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

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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. Headspace-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry (SPME-GC–MS) was used to characterize the volatile compound composition 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 beef and pork meats 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 beef and pork samples differing in fat content differ in volatile compound composition of the headspace, and that these differences facilitate discrimination between samples differing in fat content based on olfaction alone.

## 1. Introduction

Overconsumption of dietary fat can contribute to the development of overweight, obesity and non-communicable diseases (Gesta, Tseng, & Kahn, 2007). 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 (Ramirez, 1993; Takeda, Sawano, Imaizumi, & Fushiki, 2001; Xavier et al., 2016). 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 (Bolton & Halpern, 2010; Chalé-Rush, Burgess, & Mattes, 2007) and can be distinguished from each other through olfaction (Kallas & Halpern, 2011). These studies suggest that humans can smell fatty acids as well.

Several studies explored the olfactory perception of fat in foods. Boesveldt and Lundstrom (2014) found that odors of reconstituted milks differing in fat content can be distinguished through orthonasal olfaction. Le Calvé et al. (2015) further reported that retronasal olfaction is involved in discriminating fat content of milks and yogurts. Pirc et al. (2022) recently confirmed that humans can discriminate between milks differing in fat content through orthonasal and retronasal olfaction. Moreover, we (Mu, Stieger, & Boesveldt, 2022) 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

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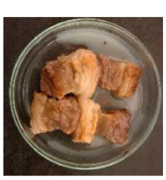
odor intensity of UHT milks may mask odor differences between UHT milks differing in fat content leading to UHT milks being undistinguishable by smell. To summarize, these studies showed that humans can discriminate between dairy foods differing in fat content based on olfaction. However, it is unknown whether this capability is limited to dairy foods or can be generalized across food categories to, for example, meats. Fernandez et al. (2000) 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 (Al-Dalali, Li, & Xu, 2022). 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 (Kerth & Miller, 2015). Fat participates in all these reactions as a precursor which influences the volatile compound composition of roasted meats (Mottram, 1998). Furthermore, the presence of fatty acids in meats also influences the odor of meats. For example, linoleic fatty acids in meats auto-oxidize and form 2-nonenal, 2,4-decadienal, 1-octen-3-one, and

**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 |
|  | 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 |

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 (Arshad et al. 2018). 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.

## 2. Materials and methods

### 2.1. 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 (Albert Heijn 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 the 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 10 g 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. Meats were unwrapped after roasting. 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)) × 3 fat levels (Low (L), Medium (M), High (H)) × 2 preparation conditions (Raw (R), rOasted (O))) were prepared. All samples were prepared one day before sensory testing and were stored in a dish covered with a lid overnight in the fridge at 4℃. Samples were taken out of the fridge one hour before testing to reach room temperature with the lid closed before tests.

### 2.2. Sensory experiments

#### 2.2.1. Participants

43 participants (mean age 25.0 ± 4.9 years; age range, 20–30 years; 38 females and 5 males; mean Body Mass Index (BMI) 21.4 ± 2.0 kg/m$^2$) were recruited from the Wageningen area and 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 (Hummel et al., 2007) 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 exempted 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 (Holm, 2013).

#### 2.2.2. 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 (select the one odd sample out of three samples of which two samples are the same) and ranking tests. Pork samples were assessed in the first session, beef and pork samples were assessed in the second and third session, the presentation orders of beef and pork samples were randomized 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 the supplementary material. Participants were blindfolded during all sensory tests to eliminate visual cues when sniffing the headspace of the samples.

#### 2.2.3. Olfactory triangle discrimination test

Olfactory triangle discrimination tests were performed to determine whether participants can discriminate the odor of meats differing in fat content. 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. Tests were performed at room temperature. 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 min between each triplet. Participants were encouraged to smell their own skin between trials to prevent adaptation during the intervals.

#### 2.2.4. Ranking test

Ranking tests were performed to determine whether participants can perceive odor differences of meats as differences in fat content. 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 min. Participants were encouraged to smell their own skin to restore smell function and prevent adaptation or olfactory fatigue during the intervals.

