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In vivo aroma release and perception of composite foods using nose space PTR–ToF–MS analysis with Temporal-Check-All-That-Apply

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ABSTRACT

In vivo aroma release and perception of complex food matrices have been underexplored. The aims of this study were to investigate the effects of (i) fat and sugar content of chocolate-hazelnut spreads on *in vivo* aroma release and perception and (ii) carrier addition (bread, wafer) on *in vivo* aroma release and perception of chocolate-hazelnut spread using dynamic nose space analysis (PTR–ToF–MS) and dynamic sensory analysis (TCATA). Carriers were combined with spreads varying in fat and sugar content and were spiked with five volatile organic compounds (benzaldehyde, filbertone, 2-methylpyrazine, delta-dodecalactone, isovaleraldehyde). TCATA profiles from a consumer panel without *in vivo* nose space analysis (n = 72) and a trained panel performing *in vivo* nose space analysis (n = 8, triplicate) were compared. TCATA profiles of the spread-carrier combinations obtained by both panels showed similarly that attributes related to the carriers were perceived at the beginning of consumption, whereas attributes related to the spreads were perceived after swallowing. Significant ($p < 0.05$) and small differences were observed for the attributes cocoa, creamy, milky, sticky and toffee between both panels. In the evaluated reformulation range, fat and sugar content of chocolate-hazelnut spreads had only a limited effect on *in vivo* aroma release and perception. In contrast, addition of carriers strongly affected *in vivo* aroma release and perception for all target molecules. The addition of carriers to spreads generally increased aroma release (duration and intensity of aroma release) and decreased aroma perception. The addition of carriers generally reduced the time to reach maximum intensity compared to when spreads were eaten alone for the five volatile organic compounds while perception decreased. We conclude that the strong effect of carrier addition on *in vivo* aroma release and perception of chocolate-hazelnut spreads highlights the importance of investigating toppings/spreads accompanied with carriers rather than in isolation.

1. Introduction

Aroma release and perception during food consumption are complex, dynamic processes influenced by physicochemical, biochemical, physiological, psychological, and cognitive phenomena (Negoias et al., 2008; Spence, 2021; van Eck et al., 2021), as well as by the composition and structure of food products and dynamic changes thereof during oral processing (Buettner & Beauchamp, 2010; Ferreira et al., 2006; Poinot et al., 2013; Pu et al., 2020; Pu et al., 2019a,b).

Proton-transfer reaction mass-spectrometry equipped with a time of flight mass analyzer (PTR-ToF-MS) is a powerful tool for real-time *in vivo* nose space analysis (NSA) during food consumption (Charles et al., 2015; Déléris et al., 2013; How et al., 2021; Mesurolle et al., 2013; Pu, Zhang, Zhang, Sun, Ren, Chen, & Xie, 2019), as it provides time-resolved information about the concentration and type of aroma compounds present in the nasal cavity by analyzing the composition of the air in the nostrils during consumption. To better understand aroma perception, PTR-ToF-MS is often coupled with dynamic sensory methods like Time-Intensity (TI) profiling (Chung et al., 2003; Pedrotti et al., 2019; van Eck et al., 2021). A drawback of TI profiling is that the intensity of only one sensory attribute is assessed over time, which may give rise to dumping effects, especially when the compared foods differ in both flavor and

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texture (Varela et al., 2018). Temporal Dominance of Sensations (TDS) and Temporal Check-All-That-Apply (TCATA) provide descriptions of the dynamic sensory properties of foods allowing simultaneous descriptions of flavor and texture properties (Castura et al., 2016). For TCATA, assessors continuously indicate the attributes that apply in describing their perception of the sample at any given moment during consumption. Multiple attributes from different modalities (e.g. taste, aroma, texture) can be selected, permitting the description of sensations that arise concurrently, and decreasing the risk of dumping effects (Castura et al., 2016). The number of assessors used in *in vivo* aroma release and perception studies that combine PTR-ToF-MS and TI profiling typically ranges from 7 to 18, as only one *in vivo* nose-space measurement can be performed at a time. Data collection with larger panels (>50 assessors), as typically used in TDS and TCATA studies, would be very time consuming and labor intensive. The limited number of assessors in studies coupling *in vivo* NSA with dynamic sensory evaluations potentially limits the quality of the sensory data.

Considerable progress has been made over the past decades to advance our understanding of the factors influencing *in vivo* aroma release and perception during food consumption. Most studies investigated single foods like candies (Déléris et al., 2011), cereal bars (Heenan et al., 2012), and white bread (Pu, Zhang, Zhang, Sun, Ren, Chen, & Xie, 2019), or model foods like model custards (Aprea et al., 2006; Gonzalez-Tomas et al., 2007), and model cheeses (Feron et al., 2014; Guichard et al., 2017). Neither single nor model foods necessarily represent a realistic context of consuming foods or meals that consist of multiple food components that differ considerably in composition, structure, and sensory properties. Most commonly consumed foods consist of different components, so-called composite foods. For example, bread or wafer (carrier foods) are commonly consumed in combination with spreads or toppings. Composition, mechanical properties, and sensory characteristics of the carrier foods differ considerably from the spreads or toppings (Scholten, 2017). Van Eck et al. (2019) demonstrated that in composite foods, carriers tend to dominate texture perception, whereas toppings drive flavor perception (van Eck et al., 2019). We recently confirmed that the addition of carriers (bread and wafer) to spreads decreased consumers' ability to discriminate between different reformulated spread compositions (Gonzalez-Estanol et al., 2022).

It was recently demonstrated that *in vivo* aroma release and perception of composite foods differ from those of single foods, as the characteristics of one component influence aroma release and perception of the other component (Scholten, 2017; van Eck et al., 2021). When different condiments were consumed and assessed alone, *in vivo* aroma release and intensity perception were positively correlated (van Eck et al., 2021). However, when condiments (e.g., mayonnaises) were combined with carriers (breads or potatoes), aroma release and perception were no longer positively correlated. The addition of carriers to condiments increased the release of volatile aroma compounds into the nasal cavity during consumption but decreased perceived aroma intensity. The authors suggested that the increase in aroma release induced by the carriers might be caused by differences in oral processing behaviors and bolus properties (van Eck et al., 2021). They argued that consistent observation of an increase in aroma intensity after swallowing (swallow breath) suggests that assessors could properly evaluate aroma intensity. Yet, they acknowledged that a potential study limitation may have been sensory dumping, as TI profiling was used to assess aroma perception of composite foods that differed considerably in texture (van Eck et al., 2021). Another study assessed the impact of size and hardness of fruit pieces added to yogurts on aroma release and perception. While pear piece size had little effect on aroma release and perception of yogurts, pear piece hardness increased the intensity and duration of aroma release. This was explained by the presence of aroma-aroma and texture-aroma interactions (Mesurolle et al., 2013).

Characterizing composite foods is gaining interest not only because of the increased sensory complexity of foods but also because doing so provides product sensory profiles that are nearer to their natural consumption contexts. To the best of our knowledge, only two studies explored aroma release and perception of composite foods (Mesurolle et al., 2013; van Eck et al., 2021). A better understanding is needed of how the release of aroma compounds during consumption of composite foods is perceived. The aims of this study were to investigate the effects of (i) fat and sugar content of chocolate-hazelnut spreads on *in vivo* aroma release and perception and (ii) carrier addition (bread, wafer) on *in vivo* aroma release and perception of chocolate-hazelnut spread using dynamic nose space analysis (PTR–ToF–MS) and dynamic sensory analysis (TCATA). We hypothesized that (i) fat has a stronger effect than sugar on aroma release and perception due to the hydrophobic nature of the target molecules and (ii) adding solid carriers to chocolate-hazelnut spreads leads to an increase of *in vivo* aroma release and decrease of aroma perception.

2. Materials and methods

2.1. Samples

Preparation and composition of chocolate-hazelnut spreads and composite foods have been described in detail elsewhere (Gonzalez-Estanol et al., 2022). Briefly, the composition of the chocolate-hazelnut spreads used herein along with their rheological properties are summarized in Supplementary Material Table 1. Three chocolate hazelnut spreads varying in fat and sugar content were used herein (Soremartec, Alba, Italy). A high fat/high sugar, control sample (C); high fat/low sugar (LS) sample with 15 % sugar reduction; low fat/high sugar (LF) sample with 15 % fat reduction were prepared; milk and inulin were used to replace fat and sugar, respectively. All spreads were reformulated to bear a close resemblance to commercial products.

All chocolate-hazelnut spreads were spiked with 0.2 % (w/w) of a food grade aroma solution (Soremartec, Alba, Italy) containing five volatile organic compounds (VOCs): benzaldehyde, filbertone, 2-methylpyrazine, delta-dodecalactone, and isovaleraldehyde (Table 1). These compounds were chosen because they present a wide range of chemical classes (aldehydes, ketones, pyrazines, and lactones), have log p values ranging from 0.21 to 3.48, and have sensory notes that have been previously described as hazelnut, milky, and cocoa, which are consistent with the sensory properties of chocolate-hazelnut spreads.

Composite foods were prepared by combining spreads with carriers with different mechanical and texture properties (bread and wafer). The carriers were chosen to simulate the consumption context of the chocolate hazelnut spreads with high ecological validity (Gonzalez-Estanol et al., 2022). A description of sample codes and sample pictures are in Table 2.

