Understanding flavour release and sensory perception of composite foods by combining dynamic sensory methods with in vivo nose space analysis

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Abstract

The aim of the study was to investigate the effect of food structure and composition on aroma release and sensory perception of composite foods. Dynamic sensory perception was assessed with Temporal-Check-All-That-Apply (TCATA) (n=72) while quantification of aroma release was done with *in vivo* nose space analysis using a commercial PTR-ToF-MS (Proton Transfer Reaction Time-of-Flight Mass Spectrometer, Ionicon Analytik, Innsbruck, Austria) (n=8, in triplicate).
Six composite foods were prepared by combining two carriers (bread and wafer) with three formulations of chocolate-hazelnut spreads varying in fat and sugar content (high fat/high sugar; high fat/low sugar; low fat/high sugar). The spreads were spiked with a known quantity of 5 aroma compounds. Evaluations of the hazelnut spreads without carriers and for the carrier-spread combinations were performed.
In general, fat and sugar content had little effect on flavour release and sensory perception of chocolate hazelnut spread. Addition of carriers increased aroma release and decreased flavour perception.
We conclude that *in vivo* nose space analysis by direct injection mass spectrometry (PTR-MS) and dynamic sensory method (TCATA) allowed to investigate aroma release and perception of real food matrices (spreads and composite foods). Flavour release and sensory perception of hazelnut chocolate spreads is strongly affected by addition of carriers. However, it seems that sensory perception of composite foods is modulated by cognitive mechanisms.

Keywords: Temporal-Check-All-That-Apply (TCATA), Aroma release, Proton Transfer Reaction–Time of Flight–Mass Spectrometry (PTR–ToF–MS), Composite food

Introduction

Even though the practice of eating is well known to all of us, the fundamental principles involved in flavour release and sensory perception of foods are not as obvious as they are normally perceived. For instance, they are both complex dynamic processes that depend on different variables. First, they depend on the aroma compounds that are released from the food matrix into the olfactory receptors located in the human nasal cavity through retronasal pathway [1]. Morover, they will also be influenced by food composition, food structure and dynamic changes thereof during oral processing [2-4].

In the past years, studies have focused on the relationship between aroma release and perception by coupling *in vivo* flavour release analysis and dynamic sensory methods [5-10]. *In vivo* nose space analysis provides information on the molecules that are interacting with our receptors by analyzing the air coming out of the nostrils [11]. Because of its high resolution, the Proton-Transfer Reaction Mass-Spectrometry equipped with a Time of Flight Mass Analyzer (PTR-Tof-MS) can be used for breath analysis and nose space measurement during consumption of food [5, 12]. To better understand flavour perception, PTR-ToF-MS is often coupled with sensory methodologies such as Time Intensity (TI) methodology or Temporal Dominance of Sensations (TDS). Nevertheless, the selection of a single attribute at a time might give rise to dumping effects [13]. Temporal Check-All-That-Apply (TCATA) extends Check-All-That-Apply (CATA) to provide a more complete description of the dynamics of the sensory characteristics of a product. The assessors' task is to indicate and continually update the attributes that apply to the sample moment to moment. Multiple attributes from different modalities (e.g. taste and texture), can be selected simultaneously, which permit the description of sensations that arise concurrently, decreasing the chance for a dumping effect [14].

However, while most of the studies involve single or model foods that do not represent the real eating context, little is known about the flavour release and sensory perception of composite foods, even though they are commonly consumed. For example, bread, or wafer biscuits (carrier foods) are commonly consumed in combination with spreads. Composition, mechanical properties, and sensory characteristics of the carrier foods differ considerably from the spreads [15, 16]. Thus, the flavour release and sensory perception of the composite

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food will be different from that of the single foods as the characteristics of one component will influence the flavour release and perception of the other components [16, 17]. For example, Van Eck et al. demonstrated that the carrier tends to dominate texture perception, whereas the condiment topping dominates flavour perception [17].

Characterizing composite foods is gaining interest not only because of the increased sensory complexity but also because they are more representative of the natural consumption context. Moreover, since flavour perception plays a key role in the liking of food and depends on different variables, a better understanding of how the release and fading of flavour compounds is perceived and how it contributes to liking, is needed. Thus, the aims of this study were (i) to investigate the effect of carrier addition on the flavour release and sensory perception of chocolate-hazelnut spread and (ii) to investigate the effect of fat and sugar content of chocolate-hazelnut spread on flavour release and sensory perception of a composite food using *in vivo* nose space analysis with PTR–ToF–MS and TCATA sensory analysis.