### 2.3. Chemical analysis

#### 2.3.1. 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 h 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 (Folch et al., 1957), 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 supplementary material. Fat content was determined in triplicate for all samples.

#### 2.3.2. 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 freshly prepared 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 h 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 (VCF) (https://www.vcf-online.nl. Van Dongen & Donders, n.d.).

### 2.4. 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.

## 3. Results

### 3.1. Olfactory discrimination ability of fat content of raw and roasted meat samples

Fig. 1 shows the results of the olfactory triangle tests for raw and roasted beef (RB and OB) (I) and pork (RP and OP) (II) differing in fat content. Both raw and roasted beef differing in fat content were distinguished through olfaction from each other [$p = 0.038$ for RBL—RBM; %Correct = 4.5 ± 0.2 vs. 24.9 ± 1.9; difference in fat content (F wt%) between samples Δ[F] = 20.4%) and RBM—RBH (%Correct = 24.9 ± 1.9 vs. 41.8 ± 0.6; Δ [F] = 16.9%), $p < 0.001$ for RBL—RBH (%Correct = 4.5 ± 0.2 vs. 41.8 ± 0.6; Δ [F] = 37.3%), OBL—OBM (%Correct = 5.2 ± 0.4 vs. 36.2 ± 3.1; Δ [F] = 31.0%), OBL—OBH (%Correct = 5.2 ± 0.4 vs. 70.1 ± 1.6; Δ [F] = 64.9%), and OBM—OBH (%Correct = 36.2 ± 3.1 vs. 70.1 ± 1.6; Δ [F] = 33.9%). However, only discriminations between pork meats with > 30% difference in fat content were possible ($p < 0.05$), whereas all discriminations between samples with < 30% difference in fat content failed (Fig. 1-II). Roasted pork with low fat content could be distinguished through olfaction from medium and high fat content [$p < 0.001$ for OPL—OPM (%Correct = 5.1 ± 0.2 vs. 43.9 ± 3.3; Δ [F] = 38.8%) and OPL—OPH (%Correct = 5.1 ± 0.2 vs. 69.3 ± 0.6; Δ

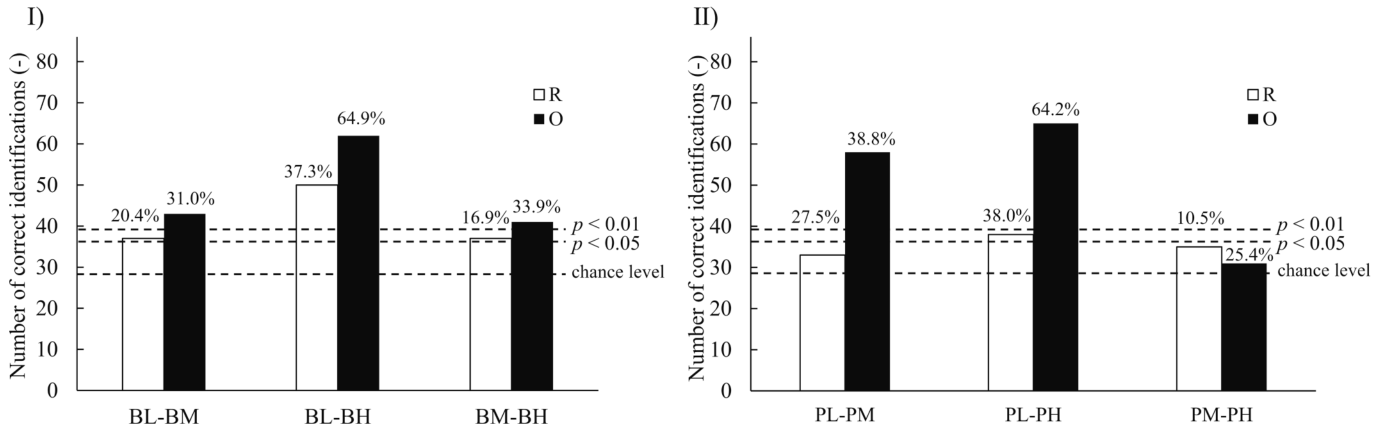



**Fig. 1.** Total number of correct identifications for each triangle discrimination test. I): olfactory discrimination for raw and roasted beef differing in fat content. II): olfactory discrimination for raw and roasted pork differing in fat content. Dotted lines indicate the minimum number of correct identifications required at chance level and 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.