All samples included 6 g chocolate-hazelnut spread and were prepared as described elsewhere. When evaluated alone, they were served on a plastic spoon. For the spread-wafer combinations, wafer biscuits (Soremartec, Alba, Italy) were pre-cut in the form of a dome with dimensions of 3x4x1 cm (1.56 ± 0.1 g) and filled with the spread. For the spread-bread combinations, commercially available bread (Morato Bruschelle, Altavilla Vicentina VI, Italy) was cut into pieces of 3 × 3 × 1cm without crust (2.23 ± 0.5 g) and spread (6 g) on top. The spread-bread composite foods were prepared just before the evaluation (<2 h).

2.2. Participants

A trained panel (n = 8) performed sensory evaluations during NSA by PTR-ToF-MS (section 2.3) (age 34.2 ± 7.4 years, body mass Index [BMI] 25.3 ± 4.3 kg/m^2), recruited as volunteers from the Edmund Mach Foundation (San Michele all'Adige, Trentino, Italy). A naive consumer panel (n = 72) performed dynamic sensory evaluation without NSA (age 22.6 ± 2.0 years, BMI 21.9 ± 2.6 kg/m^2), recruited from Wageningen University (Wageningen, the Netherlands) (Gonzalez-Estanol et al., 2022). All participants in both groups were Caucasian women who consumed chocolate-hazelnut spreads at least once a month

Table 1
Composition of the aroma solution used to spike chocolate hazelnut spreads (0.2 g/100 g) together with characteristics of the aroma compounds.

Compound	Concentration g/100 g	Chemical formula	Sensory description	t.i product ions PTR-MS (*m/z*)	Log P[a]
Benzaldehyde	0.01	C_7H_7O	Bitter almond, nutty	107.05	1.48
Filbertone	0.01	$C_8H_{14}O$	Hazelnut aroma	127.112	1.97
delta-Dodecalactone	0.01	$C_{12}H_{22}O_2$	Creamy, milky, buttery	85.0683	3.49
Isovaleraldehyde	0.02	$C_5H_{10}O$	Chocolate, nutty, cocoa	87.077	1.45
2-Methylpyrazine	0.01	$C_5H_6N_2$	Cocoa, nutty, roasted	95.0572	0.21

Table 2
Overview of all samples and their acronyms: chocolate hazelnut spreads and composite foods with bread and wafer.

Group	Spread		
	High fat/ High sugar (Control C)	Low fat / High sugar **(LF)**	**High fat/ Low sugar (LS)**
 **Alone (A)** (Spread: 6 g)	A-C	A-LF	A-LS
 **Bread- Spread (B)** 3×3x1 cm (Bread: 2.2 ± 0.5 g Spread: 6 g)	B-C	B-LF	B-LS
 **Wafer – Spread (W)** 3x4x1 cm (Wafer: 1.6 ± 0.1 g Spread: 6 g)	W-C	W-LF	W-LS

(per self-report). Other inclusion criteria were as follows: not to have any dietary restrictions, allergies, or intolerances to wheat/gluten, dairy, nuts, soybean, or eggs, not to be pregnant, not to smoke; and to have no history of self-reported oral perception disorder or olfactory impairment. Participants gave written informed consent before the start of the study and received financial compensation for their participation. The study was exempt from review by the Medical Research Ethical Committee according to the "Medical Research Involving Human Subjects Act" of The Netherlands (WMO in Dutch). The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013).

2.3. Attribute selection and determination of consumption time

The trained panel participated in four training sessions of one hour each, during which the chocolate-hazelnut spreads and spread-carrier combinations were evaluated. The first two sessions were dedicated to the generation of descriptors related to the flavor and texture of the spreads and carriers, and the most mentioned attributes were reflected in the final attribute lists. Different attribute lists were used for the evaluations of spreads alone, combinations of spread-bread and spread-wafer (Supplementary Material Table 2). Eight sensory attributes were used to describe the spreads alone and 10 attributes were used to describe the spread-wafer and spread-bread combinations. During the third and fourth sessions, participants evaluated the nine samples (Table 2**)** as if it was the actual experiment (section 2.5). The purpose of these sessions was for participants to become comfortable with the PTR-ToF-MS setting, and the swallowing and sensory protocols.

The eight participants who generated the attribute list were also used to determine the consumption time of all samples as described elsewhere (Gonzalez-Estanol et al., 2022). The average consumption time was 15 s for spreads alone and 20 s for carrier-spread combinations.

2.4. Sensory evaluation with Temporal-Check-All-That-Apply (TCATA)

TCATA evaluations of all samples were performed by a consumer panel without NSA (n = 72) to validate the TCATA results obtained from the trained panel during NSA. Sensory evaluations of the consumer panel took place in a testing room at Centrum voor Smaak Onderzoek (Wageningen, The Netherlands) under normal light conditions at room temperature (21 ± 1 °C). As described elsewhere (Gonzalez-Estanol et al., 2022), participants evaluated nine samples (Table 2) in a single 60-min session with 2-min breaks between samples. Participants received the attribute lists and their definitions (Supplementary Material Table 2) by email the day before their scheduled session and were instructed to familiarize themselves with them. Participants were asked not to eat, drink, or use any persistent flavored product for at least one hour before their session. The sensory assessment started with a warm-up sample (cracker) to allow participants to familiarize themselves with the TCATA method and software (TimeSens software version 1.1.601.0, ChemoSens, France). All samples were served at room temperature (21 ± 1 °C) in standardized bite size (Table 2). Samples were coded with random three-digit numbers and presented to the participants on three trays divided by blocks (spreads alone, spread-bread combinations, spread-wafer combinations). Each block included each of the three spread formulations (C, LS, LF). Block orders were counterbalanced and spread formulations within each tray were randomized. All attributes were presented simultaneously on the tablet and their order was randomized across participants.

As described elsewhere (Gonzalez-Estanol et al., 2022), participants were instructed to put the whole sample in their mouth, click the start button, and then immediately commence tracking sensory changes. At any time between clicking start and the end of the evaluation time, participants were asked to check the terms that apply to describe the sensory characteristics of the sample at each moment. Participants were asked to uncheck the terms when they no longer apply to describe the sample. Participants could select as many attributes as they liked, use the same attribute several times or never select an attribute.

When indicated on the screen, participants were instructed to swallow the sample. 15 s and 20 s after clicking the start button, spreads alone and carrier-spreads combinations, respectively, were swallowed (section 2.4). TCATA data collection stopped 105 s after participants clicked the start button. Between samples, participants were asked to rinse their mouth with water.

2.5. Simultaneous in vivo nose space analysis and dynamic sensory evaluation using TCATA

TCATA and *in vivo* NSA were performed simultaneously during three 60-min sessions with the trained panel (n = 8, in triplicate). During each session, participants evaluated all nine samples with short breaks in between. Participants were instructed not to eat, drink, or use any

persistent flavored products for at least one hour before each session. All measurements were performed within a two-week period.

The experimental protocol was adapted from previous PTR-ToF-MS nose space studies (Charles et al., 2015; Pedrotti et al., 2019; van Eck et al., 2021). A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used for the *in vivo* NSA. Participants' breathing patterns were traced using H_3O^+ as the precursor ion with the following drift tube parameters (Weel et al., 2002): voltage 628 V, temperature 110 °C and pressure 2.80 mbar. Acquisition was set to 1 mass spectrum per second. Nose-space sampling was carried out via disposable Teflon tubes placed in both nostrils. Teflon tubes were connected to the NASE sampling system (Ionicon Analytik GmbH, Innsbruck, Austria), which was heated to 110 °C and directly connected to the inlet of the PTR-ToF-MS system at the same temperature. Evaluations took place individually in a laboratory with filtered air. Participants arrived at the laboratory and sat down comfortably with a screen and a mouse in front of them. For each sample, participants were asked to insert the Teflon tubes in their nostrils and to start breathing normally through their nose, with their mouth closed. Their breath was sampled for 60 s after which they were instructed to put the entire sample in their mouth, click the 'start' button on the screen for the TCATA evaluation, and start chewing normally with their mouth closed. This TCATA evaluation during NSA followed the same protocol as the TCATA evaluation of the consumer panel without NSA (section 2.4) and used the same attribute lists. TCATA and *in vivo* nose-space data were acquired simultaneously for 105 s.

2.6. Data analysis

2.6.1. TCATA curves

TCATA curves from the trained panel with NSA (n = 8, in triplicate) and the consumer panel without NSA (n = 72) were constructed following the procedure described by Castura et al. (2016) (Castura et al., 2016). Citation proportions, defined as the proportion of participants who checked (or perceived) a given attribute at a given moment (every 0.1 s) during the evaluation period, were calculated for each attribute for all samples. Smoothing of TCATA curves was done via the *smoothing.spline* function in the TempR package of R software version 3.1.1. Resulting curves were then analyzed as described elsewhere (Gonzalez-Estanol et al., 2022). To evaluate the effects of formulations, for each product group (spread alone, spread-bread and spread-wafer combinations), the citation proportions of the control samples (A-C, B-C, W-C) were compared with the citation proportions of the corresponding test samples (A-LF and A-LS; B-LF and B-LS; W-LF and W-LS). Significant differences in TCATA profiles of two products were calculated for each time point and for each attribute by applying two-sided Fisher–Irwin tests to evaluate whether citation proportions for the pairs of products were statistically significant different at a 5 % significance level (Castura et al., 2016). Highlighted sections (bold lines) in the TCATA curves represent periods during which significant differences between test samples (LS or LF) and control samples were observed ($p < 0.05$).

