Double degree PhD program in

Agrifood and Environmental Sciences (University of Trento)

Engineering Science (University of Southern Denmark)

Leonardo Menghi

Understanding the role of human microbiota on sensory perception

Supervisors

Ass. Prof. Flavia Gasperi, Ass. Prof. Davide Giacalone

Tutors

Ass. Prof. Flavia Gasperi, Ass. Prof. Davide Giacalone

XXXV Cycle, 2022/2023

The PhD program in Agrifood and Environmental Sciences is managed by the
Center Agricolture Food Environment (C3A, University of Trento)
in cooperation with the Edmund Mach Foundation (FEM)

Thesis committee

Advisors

Ass. Prof. Flavia Gasperi
Center Agricolture Food Environment (C3A)
University of Trento

Ass. Prof. Davide Giacalone
Department of Technology and Innovation
University of Southern Denmark

Evaluation committee

Ass. Prof. Mathias Porsmose Clausen
Department of Green Technology, SDU Biotechnology
University of Southern Denmark

Dr. Melania Melis
Department of Biomedical Science Division of Physiology
University of Cagliari

Prof. John Prescott
Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (*DAGRI*)
University of Florence

Prof. Nicola Segata
Department of Cellular, Computational and Integrative Biology (CIBIO)
University of Trento

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 2 – SUPPLEMENTAL MATERIAL

CHAPTER 3

CHAPTER 3 – SUPPLEMENTAL MATERIAL

CHAPTER 4

CHAPTER 4 – SUPPLEMENTAL MATERIAL

CHAPTER 5

CHAPTER 5 – SUPPLEMENTAL MATERIAL

LIST OF TABLES

CHAPTER 2

CHAPTER 2 – SUPPLEMENTAL MATERIAL

CHAPTER 3

CHAPTER 4

CHAPTER 4 – SUPPLEMENTAL MATERIAL

CHAPTER 5

CHAPTER 5 – SUPPLEMENTAL MATERIAL

CHAPTER 6

LIST OF ABBREVIATIONS

ANCOM-BC	Analysis of Compositions of Microbiomes with Bias Correction
ASV	Amplicon Sequence Variant
AUC	Area under the curve
BMI	Body Mass Index
CCCRC	Connecticut Chemosensorial Clinical Research Center
CRIq	Cognitive Reserve Index questionnaire
FN	Food Neophobia
FNS	Food Neophobia Scale
GI	Gastrointestinal
GI microbiota	Gut and oral microbiota
gLMS	generalized Labeled Magnitude Scale
GPCRs	G protein-coupled receptors
LAM	Labeled Affective Magnitude scale
MFA	Multiple Factor Analysis
MoCA	Montreal Cognitive Assessment
MTs	PROP Medium Tasters
NS	Nose-space analysis
NTs	PROP Non Tasters
OD	Odor Discrimination
OI	Odor Identification
OR	Olfactory receptor
OSN	Olfactory sensory neuron
OT	Odor Threshold
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PROP	6-n-propylthiouracil
SIFT-MS	Selected-Ion Flow-Tube Mass Spectrometry
SNPs	Single nucleotide polymorphisms
STAI-T	State-Trait Anxiety Inventory Questionnaire
STs	PROP Super Tasters
TDI	Thresholds, Discrimination, Identification
TRCs	Taste receptor cells
UPSIT	University of Pennsylvania Smell Identification Test
VOCs	Volatile Organic Compounds

ABSTRACT

While consumer awareness of benefits of adequate nutrition has noticeably surged in recent years, developing countermeasures against improper eating habits still represents a public health priority in view of the growing prevalence of diet-related diseases. Eating behaviours are complex phenomena driven by a spectrum of biological and environmental factors, wherein (chemo)sensory perception is reckoned amongst the most influential. Analogously, chemosensation is affected by a myriad of determinants, and this warrants the commonly observed large variation in how tastes and smells are perceived among individuals. Given how such variability intimately relates to dietary habits, deciphering its underlying mechanisms is paramount to promoting healthier food choices.

In this vein, emerging evidence suggests that human eating behaviours can also be affected by interactions between the gastrointestinal microbiota and the chemosensory systems. Despite growing interest, the sensory-oriented microbiome field suffers from obvious limitations due to its recent emergence. As a result, little efforts has been devoted to elucidating: a) the associations between the oral microbiota and olfaction or known psychological mediators of sensory perception; b) the links between the distal gut microbiota and taste functioning; c) the consequences of interactions between chemosensation and the gastrointestinal microbiota on dietary intakes.

Against this backdrop, this thesis aimed at expanding the current knowledge on the interplays between domains of sensory perception and the gastrointestinal microbiota and how these might mirror variations in habitual food habits. In detail, four studies probing the associations a) between a psychosocial correlate of sensory perception (food neophobia), olfaction (Chapter 2) and the oral microbiota (Chapter 3); and b) between distal gut (Chapter 4) or oral (Chapter 5) microbiota, taste functioning and dietary intakes are here presented.

In Chapter 2 and 3, a healthy cohort of 83 individuals (57.8 % women; aged 22-68 yo) remotely filled out the common Food Neophobia Scale and the trait anxiety subscale of the State-Trait Anxiety Inventory prior to providing a salivary sample for subsequent metataxonomic analysis (16S rRNA gene sequencing). Next, volunteers were tested for orthonasal olfactory functioning via the Sniffin' Sticks battery, and monitored for retronasal aroma release while consuming a strawberry jelly candy by *nose-space* analysis (Selected-Ion Flow-Tube Mass Spectrometry). In Chapter 4 and 5, instead, 100 young adult volunteers (52 % women; aged 18-30 yo) attended a 7-day lasting remote protocol where responsiveness to genetically-mediated bitterness of 6-n-propylithiuracil (PROP), hedonics and intensity of oral sensations elicited by ten commercially-available food products, a battery of food-related psychological traits, a 4-day food record, and one salivary and one stool sample (sequenced by targeting the 16s rRNA gene) were collected.

Overall, results substantially strengthen past evidence suggesting: a) that pronounced neophobic tendencies translate into higher levels of (negative) emotional activation or arousal towards foods; b) the existence of homogenous groups of individuals with generalized hypergeusia towards oral stimulations; c) that hyperresponsiveness to a peculiar taste quality is a barrier to the intake of foods evoking such sensation; d) that habitual consumption of dietary fibers and simple carbohydrates can shape both the gut and oral microbial ecology, respectively. Intriguingly, food neophobia and poor olfaction were positively associated with oral microbial markers of dysbiosis (e.g., *Porphyromonas gingivalis*), whilst a *Clostridia*-enriched salivary microbiota co-occurred with low responsiveness to alarming oral sensations (astringency, bitter, sour) elicited by real foods. Similarly, an ample panel of commensal gut bacterial genera mainly allocated to the families *Lachnospiraceae* and *Ruminococcaceae* was found to be enriched in individuals exhibiting lower acuity to both tastes (bitter, salty, sour, sweet) and trigeminal sensations (astringent, pungent). Besides taxonomically

annotating a range of microbial taxa tied to sensory perception, putative metabolic pathways used by salivary and gut microbial communities to modulate taste perception were inferred and discussed.

To conclude, this thesis supports the notion that the gastrointestinal microbiota is an additional candidate to explain interindividual variations in taste and smell perception, and provides novel important insights into the aetiology of eating behaviours. More importantly, this work also offers methodological cues to robustly assess the associations between chemosensation and host-related non genetic factors, and paves the way for future interventional studies targeting the efficacy of sensory-related microbial taxa as potential modulators of dietary habits.

RIASSUNTO

Nonostante negli ultimi anni i consumatori abbiano maturato maggior consapevolezza riguardo i benefici di una sana alimentazione, lo sviluppo di contromisure contro abitudini alimentari inadeguate rappresenta ancora una priorità per la salute pubblica alla luce dell'attuale prevalenza di malattie legate allo stile di vita. I comportamenti alimentari sono fenomeni complessi guidati da una vasta gamma di fattori tanto biologici quanto ambientali, tra i quali la percezione sensoriale riveste un ruolo di primo ordine. A sua volta, anche la percezione sensoriale può essere influenzata da una moltitudine di elementi, e ciò spiega il motivo per il quale si riscontra spesso un'ampia variabilità tra individui nella percezione di odori, gusti e sapori. Dato che tale variabilità è in grado di condizionare le nostre abitudini alimentari, comprendere a fondo l'insieme dei meccanismi biologici alla base della percezione sensoriale è di rilevante importanza al fine di promuovere scelte alimentari più sane.

In tal senso, alcuni studi di recente pubblicazione suggeriscono che i comportamenti alimentari potrebbero essere influenzati anche da presunte interazioni tra il microbiota gastrointestinale e i sistemi chemosensoriali. Nonostante un interesse sempre crescente, questo nuovo filone della ricerca soffre di evidenti lacune in virtù della sua giovane età. Di conseguenza, finora è stata prestata poca attenzione allo studio: a) delle associazioni tra il microbiota orale, l'olfatto e alcuni noti mediatori psicosociali della percezione sensoriale; b) dei legami tra il microbiota intestinale e la funzione gustativa; c) del ruolo che le possibili interazioni tra il microbiota gastrointestinale e i sistemi chemosensoriali possano avere nel condizionare gli apporti dietetici quotidiani.

In questo contesto, il presente lavoro di tesi ha avuto l'obiettivo di ampliare le attuali conoscenze sui legami tra la percezione sensoriale e il microbiota gastrointestinale con il fine ultimo di comprendere meglio come questi possano spiegare le differenze individuali nelle abitudini alimentari. Specificatamente, l'elaborato presenta i risultati di quattro diversi studi concepiti per

approfondire le associazioni: a) tra un correlato psicosociale della percezione sensoriale (la neofobia alimentare), la funzione olfattiva (Capitolo 2) e il microbiota orale (Capitolo 3); e b) tra il microbiota intestinale (Capitolo 4) o orale (Capitolo 5), la funzione gustativa e gli apporti dietetici abituali.

Nei Capitoli 2 e 3, una coorte sana di 83 individui (57.8% donne; età compresa tra 22 e 68 anni) ha compilato da remoto due questionari volti alla misurazione dei livelli di neofobia alimentare (Food Neophobia Scale) e dell'ansia di tratto (trait-anxiety subscale; State-Trait Anxiety Inventory), prima di fornire un campione salivare la cui composizione microbica è stata profilata mediate tecniche metatassonomiche (sequenziamento del gene 16S rRNA). Successivamente, i volontari sono stati sottoposti ad analisi olfattometrica completa mediante la versione estesa dello Sniffin' Sticks Test prima di essere monitorati per una serie di composti volatili rilasciati per via retronasale durante il consumo di un alimento modello (caramella alla fragola) mediante analisi *nose-space* (Selected-Ion Flow-Tube Mass Spectrometry). Nei Capitoli 4 e 5, invece, un gruppo di 100 giovani adulti (52 % donne; età compresa tra i 18 e i 30 anni) ha partecipato da remoto a un protocollo della durata di 7 giorni in cui sono stati raccolti responsi legati alla percezione dell'amaro indotta dal 6-n-propiltiuracile (PROP), nonché al gradimento e all'intensità delle sensazioni orali suscitate da 10 prodotti alimentari commerciali. Inoltre, i volontari hanno compilato a una serie di test psicometrici volti alla misurazione di tratti psicologici connessi alle abitudini alimentari, un diario alimentare di 4 giorni e fornito un campione salivare e uno fecale (analizzati mediate sequenziamento del gene 16s rRNA).