[F] = 64.2%)], but the comparison of medium vs high fat content was undistinguishable through olfaction (%Correct = 43.9 ± 3.3 vs. 69.3 ± 0.6; Δ [F] = 25.4%). Moreover, the numbers of correct identifications for roasted pork comparisons were always higher than those for the corresponding raw pork comparisons (except for the PM—PH comparison).

The result of proportion of responses (discrimination based on odor intensity, quality, or unknown reasons) is shown in **Figure S1** in the 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.

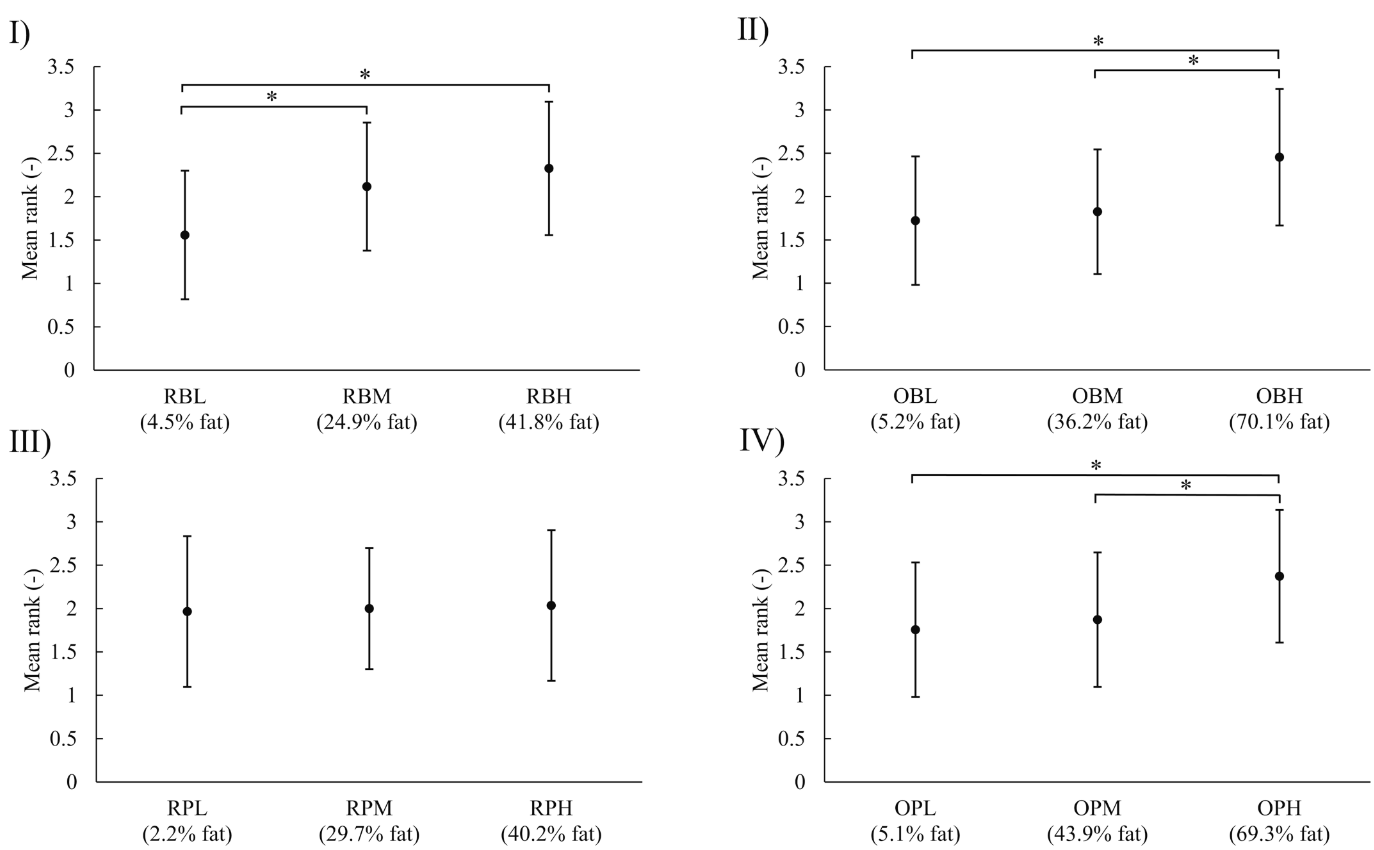



**Fig. 2.** Results of ranking test based on perceived fat content by smell ($n$ = 86, 43 participants in duplicate), mean ranks and standard error of the means are shown. I): Raw beef with low, medium, and high fat content; II): Roasted beef with low, medium, and high fat content; III): Raw pork with 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).

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

The mean and standard deviation of ranks ($n$ = 43 participants in duplicate, 86 observations) for each sample is shown in Fig. 2, the distribution of ranks is shown in **Figure S2.** Participants were able to rank samples according to their fat content based on smell for most beef and pork samples, except for raw pork. 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.

### 3.3. *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; 1-Methoxy-2-propanol, 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.

PCA was performed on the HS-SPME-GC–MS data of beef and pork separately (Fig. 3). The first and second principal components 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.

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.

### 3.4. *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 (Fig. 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 (Fig. 4-**II**).

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 VIPs of isobutyl 2-propylpentyl ester, which only identified RBM, are > 1 both in Comp1 and Comp2 (**Table S7** in the supplementary material). This indicates isobutyl 2-propylpentyl ester may be positively 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 positively correlated with correct responses for the comparisons of OBL—OBM and OBM—OBH. Octane, 2-octene, and acetic acid positively 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. Notably, the four compounds of 1-methoxy-2-propanol (OBH & OBM, increased as the fat content increased), acetonitrile (OBM, increased as the fat content increased), hexanal (OBL & OPL, reduced as the fat content increased), and benzaldehyde (OBL & OPL, reduced as the fat content increased) were commonly evaluated as important factors to discriminate between different fat-content beef and pork meats (Figs. 3, 4, **Tables S7, S8**).

