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A stepwise approach investigating salivary responses upon multisensory food cues

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ABSTRACT

Exposure to sensory food cues such as smell, vision, taste and/or texture may trigger anticipatory physiological responses such as salivation, participating on adequate metabolism of the signaled food. However, the individual contribution of each sensory modality as well as the impact of particular food products on salivation and salivary composition remains unclear. Therefore, by systematically varying sensory modalities and nutrient content of food stimuli, we investigated their effect on saliva secretion, α-amylase activity and other salivary characteristics (pH level, buffering capacity, MUC5B concentration, and total protein content). Over 3 sessions, 46 normal-weight healthy participants were exposed to 12 conditions, consisting of 4 levels of sensory stimulation (odor, odor + vision, odor + vision + taste, and odor + vision + taste + mastication) and 3 types of stimuli (bread, high-in-starch; cucumber, low-in-starch; and parafilm as non-food control) during which saliva was collected. Linear mixed models showed a significant increase in salivation with increasing levels of sensory stimulation. α-amylase secretion rate increased upon the highest level of stimulation, which involved mastication, compared to odor and odor + visual level of stimulation. Other salivary characteristics varied with the level of sensory stimulation, which might be related to the total volume of salivation. The type of stimuli did not influence the saliva composition (α-amylase concentration nor other salivary components). Our findings indicate that cumulative sensory information, rather than specific (food) product, play a vital role in anticipatory salivary responses.

1. Introduction

We are continuously exposed to sensory food cues that trigger physiological responses thereby affecting our appetite and, as a consequence, food intake [1,2]. (Multi)sensory food cues, such as sight, smell or taste of a food, may induce a rapid release of saliva in the oral cavity, this response is known as cephalic-phase salivary response [3–6].

Salivation depends on a complexity of factor such as food related cues, general health, sex, etc. [7,8]. On top of that glandular differences contribute to saliva properties. For example, saliva secreted by parotid glands is characterized by being serous and rich in α-amylase, while submandibular and sublingual glands produce viscoelastic, mucin-rich saliva [9–13]. Each component of the saliva is attuned to serve a particular function. Salivary α-amylase is involved in the digestion of starch (hydrolysis of polysaccharides into maltose and dextrin) [7,14]. Levels of electrolytes, mainly bicarbonate, increase on mastication and stimulation of the parotid glands, provide a buffering action against acidic foods in order to maintain a neutral pH. Mucins, mainly MUC5B, impact viscosity of the saliva and are responsible for lubrication of the food bolus during mastication and swallowing, and during speaking. Lastly, proteins lubricate and protect teeth surfaces by forming a thin layer (pellicle) in the oral cavity [7–9,13,15]. The secretion of mucins and protein is likely constant and its concentration may decrease upon a high saliva secretion [7].

Although some research suggests that salivation might not be conditioned to sensory cues [16] but mainly produced by muscle movements [9,17], others have shown that salivary responses could be conditioned [18–21]. Moreover, research has demonstrated that salivation increases upon (multi)sensory exposure to various foods as

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anticipatory response [22–32]. However, little is known about the influence of sensory cues on salivary composition, in particular related to food digestion. Our previous research showed that exposure to unisensory (olfactory) cues, representing foods varying in taste quality and macronutrient content, enhanced saliva secretion [33]. Yet these cues did not result in alterations in salivary viscoelasticity and α-amylase and lipase activities. A recent study has shown similar α-amylase concentration after bread odor exposure and after mastication of bread [34]. Others have shown increased levels of α-amylase secretion rate or starch hydrolysis products by modified sham feeding of high-starch food products [35–37]. Modified sham feeding encompasses all sensory modalities including smell, sight, taste, and mastication of a stimulus but the bolus is spat out before swallowing it [38]. Nevertheless, to the best of our knowledge the contribution of individual sensory modalities associated with specific food products to cephalic-phase salivary responses has not yet been investigated.

Previous reports suggest that (multi)sensory food cues may signal the nutrient composition of the foods leading to cephalic-phase salivary responses to facilitate ingestion and further digestion [1,4]. Therefore, the aim of the current study was to systematically determine the influence of different levels of sensory stimulation (2 anticipatory levels (Odor and Odor + Vision) and 2 consummatory levels (Odor + Vision + Taste and Odor + Vision + Taste + Mastication), and specific food products (bread and cucumber, which vary in their starch content) on cephalic-phase salivary response. It was hypothesized firstly, that saliva secretion rate would increase with adding levels of sensory stimulation. Secondly, we expected increased α-amylase secretion rate when exposed to bread (food high-in-starch) compared to cucumber (low-in-starch) or non-food control stimuli with higher levels of sensory stimulation. Thirdly, we expected that salivary characteristics such as pH and buffer capacity would increase, while mucin and total protein concentration would decrease with the increase of saliva secretion rate, thus upon the level of sensory stimulation.

2. Materials and methods

2.1. Participants

Healthy Dutch female participants between 18 – 35 years old were recruited in Wageningen and surrounding area. We recruited only female participants due to physiological differences in the salivary gland size between sex [39,40]. After registration, potential participants were invited to a screening session to determine their eligibility. Participants were included when having a body mass index (BMI) of 18.5 - 25 kg/m^2 (overweight and obese people tend to have greater salivation upon food cues [28]), classified as normosmic (scoring ≥12 on the Sniffin' Sticks 16 items odor identification test [41]), liked the investigated food products (>40 mm on a 100 mm visual analogue scale; VAS, anchored by "Not at all" to "Very much"; reporting in Table S1), liked and were familiar with bread and cucumber odor (>40 mm on a 100 mm VAS), and when they correctly identified these odors in a multiple forced-choice task. Smokers were excluded, as well as participants who had any allergy, intolerance or oversensitivity to the foods used in this study, used medication other than paracetamol and hormonal contraceptives, were pregnant or had the intention to become pregnant during the experiment or were currently breastfeeding, or suffered from dry mouth (score >33 out of 55, where 24 to 39 represents 'suffers moderate' to 'suffers a lot from xerostomia', we adjusted the cut-off to >33, occasional dry mouth, for our healthy participants) assessed through the Xerostomia Inventory [42,43]. After screening 64 volunteers, a total of 46 female participants were included to the study (**Table 1**).

This study was conducted in accordance with the Declaration of Helsinki (revised in 2013) and approved by the Medical Ethical Committee of Wageningen University (NL51747.081.14). Participants signed a written informed consent at the beginning of the screening session. They were compensated via a monetary voucher at the end of the study.

Table 1
Characteristics of the 46 participants in the current study.

Characteristic	Mean ± SD
Age (years)	21.9 ± 2.0
BMI (kg/m^2)	20.9 ± 1.6
Odor Identification Score	13.7 ± 1.2
Xerostomia Inventory Score*	20.9 ± 3.8

* Xerostomia Inventory classification: 11–23 = 'does not suffer' to 'suffer slightly' from xerostomia [43].

2.2. Design

This study consisted of a 3 stimuli type x 4 levels of sensory stimulation cross-over design. Over 3 test sessions, participants were exposed to 12 conditions differing in type of stimuli, including bread (food high-in-starch), cucumber (low-in-starch), and parafilm (as control), as well as levels of sensory stimulation encompassing odor, odor + vision, odor + vision + taste, and modified sham feeding (odor + vision + taste + mastication). During each test session, the participants were randomly exposed to four conditions, one of each level of sensory stimulation (see **Fig. 1**).

2.2.1. Stimuli and levels of sensory stimulation

Three types of stimuli were investigated: two food stimuli: bread (high-in-starch, 74% polysaccharides [44]) and cucumber (low-in-starch, 0% polysaccharides [44]), and parafilm (Parafilm ¨M¨, Bemis Company, Inc., North America) was used as control. Moreover, the food products were selected as they are common in a Dutch diet, and therefore familiar to the participants.

