Karina González Estañol

COMBINING FOODS, COMBINING TOOLS:

Understanding *in vivo* aroma release and sensory perception of composite foods

UNIVERSITÀ DI TRENTO

FONDAZIONE EDMUND MACH

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Thesis

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ABSTRACT

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Combining foods, combining tools.

To date, most of the published works that have attempted to relate aroma release and sensory perception are far from mimicking the real consumption context, as assessments often do not consider food oral processing during mastication and are performed on single or model foods. A multidisciplinary approach integrating *in vivo* aroma release, oral processing behaviour, and dynamic sensory methods is needed to have a better understanding of how the release, evolution, and fading of the flavour of real complex food matrices are being perceived during consumption.

The research described in this thesis aims to investigate the relative contributions that food structure and its composition and oral processing behaviour have on aroma release and perception of food matrices made up of different components and contrasting textures, so-called composite foods.

Results showed that the processes of aroma release and perception of composite foods are multidimensional phenomena and seem to be modulated by cross-modal texture-aroma interactions. While aroma release increased when a food was assessed in combination with other foods, consumer's ability to discriminate between foods was reduced and off-flavours and texture deficiencies were masked. Furthermore, the role of oral processing behaviour on the breakdown of the food impacting aroma release and perception was highlighted.

The use of a multidisciplinary approach provided more useful and accurate sensory profile representing an ecological, valid food consumption context that will ultimately offer new strategies for successfully developing healthier food products with lower fat and sugar content or elaborated with plant protein, for which sensory acceptability remains a challenge.

RESUMEN

Combinando alimentos, combinando herramientas.

Hasta la fecha, la mayoría de los trabajos publicados que han intentado relacionar la liberación de aromas y la percepción sensorial están lejos de imitar el contexto de consumo real, ya que las evaluaciones a menudo no consideran el procesamiento de los alimentos durante la masticación y se realizan usando modelos de alimentos. Es inminente un enfoque multidisciplinario que integre el análisis de la liberación de aromas y métodos sensoriales dinámicos durante la masticación de los alimentos para tener una mejor comprensión de cómo se percibe la liberación, evolución y desvanecimiento de los aromas durante la masticación de los alimentos. La investigación descrita en esta tesis tiene como objetivo investigar las contribuciones relativas que la estructura de los alimentos y su composición y el comportamiento de masticación tienen sobre la liberación y la percepción de los aromas durante el consumo de alimentos conformados por diferentes ingredientes y texturas, llamados alimentos compuestos.

Los resultados mostraron que los procesos de liberación del aroma y la percepción de los alimentos compuestos son fenómenos multidimensionales y parecen estar modulados por interacciones entre textura y aroma. Si bien la liberación de aromas aumentó cuando un alimento se evaluó en combinación con otros alimentos, la capacidad del consumidor para discriminar entre alimentos se redujo y se enmascararon los sabores desagradables y las deficiencias de textura. Además, se destacó el papel del comportamiento de procesamiento oral en la descomposición del alimento que afecta la liberación y la percepción del aroma. Este enfoque multidisciplinario proporcionó un perfil sensorial más útil y preciso que representa un contexto de consumo de alimentos válido que ofrecerá nuevas estrategias para el desarrollo de productos alimenticios más saludables con menor contenido de grasa y azúcar o elaborados con proteína vegetal, para los cuales la aceptabilidad sensorial aun es un reto

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LIST OF SYMBOLS AND ABBREVIATIONS

VOC	Volatile organic compounds
NSA	Nose-space analysis
PTRMS	proton-transfer-reaction mass spectrometry
ToF	time of flight
TCATA	Temporal – check- all-that apply
TDS	Temporal dominance of sensations
TI	Time intensity
RATA	Rate-all-that-apply
QDA	quantitative descriptive analysis
MS	Mass spectrometry
DA	Descriptive analysis
SP	Sequential profiling
BMI	Body mass index
VAS	Visual analogue scale
PCA	Principal component analysis
t.i	Tentatively identified
MFA	Multiple Factor Analysis
ANOVA	analysis of variance
AUC	Area under the curve
LMM	Linear mixed model
HSD	Honest Significant Difference
I_{max}	Maximum intensity
Cit_{max}	Maximum Citation
T_{max}	Time to reach maximum
ER	Eating rate
gLMS	Generalized Labelled Magnitude Scales
HUT	Home-Use-Test
MR	Meat replacer

CHAPTER

General introduction

INTRODUCTION

Flavour analysis has become an important field of research for the food industry. In particular, it is of interest to understand the fundamental principles involved in the release of odour-active volatile organic compounds (VOCs) during food consumption, as their release was proven to be a key factor driving flavour perception (Potineni and Peterson, 2008), which will ultimately impact food acceptability (Pico et al., 2015; Taylor and Roozen, 1996).

However, relating flavour compounds to perception is not straightforward because the mechanisms underlying flavour release and perception are complex dynamic processes that depend on the concentration of flavour compounds, the food composition and structure and dynamic changes thereof during oral processing (Buettner and Beauchamp, 2010; Poinot et al., 2013; Tournier et al., 2007).

Traditionally, instrumental methods to measure volatile release and sensory methods are used individually in flavour and aroma analysis, or when combined, studies are done on simple or model food matrices and do not consider oral processing. This thesis responds to a relatively simple premise: compared to the previous approaches, measuring the volatile compounds released by a given food matrix during mastication will likely provide a more useful and accurate sensory profile representing an ecological, valid food consumption context. Thus, a multidisciplinary approach that integrates instrumental aroma analysis (*in vivo* nose-space analysis), sensory analysis, and behavioural and physiological studies is necessary to deepen our understanding of aroma release and perception mechanisms. This will ultimately offer new strategies for successfully developing healthier food products with lower fat and sugar content or elaborated with plant protein, for which sensory acceptability remains a challenge.

1.1 Aroma release and perception

Aroma perception has proven to be a key factor in evaluating sensory food quality that drives food choices and consumer preferences (Ployon et al., 2017; Pu et al., 2020).

Aroma perception results from physical and chemical stimulation, by aroma compounds, which triggers a physiological response, and a psychological effect. The two possible pathways through which aroma stimuli can reach the olfactory epithelium are via the nose

during sniffing, referred to as orthonasal olfaction, and via the mouth during food consumption, referred to as retronasal olfaction (Negoias et al., 2008), with the latter one having a decisive role in the human aroma cognition of food (Blankenship et al., 2019) (Figure 1.1).

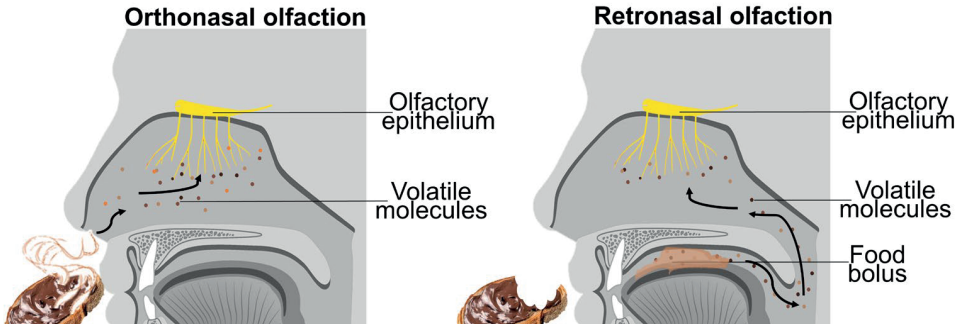

Figure 1. 1 Schematic representation of olfaction pathways

The food matrix is broken down during eating, releasing taste and aroma compounds which are mixed with saliva. Subsequently, the taste compounds are conveyed to the receptors on the tongue. By contrast, the volatile aromatic ones are transferred to the air phase in the oral cavity and eventually transported to the olfactory receptors in the nasal cavity through the retronasal pathway, either by the chewing action which pumps some air into the throat, or by swallowing, where they will interact with odour proteins further bind with the olfactory receptors to generate aroma perception (Guichard et al., 2016; Laing and Jinks, 1996).

1.2 Effect of major food components and structure on aroma release

Besides the concentration of VOCs, components in the food matrix, such as proteins, polysaccharides and lipids, not only define the sensory properties of the food, but also interact with VOCs, affecting their release kinetics from the food (Bakker et al., 1996; Guichard, 2002). This means that any modification of the product reformulation (e.g., lowering fat content or using sweeteners) affects the nature of this interaction, resulting in a change in flavour release and perception.

Even though proteins present little flavour on their own, they are known to bind aroma compounds, which results in a reduction of their release into the air phase (Tromelin et al.,

2006). The binding mechanisms depend not only on the protein structure but also on the chemical characteristics of the aroma compounds (Heng et al., 2004; Semenova et al., 2002).

Depending on the type and the amount that is present, sugars can induce physicochemical and textural changes in the matrix affecting the viscosity, specific binding to volatiles, or hydration as a result of 'salting out effects'(Hansson et al., 2001; Heenan et al., 2012; Lubbers et al., 2007; Nahon et al., 2000; Rabe et al., 2003).

Besides influencing the appearance, structure, and mouthfeel, fat also plays a key role in flavour release and perception processes. Fat acts as a carrier and reservoir especially for lipophilic aromatic compounds. Depending on its nature, quantity, and physical state, fat influences the partitioning of flavour compounds changing their temporal release profile (Boisard et al., 2014; Brauss et al., 1999; Chung et al., 2003). In fat-containing foods, lipophilic aroma compounds are bound to fat molecules by hydrophobic interactions, reducing the maximum intensity and overall release and promoting a more persistent release of the flavour (Doyen et al., 2001; Malone et al., 2003). By contrast, in the absence of fat, lipophilic flavours are poorly bound to the food matrix, resulting in an 'aroma burst' characterized by a fast and high-intensity release, which is translated into a product perceived as "unbalanced" and having lower acceptability (Plug and Haring, 1993).

Textural parameters of food, such as viscosity or hardness, also influence the release of aroma compounds by affecting their diffusion from the food matrix, into the air interface (Juteau et al., 2004; Lubbers and Guichard, 2003). It has been shown, for example, that flavour intensity decreases with an increase in viscosity or hardness (Saint-Eve et al., 2006; Weel et al., 2002).

1.3 Effect of oral behaviour on aroma release and perception

Food oral processing is the first step of food digestion. During mastication, food is broken down into smaller pieces, and VOCs are released from the food matrix into the oral and nasal cavities. Their release kinetics and perception are affected by the release of taste compounds (Pu et al., 2021), and the structure, as people adapt their mastication to texture perception (Blissett et al., 2006; Bonneau et al., 2018; Gierczynski et al., 2007; Gonzalez-Estanol et al., 2022b; Mestres et al., 2006), and by oral physiological parameters that differ greatly across individuals, such as bite force, saliva content and composition, or the

volume of the oral cavity (Canon et al., 2018; Haahr et al., 2004; Muñoz-González et al., 2021; Pionnier et al., 2004; Ployon et al., 2020). Differences in eating speed and mastication behaviour can also contribute to inter-individual differences in the release rate of aromas and sensory perception of foods (Luckett and Seo, 2017). The level of oral structural breakdown and the bolus properties induced by different chewing behaviour lead to differences in flavour and texture perception. For instance, the longer the oral chewing time, the longer the transmission time of the aroma compounds in the oral cavity. Furthermore, the food is broken down into more and smaller bolus fragments, increasing the contact surface and facilitating aroma release resulting in a higher-intensity aroma perception (How et al., 2021; Okawa et al., 2021).

1.4 Combining tools

1.4.1 Analytical methods to measure aroma release during food oral processing

While the classical study of volatiles (headspace or distillation /extract methods) considerably advanced our understanding of the total aroma composition in food or the air before eating, it fails to provide information on how the volatiles are being released, transported from the oral to the nasal cavity and sensed during oral processing. In the early 1990s, there was a huge focus on the release of aroma compounds in the mouth during food consumption (Kinsella, 1990; Overbosch et al.,1991; Taylor, 1996). Analytical tools like the oral breath sampler (Roozen and Legger-Huysman, 1994) and the nose sampler (Linforth and Taylor, 1993, 1998) were created. Advances in detection systems that allowed the direct injection of aromas,c allowed for the development of on-line analytical instruments to monitor aromas' change patterns during food consumption (Biasioli et al., 2011): selected ion flow tube-MS, atmospheric pressure chemical ionization-MS, and proton-transfer-reaction mass spectrometry (PTR-MS). Because of its high sensitivity, convenience (without pre-treatment), and rapid (real-time monitoring) properties PTR-MS equipped with a time of flight mass analyser (PTR-Tof-MS) has been widely used for monitoring the VOCs that are interacting with our receptors by analysing the air coming out of the nostrils via nose-space analysis (Biasioli et al., 2011; Déléris et al., 2013; Heenan et al., 2012; Mazzucotelli et al., 2022) (Figure 1.2) .

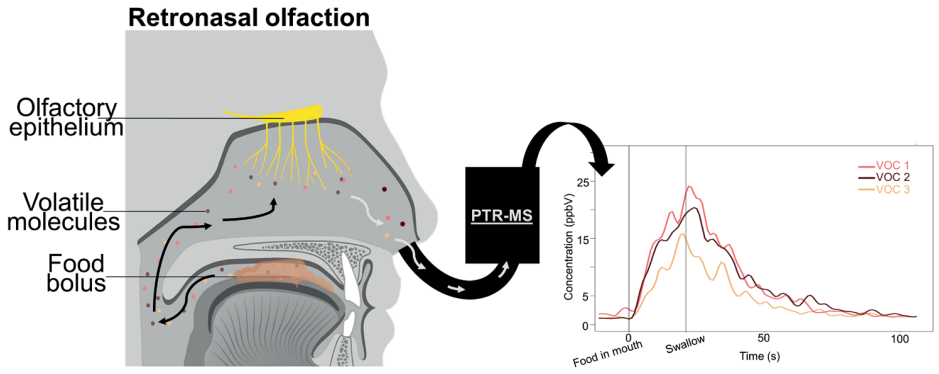

Figure 1.2 From left to right, display of retronasal olfaction during food consumption and real-time quantification of volatiles coming from the nose via PTR-MS (figure from Mazzucotelli et al., 2022).

1.4.2 Sensory perception methods

Descriptive sensory methods refer to the techniques that seek to reach a quantitative description of the sensory characteristics of a range of products and to identify the sensory attributes that differentiate them. The quantitative descriptive analysis (QDA) method has been the gold standard for many years. However, intensive panel training is required, and as a static method (overall perception), it does not necessarily capture how perception changes over time (Murray et al., 2001). Thus, the use of rapid methods such as rate-all-that-apply (RATA) and untrained panellists has increased in recent years. Interestingly, the results of the conventional descriptive analysis with trained panellists are comparable to those of rapid methods with untrained panellists (Ares et al., 2014; Ares and Varela, 2018; Dehlholm et al., 2012; Oppermann et al., 2017). These studies show that both methods can provide similar information, and that trained panels are not always necessary.

However, sensory perception during consumption is dynamic. Thus, temporal methods like time-intensity (TI), temporal dominance of sensations (TDS) and temporal check-all-that-apply (TCATA) (Figure 1.3), were developed to measure the temporal evolution of sensory attributes over time. TI allows panellists to rate the intensity of a single sensory attribute over a determined mastication time (Lee III and Pangborn, 1986). The TDS method presents assessors with a list of sensory attributes. They must select the dominant attribute (i.e., the one that catches most of the attention at each moment) until a new dominant attribute is selected (Pineau et al., 2009). Nevertheless, for most foods, multiple sensory characteristics

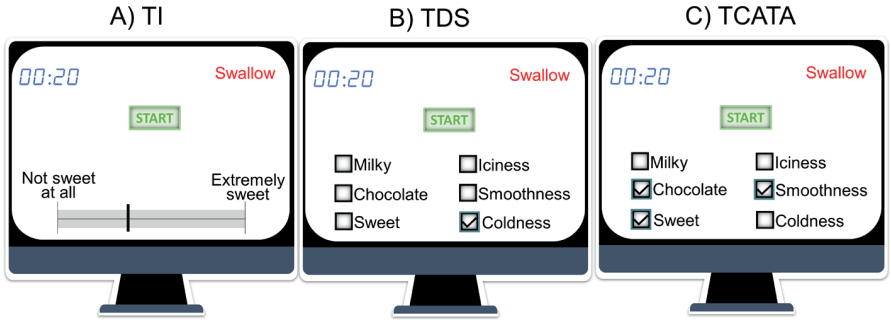

Figure 1. 3. Schematic representation of the different dynamic sensory methods. A) TI: intensity perception of a single attribute over time. B) TDS: tracking of the most dominant sensation over time C) TCATA selection of all attributes that apply to describe the sample over time

are perceived simultaneously , and selecting a single attribute at a time might give rise to dumping effects (Varela et al., 2018) in TI and TDS methods. 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 permits the description of sensations that arise concurrently, decreasing the chance for a dumping effect (Ares et al., 2015; Castura et al., 2016; Jaeger and Porcherot, 2017).

1.4.3 Measurement of oral processing behaviour

Various experimental techniques have been used to quantify oral processing behaviours and eating speed. Electromyography, electromyography combined with jaw-tracking, and ultrasonic echo-sonography have been used to assess oral processing behaviour (Boyar and Kilcast, 1986; Casas et al., 2003; Hennequin et al., 2005; Vinyard and Fiszman, 2016). Real-time magnetic resonance imaging (MRI) and video fluoroscopy have been used to observe the physiology during chewing and swallowing processes (Mioche et al., 2002, Buettner et al., 2001).Video recordings of participants consuming foods followed by post-hoc video annotations have been used frequently to code oral processing behaviours (Forde et al., 2013). Video recordings for tracking jaw movements during food consumption have also been used to characterize oral processing behaviours (Aguayo-Mendoza et al., 2020; Forde et al., 2013a; Ketel et al., 2019; van Eck et al., 2019b). Skin surface markers are placed on the consumer's face to automatically track the movement of the chin or other facial features that allow for inference of jaw movement during mastication (Figure 1.3) (Jin et al., 2022; Wilson et al.,

2016). Moreover, the combination of recording of jaw movements using a motion capture system, recording of muscle activity by EMG and image analysis for the food size distribution allowed to investigate the relationship between mastication behaviour and bolus characteristics of different cereal products (Hedjazi et al., 2013). Lastly, self-reporting of eating speed has been used to characterize oral and eating behaviours (Mochizuki et al., 2014; Petty et al., 2013).

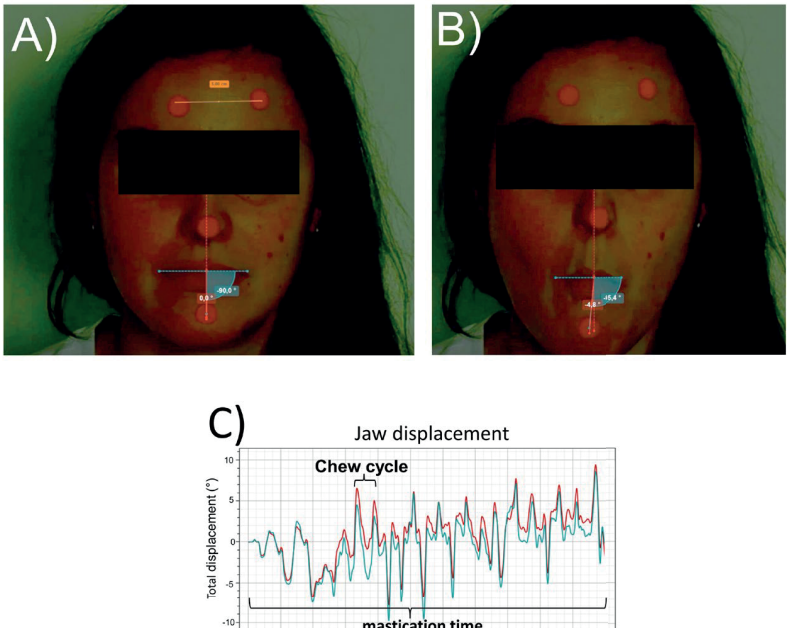

Figure 1. 4. Overview of video recordings for characterization of oral processing behaviour. Markers on the forehead are used for the calibration form pixels to cm. Marker on the nose serves as a static point and on the one on the chin as mobile point. A) Initial position before starting chewing. B) Displacement of the marker on the chin as result of mastication C) Graph showing the displacement of the marker over time, indicating number of chews (peaks) and total mastication time

1.4.4 Coupling sensory and instrumental analysis

In the early 2000s, the focus of various studies was set on model foods such as custards, gels, or egg foams (Aprea et al., 2006; Boland et al., 2006; Hansson et al., 2003; Tyapkova et al., 2016), as they proved to be a useful tool to study aroma release and therefore were a key step in understanding, for example, the role of the product (e.g. composition and structure). In the recent years, several studies have investigated the relationship between *in vivo* flavour

compounds release and sensory perception during food consumption by coupling *in vivo* nose-space analysis by PTR-MS (section 1.4.1) with temporal sensory methods (section 1.4.2) (Figure 1.5) for numerous foods such as candy (Déléris et al., 2013, 2011), coffee (Charles et al., 2015) cereal bars (Heenan et al., 2012) , wine (Arvisenet et al., 2019; Muñoz-González et al., 2019; Pittari et al., 2022), white bread (Pu et al., 2019b), and dark chocolate (Deuscher et al., 2019). Furthermore, the effects of inter-individual differences such as salivary composition (Guichard et al., 2016), oral processing parameters (How et al., 2021), interindividual retronasal aroma release (Labouré et al., 2014), ethnicity, gender, physiological parameters (Pedrotti et al., 2019), and aroma persistence in the nasal and oral cavities (Sánchez-López et al., 2016) have been studied.

1.5 Combining foods: from single to composite foods

Although great progress is undeniable regarding the understanding of aroma release and perception, most studies involve single or model foods that do not necessarily represent the real eating context. In the past few years, the focus has slowly shifted into studying food matrices made up of different components and contrasting textures, so-called composite foods (Scholten, 2017; van Eck and Stieger, 2020). These include foods almost always combined with other foods. For example, toppings, condiments, sauces and spreads are typically eaten with bread, crackers, fries, etc., rather than alone. Flavour release and sensory perception of composite foods differ from those of single foods, as the characteristic of one component will influence the flavour release and perception of the other components (van Eck et al., 2019a, 2019b). Measuring the dynamics of flavour release and sensory perception of composite foods with increased sensory complexity will lead to more representative product profiles that fit better with natural eating behaviour and, consequently, to a better understanding of consumer behaviour.

1.6 Aims and thesis outline

To date, most of the published works that have attempted to relate aroma release and sensory perception are far from mimicking the real consumption context, as assessments often do not consider food oral processing during mastication and are performed on single or model foods. Therefore, while these studies help to understand fundamental physicochemical processes, they do not necessarily represent a realistic context of consuming foods or meals that consist of multiple food components differing considerably in composition, structure, and sensory

properties and may limit the relevance and applicative outreach of the sensory and flavour release profiles obtained. A better understanding is thus needed of how the release of aroma compounds during the consumption of composite foods is perceived. The research described in this thesis aims to investigate the relative contributions that food structure and its composition and oral processing behaviour have on aroma release and perception of composite foods. Figure 1.5 provides an overview of the aims of the research papers included in this thesis.

As aroma release and perception during food consumption are both complex dynamic processes that depend on different intrinsic and extrinsic variables, a multidisciplinary approach integrating *in vivo* flavour release, oral processing behaviour, and dynamic sensory methods is needed to have a better understanding of how the release, evolution, and fading of the flavour of real complex food matrices are being perceived during consumption. **Chapters 2** and **3** are focused on the effect of the food matrix. Three chocolate hazelnut spreads varying in fat and sugar content were spiked with five VOCs consistent with sensory notes of chocolate hazelnut spreads. The spreads were combined with two carriers (bread and wafer). Specifically in **Chapter 2**, the dynamic sensory perception of the spreads alone and in combination with the carriers was determined by a consumer panel using TCATA and TDS to evaluate the effect of spread composition and carrier addition on the perception. In addition, TDS and TCATA were also used to extend the findings of previous studies comparing both methodologies from single to sensorial, more complex composite foods. In **Chapter 3**, *in vivo* nose-space analysis via PTR-ToF-MS was used to quantify aroma release and TCATA to assess dynamic sensory perception to find the relationship between flavour release and perception of composite foods.

Chapters 4 and **5** focus on factors contributing to inter-individual variation in sensory perception and aroma release. Three strawberry jams varying in sugar content and viscosity were used. Composite foods were formed by combining the jam on a piece of bread (**Chapter 4**) and sponge cake (**Chapter 5**). **Chapter 4** describes the fast vs slow eaters' effect on dynamic sensory perception of composite food. Habitual oral processing behaviour of different composite foods was quantified, and participants were divided into fast and slow eaters using a median split. The dynamics of dominant sensory attributes of the strawberry jams presented with and without bread were evaluated using TDS. The goal of **Chapter 5**

was to evaluate how composite foods' aroma release and sensory perception changed with a high and a slow chewing rate. The three strawberry jams previously described were spiked with two citrus aroma compounds. The jams served alone and in combination with bread and a sponge cake were evaluated by participants following a fast (80 chews/min) and slow (40 chews/min) chewing rate. Dynamic citrus perception of all samples, was assessed by TI methodology whereas aroma release via *in vivo* nose-space analysis was quantified with PTR-ToF-MS.

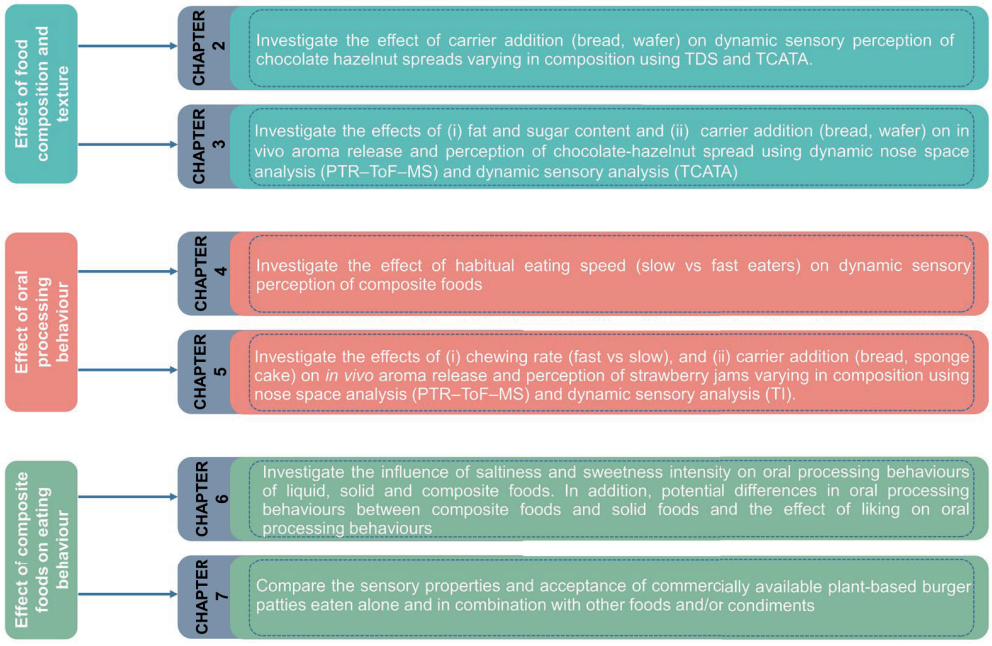

Figure 1. 5 General overview of the aims of the chapters included in this thesis.

All chapters mentioned above describe results and knowledge more representative of product profiles that fit better with natural eating behaviour than previous approaches. **Chapters 6** and **7** were designed to investigate the practical implications of composite foods on the modulation of oral processing behaviour and consumer behaviour. **Chapter 6** discusses the influence of saltiness and sweetness intensity on oral processing behaviour of liquid, solid and composite foods. Furthermore, differences in oral processing behaviour of solid and composite foods were investigated to find out whether combining different foods to form composite foods could be an effective strategy to modulate oral processing behaviour. **Chapter 7** compares the sensory perception and acceptance of plant-based patties eaten alone

and in combination with other foods to see if composite foods, could be a strategy to shift meat eaters and flexitarians towards an increased acceptance of meat alternatives.

Finally, **Chapter 8** provides a general, integrated discussion of all studies. Methodological considerations, directions for future research and general conclusions are presented.

CHAPTER

2

DIFFERENCES IN DYNAMIC PERCEPTION BETWEEN REFORMULATED HAZELNUT-CHOCOLATE SPREADS DECREASE WHEN SPREADS ARE CONSUMED WITH BREADS AND WAFERS

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Abstract

Many foods such as breads and wafers (carrier foods) are commonly consumed in combination with spreads such as jam, peanut butter and chocolate spreads. Sensory assessments are typically performed with carrier foods or spreads alone rather than with combinations thereof. The aim of this study was to investigate the effect of carrier addition (bread, wafer) on dynamic sensory perception of chocolate hazelnut spreads varying in composition. Three chocolate hazelnut spreads varying in fat and sugar content (high fat/high sugar (reference); high fat/low sugar; low fat/high sugar) were combined with two carriers (bread and wafer). Dynamic sensory perception was determined by a consumer panel using TCATA (n=72) and TDS (n=72) with three chocolate hazelnut spreads without carriers and six carrier-spread combinations. Stickiness and mouth drying were considerably more dominant for low fat/high sugar spreads compared to high fat/high sugar spreads. Addition of carriers (bread and wafer) influenced dynamic sensory perception of spreads and reduced especially stickiness. In the beginning of mastication of carrier-spread combinations, perception was dominated by sensations related to carriers while at later stages of mastication, sensations related to spreads were dominant. Addition of carriers to spreads decreased consumers' ability to discriminate between spreads differing in composition. TCATA allowed to discriminate better between spreads and TDS revealed the temporality of sensations clearer. It is concluded that dynamic perceptual differences between hazelnut chocolate spreads differing in composition diminish when spreads are consumed with breads and wafers. These findings provide product sensory profiles which are closer to the natural consumption context of spreads.

Keywords: Sensory description, Temporal methods, composite foods, sweet spreads

2.1 Introduction

Many foods such as breads, or wafer biscuits (carrier foods) are commonly consumed in combination with spreads such as jam, peanut butter, or chocolate spreads. Sensory assessments are typically performed with carrier foods or spreads alone rather than with combinations thereof which may limit the validity of the sensory profiles which are obtained. In contrast to many studies investigating sensory properties of carrier foods and spreads consumed alone, only very few studies explored sensory properties of combinations of foods (Cherdchu & Chambers IV, 2014; Nguyen & Wismer, 2020; Paulsen et al., 2012; Scarborough et al., 2012; van Eck, et al., 2019a, 2019b). This lack of data is especially surprising given that many spreads such as jam or mayonnaises are hardly ever consumed alone. Characterizing composite foods is of interest because they are more representative of the natural consumption context. Composition, mechanical properties, and sensory characteristics of the carrier foods differ considerably from those of the spreads (de Lavergne et al., 2016; Scholten, 2017). Combining carrier foods with spreads increases the sensory complexity of products, as the characteristics of one component influence sensory perception of the other (Paulsen et al., 2012; Santagiuliana et al., 2018a, 2018b; Scholten, 2017; van Eck et al., 2019b). Van Eck et al. (2019b) demonstrated that for composite foods, carriers (bread and crackers) tend to dominate texture perception, whereas condiments or toppings (firm cheese, cheese spread and mayonnaise) dominated flavour perception (van Eck et al., 2019b). In general, it has been shown that flavor intensity of sauces and condiments is decreased by addition of carriers (Cherdchu and Chambers IV, 2014; Meinert et al., 2011; Nguyen & Wismer, 2020; Paulsen et al., 2012; van Eck et al., 2021). Consequently, perceptual differences between spreads or toppings can disappear upon addition of carriers since sensitivity to detect sensory differences between them may decrease (Nguyen and Wismer, 2020; van Eck et al., 2019b). Van Eck et al. (2019b) showed that consumer sensitivity to discriminate between condiments varying in fat content and/or viscosity decreased with the addition of carriers. Nguyen et al. (2020) showed that consumer's ability to identify sensory attribute differences between regular and reformulated salt-reduced products decreased upon the addition of companion foods. This aspect is relevant for food reformulation strategies aiming at changing the nutritional composition of foods (i.e. reduction of fat and sugar content). These strategies typically require that the reformulation does not lead to changes in product sensory perception and consumer acceptance. When perceptual differences between

reformulated spreads disappear once spreads are consumed with carriers, it implies that the product reformulation space may actually be larger, so that greater reductions in fat and sugar content may be achieved without consumers perceiving differences between products (Guichard, 2002).

As sensory perception is a dynamic process (Piggott, 2000), temporal sensory evaluations became more common (Heymann and Lawless, 2013). Temporal Dominance of Sensations (TDS) has been extensively used to assess dynamic sensory perception of a broad range of single foods and beverages (de Lavergne et al., 2015; de Medeiros et al., 2020; Dinnella et al., 2012; Jourdren et al., 2016; Oberrauter et al., 2018; van Bommel et al., 2019). In addition, it was shown that with a quick familiarization and by explaining the list of attributes, TDS becomes feasible for a consumer panel (Albert et al., 2012; Brachet et al., 2014; Thomas et al., 2015; Visalli et al., 2016). TDS has been applied less to evaluate heterogeneous foods or composite foods. However, for heterogeneous foods displaying contrasting texture sensations, the selection of a single attribute at a time as dominant may give rise to dumping effects (Varela et al., 2018). Temporal-Check-All-That-Apply (TCATA) may provide a more complete description of the dynamic sensory characteristics of a product, since multiple attributes from different modalities (e.g. taste and texture) can be selected simultaneously. This permits the description of sensations that arise concurrently decreasing the likelihood for dumping effects (Castura et al., 2016). Moreover, TCATA has been also used with both expert and consumer panels (Jaeger et al., 2018; Ramsey et al., 2018; Reyes et al., 2017). Multiple studies compared TDS and TCATA and suggested that TCATA may provide a better sensory discrimination between samples than TDS (Ares et al., 2015;. Nguyen et al., 2018). The selection of an adequate dynamic sensory method depends largely on the research question and on the product properties such as food heterogeneity (i.e., composite foods) and food sensory complexity. Few studies have determined sensory properties of composite foods and the methodologies used include Quantitative Descriptive Analysis (QDA), Descriptive Analysis (DA), Temporal Dominance of Sensations (TDS), Rate-All-That-Apply (RATA), Flash Profiling (FP), Sequential Profiling (SP) and Progressive Profiling (PP) (van Eck and Stieger, 2020). To our knowledge TCATA has not been used to evaluate dynamic sensory properties of composite foods. Thus, it remains unclear which temporal sensory method (TDS or TCATA) is more adequate to capture the dynamics of sensory perception of sensorial more complex composite foods.

The aim of this study was to investigate the effect of carrier addition (bread, wafer) on dynamic sensory perception of chocolate hazelnut spreads varying in composition using TDS and TCATA. TDS was used in addition to TCATA (a) to allow for comparison of dynamic sensory properties of composite foods with previous studies which used TDS, (b) to validate the findings of previous studies comparing the TCATA and TDS methodologies and (c) to extend the findings of previous studies comparing the TCATA and TDS methodologies from single to sensorial more complex composite foods. It is hypothesized that (i) addition of carriers to spreads causes dominance of texture sensations related to carriers and decreases dominance of sensations related to spreads, (ii) discrimination sensitivity between reformulated spreads decreases when spreads are combined with carriers and (iii) TCATA provides better sensory characterization of composite foods than TDS since participants select multiple sensory attributes at the same time.

2.2 Materials and methods

2.2.1 Samples

The composition of all chocolate hazelnut spreads together with their rheological properties are summarized in Table 2. 1 Three chocolate hazelnut spreads varying in fat and sugar content (high fat/high sugar referred to as C (Control sample); high fat/ low sugar (15% sugar reduction referred to as LS); low fat/ high sugar (15% fat reduction referred to as LF) were prepared using milk and inulin to replace fat and sugar respectively. Composite foods were prepared by combining spreads with two carriers with different mechanical and texture properties (bread and wafer). Spreads have been reformulated to warrant close resemblance to real commercial products. Preparing a low fat/low sugar chocolate hazelnut spread would have changed the organoleptic characteristics of the chocolate spread to a degree that it would have no longer been comparable with the other spreads. For this reason, it was not possible to have a complete factorial design of the study.

Bread and wafer were chosen as carriers to simulate the consumption context of the chocolate hazelnut spreads with high ecological validity. The most common consumption context for chocolate hazelnut spreads is consumption with breads. The wafer filled with chocolate hazelnut spread represents a globally, commercially available composite food that

differs in mechanical and texture properties from bread. A description of sample codes is shown in Table 2.2.

All samples included of 6 g spread. The spreads evaluated alone (6 g) were served on a plastic spoon. For the spread-wafer combinations, wafer biscuits (Soremartec, Italy) were pre-cut in the form of a shell with dimensions of 3x4x1 cm (mean weight 1.56 ± 0.1g), filled with the spread (6 g) and packed.

Table 2. 1 Overview of composition of chocolate hazelnut spreads and their rheological properties.

	Chocolate hazelnut spreads		
	High fat/High sugar (Control C)	**High fat/ Low sugar (LS)**	**Low fat / High sugar (LF)**
Sugar [g/100g]	51.69	43.94	53.69
Roasted hazelnuts [g/100g]	10.20	10.20	10.20
Cocoa [g/100g]	5.48	5.48	5.48
Skimmed milk powder [g/100g]	10.95	10.95	12.13
Lecithin [g/100g]	0.43	0.43	0.43
Vanillin [g/100g]	0.02	0.02	0.02
Oil [g/100g]	21.03	21.03	17.85
Inulin [g/100g]	-	7.75	-
Aroma Mix [g/100g]	0.20	0.20	0.20
Consistency (K) [Pa*s^n]	105.5	153.9	165.5
Flow behaviour index (n)	0.45	0.37	0.34

Table 2. 2 Overview of sample labels of all stimuli.

Group \ Spread	High fat/ High sugar (Control C)	High fat/ Low sugar (LS)	Low fat / High sugar (LF)
Alone (A) (6 g)	A-C	A-LF	A-LS
Bread- Spread (B) 3x3x1cm (mean weight bread 2.23 ± 0.5g)	B-C	B-LF	B-LS
Wafer – Spread (W) 3x4x1 cm (mean weight wafer 1.56 ± 0.1g),	W-C	W-LF	W-LS

They were kept in their original package at room temperature (21±1°C), protected from sunlight, and were opened just before the evaluation. For the spread-bread combinations, commercially available bread (Morato Bruschelle, Italy) was cut into cubes of 3x3x1cm without crust (mean weight 2.23 ± 0.5g), and spread (6 g) was spread on top of the bread with a knife. The spread-bread and the spreads evaluated alone were prepared just before the evaluation (< 2 hours).

2.2.2 Rheological characterization of spreads

Flow curves of three chocolate hazelnut spreads were determined using a Physica MCR 301 Rheometer (Anton Paar GmbH, Graz, Austria) at 20°C with a smooth parallel plate geometry and stainless-steel probe (PP50/SS 61717). 2 ml of each spread was put onto the plate, which was fixed on a Peltier system with the temperature fixed at 20.0 ± 0.1 °C. The excess sample squeezed out of the gap was removed with a spatula in the trimming position and from the edge of the upper geometry. An inspection was done after trimming to avoid air remaining

in the measuring gap. The samples were equilibrated for 5 min before the analysis began. Flow curves of spreads were measured by recording shear stress values when shearing the samples with an increasing shear rate from 0.1 to 1000 s-1 and then decreasing shear rate from 1000 to 0.1 s-1 in 3.5 min . During each interval 21 data points were measured, with each point being held for 5 s. Measurements were carried out in triplicate. The Ostwald-de-Waele power law model (equation 2.1) was used to fit the flow curves to quantify consistency (*K)* and flow behaviour index (*n)*. Fitting of flow curves was done at shear rates ranging from 1 to 100 s-1.

$$\textbf{(1)}\ \eta = K * \gamma^{n}$$

2.2.3 Participants

As the focus of the study was to understand perception of composite foods varying in properties, a relatively homogeneous group of participants was selected to control as much as possible inter-individual variation. Thus, one hundred forty-four healthy women (18 -29 yrs) were recruited for this study from the consumer database of the Centrum voor Smaakonderzoek (CSO, "Center for Taste Research", Wageningen, The Netherlands). All participants were Caucasian women that consumed chocolate hazelnut spreads at least once a month (self-reported). Other inclusion criteria were to not have any dietary restrictions, nor allergy or intolerances to wheat/gluten, dairy, nuts, soybean, or eggs, to be not pregnant, non-smoking and with no history of oral perception disorders or olfactory impairments (self-reported). None of the participants had any previous training in sensory evaluation of chocolate hazelnut spreads. After inclusion, participants were randomly divided in two groups to perform sensory evaluations either with TCATA (n = 72, mean age 22.6 ± 2.0 yrs, mean BMI 21.9 ± 2.6 kg/m2) or TDS (n = 72, mean age 23.0 ± 2.0 yrs, mean BMI 22.1 ± 2.6 kg/m2). No significant differences were observed for age, BMI, desire to eat or hunger levels at the beginning of sensory evaluations between the TCATA and TDS groups according to Mann-Whitney test ($p > 0.05$). Participants gave written informed consent before the start of the study and received financial compensation for their participation.

2.2.4 Attribute selection and determination of consumption time

Sensory attributes were selected by eight women (mean age 34.2 ± 7.4 yrs., mean BMI 25.3 ± 4.3 kg/m^2) not participating in the sensory evaluation of the study. This focus group

underwent through four familiarization sessions of one hour each for the evaluation of chocolate hazelnut spreads. The first two sessions were dedicated to attribute generation focused on descriptors related to flavour and texture of the spreads and the spread-carrier combinations. The attributes that were most frequently mentioned were included in the final attribute lists used in the evaluations. Different attribute lists were used for the evaluations of spreads alone and for the carrier-spread combinations. Eight sensory attributes were included for the spreads when evaluated alone, and 10 for the spread-wafer and spread-bread combinations. The list of attributes and their definitions are shown in Table 2. 3.