For both panels, duration proportions for each participant were obtained for each sample in each TCATA evaluation as the proportion of the 105 s evaluation time that an attribute was selected. For example, if a participant selected sticky for a duration of 40 s, then the duration proportion would be obtained as 40 s/105 s = 0.38. Duration proportions ranged from 0 to 1. Data was checked for the effect of the panel on dynamic sensory perception. An analysis of variance (ANOVA) was performed with the duration proportions of each attribute as a response and panel (consumer without NSA / trained with NSA) as independent variable. A Multiple Factor Analysis (MFA) was performed on the average duration proportions of the attributes of all the samples. Product spaces and correlation plots were constructed to visualize differences and similarities in the characterization of the samples between the trained panel with NSA and the consumer panel without NSA. RV coefficients were calculated from MFA analyses to obtain the correlation between both panels.

Finally, the maximum value for citation proportions (Cit_{max}), the time to reach Cit_{max} (T_{max}), and the area under the curve (AUC) were extracted for hazelnut, milky, and cocoa attributes from the TCATA curves of both panels for a direct comparison of aroma release of the five volatile compounds used to spike the spreads. In addition to analyzing the formulation effect previously described, the effect of carrier addition was also assessed for these attributes. Thus, for each spread formulation, the citation proportions of the spreads served alone (A-C, A-LF, A-LS) were compared with the citation proportions of the spread-carrier combinations (B-C,W-C; B-LF,W-LF; and B-LS,W-LS). Pairwise product differences between the product served alone and the spread-carrier combinations were performed, where bold sections in the TCATA curves represent periods during which significant differences between samples were observed ($p < 0.05$).

2.6.2. In vivo nose-space data analysis

PTR-ToF-MS data were processed with in-house software (Department of Food Quality and Nutrition, Edmund Mach Foundation) as described elsewhere (Cappellin et al., 2011). Tentative peak identification was performed using an in-house library developed by the authors. Mass peaks corresponding to the target volatile compounds (Table 1) were extracted and their concentrations were calculated. For each selected mass peak, averaged release curves (n = 8, in triplicate) were plotted against time (s) for each sample. Time was divided into three windows: baseline (-50 s to 0 s), mastication of samples (0 s to 15 s / 20 s depending on sample type) and post-swallowing (16 s / 21 s to 105 s, depending on sample type). AUC, maximum aroma concentration (I_{max}) and mean time to reach maximum aroma concentration (T_{max}) were calculated for each compound separately.

To test the effect of chocolate hazelnut spread formulation and carrier addition, linear mixed models (LMM) were performed for each of the extracted parameters individually. Spread (C, LS, LF), carrier (Alone, Bread, Wafer), and their interactions were set as fixed factors and subjects as random effect. When $p < 0.05$, Tukey's HSD pairwise comparisons were performed.

Relative changes in AUC, I_{max} and T_{max} values were determined (%) individually for each volatile compound by calculating the difference in values between the control formulation (A-C, B-C, W-C) and the low sugar (A-LS, B-LS, W-LS) or low fat samples (A-LF, B-LF, W-LF) and then dividing this difference by the value of the control formulation (A-C, B-C,W-C). In a similar way, relative changes in AUC, I_{max} and T_{max} were determined (%) individually for each volatile compound by calculating the difference in values between the spreads evaluated alone (A-C, A-LF, A-LS) and the bread (B-C, B-LF, B-LS) or wafer (W-C, W-LF, W-LS) and then dividing this difference by the value of the spreads evaluated alone (A-C, A-LF, A-LS).

3. Results

3.1. Dynamic sensory perception of spreads alone

Fig. 1 compares the TCATA curves of both panels for the three chocolate-hazelnut spreads when consumed alone (A-C, A-LF, A-LS). The TCATA profile of the control spread obtained by the trained panel with NSA (n = 8, in triplicate) (Fig. 1**A**) was characterized by the perception of stickiness, especially before and after swallowing, reaching its highest citation proportion (0.84) at 28 s, followed by perception of cocoa reaching its highest citation proportion (0.92) at 42 s. The end of the evaluation was characterized by high citation proportions of milky and sweetness. Highlighted sections in the TCATA curves (bold lines) represent periods during which significant differences between samples were observed ($p < 0.05$). A-LF (Fig. 1**C**) was perceived to be stickier from to the middle of the evaluation, but with a short period (<3 s) during which it was significantly different from C. From the middle

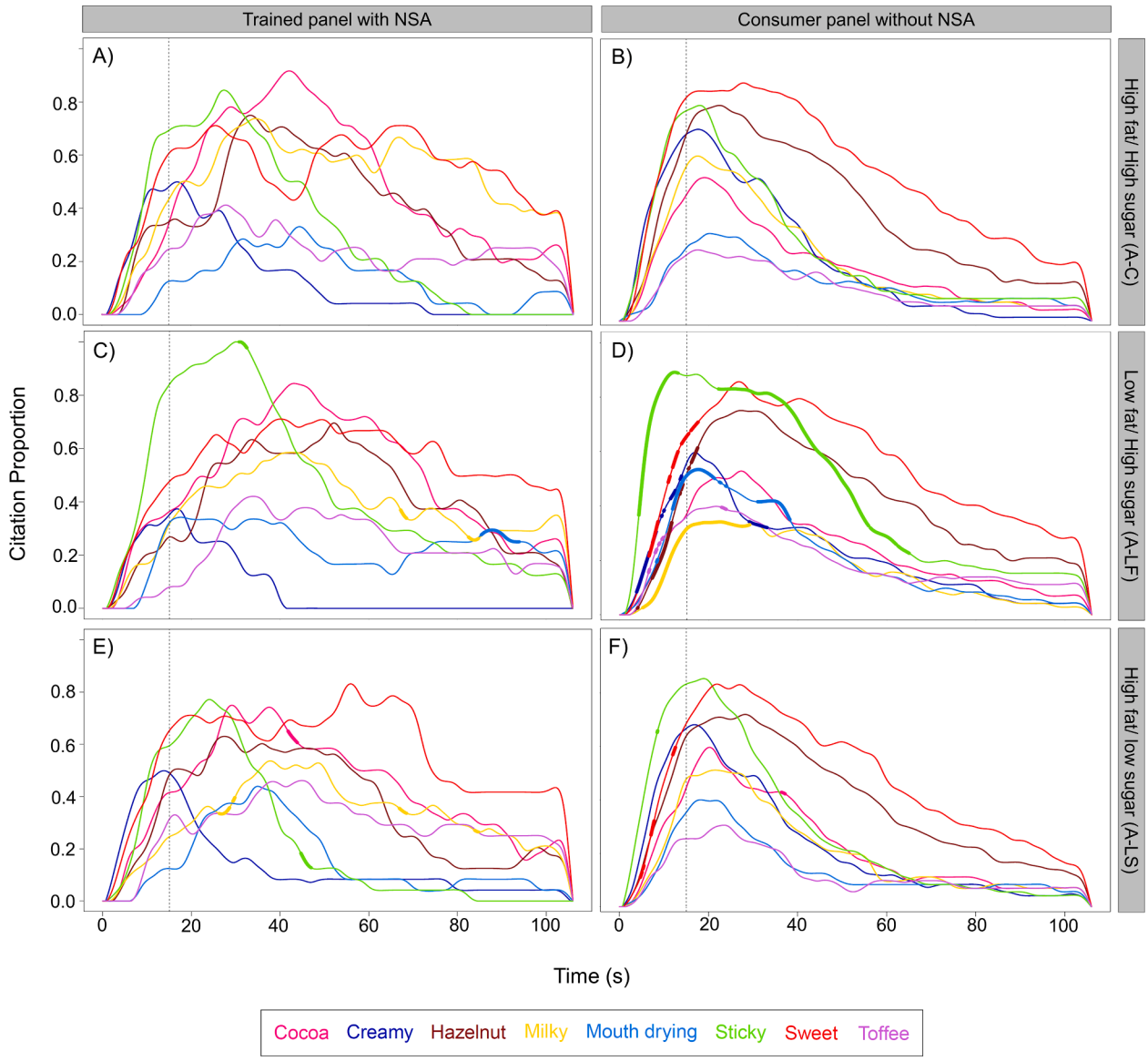

Fig. 1. Trained panel with nose space analysis (NSA) (n = 8, triplicate) and consumer panel without NSA (n = 72). TCATA curves for three chocolate-hazelnut spreads served on their own (A-C, A-LF, A-LS). Periods of significant differences ($p < 0.05$) in proportion of citations between A-LF and A-C and between A-LS and A-C are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.

through the end of the evaluation, cocoa and sweetness had the highest citation proportions. Mouth drying was higher than A-C, especially at the end of the evaluation when there was a short period (<3 s) of significant difference. Overall, milky showed a decrease in perception, with short significantly different periods around 25 s and 70 s of the evaluation. The TCATA profile of A-LS (Fig. 1**E**) was similar to the control (A-C). After swallowing, perception was characterized by sweetness, stickiness, and cocoa flavor. This was followed by high citation proportions of sweetness until the end of the evaluation.

For the TCATA evaluations with the consumer panel without NSA (n = 72), the highest citation proportions for the control spread (Fig. 1**B**) were sweetness (0.87), followed by hazelnut (0.79) and stickiness (0.79). The reduced fat spread (A-LF) (Fig. 1**D**) was perceived to be significantly ($p < 0.05$) stickier, compared with the control spread (A-C) throughout most of the evaluation period. Citation proportions of milky, sweetness, creamy, and hazelnut were significantly ($p < 0.05$) lower at the beginning of mastication and after swallowing compared with the control (A-C), whereas mouth drying citation proportions were significantly ($p < 0.05$) higher before and after swallowing. The sensory profile of the reduced sugar spread (A-LS) (Fig. 1**F**) was very similar to the control spread (A-C), except for a short period (<3 s) at the beginning of the evaluation, during which sweetness was significantly lower compared with A-C.

