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Humans possess the ability to discriminate food fat content solely based on retronasal olfaction

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ABSTRACT

Dietary fat overconsumption contributes to the development of obesity and related comorbidities; however, its sensory perception is poorly understood. Although humans can discriminate between vapor-phase fatty acids, both ortho- and retronasally, evidence of orthonasal fat discrimination in real foods is limited, and non-existent for retronasal olfaction.

In two experiments, we investigated the human ability of olfactory food fat content discrimination in dairy milk and assessed whether this ability is affected by habitual dairy intake. Participants undertook a series of DR A-not A discrimination tests (analysed with R-index analyses) coupled with perceptual ratings and a questionnaire on dairy consumption habits.

In the first experiment (n = 66), ortho- and retronasal discrimination was evaluated using dairy milk samples manipulated to contain 0%, 1.5% and 3.5% fat. Subjects could discriminate between all three fat levels orthonasally ($p < .001$), whereas retronasally they were able to do so between 0 and 1.5% ($p < .001$) and 0–3.5% ($p < .001$). The second experiment (n = 44) focused only on retronasal discrimination, using (manipulated) dairy milk samples of 3.5%, 7%, 10.5% and 14% fat. Here, discrimination was possible between 3.5 and 14% ($p < .001$) and 7–14% ($p < .05$) samples. No effects of total dairy fat intake, total dairy product intake or dairy exposure frequency were observed on discrimination ability in both experiments.

This is the first study demonstrating that humans are capable of discriminating food fat content solely based on retronasal olfaction. Results also suggest that this ability is unaffected by habitual intake.

1. Introduction

Overconsumption of dietary fat is considered a major contributing factor to the development of obesity and related comorbidities. Due to our innate inclination for energy-dense nutrients, a preference for fatty foods appears to be a universal human trait and the overconsumption of fat-laden foods is further exacerbated by the pleasurable sensory characteristics of fat (Drewnowski, 1997; Drewnowski & Almiron-Roig, 2009). Since fat consumption is exceeding intake recommendations in many Western diets, the understanding of its sensory perception is crucial in developing public health strategies aimed at reducing its excessive intake (Drewnowski & Almiron-Roig, 2009; WHO, 2018).

The alluring flavour of fat arises from a synergy between gustation, somatosensation, as well as olfaction (Drewnowski & Almiron-Roig, 2009; Zhou et al., 2016). Whereas orthonasal odours are related to food source detection and the induction of appetite during the anticipatory phase of eating, retronasal odours are considered fundamental contributors to flavour perception during food consumption and may influence intake and satiation (Boesveldt & de Graaf, 2017; Bojanowski & Hummel, 2012). An increasing body of evidence underscores the importance of olfaction in fat perception, with findings that humans are not only capable of detecting (Chale-Rush et al., 2007) and discriminating between vapour-phase fatty acids ortho- and retronasally (Bolton & Halpern, 2010; Kallas & Halpern, 2011), but also identifying different types retronasally (Chukir et al., 2013). Despite demonstrating ability for olfactory fat discrimination and identification, the ecological validity of studies using vapour-phase fatty acids as olfactory stimuli is limited: fatty acids in food are present in conjunction with other odorous constituents which can mask or influence olfactory perception. Therefore, olfactory fat perception needs to be studied in the context of real foods as well. The first to do so were Boesveldt and Lundström (2014), who demonstrated that humans can discriminate between different fat

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concentrations in dairy milk using solely orthonasal olfactory cues. To our knowledge, fat content discrimination in a real food context based solely on retronasal olfactory cues has not yet been reported. In fact, relatively little is known about the exact contribution of retronasal odours to fat perception. Yackinous and Guinard (2000) and Zhou et al. (2016), have demonstrated that retronasal odours enhance fat flavour intensity in various real foods, while Schoumacker et al. (2017) observed a decrease in fat detection and discrimination thresholds when cottage cheese varying in fat was evaluated without nose clips (with the involvement of the retronasal route). Similarly, Jervis et al. (2014) showed that inhibition of the retronasal pathway (using nose clips) diminishes the perception of creaminess in sour cream. This suggests that the perception of creaminess, which seems to be related to fat levels and considered a key driver of sensory appeal in fatty foods (Frøst & Janhøj, 2007), is assessed via retronasal olfactory mechanisms. A similar reduction in the perception of fat-related attributes was observed by Weenen et al. (2005), who demonstrated that the use of nose clips decreased the perception of creaminess and fattiness in custard deserts. Moreover, Martin et al. (2016) observed that the perception of naturally occurring cream aroma in cottage cheese was positively related to fat content and suggested that (retronasal) olfactory cues are one of the main contributors to fat perception in foods. Nevertheless, none of these studies evaluated the retronasal component in isolation, separating it from confounding factors such as gustatory, thermal, and mechanical sensations.

Chemosensory fat detection abilities in humans (Kindleysides et al., 2017; Stevenson et al., 2016; Stewart et al., 2010) and rats (Thiebaud et al., 2014) seem to be modulated by habitual fat intake to a degree, possibly via exposure effects. However, results of Boesveldt and Lundström (2014) show that olfactory fat discrimination is independent of habitual intake, suggesting that it might be an innate ability. From an evolutionary perspective this seems reasonable: An innate ability to detect fat content, and hence energy content, in foods via the olfactory system prior to and during consumption, would support energy-efficient foraging within fluctuating ancestral food environments. This line of thought is supported by findings of de Vries et al. (2020), who observed that when exposed to olfactory food cues, individuals were better at recalling locations of odours signalling high-calorie foods, compared to matched low-calorie counterparts, regardless of explicit hedonic odour evaluations or odour familiarity. Evidence therefore points towards olfaction being an effective innate mechanism for gauging the energy content of potential food sources, yet further corroboration is needed.

