Variations in oral responsiveness associate with specific signatures in the gut microbiota and modulate dietary habits

Leonardo Menghi [1,2,3)], Danny Cliceri [1)], Francesca Fava [3)], Massimo Pindo [3)], Giulia Gaudioso [3)], Erika Stefani [3)], Davide Giacalone [2)*] & Flavia Gasperi [1,3)*]

[1]Center Agriculture Food Environment, University of Trento, Via Mach 1, San Michele all'Adige, 38098, Italy;

[2]Department of Technology and Innovation, University of Southern Denmark, Campusvej 55, Odense, 5230, Denmark;

[3]Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, San Michele all'Adige, 38098, Italy.

*Correspondence to:

Davide Giacalone: dg@iti.sdu.dk; Address: Campusvej 55, Odense, 5230, Denmark

Flavia Gasperi: flavia.gasperi@unitn.it; Address: Via Mach 1, San Michele all'Adige, 38098, Italy.

Abstract

Mounting evidence suggests that ingestive behaviors may also be affected by putative interplays between taste and gut microbiota. As yet empirically unproven, we here tested the hypothesis that variations in sensory perception in foods can mirror gut microbial ecology and shape individual dietary habits. One hundred healthy participants (52% women, 18-30 y/o) remotely attended a 7-day (D) lasting protocol, and evaluated bitterness (D1) of 6-n-propylthiouracil (PROP) plus liking (D2) and intensity of sensations (D4) evoked by 5 liquid and 5 solid foods, each selected to elicit a target sensation (sweet, sour, bitter, salty, pungent). Furthermore, volunteers completed a battery of psychological questionnaires (D3), a 4-day dietary record (D1-D7), and provided one stool sample for fecal microbiota profiling by 16S rRNA gene sequencing (D4). Using a data-driven segmentation approach based on intensity scores, we identified two distinct profiles that were hypo- (CL-1, n=36, 55.5% women) and hyperresponsive (CL-2, n=64, 50% women) to oral stimulations. Moreover, CL-2 showed higher percentages of PROP Medium Tasters and pronounced pleasure-oriented attitudes. Interestingly, CL-1 exhibited higher α-diversity metrics and was enriched in 11 beneficial gut microbes (e.g., genus *Eubacterium_xylanophilum_group*), while two pro-inflammatory microbial genera (*Ruminococcus gnavus group*, *Eggerthella*) associated with CL-2. Relatedly, CL-1 declared higher intakes of fibers and vegetable proteins, whilst CL-2 habitually consumed more saturated fats. We describe the first empirical evidence that simultaneous variations in sensory acuity and gut microbial consortia imply different dietary habits, thus paving the way for unravelling the complex link between host-related non-genetic factors and aetiology of eating behaviors.

Keywords: Oral responsiveness; Taste; Gut microbiota; Diet; Liking; Psychological traits

1. Introduction

Poor dietary habits pose a serious global health threat as they are associated with the onset of many modern non-communicable diseases such as type 2 diabetes and cardiovascular diseases (e.g., Swinburn et al., 2011). Accordingly, improving the current understanding of individual food choices and preferences is essential to tackle the worldwide spreading of such diseases. Within this context, the way we experience foods and beverages through our senses is a major contributor to our eating habits (Köster, 2009). Moreover, substantial interindividual differences in responses to chemosensory (i.e., taste, smell and chemesthesis) stimuli have been reported as efficient predictors of dietary quality and health outcomes (e.g., Cox et al., 2016; Duffy, 2007).

Historically, the best-documented sources of interindividual variation in oral responsiveness revolved around genetically-induced bitterness of 6-n-propylthiouracil (PROP; Bartoshuk, 2000) and anatomic phenotypes (i.e., fungiform papillae density; Fischer et al., 2013). For years, it was widely assumed that individuals experiencing PROP as extremely bitter also housed a higher fungiform papillae density, and that this would have led to enhanced responsiveness to a wide range of oral stimuli (e.g., Essick et al., 2003; Hayes & Keast, 2011). Nevertheless, recent large scale studies have failed to corroborate this paradigm (Dinnella et al., 2018; Fischer et al., 2013; Garneau et al., 2014), though apparently confirming PROP acuity (unlike fungiform papillae density) as a proxy of generalized hypergeusia (Dinnella et al., 2018; Nolden et al., 2020). Thus, as the role of taste phenotypes still remains somehow controversial, other aspects potentially affecting the mechanisms underlying sensory perception have recently gathered special interest.

Notably, mounting evidence on eating habits and well-being has emphasized the role of the gastrointestinal microbiota (Alcock et al., 2014), a metabolically active reservoir of trillions of microbes that would jointly work with the host chemosensory systems to shape our ingestive behaviors (Alcock et al., 2014; Leung & Covasa, 2021; Schwartz et al., 2021). Relatedly, gut microbial disruption (or dysbiosis) has been reported in concomitance with unhealthy eating attitudes related to chemosensation, such as craving for high-palatable foods (Alcock et al., 2014) or binge-eating episodes (Herman & Bajaka, 2021). Thus, given that nutrient-sensing mechanisms not only operate in the oral cavity but also in the lower gastrointestinal tract (Efeyan et al., 2015), research has recently begun to deepen the links between taste and oral or distal gut microbes (e.g., Feng et al., 2018; Vascellari et al., 2020).

As an example, Cattaneo, Gargari et al. (2019) assessed the detection thresholds of a wide range of tastes (i.e., bitter, salty, sour and sweet) and the lingual bacterial populations of 59 individuals who were classified as either Super Tasters (STs) or Non Tasters (NTs) according to their PROP responsiveness. The authors found STs to be more responsive than NTs to all tastes, and to harbor greater amounts of three bacterial genera (*Actinomyces*, *Oribacterium,* *Campylobacter*) in their tongue *dorsum*. More interestingly, a follow-up study conducted on the same cohort revealed four oral microbes at genus level (*Parvimonas, Peptococcus*, *Peptostreptococcus*, *Prevotella*) to be simultaneously anticorrelated with salt taste thresholds and carbohydrate daily intake, while the opposite was true for the genus *Rothia* (Cattaneo, Riso, et al., 2019). Nevertheless, although a variety of likely pathways used by oral microbial communities to influence taste/flavor perception has been proposed (see Leung & Covasa, 2021 and Schwartz et al., 2021 for reviews), the mechanisms underlying such preliminary findings have yet to be fully clarified.

Similarly, little is known about how the gut microbiota exerts its influence on taste perception, though both factors have extensively been linked to dietary habits. It has been proposed that gut microbes would affect taste perception via modulating the host immune response and hormone secretion (Leung & Covasa, 2021). However, the afore-mentioned pathways derived evidence from animal studies (e.g., Swartz et al., 2012) or were theoretically presumed on the basis of known connections between diet and taste or diet and gut microbial communities (e.g., Turner et al., 2018, 2019). At present, we are aware of only one previous report simultaneously evaluating taste responsiveness and gut microbial composition in humans affected by Parkinson's disease (PD). In that study, Vascellari et al. (2020) observed that PROP hyporesponsive PD patients had lower gut bacterial species richness and evenness (i.e., α-diversity) and relative abundances of genus *Clostridium* compared to PROP hyperresponsive PD patients. Given how both PROP acuity and predominance of *Clostridium* species in the gut environment closely tie to the quality of the diet (e.g., Duffy, 2007; Guo et al., 2020), this study encourages further investigations on healthy individuals. Taken collectively, this initial evidence reasonably supports the hypothesis that eating habits can also be affected by a mutualistic interplay between taste perception and gastrointestinal microbes, and opens new research avenues on the aetiology of eating behaviors (Alcock et al., 2014; Leung & Covasa, 2021).

Despite mounting interest, human research relating taste to the gastrointestinal microbiota is still very much in its infancy. As a result, the current literature is affected by a few limitations.

Firstly, the majority of studies focused on the links between taste functioning and oral microbes. Beyond the exclusive profiling of the oral microbiota, these reports have mostly operationalized taste perception via detection thresholds (Besnard et al., 2018; Feng et al., 2018; Fluitman et al., 2021; Solemdal et al., 2012), which are reportedly uncorrelated with measures of taste function more relevant for actual perception of food (i.e., suprathreshold intensity measures) (e.g., Puputti et al., 2018; Webb et al., 2015). Secondly, taste assessments in previous research have exclusively been obtained in response to aqueous solutions (e.g., Besnard et al., 2018; Cattaneo et al., 2019; Feng et al., 2018) or paper strips (Fluitman et al., 2021; Solemdal et al., 2012), whilst examples collecting sensory responses from real foods are still lacking. Unlike single taste solutions or strips, actual foods permit to mimic the daily experienced interplays between taste qualities, and represent an ecologically sound alternative to identify subpopulations who are similarly responsive to oral stimulations. In this vein, this approach would also support the increasingly accepted idea about the existence of individuals with generalized hypergeusia across different sensory modalities (e.g., taste, ortho- and retronasal olfaction) (Hayes & Keast, 2011; Piochi et al., 2021; Puputti et al., 2018).

Thirdly, none of the afore-mentioned studies has considered key mediators of sensory responsiveness such as hedonics, attitudes and personality traits (e.g., Köster, 2009). Given how both liking and psychological background can mediate variations in oral acuity ultimately shaping food choices (e.g., Laureati et al., 2018; Spinelli et al., 2018), including such factors in protocols that seek to link aspects closely related to dietary habits turns out to be crucial. Lastly, only a few studies reported measures capturing individual dietary habits (e.g., Cattaneo, Riso, et al., 2019), and the minority (Fluitman et al., 2021; Solemdal et al., 2012) has considered sufficiently large cohorts in the light of the numerous confounders (demographic, dietary, environmental) affecting both chemosensation and the gastrointestinal ecosystem (e.g., Diószegi et al., 2019; Vujkovic-Cvijin et al., 2020). In this vein, a meticulous control of these covariates is pivotal to robustly detect a range of potential taste-related microbial signatures that may serve as guide for future taste-oriented microbiome studies in health and disease. Altogether, there exists a clear need to a) expand the current literature on the putative links between taste functioning and the gut microbiota, b) elucidate whether the existing knowledge can be replicated using a multidisciplinary and ecologically valid approach. Against this backdrop, we here empirically tested the hypothesis that variations in oral responsiveness to oral sensations can mirror gut microbial ecology and shape individual dietary intakes. To this

end, we carefully recruited an ethnically homogeneous cohort of 100 healthy individuals lacking evidence of a lengthy list of known taste- and gut microbiota-related confounders. Eligible participants then completed a double-blind remote design simultaneously collecting PROP responsiveness, hedonics and suprathreshold intensities in response to oral sensations evoked by 5 liquid and 5 solid real foods, attitudinal and psychological correlates of food choices, detailed information on habitual dietary intakes, and one gut microbial sample.

2. Methods

2.1 Participants

A gender-balanced healthy cohort of 100 young Italian adults (52 % women; 18-30 y/o; mean age = 23.7 ± 3.9; mean BMI = 22.5 ± 2.6) was enrolled through institutional mailing and social networks (Facebook, Instagram), word of mouth, articles published on local newspapers, and a series of public outreach events promoting the study. A detailed socio-demographic overview of our cohort is given in Supplemental Table S1.