To summarize, TCATA evaluations of the spreads alone by the trained panel with NSA and of the consumer panel without NSA were similarly to those of the reduced fat spread (A-LF), which was perceived as stickier than the control spread, especially at the beginning of mastication. Dynamic perception of hazelnut and sweetness followed similar temporal profiles for both panels.

3.2. *Dynamic sensory perception of spreads with carriers*

TCATA curves for the three chocolate-hazelnut spreads combined with bread (B-C, B-LF, B-LS), and wafer (W-C, W-LF, W-LS) are shown in Supplementary Material Figs. 1 and 2, respectively. For both panels, bread-like flavor (for spread-bread combinations) and crunchy and wafer-like (for spread-wafer combinations), were used to describe the perception of the composite foods at the beginning of mastication.

After swallowing, the evaluation of B-C by the trained panel with NSA, displayed a high citation proportion for cocoa, hazelnut, and sweetness, which lingered until the end of the evaluation. Just as with the spreads evaluated alone, comparisons were performed between the reduced fat (B-LF, W-LF) and sugar (B-LS, W-LS) spreads with their control counterparts (B-C, W-C). For the reduced fat spread (B-LF), after swallowing, there was a significant decrease in citations of cocoa and a significant increase of milky perception at the beginning of the mastication compared with B-C. Towards the end of the evaluation, sweetness was prevalent. The reduced sugar sample (B-LS) was characterized by the perception of cocoa during the middle of the evaluation. Towards the end of the evaluation, milky and sweetness were the most cited attributes.

The TCATA evaluation with the consumer panel without NSA, showed that after swallowing B-C, sweetness and hazelnut had the highest citation proportions with 0.83 and 0.72, respectively, and lingered until the end of the evaluation. While the B-LS combination displayed a very similar temporal profile as the B-C combination, the B-LF combination showed a significant increase in citation proportions of sticky, especially after swallowing, compared with B-C.

For both panels, and all samples (W-C, W-LS, W-LF), wafer-like flavor and crunchy were the characteristic attributes at the beginning of consumption until after swallowing. For the trained panel with NSA, for all samples, hazelnut, cocoa, and sweetness showed high citation proportions from the middle through the end of the evaluation. No significant differences were found between W-LF and W-C or between W-LS and W-C. For the consumer panel without NSA, all wafer combinations reached the highest citation proportions for creamy around swallowing (20 s). Sweetness and hazelnut showed a prolonged perception after swallowing and towards the end of the evaluation time. The W-LF combination displayed a significant increase in citation proportions for sticky after swallowing compared with W-C, followed by an increment of toffee flavor around the middle of the mastication period, and a decrease in cocoa flavor before swallowing. W-LS combinations showed very similar dynamic sensory profiles as W-C, with only minor significant differences during short periods (<3 s).

To summarize, TCATA profiles of the spread-carrier combinations obtained by the trained panel with NSA and the consumer panel without NSA showed similarly that the addition of carriers (bread and wafer) had a considerable effect on the dynamic sensory perception of all spreads. Attributes related to the carriers were perceived at the beginning of consumption, whereas those of the spreads were perceived after swallowing.

3.3. Comparison of TCATA profiles from consumer panel without NSA and a trained panel with NSA

The results from the ANOVA showed that the duration of citation proportions of 5 out of 8 attributes were significantly different across both panels (cocoa ($F(1, 16) = 174.51$, $p < 0.001$), creamy ($F(1, 16) = 79.51$, $p < 0.001$), milky ($F(1, 16) = 79.08$, $p < 0.001$), sticky ($F(1, 16) = 4.74$, $p < 0.05$), and toffee ($F(1, 16) = 13.12$, $p < 0.01$)). To further compare the TCATA evaluation results from the trained panel with NSA (n = 8, in triplicate; blue) and the consumer panel without NSA (n = 72; green), MFA was performed for all samples (Supplementary Material Fig. 3). The correlation circle illustrates the average duration proportions for each sensory attributes from both panels, and the individual factor map represents the average duration proportions as mean points and the variation between the trained and consumer panels. Excellent agreement between configurations would correspond to a RV coefficient close to 1 (Faye et al., 2004). With an observed RV coefficient of 0.73, it can be concluded that the overall agreement between both panels was good. Overall, this suggests that the differences observed in the temporal perception of the spreads and composite foods between the panels for the attributes cocoa, creamy, milky, sticky and toffee were significant but small. Visual inspection of the MFA plot reveals that the first dimension differentiates the spreads alone from the spread carrier combinations. The second dimension seems to differentiate mainly across chocolate hazelnut spread formulations.

Even though both panels generally perceived similar sensory attributes as applicable over time to describe the samples as observed from the MFA (Supplementary Material Fig. 3), it was decided to not average the results from both panels because the duration proportions of cocoa, creamy, milky, sticky, and toffee were significantly different across both panels ($p < 0.05$).

For further analysis and comparison of *in vivo* aroma release with aroma perception, the TCATA data from the consumer panel without NSA (n = 72) is considered in sections 3.4 and 3.5 although the data was not collected during the *in vivo* PTR-ToF-MS measurements. This is because the number of observations for the consumer panel without NSA was 72 compared to 24 for the trained panel with NSA (n = 8, triplicate), which gives the consumer panel more statistical power, and explains why the TCATA curves of the trained panel with NSA are overall less smooth than the TCATA curves of the consumer panel without NSA. Moreover, it seems that most attributes were not unselected by most of the assessors of the trained panel with NSA at the end of the evaluation in contrast to the consumer panel which unselected attributes more frequently.

3.4. Influence of spread formulation on in vivo aroma release and perception

Table 3 displays a summary of the parameters extracted from the *in vivo* aroma release curves (AUC, I_{max}, and, T_{max}) for each compound separately and the parameters extracted from the TCATA curves (AUC, Cit_{max} and T_{max}) of the attributes cocoa, hazelnut and milky. The effects of spread (Control, LS, LF), carrier (Alone, Bread, Wafer) and their interaction on the aroma release parameters were determined using linear mixed models (Table 4).

Overall, fat and sugar content of spreads had only a limited effect on *in vivo* aroma release, as it was significant ($p < 0.05$) only for benzaldehyde, filbertone and isovaleraldehyde (Table 4). Compared to the control formulation, the AUC of benzaldehyde decreased for the low sugar spreads by −7.8 % for A-LS and by −9.4 % for W-LS (Fig. 2**A**). For filbertone, AUC was reduced the most for the low sugar spreads (by −5.1 % for B-LS and by −6.8 % for W-LS compared to the control samples). I_{max} of benzaldehyde decreased the most in the low sugar spread (by −11.3 % for W-LS and by −10.7 % for A-LS).

Fig. 3**A** shows the release curves of tentatively identified (t.i.) methylpyrazine (*m/z* 95.05), benzaldehyde (*m/z* 107.05), filbertone (*m/z* 127.112) and the corresponding TCATA profiles for hazelnut obtained from the consumer panel without NSA. When the spreads were consumed alone, the A-LF displayed significantly lower citation proportions (highlighted with bold lines), compared to A-C, that lasted from the beginning of mastication and after the swallowing moment. No significant effect was observed on the perception of flavor for neither of the spread-carrier combinations.

Fig. 4**A** shows the release curves of t.i delta dodecalactone (*m/z* 85.06) and its corresponding TCATA profiles for milky flavor. When spreads were consumed on their own, milky perception of A-LF decreased significantly (bold lines) compared to A-C from the beginning of the evaluation and lasted after the swallowing moment. When carriers were added, B-LF and W-LF also displayed a decrease in perception, however not to a significant level, except for short periods (<3s) in the case of B-LF.

Lastly, a similar trend was observed for the release curves of t.i. isovaleraldehyde (*m/z* 87.07) and its corresponding TCATA profiles of the cocoa attribute (Supplementary Material Fig. 4). Significant differences of cocoa perception were found for the spread-wafer combinations. The TCATA curves of reduced fat spread (W-LF) displayed a significant decrease (bold lines) compared to W-C at the end of the mastication period and during the swallowing moment.

Table 3
(A) Area under the curve (AUC), maximum aroma concentration (I_{max}) and mean time to reach maximum aroma concentration (T_{max}) (mean ± SD) describing *in vivo* nose space release of tentatively identified delta-dodecalactone (*m/z* 85.06), isovaleraldehyde (*m/z* 87.07), methylpyrazine (*m/z* 95.05), benzaldehyde (*m/z* 107.05), and filbertone (*m/z* 127.112) determined with PTR-ToF-MS (n = 8, triplicate), and (B) maximum value for citation proportions (Cit_{max}), time to reach Cit_{max} (T_{max}), and the Area under the curve (AUC) from TCATA curves of consumer panel for attributes cocoa, hazelnut and milky.