## 4. Discussion

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

### 4.1. *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 meats through orthonasal olfaction, 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 meats. We also observed that the fat content differences of 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 (2022) who reported that olfactory discrimination between no-fat milks versus high-fat milks was 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 milks 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 orthonasal 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.

**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 |
| 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 |
| 1-Methoxy-2-propanol, | 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 |
| 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 | | – | 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 |

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Table 2 (*continued*)

| Volatiles in beef | Cas Number | RBL | RBM | RBH | OBL | OBM | OBH | Odor descriptor |
|---|---|---|---|---|---|---|---|---|
| 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 |
| 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 | | – | – | – | 38.28 ± 5.74[a] | 18.71 ± 4.8[b] | – | NK |
| **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* | | | | | | | | |
| **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* | | | | | | | | |

(*continued on next page*)

**Table 2** (*continued*)

| Volatiles in beef | Cas Number | RBL | RBM | RBH | OBL | OBM | OBH | Odor descriptor |
|---|---|---|---|---|---|---|---|---|
| 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 |
| **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 |
| 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 |

(*continued on next page*)

**Table 2** (*continued*)

| Volatiles in pork | Cas Number | RPL | RPM | RPH | OPL | OPM | OPH | Odor descriptor |
|---|---|---|---|---|---|---|---|---|
| **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 |
| 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 |
| 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 |