Table 2. 3 List of sensory attributes and definitions used for TDS and TCATA evaluations. Different attribute lists were used for evaluations of spreads alone and for carrier-spread combinations.

Attribute	Definition	Spread Alone	Bread + Spread	Wafer + Spread
Hazelnut	Flavour associated to roasted hazelnut	✓	✓	✓
Milky	Flavour associated with processed milk such as milk powder	✓	✓	✓
Sweet	Taste associated with sugar	✓	✓	✓
Toffee	Flavour associated to caramel toffee	✓	✓	✓
Cocoa	Flavour associated with cocoa powder	✓	✓	✓
Creamy	Sensation related to a full, soft and smooth texture	✓	✓	✓
Mouth drying	Sensation related to a dry and rough feeling on the tongue and oral cavity	✓	✓	✓
Sticky	Adhesion of the product to the palate, tongue and teeth	✓	✓	✓
Bread-like	Flavour associated with bread, grain flour.		✓	
Pasty	Sensation related to consistent, thick dough.		✓	
Wafer-like	Flavour associated with wafers and dry biscuits			✓
Crunchy	Auditory and tactile sensation linked to a crunching sound when chewed			✓

The eight women that generated the attribute list, also determined the consumption time for all samples. They took the sample in their mouth, masticated the samples naturally and swallowed them. They were asked to indicate when they swallowed the sample by raising their hand. The total consumption time was obtained by the researcher with a timer and was defined as the time period between taking the sample into the mouth and swallowing for each participant (i.e. raising the hand). The average total consumption time (n=8) for spreads consumed alone was 15 s and for carrier-spreads combinations 20 s. During the TCATA and TDS evaluations (section 2.6 and 2.7), participants were instructed to swallow the samples at these times.

2.2.5 Procedure

Sensory evaluations took place in a testing room under normal light conditions at room temperature (21±1°C). Participants evaluated nine samples (Table 2.2) in one session of 60 min with two minutes break in between each sample. They received the attribute lists and definitions (Table 2. 3) by email the day before their scheduled session and were instructed to familiarize themselves with them. Participants were asked not to smoke, eat, drink, or use any persistent-flavoured product for at least one hour before their session. On the day of the evaluation, a short demonstration of either the TCATA or TDS procedure was given, after which participants were asked if there were any remaining questions about the attribute lists that they received. Participants were then seated individually and were provided with the list of attributes and their definitions and a tablet on which the test was performed. The sensory assessment started with a warm-up sample (cracker) so that participants familiarized themselves with the sensory method and software that was used throughout the whole evaluation to gather data (TimeSens software version 1.1.601.0, ChemoSens, FR).

All samples were served at room temperature (21±1°C) in standardized bite size. Spreads alone (6g) were served on a plastic spoon. Spread-bread and spread-wafer combinations were placed on paper plates. Samples were coded with a random three-digit number and presented on three different trays which were presented to participants in blocks (spreads alone, bread-spread combinations, wafer-spread combinations) so that each tray had three formulations of spread (C, LS, LF). The order of trays was counterbalanced across participants and the order of spread formulations within each tray was randomized. The attributes were presented simultaneously on the tablet and their order was randomized across participants. In between samples, participants were asked to rinse their mouth with water. Participants rated their hunger level on a 100 mm Visual Analogue Scale (VAS) with end anchors ‘not at all’ to ‘extremely’ before and after all samples were assessed.

2.2.6 Temporal-Check-All-That-Apply (TCATA)

Participants (n=72) were instructed to click on a start button concurrently with taking the whole sample in their mouth. 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 and 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. Precise instructions were given regarding the moment at which assessors were asked to swallow the sample by flashing the word 'swallow' on the tablet screen. Spreads alone were swallowed 15 s and carrier-spreads combinations 20 s after clicking the start button which corresponds to the natural consumption time of these foods (Section 2.2.4). TCATA data collection was stopped automatically 105 s after participants clicked the start button.

2.2.7 Temporal Dominance of Sensations (TDS)

Participants (n=72) were instructed to click on a start button concurrently with taking the whole sample in their mouth. When participants did not perceive any sensations anymore, they were instructed to click on the "Do not perceive anything anymore" button to stop TDS data collection. Immediately after taking a sample into their mouth, participants were asked to start selecting the most dominant sensation at each moment in time. Dominant was defined as the attribute that caught most of their attention. An attribute remained dominant until another attribute was selected. Attributes could be dominant selected several times during the evaluation and not all attributes had to be selected as dominant. Similar to the TCATA evaluations, participants were instructed to swallow spreads alone after 15 s and carrier-spreads combinations after 20 s after pushing the start button by flashing the word 'swallow' on the tablet screen. These time periods correspond to the natural consumption time of these foods (section 2.2.4).

2.2.8 Data analysis

2.2.8.1 TCATA curves

TCATA curves were constructed following the procedures described by Castura et al. (2016). Proportions of citations for each attribute were calculated as the proportion of participants who checked (or perceived) a given attribute at a given moment (every 0.1 s) during the evaluation period. For a better visualization, smoothing of TCATA curves was performed using the smooth function of the TempR package of R software version 3.1.1.

For each group of products (spread alone, bread-spread and wafer-spread combinations), the citation proportions of the control samples (A-C, B-C and W-C) were compared to the citation proportions of the corresponding test samples (A-LF and A-LS; B-LS and B-LS; W-LF and W-LS). Significant differences in TCATA profiles of two products were obtained by

applying two-sided Fisher–Irwin tests for each time point and for each attribute to evaluate whether citation proportions for the pairs of products were statistically significant from zero at the 5% significance level (Castura et al., 2016). In this study, it is examined how the reformulated chocolate hazelnut spreads (High fat/ Low sugar (LS); Low fat/High sugar (LF)) differed from the reference chocolate hazelnut spread (High fat/High sugar (Control C)) regarding their dynamic sensory perception when consumed with and without carriers. Therefore, pairwise product difference between the reference product and the reformulated were performed. Highlighted sections in the TCATA curves (bold lines) represent periods during which significant differences between test sample and control sample were observed ($p < 0.05$).

2.2.8.2 TDS curves

TDS curves were constructed following the procedures described by Pineau et al., (2009). The dominance rate for each attribute at a given moment (every 0.1 s) was determined as the proportion of participants for which the given attribute was selected as dominant sensation. Chance and significance lines were calculated at $p = 0.05$ and added to the TDS curves as described by Pineau et al., (2009) (Pineau et al., 2009). For a better visualization, smoothing of TDS curves was performed using the smooth function of the TempR package of R software version 3.1.1.

For each group of products (spread alone, bread-spread and wafer-spread combinations), the dominance rates of TDS of the control samples (A-C, B-C and W-C) were compared to the dominance rates of the corresponding test samples (A-LF and A-LS; B-LS and B-LS; W-LF and W-LS). Just as with TCATA curves, limits of significance of the difference in dominance rates were obtained with Fisher's exact test (Castura et al., 2016) as the same statistical analysis as TCATA was used for pairwise comparison of the samples, independently from the significance levels of the TDS curves . Highlighted sections in the TDS curves (bold lines) represent periods during which significant differences between test sample and control sample were observed ($p < 0.05$).

2.2.8.3 Duration analysis

For each temporal method, three linear mixed models were performed by group of products separately (A, B, W) with average citation durations in the case of TCATA and dominance

durations in the case of TDS data, for a specific attribute as response. The type of formulation (C, LS, LF) was set as fixed factor and participants as random effect Three PCAs with confidence ellipses of 0.90 were obtained for each product (A, B, W) for both, TCATA and TDS data to analyse the relationships between sensory attributes within the different formulations. Only the descriptors with a significant p value ($p < 0.05$) were included in the map. Data analysis was done with XLSTAT (XLStat ver. 2021, Sensory Package, Addinsoft, Paris, France). Only the PCAs obtained with the citation durations from TCATA data were included in section 3, while the ones obtained with TDS dominance durations were included in the Supplementary Material.

2.2.8.4 Product trajectories

Mean citation proportions and dominance rates of the TCATA and TDS data respectively, were structured such that *Product x Time* was arranged in rows and Attributes in columns (Castura et al., 2016). PCA sensory trajectories describing the evolution of sensory perception over time were obtained at different time intervals. Trajectories were built and smoothed using the tempR package of R software version 3.1.1. Only the trajectories from the TDS data were included in section 3, whereas the trajectories obtained with TCATA data were included in the Supplementary Material.

2.3. Results

2.3.1 Dynamic sensory perception of spreads alone

Figure 2.1 shows the TCATA (1A,1B,1C) and TDS (1D,1E,1F) curves for the three chocolate-hazelnut spreads when consumed alone (A-C, A-LF and A-LS).

The highest citation proportions (TCATA) for the control spread were sweetness (0.87) , followed by hazelnut (0.79) and stickiness (0.79) sensations. The reduced fat spread (A-LF) was perceived significantly ($p < 0.05$) stickier, with an average increase of 0.24 of citation proportions, compared to the control spread (A-C) throughout the entire evaluation period. Citation proportions of the reduced fat spread (A-LF) of milky, decreased 0.21 ($p<0.05$) at the beginning of mastication and after the swallowing moment, compared to the control (A-C), whereas mouth drying applicability significantly increased 0.22, specially before and after swallowing for A-LF compared with A-C. Sweetness, creamy and hazelnut sensations of A-LF showed a significant decrease ($p < 0.05$) of citation proportions of 0.17, 0.17 and

0.19 respectively, compared to A-C in the beginning of mastication. The TCATA sensory profile of the reduced sugar spread (A-LS) was very similar to that of the control spread (A-C), except for short periods (<3 s) in the beginning of the evaluation during which sweetness applicability was significantly lower (0.14) for A-LS compared to A-C.

As for the TDS evaluations, the control spread (A-C) was characterized by dominance of sticky and creamy sensations during mastication. Sticky remained dominant sensation after swallowing and then, hazelnut followed by sweetness were dominant sensations after swallowing. For the reduced fat spread (A-LF), dominance of sticky sensation significantly increased 0.19 compared to the control spread (A-C), throughout most of the evaluation time and creamy sensations of the reduced fat spread (A-LF) did not reach significance during and after mastication. Sweetness was perceived as dominant in the middle and towards the end of the evaluation. The TDS sensory profile of the reduced sugar spread (A-LS) was characterized by creamy sensation in the beginning, sticky sensation before and after the swallowing moment, followed by dominance of hazelnut and sweetness after swallowing. No significant differences were observed between the TDS profiles of the reduced sugar spread (A-LS) and the control formulation (A-C).

To summarize, TCATA and TDS evaluations of the spreads alone showed similarly that dynamic sensory perception of the high fat/low sugar spread (A-LS) was very similar to the control spread (A-C) and that the low fat/high sugar spread (A-LS) was perceived stickier than the control spread (A-C) and less sweet during the mastication.

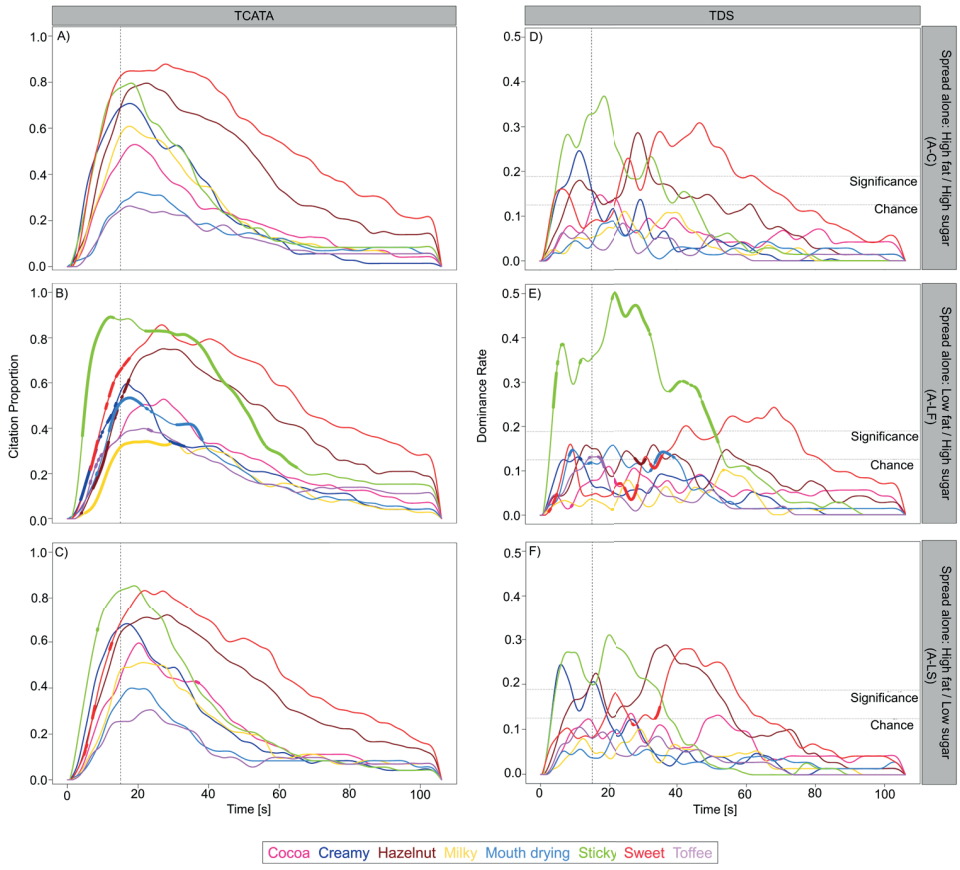

Figure 2. 1 TCATA (n=72) and TDS (n=72) curves for the three chocolate hazelnut spreads served on their own (A-C, A-LF, A-LS). Periods of significant differences ($p<0.05$) in proportion of citations (TCATA) and dominance rates (TDS), between the A-LF and A-LS compared to A-C, are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.

2.3.2 Dynamic sensory perception of spreads with carriers

Figures 2.2 and 2.3 show the TCATA and TDS curves for the three chocolate hazelnut spreads combined with bread (B-C, B-LF and B-LS, Figure 2.2) and wafer (W-C, W-LF and W-LS, Figure 2.3), respectively. As expected, the addition of carriers (bread and wafer) to spreads influenced dynamic sensory perception of all spreads considerably. For TCATA and TDS, during the beginning of consumption sensations related to the flavour and texture of the carriers, such as bread-like flavour for the bread-spread combinations, and wafer-like flavour and crunchy for the wafer-spread combinations were characteristic.

For the bread-spread combinations (B-C, B-LF and B-LS) in the TCATA evaluation, after the swallowing moment sweetness and hazelnut showed the highest citation proportions with 0.83 and 0.72 respectively and lingered until the end of evaluation time. While the B-LS combination displayed a very similar temporal profile as the B-C combination, the B-LF combination showed a significant increase of 0.21 citation proportions of sticky, especially after swallowing.

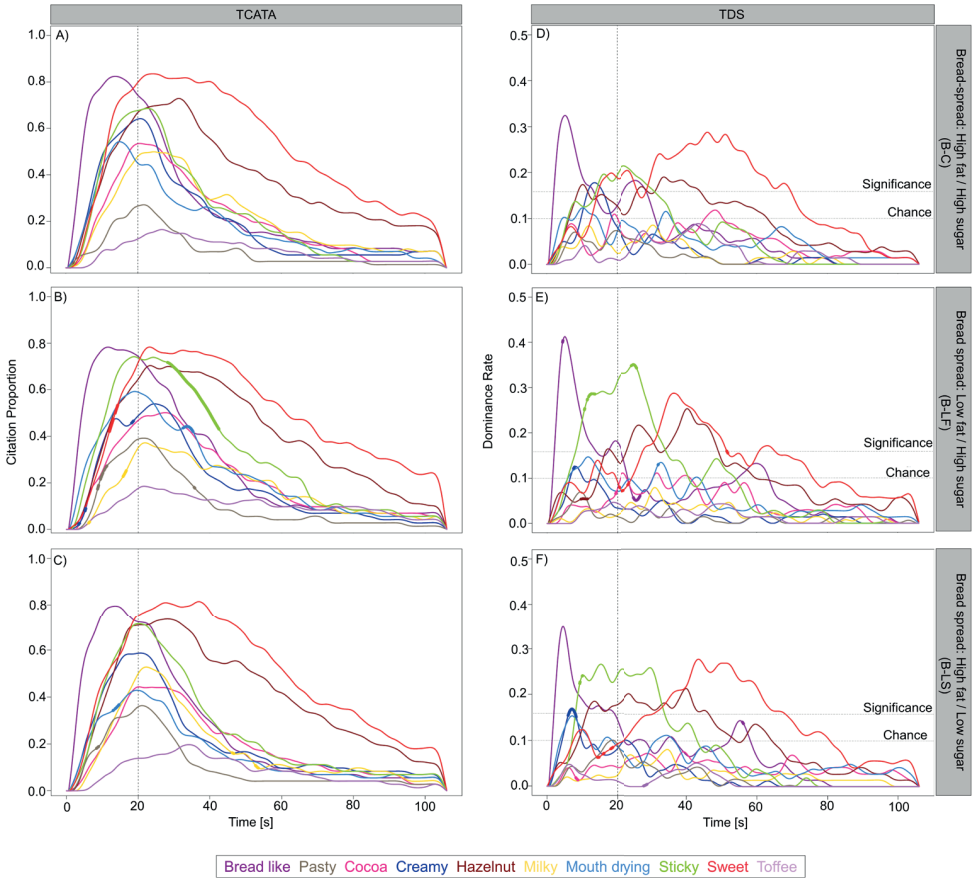

Figure 2. 2 TCATA (n=72) and TDS (n=72) curves for the three chocolate hazelnut spreads combined with bread (B-C, B-LF and B-LS). Periods of significant differences ($p<0.05$) in proportion of citations (TCATA) and dominance rates (TDS), between the B-LF and B-LS compared to B-C, are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.

For the TDS evaluation, B-C and B-LS combinations displayed similar sensory profiles. The first part of mastication was dominated by the attribute bread-like flavour. For the B-LF combinations, during the beginning of mastication time bread-like flavour was dominant sensation. Before and after the swallowing moment, dominance rate of sticky sensation increased 0.154, followed by dominance of sweetness which decreased compared to B-C.

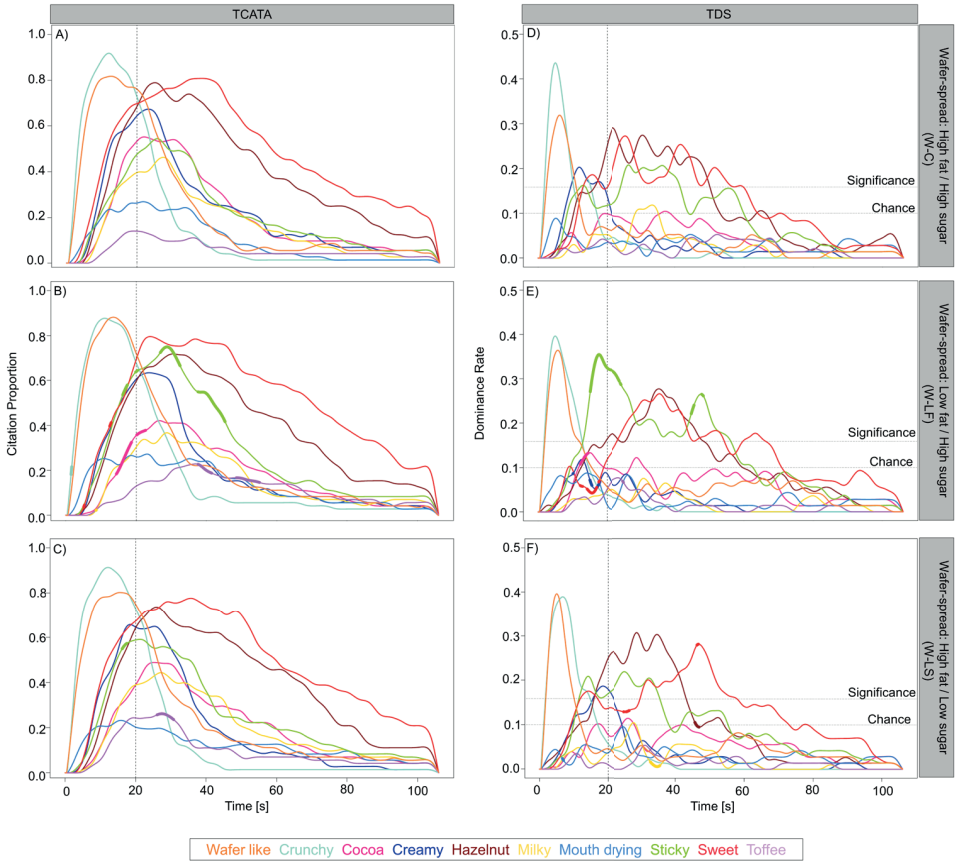

Figure 2. 3 TCATA (n=72) and TDS (n=72) curves for the three chocolate hazelnut spreads combined with wafer (W-C, W-LF and W-LS). Periods of significant differences ($p<0.05$) in proportion of citations (TCATA) and dominance rates (TDS), between the W-LF and W-LS compared to W-C, are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.

As stated before, the addition of wafer influenced the sensory perception of spreads. For TCATA and TDS evaluations, wafer-like flavour and crunchy were the characteristic attributes at the beginning of consumption time of wafer-spread combinations (W-C, W-LS, W-LF). For all combinations, creamy reached highest citation proportion in TCATA around

the swallowing moment (20s). Then, sweetness and hazelnut showed a prolonged perception after swallowing and towards the end of the evaluation time of TCATA. W-LF combination displayed a significant increase in citation proportions of 0.22 in sticky sensation after the swallowing moment, an increment of 0.11 of applicability in toffee flavour around the middle part of the mastication period, and a decrease in cocoa flavour before the swallowing moment compared to the W-C. W-LS combinations showed very similar dynamic sensory profiles as W-C with only minor significant differences being found for short time periods (< 3 s).

Crunchy and wafer-like sensations were dominant at the beginning of the TDS evaluation for W-C, followed by a short dominance period of creamy. After swallowing, hazelnut, sweetness and to a lesser extent sticky, were dominant sensations through the rest of the evaluation period before decreasing gradually. W-LF on the other hand was characterized by a significant increase of 0.20 in dominance of sticky before and after the swallowing moment followed by dominance of hazelnut and sweetness and an increase of dominance of sticky sensation in the middle of evaluation time. The beginning of the TDS profiles of W-LS were characterized by crunchy and wafer-like, followed by dominance of sticky just before the swallowing moment. There was an increase of hazelnut perception followed by sweetness. No significant differences were found between the TDS profiles of W-LS and W-C.

2.3.3 Duration analysis

Principal Component Analysis (PCA) of the durations of sensations from the TCATA data are shown in Figure 2.4 to illustrate differences between samples. The corresponding PCAs of the dominance durations from the TDS data are shown in Supplementary Material 2 1. Figure 2.4A shows the spreads when consumed alone. The first axis accounts for 82% of variability and is primarily corelated with the sensations mouth drying, toffee and stickiness. Control spread (A-C) was particularly characterized by Milky flavour and was perceived in a similar way as the A-LS spread, with their confidence ellipses overlapping. The confidence ellipse of A-LF (depicted in yellow) does not overlap with the confidence ellipses of A-C

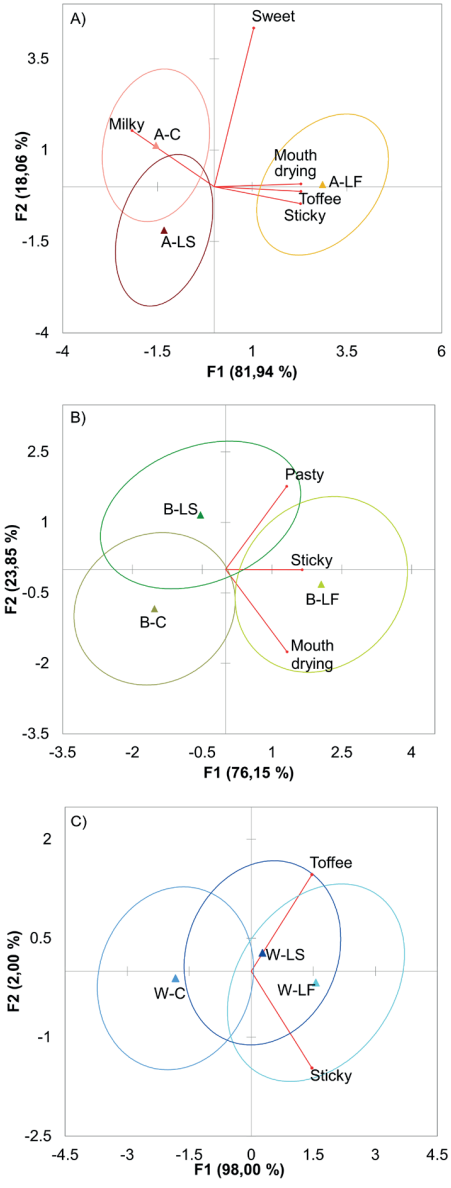

Figure 2. 4 Principal Component Analysis (PCA) of the durations of sensations from the TCATA data with confidence ellipses of 0.90. Biplot showing dimensions 1 and 2 represents the sensory profiles of the three chocolate hazelnut spreads (C, LF, LS) served alone (Figure 4A), combined with bread (Figure 4B), and combined with wafer (Figure 4C). Only the attributes that were significantly different (p<0.05) are shown.

and A-LS demonstrating that the low-fat spread was perceived significantly different from the other spreads when consumed alone.

This discrimination between spreads was influenced by the addition of bread (Figure 2.4B) and wafer (Figure 2. 4C) to the spreads. In both cases it can be observed that the sensitivity to discriminate between spreads differing in formulation decreased. Sensory profiles of carrier-spread combinations started to overlap, especially when wafer was added. For wafer-spread combinations, the confidence ellipses of W-C, W-LS and W-LF overlap suggesting that these combinations were not discriminated although spreads differed in formulation.

From the PCA it can be observed that indeed TCATA provided a more detailed description of the samples as multiple attributes can be selected at a time. In contrast, the corresponding PCAs of dominance durations from the TDS data (Supplementary Material 2.2) showed a less detailed description of the samples, as fewer attributes were statistically significant ($p < 0.05$).

2.3.4 Sensory trajectories of spreads and carrier-spread combinations

Figure 2.5 displays the temporal evolution of TDS attributes (sensory trajectories) for spreads evaluated alone, and in combination with bread and wafer. The corresponding trajectories of the TCATA attributes are provided in the

Supplementary Material 2.2. Figure 2.5A, displays the sensory trajectories of the spreads evaluated alone. The two components account for 85.57% of the total variance. At the beginning of consumption, spreads were differentiated according to the first dimension of the PCA, which was mainly related to sticky, whereas dimension 2 was negatively correlated with sweet and hazelnut. The trajectories for all spreads started in the top left. As evaluated by dominance rate, the early dominant attributes were mouth drying, creamy and sticky. As the evaluation continued, attributes hazelnut, toffee, cocoa, milky and sweet were dominant. When comparing the three spread formulations, A-C and A-LS displayed similar sensory trajectories. The sensory trajectory of A-LF was characterized by a high dominance rate of sticky and followed by creamy, milky, cocoa and toffee sensations. The addition of carriers to spreads influenced the sensory trajectories. Figure 2.5B and 2.5C display the sensory trajectories for the bread-spread and wafer-spread combinations, respectively. The first two dimensions of the PCA of the sensory trajectories for the bread-spread combinations accounted for 77.49 % of the variance observed among samples (fig. 2.4B). In the beginning of the

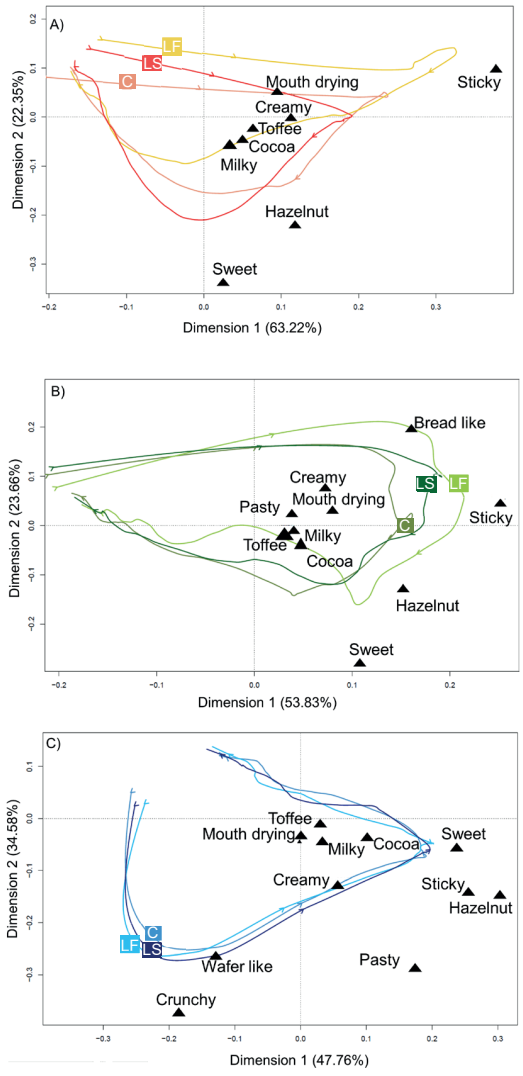

Figure 2. 5 Principal Component Analysis on the mean dominance rates from TDS data. Biplot showing dimensions 1 and 2 representing the sensory trajectories of the three chocolate hazelnut spreads (C, LF, LS) served alone (Figure 5A), combined with bread (Figure 5B), and combined with wafer (Figure 5C). Product trajectories are smoothed, and the symbol > Indicates the direction of the trajectory at different time intervals.

mastication, bread-like flavour and sticky were dominant sensations for all bread-spread combinations, with the B-LF combinations displaying a higher dominance rate of sticky. As the evaluation time continued, dominance of sensations evolved into hazelnut, cocoa and toffee for all bread-spread combinations. The sensory trajectories of the bread-spread combinations were independent of the spread formulation. Lastly, Figure2.5C, shows the sensory trajectories for the wafer-spread combinations. The two components accounted for 82.34% of the total variance. The evolution pattern from all samples was very similar across the three wafer-spread combinations. In the beginning, mastication was characterized by a high dominance rate of crunchy and wafer-like and followed by creamy, sweet, and cocoa sensations.

As mentioned previously, results indicate that TCATA provided a better discrimination between samples and less information about the temporal evolution of attributes and their dynamic sequence. This was confirmed by the corresponding trajectories of the TCATA data which are provided in the Supplementary Material 2.2, where in general all samples followed the same trajectory.

2.4 Discussion

The aim of this study was to investigate the effect of carrier addition (bread, wafer) on dynamic sensory perception of chocolate hazelnut spreads varying in composition using TDS and TCATA. It was first hypothesized that the addition of carriers to spreads causes dominance of texture sensations related to carriers and decreases sensations related to spreads. When carriers (bread and wafer) were added to chocolate hazelnut spreads, a component with a contrasting texture was added. The dynamic sensory profiles of the carrier-spreads combination in TCATA and TDS showed that the early stages of the consumption were dominated by attributes related to the carrier. In the case of bread combinations, bread-like flavour dominated the beginning of mastication. In the case of wafer combinations, not only flavour (wafer-like), but also texture sensations related to the mechanical properties (crunchy) dominated. In both cases, only during later stages of mastication attributes related to the spreads characterized the sensory profiles, thus our first hypothesis is confirmed. One explanation for this could be that participants' attention shifted from perception of one food component to the other within one single bite during the mastication process. It could also be that some texture sensations such as crunchiness are sensed with the first bite, whereas for

taste and flavour to be perceived as dominant, tastant and volatile compounds need to be released from the food matrix to reach the gustatory and olfactory receptors which is a process that is facilitated by oral breakdown of the foods and hence, it occurs at later stages of the mastication cycle. Overall, these findings agree with a recent study where carriers (bread, carrot) and mayonnaise were combined in one bite and TDS showed that the dynamic sensory profiles of the carrier-mayonnaise combinations were dominated by the texture of the solid carriers, especially in the beginning and end of consumption, whereas additional sensations related to the mayonnaises appeared in the middle stage of consumption. (van Eck et al., 2019a).

It should be noted that the lists of attributes used for the spreads alone and for the carrier-spread combinations were different. For instance, the attributes related to the carriers (e.g., bread-like flavour, and pasty for the bread combination and wafer-like, and crunchy for the wafer combination) were not present in the attribute list when spreads were consumed on their own, meaning that even if they were hypothetically perceived, they were not offered in the attribute list. The number of attributes was kept to a maximum of 10, consistent with TDS methodological recommendations (Pineau et al., 2012). It was assumed that the likelihood that a carrier related attribute such as crunchy was selected when the spread was served on its own is negligible.

Secondly, it was hypothesized that the discrimination sensitivity across reformulated spreads decreases with the addition of carriers. Previous studies have shown that the influence of mono- and disaccharides on flavour release, and consequently on flavour perception, is rather limited, especially when concentration changes remain within realistic product reformulations (Delarue and Giampaoli, 2006). This could explain why the degree of sugar reduction in our study was not sufficient to induce significant differences in dynamic sensory perception between A-LS and A-C. On the contrary, reduction of fat content led to significant differences in dynamic sensory properties between A-LF and A-C. For instance, when consumed alone, the reduced fat (A-LF) spread was perceived significantly stickier than the control (A-C) and reduced sugar (A-LS) spread throughout eating time. It is speculated that the change in viscosity of the spread (Table *2.*1) caused by fat reduction might have contributed to a higher perception of stickiness. This perceptual difference between spreads disappeared when carriers were added to spreads, as the mouthfeel attribute sticky of the

spreads decreased. Composite foods have an increased stimulus complexity, which may have caused a decrease in sensitivity to detect differences between reformulated spreads. This is in agreement with previous research where consumers' ability to identify sensory differences between regular and sodium-reduced foods decreased with the presence of the accompanying foods (Nguyen & Wismer, 2020) . Ultimately, this indicates that current practices of evaluation of single foods that are rarely consumed on their own could give an inaccurate sensory profile and mislead product development. Based on the dynamic sensory evaluation of reformulated chocolate hazelnut spreads alone, it would be concluded that the reduced fat spread (15% fat reduction) does not qualify as a reformulated spread as it does not provide a similar sensory experience as the control spread. In contrast, based on the dynamic sensory evaluation of the reformulated spreads consumed with carriers, especially wafers, it would be concluded that the reduced fat spread (15% fat reduction) qualifies as a reformulated spread as it provides a similar sensory experience as the control spread when consumed with carriers which represents a more natural consumption context. It seems that the presence of accompanying foods might distract consumer attention away from the (reformulated) product of interest, which, in fact, could give more flexibility in the design of healthier foods (e.g., low in calories, reduced fat, reduced sugar, reduced salt) as it increases the reformulation space.

In addition, an effect of the type of carrier was observed as the perception of certain attributes of spreads were affected in different ways by bread compared to wafer. For instance, decrease of stickiness was more pronounced for the wafer-spread combinations than for the bread-spread combinations. On the other hand, the presence of bread but not wafer, increased the perception of mouth drying, while the perception of milky, hazelnut and sweetness perception were reduced by addition of carrier. Even though the swallowing moment was fixed for all groups of foods, it may be that oral processing behaviour and bolus formation were affected differently by bread and wafer leading to different taste, flavour, and texture sensations. This may indicate that cross-modal interactions may play a role, since the addition of a carrier with a hard texture might impact the flavour perception of a semi-solid spread (Cherdchu and Chambers IV, 2014; Meinert et al., 2011; Paulsen et al., 2012). Overall, these findings corroborate the results of van Eck (2021) who observed a decrease in taste and flavour intensities of mayonnaise when it was combined with bread and potato (van Eck et al., 2021). It should be noted, however, that even though there were clear differences in the mechanical

and texture properties of both carriers, no further analysis to characterize them was performed.

Lastly, it was hypothesized that TCATA provides better characterization of composite foods than TDS since participants can select multiple sensory attributes from different modalities (flavour and texture) at the same time. For the carrier-spreads combinations, the sensory attributes with highest citation proportions observed with TCATA were also significantly dominant in TDS, indicating that TDS and TCATA characterized products in a similar way. In both methodologies, peaks related to the attributes from the carrier reached highest citation proportions and dominance rate in TCATA and TDS respectively, in the beginning of the evaluation and across all spread formulations. However, it seems that TDS provides a better description of the temporal changes in sensory perception, as the sensory attributes appear and disappear above the significance line over the course of the evaluation. In contrast, in TCATA, only the attributes sticky, sweet and hazelnut showed somewhat clear peaks in citation proportions at specific moments in time. A general bell-shaped curve for all attributes was seen in TCATA peaking around the swallowing moment with limited variation in peak time between attributes. TCATA provided a more detailed product description compared to TDS. The PCAs built from the TCATA data show that the difference between the spreads disappear when they are combined with carriers (especially with wafers). Looking at the sensory trajectories built from the TDS data, they show similar trajectories for all reformulated spreads even when consumed alone, suggesting that TCATA indeed provides a better discrimination between products than TDS, which validates previous studies that have compared both methodologies (Ares et al., 2015, 2017; Nguyen et al., 2018) in single foods. Our study demonstrates that previous findings of studies comparing TCATA, and TDS can be extended from single foods to sensorial more complex composite foods which confirms our last hypothesis.

2.5. Conclusions

This study demonstrated that dynamic sensory perception of hazelnut chocolate spreads differing in composition was strongly affected by addition of carriers. Perceptual differences between spreads diminish when spreads are consumed with breads and wafers. These findings indicate that sensory perception of composite foods is complex, and sensory characteristics of one food are influenced by the specific properties of the other food present.

Consequently, considering sensory assessments not only with consumers but also with the most frequently used accompanying foods will give a more representative sensory profile of the real consumption context. Ultimately, the use of carriers might also help in the development of healthier foods (e.g., low in calories, reduced fat, reduced sugar, reduced salt), due to organoleptic complexity of the product. Finally, TCATA and TDS both gave complementary information; while TCATA allowed to discriminate better between spreads, TDS revealed the temporality of sensations clearer.

CHAPTER

3

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- 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 effected *in vivo* aroma release and perception for all target molecules. The addition of carriers to spreads generally increased *in vivo* 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. It is concluded 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.

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

3.1. 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., 2019).

Proton-transfer reaction mass-spectrometry equipped with a time of flight mass analyser (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 et al., 2019b), as it provides time-resolved information about the concentration and type of aroma compounds present in the nasal cavity by analysing 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 (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 flavour and 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 flavour 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 labour 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 et al., 2019b), 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 that are consumed together, 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. (2019b) demonstrated that in composite foods, carriers tend to dominate texture perception, whereas toppings drive flavour perception(van Eck et al., 2019b). It was 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., 2022a).

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 behaviours 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). It is 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.

3.2. Materials and Methods

3.2.1 Samples

Preparation and composition of chocolate-hazelnut spreads and composite foods have been described in detail elsewhere (Gonzalez-Estanol et al., 2022a). Briefly, the composition of the chocolate-hazelnut spreads used herein along with their rheological properties are summarized in Table 2. 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 3.1). 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.

Table 3. 1 Composition of the aroma solution used to spike chocolate hazelnut spreads (0.2 g/100g) together with characteristics of the aroma compounds.

Compound	Concentration g/100g	Chemical formula	Sensory description	Tentatively identified 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	2.56-2.94
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

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., 2022a). A description of sample codes and sample pictures are in Table 2.2. 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) filled with the spread. For the spread-

bread combinations, commercially available bread (Morato Bruschelle, Altavilla Vicentina VI, Italy) was cut into pieces of 3x3x1cm 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 hours).

3.2.2 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., 2022a). All participants in both groups were Caucasian women who consumed chocolate-hazelnut spreads at least once a month (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).

3.2.3 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 flavour 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 (Table 2. 3). 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 2.2) as if it was the actual experiment (section 3.2.5). 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., 2022a). The average consumption time was 15s for spreads alone and 20s for carrier-spread combinations.

3.2.4 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., 2022a), participants evaluated nine samples (Table 2.2) in a single 60-min session with 2-min breaks between samples. Participants received the attribute lists and their definitions (Table 2. 3) 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 flavoured 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 2.2). 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., 2022a), 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 3.2.4). TCATA data collection stopped 105 s after participants clicked the start button. Between samples, participants were asked to rinse their mouth with water.

3.2.5 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 flavoured 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 H3O+ as the precursor ion with the following drift tube parameters (Weel et al., 2002): voltage 628V, 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 60s 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 3.2.4) and used the same attribute lists. TCATA and *in vivo* nose-space data were acquired simultaneously for 105 s.

3.2.6 Data analysis

3.2.6.1 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 analysed as described elsewhere (Gonzalez-Estanol et al., 2022a).

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 in the TCATA curves (bold lines; Figures 3.1, 3.3 and 3.4) 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 40s/105s = 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 of citation 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 analysing 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 ($p < 0.05$).

3.2.6.2 *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 3.1) 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).

3.3. Results

3.3.1 Dynamic sensory perception of spreads alone

Figure 3.1 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) (Figure 3.1A) 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 (Figure 3.1C) 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 through the end of the evaluation, cocoa and sweetness were 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.