Experimental Design

Samples

Three chocolate hazelnut spreads varying in fat and sugar content (high fat/high sugar (control); high fat/ low sugar (15% reduction); low fat (15% reduction)/ high sugar), were used. All formulations were spiked with 0.2% (w/w) of an aroma solution containing 5 compounds: Benzaldehyde, Filbertone, delta-Dodecalactone, Isovaleraldehyde, and 2-Methylpyrazine (Table 1). These molecules were chosen because they present a rather wide range of chemical classes, and because their sensory properties are in line with chocolate-hazelnut spread.

Table 1. Characteristics of selected compounds used to spike the chocolate hazelnut spreads

Compound	Chemical formula	Sensory Attributes	Fragmentation pattern by PTR-MS:
Benzaldehyde	C_7H_7O	Similar to bitter almond	107.05
Filbertone	$C_8H_{14}O$	Hazelnut aroma	127.112
delta-Dodecalactone	$C_{12}H_{22}O_2$	creamy, milky, buttery	199.1727/ 85.0683
Isovaleraldehyde	$C_5H_{10}O$	Chocolate, nutty, cocoa	87.077
2-Methylpyrazine	$C_5H_6N_2$	Cocoa, nutty, roasted	95.0572

Composite foods were formed by combining all spreads with two different carriers (bread and wafer). Evaluations of the chocolate-hazelnut spreads without carriers and for the carrier-spread combinations were performed (n=9). All samples consisted of 6 grams of spread. The spreads evaluated alone were served on a plastic spoon. For the spread-wafer samples, wafer biscuits were pre-cut in the form of a shell with dimensions of 3cm*4cm (mean weight 1.56 ± 0.10 g), filled with the spread and packed. For the spread-bread combinations, bread (Morato Bruschelle, Italy) was cut in squares of 3cm*3cm without crust (mean weight 2.23 ± 0.50 g), and spread was served on top.

Subjects

Eight women (mean age 34.2 ± 7.4 years) were recruited for the nose space analysis and 72 (mean age 22.6 ± 2.0 years) for the sensory evaluation. Both cohorts consisted of volunteers from Edmund Mach Foundation (San Michele all'Adige (TN), Italy) and Wageningen University (Wageningen, The Netherlands), respectively. All participants were Caucasian women that consume hazelnut spread on regular basis. Other inclusion criteria were the following: not to have any dietary restrictions, non-pregnant, non-smoking, with no history of oral perception disorders or olfactory impairments (self-reported), no intolerance/allergy to any ingredient in chocolate-hazelnut spread. Participants gave a written informed consent before the start of the study.

In vivo nose space analysis

As part of a bigger project, the selected 8 panellists went through four training sessions of 60 min each. The experimental set up was adapted from previous PTR-ToF-MS nose space studies [6, 7]. A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used for the *in vivo* nose space analysis. The ionization conditions, with H_3O^+ to trace panellist breath [9], were the following: drift voltage 628V, at 110°C, and pressure of 2.80 mbar. Acquisition was set to 1 mass spectrum per second. Nose space sampling was carried out via two Teflon tubes placed in both nostrils of the assessors and connected to a heated device (N.A.S.E, IONICON at 110°C) which was directly connected into the inlet of the PTR-ToF-MS system. Evaluations took place in a laboratory with filtered air. Panellists were asked to insert the Teflon tubes in their nostrils and to start breathing normally through the nose keeping their mouth closed. Their breath was sampled for 60 s after which they were instructed to put the entire sample in their mouth and chew normally with their mouth closed. The time of the swallowing was standardized to 15 seconds for the spread served on its own, and 20 seconds for the bread and wafer combinations. Nose space data were acquired for 1 minute and 45 seconds. Between each sample, panellists were asked to clean their mouth with warm water. Panellists' breath was retested before each new measurement.

