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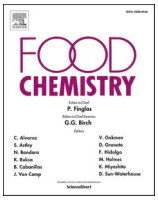

Olfactory discrimination of fat content in milks is facilitated by differences in volatile compound composition rather than odor intensity

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ABSTRACT

The mechanisms underlying the ability of humans to olfactorily discriminate fat content in milks remain unknown. In this study, we found that fat contents (0.5, 1.5 and 3.5% fat) can be discriminated by olfaction in commercially available pasteurized milks ($p < 0.05$) but not in ultra-high temperature processed (UHT) milks. The composition of volatile compounds of pasteurized milks differed with fat content, whereas that of UHT milks differing in fat content was similar. Principal component analysis revealed that differences in volatile compound composition of pasteurized milks differing in fat content contribute to olfactory discrimination. In UHT milks, acetoin and 2-heptanone may mask odor differences leading to indistinguishable odors. No differences were observed regarding perceived odor intensity of pasteurized milks or UHT milks differing in fat content. We conclude that the olfactory discrimination of fat content in pasteurized milks is facilitated by differences in volatile compound composition rather than odor intensity.

1. 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 (Frøst et al., 2001; Galindo et al., 2012; Kindleysides et al., 2017). Fat also impacts odor perception as it can act as a reservoir retaining lipophilic volatile compounds and repel hydrophilic volatile compounds (Brauss et al., 1999). In contrast to numerous studies exploring gustatory perception of fat and fatty acids (Galindo et al., 2012; Keast & Costanzo, 2015; Mattes, 2005), 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 (Xavier et al., 2016), surgery to remove olfactory bulbs (Ramirez, 1993), zinc sulfate treatment (Takeda et al., 2001) or sectioning olfactory nerve (Kinney & Antill, 1996), lost their preference for high fat content feeds. Humans can detect fatty acids (linoleic, oleic, and stearic acid), both orthonasally and retronasally (Bolton & Halpern, 2010). Humans can retronasally detect the presence of fat in milks (Le Calvé et al., 2015) and discriminate between different concentrations of fat in milk (0, 1.5 and 3.5%) based on orthonasal smell (Boesveldt & Lundstrom, 2014). Descriptive sensory analysis of margarines showed that with increasing fat content, butter and cheese odor intensity increased while creamy odor intensity decreased (Dadalı & Elmacı, 2019). 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.

Dairy fats are mainly composed of saturated triglycerides, most of which have low volatility and are thus difficult 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. More than 40 different volatile compounds were identified in the headspace of commercial pasteurized and UHT milks, including hexanal, heptanal, octanal, nonanal and 2-heptanone (Chi et al., 2021; Karatapanis et al., 2006; Valero et al., 2001). These volatile compounds were reported to be metabolized from milk fat and were suggested to contribute to perception of fat-related sensory attributes (Francis et al., 2005). It is still unknown whether or how these compounds or other volatile compounds 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 volatile compound composition and odor perception of milks. Many volatile compounds generated

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during the thermal processing of milk have been associated with cooked, stale, and sulfurous notes and are considered off-flavors (Al-Attabi et al., 2008). Dimethyl sulfide, 2-heptanone, 2-nonanone, 2-undecanone, 3-methylbutanal, and nonanal were found in ultra-high temperature processed (UHT) milks and could therefore be important contributors to the off-flavor of UHT milk (Bottiroli et al., 2020). To summarize, the volatile composition of milks is influenced by the thermal 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 volatile compound 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 volatile compound 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.

2. Materials and methods

2.1. Materials

Three commercially available pasteurized milks (De Zaanse Hoeve, The Netherlands, 0.5, 1.5 and 3.5% fat) and three commercially available UHT milks (AH Houdbare, The Netherlands, 0.5, 1.5 and 3.5% fat) were purchased from a local supermarket (Albert Heijn, Wageningen, The Netherlands). These three fat levels were chosen for both milks because they represent the most commonly consumed fat levels in commercial milks in 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).

2.2. Sensory experiments

2.2.1. 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 (Hummel et al., 2007). 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) was collected through an online questionnaire. All participants were provided written informed consent prior to participation and were paid €25 after finishing all sessions. The Medical Ethics Review Committee of Wageningen University approved the study (NL51747.081.14; ABR 51747).

2.2.2. Study procedure.

All sensory assessments were conducted in individual sensory booths at Wageningen University and Research, The Netherlands. The sensory booths were well-ventilated to ensure an odorless environment. Participants attended three sessions of 30–50 min. In the first session, a dairy food frequency questionnaire (DFFQ, shown in Table S5 in supplementary material) was filled in and perceptual rating tests were performed. The DFFQ collects information about dairy intake habits such as dairy consumption frequency (DCF), dairy intake (DI), and dairy fat intake (DFI). 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.