To reliably isolate potential interplays between orosensory responsiveness and gut bacterial composition, we aimed at recruiting individuals not presenting the majority of conditions reportedly impairing or affecting perceptual abilities and/or the gut microbial consortium. Among others, we excluded interested volunteers with ongoing or historical diagnosis of COVID-19 or gastrointestinal chronic diseases (e.g., coeliac disease), or who were habitual smokers or consumed (pre-) probiotics or antibiotics 6 months before the study. The full list of inclusion/exclusion criteria here employed (Supplemental Table S2) mostly relies on the protocol used by the Human Microbiome Project (Turnbaugh et al., 2007) to target the core human gut microbiota in health.

2.2 Overview of data collection

Interested participants were invited to remotely fill in a logic-based questionnaire designed to grant eligibility only to those who simultaneously met the inclusion criteria and none of the exclusion criteria. Eligible participants were then automatically directed to a video that introduced the whole experimental design, and thus asked to electronically provide their informed consent. Our cohort attended a double-blind 7-day (D-) lasting remote protocol aimed at collecting a large variety of sensory and psychometric measures, a food diary, and one stool sample (Figure 1). Particularly, data collection occurred in four working sessions (D1, D2, D3,

D4) to be finalized in four days within a week period, which was employed to increase both participants' compliance and reliability of dietary recording. Beyond the four working sessions, volunteers also completed a 4-day dietary record within the 7 days expected by our design (Figure 1).

Eligible participants were firstly asked to collect a bag storing all the equipment needed to complete the study (Supplemental Figure S1) from different pick-up points located in the Autonomous Province of Trento (Italy). Once the bag was collected, participants accessed a first working session (D1) revolving around the measurement of PROP responsiveness. To this end, they were extensively trained on the use of the generalized Labeled Magnitude Scale (gLMS; Bartoshuk et al., 2004) before rating the bitterness elicited by two PROP impregnated taste strips. D2 was then devoted to collecting hedonic responses to 5 liquid and 5 solid foods, each selected to elicit a target taste (i.e., sweet, sour, bitter, salty) or sensation (i.e., pungent). This session was preceded by detailed instructions on the use of the Labeled Affective Magnitude scale (LAM; Schutz & Cardello, 2001). At the end of the liking task, volunteers were asked to rate their familiarity (5-point Likert scale; 1 = Not at all familiar, 5 = Extremely familiar), and their weekly frequency of consumption (5-point Likert scale; 1 = Never, 5 = Five or more times/week) of the evaluated food product categories.

At D3, participants filled in a battery of questionnaires aimed at collecting a variety of psychological and personality traits, food-related attitudes, and demographics. At D4, volunteers were asked to attend one last working session including the collection of one fecal sample, and the rating of perceived intensities (gLMS) in response to oral sensations evoked by the same series of foods evaluated on D2. Participants were asked to provide their stool sample before starting the session. Once the sample was collected, they were again introduced to the gLMS just prior to finalizing the intensity task that ended the last working session. Upon completion of D4, volunteers were asked to confirm they concluded all the expected tasks before being invited to deliver (D4-D7) their fecal sample at one of the pick-up points available.

Along the entire design, participants were guided by a logic-based system ensuring that working sessions were completed in the expected order (D1, D2, D3, D4), and that commonly used good practices in sensory evaluations were respected. Access to the online platforms used for data collection was granted only when volunteers confirmed to properly comply with the instructions. In detail, they were instructed to: refrain from eating, drinking (except water) and brushing their teeth during the 3 h preceding the evaluations; set-up a sufficiently large

working-station in a quiet and well-illuminated room devoid of cooking smells or home fragrances; be alone during the whole test (Dinnella et al., 2022).

All measures were collected via Eye Question (Elst, The Netherlands) and Alchemer (Louisville, CO, USA), whereas a dietetic software package (Dietosystem®, DS Medica, Milan, Italy) was employed to collect and process dietary records. Remote data collection occurred between May 2021 and (early) January 2022, a relatively restriction-free COVID-19 era in Italy. Nevertheless, we favored remote testing as it ensured participants' safety and, if meticulously planned, constituted a promising and ecologically valid alternative to common lab settings (Dinnella et al., 2022). Lastly, the study was reviewed and approved by the Research Ethics Committee of the University of Trento (n° prot. 2020-040, approved on 08/02/2021), and performed in adherence with the principles laid down in the Declaration of Helsinki.

The next sections provide extensive details on food stimuli, scales training, sensory and psychometric assessments, dietary recording, and fecal samples collection/processing.

2.3 Sensory stimuli, training and evaluations

2.3.1 Food stimuli

Food stimuli were selected looking at the following criteria: a) being liquid and solid foods each evoking a clearly and easily recognizable target taste (i.e., sweet, sour, bitter, salty) or sensation (i.e., pungent) at an expected moderate/very strong level on a gLMS; b) being common/familiar and widely distributed within the Italian market; c) being ready-to-use, easy to portion foods and suitable to be consumed at room temperature.

Five liquid and five solid commercially available foods were thus selected, and tested with pilot studies (n = 3) to confirm their appropriateness. Specifically, pilot tests aimed at defining a ballot of relevant and easy-to-evaluate sensory attributes (Pilot 1; n = 17; 82 % men; 18-30 y/o), then confirmed on its effectiveness and accuracy by a second cohort (Pilot 2; n = 20; 80 % men; 18-30 y/o). The same cohort was also checked for perceptual differences potentially induced by a lab (Pilot 2) or remote (Pilot 3) testing condition at an interval of 2 weeks. Overall, each target sensation was similarly perceived at the expected gLMS range in both conditions (Supplemental Figure S2), and the scores given to the sensory ballot were strongly correlated (Supplemental Figure S3) thus corroborating the reliability of the remote protocol. Table 1 lists relevant information on food matrices and the ballot of sensory attributes here used.

2.3.2 Scales training

Before each tasting session, volunteers were extensively trained on the use of the gLMS (0 = no sensation, 100 = the strongest imaginable sensation of any kind; D1 and D4) or the LAM (0 = greatest imaginable dislike, 100 = greatest imaginable like; D2) scale according to standard procedures (Bartoshuk et al., 2004; Schutz & Cardello, 2001; Webb et al., 2015). Particularly, to avoid potential idiosyncratic use of the gLMS, participants were firstly invited to watch a video designed to emphasize the meaning of the anchors (e.g., the strongest imaginable sensation of any kind), and the continuous nature of the scale to stem common categorial behaviors (Bartoshuk et al., 2004; Hayes et al., 2013; Webb et al., 2015). Moreover, they were also trained to adapt their use of the scale as a function of the magnitude of perceptions habitually experienced across different sensory modalities (Webb et al., 2015).

To this end, volunteers rated the intensities of five recalled extraoral stimuli (D1; Figure 1), each selected to theoretically represent different rating ranges along the scale (Hayes et al., 2013). For individual orientation, we developed a logic-based system that automatically alerted participants about erroneous use of the scale (i.e., ratings out of the expected ranges) and provided clarifications to calibrate its use. Overall, the stimuli were evaluated using different ranges of the gLMS (Supplemental Figure S4), and the effectiveness of the gLMS training was further corroborated by the low percentage (7.7 %) of theoretically misleading correlations between the intensity ratings given to the recalled extraoral stimuli and to the actual foods (Supplemental Figure S5), and by widely-known correlations between the perceived intensity of innately (dis)liked oral sensations and hedonic responses (Supplemental Figure S6).

2.3.3 Sensory evaluations

After scales training, volunteers were given access to the tasting sessions. On D1 (Figure 1), PROP responsiveness was evaluated in duplicate via taste impregnated strips (3-5 µg, MediSens, Groningen, The Netherlands). Briefly, participants were trained to place each strip in the middle of their tongue before pushing it to the palate and around the oral cavity (Smutzer et al., 2013) to spread the sensation. After 10 s, they were asked to expectorate, and then to wait again for 5 s prior to rating the bitterness elicited by the strip (gLMS).

While PROP responsiveness varies along a continuum, discrete grouping is a common approximation of this trait (e.g., Dinnella et al., 2018; Laureati et al., 2018) as functional to easily investigate the host-related features of similarly responsive individuals. Accordingly, the average of bitterness ratings across the two strips was individually considered to group

volunteers falling into the lowest (gLMS < 9.5), the second and the third (9.5 ≥ gLMS ≤ 31.3), and the highest (gLMS > 31.3) quartiles of our cohort's score distribution as Non, Medium and Super Tasters, respectively.

On D2 and D4 (Figure 1), instead, food stimuli were evaluated in two independent sets (Table 1), each including 5 liquid (Set 1) and 5 solid (Set 2) samples presented in a fixed order across individuals. Specifically, foods selected to elicit sweet as target taste (Table 1) were always evaluated as first then followed by sour, bitter, salty, and pungent stimuli as last. In this way, we sought to stem potential carry-over effects led by long-lasting sensations of pungent stimuli, and to simultaneously induce the same perceptual biases across individuals to make interindividual variations more easily comparable. For the same reason, volunteers always rated the perceived intensities of target sensations just prior to evaluating other relevant product-specific taste qualities, and flavors as last (Table 1).

To maximize the reliability of the entire tasting protocol, all stimuli were properly anonymized (e.g., removing brand information), and individually stored in paper-based packages. Each package was supplemented with a random 3-digit code and with a colored label used as a diagnostic check (by asking the color of the label after evaluation) of whether individuals tasted the correct sample. Moreover, each food evaluation (on D2 and D4) was preceded by videos designed to train volunteers to easily portion the planned amount of the stimulus (Table 1) by using the supports provided (i.e., spoons and graduated plastic cups). Lastly, a 90 s break was enforced after each tasting (D1, D2, D4), and mineral water plus plain crackers were used to remove residual sensations from previous evaluations. Similarly, the assessment of each food set (Set 1, Set 2) was interspersed with a 5 min break.

2.4 Psychometric and demographic measures

On D3 (Figure 1), volunteers completed a battery of questionnaires assessing their food neophobia, trait anxiety, health- and taste-oriented food attitudes, eating behaviors, domains of personality, and demographics. To this end, we used the validated Italian versions of the Food Neophobia Scale (Laureati et al., 2018; Pliner & Hobden, 1992), the trait anxiety subscale of the State-Trait Anxiety Inventory Questionnaire (Pedrabissi & Santinello, 1989; Spielberger, 1983), the Health and Taste Attitude Scale (Roininen & Tuorila, 1999; Saba et al., 2019), the Dutch Eating Behavior Questionnaire (Monteleone et al., 2017; van Strien et al., 1986), and the Big Five Inventory (Fossati et al., 2011; John et al., 2008), respectively. Additionally, participants

were asked to indicate their own gender, age, weight and height (later used to calculate the BMI as Kg/m^2), educational level, job occupation, yearly income, and diet choice. Dietary habits were measured and eating patterns (omnivores, flexitarians, vegetarians, vegans) defined as previously proposed (De Backer & Hudders, 2015). All psychometric measures exhibited satisfactory ($\alpha = 0.658$; Pleasure domain in the Health and Taste Attitude Scale) up to excellent ($\alpha = 0.941$; Trait anxiety Inventory) internal reliability (ordinal Cronbach's α). Further details on questionnaires, items (domains), rating scales, scores computation strategy, and internal reliability are given in Supplemental Table S3.