Nel complesso, i risultati hanno rafforzato alcune conoscenze preesistenti secondo cui: a) la presenza di tratti neofobici pronunciati implica livelli più elevati di attivazione emotiva negativa nei confronti del cibo; b) esisterebbe una fetta della popolazione che mostra ipergeusia generalizzata nei confronti delle sensazioni orali; c) l'iperreattività a una determinata qualità gustativa ostacola l'assunzione di alimenti in grado di suscitarla; d) il consumo abituale di fibre alimentari e di carboidrati

semplici può modellare rispettivamente l'ecologia microbica intestinale e orale. Inoltre, il presente elaborato ha anche evidenziato che tanto la presenza di spiccate tendenze neofobiche quanto di ridotte abilità olfattive si associa positivamente a marcatori microbici orali di disbiosi (ad esempio, *Porphyromonas gingivalis*), mentre gli individui con un microbiota salivare arricchito di microorganismi afferenti alla classe *Clostridia* tendono a percepire un insieme di sensazioni orali di allarme (acido, amaro, astringente) come meno intense. Similmente, un insieme di generi batterici intestinali commensali, principalmente allocati alle famiglie *Lachnospiraceae* e *Ruminococcaceae*, è risultato più abbondante in coloro che mostravano una minore acuità sia ai gusti (acido, amaro, dolce, salato) che alle sensazioni trigeminali (astringente, pungente) elicitate dai 10 prodotti alimentari impiegati. Oltre ad aver classificato tassonomicamente un serie di microrganismi associati alla percezione sensoriale, il presente lavoro di tesi specula e discute una serie di presunte vie metaboliche potenzialmente usate dalle comunità microbiche salivari e intestinali per modulare la percezione.

In conclusione, questa tesi supporta l'idea che il microbiota gastrointestinale possa contribuire alla spiegazione della vasta variabilità individuale nella percezione di gusti e odori e fornisce un nuovo importante contributo alla comprensione dell'eziologia dei comportamenti alimentari. Inoltre, questo lavoro offre spunti metodologici per valutare in modo robusto le associazioni tra la percezione sensoriale e i fattori legati all'ospite di natura non genetica oltre ad aprire la strada per futuri studi d’intervento volti a verificare l’efficacia nell’utilizzo di marcatori microbici della percezione sensoriale come potenziali modulatori delle abitudini alimentari.

RESUMÉ

Mens forbrugernes bevidsthed om fordelene ved passende ernæring er steget markant i de seneste år, er det stadig en prioritet for folkesundheden at udvikle modforanstaltninger mod forkerte spisevaner i lyset af den stigende forekomst af kostrelaterede sygdomme. Spiseadfærd er et komplekst fænomen, der drives af en række biologiske og miljømæssige faktorer, hvor (kemo)sensorisk perception regnes for at være en af de mest indflydelsesrige. Tilsvarende påvirkes kemosensationen af et utal af determinanter, og dette begrunder den almindeligt observerede store variation i den måde, hvorpå smags- og lugtoplevelser opfattes af forskellige individer. Da en sådan variation er tæt forbundet med kostvaner, er det af afgørende betydning for at fremme sundere fødevarevalg at afkode de underliggende mekanismer.

I den forbindelse tyder nye beviser på, at menneskers spiseadfærd også kan påvirkes af formodede gensidige forbindelser mellem den gastrointestinale mikrobiota og de kemosensoriske systemer. På trods af den stigende interesse lider det sensorisk orienterede mikrobiomfelt under åbenlyse begrænsninger på grund af dets nylige opståen. Som følge heraf er der kun gjort en lille indsats for at belyse: a) forbindelserne mellem den orale mikrobiota og lugtesansen eller kendte psykologiske mediatorer for sanseopfattelse, b) forbindelserne mellem den distale tarmmikrobiota og smagsfunktionen, c) konsekvenserne af interaktioner mellem kemosensationen og den gastrointestinale mikrobiota for kostindtag.

På denne baggrund havde denne afhandling til formål at udvide den nuværende viden om samspillet mellem områder af sanseopfattelse og den gastrointestinale mikrobiota, og hvordan dette kan afspejle variationer i vanemæssige madvaner. I detaljer præsenteres her fire Kapitel, der undersøger forbindelserne a) mellem psykosociale korrelater af sanseopfattelse, lugtesansen (Kapitel

2) og den orale mikrobiota (Kapitel 3); og b) mellem distal tarm (Kapitel 4) eller oral (Kapitel 5) mikrobiota, smagsfunktion og kostindtag.

I Kapitel 2 og Studie 3 udfyldte en sund kohorte af 83 personer (57.8 % kvinder; alder 22-68 år) hjemmefra den almindelige Food Neophobia Scale (skalaen for fødevarefobi) og på en delskala af State-Trait Anxiety Inventory (skalaen for generaliseret angst), før de afgav en spytprøve til efterfølgende metataxonomisk analyse (16S rRNA-gen-sekventering). Dernæst blev frivillige testet for ortonasal olfaktorisk funktion via Sniffin' Sticks og overvåget for retronasal aromafrigivelse under indtagelse af en jordbær vingummi ved hjælp af *næse-hulrums* analyse *(nose-space* analysis) (Selected-Ion Flow-Tube Mass Spectrometry). I Kapitel 4 og 5 deltog i stedet 100 unge voksne frivillige (52 % kvinder; 18-30 år) i en 7-dages langvarig fjernprotokol, hvor der blev indsamlet respons på genetisk formidlet bitterhed af 6-n-propylithiuracil (PROP), hedonisk og intensitet af orale fornemmelser fremkaldt af ti kommercielt tilgængelige fødevarer, talrige fødevarerelaterede psykologiske egenskaber, en 4-dages madoptegnelse og en spyt- og en afføringsprøve (sekventeret ved at målrette 16s rRNA-genet) blev indsamlet.

Samlet set forstærkede resultaterne i væsentlig grad tidligere rapporter, der tyder på: a) at udpræget neofobiske tendenser udmønter sig i højere niveauer af (negativ) følelsesmæssig aktivering eller ophidselse over for fødevarer; b) eksistensen af homogene grupper af personer med generaliseret hypergeusi over for orale stimulationer; c) at hyperresponsivitet over for en bestemt smagskvalitet er en barriere for indtagelse af fødevarer, der fremkalder en sådan fornemmelse; d) at sædvanligt forbrug af kostfibre og simple kulhydrater kan henholdsvis forme både tarmen og den orale mikrobielle miljø. Det er interessant at se, at neofobi for fødevarer og dårlig lugtesans var positivt forbundet med orale mikrobielle markører for dysbiose (f.eks. *Porphyromonas gingivalis*), mens en *Clostridia*-beriget spytmikrobiota var sammenfaldende med lav responsivitet over for alarmerende orale fornemmelser (astringerende, bitter, sur) fremkaldt af ægte fødevarer. På samme måde viste det sig, at et omfattende

panel af bakterieslægter fra tarmbakterier, der hovedsageligt tilhører familierne *Lachnospiraceae* og *Ruminococcaceae*, var beriget hos personer, der udviste en lavere skarphed over for både smagsoplevelser (bitter, salt, sød, sur) og trigeminale fornemmelser (astringerende, skarp). Ud over taxonomisk annotering af en række mikrobielle taxa, der er knyttet til sensorisk perception, blev der udledt og diskuteret formodede metaboliske veje, der anvendes af spyt- og tarmmikrobielle samfund til at modulere smagsopfattelsen.

Afslutningsvis støtter denne afhandling tanken om, at den gastrointestinale mikrobiota er en yderligere kandidat til at forklare interindividuelle variationer i smags- og lugtopfattelse, og den giver ny vigtig indsigt i ætiologien af spiseadfærd. Endnu vigtigere er det, at dette arbejde også giver metodologiske signaler til robust vurdering af forbindelserne mellem kemosensation og værtsrelaterede ikke genetiske faktorer og baner vejen for fremtidige interventions, der er rettet mod effektiviteten af sensorisk relaterede mikrobielle taxa som potentielle modulatorer af kostvaner.

Chapter 1

Introduction

CHAPTER 1:

INTRODUCTION

1.1 | Basics of sensory perception

Sense organs are vital to synchronize humans with their surroundings and serve as a gateway to fathom the reality. Evolutionary speaking, sensory processing permits us to capture a myriad of environmental stimuli, and to endorse adaptive actions in the pursuit of survival [1]. Such paradigm directs the activities of daily living, and reaches one of its greatest expressions in the dynamics underlying ingestive behaviors. While food choices intimately tied to complex crosstalks among the five senses, it sounds axiomatic that smell and taste exert a role of considerable importance.

1.1.1 | Basics of smell perception

Olfaction is a chemical sense [2], and it is primary devoted to tracing and decoding an ample spectrum of airborne chemicals that act as precursors of safe or harmful scenarios [3]. Consequently, the olfactory system endows us with abilities aimed at safeguarding each domain of life, from promoting social interactions to locating useful nutritional sources [3,4]. The anticipatory messages send by olfaction originate as soon as odorants are detected by the vast panel of G-protein-coupled receptors (GPCRs) expressed by the olfactory sensory neurons (OSNs) colonizing the olfactory epithelium lining the nasal cavity [5,6]. Odorous molecules can take advantage of two distinct routes (Figure 1.1) to trigger the olfactory system, depending on whether volatile chemicals reach the olfactory epithelium by travelling via the nostrils (when inhaled; orthonasal olfaction) or via the nasopharynx (when exhaled in mouth while eating; retronasal olfaction) [7].

Regardless of the passage, ligands are firstly dissolved in the mucus secreted by the olfactory epithelium and bonded to odorant-binding proteins [8] to ease the contact with luminal protrusions of the OSNs (cilia), each equipped with highly specialized odorant receptors [5,6] (Figure 1.1). As a result, each free nerve endings expresses a single multimolecular-transducing receptor [5], which gives olfaction the capacity to elaborate a tremendous number of chemicals including irritants [6].

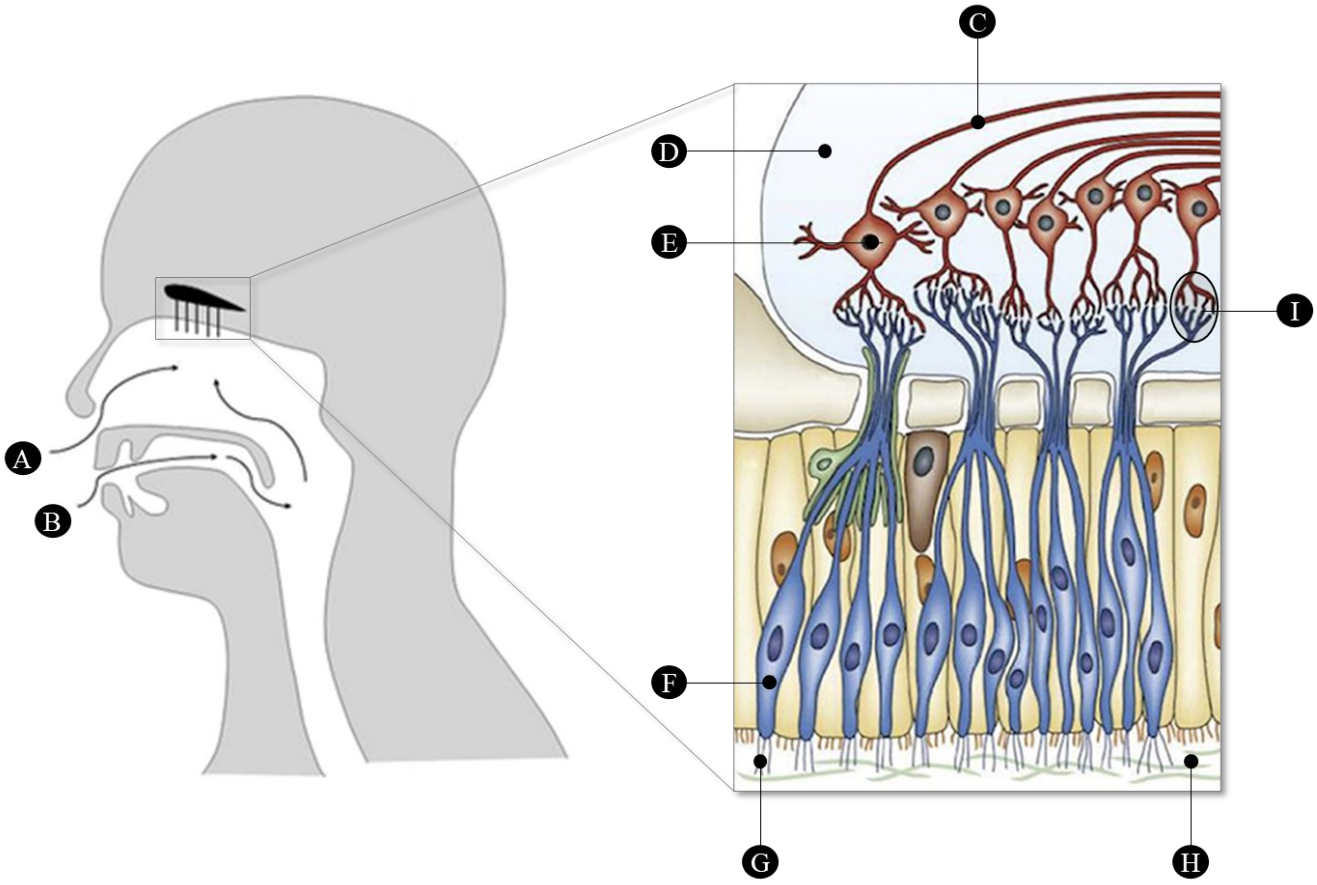

Figure 1.1: Physiology of the human olfactory system. **A.** Orthonasal olfaction; **B.** Retronasal olfaction; **C.** Axons of mitral cells; **D.** Olfactory bulb; **E.** Mitral cells; **F.** Olfactory receptor cell; **G.** Cilia; **H.** Mucus layer; **I.** Glomerulus. Adapted from [9] (left panel; **A-B**), and from [10] (right panel; **C-I**).