2.2.3. Perceptual rating test.

Participants (N = 33) rated the perceived intensity and pleasantness of each milk samples 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 anchor. 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 random three-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 min.

2.2.4. 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 30 g of milk per vial. 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.

2.3. Characterization of headspace volatile compound composition

The headspace volatile compound composition of all milks was analyzed using 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, Thermo, 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 vail 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.

A gas chromatograph system (Trace GC Ultra, Thermo, USA) coupled with a mass spectrometer (DSQ II, Thermo, USA) was used. Samples were analyzed on a Stabilwax DA capillary column (30 m × 0.25 mm ID × 0.25 μm, Munich, Germany). 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 spitless 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 compounds 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 volatile compound 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 volatile compounds

were obtained from the Volatile Compounds in Food (VCF) online database (www.vcf-online.nl, Van Dongen & Donders, n.d.).

To estimate whether concentrations of volatile compounds in the headspace of milks were higher than odor detection thresholds, odor activity values (OAV) were calculated as the ratio between relative concentration of the compound and its' detection threshold. OAVs of volatile compounds are reported in Table 1 and detailed OAV data is shown in Table S2 in the supplementary data.

2.4. 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 volatile compound compositions between samples, PCA was performed based on the relative concentrations of all volatile compounds in samples. Pearson correlation analysis was performed among volatiles found in all milks differing in fat content (pasteurized milks and UHT milks were analyzed independently) to explore the correlations between fat content and headspace volatiles. The correlation matrix as well as p-value matrix were reported in Table S3 and Table S4 in supplementary materials. Only the volatile compounds of which concentrations were higher than their thresholds were included in the following analysis. To explore the volatile compounds 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 OAVs in samples and y variables were rating scores for perceived intensity and pleasantness. To investigate volatile compounds that influence the olfactory judgement of fat content, a partial least squares discrimination analysis (PLS-DA) 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 olfactory discrimination ability of that sample comparison was used in PLS-DA. Variable importance in the projection (VIP) of variables were calculated and were reported in Table S5 in supplementary material.

3. Result

3.1. Pasteurized milks differing in fat content can be olfactory distinguished but UHT milks cannot

Fig. 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).

3.2. Odor of UHT milks is perceived more intense than odor of pasteurized milks

Fig. 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 for 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, DCF, DI, and DFI did not significantly influence the olfactory discrimination ability of milks differing in fat content.

3.3. Compositions of volatile compounds in pasteurized and UHT milks differing in fat content

The volatile compounds detected in the pasteurized and UHT milks differing in fat content are shown in Table 1. 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 *P*3.5. Tetrahydrofuran, 2-pentanone, acetoin, 2-butanone, 2-nonanone, dimethyl sulfone, 1-pentanol, butanoic acid, 1-hexanol, and ethanol were found in all three pasteurized milks differing in fat content.

Pearson correlation analyses (results are showed in Table S3 and Table S4 in supplementary material) indicated that concentration of 2-pentanone, 1-pentanol, and butanoic acid have linear positive correlations with fat content of pasteurized milks. For UHT milks, tetrahydrofuran, 2-pentanone, acetoin, 2-butanone, 2-nonanone, acetone, 2-heptanone, and benzaldehyde were found in all milks. Concentrations of acetoin, 2-nonanone, and 2-heptanone were linear positively correlated with fat content. PCA was performed on the HS-SPME- GC-MS data and the results are shown in Fig. 3. The first and second principal component explain 64.59% (33.29% for F1 and 31.30 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 ellipse areas, indicating similar volatile compound compositions of UHT milks differing in fat content. Their volatile compound 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 area 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 volatile compound composition of P0.5 differs strongly from the volatile compound composition of P1.5 and P3.5. The confidence ellipses of P1.5 and P3.5 overlap only slightly suggesting that the composition differs between P1.5 and P3.5 for several volatile compounds (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 volatile compounds (acetoin, 1-pentanol, and ethanol; see Table 1) no significant difference are observed. Overall, P0.5 is characterized by 2-methy-butanal, 1-decanol, 2-methy-1-butanol, and 2-undecanone; P1.5 is characterized by high concentrations of 1-hexanol; and P3.5 is characterized by high concentrations of 2-nonanone, butanoic acid, hexanoic acid, 1-octanol, octanoic acid, 3-methyl-butanoic acid.