2.5 Dietary intakes assessment

Along the 7-day lasting protocol, volunteers also completed a food record aimed at gathering detailed dietary information. While multiple administrations of food records are frequently needed to assess habitual nutrient intakes, prolonged recording (> 4 days) reportedly affects the reliability of data due to participant fatigue (Thompson & Subar, 2017). Hence, a 4-day period (3 week days and 1 w-end day) was chosen as an appropriate trade-off between accuracy and participant burden.

Volunteers were given video instructions on how to fill in the food recording (with practical examples), and invited to be as precise as possible in listing recipes, amounts and types of foods consumed. To improve data accuracy, participants were also granted access to a comprehensive photographic food atlas (Istituto Scotti Bassani, Milan, Italy), based on the Italian food composition database (https://www.ieo.it/bda), to be used as reference to easily estimate portion sizes.

Data were collected using a mobile dietary record app (Dietosystem®, DS Medica, Milan, Italy), and later processed through the dietetic software Terapia Alimentare Dietosystem® (version 17.00.02, DS Medica, Milan, Italy). This platform enabled us to calculate both daily caloric intake (as Kcal) and the quantities of a large variety (n = 93) of macronutrients (e.g., main type of carbohydrates, fats, proteins and fibers) and micronutrients (e.g., hydrosoluble and liposoluble vitamins, minerals). Lastly, to reliably estimate interindividual differences in single nutrient intakes unaffected by known confounding factors (gender, BMI, physical activity), dietary data were energy-adjusted by residual method as previously recommended (Poslusna et al., 2009) and then individually averaged.

2.6 Stool samples

2.6.1 Stool collection and preprocessing

Prior to starting the last session (D4; Figure 1), volunteers were instructed (via textual and video tutorials) to collect one stool sample using OMNIgene®•GUT (OM-200.100, DNA Genotek Inc., Ottawa, Canada), a widely-used commercially available kit optimized for autonomous feces collection and preservation of bacterial DNA up to 60 days at ambient temperature. Volunteers delivered their sample within 1 day (mean = 1.09 ± 2.27 days) after collection. Upon delivery, the tubes were vigorously shaken for 30 s to further homogenize and liquefy the samples, and 750 µL aliquots were then stored at -80 °C until subsequent downstream applications.

2.6.2 Stool DNA extraction, amplification and sequencing

Next, total microbial DNA was extracted from fecal specimens (250 µL) using the QIAamp® PowerFecal® Pro DNA Kit (Qiagen, Hilden, Germany) with a minor deviation from the manufacturer instructions. Specifically, the Qiagen Spin column tube was eluted twice with DEPC-treated water (Thermo Fisher Scientific, Waltham, MA, USA) to a final volume of 100 µL to optimize bacterial DNA quality and concentration. High-quality microbial DNA was then stored again at -80 °C until the succeeding Polymerase Chain Reaction (PCR) application.
PCR amplification was performed by targeting 16S rRNA gene V3-V4 hypervariable regions using the specific bacterial primer set 341 F (5' CCTACGGGNGGCWGCAG 3') and 806 R (5' GACTACNVGGGTWTCTAATCC 3') with overhang Illumina adapters (Apprill et al., 2015; Klindworth et al., 2013). Amplicons were then purified, and libraries prepared as described by Gaudioso et al. (2021) prior to being sequenced using the Illumina® MiSeq (PE300) platform (San Diego, CA, USA).

2.6.3 Bioinformatics

Forward and reverse raw sequences were firstly demultiplexed before being trimmed (~265 bp; PHRED score > 20), and filtered for chimeric sequences, primers, and potential sequencing artifacts via DADA2 (Callahan et al., 2016). High-quality sequences were thus resolved into amplicon sequence variants (ASVs) and then mapped against the SILVA database (version 138; Quast et al., 2013) for taxonomic annotation up to the genus level at 99 % of similarity. Bioinformatics were carried out using the Quantitative Insights Into Microbial Ecology 2 (QIIME 2™; Bolyen et al., 2019), while subsequent computation of intra-sample (α-) diversity metrics

(i.e., Chao-1, Shannon, Simpson, Inverse Simpson, and Fischer indices) was performed at genus level through the R package *phyloseq* (McMurdie & Holmes, 2013).

2.7 Data analysis

2.7.1 Taste profiles derivation and characterization

We firstly aimed at identifying groups of volunteers homogenous for their overall orosensory responsiveness in actual foods (hereafter, "taste profiles"). To this end, perceived intensity responses (gLMS; D4) relevant for each product (Table 1) were organized in as many groups as the stimuli evaluated (n = 10). A Multiple Factor Analysis (MFA) was then computed to have a spatial configuration of individuals who were similarly responsive to all target and other relevant sensations (e.g., flavors) evoked by each stimulus.

To derive distinct taste profiles, we employed a data-driven segmentation approach determining both algorithm and number of clusters best fitting the data in adherence with previous guidelines (Kassambara, 2017). Specifically, six algorithms (i.e., K-means, Hierarchical Agglomerative, PAM, SOTA, CLARA, and DIANA clustering) along an increasing number of clusters from n = 2 to n = 10 were tested, and optimal partitioning was defined in the light of the lowest cluster connectivity and the highest silhouette width and Dunn index observed (Brock et al., 2008). As input, we used the factor scores produced by the first three dimensions of the MFA model as suggested by the Kaiser criterion (eigenvalues > 1; Kaiser, 1960). Differences between taste profiles as a function of sensory-related (e.g., intensity and liking data), psychometric, and dietary measures were then calculated via permutational Wilcoxon rank sum test (n = 10000), which gives the advantage to accurately estimate exact rates of significance when groups, as in our case, vary greatly in size (Endrizzi et al., 2022).

2.7.2 Differences in gut microbial ecology between taste profiles

Given the intrinsic compositional nature of sequencing products (Gloor et al., 2017), dissimilarities in gut bacterial ecology between taste profiles were tested at genus level using a compositional data approach, which allows to reliably draw inferences based on ratios between taxa (Gloor et al., 2017). First, to deal with the high sparsity of high-throughput data, zeros were imputed with sensible counts by geometric Bayesian-multiplicative replacement (Gloor et al., 2017; Palarea-Albaladejo & Martín-Fernández, 2015). Next, ASVs were centered log ratio transformed before computing the Euclidean (i.e., Aitchison) distance between samples as

index of compositional inter-sample (β-) diversity (Gloor et al., 2017). Differences in α- and β-diversity metrics between taste profiles were then checked via permutational Wilcoxon rank sum test (as previously described in section 2.7.1) and permutational multivariate analysis of variance (PERMANOVA; n = 10000), respectively. β-dissimilarities were also graphically represented using Principal Component Analysis (Gloor et al., 2017).

Lastly, raw ASV counts were filtered for taxa present in at least 10 % of participants before differential abundance analysis as previously recommended (Nearing et al., 2022). Differentially abundant taxa between taste profiles were thus defined at different taxonomic levels (phylum, class, order, family, genus) via Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC; Lin & Peddada, 2020), a compositionally aware method reportedly reducing the occurrence of false discovery rates (Lin & Peddada, 2020; Nearing et al., 2022). Data are expressed as median ± interquartile range (IQR), and as mean ± standard deviation (SD) whenever stated. All tests were two-tailed, and a p value < 0.05 (after permutation test or Benjamini-Hochberg adjustment in ANCOM-BC) was considered statistically significant.

2.7.3 Software

Statistics were calculated using R 4.2.0 (R Core Team, 2019). Particularly, MFA model computation and visualization was carried out via *FactoMineR* (Husson et al., 2018), while the R packages *NbClust* (Charrad et al., 2014) and *clValid* (Brock et al., 2008) were employed within the data-driven segmentation approach. Lastly, the R packages *zCompositions* (Palarea-Albaladejo & Martín-Fernández, 2015), *vegan* (Dixon, 2003), and *ANCOMBC* (Lin & Peddada, 2020) were used for zeros replacement, β-dissimilarity and differential abundance analysis, respectively.

3. Results

3.1 Optimal partitioning and taste profiles characterization

Assuming that individuals would show similar patterns of responsiveness across different sensory modalities (Hayes & Keast, 2011; Nolden et al., 2020; Piochi et al., 2021), relevant intensity ratings within each food stimulus (n = 10) were separately grouped and submitted to a MFA to derive taste profiles homogenous for their global orosensory responsiveness. Overall, participants were uniformly distributed over the first two dimensions of the MFA score plot (31.0 % of variance; Supplemental Figure S7a), and the sensory ballot positively associated

with the first component of the model (Supplemental Figure S7b). Along Dim. 2, warning sensations (e.g., bitter, pungent) tended to be oppositely distributed to innately liked tastes (e.g., sweet, salty), whilst flavors seemed to be positively or negatively associated with taste qualities as a function of taste-flavor congruence (e.g., bitter-coffee or sweet-cocoa).

Based on the factor scores from the first three MFA dimensions (39.7 % of variance), we then sought to define both the algorithm and the partitioning best fitting the data. Results from the data-driven segmentation approach revealed that cluster solutions derived via K-means clustering best suited the data (Supplemental Figure S8), and thus it was selected for our purposes. Nevertheless, while both connectivity and silhouette index suggested n = 2 clusters as the best partition, we found the highest Dunn index value when parsing into 6 clusters (Supplemental Figure S8). Hence, to conclusively define the optimal partitioning, we used the 26 cluster validation indices implemented in the R package *NbClust* (Charrad et al., 2014), and found n = 2 as the cluster number supported by the majority of these indices (Supplemental Figure S9).

The two distinct taste profiles (CL-1 CL-2) thus derived (K-means clustering) were not different for gender proportion, BMI, age, dietary styles, level of food neophobia, trait-anxiety, and domains of personality. Interestingly, we found CL-2 populated by a higher proportion of PROP Medium Tasters (and fewer PROP Non Tasters) showing higher external eating behaviors (Dutch Eating Behaviour Questionnaire; van Strien et al., 1986) and proneness to use food as a source of reward (Health and Taste Attitudes Scale; Roininen & Tuorila, 1999). Table 2 lists baseline demographics, attitudes and psychological traits, and PROP taste phenotypes distribution across taste profiles.

3.2 Differences in orosensory responsiveness, liking, familiarity and frequency of consumption between taste profiles

As expected, we found CL-2 to be more responsive ($p < 0.05$) to the majority of oral sensations measured in both liquid (Figure 2) and solid (Figure 3) foods. Except for bitterness in PR-08 (Figure 3), CL-2 was hyperresponsive to all target tastes (i.e., sweet, sour, bitter, salty), and this effect went beyond differences on textural properties of stimuli. Relatedly, CL-2 reported higher intensity ratings for somatosensory sensations, like pungency and astringency, and for flavors. Also, CL-2 seemed to rate bitterness at higher extent especially in simple matrices (i.e., PR-03 = coffee) not eliciting concomitant suppressive (i.e., sweet in PR-08) or warning (i.e.,

sour in PR-02) oral sensations. Noteworthily, variations in oral acuity could not be imputed to an idiosyncratic use of the gLMS, as taste profiles similarly rated the recalled intensities evoked by the extraoral stimuli used within the training (Supplemental Figure S10).