Upon an odorant binds its receptor neuron, an immediate ciliary membrane depolarization signals the beginning of conversion of the olfactory stimulus into percept [5,6]. Indeed, such membranal voltage reduction fires action potentials ultimately reaching the site of primary processing of the input

(the olfactory bulb) via single unbranched axons (olfactory nerve, cranial nerve I), whose apical projections coalesce into small spherical structures named glomeruli [5,6] (Figure 1.1). In turn, these axonal repertoires are innervated by the dendrites of second-order olfactory bulb neurons (Figure 1.1; mitral and tufted cells), which form synaptic connections running through a bundle of nerve fibers (the olfactory tract) to convey information to various cortical targets [5,6,11–13]. The signal transduction will thus resolve to a cluster of interconnected cortical regions, including the primary olfactory cortex and areas of the limbic system (hippocampus, amygdala), each eliciting qualitative or quantitative features of odorants that will lead to the conscious perception of smell [6,11–13].

1.1.2 | Basics of taste perception

Akin to olfaction, the evolutionary purpose of taste is widely established [14]. In its absence, humanity survival would have been jeopardized by the inability to properly detect and distinguish useful from noxious or indigestible nutritional sources. To this end, taste elicits sentinel indicators of food quality, and integrates with other sensory modalities (olfaction, somatosensation) to generate holistic sensations (flavors) that amplify the message and signal the palatability of food. Further, taste guides the metabolic fate of nutrients, as combines with smell and vision to activate a series of biological pathways (named cephalic phase) optimizing how food will later be digested and absorbed [15]. Hence, the evolutionary benefits offered by the sense of taste are indissolubly linked to a complex multimodal process, in which each sensory modality decodes different environmental inputs collectively forming the elements of a unitary experience [16].

It is conventionally accepted that humans can experience five taste qualities (sweet, salty, umami, sour and bitter), albeit additional oral sensations (fat, metallic) have been proposed to be added in this list [15,17]. Remarkably, each taste is responsible for making explicit the dietary value attributable to the ingestion of a certain food [14,15]. Specifically, sweet taste signals energy-dense nutritional

sources, salty associates with minerals and contributes to their homeostasis, whilst umami is thought to be indicative of the amino-acid (especially L-glutamate) and protein food content [15]. Conversely, sour and bitter tastes are prodromal signs of potentially aversive post-ingestion effects, and act as deterrents to the consumption of spoiled foods (sour) and poisons (bitter) [15]. Interestingly, these messages can even be further magnified by the array of sensations (e.g., chemesthesis) elicited by the trigeminal system, as amongst the major determinants of flavor perception [16].

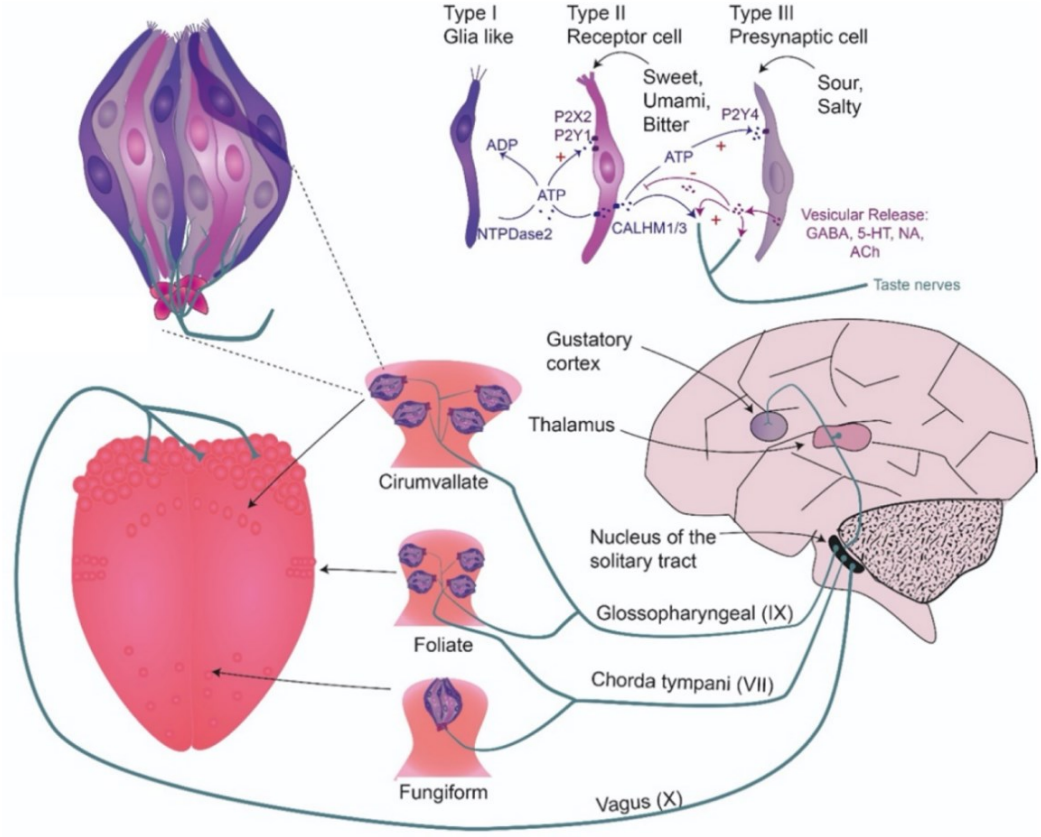

Figure 1.2: Overview of pathways (lines) and anatomic units (images) implicated in chemoperception. Taste-active substances are bonded to tastant-specific receptors expressed by clusters (upper left) of apical evaginations of taste cells (upper right), which reside in little bumps (circumvallate, foliate, and fungiform papillae; center) of the tongue (lower left). Once initially encoded, taste signals transit (lower right) various cranial nerves (VII, IX, and X), the nucleus of the solitary tract, and the thalamus prior to triggering the gustatory cortex and eliciting a conscious taste perception. Adapted from [18].

The salivary dissolution of taste-active water-soluble molecules marks the initial step of the taste signal transduction (Figure 1.2), which is operated by guilds of neuroepithelial cells (known as

taste buds) mostly harbored by small visible protrusions named papillae [15,19]. In turn, taste-detecting papillae are located in the tongue, and show morphological and functional variations resolving into three different anatomic structures (Figure 1.2; circumvallate, foliate, fungiform papillae) [19]. Among these, the ensemble of fungiform papillae decorating the lingual anterior section (Figure 1.2) is the primary responsible of signal transduction elicited by tastants or irritants (e.g., pungent stimuli), as co-innervated by both gustatory (cranial nerve VII) and trigeminal (cranial nerve V) nerves [20].

Once a salivary-dissolved taste stimulus navigates nearby fungiform papillae, it encounters taste receptor cells' (TRCs) apical microvillar processes projecting into excavations of the tongue epithelium called taste pores [14,15]. Here, the ligand binds to the receptors expressed by the 50-100 TRCs embedded into each taste bud (Figure 1.2) to initiate the peripherical decoding of the message [21,22]. Particularly, such event is promoted by three (Figure 1.2) morphologically and functionally different subtypes of TRCs: the Type I, II, and III cells [15].

Type I cells have supportive or glia-like functions, as coating both Type II and III cells of laminar processes [23] and contributing to clearance of various neurotransmitters [15,22]. While their putative role in sensing salt stimuli is largely unclear [15], novel research suggests that these cells benefit from excitement of adjacent taste-transducing cells to influence taste neurotransmission [23]. Conversely, a broader consensus underpins the role of Type II G protein-coupled receptor (GPCRs) cells on integration of sweet, umami, and bitter perception [15,21]. In this vein, two superfamilies of GPCRs are independently activated in response to these ligands, and operate as dimers (T1Rs) or monomers (T2Rs) [21]. Notably, heterodimeric complexes of T1Rs family function as transducers of sweet (T1R2 plus T1R3) or umami (T1R2 plus T1R3) stimuli, whereas 25 different T2Rs (or TAS2Rs) have been described to decipher multiple bitter-eliciting compounds [15,19,21]. Lastly, Type III presynaptic cells are the only forming canonical synapses with gustatory-afferent nerve terminals [15], and are able to elaborate both sour [24] and salt [25] tastants via ionotropic receptors.

Regardless of the stimulus, binding of taste-active molecules to their tuning receptor (GPCRs or ionotropic channels) will resolve into TRCs membrane depolarizations and subsequent exocytosis of neurotransmitters peripherally synapsing with axons of the gustatory neurons [26]. The consequent postsynaptic potentials will travel along cranial nerves VII (Facial), IX (Glossopharyngeal), and X (Vagus) prior to reaching the solitary nucleus in the brain stem (Figure 1.2) [14]. Here, taste signals are directed to the ventral posterior medial nucleus of the thalamus, whose nerve projections will synapse with afferent nerve fibers of diverse regions of the gustatory cortex (opercular, insular, orbitofrontal; Figure 1.2) to conclusively generate the percept [14].

1.2 | Confounders of smell and taste perception

Everyday olfactory and gustatory experiences are far from identical among individuals. Consequently, extensive research has been conducted to elucidate the myriad factors acting as mediators of inter-individual variations in chemosensory abilities. Given the foundational role of smell and taste perception in eating habits (see for a review [27]) as well as the negative health outcomes caused by alterations to the senses organs (see for a review [28]), these experimental efforts are more than warranted.

1.2.1 | Biological correlates of sensory perception

The largest body of the literature examining how food smells and tastes differently among individuals deals with the genetic roots of perception, which show patterns of heritability (see for reviews [29,30]) and revolve around polymorphic variants in genes coding for chemosensory receptors. In this regard, two superfamilies of olfactory and gustatory GPCRs have attracted much attention over the past decades: the olfactory receptors (ORs) and the bitter taste receptors (TAS2Rs).