For all three stimuli, participants were exposed to four different levels of sensory stimulation. **Odor (O) -** 15 mL of the food odors were placed in amber opaque glass bottles: bread flavor (205361- Symrise, Holzmiden, Germany, 8% in PG) and cucumber flavor (15311331- International Flavors & Fragrances, IFF, New York, USA, 100%). For the control, 0.3 g (5 × 5 cm sheet) of parafilm was placed in amber opaque glass bottles. By orthonasal olfaction, participants smelled the odor stimulus and their whole mouth saliva was collected simultaneously for 5 min (spitting every 30 s and kept on ice, as described in more detail in Section 2.3). **Odor + Vision (O+V) –** The odor stimuli described above were combined with a computer screen showing matching pictures from the 'Food-pics_extended' database [45]): white bread (#439) and cucumber with slices (#267). A picture of the parafilm was created by the researchers (see **Fig. 1**). Whole mouth saliva was collected during 5 min of simultaneous exposure to an odor stimulus and a matching picture on a computer screen. **Odor + Vision + Taste (O +V+T) –** We standardized weight and diameter of real products to minimize differences in oral experience of sample size. All stimuli were cut in a 4.5 cm diameter circle, representing a normal bite-size. Bread (white casino bread, Jumbo, Veghel, the Netherlands) was toasted 2 min before presenting it using a toaster (Tefal Principio, Groupe SEB, Rumilly, Haute-Savoie, France) set to level one. One piece of bread without crust was 3.2 $g \pm 0.2$. A slide of cucumber was 3.5 $g \pm 0.2$. A double layer of a round (4.5 cm diameter) piece of parafilm was 0.3 g. Participants held the stimulus against the palate with their tongue during the first 15 s. In the next 20 s they expectorated the stimulus in a pre-weighted cardboard cup, rinsed their mouth with tap water and swallowed once more before saliva collection. Whole mouth saliva was collected during the subsequent 1 min (spitting every 30 s). This procedure was repeated five times to obtain a total of 5 min saliva collection, and based on previous literature [35,46–48]. As shown in **Fig. 1**, stimuli were presented on a plate containing 5 round cut pieces of the stimulus plus a 'full version' of the stimulus (a slice of bread, half of a cucumber, two 5 × 5 cm sheets of parafilm) to maintain odor and

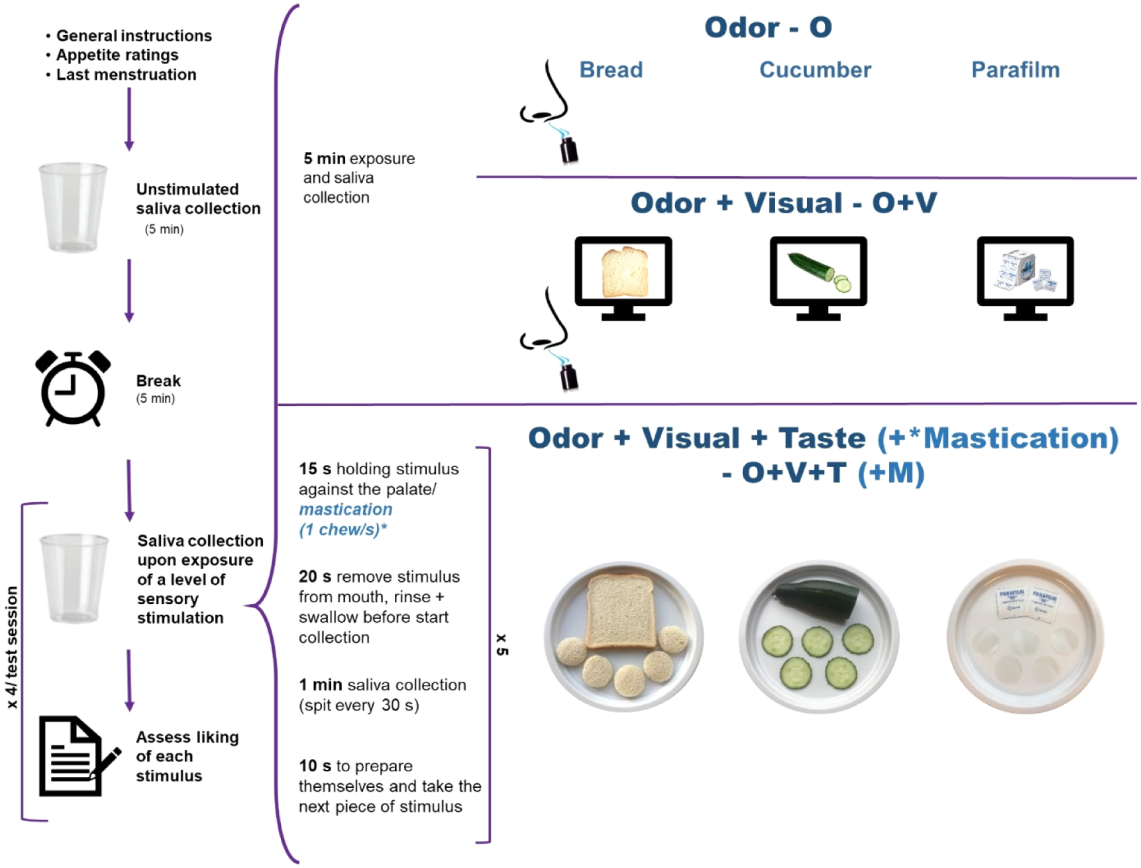

Fig. 1. Schematic overview of each test session. Bread and cucumber pictures in the O + V condition were taken from food-pics_extended database [45].

visual stimulation during the 5 min saliva collection. **Odor + Vision + Taste + Mastication (O + V + T + M) –** We used the same real products described above. Participants were asked to chew the stimulus during the first 15 s (1 chew/s), instead of being held against their palate as described for O + V + T. The following steps were the same as described for O + V + T.

2.3. Exposure to multisensory cues and saliva collection

The test sessions followed a similar procedure as described previously [33] and were held at the sensory booths at Wageningen University & Research during the morning (9.30 to 11.30 h). Participants were scheduled at the same time during the three test sessions with at least one day in between. They were asked to refrain from drinking alcohol 12 h before the study; to avoid wearing fragranced products on the day of the test session; to avoid vigorous exercise on the morning of the test session; refrain from acid and caffeinated beverages 4 h before the study; avoid eating and drinking anything except water in the 2 h prior to the test session; refrain from daily dental hygiene measures including use of mouthwash or chewing gum 2 h before the study; avoid stressful activities at least 30 min before the test session. Each test session lasted around 60 min and instructions were given through EyeQuestion® (Version 3.11.1, Logic8 BV, Elst, the Netherlands).

On arrival to each test session, participants rated their appetite by assessing their hunger, fullness, prospective consumption, and desire to eat on 100 mm VAS anchored by 'not at all'- 'very much'. They then indicated the first day of their last menstruation cycle, which was considered as a potential covariate because it may influence certain salivary outcomes. Next, they were asked to rinse their mouth with deionized, distilled water, empty their mouth in a plastic container and to wait for one minute. On the first test session, they underwent a 30 s trial to get familiar with the collection technique, 'passive drooling' method [49]. Instructions on the screen indicated them to sit down with a slightly tilted head, allowing the saliva to gather in the mouth and to expectorate saliva into a container once in every 30 s. The average weight of those containers was determined in advance. They were instructed to avoid swallowing or moving their mouth or tongue and not to speak during the collection. After 1-min break, participants were asked to collect their unstimulated saliva for 5 min, spitting every 30 s, into an empty polypropylene 25 mL container (Böttger, Germany), After collecting unstimulated saliva, and between conditions, participants had a 5-min break. They were then randomly exposed to a condition, for which they received a stimulus and an empty ~~pre-weighed~~ container to collect their saliva as specified above, depending on the level of sensory stimulation. The containers were kept on ice during the saliva collection. After each condition, participants assessed liking of the stimulus on a 100 mm VAS anchored by 'not at all - very much' (see supplementary material **Table S1**). An overview of the procedure is presented in Fig. 1. A total of 690 samples were collected. All the saliva samples were immediately weighed and kept on ice until the determination of pH and buffer capacity. After these measurements, samples were clarified by centrifugation (10 min, 4 °C, 10,000 *g*) to remove cellular debris and food residues. The clarified saliva was diluted 1:1 v:v with 150 mM NaCl, to avoid aggregation and precipitation of proteins, aliquoted, and stored at −20 °C until α-amylase and mucin analysis [40]. Depending on the total amount of saliva collected, we kept at least 1 aliquot of ≤1.5 mL per sample.

2.4. Measurements

2.4.1. Saliva secretion rate

Whole mouth saliva was weighed shortly after collection by an analytical gravimetric scale (Adventurer™ Pro, OHAUS Europe GmbH, Greifensee, Switzerland), assuming that 1 g is equal to 1 mL [50]. The

average weight of the containers was subtracted from the final container weight with the collected saliva. Saliva secretion was divided by the 5-min of collection time and therefore we used mL/min as final units.

2.4.2. Salivary α-amylase concentration and secretion rate

Salivary α–amylase was measured by analysis of colorimetric-based enzymatic activity. Saliva samples were diluted 1:50 with HPLC-grade water. Ten μl of diluted saliva and 90 μl of amylase substrate, 2-chloro-4-nitrophenyl-α-D-maltotrioside (Sigma-Aldrich, St Louis, MO, USA), were added in each well of 96 wells ELISA-microplate (655101, Greiner Bio-One B.V, Alphen aan den Rijn, the Netherlands). α–amylase cleaves the substrate into 2-chloro-4-nitrophenol, a yellow compound. The absorbance of this compound was measured by Multiskan™ FC Microplate Photometer using SkanIt™ Software 3.1 (Thermo Fisher Scientific, Waltham, MA, USA) at 405 nm directly after the addition of amylase substrate for 15 min. A reference of a known concentration of 3 U α–amylase standard was included in each plate. All measurements were performed in duplicate. Salivary α-amylase concentration was expressed as units per milliliter (U/mL). Additionally, we calculated the α-amylase secretion rate (U/min) considering the salivation rate (mL/min).