To check for potential mediators of sensory responsiveness, we then looked into the differences between taste profiles in terms of liking, familiarity and frequency of consumption (Table 3). Overall, we found no differences for 7 out of 10 samples for liking and familiarity. Moreover, both clusters declared to consume all food categories evaluated equally often. Interestingly, CL-2 reported higher liking or familiarity ratings for energy-dense foods (e.g., PR-09 = fries) eliciting innately liked oral sensations (e.g., salty in PR-04 or PR-09; sweet in PR-01 or PR-06).

3.3 Differences in dietary intakes between taste profiles

Next, we examined variations in habitual dietary intakes between taste profiles. To this end, total energy intake (as Kcal) and the large variety of macro- and micronutrients (n = 93) extracted from diary records were considered. Overall, CL-1 reported a 4.3 % (Proteins; $p = 0.038$) up to 33.7 % (tartaric acid; $p = 0.015$) higher intakes of several beneficial macro- (e.g., vegetable proteins) and micronutrients (e.g., a variety of B vitamins and minerals). Oppositely, CL-2 declared to habitually consume higher amounts of saturated fats (+ 5.7 %; $p = 0.005$). Particularly, CL-1 habitually assumed larger quantities of macro- and micronutrients commonly included in plant-based foods. Among others, we found CL-1 relating to greater intakes of total fibers (+ 7.2 %; $p = 0.001$), magnesium (+ 5.6 %; $p = 0.008$) or retinol (Vit. A; + 12.6 %; $p = 0.039$). Simultaneously, CL-1 also reported a higher consumption of compounds included in legumes, oily fish and meat-based products (i.e., purines; + 15.4 %; $p = 0.006$). More interestingly, the hyporesponsive cluster also showed significantly higher ($p < 0.05$) habitual intakes of molecules supposed to elicit sweetness (i.e., glucose = + 21.9 %; fructose = + 26.8 %) or sourness (i.e., Vit. C (ascorbic acid) = + 15.0 %; tartaric acid = + 33.7 %; malic acid = + 30.1 %). Figure 4 illustrates significant ($p < 0.05$) variations in percentages of habitually consumed nutrients between taste profiles, whilst exact quantities of significantly different dietary components of groups' habitual diet are listed in Supplemental Table S4.

3.4 Taste profiles differed in gut microbial diversity and composition

After discarding mitochondrial and *Cyanobacteria* reads, a total of 7635757 (mean = 76357.6 ± 12292.8 per sample) high-quality sequences were conclusively generated. In line with numerous reports (e.g., Rinninella et al., 2019), the gut microbial consortium was on average

dominated by the phyla *Firmicutes* (59.9 ± 8.0 %), *Bacteroidetes* (31.4 ± 7.5 %), *Actinobacteria* (5.0 ± 4.0 %), *Proteobacteria* (2.6 ± 1.5 %) and *Verrucomicrobia* (0.8 ± 1.7 %), which represented over 99 % of taxa detected in our cohort.

We then evaluated the differences between taste profiles as a function of gut microbial α- and β- diversity metrics. Compared to CL-2, CL-1 exhibited higher taxonomic richness (e.g., Chao-1; CL-1 = 104 ± 13.8; CL-2 = 95 ± 24.8; $p = 0.003$) and evenness (e.g., Shannon index; CL-1 = 3.3 ± 0.3; CL-2 = 3.2 ± 0.6; $p = 0.017$), as corroborated by five different intra-sample diversity metrics (Supplemental Figure S11). Next, we tested the extent of β-dissimilarities between fecal microbial communities of groups using Aitchison distances (Gloor et al., 2017), and found both taste profiles effectively separated (PERMANOVA; $R^2 = 0.026$; $p = 0.001$). More interestingly, Aitchison distances within members of CL-1 were significantly shorter than in CL-2 (CL-1 = 38.4 ± 6.0; CL-2 = 41.0 ± 9.1; $p < 0.001$), thus suggesting that the hyporesponsive cluster housed a more homogenous gut bacterial composition (Supplemental Figure S12).

3.5 Taste profiles associated with specific signatures in the gut microbiota

Lastly, we evaluated differentially abundant gut bacterial taxa between taste profiles at five taxonomic levels (phylum, class, order, family, genus) via ANCOM-BC (Lin & Peddada, 2020). Overall, taste profiles showed no significantly different ($p_{adj} > 0.05$) gut microbial abundances at the highest taxonomic levels (phylum, class, order, family). The gut microbiota of both groups was on average dominated by the phyla *Firmicutes* (CL-1 = 62.5 ± 6.8 %; CL-2 = 58.4 ± 8.3 %) and *Bacteroidetes* (CL-1 = 29.4 ± 7.1 %; CL-2 = 32.6 ± 7.4 %), which represented over 90 % of their gut microbial consortium. Moreover, among the 171 genera observed, *Bacteroides* was the most abundant ($p_{adj} > 0.05$) both in CL-1 (16.4 ± 6.8 %) and CL-2 (21.8 ± 9.1 %), as commonly documented in healthy individuals (Rinninella et al., 2019). Top abundant phyla (n = 10) and genera (n = 20) by taste profiles are depicted in Supplemental Figure S13. Nevertheless, several differences emerged when it came to evaluate the differently abundant gut microbial genera between groups. Results (Figure 5) revealed abundances of 11 gut taxa at genus level (phylum Firmicutes) to be significantly higher in CL-1 relative to CL-2. These included *[Eubacterium] coprostanoligenes group* ($p_{adj} = 0.009$), *[Eubacterium] eligens group* ($p_{adj} = 0.020$), *[Eubacterium] xylanophilum group* ($p_{adj} < 0.001$), *Family XIII UCG-001* ($p_{adj} = 0.006$), *Marvinbryantia* ($p_{adj} = 0.004$), *Ruminiclostridium 6* ($p_{adj} = 0.004$), *Ruminococcaceae NK4A214 group* ($p_{adj} = 0.019$), *Ruminococcaceae UCG-002* ($p_{adj} = 0.008$), *Ruminococcaceae*

UCG-005 (p_{adj} = 0.005), *Ruminococcus 1* (p_{adj} = 0.004), and one uncultured bacterium assigned to the family *Clostridiales vadinBB60 group* (p_{adj} = 0.003). Conversely, we found two taxa to be significantly more abundant in the gut microbiota of CL-2, namely the genera *[Ruminococcus] gnavus group* (phylum *Firmicutes*; p_{adj} = 0.039) and *Eggerthella* (phylum *Actinobacteria*; p_{adj} = 0.029). Relative abundances of significantly different gut microbial genera between taste profiles are listed in Supplemental Table S5.

4. Discussion

4.1 Supporting the existence of individuals with generalized hypergeusia

In this study, we empirically tested the hypothesis that variations in oral responsiveness would translate into different gut microbial consortia and modulate dietary habits. Our findings largely confirmed this assumption, as individuals differing for their oral responsiveness in actual foods went along with a distinctive gut microbial composition and differences in habitual consumption of macro- and micronutrients.

Motivated by previous reports (e.g., Hayes & Keast, 2011; Nolden et al., 2020; Piochi et al., 2021), we firstly aimed at segmenting our cohort in homogenous groups of individuals according to their global orosensory responsiveness to the ten foods here tested. To this end, relevant intensity ratings within each food matrix (n = 10) were grouped separately and submitted to a MFA model. The MFA factor scores were thus employed to derive clusters using a variety of quantitative criteria to objectively define the best partition. Overall, we found two distinct groups (named taste profiles throughout the paper), which were, respectively, hypo- (CL-1) and hyperresponsive (CL-2) to nearly all tastes, somatosensory sensations or flavors elicited by the ten foods.

Importantly, differences in orosensory perception between taste profiles were consistently observed regardless of the textural properties of the stimuli. As a result, hyperresponsive individuals systematically showed enhanced acuity to tastes or sensations in both liquid and solid foods, and this leads us to think that taste profiles may also differ on acuity towards textural properties. However, as currently accepted positive relationships between oral responsiveness and tactile acuity (Breen et al., 2019; Essick et al., 2003; Linne & Simons, 2017) have recently been questioned (Mani et al., 2022), we encourage further investigations to conclusively (dis)confirm such link into real foods. Taken collectively, our findings fall into the existing literature supporting the existence of groups of individuals with generalized

hypergeusia (Dinnella et al., 2018; Hayes & Keast, 2011; Nolden et al., 2020; Piochi et al., 2021; Puputti et al., 2018).

However, a surprising result also emerged. Against expectations, the proportion of PROP Super Tasters was similar (25 %) across taste profiles. This result could tentatively be linked to methodological concerns on operationalizations of PROP responsiveness via paper strips (relative to using aqueous solutions). Indeed, impregnated strips reportedly tend to generate high false positive rates from individuals insensitive to PROP (Lawless, 1980), and may not guarantee consistent quantities of PROP across the strip thus inducing biases on phenotypic assignment (Zhao et al., 2003). Furthermore, though extensively trained, participants may have faced difficulties in complying with the unfamiliar tasting protocol, which could inadvertently have promoted differences on the amount of PROP delivered across individuals. Nevertheless, the hyperresponsive group was populated by significantly more Medium Tasters (59.4 % vs 33.3 % in the hyporesponsive group) but fewer Non Tasters (15.6 % vs 41.7 %), thus reasonably suggesting that oral hyperresponsiveness also corresponds to enhanced PROP acuity (e.g., Dinnella et al., 2018).

4.2 Role of hedonics, familiarity and psychological traits on variations in oral responsiveness across taste profiles

While taste profiles were largely similar in terms of liking and familiarity (Table 3) or demographics, dietary styles and psychological traits (Table 2), the few differences observed favor a deeper understanding of variations in acuity above mentioned. Particularly, we noticed the hyperresponsive group giving higher liking ratings for samples evoking innately liked tastes like sweet (e.g., PR-06 = biscuit) and salty (e.g., PR-09 = fries), and found the same tendency for familiarity albeit in different samples (e.g., PR-01 = pear juice). Thus, given how these foods associated with rewarding sensory properties, it was unsurprising to observe most responsive individuals exhibiting higher pleasure-oriented attitudes (Burton et al., 2007). Furthermore, these results overlap those by Hayes, Sullivan, and Duffy (2010) who observed that liking for energy-dense snacks (chips, pretzels) went along with perceived saltiness in PROP Super Tasters.

Interestingly, we evidenced very few cases of no differences in sour or bitter responsiveness between taste profiles (Figure 2 and 3). Noteworthily, these mostly occurred in palatable (LAM > 50; Table 3) and energy-dense matrices simultaneously eliciting rewarding sensations as

sweet (i.e., PR-01 and PR-08). This suggests that the few circumstances of no variation in oral acuity between taste profiles may be ascribed to the hedonic orientation of the hyperresponsive group, which would have deviated volunteers' attention towards a sensation more frequently experienced and thus liked (e.g., Burton et al., 2007). Nevertheless, given the substantial background homogeneity across clusters, we can reasonably conclude that variations in sensory responsiveness here observed can mostly be allocated to physiological rather than attitudinal factors.