Compared to the human genome, a surprising number of OR-related genes are non-functional [31]. Interestingly, this pool of "*natural knock-outs*" [32] varies greatly among individuals, thus supporting the existence of unique potentially active OR repertoires from person to person [29,33]. For example, a genetic variant in OR5A1 (rs6591536) explained 96.3 % of the variance in sensitivity for a floral odorant (β-ionone) as well as the preference and emotions aroused by foods containing that compound [34]. Similarly, a recent large-scale report (n = 11.326) unveiled that single nucleotide polymorphisms (SNPs) in OR6C70 occurred in concomitance with higher abilities in naming odors like licorice and cinnamon, while a genotype in TAAR5 was inversely correlated with acuity or liking for fish-evoking odors [35]. Other authors have instead focused on a SNP (rs2590498) coding for the sole odorant-binding protein (OBPIIa) reported in humans [8], and noted healthy individuals carrying in homozygosis the A allele to show higher olfactory abilities [36] and bitter taste intensity ratings for oleic acid [37] relative to haplotypes A/G and G/G. Nevertheless, though genetic fingerprints clearly differentiate how people interact with olfactory stimuli, their contribution is insufficient to capture the whole individuality of smell perception [29].

The most well-studied genetic signature of variability in taste perception instead centers on the extent to which individuals sense a series of bitter-tasting thiourea compounds (see for a review [30]), particularly 6-n-propylthiouracil (PROP). It is now widely acknowledged that PROP perception relates to three SNPs (rs713598, rs1726866, rs10246939) in the coding region of the TAS2R38 gene, which produce two major non-functional (AVI) and functional (PAV) haplotypes [30,38,39]. Typically, homozygosity for the AVI and the PAV haplotypes translates into low and extreme responsiveness to PROP, respectively, whilst the AVI/PAV diplotype generally associates with intermediate responses to PROP stimulation. Therefore, those who experience a blind (AVI/AVI), intermediate (AVI/PAV) or extremely intense (PAV/PAV) bitterness from PROP are commonly referred to as Non, Medium and Super Tasters, respectively [30,38,39].

It is noteworthy that a plethora of studies has consistently observed PROP responsiveness to go along with acuity for a wide range of oral sensations [40–48], thus supporting its candidature as a marker of generalized hypergeusia. Further corroborations to this clue derived from several evidence positively linking psychophysical responses to PROP to fungiform papillae density, which in turn has been thought for years to be an additional predictor of chemosensory and somatosensory abilities [44,48–51]. Nonetheless, novel large-scale studies have failed to corroborate such relationship [40,52–54], and have unpacked fungiform papillae density from oral responsiveness [40,53,54]. Moreover, canonical associations between TAS2R38 haplotypes and patterns of oral acuity have recently been questioned [39,42,43]. Hence, while PROP taste phenotyping (unlike fungiform papillae density) apparently remains a valid approximation of inter-personal variations in chemo- and somatosensation, it turns to be evident that its predictive value should be reformulated (e.g., [40,42]).

Beyond genetic and anatomic correlates, smell and taste perception is also inevitably connected to the composition of saliva. Indeed, salivary medium exerts key roles on both oral health and maintenance of homeostasis of the gustatory system [55]. Most importantly, its constituents interact with food during mastication and largely condition the peri-receptor events leading to taste or flavor signal transduction, as well as the basics of perception of fat, texture, and trigeminal sensations (see for reviews [55–57]). In this sense, archetypical examples include the influence of salivary: a) mucin and proline-rich protein content on bitterness and astringency; b) electrolytes on lowering the sensitivity for salty, umami and sour via adaptation; c) enzymes and mucins on decreasing the volatility of odorants and their subsequent elicitation [55–57]. Intriguingly, recent evidence has illuminated putative relationships between the salivary microbiota and chemosensation (see for reviews [58,59]), although the underlying mechanisms are vastly unexplored. Since this subject is of particular interest to this thesis, the reader may refer to section 1.5 for further details on current knowledge.

1.2.2 | Demographic and environmental correlates of sensory perception

From the early stages of research on human chemosensation, multiple researchers wondered whether gender-dependent differences in sensory perception existed. Traditionally, it is assumed that women have superior olfactory functions than man [60–64], and such advantage would either be preparatory to mate selection [65] and fetal protection [66], or would be related to fluctuations in gonadal hormones [66]. Nevertheless, the extent to which women would outperform man in olfactory performances appears to be subtle [60], and although numerous studies have noted prominent gender-specific anatomic and neurophysiological deviations in the gustatory system that would support greater acuity in females (see for a review [67]), the debate is still alive.

Conversely, the detrimental effects of ageing on sensory functioning have considerably been clarified. Age-dependent impairments of the sense of smell have been linked to a series of events, which lead (among others) to: a) serious anatomic and functional damages of the olfactory neuroepithelium; b) reduced activation of cortical areas involved in smell processing; c) higher proneness to develop nasal and sinus disorders; d) decrements in immunoenzymatic pathways defending the integrity of the olfactory mucosa; and e) cognitive deficits [68–70]. Similarly, severe declines of taste functions are also common in the later stages of life, and are mostly ascribable to: a) degeneration of taste buds and gustatory peripheral tissues; b) collateral defects in olfaction; c) tooth loss; and d) alterations in taste neurotransmission [70,71].

Rounding out the list of traditional descriptive variables affecting smell and taste perception is the weight status. In light of the remarkable contribution of sensory perception in dietary habits [27] and the ongoing global obesity epidemic [72], scientists have always been interested in probing whether overnutrition was concomitant to changes in sensory acuity. However, current knowledge is largely inconsistent and inconclusive [73], as overweight and obesity (relative to healthy weight) have been

linked to either decreased [74–78], identical [79], or enhanced smell and taste functions [73,80]. In this vein, some authors support the notion that lower chemosensory abilities would favor augmented intakes of high-calorie foods [74–78,81], whilst others suggest the opposite, since these foods are known to evoke innately liked sensations [73,80]. Altogether, it is apparent that the link between sensory perception and weight deserves further investigation to be fully uncovered.

To conclude, the perception of odorants and tastants can also be impacted by cultural influences, or by environmental insults (e.g., smoking) and habitual drug intake (see for reviews [82,83]). As an example, Pedrotti *et al.* [84] noted that Asians showed a greater extent of retronasal aroma release than Europeans while consuming a mint-flavored gum, and this went along with heightened psychophysical responses to sweetness and overall mint flavor. Similarly, Asians have also been reported to outperform Caucasians in responsiveness to sour taste and metallic sensation evoked by aqueous solutions [85], whereas Hispanics and African Americans exhibited higher intensity responses to taste-active molecules (administered by swabbing various sites of the oral cavity) when compared to non-Hispanic Whites [86]. Notwithstanding, whether the afore-mentioned cross-cultural differences can be disentangled from additional psychological or physiological confounders of retronasal and taste functioning has yet to be clarified. Taken collectively, smell and taste perception is anything but autonomous phenomena. However, beyond the established deleterious effects of ageing and exogenous toxicants on chemosensory abilities, the actual weight of other relevant demographic confounders is still somewhat inconclusive.

1.2.3 | Psychological correlates of sensory perception

The idea that attitudes and psychological traits would affect the way we experience the chemosensory world is fascinating, and has attracted significant interest since the 1980s [87]. Not surprisingly, a variety of constructs dealing with facets of our psychological sphere has been linked

to sensory perception, though most of the literature has addressed the issue by focusing more on preferences rather than on psychophysics (see for a review [88]). For instance, individuals with pronounced lack of emotional self-awareness (alexithymia) were found to be hyperresponsive to 13 common odorants [89], and to a few alarming sensations (pungent, sour) evoked by a tomato juice [46]. Interestingly, the latter authors also noted similar patterns of oral acuity in those exhibiting strong sensitivity to disgust or in females particularly sensitive to punishment [46]. Similarly, proneness to experience negative emotions (neuroticism) was early linked to olfactory [90,91] and bitter tasting [92] abilities, albeit its direct effect on chemosensation was later questioned [93,94].

Notable insights into the psychometric correlates of sensory perception also emerged from a widely common and documented trait, namely Food Neophobia (FN). FN refers to those unwilling to try novel and/or unfamiliar foods [95], and constitutes a source of public health concern as fostering unhealthy dietary habits and overweight across the lifespan [74,96–99]. Thus, much research has been devoted (especially in toodlers) to clarifying whether the restrictive behaviors underpinning neophobic tendencies had a sensory-related origin. It turned out that the links between FN and chemosensation may converge in the way neophobics cope with the physiological and psychological alert states triggered by odorants or tastants (hereafter arousal [100]). In this vein, the arousal hypothesis [101] assumes that chemosensory stimuli would act as elicitors of negative physiological and emotional states, which would lead high neophobic individuals to reject food items especially if presenting a certain degree of novelty. Consistently, food neophobics were more vigilant than neophilics when confronted with food stimuli [102], or showed features of physiologically-increased arousal (e.g., increased pulse rate) at the sight of food pictures [103]. Further, FN inversely associated with pleasantness of (un-) familiar odorants and sniff vigor [104], which could underlie a protective behavior against potentially unpleasant olfactory experiences [105]. Lastly, FN frequently co-occurred with psychological drivers of negative arousal states such as anxiety [95,103,106] and reluctance to seek novel

sensations [95,102]. However, while the link between arousal and FN behaviors appears to be clear, the mediating effect of sensory perception within this relationship is still uncertain (especially in adults).

Indeed, studies in toddlers evidenced that high neophobic traits generally corresponded to enhanced sensitivity to recalled olfactory and gustatory experiences [107–110]. Conversely, recent large-scale reports argued that adult neophobics (relative to neophilics) were not endowed with superior chemosensory functions [111], and did not attribute the observed positive associations between strong neophobic traits and acuity for pungency evoked by a capsaicin-spiked tomato juice to increased taste functioning [46]. A tentative explanation of such discrepancies has been proposed by Zickgraf and Elkins [110], who noted that sensory sensitivity was much more correlated with FN in toodlers (8-13 y/o) rather than in young adults (18-22 y/o), thus suggesting that the magnitude of this relationship may depend on developmental stages. Hence, in view of the negative health consequences led by high neophobic tendencies, further research addressing this research topic is urged. Particularly, while the associations between adult FN, oral acuity and arousal have been at least deduced [46,111], whether these also replicated in ortho- and retronasal olfaction has not yet been empirically probed.

1.3 | Methods to assess smell and taste functioning

1.3.1 | Assessment of smell function

Quantifying subjective representations of reality that humans can only communicate is what makes the assessment of smell and taste functions extremely challenging. And to complicate matters further, senses organs intertwine with other sensory modalities and domains of cognition to originate a percept, which raises the need for a variety of tools that can robustly capture the multiple facets of perception (see for reviews [112,113]). Just think to olfaction: one might be interested in how people differ in perceptual abilities and/or those closely related to the cognitive sphere, such as naming an odor. In this regard, scientists can take advantage of qualitative or quantitative measures to address

these research issues. The former mostly rely on self-reports of one's olfactory abilities, whereas the latter comprise tests gathering neurophysiological outcomes on olfactory system function or psychophysical responses indicative of conscious smell perceptions [112]. Nonetheless, since self-reporting generally tends to produce spurious results and neurophysiological assessments are expensive, time-consuming, and poorly portable [112], psychophysical methods set a benchmark for research frameworks.

Among the countless number of tools developed (see for a review [112]), the Sniffin' Sticks battery (Figure 1.3) is counted amongst the most frequently used and validated kit to measure olfactory function [112,114]. It comprises 48 commercial felt-pens dispensing odors [115], which are used in three different subtasks aimed at targeting: a) the minimum detectable concentration of an airborne chemical (*n*-butanol or 2-phenylethanol) under a triple-forced-choice paradigm governed by a single staircase procedure (Odor Thresholds); b) the ability to detect the odd odorant elicited by each of the 16 stick triplets provided by the kit in a forced-choice triangle test (Odor Discrimination); c) the capacity to name smells (n = 16) from a list of 4 visual-worded alternatives (Odor Identification) [112,115]. As a result, each subtask yields to separate scores, whose sum is commonly computed to get a global index of the olfactory performance, known as TDI (Odor Threshold, Discrimination, Identification) [115].

Figure 1.3: The Sniffin' Sticks battery (Burghardt®, Wedel, Germany). Retrieved from [115].