3.4. Volatile compound 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

Table 1
Relative abundance, odor threshold and odor quality of volatile compounds 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	*P*1.5	*P*3.5	U0.5	U1.5	U3.5		
Ketones								
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
acetone	16.015 ± 2.548^{b}	–	–	26.979 ± 3.548^{a}	22.506 ± 4.574^{ab}	20.991 ± 3.578^{ab}	OW, R: 48.000 OW, R: 78.000	chemical, ether, nauseating, pungent
2-butanone	**7.651 ± 1.356^{d}**	**13.320 ± 2.548^{c}**	**0.757 ± 0.157^{e}**	**34.436 ± 5.658^{a}**	**27.391 ± 5.223^{ab}**	**20.401 ± 3.875^{b}**	OW, D: 5.800 OA, r: 16.000	ether, fragrant, fruit, pleasant, sweet
2-heptanone	–	**0.289 ± 0.015^{b}**	**5.960 ± 1.589^{a}**	**3.658 ± 0.897^{a}**	**4.845 ± 0.875^{a}**	**6.802 ± 1.857^{a}**	OW, D: 0.023	blue cheese, cinnamon, green, nut
2-nonanone	**0.514 ± 0.054^{a}**	**0.167 ± 0.034^{b}**	**0.695 ± 0.085^{a}**	**0.200 ± 0.036^{c}**	**0.645 ± 0.087^{a}**	**0.673 ± 0.105^{a}**	OA, D: 0.032	fragrant, fruit, hot milk, pleasant
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
2-undecanone	**0.232 ± 0.008**	–	–	–	–	–	OW, D: 0.0055	fresh, green, orange, pineapple, rose
Acids								
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
hexanoic acid	**–**	**3.407 ± 0.987^{a}**	**4.452 ± 0.753^{a}**	–	–	–	OW, D: 0.048	acid, cheese, goat, pungent, rancid
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: D: 0.001–0.002	cheese, fecal, putrid fruit, rancid, sweat
Alcohols								
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
1-butanol	0.628 ± 0.095^{a}	–	0.521 ± 0.185^{a}	–	–	–	OA, R: 45.000	alcohol, fruit, medicine, phenol, solvent
1-decanol	0.320 ± 0.085	–	–	–	–	–	OW, R: 19.400	fat, oil, plastic
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
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
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^{a}**	OW, D: 0.153	balsamic, fruit, green, medicine, yeast
2-methyl-1-butanol	**0.257 ± 0.007**	–	–	–	–	–	OW, D: 0.140 OW, R: 0.830	banana, fuel oil, green, malt, medicine
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
Aldehydes								
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
2-methyl-butanal	**0.028 ± 0.009**	–	–	–	–	–	OW, D: 0.0015 OW, R: 0.0044	almond, cocoa, fermented, hazelnut, malt
2-methyl-propanal	**0.454 ± 0.015**	–	–	–	–	–	OW, D: 0.140 OW, R: 0.410	caramel, cocoa, green, malt, nut
Others								
allyl isothiocyanate	0.184 ± 0.015^{a}	–	–	0.201 ± 0.036^{a}	–	–	OW, D: 0.375	garlic, mustard, pungent, sulfur
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
dimethyl sulfone	0.193 ± 0.023^{a}	0.107 ± 0.018^{b}	0.113 ± 0.068^{b}	–	0.253 ± 0.025^{a}	0.243 ± 0.008^{a}	N	burnt, sulfur/flavorless
pyrrole	0.255 ± 0.009	–	–	–	–	–	OW, D: 20.000	nut, sweet
styrene	–	–	–	0.180 ± 0.094^{a}	0.114 ± 0.067^{a}	–	OA, D: 26.400	balsamic, gasoline, plastic, rubber, solvent
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
trichloromethane	–	–	–	–	1.018 ± 0.057^{b}	1.690 ± 0.82^{a}	OW, D: 3700	misleadingly pleasant ethereal odor, leading to olfactory fatigue

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 the VCF online database (www.vcf-online.nl).

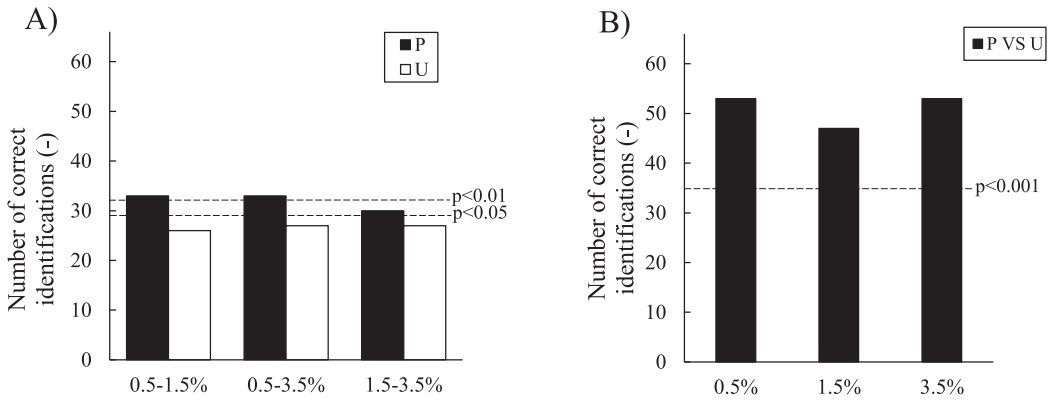

Fig. 1. Total number of correct answers for each triangle discrimination test. A) olfactory discrimination between 0.5 vs 1.5%, 0.5 vs 3.5%, and, 1.5 vs 3.5% fat content in pasteurized and UHT milks. B) olfactory discrimination between pasteurized and UHT milk with 0.5%, 1.5%, and 3.5% fat content. Dotted 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.