4.3 Simultaneous variations in oral responsiveness and gut microbial ecology mirror dietary habits

The main novel contribution of the current study lies in the observed differences between taste profiles in terms of gut microbiota composition and, ultimately, habitual dietary intakes. Indeed, the hyporesponsive group showed a more diverse, complex and homogeneous gut microbial environment over the hyperresponsive group. Moreover, strong (β-) dissimilarities in the overall genus-level composition of the gut microbiota significantly distinguished both groups. In detail, hyporesponsive individuals were found to harbor significantly higher abundances of 11 beneficial gut microbial genera, while the gut microbial consortium of the hyperresponsive group was enriched in two dysbiotic genera (*[Ruminococcus] gnavus group* and *Eggerthella*). Also, oral hyporesponsiveness went along with higher habitual intakes of vegetable proteins, fibers, simple carbohydrates, and several vitamins and micronutrients, whilst oral hyperresponsiveness associated with a higher habitual consumption of saturated fats. Interestingly, the majority of differentially enriched taxa observed in the hyporesponsive group belonged to the families *Lachnospiraceae* and *Ruminococcaceae*. These two reservoirs of commensal gut taxa reportedly hydrolyze plant polysaccharides to produce a range of short chain fatty acids (Vacca et al., 2020), and relate to plant-oriented diets (Cronin et al., 2021). As an example, *[Eubacterium] xylanophilum group* positively associated with long-term consumption of healthful fiber sources such as fruits and vegetables (Miao et al., 2022), while a resistant starch-supplemented diet promoted increased abundances of *Ruminococcaceae UCG-005* (Zhang et al., 2019). Similarly, Ma et al. (2021) longitudinally (~30 years) screened the gut microbiota and diet quality of a large cohort of 5936 individuals, and found *[Eubacterium] eligens group* and *Ruminococcus 1* consistently associated with healthier dietary patterns (e.g., fiber-, legume- and whole grain-rich diets). Noteworthily, the same follow-up study observed

the pro-inflammatory *[Ruminococcus] gnavus group* systemically anticorrelated with diet quality (Ma et al., 2021), thus further explaining the habitual diet (high in saturated fats and low in plant-based components) declared by the hyperresponsive group. Altogether, given how plant-oriented diets can positively boost gut bacterial richness and evenness (Cronin et al., 2021; Wolters et al., 2019), our findings from both ecological (α- and β-diversity) and differential abundance analysis reinforce an extensive literature pointing out evident interplays between dietary habits and the gut microbiota.

In the same vein, expected associations between sensory perception, psychological traits and dietary intakes also emerged. First, oral hyperresponsiveness translated into lower intakes of nutrients (in)directly linkable to sweetness (e.g., glucose and fructose), sourness (e.g., malic acid) or bitterness (e.g., total fibers). Second, it corresponded to higher intakes of saturated fats, likely due to the mediating effect of pleasure-oriented tendencies (Burton et al., 2007). Hence, our findings substantially agree with previous reports suggesting how an enhanced oral acuity for a certain sensation tend to minimize its consumption (e.g., Cattaneo, Riso, et al., 2019), but motivate future studies to increasingly consider key mediators of taste perception when it comes to evaluate its relationships with dietary patterns.

4.4 Potential interplays between taste perception and gut microbiota in modulating dietary intakes

At present, the most reasonable paradigm underlying our findings would presume that oral responsiveness and its psychological covariates affect dietary patterns thus promoting a cascade system ultimately shaping the gut microbiota (e.g., Cronin et al., 2021; Köster, 2009; Monteleone et al., 2017; Wolters et al., 2019). However, an alternative model focused on a putative mutualistic interplay between taste perception and gut communities in modulating dietary habits could also be speculated.

Gut microbiota has previously been proposed as a reservoir of microbes actively influencing our food choices (also) via taste perception to selectively dominate the gut environment (Alcock et al., 2014). A variety of potential mechanisms have been discussed, including the modulation of the host immune system and hormone secretion (see Leung & Covasa, 2021 for a review). Interestingly, inflammation appears to play a key role in these pathways. Indeed, bacterial lipopolysaccharides would play in concert with gut lumen Tool Like Receptors to induce systemic circulation of inflammatory cytokines (e.g., TNF-α), which ultimately would reach the

sites of taste transduction in the tongue and jeopardize the expression of taste receptors (Leung & Covasa, 2021).

In this context, a key difference here observed among the differentially abundant microbes between the hypo- and hyperresponsive group sits into their anti- or pro-inflammatory activities. Notably, the gut microbiota of less responsive individuals harbored greater proportions of gut microbial genera with anti-inflammatory related activities such as short-chain fatty acids production (e.g., *[Eubacterium] xylanophilum group*), cholesterol reduction (i.e., *[Eubacterium] coprostanoligenes group*) or promotion of potent anti-inflammatory effects (i.e., *[Eubacterium] eligens group*) (Cronin et al., 2021; Kenny et al., 2020; Ohira et al., 2017; Vacca et al., 2020). Conversely, the hyperresponsive group showed higher relative abundances of *[Ruminococcus] gnavus group* and *Eggerthella*, two bacterial genera widely associated with inflammatory bowel disease (Henke et al., 2019; Pascal et al., 2017). Moreover, the same group housed a less complex and diverse gut microbial composition, which is reportedly (also) a proxy of both local and systemic inflammation (e.g., Le Chatelier et al., 2013; Zouiouich et al., 2021). Noteworthily, these differences parallelly corresponded to hypo- or hyperresponsiveness to oral stimuli and distinct dietary patterns. Thus, it might be possible that a simultaneous enrichment or depletion in gut microbial taxa (and/or diversity) promoting (anti-)inflammation could have manipulated the expression of taste receptors (Leung & Covasa, 2021). Within this context, the consequent decreased or enhanced taste responsiveness would putatively have induced the host to select nutritional sources that these taxa needed to ensure their dominance within the gut environment (Alcock et al., 2014). However, mechanisms underlying potential interplays between taste perception and gut microbial ecology are far to be conclusively understood. Relatedly, to infer potential metabolic pathways, future studies should firstly aim at unraveling a consistent narrow circle of gut biomarkers related to oral acuity in actual foods by coupling deeper sequencing coverages (i.e., shotgun sequencing) to promising marker-based approaches like metabarcoding (Ranjan et al., 2016; Taberlet et al., 2012). However, such experimental efforts would be poorly resolutive unless included in large-scale multidisciplinary designs. Beyond generalizability of findings, such studies will be pivotal to reliably estimate the actual weight of key mediators of taste perception and/or gut microbial composition (e.g., age, weight status, gender, psychological traits) within their interplay.

4.5 Strengths, limitations and conclusions

To our knowledge, this is the first study empirically supporting that variations in responsiveness towards a large variety of oral stimuli in foods correspond to parallel changes in gut bacterial ecology and dietary intakes. The strengths of this study include the comprehensive experimental design, the use of real foods, and the ecological validity of outcomes. Also, we provided evidence on the accuracy and feasibility of collecting sensory data remotely. In line with recent guidelines (Dinnella et al., 2022), the success of remote testing mostly sits in meticulously planned working sessions enriched in a range of measures guaranteeing the respect of good practices in sensory analysis and the validation of the tasting protocol. Lastly, another important strength of the current study is the high background homogeneity and size (compared to previous reports) of our cohort. While limiting the generalizability of results, such strategy permitted us to reliably draw inferences minimally affected by known mediators of the factors under-investigation, and to speculate potential mechanistic explanations underlying the differences observed.

However, we should also acknowledge a few limitations. In the light of the restricted ethnic and age range here employed, we can not conclude that our results are generalizable to broader populations. Moreover, while commonly employed in consumer studies, our sample size was still relatively small to highlight deeper variations in patterns of sensory responsiveness. Indeed, given the low variance explained by MFA factor scores (39.7 %), the data-driven segmentation approach has probably merged groups of individuals with differently enhanced (e.g., intermediate vs high) oral responsiveness (e.g., Piochi et al., 2021; Puputti et al., 2018) for the sake of clustering reliability and stability. Nevertheless, objective clustering largely outperforms commonly used arbitrary criteria (e.g., Sauvageot et al., 2017), and should increasingly be used in future studies (possibly) along with larger samples to reproducibly target groups of differentially responsive individuals. Lastly, although dietary records represent the gold standard in nutritional epidemiological research (Thompson & Subar, 2017), these measures still rely on self-reporting. Hence, potential over- or underestimations in intakes due to participants' fatigue or self-presentation biases may also be possible (Grant et al., 2021; Thompson & Subar, 2017), though our dietary-related findings largely agree with the current literature.

To conclude, we described the first empirical evidence pointing out, in healthy individuals, a potential mutualistic interplay between sensory responsiveness and gut bacterial ecology in shaping dietary patterns. Given how both factors intimately correlate with eating habits, the results of this study shed new light into the aetiology of eating behaviors and can hopefully

pave the way towards further research on the conjoint effects of host-related non-genetic factors and sensory perception.

5. Acknowledgements

The authors are grateful to all volunteers who attended the study. Special thanks to Elisa Facen, Katia Franzoso, Carola Pedrinolla, Giulia Pirrello, Michele Ricci, Elena Santoro and Jessica Zambanini for their invaluable help and generous cooperation along the phases of the study. We thank Istituto Scotti Bassani (Milan, Italy) for granting our cohort access to the food atlas and, particularly, Dr. Francesca Passannanti for her assistance. We also would like to thank Perfetti Van Melle S.p.A (Italy) for providing one of the test samples and, in particular, Dr. Maria Laura Corollaro for her precious support. Last but not least, we thank Giract (Geneva, Switzerland) for honoring Leonardo Menghi with the 1st year PhD Student European Flavour Research Award for his PhD project.

6. Author contributions

Leonardo Menghi: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization; **Danny Cliceri**: Conceptualization, Methodology, Writing – Review & Editing, Supervision; **Francesca Fava:** Conceptualization, Methodology, Writing - Review & Editing, Supervision; **Massimo Pindo:** Methodology, Investigation, Writing - Review & Editing; **Giulia Gaudioso:** Methodology, Writing - Review & Editing; **Erika Stefani:** Investigation; **Davide Giacalone:** Conceptualization, Methodology, Writing – Review & Editing, Supervision, Funding acquisition; **Flavia Gasperi:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

7. Fundings

This work was funded by the University of Trento - Center Agriculture, Food and Environment (Italy) and the University of Southern Denmark - Faculty of Engineering (DK) as part of the double degree PhD programme of Leonardo Menghi, and by funds assigned to Flavia Gasperi by Fondazione Edmund Mach under the ADP 2021 Program Agreement (Autonomous Province of Trento, Italy).

8. Declaration of interest

None.

9. Data statement

For ethical restrictions, all data here generated or analyzed is confidential and thus not publicly shareable. Nevertheless, aggregate data are available from the corresponding authors upon reasonable requests.

10. Figure captions

Figure 1: Graphical overview of data collection.

Figure 2: Differences in oral responsiveness in liquid foods between CL-1 (dark-blue; n = 36) and CL-2 (orange; n = 64). The raincloud plot graphically represents data distribution (the "cloud"), individual raw observations (the "rain"), and the median (filled circle) ± IQR (perpendicular black line) within each taste profile. Statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10000) are depicted (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Figure 3: Differences in oral responsiveness in solid foods between CL-1 (dark-blue; n = 36) and CL-2 (orange; n = 64). The raincloud plot graphically represents data distribution (the "cloud"), individual raw observations (the "rain"), and the median (filled circle) ± IQR (perpendicular black line) within each taste profile. Statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10000) are depicted (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Figure 4: Circular heatmap depicting variations (%) in habitual nutrient intakes between CL-1 (n = 36; outer circumference) and CL-2 (n = 64; inner circumference), as calculated by the proportional difference between the medians across taste profiles. Macronutrients, essential amino acids (AA), organic compounds, minerals, and vitamins (Vit.) are plotted. Moreover, statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10000) are given (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). † to be considered as a semi-essential amino acid.