The contribution of olfaction, retronasal olfaction in particular, to fat perception remains to be clarified. The first step in filling this knowledge gap is to assess whether humans possess the ability to retronasally discriminate fat content in real foods. The aim of the present study was therefore to explore whether humans can discriminate fat content in different versions of dairy milk and assess whether this ability is dependent on habitual dairy intake. To confirm findings on orthonasal discrimination of fat content in food by Boesveldt and Lundström (2014), and extend those of Bolton and Halpern (2010) on retronasal fatty acid discrimination, two experiments were carried out. In the first experiment, we determined ortho- and retronasal discrimination ability between three milk samples manipulated to contain ecologically relevant fat levels. To gain insight on the sensory differences between the samples and allow for a more in-depth comparison between the two olfaction routes, ratings of fat odour intensity and liking were evaluated as well. In the second experiment, we focused solely on retronasal olfaction while expanding the fat sample range. In the attempt to better understand the differences in discrimination ability between the fat concentrations, perceptual ratings of creaminess were evaluated as well. Potential effects of habitual dairy consumption on discrimination ability were assessed in both experiments.

2. Materials & methods

All participants were informed about the experimental protocol and provided written informed consent in accordance with the Declaration of Helsinki prior to participation. All study aspects were approved by the Wageningen University Medical Ethics Review Board. Data that support the findings of this study are available on the Open Science Framework Repository with the identifier DOI: https://doi.org/10.17605/OSF.IO/NXFQZ (Pirc et al., 2021).

2.1. Experiment 1

The main aim of experiment 1 was to assess ortho- and retronasal discrimination ability in dairy milk consumers, using dairy milk samples containing 0%, 1.5% and 3.5% fat. Effects of habitual dairy consumption, along with perceptual ratings of fat odour intensity and liking were assessed as well.

2.1.1. Participants

A total of 66 participants ($M_{Age} = 24 \pm 3.3$ years; $M_{BMI} = 22.7 \pm 2.4$ kg/m^2; 31 males) recruited from Wageningen (The Netherlands) and its surroundings took part in the study. All were consumers of dairy milk and met eligibility criteria of being between 18 and 55 years of age, healthy, non-smoking, normosmic (assessed with the Sniffin' Sticks 16-item odour identification test (Hummel et al., 2007)), non-dieting currently or in the past two months, non-pregnant, non-lactating, not being lactose-intolerant or having any other dairy-related allergies.

2.1.2. Stimuli & stimulus presentation

Three versions of dairy milk, containing fat levels resembling those found in commercially available skimmed, semi-skimmed and whole milk, respectively, were used as odour stimuli: 0% (F_0), 1.5% ($F_{1.5}$) and 3.5% ($F_{3.5}$). They were produced by combining fresh, pasteurised skimmed milk (0% fat - AH Magere melk, Albert Heijn B.V.) with fresh, pasteurised full-fat cream (35% fat - AH Verse Slagroom, Albert Heijn B.V.), both processed within the same dairy processing facility (Arla Foods B.V., Nijkerk, Netherlands – EC approval number: NL Z0055 EG), to minimise between-sample variation. Sample mixtures were prepared fresh at the beginning of each testing day with the use of a magnet stirrer and kept in air-tight containers until presented. To ensure sample stability, 0.5% kappa (κ) carrageenan water-based solution was added to all three milk versions. Sample ingredients and corresponding nutritional values can be found in Table A1 in the supplementary material.

Samples were presented in 60 ml amounts at 20 ± 1 °C, using containers adapted from the design used by Bolton and Halpern (2010) (see Fig. 1). They consisted of an opaque, black polypropylene cup (Ø 95 mm × H 40 mm; volume 150 ml), covered with a black, reusable silicone coffee cup lid. A 2-ml micro tube with its bottom portion cut away (Ø 10 mm × H 25 mm) was inserted into the lid's drinking hole to serve as an air inlet. The retronasal container version had a single drinking straw

Fig. 1. Retronasal (left) and orthonasal (right) delivery containers.

piece inserted into the silicone lid, whereas the orthonasal version had two (12 mm apart). Straw pieces were 65 mm long and inserted into holes made in the lid with a hole punch (Ø 5 mm), with 48 mm protruding above the lid surface. Due to elasticity of silicone all elements fit tightly, with the straws being adjustable in angle. When not in use, all openings were covered with caps.

2.1.3. Study design and procedures

Participants attended three sessions, spread across separate days and carried out in sensory booths. They were given instructions not to consume anything other than water two hours prior to testing and to avoid using any scented products on testing days.

The first session included bodyweight and height measurements, followed by an olfactory function assessment and a short training procedure. Participants were instructed not to lift the containers or blow air into the straws and to make steady, moderately intense inhalations, lasting approximately two seconds. The importance of producing consistent inhalations across all trials was emphasised. For orthonasal inhalation, they were instructed to insert straw tips into the nostrils, inhale, remove straws from the nostrils and exhale through the nose. For retronasal inhalation, they were instructed to put on a nose clip before inserting the straw tip into their mouth, inhale, remove the nose clip and exhale through the nose, while keeping the mouth closed. A demonstration on proper container handling and inhalation techniques was also given at this point.

The training procedure was followed by two blocks of intensity and liking ratings – a retronasal and an orthonasal one (order counterbalanced across participants). In both blocks, participants were presented with the three milk samples (one at a time, in a random order), instructed to smell them and rate the perceived odour intensity and liking on 100-unit Visual Analogue Scales (VAS). To prevent olfactory adaptation Pellegrino et al. (2017), samples were separated by 30-s pauses, whereas a 5-min break was implemented between the two blocks. The session concluded with a dairy food frequency questionnaire (DFFQ) (adapted from Boesveldt and Lundström (2014)), containing questions about participants' habitual dairy product consumption.

The remaining two sessions – one orthonasal, the other retronasal, with the order counterbalanced across participants, both comprised of discrimination testing. Participants undertook the dual reminder A-not A (DR A-not A) test (see Mun et al. (2019)) with a pairwise design (Hautus et al., 2018). In this version of the A-not A test, two reference stimulus presentations precede a single test stimulus presentation. Participants thus had to smell the reference sample twice prior to smelling the test sample once and responding whether the test sample was the reference (S_A) or not ($S_{not\ A}$). Each discrimination testing block began with a familiarisation procedure, during which participants were presented with both stimuli used in that block. They were told which sample was the reference and which was different from the reference, and instructed to smell them twice, in an alternating manner (S_A, $S_{not\ A}$, S_A, $S_{not\ A}$). This was implemented to stabilise participants' cognitive decision criteria (Lee, van Hout, & O'Mahony, 2007). They then completed three blocks of four tests, each block consisting of only two stimulus levels: either 0% and 1.5% ($F_{0\text{-}1.5}$); 0% and 3.5% ($F_{0\text{-}3.5}$); or 1.5% and 3.5% ($F_{1.5\text{-}3.5}$). Block order was randomised. The sample with the lower fat concentration always served as the reference, whereas the test sample could be either of the stimuli in that pair. For each stimulus level combination, there were two possible presentation sequences: S_A – S_A – S_A or S_A – S_A – $S_{not\ A}$. Within a block, each presentation sequence was provided twice, in a random order. To counteract olfactory adaptation, inter-test and inter-block intervals of approximately 30 and 3 min were implemented, respectively. Responses were collected in terms of six categories: "it is the reference – I am sure", "it is the reference – I am unsure", "I am guessing it is the reference", "I am guessing it is not the reference", "it is not the reference – I am unsure", "it is not the reference – I am sure". See Fig. 2 for an overview of the first experiment.