2.4.3. Other salivary characteristics

2.4.3.1. pH and buffering capacity. Before pH was measured, the samples were homogenized for 20 s by vigorous vortexing. Subsequently, the buffering capacity of the saliva was assessed by adding 1 mL of 0.01 M HCl (pH = 2) to 1 mL of saliva and briefly vortexed [51]. The buffering capacity could be assessed only when the collected saliva was ≥ 2 mL (554 out of the 690 samples). The pH of this solution was measured 30 s after mixing. A digital pH meter was used to assessed pH and buffer capacity (VOS-10001, VOS instrumenten B.V., Gelderland, the Netherlands).

2.4.3.2. Mucin 5B (MUC5B) concentration. An enzyme-linked immuno sorbent assay (ELISA) was performed to determine the amount of MUC5B in each of the saliva samples following the procedure described by Veerman and collaborators [52]. Samples were diluted 1:100 with coating buffer (0.1 M Na_2CO_3; pH = 9.6) and 200 μl was pipetted into the wells of a 96-well ELISA Microlon F-shape Microplate (Greiner Bio-One B.V, Alphen aan den Rijn, the Netherlands). Two-fold serial dilutions of each saliva sample were prepared in the previous coating buffer, in separate wells. The last row of the microplate was used as blank and only coating buffer was added. The microplates were incubated overnight at 4 °C to allow the adherence of the mucins to microplate surface. The next day the microplates were rinsed 3 times with phosphate buffered saline supplemented with 0.1% tween-20 (PBS-T) and blocked with 1% gelatin dissolved in PBS-T (PBS-T-G) and incubating at 37 °C for 1 h in a mini shaking incubator, with gentle shaking. Next, the microplates were washed with PBS-T to remove the unbound gelatin. Then 100 μl of the mouse-antibody F2 diluted 1:40 in PBS-T-G was added in each well [53]. After 1 h of incubation at 37 °C, the microplates were rinsed 3 times with PBS-T to remove unbound F2 antibodies. Next, 100 μl of conjugant rabbit α-Mouse – HRP (GeneTex, Inc., CA, USA) diluted 1:2000 in PBS-T-G was added to each well and the microplates were incubated for 1 h at 37 °C. Then the microplates were rinsed 5 times with PBS-T and once with demineralized water. The substrate solution used was a TMB buffer mixture (3.75 mL 3,3′,5,5′-Tetramethylbenzidene (TMB) in dimethylsulfoxide (DMSO) dissolved in 150 mL TMB buffer supplemented with 30 μL H_2O_2). A total of 100 μl of this solution was added to each well. The reaction was stopped after all the wells turned into a light blue color (< 5 min) by adding 50 μl of 0.1 M H_2SO_4 to each well. The absorption was measured using a Multiskan™ FC Microplate Photometer using SkanIt™ Software 3.1 at 492 nm (Thermo Fisher Scientific, Waltham, MA, USA). A reference saliva sample (from a pool of unstimulated saliva from 10 healthy volunteers) was added to each microplate. The concentration of MUC5B is a relative difference taking the reference sample into account and is reported as absorbance units (AU). All measurements were done in duplicate.

2.4.3.3. Total protein concentration and secretion rate. Total protein concentration was measured by means of a Protein Assay Kit (Pierce BCA, Thermo Fisher Scientific, Waltham, MA, USA), according to the recommendations of the manufacturer. Samples were diluted 1:1 with HPLC-grade water and 20 μl was pipetted into the wells of a 96-well polystyrene microplate (Greiner Bio-One B.V, Alphen aan den Rijn, the Netherlands). A standard curve of bovine serum albumin was performed in the first two rows of each microplate. The reaction was made by adding 180 μl of BCA reagents (50:1, BCA reagent A:B) into each well and incubating at 37 °C for 30 min in a mini shaking incubator. The absorbance was measured by Microplate Photometer using SkanIt™ Software 3.1 at 562 nm (Multiskan™ FC, Thermo Fisher Scientific). All measurements were performed in duplicate. The total protein content was expressed as mg/mL. Due to the limited amount of saliva, this experiment was performed in 70% of the total samples (483 samples out of the 690 samples). Total protein concentration was expressed in mg/mL and we calculated the total protein secretion rate (mg/min) taking the salivation rate (mL/min) into account.

2.4.4. Food recovery

Food recovery in O + V + T and O + V + T + M sensory levels was measured to confirm the compliance of the participants to refrain from swallowing the stimuli. Food recovery was measured taking into account the final weight of the cardboard cups where participants spat out the stimuli (held or masticated stimuli with saliva), the weight of the 5 stimuli which were weighed while preparing the plate, and the average weight of cardboard cups. Food recovery of the stimuli after O + V + T level was 115.8% ± 2.1 for bread, 98.1% ± 0.7 for cucumber and 119.9% ± 1.8 for parafilm. The recovery after O + V + T + M level was 132.6% ± 2.5 for bread, 109.7% ± 2.7 for cucumber and 127.7% ± 2.2 for parafilm. Most of the food recoveries exceeded the 100% due to saliva expectorated with the stimuli.

2.5. Statistical analysis

Parameters are shown as mean and standard deviation, unless otherwise specified. Results were considered statistically significant when $p < 0.05$. All statistical analyses were carried out with R (version 0.99.902, RStudio Inc, Boston, MA, USA [54]) and graphs were performed using Prism GraphPad 5.0 (GraphPad Prism Software). The R codes for all final models can be found in the supplementary materials.

After data collection, 8 outliers were removed from the whole data set due to low or high percentage of food recovery (6 data points from cucumber in O + V + T + M were ≤85% of food recovery, and 2 data points from parafilm in O + V + T + M were ≥200% of food recovery; [55]).

Within the same test session, unstimulated saliva secretion rate was subtracted from the saliva secretion rate upon each condition obtaining "change in saliva secretion rate" as final outcome.

Linear mixed models using *lme4* package [56] were performed to analyze liking of the stimuli, change in saliva secretion rate, salivary α-amylase concentration and secretion rate, pH, buffer capacity, MUC5B concentration and total protein concentration and secretion rate. The modeling followed a backward approach and the most parsimonious models were selected by comparing AIC and log-likelihood of the models. For each individual model, homoscedasticity and normal distribution of error terms, and correct specifications of the fixed and random parts of the model were checked. Square root transformation was performed when the model violated these assumptions, this was done for the α-amylase concentration and secretion rate models. In a first stage of the analyses, the level of sensory stimulation, was included

as fixed factor. In a second stage of the analyses, data was divided into the level of sensory stimulation. Individual models were performed for each level of sensory stimulation, with 'type of stimuli' as fixed factor. Participants and evaluation order nested in test sessions were considered as random factors, indicated by (1|*random effect*) in the results. However, after checking the random part of each model, most of the final models only included participants as random factors, except when indicated. potential covariates were systematically removed following this order: 1) participants characteristics (age, BMI, dry mouth sensation score, and phase of menstrual cycle which was categorized depending on the day of cycle in follicular, ovulation or luteal phase); 2) appetite ratings; 3) liking of the stimuli; 4) for α-amylase concentration and secretion rate, pH, and buffer capacity, their respective unstimulated data was also added as covariate; saliva secretion rate was added as covariate in the MUC5B concentration models. The unstimulated data of MUC5B concentration and total protein concentration and secretion rate were considered as a level of sensory stimulation condition. Post-hoc test with Bonferroni correction using *lsmeans* package [57] were performed when the fixed factor was significant. Pearson correlation analyses were done to determine the correlation between pH/buffer capacity and change in saliva secretion rate, on saliva measurements corrected for participants. First, we corrected change in saliva secretion rate by using a mixed model with change in saliva secretion rate as a dependent variable and participants as random effects (Correction_Saliva = lmer(ChangeinSaliva ~ (1|Participant)). The residuals of that model (Correction_Saliva) were saved and used to correlate with pH/buffer capacity.

3. Results

3.1. Change in saliva secretion rate

Levels of sensory stimulation had a significant impact on change in saliva secretion rates ($F(3,495) = 156.7$, $p < 0.0001$, change in saliva secretion rate ~ level of sensory stimulation + liking of the stimuli + (1| participants); **Fig. 2A**). O and O + V stimulation resulted in the lowest saliva secretion rate and did not differ significantly from each other; while O + V + T and O + V + T + M stimulation produced significantly higher saliva secretion rates.

Analyzing the data within each level of sensory stimulation, change in saliva secretion rate differed significantly between the different stimuli (O: $F(2,90) = 4.2$, $p = 0.019$; O + V: $F(2,90) = 3.6$, $p = 0.031$; O + V + T: $F(2,90) = 8.9$, $p < 0.001$; O + V + T + M: $F(2,83) = 36.7$, $p < 0.0001$, change in saliva secretion rate ~ type of stimuli + (1| participants); **Fig. 2B**). In general, salivation was highest upon exposure to bread, then cucumber and then parafilm.