		A) *in vivo* aroma release (PTR-ToF-MS)						B) Sensory perception (TCATA)		
		Delta-dodecalactone	**Isovaleraldehyde**	**Methylpyrazine**	**Benzaldehyde**	**Filbertone**		**Cocoa**	**Hazelnut**	**Milky**
A-C	MaxI (ppbV)	1.0 ± 0.4	25.9 ± 11	1.7 ± 1	2.4 ± 1.1	1.5 ± 0.5	MaxCit (-)	0.5	0.8	0.6
	Tmax (s)	34 ± 18	33 ± 20	41 ± 30	35 ± 18	37 ± 19	Tmax (s)	19	23	18
	AUC (ppbV*s)	19 ± 10	443 ± 243	39 ± 25	53 ± 28	34 ± 13	AUC	21	42	24
A-LF	MaxI (ppbV)	0.9 ± 0.4	25.5 ± 13.9	1.3 ± 0.7	2.2 ± 1.1	1.4 ± 0.6	MaxCit (-)	0.5	0.8	0.3
	Tmax (s)	35 ± 19	33 ± 22	42 ± 31	33 ± 16	32 ± 24	Tmax (s)	27	27	23
	AUC (ppbV*s)	19 ± 12	520 ± 349	33 ± 26	53 ± 29	34 ± 18	AUC	25	43	17
A-LS	MaxI (ppbV)	0.8 ± 0.4	24.1 ± 14.8	1.3 ± 0.8	2.2 ± 1.1	1.3 ± 0.5	MaxCit (-)	0.6	0.7	0.5
	Tmax (s)	27 ± 12	25 ± 13	45 ± 31	31 ± 17	31 ± 17	Tmax (s)	20	28	22
	AUC	15 ± 10	394 ± 256	35 ± 25	46 ± 23	27 ± 15	AUC	24	40	21
B-C	MaxI (ppbV)	4.3 ± 2.1	38 ± 19.2	1.2 ± 0.5	2.8 ± 1	1.8 ± 0.5	MaxCit (-)	0.5	0.7	0.5
	Tmax (s)	32 ± 11	26 ± 9	35 ± 17	35 ± 11	36 ± 13	Tmax (s)	21	32	25
	AUC (ppbV*s)	149 ± 72	777 ± 319	42 ± 20	74 ± 31	50 ± 17	AUC	21	40	23
B-LF	MaxI (ppbV)	4.1 ± 2.6	34.6 ± 13.1	1.2 ± 0.8	2.5 ± 1	1.5 ± 0.4	MaxCit (-)	0.5	0.7	0.4
	Tmax (s)	31 ± 11	25 ± 7	40 ± 25	35 ± 13	33 ± 10	Tmax (s)	28	23	22
	AUC (ppbV*s)	144 ± 93	776 ± 350	34 ± 19	72 ± 37	46 ± 20	AUC	22	41	18
B-LS	MaxI (ppbV)	4.2 ± 2.9	36.5 ± 15	1.4 ± 0.9	3 ± 1.5	1.7 ± 0.6	MaxCit (-)	0.4	0.7	0.5
	Tmax (s)	29 ± 13	23 ± 8	40 ± 31	31 ± 10	30 ± 12	Tmax (s)	20	28	22
	AUC (ppbV*s)	129 ± 86	686 ± 336	37 ± 24	70 ± 39	41 ± 18	AUC	19	42	19
W-C	MaxI (ppbV)	1.2 ± 0.5	34.8 ± 14.4	1.7 ± 1.2	2.7 ± 1.5	1.6 ± 0.6	MaxCit (-)	0.6	0.8	0.5
	Tmax (s)	29 ± 12	27 ± 11	38 ± 33	34 ± 14	34 ± 19	Tmax (s)	22	25	28
	AUC (ppbV*s)	29 ± 14	818 ± 351	60 ± 45	70 ± 42	47 ± 19	AUC	22	40	17
W-LF	MaxI (ppbV)	1.1 ± 0.4	35.5 ± 12.9	1.8 ± 1.3	2.7 ± 1.2	1.6 ± 0.7	MaxCit (-)	0.4	0.7	0.4
	Tmax (s)	27 ± 11	26 ± 11	42 ± 28	35 ± 11	34 ± 15	Tmax (s)	27	31	29
	AUC (ppbV*s)	28 ± 12	846 ± 341	59 ± 37	71 ± 30	46 ± 18	AUC	18	39	16
W-LS	MaxI (ppbV)	1 ± 0.4	28.1 ± 10.2	1.6 ± 0.9	2.3 ± 0.9	1.3 ± 0.5	MaxCit (-)	0.5	0.7	0.4
	Tmax (s)	32 ± 16	32 ± 16	30 ± 23	32 ± 11	35 ± 15	Tmax (s)	25	26	27
	AUC (ppbV*s)	24 ± 10	673 ± 263	52 ± 36	60 ± 31	40 ± 16	AUC	20	39	20

Table 4
Effect of carrier addition to spreads (carrier), reformulation of chocolate hazelnut spreads (formula) and their interaction effect (linear mixed model) on AUC, MaxI and Tmax values obtained from *in vivo* nose space aroma release of t.i. (A) methylpyrazine, (B) benzaldehyde, (C) filbertone, (D) delta-dodecalactone, and (E) isovaleraldehyde.

A) Methylpyrazine	**AUC**		**I_{max}**		**Tmax**	
Source	**F**	**p value**	**F**	**p value**	**F**	**p value**
Carrier	32.99	**<0.001**	12.44	**<0.001**	0.99	0.37
Formula	2.22	0.11	0.95	0.39	0.46	0.63
Formula * Carrier	0.62	0.65	1.96	0.10	0.79	0.53
B) Benzaldehyde	**AUC**		**I_{max}**		**Tmax**	
Source	**F**	**p value**	**F**	**p value**	**F**	**p value**
Carrier	40.26	**<0.001**	11.53	**<0.001**	0.28	0.75
Formula	4.70	**0.01**	1.89	0.15	2.80	0.06
Formula * Carrier	0.48	0.75	3.17	**0.01**	0.34	0.85
C) Filbertone	**AUC**		**I_{max}**		**Tmax**	
Source	**F**	**p value**	**F**	**p value**	**F**	**p value**
Carrier	36.64	**<0.001**	10.39	**<0.001**	0.27	0.76
Formula	10.21	**<0.001**	5.25	**<0.001**	1.98	0.14
Formula * Carrier	0.16	0.96	2.05	0.09	0.66	0.62
D) Delta-dodecalactone	**AUC**		**I_{max}**		**Tmax**	
Source	**F**	**p value**	**F**	**p value**	**F**	**p value**
Carrier	190.73	**<0.001**	149.21	**<0.001**	0.85	0.43
Formula	1.09	0.34	0.40	0.67	0.89	0.41
Formula * Carrier	0.28	0.89	0.07	0.99	2.13	0.08
E) Isovaleraldehyde	**AUC**		**I_{max}**		**Tmax**	
Source	**F**	**p value**	**F**	**p value**	**F**	**p value**
Carrier	81.72	**<0.001**	23.49	**<0.001**	3.59	**0.03**
Formula	11.35	**<0.001**	1.99	0.14	0.84	0.43
Formula * Carrier	0.78	0.54	1.49	0.20	2.81	**0.03**

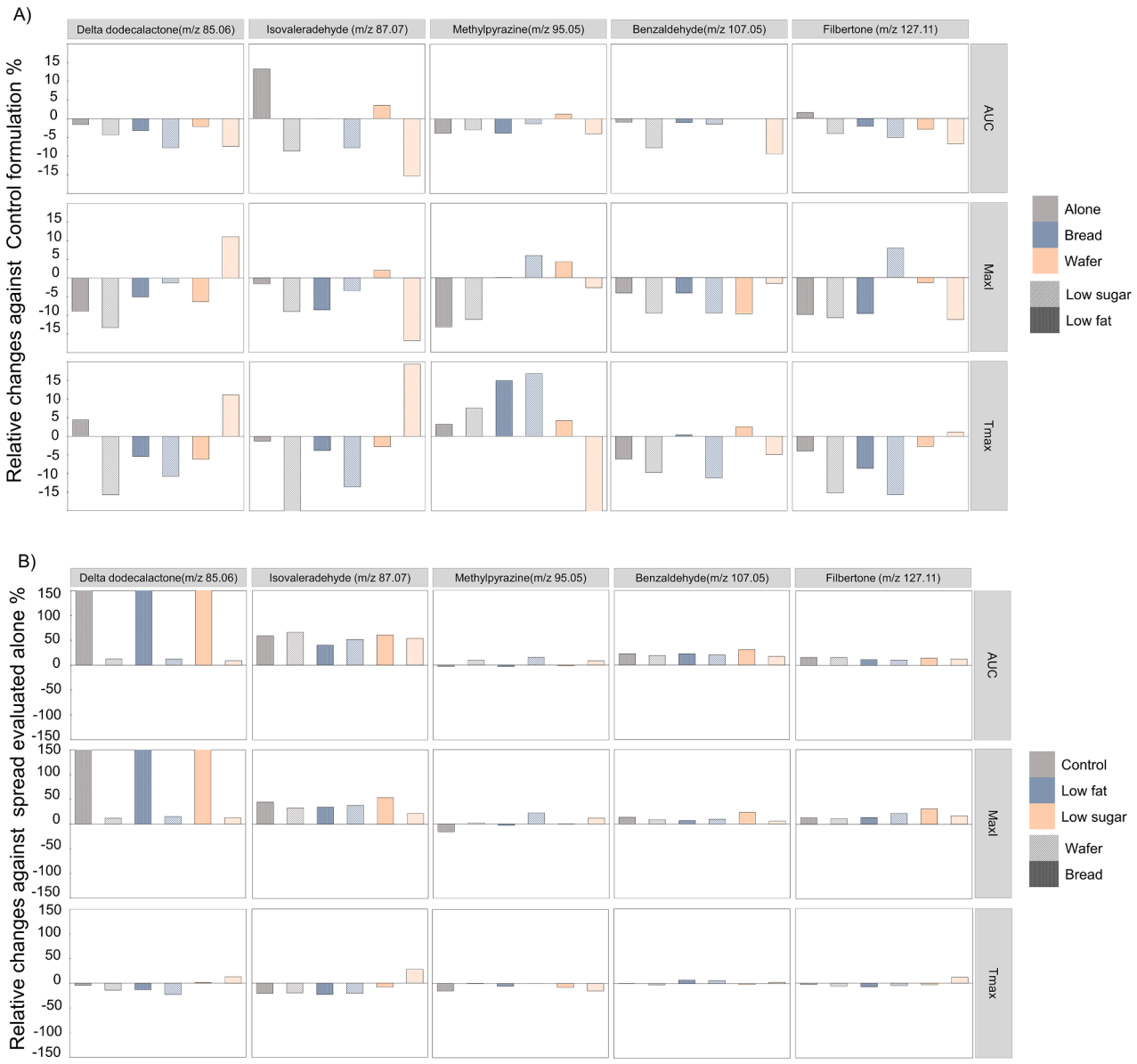

Fig. 2. Relative intensity changes in AUC, I_{max} and T_{max} (%) for each volatile compound. (A) describes the relative changes against the control formulation in comparison to reduced fat and reduced sugar spreads. (B) describes the relative changes against the spreads evaluated alone compared to spreads combined with bread or wafer.