Figure 5: Differently abundant taxa between taste profiles. The plot illustrates the main outcome produced by ANCOM-BC (W statistic), which summarizes the ratio between the effect size (log fold change) and the standard error (95 % confidence interval) underlying the

differences observed (Lin & Peddada, 2020). Genera found to be significantly ($p < 0.05$) more abundant in CL-1 ($n = 36$) are depicted in the dark-blue side of the plot (left), whereas the orange band (right) houses differentially abundant microbial genera that were significantly enriched in CL-2 ($n = 64$). Colored bars (dark-blue and orange) show the magnitude of the effect size (log fold change), whilst colored circles represent the rates of significance after Benjamini-Hochberg adjustment (orange: $p < 0.05$; white: $p < 0.01$; dark-blue: $p < 0.001$).

11. Tables

Acronym	Set	Order	Product (Brand)	Amount	Consistency	Target sensation	Other sensations	Flavor
PR-01	1	1	Pear juice (Yoga, Italy)	10 mL	Liquid	Sweet	Sour	Pear
PR-02	1	2	Grapefruit juice (Derby Blue, Italy)	10 mL	Liquid	Sour	Bitter	Grapefruit
PR-03	1	3	Ready to drink coffee (Pocket Bar, Italy)	10 mL	Liquid	Bitter	/	Coffee
PR-04	1	4	Olive pate (Madama Oliva S.r.l, Italy)	10 mL	Liquid	Salty	/	Olive
PR-05	1	5	Tomato juice (Industrie Montali S.r.l, Italy)	10 mL	Liquid	Pungent	/	Tomato
PR-06	2	6	Biscuit (Lotus Bakeries NV, Belgium)	1 unit	Solid	Sweet	/	Caramel
PR-07	2	7	Lemon candy (Perfetti Van Melle S.p.A, Italy)	1 unit	Solid	Sour	Sweet	Lemon
PR-08	2	8	Dark chocolate (Venchi S.p.A, Italy)	1 unit	Solid	Bitter	* Sweet, Astringent	Cocoa
PR-09	2	9	Fries (Saiwa S.r.l, Italy)	4 units	Solid	Salty	/	Potato
PR-10	2	10	Ginger candy (Euro Company S.r.l, Italy)	2 units	Solid	Pungent	Sweet	Ginger

Table 1: Food matrices and ballot of sensory attributes used in the current study. Acronyms, set and order of evaluation, food products (brands), quantities employed (Amount), textural properties of samples (Consistency), target sensations (i.e., sweet, sour, bitter, salty, pungent) and other measured relevant oral sensations (Other sensations; Flavor) are listed. * In PR-08, sweetness was evaluated before astringent, and cocoa flavor as last.

	CL-1 (n = 36)	CL-2 (n = 64)	p.value
Gender (n)			
Women	20	32	0.593†
Men	16	32	
Age (mean ± SD)	24.6 ± 3.4	23.2 ± 4.1	0.071††
BMI (mean ± SD)	22.7 ± 2.7	22.3 ± 2.6	0.555††
Diet (n)			
Omnivores	23	39	
Flexitarians	8	20	0.430†
Vegetarians	4	5	
Vegans	1	0	
Food Neophobia Scale (median ± IQR)	23.5 ± 11.0	24.0 ± 10.0	0.822
Trait Anxiety Inventory	44.5 ± 11.7	44.0 ± 13.5	0.913
Health and Taste Attitude Scale			
General health interest	4.5 ± 1.1	4.4 ± 1.3	0.564
Light product interest	4.1 ± 1.4	3.8 ± 1.5	0.862
Natural product interest	4.0 ± 1.5	3.7 ± 1.7	0.891
Craving for sweet foods	4.9 ± 1.9	5.4 ± 1.7	0.072
Using food as reward	4.3 ± 1.2	5.1 ± 1.4	**0.016**
Pleasure	4.7 ± 0.9	4.8 ± 1.3	0.554
Dutch Eating Behaviour Questionnaire			
Restrained Eating	2.7 ± 1.3	2.6 ± 0.9	0.942
Emotional Eating	2.4 ± 0.9	2.5 ± 0.8	0.421
External Eating	3.2 ± 0.5	3.5 ± 0.8	**0.003**
Big Five Inventory			
Extraversion	3.1 ± 1.2	3.3 ± 1.0	0.362
Agreeableness	3.7 ± 0.9	3.7 ± 0.6	0.923
Conscientiousness	3.7 ± 1.1	3.6 ± 0.9	0.487
Neuroticism	3.3 ± 1.0	2.9 ± 1.3	0.416
Openness	3.7 ± 0.9	3.9 ± 0.8	0.479
PROP Taster Status (n)			
Non Tasters	15	10	
Medium Tasters	12	38	**0.009**†
Super Tasters	9	16	

Table 2: Baseline demographics, dietary styles, attitudes and psychological traits, and PROP taste phenotypes distribution among taste profiles (CL-1, CL-2). Data are summarized as raw observations (n), mean ± SD (Age, BMI) or median ± IQR whenever appropriate. Differences between CL-1 (n = 36) and CL-2 (n = 64) are also tabulated (p.value), and calculated via chi-squared test (†), unpaired t-test (††) or permutational Wilcoxon rank sum test (n = 10000). Values in bold are intended as statistically significant ($p < 0.05$).

Sample	Liking		p.value	Familiarity		p.value	Consumption		p.value
	CL-1	CL-2		CL-1	CL-2		CL-1	CL-2	
PR-01	67.6 ± 13.5	69.3 ± 14.2	0.349	3 ± 2	4 ± 1	**0.041**	2 ± 0	2 ± 0	0.587
PR-02	45.0 ± 22.6	40.6 ± 24.0	0.112	2 ± 2	2 ± 2	0.974	2 ± 1	2 ± 1	0.456
PR-03	34.0 ± 22.1	36.5 ± 28.1	0.657	4 ± 1	5 ± 2	1	5 ± 1	4 ± 2	0.384
PR-04	56.2 ± 30.6	68.9 ± 22.1	**0.011**	2 ± 2	2 ± 1	0.267	2 ± 1	2 ± 1	0.264
PR-05	64.8 ± 18.4	68.0 ± 22.4	0.276	4 ± 1	5 ± 1	0.134	3 ± 1	3 ± 1	0.728
PR-06	76.6 ± 22.2	78.8 ± 12.3	**0.029**	5 ± 1	5 ± 1	0.274	3 ± 2	4 ± 2	0.093
PR-07	68.7 ± 20.6	69.5 ± 16.2	0.547	3 ± 2	4 ± 2	**0.027**	2 ± 1	2 ± 2	0.149
PR-08	64.4 ± 21.8	62.0 ± 24.5	0.567	4 ± 1	5 ± 1	0.137	3 ± 2	3 ± 1	0.279
PR-09	72.9 ± 22.0	77.2 ± 9.4	**0.007**	3 ± 2	4 ± 2	**0.032**	2 ± 1	2 ± 1	0.174
PR-10	44.5 ± 29.7	46.9 ± 46.3	0.657	2 ± 1	2 ± 2	0.299	1 ± 1	1 ± 1	0.607

Table 3: Differences between CL-1 (n = 36) and CL-2 (n = 64) as a function of liking, familiarity and weekly frequency of consumption for the n = 10 foods (Sample) here employed. Values are summarized as median ± IQR, and statistically significant ($p < 0.05$) differences (p.value) according to permutational Wilcoxon rank sum test (n = 10000) are depicted in bold.

12. References

Alcock, J., Maley, C. C., & Aktipis, C. A. (2014). Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. In *BioEssays* (Vol. 36, Issue 10, pp. 940–949). https://doi.org/10.1002/bies.201400071

Apprill, A., Mcnally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

Bartoshuk, L. M. (2000). Comparing sensory experiences across individuals: Recent psychophysical advances illuminate genetic variation in taste perception. *Chemical Senses*, *25*(4), 447–460. https://doi.org/10.1093/chemse/25.4.447

Bartoshuk, L. M., Duffy, V. B., Green, B. G., Hoffman, H. J., Ko, C. W., Lucchina, L. A., Marks, L. E., Snyder, D. J., & Weiffenbach, J. M. (2004). Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. *Physiology and Behavior*, *82*(1), 109–114. https://doi.org/10.1016/j.physbeh.2004.02.033

Besnard, P., Christensen, J. E., Brignot, H., Bernard, A., Passilly-Degrace, P., Nicklaus, S., Pais De Barros, J. P., Collet, X., Lelouvier, B., Servant, F., Blasco, V., Verges, B., Lagrost, L., Feron, G., & Burcelin, R. (2018). Obese Subjects with Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-24619-1

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Breen, S. P., Etter, N. M., Ziegler, G. R., & Hayes, J. E. (2019). Oral somatosensatory acuity is related to particle size perception in chocolate. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-019-43944-7

Brock, G., Pihur, V., Datta, S., & Datta, S. (2008). ClValid: An R package for cluster validation.

784 *Journal of Statistical Software*, *25*(4), 1–22. https://doi.org/10.18637/jss.v025.i04

785 Burton, P., J. Smit, H., & J. Lightowler, H. (2007). The influence of restrained and external
786 eating patterns on overeating. *Appetite*, *49*(1), 191–197.
787 https://doi.org/10.1016/j.appet.2007.01.007

788 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P.
789 (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature*
790 *Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

791 Cattaneo, C., Gargari, G., Koirala, R., Laureati, M., Riso, P., Guglielmetti, S., & Pagliarini, E.
792 (2019). New insights into the relationship between taste perception and oral microbiota
793 composition. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-40374-3

794 Cattaneo, C., Riso, P., Laureati, M., Gargari, G., & Pagliarini, E. (2019). Exploring associations
795 between interindividual differences in taste perception, oral microbiota composition, and
796 reported food intake. *Nutrients*, *11*(5), 1167. https://doi.org/10.3390/nu11051167

797 Charrad, M., Ghazzali, N., Boiteau, V., & Niknafs, A. (2014). Nbclust: An R package for
798 determining the relevant number of clusters in a data set. *Journal of Statistical Software*,
799 *61*(6), 1–36. https://doi.org/10.18637/jss.v061.i06

800 Cox, D. N., Hendrie, G. A., & Carty, D. (2016). Sensitivity, hedonics and preferences for basic
801 tastes and fat amongst adults and children of differing weight status: A comprehensive
802 review. In *Food Quality and Preference* (Vol. 48, pp. 359–367). Elsevier.
803 https://doi.org/10.1016/j.foodqual.2015.01.006

804 Cronin, P., Joyce, S. A., O'toole, P. W., & O'connor, E. M. (2021). Dietary fibre modulates the
805 gut microbiota. *Nutrients*, *13*(5). https://doi.org/10.3390/nu13051655