3.2. Salivary α-amylase concentration and secretion rate

Square root of α-amylase concentration (U/mL) of the secreted saliva significantly decreased upon the level of sensory stimulation ($F(3,494) = 18.44$, $p < 0.0001$, sqrt (α-amylase concentration) ~ level of sensory stimulation + sqrt (α-amylase concentration) + (1| participants); for back-transformed data see **Fig. 3A**). However, square root of α-amylase secretion rate (U/min) significantly increased upon the level of sensory stimulation ($F(3,493) = 3.75$, $p = 0.011$, sqrt (α-amylase secretion rate) ~ level of sensory stimulation + sqrt (α-amylase secretion rate) + liking of the stimuli + (1| participants); for back-transformed data see **Fig. 3B**). O + V + T + M stimulation produced significantly higher α-amylase secretion rate (U/min) compared to O ($p = 0.046$) and O + V stimulation ($p = 0.011$).

When the data was analyzed within each level of sensory stimulation, only α-amylase secretion rate (U/min) after exposure to O + V + T was significantly different between the stimuli ($F(2,97) = 5.16$, $p = 0.007$, sqrt (α-amylase secretion rate) ~ type of stimuli + sqrt (α-amylase secretion rate) + liking of the stimuli + (1| participants); for back-transformed data see **Table 2**), where the exposure of parafilm and bread significantly increased the α-amylase secretion rate (U/min) compared to cucumber ($p = 0.016$ and $p = 0.027$, respectively).

3.3. Other salivary characteristics

3.3.1. pH level and buffering capacity

Similar to saliva secretion rate, level of sensory stimulation had an impact on salivary pH ($F(3,447) = 38.63$, $p < 0.0001$, pH ~ level of sensory stimulation + pH from the unstimulated saliva + (1| participants)) and salivary buffering capacity ($F(3,349) = 14.52$, $p < 0.0001$, buffer capacity ~ level of sensory stimulation + buffer capacity from the unstimulated saliva + liking of the stimuli + (1| participants)). The pH and buffering capacity after exposure to O + V + T + M were significantly higher compared to the other levels of stimulation (pH: *O* = 7.01 ± 0.03; O + V = 7.00 ± 0.03; O + V + T = 7.14 ± 0.03; O + V + T + M = 7.26 ± 0.03, $p < 0.0001$; buffering capacity: *O* = 3.98 ± 0.10; O + V = 3.88 ± 0.10; O + V + T = 3.94 ± 0.10; O + V + T + M = 4.47 ± 0.10, $p < 0.0001$). Moreover, saliva secretion rate was positively correlated with pH ($r(498) = 0.26$, $p < 0.0001$) and buffer capacity ($r(443) = 0.20$, $p < 0.0001$; cor.test(lmer(change in saliva secretion rate ~ (1| participants)), pH/buffer capacity)).

Within each level of sensory stimulation, with the exception of O + V, pH level of saliva differed after exposure to the different stimuli (**Table 2**; O: $F(2,83) = 3.32$, $p = 0.041$; O + V: $F(2,80) = 0.18$, $p = 0.84$; O + V + T: $F(2,82) = 25.71$, $p < 0.0001$; O + V + T + M: $F(2,78) = 9.98$, $p = 0.0001$; pH ~ type of stimuli + pH from the unstimulated saliva + (1| participants) for all the models, also desire to eat and

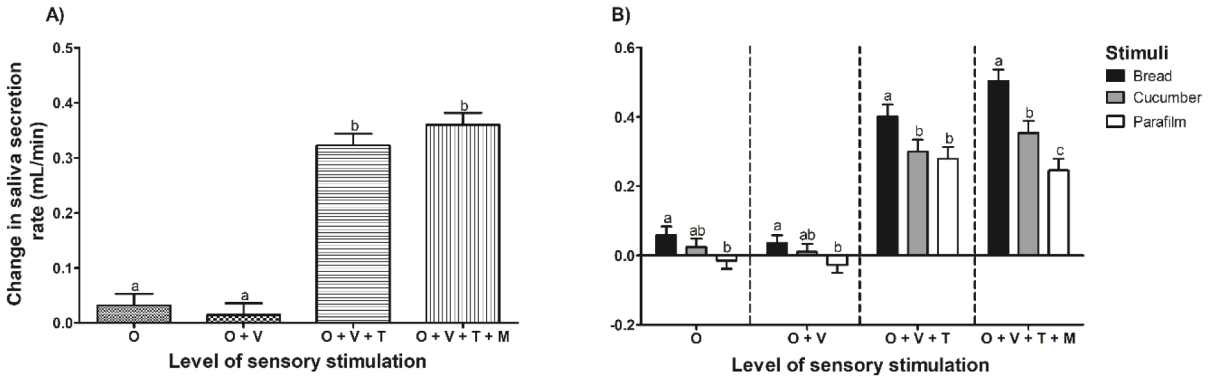

Fig. 2. Change in saliva secretion rate (mL/min; corrected for unstimulated saliva (mean 0.60 ± 0.02 mL/min)) upon different levels of sensory stimulation (A) and upon different stimuli within each level of sensory stimulation (B). Values are expressed as mean and standard error. Similar letters indicate no significant differences ($p > 0.05$) across level of sensory stimulation (A) and across stimuli type within each level of sensory stimulation (B). *O* = odor; O + V = odor + vision; O + V + T = odor + vision + taste; O + V + T + M = odor + vision + taste + mastication.

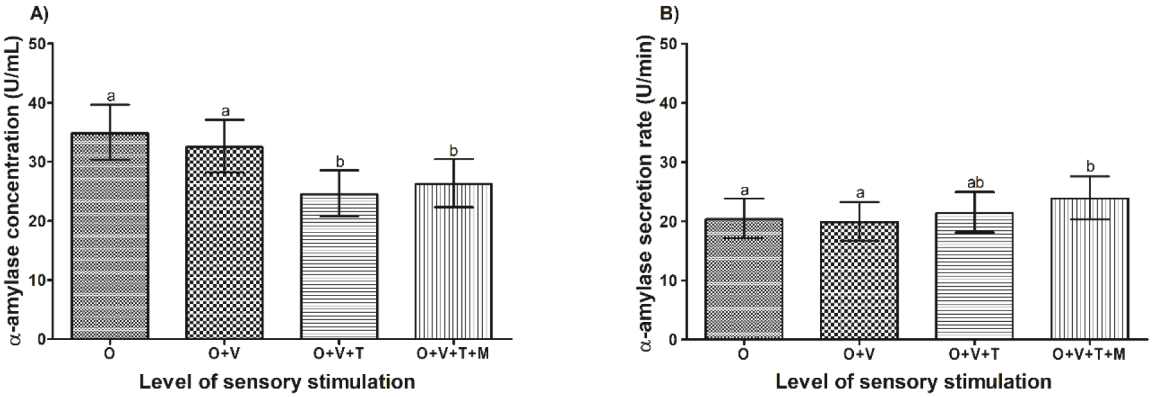

Fig. 3. Back-transformed of α-amylase concentration (U/mL; A) and secretion rate (U/min; B) of secreted saliva upon different levels of sensory stimulation. The data was analyzed in a square root scale; however, the data has been back-transformed by means of square, values are thus expressed as median (mean of square root data^2) and 95%CI ((mean ± 1.96*SE)^2). Similar letters indicate no significant differences ($p > 0.05$). *O* = odor; O + V = odor + vision; O + V + T = odor + vision + taste; O + V + T + M = odor + vision + taste + mastication.

Table 2
pH, buffer capacity, back-transformed α-amylase concentration (U/mL) and secretion rate (U/min), MUC5B concentration (AU) and total protein concentration (mg/mL) and secretion rate (mg/min) of the secreted saliva upon all exposure conditions. Values are expressed in median (mean of square root data^2) and 95%CI ((mean ± 1.96*SE)^2) for α-amylase data and in mean and standard error for the other outcomes. Similar letters indicate no significant differences ($p > 0.05$) within level of sensory stimulation.

