-pentanol, and octane were observed to be responsible for raw beef discriminations (**Table S7**). These volatile compounds are likely mainly generated from microbial activity and lipid oxidation of raw beef [18, 19]. Moreover, we also observed that the peak area of acetic acid, which has a pungent odor, had a positive correlation with fat content whereas a negative correlation was observed for octane (**Table S3**), which is in line with a previous study on beef patties [20]. The different volatile compound compositions may help

participants distinguish between beefs differing in fat content. However, not all perceptible odor differences contributed to accurate fat content ranking, as only raw beef with low fat content was correctly ranked for its' fat content.

As for raw pork, all raw pork samples differing in fat content had similar volatile profiles, characterized by 1-pentanol, isopropyl alcohol, 3-methylbutanal, dodecane, diethyl phthalate, acetoin, 2,3-butanedione, and carbon disulfide. Furthermore, they were found in different relative concentrations (peak areas) between pork samples differing in fat content, likely resulting in different odor intensities. This is in line with participants' responses for raw pork **Figure S1(II)** that they based their discrimination on differences in intensity between the samples. However, the difference in odor intensity of raw pork seems difficult to be detected as two of three raw pork comparisons were considered as olfactory indistinguishable. The detectable odor difference between raw pork with low and high fat content may be due to lipid oxidation, e.g., 2,3-butanedione and acetoin, which were found with larger peak area in raw pork with high fat content. These compounds are typically considered sour and pungent off-flavors, indicators of spoiled pork [21], which explains why the perceived odor differences were not associated with fat content itself in raw pork, as shown from the ranking results.

Abundant volatile compound compositions contribute to fat content ranking in roasted samples

Participants showed the ability to distinguish roasted beef and pork differing in fat content through olfaction, except for one roasted pork comparison, medium vs high fat content, which had the smallest fat content difference (25.4%) among roasted sample comparisons. The ability to discriminate between roasted samples differing in fat content by smell might be facilitated by the volatile compound compositions formed during heating of samples. The volatile compounds of roasted samples are mainly generated from lipid reaction and Maillard reaction, which were both influenced by fat [15]. Furthermore, the heating in our study was in moist condition as meat samples were wrapped in foil and heated in an oven, which may greatly favor lipid degradation [14]. Our GC-MS data (**Table 2**) also confirmed this, as relatively few Strecker aldehydes and pyrazines -which are normally associated with high, direct heat-induced Maillard reaction- were identified in our study. Our study observed that pork and beef samples differing in fat content had different volatile compound compositions,

resulting in different odor intensities and odor qualities, both of which contributed to their olfactory discrimination, according to participants' responses (**Figure S1**).

Both roasted beef and pork with the highest fat content (70.1% and 69.3%, respectively) were correctly ranked for their fat content through olfaction. This might be because roasted samples with the highest fat content had the most complex volatile composition. Higher fat content can facilitate richer volatile compositions of samples during cooking [22, 23]. More abundant aldehydes and ketones were identified in beef samples with higher fat content in our study. For roasted beef, 2-methyl-propanal, benzaldehyde, 1-hydroxy-2-propanone, 2,3-pentanedione, 2,5-octanedione, 2-butanone were observed to be correlated with detectable odor difference (**Table S7**), all of them except benzaldehyde and 2-butanone were observed with larger peak area in beef with higher fat content, 2-methyl-propanal even had a positive linear correlation with fat content. Aldehydes usually form through lipid degradation and oxidation during heating [24] whereas ketones are usually formed through Maillard reactions [25]. Both types of volatile compounds are identified to contribute to beef odor [26].

As for pork, the samples with highest fat content (69.3%) also had the most abundant volatile composition, and several fatty acids, including *cis*-13 octadecenoic acid, hexanoic acid, nonanoic acid, and *trans*-13-octadecenoic acid, were only identified in samples with highest fat content. Furthermore, Strecker aldehydes, including 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, were found with larger peak areas for roasted pork with higher fat content. Strecker aldehydes are usually the final aroma compounds that are generated from Strecker degradation during the Maillard reaction and can contribute to the aroma [27]. Fuentes et al [28] also reported that 3-methylbutanal and 2-methylbutanal exhibited higher concentrations in dry-cured ham with higher fat content at the beginning of storage. In summary, the abundant composition of fatty acids, aldehydes, and ketones in roasted sample with the highest fat content may enrich the overall odor perception and participants associated it with high-fat content.

Limitations and recommendations

In this study, we quantified peak area in HS-SPME-GC-MS analysis for each volatile compound rather than (absolute) concentrations because we could not add and evenly distribute internal standards into an intact meat matrix without destroying it. Several studies quantified the concentration of volatile compounds in minced meat by adding internal standards. We aimed to mimic what participants sniffed and smelled during the sensory test,

and thus did not mince the meats to add a standard as this would alter the protein-fat structure and thereby influence the release of volatile compounds and aroma. Since volatile compounds only contribute to odor perception when their concentration surpasses their detection threshold, we can only speculate whether the obtained volatile compounds actually influenced the olfactory perception of fat. Since we determined area under the curve rather than (absolute) concentration for all compounds, we could not obtain odor activity values. Solid evidence such as odor activity values, which quantify the odor contribution of volatile compounds, is needed to verify our findings.

As the aim of our study was to investigate the olfactory perception of beef and pork, we have solely profiled the volatile composition, rather than individual fatty acids, in our study. Many studies have emphasized the significant impact of fatty acid composition on flavor perception of meat [29, 30]. Fatty acids present in meat generally exhibit long carbon chains (C16-C20) and low volatility. Therefore, fatty acids are presumed to have little contribution to the olfactory profile by themselves. However, given that fatty acids serve as both precursors and reservoirs of volatile compounds in meat, identification of the individual fatty acid composition in future studies may help us comprehend the impact of fat content on the formation of volatile compounds in meats.

Although several earlier studies, including our own, showed that humans can perceive fat through olfaction and olfactory perception plays an important role in flavor perception of food, it is still unknown how this ability influences food intake and choice behaviors of humans. Further studies should explore how the olfactory perception of fat affects eating behavior and food choice.

Conclusions

Our study demonstrates that humans can discriminate between beef samples (raw, roasted) and pork samples (raw, roasted) varying in fat content (muscle tissue, muscle tissue with lard, lard) based on smell. Perceived odor differences did not always contribute to olfactory identification of fat content in raw and roasted samples. Different headspace volatile compound compositions were observed for meat samples differing in fat content except for raw pork. Fatty acids, aldehydes and ketones facilitated the olfactory discrimination between roasted samples differing in fat content. These findings contribute to a better understanding of the mechanisms underlying the olfactory perception and sensory identification of fat in meat and may support the development of strategies to enrich flavor of low-fat meats using odor-induced enhancement strategies.

CRediT authorship contribution statement

Shuo Mu: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Nan Ni**: Methodology, Investigation, Data curation, Formal analysis, Visualization. **Yuting Zhu**: Methodology, Investigation, Data curation, Formal analysis, Visualization, **Markus Stieger**: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration. **Sanne Boesveldt:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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Supplementary material

Table S1. Nutritional composition of beef and pork meats, these values apply to the unprepared product.

Nutritional composition	Per 100 Gram (Pork)	Per 100 Gram (Beef)
Energy	1325 kJ (320 kcal)	691 kJ (165 kcal)
Fat	28.0 g	8.1 g
saturated	11.0 g	3.5 g
unsaturated	16.0 g	3.6 g
polyunsaturated	3.4 g	0.0 g
Carbohydrates	0.0 g	0.0 g
sugars	0.0 g	0.0 g
Dietary fiber	0.0 g	0.0 g
Protein	17.0 g	23.0 g
Salty	0.15 g	0.13 g

Table S2. One example of detailed sample comparisons for each session. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The italics are considered as duplicate comparisons.

Session 1	Sniffin' Sticks test		
	Triangle test		
	RPL	RPL	RPM
	RPM	RPH	RPM
	RPH	RPL	RPH
	OPL	OPM	OPL
	OPH	OPM	OPM
	OPH	OPH	OPL
Session 2	Triangle test		
	RPM	RPM	RPL
	RPH	RPM	RPH
	RPH	RPL	RPL
	RBL	RBL	RBM
	RBM	RBH	RBM

	RBL	RBH	RBH
	OBL	OBM	OBL
	OBM	OBH	OBM
	OBH	OBL	OBH
	Ranking test		
	RPL	RPH	RPM
	OPH	OPM	OPL
	RBL	RBH	RBM
	OBL	OBH	OBM
Session 3	Triangle test		
	OPM	OPM	OPL
	OPH	OPH	OPM
	OPH	OPL	OPL
	RBL	RBM	RBL
	RBM	RBH	RBM
	RBH	RBH	RBL
	OBL	OBM	OBL
	OBM	OBH	OBM
	OBH	OBH	OBL
	Ranking test		
	RPL	RPM	RPH
	OPH	OPM	OPL
	RBH	RBL	RBM
	OBM	OBL	OBH

Method S1. Total fat content determination according to the Folch method.