806 De Backer, C. J. S., & Hudders, L. (2015). Meat morals: relationship between meat consumption
807 consumer attitudes towards human and animal welfare and moral behavior. *Meat Science*,
808 *99*, 68–74. https://doi.org/10.1016/J.MEATSCI.2014.08.011

809 Dinnella, C., Monteleone, E., Piochi, M., Spinelli, S., Prescott, J., Pierguidi, L., Gasperi, F.,
810 Laureati, M., Pagliarini, E., Predieri, S., Torri, L., Barbieri, S., Valli, E., Bianchi, P., Braghieri,
811 A., Del Caro, A., Di Monaco, R., Favotto, S., & Moneta, E. (2018). Individual variation in
812 PROP status, fungiform papillae density, and responsiveness to taste stimuli in a large

population sample. *Chemical Senses*, *43*(9), 697–710. https://doi.org/10.1093/chemse/bjy058

Dinnella, C., Pierguidi, L., Spinelli, S., Borgogno, M., Gallina Toschi, T., Predieri, S., Lavezzi, G., Trapani, F., Tura, M., Magli, M., Bendini, A., & Monteleone, E. (2022). Remote testing: Sensory test during Covid-19 pandemic and beyond. *Food Quality and Preference*, *96*, 104437. https://doi.org/10.1016/j.foodqual.2021.104437

Diószegi, J., Llanaj, E., & Ádány, R. (2019). Genetic Background of Taste Perception, Taste Preferences, and Its Nutritional Implications: A Systematic Review. *Frontiers in Genetics*, *10*, 1272. https://doi.org/10.3389/fgene.2019.01272

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Duffy, V. B. (2007). Variation in oral sensation: Implications for diet and health. *Current Opinion in Gastroenterology*, *23*(2), 171–177. https://doi.org/10.1097/MOG.0b013e3280147d50

Efeyan, A., Comb, W. C., & Sabatini, D. M. (2015). Nutrient-sensing mechanisms and pathways. *Nature*, *517*(7534), 302–310. https://doi.org/10.1038/nature14190

Endrizzi, I., Cliceri, D., Menghi, L., Aprea, E., Charles, M., Monteleone, E., Dinnella, C., Spinelli, S., Pagliarini, E., Laureati, M., Torri, L., Bendini, A., Toschi, T. G., Sinesio, F., Predieri, S., & Gasperi, F. (2022). Relationships between intensity and liking for chemosensory stimuli in food models: A large-scale consumer segmentation. *Foods*, *11*(1), 5. https://doi.org/10.3390/foods11010005

Essick, G. K., Chopra, A., Guest, S., & McGlone, F. (2003). Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects. *Physiology and Behavior*, *80*(2–3), 289–302. https://doi.org/10.1016/j.physbeh.2003.08.007

Feng, Y., Licandro, H., Martin, C., Septier, C., Zhao, M., Neyraud, E., & Morzel, M. (2018). The Associations between Biochemical and Microbiological Variables and Taste Differ in Whole Saliva and in the Film Lining the Tongue. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/2838052

Fischer, M. E., Cruickshanks, K. J., Schubert, C. R., Pinto, A., Klein, R., Pankratz, N., Pankow, J.

S., & Huang, G. H. (2013). Factors related to fungiform papillae density: The beaver dam offspring study. *Chemical Senses*, *38*(8), 669–677. https://doi.org/10.1093/chemse/bjt033

Fluitman, K. S., van den Broek, T. J., Nieuwdorp, M., Visser, M., IJzerman, R. G., & Keijser, B. J. F. (2021). Associations of the oral microbiota and Candida with taste, smell, appetite and undernutrition in older adults. *Scientific Reports*, *11*(1), 1–11. https://doi.org/10.1038/s41598-021-02558-8

Fossati, A., Borroni, S., Marchione, D., & Maffei, C. (2011). Reliability and validity of its italian translation in three independent nonclinical samples. *European Journal of Psychological Assessment*, *27*(1), 50–58. https://doi.org/10.1027/1015-5759/a000043

Garneau, N. L., Nuessle, T. M., Sloan, M. M., Santorico, S. A., Coughlin, B. C., & Hayes, J. E. (2014). Crowdsourcing taste research: Genetic and phenotypic predictors of bitter taste perception as a model. *Frontiers in Integrative Neuroscience*, *8*(MAY), 33. https://doi.org/10.3389/fnint.2014.00033

Gaudioso, G., Marzorati, G., Faccenda, F., Weil, T., Lunelli, F., Cardinaletti, G., Marino, G., Olivotto, I., Parisi, G., Tibaldi, E., Tuohy, K. M., & Fava, F. (2021). Processed animal proteins from insect and poultry by-products in a fish meal-free diet for rainbow trout: Impact on intestinal microbiota and inflammatory markers. *International Journal of Molecular Sciences*, *22*(11), 5454. https://doi.org/10.3390/ijms22115454

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*(NOV), 2224. https://doi.org/10.3389/fmicb.2017.02224

Grant, F., Scalvedi, M. L., Scognamiglio, U., Turrini, A., & Rossi, L. (2021). Eating habits during the covid-19 lockdown in italy: The nutritional and lifestyle side effects of the pandemic. *Nutrients*, *13*(7). https://doi.org/10.3390/nu13072279

Guo, P., Zhang, K., Ma, X., & He, P. (2020). Clostridium species as probiotics: Potentials and challenges. *Journal of Animal Science and Biotechnology*, *11*(1), 1–10. https://doi.org/10.1186/s40104-019-0402-1

Hayes, J. E., Allen, A. L., & Bennett, S. M. (2013). Direct comparison of the generalized visual analog scale (gVAS) and general labeled magnitude scale (gLMS). *Food Quality and*

Preference, *28*(1), 36–44. https://doi.org/10.1016/j.foodqual.2012.07.012

Hayes, J. E., & Keast, R. S. J. (2011). Two decades of supertasting: Where do we stand? *Physiology and Behavior*, *104*(5), 1072–1074. https://doi.org/10.1016/j.physbeh.2011.08.003

Hayes, J. E., Sullivan, B. S., & Duffy, V. B. (2010). Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiology and Behavior*, *100*(4), 369–380. https://doi.org/10.1016/j.physbeh.2010.03.017

Henke, M. T., Kenny, D. J., Cassilly, C. D., Vlamakis, H., Xavier, R. J., & Clardy, J. (2019). Ruminococcus gnavus, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(26), 12672–12677. https://doi.org/10.1073/pnas.1904099116

Herman, A., & Bajaka, A. (2021). The role of the intestinal microbiota in eating disorders – bulimia nervosa and binge eating disorder. In *Psychiatry Research* (Vol. 300, p. 113923). Elsevier. https://doi.org/10.1016/j.psychres.2021.113923

Husson, F., Josse, J., Le, S., & Mazet, J. (2018). FactoMineR: multivariate exploratory data analysis and data mining. *J. Stat. Softw*. http://factominer.free.fr

John, O. P., Naumann, L. P., & Soto, C. J. (2008). Paradigm shift to the integrative Big Five Trait taxonomy. In *Handbook of personality: Theory and research* (Vol. 2, Issue 510). Guilford Press. https://doi.org/10.1016/S0191-8869(97)81000-8

Kaiser, H. F. (1960). The Application of Electronic Computers to Factor Analysis. *Educational and Psychological Measurement*, *20*(1), 141–151. https://doi.org/10.1177/001316446002000116

Kassambara, A. (2017). Practical guide to cluster analysis in R: unsupervised machine learning. In *Journal of Computational and Graphical Statistics*. https://pe56d.s3.amazonaws.com/o_1b64qp8fdsg81t4k1fongljq24m.pdf?AWSAccessKeyId=AKIAIP3NFJ5OR5FTFOQQ&Signature=ekwWQ2FpwaTjBwVMF20SsNGBSR4%3D&Expires=1522680996&response-content-disposition=inline;filename=%22preview.pdf%22&response-content-type=applicati

Kenny, D. J., Plichta, D. R., Shungin, D., Koppel, N., Hall, A. B., Fu, B., Vasan, R. S., Shaw, S. Y., Vlamakis, H., Balskus, E. P., & Xavier, R. J. (2020). Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level. *Cell Host and Microbe*, *28*(2), 245-257.e6. https://doi.org/10.1016/j.chom.2020.05.013

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), e1–e1. https://doi.org/10.1093/nar/gks808

Köster, E. P. (2009). Diversity in the determinants of food choice: A psychological perspective. *Food Quality and Preference*, *20*(2), 70–82. https://doi.org/10.1016/j.foodqual.2007.11.002

Laureati, M., Spinelli, S., Monteleone, E., Dinnella, C., Prescott, J., Cattaneo, C., Proserpio, C., De Toffoli, A., Gasperi, F., Endrizzi, I., Torri, L., Peparaio, M., Arena, E., Bonello, F., Condelli, N., Di Monaco, R., Gatti, E., Piasentier, E., Tesini, F., & Pagliarini, E. (2018). Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Quality and Preference*, *68*, 113–124. https://doi.org/10.1016/j.foodqual.2018.02.007

Lawless, H. (1980). A comparison of different methods used to assess sensitivity to the taste of phenylthiocarbamide (PTC). *Chemical Senses*, *5*(3), 247–256. https://doi.org/10.1093/chemse/5.3.247

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J. M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jørgensen, T., Brandslund, I., Nielsen, H. B., Juncker, A. S., Bertalan, M., … Yamada, T. (2013). Richness of human gut microbiome correlates with metabolic markers. *Nature*, *500*(7464), 541–546. https://doi.org/10.1038/nature12506

Leung, R., & Covasa, M. (2021). Do gut microbes taste? *Nutrients*, *13*(8), 2581. https://doi.org/10.3390/nu13082581

Lin, H., & Peddada, S. Das. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*(1), 1–11. https://doi.org/10.1038/s41467-020-17041-7

Linne, B., & Simons, C. T. (2017). Quantification of oral roughness perception and comparison with mechanism of astringency perception. *Chemical Senses*, *42*(7), 525–535. https://doi.org/10.1093/chemse/bjx029

Ma, E., Maskarinec, G., Lim, U., Boushey, C. J., Wilkens, L. R., Setiawan, V. W., Le Marchand, L., Randolph, T. W., Jenkins, I. C., Curtis, K. R., Lampe, J. W., & Hullar, M. A. J. (2021). Long-term Association between Diet Quality and Characteristics of the Gut Microbiome in the Multiethnic Cohort Study. *British Journal of Nutrition*, *128*(1), 93–102. https://doi.org/10.1017/S0007114521002968

Mani, E., Ford, R., Pierguidi, L., Spinelli, S., Ramsey, I., Monteleone, E., & Dinnella, C. (2022). Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness. *Journal of Texture Studies*, *53*(3), 345–356. https://doi.org/10.1111/jtxs.12683

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Miao, Z., Du, W., Xiao, C., Su, C., Gou, W., Shen, L., Zhang, J., Fu, Y., Jiang, Z., Wang, Z., Jia, X., Zheng, J.-S., & Wang, H. (2022). Gut microbiota signatures of long-term and short-term plant-based dietary pattern and cardiometabolic health: a prospective cohort study. *BMC Medicine*, *20*(1), 1–15. https://doi.org/10.1186/s12916-022-02402-4

Monteleone, E., Spinelli, S., Dinnella, C., Endrizzi, I., Laureati, M., Pagliarini, E., Sinesio, F., Gasperi, F., Torri, L., Aprea, E., Bailetti, L. I., Bendini, A., Braghieri, A., Cattaneo, C., Cliceri, D., Condelli, N., Cravero, M. C., Del Caro, A., Di Monaco, R., … Tesini, F. (2017). Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Quality and Preference*, *59*, 123–140. https://doi.org/10.1016/j.foodqual.2017.02.013

Nearing, J. T., Douglas, G. M., Hayes, M. G., MacDonald, J., Desai, D. K., Allward, N., Jones, C. M. A., Wright, R. J., Dhanani, A. S., Comeau, A. M., & Langille, M. G. I. (2022). Microbiome differential abundance methods produce different results across 38 datasets. *Nature Communications*, *13*(1), 1–16. https://doi.org/10.1038/s41467-022-28034-z

Nolden, A. A., McGeary, J. E., & Hayes, J. E. (2020). Predominant Qualities Evoked by Quinine, Sucrose, and Capsaicin Associate with PROP Bitterness, but not TAS2R38 Genotype.