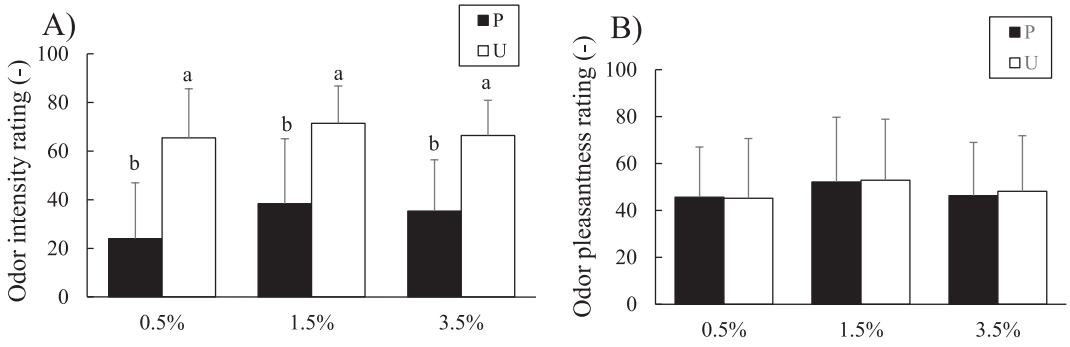

Fig. 2. Odor intensity (A) and odor pleasantness (B) ratings of milk samples differing in fat content measured on 100 mm VAS. P denotes pasteurized milk; U denotes UHT milks. Different letters above bars denote significant differences between means ($p < 0.05$). Error bars denote standard deviation.

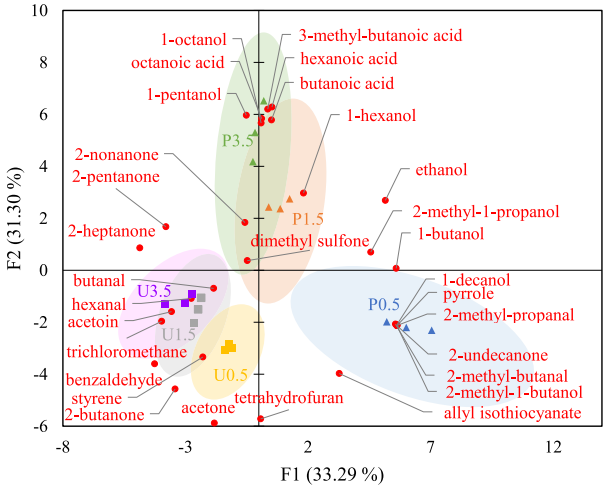

Fig. 3. PCA of volatile compounds in pasteurized and UHT milks differing in fat content. P0.5 denotes pasteurized milk with 0.5% fat content; P1.5 denotes pasteurized milk with 1.5% fat content, P3.5 denotes pasteurized milk with 3.5% fat content; U0.5 denotes UHT milk with 0.5% fat content; U1.5 denotes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

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.

3.5. *Volatile compound compositions responsible for odor intensity and pleasantness of pasteurized and UHT milks differing in fat content*

Fig. 5 shows the result of PLSR correlating the volatile compound 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%

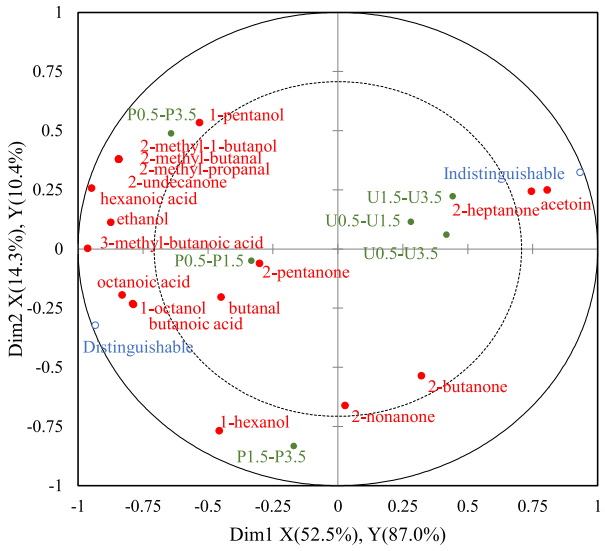

Fig. 4. PLS-DA of VOC compositions and triangle test results. The PLS-DA was performed among olfactory distinguishablity of each sample comparison and absolute difference of volatile compound content in each sample comparison. P0.5 denotes pasteurized milk with 0.5% fat content; P1.5 denotes pasteurized 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 distinguishablity, green dots represent sample comparisons, and red dots represent volatile compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