2 g of sample powder was defrosted and placed in 100 mL glass centrifuge tubes. 50 mL of dichloroform: methanol (2:1, vol/vol) mixture was added to each glass tube and homogenized using an ultraturrax (Ultra-Turrax® T 25, IKA, Germany) at 11.000 rpm for 2 min and centrifuged (Heraeus Multifuge X3R, Thermo Fisher Scientific, Germany) for 10 min at 3000 rpm. After that, the solution was filtered through a filter (Grade 595½ Pleated Filter Paper Cytiva, Germany) into 100 mL glass centrifuge tubes. 2.5 mL of the 0.9% NaCl solution was added to the tube and mixed. The biphasic mixture was separated by 1200 g-force for 10 min through centrifugation. The upper aqueous layer was removed and filtered again into a 100 mL beaker filled with 2 g of anhydrous sodium sulfate. After settling for 5 min, the chloroform layer was poured into a pre-weighed 100 mL flat-bottomed flask without sodium sulfate. The chloroform was evaporated entirely in a nitrogen atmosphere using a rotary evaporator (Büchi R-200 Rotavapor System, Büchi Labortechnik AG, Switzerland) with an end vacuum of 100 mbar. After washing with 10 mL of acetone three times, all solvent and water residues were removed, and fat content was quantified gravimetrically. All chemicals were purchased from Sigma-Aldrich Company, Ltd (Amsterdam, the Netherlands).

Figure S1. Reasons that participants provided for olfactory discrimination between low vs medium, low vs high, and medium vs high fat content in beef and pork. I): beef samples. II): pork samples. All results were calculated based on correct response; *n* indicates the correct response for each sample comparison; B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content.

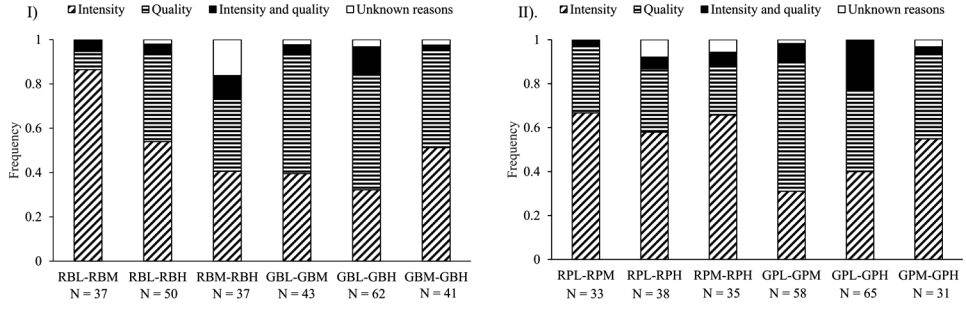

Table S3, Pearson correlation analysis between individual volatiles and the fat contents of raw beef. Bold fonts indicate that the *p*-values of the correlations are < 0.05.

Variables	Coefficients of Correlation	*p*-value
acetic acid	**0.892**	**0.001**
butanoic acid	**0.885**	**0.002**
mercaptoacetic acid, 2tms derivative	0.405	0.280
1-pentanol	-0.584	0.099
heptanal	**-0.801**	**0.009**
diethyl phthalate	-0.165	0.672
2-octene	**-0.924**	**0.003**
octane	**-0.829**	**0.006**
2,3-butanedione	**0.888**	**0.001**
acetoin	0.575	0.105
carbon disulfide	**-0.889**	**0.001**

Table S4, Pearson correlation analysis between individual volatiles and the fat contents of roasted beef. Bold fonts indicate that the *p*-values of volatile compounds are < 0.05.

Variables	Coefficients of Correlation	*p*-value
mercaptoacetic acid, 2tms derivative	0.238	0.537
nonanoic acid	**-0.873**	**0.002**
1-octen-3-ol	**-0.830**	**0.006**
1-pentanol	**-0.861**	**0.003**
1-penten-3-ol	**-0.852**	**0.004**
2-propanol, 1-methoxy-	**0.896**	**0.001**
acetaldehyde	-0.435	0.242
2-methyl-butanal	**0.957**	**< 0.0001**
3-methyl-butanal	**0.889**	**0.001**
hexanal	**-0.953**	**< 0.0001**
pentanal	**-0.800**	**0.010**
trichloromethane	**-0.946**	**0.000**
diethyl phthalate	0.404	0.280
2-octene	**-0.908**	**0.001**
2,3,4-trimethyl-hexane	**-0.956**	**< 0.0001**
3-methyl-hexane	**-0.896**	**0.001**
octane	**-0.944**	**0.003**
toluene	**0.781**	**0.013**
2,3-pentanedione	-0.393	0.295
2-butanone	**-0.960**	**< 0.0001**

acetoin	**-0.825**	**0.006**
acetonitrile	**0.976**	**< 0.0001**
carbon disulfide	**-0.891**	**0.001**

Table S5, Pearson correlation analysis between individual volatiles and the fat contents of raw pork. Bold fonts indicate that the *p*-values of volatile compounds are < 0.05.

Variables	Coefficients of Correlation	*p*-value
carbon disulfide	**-0.903**	**0.001**
diethyl phthalate	0.531	0.142
chloroform	**-0.955**	**< 0.0001**
carbon dioxide	**0.945**	**0.000**
1-pentanol	**-0.869**	**0.002**
isopropyl alcohol	-0.185	0.634
3-methylbutanal	**-0.942**	**0.000**
acetone	-0.234	0.545
acetoin	**0.836**	**0.005**

Table S6, Pearson correlation analysis between individual volatiles and the fat contents of roasted pork. Bold fonts indicate that the *p*-values of volatile compounds are < 0.05.

Variables	Coefficients of Correlation	*p*-value
carbon disulfide	**0.796**	**0.010**
dimethyl sulfone	**0.883**	**0.002**
diethyl phthalate	0.367	0.331
chloroform	**-0.976**	**< 0.0001**
1-pentanol	**-0.943**	**0.000**
1-methoxy-2-propanol	**0.836**	**0.005**
hexanal	**-0.943**	**0.002**
3-methylbutanal	**0.889**	**0.001**
2-methylbutanal	**0.855**	**0.003**
acetoin	**0.928**	**0.000**
acetonitrile	**0.870**	**0.002**
pentane	**0.982**	**< 0.0001**
n-hexane	-0.542	0.132
octane	-0.432	0.245

Table S7. Variable importance in the projection for PLSR performed among GC-MS data and olfactory discrimination ability of beef samples. Bold fonts indicate that the VIPs of volatile compounds are > 1 in both 1 Comp and 2 Comp, meaning they contribute to the olfactory discrimination between beefs differing in fat content.