2.1.4. Statistical analyses

Discrimination ability was assessed with R-index analyses carried out in accordance with the protocols described by Lee and van Hout (2009). To account for replicated testing, R-indices were computed based on weighted means of individual R-index values (derived from 4 signal/noise tests per judge) (Bi, 2015). Statistical significance was established by calculating the R-index critical value, using R statistical software (R-Core Team, 2020) and the code provided by Bi and O'Mahony (2020). The R-index critical value for 132 control and 132 test samples in a one-sided test at the 0.05 significance level amounts to 55.81. Apart from R-index analyses, all other statistical procedures were carried out using IBM SPSS Statistics, version 27. Differences in discrimination ability (mean individual R-index values) between olfaction routes for each of the fat concentration comparisons were analysed using Wilcoxon signed-ranks tests. Potential learning or warm-up effects during discrimination testing were assessed by evaluating frequencies of hits, misses, correct rejections and false alarms across the test repetitions, using chi-square tests of independence.

Effects of olfaction route and fat concentration on perceived odour intensity and liking were analysed with linear mixed models (LMM), using intensity or liking as dependent variables, milk fat sample concentrations and olfaction routes as fixed factors, and subjects as a random one. For significant main effects, post-hoc pairwise comparisons with Bonferroni corrections were applied to compare ratings between

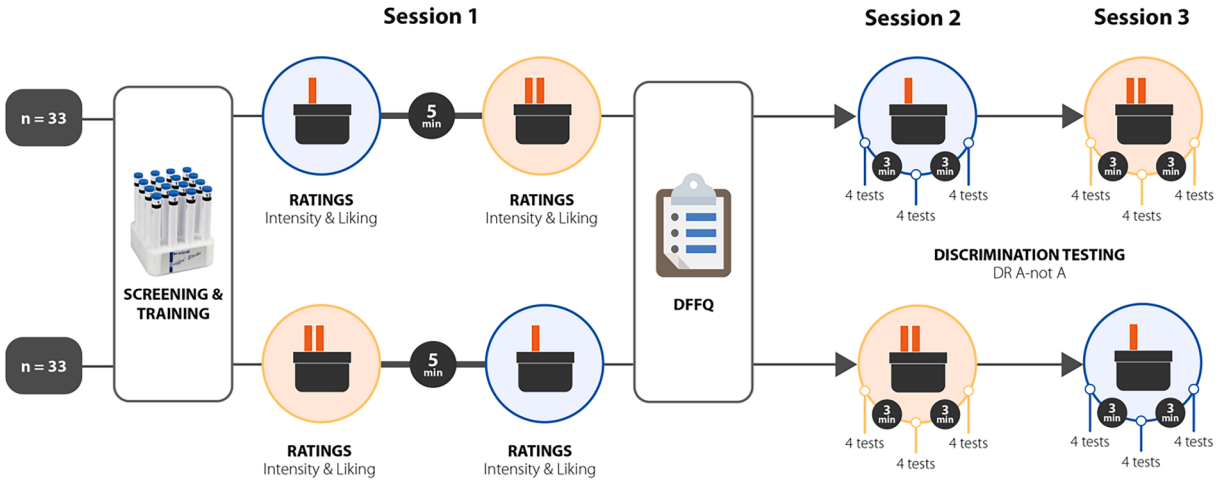

Fig. 2. An overview of experiment 1. Retronasal trials are coloured blue; orthonasal trials are coloured orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

olfaction routes and fat concentrations.

To assess habitual dairy consumption, DFFQ responses were converted into total dairy product intakes (in g/day), total dairy fat intakes (in g/day) and dairy product consumption frequencies (number of times/day). This was done with the help of the Dutch Food Composition Database (NEVO), published by the Dutch National Institute for Public Health and Environment (RIVM). Effects of these habitual dairy consumption parameters on discrimination ability were evaluated with LMM analyses, using R-indices as dependent variables, either total dairy product intakes, total dairy fat intakes or dairy product consumption frequencies as fixed factors and subjects a random one.

2.2. Experiment 2

Results from experiment 1 confirmed previous findings of Boesveldt and Lundström (2014) on orthonasal fat content discrimination and revealed that fat content discrimination is also possible retronasally. The main aim of experiment 2 was to further explore retronasal discrimination ability, by evaluating whether and how it is affected by larger fat concentration magnitude differences. The fat sample range was expanded to contain dairy milk samples with 3.5%, 7%, 10.5% and 14% fat. Effects of habitual dairy consumption on discrimination ability, along with perceptual ratings of fat odour intensity, creaminess and liking were assessed as well. Creaminess was added as an attribute following multiple reports from participants taking part in the first experiment, claiming that their discrimination testing decision was based on differences in creaminess between the samples.

2.2.1. Participants

A total of 44 participants (mean age 23.8 ± 3.2 years; 21 men; mean BMI 22.2 ± 2.1 kg/m^2) recruited from Wageningen (the Netherlands) and its surroundings participated in the study. All met the same inclusion criteria as described for Experiment 1 (see section 2.1.1).

2.2.2. Stimuli & stimulus presentation

Four versions of dairy milk, containing fat levels resembling those found in commercially available whole milk, quark, sour cream and reduced-fat cooking cream, respectively, were used as odour stimuli: 3.5% ($F_{3.5}$), 7% (F_7), 10.5% ($F_{10.5}$) and 14% (F_{14}). They were produced by combining fresh, pasteurised skimmed milk (0% fat content) (AH Magere melk, Albert Heijn B.V.) with fresh, pasteurised full fat cream (35% fat content) (AH Verse Slagroom, Albert Heijn B.V.). Sample mixtures were prepared fresh at the beginning of each testing session, using a dispersing machine (T 25 digital Ultra-Turrax, IKA®-Werke GmbH & Co. KG) set at 4000 rpm for 2 min. They were presented as described for Experiment 1 (see section 2.1.2). Sample ingredients and corresponding nutritional values can be found in Table A2 in the supplementary material.