Sensory evaluation

Sensory evaluations took place in a testing room at Centrum voor Smaak Onderzoek, (Wageningen, The Netherlands), under normal light conditions at room temperature. Panellists evaluated the nine samples in one session of 60 minutes with short breaks in between. They received a pre-made attribute lists with definitions by email and were instructed to familiarize themselves with them. They were also asked not to smoke, eat, drink, and use any persistent-flavoured product for at least one hour before their session. Subjects seated individually and were provided with a tablet which the test was displayed on. For each sample, subjects were instructed to click on a start button concurrently with taking the whole sample in their mouth, and to immediately start tracking sensory changes. At any time between clicking start and the end of the evaluation time, they were free to check the terms that applied to describe the sensory characteristics of the sample at each moment and to uncheck the terms when they were no longer applicable. Just as in the nose space assessment, precise instructions were given regarding the moment at which assessors should swallow the sample and the total duration of the evaluation (1 minute and 45 seconds).

Results and discussion

In vivo nose space analysis

Mean time of maximum aroma concentration (Tmax) and area under the curve (AUC) were calculated for each compound separately. To test how these parameters differed across formulas and carriers, mixed model ANOVA was performed on Tmax and AUC, with formula, carrier and their interaction as fixed factors and subject as random one. Upon significance of the ANOVA, Tukey's HSD pairwise comparison was performed. Tmax was not affected by neither formula nor carrier, except for Isovaleraldehyde (m/z 87.0811), where there was a significant effect in the interaction between formula and carrier ($p<0.05$). AUC of all compounds was significantly affected by the addition of carrier ($p< 0.05$). In general, when bread and wafer were added to the spread, there was an increase of the AUC. For example, in case of delta-Dodecalactone (m/z 85.0683), AUC increased of 165.8% on average after addition of bread across all formulations ($p<0.05$) (Figure 1A). The same was observed for Filbertone (m/z 127.1140), where there was a significant increase of AUC of 14.0% and 12.9% in average across all formulations, with the addition of bread and wafer, respectively ($p<0.05$). Finally, Isovaleraldehyde showed as well a significant increase of 53.1% and 56.8% with bread and wafer addition, respectively ($p<0.05$). Overall, fat and sugar content did not affect flavour release significantly ($p> 0.05$), except for Isovaleraldehyde, where there was a significant decrease of AUC of 10.6% with the low sugar formulation, across all carriers.

Dynamic Sensory Perception

The addition of carrier (bread and wafer) influenced the sensory perception of the different spreads. For instance, citation proportion of the attribute *Milky*, which could be partly related with the release of delta-Dodecalatone, decreased significantly ($p<0.05$) when bread and wafer were added, especially in the beginning of the consumption (Figure 1B). This was clearly observed in the control formulation and to a lesser extent in the low fat and low sugar formulations. Similar trends were observed for the attributes *Hazelnut* and *Cocoa*, which could be partly related to the release of Filbertone and Isovaleraldehyde, respectively. In both cases, the addition of a carrier led to a significant decrease ($p<0.05$) in the perception of the attributes across the three formulations, in the beginning of the consumption time. Overall, fat and sugar content had little effect on the sensory perception of spreads ($p>0.05$).