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
acetic acid	**1.512**	**0.595**	**-0.017**	**3.041**	**1.545**	**0.500**	**0.260**	**2.831**
butanoic acid	0.678	0.906	-1.652	3.007	1.348	0.818	-0.756	3.451
octane	**1.407**	**0.740**	**-0.494**	**3.309**	**1.339**	**0.525**	**-0.010**	**2.688**
2-octene	**1.397**	**0.669**	**-0.323**	**3.116**	**1.303**	**0.517**	**-0.025**	**2.632**
n-hexadecanoic acid	0.487	0.635	-1.145	2.120	1.238	0.477	0.011	2.465
1-propen-2-ol, acetate	0.487	0.635	-1.145	2.120	1.238	0.477	0.011	2.465
benzene, 1,3-dimethyl-	0.487	0.635	-1.145	2.120	1.238	0.477	0.011	2.465
1-pentanol	**1.530**	**0.291**	**0.782**	**2.279**	**1.189**	**0.398**	**0.165**	**2.213**
2-methyl-butanal	**1.445**	**0.389**	**0.444**	**2.445**	**1.128**	**0.455**	**-0.041**	**2.297**
benzaldehyde	**1.445**	**0.385**	**0.456**	**2.434**	**1.128**	**0.452**	**-0.035**	**2.290**
ethylbenzene	**1.444**	**0.380**	**0.467**	**2.421**	**1.127**	**0.449**	**-0.028**	**2.282**
acetonitrile	**1.441**	**0.412**	**0.382**	**2.500**	**1.125**	**0.468**	**-0.077**	**2.328**
2,5-octanedione	**1.441**	**0.413**	**0.379**	**2.502**	**1.125**	**0.468**	**-0.078**	**2.329**
glycerol 1,2-diacetate	**1.435**	**0.425**	**0.344**	**2.527**	**1.121**	**0.474**	**-0.098**	**2.340**
2,3-pentanedione	**1.433**	**0.349**	**0.536**	**2.331**	**1.118**	**0.430**	**0.014**	**2.222**
1-penten-3-ol	**1.400**	**0.533**	**0.030**	**2.771**	**1.116**	**0.510**	**-0.195**	**2.428**
decane	0.654	0.704	-1.155	2.463	1.116	0.557	-0.315	2.547
nonanoic acid	**1.413**	**0.320**	**0.590**	**2.235**	**1.101**	**0.409**	**0.050**	**2.153**
2,2,6-trimethyl-octane	0.304	0.568	-1.157	1.764	1.096	0.440	-0.035	2.227
2,5-dimethyl-pyrazine	**1.397**	**0.462**	**0.209**	**2.586**	**1.093**	**0.491**	**-0.170**	**2.355**
trichloromethane	**1.400**	**0.247**	**0.766**	**2.034**	**1.088**	**0.362**	**0.157**	**2.020**

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
phthalic acid, isobutyl 2-propylpentyl ester	**1.300**	**1.432**	**-2.382**	**4.982**	**1.069**	**0.911**	**-1.273**	**3.412**
2-methyl-propanal	**1.364**	**0.480**	**0.130**	**2.599**	**1.067**	**0.497**	**-0.211**	**2.346**
2-propanol, 1-methoxy-	**1.358**	**0.483**	**0.117**	**2.599**	**1.063**	**0.498**	**-0.218**	**2.343**
2-butanone	**1.360**	**0.275**	**0.652**	**2.068**	**1.059**	**0.374**	**0.098**	**2.020**
hexanal	**1.356**	**0.273**	**0.654**	**2.059**	**1.057**	**0.372**	**0.100**	**2.013**
1-hydroxy-2-propanone	**1.330**	**0.493**	**0.062**	**2.598**	**1.041**	**0.501**	**-0.245**	**2.328**
benzene, 1,3-bis(1,1-dimethylethyl)-	0.023	0.841	-2.139	2.185	1.037	0.611	-0.534	2.607
2,3,4-trimethyl-hexane	0.593	0.853	-1.599	2.784	1.025	0.623	-0.576	2.626
phthalic acid, 5-methylhex-2-yl isobutyl ester	0.279	0.508	-1.026	1.584	1.006	0.418	-0.067	2.079
1-octen-3-ol	**1.284**	**0.506**	**-0.017**	**2.585**	**1.006**	**0.502**	**-0.286**	**2.297**
3-methyl-butanal	**1.286**	**0.497**	**0.007**	**2.564**	**1.005**	**0.501**	**-0.283**	**2.293**
dibutyl phthalate	0.905	0.559	-0.532	2.342	0.955	0.539	-0.431	2.341
2,2,7,7-tetramethyloctane	1.219	0.214	0.668	1.769	0.948	0.309	0.154	1.742
phthalic acid, hex-3-yl isobutyl ester	1.219	0.214	0.668	1.769	0.948	0.309	0.154	1.742
1-hexanol	1.219	0.214	0.668	1.769	0.948	0.309	0.154	1.742
2,5,6-trimethyl-octane	1.219	0.214	0.668	1.769	0.948	0.309	0.154	1.742
octanal	1.219	0.214	0.668	1.769	0.948	0.309	0.154	1.742
pentanal	1.184	0.523	-0.160	2.528	0.929	0.500	-0.357	2.215
acetaldehyde	1.169	0.525	-0.180	2.517	0.917	0.499	-0.367	2.201
carbon disulfide	0.059	0.607	-1.500	1.619	0.913	0.499	-0.369	2.196
p-xylene	1.158	0.193	0.662	1.654	0.911	0.287	0.174	1.648
2,3-butanedione	0.250	0.400	-0.779	1.279	0.904	0.368	-0.042	1.851
hexanoic acid	0.221	0.738	-1.676	2.118	0.901	0.541	-0.491	2.292

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
nonanal	1.100	0.183	0.631	1.569	0.882	0.266	0.199	1.564
trans-2-octene	0.244	0.384	-0.743	1.231	0.881	0.361	-0.045	1.808
1-undecanol	0.244	0.384	-0.743	1.231	0.881	0.361	-0.045	1.808
2,3,4-trimethylhexane	0.244	0.384	-0.743	1.231	0.881	0.361	-0.045	1.808
decane, 2,5,6-trimethyl-	0.244	0.384	-0.743	1.231	0.881	0.361	-0.045	1.808
2-octanamine	0.244	0.384	-0.743	1.231	0.881	0.361	-0.045	1.808
nonane	0.244	0.382	-0.737	1.225	0.877	0.377	-0.093	1.848
4-amino-1-pentanol	0.244	0.382	-0.737	1.225	0.877	0.377	-0.093	1.848
2-ethyl-oxetane	0.244	0.382	-0.737	1.225	0.877	0.377	-0.093	1.848
2-propen-1-ol	0.244	0.382	-0.737	1.225	0.877	0.377	-0.093	1.848
ethanol	0.244	0.382	-0.737	1.225	0.877	0.377	-0.093	1.848
heptanal	0.276	0.345	-0.611	1.163	0.872	0.321	0.047	1.698
1-methyldodecylamine	0.412	0.438	-0.715	1.539	0.839	0.167	0.410	1.268
toluene	0.946	0.194	0.447	1.445	0.831	0.210	0.290	1.372
benzeneacetaldehyde	1.056	0.532	-0.312	2.424	0.830	0.491	-0.432	2.093
2-methyl-pyrimidine	1.056	0.532	-0.312	2.424	0.830	0.491	-0.432	2.093
methylpent-4-enylamine	0.935	0.507	-0.368	2.238	0.814	0.271	0.118	1.510
5-ethyl-2,2,3-trimethyl-heptane	0.863	0.464	-0.329	2.055	0.801	0.200	0.287	1.314
acetoin	0.843	0.492	-0.422	2.108	0.788	0.223	0.214	1.362
acetic acid ethenyl ester	0.342	0.504	-0.954	1.637	0.771	0.318	-0.046	1.588
diethyl phthalate	0.076	0.311	-0.723	0.876	0.748	0.515	-0.575	2.071
3-methyl-hexane	0.115	0.508	-1.191	1.421	0.548	0.489	-0.708	1.804
mercaptoacetic acid, 2tms derivative	0.654	0.683	-1.101	2.409	0.522	0.560	-0.916	1.961

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
n-hexane	0.479	0.796	-1.566	2.525	0.398	0.433	-0.714	1.510

Table S8. Variable importance in the projection for PLS-DA performed among GC-MS data and olfactory discrimination ability of pork samples. Bold fonts indicate that the VIPs of volatile compounds are > 1 in both 1 Comp and 2 Comp, meaning they contribute to the olfactory discrimination between pork differing in fat content.

Variable	VIP 1 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP 2 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)
isopropyl alcohol	**1.499**	**0.866**	**-0.906**	**3.905**	**1.842**	**1.127**	**-1.287**	**4.970**
tetradecane	**1.466**	**0.836**	**-0.856**	**3.788**	**1.777**	**1.068**	**-1.188**	**4.741**
dodecane	**1.443**	**0.818**	**-0.829**	**3.715**	**1.734**	**1.030**	**-1.127**	**4.594**
carbon dioxide	0.922	1.530	-3.327	5.171	1.577	1.588	-2.831	5.985
acetoin	0.498	1.215	-2.875	3.871	1.376	1.318	-2.285	5.036
1-pentanol	**1.594**	**0.487**	**0.242**	**2.946**	**1.358**	**0.569**	**-0.220**	**2.937**
carbon disulfide	**0.509**	**1.226**	**-2.895**	**3.914**	**1.314**	**1.234**	**-2.111**	**4.739**
dimethyl disulfide	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
acetic acid ethenyl ester	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
oct-1-en-3-ol	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
pentanal	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
nonanal	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
benzaldehyde	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
heptanal	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
ethanal	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
2-methyl-3-octanone	**1.383**	**0.720**	**-0.615**	**3.381**	**1.232**	**0.591**	**-0.408**	**2.871**
acetonitrile	**1.404**	**0.703**	**-0.549**	**3.357**	**1.232**	**0.634**	**-0.528**	**2.991**