2.2.3. Study design and procedures

Participants attended four sessions spread across separate days. Apart from excluding orthonasal inhalation procedures, the timeline of the first session, provided instructions and training were as described for Experiment 1 above. After training, participants were presented with the four milk sample versions, instructed to inhale them retronasally and rate the perceived odour intensity, creaminess and liking on 100-unit VAS. Samples were presented in a random order, one at a time, with 45-s pauses in between. The session concluded with the DFFQ.

The remaining three sessions involved discrimination testing, using the DR A-not A methodology as described for Experiment 1 (see Section 2.1.3). Each discrimination testing session comprised of two blocks of six tests, with each block consisting of two stimulus levels: either 3.5% and 7% ($F_{3.5\text{-}7}$); 3.5% and 10.5% ($F_{3.5\text{-}10.5}$); 3.5% and 14% ($F_{3.5\text{-}14}$); 7% and 10.5% ($F_{7\text{-}10.5}$); 7% and 14% ($F_{7\text{-}14}$); 10.5% and 14% ($F_{10.5\text{-}14}$). Inter-test and inter-block intervals of 45 s and 5 min were implemented to counteract olfactory adaptation. All other aspects of discrimination testing procedures were identical to those described for Experiment 1. See Fig. 3 for an overview of the second experiment.

2.2.4. Statistical analyses

Discrimination ability was assessed with R-index analyses as described for Experiment 1 (see section 2.1.4). Potential learning or warm-up effects during discrimination testing were assessed as described for Experiment 1. Effects of fat concentration on perceived odour intensity, creaminess and liking were analysed with LMM, using intensity, creaminess or liking as dependent variables, fat concentrations as fixed factors and subjects as random ones. For significant main effects, post-hoc pairwise comparisons with Bonferroni corrections were applied to compare these ratings between fat concentrations. Habitual dairy consumption and its effect on discrimination ability were analysed as described for Experiment 1.

3. Results

3.1. Experiment 1

3.1.1. Discrimination ability

Results of R-index analyses (Fig. 4) show that orthonasally, participants were able to discriminate between all three fat sample comparisons: $F_{0\text{-}1.5}$ ($M_{R\text{-}index} = 68.4 \pm 29.1$, $p < .001$); $F_{0\text{-}3.5}$ ($M_{R\text{-}index} = 74.8 \pm 32$, $p < .001$); $F_{1.5\text{-}3.5}$ ($M_{R\text{-}index} = 58.5 \pm 32.2$, $p < .01$). Retronasally, they were able to do so between $F_{0\text{-}1.5}$ ($M_{R\text{-}index} = 72 \pm 31.2$, $p < .001$); $F_{0\text{-}3.5}$ ($M_{R\text{-}index} = 65.3 \pm 32.4$, $p < .001$); but not between $F_{1.5\text{-}3.5}$ ($M_{R\text{-}index} = 53.6 \pm 31$, $p > .05$).

No statistically significant differences in mean individual R-index values within fat sample comparisons were observed between ortho- and retronasal conditions ($F_{0\text{-}1.5}$: Z = -0.675, $p = .499$; $F_{0\text{-}3.5}$: Z = −1.936, $p = .053$; $F_{1.5\text{-}3.5}$: Z = −0.827, $p = .408$), indicating that discrimination ability was similar between the two olfaction routes for all fat sample comparisons.

No learning or warm-up effects were observed across the four test repetitions per participant, for any of the fat sample comparisons (see Table A3 in the supplementary material).

3.1.2. Intensity and liking ratings

Mean odour intensity and liking ratings per fat sample comparison, for both olfaction routes, are shown in Fig. 5.

LMM analyses show that fat concentration and olfaction route had main effects on intensity (fat concentration: $F(2, 327) = 23.45$, $p < .001$; olfaction route: $F(1, 327) = 321.02$, $p < .001$) and liking (fat concentration: $F(2, 327) = 13.36$, $p < .001$; olfaction route: $F(1, 327) = 92.61$, $p < .001$). No interactions were observed between olfaction route and fat concentration for both, intensity ($F(2, 325) = 0.97$, $p = .380$) and liking ($F(2, 325) = 0.02$, $p = .984$). For both olfaction routes, intensity of the F_0 sample ($M_{orthonasal} = 46.4 \pm 23.2$; $M_{retronasal} = 16.4 \pm 15.6$) was rated significantly lower ($p < .001$) than intensities of $F_{1.5}$ ($M_{orthonasal} = 58.6 \pm 20.7$; $M_{retronasal} = 30.3 \pm 21.6$) and $F_{3.5}$ samples ($M_{orthonasal} = 60.3 \pm 22.2$; $M_{retronasal} = 26.3 \pm 19.8$). No significant differences in intensity ratings were observed between $F_{1.5}$ and $F_{3.5}$ samples for both olfaction routes ($p = 1.000$). Similarly, the F_0 sample ($M_{orthonasal} = 55.7 \pm 20.8$; $M_{retronasal} = 40.4 \pm 20.6$) was rated as being significantly less liked ($p < .001$) than $F_{1.5}$ ($M_{orthonasal} = 64.9 \pm 16.8$; $M_{retronasal} = 49.2 \pm 19.4$) and $F_{3.5}$ samples ($M_{orthonasal} = 64.8 \pm 20.5$; $M_{retronasal} = 48.8 \pm 19.1$) in both olfactory conditions. Liking ratings between $F_{1.5}$ and $F_{3.5}$ samples did not differ significantly between the routes ($p = .893$). Intensity of all three fat samples was rated as being lower in the retronasal condition ($p < .001$). Likewise, the three fat samples were less liked in the retronasal condition ($p < .001$).