Chemical Senses, *45*(5), 383–390. https://doi.org/10.1093/chemse/bjaa028

Ohira, H., Tsutsui, W., & Fujioka, Y. (2017). Are short chain fatty acids in gut microbiota defensive players for inflammation and atherosclerosis? *Journal of Atherosclerosis and Thrombosis*, *24*(7), 660–672. https://doi.org/10.5551/jat.RV17006

Palarea-Albaladejo, J., & Martín-Fernández, J. A. (2015). ZCompositions - R package for multivariate imputation of left-censored data under a compositional approach. *Chemometrics and Intelligent Laboratory Systems*, *143*, 85–96. https://doi.org/10.1016/j.chemolab.2015.02.019

Pascal, V., Pozuelo, M., Borruel, N., Casellas, F., Campos, D., Santiago, A., Martinez, X., Varela, E., Sarrabayrouse, G., Machiels, K., Vermeire, S., Sokol, H., Guarner, F., & Manichanh, C. (2017). A microbial signature for Crohn's disease. *Gut*, *66*(5), 813–822. https://doi.org/10.1136/gutjnl-2016-313235

Pedrabissi, L., & Santinello, M. (1989). Verifica della validità dello STAI forma Y di Spielberger. [Verification of the validity of the STAI, Form Y, by Spielberger.]. *Giunti Organizzazioni Speciali*, *191–192*, 11–14.

Piochi, M., Dinnella, C., Spinelli, S., Monteleone, E., & Torri, L. (2021). Individual differences in responsiveness to oral sensations and odours with chemesthetic activity: Relationships between sensory modalities and impact on the hedonic response. *Food Quality and Preference*, *88*. https://doi.org/10.1016/j.foodqual.2020.104112

Pliner, P., & Hobden, K. (1992). Development of a scale to measure the trait of food neophobia in humans. *Appetite*. https://doi.org/10.1016/0195-6663(92)90014-W

Poslusna, K., Ruprich, J., De Vries, J. H. M., Jakubikova, M., & Van'T Veer, P. (2009). Misreporting of energy and micronutrient intake estimated by food records and 24hour recalls, control and adjustment methods in practice. *British Journal of Nutrition*, *101*(SUPPL. 2), S73–S85. https://doi.org/10.1017/S0007114509990602

Puputti, S., Aisala, H., Hoppu, U., & Sandell, M. (2018). Multidimensional measurement of individual differences in taste perception. *Food Quality and Preference*, *65*, 10–17. https://doi.org/10.1016/j.foodqual.2017.12.006

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F.

O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1). https://doi.org/10.1093/nar/gks1219

R Core Team. (2019). A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*, *2*(1), https://www.R--project.org. http://www.r-project.org

Ranjan, R., Rani, A., Metwally, A., McGee, H. S., & Perkins, D. L. (2016). Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochemical and Biophysical Research Communications*, *469*(4), 967–977. https://doi.org/10.1016/j.bbrc.2015.12.083

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, *7*(1), 14. https://doi.org/10.3390/microorganisms7010014

Roininen, K., & Tuorila, H. (1999). Health and taste attitudes in the prediction of use frequency and choice between less healthy and more healthy snacks. *Food Quality and Preference*, *10*(4–5), 357–365. https://doi.org/10.1016/s0950-3293(98)00057-3

Saba, A., Sinesio, F., Moneta, E., Dinnella, C., Laureati, M., Torri, L., Peparaio, M., Saggia Civitelli, E., Endrizzi, I., Gasperi, F., Bendini, A., Gallina Toschi, T., Predieri, S., Abbà, S., Bailetti, L., Proserpio, C., & Spinelli, S. (2019). Measuring consumers attitudes towards health and taste and their association with food-related life-styles and preferences. *Food Quality and Preference*, *73*, 25–37. https://doi.org/10.1016/j.foodqual.2018.11.017

Sauvageot, N., Schritz, A., Leite, S., Alkerwi, A., Stranges, S., Zannad, F., Streel, S., Hoge, A., Donneau, A. F., Albert, A., & Guillaume, M. (2017). Stability-based validation of dietary patterns obtained by cluster analysis. *Nutrition Journal*, *16*(1), 1–13. https://doi.org/10.1186/s12937-017-0226-9

Schutz, H. G., & Cardello, A. V. (2001). A labeled affective magnitude (LAM) scale for assessing food liking/disliking. *Journal of Sensory Studies*, *16*(2), 117–159. https://doi.org/10.1111/j.1745-459X.2001.tb00293.x

Schwartz, M., Canon, F., Feron, G., Neiers, F., & Gamero, A. (2021). Impact of oral microbiota

on flavor perception: From food processing to in-mouth metabolization. In *Foods* (Vol. 10, Issue 9). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods10092006

Smutzer, G., Desai, H., Coldwell, S. E., & Griffith, J. W. (2013). Validation of edible taste strips for assessing PROP taste perception. *Chemical Senses*, *38*(6), 529–539. https://doi.org/10.1093/chemse/bjt023

Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M., & Hummel, T. (2012). The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS ONE*, *7*(5), e36557. https://doi.org/10.1371/journal.pone.0036557

Spielberger, C. D. (1983). Manual for the State-Trait Anxiety Inventory. In *Palo Alto, CA: Consulting Psychologists Press; Vol. IV*.

Spinelli, S., De Toffoli, A., Dinnella, C., Laureati, M., Pagliarini, E., Bendini, A., Braghieri, A., Gallina Toschi, T., Sinesio, F., Torri, L., Gasperi, F., Endrizzi, I., Magli, M., Borgogno, M., di Salvo, R., Favotto, S., Prescott, J., & Monteleone, E. (2018). Personality traits and gender influence liking and choice of food pungency. *Food Quality and Preference*, *66*, 113–126. https://doi.org/10.1016/j.foodqual.2018.01.014

Swartz, T. D., Duca, F. A., De Wouters, T., Sakar, Y., & Covasa, M. (2012). Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota. *British Journal of Nutrition*, *107*(5), 621–630. https://doi.org/10.1017/S0007114511003412

Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., Moodie, M. L., & Gortmaker, S. L. (2011). The global obesity pandemic: Shaped by global drivers and local environments. *The Lancet*, *378*(9793), 804–814. https://doi.org/10.1016/S0140-6736(11)60813-1

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Thompson, F. E., & Subar, A. F. (2017). Dietary assessment methodology. *Nutrition in the Prevention and Treatment of Disease*, 5–48. https://doi.org/10.1016/B978-0-12-802928-

2.00001-1

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The Human Microbiome Project. In *Nature* (Vol. 449, Issue 7164, pp. 804–810). https://doi.org/10.1038/nature06244

Turner, A., Chijoff, E., Veysey, M., Keely, S., Scarlett, C. J., Lucock, M., & Beckett, E. L. (2019). Interactions between taste receptors and the gastrointestinal microbiome in inflammatory bowel disease. In *Journal of Nutrition and Intermediary Metabolism* (Vol. 18). https://doi.org/10.1016/j.jnim.2019.100106

Turner, A., Veysey, M., Keely, S., Scarlett, C., Lucock, M., & Beckett, E. L. (2018). Interactions between bitter taste, diet and dysbiosis: Consequences for appetite and obesity. *Nutrients*, *10*(10), 1336. https://doi.org/10.3390/nu10101336

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., & De Angelis, M. (2020). The controversial role of human gut lachnospiraceae. *Microorganisms*, *8*(4). https://doi.org/10.3390/microorganisms8040573

van Strien, T., Frijters, J. E. R., Bergers, G. P. A., & Defares, P. B. (1986). The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*, *5*(2), 295–315. https://doi.org/10.1002/1098-108X(198602)5:2<295::AID-EAT2260050209>3.0.CO;2-T

Vascellari, S., Melis, M., Cossu, G., Melis, M., Serra, A., Palmas, V., Perra, D., Oppo, V., Fiorini, M., Cusano, R., Morelli, M., Manzin, A., & Barbarossa, I. T. (2020). Genetic variants of TAS2R38 bitter taste receptor associate with distinct gut microbiota traits in Parkinson's disease: A pilot study. *International Journal of Biological Macromolecules*, *165*, 665–674. https://doi.org/10.1016/j.ijbiomac.2020.09.056

Vujkovic-Cvijin, I., Sklar, J., Jiang, L., Natarajan, L., Knight, R., & Belkaid, Y. (2020). Host variables confound gut microbiota studies of human disease. *Nature*, *587*(7834), 448–454. https://doi.org/10.1038/s41586-020-2881-9

Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E., & Keast, R. (2015). The Relationships Between Common Measurements of Taste Function. *Chemosensory Perception*, *8*(1), 11–18. https://doi.org/10.1007/s12078-015-9183-x

Wolters, M., Ahrens, J., Romaní-Pérez, M., Watkins, C., Sanz, Y., Benítez-Páez, A., Stanton, C., & Günther, K. (2019). Dietary fat, the gut microbiota, and metabolic health – A systematic review conducted within the MyNewGut project. *Clinical Nutrition*, *38*(6), 2504–2520. https://doi.org/10.1016/j.clnu.2018.12.024

Zhang, L., Ouyang, Y., Li, H., Shen, L., Ni, Y., Fang, Q., Wu, G., Qian, L., Xiao, Y., Zhang, J., Yin, P., Panagiotou, G., Xu, G., Ye, J., & Jia, W. (2019). Metabolic phenotypes and the gut microbiota in response to dietary resistant starch type 2 in normal-weight subjects: a randomized crossover trial. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-018-38216-9

Zhao, L., Kirkmeyer, S. V., & Tepper, B. J. (2003). A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. *Physiology and Behavior*, *78*(4–5), 625–633. https://doi.org/10.1016/S0031-9384(03)00057-X

Zouiouich, S., Loftfield, E., Huybrechts, I., Viallon, V., Louca, P., Vogtmann, E., Wells, P. M., Steves, C. J., Herzig, K. H., Menni, C., Jarvelin, M. R., Sinha, R., & Gunter, M. J. (2021). Markers of metabolic health and gut microbiome diversity: findings from two population-based cohort studies. *Diabetologia*, *64*(8), 1749–1759. https://doi.org/10.1007/s00125-021-05464-w