3.5. Influence of carrier addition to spreads on in vivo aroma release and perception

Contrary to the small effect of spread reformulation on *in vivo* aroma release and perception, there was a strong effect of the addition of carrier on both aroma release and perception for all target molecules. Overall, the AUC and I_{max} of the spreads consumed alone showed a significant increase ($p < 0.05$) with the addition of bread and wafer (Table 3, Table 4). In the case of delta dodecalactone, its release was only affected by the addition of bread ($p < 0.05$), which increased AUC by 170 % for the control formulation, by 166 % for the low fat formulation and by 160 % for the low sugar formulation compared to when the corresponding spreads were eaten alone (Fig. 2**B**). I_{max} increased by 214 % with the addition of bread for the control formulation, by 228 % for the low fat formulation and by 258 % for the low sugar formulation. For the release of the five VOC's, the addition of carriers reduced the time to reach maximum intensity compared to when spreads were eaten alone, but only in the case of isovaleraldehyde this reduction was significant ($p < 0.05$) when bread was added to the control formulation (−20.7 %) and to the low fat formulation (–22.7 %) (Tables 3 and 4, and Fig. 2**B**).

Fig. 3**B and 4B** display the carrier effect of aroma release of t.i. methylpyrazine, t.i. benzaldehyde, t.i. filbertone, and t.i. delta dodecalactone and their corresponding TCATA profiles obtained from the consumer panel without NSA for their corresponding flavor attributes (hazelnut and milky). In all cases, contrary to the increase of aroma release observed upon addition of carriers to spreads, aroma perception decreased, especially in the beginning of mastication. For the control and the reduced sugar spreads, hazelnut perception significantly decreased with the addition of wafer (bold lines). For milky, this decrease was more pronounced in the control spreads where the decrease was significant (bold lines) with bread and wafer addition. Lastly, in the case of cocoa, wafer addition led to a significant decrease (bold lines) in perception at the beginning of mastication in all formulations.

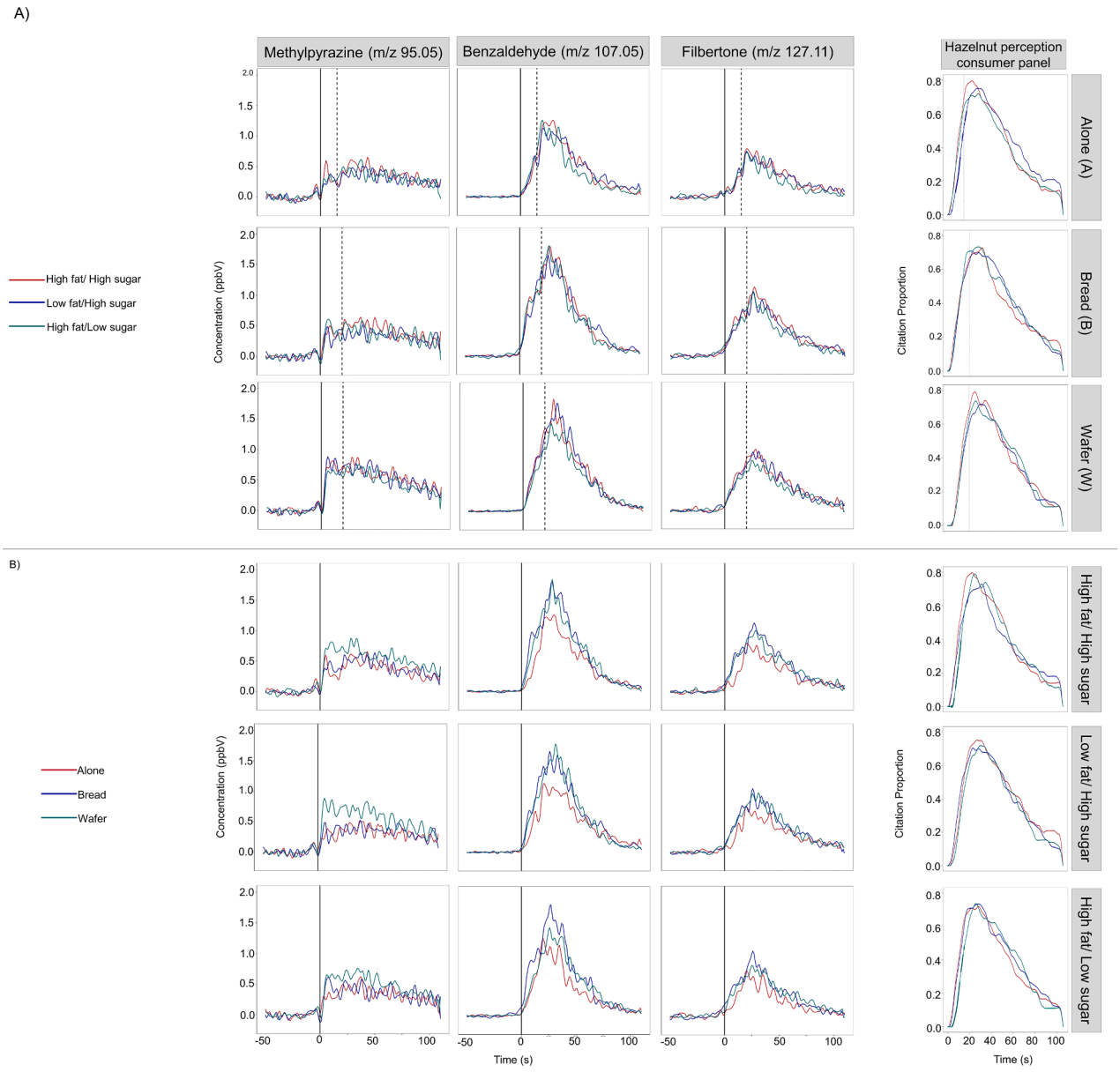

Fig. 3. Averaged *in vivo* nose space release of methylpyrazine, benzaldehyde and filbertone (n = 8, triplicate) and TCATA proportion of citations of the consumer panel without NSA (n = 72) for the attribute hazelnut comparing effects of formula (A) and carrier (B) on aroma release and perception. Black continuous lines represent moment when samples were put in mouth, and dotted lines represent swallowing moment. Periods of significant differences ($p < 0.05$) in proportion of citations in TCATA curves, compared to control formula (A) and spread alone (B) are indicated by highlighted thick sections.

4. Discussion

While many studies investigated the relationship between *in vivo* aroma release and perception of single or model foods, little attention has been paid to *in vivo* aroma release and perception of complex food matrices that could be more representative of the real consumption context. The aims of this study were (i) to investigate the effect of fat and sugar content of chocolate-hazelnut spread on *in vivo* aroma release and sensory perception of a composite food and (ii) to investigate the effect of carrier addition on the aroma release and sensory perception of chocolate-hazelnut spread using *in vivo* nose space analysis with PTR–ToF–MS and TCATA sensory analysis. From a methodological point of view, the trained panel with nose space analysis does not warrant smooth TCATA data due to the limited number of observations (n = 8, in triplicate). Consequently, a TCATA evaluation was done with consumers without nose space analysis (n = 72) to validate the TCATA results obtained from the trained panel with NSA. Overall, the TCATA profiles obtained with the trained panel during nose space analysis and the consumer panel without nose space analysis showed good agreement (RV coefficient = 0.73). Therefore, the TCATA profiles obtained from the consumer panel without nose space analysis were used for the comparisons of aroma release and perception.

It was first hypothesized that fat has a stronger effect than sugar on aroma release and perception due to the hydrophobic nature of the target molecules. Our results showed that modulations of fat and sugar content of spreads had little effect on aroma release and perception. It is important to note that fat and sugar reduction remained within realistic product reformulations (Supplementary Material Table 1), so had high ecological validity but were small. This could partly explain why the degree of reformulation of spreads in our study was not sufficient to cause larger differences in aroma release and perception depending on fat and sugar content of the spreads.