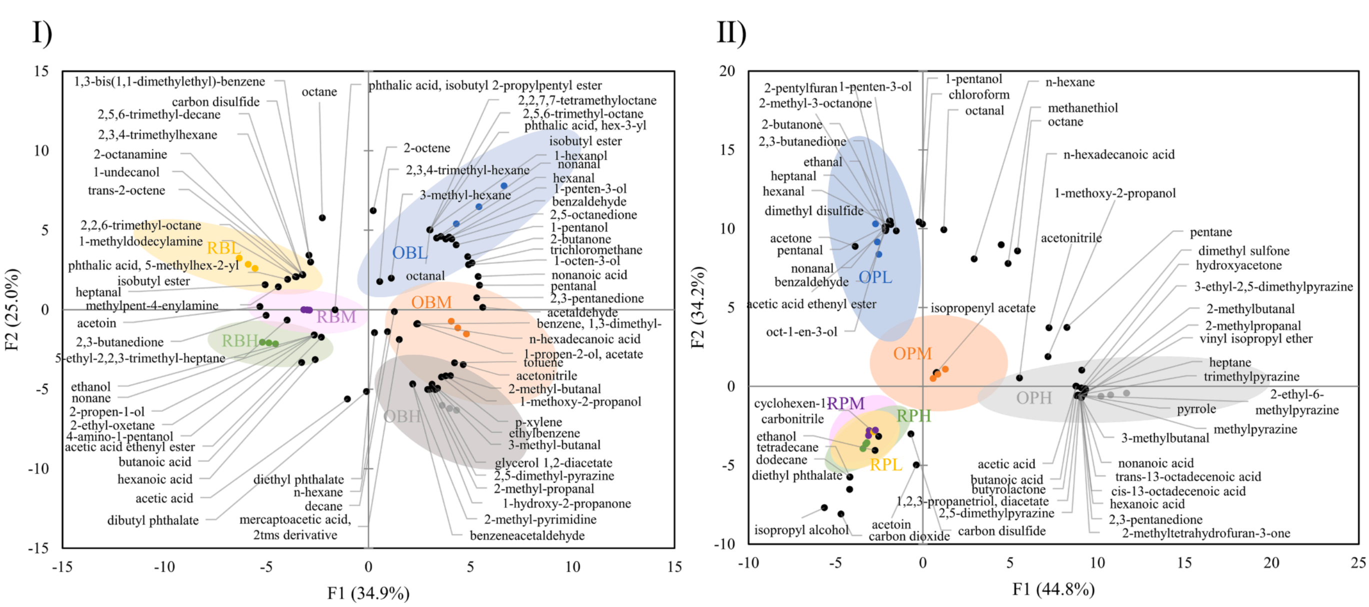


**Fig. 3.** PCA of headspace volatile compound composition of raw and roasted beef and pork meats differing in fat content. I): PCA of beef meats. II): PCA of pork meats. B = Beef; P = Pork; R = Raw; O = rOasted. L = Low fat content; M = Medium fat content; H = High fat content. The confidence ellipses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