Variable	VIP 1 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP 2 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)
chloroform	**1.352**	**0.596**	**-0.302**	**3.005**	**1.202**	**0.510**	**-0.214**	**2.618**
acetone	**1.237**	**0.829**	**-1.066**	**3.539**	**1.200**	**0.394**	**0.105**	**2.294**
hexanal	**1.303**	**0.788**	**-0.885**	**3.491**	**1.170**	**0.589**	**-0.466**	**2.806**
1-methoxy-2-propanol	**1.263**	**0.816**	**-1.004**	**3.529**	**1.139**	**0.587**	**-0.491**	**2.769**
ethanol	0.376	1.084	-2.632	3.385	1.071	1.075	-1.914	4.057
2,3-butanedione	0.376	1.084	-2.632	3.385	1.071	1.075	-1.914	4.057
cyclohexen-1-carbonitrile	0.376	1.081	-2.624	3.377	1.004	1.006	-1.791	3.798
octanal	1.065	0.921	-1.492	3.622	0.983	0.562	-0.576	2.543
n-hexadecanoic acid	0.983	0.923	-1.581	3.546	0.918	0.546	-0.597	2.433
butanoic acid	0.959	0.899	-1.536	3.454	0.899	0.540	-0.601	2.399
2-pentylfuran	0.927	0.868	-1.482	3.337	0.874	0.533	-0.607	2.354
acetic acid	0.925	0.866	-1.479	3.330	0.872	0.533	-0.607	2.352
2,3-pentanedione	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
vinyl isopropyl ether	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
2,5-dimethylpyrazine	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
3-ethyl-2,5-dimethylpyrazine	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
trimethylpyrazine	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
nonanoic acid	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
cis-13-octadecenoic acid	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
pyrrole	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
butyrolactone	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
1,2,3-propanetriol, diacetate	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
methylpyrazine	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
2-ethyl-6-methylpyrazine	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665

Variable	VIP 1 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)	VIP 2 Comp	Standard deviation	Lower bound (95%)	Upper bound (95%)
trans-13-octadecenoic acid	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
hexanoic acid	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
2-methyltetrahydrofuran-3-one	0.922	0.785	-1.258	3.102	0.784	0.677	-1.097	2.665
octane	0.792	0.758	-1.313	2.896	0.766	0.498	-0.618	2.150
2-butanone	0.770	0.744	-1.296	2.837	0.750	0.492	-0.618	2.117
2-methylbutanal	0.761	0.813	-1.496	3.019	0.648	0.662	-1.189	2.485
2-methylpropanal	0.755	0.858	-1.627	3.136	0.646	0.668	-1.208	2.499
pentane	0.615	0.679	-1.269	2.500	0.629	0.444	-0.603	1.860
hydroxyacetone	0.533	0.669	-1.325	2.391	0.565	0.413	-0.581	1.711
methanethiol	0.469	0.674	-1.401	2.340	0.517	0.386	-0.555	1.589
3-methylbutanal	0.528	0.611	-1.167	2.223	0.450	0.480	-0.882	1.782
isopropenyl acetate	0.376	0.698	-1.561	2.314	0.450	0.340	-0.495	1.395
n-hexane	0.481	0.617	-1.234	2.195	0.409	0.458	-0.863	1.682
diethyl phthalate	0.445	0.654	-1.370	2.261	0.403	0.336	-0.529	1.335
dimethyl sulfone	0.240	0.475	-1.080	1.560	0.287	0.266	-0.452	1.026
1-penten-3-ol	0.154	0.396	-0.945	1.254	0.256	0.184	-0.255	0.768
heptane	0.014	0.389	-1.067	1.095	0.254	0.107	-0.044	0.552


6

Chapter 6

General Discussion

This thesis aimed to investigate how olfaction contributes to the perception of fat/fatty acids, and to identify differences in volatile compound composition between foods differing in fat content, by answering the following three research questions:

1. Can humans perceive fatty acids and tastants through olfaction?
2. Can humans discriminate between foods differing in fat content solely based on olfaction?
3. What volatile compounds facilitate the olfactory discrimination between foods differing in fat content?

In the general discussion, the aims, approaches, and main findings of each chapter are summarized. Then the three research questions are addressed and methodological considerations discussed. Finally, the relevance of findings and recommendations for further research are discussed and overall conclusions provided.

Summary of main findings

Table 1 provides an overview of the main findings of the thesis.

Table 1. Aims, approach, and main findings of each chapter.

Chapter	Aim	Approach	Main Findings
2	Explore the ortho- and retronasal olfactory perception of **tastant and fatty acids.**	Olfactory triangle discrimination test. ITEX-GC-MS	Fatty acids (and tastants) can be discriminated from blanks through orthonasal olfaction. Perceived odor of tastant solutions is not associated with their taste quality. Perceived odor of fatty acid solution is associate with fat. Fatty acids present different odor qualities when present ortho- vs retronasally. Fat oxidation compounds contribute to olfactory discrimination of fatty acids.
3	Summarize current knowledge of **olfactory fat perception** and identify research gaps.	Systematic scoping review	Olfaction contributes to fat perception independent of other sensory modalities. Dietary fat can be detected, discriminated, and identified via olfaction. Fat-related odors can enhance sensations of other sensory modalities.
4	Investigate whether humans can discriminate fat content differences in **dairy milk matrices** through orthonasal olfaction.	Olfactory triangle discrimination test. SPME-GC-MS	Differences in fat content can be detected through olfaction in pasteurized milks, but not in UHT milks. Pasteurized milks differing in fat content differ in volatile compound composition. UHT milks are perceived more intense than pasteurized milks. Perception of 2-heptanone and acetoin may limit odor discrimination of UHT milks.
5	Investigate whether human can discriminate fat content difference in **beef and pork matrices** through orthonasal olfaction.	Olfactory triangle discrimination test. SPME-GC-MS	Humans can discriminate meats differing in fat content through olfaction. Perceived odor differences do not always allow to rank fat content correctly. Raw pork meats differing in fat content have similar volatile compound compositions. Fatty acids, aldehydes and ketones contribute to odor discrimination of roasted meats.

1. Can humans perceive fatty acids and tastants through olfaction?

Although fat taste has been suggested to be the sixth basic taste quality and fatty acids as taste stimuli responsible, one factor that remains controversial is that olfaction may also be involved in the perception of fat, implying that the oral perception of fat or fatty acids may not be classified as taste perception [1-4]. Fatty acids have an odor whereas basic tastants are usually considered odorless. However, only few preliminary studies observed that tastant solutions can have a perceivable odor [5, 6], which indicates that the oral perception of tastants might actually also involve some/a certain level of retronasal olfactory input and thus goes beyond sole taste perception of non-volatiles. To our surprise, Chapter 2 indicates that all tastant solutions do have an odor, but their quality is not attributable to their taste quality. Our chemical data (**Chapter 2**) may explain this partly: ethyl dichloroacetate, methylene chloride, and acetone were identified in the headspace of sucrose, MSG and quinine solutions but not in water, which could be impurities included in tastants during processing. However, no differences in headspace volatile compound composition of NaCl and citric acid solutions and water were observed although these tastant solutions were discriminated from water by olfaction. As discussed in **Chapter 2,** the analytical methodologies applied to characterize the headspace composition of tastant solutions may not have been sensitive enough to potentially capture all the difference in volatile compound composition between solutions and blank water.

In line with studies [7-9] reviewed in **Chapter 3**, our observations regarding fatty acids (**Chapter 2**) demonstrate that both oleic and linoleic acid solutions can be discriminated from blank mineral oil through ortho- and retronasal olfaction. In contrast to tastants, where the perceived odor quality is not linked to the taste quality of the solution, the perceived odor qualities of fatty acids can be associated with fat taste. This association seems to be related to the volatile compound composition in the headspace, which contained various fat oxidation compounds, including aldehydes, ketones, alcohols, and hydrocarbons. Fatty acids, especially unsaturated ones such as oleic and linoleic acids, are susceptible to oxidation when exposed to oxygen, heat, and light, leading to the breakdown of the fatty acid molecules and the formation of various volatile oxidation products [10, 11]. Additionally, fatty acids are amphiphilic molecules, they have the capacity to act as solvents for volatile compounds, and thus present certain odor. Further, these volatile compounds may present different odor quality between ortho- and retronasal olfaction (**Chapter 2**). In contrast, tastants are

relatively more stable and less prone to degradation under typical storage conditions, which could explain the lack of compounds detected in the headspace of tastant solutions. The olfactory perception of fatty acids suggests that oral perception of fatty acids cannot be simply categorized as taste perception; instead, it likely represents a more complex multimodal experience, such as flavor perception.