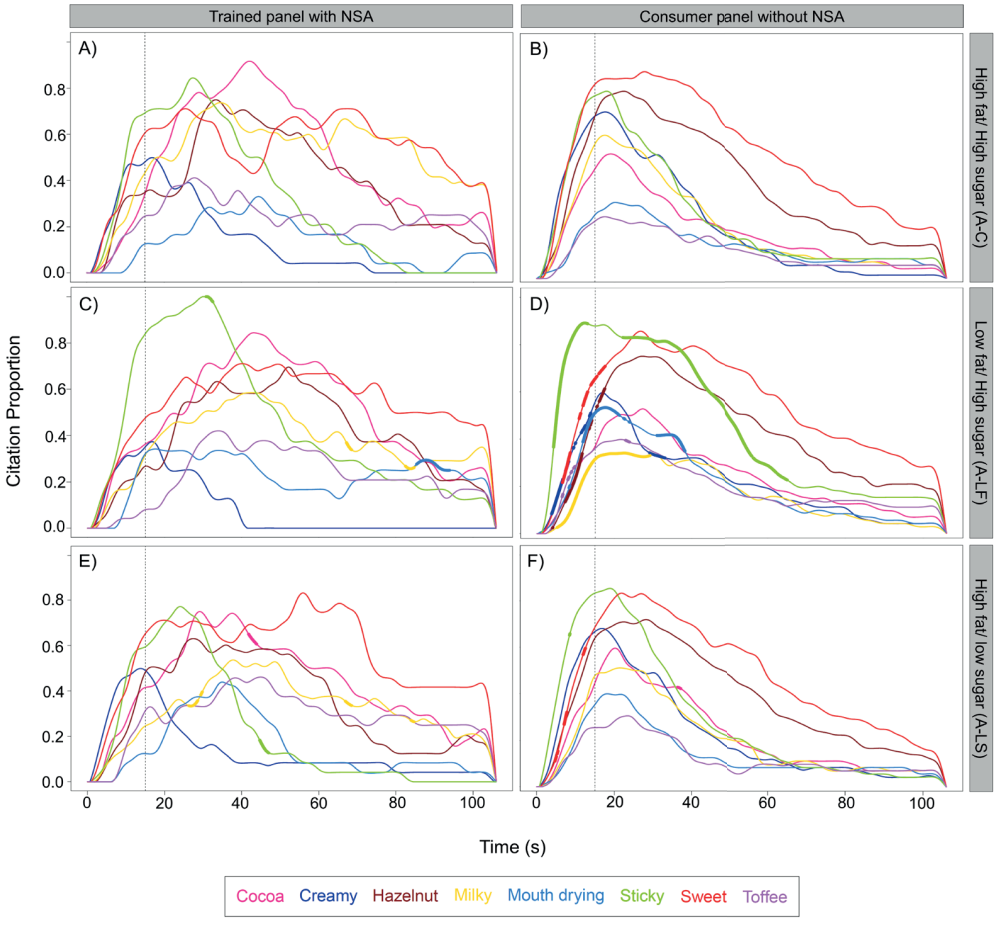

Figure 3. 1 Trained panel with nose space analysis (NSA) (n = 8, triplicate) and consumer panel without NSA (n = 72) TCATA curves for three chocolate hazelnut spreads served on their own (A-C, A-LF, A-LS). Periods of significant differences ($p < 0.05$) in proportion of citations between A-LF and A-C and between A-LS and A-C are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.

Overall, milky sensation 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 (Figure 3.1E) was similar to the control (A-C). After swallowing, perception was characterized by sweetness, stickiness, and cocoa flavour. 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 (Figure 3.1B) were sweetness (0.87), followed by

hazelnut (0.79) and stickiness (0.79). The reduced fat spread (A-LF) (Figure 3.1D) 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) (Figure 3.1F) 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.

3.3.2 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 3.1 and 3.2, respectively. For both panels, bread-like flavour (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. Toward 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 showed 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 flavour 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 flavour around the middle of the mastication period, and a decrease in cocoa flavour 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.

3.3.3 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 3.3). 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 3.3), 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 the comparison of *in vivo* aroma release with aroma perception, the TCATA data from the consumer panel without NSA (n=72) is considered in sections 3.3.4 and 3.3.5 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.

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

Table 3.2 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 3.3)

Table 3. 2. (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	I_{max} (ppbV)	1.0±0.4	25.9±11	1.7±1	2.4±1.1	1.5±0.5	Cit_{max} (-)	0.5	0.8	0.6
	T_{max} (s)	34±18	33±20	41±30	35±18	37±19	T_{max} (s)	19	23	18
	AUC (ppbV*s)	19±10	443±243	39±25	53±28	34±13	AUC	21	42	24
A-LF	I_{max} (ppbV)	0.9±0.4	25.5±13.9	1.3±0.7	2.2±1.1	1.4±0.6	Cit_{max} (-)	0.5	0.8	0.3
	T_{max} (s)	35±19	33±22	42±31	33±16	32±24	T_{max} (s)	27	27	23
	AUC (ppbV*s)	19±12	520±349	33±26	53±29	34±18	AUC	25	43	17
A-LS	I_{max} (ppbV)	0.8±0.4	24.1±14.8	1.3±0.8	2.2±1.1	1.3±0.5	Cit_{max} (-)	0.6	0.7	0.5
	T_{max} (s)	27±12	25±13	45±31	31±17	31±17	T_{max} (s)	20	28	22
	AUC	15±10	394±256	35±25	46±23	27±15	AUC	24	40	21
B-C	I_{max} (ppbV)	4.3±2.1	38±19.2	1.2±0.5	2.8±1	1.8±0.5	Cit_{max} (-)	0.5	0.7	0.5
	T_{max} (s)	32±11	26±9	35±17	35±11	36±13	T_{max} (s)	21	32	25
	AUC (ppbV*s)	149±72	777±319	42±20	74±31	50±17	AUC	21	40	23
B-LF	I_{max} (ppbV)	4.1±2.6	34.6±13.1	1.2±0.8	2.5±1	1.5±0.4	Cit_{max} (-)	0.5	0.7	0.4
	T_{max} (s)	31±11	25±7	40±25	35±13	33±10	T_{max} (s)	28	23	22
	AUC (ppbV*s)	144±93	776±350	34±19	72±37	46±20	AUC	22	41	18
B-LS	I_{max} (ppbV)	4.2±2.9	36.5±15	1.4±0.9	3±1.5	1.7±0.6	Cit_{max} (-)	0.4	0.7	0.5
	T_{max} (s)	29±13	23±8	40±31	31±10	30±12	T_{max} (s)	20	28	22
	AUC (ppbV*s)	129±86	686±336	37±24	70±39	41±18	AUC	19	42	19
W-C	I_{max} (ppbV)	1.2±0.5	34.8±14.4	1.7±1.2	2.7±1.5	1.6±0.6	Cit_{max} (-)	0.6	0.8	0.5
	T_{max} (s)	29±12	27±11	38±33	34±14	34±19	T_{max} (s)	22	25	28
	AUC (ppbV*s)	29±14	818±351	60±45	70±42	47±19	AUC	22	40	17
W-LF	I_{max} (ppbV)	1.1±0.4	35.5±12.9	1.8±1.3	2.7±1.2	1.6±0.7	Cit_{max} (-)	0.4	0.7	0.4
	T_{max} (s)	27±11	26±11	42±28	35±11	34±15	T_{max} (s)	27	31	29
	AUC (ppbV*s)	28±12	846±341	59±37	71±30	46±18	AUC	18	39	16
W-LS	I_{max} (ppbV)	1±0.4	28.1±10.2	1.6±0.9	2.3±0.9	1.3±0.5	Cit_{max} (-)	0.5	0.7	0.4
	T_{max} (s)	32±16	32±16	30±23	32±11	35±15	T_{max} (s)	25	26	27
	AUC (ppbV*s)	24±10	673±263	52±36	60±31	40±16	AUC	20	39	20

Table 3. 3 Effect of carrier addition to spreads (carrier), reformulation of chocolate hazelnut spreads (formula) and their interaction effect (linear mixed model) on AUC, I_{max} and T_{max} 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}		T_{max}	
Source	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
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}**		**T_{max}**	
Source	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
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}**		**T_{max}**	
Source	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
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}**		**T_{max}**	
Source	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
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}**		**T_{max}**	
Source	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
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**

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. 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 (Figure 3.2A). 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).

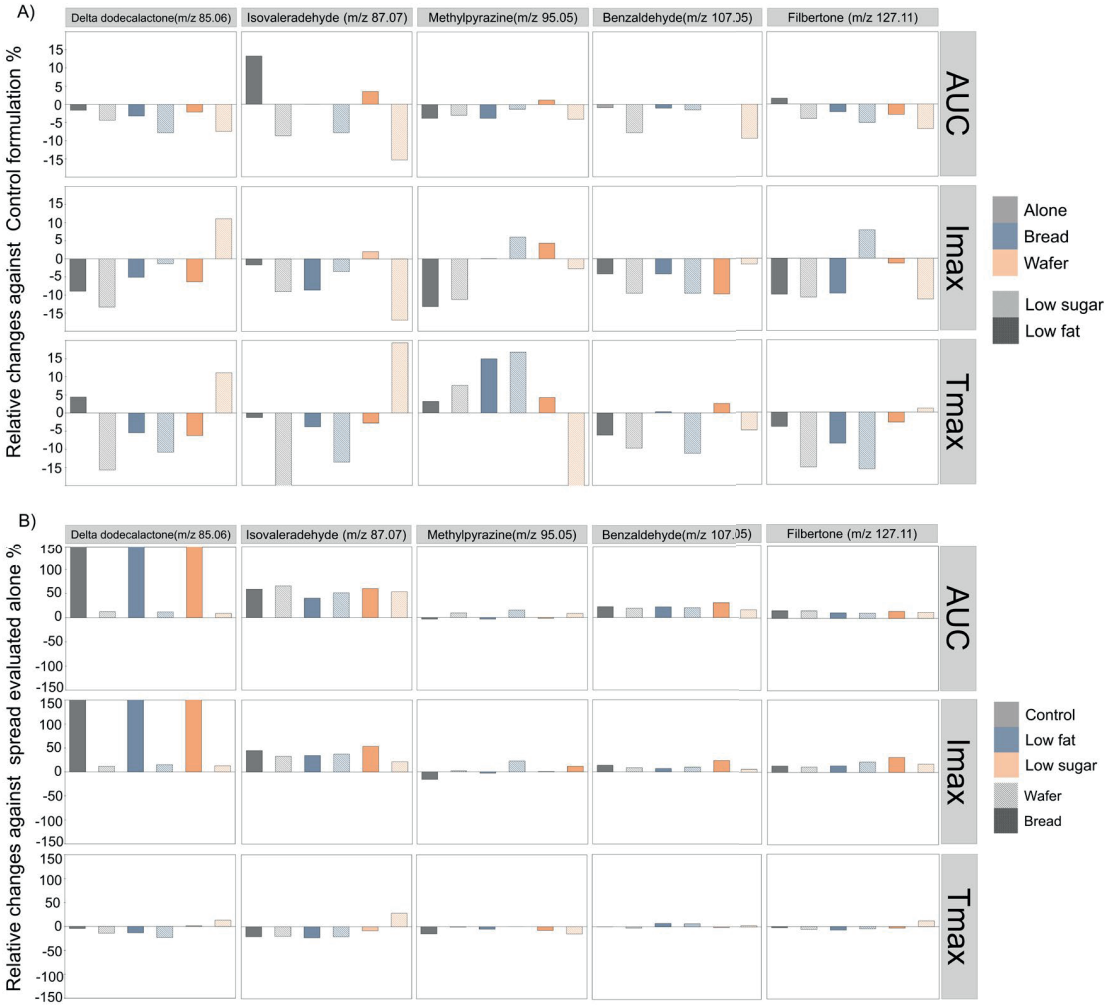

Figure 3. 2 Relative intensity changes in AUC, I_{max} and T_{max} (%) for each volatile compound. (A) describes the relative changes against the control formulation in comparison to reduced fat and reduced sugar spreads. (B) describes the relative changes against the spreads evaluated alone compared when spreads were combined with bread or wafer.

Figure 3.3A 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.

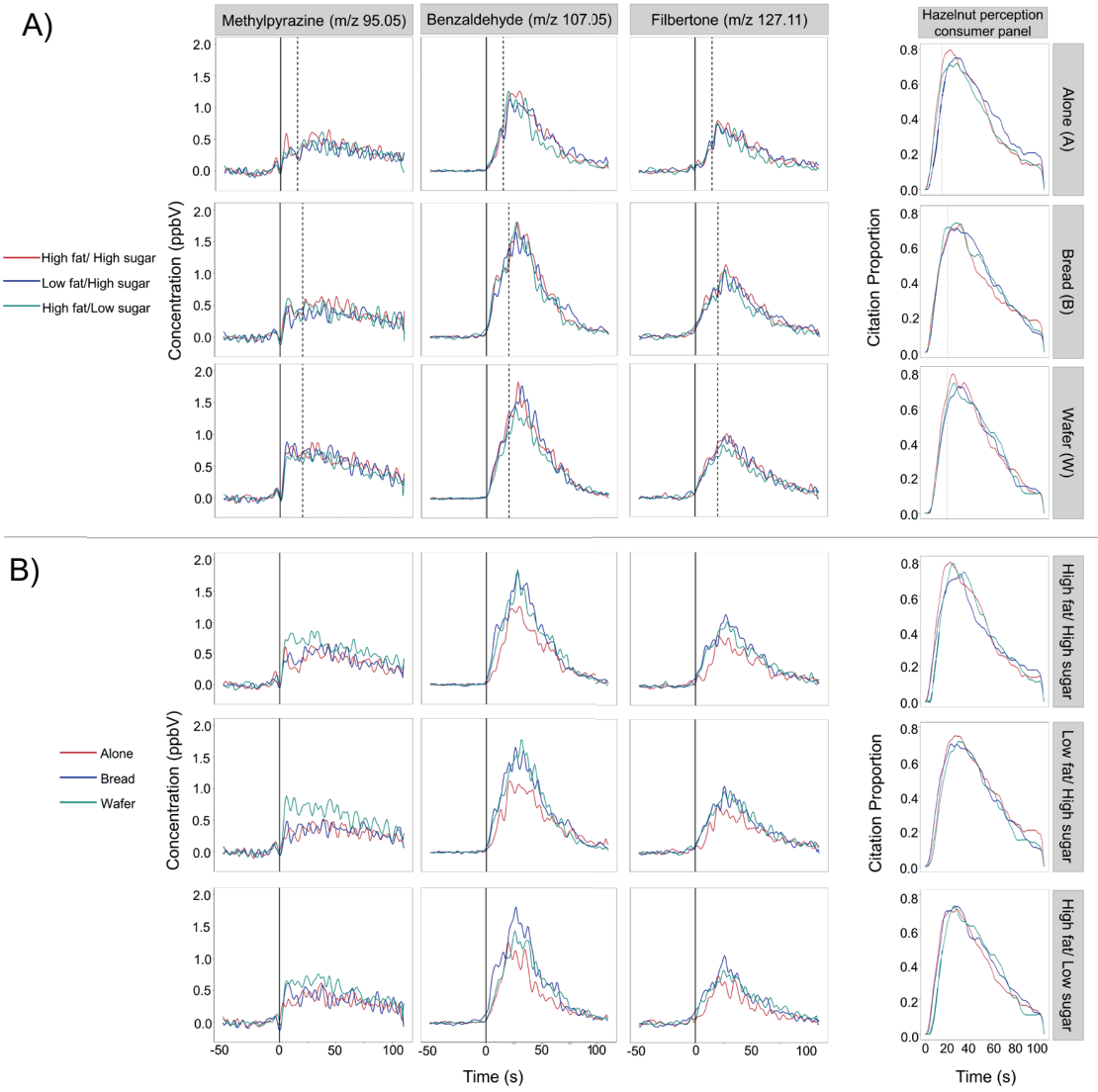

Figure 3. 3 Averaged in vivo nose space release of methylpyrazine, benzaldehyde and filbertone (n=8, triplicate) and TCATA proportion of citations of the consumers panel without NSA (n=72) for attribute hazelnut comparing effects of formula (A) and carrier (B) on aroma release and perception. Black continuous 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.

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 flavour for neither of the spread-carrier combinations.

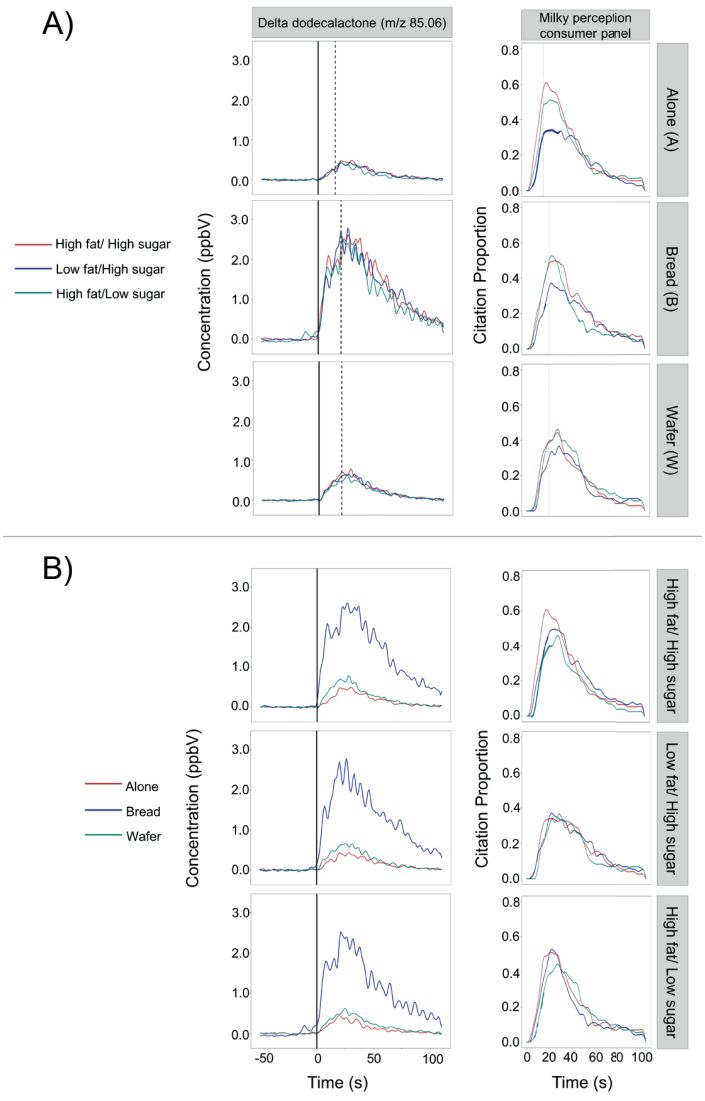

Figure 3. 4 Averaged *in vivo* nose space release of delta-dodecalactone (n=8, triplicate) and TCATA proportion of citations of the consumers panel without NSA (n=72) for attribute milky comparing effects of formula (A) and carrier (B) on aroma release and perception. Black continuous lines represent 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.

Figure 3.4A shows the release curves of t.i delta dodecalactone (m/z 85.06) and its corresponding TCATA profiles for milky flavour. 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 that 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 3.4). 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.

3.3.5 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. 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 (Figure 3.2B). 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%) (Table 3.2 and Table 3.3 and Figure 3.2B).

Figure 3.3B and 3.4B 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 and the trained panel with NSA for their corresponding flavour attributes (hazelnut and milky). In all cases, contrary to the increase of flavour release observed upon addition of carriers to spreads, flavour 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.

3.4. 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 naive panel without nose space analysis showed good agreement (RV coefficient = 0.73). Therefore, the TCATA profiles obtained from the naive 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 3.1), 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 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 and 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 and Taylor, 2001; Van Ruth and Roozen, 2000), which could explain its lower release, despite being an aldehyde. Even though all aroma molecules were hydrophobic (Table 3.1) 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 (Table 2.1). 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 flavors 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 and 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 leads 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 behaviour 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 an important factor 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 flavour 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. It is acknowledged that monitoring specific odor-active compounds and knowing their individual flavour quality does not necessarily translate to the contribution of the individual compound to the overall flavour 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.

3.5. 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 sensory perception during food consumption.

CHAPTER

DIFFERENCES IN HABITUAL EATING SPEED LEAD TO SMALL DIFFERENCES IN DYNAMIC SENSORY PERCEPTION OF COMPOSITE FOODS

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Abstract

Previous studies demonstrated that variability in oral processing behaviours impacts bolus properties and consequently texture and flavour perception. However, most studies followed a prescribed mastication protocol during the products' sensory evaluations. A better understanding of how variability in habitual eating behaviour impacts sensory perception of foods is needed. The aim of this study was to investigate the effect of habitual eating speed (slow vs fast eaters) on dynamic sensory perception of composite foods. Habitual oral processing behaviour of different composite foods was quantified in n=105 participants. Participants were divided in fast (n=53) and slow (n=52) eaters using a median split. Three formulations of strawberry jam varying in viscosity and sugar content (High Sugar/Low Pectin (Control), High sugar/High Pectin, Low Sugar/Low Pectin) were used which resembled commercial strawberry jam formulations. Composite foods were prepared by spreading jams on breads. Dynamics of dominant sensory attributes of strawberry jams presented with and without breads was evaluated using Temporal Dominance of Sensations (TDS). Dynamic sensory perception of jams and jam-bread combinations differed only slightly for short periods of time between habitual slow and fast eaters. The addition of breads to jams reduced especially the ability of the fast eaters to discriminate between jams differing in formulation. Slow eaters discriminated between different formulations of jams better than fast eaters, regardless of whether jams were presented alone or in combination with breads. It is concluded that differences in habitual eating speed between consumers lead to small differences in dynamic sensory perception and discrimination ability of composite foods.

Keywords : Oral processing behaviour, TDS, composite foods, eating speed

4.1. Introduction

It is well known that sensory perception of foods varies greatly across individuals (Bachmanov and Boughter, 2012; Hayes and Keast, 2011). Factors contributing to inter-individual variation in sensory perception include age, gender, ethnic origin and phenotypes such as 6-n-propylthiouracil (PROP) and thermal taster status (Bajec and Pickering, 2008; Hirokawa et al., 2006; Lim et al., 2008; Mojet et al., 2003; Pickering et al., 2010; Yang et al., 2014). Eating behaviours emerge and stabilize in early life (WHO, 2016). Eating speed is defined as the mass of food (g) consumed per time unit (min) and is expressed as eating rate (g/min). Variations in eating speed can already been observed at 2-4 weeks postpartum (Agras et al., 1990), are influenced by parental feeding practices (Fogel et al., 2019), are generally stable over time at an individual level and tend to be the same across foods (McCrickerd and Forde, 2017). There are many and very diverse factors leading to different habitual eating speeds, including genetics (Brunkwall et al., 2013; Dubois et al., 2013), energy requirements and basal metabolic rate (Henry et al., 2018), or lifestyle habits and environmental factors (Keski-Rahkonen et al., 2004; Wing et al., 2001). Research has shown that variations in oral processing behaviours and saliva properties may influence expected fullness, sensory perception (Ferriday et al., 2016; Ketel et al., 2019). Furthermore, a faster eating rate has been associated with higher energy intake (Robinson et al., 2014), and may also affect glycaemic and insulin responses and post-meal satiety (Goh et al., 2021; Ranawana et al., 2011; Vega-López et al., 2007; Zhu et al., 2013). A broad variety of experimental techniques have been used to quantify oral processing behaviours and eating speed. Electromyography (EMG), electromyography combined with jaw-tracking (EMG-JT), videofluorography and ultrasonic echo-sonography have been used to assess oral processing behaviours (Boyar and Kilcast, 1986; Casas et al., 2003; Hennequin et al., 2005; Mioche et al., 2002; Vinyard and Fiszman, 2016). Video recordings of participants consuming foods followed by post-hoc video annotations have been used frequently to code oral processing behaviours (C. G. Forde et al., 2013). The use of video recordings for tracking jaw movements during food consumption has also been used to characterize oral processing behaviours (Aguayo-Mendoza et al., 2020; C G Forde et al., 2013a; Ketel et al., 2019; van Eck et al., 2019b). Skin surface markers are placed on the consumer's face to track automatically the movement of the chin or other facial features inferring the movement of the jaw during mastication (Jin et al., 2022; Wilson et al., 2016). Lastly, self-reporting of

eating speed has been used to characterize oral and eating behaviours (Mochizuki et al., 2014; Petty et al., 2013).

Differences in eating speed and mastication behaviour can also contribute to inter-individual differences in sensory perception of foods. To date, only few studies addressed this matter. Tarrega et al. (2008) demonstrated that the number of chews, chewing work and chewing strength correlated positively with flavour intensity of model cheeses, while chewing duration correlated negatively with flavour intensity (Tarrega et al., 2008). Luckett et al. (2016 and 2017) showed that the number of chews and chewing rate modulate the temporal dynamics of flavour perception of potato chips (Luckett et al., 2016; Luckett and Seo, 2017). Doyennette et al. (2019) showed that 'chewers' consumed ice creams with a shorter consumption time and perceived aromas earlier and longer compared to 'melters', who consumed ice creams slower (Doyennette et al., 2019). These studies demonstrated that variability in oral processing behaviours impacts flavour and texture perception of foods. These studies have in common that participants were instructed to follow a prescribed mastication protocol during the products' sensory evaluations. This suggests that in these studies, the level of oral structural breakdown of foods differed during sensory evaluations depending on the instructed chewing behaviour. These differences in oral structural breakdown and bolus properties induced by the different prescribed mastication protocols are likely to be sensed by the consumer, leading to differences in flavour and texture perception.

Even though it has been shown that eating speed is relatively consistent within individuals across different foods and meals (McCrickerd and Forde, 2017), little is known about how differences in habitual eating behaviour affect sensory perception of foods. To the best of our knowledge, only two studies characterized the impact of habitual eating speed on dynamic sensory perception. Devezeaux de Lavergne et al. (2015) showed that dynamic texture perception of sausages was similar between habitual slow and fast eaters during the first half of mastication but differed between groups in the second half of mastication. Differences in sausage bolus properties were caused by differences in eating speed and led to differences in dynamic texture perception (Devezeaux de Lavergne et al., 2015). Aguayo-Mendoza et al. (2020) showed that dynamic texture perception of sausages was similar for consumers differing in age, gender and ethnicity at the beginning and end of mastication and strongly correlated with bolus properties which were mediated by mastication time. Minor differences

in dynamic texture perception between consumer groups were observed only during the middle stages of mastication with low dominance rates. It was concluded that variations in habitual consumption time led to considerable differences in bolus properties but only small differences in dynamic texture perception of sausages (Aguayo-Mendoza et al., 2020). In both studies (Aguayo-Mendoza et al., 2020; Devezeaux de Lavergne et al., 2015) participants were characterized according to their habitual eating speed, which decreased when sensory evaluations were performed. A better understanding of how variability in naturally occurring mastication behaviour impacts sensory perception of foods is needed. The aim of this study was to investigate the effect of habitual eating speed (slow vs fast eaters) on dynamic sensory perception of composite foods. Composite foods are defined as the combination of single foods differing in composition and properties (Scholten, 2017). In this study it was first categorized consumers based on their habitual eating speed of two composite foods (jam on bread and wafer filled with chocolate spread) as slow and fast eaters.

Then it was determined (i) how dynamic sensory perception of foods differs between consumers differing in habitual eating speed and (ii) how participants adapt their oral behavior during sensory evaluations. It is hypothesized that dynamic sensory perception is affected by habitual eating speed as habitual slow eaters break down the food bolus into more and smaller bolus fragments, which facilitates flavour release from the food, compared to fast eaters.

4.2. Materials and Methods

4.2.1 Samples

Table 4.1 provides an overview of all samples used in the study. Three strawberry jams varying in sugar content and viscosity were used (Menz & Gasser, Italy). All jams consisted of strawberries, glucose-fructose syrup, sugar, pectin, citric acid, and elderberry juice concentrate. The strawberry jam used as control (C) had a sugar content of 60 ° Brix and a viscosity at a shear rate of $1s^{-1}$ of $\eta_{1s-1} = 48 \pm 6$ Pa·s and is categorized as High sugar-Low pectin jam (Table 4.1). The High sugar-High pectin (HP) strawberry jam had a sugar content of 60 ° Brix and a viscosity of $\eta_{1s-1} = 78 \pm 2$ Pa·s. The Low sugar-Low pectin (LS) jam had a sugar content of 45 ° Brix and a viscosity of $\eta_{1s-1} = 18 \pm 3$ Pa·s (Table 4.1).

Table 4. 1 Overview of strawberry jams and composite foods (jam-bread combinations)

Group \ Jams	High sugar/ Low pectin (Control C)	High sugar/ High pectin (HP)	Low sugar / Low pectin (LS)
° Brix	60	60	45
Viscosity at shear rate $1s^{-1}$ (Pa·s)	48 ± 6	78 ± 2	18 ± 3
Jam alone (A) (mean weight: 6.0± 0.3 g)	A-C	A-HP	A-LS
Jam-bread (B) 3x3x1 cm (Bread: 2.2 ± 0.5 g Spread: 6 g)	B-C	B-HP	B-LS

Reformulation of the strawberry jams remained within realistic product reformulation boundaries to warrant close resemblance to reformulated, commercially available products. To obtain composite foods, 6.0 g of strawberry jam was spread on a piece of white bread without crust (3 x 3 x 1 cm; 2.2 g; Bruschelle mini, Morato, Italy).

4.2.2 Participants

For this study 124 participants were recruited. Recruitment was done through posters, social media posts and emails. An online questionnaire was used to exclude participants with swallowing, mastication, olfactory and gustatory disorders. Inclusion criteria included good dental health, non-smoking, no missing teeth (except wisdom teeth), no piercings or braces in the mouth, no recent dental surgery, not being on a calorie restricted diet, no food allergy to any of the ingredients present in bread, wafer, jam, no partial nor complete denture, and not being pregnant nor lactating. Out of the 124 participants, n=105 (age 24.8 ± 4.73 years, BMI 22.8 ± 2.49 kg/m^2, 38% male) completed the study. Using a median split, participants were divided in two groups according to their natural eating speed (consumption time per

bite) of standardized bite sizes of different composite foods (section 2.3). A group referred to as fast eaters (n = 53, age 24.5 ± 4.83 years, BMI 22.6 ± 2.16 kg/m^2, 43.4% male) and a group referred to as slow eaters (n=52, age 25.2 ± 4.65 years, BMI 23.0 ± 2.80 kg/m^2 28.8% male) were obtained. No significant differences were observed for age and BMI between fast and slow eaters according to Welch's t-test ($p > 0.05$). All participants were familiar with the test foods. All participants gave written informed consent prior to the study and received financial compensation for their participation. The study did not meet the requirements to be reviewed by the Medical Research Ethical Committee of The Netherlands 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).

4.2.3 Characterization of habitual eating speed

Habitual oral processing behaviour of three standardized bite sizes of different foods was quantified in n=105 participants using a video camera (Canon IXUS-180) placed on a tripod. The first food used to determine habitual eating speed consisted of 6 g of commercial cherry jam (Zwarte Kers, Hero, Netherlands) and was served on a spoon, the second food (bite size 8.2 ± 0.7g) consisted of 6 g of cherry jam spread on a piece of white bread without crust (2.8 g; 3.5 x 3.5 x 1.0 cm; Casino Wit, Jumbo, Netherlands) and the third food (bite size 7.6 ± 0.5 g) consisted of a piece of wafer (1.6 g; 1.0 x 4.0 x 2.0 cm) filled with 6 g of hazelnut chocolate spread (B-ready, Fererro, Italy). The three samples were presented to the participants in fixed bite sizes in randomized order. Participants were seated individually in front of the video camera, instructed to put the whole sample in their mouth and to start chewing as they normally do. Participants were asked to raise their hand when they swallowed. Between samples participants could drink a sip of water.

To measure oral processing behaviours, stickers were placed on the face of participant following the procedure previously described (Aguayo-Mendoza et al., 2018; Ketel et al., 2019; van Eck et al., 2019b). Two stickers were placed 5 cm apart on the forehead of participants to calibrate the software with the number of pixels that represented 5 cm. Two additional stickers were placed on the nose and chin. The sticker on the nose was used as reference point and the one on the chin as mobile point. During the recording, participants were asked to not block the stickers with their hands and limit their head movements while

eating the sample. Videos were analysed using Kinovea software (version 0.8.15) a motion analysis software that tracks changes in the spatial position of specific markers in video recordings. The movement of the nose and chin stickers relative to each other was extracted as X-Y coordinates over time. Consumption time per bite (s) defined as average time from putting the sample in the mouth until swallowing was extracted from video recordings and eating rate (g/min) was calculated for each sample. The habitual eating rate *ER* (g/min) of both composite foods (wafer filled with hazelnut chocolate spread; cherry jam on white bread without crust) was calculated for each participant by adding the weights *w* (g) of the composite foods and dividing it by the sum of the consumption times *t* (min) of the composite foods.

Categorization of participants as slow and fast eaters was based on the median split of the habitual eating rate *ER* of the wafer filled with hazelnut chocolate spread and the cherry jam on white bread combination. Contrary to the cherry jam served alone, these foods required mastication as they included a solid food component. It was decided to use the habitual eating rate obtained from consumption of both composite foods (wafer filled with hazelnut chocolate spread; cherry jam on white bread without crust) instead of averaging the eating rate over both composite foods since these composite foods were very different in mechanical and texture properties. The two composite foods used to determine habitual eating speed (wafer filled with hazelnut chocolate spread; cherry jam on white bread without crust) were different from the foods used in the sensory evaluation (strawberry jams differing in sugar content and viscosity served alone and with bread) but belonged to the same product category of sweet spreads.

4.2.4 Dynamic sensory perception

4.2.4.1 Attribute list

Sensory attributes to describe jam and bread were obtained from previous studies (Alves et al., 2008; Kurotobi et al., 2018; Oliver et al., 2018; Panouille et al., 2014; van Eck et al., 2019b) and validated with a pilot test with consumers (n=10 women, age: 21.8 ± 1.3 years) not participating in the main study. A Check-All-That-Apply (CATA) methodology was used. The bread and the control strawberry jam were evaluated individually. Participants of the pilot test were given two lists of attributes, one for each product. The attributes that were

most frequently selected were included in the final attribute list used in the main study (Table 4.2). Eight attributes were selected for the evaluation of jam alone and ten for the jam-bread evaluation. The list of attributes and their definitions are summarized in Table 4.2.

Table 4. 2. List of sensory attributes and definitions used for the TDS evaluation of jam and jam-bread combinations.

Attribute	Definition	Jam	Jam-bread
Sweet	Sweet taste, associated with sugar.	x	x
Sour	Sour taste, associated with sour ingredients such as lemon.	x	x
Strawberry flavor	Strawberry flavor associated with strawberries.	x	x
Fruity flavor	Fruity flavor associated with all fruits except strawberry.	x	x
Bread flavor	Flavor associated with bread and grain flour.		x
Sticky	Feeling of stickiness in the mouth. The jam sticks to the mouth.	x	x
Smooth	Sensation of smoothness, defined as the feeling of smooth jelly in the mouth.	x	x
Melting	The speed of the jam mixing with saliva in the mouth.	x	x
Soft	Sensation of softness described as no or little force required to deform the jam.	x	x
Chewy	Sensation of chewiness described as resistance to chewing/breakdown.		x

4.2.4.2 Temporal Dominance of Sensations (TDS)

Dynamic sensory perception was assessed using Temporal Dominance of Sensations (TDS). Dominance was defined as the most striking sensation, which catches the attention of the assessor at a given moment in time (Pineau et al., 2009). TDS data collection started the moment participants put the sample in their mouth and pressed the start button and ended 90 s thereafter. An attribute was dominant until another attribute was chosen. Attributes could be dominant several times during the evaluation and not all attributes had to be selected as dominant. Participants were instructed to indicate the moment they swallowed the sample by clicking on a 'swallow' button. After swallowing, participants could continue selecting the most dominant attributes. When they did not perceive anything anymore, they were instructed to click on "Do not perceive anything anymore" button and wait until time was up. No chewing protocol was prescribed during the TDS evaluations. Attributes were presented in randomized order across participants but maintained for each panellist between samples.

4.2.4.3 Procedure

Sensory sessions took place at Centrum voor Smaakonderzoek Wageningen (CSO, The Netherlands) and took approximately 30 min. Samples were labelled with random three-digit-codes and served at room temperature. The order of samples was counterbalanced, so

that half of the participants evaluated the strawberry jam without carrier first and the other half started with the strawberry jam-bread combinations. The order of the different formulations, control (C), high pectin (HP) and reduced sugar (LS), was randomized across participants. When served on its own, jam was served on a spoon, while the jam-bread combinations were served on a paper plate. Participants were seated individually with 1.5 m distance from each other. They were instructed to not eat, drink, or brush their teeth two hours prior to the session.

At the beginning of the sensory session a short introduction was given to explain the principle of the TDS methodology. The attribute list was provided, and participants were instructed to familiarize themselves with the attributes before starting the evaluation. Each participant was given a tablet on which the test was performed.

Before the main TDS evaluation, participants performed a practice TDS evaluation with a cracker. In this way, participants familiarized themselves with the TDS methodology and the software used. After the familiarization phase, they continued with the TDS evaluation of the test samples. For each sample, participants were instructed to put the whole sample in their mouth, click on the start button and start selecting the most dominant attribute during consumption. After each TDS evaluation, participants evaluated liking of the sample using a 100 mm VAS anchored with the words 'extremely dislike' to 'extremely like'. Participants were instructed to cleanse their palate with water between samples. Data was acquired using TimeSens software (Version October 2020, ChemoSens, France).

4.2.5 Data Analysis

4.2.5.1 Characterization of oral processing behaviour

To determine whether habitual oral behaviour captured with video recordings differed between slow and fast eaters, mean consumption time and eating rates were calculated and a linear mixed model (LMM) was performed for each parameter individually. Samples (cherry jam, cherry jam with bread, wafer filled with hazelnut chocolate spread and both composite foods), group (slow/fast), gender and the interaction between food and group were considered as fixed effects while participant was treated as random effect. When $p < 0.05$, post- hoc pairwise comparisons with Tukey's correction were used to determine where differences existed between groups.

4.2.5.2 TDS curves

TDS curves were constructed following the procedure described by Pineau et al. (2009). The dominance rate for each attribute at a given moment (every 0.1 s) was determined as the proportion of participants for which the given attribute was selected as dominant sensation. TDS curves were not standardized for time. Chance and significance lines were calculated at $\alpha = 0.05$ and added to the TDS curves as described by Pineau et al. (2009). For a better visualization, smoothing of TDS curves was performed using the smooth function of the TempR package of R (software version 3.1.1).

TDS curves are presented per sample. Pairwise comparison between the dominance rates of the slow and fast eaters was done using a Fisher's exact test (Castura et al., 2016), independent of the significance level of the TDS attribute curves. Highlighted sections in the TDS curves (bold lines) represent periods during which significant differences between slow and fast eaters were observed ($p < 0.05$). The TDS curves of the control jam alone (A-C) and the control jam combined with bread (B-C) are shown in figure 1 whereas the TDS curves of the four other samples (A-HP, B-HP, A-LS, B-LS) are shown in the supplementary materials (4.1 and 4.2).

4.2.5.3 Duration analysis

For each group (slow and fast eaters) a LMM was performed with mean dominance durations for a specific attribute as response. Samples (n=6) were set as fixed factor and participants as random factor. Principal component analysis (PCA) with confidence ellipses of 0.90 were obtained for each product for slow and fast eaters separately to analyse the relationships between sensory attributes within the different formulations and carrier addition. Only attributes that were significantly different ($p < 0.05$) are shown in the PCA.

4.2.5.4 Comparison of eating speed between video recordings and TDS

To determine differences in eating rate across eating speed groups and between video recordings (habitual eating) and TDS evaluations, means of eating rate of cherry jam and cherry jam with bread from the video recordings were compared to the eating rates calculated from the consumption time of strawberry jam (A-C) and strawberry jam with bread (B-C) during the TDS evaluation. A LMM was performed with group (slow/fast), gender, method (video recording/TDS), and their interactions as fixed effects while participant was treated as

random effect. Pairwise comparisons were conducted using Tukey Post-hoc tests when significant differences were present ($p<0.05$).

4.2.5.5 Liking scores

To determine differences in liking between slow and fast eaters, a LMM was performed with liking scores as response. Samples (n=6), gender, group (slow/fast) and the interaction between samples and group were set as fixed factors and participants as random factor. When $p <0.05$, post- hoc pairwise comparisons with Tukey's correction were used to determine where differences existed between groups.

4.3. Results and Discussion

4.3.1 Characterization of participants according to their habitual eating speed of composite foods

The median split that categorized participants as slow or fast eaters was based on their habitual eating rate of two composite foods (cherry jam with bread and wafer with hazelnut-chocolate spread (Section 4.2.3). Consumption time (s) and eating rate (g/min) of cherry jam, cherry jam with white bread, wafer with hazelnut chocolate spread and both composite foods combined (used to categorize participants as slow and fast eaters based on median split) are summarized in Table 4.3.

Table 4. 3 Oral processing parameters extracted from video recordings of n=105 participants for cherry jam alone (bite size 6.0 ± 0.2 g), cherry jam-bread combination (bite size 8.2 ± 0.7 g), wafer filled with hazelnut-chocolate spread (bite size 7.6 ± 0.5 g), both composite foods (bite size 15.79 g) and Tukey's Student t-tests for pairwise comparisons of the interaction effect sample*group (slow/fast eaters) from LMM.

	Slow eaters (n=52)		Fast eaters (n=53)		Consumption time		Eating rate	
Sample	**Consumption time (s)**	**Eating rate (g/min)**	**Consumption time (s)**	**Eating rate (g/min)**	***t ratio***	***p***	***t ratio***	***p***
Cherry jam alone	11±5	42±25	6±3	71±38	-2.90	0.08	8.91	<.001
Cherry jam with bread	22±7	24±6	12±3	45±11	-7.23	<.001	6.45	<.001
Wafer with chocolate spread	31±9	16±4	19±4	25±5	-8.13	<.001	2.91	0.07
Both composite foods (Jam-bread and wafer with chocolate spread)	52±15	19±4	30±4	32±5	-15.50	<.001	4.04	0.001

For consumption time and eating rate, the interaction between samples and group was significant (consumption time $F(3,309)=76.09$, $p <0.001$, eating rate $F(3,309)=8.41$, $p <0.001$). Except for jam alone, consumption time of all samples differed significantly between slow and fast eaters ($p <0.05$).

In general, eating rate differed significantly ($p <0.05$) between slow and fast eaters except for wafer filled with hazelnut chocolate spread. Fast eaters habitually consumed jam alone, jam with bread, wafer filled with hazelnut-chocolate spread and both composite foods at higher eating rates (69, 87, 56 and 68% higher ER, respectively) than slow eaters. This demonstrates that the slow and fast eaters differed considerably in their habitual eating speed of these foods.

As expected, cherry jam alone was consumed at highest eating rate by the slow and fast eaters compared to the composite foods (cherry jam with bread; wafer with hazelnut chocolate spread) which contained a solid carrier ($p <0.05$). Cherry jam with bread was consumed at significantly higher eating rate than the wafer with hazelnut-chocolate spread ($p <0.05$) by the slow and fast eaters.