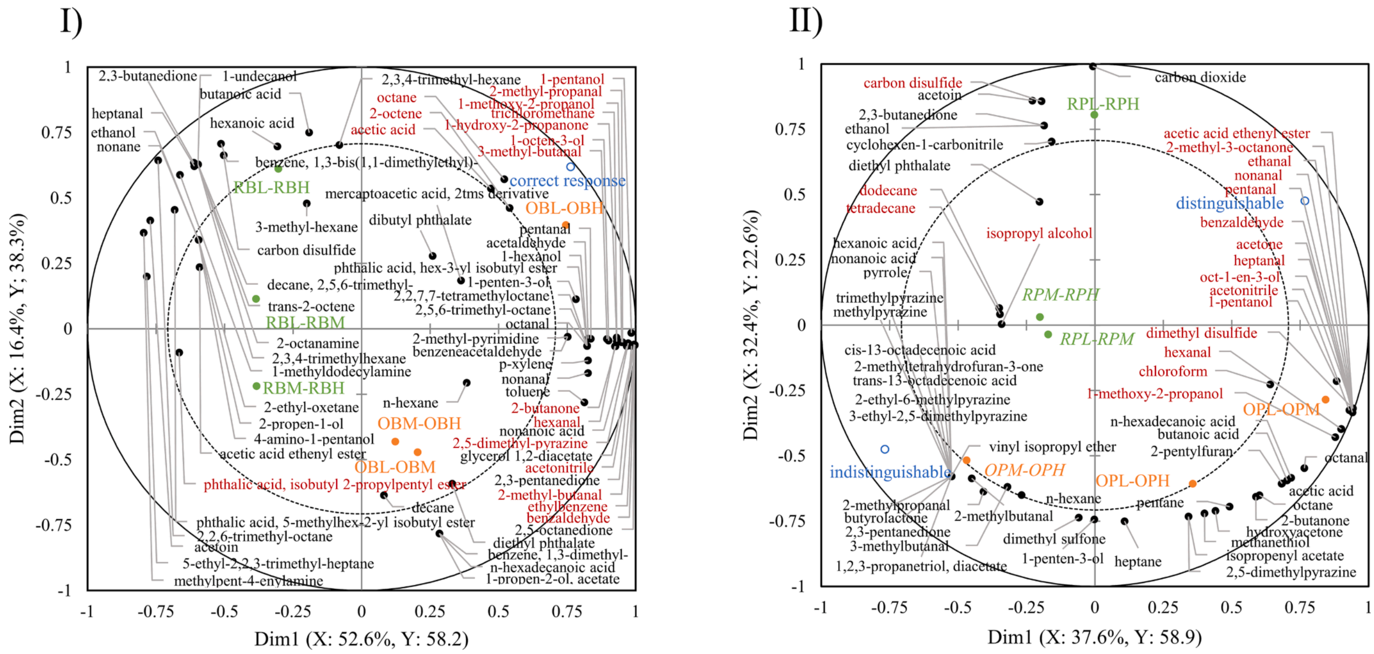


**Fig. 4.** I) PLSR of volatile compound compositions and triangle test results of beef meats. The PLSR was performed among number of correct identifications of each sample comparison and absolute difference in peak area of volatile compound in each sample comparison. II): PLS-DA of volatile compound 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 interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 (Frank et al., 2020; Narváez-Rivas, Gallardo, & León-Camacho, 2012). 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 (El-Magoli, Laroia, & Hansen, 1996). 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 (Sun, Fu, Li, & Peng, 2018), which explains why the perceived odor differences were not associated with fat content itself in raw pork, as shown from the ranking results.

### *4.2. 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 (Mottram, 1998). Furthermore, the heating in our study was in moist condition as beef and pork samples were wrapped in foil and heated in an oven, which may greatly favor lipid degradation (Kerth & Miller, 2015). Our SPME-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 (Domínguez, Gómez, Fonseca, & Lorenzo, 2014; Xu et al., 2011). 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 (Elmore et al. 1999) whereas ketones are usually formed through Maillard reactions (Martins et al. 2001). Both types of volatile compounds are identified to contribute to beef meat odors (Celia Resconi, del Mar Campo, Montossi, Ferreira, Sanudo, & Escudero, 2012; Machiels et al., 2003).

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 (Rizzi, 2008). Fuentes et al (2014) 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.

### 4.3. 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 meats, 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 (Hunt et al., 2016; Legako et al., 2015). 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. Furthermore, lipid and protein oxidation, denaturation, and breakdown may have happened when roasted meats were kept refrigerated. All samples in our experiments were prepared following a standardized protocol so that a comparable level of chemical changes across samples can be assumed. Future studies could focus on the relationships between the volatile compounds brought on by aging and those brought on by lipid content, which may give more insightful information of volatile compounds that contribute to fat perception.

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.

## 5. Conclusions

Our study demonstrates that humans can discriminate between raw or roasted beef or pork meats differing in fat content through orthonasal odors which differed in volatile compound composition. 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 beef and pork 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. **Sanne Boesveldt:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Markus Stieger:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing, Project administration.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that has been used is confidential.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113637.

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