To summarize, humans can perceive fatty acids and tastant solutions through olfaction. The oxidation characteristics and the role of fatty acids as solvents for volatile compounds are primarily responsible for the differences compared to basic tastants, and play an important role in the olfactory perception of fatty acids.

2. Can humans discriminate between foods differing in fat content solely based on olfaction?

Chapter 3 summarizes the current knowledge about olfactory fat perception. Our systematic scoping review showed that fat in food matrices presents with odor(s) that can be detected by humans, and perceived odor quality can be associated with fat. Based on these findings, we are wondering whether such fat-related odor can help humans to discriminate fat content, thus the energy content, in real food matrices through orthonasal olfaction. we subsequently explored this in various (liquid) dairy (**Chapter 4**) and (solid) meat (**Chapter 5**) matrices and found that, although humans possess the ability to discriminate some foods differing in fat content solely based on olfactory cues, the perceived odor differences were not always associated with its fat content.

Olfactory discrimination of fat content is more sensitive in milks compared with meats.

We observed that humans can discriminate fat content in food through orthonasal olfaction; however, the absolute differences in fat content that could be distinguished appears to differ between food matrices. In pasteurized milk matrices, we found that a relatively small difference in fat content can be discriminated. For instance, participants were able to discriminate a fat content difference of 1.5% - 2% in pasteurized milks (milks with fat content of 0.5% VS 1.5% and 1.5% VS 3.5%) through orthonasal olfaction. Other studies using reconstituted milks [12, 13] similarly observed that a fat content difference ranging from 1.0% - 3.5% can be discriminated through orthonasal olfaction. However, in UHT milk, such olfactory discrimination ability for fat content differences of 1.0% - 3.0% was not observed.

A previous study [13] observed that a larger fat content difference (>7%) was needed to be facilitate discrimination of reconstituted milks by retronasal olfaction, suggesting that larger fat content differences in UHT milks may be olfactory discriminable. In **Chapter 4** we only used commercially available UHT milks with a maximum fat content difference of 3%. Future studies could process UHT milks with higher fat content differences and explore olfactory discriminable fat content difference in UHT milks. A Previous study compared oral discrimination ability of fat content in milks with and without olfaction [14]. They found that fat content differences of 1.1%, 2.7%, and 3.8% cannot be discriminated by means of only gustatory exposure (blocking olfaction) whereas the addition of olfactory input enabled discrimination of fat content differences of 2.7% and 3.8%. Besides, another study investigated the contribution of flavor volatiles to the perception of fat in a milk model system and observed that the addition of powdered natural cream flavor could diminish the perceptual difference between 5% fat milk and 10% fat milk [15]. These results indicate that compared to gustatory perception, olfactory perception maybe more sensitive to discriminate difference in fat content in milks.

When exploring the orthonasal olfactory discrimination ability in meat matrices, we observed that a relatively larger fat content differences was needed for olfactory discrimination in these matrices. Specifically, in raw meat matrices, participants could discriminate a fat content difference of 17% in raw beef, while a minimum fat content difference of 38% was required for discrimination in raw pork. This difference can be attributed to the observed volatile composition in the headspace of the meat samples, with relatively more abundant volatile compounds detected in raw beef compared to raw pork samples (**Chapter 5**). As for roast meat matrices, we found olfactory discriminable fat content differences of 31% and 39% for beef and pork, respectively. For roast pork, the minimum fat content difference was 25%, which was not discriminable through olfaction. Therefore, the minimum olfactory discriminable fat content difference should fall within the range of 25% to 39%. It has to be noted that in **Chapter 5** we used commercially available meats and manually cut the meats into low (muscle tissue), medium (muscle tissue with lard), and high fat content (lard). Thus, the differences in fat content are depend on meat products themselves. The minimum olfactory discriminable fat content difference for raw and roast beef maybe less than 17% and 31%, respectively, as these values were the minimal fat content difference in our experiment. Future studies could produce minced beef by mixing different portion of muscle and lard to achieve (lower) desirable fat content differences and explore

the minimal olfactory discriminable fat content difference of raw and roast beef. Overall, from current results, the olfactory discriminable fat content differences in meat, whether raw or roasted, are much larger than those observed in dairy milks. Such difference maybe because liquid and solid food matrices have different influence on the release of volatile compounds. When oral process is not involved, which is our experiment environment, volatile compounds might release more easily from liquid matrices than from solid matrices. Compared with solid food matrices, liquid food matrices have higher mobility and less structural hindrance for the release of volatile compounds [16-18].

olfactory fat content discrimination is based on differences in odor quality.

Odor quality and intensity are main factors contributing to olfactory discriminations [19, 20]. In our study, we observed that these factors had different contributions to the olfactory discrimination in milk and meat matrices. In milk matrices, participants perceived similar odor intensities for pasteurized milks with differing fat content, suggesting that the difference in odor quality played a significant role in the olfactory discrimination. In contrast, in meat matrices, participants attributed the olfactory discrimination to both odor quality and odor intensity, particularly in raw beef and pork, where more than half of the participants indicated the difference in odor intensity as a key factor. This may explain why, as indicated above, relatively smaller fat content differences can be discriminated through olfaction in pasteurized milks compared to meat matrices, as differences in odor quality might be more easily detected through olfaction [20].

It has to be noted that the comparison of odor intensity and quality contribution between milk and meat matrices is "indirect" since we employed different experimental designs for the milk and meat studies. Specifically, in the milk study, we did not explicitly ask participants to indicate whether odor intensity or odor quality contributed to the olfactory discrimination, whereas in the meat studies, this question was asked. Despite this limitation, when considering that pasteurized milks with differing fat content exhibited different volatile compositions but were scored similarly for perceived odor intensity, we deduce that the difference in odor quality was a crucial factor contributing to the olfactory discrimination of pasteurized milks. This "indirect" evidence indicates that humans are inclined to discriminate fat content through olfaction in foods that present distinct odor qualities.

The evidence from chemical data also indicates that olfactory discrimination of fat content in milks and meats may be due to the different perceived odor qualities. The PCA performed

on the HS-SPME-GC-MS data of milks (**Figure 3**, **Chapter 4**) and meats (**Figure 3, Chapter 5**) showed that olfactory discriminable sample comparisons presented different volatile compound compositions. These different volatile compositions, though their odor perceptions still need to be validated, likely present with different odor quality that contribute to olfactory discrimination of fat content in milks and meats.

To summarize, this thesis has revealed that humans can discriminate fat content in real food matrices based on smell only; furthermore, the olfactory discrimination of fat content in milks is more sensitive than in meats. In contrast to differences in odor intensity, humans are more inclined to detect the disparity in odor quality, even when it is presented through a small fat content difference.

3. What volatile compounds facilitate the olfactory discrimination between foods differing in fat content?

Milk contains a type of fat known as milk fat, primarily composed of triglycerides with glycerol and three fatty acid chains. The composition of milk fat can vary depending on factors like animal species, breed, and diet. Generally, bovine milk fat contains a mixture of saturated, monounsaturated, and polyunsaturated fatty acids, including palmitic acid, oleic acid, stearic acid, and linoleic acid [21]. In contrast, the fat in beef mainly comprises saturated fatty acids, with palmitic acid and stearic acid being the most abundant [22]. Pork fat, commonly known as lard, contains a higher proportion of monounsaturated fatty acids compared with beef, particularly oleic acid [23]. These differences in fat and fatty acids influence the volatile compound composition as well as olfactory perception of fat. Besides, the fat related perception differs between food matrices. For example, dairy fat is usually associated with sweet taste and creamy flavor and texture of dairy product such as yogurt and milks [24], whereas fat in meat is usually associated with savory taste, meaty flavor and perception of fatty and greasy texture [25]. Furthermore, the processing of food plays a pivotal role in determining the volatile compound composition, consequently influencing the olfactory perception and discrimination of fat content in various food matrices. Among the different food processing methods, thermal processing stands out as a prominent technique.