It is well known that food texture and degree of lubrication affect eating rate (Bolhuis et al., 2014; Forde et al., 2013; Lasschuijt et al., 2017; McCrickerd et al., 2017; Zijlstra et al., 2009). Foods that require more chewing and lubrication take more time to be orally processed and are therefore consumed with lower eating rates. In our study, it may be that due to its lower moisture content, the wafer might have required an increased mastication time. Overall, our findings are in agreement with previous studies which demonstrated that toppings assisted saliva in bolus formation of different solid foods (bread, crackers, pasta, milk gels), leading to shorter oral processing times (Gonzalez-Estanol et al., 2022b; van Eck et al., 2019b).

4.3.2 Impact of habitual eating speed on dynamic sensory perception

Figure 4.1 displays the TDS curves for the control strawberry jam alone (A-C) and control strawberry jam-bread combination (B-C) for slow and fast eaters. The vertical black line represents the average swallowing moment of the samples during the TDS evaluations for each group. It should be noted that the TDS curves were not time standardized. Dynamic

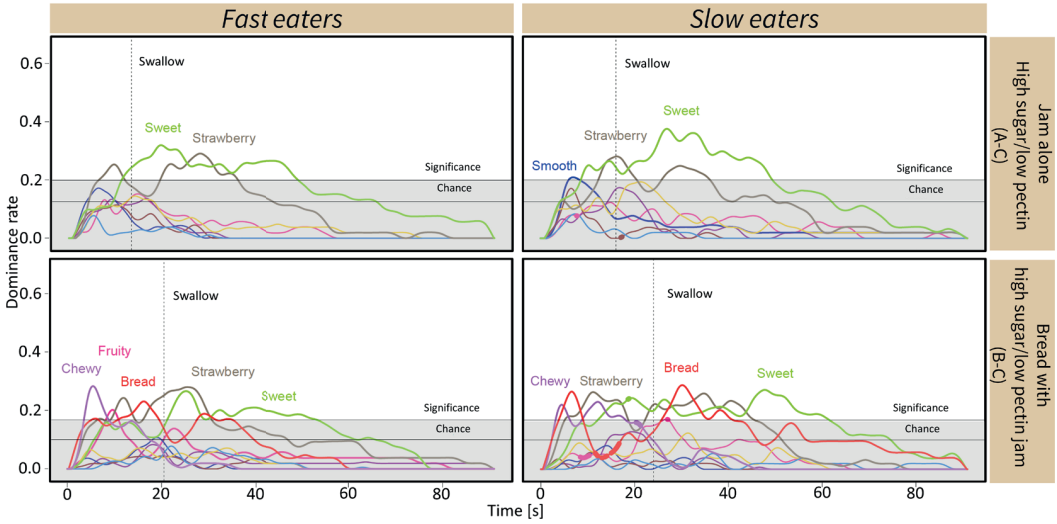

Figure 4. 1 TDS curves for fast (n=53) and slow (n=52) eaters for the strawberry jam served alone (A-C) and jam-bread combination (B-C). Periods of significant differences ($p < 0.05$) of dominance rates between slow and fast eaters are indicated by highlighted thick sections. The vertical dotted line represents the average swallowing moment.

sensory perception of the control strawberry jam without carrier (A-C) was characterized by prolonged dominance of strawberry and sweetness sensations with only minor significant differences for short periods of time between slow and fast eaters. Sweetness was the dominant sensation after swallowing for the fast eaters, while it was the dominant sensation before and after swallowing for the slow eaters. Similarly, strawberry flavour was the dominant sensation before and after swallowing for fast eaters while it was only the dominant sensation around the swallowing moment for slow eaters. Similar to the control jam alone (A-C), the TDS curves for the reduced sugar (A-LS) and high viscosity (A-HP) jams alone showed only minor differences in dominant sensations for short periods of time between slow and fast eaters (Supplementary Material 4.1). Fast eaters perceived texture sensations such as soft and sticky as dominant sensation at the beginning of mastication for the reduced sugar (A-LS) and high viscosity (A-HP) jams, respectively. Slow eaters perceived sour as the dominant sensation for a short period of time after swallowing for the reduced sugar (A-LS) and high viscosity (A-HP) jams. For both groups, strawberry and sweetness were the dominant sensations for the rest of the evaluation time. It is hypothesized that differences in habitual eating speed between slow and fast eaters lead to differences in dynamic sensory perception. Overall, it was observed only minor differences, so differences that were

significant but only for short time periods, typically for less than 4 s, in dynamic sensory perception of jams and jam-bread combinations between habitual slow and fast eaters.

This is in general agreement with Aguayo-Mendoza et al. (2020) who showed that dynamic texture perception of sausages was similar for consumers differing in age, gender and ethnicity at the beginning and end of mastication and only minor differences in dynamic texture perception between consumer groups were observed during the middle stages of mastication with low dominance rates (Aguayo-Mendoza et al., 2020). It was speculated that variations in habitual consumption time might cause considerable differences in bolus properties but do not necessarily lead to considerable difference in dynamic sensory perception. It is important to note that sugar reduction and addition of pectin remained within realistic product reformulation boundaries and were small. This could partly explain why the degree of reformulation in our study was not sufficient to cause larger differences in perception of dominant sensations between products. The main objective of the study was to investigate the effect of habitual eating speed (slow vs fast eaters) on dynamic sensory perception of composite foods rather than to quantify the impact of product formulation on dynamic sensory perception.

As expected, the dynamic sensory perception of the jam was strongly affected by the addition of bread (B-C) for slow and fast eaters. In the beginning of mastication, perception was dominated by sensations related to the carrier (chewy and bread flavour). Slow eaters perceived these sensations as dominant for slightly longer periods than fast eaters. At later stages of mastication, sensations related to the jams (strawberry and sweetness) were dominant. Overall, similar results were obtained for the low sugar and high viscosity jams combined with bread (B-LS and B-HP) for slow and fast eaters (Supplementary Material 4.2). In general, our findings regarding the temporal sequence of dominant sensations of composite foods are in agreement with previous studies where different solid carriers (bread, wafer, carrots) were combined with various toppings (chocolate hazelnut spread, mayonnaise). At the beginning of consumption, sensations related to the flavour and texture of the carriers were dominant, whereas sensations related to the toppings were dominant at later stages of consumption (Gonzalez-Estanol et al., 2022a; van Eck et al., 2019b).

To determine differences in eating rate across eating speed groups and between video recordings and TDS evaluations, means of eating rate of cherry jam alone and cherry jam

with bread from the video recordings were compared to the eating rates of strawberry jam (A-C) and strawberry jam with bread (B-C) from the TDS evaluation (Table 4.4). For both sets of products, the interaction between group (slow/fast) and method (video recording/TDS) was significant (jam with bread $F(3,309)=8.41$, $p < 0.001$, jam alone $F(1,101)= 11.78$, $p < 0.001$). During the video recordings, habitual eating rate of cherry jam and cherry jam with bread were significantly different between slow and fast eaters ($p < 0.05$). During the TDS evaluation, slow and fast eaters increased their consumption time which implies that their eating rate decreased when performing an analytical sensory evaluation compared to their habitual eating rate. This is in line with previous studies which demonstrated that when participants focus on an analytical sensory task which they presumably want to perform well, they extend their consumption time (Devezeaux de Lavergne et al., 2015, 2016). Consequently, differences in eating rate of jams alone and jam-bread combinations between slow and fast eaters were reduced from 68 and 86% during habitual mastication to 37 and 20% during TDS evaluation, respectively (Table 4.4). Thus, the differences in eating rate of jams alone between slow and fast eaters were no longer significant ($p > 0.05$).

Table 4. 4. Summary of eating rate (mean ± SD) obtained from video recordings (habitual consumption) and from TDS evaluations for jams served alone and jam-bread combinations. Superscript letters indicate significant differences ($p < 0.05$) between means within each sample obtained from Tukey's Student t-tests for pairwise comparisons of the interaction effect group (slow/fast) * method (Video recording/TDS) from LMM.

		Eating rate (g/min) Video recording	Eating rate (g/min) TDS
Jam	**Slow**	42+25 ^{B}	26+10 ^{C}
	Fast	71+38 ^{A}	33+14 ^{BC}
Jam-bread	**Slow**	24+6 ^{bc}	22+7 ^{c}
	Fast	45+11 ^{a}	27+8 ^{b}

Upper cases: comparison between jam samples
Lower cases: comparison between jam-bread samples

Moreover, there were significant differences in eating rate of jam alone and jam with bread between video recordings and TDS evaluation for fast eaters ($p < 0.05$). On the other hand, only the eating rate of the jam alone was significantly different between video recordings and TDS evaluation for the slow eaters ($p < 0.05$), which suggests that fast eaters adapted their habitual eating speed more during the sensory evaluation than slow eaters. These changes in eating rate differences between habitual slow and fast eaters may explain the limited differences in dynamic sensory perception observed between these groups.

Liking of all jams and composite foods did not differ significantly between slow and fast eaters ($F(5,505.68)= 0.75$, $p =0.6$) (Table 4.5). The absence of differences in liking between slow and fast eaters is consistent with the absence of large differences in dynamic sensory perception between the two groups.

Table 4. 5 Liking scores (mean ± SD) of all samples for slow and fast eaters and Tukey's Student t-tests for pairwise comparisons of the interaction effect samples (A-C, A-LS, A-HP, B-C, B-LS, B-HP) * group (slow/fast) from LMM.

Sample	Slow eaters (n=52)	Fast eaters (n=53)	*t ratio*	*p*
A-C	6.0 ± 2.0	6.2 ± 2.2	0.84	0.99
A-LS	6.0 ± 2.1	6.2 ± 2.1	0.47	1
A-HP	5.7 ± 2.2	6.1± 2.1	0.92	0.99
B-C	6.9 ± 1.8	5.6 ± 2.1	0.09	1
B-LS	6.0 ± 1.7	5.8 ± 1.9	-0.57	1
B-HP	5.8 ± 1.7	5.8 ± 1.9	-0.33	1

4.3.3 Duration analysis

Principal Component Analysis (PCA) of the mean dominance durations for slow and fast eaters are shown in Figure 4.2 to summarize perceptual differences between samples. The correlation circles (Figure 4.2A and 4.2C) visualize the mean dominance durations of the sensory attributes that were significantly different ($p < 0.05$) between samples for the fast and slow eaters, respectively. The individual factor maps (Figure 4.2B and 4.2D) show the representation of all samples with confidence ellipses of 0.90. For both groups, control jam (A-C) was perceived similar to the low sugar jam (A-LS) as indicated by the overlapping confidence ellipses, while the high viscosity (A-HP) jam (depicted in pink) was perceived significant differently from A-C and A-LS as indicated by non-overlapping confidence ellipses. However, this discrimination between jams was influenced in different ways by the addition of bread across groups. Fast eaters' ability to discriminate between jams differing in formulation decreased, as the confidence ellipses of B-C, B-LS and B-HP overlap (Figure 4.2B). This is in line with previous studies where perceptual differences between spreads or toppings disappeared upon addition of carriers since sensitivity to detect sensory differences between them may decrease (Cherdchu and Chambers IV, 2014; Gonzalez-Estanol et al., 2022a; Nguyen and Wismer, 2020; van Eck et al., 2021). In contrast, slow eaters were able to better discriminate between the different jam formulations when the jams were combined with breads (Figure 4.2D). Slow eaters selected on average one attribute more as dominant

sensation than fast eaters (slow eaters: 8.0 ± 3.2, fast eaters: 7.1 ± 4.4), suggesting that habitual slow eaters described the dynamic texture perception of the samples in more detail.

While previous studies with specific chewing protocols demonstrated that eating speed had a strong effect on bolus properties, flavour release, and consequently on texture and flavour perception (Luckett and Seo, 2017; Tarrega et al., 2008), our study shows that large differences in habitual eating speed have limited effect on dynamic sensory perception, which is in line with the previous studies that categorized consumers according to their natural consumption time (Aguayo-Mendoza et al., 2020; Devezeaux de Lavergne et al., 2015). Even though habitual slow eaters showed a better discrimination ability than habitual fast eaters, it seems that eating speed explains inter-individual variability in dynamic sensory perception of foods only to a limited extent.

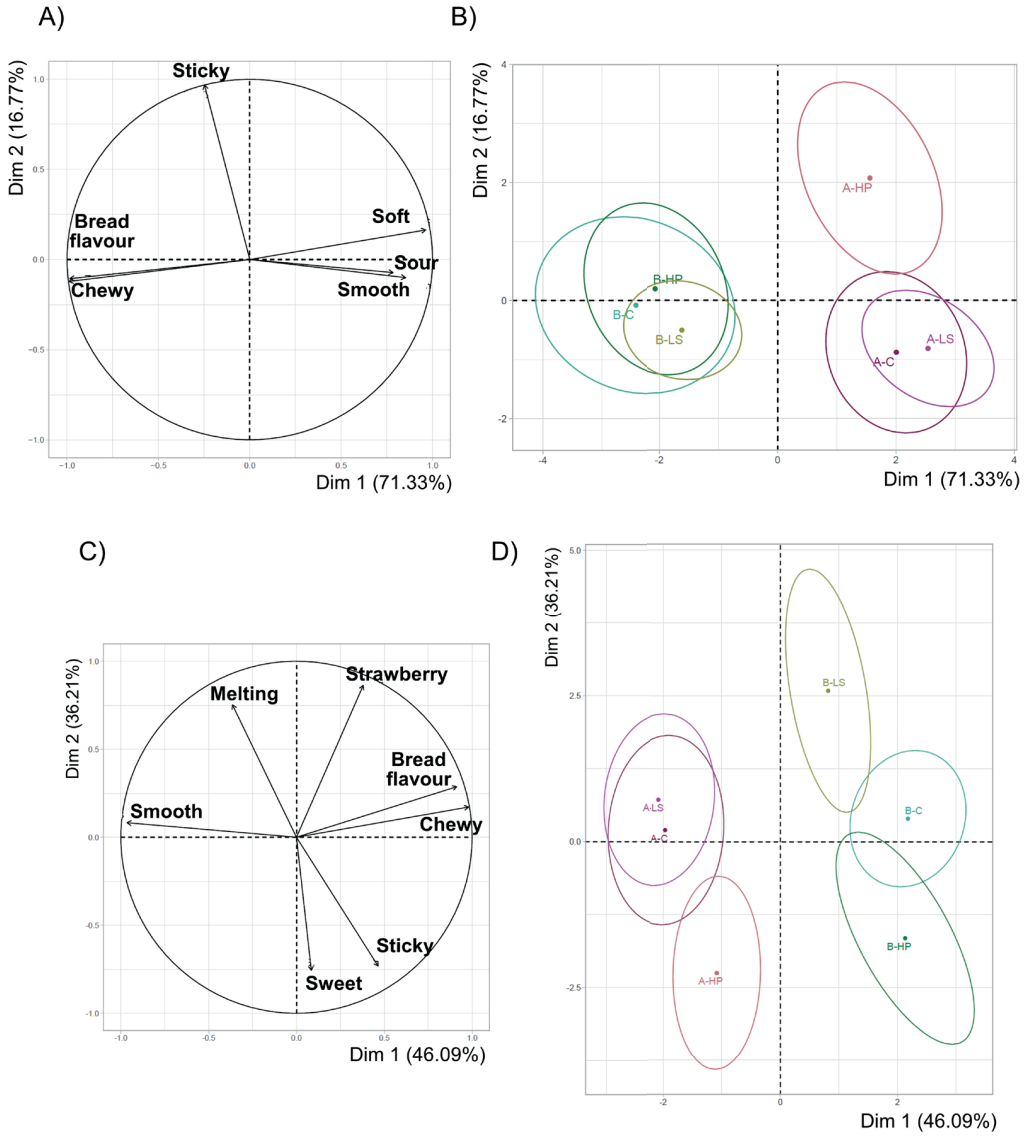

Figure 4. 2 Principal Component Analysis (PCA) of the durations of dominant sensations from the TDS data with confidence ellipses of 0.90. Biplot showing dimensions 1 and 2 represents the sensory profiles of the jams and jam-bread combinations (A-C, A-LS, A-HP, B-C, B-LS, B-HP) for fast eaters (A, B) and slow eaters (C, D). Only the attributes that were significantly different ($p < 0.05$) are shown.

As mentioned in section 4.2.3, a median split was applied to categorize participants (n=105) into slow and fast eaters. It is worth noting that a quartile split of participants was performed, too. The lowest and highest quartile consisted of participants with lowest eating rate (n=26,

15.89±3.06 g/min) and highest eating rate (n=26, 35.91±3.31g/min), respectively. When TDS curves were compared for the various foods between the slow and fast eaters obtained by quartile split (data not shown), the results and conclusions were very similar to those obtained from the median split. The number of observations for the median split was n=53 per group compared to n=26 per group for the quartile split. Thus, it was decided to use the median split classification for the study.

4.4. Conclusions

Previous studies demonstrated that eating speed impacts bolus properties and consequently texture and flavour perception. Investigating the relationships between eating speed and sensory perception of foods further may assist in better understanding inter-individual differences in sensory perception. Without any prescribed eating speed protocol and based on the habitual consumption time of participants, it was demonstrated that dynamic sensory perception of jams and jam-bread combinations showed only minor differences (significant difference for very short periods of time) in dominant sensations between habitual slow and fast eaters. Slow eaters discriminated better between different formulations of jams, regardless of whether the jam was presented alone or in combination with bread, than fast eaters. It is concluded that differences in habitual eating speed of consumers have limited effect on dynamic sensory perception of composite foods.

CHAPTER

5

INFLUENCE OF CHEWING RATE AND FOOD COMPOSITION ON AROMA RELEASE AND PERCEPTION OF COMPOSITE FOODS

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In preparation

Abstract

The release of volatile organic compounds (VOCs) from the food matrix into the oral and nasal cavity during consumption has proven to be critical for aroma perception. The aim of this study was to investigate the effect of chewing rate, food structure and composition on aroma release and perception of composite foods. Three strawberry jams differing in sugar content and viscosity were spiked with citral 0.4% (w/w) and limonene 0.4% (w/w). They were evaluated alone and in combination with two carriers (bread, sponge cake). In vivo release of citral and limonene were characterised using Proton-Transfer-Reaction-Time-of-Flight-Mass-Spectrometry (PTR-ToF-MS) and simultaneously the corresponding aroma perception (citrus aroma intensity) was determined using Time-Intensity (TI) (n=8, triplicate). Standardised fast and slow chewing protocols (chewing at a chewing rate of 1.33 chews/s (fast) or 0.66 chews/s (slow) for 25 s) were applied. Carriers' addition to jams increased aroma release while perceived citrus aroma intensity decreased regardless of the chewing rate. A faster chewing rate led to higher aroma release compared to a slower chewing rate for composite foods (carriers with jams) without impacting dynamic perception of citrus aroma intensity. This suggests that the aroma release increase, probably caused by an increase in structural breakdown (total surface area of bolus fragments) of the composite food during mastication, was too limited to cause considerable changes in aroma perception. Jams sugar content had negligible effects on both aroma release of citral and limonene and citrus aroma perception of the composite foods. Increasing the jams viscosity decreased the citrus aroma intensity significantly. The simultaneous coupling of PTR-ToF-MS with TI profiling allowed to reveal the presence of cross-modal interactions between food texture and aroma perception in complex, composite food matrices and to quantify the impact of mastication behaviour on in vivo aroma release and perception. This study confirms that (a) carrier addition (bread, sponge cake) to strawberry jams strongly influenced both aroma release and perception and (b) chewing rate impacted aroma release without impacting aroma perception of composite foods.

Keywords : Oral processing behaviour, TI, PTR-MS composite foods, chewing rate

5.1 Introduction

The *in vivo* release of volatile organic compounds (VOCs) from the food matrix into the oral and nasal cavity during consumption is critical for aroma perception. The combined actions of mastication and salivation lead to the breakdown of foods and formation of safe-to-swallow bolus (Feron & Salles, 2018; Salles et al., 2011). During oral processing of many semi-solid and solid foods, the number of bolus fragments increases and their size decreases, leading to an increase of total surface area of food bolus particles, which facilitates the release of taste and aroma compounds from the food matrix enhancing taste and aroma perception (Braud & Boucher, 2020; Mosca et al., 2015).

Several studies explored the impact of oral processing behaviours on sensory perception (de Lavergne et al., 2015; Doyennette et al., 2019; Gonzalez-Estanol et al., 2023) and the effect of mastication on *in vivo* aroma release separately. Tarrega et al. (2008) monitored aroma release and chewing activity during consumption of cheeses. The number of chews, chewing work and chewing strength were positively correlated with the maximum concentration of released aroma compounds and time to reach maximum concentration was correlated with chewing time (Tarrega et al., 2008). Feron et al. (2014) demonstrated that aroma release from various cheeses was mostly explained by masticatory behaviour whatever the cheese and the aroma, with a specific influence of mean chewing amplitude on aroma release after swallowing (Feron et al., 2014). Repoux et al. (2012) showed that an increase in firmness of processed cheeses induced an increase in chewing duration leading to an increased *in vivo* aroma release (Repoux et al., 2012) . More recently, Okawa et al. (2021) showed positive correlations between the maximum aroma concentration released into the nasal cavity and the number of chewing strokes and salivary flow rate during mastication of gummy jellies (Okawa et al., 2021). How et al. (2021), demonstrated that aroma release was dependent on the particle breakdown pathways, showing that a bolus with smaller pieces led to a higher aroma release from white cooked rice (How et al., 2021). Lastly, it was also shown that coffee sip volumes caused different release of some aroma compounds under mouth simulated conditions (Genovese et al., 2014). These studies consistently demonstrated that variability in oral processing behaviours affects aroma release, but the impact of oral processing behaviours on aroma perception was not quantified, so it is not evident from these studies how differences in aroma release caused by differences in oral processing behaviours lead to

differences in aroma perception. Luckett et al. (2016 and 2017) showed that the number of chews and chewing rate modulate the temporal dynamics of flavour perception of potato chips (Luckett et al., 2016; Luckett & Seo, 2017). In another study, the rate of flavour release in the mouth from traditional dry-cured pork was evaluated and showed that the interaction between food and saliva causes significant changes in sensory perception. (Tian et al., 2023). Doyennette et al. (2019) showed that 'chewers' consumed ice creams with a shorter consumption time and perceived aromas earlier and longer compared to 'melters', who consumed ice creams slower (Doyennette et al., 2019).

While these studies determined the impact of oral processing behaviours on either aroma release or aroma perception, none of them determined the influence of oral processing behaviours on aroma release and perception simultaneously. Only few studies have applied such approach. Deleris *et al.* (2011) demonstrated that chewing or letting gelatine melt in the mouth had different temporality of aroma release and perception (Déléris et al., 2011). Leclercq (2012) investigated the benefits of imposing a strict chewing and swallowing pattern on aroma release and perception of flavoured gelled candies. The results highlighted the effect of inter-individual variability and the presence of taste and texture cross-modal interaction with aroma perception (Leclercq & Blancher, 2012). Recently, it was shown that chewing rate directly impact aroma release and perception in grilled eel (Huang et al., 2022).

Although these studies demonstrated that variability in oral processing behaviours impacts aroma release or aroma perception of foods, they do not necessarily represent foods that consist of multiple food components differing considerably in composition, structure, and sensory properties and may limit the relevance and applicative outreach of the sensory and aroma release profiles obtained. Most consumed foods consist of different components that are consumed together, 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).

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 *in vivo* aroma release and perception of composite foods (Mesurolle et al., 2013; van Eck et al., 2021). However, these studies did not assess or control for the effects of oral processing behaviour on *in vivo* aroma release and perception.

A better understanding is needed of how variability in chewing rate impacts the release and perception of aroma compounds during consumption of composite foods. Thus, the aims of this study were to investigate the effects of (i) chewing rate (fast vs slow), and (ii) carrier addition (bread, sponge cake) on *in vivo* aroma release and perception of strawberry jams varying in composition using nose space analysis via Proton-Transfer-Reaction-Time-of-Flight-Mass-Spectrometry (PTR-ToF-MS) combined with Time-Intensity (TI). It is hypothesised that (i) aroma release and perception is affected by chewing rate as faster chewing rate leads to the breakdown of the food bolus into more and smaller fragments facilitating aroma release from the food matrix into the nasal cavity leading to higher perception of aroma intensity compared to a slower chewing rate, and that (ii) adding solid carriers to strawberry jams leads to an increase of aroma release and decrease of aroma perception.

5.2. Materials and Methods

5.2.1 Samples

Three strawberry jams varying in sugar content and viscosity, namely high sugar/ medium viscosity (HS/MV); high sugar/high viscosity (HS/HV) and low sugar/ low viscosity (LS/LV) strawberry jams, were prepared (Menz & Gasser, Novaledo Italy). Sugar content and rheological properties of the three jams are displayed in Table 5.1. Reformulation of the strawberry jams remained within narrow and realistic product reformulation boundaries to warrant close resemblance to commercially available products. All strawberry jams were spiked with 0.4% (w/w) citral (Sigma-Aldrich, USA) and 0.4% (w/w) limonene (Sigma-Aldrich, USA). Citral and limonene were chosen based on a previous *in vivo* aroma release and perception studies (van Eck et al., 2021). While both compounds are characterised by a citrus aroma, they differ in physicochemical properties (citral: Mw= 152g/mol, logP=2.76; limonene: Mw=136g/mol, logP=4.20). Composite foods were prepared by combining the three strawberry jams with two carriers (bread and sponge cake). They were chosen to

simulate the typical consumption context of strawberry jams and because of their differences in mechanical properties (Table 5.1).

Table 5. 1 Sugar content and rheological characteristics of strawberry jams and mechanical properties of carriers

	Strawberry jams			Carriers	
	High sugar/ medium viscosity (HS/MV)	**Low sugar/ low viscosity (LS/LV)**	**High sugar/ high viscosity (HS/HV)**	**Bread (B)**	**Sponge cake (SC)**
Sugar content (g/100g)	52	39	52		
Brix	60	45	60	-	-
Viscosity at shear rate 1.5s^{-1} (Pa·s)	51.8	18.2	97.5	-	-
Hardness (N)	-	-	-	5095±37	10±1

For the jam-bread combinations, commercially available white bread (Bruschelle mini, Morato, Italy) was used. The bread ingredients are soft wheat flour, olive oil 1.8%, dehydrated soft wheat flour sourdough, salt, yeast, and malt flour. Bread was cut in pieces of 3 x 3 x 1 cm without crust (2.8 ± 0.6 g) and strawberry jam (4.0 ± 0.3 g) was spread on top. For the jam-sponge cake combinations, pre-packed sponge cakes (Soremartec, Alba, Italy) were used. Ingredients of the cake are flour mix, milk proteins, sugar, egg yolk, sunflower oil, brewer's yeast, and salt. Sponge cakes were cut in pieces of 3.5 x 3.0 x 2.5cm without crust (4.1± 0.56 g) and strawberry jam (6.0 ± 0.5 g) was spread on top. The mass ratios bread:jam and sponge cake:jam were determined in a preliminary study (data not shown) in which participants were asked to spread strawberry jams on top of breads or sponge cakes as they normally do. All samples were prepared shortly before serving (< 30 min). A description of sample codes and pictures are provided in Figure 5.1.

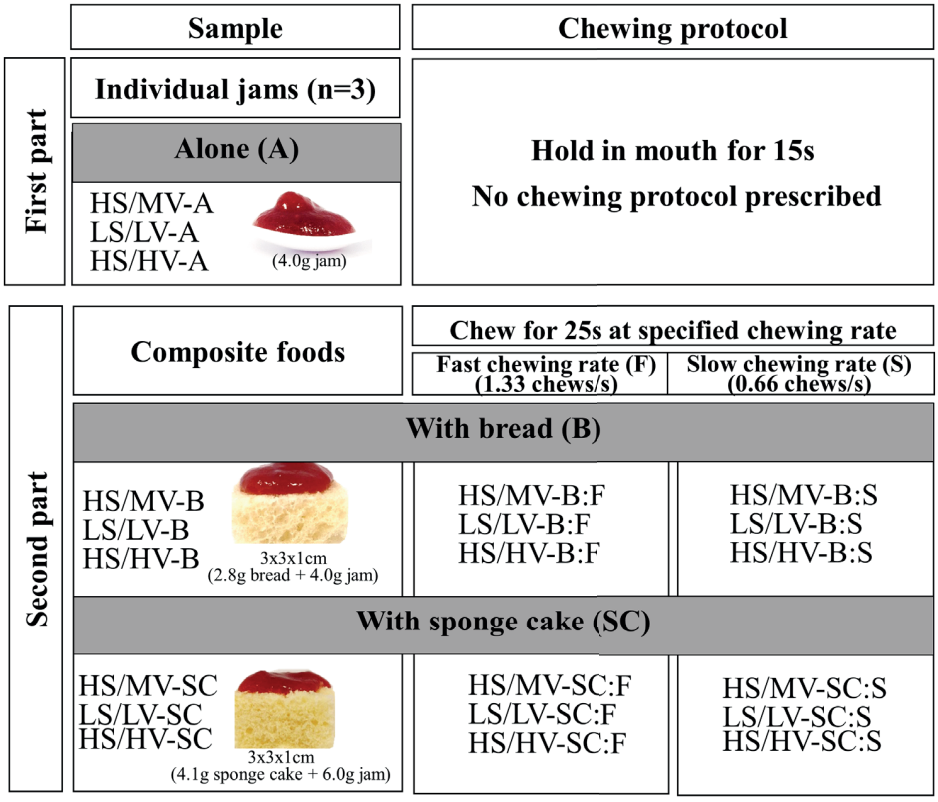

Figure 5. 1 Experimental design outlining the different samples used. In part one, jams varying in sugar and pectin content were evaluated on their own without standardised chewing rate. In part two, composite foods were evaluated, using two different chewing rates. As a within subjects' design, all participants evaluated all samples (n=15, in triplicate).

5.2.2 Participants

Nine participants were recruited for this study from the Edmund Mach Foundation (San Michele all'Adige, Trentino, Italy). All participants were Caucasian women. Other inclusion criteria were no allergies or intolerances to wheat/gluten, dairy, nuts, soybeans, eggs, not pregnant nor lactating, no history of oral perception disorders or olfactory impairments and not being on a calorie restricted diet (self-reported). Participants gave written informed consent prior to the study and received financial compensation for their time in the form of a gift card after completing the study. The study was conducted in agreement with the ethics regulations laid out in the Declaration of Helsinki (2013). Out of the nine recruited participants, n=8 participants (age 27±5 years, all female, BMI 20.9±1.9 kg/m^2) completed the study.

5.2.3 Determination of bite size, consumption time and chewing protocol

Bite size was based on previous studies (Gonzalez-Estanol et al., 2023) and validated by a focus group of n=10 women not participating in the study. This focus group was also used to determine the total consumption time and chewing rate protocols. Individual video recordings were done to characterise oral processing behaviours. For this only fixed bite sizes of jam alone (4.0 g) and jam-bread combinations (6.2 g) were presented to the participants. They were instructed to put the whole sample in their mouth and start chewing as they normally do. Participants were asked to raise their hand when they swallowed. To measure oral processing behaviours, stickers were placed on the face of the participant, following the procedure previously described (Aguayo-Mendoza et al., 2018; Gonzalez-Estanol et al., 2022; Ketel et al., 2019). Consumption time (s), defined as average time from putting the sample in the mouth until swallowing, and number of chews per bite (-), calculated from vertical jaw displacement, were extracted from video recordings and chewing rate (chews/s) was calculated by dividing the number of chews by the consumption time. The average of these parameters was used to fix the chewing protocol for the main study. The total consumption time was set to 15 s for jams consumed alone and 25 s for jam-carrier combinations. Chewing rates of the composite foods were set to 1.33 and 0.66 chews/s corresponding to fast and slow chewing, respectively.

5.2.4 Familiarisation sessions

The panel underwent two training sessions of one hour each. The first session began with the recognition and evaluation of strawberry jams with different citrus aroma intensities. Two references were used for identification of citrus aroma intensity: a strawberry jam without added citrus aroma (not citrus at all) and a strawberry jam spiked with 1.25% w/w of limonene and citral (extremely citrus). Participants were then explained the Time-Intensity (TI) methodology (section 5.2.5) and were served strawberry jam spiked with 0.2% w/w citral and 0.2% w/w limonene to get familiar with the use of the intensity scale and the TI procedure. Lastly, the different chewing protocols were practised on chewing gums, using a metronome to set the chewing pace. During the second session, participants evaluated the samples as if it was the actual experiment (section 5.2.6) to become comfortable with the whole set up.

5.2.5 Sensory evaluation with Time-Intensity (TI)

Dynamic citrus aroma intensity of strawberry jams was measured using the TI methodology. Citrus aroma was defined as "the combination of aromas associated with citrus fruits such as lemon, lime, orange, tangerine and grapefruit." Participants were instructed to put the sample in their mouth, click the 'start' button on a screen placed in front of them, and to immediately commence tracking citrus aroma intensity. Whenever they felt any differences between clicking start and the end of the evaluation time, participants were asked to move a cursor along a 100 mm unstructured horizontal line scale, anchored from not at all to extreme citrus aroma intensity (EyeQuestion software, version 5). Precise instructions on the chewing rate (1.33 or 0.66 chews/s) and the swallowing moment (15 s for jam alone, 25 s for composite foods) were given with a metronome and with a visual prompt that was displayed on a computer screen (section 2.7). TI data was recorded every second and stopped 135 s after participants clicked the 'start' button. Between samples, participants were asked to rinse their mouth with water.

5.2.6 Simultaneous in vivo nose space analysis and dynamic sensory evaluation with TI

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 Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used to measure the in vivo nose space release (NSA) of citral and limonene from strawberry jams. The ionisation conditions to perform the analysis were 628 V drift voltage, 110 °C drift temperature and 2.80 mbar drift pressure which leads to E/N = 130. Data acquisition was set to one spectrum per second with an inlet flow of 500 sccm. The NASE sampling system (Ionicon Analytik GmbH, Innsbruck, Austria) was used to sample the air coming from both nostrils. It was heated to 110 °C and directly connected to the PEEK inlet of the PTR-ToF-MS system at the same temperature. Evaluations took place individually in a laboratory with filtered air. 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, and to start the TI evaluation, with their mouth closed. TI and *in vivo* nose-space data were acquired simultaneously for 135s.

5.2.7 Procedure

TI and *in vivo* PTR-ToF-MS were performed simultaneously during five sessions (60 min per session) with the panel (n=8, in triplicate). The five sessions were divided in two parts **(**Figure 5.1). The first part entailed one session during which participants evaluated the three jams without carries in triplicate. Samples were divided into blocks of three jam formulations (HS/MV-A, LS/LV-A, HS/HV-A) and the order of jam formulations within each block was randomised within participants. Participants evaluated the samples monadically. For the TI evaluation, participants were instructed to keep the jam in the mouth for 15 s before swallowing. No chewing protocol was described for the evaluations of the strawberry jams without carrier foods.

The second part entailed four session (60 min per session) during which participants evaluated the composite foods (jam-bread, jam-sponge cake) following the two chewing protocols (chewing rate of 1.33 or 0.66 chews/s for 25 s). One session was used for each carrier-chewing rate combination (jam-bread/fast, jam-bread/slow, jam-sponge cake/fast, jam-sponge cake/slow). In each session, participants evaluated three samples in triplicate divided into blocks. Each block included each of the three jam formulations (HS/MV-A, LS/LV-A, HS/HV-A). The jam formulations were randomised within each block. The order of these four sessions was counterbalanced within participants. For the TI evaluation, participants were instructed to chew the composite foods for 25 s before swallowing following the different chewing rates with the help of a metronome and the visual prompts.

All samples were served at room temperature (21 ± 1°C) in standardised bite size (Figure 1) and were coded with random three-digit numbers. In between samples, participants took a 3-minutes break and could take a sip of water and eat a cracker to cleanse their palate. For all sessions, participants were asked to refrain from eating, drinking coffee, or use of any persistent flavoured product for at least 1 hour before the start of the session. Between the first and second part of the study, there was a washout period of at least two days.

5.2.8 Data analysis

5.2.8.1 TI data

Means are reported together with standard deviations. The area under the curve (AUC), maximum aroma intensity (I_{max}) and time to reach maximum aroma intensity (T_{max}) were

extracted from the TI profiles of each measurement. To test for the effect of chewing rate on AUC, I_{max} and T_{max}, a linear mixed model (LMM) with the composite foods was performed for each extracted parameter with chewing rate (fast, slow) as fixed effect and participant as a random effect.

To test for the effects of carrier addition and jam formulation on AUC, I_{max} and T_{max} , a second LMM was performed with individual and composite foods. Carrier (bread, sponge cake, non), jam formulation (HS/MV-A, LS/LV-A, HS/HV-A) and their interaction were set as fixed effects and participant as a random effect. None of the interactions within fixed effects were significant, and thus they were removed from the LMM. In case of significant differences, post-hoc tests were performed with Tukey's Honest Significant Difference test (HSD) at 95% confidence level.

Values of citrus aroma intensity were averaged over participants (n=8; triplicate) for every second of the evaluation period to obtain TI curves. This was done for each replicate, sample and chewing rate. Smoothing of the curves was done using the *smoothing.spline* function in the TempR package of R software (version 3.1.1).

Significant differences in TI profiles between two samples were calculated by applying a paired t-test for citrus aroma intensity at each second to evaluate the moments in the evaluation where mean intensities were significantly different at a 5% significance level. Highlighted sections on the averaged TI curves (bold lines) represent periods of time during which significant differences between the compared conditions were observed ($p<0.05$) (Gonzalez-Estanol et al., 2022).

5.2.8.2 *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). Peak identification was performed using an in-house library developed by the authors. Mass peaks corresponding to limonene (m/z 138.13) and citral (m/z 153.13) 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 to 15 s for jams alone; 0 to 25 s

for composite foods) and post-swallowing (16 to 135 s for jams alone, 25 to 135 s for composite foods). AUC, maximum aroma concentration (I_{max}) and time to reach maximum aroma concentration (T_{max}) were calculated for each compound and sample separately.

Similar to the data analysis of the TI data (section 2.8.1), to test for the effect of chewing rate, on AUC, I_{max} and T_{max}, a LMM with the composite foods was performed for AUC, I_{max}, and T_{max} individually. Chewing rate (fast, slow) was set as fixed effect and participant as a random effect. To test for the effects of carrier addition and jam formulation on AUC, I_{max} and T_{max} , a second LMM was performed with individual and composite foods. Carrier (bread, sponge cake, non) and jam formulation (HS/MV-A, LS/LV-A, HS/HV-A) were set as fixed effects and participant as a random effect.

None of the interactions within fixed effects were significant, and thus they were removed from the LMM. When $p<0.05$, Tukey's HSD pairwise comparisons were performed. All analysis was done using R software (R Core Team, 2020).

5.3. Results

5.3.1 Influence of chewing rate on in-vivo aroma release and perception

Table 5.2 displays a summary of all parameters extracted from the aroma release curves (AUC, I_{max}, T_{max}) for limonene and citral, and all parameters extracted from the TI curves describing temporal citrus aroma perception (AUC, I_{max}, T_{max}). Figure 5.2 shows the chewing rate effect on release of limonene (m/z 138.13), citral (m/z 153.13) and the corresponding perception of citrus aroma intensity. The effect of chewing rate (fast, slow), carrier addition (bread, sponge cake) and formula (HS/MV, LS/LV, HS/HV) on the parameters extracted from aroma release and TI curves of the composite foods was determined using a LMMM.

Table 5. 2 Maximum aroma concentration/intensity (Imax) , time to reach maximum aroma concentration/intensity (Tmax), and area under the curve (AUC), (Mean ± SD) describing in vivo nose space release of limonene (m/z 138.134), and citral (m/z 153.128) determined with PTR-ToF-MS and citrus aroma perception from TI curves.

	Maximum aroma intensity (I_{max})			Maximum aroma concentration/intensity (T_{max})			Area under the curve (AUC)		
	Limonene	Citral	Citrus aroma perception	Limonene	Citral	Citrus aroma perception	Limonene	Citral	Citrus aroma perception
HS/MV-A	179±103	71±27	59±29	16±7	19±7	22±7	1620±955	1030±424	2015±1207
LS/LV-A	173±112	65±27	67±27	17±7	20±6	25±12	1666±1086	1135±611	3230±2334
HS/HV-A	163±131	66±43	54±24	18±8	21±7	24±8	1719±1290	1077±631	2104±2050
HS/MV-B:F	206±153	60±41	60±28	12±7	14±8	21±11	2880±1710	1382±725	3339±2760
LS/LV-B:F	179±94	56±26	51±23	8±4	11±6	20±12	2396±1146	1213±474	3039±2341
HS/HV-B:F	160±109	62±45	56±28	10±6	12±6	25±11	2333±1409	1308±766	2349±1347
HS/MV-B:S	154±68	55±19	55±25	16±8	18±9	23±12	2451±817	1336±556	3035±2536
LS/LV-B:S	174±114	55±31	61±25	11±5	15±7	26±14	2593±1452	1326±687	2592±1549
HS/HV-B:S	148±112	52±28	48±27	16±7	19±7	26±12	2343±1316	1308±682	3024±2584
HS/MV-SC:F	131±117	47±25	52±27	9±4	10±5	14±7	1466±986	808±352	2728±2078
LS/LV-SC:F	111±87	46±22	55±24	7±3	9±5	12±7	1337±883	775±378	3655±2862
HS/HV-SC:F	108±126	40±28	47±29	9±6	11±7	16±10	1306±1351	722±511	2363±1397
HS/MV-SC:S	104±64	44±18	51±27	10±4	12.2±5	18±9	1525±612	946±388	2809±2849
LS/LV-SC:S	121±73	47±16	50±28	11±8	13.1±8	20±9	1384±652	820±289	2635±1778
HS/HV-SC:S	100±81	41±20	54.±31	11±7	12.4±7	22±10	1317±845	802±416	2704±2196

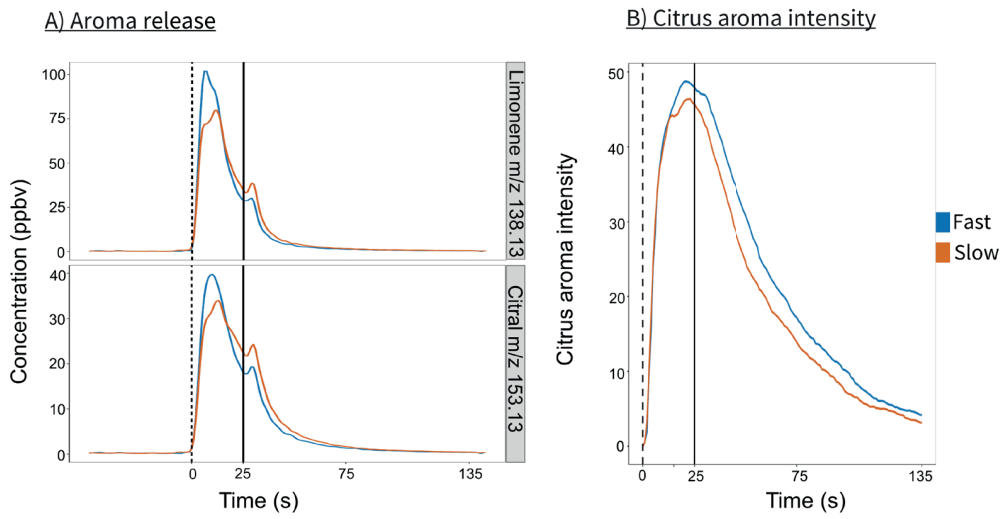

Figure 5. 2 (A) In vivo nose space release of limonene and citral and (B) citrus aroma intensity for fast (blue) and slow (orange) chewing rate (fast: chewing rate of 1.33 chews/s for 25 s; slow: chewing rate of 0.66 chews/s for 25 s) averaged across jam formulations, composite foods (bread with jams and sponge cakes with jams) and participants (n=8, in triplicate) . Black dotted lines represent moment when samples were put in mouth and solid lines indicate the moment of swallowing.