3.1.3. Effects of habitual dairy consumption on discrimination ability

Mean reported daily dairy fat and dairy product intakes of participants were 8.4 ± 5.5 g/day and 364.1 ± 188.7 g/day, respectively. The

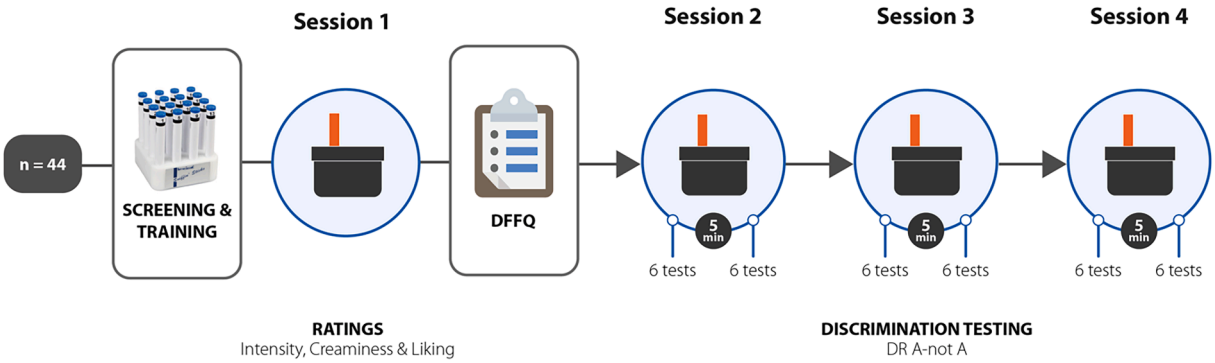

Fig. 3. An overview of experiment 2.

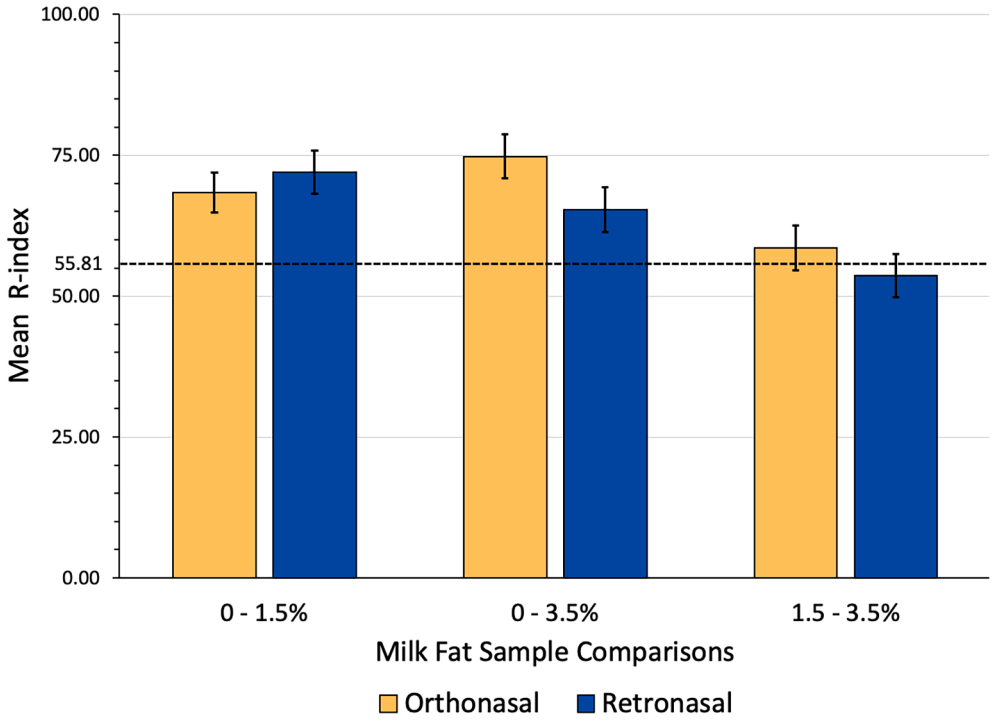

Fig. 4. R-index analyses results of Experiment 1. The dashed line indicates discrimination above statistical significance at p = 0.05 (error bars represent ± 1 SE).

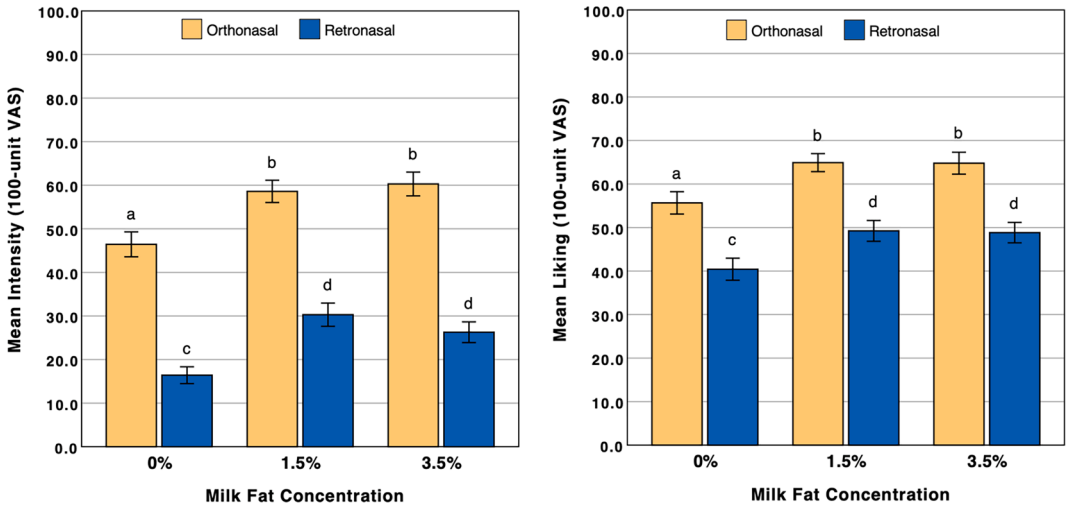

Fig. 5. Mean odour intensity and liking ratings for the three fat concentrations from Experiment 1, per olfaction route (error bars represent ± 1 SE). Mean differences between elements denoted with different letters (a, b, c, d) are statistically significant at p = 0.05.

average reported dairy consumption frequency amounted to 2.3 ± 0.9 times/day. No effects of total dairy fat intake ($F(1, 62) = 0.008$, $p = .927$), total dairy product intake ($F(1, 62) = 0.434$, $p = .512$) or dairy consumption frequency ($F(1, 62) = 0.036$, $p = .849$) were observed on discrimination ability.