Despite the inter- and intra- individual differences, the five t.i. VOC's (methylpyrazine, benzaldehyde, filbertone, delta dodecalactone and

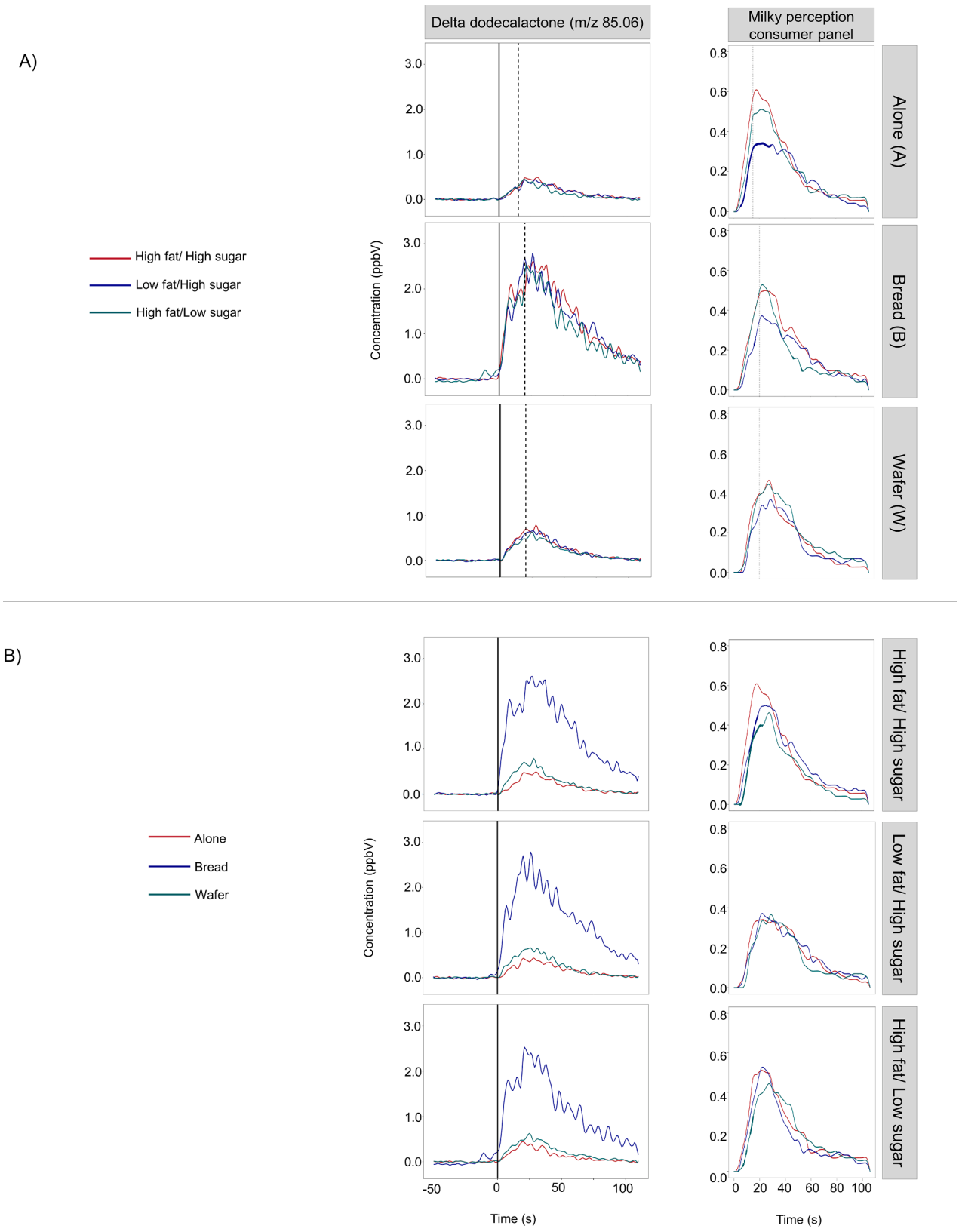

Fig. 4. Averaged *in vivo* nose space release of delta-dodecalactone (n = 8, triplicate) and TCATA proportion of citations of the consumer panel without NSA (n = 72) for attribute milky comparing effects of formula (A) and carrier (B) on aroma release and perception. Black continuous lines represent moment when samples are put in mouth, and dotted lines represent swallowing moment. Periods of significant differences ($p < 0.05$) in proportion of citations in TCATA curves compared to control formula (A) and spread alone (B) are indicated by highlighted thick sections.

isovaleraldehyde) used to spike the spreads displayed similar *in vivo* release patterns with the maximum release being reached after the swallowing moment. This is in accordance with literature (Déléris et al., 2011; Hodgson et al., 2003) which demonstrated an increase in aroma release after swallowing (swallow breath). Isovaleraldehyde displayed the highest in-nose concentration. In previous studies comparing volatility of different chemical classes, esters showed the highest volatility, followed by aldehydes, ketones and alcohols (Naknean & Meenune, 2010). On the other hand, saliva affects odorant concentration by chemical and biochemical reactions between its components and food volatiles. It has been shown that benzaldehyde can be affected by an interaction with mucins (Friel & Taylor, 2001; Van Ruth & Roozen, 2000), which could explain its lower release, despite being an aldehyde. Even though all aroma molecules were hydrophobic (Table 1) our results are not in line with the general concept that the lipid phase acts as a reservoir for aroma compounds resulting in a decrease of aroma release with increasing lipid content in the food matrix (Arancibia et al., 2011; Frank et al., 2011; Gonzalez-Tomas et al., 2007; Guichard, 2002). This discrepancy could be because the reduced fat spread had the highest viscosity of all spreads (Supplementary Material Table 1). As a direct consequence of a change in viscosity, sensory perception of the reduced fat spread was characterized by sticky perception, decreasing the perception of aromas associated to the molecules of interest. This is in line with a previous study reporting that an increase in viscosity induced a decrease in aroma perception (Baines & Morris, 1987). Similarly, it was reported that decreasing fat content led to a reduction of perceived lemon intensity in mayonnaises (van Eck et al., 2021; Wendin et al., 1997).

Secondly, it was hypothesized that adding solid carriers to hazelnut-chocolate spreads lead to an increase in aroma release and decrease in aroma perception. From our results, when the spreads were combined with carriers, *in vivo* aroma release and perception correlated negatively. In general, addition of bread or wafer enhanced aroma release, which could be explained by the introduction of chewing by the carriers and an adaptation of the masticatory behavior to the hardness of the carriers, which caused the breakdown of the food into many particles increasing the surface area and thus, the mass transfer from the bolus to the saliva and air. Differences within carriers were observed for delta dodecalactone as the addition of bread significantly increased its release, but the addition of wafer had no effect on delta dodecalactone release. This lactone is naturally found in butter, cheese, apricot, coconut, peach, pineapple, strawberry and meat (Sánchez-Palomo et al., 2008; Zhang et al., 2020), so its presence in bread can be neglected. Even if aroma compound properties can account for the diversity in the shape of release curves kinetics, the high inter-individual physiological variability (e.g volumes of the naso-oropharyngeal cavities, dentition, tongue movements during each chew, variations in saliva flow and salivary protein composition, velum opening and different breathing rates) (Deleris et al., 2015; Labouré et al., 2014) and product characteristics remain important factors that could explain the differences that were observed. In this case, it may be that the bolus formation process of bread led to an increase of volatile release.

In contrast to the increase of aroma release, addition of solid carriers resulted in a decrease of aroma perception. The addition of a carrier food to a spread created a contrasting texture, and perception may have shifted from one food to another within one single bite and during the mastication process. So, carrier addition modulates aroma perception of composite foods by cross-modal texture-aroma interactions. This is in line with previous findings, where it was shown that the texture perception of food dominates the duration from early to middle chewing time in cereal products such as biscuits (Laguna et al., 2013). Our results are also in line with previous studies on composite foods. For instance, the study of van Eck et al. (2021) combined bread and crackers with different mayonnaises. Their study found that carriers contribute to the texture perception, whereas toppings drive overall flavor perception. More recently, in another study, when different mayonnaises were consumed and assessed alone, aroma release and intensity perception were positively correlated, however, when they were combined with different carriers, release of aroma into the nasal cavity during consumption increased, but the perceived aroma intensity decreased (van Eck et al., 2021). Thus, our second hypothesis is confirmed. We acknowledge that monitoring specific odor-active compounds and knowing their individual flavor quality does not necessarily translate to the contribution of the individual compound to the overall flavor of the food due to perceptual interactions between different aroma volatiles. Therefore, the relationships between the *in vivo* aroma release of the specific volatile compounds and their aroma perception reported in this study should be interpreted with caution.

5. Conclusions

To conclude, this study demonstrated that fat and sugar content of chocolate-hazelnut spreads, in the studied reformulation range, had only limited effect on *in vivo* aroma release and perception. The addition of carriers to the chocolate-hazelnut spreads generally increased the duration and intensity of *in vivo* aroma release and decreased aroma perception. This strong effect of carrier addition on aroma release and perception of chocolate-hazelnut spreads highlights the importance of investigating toppings/spreads accompanied with carriers rather than in isolation as it could give an inaccurate sensory profile and mislead product development. Ultimately, the presence of accompanying foods could be a strategy in the design of healthier foods (e.g. low in calories, reduced fat, reduced sugar, reduced salt) without the consumer perceiving differences between the reformulated products. Finally, this work stressed the fact that aroma release and sensory perception of complex food matrices during consumption are multidimensional phenomena highlighting the need to develop multidisciplinary approaches to better understand the mechanisms governing aroma release and perception during food consumption.

CRediT authorship contribution statement

Karina Gonzalez-Estanol: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Investigation, Writing – original draft. **Iuliia Khomenko:** Methodology, Data curation, Formal analysis, Writing – review & editing. **Danny Cliceri:** Conceptualization, Methodology, Visualization, Data curation, Writing - review & editing. **Franco Biasioli:** Conceptualization, Methodology, Supervision. **Markus Stieger:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

The data that has been used is confidential.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112726.

References

Aprea, E., Biasioli, F., Gasperi, F., Märk, T. D., & van Ruth, S. (2006). In vivo monitoring of strawberry flavour release from model custards: Effect of texture and oral processing. *Flavour and Fragrance Journal, 21*(1), 53–58.

Arancibia, C., Jublot, L., Costell, E., & Bayarri, S. (2011). Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behavior. *Food Research International, 44*(6), 1632–1641.