Stimuli	Level of sensory stimulation			
	O	O + V	O + V + T	O + V + T + M
α-amylase concentration (U/mL)				
Unstimulated saliva		35.28 (29.70, 41.34)		
Bread	37.58 (31.88, 43.75)	36.24 (30.54, 42.43)	23.52 (19.37, 28.08)	23.23 (19.12, 27.74)
Cucumber	39.56 (33.71, 45.89)	39.56 (33.80, 45.78)	21.72 (17.78, 26.04)	24.21 (19.61, 29.29)
Parafilm	33.18 (27.90, 38.92)	34.81 (28.97, 41.18)	25.70 (21.37, 30.44)	33.06 (26.50, 40.34)
α-amylase secretion rate (U/min)				
Unstimulated saliva		20.16 (16.79, 23.83)		
Bread	22.56 (19.01, 26.42)	21.34 (17.50, 25.57)	21.81 (17.90, 26.10) [a]	24.98 (20.64, 29.73)
Cucumber	22.37 (18.85, 26.19)	23.26 (19.39, 27.48)	17.06 (13.41, 21.15) [b]	22.80 (18.28, 27.82)
Parafilm	18.40 (15.26, 21.85)	19.72 (15.89, 23.97)	26.11 (20.84, 31.98) [a]	29.48 (23.24, 38.48)
pH				
Unstimulated saliva		7.06 ± 0.03		
Bread	7.00 ± 0.03 [ab]	7.01 ± 0.04	7.26 ± 0.04 [a]	7.36 ± 0.03 [a]
Cucumber	7.08 ± 0.03 [a]	6.99 ± 0.04	6.97 ± 0.04 [b]	7.17 ± 0.03 [b]
Parafilm	6.97 ± 0.03 [b]	6.98 ± 0.04	7.16 ± 0.04 [a]	7.25 ± 0.03 [b]
Buffering capacity				
Unstimulated saliva		3.93 ± 0.07		
Bread	3.99 ± 0.11	4.01 ± 0.11	4.43 ± 0.15 [a]	5.09 ± 0.16 [a]
Cucumber	4.01 ± 0.11	3.70 ± 0.11	3.85 ± 0.14 [b]	4.32 ± 0.16 [b]
Parafilm	3.88 ± 0.12	3.83 ± 0.11	3.66 ± 0.14 [b]	3.98 ± 0.16 [b]
MUC5B concentration (AU)				
Unstimulated saliva		1513.59 ± 23.62		
Bread	1439 ± 34.80	1435 ± 35.40 [ab]	1337 ± 39.50	1336 ± 40.40
Cucumber	1433 ± 34.80	1386 ± 36.00 [a]	1330 ± 39.40	1354 ± 41.80
Parafilm	1420 ± 34.50	1476 ± 35.70 [b]	1365 ± 39.30	1339 ± 41.20
Total protein concentration (mg/mL)				
Unstimulated saliva		1.08 ± 0.05		
Bread	0.93 ± 0.07	0.95 ± 0.07	0.54 ± 0.04	0.70 ± 0.04
Cucumber	1.00 ± 0.07	0.89 ± 0.08	0.64 ± 0.04	0.68 ± 0.05
Parafilm	0.87 ± 0.07	0.96 ± 0.08	0.55 ± 0.04	0.63 ± 0.05
Total protein secretion rate (mg/min)				
Unstimulated saliva		0.68 ± 0.05		
Bread	0.69 ± 0.06	0.61 ± 0.06	0.58 ± 0.05	0.79 ± 0.05 [a]
Cucumber	0.67 ± 0.06	0.62 ± 0.06	0.61 ± 0.05	0.67 ± 0.06 [ab]
Parafilm	0.58 ± 0.06	0.57 ± 0.06	0.51 ± 0.05	0.54 ± 0.06 [b]

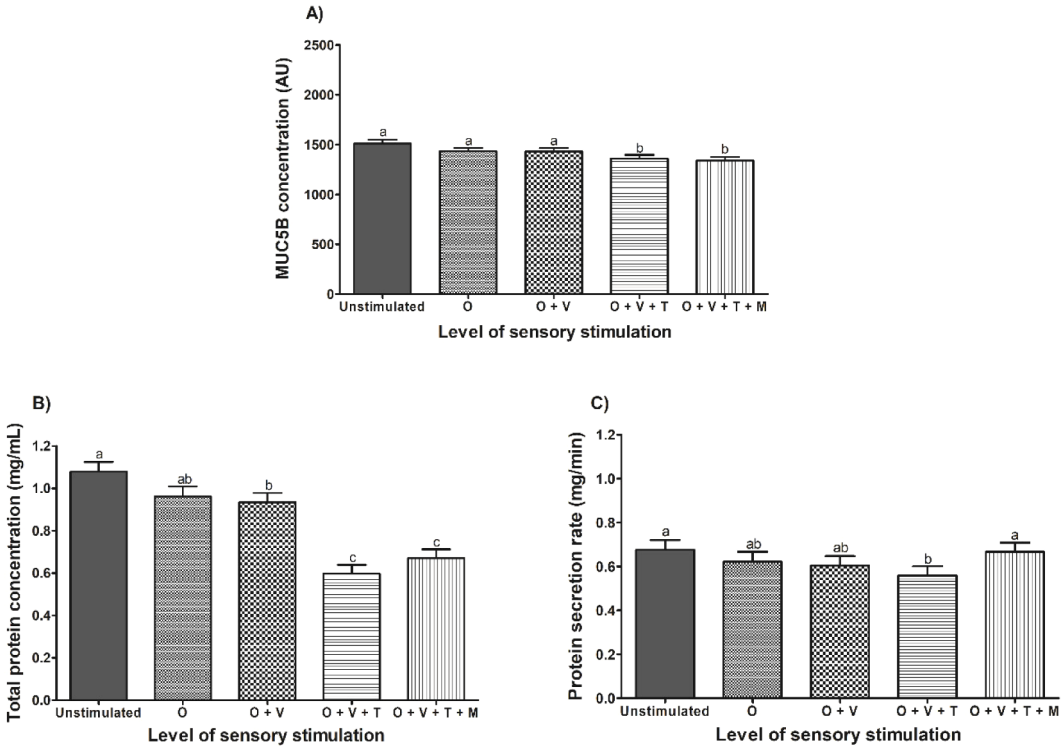

Fig. 4. MUC5B concentration (AU; A), total protein concentration (mg/mL; B) and protein secretion rate (mg/min; C) of secreted saliva upon different levels of sensory stimulation. Values are expressed as mean and standard error. Similar letters indicate no significant differences ($p > 0.05$). *O* = odor; O + V = odor + vision; O + V + T = odor + vision + taste; O + V + T + M = odor + vision + taste + mastication.

satiety were respectively included in O and O + V model). Buffering capacity of secreted saliva was significantly different between the different stimuli after exposure to O + V + T ($F(2,65) = 10.12$, $p = 0.0002$) and O + V + T + M ($F(2,60) = 21.42$, $p < 0.0001$; Table 2, all models: buffer capacity ~ type of stimuli + buffer capacity from the unstimulated saliva + (1| participants)). In both level of stimulation, the exposure of bread increased the buffer capacity of the saliva compared to cucumber and parafilm.

3.3.2. Mucin 5B (MUC5B) concentration

The concentration of MUC5B in the secreted saliva decreased significantly upon increasing levels of sensory stimulation ($F(4,77) = 12.58$, $p < 0.0001$, mucin concentration ~ level of sensory stimulation + satiety + prospective consumption + (1| participants) + (1| test session/evaluation order)). MUC5B concentration was significantly lower after O + V + T and O + V + T + M exposure compared to O and O + V exposure and unstimulated saliva. Moreover, MUC5B concentration in O and O + V-stimulated saliva did not differ from unstimulated saliva (Fig. 4**A**). MUC5B concentration significantly differed among the stimuli in the O + V sensory level ($F(2,86) = 3.12$, $p = 0.049$, mucin concentration ~ type of stimuli + (1| participants)), showing that the exposure of parafilm secreted a saliva with higher MUC5B concentration compared to the exposure of cucumber (Table 2, all models: mucin concentration ~ type of stimuli + (1| participants)).

3.3.3. Total protein concentration and secretion rate

Total protein concentration of secreted saliva significantly decreased upon increasing levels of sensory stimulation ($F(4,423) = 46.92$, $p < 0.0001$, total protein concentration ~ level of sensory stimulation + (1| participants)). Unstimulated and O and O + V-stimulated saliva had a higher protein concentration compared to O + V + T and O + V + T + M (Fig. 4**B**).

Protein secretion rate of secreted saliva is modified upon the levels of sensory stimulation ($F(4,421) = 3.68$, $p = 0.006$, protein secretion rate ~ level of sensory stimulation + (1| participants), Fig. 4**C**). Post-hoc testing revealed that the total protein secretion rate upon O + V + T sensory level was significantly lower compared to the total protein secretion rate upon unstimulated saliva and upon O + V + T + M (both $p = 0.02$). However, total protein secretion rate upon unstimulated, O, O + V and O + V + T + M did not differ significantly.

Within each level of sensory stimulation, total protein concentration was not affected by the different stimuli (Table 2, total protein concentration ~ type of stimuli + (1| participants) for all models). For protein secretion rate, there was a significant difference among the stimuli in the O + V + T + M sensory level ($F(2,69) = 9.80$, $p = 0.0002$, no covariates contributed to the fit of this mixed model), showing that the saliva secreted upon bread contained more protein compared to parafilm (Table 2, all the models: protein secretion rate ~ type of stimuli + (1| participants)).