The fast-chewing rate showed a significant reduction in the time to reach maximum intensity (Tmax) by 42% for limonene ($F= 33.54$, $p<0.001$), by 23% for citral ($F= 17.72$, $p<0.001$), and by 14% for citrus aroma intensity ($F=7.92$, $p<0.001$) compared to the slow chewing rate. The fast-chewing rate tended to increase Imax by 27% and AUC by 0.9% for limonene and Imax by 17% and reduced AUC by 5% for citral compared to slow chewing rate, although these differences were not significant ($p>0.05$). This tended aroma release increase did not have a significant influence on perception of citrus aroma intensity since Imax and AUC showed not significant differences. AUC was not significantly affected by chewing rate for limonene, citral and citrus aroma intensity ($p>0.05$).

5.3.2 Influence of carrier addition on in-vivo aroma release and perception

A strong effect of the addition of carrier on aroma release and perception was observed (Figure 5.3). Significant effects of carrier addition on limonene release [I_{max} ($F=22.14$, $p<0.001$), T_{max} ($F=48.71$, $p<0.001$) and AUC ($F=49.51$, $p<0.001$)] and citral release [I_{max} ($F=30.68$, $p<0.001$), T_{max} ($F=51.83$, $p<0.001$) and AUC ($F=44.67$, $p<0.001$)] were observed. Bread addition increased the AUC by 50% for limonene and by 22% for citral. The addition

of sponge cake decreased the AUC by 17% for limonene and by 25% for citral. The addition of bread increased I_{max} by 17% for limonene but decreased I_{max} by 7.0% for citral. The addition of sponge cake decreased I_{max} by 17% for limonene and by 29% for citral (Figure 3A). Lastly, the addition of bread reduced T_{max} by 48% for limonene and by 50% for citral while the sponge cake addition reduced T_{max} by 72% for limonene and by 64% for citral.

Regarding the citrus aroma intensity perception, I_{max} ($F=10.34$, $p<0.001$), and T_{max} ($F=25$, $p<0.001$) were significantly affected by the addition of carriers. Specially, cake addition reduced I_{max} and T_{max} by 23% and by 42%, respectively, compared to bread which its addition reduced I_{max} by 14% but increased T_{max} by 29%. The TI curves (Figure 3B) showed that during the first 10 s of consumption, intensity of citrus aroma increased steeper for jam-carrier combinations than for jams evaluated alone (bold lines), however intensity of jam-carrier combinations significantly decreased around the swallowing moment in the case of bread, and for the rest of the evaluation in the case of sponge cake (bold lines).

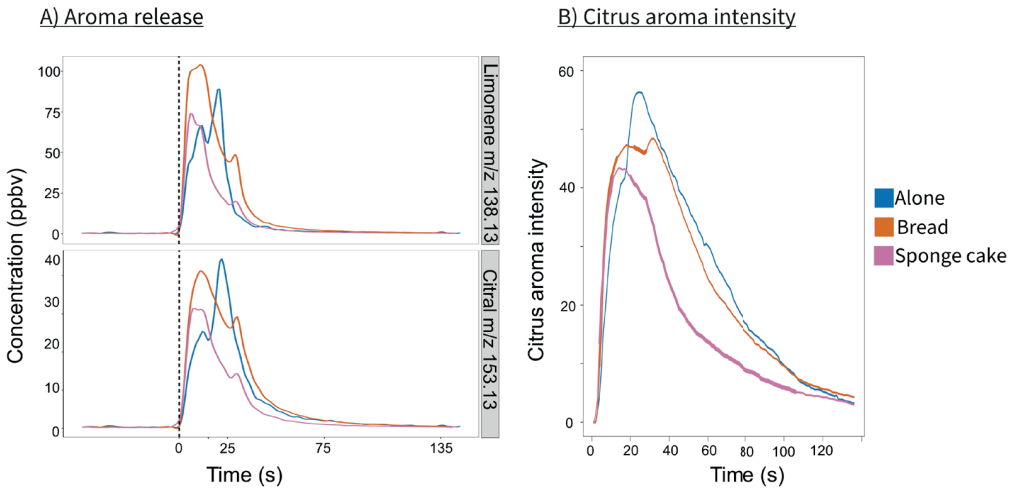

Figure 5. 3 (A) Nose space release of limonene and citral and (B) citrus aroma intensity for jams alone (blue), breads with jams (brown) and sponge cake with jams (magenta) averaged across jam formulations, chewing rates, and participants (n=8, in triplicate). Black dotted lines represent moment when samples were put in mouth. Periods of significant differences ($p<0.05$) in TI curves of jam-bread and jam-sponge cake combinations compared to jams evaluated alone are indicated by highlighted thick sections.

Overall, before swallowing, composite foods show a higher aroma release than jams evaluated alone. It is only that after the swallowing moment (known as swallow breath), that aroma release from the jams alone shows a pronounced increase compared to the one of the composite foods. In contrast, perception of citrus aroma intensity of jam—carrier combinations were significantly lower (highlighted with bold lines) than those of jams alone.

5.3.3 Influence of sugar and viscosity on in-vivo aroma release and perception

Jams reformulation only had a significant effect on T_{max} of limonene ($F=5.65$, $p=0.02$). LS/LV formulation needed less time to reach maximum intensity compared to the HS/MV (Figure 5.4A). In contrast, perception of citrus aroma intensity was significantly reduced (highlighted with bold lines Figure 5.4B) in the jam with higher viscosity (HS/HV), consequently, I_{max} was also significantly decreased ($F=4.083$, $p<0.05$).

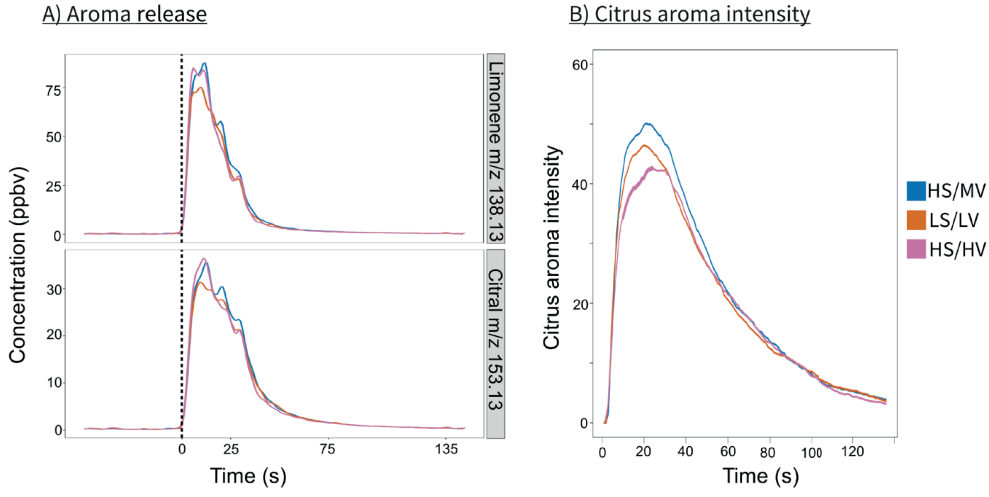

Figure 5. 4 (A) In vivo nose space release of limonene and citral and (B) citrus aroma intensity for high sugar/medium viscosity (HS/MV), low sugar/low viscosity (LS/LV) and high sugar/high viscosity (HS/HV) jams averaged across carriers, chewing rates and participants (n=8, in triplicate). Black dotted lines represent moment when samples were put in. Periods of significant differences ($p<0.05$) in TI curves of low sugar/low viscosity (LS/LV) and high sugar/high viscosity (HS/HV) jams compared to the high sugar/medium viscosity (HS/MV) jam are indicated by highlighted thick sections.

5.4. Discussion

It was first hypothesized that aroma release and perception is affected by chewing rate. While previous studies demonstrated that chewing speed had a strong effect on aroma release and bolus properties, and thus on sensory perception (Luckett & Seo, 2017; Tarrega et al., 2008), this study showed that a faster chewing rate influenced Tmax and Imax of limonene and citral. This is probably due to the breakdown of the food bolus into more and smaller fragments facilitating aroma release from the food matrix into the nasal cavity. However, the increase in aroma release did not lead to a higher perception of citrus aroma intensity compared to a slower chewing rate. It could be that these changes in aroma release were too small to cause perceivable changes in aroma perception. These results are in line with previous studies where differences in eating speed between consumers led to small differences in dynamic sensory perception (Aguayo-Mendoza et al., 2020; de Lavergne et al., 2015; Gonzalez-Estanol et al., 2023).

Secondly, it was hypothesized that adding solid carriers to strawberry jams leads to an increase of aroma release and decrease of aroma perception. When the jams were combined with bread, aroma release was enhanced during mastication. This may be partly attributed to the difference of oral processing time between jams alone (15 s) and composite foods (25 s). In addition, jams alone do not require chewing and they were just swirled around the mouth not following a prescribed mastication protocol, while jam-carrier combinations required chewing to breakdown the food, thus, inducing more aroma release. Besides, the oral processing increased the surface area of spread-carrier combinations allowing a higher transfer of aroma compounds from the jam into to the vapor phase when compared to jams consumed alone(van Eck et al., 2021). Instinctively, it would be expected that this increase in *in vivo* aroma release increases aroma perception. However, addition of a carrier, led to a decrease in citrus aroma intensity. This decrease is in line with previous studies where aroma intensity of a sauce or topping decreased with the addition of other solid food components. For example, Meinert et al. (2011) showed that the addition of gravy to vegetables (broccoli, cauliflower and potato) reduced flavour intensity of the vegetables (Meinert et al., 2011). Similarly, Paulsen et al. (2012) showed that addition of sauces to salmon reduced salmon flavour intensity (Paulsen et al., 2012). Van Eck et al. (2019b) showed that toppings (cheese, cream cheese and mayonnaise) affected the sensory perception of carriers (bread, cracker)

(van Eck 2019b). This reduction in aroma perception is not due to a lower release of aromatic compounds into the nose space, so other factors may play a role. It could be 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. In a similar way, as a direct consequence viscosity, aroma perception was decreased without affecting aroma release. This suggests a perceptual cross-modal effect of viscosity on aroma perception of the jams. This is in line with a previous study reporting that increased gel rigidity resulted in decreased perception of odour, strawberry flavour and sweetness; in strawberry flavour gels (Boland et al., 2006). Thus, the second hypothesis is confirmed.

Imax and the temporal release pattern were both affected by carriers addition. In contrast to the composite foods, strawberry jams assessed alone displayed the maximum release after the swallowing moment. This confirms other observations from Ruijschop *et al.* (2009) who reported that solid foods had an immediate and prolonged retro-nasal aroma release in contrast to (semi)liquid foods which displayed a spiked aroma release pattern (Ruijschop et al., 2009).

Two different types of tongue-velum behaviours that transfer VOCs into the olfactory receptors during the mastication and deglutition processes have been proposed. The first mechanism displays a profile with relatively continuous aroma transfer. During mastication, respiration is maintained at an altered rate which causes the intermittent elevation of the velum, particularly during expiration (Hodgson et al., 2003; Matsuo et al., 2010). This allows air transfer between the oral and nasal cavities and is associated with the chewing movements during consumption of solid foods. As a result, pulses of air with VOCs are continuously 'pumped' into the nose (Hodgson et al., 2003), which could explain the high aroma release during mastication. The second mechanism relates to a single-phase aroma transfer profile. The VOCs are blocked by the closing of the fauces during consumption and reach the olfactory receptor only after swallowing. At this time, the reopening of the sphincters is followed by a short pulse of respiration: the ''swallow breath'' (Buettner et al., 2001; Hodgson et al., 2003; Land, 1996) which explains the steep release of the jams alone which do not require chewing.

5.5. Conclusions

This study demonstrated that different chewing rates influenced aroma release of composite foods without impacting aroma intensity perception. Carrier addition (bread, sponge cake) to strawberry jams influenced aroma release and perception in different ways. While both carriers decreased aroma perception, bread increased the duration and intensity of aroma release more than sponge cake during the mastication period. This strong effect of carrier addition highlights the importance of investigating toppings/jams accompanied with carriers rather than in isolation as it could give an inaccurate sensory profile and mislead product development.

Furthermore, having a prescribed chewing protocol and a specific swallowing time allowed for a direct comparison of aroma release during consumption of a solid and of a semi-solid food, highlighting the effect of physiological parameters on aroma release during consumption.

Finally, this work stressed the fact that aroma release and sensory perception of complex food matrices during consumption are multidimensional phenomena, and as such, investigating aroma release and perception simultaneously will provide better understanding of the mechanisms governing aroma release and sensory perception during food consumption.

CHAPTER

6

ORAL PROCESSING BEHAVIOURS OF LIQUID, SOLID AND COMPOSITE FOODS ARE PRIMARILY DRIVEN BY TEXTURE, MECHANICAL AND LUBRICATION PROPERTIES RATHER THAN TASTE INTENSITY

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Abstract

The aim of this study was to understand the influence of saltiness and sweetness intensity on oral processing behaviours of liquid, solid and composite foods. As salty foods, tomato sauce (liquid), penne pasta (solid) and their combination (composite food) were used at two levels of saltiness intensity (low/high). As sweet foods, strawberry sauce (liquid), milk gels (solid) and their combination (composite foods) were used at two levels of sweetness intensity (low/high). Saltiness, sweetness, hardness, chewiness, and liking were quantified using Generalized Labelled Magnitude Scales (gLMS). Oral processing behaviours were determined using video recordings (n=39, mean age 25 ± 3 yrs) in a Home-Use-Test (HUT) providing fixed bite sizes for all foods. As expected, taste intensity differed significantly between samples within the same food category. No significant effects of taste intensity on oral processing behaviours were found for sweet and salty foods. As expected, consistency strongly affected consumption time per bite, number of chews per bite, number of chews per gram and eating rate. Solid foods were masticated for the longest time with the highest number of chews per bite, followed by composite foods as the liquid added to the solid foods enhanced lubrication. Liquid foods were masticated for the shortest time. It is concluded that large differences in saltiness and sweetness intensity of liquid, solid and composite foods cause no differences in oral processing behaviours. It is suggested that oral processing behaviours are primarily driven by texture, mechanical and lubrication properties of foods rather than their taste intensity.

Keywords: Oral processing behaviour, texture, taste intensity, composite foods

6.1. Introduction

Food oral processing behaviour plays an important role in taste and texture perception of foods (Chen, 2009; de Lavergne et al., 2015). The combined action of mastication and salivation leads to breakdown of foods and bolus formation allowing a safe swallow (Feron and Salles, 2018; Salles et al., 2011). During oral processing of many solid foods, the total surface area of food particles increases, which facilitates the release of taste compounds from the food matrix into saliva, enhancing taste perception (Braud and Boucher, 2020; Liu et al., 2017; Mosca et al., 2015). Differences in oral processing behaviours can cause changes in sensory perception of foods. Doyennette et al. (2019) showed that dynamic texture and flavour perception of ice creams depends on consumption style (Doyennette et al., 2019). Similarly, Devezeaux de Lavergne et al. showed that dynamic sensory perception of sausages differs between consumers varying in eating speed (de Lavergne et al., 2015).

Oral processing behaviours not only depend on anatomical and physiological consumer characteristics (de Wijk et al., 2012; Ketel et al., 2019) but also depend on physicochemical, rheological, mechanical and tribological properties of foods (Chen and Engelen, 2012; Muñoz-González et al., 2021). Wee et al. (2018) determined instrumental texture properties and oral processing behaviours of a wide range of solid foods (n=59). They reported that less adhesive, more springy, chewy and resilient solid foods require a greater number of chews. Aguayo-Mendoza et al. (2018) showed that for liquid and semi-solid foods, oral behaviours were driven by rheological food properties. Consumption time of liquid and semi-solid foods correlated positively with consistency (viscosity at shear rate of $1s^{-1}$), while for solid foods, positive correlations between oral behaviours such as number of chews and eating rate

and Young's modulus were found (Aguayo-Mendoza et al., 2018). Other authors found positive relationships between hardness of solid foods and oral behavioural parameters such as masticatory force, jaw muscle activity, movement of lower jaw and chewing cycle duration (Chen and Engelen, 2012; Horio and Kawamura, 1989; Plesh et al., 1986). It was also shown that dry foods require more chewing cycles compared to moist foods, since incorporation of saliva into the bolus is needed to prepare a food bolus that is sufficiently lubricated to be swallowed safely (Anderson et al., 1985; Chen and Engelen, 2012). Mechanical properties of foods do not only impact oral behaviours, but also sensory properties. For a variety of foods, increasing food hardness resulted in decreased flavour intensity (Baines and Morris,

1987; Christensen, 1980; Mälkki et al., 1993; Moskowitz and Arabie, 1970; Pangborn et al., 1973; Vaisey et al., 1969). While it is well known that rheological, mechanical, texture and lubrication properties of foods strongly affect oral behaviours and sensory perception, less attention has been paid to the effect of taste properties on oral processing behaviours. It has been shown that mild bitterness of model gels does not effect chewing muscle activity and chewing frequency (Alfonso et al., 2002). In a follow up study, Neyraud et al. (2005) used viscoelastic gels with varying concentration of quinine and measured masticatory muscle activities. A shorter mastication time (fewer bites, less muscle effort) and longer clearance time was observed with increasing bitterness(Neyraud et al., 2005). Using salted chewing gums, Neyraud et al. (2003) observed that with incrementing saltiness intensity, chewing activity and salivation increased (Neyraud et al., 2003). De Wijk et al. (2006) used M-mode ultrasound imaging to characterize oral movements during the consumption of custards differing in viscosity and sweetness. A higher sweetness intensity resulted in an increased oral processing time and oral movements.

However, oral movements were quantified as the relative change in the ultrasound images from one moment to the next and were not reported in terms of numbers of chews or eating rate (de Wijk et al., 2006). Bolhuis et al. (2011) showed that oro-sensory exposure time was not influenced by saltiness intensity of soups (Bolhuis et al., 2011). More recently, Lasschuijt et al. (2017) demonstrated that neither number of chews, chewing duration, chewing rate nor eating rate were affected by sweetness intensity of model gels (Lasschuijt et al., 2017). The afore summarized literature shows that the mechanisms underlying the influence of taste intensity on oral processing behaviours are not fully understood and largely depend on the food studied, stressing the complexity of the phenomena involved. When modifications of the taste properties are accompanied by changes in the texture or mechanical properties of the foods, then an increase in muscular activity or changes in oral processing behaviour can be caused by an adaptation of oral behaviour to the changes in food texture. When modifications of the taste properties are realized while maintaining texture or mechanical properties of the foods, then peripheral information originating from temperature and taste receptors might be signalled in the brain which could affect mastication behaviour (Schwartz and Lund, 1995). Thus, it has been suggested that cognitive processes, in addition to reflex control, can influence oral processing and mastication behaviour of foods with similar texture

and mechanical properties but differing in taste properties (Campbell et al., 2017; de Wijk et al., 2006; Woda et al., 2006). Nevertheless, only few studies accounted for food liking and/or familiarity when assessing oral processing behaviours. Results have been inconsistent and largely depend on the food that was studied. Forde et al. (2017) and Bellisle and Le Magnen (1980) showed that for solid foods, liking was negatively correlated with chews per bite and chewing time (Bellisle and Le Magnen, 1980; Forde et al., 2017).

However, other studies showed no relationships between liking and oral processing behaviours (Ferriday et al., 2016; C. G. Forde et al., 2013). Brown and Braxton (2000) identified that individuals use different mechanisms for the oral breakdown of food, and thus suggested that individual differences in the ability to manipulate and manage the product in the mouth may influence individual preferences of food (Brown and Braxton, 2000). Bellisle et al (2000) showed that highly palatable foods were swallowed after minimal chewing, although the meal duration was longer with more palatable foods (Bellisle et al., 2000). More recently, Aguayo-Mendoza et al (2018) showed that liking, familiarity, and consumption frequency had little impact on oral processing behaviours in comparison to rheological and mechanical properties of foods (Aguayo-Mendoza et al., 2018).

Most studies investigating the relations between oral processing behaviours and sensory properties of foods have been conducted with model foods or single foods. However, in reality, foods are most commonly consumed in combination with other foods, thus studying heterogeneous foods, composite foods and meals and their impact on oral processing behaviours resembles closer the natural consumption context (van Eck and Stieger, 2020). Composite foods are usually characterized by a product composed of various single foods which differ in composition, mechanical properties and sensory characteristics (van Eck et al., 2019b). Oral processing behaviours of composite foods differ from those of single foods (van Eck et al., 2019b). Differences are attributed to the influence that one food component can have on the breakdown and lubrication properties of the accompanying food during oral processing (Hutchings et al., 2011; Larsen et al., 2016). For example, the addition of spreads such as butter, cheese spread and mayonnaise to a dry carrier such as bread, toast or cracker, decreased number of chewing cycles and increased eating rate compared to the dry carriers alone (Engelen et al., 2005; van Eck et al., 2019b). It was also shown that an increase in

hardness and viscosity of composite foods reduced eating rate (Bolhuis et al., 2014; van Eck et al., 2019b; van Eck and Stieger, 2020).

To the best of our knowledge oral processing behaviours of liquid, solid and composite foods varying in taste intensity have not been studied systematically yet. The aim of this study was to understand the influence of saltiness and sweetness intensity on oral processing behaviours of liquid, solid and composite foods. In addition, potential differences in oral processing behaviours between composite foods and solid foods and the effect of liking on oral processing behaviours were explored in a Home-Use-Test (HUT). It is hypothesized that (i) taste intensity influences oral behaviours of liquid foods as texture properties are less dominant in liquid foods, and that (ii) taste intensity has no effect on oral behaviours of solid and composite foods as their mechanical properties are the main driver of oral behaviour. Lastly, it is hypothesized that (iii) solid foods require longer chewing time per bite and greater number of chews per bite compared to composite foods, as the liquid food assists in lubricating the bolus.

6.2. Materials and Methods

6.2.1 Samples

An overview of all foods and their bite sizes is provided in Table 6.1. Two sets of foods differing in taste modality (sweet, salty) were investigated. Within each set, foods varying in consistency (3 levels: liquid, solid, composite) and tastant concentration (2 levels: low, high) were prepared. As salty foods, tomato sauce (liquid), penne pasta (solid) and their combination (composite food) were used at two levels of saltiness intensity (low, high). As sweet foods, strawberry sauce (liquid), milk gels (solid) and their combination (composite foods) were used at two levels of sweetness intensity (low, high).

Table 6. 1 Overview of salty and sweet foods together with sample codes and size of one bite/sip

Taste modality	Consistency	Food	Taste intensity	Code	Bite/sip size
Salty foods	Liquid	Tomato sauce	Low	T_LS	10 mL
			High	T_HS	10 mL
	Solid	Pasta penne	Low	P_LS	Three pieces of penne (9 g in total)
			High	P_HS	Three pieces of penne (9 g in total)
	Composite	Pasta penne + Tomato sauce	Low	PT_LS	Three pieces of penne +10 mL of sauce (19 g in total)
			High	PT_HS	Three pieces of penne +10 mL of sauce (19 g in total)
Sweet foods	Liquid	Strawberry sauce	Low	S_LSW	10 mL
			High	S_HSW	10 mL
	Solid	Milk gel	Low	M_LSW	One milk gel cube (8 g)
			High	M_HSW	One milk gel cube (8 g)
	Composite	Milk gel + Strawberry sauce	Low	MS_LSW	One milk gel cube +10 mL of sauce (18 g in total)
			High	MS_HSW	One milk gel cube +10 mL of sauce (18 g in total)

Figure 6.1 displays how foods were provided for the Home-Use-Test (HUT).Commercially available tomato sauce was used (Albert Heijn, Tomaten Passata). For the low salt condition, the original tomato sauce was used (0.5 w/w% NaCl). For the high salt condition, table salt was added to the tomato sauce (3.0 w/w% NaCl). Both tomato sauces were cooked in a pot until boiling, after which they were left at room temperature to cool down, then refrigerated overnight (4°C) and poured into serving cups (10 mL) for the evaluations. Commercially available pasta penne were used (Albert Heijn, Penne). 500 g of pasta penne (4.5 x 1.4 cm (length x diameter); 3 g/piece) were cooked for 11 minutes in 2 L of boiling water (100°C) and sieved. For the low salt condition, 250 g of the cooked pasta penne were put in 62.5 mL of salted water (1% w/w NaCl), cooled to room temperature, refrigerated overnight (4°C), sieved and put in serving containers (9 pieces, 27 g in total) for HUT evaluations. For the high salt condition, 250 g of the cooked pasta was put in 62.5 mL of salted water (10% w/w

NaCl), let cool at room temperature, refrigerated overnight (4°C), sieved and put in serving containers (9 pieces, 27 g in total) for HUT evaluations.

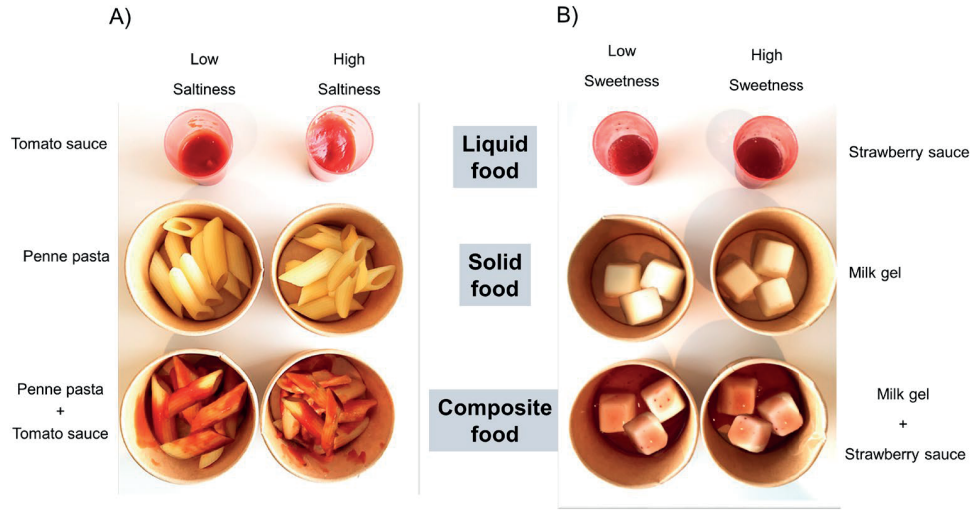


Figure 6. 1 Visualization of all salty (A) and sweet (B) foods used in the Home-Use-Test (HUT)

To prepare the salty composite foods, 9 pieces of penne (27 g) were combined with 30 mL of tomato sauce. Pasta penne were always combined with tomato sauce of the same saltiness intensity condition, so low/low or high/high. To avoid changes in the consistency of the pasta during storage caused by addition of tomato sauce, composite foods were placed in separate containers and were only mixed right before tasting by the participant at their home.

To prepare liquid strawberry sauces differing in sweetness, an aqueous solution of 0.5 w/w% (low sweetness condition) or 3.5 w/w% (high sweetness condition) sweetener (Stevia, Albert Heijn, The Netherlands) was added to commercially available strawberry jam (Céréal, Glucoregul) in a 1:1 ratio and mixed with an electric mixer (Bourgini, 500W) for 10 s. The strawberry sauce was poured into containers (10 mL) for HUT evaluations. As sweet solid foods, milk gel cubes (2 x 2 x 2 cm (length x width x height); 8 g/cube) were prepared. Full fat milk (Albert Heijn, The Netherlands) was boiled with 4 w/w % granulated sugar (Albert Heijn, The Netherlands) and 5 w/w % gelatine (Dr. Oetker, The Netherlands) in a pot. Low sweetness milk gel cubes were prepared by adding 0.3 w/w% stevia (Albert Heijn, The Netherlands) and high sweetness milk gel cubes by adding 3.5 w/w% stevia to the hot liquid.

The hot, liquid mixture was poured into cubic moulds, solidified in the refrigerator (4°C) overnight and put in the evaluation containers (3 cubes, 24g in total). Composite foods were prepared by adding 30 mL of strawberry sauce to three milk gel cubes (24 g in total) of similar sweetness intensity (low/low; high/high). To avoid any changes in consistency of solid foods, composite foods were kept separately and were only mixed by the participants at home immediately before tasting.

All salty and sweet foods were placed in separate closed containers and kept in the refrigerator (4°C) until the testing session. The bite size for the evaluation of each sample was fixed (Table 6.1) and determined in a preliminary study with a focus group who was not taking part in the main study (n=10, 60% female). In the preliminary study participants were served with larger amounts of all foods and were asked to consume the solid and composite foods with a fork as they would normally do and to consume the liquid foods by drinking from a cup as they would normally do. The weight balance before and after consumption was determined to obtain the natural bite size for each food in the preliminary study which was then fixed for the main study (Table 6.1).

6.2.2 Participants

Fifty-five naive participants were recruited for this study via social media and posters. After recruitment, participants filled in an online screening questionnaire (Qualtrics, USA, September 2020). Inclusion criteria included to be non-smoker, good dental health, no missing teeth (except wisdom teeth), no piercings or braces in the mouth, no dental surgery in the last six months, no food allergies to the products of the study (i.e. no lactose intolerance) and not being pregnant nor lactating. Participants should not have a history of olfactory, gustatory, swallowing or chewing disorders (self-reported). After inclusion, participants gave written informed consent before the start of the study and received financial compensation for their participation. From the fifty-five recruited participants, six were not eligible. From the forty-nine eligible, n=39 naive and untrained participants completed the HUT study (25 ± 3 yrs; 28 females). Most of the participants were students of Wageningen University.

6.2.3 Experimental Design

The study consisted of two HUT sessions: one for the sensory assessment (section 6.2.3.1) and one for the characterization of oral processing behaviours (section 6.2.3.2). The order of both HUT sessions was counterbalanced across participants and there was a wash out period of one week in between HUT sessions.

On the day of each HUT session, participants were asked to collect the samples at Wageningen University campus and to perform the test within 2 hours at their home. In each HUT session, participants evaluated all samples (six salty foods, six sweet foods). Participants were randomly assigned to start either with the salty or the sweet foods and were instructed to take a 10 min break between the two sets. Within each set, the order of samples was randomised across food consistency (liquid, solid and composite food) and tastant concentration (high and low). All samples were coded with random three-digit codes.

*6.*2.3.1 Sensory evaluation

To confirm whether the modifications in salt and sweetener concentration led to perceivable differences in saltiness and sweetness with no differences in texture, a HUT sensory test was performed using Qualtrics survey software (version September 2020, Qualtrics, USA). At the beginning of the evaluation, participants had to indicate their level of hunger using a General Labelled Magnitude Scale (gLMS) with the anchor words from “Not at all” to “Extremely high”. Participants were then instructed to take the sample in their mouth and eat it as they would naturally do. Participants were asked to rate the intensity of saltiness, sweetness, hardness and chewiness on a gLMS with anchor words from “Barely detectable” to “Strongest imaginable” (Green et al., 1996). Then liking was assessed using a Labelled Magnitude Scale (LMS) for hedonic rating (Kalva et al., 2014) anchored with the words ‘greatest imaginable dislike’ to ‘greatest imaginable like’. No specific instructions were given on the amount of food that the participants could taste for the HUT sensory evaluations. Table 6.1 lists the number of foods offered for the sensory evaluations. In between samples participants were instructed to wait one minute, rinse their mouth with water and eat a piece of cracker.

6.2.3.2 Oral processing behaviour characterization

Oral processing behaviours were characterized by video recordings (Pipe software, available at: https://addpipe.com/), which were done by the participants (n=39) in their homes (HUT). Precise instructions were given how to record videos when consuming the samples. Before starting, participants had to place two stickers on their face, one on the chin and one on the tip of the nose. These points were used as reference points for post hoc video analysis. Participants were told to sit still in front of the camera and to not to cover their face with their hands while eating. Participants were instructed to put the whole sample in their mouth which corresponded to the bite/sip size shown in Table 6.1, to start chewing according to their natural eating behaviour and to raise their hand the moment they swallowed the food. This was repeated three times for each food (n=39, triplicate). After the three bites were recorded, participants were instructed to wait one minute, rinse their mouth with water and eat a piece of cracker. This procedure was done until all products were masticated. At the end of the HUT session participants had to close the software window to stop the video recording. Video recordings were analysed by a researcher using Kinovea software (version 0.8.15) to extract parameters describing oral processing behaviour of all participants (n=39, triplicate) for each food product. Oral processing movements were tracked by the two stickers on the face of the participants similar as previously reported (Aguayo-Mendoza et al., 2018; Ketel et al., 2019). The sticker on the nose was used as a reference point and the sticker on the chin as a mobile point. Consumption time per bite (s), defined as average time period from putting the sample in the mouth until swallowing, consumption time per gram (s/g), number of chews per bite (-) defined as the number of chews per bite calculated from vertical jaw displacement, chewing rate (chews/s), number of chews per gram (-/g) and eating rate (g/min) were extracted from the video recordings. Number of chews per bite was calculated by plotting the jaw displacement over time, where each peak indicated a single chewing cycle. When peaks were not clear, visual inspection of the video recording was used for clarification. Consumption time per bite and per gram, and eating rate were obtained for all food consistencies (liquid, solid and composite), while number of chews per bite, chewing rate and chews per gram were only obtained for solid and composite foods. Validation of the video analysis was done with six videos which were analysed separately by two researchers, after which they compared their findings until agreement was met. When participants took

multiple swallows during mastication, only the moment of the main swallow was used for analysis.

6.2.4 Data analysis

Normality of all dependent variables was checked, and non-normal distributed data was subjected to either square or log10 transformation for data analysis (Gałecki and Burzykowski, 2013). All results are reported as mean and standard deviation on the original data as they are considered more representative than the transformed data used for the statistical data analysis. All analyses were done separately for salty and sweet foods. Unless stated otherwise, statistical analysis was done using R software (R Core Team, 2020). A level of significance of $p < 0.05$ was chosen. Data were checked for the effect of presentation order in which the sensory or oral behaviour characterization HUT session was done and for hunger level before evaluations and neither had a significant effect on any of the sensory attributes ($p > 0.05$). To determine whether saltiness and sweetness manipulations affected taste and perceived texture (Gałecki and Burzykowski, 2013), mean intensity scores of each of the sensory attributes (saltiness, sweetness, hardness, chewiness) were calculated and a linear mixed model (LMM) (Bates et al., 2014) was performed for each attribute individually for salty and sweet foods. Food consistency (liquid, solid, composite foods), gender (female, male), level of tastant concentration (high, low), liking scores and the interaction between food consistency and tastant concentration, were considered as fixed effects while participant was treated as random effect. Pairwise comparisons were conducted using Tukey Post-hoc tests when significant differences were present. To determine whether liking scores were affected by perceived taste and consistency, mean liking scores of each sample were calculated. LMM was performed individually for salty and sweet foods. Food consistency (liquid, solid, composite foods), gender (female, male), level of tastant concentration (high, low), and the interaction between food consistency and tastant concentration were considered as fixed effects while participant was treated as random effect. Pairwise comparisons were conducted using Tukey Post-hoc tests when significant differences were present.

To determine whether tastant intensity manipulation affected oral behaviours, means of consumption time per bite (s), consumption time per gram (s/g), chewing rate (-/s), number of chews per bite (-), number of chews per gram (-/g) and eating rate (g/min) were calculated and analysed individually using LMM. For each model, tastant concentration (high, low),

food consistency (liquid, solid, composite), gender (female, male), liking scores and the interaction between tastant intensity and food consistency were set as fixed effects and participants as random effect. Pairwise comparisons were conducted using Tukey Post-hoc tests when significant differences were present. Pearson correlation coefficients were calculated to assess inter-relationships between all oral processing behaviour variables.

6.3 Results

6.3.1 Sensory evaluation

Saltiness intensity was significantly and considerably higher for the high salt liquid, solid and composite foods compared to their low salt counterparts (Figure 6.2A) ($F(1,200.84) = 513.06$, $p < 0.001$). The interaction between sweetness intensity and consistency was significant ($F(2,186.09) = 12.42$, $p < 0.001$). High sweet liquid and solid foods were

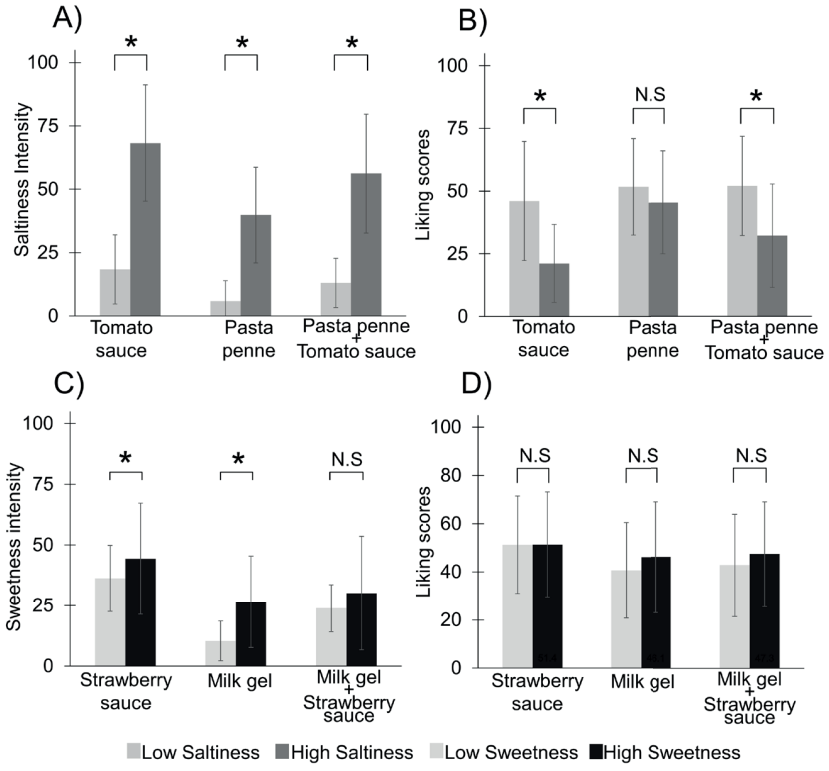


Figure 6. 2 **Mean scores and standard deviations of taste intensity and liking scores for salty (A, B) and sweet (C, D) foods. Data analysis was conducted on the squared transformed data. Means and standard deviations are reported on original data. N.S. - Means are not significantly different according to linear mixed model ($p \geq 0.05$). * means are significantly different according to linear mixed model ($p < 0.05$).**

perceived significantly ($p < 0.05$) sweeter than their low sweet counterparts (Figure 6.2C) whereas sweetness did not differ significantly ($p > 0.05$) between high and low sweet composite foods. Sweet foods differed to a lesser extent in taste intensity compared to salty foods (Figure 6.2A and C). For salty foods, the interaction between liking scores and consistency was significant ($F(2,190) = 6.40$, $p = 0.002$). For liquid and composite foods, liking was significantly higher ($p < 0.001$) for the low salt foods compared to their high salt counterparts. For solid salty foods, no significant difference in liking was found between the high and low salty foods (p = 0.47). (Figure 6.2B). For sweet food, the interaction between sweetness intensity and consistency did not significantly affect liking so that the high and low sweet foods were liked similarly ($F(2,190) = 0.8324$, $p = 0.43$) (Figure 6.2D).