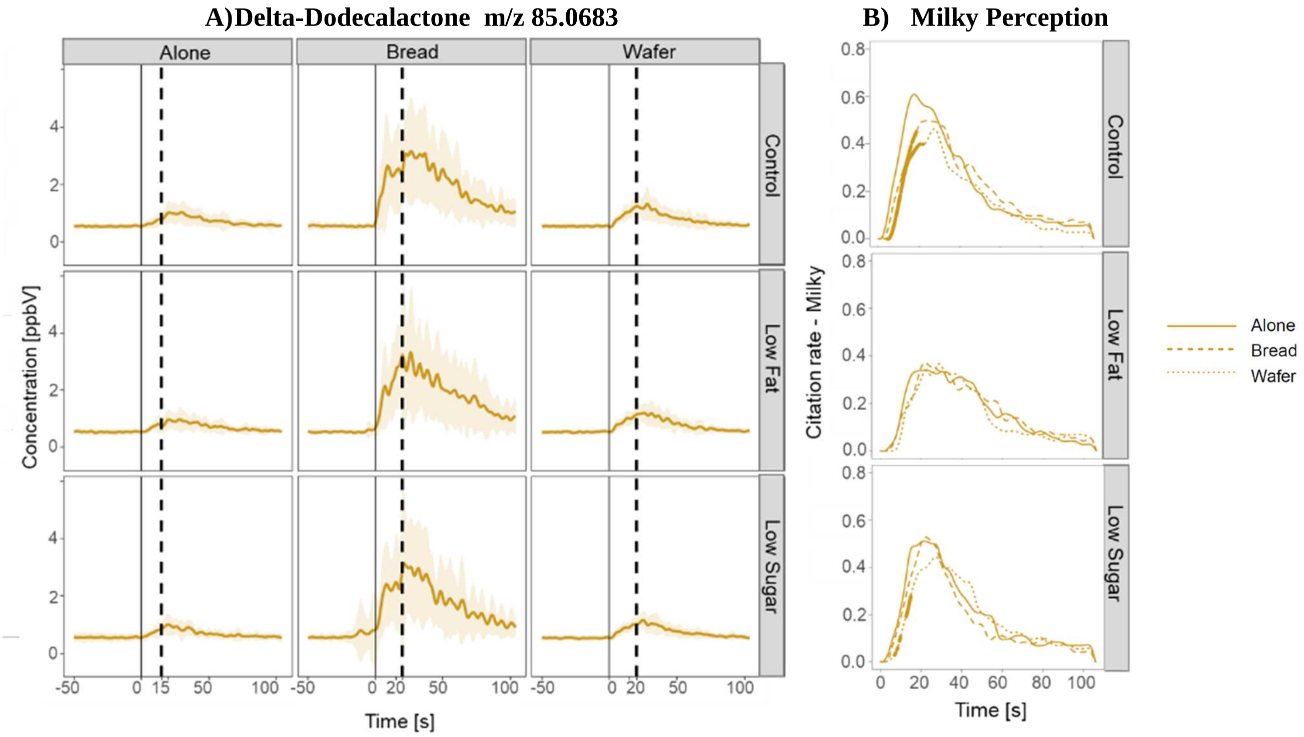

Figure 1: *Averaged in-nose release (n=8 subjects in triplicate) of delta-Dodecalactone (a) and smoothed TCATA curves (n=72) for the attribute Milky (b). Black continuous and dotted lines represent moment when samples are put in the mouth, and swallowing moment, respectively. Shaded areas represent standard error of the mean. Periods of significant differences (p<0.05) in proportion of citations in TCATA curves, compared to Spread Alone are indicated by highlighted thick sections.*

To summarize, neither the composition nor the addition of carrier influenced time of maximum aroma concentration of any of the aroma molecules used to spike the spreads. Fat and sugar content had little effect on flavour release and sensory perception. When chocolate-hazelnut spreads were combined with a carrier (bread or wafer) aroma release was enhanced. This may be partly attributed to the difference of oral processing time between spreads alone (15 seconds) and spreads in combination with a carrier (20 seconds). In addition, spreads alone do not require chewing and they are just swirled around the mouth, while spread/carrier combinations require more chewing to breakdown the food, thus, inducing more aroma release. Besides, because of chewing and mixing, the surface area of spread-carrier combinations probably increased, allowing a higher transfer of aroma compounds from the spread into to the vapour phase [19]. Instinctively, it would be expected that this increase would also increase the sensory perception. However, the addition of a carrier, led to a decrease in sensory perception. This decrease is in line with previous studies where flavour intensity of a sauce/topping decreased with the addition of a solid food [19,18]. This reduction in perception is not due to a lower release of aromatic compounds into the nose space, but rather other factors may play a role. It has been suggested that cognitive effects play a role in the modulation of condiment-carrier perception, *i.e.* consumers pay more attention to texture and/or chewing with the presence of carriers [19].

Conclusion

We conclude that *in vivo* nose space analysis by direct injection mass spectrometry (PTR-ToF-MS) and dynamic sensory methods (TCATA) allowed to investigate aroma release and perception of real food matrices which not only increases the complexity of the food consumed, but also is more representative of the common consumption context. Moreover, coupling nose space with TCATA analysis, underlined the presence of cross modal associations between food texture and aroma perception in complex, real food matrices. Flavour release and sensory perception of hazelnut chocolate spreads is strongly affected by addition of carriers such as bread and wafer. Finally, it seems that perception of composite foods is modulated by cognitive effects.

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