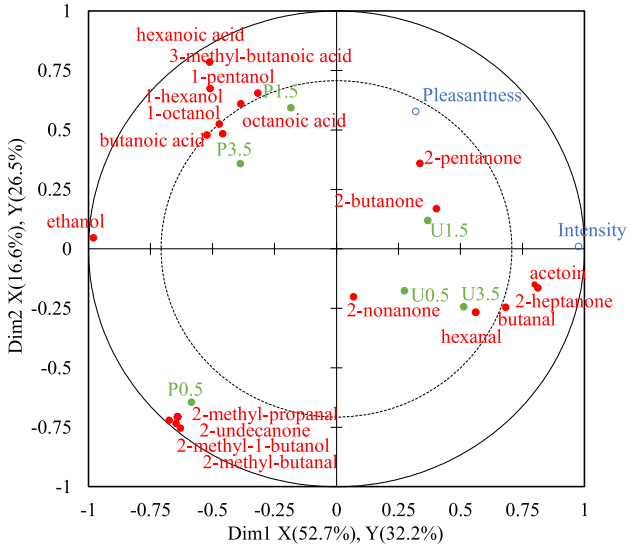

Fig. 5. PLRS correlation matrix of OAV results and perceptual rating scores of pasteurized and UHT milks differing in fat content. P0.5 denotes pasteurized milk with 0.5% fat content; P1.5 denotes pasteurized milk with 1.5% fat content, P3.5 denotes pasteurized milk with 3.5% fat content; U0.5 denotes 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 conten. Blue dots represent sensory attributes, green dots represent milk samples, and red dots represent volatile compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 (Fig. 2B). Acetoin and 2-heptanone are located close to intensity, indicating that these two volatile compounds may contribute strongly to perceived odor intensity of milks.

4. 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 compound 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 is not related to any demographic characteristics or dairy consumption frequency. Different volatile compound 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 volatile compound compositions and not by perceived odor intensity.

4.1. Different 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 (2014) previously reported that humans can orthonasally detect difference in fat content of reconstituted milks. Pirc et al. (2022) 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 volatile compound compositions. Pasteurized milks cover 70% of the global milk market share and >90% of the countries in the world mainly consume pasteurized milk (Research and Markets, 2014). 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 (Boesveldt & Lundstrom, 2014; Pirc et al., 2022). However, for non-dairy foods, Kindleysides et al. (2017) 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 taking 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.

4.2. 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 triglycerides form and not volatile, thus, 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 (Van Hekken et al., 2019). Most volatile compounds detected in pasteurized and UHT milks in our study have been previously reported (Bendall, 2001; Moid et al., 1994; Solano-Lopez et al., 2005). Our results showed that the concentration of volatile compounds 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 (Contarini, Povolo, Leardi, & Toppino, 1997; Valero, Villamiel, Miralles, Sanz, & Martınez-Castro, 2001) 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 (Van Boekel & Lindsay, 1992).

In pasteurized milks with low fat content (P0.5) we found several unique volatile compounds 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 volatile compounds are higher than 0, which means these compounds are lipophilic. The absence of these volatile compounds 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 (Wilkes et al., 2000). Similar results were reported previously (Hougaard et al., 2011), in which lipophilic volatile compounds 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 hexanoic acid which is absent 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 volatile compound composition which may have contributed to its' distinguishable odor. When comparing the volatile compounds between P1.5 and P3.5, the concentration as well as OAV of each volatile compound in the two milks were different. Higher OAVs of 2-heptanone, butanoic acid, 1-octanol, octanoic acid, and 2-nonanone were found in P3.5 whereas higher OAV of 1-hexanol and 2-butanone were found in P1.5. These differences in the volatile compound composition may have contributed to the olfactory discrimination between P1.5 and P3.5. Result of PLS-DA also confirmed that 1-hexanol is the key volatile compound that is responsible for olfactory discrimination of *P*1.5-*P*3.5.