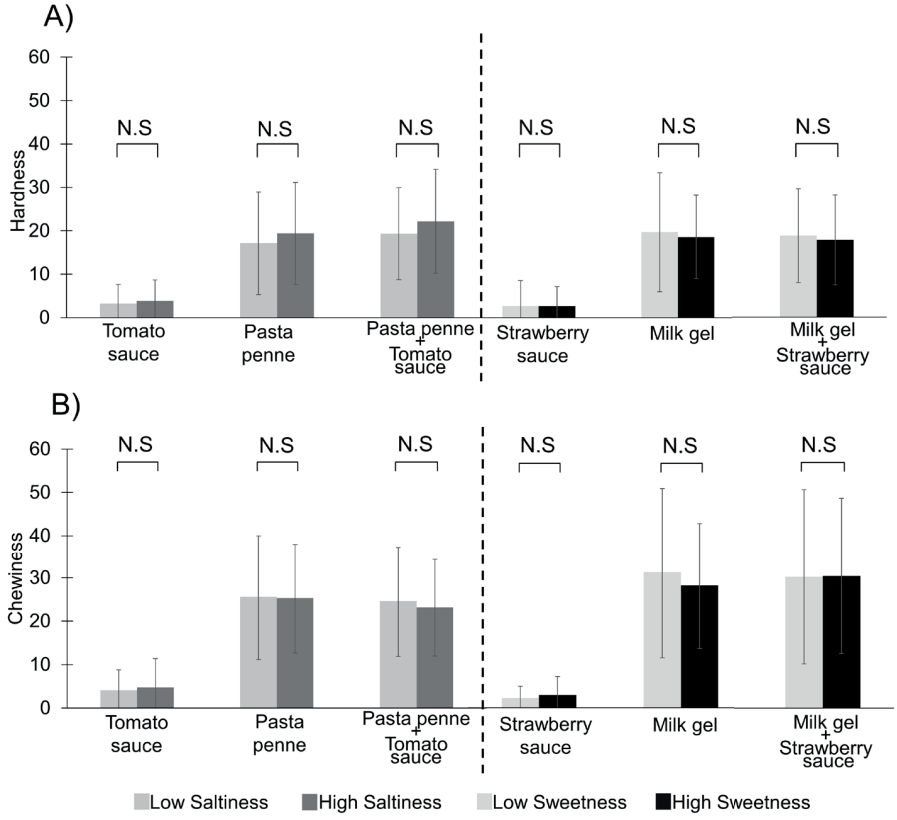


Figure 6. 3 Mean scores and standard deviations of hardness (A) and chewiness (B) intensity for salty and sweet foods. Data analysis was conducted on the squared transformed data. Means and standard deviations are reported on original data. N.S. - Means are not significantly different according to linear mixed model ($p \geq 0.05$). * means are significantly different according to linear mixed model ($p < 0.05$).

No significant differences were found for hardness and chewiness intensity between the high and low taste intensity of salty (hardness: $F(2,190.815) = 0.67$, $p = 0.93$; chewiness: $F(2,190.27) = 0.247$, $p = 0.78$) and sweet foods (hardness: $F(2,189.80) = 0.06$, $p = 0.94$; chewiness: $F(2,189.76) = 0.41$, $p = 0.66$) across all food consistencies (Figures 6.3A and 6.3B).

These results confirm that samples differed in taste intensity between the low and high tastant concentration condition as intended apart from the sweet composite foods (milk gel cubes with strawberry sauce). As intended, texture perception was not affected by the taste manipulations. As food texture is known to modulate food oral processing behaviours, it was important to demonstrate that sensory texture was not affected by taste manipulations.

6.3.2 Oral processing behaviours

6.3.2.1 Effect of taste intensity and food consistency on number of chews per bite

The bite/sip sizes used for quantification of oral processing behaviours was fixed for all foods (Table 6.1). Figure 6.4A shows the number of chews per bite for salty foods. The interaction between salt intensity and food consistency showed no significant effect ($F(1, 107.90) = 0.12$, $p = 0.72$). When looking at the main effects, taste intensity revealed a significant difference ($F(1, 108.39) = 7.35$, $p = 0.007$). Food consistency had a significant effect on number of chews per bite ($F(1, 108.12) = 43.46$, $p < 0.001$), with composite foods requiring 12% (3.2 chews) fewer chews per bite compared to solid foods.

Figure 6.4G displays the number of chews per bite for sweet foods. No significant interaction effect between sweetness intensity and food consistency on number of chews per bite was found ($F(1, 108.167) = 0.1233$, $p = 0.73$), nor was the main effect of taste intensity significant ($F(1, 109.45) = 0.4946$, $p = 0.48$). Food consistency displayed a significant effect on number of chews per bite ($F(1, 108.77) = 18.41$, $p < 0.001$) with composite foods requiring 10% (2.9 chews) fewer chews per bite than solid foods.

6.3.2.2 Effect of taste intensity and consistency on chewing rate

Figure 6.4B and 6.4H display the chewing rate (chews/s) of salty and sweet foods, respectively. For salty foods, neither the interaction between taste intensity and food

consistency ($F(1, 108.471) = 0.015$, $p = 0.90$), nor the main effects of tastant intensity ($F(1, 109.37) = 3.26$, $p = 0.07$) and consistency ($F(1, 108.871) = 0.002$, $p = 0.96$) were significant. On the contrary, for the sweet foods, the interaction between sweetness intensity and consistency displayed a significant effect on chewing rate ($F(1, 108.474) = 6.018$, $p = 0.01$). No significant effects were found for the main effects of sweetness intensity ($F(1, 110.582) = 0.8438$, $p = 0.36$) and food consistency ($F(1, 109.483) = 0.531$, $p = 0.47$).

6.3.2.3 Effect of taste intensity on number of chews per gram

Figure 6.4C and 6.4I show the number of chews per gram for salty and sweet foods, respectively. For salty foods, the interaction between tastant concentration and consistency showed no significant effect ($F(1, 107.9) = 0.12$, $p = 0.73$). However, there were significant main effects of tastant intensity ($F(1, 108.4) = 7.35$, $p = 0.007$) and consistency ($F(1, 108.12) = 1576.7$, $p < 0.001$) with salty composite foods being masticated with 58% (1.8 chews/g) fewer chews per gram compared to salty solid foods.

As for the sweet foods, neither the interaction between tastant concentration and consistency ($F(1, 108.17) = 0.12$, $p = 0.72$) nor the main effect of tastant concentration ($F(1, 109.45) = 0.49$, $p = 0.48$) were significant. Nevertheless, consistency had a significant effect ($F(1, 108.77) = 1690.44$, $p < 0.001$) on number of chews per gram, with sweet composite foods being masticated with 60% (2.2 chews/g) fewer chews per gram than solid sweet foods.

6.3.2.4 Effect of taste intensity and consistency on eating rate

Figure 6.4D and 6.4J display the eating rate (g/min) for salty and sweet foods, respectively. For the salty foods, neither the interaction between taste intensity and consistency ($F(2, 181.48) = 0.032$, $p = 0.96$) nor the main effect of taste intensity ($F(1, 186.23) = 0.835$, $p = 0.36$) were significant. However, there was a significant effect of consistency on eating rate ($F(2, 182.64) = 258.65$, $p < 0.001$). Eating rate of salty composite foods was 139% (41.7 g/min) higher than for salty solid foods. Eating rate of salty liquid foods was 269% (80.6 g/min) and 54% (39 g/min) higher compared to salty solid and composite foods, respectively.

For sweet foods there was no significant effect of the interaction between taste intensity and consistency ($F(2, 178.17) = 0.16$, $p = 0.85$) nor the main effect of taste intensity ($F(1, 179.36) = 0.002$, $p = 0.96$). The main effect of consistency on eating rate was significant ($F(2,$

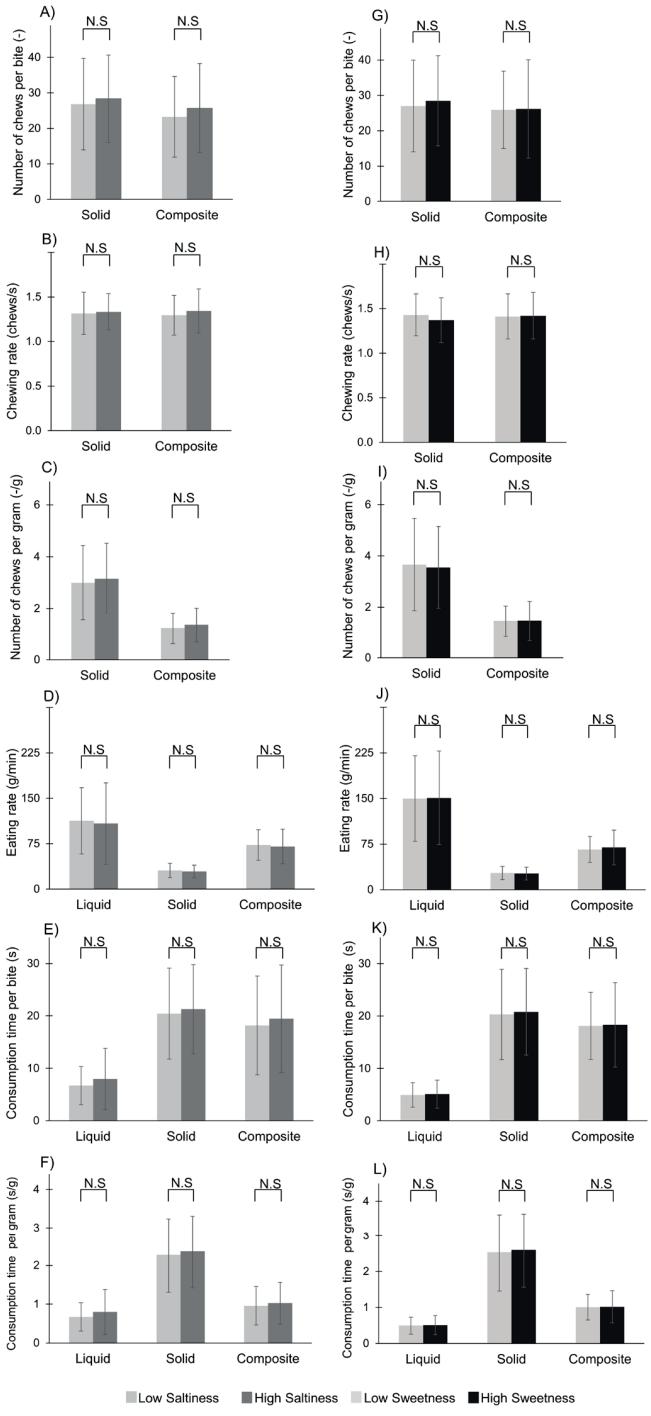
181.96) = 484.44, $p < 0.001$). Eating rate of sweet composite foods was 151% (40.8 g/min) higher compared to sweet solid foods. Eating rate of sweet liquid foods was 456% (123.3 g/min) and 122% (82.5 g/min) higher compared to sweet solid and composite foods, respectively.

6.3.2.5 Effect of taste intensity and food consistency on consumption time per bite

Figure 6.4E and 6.4K illustrate the consumption time per bite/sip for salty and sweet foods, respectively. There was no significant effect of the interaction between salty intensity and consistency (F(2, 182.418) = 0.047, $p = 0.95$) nor the main effect of taste intensity (F(1, 187.24) = 0.761, $p = 0.383$). On the contrary, the main effect of consistency was significant (F(2, 183.59) = 324.97, $p < 0.001$). Composite salty foods required 10% (2.0 s) less consumption time per bite than salty solid foods. Salty liquid foods were consumed the fastest with 65% (13.5 s) and 61% (11.5 s) less time per bite/sip than salty solid and composite foods, respectively. For the sweet foods, there was no significant effect of the interaction between taste intensity and consistency (F(2, 177.85) = 0.236, $p = 0.79$) nor taste intensity (F(1, 178.89) = 0.018, $p = 0.89$). Comparable to salty foods, there was a significant effect of consistency on consumption time per bite (F(2, 181.19) = 524.51 $p < 0.001$). Sweet composite foods were consumed in 11% (2.3 s) less time compared per bite than sweet solid foods and sweet liquid foods were consumed with 76% (15.5 s) and 72% (13.2 s) less time per bite than sweet solid and composite foods, respectively.

6.3.2.6 Effect of taste intensity and food consistency on consumption time per gram

Figure 6.4F and 6.4L display the consumption time per gram for salty and sweet foods, respectively. For salty foods, there was no significant effect of taste intensity on consumption time per gram (F(1, 187.24) = 0.76, $p = 0.38$). On the contrary, there was a significant effect of consistency on consumption time per gram (F(2, 183.6) = 343.71, $p < 0.001$). Composite foods required 57% (1.3 s/g) and liquid foods 68% (1.6 s/g) less time per gram compared to solid foods. Composite foods required 35% more time per gram (0.3 s/g) compared to liquid



foods (Figure 6.4F). A similar trend was observed for sweet foods. No significant effect of taste intensity on consumption time per gram was observed ($F(1, 178.9) = 0.019$, $p = 0.89$).

Consistency of the foods had a significant effect on consumption time per gram ($F(2, 181.19) = 574.08$, $p < 0.001$). Solid sweet foods required the longest time per gram to be consumed. Sweet composite and liquid foods required 61% (1.6 s/g) and 80% (2.1 s/g) less time per gram than solid foods, respectively. Liquids required 50% (0.5s/g) less time

Figure 6. 4 Mean scores and standard deviations of number of chews per bite, chewing rate, number of chews per gram, eating rate, consumption time per bite and consumption time per gram for salty (A, B, C, D, E, F) and sweet (G, H, I, J, K, L) foods. Data analysis was conducted on the log10 transformed data. Means and standard deviations are reported on original data. Means and standard deviations are reported on original data. N.S. - Means are not significantly different according to linear mixed model ($p \geq 0.05$). * means are significantly different according to linear mixed model ($p < 0.05$).

per grams to be consumed than composite foods (Figure 6.4L).

6.3.2.7 Inter-relationships between oral processing behaviour parameters

Pearson correlation coefficients were calculated to assess the relationships between parameters describing oral processing behaviors (Figure 6.5). Interrelationships between oral processing parameters have been previously described in detail (Chen, 2009; Fogel et al., 2017). All oral processing parameters were significantly and strongly correlated with each other ($p < 0.05$) since several parameters are derived from other oral processing parameters and bite size was fixed in this study. Eating rate was significantly and negatively correlated with consumption time per bite (r=-0.95, $p < 0.05$), number of chews per bite (r=-0.92, $p < 0.05$), chewing rate (r=-0.88, $p < 0.05$), number of chews per gram (r=-0.94, $p < 0.05$), and consumption time per grams (r=-0.89, $p < 0.05$). Consumption time per bite, number of chews per bite, chewing rate, number of chews per gram and consumption time per grams were all significantly and positively correlated with each other ($0.66 \leq r \leq 0.99$, $p < 0.05$).

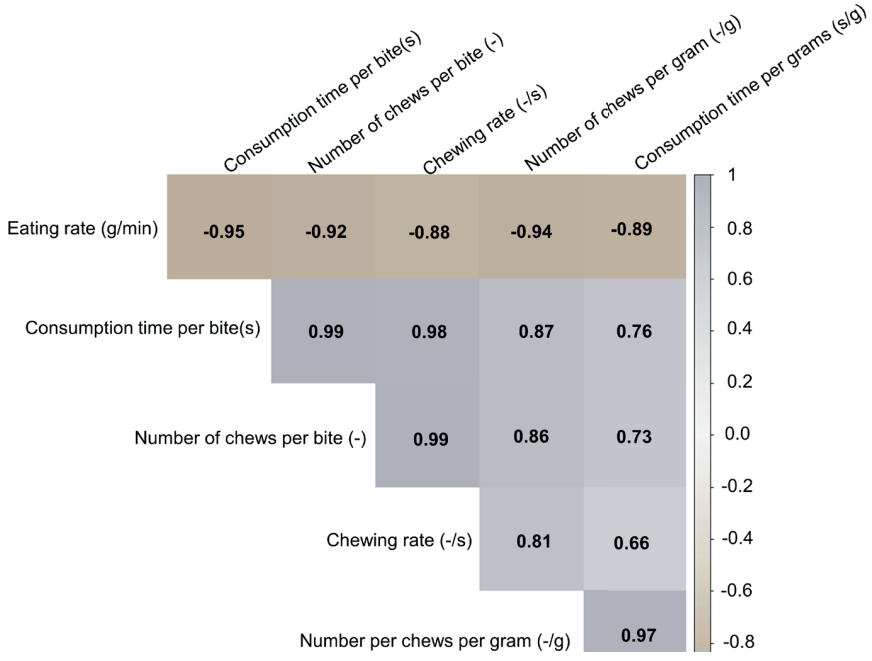


Figure 6.5 Pearson correlation coefficients for the parameters describing oral processing behaviors. All correlations are significant at p <0. 05.

6.3.2.8 Oral processing behaviours and liking

When looking at the relationships between liking scores and oral processing behaviour, liking did not have a significant effect on neither salty nor sweet foods on number of chews per bite (salty foods: $F(1, 112.06) = 0.444$, $p = 0.50$; sweet foods: $F(1, 126.33) = 1.36$, $p = 0.24$), chewing rate (salty foods: $F(1, 116.18) = 0.49$, $p = 0.48$; sweet foods: $F(1, 137.84) = 0.248$, $p = 0.61$), eating rate (salty foods: $F(1, 202.81) = 0.517$, $p = 0.47$; sweet foods: $F(1, 206.43) = 0.374$, $p = 0.54$) and consumption time per bite (salty foods: $F(1, 203.95) = 0.646$, $p = 0.42$; sweet foods: $F(1, 213.134) = 0.0005$, $p = 0.98$).

6.4. Discussion

Many studies investigated the effect of food texture on oral processing behaviours while little attention has been paid on how taste could impact oral processing behaviours. The aim of this study was to understand the influence of saltiness and sweetness intensity on oral processing behaviours of liquid, solid and composite foods. In addition, potential differences in oral processing behaviours between composite foods and solid foods and the effect of liking on oral processing behaviours were explored in a Home-Use-Test (HUT).

Is was hypothesized that (i) taste intensity influences oral behaviours of liquid foods as texture properties are less dominant in liquid foods. For both, liquid salty and sweet foods, consumption time per bite and eating rate were not affected by taste intensity, thus our first hypothesis is rejected. This finding is in line with a study of Bolhuis et al. in which oro-sensory exposure time was not influenced by saltiness intensity of soups (Bolhuis et al., 2011). As for solid and composite foods, it was hypothesized that (ii) taste intensity has no effect on oral processing behaviours since their mechanical, texture and lubrication properties are the main drivers of oral behaviour. It was not found differences in number of chews per bite, chewing rate, chews per gram, eating rate nor consumption time per bite between high and low taste intensities of solid and composite sweet and salty foods. This is in accordance with a study of Lasschuijt et al. where neither number of chews, chewing duration, chewing rate nor eating rate, were significantly affected by sweetness intensity of model gels (Lasschuijt et al., 2017). On the contrary, food consistency displayed a large effect on oral processing behaviours. As expected, liquid foods were consumed with the shortest consumption time per bite, as oral processing behaviour of liquid foods requires minimal

effort, consisting mainly of moving the fluid from the front to the back of the mouth (Aguayo-Mendoza et al., 2018; Hiiemae, 2004; Hutchings et al., 2011; Ketel et al., 2019). In contrast, an increase in consumption time per bite for solid foods was expected as solid foods require to be fragmented into smaller particles during mastication and to be lubricated with saliva until a bolus is formed that is safe to be swallowed (Aguayo-Mendoza et al., 2018). As expected, there was no effect of food consistency on chewing rate (chews/s) in agreement with previous studies (Aguayo-Mendoza et al., 2018; Bellisle et al., 2000). Our results clearly demonstrate that mainly rheological, mechanical, texture and lubrication properties of foods determine oral behaviours rather than taste properties, thus our second hypothesis is accepted.

Furthermore, it was hypothesized (iii) that oral behaviours of solid foods require longer chewing time per bite and greater number of chews per bite compared to composite foods, as the liquid foods assist in bolus formation. Indeed, our findings showed a decrease of 11% and 10% in consumption time per bite of sweet and salty composite foods compared to the solid foods. A similar trend was also observed for number of chews per bite. Lastly, chews needed per gram of composite foods decreased by more than 50% for salty and sweet foods compared to solid foods. This implies that liquids influenced the mastication behaviour of solid foods. These results are in line with those of Gavião et al. (2004) and Engelen et al. (2005) who showed that spreading butter on toast decreased the time and number of chewing cycles until swallowing. Similarly, van Eck et al. (2019b), showed that bread and crackers, when combined with cheese spread and mayonnaise, were chewed shorter and with fewer chews than single bread and crackers as these toppings contributed to a faster bolus formation by providing moisture, so that less saliva was incorporated into the bolus during mastication. In those studies, it was hypothesized that toppings facilitated saliva in the bolus formation of carriers leading to boli that are broken down and lubricated enough to be safely swallowed after shorter mastication times and less chews (Engelen et al., 2005; Gavião et al., 2004; van Eck et al., 2019b). This is confirmed by the results of our current study. Our results show that the liquid component in the composite foods assists in lubrication and bolus formulation, and consequently less time had to be spent on reducing structure and increasing lubrication to reach the swallowing threshold.

It is interesting to note that the bite size of the solid foods was 9 g for the salty and 8 g for the sweet foods while the bite size of the composite foods was roughly twice the weight (19

g for the salty and 18 g for the sweet composite foods) (Table 6.1). Thus, even though addition of the liquid to the solid foods doubled the weight-based bite size, sweet and salty composite foods were masticated with fewer chews per bite and shorter consumption time per bite than solid foods. Eating rate of sweet and salty composite foods also increased by more than two-fold compared to solid foods (salty foods 139%, sweet foods 151%) demonstrating the strong effect that addition of a liquid lubricant has on oral processing behaviours.

It is well known that food texture and degree of lubrication affect eating rate (Bolhuis et al., 2014; C. G. Forde et al., 2013; Lasschuijt et al., 2017; McCrickerd et al., 2017; Zijlstra et al., 2009). Foods that require more chewing and lubrication will take more time to be orally processed and therefore will have a lower eating rate, since liquid foods facilitate lubrication of solid foods. It is reasonable that eating rate increases for composite foods compared with solid foods, which is in line with the reduction of number of chews per bite and consumption time per bite even though weight of the bite size was doubled. For the relationships between taste intensity and eating rate, some studies have shown that taste intensity is inversely associated with eating rate. Bolhuis et al. (2011) showed that longer oro-sensory exposure and higher saltiness intensity both decreased food intake, although oro-sensory exposure had more impact than taste intensity. In the current study taste intensity had no effect on eating rate, as described by Lasschuijt et al. (2017) where it was demonstrated that oral behaviour was not affected by sweetness intensity of model gels (Bolhuis et al., 2011; C G Forde et al., 2013b; Forde et al., 2017; Lasschuijt et al., 2017).

For the concentrations considered in our study, differences in saltiness intensity between salty foods were larger than differences in sweetness intensity between sweet foods. However, not even a rather strong dislike of the high saltiness tomato sauce influenced oral processing behaviours. While significant differences in liking between the low and high saltiness foods were observed, it is acknowledged that the study might have lacked power to find smaller differences in liking between the low and high sweetness stimuli to be significant. Forde et al. (2017) and Bellisle and Le Magnen (1980) reported that as liking increased, chewing time decreased and fewer chews were needed for mastication of solid foods (Bellisle and Le Magnen, 1980; Forde et al., 2017). Bellisle et al., (2000) and Aguayo-Mendoza et al., (2018) showed a similar behaviour (Aguayo-Mendoza et al., 2018; Bellisle et al., 2000). However,

other studies reported no relation between food liking and oral processing behaviours (Ferriday et al., 2016; C. G. Forde et al., 2013). It should be acknowledged that mechanical and rheological properties of the foods were not measured, so it cannot be excluded with certainty that foods differing in taste intensity differed in rheological and mechanical properties. However, our sensory data strongly suggests that potential differences in mechanical properties were not perceivable, thus it is assumed they can be neglected.

In our study, sensory data and the oral processing data were collected in a Home Use Test (HUT). To the best of our knowledge, it is the first time that a HUT approach was used to collect oral processing behaviours of foods. Our data demonstrates that differences in oral processing behaviours between liquid, solid and composite foods were observed in the HUT study (n=39, triplicate) in agreement with previous studies that determined oral processing behaviours in research facilities. This suggests that HUT is a valid approach to characterize oral processing behaviours of consumers. The HUT approach allows to study oral processing behaviours in a somewhat more realistic setting than sensory booths or laboratory settings, since consumers eat the foods at home while sitting in front of a computer with a camera. A limitation of the HUT approach in our study was that the dropout rate was fairly high (20%) mainly because participants felt the task was very long and lost motivation in the course of the study. Furthermore, participants were instructed to taste three times samples of a fixed bite size. This was done to avoid that participants had to weigh the samples at home to determine bite size, which might have provided stronger cues towards the aim of the study. Bite size can vary across consumers, depending on liking, familiarity and satiation as well as across food type, sensory stimuli and digestive responses (de Wijk et al., 2012). In our study, these interindividual variations in bite size were excluded as bite size was fixed. Future studies should explore the potential of HUT approaches to quantify oral processing and eating behaviours further.

6.5. Conclusions

It was investigated oral processing behaviours with real foods and composite foods in a HUT and demonstrated that oral processing behaviours are determined primarily by rheological, mechanical, texture and lubrication properties of foods rather than by taste intensity. It was shown that large differences in saltiness and sweetness intensity of liquid, solid and composite foods cause no or very small differences in oral processing behaviours.

Differences in oral processing behaviours between solid and composite foods were highlighted, validating that consumption time per bite and number of chews per bite of composite foods is shorter compared to solid foods as the added liquid foods provide lubrication and reduce mastication time needed to form a safe to swallow bolus. The results suggest that texture manipulations or combining different foods to composite foods can be an efficient strategy to modulate oral processing behaviours while modulations of taste properties have a negligible effect on oral processing behaviours. It is well-known that oral processing behaviours impact satiation and food intake, so future research should focus on understanding the role that each single food plays in a heterogenous food or a meal context in oral processing behaviour.

This will further help to capture the complex nature of the mastication process and support the design of foods that can lead to lower overall energy intake while maintaining good sensory characteristics, fullness and satiety.

CHAPTER

CAN FLAVOUR AND TEXTURE DEFECTS OF PLANT-BASED BURGER PATTIES BE MITIGATED BY COMBINING THEM WITH BUNS AND TOMATO SAUCE?

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Abstract

Common challenges for plant-based meat replacers are undesired (inherent) off-flavours (flavour defects) such as beany flavour or bitter taste which are often associated with the use of plant proteins in meat replacers. Furthermore, plant-based meat replacers are often perceived as dry and lack juiciness and tenderness (texture defects). However, many foods are not consumed in isolation and sensory properties, and their acceptance, can be altered by the addition of other food components or condiments.

The study aimed to compare the sensory properties and acceptance of commercially available plant-based burger patties eaten alone and in combination with other foods and/or condiments. Liking and sensory properties of a beef and two plant-based (soy and hemp) patties consumed alone, with a bun, with tomato sauce, and with a bun and tomato sauce were determined using a hedonic scale and Rate-All-That-Apply (RATA) methodology, respectively.

For both plant-based patties (hemp, soy) bitter taste, beany and nutty flavour intensity remained but decreased with addition of a bun and/or sauce, but positive liking drivers such as meaty and fat flavour were also reduced. This suggests that these off-flavours can only be partly mitigated by the addition of a bun and/or sauce, but clearly cannot be masked completely. Hemp patties were perceived to have a high amount of chunks, which was a driver of disliking. This texture defect decreased with the addition of a bun and a bun with sauce. Off-flavours and texture defects of the hemp- and soy-based patties were too intense to be fully masked and could only be mitigated to a limited extent by the addition of a bun and/or tomato sauce. Notably, these reductions in off-flavour and texture defects were not sufficient to impact liking. The results highlight the importance of understanding product performance in realistic consumption scenarios. Addition of further ingredients and condiments, as is typical in a burger context, may be advantageous, but further research is required to test such a hypothesis.

Keywords: Plant-based, RATA, sensory perception, composite foods

7.1. Introduction

Animal proteins (e.g. meat and dairy proteins) are increasingly challenged for environmental sustainability, health, safety, and animal rights/welfare concerns (Pluhar, 2010; Ruby, 2012; Tilman and Clark, 2014). A transition towards decreased meat and increased plant-based foods consumption has been suggested to be beneficial for biodiversity, land use, water use, climate, human health and animal welfare (Aiking, 2011; Lamb et al., 2016; Leip et al., 2015). Despite the potential benefits of this transition, meat consumption remains high and acceptability of plant-based meat replacers (MR) is limited in most western societies (Latvala et al., 2012; Lea et al., 2006; Schösler et al., 2015, 2012).

Traditionally, plant-based foods such as pulses, tofu, and textured vegetable protein have been used to replace meat. Such products are generally accepted among vegan and vegetarian consumers but are less appealing to flexitarians and omnivores due to their low sensory appeal (Fiorentini et al., 2020; Hoek et al., 2011). In more recent times, a broad range of innovative meat replacers became available that aim to closely mimic the sensory properties of conventional meat products in terms of functionality, nutrient composition, and sensory characteristics (Tso et al., 2020). For omnivores the primary motivation to consume meat is the sensory pleasure derived from consumption (Mullee et al., 2017). Therefore, these new generation meat replacers have potential to aid omnivores in reducing their meat consumption. However, for many of these products the sensory experience is very different from meat as recreating the unique texture, mouthfeel, and juiciness of traditional meat is an ongoing challenge (Fiorentini et al., 2020). As such, low sensory appeal of meat replacers has been identified as the primary barrier to their acceptance (Michel et al., 2021).

The importance of consumption context, including situational and /or meal context, for product acceptance has been previously highlighted (Elzerman et al., 2011; Jaeger & Porcherot, 2017; Meiselman, 2008; Piqueras-Fiszman & Jaeger, 2015). In particular, in the study conducted by Elzerman (2011), the evaluation of meat substitutes consumed both individually and as part of a dish was examined. Meat substitutes that had similar shapes, but differed in flavour and texture, differed in liking when evaluated individually. However, these differences in liking vanished when meat substitutes were incorporated into a rice dish. Similarly, meat substitutes with the same flavour and texture characteristics, but differing in shape (pieces and minced), differed in liking when assessed individually, but these

differences in liking diminished when meat substitutes were included as part of a meal (rice, spaghetti, soup, and salad) (Elzerman et al., 2011) . This is unsurprising since combining different foods increases stimulus complexity and decreases sensitivity to perceive sensory differences between foods (Carpenter et al., 2000; Kroll and Pilgrim, 1961; Lawless and Heymann, 2010; Stone et al., 2020). Foods that differ in composition, meaning they have variation in their formulation and ingredients, may be distinguishable when consumed individually. However, these perceptual differences might disappear when they are consumed alongside other foods, condiments, or as part of a meal. For example, consumer ability to identify sensory attribute differences between regular and sodium-reduced products, such as salsa, soy sauce, and tomato sauce, was found to decrease when these products were consumed alongside foods like corn chips, cooked rice, and 'tater tots' (Nguyen & Wismer, 2020). A similar effect was observed when different types of mayonnaises with varying fat content and viscosity were consumed alone or combined with bread and carrots. When consumed individually, consumers were able to distinguish between mayonnaises based on perception. However, when paired with bread or carrots, the impact on ability to differentiate between them varied. Specifically, the presence of bread caused the sensory profiles of the mayonnaises to overlap, making it more difficult to distinguish between them. In contrast, the presence of carrots did not affect discrimination of mayonnaise characteristics, underscoring how consumer perception of specific products can be influenced by the foods they are paired with, ultimately affecting sensory sensitivity (van Eck et al., 2019a). More recently, it was demonstrated that dynamic sensory perception of hazelnut chocolate spreads differing in fat and sugar content were strongly affected by addition of carriers, as perceptual differences between spreads decreased when consumed with breads and wafers (Gonzalez-Estanol et al., 2022). To summarise, these studies demonstrate that the sensory properties of foods and their acceptance can be altered by the addition of other food components or condiments and that perceptual differences between foods can disappear when they are consumed with other foods.

Common challenges for plant-based meat replacers are undesired (inherent) off-flavours (flavour defects) such as beany flavour or bitter taste which are often associated with the use of plant proteins in meat replacers (Chigwedere et al., 2022; Roland et al., 2017; Wang et al., 2022). Furthermore, plant-based meat replacers are often perceived as dry and lack juiciness

and tenderness (texture defects) (Sha and Xiong, 2020). This raises the question whether flavour and texture defects of plant-based meat replacers such as plant-based burger patties can be mitigated or even fully masked by combining them with other food components such as tomato ketchup (referred to as tomato sauce here) and a bun which provide different desired flavour and texture sensations. Investigating sensory perception of plant-based meat replacers in composite foods instead of in isolation also provides knowledge that is more representative of the natural consumption context of patties. This study aimed to compare the sensory properties and acceptance of commercially available plant-based burger patties eaten alone and in combination with other foods and/or condiments. Liking and sensory properties of a beef and two plant-based (soy and hemp) patties consumed alone, with a bun, with tomato sauce, and with a bun and tomato sauce were determined using a hedonic scale and Rate-All-That-Apply (RATA) methodology, respectively. It was hypothesised that combining plant-based burger patties with a bun and/or tomato sauce (i) masks off-flavours of plant-based burger patties, (ii) decreases texture defects such as lack of juiciness and (iii) increases liking.

7.2. Materials and methods

7.2.1 Samples preparation

Three commercially available burger patties were selected for this study: hemp-based patties (Bean Supreme, New Zealand), soy-based patties (Veggie Delights, New Zealand), and minced beef patties (Countdown, New Zealand). These patties were selected as they represented a wide variation in sensory characteristics across the New Zealand plant-based burger market and are made from different plant protein sources (hemp and soy). Composite foods were prepared by combining the patties with sauce (Tomato Sauce, Heinz Wattie's, New Zealand) and/or a bun (Nature's fresh, Goodman Fielder, New Zealand). The combinations of patty with sauce and patty with bun were chosen to systematically build up the complexity of the burger. This enabled the evaluation of the interactions between each ingredient and provided insights into their (potential) synergistic contribution in off-flavour reduction in simplified model burgers. The combination of patty with tomato sauce and bun represents a consumption context that is relevant for patties (Chamie, 2023), while we acknowledge that it represents a model burger rather than a full burger in which patties are

often combined with more components such as buns, sauces, pickles, salad and cheese. All samples were bought in local supermarkets in New Zealand.

Patties were evaluated alone and in combination with a bun, with tomato sauce and with a bun and tomato sauce. The sensory properties and liking of all patties and their combinations were determined. Liking of buns and sauce was also determined. An overview of all used samples is shown in Table 7.1 The patties (n=3; hemp, soy and beef) were kept frozen in their original package until a day before the evaluation, then defrosted overnight in the refrigerator. Before the evaluation, they were taken out of the fridge, were lightly brushed with Rice Bran Oil (Countdown, New Zealand) on both sides and cooked on a commercial grill (Grill Station, Roband, Australia) where they were heated at 180 °C between a top and bottom cooking plate. The hemp and soy patties were cooked for 5 min, and the beef patties for 7 min. All patties were flipped half-way through the cooking time. Once cooked, patties were cut into thirds (beef 24.6 ± 2.0 g; hemp 29.8 ± 2.3 g; 24.9 ± 2.5 g) (Table 1) and wrapped individually in aluminium foil. Patties were cooked prior to each sensory session and held in a food warmer (E84 Food Warmer, Bakbar, New Zealand) heated to 50 ± 5 °C for a maximum of 30 min before serving. Buns were cut into thirds (31.1±2.0 g), wrapped in aluminium foil, and kept at room temperature for a maximum 30 min before serving. The tomato sauce (5.4 ± 0.3 g) was poured into containers, covered with a lid and kept at room temperature until serving.

Table 7. 1 Overview of foods with sample codes and photos illustrating the foods used for sensory and liking evaluations.

Group	Food	Codes	
Individual	Bun	B	
	Tomato sauce	T	
	Soy patty	SP	
	Hemp patty	HP	
	Beef patty	BP	
Composite	Soy patty + bun	SP+B	
	Soy patty + tomato sauce	SP+T	
	Soy patty + tomato sauce + bun	SP+B+T	
	Hemp patty + bun	HP+B	
	Hemp patty + tomato sauce	HP+T	
	Hemp patty + tomato sauce + bun	HP+B+T	
	Beef patty + bun	BP+B	
	Beef patty + tomato sauce	BP+T	
	Beef patty + tomato sauce + bun	BP+B+T	

To determine tomato sauce quantity participants were served a third of a patty in a preliminary study and asked to take a regular tomato sauce container (Tomato Sauce, Heinz Wattie's, New Zealand) and spread it on top of the patty as they would normally do if they were going to eat a burger. The average weight of sauce was obtained (5.4 ± 0.3 g) and was fixed for the main study. Composite foods were wrapped in aluminium foil and placed in 'burger boxes' to keep warm and add to context authenticity (Kraft Clam Shell Burger Box, NZ Safety Blackwoods, New Zealand). Burger boxes were held in a food warmer (E84 Food Warmer, Bakbar, New Zealand) heated to 50 ± 5 °C until served. To avoid tomato sauce soaking into the bun, the sauce was portioned into containers and spread on top of the patty with a spoon by the participants immediately before tasting. Participants could take multiple bites of all samples to perform the sensory evaluation. All samples were coded with random three-digit codes.

7.2.2 Participants

Ninety-one consumers were recruited for this study from the Food Experience and Sensory Testing (Feast) Lab (Palmerston North, New Zealand) consumer data base. All participants self-reported consumption of meat and burgers at least once a month. Other inclusion criteria were no allergies or intolerances to wheat/gluten, dairy, nuts, soybeans, eggs, not pregnant nor lactating, being able to fully communicate in English and no history of oral perception disorders or olfactory impairments (self-reported). Participants were asked to self-report their meat and burger consumption frequency using a multiple-choice questionnaire with 'daily', '2-3 times a week', 'once a week', 'once a fortnight' and 'once a month' as possible answers. The study was submitted according to the Massey University's Human Ethics procedure (reference number 4000026311) and was deemed to be low risk. Participants gave written informed consent prior to the study and received financial compensation for their time in form of a gift card after completing the study. Out of ninety-one participants volunteering, n=87 participants (60% women, 39.1±11.9 yrs) completed the study.

7.2.3 Attribute selection

Sensory attributes to describe sensory properties of beef and plant-based patties were identified from literature (Carvalho et al., 2015; De Angelis et al., 2020; Grasso et al., 2022, 2019). Three focus groups (n=22) were held to determine if all sensory attributes from the

compiled list were relevant to describe a wide variety of beef and plant-based patties. Attributes related to the evaluation of sauce and breads were selected from literature and validated with a focus group of participants (n=4) that did not take part in the main study. The final list of attributes and definitions used for Rate-all-that-apply (RATA) evaluation is shown in Table 7.2. This list included 18 attributes covering taste, flavour, mouthfeel, texture, and aftertaste modalities.

Table 7. 2 List of sensory attributes with definitions used for the RATA evaluation of patties evaluated alone and in combination with a bun and/or sauce

Attribute	Definition
Juiciness	Moisture/juice is released from the sample during chewing
Greasiness/oiliness	Sensation related to an oily or greasy coating in the mouth
Tenderness	The sample is easy to chew
Chunkiness	The sample has thick or lumpy pieces
Mouth dryness	The dry, puckering mouthfeel sensation
Saltiness	Basic taste associated with salt/ sodium chloride
Sweetness	Basic taste associated with sugar/ sucrose
Bitterness	Basic bitter taste associated with caffeine
Savoury/ Umami	Taste of glutamate (MSG) e.g., soy sauce, broth-like
Smoky/ grilled	Flavour of smoke e.g., ashy/ woody/ burnt
Meaty flavour	Flavour associated to cooked beef/meat
Wheaty flavour	flavour associated with many wheat-based cereals
Fat flavour	The flavour of cooked animal fat
Beany flavour	The flavour of beans e.g., Soybean, includes musty/earthy
Nutty flavour	The flavour of nuts (unspecific)
Tomato flavour	The flavour of crushed tomatoes
Aromatic Spices	The flavour of aromatic spices e.g., cloves, cumin, fennel, cinnamon, nutmeg, allspice, Paprika, coriander seed
Aftertaste	Described as the product taste/flavour intensity that remains in the mouth

7.2.4 Procedure

The study was carried out in a recreated eating context of a bar restaurant**.** (Supplementary Material 7.1) to simulate a common consumption context of burgers. To create the immersive eating context, 360-degree images of a bar restaurant (purchased from Shutterstock [https://www.shutterstock.com]) were projected on the walls of the testing room using Igloo immersive technology (Igloo Vision Ltd, Australia). In addition, audio of bar ambiance and background music was played (Sleep Sounds Express, 2015).

The study consisted of four sessions divided in two parts (Figure 7.1). The first part entailed one session of 20 min during which participants evaluated all individual foods (patties alone; sauce alone; bun alone). All participants underwent the first part of the study. After that, they proceeded to the second part, which comprised the composite foods evaluation (patties with bun; patties with sauce; patties with bun and sauce) and involved three separate sessions of 30 min each, one for each type of patty (hemp, beef, soy). Between the two parts, there was a wash-up period of at least two days, which allowed for some time to elapse between the two parts of the study. However, it is acknowledged that this wash-up period of at least two days may not have entirely eliminated the possibility of a potential order effect. This block design was selected for logistical convenience, as samples were prepared immediately prior to the evaluation sessions. The four different sessions encompassed liking assessments and descriptive sensory profiling using RATA and took place across two weeks.

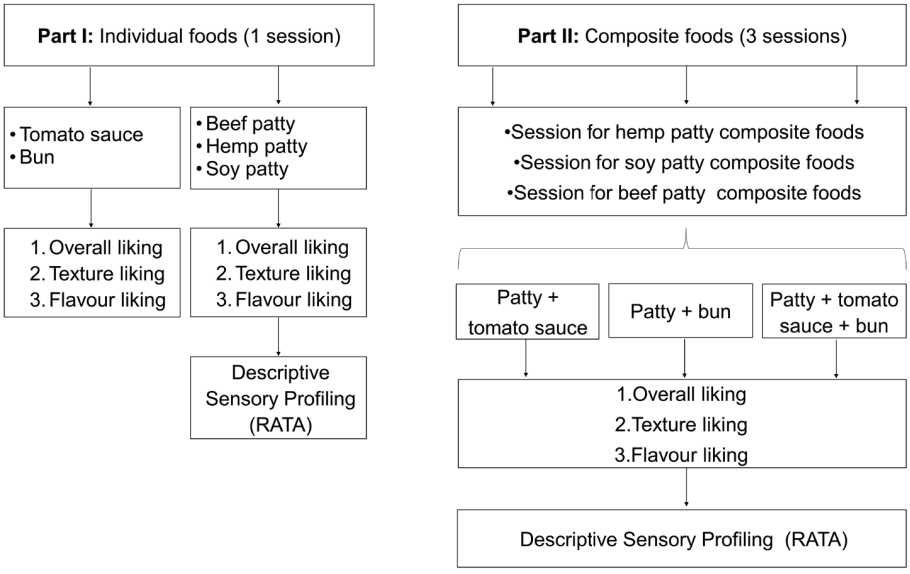


Figure 7. 1 Flow diagram representing organization of sessions evaluating individual foods and composite foods.