4. Discussion

The aim of our study was to investigate different levels of sensory stimulation and specific food products (differing in starch content) in relation to saliva secretion, α-amylase (concentration and secretion rate) and other salivary characteristics including pH and buffering capacity, MUC5B concentration and total protein (concentration and secretion rate). Our results confirm that saliva secretion increases upon the level of sensory stimulation. Moreover, the highest level of sensory stimulation (Odor + Vision + Taste + Mastication) induces the greatest α-amylase secretion rate compared to anticipatory sensory cues (Odor and Odor + Vision). Interestingly, our results suggest that the level of sensory stimulation may play a more critical role for α-amylase concentration and secretion rate rather than the specific food product. Salivary characteristics such as pH and buffer capacity increased while other characteristics such as MUC5B and total protein concentration decreased upon the level of sensory stimulation and the secretion of saliva. Moreover, protein secretion rate seems to remain similar across

the levels of sensory stimulation, except of O + V + T which secreted significantly lower protein compared to unstimulated saliva and O + V + T + M.

As expected, saliva secretion rate increased upon the levels of sensory stimulation. Remarkably, we observed a clear distinction difference between anticipatory sensory cues (Odor and Odor + Vision) versus consummatory sensory cues (Odor + Vision + Taste and Odor + Vision + Taste + Mastication). These results are in line with previous research that showed a higher saliva secretion upon taste or mastication compared to smell and sight [1758]. Saliva secretion rate did not increase from the Odor to the Odor + Vision condition, even though extra sensory information was added. Previous studies have shown that salivation upon exposure to food pictures is similar to unstimulated saliva [[17],[21],[59],[60]]. However, when using a real food product as visual stimulation, salivation is significantly higher compared to unstimulated saliva or to odor exposure [[17],[24],[29],[30],[32],[61]]. We postulate that a digital picture may not add additional anticipatory information on top of the odor cue, whereas the use of a real food product could be related to more realistic expectations of consumption. Moreover, the combination of Odor + Vision + Taste may provide sufficient information to secrete a higher amount of saliva and adding more sensory information (by mastication) would not increase it any further. It is noteworthy to mention that the similar results between the two consummatory levels (Odor + Vision + Taste and Odor + Vision + Taste + Mastication) might be related to the procedure. We collected the saliva after "the activation of the saliva" upon the stimuli rather than during the tasting and chewing of the stimuli. There is no golden standard procedure for the collection of saliva during/after taste or mastication exposure, which is a complex procedure. The experiment was designed based on our research questions, and the selected procedure was based on previous literature [35], [46–48]. Moreover, previous research performed on taste solutions or mastication (food or non-food stimuli) has shown an increase in salivation [e.g. [17],[44],[61]]. Although we strictly controlled the (multi) sensory exposure in each level of sensory stimulation (e.g., participants were simultaneously exposed to the smell and sight of the stimuli with a plate containing the stimuli in front of them, while having a stimulus in the oral cavity), it is possible that results from the consummatory levels are driven solely by taste and/or mastication, rather than the added combination of all levels of sensory stimulation.

Bread (food high-in-starch) enhanced salivation compared to the non-food control (parafilm), regardless of the level of sensory stimulation. However, only for both consummatory levels of stimulation (Odor + Vision + Taste and Odor + Vision + Taste + Mastication) did saliva secretion increase upon exposure to bread compared to cucumber (low-in-starch). These results may be explained by the fact that these food products differed in their water content. Bread is a dry food product which contains 7.5 and 37.3 g of water/100 g of product for toasted and untoasted bread, respectively; while cucumber contains 96.7 g of water/100 g of product [44]. It has been suggested that dry food products, such as bread, toast, etc., may require longer mastication cycles, increasing saliva production for a proper lubrication, bolus formation and further swallowing [35],[62–65]. Moreover, others have suggested that liking of a food product plays a role in salivation [29], [61]. However, both food products were selected to be moderately liked (>60 mm on a 100 mm VAS), and individual liking ratings were considered in data analyses, thus discounting this as potential confounding factor.

Salivary α-amylase concentration (U/mL) decreased upon the level of sensory stimulation while α-amylase secretion rate (U/min) increased after Odor + Vision + Taste + Mastication sensory stimulation compared to the anticipatory levels (Odor and Odor + Vision). Carreira and colleagues showed a similar α-amylase concentration after bread odor exposure and after mastication of bread compared to unstimulated saliva [34]. The α-amylase secretion rate (U/min) results are in line with previous research which also compared modified sham feeding to smell and sight stimulation and showed a higher secretion of gastric acid with consummatory stimulation compared to the anticipatory ones [66]. Perhaps surprisingly, this study has not been able to demonstrate a specific effect of starch content on α-amylase concentration nor on secretion rate. Mackie and Pangborn showed a higher α-amylase secretion rate (U/min) upon chewing food high-in-starch (bread) for 15 s compared to food low-in-starch (celery) and parafilm (non-food control). However, the α-amylase concentration (U/mL) was similar across the different conditions [35]. Additionally, in contrast to the present findings, they collected unilateral parotid saliva, while we collected whole mouth saliva. α-amylase is one of the most abundant proteins in saliva and is particularly secreted by the parotid gland [9]. α-amylase can be directly collected from the parotid gland, where the highest percentage of α-amylase is produced, by means of Lashley cups [35],[49],[67]. However, we focused on whole mouth saliva secretion to test our hypotheses that involved salivation from the different salivary glands. Moreover, the 'passive drooling' method (collection method for the whole mouth saliva) is less complicated to collect and less invasive for participants compared to the use of the Lashley cup [49].

Further research could analyze the starch breakdown of the food products to give more insights about the amount of starch that could have already hydrolyzed by the α-amylase. Some researchers suggest that around half of the total starch content in food (e.g., bread and wheat) is hydrolyzed into oligosaccharides upon a short modified sham feeding exposure [37],[47],[48].

Regarding the salivary characteristics, we found a positive weak correlation between saliva secretion and pH/ buffering capacity. Our results are in line with previous studies that showed a linear relation between salivation and the release of bicarbonate ions, modifying the pH and buffering capacity of secreted saliva [68–70]. Upon activation of the parotid gland through the consummatory levels of stimulation, the levels of bicarbonate increase leading to a slight increased pH and stronger increased buffer capacity. The bicarbonate ions are converted upon the release of the watery portion of the saliva through the ducts [71]. These bicarbonate ions produce a more basic environment, increasing the pH which supports prevention of enamel demineralization [8].

Moreover, MUC5B concentration significantly decreased upon exposure to the more consummatory levels of stimulation compared to the anticipatory sensory cues and unstimulated saliva. As reported in literature, unstimulated saliva is more visco-elastic, suggesting a saliva richer in mucins, compared to stimulated saliva [12],[72]. Also, saliva upon chewing exposure has been found to be significantly less elastic compared to saliva upon citric acid exposure and unstimulated saliva [10]. MUC5B may be continuously secreted and less prone to stimulation [10],[13]. Our results suggest that the composition of saliva stimulated by anticipatory sensory cues (Odor and Odor + Vision) is similar in mucin concentration to unstimulated saliva.

Furthermore, the total protein concentration decreased significantly upon exposure to the consummatory levels of stimulation (Odor + Vision + Taste and Odor + Vision + Taste + Mastication) compared to the anticipatory sensory cues and unstimulated saliva. Carreira and colleagues showed that the protein concentration of the unstimulated saliva was significantly higher compared to the saliva upon bread odor exposure but similar after the mastication of bread or rice [34]. Except for the protein secretion rate in the saliva upon Odor + Vision + Taste, our results suggest that the protein secretion rate remains stable over the different levels of sensory stimulation. It could be argued that the decreased protein secretion rate in the saliva upon Odor + Vision + Taste were due to the saliva secretion rate induce for that level of sensory stimulation

The proteins predominantly present in saliva are α-amylase, proline-rich proteins and mucins. Stimulation of saliva by means of sensory cues can immediately enhance the secretion of water but not of other components, resulting in a watery and serous saliva with low

percentage of proteins and other components [7]. Some researchers have suggested that the decrease of total protein concentration upon some sensory cues is related to a dilution effect [34],[73]. Therefore, a comparison between the total protein concentration and the protein secretion rate results may suggest that the decrease in the total protein concentration for the consummatory levels is in fact a dilution effect. A moderate negatively correlation between total protein concentration and salivary secretion rate upon stimulation has been reported [7],[40], [73]. A recent systematic review suggests that mastication has little or negative effect on salivary proteins concentration (α-amylase, mucin and total protein), which could be mainly affected by salivation flow [74].

5. Conclusion

Exposure to multisensory consummatory cues induces larger changes in saliva secretion rate and its composition than anticipatory sensory cues. However, changes in composition may be influenced by the combination of several sensory modalities (mainly gustatory and mechanical, via mastication) rather than by specific (food) products. This study has provided deeper insight into the role of (multi)sensory food cues in anticipatory eating responses.

Author contributions

P. Morquecho-Campos, F.J. Bikker, C. de Graaf, M.L. Laine, and S. Boesveldt jointly conceptualized and developed the study design. P. Morquecho Campos collected and analyzed the data. K. Nazmi and P. Morquecho-Campos determined the salivary components. P. Morquecho-Campos drafted the manuscript under the supervision of S. Boesveldt, F.J. Bikker, K. Nazmi, and M.L. Laine.

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Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2020.113116.