Baines, Z. V., & Morris, E. R. (1987). Flavour/taste perception in thickened systems: The effect of guar gum above and below c*. *Topics in Catalysis, 1*(3), 197–205. https://doi.org/10.1016/S0268-005X(87)80003-6

Buettner, A., & Beauchamp, J. (2010). Chemical input–Sensory output: Diverse modes of physiology–flavour interaction. *Food Quality and Preference, 21*(8), 915–924.

Cappellin, L., Biasioli, F., Granitto, P. M., Schuhfried, E., Soukoulis, C., Costa, F., … Gasperi, F. (2011). On data analysis in PTR-TOF-MS: From raw spectra to data mining. *Sensors and Actuators B: Chemical, 155*(1), 183–190.

Castura, J. C., Antúnez, L., Giménez, A., & Ares, G. (2016). Temporal Check-All-That-Apply (TCATA): A novel dynamic method for characterizing products. *Food Quality and Preference, 47*, 79–90.

Charles, M., Romano, A., Yener, S., Barnabà, M., Navarini, L., Märk, T. D., … Gasperi, F. (2015). Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis. *Food Research International, 69*, 9–20.

Chung, S., Heymann, H., & Grün, I. U. (2003). Temporal release of flavor compounds from low-fat and high-fat ice cream during eating. *Journal of Food Science, 68*(6), 2150–2156.

Déléris, I., Saint-Eve, A., Dakowski, F., Sémon, E., Le Quéré, J.-L., Guillemin, H., & Souchon, I. (2011). The dynamics of aroma release during consumption of candies of different structures, and relationship with temporal perception. *Food Chemistry, 127*(4), 1615–1624.

Deleris, I., Saint-Eve, A., Kauffmann, M., Feron, G., & Souchon, I. (2015). Experimental approaches to better understand the retention of aroma compounds in oro-naso-pharyngeal cavities. In *The Chemical Sensory Informatics of Food: Measurement, Analysis, Integration* (pp. 147–170). ACS Publications.

Déléris, I., Saint-Eve, A., Sémon, E., Guillemin, H., Guichard, E., Souchon, I., & Le Quéré, J. (2013). Comparison of direct mass spectrometry methods for the on-line analysis of volatile compounds in foods. *Journal of Mass Spectrometry, 48*(5), 594–607.

Faye, P., Brémaud, D., Daubin, M. D., Courcoux, P., Giboreau, A., & Nicod, H. (2004). Perceptive free sorting and verbalization tasks with naive subjects: An alternative to descriptive mappings. *Food Quality and Preference, 15*(7–8), 781–791.

Feron, G., Ayed, C., Qannari, E. M., Courcoux, P., Laboure, H., & Guichard, E. (2014). Understanding aroma release from model cheeses by a statistical multiblock approach on oral processing. *PloS One, 9*(4), e93113.

Ferreira, V., Pet'ka, J., & Cacho, J. (2006). Intensity and persistence profiles of flavor compounds in synthetic solutions. Simple model for explaining the intensity and persistence of their aftersmell. *Journal of Agricultural and Food Chemistry, 54*(2), 489–496.

Frank, D., Appelqvist, I., Piyasiri, U., Wooster, T. J., & Delahunty, C. (2011). Proton transfer reaction mass spectrometry and time intensity perceptual measurement of flavor release from lipid emulsions using trained human subjects. *Journal of Agricultural and Food Chemistry, 59*(9), 4891–4903.

Friel, E. N., & Taylor, A. J. (2001). Effect of salivary components on volatile partitioning from solutions. *Journal of Agricultural and Food Chemistry, 49*(8), 3898–3905.

Gonzalez-Estanol, K., Cliceri, D., Biasioli, F., & Stieger, M. (2022). Differences in dynamic sensory perception between reformulated hazelnut chocolate spreads decrease when spreads are consumed with breads and wafers. *Food Quality and Preference, 104532*.

Gonzalez-Tomas, L., Bayarri, S., Taylor, A. J., & Costell, E. (2007). Flavour release and perception from model dairy custards. *Food Research International, 40*(4), 520–528.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*(1), 49–70.

Guichard, E., Repoux, M., Qannari, E. M., Labouré, H., & Feron, G. (2017). Model cheese aroma perception is explained not only by in vivo aroma release but also by salivary composition and oral processing parameters. *Food & Function, 8*(2), 615–628.

Heenan, S., Soukoulis, C., Silcock, P., Fabris, A., Aprea, E., Cappellin, L., … Biasioli, F. (2012). PTR-TOF-MS monitoring of in vitro and in vivo flavour release in cereal bars with varying sugar composition. *Food Chemistry, 131*(2), 477–484.

Hodgson, M., Linforth, R. S. T., & Taylor, A. J. (2003). Simultaneous real-time measurements of mastication, swallowing, nasal airflow, and aroma release. *Journal of Agricultural and Food Chemistry, 51*(17), 5052–5057.

How, M. S., Jones, J. R., Morgenstern, M. P., Gray-Stuart, E., Bronlund, J. E., Saint-Eve, A., … Souchon, I. (2021). Modelling the role of oral processing on in vivo aroma release of white rice: Conceptual model and experimental validation. *LWT, 141*, Article 110918.

Labouré, H., Repoux, M., Courcoux, P., Feron, G., & Guichard, E. (2014). Inter-individual retronasal aroma release variability during cheese consumption: Role of food oral processing. *Food Research International, 64*, 692–700.

Laguna, L., Varela, P., Salvador, A., & Fiszman, S. (2013). A new sensory tool to analyse the oral trajectory of biscuits with different fat and fibre contents. *Food Research International, 51*(2), 544–553.

Mesurolle, J., Saint-Eve, A., Déléris, I., & Souchon, I. (2013). Impact of fruit piece structure in yogurts on the dynamics of aroma release and sensory perception. *Molecules, 18*(5), 6035–6056.

Naknean, P., & Meenune, M. (2010). Factors affecting retention and release of flavour compounds in food carbohydrates. *International Food Research Journal, 17*(1), 23–34.

Negoias, S., Visschers, R., Boelrijk, A., & Hummel, T. (2008). New ways to understand aroma perception. *Food Chemistry, 108*(4), 1247–1254.

Pedrotti, M., Spaccasassi, A., Biasioli, F., & Fogliano, V. (2019). Ethnicity, gender and physiological parameters: Their effect on in vivo flavour release and perception during chewing gum consumption. *Food Research International, 116*, 57–70.

Poinot, P., Arvisenet, G., Ledauphin, J., Gaillard, J.-L., & Prost, C. (2013). How can aroma–related cross–modal interactions be analysed? A review of current methodologies. *Food Quality and Preference, 28*(1), 304–316.

Pu, D., Duan, W., Huang, Y., Zhang, Y., Sun, B., Ren, F., … Tang, Y. (2020). Characterization of the key odorants contributing to retronasal olfaction during bread consumption. *Food Chemistry, 318*, Article 126520.

Pu, D., Zhang, H., Zhang, Y., Sun, B., Ren, F., Chen, H., & He, J. (2019). Characterization of the aroma release and perception of white bread during oral processing by gas chromatography-ion mobility spectrometry and temporal dominance of sensations analysis. *Food Research International, 123*, 612–622.

Pu, D., Zhang, H., Zhang, Y., Sun, B., Ren, F., Chen, H., & Xie, J. (2019). Characterization of the oral breakdown, sensory properties, and volatile release during mastication of white bread. *Food Chemistry, 298*, Article 125003.

Sánchez-Palomo, E., Pérez-Coello, M. S., & Cabezas, L. (2008). Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. *Dairy Science and Technology, 88*(3), 355–367.

Scholten, E. (2017). Composite foods: From structure to sensory perception. *Food & Function, 8*(2), 481–497.

Spence, C. (2021). What is the relationship between the presence of volatile organic compounds in food and drink products and multisensory flavour perception? *Foods, 10*(7), 1570.

van Eck, A., Hardeman, N., Karatza, N., Fogliano, V., Scholten, E., & Stieger, M. (2019). Oral processing behavior and dynamic sensory perception of composite foods: Toppings assist saliva in bolus formation. *Food Quality and Preference, 71*, 497–509.

van Eck, A., Pedrotti, M., Brouwer, R., Supapong, A., Fogliano, V., Scholten, E., … Stieger, M. (2021). In Vivo Aroma Release and Dynamic Sensory Perception of Composite Foods. *Journal of Agricultural and Food Chemistry*.

Van Ruth, S. M., & Roozen, J. P. (2000). Influence of mastication and saliva on aroma release in a model mouth system. *Food Chemistry, 71*(3), 339–345.

Varela, P., Antúnez, L., Carlehög, M., Alcaire, F., Castura, J. C., Berget, I., … Ares, G. (2018). What is dominance? An exploration of the concept in TDS tests with trained assessors and consumers. *Food Quality and Preference, 64*, 72–81.

Weel, K. G. C., Boelrijk, A. E. M., Alting, A. C., Van Mil, P. J. J. M., Burger, J. J., Gruppen, H., … Smit, G. (2002). Flavor release and perception of flavored whey protein gels: Perception is determined by texture rather than by release. *Journal of Agricultural and Food Chemistry, 50*(18), 5149–5155.

Wendin, K., Aaby, K., Edris, A., Ellekjaer, M. R., Albin, R., Bergenståhl, B., … Solheim, R. (1997). Low-fat mayonnaise: Influences of fat content, aroma compounds and thickeners. *Food Hydrocolloids, 11*(1), 87–99.

Zhang, H., Chen, H., Wang, W., Jiao, W., Chen, W., Zhong, Q., … Chen, W. (2020). Characterization of volatile profiles and marker substances by HS-SPME/GC-MS during the concentration of coconut jam. *Foods, 9*(3), 347.