Beyond the Sniffin' Sticks test, other popular psychophysical toolboxes conceived to assess dimensions of the olfactory function include the University of Pennsylvania Smell Identification Test (UPSIT) [116], and the Connecticut Chemosensorial Clinical Research Center olfaction test (CCCRC) [117]. Nevertheless, given that the UPSIT only evaluates the ability to identify 40 odorants impregnated in paper strips [116] and the CCCRC lacks of a discrimination test [117], the Sniffin' Sticks battery can reasonably be considered the gold standard measure when variations in overall orthonasal olfactory functioning are of research interest [115].

Unfortunately, psychophysical testing of retronasal olfactory function is very much less developed [118]. Historically, retronasal olfaction has mainly been operationalized via encapsulated odorants designed to elicit a sensation only when exhaled through the nose [119], or via commercially-available powders [120] and flavored candies [121,122] devoid of perceivable tastants [112,118]. Regardless of the method, participants are asked to identify the target flavor from lists of items [118]. However, these methodologies are reportedly poorly standardized (in terms doses and concentrations of stimuli employed) and cross-culturally adapted [118], thus justifying their infrequent use in academic contexts.

Psychophysical responses to retronasal stimulations are undoubtedly affected by the amount of volatile organic compounds (VOCs) reaching the olfactory epithelium during consumption. Hence, quantifying such extent of VOCs may be a proxy to evaluate the retronasal olfactory function. However, as no instrument designed for directly measuring the VOCs triggering the olfactory system apparently exists, the assessment of VOCs exhaled by the nasal cavity while eating could be the sole viable alternative (see for a review [56]).

In this vein, a number of studies has evidenced important physiologically- and behaviorally-related sources of inter-individual variability in aroma release, and discussed their impact on actual flavor perception [56,84,123–128]. While the relationships between the VOCs released in the retronasal space and chemosensation have yet to be fully proven, a key advantage of such approach over

canonical tools lies in its temporal nature. Indeed, *on-line* aroma release measurements (named *nose-space* tasks) are thought to monitor the volume of VOCs exhaled throughout the entire oral processing, thus potentially permitting a comprehensive tracing of changes in flavor perception over time [129].

Traditionally, participants are invited to taste a model food while breathing into ergonomic nosepieces connected to a high resolution mass spectrometer equipped with direct injection units (e.g., Proton Transfer Reaction Mass Spectrometry or Selected Ion Flow-Tube Mass Spectrometry), which reliably captures a panel of exhaled VOCs according to the research question at hand [129]. Additionally, *nose-space* analysis is frequently coupled with temporal sensory methods (e.g., Time-Intensity, Temporal Dominance of Sensations) to probe the links between the released VOCs and changes over time in psychophysical responses to flavors [84,126,128]. As a result, *on-line* monitoring of volatiles travelling the retronasal route resolves into a lengthy list of time-intensity- (e.g., area under the curve) or behaviorally-related (e.g., breathing rates) parameters, which can give useful clues to reveal distinct aspects of retronasal functioning.

1.3.2 | Assessment of taste function

Similar to olfaction, there is a plethora of tests from which one can choose to explore taste functioning (see for reviews [113,130]). Conventionally, two major functionalities of the gustatory system are evaluated: thresholds and supra-threshold intensities. The former refers to the lowest perceivable concentration of a chemical, which can be delivered either via (usually deionized) aqueous solutions or paper impregnated strips, or via electronic devices. Notably, taste-active molecules can not only be detected at very low concentrations, but can also be simultaneously attributed to specific taste qualities. Such abilities are operationalized via detection and recognition threshold tests, respectively. In routine research practices, both threshold tasks use a series of concentrations (from 6

to 12) of tastants presented in descending order (method of limits) or adaptive up-down staircase paradigms, whose results are intended as indices of taste sensitivity [113,130].

Instead, the latter deal with the magnitude of sensations elicited by chemicals embedded in artificial dispensers (water solution or paper strips) or actual foods (e.g., [131,132]) at above threshold concentrations [113,130]. Here, participants are usually asked to rate the intensity of perceived oral sensations using psychophysical scales, whose length indicates a progressively higher magnitude of the stimulus. To this end, different scaling methods (Figure 1.4) are extensively used by the sensory science community [113], including categorical (e.g., 9-point scale) and line scales (or visual analog scales), or the common general Labeled Magnitude Scale [133]. While none of the aforementioned methods can comprehensively represent the gustatory function [134], supra-threshold intensities possess the key advantage (over thresholds) of mirroring actual perceptions of food and being valid predictors of habitual dietary intakes (e.g., [113,134,135]).

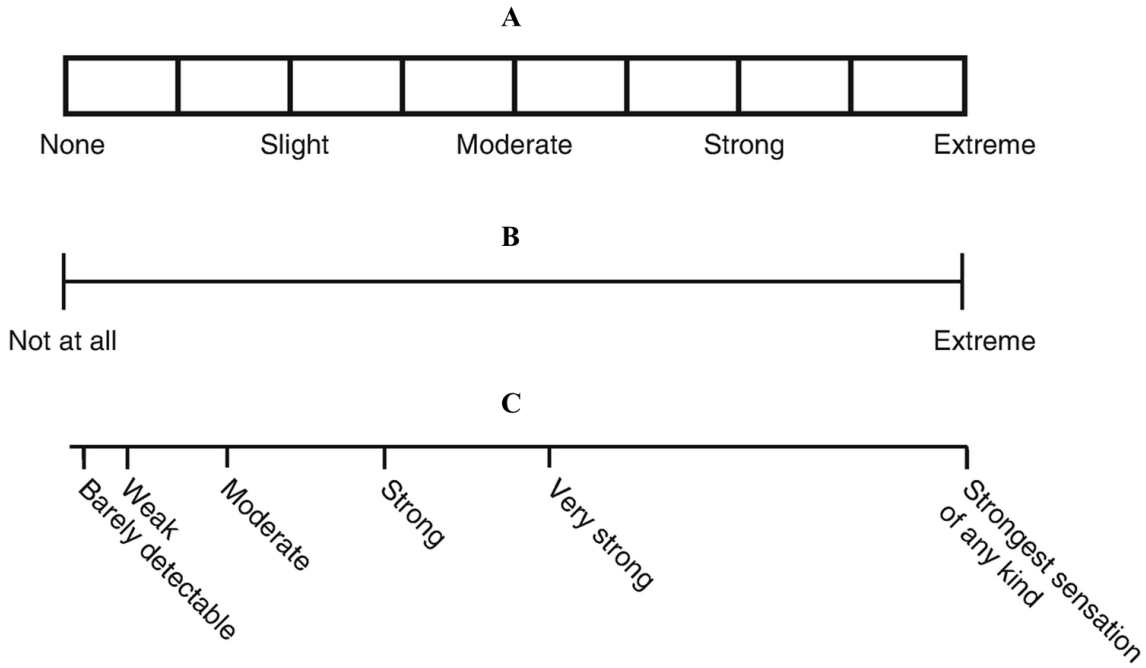

Figure 1.4: Examples of psychophysical methods commonly employed in supra-threshold intensity tests. **A:** Categorical scale. **B:** Visual analog scale. **C:** General Labeled Magnitude Scale [133]. Adapted from [113].

Nonetheless, it is worth mentioning that supra-threshold intensities are most frequently operationalized with aqueous solutions instead of food matrices, which can limit their usefulness in ascertaining the taste-related effects on eating habits [113]. Hence, as recently advocated by the US National Institutes of Health [136], real food products should increasingly be preferred to traditional model systems to answer this latter research question and maximize the external (ecological) validity of results. In this regard, the recent COVID-19 pandemic has dramatically imposed a sabbatical leave from lab testing, and pushed a global transition towards remote research [87]. Accordingly, much effort has been spent on adapting laboratory-born experimental techniques to natural contexts, resulting in precious guidelines [137] that the sensory community ought to benefit from to greatly improve the ecological value of outcomes and to expand the current knowledge on aetiology of eating behaviors.

1.4 | Smell and taste modulate eating behaviors and dietary habits

Smell and taste are among the most influential factors that guide our food choices and behavior [138]. As above mentioned (section 1.1), chemosensory organs are in charge of transducing several exogenous chemicals, which operate as detectors of the nutrient content provided by a food source. Particularly, odors punctuate the preliminary stages of the meal, and combines with visual cues to trigger distinct physiological pathways (e.g., appetite, cephalic phase) that should prepare our body for food ingestion [27]. In contrast, tastants and flavors make explicit the anticipatory signals send by olfaction, by revealing the nutritional value and safety of food, and cooperate with textural properties to scan meal duration and termination [27]. Thus, while the role of sensory perception on food choices appears to be apparent, it should always be placed in a multidimensional context that is affected by the conditioning of myriad factors tied to the hedonic and psychological spheres [139]. As further confirmation, it is unsurprising that the actual weight of chemosensation on habitual dietary intakes is still debated.

Against expectations, odors would apparently be better predictors of food intakes when perceived unconsciously rather than at suprathreshold levels, even though odorants are known to induce appetite for products that will later be consumed [4,27]. In this context, some authors have proven that unaware exposure to fruity odors, like melon or pear, favored subsequent choices of desserts eliciting congruent sensations [140], whilst participants exposed to a barely detectable "*pain au chocolate*" odor were more prone to select energy-dense desserts than a control group [141]. Similarly, unconscious exposure to odorants linkable to high-calorie foods was positively associated with both salivation and *ad libitum* intakes of chocolate rice [142], while a priming experiment confirmed that obese women may consume greater amounts of a vegetable soup when unobtrusively exposed to a bread odor [143].

Instead, given that long-lasting exposure to oral sensations commonly reduce food intake and promote satiety, it was early believed that prolonged retronasal stimulation would exert similar conditioning effects (see for a review [27]). However, while literature substantially agrees that *ad libitum* food intake can only be marginally affected by odorants persistently triggering the retronasal space [27,144–147], a few authors argued that the extent of retronasal exposure to odorants may reduce the desire to eat congruent food categories [145] and operate as satiety enhancer [144,146]. Hence, the role of retronasal smell on ingestive behaviors does not seem to be limited to discernment of palatability and food safety, but it may extend to the biological mechanisms preparatory to meal termination.

Conversely, whether orosensation strictly affects eating behaviors is now widely accepted [27]. Ample evidence states that orosensory persistence of taste qualities marks meal duration, since it triggers satiety responses that ultimately lower eating rates [27,148–150]. Accordingly, given that taste acuity closely relates to satiation [151] and high individuality in chemosensation exists, research has spent a great deal of energy clarifying how patterns of sensory perception can shape food intakes.

In this vein, the best documented taste phenotype in relation to dietary habits is the PROP taster status. Being considered as a marker of generalized hypergeusia [40–48], hyperresponsiveness to PROP is a proxy for rejection of those foods eliciting innately disliked taste qualities (see for a review [30]). Compared to non-tasters, PROP tasters frequently declare lower intake of bitter- or sour-evoking fruits and vegetables [30], which is reportedly linked to higher mortality risk [152]. Notwithstanding, PROP acuity may also prove protective against food sources whose overconsumption is correlated with deleterious health outcomes, such as alcoholic beverages [153], dietary fats [30] or high salt intakes [132]. While one might expect this paradigm to extend to sugary foods, literature provides mixed results and highlights negative or no associations between phenotypic responses to PROP and sweet taste preference or sweet food intake [30]. It is worth mentioning that the mediating effect of orosensation on dietary outcomes is unlikely to be decoupled (among others) from liking, as outlined by two recent reviews arguing that the intake of sweetened [154] and salty [155] foods can more easily be predicted by hedonic rather than chemosensory responses. Hence, according to what discussed in the previous sections, it is clear that the links between food choices and chemosensation can only be fully quantified when framed in multidisciplinary contexts designed to consider the variety of confounders shaping our perceptions.

1.5 | What if chemosensation was affected by the gastrointestinal microbiota?