3.2. Experiment 2

3.2.1. Discrimination ability

Results of R-index analyses (Fig. 6) show that participants were able to retronasally discriminate between $F_{3.5\text{-}14}$ ($M_{R\text{-}index} = 60.9 \pm 26.1$, $p < .001$) and $F_{7\text{-}14}$ ($M_{R\text{-}index} = 56.6 \pm 27$, $p < .05$), but not between $F_{3.5\text{-}7}$ ($M_{R\text{-}index} = 54.2 \pm 22.7$, $p > .05$), $F_{3.5\text{-}10.5}$ ($M_{R\text{-}index} = 54.4 \pm 28.1$, $p > .05$), $F_{7\text{-}10.5}$ ($M_{R\text{-}index} = 54.7 \pm 23.6$, $p > .05$) and $F_{10.5\text{-}14}$ ($M_{R\text{-}index} = 44.9 \pm 24.7$; $p > .05$).

No learning or warm-up effects were observed across the four test repetitions per participant for any of the fat concentration comparisons (see Table A4 in the supplementary material).

3.2.2. Intensity, creaminess and liking ratings

Mean odour intensity, creaminess and liking ratings per milk fat sample comparison are displayed in Fig. 7. See Table B1 in the supplementary material for means with SD.

Based on LMM analyses, fat concentration had no main effect on intensity ($F(3, 129) = 1.154$, $p = .330$) or creaminess ($F(3, 129) = 2.160$, $p = .096$). It did, however, have an effect on liking ($F(3, 129) = 3.855$, $p = .011$). The F_{14} ($M = 45.2 \pm 21.9$) sample was significantly ($p = .011$) more liked than the F_7 ($M = 35.9 \pm 18.6$) sample. No differences in liking were observed between other fat concentrations ($p > .05$).

Mean reported daily dairy fat and dairy product intakes of participants were 8.8 ± 5.9 g/day and 288 ± 226 g/day, respectively. The average reported dairy consumption frequency amounted to 2.1 ± 1.1 times/day. No effects of total dairy fat intake ($F(1, 40) = 0.376$, $p = .543$), total dairy product intake ($F(1, 40) = 0.154$, $p = .679$) or dairy consumption frequency ($F(1, 40) = 1.097$, $p = .301$) were observed on discrimination ability.

4. Discussion

The present research aimed at gaining insight on the human ability of retronasal fat content discrimination, using an ecologically relevant olfactory stimulus – dairy milk samples varying in fat concentration. This is the first study to demonstrate that humans are capable of discriminating fat content in a real food product, using solely retronasal olfactory cues. Furthermore, this ability does not appear to be related to habitual dairy intake. Although samples were perceived as being less intense and less liked in the retronasal condition, fat content discrimination between the two olfactory routes was comparable.

Previous research on ortho- and retronasal perception of vapour-phase fatty acids (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011) and orthonasal perception of fat levels in dairy milk (Boesveldt & Lundström, 2014) has indicated that humans possess a functional olfaction-based system for detecting food fat content. The present research replicates findings on orthonasal fat content discrimination in real foods and, more importantly, extends those on retronasal perception of vapour-phase fatty acids to a real-food context. Not only were subjects in our experiments able to retronasally discriminate between non-fat and fat-containing samples, they were able to do so between different levels of fat as well. Furthermore, the fact that we separated the olfactory component from confounding effects of taste and mouthfeel sensations, clearly demonstrates that retronasal olfaction in isolation is sufficient for discriminating fat levels in food and further emphasises its importance in fat perception.

Subjects were able to orthonasally discriminate between all three fat level comparisons used in our first experiment. This is in line with the study of Boesveldt and Lundström (2014), who observed the same in a comparable set of samples, albeit with some inconsistencies: in two of the three experiments participants could not discriminate semi-skimmed milk from whole milk; in one experiment they were unable to discriminate skimmed milk from semi-skimmed milk. Disparities between the latter and our study might have occurred due to differences in fat concentration steps between the experiments, or different methodological approaches to discrimination testing. Whereas Boesveldt and Lundström (2014) applied the triangle discrimination method, the current study implemented the DR A-not A approach. The A-not A method not only tends to be more powerful than the triangle procedure (Bi & Ennis,

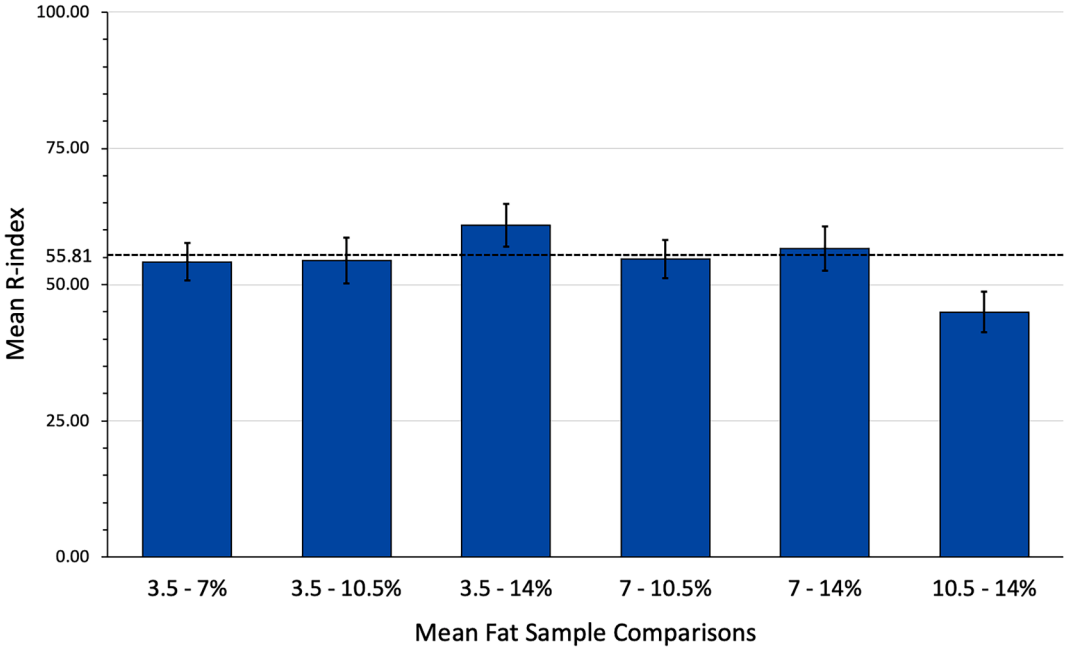

Fig. 6. R-index analyses results of Experiment 2. The dashed line indicates discrimination above statistical significance at p = 0.05 (error bars represent ± 1 SE).