For the first part, the five individual foods (bun, sauce, hemp patty, soy patty and beef patty) were divided into two blocks, the sauce and bun were in one block and the three patties were grouped in another block. Block order was counterbalanced across participants and the sample order within each block was determined in accordance to William's Latin Square

design. In between samples, participants were asked to take a sip of water and a cracker (Arnott's water cracker original, Australia) to cleanse their palate.

The samples were given to participants monadically, and each followed a specific evaluation procedure. In the sauce and bun block, participants tasted the sample and rated their overall liking, liking of texture, and liking of flavour on a 9-points hedonic scale anchored with the words 'extremely dislike' to 'extremely like'. Participants then rinsed their palate with water and a cracker before proceeding to the next sample. The process was repeated for each sample.

Similarly, in the block with the patties, samples were also given monadically, and participants followed a specific evaluation procedure for each sample. Using the same 9-point hedonic scale, participants tasted the patty in front of them and rated overall liking, liking of texture and liking of flavour. Following this, participants took a minimum of a one-min break during which they rinsed their palate with a sip of filtered water and a bite of cracker. Participants were then asked to taste the same sample again and conduct the sensory RATA evaluation. Before moving on to the next sample, participants rinsed their palate again with water and a cracker. This entire process was repeated for each sample. No specific instructions were given about the amount of sample that participants could taste.

Part two (Figure 1) consisted of three sessions, one for each type of patty (beef, soy, and hemp). In each session, participants evaluated the composite foods (patty with tomato sauce; patty with bun; patty with tomato sauce and bun). Similar to the first session, samples were given monadically, and participants followed the previously described evaluation procedure. During each session, participants tasted the sample in front of them and rated overall liking, liking of texture and liking of flavour on a 9-points hedonic scale anchored with the words 'extremely dislike' to 'extremely like'. Following the initial tasting, participants took a minimum of a one-min break to rinse their palate with a sip of water and a piece of cracker. Subsequently, participants were asked to taste the same sample again and conduct the sensory RATA evaluation. Finally, they rinsed their palate once more with water and a cracker. This entire process was repeated for each sample. The order of the type of patties was counterbalanced across participants and the order of composite foods was in accordance with William's Latin Square design. While no specific instructions were given regarding the

quantity of sample that participants could taste, they were instructed to bite through all components of the composite foods.

For all sessions, participants were asked to refrain from eating, and drinking anything except for water for at least one hour before the start of the session. At the beginning of each session, participants were provided with attributes list and their definitions, and a short explanation of the RATA assessment procedure was given.

7.2.5 Rate-All-That-Apply (RATA)

In line with previous RATA studies, participants were asked to select whether an attribute (Table 7.2) applied to describe the sample being tasted, and only if so, to rate its perceived intensity (Ares et al., 2014; Giacalone and Hedelund, 2016) using a 9-box scale ranging from 'low' to 'high'. The order of attribute presentation was grouped by modality and randomized across participants but maintained for each participant between samples. All data was collected using computerized data acquisition software (Compusense Cloud, Guelph, Ontario, Canada).

7.2.6 Data Analysis

7.2.6.1 Liking scores

Throughout the manuscript, the term "food combinations" refers to (1) the patties alone, (2) the combination of patties with a bun , (3) the combination of tomato sauce spread on top of patties and (4) the combination of patties with tomato sauce spread on top with a bun (Table 7.1). Mean scores and standard deviations for overall, flavour and texture liking scores of all food combinations and type of patty were established. To determine significant differences and effects between samples liking scores, three Linear Mixed Models (LMM) with each liking score (overall, texture, and flavour) as the response variable were determined. Type of patty (beef, hemp, soy), food combination (patty alone; patty with bun; patty with sauce; patty with sauce and bun), and the food combination*patty type interaction were set as fixed factors and participants as a random factor. When the food combination*patty type interaction was not significant, the interaction was removed from the model and a LMM was carried out without it. Significance level was set at $\alpha < 0.05$. Tukey Post-hoc pairwise comparison tests were conducted when significant effects were identified. Pearson correlation coefficients were calculated to assess inter-relationships between liking scores

and sensory variables. Three LMM were performed for liking scores with elements (sauce, bun) set as fixed factors and participants as random factor. Analyses were done using lme4, emmeans, and lmerTest packages of R software version 3.1.1 (R Core Team, 2020).

To evaluate how addition of buns and/or sauce impacted patty liking, relative changes in liking scores were determined (%) by calculating the difference in liking between patties evaluated alone ($L_{Patty\ alone}$) and patties combined with buns ($L_{Patty\ with\ bun}$) and/or sauce ($L_{Patty\ with\ sauce}$; $L_{Patty\ with\ bun\ and\ sauce}$) and dividing the liking difference by the liking of the patties evaluated alone ($L_{Patty\ alone}$) (1).

(1) $$\text{Relative liking change (\%)} = \frac{L_{Patty\ alone} - L_{Patty\ with\ bun\ and/or\ sauce}}{L_{Patty\ alone}} \times 100$$

7.2.6.2 RATA scores

RATA data was interpreted as a 10-point scales considering "not applicable" as intensity = 0. Intensity scores were treated as continuous data. Results for each attribute per sample are reported as mean with standard deviation. To determine significant differences between samples, a LMM was performed with food combination (patty alone; patty with bun; patty with sauce; patty with bun and sauce), patty type (hemp; soy; beef) and the food combination*patty type interaction as fixed factors and participant as a random factor for each sensory attribute to assess differences between samples. In case of significant differences, post-hoc tests were performed with Tukey's Honest Significant Difference test (HSD) at 95% confidence level. When the food combination*patty type interaction was not significant, the interaction was removed from the model and a LMM was carried out without it.

To evaluate how addition of bun and/or sauce impacted the sensory properties of the patties, relative changes in intensity ratings were determined (%) for each attribute by calculating the difference in intensity between the patties evaluated alone ($I_{Patty\ alone}$) and the patties combined with buns ($I_{Patty\ with\ bun}$) and/or sauce ($I_{Patty\ with\ sauce}$; $I_{Patty\ with\ bun\ and\ sauce}$), and dividing this intensity difference by the intensity of the patties evaluated alone ($I_{Patty\ alone}$) (2).

(2) $$\text{Relative intensity change (\%)} = \frac{I_{Patty\ alone} - I_{Patty\ with\ bun\ and/or\ sauce}}{I_{Patty\ alone}} \times 100$$

Table 7. 3 Summary of liking scores (mean ±SD) of individual patties and composite foods with patties. BP denotes Beef Patty, SP Soy Patty, HP Hemp Patty, B Bun and T denotes Tomato sauce. F and p values derived from linear mixed models for each liking score for food combination and patty type as fixed effects and participants as random effect. P values presented as * (p<0.001),** (p<0.01), * (p<0.05), and N.S (not significant). Lowercase letters indicate significant differences between means across samples obtained from Tukey's Student t-tests for pairwise comparisons. The food combination*patty type interaction was not significant and is therefore not included in the model.**

	Beef				Soy				Hemp				Food combination		Patty type	
	BP	BP+B	BP+B+T	BP+T	SP	SP+B	SP+B+T	SP+T	HP	HP+B	HP+B+T	HP+T	F	p	F	p
Overall liking	7.3 ± 0.9e	6.7 ± 1.2d	7.3 ± 1.1e	7.3 ± 1.1e	6.6 ± 1.5d	6.1 ± 1.6c	6.7 ± 1.3d	6.6 ± 1.5d	5.0 ± 2.0b	4.7 ± 1.7a	5.3 ± 1.7b	5.2 ± 1.7b	12.4	***	267.8	***
Texture liking	6.9 ± 1.3cfg	6.6 ± 1.4ef	7.2 ± 1.1g	7.1 ± 1.2fg	6.1 ± 1.6cd	6.0 ± 1.6c	6.5 ± 1.4de	6.4 ± 1.6cde	4.4 ± 2.0ab	4.3 ± 1.6a	4.9 ± 1.8b	4.6 ± 1.7ab	7.3	***	302.5	***
Flavour liking	7.3 ± 1.1e	6.6 ± 1.3d	7.3 ± 1.2e	7.3 ± 1.1e	6.5 ± 1.7d	6.0 ± 1.6c	6.5 ± 1.4d	6.5 ± 1.6d	5.2 ± 2b	4.8 ± 1.6a	5.5 ± 1.7b	5.3 ± 1.6b	11.0	***	192.8	***

Principal component analysis (PCA) with confidence ellipses of 0.90 was carried out on average intensity ratings to analyse the relationships between sensory attributes and samples. Only attributes that were significantly different ($p < 0.05$) are shown in the PCA (SensoMineR package).

7.3. Results

7.3.1 Sensory properties and liking of meat and plant-based patties

Flavour and texture liking of patties and their combination with a bun and/or sauce are summarised in Table 7.3. No significant food combination*patty type interaction was found for any liking score and thus the interaction effect was removed from the LMM. Beef samples scored highest for texture liking, flavour and overall liking followed by the soy patties. Hemp patties were liked the least, the texture liking of hemp patties was particularly lower compared to soy and beef patties. There was a significant effect of the food combination and patty type on the three liking scores.

The mean intensity sensory scores for all samples are summarised in Table 7.4. Considering patty type (beef, hemp, soy) as a main effect from the LMM, beef patties scored significantly higher in tenderness intensity ($F(2, 948.7) = 169.8$, $p < 0.001$) and meaty flavour intensity ($F(2, 951.3) = 1069$, $p < 0.001$). Hemp patties were perceived as the most bitter ($F(2, 944.8) = 59.2$, $p < 0.001$) and having the highest beany ($F(2, 951.5) = 631.9$, $p < 0.001$) and nutty flavour intensity ($F(2, 951.5) = 502.4$, $p < 0.001$) compared with the other patties. Smoky ($F(2, 949.6) = 222.2$, $p < 0.001$), aromatic spices ($F(2, 950.4) = 144.1$, $p < 0.001$) and saltiness intensity ($F(2, 951.2) = 76.2$, $p < 0.001$) were significantly higher for soy patties than the other patties.

Table 7. 4 Intensity scores (mean ±SD) obtained from RATA evaluations (n=87 participants) of food combinations. BP denotes Beef Patty, SP Soy Patty, HP Hemp Patty, B Bun and T denotes Tomato sauce. F and p values derived from linear mixed models for each attribute with food combination, patty type and food combination*patty type interaction as fixed effects and participants as random effect. P values are presented as * (p<0.001),** (p<0.01), * (p<0.05), and N.S (not significant). Lowercase letters indicate significant differences between means across samples obtained from Tukey's Student t-tests for pairwise comparisons.**

Beef				Soy				Hemp				Food combination		Patty type		Food c *Pa
BP	BP+B	BP+B+T	BP+T	SP	SP+B	SP+B+T	SP+T	HP	HP+B	HP+B+T	HP+T	F	p	F	p	F
5.4 ± 2.4d	4.1 ± 2.3c	5.1 ± 2d	5.6 ± 2.2d	3.4 ± 2.4c	2.4 ± 1.8b	3.4 ± 2c	3.8 ± 2.2c	1.3 ± 1.3a	1.2 ± 1a	1.8 ± 1.4ab	1.8 ± 1.5ab	29.5	***	457.0	***	2.9
5.2 ± 2.1f	3.9 ± 2.2d	4 ± 2.1de	4.6 ± 2.2ef	4.7 ± 2.1f	2.5 ± 1.7bc	2.8 ± 1.8c	3.6 ± 2d	1.9 ± 1.4ab	1.4 ± 0.9a	1.6 ± 1.1fa	1.9 ± 1.4ab	46.0	***	293.9	***	6.4
6.1 ± 2.2e	6.0 ± 1.9cde	6.3 ± 1.6de	6.0 ± 1.7cde	5.9 ± 2.2bcd	5.2 ± 2.1b	5.4 ± 2.2bc	5.2 ± 2.2b	3.9 ± 2.6a	3.8 ± 2.3a	4 ± 2.4a	3.8 ± 2.4a	2.9	*	169.9	***	
5.0 ± 2.3cd	4.2 ± 2.2ab	4.3 ± 2.3ab	4.6 ± 2.3abcd	4.8 ± 2.1bd	4.3 ± 2.1ac	4.4 ± 2.2ac	5 ± 2.2abcd	6.5 ± 2f	6.1 ± 1.9e	6 ± 2.1e	6.5 ± 1.8ef	7.3	***	107.9	***	
2.5 ± 2.1a	3.3 ± 2.4b	2.6 ± 2.1a	1.8 ± 1.7a	3.6 ± 2.3bc	5.2 ± 2.5d	3.8 ± 2.3bc	3.7 ± 2.4b	4.8 ± 2.5d	5.6 ± 2.6e	4.8 ± 2.4d	4.7 ± 2.3cd	24.4	***	140.2	***	
4.1 ± 2e	3.1 ± 1.8bc	3.1 ± 1.9bc	3.5 ± 2cd	4.8 ± 2.1f	3.9 ± 2.3de	3.9 ± 2de	4.2 ± 2.1e	3.4 ± 2.1cd	2.5 ± 1.7a	2.7 ± 1.7a	2.9 ± 1.9ab	22.3	***	76.2	**	
1.8 ± 1.9a	1.9 ± 1.7ab	3.2 ± 2.2c	3.1 ± 2.4c	2.1 ± 1.9cb	2.0 ± 1.9cb	3.3 ± 2.2c	3.3 ± 2.2c	2.6 ± 2.1bc	1.9 ± 1.6ab	2.9 ± 2c	2.9 ± 2.2c	41.2	***	0.9	N.S	2.4
0.9 ± 1.2bbc	0.8 ± 1.2ab	0.6 ± 0.9a	0.9 ± 1.2ab	1.9 ± 2.1ef	1.7 ± 1.8def	1.3 ± 1.6cd	1.7 ± 1.8def	2.1 ± 2.4f	1.7 ± 2def	1.6 ± 1.9de	1.7 ± 1.9def	5.6	**	59.2	***	
5.1 ± 2.6de	4.4 ± 2.6bc	4.6 ± 2.6c	4.9 ± 2.5c	5.6 ± 2.5e	4.7 ± 2.4c	4.6 ± 2.4cd	4.9 ± 2.5cd	4.5 ± 2.4c	3.4 ± 2.3a	3.7 ± 2.4a	3.7 ± 2.4ab	16.1	***	44.4	***	
4.9 ± 2.4fg	4.1 ± 2.4d	4.4 ± 2.5de	5 ± 2.2ef	6 ± 2.4h	4.9 ± 2.2ef	4.8 ± 2.4fg	5.5 ± 2.3gh	3.1 ± 2.2c	2.3 ± 1.8a	2.4 ± 1.8ab	2.7 ± 1.9bc	15.2	***	222.2	***	
7.7 ± 1.4f	6.7 ± 1.7e	7.1 ± 1.7ef	7.6 ± 1.4f	5.0 ± 2.5d	4.3 ± 2.2c	4.7 ± 2.3cd	4.7 ± 2.4d	1.9 ± 1.7b	1.5 ± 1.7a	1.7 ± 1.9ab	1.9 ± 1.8b	9.9	***	1069	***	
1.7 ± 1.7b	3 ± 2.4cd	2.5 ± 2.3bc	1.2 ± 1.3a	3.8 ± 2.4ef	4.2 ± 2.6gh	3.9 ± 2.4efgh	2.9 ± 2.5cd	4.2 ± 2.5fg	4.3 ± 2.5h	4.1 ± 2.4fgh	3.4 ± 2.4de	24.4	***	109.7	***	
5.8 ± 2.2d	4.6 ± 2.2c	4.7 ± 2c	5.1 ± 1.9cd	4.4 ± 2.3c	2.6 ± 1.9b	2.8 ± 1.8b	3.1 ± 2b	1.8 ± 1.5a	1.3 ± 1a	1.6 ± 1.3a	1.7 ± 1.3a	32.3	***	473.3	***	3.9
1.3 ± 1.5b	1.2 ± 1.6ab	0.9 ± 1.3a	0.7 ± 1.1a	3.8 ± 2.5d	3.3 ± 2.6cd	3.1 ± 2.5c	3.3 ± 2.7c	6.3 ± 2.5f	5.8 ± 2.5ef	5.5 ± 2.4e	5.6 ± 2.4e	6.12	***	631.9	***	
1.6 ± 1.9b	1.1 ± 1.6a	1.1 ± 1.4a	1.2 ± 1.5ab	3.3 ± 2.5d	2.7 ± 2.4c	2.6 ± 2.4c	3.1 ± 2.7cd	6.0 ± 2.6f	5.0 ± 2.5e	5.0 ± 2.5e	5.2 ± 2.5ef	9.9	***	502.4	***	
1.1 ± 1.4ab	0.7 ± 1.3a	5.2 ± 2.2def	5.8 ± 2.4ef	2.1 ± 2.1c	0.9 ± 1.3a	5 ± 2.1de	5.9 ± 2.1f	1.9 ± 1.9bc	0.7 ± 1.1a	4.8 ± 2.1d	5.4 ± 2.2def	545.5	***	2.9	N.S	2.9
3.3 ± 2.3c	2.5 ± 2a	2.7 ± 2fa	3.1 ± 2.2ab	6.0 ± 2.3f	5.0 ± 2.4de	4.6 ± 2.4de	5.2 ± 2.3e	4.5 ± 2.4d	3.4 ± 2.5bc	3.8 ± 2.5bc	3.8 ± 2.2c	16.6	***	144.1	***	
4.5 ± 2.5ef	3.7 ± 2.3ac	4.1 ± 2.4abcd	4.4 ± 2.5bde	5.8 ± 2.3h	4.9 ± 2.1ef	5 ± 2.2fg	5.6 ± 2.2gh	4.8 ± 2.5ef	3.7 ± 2.2ab	3.9 ± 2.2abcd	4.1 ± 2.2cde	15.8	***	53.5	***	

Table 7. 5 Pearson correlation coefficients for liking scores and sensory attributes. *Correlation is significant at p<0.05

	Juicy	Greasy/oily	Tender	Chunky	Mouth drying	Salty	Sweet	Bitter	Savoury umami	Smoky/ grilled	Meaty flavour	Wheaty flavour	Fat flavour	Beany flavour	Nutty flavour	Tomato flavour	Aromatic spices	Aftertaste
Overall liking	0.97*	0.91*	0.97*	-0.84*	-0.90*	0.55	0.15	-0.74*	0.75*	0.81*	0.97*	-0.76*	0.92*	-0.95*	-0.93*	0.21	-0.16	0.27
Texture liking	0.95*	0.86*	0.96*	-0.89*	-0.86*	0.49	0.16	-0.77*	0.69*	0.79*	0.96*	-0.73*	0.88*	-0.96*	-0.96*	0.20	-0.19	0.23
Flavour liking	0.98*	0.91*	0.95*	-0.80*	-0.93*	0.50	0.16	-0.77*	0.73*	0.76*	0.97*	-0.81*	0.93*	-0.94*	-0.92*	0.23	-0.21	0.22

The Pearson's correlation coefficients are presented in Table 7.5 to assess relationships between sensory attributes and texture, flavour, and overall liking. Regarding the taste and flavour attributes, bitter (r=-0.74, $p<0.05$), wheaty flavour (r=-0.76, $p<0.05$), beany (r=-0.95, $p<0.05$), and nutty (r=-0.93, $p<0.05$) flavour were significantly negatively correlated with overall liking. In contrast, fat flavour (r=0.92, $p<0.05$), greasy (r=0.92, $p<0.05$), smoky (r=0.81, $p<0.05$), and meaty flavour (r=0.97, $p<0.05$) were significantly positively correlated with overall liking. Regarding the texture attributes, mouth drying (r=-0.90, $p<0.05$) and chunky (r=-0.84, $p<0.05$) were significantly negatively correlated with overall liking, in contrast to juiciness which was significantly positively correlated with overall liking (r=0.97, $p<0.05$).

Figure 7.2 shows the Principal Component Analysis (PCA) of the mean intensity scores of all attributes, visualising the differences indicated by the LMMs. The individual factor map (Figure 7.2A) shows the representation of all samples with confidence ellipses of 0.90. The correlation circles (Figure 7.2B) visualise the mean intensity scores of the sensory attributes with all liking scores presented as supplementary variables. The first two components explain 61.5% and 22.1% of the total variance. The first dimension distinguished between the different types of patties.

In general, hemp samples (depicted in shades of green) were characterised by higher bitter, beany, nutty and mouth drying intensities compared to the soy and beef samples. The beef samples (depicted in shades of brown) were characterised by meaty, fat flavour, tender and juicy sensations. Lastly, the soy-based samples (depicted in shades of yellow) were described by after taste, salty, savoury and flavour of aromatic spices sensations (Figure 7.2A).

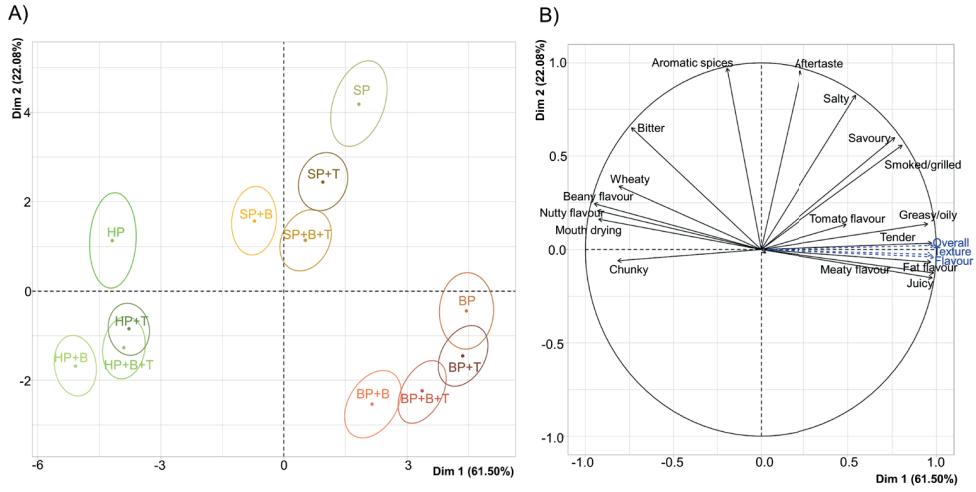


Figure 7. 2 Principal Component Analysis (PCA) of the RATA intensity scores for all samples. Map (A) displays samples with confidence ellipses of 0.90. Different colour shades indicate different type of patties (beef; depicted in shades of brown ,hemp; depicted in shades of green and soy; depicted in shades of yellow). Correlation circles (B) display sensory attributes with overall liking, flavour and texture liking scores as supplementary variables (blue hashed arrows).

The correlation circle (Figure 7.2B) displays the liking scores projected as supplementary variables (blue dashed lines) into the sensory profiles of the samples. The positive side of the first dimension is correlated with meaty and fatty flavour and with tender and juicy texture (highlighted in green) indicating they are the flavour and texture attributes driving overall liking, flavour and texture liking. In contrast, the negative side of the first dimension is correlated with beany, nutty and wheaty flavours and with chunky and mouth drying texture indicating these are undesired sensory characteristics, off-flavours and texture defects driving disliking of the samples. These findings are, as expected, in agreement with the results of the Pearson correlation coefficients relating sensory attributes to texture, flavour, and overall liking (Table 7.5).

7.3.2 Effect of bun and/or tomato sauce addition on flavour perception of plant-based patties

For the off-flavours beany, nutty and wheaty flavour, the LMM showed that the food combination (beany ($F(3, 953.3) = 6.12$, $p < 0.001$), nutty ($F(3, 952.9) = 9.97$, $p < 0.001$), wheaty ($F(3, 952.7) = 24.45$, $p < 0.001$)) and patty type (beany ($F(2, 951.5) = 631.9$, $p < 0.001$), nutty ($F(2, 951.5) = 502.43$, $p < 0.001$), wheaty ($F(2, 950.9) = 109.8$, $p < 0.001$))

effects were significant (Table 7.4). Within patty type, no significant differences were observed ($p>0.05$). However, off-flavours of hemp and soy patties tended to be reduced when patties were combined with a bun, tomato sauce or both (Figure 7.3). The largest relative reduction of beany intensity (-18.4%) was achieved when soy patties were combined with a bun and tomato sauce, whereas combining the soy patties with a bun (-13.2%) or tomato sauce (-13.2%) led to smaller relative reductions in beany intensity. In the case of hemp patties, beany intensity was reduced by -12.7% when patties were combined with a bun and tomato sauce, by -11.1% when combined with sauce and by -7.9% when combined with a bun. For both, hemp and soy patties, nutty intensity was reduced the most when combined with a bun and sauce. For soy patties, nutty intensity decreased by -21.2% and for hemp by -16.7%. When soy patties were combined with a bun, nutty intensity decreased by -18.2% and when combined with sauce by -6.1%. For hemp patties, nutty intensity decreased by -16.7% with the addition of a bun and by -13.3% with the addition of sauce and a bun. Wheaty flavour increased by 10.5% for soy and by 2.4% for hemp patties when buns were added. Wheaty flavour intensity decreased by -23.7% for soy and by -19.0% for hemp when sauce was added. Regarding the positive drivers of liking, the food combination* type of patty interaction was significant for fatty flavour ($F(6, 945.16) = 3.9$, $p< 0.001$) while for meaty flavour, food combination ($F(3, 953.09) = 9.95$, $p< 0.001$) and patty type ($F(2, 951.28) = 1069$, $p< 0.001$) were both significant (Table 7.3). Regardless of having no significant effects within each type of patty, meaty flavour intensity was reduced by -14.0% for soy and by -21.1% for hemp when patties were combined with a bun, while fatty flavour intensity was reduced by -40.9% for soy and by -27.8% for hemp when a bun was added to the patties (Figure 7.3). Lastly, the LMM showed that the food combination*patty type interaction was significant for tomato flavour ($F(6,945.43)=2.9$, $p<0.01$) as the addition of sauce increased the tomato flavour intensity of all three patties but to a different extent. With addition of tomato sauce, tomato flavour intensity increased by 181% and by 184% in soy and hemp patties, respectively. When a bun and sauce were added, tomato flavour increased by 138% and by 152% in soy and hemp patties, respectively.

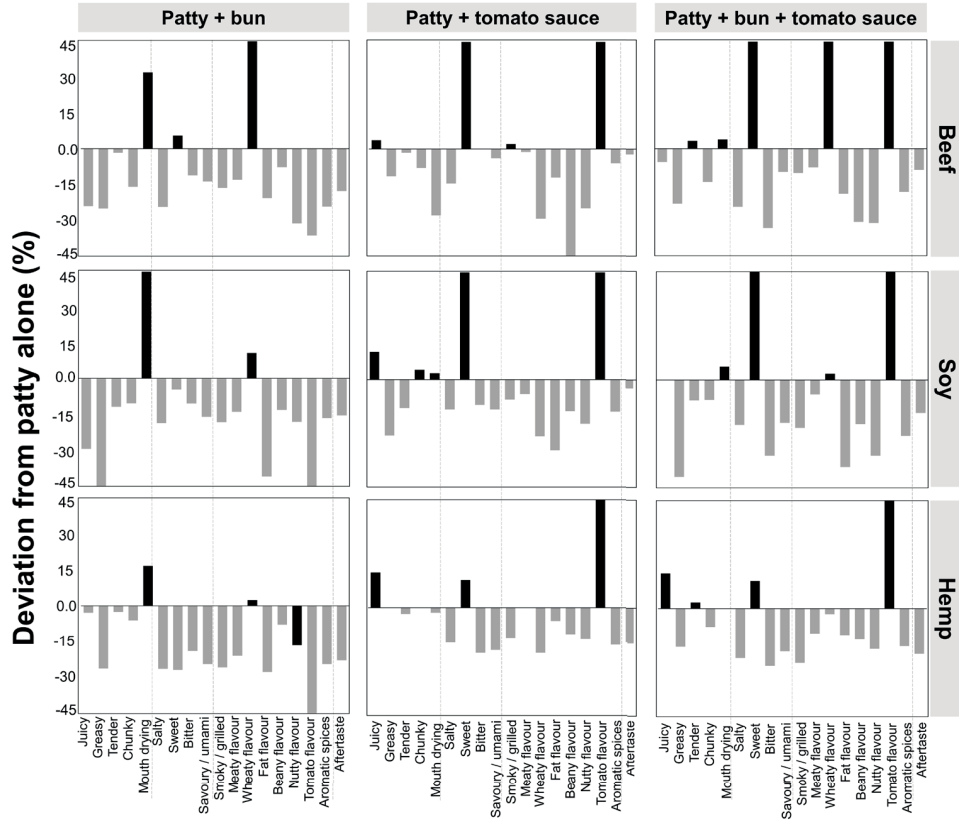


Figure 7. 3 Relative intensity change (%) for each attribute when patties were combined with buns and/or sauce in comparison to patties consumed alone [Relative intensity change (%) = ($I_{Patty\ alone} - I_{Patty\ with\ bun\ and/or\ sauce}$) / $I_{Patty\ alone}$ × 100].

7.3.3 Effect of bun and/or tomato sauce addition on texture perception of plant-based patties

Figure 7.2 and Table 7.5 highlighted that chunky and mouth drying texture are negative drivers of liking. The LMM showed a significant effect of food combination (chunky ($F(3, 953.06) = 7.38$, $p < 0.001$), mouth drying ($F(3, 953.06) = 7.38$, $p < 0.001$)) and patty type (chunky ($F(2, 951.19) = 107.96$, $p < 0.001$), mouth drying ($F(2, 948.91) = 140.19$, $p < 0.001$) but no significant differences within type of patty (Table 7.4). Regardless, when hemp and soy patties were combined with a bun and/or sauce, the intensities of chunky and mouth drying tended to decrease (Figure 7.3). Chunky intensity was reduced by -10.4% for soy and by -7.7% for hemp patties when a bun was added. Mouth drying intensity increased by 44.4% for soy and by 16.7% for hemp patties when a bun was added. Positive drivers of liking such as juicy, greasy and tender (Figure 7.2 and Table 7.5) were also influenced by bun and/or

sauce addition. The interaction of food combination*type was significant for juiciness ($F(6, 944.75) = 2.9$, $p < 0.01$). Juiciness intensity decreased by -29.4% for soy and by -7.7% for hemp patties when a bun was added. Juiciness intensity increased by 11.8% for soy and by 38.5% for hemp patties when sauce was added. Juiciness intensity increased by 38.5% for hemp but did not change for soy patties when a bun and sauce were added. The interaction between food combination* patty type was also significant for greasiness ($F(6, 944.87) = 6.4$, $p < 0.001$). Relative changes within samples showed that addition of a bun decreased perception of greasiness by -46.8% for soy but only -26.3% for hemp. When tomato sauce was added, greasiness perception was reduced by -23.4% for soy but it did not change for hemp. When both components were added, greasiness perception was reduced by -40.4% for soy and by -15.8% for hemp. Lastly, tender perception was significantly affected by food combination ($F(3, 950.26) = 2.9$, $p < 0.01$) and patty type ($F(2, 948.75) = 169.9$, $p < 0.001$). Relative changes within samples showed that when a bun was added, tender intensity decreased by -11.9%.

7.4. Discussion

This study aimed to compare the sensory properties and acceptance of commercially available plant-based burger patties eaten alone and in combination with other foods and/or condiments. It was hypothesised that (i) combining plant-based burger patties with a bun and/or sauce masks off-flavours of the plant-based burger patties. For both plant-based patties (hemp, soy) negative drivers of liking such as bitter taste, beany and nutty flavour intensity decreased with the addition of a bun and/or sauce, but positive drivers of liking such as meaty and fat flavour were also reduced, only partly confirming the hypothesis. While these off-flavours were reduced in soy and hemp patties by the addition of a bun and/or sauce, the perception of these off-flavours did not disappear. This suggests that the off-flavours can only be partly mitigated by the addition of a bun and/or sauce, but clearly cannot be supressed completely. This demonstrates that inherent off-flavours in the soy and hemp patties were strongly pronounced and the addition of tomato sauce with a strong desirable flavour was insufficient to mask the off-flavour..

Previous studies reported that the sensory perception of individual food components was affected by addition of other components (Gonzalez-Estanol, Cliceri, et al., 2022; Meinert et al., 2011; Paulsen et al., 2012; van Eck et al., 2021; van Eck et al., 2019a). The overall flavour

intensity of the tomato sauce is likely to be higher than the overall flavour intensity of the bun, so it could be argued that the stronger tomato sauce flavour mitigates the off-flavours more than the blander flavoured bun. Remarkably, the reduction in off-flavour intensities was larger upon addition of a bun than upon addition of sauce. It could be that the dry character of the bun and the difference in weight (31.1±2.0 g) and volume of the bun compared to tomato sauce (5.4 ± 0.3 g) limited aroma release in the oral cavity and thus inhibited perception. Further studies are needed to better understand why a bun mitigated the off-flavour more than the tomato sauce.

Overall, the hemp patty had more intense off-flavours than the soy patty and masking by addition of a bun and/or sauce was also different across these two patties, highlighting that masking effects are product dependent. This is in line with previous studies showing that the addition of different carriers affected the sensory perception of the product of interest in different ways. For example, Cherdchu & Chambers IV (2014) investigated the effect of carrier additions on the perception of soy sauce. They found that solid white rice and grilled chicken had a stronger effect than chicken broth on the sensory perception of the sauce (Cherdchu & Chambers IV, 2014). The presence of a companion food reduced consumers' ability to discriminate sensory attributes between regular and sodium-reduced tomato sauce but not in regular and sodium-reduced soy sauce (Nguyen & Wismer, 2020). It should be noted that when interpreting relative comparisons between plant-based patties, these patties were commercially available and differed in composition not only regarding hemp and soy.

Secondly, it was hypothesised that (ii) combining plant-based burger patties with a bun and/or sauce masks texture defects of plant-based burger patties. As expected, mouth drying increased while juiciness decreased upon addition of a bun to patties. The addition of a dry bun absorbed moisture and saliva during consumption, which reduced the lubricity of the bolus and led to an increase of mouth drying and a decrease of juiciness. Texture defects such as dryness and lack of juiciness and tenderness have been shown to have a strong negative impact on hedonic response to plant-based products (Carter et al., 2020; Chigwedere et al., 2022; Sha and Xiong, 2020; Yeom et al., 2020).

Tenderness and juiciness are attributes related to high quality and influence overall liking of meat and analogue products such as patties, steaks and sausages (Chen et al., 2022;

Schreuders et al., 2021). Their perception in meat is partly impacted through lubrication by fats, oils, water and stimulation of saliva during mastication (Calkins and Sullivan, 2007; Legako, 2019). When plant-based patties were combined with sauce, juiciness increased, suggesting the addition of sauce assisted in the lubrication of the patty. This increased lubrication by a liquid component in composite foods resulting in a reduced dryness perception has been previously described (Gonzalez-Estanol, et al., 2022; van Eck et al., 2019b). However, addition of tomato sauce did not increase tenderness perception, indicating that tenderness is more related to in-mouth sample fragmentation, while juiciness might be related to saliva incorporated into the bolus and product moistness (Rizo et al., 2019).

Lastly, chunky perception was most present in the hemp patties. Hemp patties displayed a very heterogeneous structure as exemplified in Table 1. These structural heterogeneities likely restricted matrix breakdown during mastication preventing formation of a cohesive bolus, ultimately leading to the perception of chunky texture. This texture defect decreased with the addition of a bun and a bun and sauce but not with the addition of sauce. It is speculated that during mastication the bun acted as glue binding the hemp patty particles together to form a more cohesive bolus, decreasing the perception of chunky texture, which did not happen with the addition of sauce.

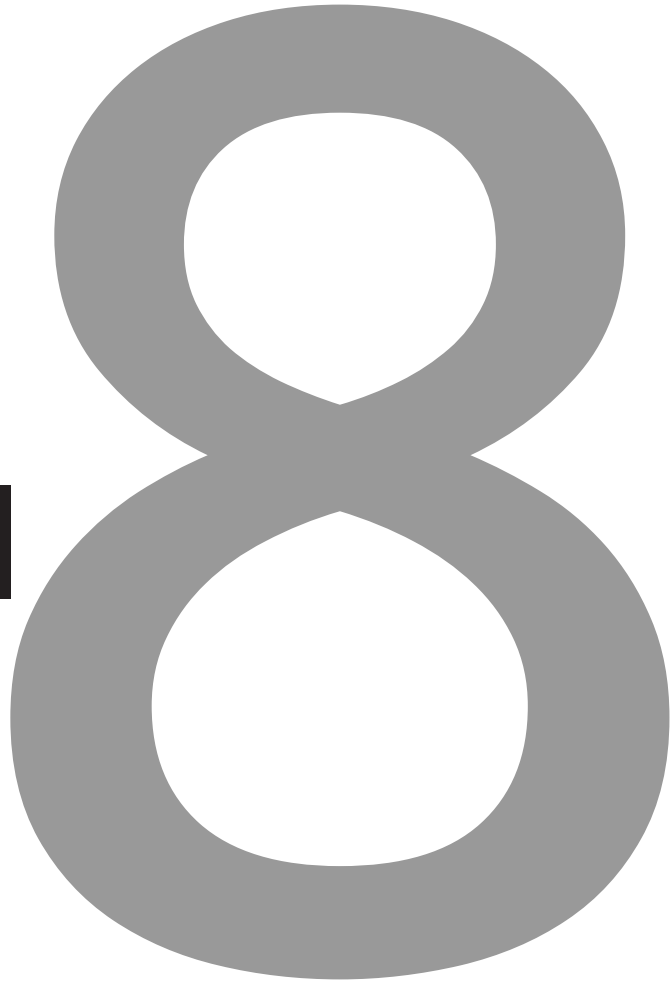
Thirdly, it was hypothesised that (iii) combining plant-based burger patties with a bun and/or sauce increases liking of the plant-based burger patties. Liking scores did not change for the plant-based patties when patties were combined with a bun and/or sauce. This was unexpected as it was speculated that liking increases with increasing sensory complexity and with increasing texture and flavour contrast by addition of a bun and/or sauce. Although only one patty type was assessed within a session, allowing for direct comparison between samples by the participants, only differences in liking between patties which were assessed in different sessions were significant with beef patties having the highest liking followed by soy and hemp patties. Here, adding a bun and tomato sauce to patties did not change liking. As described above, flavour and texture defects of these plant-based burger patties could only be mitigated to a limited extent by combining them with a bun and tomato sauce. Notably, these reductions in off-flavour and texture defects were not sufficient to impact liking. Addition of other burger components such as mayonnaise, pickles, lettuce and tomatoes may increase liking of the burger compared to the patty consumed alone.

7.5. Conclusions

This is the first study to explore the impact of the addition of two components (bun and tomato sauce) to beef and plant-based burger patties to create a simplified burger model for assessing patty sensory properties and liking. This study compared sensory perception and liking of beef and plant-based patties consumed alone and in combination with a bun and/or tomato sauce. When combined with a bun and/or sauce, inherent off-flavours and texture defects of the hemp- and soy-based patties could be mitigated but only to a limited extent. The off-flavours and texture defects of the hemp- and soy based patties were too strong to be fully masked by the addition of a bun and/or tomato sauce. Flavour and texture defects of plant-based burger patties can only be mitigated to a limited extent by combining them with a bun and sauce. Reductions in off-flavour and texture defects of the plant-based patties were not sufficient to impact liking but the data demonstrates the importance of understanding product performance in realistic consumption scenarios.

This study did not explore the effect of combining patties with additional components such as mayonnaise, pickles, salad, onions, tomatoes, and cheeses into a burger. Addition of further components with strong and desired flavours and texture might be beneficial to reduce off-flavours and texture defects further and consequently increase liking. Future studies should explore the impact of addition of these components to plant-based patties on off-flavours and texture defects.

CHAPTER



GENERAL DISCUSSION

GENERAL DISCUSSION

The research described in this thesis aims to integrate instrumental aroma analysis (*in vivo* nose-space analysis), sensory analysis and oral processing characterization to investigate the aroma release and perception of composite foods. Figure 8.1 shows a schematic summary of the individual and composite foods that were used (depicted in orange), the different effects that were studied; food structure and composition (in turquoise), oral behaviour (in pink), practical applications (in green) and the methodologies that were used (in scales of grey) through all chapters. Methodological aspects are summarized in Section 8.1, the main findings and interpretations are summarized and discussed in Section 8.2, methodological challenges, and future prospects (Section 8.3) and main conclusions (Section 8.4) are described.

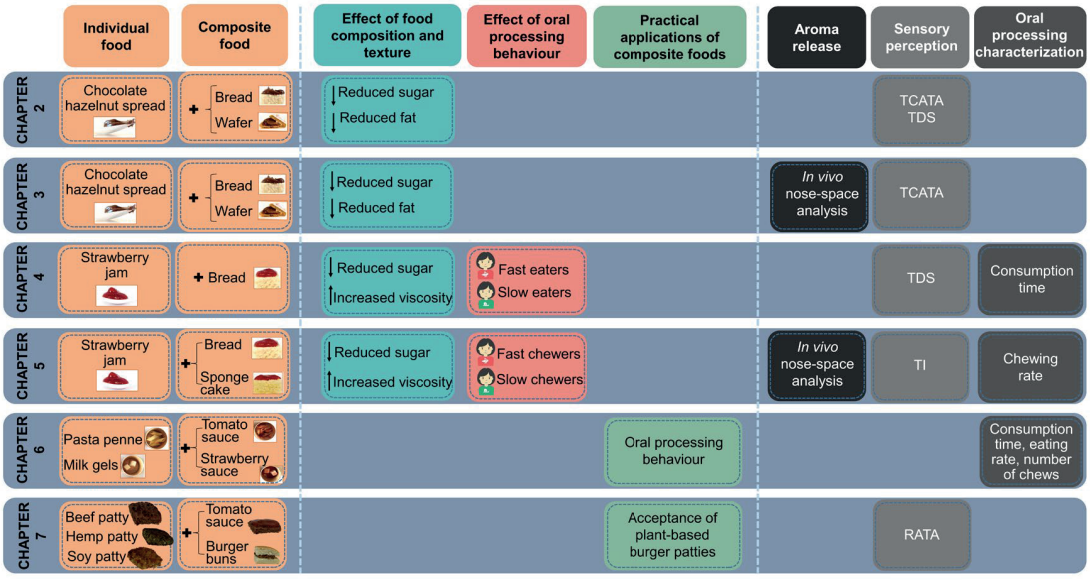


Figure 8. 1 Schematic overview illustrating the samples, varied and studied properties, and methodologies employed in each chapter.