Now more than ever, the scientific community has been concentrating its resources on how we interface with the trillions of microorganisms harbored by the gastrointestinal (GI) tract (oral and gut microbiota). Academically, the ensemble of microbial inhabitants of a circumscribed environment is termed microbiota, which collectively forms the microbiome when its structural/metabolic features and surroundings are considered [156]. Nearly overlapping the number of humans cells [157], the GI microbiota exerts critical roles for host homeostasis, ranging from immune regulation to nutrient

metabolism [158–160]. Also, this is corroborated by the array of systemic diseases related to imbalances (or *dysbiosis*) of GI microbial consortia, which are commonly dominated by commensal bacterial members posing a symbiotic relationship with the host [158–160].

It is widely established that diet is amongst the most impacting exogenous factors modeling the composition of the GI microbiota (see for reviews [158,159]). Intriguingly, this seemingly unilateral relationship has recently been expanded by Alcock *et al.* [161], who has speculated main symbionts to modulate host eating behaviors to meet their nutritional needs [161]. In support of this theory, vast research has argued that the GI microbiota may intervene in dynamics underlying craving for high-palatable foods [161], appetite [162] or eating disorders [163], and has documented its impact on taste function preservation [164] or taste receptor expression [165]. Further, taste receptor cells widely line the distal sites of the GI tract, and serve as primary initiators of hormonal signaling pathways ultimately conditioning (among others) nutrient uptake and glucose homeostasis [166,167]. Accordingly, the idea that the human GI microbiota may putatively play in concert with chemosensation to mediate ingestive behaviors appears to be reasonable [58,161].

The first evidence linking domains of gustatory function to GI microbial communities dates back to 2012. In that study, Solemdal *et al.* [168] probed the links between oral health and taste recognition thresholds in a cohort of 174 acutely hospitalized elderly, and found sour sensitivity to anticorrelate with the growth of salivary acid-producing bacteria (*Lactobacilli*). Tentatively, the authors hypothesized that such ability would increase acid concentrations nearby taste buds, thus promoting sensory adaptation phenomena caused by less reactive receptor activations in response to sour stimuli [168]. A few years later, the ever growing awareness on diet-host-microbiota interactions has fostered the real beginning of the sensory-oriented microbiome field (see for reviews [58,59]). Unsurprisingly, the majority of reports generated by this fledging research area tackled the subject by testing whether variations in taste sensitivity corresponded to different tongue *dorsum* microbiota. For

instance, lingual bacterial families *TM7* and *Lactobacillaceae* positively associated with the ability to detect linoleic acid in aqueous solution [169], whilst the abundances of the phylum *Bacteroidetes* were negatively correlated with bitter detection abilities [170]. Increasingly, PROP Super Tasters (relative to Non-Tasters) were found to be hypersensitive to a range of tastants (bitter, salty, sour, sweet) and to harbor greater amount of three microbial genera (*Actinomyces*, *Oribacterium*, *Campylobacter*) inhabiting the tongue biofilm [171]. Notably, the same authors later used a correlative approach to relate either a *Clostridia*-enriched lingual microbiota to high salt sensitivity and to a fat/protein-rich diet, or the abundances of genus *Prevotella* to higher habitual intakes of fibers [172]. Lastly, a few reports have also evidenced a spectrum of salivary microbial signatures linkable to taste functions in both children [173] and PROP taste phenotypes [174]. However, empirical mechanistic explanations underlying these outcomes have yet to be provided.

In this vein, some previously proposed anatomical- or metabolic-driven theories may help to further clarify the picture. Indeed, the peculiar concave morphology of the tongue *dorsum* represents a suitable environment for microbial biofilm formation and subsequent adherence, which may hinder the flux of tastants in the taste peri-receptor space and prevent its activation. Alternatively, oral microbes may produce taste-active secondary metabolites, which would spread nearby taste receptors and alter taste sensitivity via adaptation [58,59,168–171,175,176]. Nonetheless, the sole empirical support to these hypotheses comes from Gardner *et al.* [175], who observed that salivary specimens from low responsive individuals to sweetness more efficiently catabolized pyruvate in lactate after exogenous sucrose exposure. In turn, this would have augmented the amount of sweet-tasting metabolites nearby taste receptors and led to diminished signal transduction [175]. However, whether these pathways are driven by specific microbial consortia has yet to be confirmed, though hypothetically attributed to bacterial members of the genus *Streptococcus* [175].

Unfortunately, even less is known about how smell and taste perception may be affected by the nasal or gut microbiota. To the best of the author knowledge, olfaction has only been linked to GI bacterial ecosystems by two previous studies [177,178]. Firstly, Koskinen *et al.* [178] tested the olfactory performances of 67 healthy individuals via the Sniffin' Sticks battery and sampled the microbiota housed by the olfactory mucosa located at the ceiling of the nasal cavity. Interestingly, they noted that several butyric acid-producing microbial residents of the nasal epithelium (e.g., genus *Porphyromonas)* were more abundant in those exhibiting poor olfactory acuity [178]. Secondly, impaired olfactory functioning was negatively associated with the lingual intra-sample bacterial (α-) diversity and proportions of species *Streptococcus salivarius* in a large cohort of 356 adults (65-93 y/o) by Fluitman *et al.* [177], who also linked the amount of fungal species *Candida albicans* to poor smell discrimination abilities. Lastly, the distal gut microbiota is paradoxically the least investigated reservoir of microbes in relation to human sensory perception. Indeed, most of the literature concerns murine models (e.g., [164,165]), whilst human studies are limited to a recent report showing hyporesponsiveness to PROP to inversely associate with gut microbial α-diversity and abundances of the genus *Clostridium* in adults with Parkinson's disease [179]. Again, the biological reasons behind these findings remain poorly understood.

To conclude, it is evident that the sensory-oriented microbiome field is in its infancy and is inevitably affected by a few limitations that require a multidisciplinary design to be addressed. These include: a) little attention to potential interplays between GI microbial consortia and ortho- and (especially) retronasal olfaction [177,178]; b) almost total interest in the tongue *dorsum* microbiota [169–172,177,180] and less frequent sampling of both salivary [168,170,173,174] and (especially) gut microbiota [179]; c) exclusive testing of gustatory function via thresholds in response to artificial model systems (aqueous solutions, paper strips) [168–173,175,177], even though collection of supra-threshold intensities from food matrices is advocated to reliably capture everyday sensory perceptions and eating behaviors

[134–136]; d) rare assessment of food-related attitudes and psychological traits [173], although their role on eating behaviors is widely acknowledged (e.g., [139]); e) poor monitoring of detailed dietary habits [172]; and f) involvement of relatively low sample sizes [170–174,178,180] in view of the myriad factors affecting both chemosensation and the GI microbial composition (e.g., [30,181]). Addressing these issues is therefore critical to expanding current knowledge and paving the way for the ultimate understanding of eating behaviors.

1.6 | Rationale and aims of the thesis

The dramatic global spread of diet-related noncommunicable diseases [72,182] places elucidation of the mechanisms underpinning eating behaviors at the forefront of public interest research. The multidimensional nature of food choices is well established, and distinct biological, environmental, and psychological factors play in concert to guide food-decision making processes [139]. Among these, sensory perception is amongst the most influential [138], and people have been shown to differ greatly in how they interface with the chemosensory world. Since individuality in chemosensation is regarded one of the major predictors of food choices, uncovering its mechanistic basis represents an important research frontier to promote healthier diets and counter the spread of such diseases.

However, dissecting interpersonal differences in smell and taste perception is a daunting task, as a huge number of confounders mediate what we sense from foods. In this regard, our psyche strongly models our view of reality, and this also applies to perceptions [88]. Despite a few decades of experimental efforts, adult food neophobia [95] is still a trending topic within the sensory science community [87] in view of its negative health consequences [74,96–99]. For years, it has been thought that this avoidance/restrictive behavior could be paralleled by enhanced chemosensory abilities. However, recent large-scale reports have questioned such paradigm [46,111], and suggested that adult neophobic tendencies are most likely attributable to higher levels of negative arousal in response to tastants than

to superior chemosensory functions [46,111]. Unfortunately, whether this is also true for odors and (especially) retronasally perceived odorants has yet to empirically documented, thus resulting a research subject of outstanding importance.

On the other hand, we are probably now living a golden age of microbiome research, which is tremendously increasing public awareness of the key roles that the GI microbiota exerts on health and disease states across the lifespan. Of note, GI microbial communities have also been proposed as active modulators of eating behaviors [161], and this has recently encouraged the sensory community to probe the hypothesis that microbes can also be involved in mechanisms underlying chemosensation [160,167–171,173-174,176-177,179]. Nonetheless, research is still on its early stages, and several gaps have yet to be filled (see section 1.5) prior to elucidating the nature of such link. Altogether, given how both sensory perception and the GI microbiota intimately tied to eating habits (e.g., [27,158,159]), there is a clear need to provide novel insights into the complex crosstalks between chemosensation and host-related non-genetic factors.

Against this backdrop, the current thesis had the general aim of expanding current knowledge on the putative associations between inter-individual variations in sensory perception and the composition of distinct GI ecological niches (saliva and gut microbiota), and how these might translate into different habitual dietary habits. To this end, this work employed a multidisciplinary approach designed to capture a multitude of correlates of the phenomena under-investigation. Among these, particular emphasis was given to food neophobia, with the additional aim of clarifying the mediating role of arousal on olfactory performances.

1.7 | Thesis outline

This thesis is structured in two main sections (Section I & II; Figure 1.5), each focusing on a specific sensory modality (Olfaction: **Chapter 2-3**; Taste: **Chapter 4-5**), followed by a general

discussion of findings (**Chapter 6**). Specifically, **Chapter 2** and **3** detail the results of two distinct studies, whose aims were to elucidate the associations between olfactory functioning, food neophobia and salivary microbial ecology. **Chapter 2** probed the hypothesis that variations in olfactory performances between adults with different degree of food neophobia were more likely to be ascribable to differences in levels of negative arousal elicited by odorants rather than to physiological abilities. Instead, **Chapter 3** was conceived with a two-fold purpose. First, given the deleterious effect of food neophobia on both diet quality and variety, it appeared plausible that this behavioral trait could also promote undesirable changes in salivary microbial ecology. Second, the links between orthonasal and retronasal olfaction and the oral bacterial communities were undoubtedly little investigated and urged to be further examined.

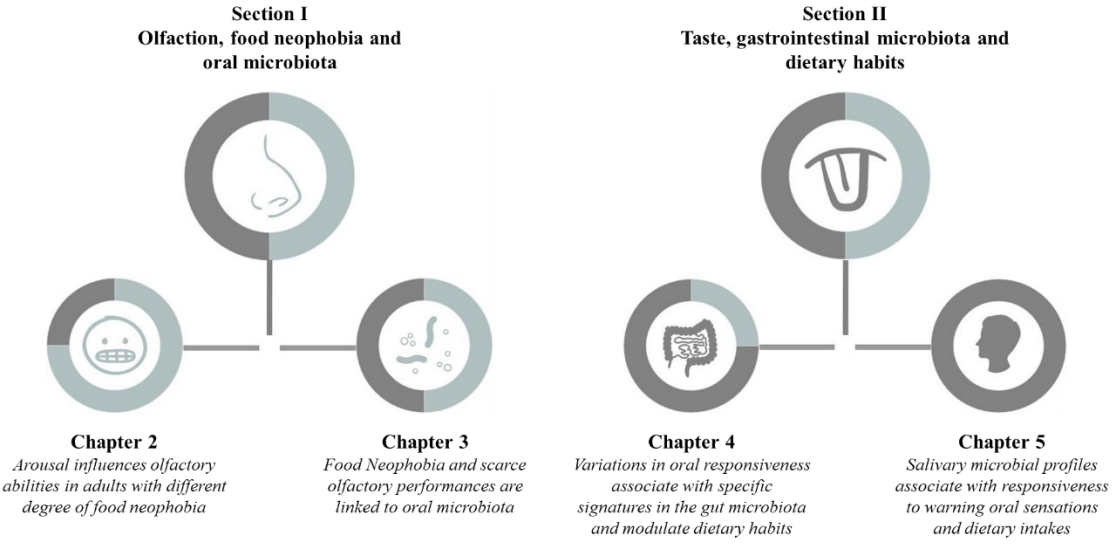

Figure 1.5: Graphical overview of the thesis. Chapter 2 and 3 focused on the links between olfaction, food neophobia and oral microbiota, whilst Chapter 4 and 5 examined the associations between taste, GI microbiota and habitual dietary intakes.