For UHT milks, unlike pasteurized milks, the least abundance of volatile compounds was observed in U0.5, dimethyl sulfone and 1-pentanol were not found in U0.5 but were present in U1.5 and U3.5. Acetoin, 2-nonanone, and 2-heptanone were positively linear correlated with fat content. 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 (Valero et al., 2001). The concentration of these dairy fat related volatile compounds 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 (Valero et al., 2001). 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 (Jensen et al., 2015; Valero et al., 2001; Van Hekken et al., 2019). All these ketones can be generated from fatty acids, formed by β-ketoacid decarboxylation (Calvo & de la Hoz, 1992) while acetoin was reported to be generated from citrate, which can be generated from triglycerides in dairy products (Cheng, 2010). We also observed from PLS-DA that acetoin and 2-heptanone were responsible for the indistinguishable odor of UHT milk comparisons. Although the volatile compound 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 (Dadalı & Elmacı, 2019; Tian et al., 2020), 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 volatile compounds 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 volatile compound 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 volatile compounds. Higher odor intensity of UHT milks differing in fat content may have masked odor differences and may have disabled their olfactory discrimination.

4.3. UHT milks have stronger odor intensity than pasteurized milk with same fat content

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 volatile compound compositions, which in turn leads to different odor perceptions. Many studies indicated that thermal processing has a major impact on the volatile compound composition of milks, and that the key changes in volatile compound composition of milks during thermal treatment have been associated with Maillard reactions (Colahan-Sederstrom & Peterson, 2005). Such changes in volatile compound composition can lead to stronger flavor of UHT milks compared to pasteurized milks (Perkins & Deeth, 2001), 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 volatile compounds 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 have milky/buttery/creamy odor, 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. All these results indicate that 2-heptanone and acetoin are potential aroma compounds that might allow to enhance the odor intensity and perception of fat related sensory attributes in UHT milks. It might be possible to reduce fat content in milks while maintaining fat related sensory perceptions by modifying the volatile compound composition. Further studies are needed to confirm these hypotheses.

4.4. Limitations and future recommendations

To investigate the volatile compound 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 volatile compounds from the milks. Although this is a frequently used method to quantify volatile compounds in foods (Karatapanis, Badeka, Riganakos, Savvaidis, & Kontominas, 2006; Vazquez-Landaverde, Velazquez, Torres, & Qian, 2005; Yin et al., 2021), limited absorption space in the SPME fiber may have led to competitive adsorption among volatile compounds, which can decrease quantification precision. OAVs were included in our study to

estimate olfactory contribution of each volatile compound to the odor, but this approach has limitations as the correlations between OAV and perceived intensity are non-linear. Furthermore, perceptual interactions between volatile compounds are ignored by this approach as the presence of one volatile compound can influence the odor perception of other volatile compounds. 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.

5. Conclusions

Our study demonstrates that humans can discriminate between varying fat contents in pasteurized milks, but not in UHT milks, based on smell. The unique volatile compound composition of pasteurized milk with low fat content (0.5 %) which is different compared to pasteurized milks with higher fat content (1.5 and 3.5 %) contributes to its' distinguishable odor, whereas 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. These findings may aid in the development of strategies to use odor-induced enhancement of fat related sensations in foods and by those means may potentially help to lower fat content of foods while maintaining sensory properties and hedonics.

CRediT 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.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133357.

References

Al-Attabi, Z., D'arcy, B., & Deeth, H. (2008). Volatile sulphur compounds in UHT milk. *Critical Reviews in Food Science and Nutrition, 49*(1), 28–47. https://doi.org/10.1080/10408390701764187

Bendall, J. G. (2001). Aroma compounds of fresh milk from New Zealand cows fed different diets. *Journal of Agricultural and Food Chemistry, 49*(10), 4825–4832. https://doi.org/10.1021/jf010334n

Boesveldt, S., & Lundstrom, J. N. (2014). Detecting fat content of food from a distance: Olfactory-based fat discrimination in humans. *PloS one, 9*(1), e85977.

Bolton, B., & Halpern, B. P. (2010). Orthonasal and retronasal but not oral-cavity-only discrimination of vapor-phase fatty acids. *Chemical Senses, 35*(3), 229–238. https://doi.org/10.1093/chemse/bjq002

Bottiroli, R., Dario Troise, A., Aprea, E., Fogliano, V., Vitaglione, P., & Gasperi, F. (2020). Chemical and sensory changes during shelf-life of UHT hydrolyzed-lactose milk produced by "in batch" system employing different commercial lactase preparations. *Food Research International, 136*, 109552. https://doi.org/10.1016/j.foodres.2020.109552

Brauss, M. S., Linforth, R. S., Cayeux, I., Harvey, B., & Taylor, A. J. (1999). Altering the fat content affects flavor release in a model yogurt system. *Journal of Agricultural and Food Chemistry, 47*(5), 2055–2059. https://doi.org/10.1021/jf9810719

Calvo, M. M., & de la Hoz, L. (1992). Flavour of heated milks. A review. *International Dairy Journal, 2*(2), 69–81. https://doi.org/10.1016/0958-6946(92)90001-3