In **Chapters 4 and 5**, we extensively discussed the impact of fat type and thermal processing on the volatile compound composition of milk and meat matrices independently. Now, our objective is to amalgamate the findings from those chapters and elucidate whether any volatile compounds exist that may influence olfactory perception of fat across different

food matrices. By combining the data from previous chapters, we identified volatile compounds present in both milk and meat matrices. The relevant information, such as odor quality, threshold, and logP value for each compound, is presented in **Table 2**. We found that the volatile compound composition in milk and beef matrices exhibited notable differences. However, intriguingly, we found a subset of compounds, including benzaldehyde, butyric acid, hexanoic acid, acetoin, ethanol, hexanal, chloroform, 1-pentanol, 2-methyl-propanal, 2-butanone, and 2-methylbutanal, which were present in both milk and meat matrices. Furthermore, butyric acid, hexanoic acid, 2-methyl-propanal, and 2-methylbutanal were observed to influence the olfactory discrimination of fat content in milks and meats (**Chapters 4 and 5**). All these compounds, except for acetoin and ethanol, are lipophilic in nature (have a positive LogP value), suggesting their affinity for fat-containing environments.

Table 2. Volatile compounds that were identified in milk and meat matrices. OA: odor threshold in air (mg/m^3); OW: odor threshold in water (mg/L); d: detection threshold; r: recognition threshold

Compound name	CAS number	Odor quality	Threshold (if applicable)	LogP value
Benzaldehyde	100-52-7	almond, berry, bitter, almond, burnt sugar, cherry, fruit, malt, roasted pepper, spice, sweet	OA, d: 0.44 - 4.3 OA, r: 0.50 - 4.1 OW, d: 0.35 - 4.6	1.69
Butyric acid	107-92-6	butter, cheese, must, rancid, sour, sweat	OA, d: 0.0004 – 0.018 OA, r: 0.016 – 3.6 OW, d: 0.24 – 4.8 OW, r: 7.7	0.79
Hexanoic acid	142-62-1	acid, cheese, fermented, goat, pungent, rancid, sweat	OA, d: 0.012 – 0.52 OA, r: 0.23 – 3.5 OW, d: 0.093 – 3	1.81
Acetoin	513-86-0	butter, cream, green pepper, rancid, sour, sweat	OW, d: 0.014 – 8	-0.14
Ethanol	64-17-5	alcohol, floral, ripe apple, sweet	OA, d: 2 – 302 OA, r: 8.7 – 665 OW, d: 100 – 950 OW, r: 2000 – 11100	−0.18
Hexanal	66-25-1	apple, cut grass, fresh, fruit, grass, green, oil	OA, d: 0.0014 – 0.33 OA, r: 0.02 – 0.16 OW, d: 0.0045 - 0.005 OA, r: 0.01	1.65
Chloroform	67-66-3	Hay	OA, d: 30 – 1350 OA, r: 480 – 622 OW, d: 0.1 – 1	1.83
1-Pentanol	71-41-0	almond, balsamic, fruit, green, medicine, yeast	OA, d: 0.8 – 35 OA, r: 30 – 80 OW, d: 4 – 70	1.25

Compound name	CAS number	Odor quality	Threshold (if applicable)	LogP value
2-methyl-propanal	78-84-2	caramel, cocoa, floral, fresh, green, malt, nut	OA, d: 0.015 – 0.14 OA, r: 0.41 OW, d: 0.0015 – 0.0435	0.6
2-Butanone	78-93-3	butterscotch, ether, fragrant, fruit, pleasant, solvent, sweet	OA, d: 1.3 – 250 OA, r: 16 – 163 OW, d: 17 – 35	0.81
2-Methylbutanal	96-17-3	almond, chocolate, cocoa, fermented, hazelnut, malt, nut	OW, d: 0.001 – 0.0125 OW, r: 0.004	1.31

Although these compounds were identified in milk and meat matrices, their metabolic sources maybe different. For example, short chain fatty acids such as butyric acid and hexanoic acid may derived from the fermentation of dietary fiber by gut bacteria in the cow's digestive system and be absorbed into the bloodstream and can eventually end up in the milk fat of lactating cows [26]. While in pork and beef, expect the pervious resource, they can also be formed through the elongation and desaturation of shorter-chain fatty acids in animal tissue [27]. Aldehydes and alcohols such as hexanal, 1-pentanol, and 2-methyl-propanal, are the Maillard reaction products of thermal sterilization of milks, while in beef and pork, they can also be formed from oxidation of fatty acids in animal tissues and Maillard reactions [28-30]. Moreover, it is noteworthy that all these compounds, with the exception of ethanol and chloroform, have a threshold concentration in air below 5 mg/m^3, indicating that their odors may be perceivable by humans through olfaction at low concentrations. The unique odor perceptions associated with each of these compounds are of particular interest. Notably, butyric acid, hexanal, and acetoin contribute to buttery and creamy odors, closely resembling the perception of fat content [31]. Additionally, two acids, butyric acid and hexanoic acid, exhibit odors reminiscent of fat decay, such as sour and rancid aromas, which could also contribute to the overall perception of fat in food matrices. The presence of these shared volatile compounds in both milk and meat matrices suggests the existence of common odor profiles that may contribute to the perception of fat in diverse food matrices. However, more studies employing different types of fat or food matrices, such as vegetable oil, chicken, lamb, and sea food, are needed to validate whether there are common volatiles or volatile compositions that facilitate the discrimination of foods differing in fat content by olfaction.

Relevance and Implications

This thesis delves into the role of olfaction in perceiving tastants and fatty acids, providing substantial evidence to confirm the involvement of olfaction in perceiving fatty acids and fat in foods. By examining the influence of olfaction on perception of tastant and fatty acids, our research contributes to a deeper understanding of how the sense of smell plays a crucial role in shaping our overall sensory experience. Furthermore, these findings add to the ongoing discussion surrounding the consideration of fat as the sixth basic taste. Our findings suggest that the oral perception of fatty acids cannot be simplified as one (basic) taste perception as fatty acids present a distinguishable odor. The oral perception of fatty acids may thus be a more complicated sensory perception.

In addition to investigating the role of olfaction in tastant and fatty acid perception, we also identified orthonasal olfactory discriminable fat content differences in milk and meat matrices. Such knowledge could be beneficial for food industry, as humans did not notice a (olfactory) difference when fat content in food was reduced at certain amount (e.g., 3% fat content difference in UHT milk; 25% fat content difference in roast pork). Besides, our study analyzed the composition of volatile compounds that potentially impact the olfactory perception of fat. Such knowledge can be applied to enhance fat related perception in low fat content foods or reduce fat content in foods without sacrificing hedonic evaluations/properties by adding fat odor related compounds into a food matrix. Furthermore, the identification of fat odor related compounds across food matrices that relate to fat perception pave the way for the mimicry of fat flavor in plant-based products, which is a key and difficult problem for the innovation of meat analogs.

Limitations and future perspective

Supra-additive effect of tastant odor

Previous researchers observed that blockage of olfactory input can decrease the perceived taste intensity of tastant solutions [5, 32]. This may be because of the "supra-additive" effect of sensory input. Specifically, when different sensory modalities, such as olfactory, gustatory, and tactile stimuli, are presented together, they can interact with each other and create an enhanced overall perception stronger than any of the single sensory modalities [33]. For example, the perception of sweet taste is rated higher when vanillin odor was added, and similarly the other way around, the vanillin flavor was rated higher when sweet taste input

was increased [34, 35]. However, this synergistic effect is usually observed for tastes and odors that are congruent with each other [36]. It seems that even if the odor quality of tastant solutions is not associated with their relative taste (**Chapter 2**), the blockage of such odors may still decrease perceived taste intensity [5, 32]. More studies are needed to validate the odor quality of tastant solution and explore whether such odor influence the perception of taste intensity.

Aerosols in oral cavity

In **Chapter 2**, we investigated the orthonasal and retronasal olfactory perception of tastants and fatty acids. It is important to note that our exploration focused solely on the (ortho- and retronasal) olfactory perception of the volatile composition in the headspace. Thus, oral processing, as well as related gustatory and textural perception, were not involved in our experiments. Since the used tastants themselves are non-volatile, they are not present in the headspace and, therefore, do not directly contribute to olfactory perception. However, aerosols can be formed during the oral processing of liquid food, specifically through processes like sipping, swishing, or spraying, which leads to the generation of small droplets of liquid suspended in the air as aerosols in the oral cavity [37]. Considering limited volatile compounds were identified in Chapter 2, we hypothesize that non-volatile compounds, such as tastants and fatty acids themselves, may be carried by aerosols and transferred to the nasal cavity through the mouth cavity during breathing. These transfer pathways may offer non-volatile components the opportunity to trigger olfactory perception. However, this hypothesis requires further validation with experimental studies. For example, there is currently no direct evidence to prove that aerosols can be formed and transferred into the nasal cavity during the consumption of liquid food. Furthermore, even if the aerosols can carry non-volatile components such as tastants into the nasal cavity, it remains unknown whether humans can perceive those non-volatiles in the nasal cavity. Previous studies indicated that taste receptors are present in other parts of the human body beyond the mouth [38, 39], however, they do not seem to contribute to taste perception. There could also be other receptors or channels in nasal cavity that contribute to the perception of non-volatile components, but more human and animal studies on molecular level are needed to verify this hypothesis.