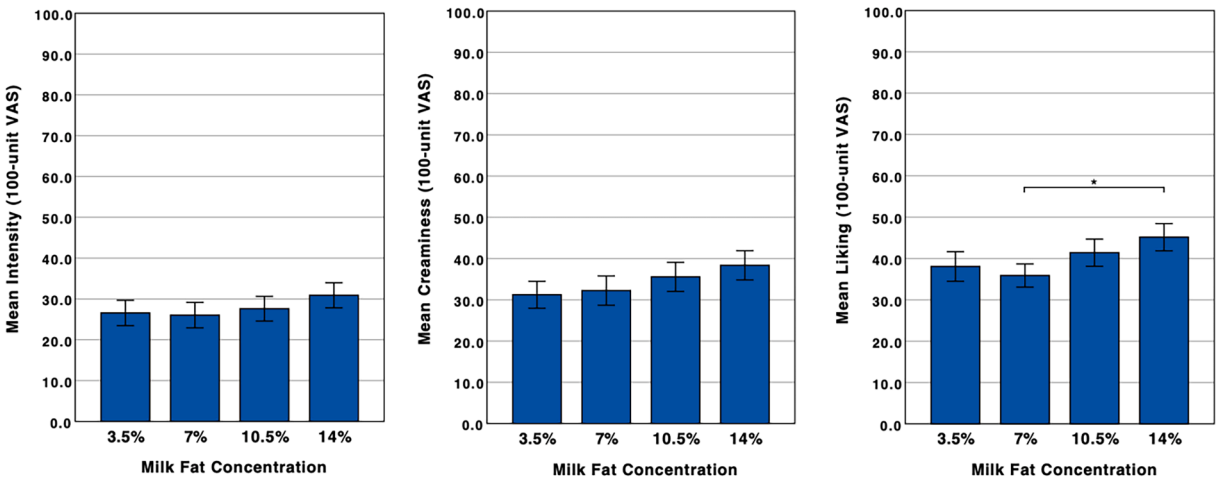

Fig. 7. Mean odour intensity, creaminess and liking ratings for the four fat concentrations used in Experiment 2 (error bars represent ± 1 SE).

2001), sensitivity variations between the two discrimination approaches are also to be expected (Lee, van Hout, & Hautus, 2007; Mun et al., 2019).

Discrimination ability between the two olfaction routes was similar overall, however, individual comparisons revealed that in contrast to the orthonasal condition, subjects were not able to retronasally discriminate between $F_{1.5\text{-}3.5}$. This could be because retronasal detection thresholds are generally higher than orthonasal ones (Goldberg et al., 2018), which also seems to be the case for fatty stimuli (Chale-Rush et al., 2007). Our second experiment demonstrated that retronasal discrimination between different fat levels, not just between non-fat and fat-containing samples, is also possible, as subjects were able to discriminate between $F_{3.5\text{-}14}$ and $F_{7\text{-}14}$ comparisons. It has to be acknowledged that despite a comparable absolute, but smaller relative difference in fat levels, the $F_{7\text{-}14}$ comparison could be discriminated, while the $F_{3.5\text{-}10.5}$ could not. There is a possibility that the sample size implemented in our experiment was insufficient, resulting in the lack of statistical power for this particular comparison. Alternatively, perhaps quality differences between stimuli are more relevant than their intensities when it comes to olfactory fat discrimination. Indeed, as recently demonstrated by Ravia et al. (2020), quality differences between odorant pairs might be key to olfactory discrimination. Since unlike for JNDs in odour intensity (Cain, 1977), no framework for JND in odour quality exists, this remains to be elucidated. Future studies should therefore aim at establishing JND types and ranges relevant for fat odour discrimination and ensure sufficient sample sizes. Although a combination of the aforementioned causes is likely to have influenced our results, overall, they clearly show that humans can retronasally discriminate between various levels of fat in food and indicate that this ability seems to be comparable between the two olfaction routes. It has to be noted, however, that discrimination between non-fat-containing and fat-containing samples seems to be relatively straightforward, whereas larger fat difference magnitudes are seemingly required for discrimination between fat-containing samples. Based on the outcomes, it seems relevant for future studies to focus on individual sensitivity measurements and individual factors that might affect discrimination ability.

No perceptual rating differences were observed between the two fat-containing samples in our first experiment; however, they were both perceived as more intense and more liked compared to the non-fat sample. In comparison, Boesveldt and Lundström (2014), using a set of samples comparable to the one described here, observed a decrease in pleasantness with increasing fat content in one, but not their other two experiments. In congruence with the notion that orthonasal stimuli are generally perceived as more intense than retronasal ones (Goldberg et al., 2018), orthonasal intensity and liking for all three fat levels in our experiment was higher compared to the retronasal condition. Despite olfactory route-dependent perceptual rating differences and the lack of perceptual rating differences between the two fat-containing samples which could be discriminated orthonasally, discrimination ability was similar between the two conditions. This suggests that discrimination likely did not depend on intensity differences between the samples and supports the idea that quality, not intensity differences between stimuli might be crucial for olfactory fat discrimination, as mentioned in the previous paragraph.

This reasoning was also put forward by Boesveldt and Lundström (2014), who suggested that the addition of other relevant perceptual descriptors, namely creaminess, could help elucidate perceptual differences responsible for olfactory fat content discrimination. Therefore, creaminess was added as a perceptual rating in our second experiment. In contrast to the first experiment, we observed no perceptual differences between the samples, apart from a difference in liking between F_7 and F_{14}. Considering this was the only perceptual difference among our set of samples and three perceptual variables, we speculate it is likely a coincidental finding. All in all, it is plausible that intensity differences contributed towards discrimination results between non-fat and fat-containing samples in our experiment, however, perceptual differences responsible for discrimination between fat-containing ones remain unclear. Perhaps a larger sample size or the addition of other fat-related descriptors might reveal perceptual differences accounting for the current discrimination results.