To these ends, within the framework of large-scale project aimed at elucidating the prevalence of olfactory disorders in Italy [61,183], 83 healthy individuals filled the common Food Neophobia Scale prior to providing a saliva sample, whose bacterial composition was profiled by 16s rRNA gene

sequencing. Next, volunteers were assessed for full orthonasal olfactory functioning through the extended Sniffin' Sticks Test [114], and monitored in real-time for the retronasal aroma release of 7 VOCs exhaled while consuming a commercial strawberry jelly candy via *nose-space* analysis.

Conversely, **Chapter 4** and **5** summarize the results from a 7-day double-blind cross-sectional remote study designed to test whether variations in taste functioning mirrored specific gut (**Chapter 4**) or salivary (**Chapter 5**) microbial ecologies and how these links putatively associated with eating habits. Notably, **Chapter 4** was devoted to inquiring whether homogenous groups of similarly responsive individuals to oral stimulation could harbor different gut microbial consortia and how this was associated with psychological background and habitual food intake. Likewise, in **Chapter 5** we probed whether patterns of bacterial co-habitation in the salivary microbiota (named salivary microbial profiles) might translate into variations in orosensory abilities, psychological traits, and diet. To address these research questions, 100 healthy young adults provided data on: PROP acuity, hedonics and supra-threshold intensities in response to a wide range of oral sensations evoked by 5 liquid and 5 solid real foods, food-related attitudinal and psychological traits, and habitual dietary intakes (4-day food diary). Furthermore, participants self-collected and delivered one fecal and one saliva sample for subsequent metataxonomic analysis (16s rRNA gene sequencing).

Lastly, **Chapter 6** is intended as a general discussion of outcomes and centers on the novel contribution given by this work to the sensory-oriented microbiome research field. Future directions, methodological considerations for future studies, and main conclusions are also discussed.

Note for the reader: while all manuscripts here included were adapted to make the layout of the current thesis as homogenous as possible, their content is faithful to that originally published. Further, note that figures and tables are linked to related images throughout the text. Hyperlinks are marked with underlined text (e.g., Figure 3.1).

1.8 | References

1. Harricharan, S., McKinnon, M. C. & Lanius, R. A. How Processing of Sensory Information From the Internal and External Worlds Shape the Perception and Engagement With the World in the Aftermath of Trauma: Implications for PTSD. *Front. Neurosci.* **15**, 360 (2021).
2. Hettinger, T. P. Olfaction is a chemical sense, not a spectral sense. *Proc. Natl. Acad. Sci. U. S. A.* **108**, E349–E349 (2011).
3. Sarafoleanu, C., Mella, C., Georgescu, M. & Perederco, C. The importance of the olfactory sense in the human behavior and evolution. *J. Med. Life* **2**, 196–198 (2009).
4. Boesveldt, S. & Parma, V. The importance of the olfactory system in human well-being, through nutrition and social behavior. *Cell Tissue Res.* **383**, 559–567 (2021).
5. DeMaria, S. & Ngai, J. The cell biology of smell. *J. Cell Biol.* **191**, 443–452 (2010).
6. Nagappan, P. G., Subramaniam, S. & Wang, D. Y. Olfaction as a soldier-- a review of the physiology and its present and future use in the military. *Mil. Med. Res.* **4**, 1–12 (2017).
7. Rozin, P. 'Taste-smell confusions' and the duality of the olfactory sense. *Percept. Psychophys.* **31**, 397–401 (1982).
8. Briand, L. *et al.* Evidence of an odorant-binding protein in the human olfactory mucus: Location, structural characterization, and odorant-binding properties. *Biochemistry* **41**, 7241–7252 (2002).
9. Altundag, A., Salihoglu, M., Cayonu, M., Tekeli, H. & Kayabasoglu, G. The effect of anatomic clearance between tongue and soft palate on retronasal olfactory function. *Chemosens. Percept.* **7**, 40–45 (2014).
10. Gladwin, K. & Choi, D. Olfactory ensheathing cells: Part i - Current concepts and experimental laboratory models. *World Neurosurg.* **83**, 114–119 (2015).
11. Zhou, G., Lane, G., Cooper, S. L., Kahnt, T. & Zelano, C. Characterizing functional pathways of the human olfactory system. *Elife* **8**, (2019).
12. Feher, J. *Quantitative Human Physiology An Introduction Book*. *Academic press* (Academic press,

2017).

13. Gottfried, J. A. Central mechanisms of odour object perception. *Nat. Rev. Neurosci.* **11**, 628–641 (2010).
14. Breslin, P. A. S. An evolutionary perspective on food and human taste. *Curr. Biol.* **23**, R409–R418 (2013).
15. Chaudhari, N. & Roper, S. D. The cell biology of taste. *J. Cell Biol.* **190**, 285–296 (2010).
16. Small, D. M. & Prescott, J. Odor/taste integration and the perception of flavor. *Exp. Brain Res.* **166**, 345–357 (2005).
17. Besnard, P., Passilly-Degrace, P. & Khan, N. A. Taste of fat: A sixth taste modality? *Physiol. Rev.* **96**, 151–176 (2015).
18. von Molitor, E., Riedel, K., Hafner, M., Rudolf, R. & Cesetti, T. Sensing senses: Optical biosensors to study gustation. *Sensors (Switzerland)* **20**, 1811 (2020).
19. Scott, K. Taste recognition: Food for thought. *Neuron* **48**, 455–464 (2005).
20. Mistretta, C. M. & Bradley, R. M. The fungiform papilla is a complex, multimodal, oral sensory organ. *Curr. Opin. Physiol.* **20**, 165–173 (2021).
21. Boughter, J. D. & Munger, S. D. Taste Receptors. *Encycl. Biol. Chem. Second Ed.* 366–368 (2013) doi:10.1016/B978-0-12-378630-2.00397-2.
22. Roper, S. D. & Chaudhari, N. Taste buds: Cells, signals and synapses. *Nat. Rev. Neurosci.* **18**, 485–497 (2017).
23. Rodriguez, Y. A. *et al.* 'Tripartite Synapses' in Taste Buds: A Role for Type I Gliallike Taste Cells. *J. Neurosci.* **41**, 9860–9871 (2021).
24. Huang, Y. A., Maruyama, Y., Stimac, R. & Roper, S. D. Presynaptic (Type III) cells in mouse taste buds sense sour (acid) taste. *J. Physiol.* **586**, 2903–2912 (2008).
25. Lewandowski, B. C., Sukumaran, S. K., Margolskee, R. F. & Bachmanov, A. A. Amiloride-insensitive salt taste is mediated by two populations of type III taste cells with distinct transduction mechanisms. *J. Neurosci.* **36**, 1942–1953 (2016).
26. Taruno, A. *et al.* Taste transduction and channel synapses in taste buds. *Pflugers Arch. Eur. J. Physiol.*

473, 3–13 (2021).

27. Boesveldt, S. & de Graaf, K. The differential role of smell and taste for eating behavior. *Perception* **46**, 307–319 (2017).
28. Malaty, J. & Malaty, I. A. C. Smell and taste disorders in primary care. *Am. Fam. Physician* **88**, 852–859 (2013).
29. Logan, D. W. Do you smell what i smell? Genetic variation in olfactory perception. *Biochem. Soc. Trans.* **42**, 861–865 (2014).
30. Diószegi, J., Llanaj, E. & Ádány, R. Genetic Background of Taste Perception, Taste Preferences, and Its Nutritional Implications: A Systematic Review. *Front. Genet.* **10**, 1272 (2019).
31. MacArthur, D. G. *et al.* A systematic survey of loss-of-function variants in human protein-coding genes. *Science (80-.).* **335**, 823–828 (2012).
32. Trimmer, C. *et al.* Genetic variation across the human olfactory receptor repertoire alters odor perception. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 9475–9480 (2019).
33. Olender, T. *et al.* Personal receptor repertoires: olfaction as a model. *BMC Genomics* **13**, 1–16 (2012).
34. Jaeger, S. R. *et al.* A mendelian trait for olfactory sensitivity affects odor experience and food selection. *Curr. Biol.* **23**, 1601–1605 (2013).
35. Gisladottir, R. S. *et al.* Sequence Variants in TAAR5 and Other Loci Affect Human Odor Perception and Naming. *Curr. Biol.* **30**, 4643-4653.e3 (2020).
36. Sollai, G. *et al.* Association between the rs2590498 polymorphism of Odorant Binding Protein (OBPIIa) gene and olfactory performance in healthy subjects. *Behav. Brain Res.* **372**, 112030 (2019).
37. Tomassini Barbarossa, I. *et al.* Variant in a common odorant-binding protein gene is associated with bitter sensitivity in people. *Behav. Brain Res.* **329**, 200–204 (2017).
38. Risso, D. S. *et al.* Global diversity in the TAS2R38 bitter taste receptor: Revisiting a classic evolutionary PROPosal. *Sci. Rep.* **6**, 1–9 (2016).
39. Robino, A. *et al.* Combined influence of TAS2R38 genotype and PROP phenotype on the intensity of basic tastes, astringency and pungency in the Italian taste project. *Food Qual. Prefer.* **95**, 104361 (2022).

40. Dinnella, C. *et al.* Individual variation in PROP status, fungiform papillae density, and responsiveness to taste stimuli in a large population sample. *Chem. Senses* **43**, 697–710 (2018).

41. Piochi, M., Dinnella, C., Spinelli, S., Monteleone, E. & Torri, L. Individual differences in responsiveness to oral sensations and odours with chemesthetic activity: Relationships between sensory modalities and impact on the hedonic response. *Food Qual. Prefer.* **88**, (2021).

42. Nolden, A. A., McGeary, J. E. & Hayes, J. E. Predominant Qualities Evoked by Quinine, Sucrose, and Capsaicin Associate with PROP Bitterness, but not TAS2R38 Genotype. *Chem. Senses* **45**, 383–390 (2020).

43. Fischer, M. E. *et al.* The associations between 6-n-propylthiouracil (PROP) intensity and taste intensities differ by TAS2R38 haplotype. *J. Nutrigenet. Nutrigenomics* **7**, 143–152 (2014).

44. Essick, G. K., Chopra, A., Guest, S. & McGlone, F. Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects. *Physiol. Behav.* **80**, 289–302 (2003).

45. Hayes, J. E. & Keast, R. S. J. Two decades of supertasting: Where do we stand? *Physiol. Behav.* **104**, 1072–1074 (2011).

46. Spinelli, S. *et al.* Personality traits and gender influence liking and choice of food pungency. *Food Qual. Prefer.* **66**, 113–126 (2018).

47. Yackinous, C. & Guinard, J. X. Relation between PROP taster status and fat perception, touch, and olfaction. *Physiol. Behav.* **72**, 427–437 (2001).

48. Bajec, M. R. & Pickering, G. J. Thermal taste, PROP responsiveness, and perception of oral sensations. *Physiol. Behav.* **95**, 581–590 (2008).

49. Bartoshuk, L. M., Duffy, V. B. & Miller, I. J. PTC/PROP tasting: Anatomy, psychophysics, and sex effects. *Physiol. Behav.* **56**, 1165–1171 (1994).

50. Melis, M. *et al.* The Gustin (CA6) Gene Polymorphism, rs2274333 (A/G), as a Mechanistic Link between PROP Tasting and Fungiform Taste Papilla Density and Maintenance. *PLoS One* **8**, e74151 (2013).