References

[1] S. Boesveldt, K. de Graaf, The differential role of smell and taste for eating behavior, Perception 46 (2017) 307–319, https://doi.org/10.1177/0301006616685576.
[2] K. McCrickerd, C.G. Forde, Sensory influences on food intake control: moving beyond palatability, Obes. Rev. 17 (2016) 18–29, https://doi.org/10.1111/obr.12340.
[3] R.D. Mattes, Physiologic responses to sensory stimulaion by food: nutritional implications, J. Am. Diet. Assoc. 97 (1997) 406–410, https://doi.org/10.1016/S0002-8223(97)00101-6 413.
[4] P. Smeets, A. Erkner, C. de Graaf, Cephalic phase responses and appetite, Nutr. Rev. 68 (2010) 643–655, https://doi.org/10.1111/j.1753-4887.2010.00334.x.
[5] M.A. Zafra, F. Molina, A. Puerto, The neural/cephalic phase reflexes in the physiology of nutrition, Neurosci. Biobehav. Rev. 30 (2006) 1032–1044, https://doi.org/10.1016/j.neubiorev.2006.03.005.
[6] C. Spence, Mouth-watering: the influence of environmental and cognitive factors on salivation and gustatory/flavor perception, J. Texture Stud. 42 (2011) 157–171, https://doi.org/10.1111/j.1745-4603.2011.00299.x.
[7] M. Levine, Saliva, Top. Dent. Biochem, Springer Science & Business Media, 2010, pp. 203–230, https://doi.org/10.1007/978-3-540-88116-2_12.
[8] A. Pedersen, C. Sørensen, G. Proctor, G. Carpenter, Salivary functions in mastication, taste and textural perception, swallowing and initial digestion, Oral Dis. 24 (2018) 1399–1416, https://doi.org/10.1111/odi.12867.
[9] G.H. Carpenter, The secretion, components, and properties of saliva, Annu. Rev. Food Sci. Technol. 4 (2013) 267–276, https://doi.org/10.1146/annurev-food-030212-182700.
[10] J.R. Stokes, G.A. Davies, Viscoelasticity of human whole saliva collected after acid and mechanical stimulation, Biorheology 44 (2007) 141–160.
[11] A. Pedersen, A. Bardow, S.B. Jensen, B. Nauntofte, Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion, Oral Dis. 8 (2002) 117–129, https://doi.org/10.1034/j.1601-0825.2002.02851.x.
[12] H. Mese, R. Matsuo, Salivary secretion, taste and hyposalivation, J. Oral Rehabil. 34 (2007) 711–723, https://doi.org/10.1111/j.1365-2842.2007.01794.x.
[13] C. Dawes, A.M.L. Pedersen, A. Villa, J. Ekström M E, G.B. Proctor, A. Vissink, D. Aframian, R. Mcgowan, A. Aliko, N. Narayana, Y.W. Sia, R.K. Joshi, S.B. Jensen, A.R. Kerr, A. Wolff, The functions of human saliva: a review sponsored by the World Workshop on Oral Medicine VI, Arch. Oral Biol. 60 (2015) 863–874, https://doi.org/10.1016/j.archoralbio.2015.03.004.
[14] R.D. Mattes, Nutritional implications of the cephalic-phase salivary response, Appetite 34 (2000) 177–183, https://doi.org/10.1006/appe.1999.0278.
[15] S.P. Humphrey, R.T. Williamson, A review of saliva: normal composition, flow, and function, J. Prosthet. Dent. 85 (2001) 162–169, https://doi.org/10.1067/mpr.2001.113778.
[16] K.S. Lashley, The human salivary reflex and its use in psychology, Psychol. Rev. 23 (1916) 446–464, https://doi.org/10.1037/h0070181.
[17] Y. Ilangakoon, G.H. Carpenter, Is the mouthwatering sensation a true salivary reflex? J. Texture Stud. 42 (2011) 212–216, https://doi.org/10.1111/j.1745-4603.2011.00290.x.
[18] J.C. Kershaw, C.A. Running, Conditioning of human salivary flow using a visual cue for sour candy, Arch. Oral Biol. 92 (2018) 90–95, https://doi.org/10.1016/j.archoralbio.2018.05.010.
[19] R. Holland, B. Matthews, Conditioned reflex salivary secretion in man, Arch. Oral Biol. 15 (1970) 761–767, https://doi.org/10.1016/0003-9969(70)90039-7.
[20] K.D. White, Salivation: the significance of imagery in its voluntary control, Psychophysiology 15 (1978) 196–203, https://doi.org/10.1111/j.1469-8986.1978.tb01363.x.
[21] T. Hayashi, Experimental evidence of the second signaling system of man, Cond. Reflex 3 (1968) 18–28.
[22] R. Pangborn, S. Witherly, F. Jones, Parotid and whole-mouth secretion in response to viewing, handling, and sniffing food, Perception 8 (1979) 339–346, https://doi.org/10.1068/p080339.
[23] I. Shannon, Effects of visual and olfactory stimulation on parotid secretion rate in the human, Proc. Soc. Exp. Biol. Med. 146 (1974) 1128–1131.
[24] B.J. Sahakian, M.E.J. Lean, T.W. Robbins, W.P.T. James, Salivation and insulin secretion in response to food in non-obese men and women, Appetite 2 (1981) 209–216, https://doi.org/10.1016/S0195-6663(81)80043-8.
[25] V.M. Lee, R.W. Linden, An olfactory-submandibular salivary reflex in humans, Exp. Physiol. 77 (1992) 221–224, https://doi.org/10.1113/expphysiol.1992.sp003578.
[26] M. Keesman, H. Aarts, S. Vermeent, M. Häfner, E.K. Papies, Consumption simulations induce salivation to food cues, PLoS ONE 11 (2016) 1–16, https://doi.org/10.1371/journal.pone.0165449.
[27] R.M. Pangborn, Parotid flow stimulated by the sight, feel, and odor of lemon, Percept. Mot. Skills 27 (1968) 1340–1342, https://doi.org/10.2466/pms.1968.27.3f.1340.
[28] D. Ferriday, J.M. Brunstrom, I just can"t help myself': effects of food-cue exposure in overweight and lean individuals, Int. J. Obes. 35 (2011) 142–149, https://doi.org/10.1038/ijo.2010.117.
[29] F. Klajner, C.P. Herman, J. Polivy, R. Chhabra, Human obesity, dieting, and anticipatory salivation to food, Physiol. Behav. 27 (1981) 195–198, https://doi.org/10.1016/0031-9384(81)90256-0.
[30] P.J. Rogers, A.J. Hill, Breakdown of dietary restraint following mere exposure to food stimuli: interrelationships between restraint, hunger, salivation, and food intake, Addict. Behav. 14 (1989) 387–397, https://doi.org/10.1016/0306-4603(89)90026-9.
[31] C. Proserpio, C. de Graaf, M. Laureati, E. Pagliarini, S. Boesveldt, Impact of ambient odors on food intake, saliva production and appetite ratings, Physiol. Behav. 174 (2017) 35–41, https://doi.org/10.1016/j.physbeh.2017.02.042.
[32] S.C. Wooley, O.W. Wooley, Salivation to the sight and thought of food: a new measure of appetite, Psychosom. Med. 35 (1973) 136–142, https://doi.org/10.1097/00006842-197303000-00006.
[33] P. Morquecho-Campos, F.J. Bikker, K. Nazmi, K. de Graaf, M.L. Laine, S. Boesveldt, Impact of food odors signaling specific taste qualities and macronutrient content on saliva secretion and composition, Appetite 143 (2019) 104399, , https://doi.org/10.1016/J.APPET.2019.104399.
[34] L. Carreira, P.M. Castelo, C. Simões, F. Capela, e Silva, C. Viegas, E. Lamy, Changes