Future studies could employ technologies such as endoscopy coupled with high-speed cameras [40, 41] to capture generation and movements of aerosol in the oral and nasal cavities during oral processes, providing more direct evidence of aerosol behavior during

oral processing. Considering the gustatory input in the oral cavity, future studies could begin by generating *in vitro* tastant aerosols using aerosol generators and subsequently explore the orthonasal olfactory perception of tastants. Additionally, the characteristics of aerosols, such as temperature, size, surface area, and composition, generated through the oral process might differ from that through in vitro generators. Therefore, more studies are needed to characterize aerosols generated during oral processing to ensure accurate in vitro experiments.

Study population and individual difference

Despite the established contribution of olfaction to fat perception, there remains an ongoing scientific discussion regarding potential individual differences in olfactory sensitivity to fat. Our experimental observations revealed no effect of individual differences on olfactory fat content discrimination ability. However, it is essential to acknowledge that the majority of participants in our experiments (**Chapter 2, Chapter 4, and Chapter 5**) were students on campus, which may limit the diversity in demographics, such as age and BMI. Moreover, their educational and knowledge backgrounds could potentially influence their behaviors in discrimination tasks.

While some studies [12, 13] have explored the influence of demographics on the ability to discriminate fat content through olfaction, the focus has been somewhat limited. For instance, one study specifically investigated the difference between normal-weight and overweight populations [12] but found no differences between these groups in olfactory fat perception. However, compared to the considerable number of studies examining the effect of demographics on gustatory perception [42, 43], the population and sample size in studies exploring the impact of demographics on olfactory perception, including ours, remain limited. Considering lean populations were observed to have higher sensitivity for gustatory perception of fatty acids compared with obese population [44, 45], similar effect could also be expected for olfactory perception of fatty acids. To gain a comprehensive understanding of how demographic variability influences olfactory perception of fat, further research with a wider range of ages and BMI levels is necessary. Furthermore, the difference in olfactory fat perception sensitivity between individuals may also lead to different eating behavior. For example, individuals with low fat odor sensitivity may ingest Fat-rich foods to reach certain hedonic perspective compared with individuals with high fat odor sensitivity. It could also be that individuals with low fat odor sensitivity consume more fat without notice. Future studies could explore the relationship between olfactory fat perception sensitivity and dietary

intake habits, to gain a better understanding of how olfactory perception of fat influence dietary intake.

In **Chapter 4**, we investigated the influence of dairy fat intake on olfactory fat content discrimination ability and found no discernible effect. However, it is crucial to note that dietary fat types are diverse in various food sources, and focusing solely on dairy fat may not be sufficient to conclude that the intake of fat does not influence olfactory perception of fat. Previous studies have indicated that the intake of fat-rich nuts may positively affect sensitivity to fatty acid odors [46]. However, the discussion remains ongoing, as limited studies have investigated whether and how the intake of fat or other nutrients influences olfactory perception of fat. More research including different types of fat is needed to provide a more comprehensive understanding of the potential influence of dietary factors on olfactory fat perception.

Chemical analysis

There are some methodological considerations in the chemical analysis of volatile compound composition. In **Chapter 4 and 5** we used HS-SPME to extract the volatile compounds from the headspace of samples. Although this is a frequently used method to quantify VOCs in foods [47, 48], limited absorption capacity of the SPME fiber may have led to competitive adsorption among volatile compounds, which can decrease the accuracy of measurements. Furthermore, in **Chapter 5,** we did not quantify the (relative) concentration of volatile compounds in the headspace because we could not add and evenly distribute internal standards into an intact meat matrix without destroying it, so we cannot state that all compounds identified in our experiment have an olfactory contribution. Previous studies used to quantify volatile compounds in meat by adding internal standards into minced meat to distribute the internal standards evenly [49-51]. This is not appliable for our study as mincing would break the original structure of meats and thus influence the release of volatile compounds, which may lead to a different volatile compound composition compared with the headspace of the sensory experiments. Furthermore, in this thesis we only profiled the volatile compound compositions in the headspace of samples and did not verify the olfactory perception of these volatile compound and compositions. Future studies could use GC-O or GC-O-MS to identify the key odor active compounds that are responsible for olfactory fat perception.

Throughout this thesis, we have identified a subset of common compounds that are consistently present across different food matrices, including milk and meat products. These compounds, such as butyric acid, hexanal, and acetoin, are known to contribute to the characteristic sensory attributes of fat-containing foods. However, it is essential to acknowledge that the perception of these compounds in real food matrices is not isolated but rather subject to the influence of other volatile compounds present in the mixture. The olfactory perception of volatile compound composition, as well as the perception of fat in real food matrices, is a complex perception influenced by the interactions between compounds. When multiple odorants are present together, they can interact with each other, profoundly shaping the overall perception of the aroma. This interaction of compounds can lead to various outcomes, such as enhanced perception, new odor quality perceptions, masking of certain odors, and even modulation of overall perception [52]. Especially in daily life, the olfactory perceptions of foods are usually based on combined, interacting volatile compound compositions. Therefore, it is crucial to recognize the importance of interaction between volatile compounds when investigating the olfactory perception of fat in various food matrices [53, 54]. Future validation and studies should encompass the complex interactions between volatile compounds to gain a comprehensive understanding of how different volatile compounds collectively contribute to the overall olfactory perception of fats. Such knowledge will enable researchers and food industry professionals to craft more precise and nuanced approaches the hedonic experience of low-fat content foods by adding fat odors.

Conclusions

To answer the three research questions of this thesis, we conclude that humans can discriminate the odor of tastant and fatty acid solutions from blank through olfaction; that humans also possess the ability to discriminate fat content in real food matrices such as pasteurized milks, beef, and pork; that volatile compound generated through fat oxidation, degradation, and Maillard reaction during thermal processing of food contribute to olfactory fat content discrimination.

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Summary

The perception of fat taste has been proposed as the sixth basic taste, with fatty acids suggested as the responsible tastants. A significant distinction between traditional tastants and fatty acids is that tastants are generally considered odorless, while fatty acids may exhibit certain odors. Nonetheless, the role of olfaction in the perception of fatty acids and tastants remains unclear. Moreover, if fat does emit odor, the question arises whether this can serve as a signal for detecting fat content in foods from a distance. Consequently, this thesis aims to explore the role of olfaction in perceiving and discriminate fat content in actual food matrices by addressing three research questions: 1) Can humans perceive fatty acids (and tastants) through olfaction? 2) Can humans discriminate fat content in real food matrices? 3) What volatile compounds facilitate the olfactory discrimination between foods differing in fat content?

In **Chapter 2**, olfactory triangle discrimination tests were performed to explore whether humans could discriminate solutions of basic tastants and fatty acids form blank through orthonasal and retronasal olfaction. ITEX-GC-MS were employed to examine what volatile odor compounds underlie the discrimination ability. We found that participants were able to distinguish all tastant and fatty acid solutions from blank through orthonasal olfaction. In addition, sucrose, sodium chloride, oleic acid, and linoleic acid were distinguished from blank by retronasal olfaction. The perceived odor of tastant solutions was not associated with their taste quality, whereas the odor of fatty acids could be associated to fat perception. Ethyl dichloroacetate, methylene chloride, and acetone were identified in the headspace of sucrose, MSG and quinine solutions but not in water (blank). Fat oxidation compounds such as alcohols and aldehydes were detected in the headspace of the oleic and linoleic acid solutions and could be responsible for their identifiable odor.

Next, **in Chapter3**, a systematic scoping literature review was performed to identify and summarize relevant evidence on the contribution of olfaction to dietary fat perception and highlight relevant knowledge gaps. Overall, 42 articles were included based on our search, and findings from those articles were consistent with the notion that olfaction plays a role in the perception of dietary fat in rodents and humans. Rodents can perceive dietary fat via olfactory cues, and this ability may affect their preference for fat-containing feed. Humans can detect, discriminate, and identify fat and its constituents solely by olfaction, even when embedded within a complex food matrix. Food fat content can modulate the perception of various fat- and non-fat olfactory qualities, depending on the food matrix and odorant physio-chemical properties. On the other hand, the presence of fat-related odors can modify the

perception of olfactory and non-olfactory sensory qualities (e.g., mouthfeel). However, the nature of chemical signals underlying olfactory fat perception remains unknown.