8.1 Methodological aspects

8.1.1 Combining foods

Although sensory evaluations are typically performed on a single food, this thesis investigated the underexplored area of aroma release and perception of composite foods, which is more representative of daily life consumption contexts. Throughout the thesis,

different single foods varying in composition, mechanical properties and sensory characteristics were combined to form commonly consumed composite foods (Figure 8.2). Through Chapters 2 to 5, a fat (chocolate-hazelnut spread) and a sugar (strawberry jam) based products belonging to the sweet spreads market were used. Properties such as fat, sugar and viscosity were varied. Composite foods were formed by combining them with a wafer biscuit, or with a sponge cake, both of which represent commercial products, and with bread, which is one of the most common ways to consume sweet spreads. In Chapter 6, common salty and sweet composite foods were formed by combining pasta with tomato sauce and milk gels with strawberry sauce (pannacotta dessert) . Lastly, in Chapter 7, beef, hemp, and soy burger patties were combined with burger buns and/or tomato sauce to create a simplified burger model. This 'composite foods' approach, provided more representative sensory profiles that fit better with natural eating contexts than when using individual foods.

8.1.2 Combining tools

Throughout this thesis a multidisciplinary approach was used, allowing for a better understanding of aroma release and perception mechanisms during the mastication of composite foods. Regarding sensory perception, TDS (Chapters 2 and 4), TCATA (Chapters 2 and 3), and TI (Chapter 5) were used to assess the dynamic sensory perception of single bites of composite foods. In Chapter 2, it was shown that TCATA and TDS methodologies could be extended from single foods evaluations (Ares et al., 2015, 2017; Nguyen et al., 2018) to sensorial analysis of more complex composite foods. TCATA and TDS both gave complementary information; while TCATA allowed better discrimination between spreads, and TDS more clearly revealed the temporality of sensations. In Chapter 7, RATA methodology was used to evaluate the sensory properties of beef- and plant-based burger patties.

In Chapters 3 and 5, to understand aroma perception better, *in-vivo* nose-space analysis with PTR-MS coupled with dynamic sensory perception (TCATA and TI) was used to track the release and perception of target molecules. The simultaneous coupling of PTR-ToF-MS with TI/TDS profiling allowed detection of the presence of cross-modal interactions between food texture and aroma perception in complex, composite food matrices and to quantify the impact of mastication behaviour on in vivo aroma release and perception.

With respect to oral processing behaviours, in Chapters 4 and 6, video recordings were used to track jaw movements during food consumption. Following previous studies, (Aguayo-Mendoza et al., 2020; C G Forde et al., 2013a; Ketel et al., 2019; van Eck et al., 2019b), it was possible to assess how different products affect the chewing process and parameters such as the number of chews, chewing rate, total consumption time, and eating rate, were extracted. Furthermore, in Chapter 6 it was demonstrated that HUT is a valid approach to characterize the oral processing behaviours of consumers. The HUT approach allows studying oral processing behaviours in a somewhat more realistic setting than sensory booths or laboratory settings, because consumers eat the foods at home while sitting in front of a computer with a camera.

Regarding the type of panels used for the sensory evaluations, most of the studies were done with largely naïve consumers (Chapters 2, 4, 6 and 7). In Chapter 3, a trained panel consisting of eight assessors, was used for the simultaneous measurement of *in vivo* nose-space and TCATA evaluation, which is uncommon during TCATA evaluations where a larger number of observations are typically used. However, in this study it was demonstrated that the TCATA data from a consumer panel (n= 72) and from a trained panel during nose-space analysis (n= 8, in triplicate) generally perceived similar sensory attributes as applicable over time. Consequently, a direct comparison of aroma release and perception was possible using the TCATA data from the consumer panel, which showed a higher statistical power because of the number of observations.

8.2 Main findings and interpretations

The main findings of this thesis are summarized based on the effects of food composition and structure, carrier addition, and oral processing behaviours on aroma release and perception and on the practical applications of composite foods (Figure 8.2).

8.2.1 Effect of food composition and texture on aroma release and perception of composite foods

As shown in Figure 8.2, modulation of sugar content (in chocolate-hazelnut spread (Chapters 2 and 3), and in strawberry jam (Chapters 4 and 5)) had limited effect on aroma release and

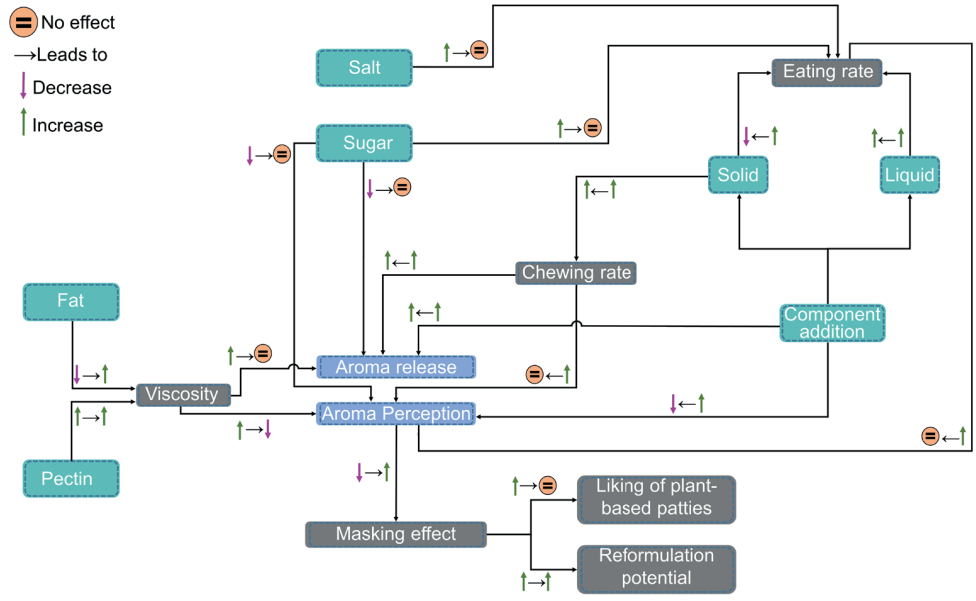


Figure 8. 2 Flow diagram illustrating the impact of altering a specific variable on aroma release and perception.

perception, because they displayed similar profiles to their references' counterparts. It has been shown that the influence of mono- and disaccharides on flavour release, and consequently on flavour perception, is rather limited, especially when concentration changes remain within realistic product formulations (Delarue and Giampaoli, 2006). This could partly explain why the degree of sugar reduction was not sufficient to induce significant differences in in vivo aroma release and perception between the reduced-sugar products and the references. In the case of fat, it was expected that it would have a strong effect on aroma release and perception due to the hydrophobic nature of the target molecules used in the chocolate-hazelnut spread. Although the effect on aroma release was not significant, fat reduction caused an increase in viscosity (Figure 8.2), leading to stickiness perception, and consequently, perception of aromas associated with the chocolate-hazelnut spread decreased (Chapters 2 and 3). Similarly, strawberry jams with a higher pectin content, led to an increase in viscosity, resulting in an overall increment of stickiness perception, a delay in the perception of strawberry-related attributes (Chapter 4) and in a decrease of citrus aroma perception (Chapter 5). These results are in line with previous studies reporting that an increase in viscosity induced a decrease in aroma perception (Baines and Morris, 1987; van Eck et al., 2021; Wendin et al., 1997).

It is important to note that sugar and fat reductions and the addition of pectin remained within realistic product formulation boundaries. As stated earlier, this could partly explain why the degree of reformulation in these studies was not sufficient to cause larger differences in aroma release and perception.

8.2.2 Effect of component addition on aroma release and perception of composite foods

Contrary to the relatively small effects observed with product formulation, there was a strong effect of component addition (composite foods) in *in vivo* aroma release and perception (Figure 8.2). When bread, wafer, sponge cake (Chapters 2, 3, 4 and 5), and burger buns and/or tomato sauce (Chapter 7), were combined with sweet spreads, jams and burger patties, respectively, a contrasting texture was added to the product. It has been shown that *in vivo* aroma release and perception of foods made of different components (composite foods) differ from those of single foods, because the characteristics of one component influence aroma release and perception of the other component (Scholten, 2017; van Eck et al., 2021). For instance, the dynamic sensory profiles of carrier-spread/jam combinations in TCATA, TDS and TI showed that the early stages of the consumption were dominated by attributes related to the flavour of the solid component (carrier) (e.g., bread-like, wafer-like), and to its mechanical properties (e.g. crunchy, pasty). It was only during the later stages of mastication that sensations related to the spreads/jams characterized the sensory profiles. One explanation for this could be that participants' attention shifted from the perception of one food component to the other within one single bite during the mastication process. It could also be that some texture sensations such as crunchiness are sensed with the first bite, whereas for taste and flavour to be perceived, the tastant and volatile compounds need to be released from the food matrix to reach the gustatory and olfactory receptors, which is a process that is facilitated by the oral breakdown of the foods and hence, it only occurs at later stages of the mastication cycle.

Overall, these findings agree with a study where carriers (bread, carrot) and mayonnaise were combined in one bite and TDS showed that the dynamic sensory profiles of the carrier-mayonnaise combinations were dominated by the texture of the solid carriers, especially in the beginning and end of consumption, whereas additional sensations related to the

mayonnaises appeared in the middle stage of consumption. (van Eck et al., 2019a). Besides a delay, a decrease in intensity aroma perception was also observed when a component with contrasting texture was added, possibly due to the distraction of the consumer's attention away from the product of interest. These results suggest the presence of cross-modal texture-aroma interactions in the role of aroma perception of composite foods. (Cherdchu and Chambers IV, 2014; Meinert et al., 2011; Paulsen et al., 2012). As demonstrated through Chapters 2, 3, 4 and 5, composite foods have an increased stimulus complexity, which may have caused a decrease in sensitivity to detection of differences between reformulated chocolate-hazelnut spreads and strawberry jams. This is in line with previous studies where perceptual differences between spreads or toppings disappeared upon the addition of carriers because sensitivity to detect sensory differences between them decreased (Cherdchu and Chambers IV, 2014; Gonzalez-Estanol et al., 2022a; Nguyen and Wismer, 2020; van Eck et al., 2021). A similar effect was also observed with plant-based patties (Chapter 7). The addition of buns and/or tomato sauce managed to mitigate, to some extent, inherent off-flavour and texture deficiencies of soy and hemp burger patties. This was in agreement with Meinert et al. (2011) who showed that the addition of gravy to vegetables (broccoli, cauliflower and potato) reduced the flavour intensity of vegetables (Meinert et al., 2011). Similarly, Paulsen et al. (2012) showed that the addition of sauces to salmon reduced salmon flavour (Paulsen et al., 2012).

Ultimately, these results mean that there could be more flexibility in the design of healthier foods (e.g. low in calories, reduced fat, reduced sugar, reduced salt, plant-based products) because it increases the reformulation space (Figure 8.2). For example, based on the dynamic sensory evaluation of reformulated chocolate-hazelnut spreads alone, it would be concluded that the reduced fat spread (15% fat reduction) does not qualify as a reformulated spread because it does not provide a similar sensory experience as the control spread. In contrast, based on the dynamic sensory evaluation of the reformulated spreads-carrier combination, it would be concluded that the reduced fat spread (15% fat reduction) qualifies because a reformulated spread as it provides a similar sensory experience as the control spread when consumed with carriers, which in fact, represents a more natural consumption context (Chapter 2). In the case of plant-based meat replacers, which are often perceived with off-

flavour and texture defects, by combining them with other food components, these deficiencies could be mitigated or even fully masked (Chapter 7).

Contrary to the decrease in aroma perception, *in vivo* aroma release was generally enhanced (Chapters 3 and 5) by the addition of carriers (Figure 8.2). This could be explained by the introduction of chewing and adaptation of the masticatory behaviour to the hardness of the carriers, causing the breakdown of the food into many particles, thus, increasing the surface area and the aroma transfer from the bolus to the saliva and air. This increment in aroma release and decrease in the perception of aroma intensity of composite foods was also observed in a previous study where mayonnaises were combined with carriers (van Eck et al., 2021).

An effect of the carrier type was also observed. For instance, the perception of certain attributes of chocolate-hazelnut spread and strawberry jams was affected in different ways by the addition of wafers or bread or by the addition of bread or sponge cake, respectively. For example, the decrease of stickiness perception in chocolate-hazelnut spreads was more pronounced in the spread-wafer combinations than in the spread-bread combinations (Chapter 2). In contrast, the presence of sponge cake decreased the perception of citrus aroma in strawberry jams more than the presence of bread (Chapter 5). Lastly, in the case of plant-based patties, the addition of burger buns decreased the intensity of nutty and beany flavours more than the addition of tomato sauce (Chapter 7). It may be that oral processing behaviour and bolus formation were affected differently by each component, leading to different taste, aroma, and texture sensations. This is in agreement with the study from Van Eck et al. (2019a) who showed that toppings (cheese, cream cheese and mayonnaise) affected the sensory perception of carriers (bread, cracker) by influencing structural transitions during oral processing (van Eck and Stieger, 2020).

8.2.3 Effect of oral processing behaviour on aroma release and perception of composite foods

Even if aroma compound properties can account to some extent for diversity in the shape of the release curves kinetics, interindividual differences in oral processing behaviours remain an important factor in understanding aroma release and perception. It is during oral processing, especially of many semisolid and solid foods, that the total surface area of food

particles increases, facilitating the release of taste and volatile compounds from the food matrix into the mouth and nose, respectively, enhancing the taste perception (Braud and Boucher, 2020; Mosca et al., 2015). In Chapter 4 and 5 it was shown that large differences in habitual eating speed and in chewing rate (fast, slow) had limited effect on dynamic sensory perception of composite foods. This is in general agreement with a previous study where it was shown that short- and long- duration eaters perceived the same sausage similarly at the beginning of consumption but perceived the texture of the sausage differently from the middle towards the end of the evaluation (de Lavergne et al., 2015). Regardless of the lack of effect on perception, a faster chewing rate led to a higher *in vivo* aroma release compared to a slower chewing rate (Chapter 5). This suggests that the increase in *in vivo* aroma release probably caused by an increase in structural breakdown (total surface area of bolus fragments) due to increased mastication being too limited to cause considerable changes in aroma perception.

8.2.4 Effect of composite foods on eating behaviour

It is well-known that food texture and degree of lubrication affect eating rate (Bolhuis et al., 2014; C. G. Forde et al., 2013; Lasschuijt et al., 2017; McCrickerd et al., 2017; Zijlstra et al., 2009). Foods that require more chewing and lubrication take more time to be orally processed and, therefore, are consumed with lower eating rates. In Chapters 4 and 6 it was demonstrated that the addition of liquid components influenced the mastication behaviours of solid foods, by decreasing consumption time and increasing eating rate (Figure 8.2) , possibly because the added liquid component provided lubrication and reduced mastication time needed to form a safe-to-swallow bolus . These results are in line with those of Gavião et al. (2004) and Engelen et al. (2005) who showed that spreading butter on toast decreased the time and number of chewing cycles until swallowing (Engelen et al., 2005; Gavião et al., 2004). Similarly, van Eck et al. (2019b), showed that bread and crackers, when combined with cheese spread and mayonnaise, were chewed shorter and with fewer chews than single bread and crackers (van Eck et al., 2019b). Through Chapters 4, 5 and 6, the effect of food texture (addition of carriers) on oral processing behaviour was shown. Specifically, in Chapter 6, it was demonstrated that modulations of taste properties (salty, sweet) have a negligible effect on oral processing behaviours. This was in accordance with a study by Lasschuijt et al. (2017)

where neither number of chews, chewing duration, chewing rate nor eating rate, were significantly affected by the sweetness intensity of model gels (Lasschuijt et al., 2017).

As stated in Section 8.1.2, composite foods have an increased stimulus complexity, which may mask certain attributes detected if the product was consumed on its own. In Chapter 7, it was shown that combing plant-based patties with other foods (burger buns and/or tomato sauce), inherent off-flavours and texture defects of the hemp and soy-based patties could be mitigated. However, reductions in these attributes were not sufficient to impact the liking of the patties (Figure 8.2), which highlights the difficulty of masking off-notes in these novel products.

8.3 Methodological challenges and future research

Although this thesis went a step further and investigated foods that consist of multiple components that better represent the real consumption context, in five out of six studies single-bite assessments were used. Because perception is dynamic, it might be that a single bite may not be sufficient to capture the whole consumption experience. Although single-bite assessments were used for practical reasons, future research should consider not only the evaluation of composite foods rather than their individual components (this thesis) but also evaluations of multiple bites because they could have the potential to evaluate more accurately the dynamic profile of products.

Factors such as interindividual and environmental factors have been shown to influence eating behaviour and perception. As the focus of the first part of the thesis (Chapters 2 and 3) was to understand perception of composite foods varying in properties, these studies were performed with young, healthy, Caucasian women. On one hand, this raises the question of whether results could be generalized to other population groups. For example, Chapter 4 studied the natural oral processing behaviour of a relatively homogeneous group, n=105 (age 24.8 ± 4.73 years, BMI 22.8 ± 2.49 kg/m^2, 38% male). However, the magnitude of the effects that were observed might be different in different population groups, for example in an elderly population where taste and eating capabilities (e.g. changes in salivary flow and composition, tooth loss and/or reduced jaw muscle strength) (Aguayo-Mendoza et al., 2020).

On the other hand, it has been demonstrated that a high interindividual 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), lead to differences in aroma release from the food, impacting perception. Future research should take these interindividual variations into account when performing *in vivo* aroma release studies.

It is well-known that oral processing behaviours impact satiation and food intake, so future research should focus on understanding the role that every single food plays in a heterogenous food or a meal context in oral processing behaviour. This will further help to capture the complex nature of the mastication process and support the design of foods that can lead to lower overall energy intake while maintaining good sensory characteristics, fullness, and satiety.

8.4 Conclusions

To date, most of the published works that have attempted to relate aroma release and sensory perception are far from mimicking the real consumption context, because assessments often do not consider food oral processing during mastication and are performed on single or model foods. The research described in this thesis aimed to use a multidisciplinary approach to investigate the relative contributions that food structure and its composition and oral processing behaviour have on aroma release and perception of food matrices made up of different components and contrasting textures, so-called composite foods.

The results in this thesis showed that the processes of aroma release and perception of composite foods are multidimensional phenomena and seem to be modulated by cross-modal texture-aroma interactions. Consumers' ability to discriminate between foods was reduced and off-flavours and texture deficiencies were masked when a food was assessed in combination with other foods. Furthermore, the role of oral processing behaviour on the breakdown of the food impacting aroma release and perception was highlighted.

The use of a multidisciplinary approach provided more useful and accurate sensory profile representing an ecological, valid food consumption context. It was highlighted that not only food composition and structure, but also cognitive factors and eating behaviours should be

considered for successfully developing healthier food products with lower fat and sugar content or elaborated with plant protein. Furthermore, composite foods could be an strategy to help modulate eating behaviour, (e.g. Development of long chewable food structures to increase transit time in the oral cavity leading to a reduction in eating rate).

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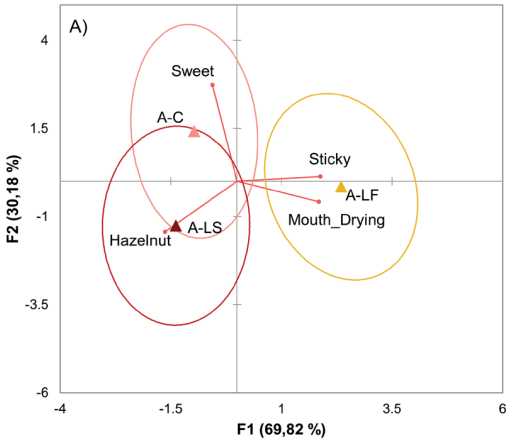
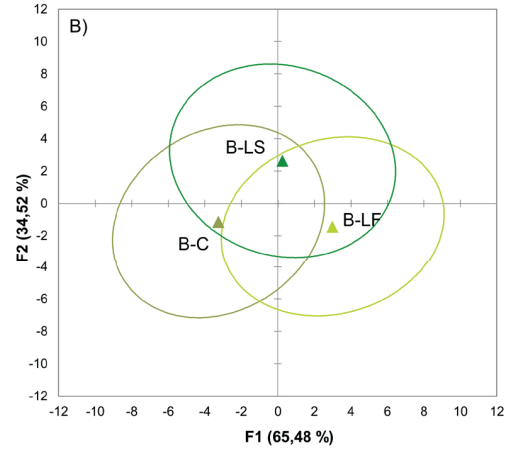
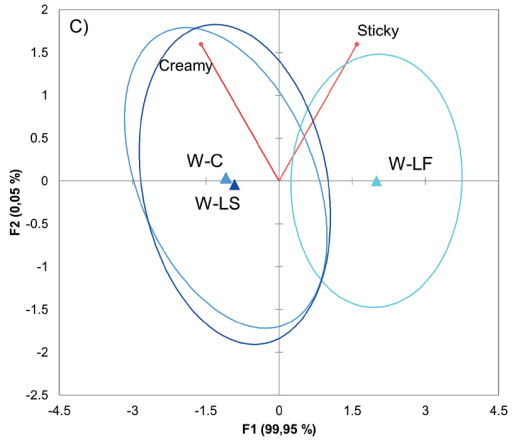
SUPPLEMENTARY MATERIALS

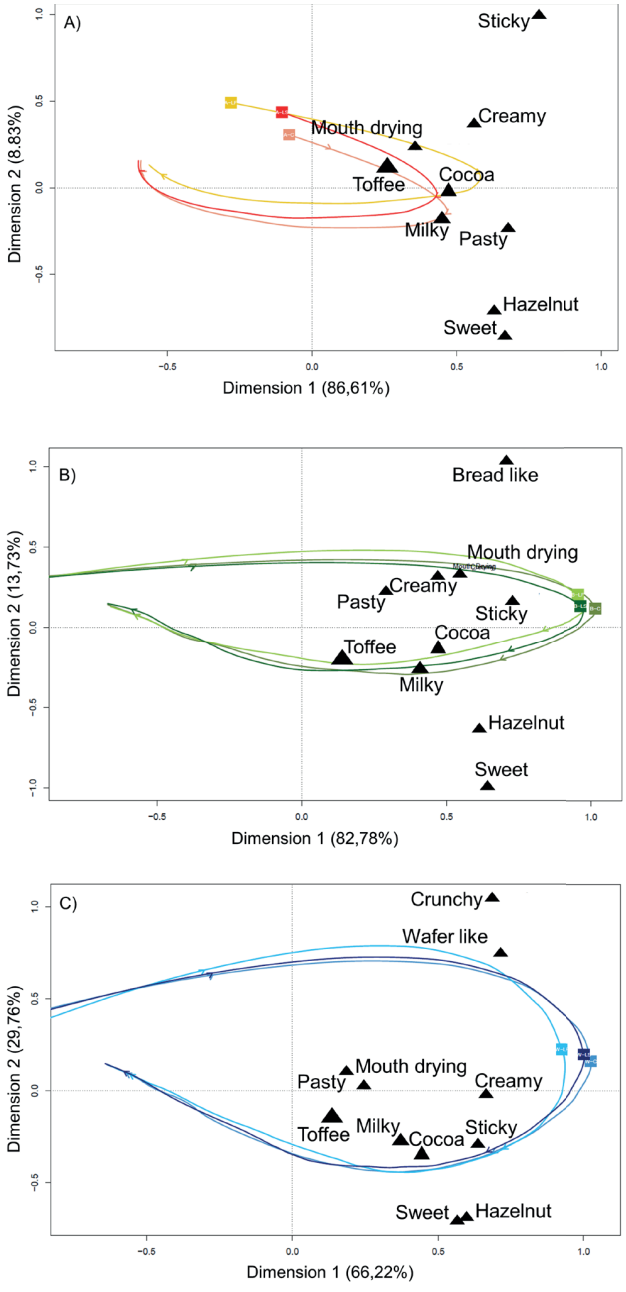
Supplementary Materials



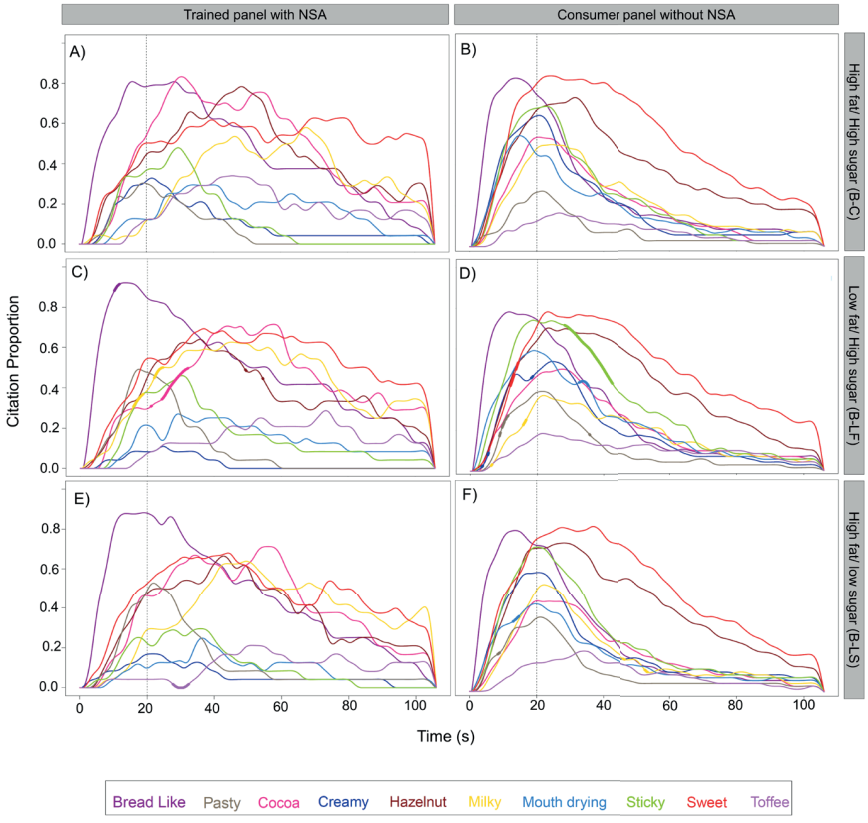




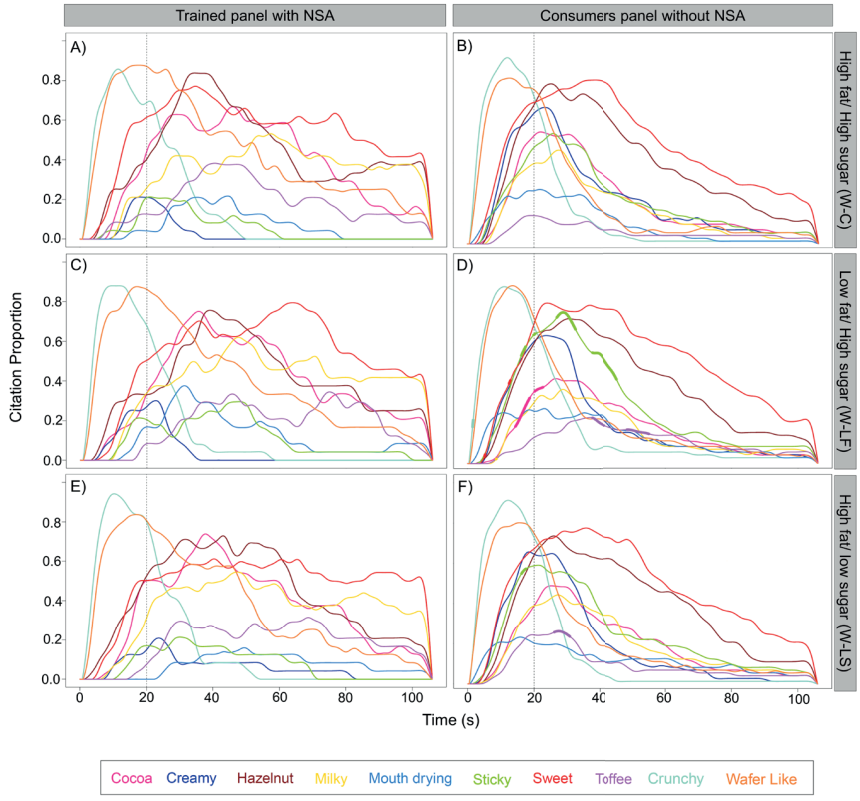
SM 2. 1 Principal Component Analysis (PCA) of the durations of dominant sensations from the TDS data with confidence ellipses of 0.90. Biplot showing dimensions 1 and 2 represents the sensory profiles of the three chocolate hazelnut spreads (C, LF, LS) served alone (A), combined with bread (B), and combined with wafer (C). Only the attributes that were significantly different ($p<0.05$) are shown



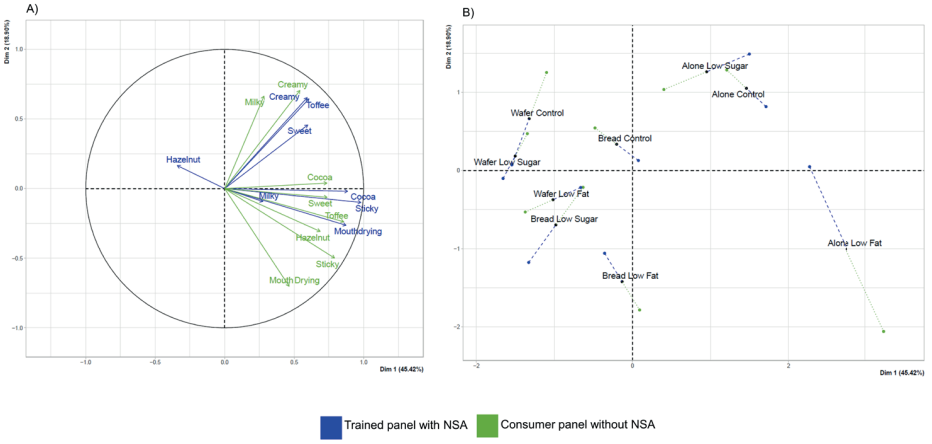
SM2. 2 Principal Component Analysis on the mean citation proportions from TCATA data. Biplot showing dimensions 1 and 2 representing the sensory trajectories of the three chocolate hazelnut spreads (C, LF, LS) served alone (A), combined with bread (B), and combined with wafer (C). Product trajectories are smoothed, and the symbol > Indicates the direction of the trajectory at different time intervals.



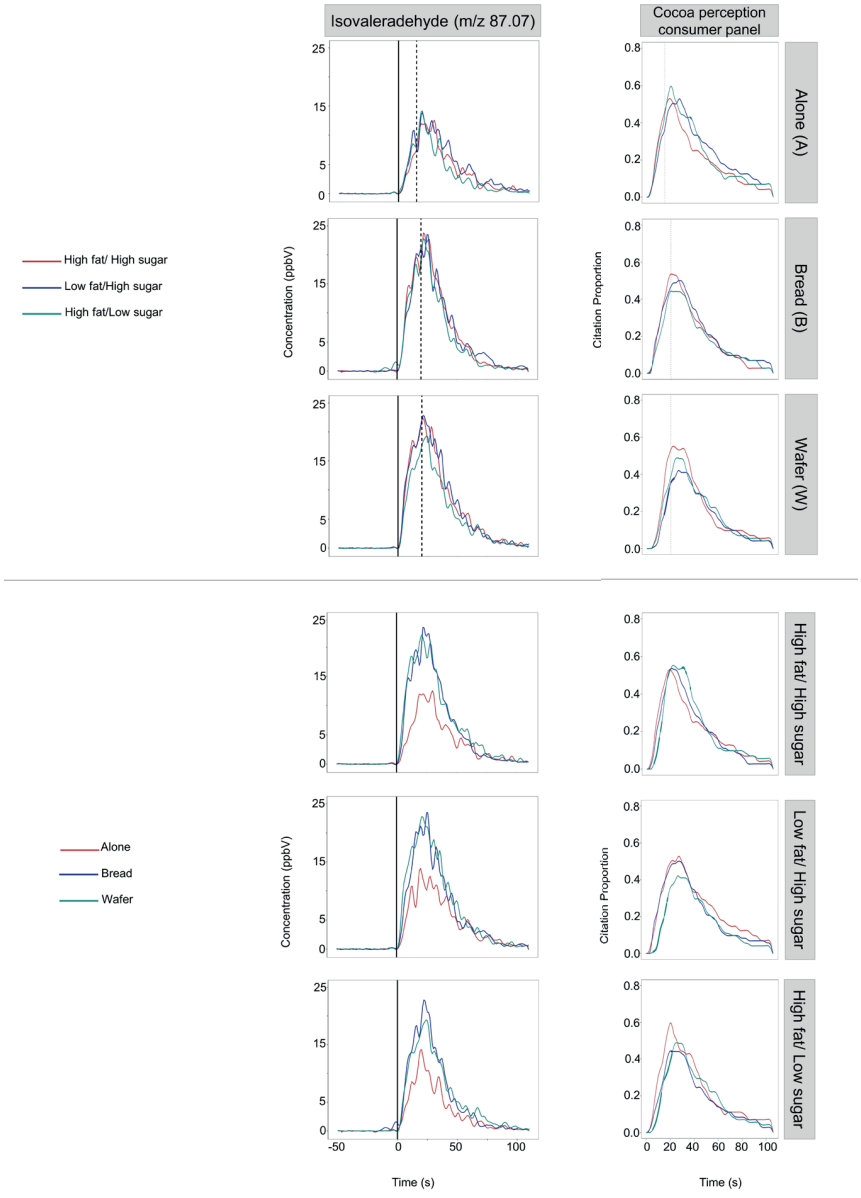
SM 3. 1 Trained panel with nose space analysis (NSA) (n=8,triplicate) and consumers panel without NSA (n=72) TCATA curves for three chocolate hazelnut spreads served on bread (B-C, B-LF, B-LS). Periods of significant differences ($p<0.05$) in proportion of citations between B-LF and BC and between B-LS and B-C are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.



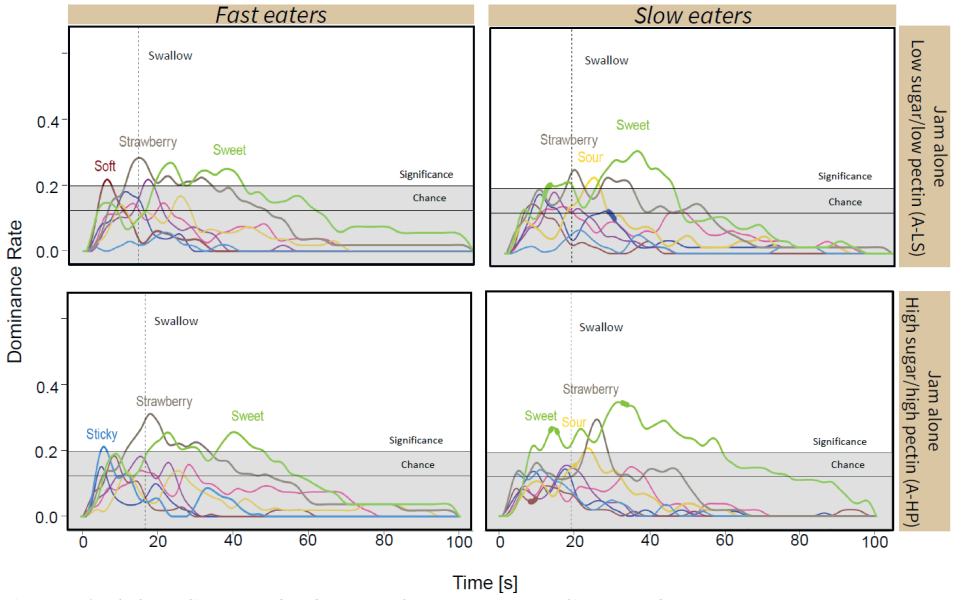
SM 3. 2 Trained panel with NSA (n=9, triplicate) and consumers panel without NSA (n=72). TCATA curves for the three chocolate hazelnut spreads served on wafer (W-C, W-LF, W-LS). Periods of significant differences (p<0.05_ in proportion of citations between W-LF and W-C and between W-LS and W-C are indicated by highlighted thick sections. The vertical dotted line represents the swallowing moment.



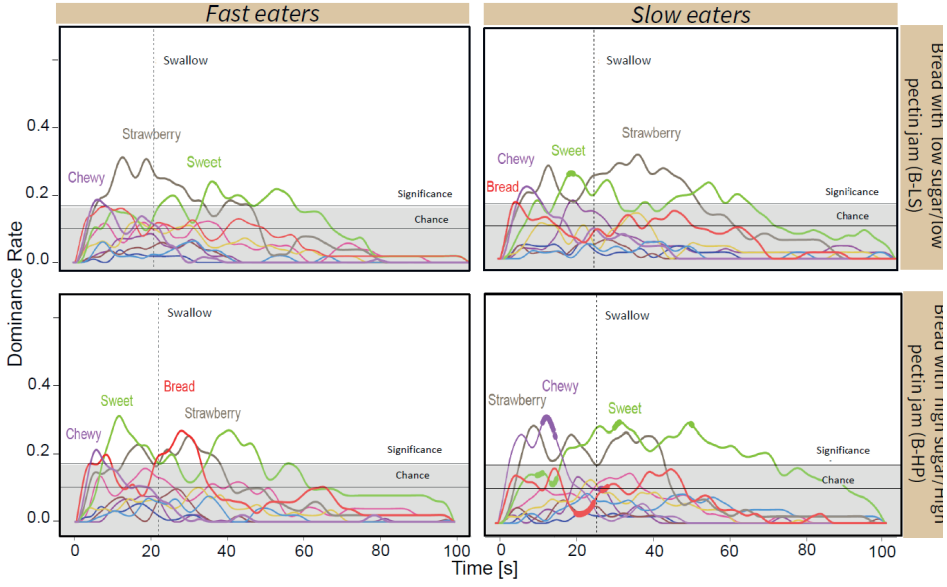
SM 3. 3 Representation of the first two dimensions of the MFA space showing correlation circle(A), and individual factor map (B) for sensory descriptors based on AUC values from TCATA with the trained panel with nose space analysis (NSA;n=8, triplicate; blue) and consumers panel without NSA (n=72;green).



SM 3. 4 Averaged in vivo nose space release of Isovaleraldehyde (n=8 triplicate) and TCATA curves of the consumers panel without NSA (n=72) for attribute Cocoa comparing effects of carrier and formula on aroma release and perception Black continuous lines represent moment when samples were put in the mouth, and dotted lines represent swallowing moment Periods of significant differences (P<0 05) in proportion of citations in TCATA curves, compared to spread alone (and control formula (are indicated by highlighted thick sections



Appendix 4. 2 TDS curves for fast (n=53) and slow (n=52) eaters for the reduced sugar strawberry jam served alone (A-LS) and high pectin strawberry jam served alone (A-HP). Periods of significant differences ($p < 0.05$) of dominance rates between slow and fast eaters are indicated by highlighted thick sections. The vertical dotted line represents the average swallowing moment.



SM4. 1 TDS curves for fast (n=53) and slow (n=52) eaters for the reduced sugar strawberry jam with bread (B-LS) and high pectin strawberry jam with bread (B-HP). Periods of significant differences ($p < 0.05$) of dominance rates, between slow and fast eaters are indicated by highlighted thick sections. The vertical dotted line represents the average swallowing moment.

ABOUT THE AUTHOR

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Karina Gonzalez Estanol was born on the 4th of November 1992 in Mexico City, Mexico. In 2011 she enrolled in the BSc program of Food Engineering at Universidad Iberoamericana in Mexico City. In 2016, Karina enrolled in the MSc of Food Technology with specialization in Sensory Science at Wageningen University in The Netherlands. For her MSc thesis she collaborated with Unilever and investigated the sensory perception of ice creams differing in sugar content using a multiple-bites approach. She had the opportunity to do her internship at the Riddet Institute in New Zealand where she investigated how the sensory perception of chocolate milk changed when it was sipped directly from the cup or using a straw. In November 2019, Karina was appointed as a PhD candidate at Center of Agri-food and Environmental Sciences, Trento University and Research and Innovation Centre, Edmund Mach Foundation with a partnership with the department of Food Quality and Design, Wageningen University. Her research focused on understanding aroma release and perception of composite foods during consumption. During her PhD project, Karina attended various courses and presented her work at several international conferences. Furthermore, she was involved in teaching and supervising MSc theses. Currently, she is the chair of the Next Generation group of the European Sensory Society.

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A

LIST OF PUBLICATIONS

Papers in peer-reviewed Journals

Gonzalez-Estanol, K., Libardi, M, Biasioli, F., & Stieger, M. (2022).Oral processing behaviours of liquid, solid and composite foods are primarily driven by texture, mechanical and lubrication properties rather than taste intensity . Food and Function.

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Gonzalez-Estanol, K.; Cliceri, D.; Khomenko,I; Biasioli, F.; Stieger, M; (2022). In vivo aroma release and perception of composite foods using nose space PTR–ToF–MS analysis with Temporal-Check-All-That-Apply. Submitted for publication to Food Research International

Gonzalez-Estanol,K.; Orr,R.; Hort,J.; Stieger,M.;(2022) Can flavour and texture defects of plant-based burger patties be mitigated by combining them with buns and tomato sauce? Submitted for publication to Food Quality and Preference

Gonzalez-Estanol, K., Fontova-Cerda, M., Biasioli, F., & Stieger, M. (2022). In vivo monitoring of flavour release using PTR-MS: effect of chewing rate and food composition. In preparation

Poster/Oral Presentations at conferences

Gonzalez-Estanol, Karina; Khomenko, Iuliia; Fontova-Cerda, Monica; Stieger, Markus; Biasioli, Franco. (2022). In vivo monitoring of flavour release using PTR-MS: effect of

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Awards

2021 Fonterra award to best poster presented at the Food Oral Processing Conference 2021 entitled “Differences in consumption time of composite foods lead to small differences in dynamic sensory perception”

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