in salivary proteome in response to bread odour, Nutrients 12 (2020) 1002, https://doi.org/10.3390/nu12041002.
[35] D. Mackie, R. Pangborn, Mastication and its influence on human salivary flow and alpha-amylase secretion, Physiol. Behav. 47 (1990) 593–595, https://doi.org/10.1016/0031-9384(90)90131-M.
[36] G. Kusuma Aji, F.J. Warren, E. Roura, Salivary α-amylase activity and starch-related sweet taste perception in humans, Chem. Senses 44 (2019) 249–256, https://doi.org/10.1093/chemse/bjz010/5308913.
[37] J.W. Woolnough, A.R. Bird, J.A. Monro, C.S. Brennan, The effect of a brief salivary α-amylase exposure during chewing on subsequent in vitro starch digestion curve profiles, Int. J. Mol. Sci. 11 (2010) 2780–2790, https://doi.org/10.3390/ijms11082780.
[38] M.D. Robertson, K.G. Jackson, C.M. Williams, B.A. Fielding, K.N. Frayn, Prolonged effects of modified sham feeding on energy substrate mobilization, Am. J. Clin. Nutr. 73 (2001) 111–117.
[39] H. Inoue, K. Ono, W. Masuda, Y. Morimoto, T. Tanaka, M. Yokota, K. Inenaga, Gender difference in unstimulated whole saliva flow rate and salivary gland sizes, Arch. Oral Biol. 51 (2006), https://doi.org/10.1016/j.archoralbio.2006.06.010 1055–1.
[40] A. Prodan, H.S. Brand, A.J.M. Ligtenberg, S. Imangaliyev, E. Tsivtsivadze, F. van der Weijden, W. Crielaard, B.J.F. Keijser, E.C.I Veerman, Interindividual variation, correlations, and sex-related differences in the salivary biochemistry of young healthy adults, Eur. J. Oral Sci 123 (2015) 149–157, https://doi.org/10.1111/eos.12182.
[41] A. Oleszkiewicz, V.A. Schriever, I. Croy, A. Hähner, T. Hummel, Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects, Eur. Arch. Oto-Rhino-Laryngol. 276 (2018) 719–728, https://doi.org/10.1007/s00405-018-5248-1.
[42] W.M. Thomson, Dry mouth and older people, Aust. Dent. J. 60 (2015) 54–63, https://doi.org/10.1111/adj.12284.
[43] W.M. Thomson, J.M. Chalmers, A.J. Spencer, S.M. Williams, The Xerostomia inventory: a multi-item approach to measuring dry mouth, Commun. Dent. Health 16 (1999) 12–17.
[44] RIVM, NEVO Online Version, (2019) http://nevo-online.rivm.nl/Default.aspx.
[45] J. Blechert, A. Lender, S. Polk, N.A. Busch, K. Ohla, Food-pics_extended-an image database for experimental research on eating and appetite: additional images, normative ratings and an updated review, Front. Psychol. 10 (2019) 1–9, https://doi.org/10.3389/fpsyg.2019.00307.
[46] D. Froehlich, R. Pangborn, J. Whitaker, The effect of oral stimulation on human parotid salivary flow rate and alpha-amylase secretion, Physiol. Behav 41 (1987) 209–217, https://doi.org/10.1016/0031-9384(87)90355-6.
[47] C. Hoebler, A. Karinthi, M.-.F. Devaux, F. Guillon, D.J.G. Gallant, B. Bouchet, C. Melegari, J.-.L. Barry, Physical and chemical transformations of cereal food during oral digestion in human subjects, Br. J. Nutr. 80 (1998) 429–436, https://doi.org/10.1017/S0007114598001494.
[48] C. Hoebler, M.-.F. Devaux, A. Karinthi, C. Belleville, J.-.L. Barry, Particle size of solid food after human mastication and in vitro simulation of oral breakdown, Int. J. Food Sci. Nutr. 51 (2000) 353–366, https://doi.org/10.1080/096374800426948.
[49] M. Navazesh, Methods for collecting saliva, Ann. New York Acad. Sci. 694 (1993) 72–77, https://doi.org/10.1111/j.1749-6632.1993.tb18343.x.
[50] J.L. Chicharro, A. Lucía, M. Pérez, A.F. Vaquero, R. Ureña, Saliva composition and exercise, Sport. Med. 26 (1998) 17–27, https://doi.org/10.2165/00007256-199826010-00002.
[51] H. Brand, A. Ligtenberg, C. Bots, A. Nieuw Amerongen, Secretion rate and buffer capacity of whole saliva depend on the weight of the mechanical stimulus, Int. J. Dent. Hyg. 2 (2004) 137–138, https://doi.org/10.1111/j.1601-5037.2004.00081.x.
[52] E.C.I. Veerman, J.G.M. Bolscher, B.J. Appelmelk, E. Bloemena, T.K. Van Den Berg, A.V.N. Amerongen, A monoclonal antibody directed against high M r salivary mucins recognizes the SO 3 -3Gal(Jl-3GlcNAc moiety of sulfo-Lewis: a histochemical survey of human and rat tissue, Glycoblology 7 (1997) 37–43, https://doi.org/10.1093/glycob/7.1.37.
[53] E.C.I. Veerman, P.A.M. Van Den Keijbus, K. Nazmi, W. Vos, J.E. Van Der Wal, E. Bloemena, J.G.M. Bolscher, A.V.N. Amerongen, Distinct localization of MUC5B glycoforms in the human salivary glands, Glycobiology 13 (2003) 363–366, https://doi.org/10.1093/glycob/cwg037.
[54] RStudio Team, RStudio: Integrated Development Environment for R, (2016).
[55] A.G. Wijlens, A. Erkner, M. Mars, C. de Graaf, Longer oral exposure with modified sham feeding does not slow down gastric emptying of low- and high-energy-dense gastric loads in healthy young men, J. Nutr. 145 (2015) 365–371, https://doi.org/10.3945/jn.114.200584.
[56] D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4, J. Stat. Softw. 67 (2015) 1–48, https://doi.org/10.18637/jss.v067.i01.
[57] R.V. Lenth, Least-squares means: the *R* package **lsmeans**, J. Stat. Softw. 69 (2016) 1–33, https://doi.org/10.18637/jss.v069.i01.
[58] R. Pangborn, S. Witherly, F. Jones, Parotid and whole-mouth secretion in response to viewing, handling, and sniffing food, Perception 8 (1979) 339–346, https://doi.org/10.1068/p080339.
[59] C.T. Richardson, M. Feldman, Salivary response to food in humans and its effect on gastric acid secretion, Am. J. Physiol. 250 (1986) G85–G91 http://ajpgi.physiology.org/content/ajpgi/250/1/G85.full.pdf accessed May 4, 2017.
[60] D. Birnbaum, J.E. Steiner, F. Karmeli, M. Ilsar, Effect of visual stimuli on human salivary secretion, Psychophysiology 11 (1974) 288–293, https://doi.org/10.1111/j.1469-8986.1974.tb00546.x.
[61] C.M. Christensen, M. Navazesh, Anticipatory salivary flow to the sight of different foods, Appetite 5 (1984) 307–315, https://doi.org/10.1016/S0195-6663(84)80003-3.
[62] R. Pangborn, B. Lundgren, Salivary secretionin response to crisp bread, J. Texture Stud. 8 (1977) 463–472, https://doi.org/10.1111/j.1745-4603.1977.tb01196.x.
[63] M. Gavião, L. Engelen, A. van der Bilt, Chewing behavior and salivary secretion, Eur. J. Oral Sci. Oral Sci. 112 (2004) 19–24, https://doi.org/10.1111/j.0909-8836.2004.00105.x.
[64] L. Engelen, A. Fontijn-Tekamp, A. Van Der Bilt, The influence of product and oral characteristics on swallowing, Arch. Oral Biol. 50 (2005) 739–746, https://doi.org/10.1016/j.archoralbio.2005.01.004.
[65] J.X. Guinard, C. Zoumas-Morse, C. Walchak, Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods, Physiol. Behav. 63 (1998) 109–118, https://doi.org/10.1016/S0031-9384(97)00399-5.
[66] M. Feldman, C.T. Richardson, Role of thought, sight, smell, and taste of food in the cephalic phase of gastric acid secretion in humans, Gastroenterology 90 (1986) 428–433.
[67] K.S. Lashley, Reflex secretion of the human parotid gland, J. Exp. Psychol. 1 (1916) 461–493, https://doi.org/10.1037/h0073282.
[68] J. Thaysen, N. Thorn, I. Schwartz, Excretion of sodium, potassium, chloride and carbon dioxide in human parotid saliva, Am. J. Physiol. 178 (1954) 155–159, https://doi.org/10.1152/ajplegacy.1954.178.1.155.
[69] A. Bardow, J. Madsen, B. Nauntofte, The bicarbonate concentration in human saliva does not exceed the plasma level under normal physiological conditions, Clin. Oral Investig. 4 (2000) 245–253, https://doi.org/10.1007/s007840000077.
[70] A. Bardow, D. Moe, B. Nyvad, B. Nauntofte, The buffer capacity and buffer systems of human whole saliva measured without loss of CO_2, Arch. Oral Biol. 45 (2000) 1–12, https://doi.org/10.1016/S0003-9969(99)00119-3.
[71] M. Edgar, C. Dawes, D. O'Mullane, Saliva and Oral Health, fourth ed, Stephen Hancocks Limited, London, 2012https://pdfs.semanticscholar.org/e483/571214ba40121071a143f100e2fe9f7fc7d8.pdf accessed November 7, 2018.
[72] P.J.F. Rantonen, J.H. Meurman, Viscosity of whole saliva, Acta Odontol. Scand. 56 (1998) 210–214, https://doi.org/10.1080/00016359850142817.
[73] E. Neyraud, C.I. Heinzerling, J.H.F. Bult, C. Mesmin, E. Dransfield, Effects of different tastants on parotid saliva flow and composition, Chem. Percept. 2 (2009) 108–116, https://doi.org/10.1007/s12078-009-9041-9.
[74] K. Al-Manei, N. Almotairy, N. Bostanci, A. Kumar, A. Grigoriadis, Effect of chewing on the expression of salivary protein composition: a systematic review, Proteomics (2019) 1900039, , https://doi.org/10.1002/prca.201900039.