In **Chapter 4,** olfactory triangle discrimination tests were performed to investigate whether humans can discriminate fat content in pasteurized milks and UHT milks differing in fat content. HS-SPME-GC-MS was used to characterize the volatile compound compositions of the headspace of samples differing in fat content. We found that fat contents can be discriminated by olfaction in commercially available pasteurized milks but not in UHT milks. UHT milks are perceived higher in odor intensity than pasteurized milks. The compositions of volatile compounds of pasteurized milks differed with fat content, whereas the volatile compound compositions of UHT milks differing in fat content were similar. PLSR performed among sensory and chemical data revealed that the olfactory discrimination of fat content in pasteurized milks is facilitated by differences in volatile compound composition rather than odor intensity. As for UHT milks, the perception acetoin and 2-heptanone may mask odor differences, leading to their indistinguishable odors.

In **Chapter 5**, olfactory fat content discrimination ability was examined in beef and pork differing in fat content. Following similar methodology as in **Chapter 4,** we found that fat content in both raw and roasted samples can be distinguished through orthonasal olfaction, but perceived odor differences did not always contribute to olfactory fat content ranking. Roasted samples with higher fat content had more abundant fatty acids, aldehydes, and ketones in their headspace. Phthalic acid, isobutyl 2-ropylpentyl ester, and carbon disulfide facilitated the olfactory discrimination of fat content in raw pork and beef samples. 2-Methyl-propanal, benzaldehyde, 1-hydroxy-2-propanone, 2,3-pentanedione, 2,5-octanedione, and 2-butanone contributed to odor differences of roasted beef samples differing in fat content.

In summary, this thesis found that human possess the ability to detect fatty acids and to discriminate fat content in real food matrices such as pasteurized milks, beef, and pork through olfaction, the olfactory, smaller olfactory discriminable fat content differences were observed for milks compared with meats. Volatile compounds generated through fat oxidation, degradation, and Maillard reaction during thermal processing of food contribute to olfactory perception of fat. These findings contribute to a better understanding of the role of olfaction in perceiving fat in foods. Identification of olfactory discriminable fat content difference may aid in reducing fat content in food without losing hedonic perception, while the identification of volatile compound compositions that contribute to fat odor may help enrich the enjoyable fat flavor of low-fat and/or plant-based foods. However, more studies

are needed to further validate the olfactory perception of volatile compound compositions identified to in our studies before applying this knowledge towards mimicking fat flavor in food products. Future studies should also focus on how the ability of smelling fat influence human intake and eating behavior.

Acknowledgement
About the author
List of publications
Overview of completed training activities

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About the author

Shuo Mu was born on November 20, 1994, in Baotou, Inner Mongolia, China. With strong interest and enthusiasm in food, he started his study in university.

In 2013, Shuo started his BSc study in food science major in Food Quality and Safety at China Agricultural University and obtained his bachelor's degree in 2017. He continued his study at China Agricultural University and followed the master project. During his master's project, Shuo studied at the Key Laboratory of Functional Dairy in Beijing and worked on the topic "creamy feeling in fermented dairy products". Shuo finished his master program in 2019 and obtained a master degree of Agriculture, specialty of Food Processing and Safety.

In 2020, Shuo received financial support from the Chinese Scholarship Council and started his PhD at the Division of Human Nutrition and Health at Wageningen University & Research. He was supervised by Dr. Sanne Boesveldt and Prof. Dr. Markus Stieger and worked on the research project "the olfactory perception of fat in food". During his PhD project, Shuo attended various courses and he presented his work at serval international conferences. He was involved in assisting course of Food Properties and Functions and supervising MSc students. He was a member of PhD committee in division of Human Nutrition and Health. He was also a part of the organizing committee of the PhD tour to Italy and Switzerland in 2022.

List of publications

Publications in peer-reviewed journals

Mu, S., Pirc, M., Frissen, G., Stieger, M., & Boesveldt, S. (2023). Smells like fat: A systematic scoping review on the contribution of olfaction to fat perception in humans and rodents. *Food Quality and Preference*, 104847. DOI: https://doi.org/10.1016/j.foodqual.2023.104847

Mu, S., Stieger, M., & Boesveldt, S. (2022). Olfactory discrimination of fat content in milks is facilitated by differences in volatile compound composition rather than odor intensity. *Food Chemistry*, 393, 133357. DOI: https://doi.org/10.1016/j.foodchem.2022.133357

Mu, S., Ni N., Zhu Y., Boesveldt, S. & Stieger. M. How volatile composition facilitates olfactory discrimination of fat content in beef and pork. *Food Research International.* Being accepted for publication.

Mu, S., Ren, F., Shen, Q., Zhou, H., & Luo, J. (2022). Creamy mouthfeel of emulsion-filled gels with different fat contents: Correlating tribo–rheology with sensory measurements. *Food Hydrocolloids,* 131, 107754. DOI: https://doi.org/10.1016/j.foodhyd.2022.107754

Mu, S., Liu, L., Liu, H., Shen, Q., & Luo, J. (2021). Characterization of the relationship between olfactory perception and the release of aroma compounds before and after simulated oral processing. *Journal of Dairy Science*, 104(3), 2855-2865. DOI: https://doi.org/10.3168/jds.2020-19026

Mu, S., Liu, X., Luo, J., Ren, F., Li, B., Wang, N., . . . Ge, K. (2019). Effect of raw milk somatic cell count on protein hydrolysis and flavor and texture quality of hard cheese. *Shipin Kexue/Food Science*, 40(15), 64-70. https://www.spkx.net.cn/EN/Y2019/V40/I15/64

Mu, S., Lu, Y., Gao, Y., & Mao, L. (2018). Effect of pectin and preheating on the structure and stability of mixed vitamins in protein emulsion gels. *Shipin Kexue/Food Science*, 39(18), 29-34. https://www.spkx.net.cn/EN/Y2018/V39/I18/29

Bing, Z., Tian-shu, L., **Shuo, M.,** Peng-jie, W., Qing-wu, S., & Jie, L. (2021). Using Spectroscopy Methods to Analyze the Key Textural Characteristics of Fermented Milk with High Creaminess Intensity. *Spectroscopy and Spectral Analysis*, 41(4), 1194-1198. http://www.gpxygpfx.com/EN/Y2021/V41/I04/1194

Publications under review

Mu, S., Stieger, M., & Boesveldt, S. Can humans smell tastants?

Overview of completed training activities

Discipline specific course and activities

Name	Organizer and location	Year
Eurosense 2020 (oral presentation)	Elsevier; online, NL	2020
Dairy science and technology symposium (oral presentation)	FOOD Arahus University; online, NL	2021
Weurman Flavour Research Symposium 2020 (oral presentation)	Weurman; online, NL	2021
ECRO 2022 (poster presentation)	European Chemoreception Research Organization; Berlin,DE	2022
AChems annual meeting XLV (poster presentation)	Association for Chemoreception Sciences	2023
Olfactometer training	Burgart; Ede, NL	2020
Principles of Sensory Science	VLAG; Wageningen, NL	2020
Sensory perception & food preference:Into the future!	VLAG; Wageningen, NL	2021
Advanced food analysis	VLAG; Wageningen, NL	2022

General courses

Name	Organizer and location	Year
PhD week	VLAG; Baarlo, NL	2020
Scientific Writing 7	WGS; Wageningen, NL	2020
Introduction to R	VLAG; Wageningen, NL	2020
Reviewing a Scientific Manuscript	WGS; Wageningen, NL	2022
Critical thinking and argumentation	WGS; Wageningen, NL	2022
Scientific Publishing	WGS; Wageningen, NL	2022
Philosophy and Ethics of Food Science and Technology	VLAG; Wageningen, NL	2023

Optional courses and activities

Name	Organizer and location	Year
Preparation of research proposal	VLAG; Wageningen, NL	2020
Tasty Talks	HNH; Wageningen, NL	2020-2023
Club Sense	HNH; Wageningen, NL	2020-2023
PhD committee	HNH; Wageningen, NL	2020-2022
Organization PhD tour	HNH; Wageningen, NL	2021-2022
PhD tour Switzerland, Italy	HNH; Wageningen, NL	2022
Teaching Assistant in course *FCH-22308 Food Properties and Functions* Supervising MSc students	WUR; Wageningen, NL	2020-2023

Colophon

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