Cheng, H. (2010). Volatile flavor compounds in yogurt: A review. *Critical Reviews in Food Science and Nutrition, 50*(10), 938–950. https://doi.org/10.1080/10408390903044081

Chi, X., Shao, Y., Pan, M., Yang, Q., Yang, Y., Zhang, X., … Sun, B. (2021). Distinction of volatile flavor profiles in various skim milk products via HS-SPME–GC–MS and E-nose. *European Food Research and Technology, 247*(6), 1539–1551. https://doi.org/10.1007/s00217-021-03730-0

Colahan-Sederstrom, P. M., & Peterson, D. G. (2005). Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. *Journal of Agricultural and Food Chemistry, 53*(2), 398–402. https://doi.org/10.1021/jf0487248

Contarini, G., Povolo, M., Leardi, R., Toppino, P., et al. (1997). Influence of heat treatment on the volatile compounds of milk. *Journal of Agricultural and Food Chemistry, 45*(8), 3171–3177. https://doi.org/10.1021/jf960849s

Dadalı, C., & Elmacı, Y. (2019). Characterization of volatile release and sensory properties of model margarines by changing fat and emulsifier content. *European Journal of Lipid Science and Technology, 121*(6), 1900003. https://doi.org/10.1002/ejlt.201900003

Francis, L. L., Chambers, D. H., Kong, S. H., Milliken, G. A., Jeon, I. J., & Schmidt, K. A. (2005). Serving temperature effects on milk flavor, milk aftertaste, and volatile compound quantification in nonfat and whole milk. *Journal of Food Science, 70*(7), s413–s418. https://doi.org/10.1111/j.1365-2621.2005.tb11485.x

Frøst, M. B., Dijksterhuis, G., & Martens, M. (2001). Sensory perception of fat in milk. *Food Quality and Preference, 12*(5–7), 327–336. https://doi.org/10.1016/S0950-3293(01)00018-0

Galindo, M. M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J.-D., Hofmann, T., … Behrens, M. (2012). G protein–coupled receptors in human fat taste perception. *Chemical Senses, 37*(2), 123–139. https://doi.org/10.1093/chemse/bjr069

Hougaard, A. B., Vestergaard, J. S., Varming, C., Bredie, W. L., & Ipsen, R. H. (2011). Composition of volatile compounds in bovine milk heat treated by instant infusion pasteurisation and their correlation to sensory analysis. *International Journal of Dairy Technology, 64*(1), 34–44. https://doi.org/10.1111/j.1471-0307.2010.00641.x

Hummel, T., Kobal, G., Gudziol, H., & Mackay-Sim, A. (2007). Normative data for the "Sniffin'Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: An upgrade based on a group of more than 3,000 subjects. *European Archives of Oto-Rhino-Laryngology, 264*(3), 237–243. https://doi.org/10.1007/s00405-006-0173-0

Jensen, S., Jansson, T., Eggers, N., Clausen, M. R., Larsen, L. B., Jensen, H. B., … Bertram, H. C. (2015). Storage-induced changes in the sensory characteristics and volatiles of conventional and lactose-hydrolyzed UHT processed milk. *European Food Research and Technology, 240*(6), 1247–1257. https://doi.org/10.1007/s00217-015-2427-9

Karatapanis, A. E., Badeka, A. V., Riganakos, K. A., Savvaidis, I. N., & Kontominas, M. G. (2006). Changes in flavour volatiles of whole pasteurized milk as affected by packaging material and storage time. *International Dairy Journal, 16*(7), 750–761. https://doi.org/10.1016/j.idairyj.2005.06.008

Keast, R. S., & Costanzo, A. (2015). Is fat the sixth taste primary? Evidence and implications. *Flavour, 4*(1), 1–7. https://doi.org/10.1186/2044-7248-4-5

Kindleysides, S., Beck, K. L., Walsh, D. C., Henderson, L., Jayasinghe, S. N., Golding, M., & Breier, B. H. (2017). Fat sensation: Fatty acid taste and olfaction sensitivity and the link with disinhibited eating behaviour. *Nutrients, 9*(8), 879. https://doi.org/10.3390/nu9080879

Kinney, N. E., & Antill, R. W. (1996). Role of olfaction in the formation of preference for high-fat foods in mice. *Physiology & Behavior, 59*(3), 475–478. https://doi.org/10.1016/0031-9384(95)02086-1

Le Calvé, B., Saint-Léger, C., Babas, R., Gelin, J. L., Parker, A., Erni, P., & Cayeux, I. (2015). Fat perception: How sensitive are we? *Journal of Texture Studies, 46*(3), 200–211. https://doi.org/10.1111/jtxs.12120

Mattes, R. D. (2005). Fat taste and lipid metabolism in humans. *Physiology & Behavior, 86*(5), 691–697. https://doi.org/10.1016/j.physbeh.2005.08.058

Moid, L., Etievant, P., Langlois, D., Dekimpe, J., & Addeo, F. (1994). Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis. *Journal of Dairy Research, 61*(3), 385–394. https://doi.org/10.1017/S0022029900030806

Perkins, M. L., & Deeth, H. C. (2001). A survey of Australian consumers' attitudes towards UHT milk. *Australian Journal of Dairy Technology, 56*(1), 28. https://www.proquest.com/scholarly-journals/survey-australian-consumers-attitudes-towards-uht/docview/199511320/se-2?accountid=27871.