The ability to discriminate between fat levels was not affected by habitual dairy consumption in either of our experiments. This is consistent with findings of Boesveldt and Lundström (2014), who observed no associations between BMI or dairy consumption habits and orthonasal fat discrimination. Similarly, Stevenson et al. (2016) reported no associations of a Western-style diet, rich in fat and sugar, on general odour discrimination or olfactory thresholds. They did, however, find that consumers of a Western-style diet performed worse during odour identification trials and were poorer at discriminating fat levels during multisensory testing. Relatedly, Kindleysides et al. (2017) observed that a higher intake of fatty foods, namely seeds, nuts and nut spreads, was associated with a higher olfactory sensitivity to oleic acid. An additional observation, supporting our findings of olfactory fat content discrimination being independent of past exposure, at least in the short term, is that no learning or warm-up effects were observed during discrimination testing trials in the current study. However, since subjects in our experiments were dairy consumers, the possibility of long-term past exposure having an influence on fat odour discrimination cannot be ruled out either. Moreover, the DFFQ utilised in the current study might not have been the optimal approach for assessing habitual dairy consumption: increasing the range of response options, along with the range of dairy products it covers, could improve its accuracy.

Furthermore, perhaps instead of looking into dairy consumption habits, information about overall fat consumption, beyond dairy, could help reveal potential effects on discrimination ability. To date, only a handful of studies investigated the relationship between olfactory fat detection and habitual intake, yielding somewhat mixed results. Further research on the nature of olfactory fat detection abilities is therefore warranted.

Despite going beyond vapour-phase fatty acids, utilising actual food as an olfactory stimulus, the ecological validity of the current study should not be overstated. It must be acknowledged that inhalation via containers resulted in a retronasal stimulus transportation path not likely to occur during food consumption. When odours are inhaled orally, in the absence of food, they first travel to the lungs before ultimately reaching the olfactory epithelium. This results in varying degrees of lung retention (mainly depending on the type of odorant), which not only reduces the odour mixture concentration, but can also potentially alter the relative composition of the originally inhaled mixture. This is in contrast to what happens during actual food intake, where swallowing closes the trachea, thereby forcing odorants through the nasopharynx into the olfactory mucosa (Verhagen, 2015). Nevertheless, despite the highly likely occurrence of odorant lung retention in our experiments, odorant intensities were sufficient for the subjects to detect and discriminate between. We speculate that the effect of these odorants is more pronounced in normal eating situations.

Another point that needs to be addressed is the nature of chemical signals that are being perceived when "smelling fat". Since triglycerides – the most common form of dietary fat (Lichtenstein et al., 1998), are not known to be volatile, it is highly unlikely that they are directly responsible for the smell differences between our samples. However, since triglycerides can act as carriers of flavour compound reservoirs (McSweeney & Sousa, 2000), it is likely that compounds bound to them elicited the smell differences. As demonstrated by Roberts and Pollien (2000) and Roberts, Pollien, and Watzke (2003), the amount of aroma compound retention in dairy milk mainly depends on the fat content, with higher fat samples absorbing more aroma compounds than low fat ones. Furthermore, food matrix manipulations, such as the ones done in our experiments, lead to changes in lipophilicity which can potentially alter flavour release (Roberts et al., 2003). These factors might have caused qualitative shifts in odour characters between the samples in our experiments and could potentially be the key underlying mechanism by which subjects could discriminate between the samples. Furthermore, fatty acids, which are present in trace quantities in dairy milk (Parodi, 2004) and were demonstrated to be effective olfactory stimuli (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011), could also have caused perceptual differences. Effects of fat oxidation by-products cannot be ruled out either. For a better understanding of the mechanisms behind olfactory fat perception, more work is needed in on identifying the source of fat-odour-related chemical signals.

While orthonasal odours seem to aid in guiding towards potential (fat) food sources during the anticipatory phase of food consumption (de Vries et al., 2020), the behavioural relevance of (discriminating) retronasal odours in fat perception is less evident. Nevertheless, the ability to retronasally detect differences in food fat content points towards retronasal fat odours being behaviourally relevant in the consummatory phase of eating, likely beyond their contribution to flavour. Perhaps they serve to reinforce choice and intake of fat-rich food sources via reward mechanisms. The influence of retronasal odour exposure on food intake has been studied before (Raemaekers, 2014; Ramaekers et al., 2014; Ruijschop et al., 2010; Ruijschop et al., 2008), yet the observed effects were minor. The studies, however, used either non-fat odours or fat-related aromas (Raemaekers, 2014), rather than fat itself. The olfactometer-based delivery method employed in these studies, which can be considered rather unnatural when studying behaviour, possibly affected results as well. Notwithstanding, studying the effects of retronasal odours on behaviour is inherently difficult, mainly due to limited and often invasive options of stimulus delivery, and interactions with other senses involved in flavour perception (Bojanowski & Hummel, 2012; Goldberg et al., 2018). In view of these limitations, perhaps investigating underlying neural responses to olfactory fat exposure could shed light on potential behavioural correlates. Considering that the neural underpinnings of olfactory fat remain unexplored, neuroimaging techniques could be utilised to map involved brain regions and explore activation patterns in response to fat exposure (fat source, concentration, and exposure duration) for both olfaction routes.

To conclude, the current study represents an important step towards understanding olfactory fat perception, as our results clearly demonstrate that humans are capable of not only detecting the presence of fat retronasally, but also discriminating between its levels in a real food product. Additionally, this ability does not appear to be affected by habitual intake. The next important step, besides investigating individual factors that might affect discrimination ability and unravelling if and how retronasal fat perception affects food intake and choice, is to identify which chemical signals are responsible for the smell of fat. Doing so would provide opportunities to reduce fat content in a range of fat-laden foods, while maintaining their pleasurable sensory characteristics via the addition of compounds responsible for the alluring flavour.

CRediT authorship contribution statement

Matjaž Pirc: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Pim Maas:** Investigation, Data curation, Formal analysis, Writing – review & editing. **Kees De Graaf:** Supervision, Project administration, Writing – review & editing. **Hye-Seong Lee:** Methodology, Writing – review & editing. **Sanne Boesveldt:** Conceptualization, Methodology, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodqual.2021.104449.

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