Pirc, M., Maas, P., De Graaf, K., Lee, H.-S., & Boesveldt, S. (2022). Humans possess the ability to discriminate food fat content solely based on retronasal olfaction. *Food Quality and Preference, 96*, 104449. https://doi.org/10.1016/j.foodqual.2021.104449

Ramirez, I. (1993). Role of olfaction in starch and oil preference. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 265*(6), R1404–R1409. https://doi.org/10.1152/ajpregu.1993.265.6.R1404

Research and Markets. (2014). Research and Markets: Analysis and Forecast Report on Pasteurized Milk Market in China to 2020. BUSINESS WIRE. Retrieved from https://www.businesswire.com/news/home/20140313006274/en/Research-and-Markets-Analysis-and-Forecast-Report-on-Pasteurised-Milk-Market-in-China-to-2020.

Solano-Lopez, C. E., Ji, T., & Alvarez, V. B. (2005). Volatile compounds and chemical changes in ultrapasteurized milk packaged in polyethylene terephthalate containers. *Journal of Food Science, 70*(6), c407–c412. https://doi.org/10.1111/j.1365-2621.2005.tb11438.x

Takeda, M., Sawano, S., Imaizumi, M., & Fushiki, T. (2001). Preference for corn oil in olfactory-blocked mice in the conditioned place preference test and the two-bottle choice test. *Life Sciences, 69*(7), 847–854. https://doi.org/10.1016/S0024-3205(01)01180-8

Tian, H., Yu, B., Yu, H., & Chen, C. (2020). Evaluation of the synergistic olfactory effects of diacetyl, acetaldehyde, and acetoin in a yogurt matrix using odor threshold, aroma intensity, and electronic nose analyses. *Journal of Dairy Science, 103*(9), 7957–7967. https://doi.org/10.3168/jds.2019-17495

Valero, E., Villamiel, M., Miralles, B., Sanz, J., & Martınez-Castro, I. (2001). Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry, 72*(1), 51–58. https://doi.org/10.1016/S0308-8146(00)00203-X

Van Boekel, M. S., & Lindsay, R. (1992). Partition of cheese volatiles over vapour, fat and aqueous phases. *Nederlands melk en Zuiveltijdschrift, 46*(3–4), 197–208.

Van Dongen, W. D., & Donders, J. J. H. (n.d.) *VCF Volatile Compounds in Food: database, Version 16.8.* Retrieved July 13, 2021, from https://www.vcf-online.nl.

Van Hekken, D. L., Iandola, S., & Tomasula, P. M. (2019). Short communication: Volatiles in microfluidized raw and heat-treated milk. *Journal of Dairy Science, 102*(10), 8819–8824. https://doi.org/10.3168/jds.2018-15776

Vazquez-Landaverde, P. A., Velazquez, G., Torres, J. A., & Qian, M. C. (2005). Quantitative Determination of Thermally Derived Off-Flavor Compounds in Milk Using Solid-Phase Microextraction and Gas Chromatography. *Journal of Dairy Science, 88*(11), 3764–3772. https://doi.org/10.3168/jds.S0022-0302(05)73062-9

Wilkes, J. G., Conte, E. D., Kim, Y., Holcomb, M., Sutherland, J. B., & Miller, D. W. (2000). Sample preparation for the analysis of flavors and off-flavors in foods. *Journal of Chromatography A, 880*(1–2), 3–33. https://doi.org/10.1016/S0021-9673(00)00318-6

Xavier, A. M., Ludwig, R. G., Nagai, M. H., De Almeida, T. J., Watanabe, H. M., Hirata, M. Y., … Glezer, I. (2016). CD36 is expressed in a defined subpopulation of neurons in the olfactory epithelium. *Scientific Reports, 6*, 25507. https://doi.org/10.1038/srep25507

Yin, X., Lv, Y., Wen, R., Wang, Y., Chen, Q., & Kong, B. (2021). Characterization of selected Harbin red sausages on the basis of their flavour profiles using HS-SPME-GC/MS combined with electronic nose and electronic tongue. *Meat Science, 172*, 108345. https://doi.org/10.1016/j.meatsci.2020.108345