51. Yeomans, M. R., Tepper, B. J., Rietzschel, J. & Prescott, J. Human hedonic responses to sweetness:

Role of taste genetics and anatomy. *Physiol. Behav.* **91**, 264–273 (2007).

52. Garneau, N. L. *et al.* Crowdsourcing taste research: Genetic and phenotypic predictors of bitter taste perception as a model. *Front. Integr. Neurosci.* **8**, 33 (2014).

53. Fischer, M. E. *et al.* Factors related to fungiform papillae density: The beaver dam offspring study. *Chem. Senses* **38**, 669–677 (2013).

54. Piochi, M., Dinnella, C., Prescott, J. & Monteleone, E. Associations between human fungiform papillae and responsiveness to oral stimuli: Effects of individual variability, population characteristics, and methods for papillae quantification. *Chem. Senses* **43**, 313–327 (2018).

55. Neyraud, E. Role of saliva in oral food perception. *Monogr. Oral Sci.* **24**, 61–70 (2014).

56. Salles, C. *et al.* In-mouth mechanisms leading to flavor release and perception. *Crit. Rev. Food Sci. Nutr.* **51**, 67–90 (2011).

57. Laguna, L., Fiszman, S. & Tarrega, A. Saliva matters: Reviewing the role of saliva in the rheology and tribology of liquid and semisolid foods. Relation to in-mouth perception. *Food Hydrocoll.* **116**, 106660 (2021).

58. Leung, R. & Covasa, M. Do gut microbes taste? *Nutrients* **13**, 2581 (2021).

59. Schwartz, M., Canon, F., Feron, G., Neiers, F. & Gamero, A. Impact of oral microbiota on flavor perception: From food processing to in-mouth metabolization. *Foods* vol. 10 (2021).

60. Sorokowski, P. *et al.* Sex differences in human olfaction: A meta-analysis. *Front. Psychol.* **10**, 242 (2019).

61. Masala, C. *et al.* Correlation between olfactory function, age, sex, and cognitive reserve index in the Italian population. *Eur. Arch. Oto-Rhino-Laryngology* **279**, 4943–4952 (2022).

62. Oleszkiewicz, A., Schriever, V. A., Croy, I., Hähner, A. & Hummel, T. Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. *Eur. Arch. Oto-Rhino-Laryngology* **276**, 719–728 (2019).

63. Hummel, T., Kobal, G., Gudziol, H. & Mackay-Sim, A. Normative data for the 'Sniffin'' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: An upgrade based

on a group of more than 3,000 subjects'. *Eur. Arch. Oto-Rhino-Laryngology* **264**, 237–243 (2007).

64. Husain, S., Hamid, I. A., Zahedi, F. D. & Wan Hamizan, A. K. Normative data of olfactory abilities using cultural adaption Sniffin' sticks smell test in different age groups. *Saudi Med. J.* **42**, 1209–1216 (2021).

65. Herz, R. S. & Inzlicht, M. Sex differences in response to physical and social factors involved in human mate selection. The importance of smell for women. *Evol. Hum. Behav.* **23**, 359–364 (2002).

66. Doty, R. L. & Cameron, E. L. Sex differences and reproductive hormone influences on human odor perception. *Physiol. Behav.* **97**, 213–228 (2009).

67. Martin, L. J. & Sollars, S. I. Contributory role of sex differences in the variations of gustatory function. *J. Neurosci. Res.* **95**, 594–603 (2017).

68. Doty, R. L. Age-Related Deficits in Taste and Smell. *Otolaryngol. Clin. North Am.* **51**, 815–825 (2018).

69. Doty, R. L. & Kamath, V. The influences of age on olfaction: A review. *Front. Psychol.* **5**, 20 (2014).

70. Boyce, J. M. & Shone, G. R. Effects of ageing on smell and taste. *Postgrad. Med. J.* **82**, 239–241 (2006).

71. Iannilli, E., Broy, F., Kunz, S. & Hummel, T. Age-related changes of gustatory function depend on alteration of neuronal circuits. *J. Neurosci. Res.* **95**, 1927–1936 (2017).

72. Chooi, Y. C., Ding, C. & Magkos, F. The epidemiology of obesity. *Metabolism.* **92**, 6–10 (2019).

73. Herz, R. S., Van Reen, E., Gredvig-Ardito, C. A. & Carskadon, M. A. Insights into smell and taste sensitivity in normal weight and overweight-obese adolescents. *Physiol. Behav.* **221**, 112897 (2020).

74. Proserpio, C., Laureati, M., Invitti, C. & Pagliarini, E. Reduced taste responsiveness and increased food neophobia characterize obese adults. *Food Qual. Prefer.* **63**, 73–79 (2018).

75. Peng, M., Coutts, D., Wang, T. & Cakmak, Y. O. Systematic review of olfactory shifts related to obesity. *Obes. Rev.* **20**, 325–338 (2019).

76. Duffy, V. B. Variation in oral sensation: Implications for diet and health. *Curr. Opin. Gastroenterol.* **23**, 171–177 (2007).

77. Proserpio, C., Laureati, M., Bertoli, S., Battezzati, A. & Pagliarini, E. Determinants of obesity in Italian adults: The role of taste sensitivity, food liking, and food neophobia. *Chem. Senses* **41**, 169–176 (2016).

78. Besser, G. *et al.* Body-mass-index associated differences in ortho-and retronasal olfactory function and the individual significance of olfaction in health and disease. *J. Clin. Med.* **9**, (2020).

79. Martinez-Cordero, E., Malacara-Hernandez, J. M. & Martinez-Cordero, C. Taste perception in normal and overweight Mexican adults. *Appetite* **89**, 192–195 (2015).

80. Hardikar, S., Höchenberger, R., Villringer, A. & Ohla, K. Higher sensitivity to sweet and salty taste in obese compared to lean individuals. *Appetite* **111**, 158–165 (2017).

81. Donaldson, L. F., Bennett, L., Baic, S. & Melichar, J. K. Taste and weight: Is there a link? *Am. J. Clin. Nutr.* **90**, 800S-803S (2009).

82. Genter, M. B. & Doty, R. L. Toxic exposures and the senses of taste and smell. *Handb. Clin. Neurol.* **164**, 389–408 (2019).

83. Da Ré, A. F. *et al.* Tobacco influence on taste and smell: Systematic review of the literature. *Int. Arch. Otorhinolaryngol.* **22**, 81–87 (2018).

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

85. Yang, Q., Williamson, A. M., Hasted, A. & Hort, J. Exploring the relationships between taste phenotypes, genotypes, ethnicity, gender and taste perception using Chi-square and regression tree analysis. *Food Qual. Prefer.* **83**, 103928 (2020).

86. Williams, J. A., Bartoshuk, L. M., Fillingim, R. B. & Dotson, C. D. Exploring ethnic differences in taste perception. *Chem. Senses* **41**, 449–456 (2016).

87. Meiselman, H. L., Jaeger, S. R., Carr, B. T. & Churchill, A. Approaching 100 years of sensory and consumer science: Developments and ongoing issues. *Food Qual. Prefer.* **100**, 104614 (2022).

88. Spence, C. What is the link between personality and food behavior? *Curr. Res. Food Sci.* **5**, 19–27 (2022).

89. Lombion, S., Bechetoille, B., Nezelof, S. & Millot, J. L. Odor perception in alexithymic patients. *Psychiatry Res.* **177**, 135–138 (2010).

90. Pause, B. M., Ferstl, R. & Fehm-Wolfsdorf, G. Personality and Olfactory Sensitivity. *J. Res. Pers.* **32**, 510–518 (1998).

91. Larsson, M., Finkel, D. & Pedersen, N. L. Odor identification: Influences of age, gender, cognition, and personality. *Journals Gerontol. - Ser. B Psychol. Sci. Soc. Sci.* **55**, P304–P310 (2000).

92. Corlis, R., Splaver, G., Wisecup, P. & Fischer, R. Myers-briggs type personality scales and their relation to taste acuity [42]. *Nature* **216**, 91–92 (1967).

93. Chen, D. & Dalton, P. The effect of emotion and personality on olfactory perception. *Chem. Senses* **30**, 345–351 (2005).

94. Samant, S. S. & Seo, H. S. Personality traits affect the influences of intensity perception and emotional responses on hedonic rating and preference rank toward basic taste solutions. *J. Neurosci. Res.* **97**, 276–291 (2019).

95. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).

96. Jaeger, S. R., Rasmussen, M. A. & Prescott, J. Relationships between food neophobia and food intake and preferences: Findings from a sample of New Zealand adults. *Appetite* **116**, 410–422 (2017).

97. Knaapila, A. J. *et al.* Food neophobia associates with lower dietary quality and higher BMI in Finnish adults. *Public Health Nutr.* **18**, 2161–2171 (2015).

98. Dovey, T. M., Staples, P. A., Gibson, E. L. & Halford, J. C. G. Food neophobia and 'picky/fussy' eating in children: A review. *Appetite* vol. 50 181–193 (2008).

99. Marcontell, D. K., Laster, A. E. & Johnson, J. Cognitive-behavioral treatment of food neophobia in adults. *J. Anxiety Disord.* **16**, 341–349 (2002).

100. Duffy, E. The psychological significance of the concept of 'arousal' or 'activation'. *Psychol. Rev.* **64**, 265–275 (1957).

101. Jaeger, S. R., Chheang, S. L. & Prescott, J. Variations in the strength of association between food neophobia and food and beverage acceptability: A data-driven exploratory study of an arousal hypothesis. *Nutrients* **13**, 3657 (2021).

102. Pliner, P. & Melo, N. Food neophobia in humans: Effects of manipulated arousal and individual differences in sensation seeking. *Physiology and Behavior* vol. 61 331–335 (1997).

103. Raudenbush, B. & Capiola, A. Physiological responses of food neophobics and food neophilics to food and non-food stimuli. *Appetite* **58**, 1106–1108 (2012).

104. Raudenbush, B., Schroth, F., Reilley, S. & Frank, R. A. Food neophobia, odor evaluation and exploratory sniffing behavior. *Appetite* **31**, 171–183 (1998).

105. Prescott, J., Burns, J. & Frank, R. A. Influence of odor hedonics, food-relatedness, and motivational state on human sniffing. *Chemosens. Percept.* **3**, 85–90 (2010).

106. Galloway, A. T., Lee, Y. & Birch, L. L. Predictors and consequences of food neophobia and pickiness in young girls. *J. Am. Diet. Assoc.* **103**, 692–698 (2003).

107. Farrow, C. V. & Coulthard, H. Relationships between sensory sensitivity, anxiety and selective eating in children. *Appetite* **58**, 842–846 (2012).

108. Monnery-Patris, S. *et al.* Smell differential reactivity, but not taste differential reactivity, is related to food neophobia in toddlers. *Appetite* **95**, 303–309 (2015).

109. Coulthard, H. & Blissett, J. Fruit and vegetable consumption in children and their mothers. Moderating effects of child sensory sensitivity. *Appetite* **52**, 410–415 (2009).

110. Zickgraf, H. F. & Elkins, A. Sensory sensitivity mediates the relationship between anxiety and picky eating in children/ adolescents ages 8–17, and in college undergraduates: A replication and age-upward extension. *Appetite* **128**, 333–339 (2018).

111. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–124 (2018).

112. Parma, V. & Boesveldt, S. Measurement of Olfaction: Screening and Assessment. *Sens. Sci. Chronic Dis.* 45–63 (2021) doi:10.1007/978-3-030-86282-4_3.

113. Duffy, V. B., Rawal, S. & Hayes, J. E. Measurement of Gustation: From Clinical to Population-Based Methods. *Sens. Sci. Chronic Dis.* 65–102 (2021) doi:10.1007/978-3-030-86282-4_4.

114. Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E. & Kobal, G. 'Sniffin' sticks'. Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem. Senses* **22**, 39–52 (1997).

115. Rumeau, C., Nguyen, D. T. & Jankowski, R. How to assess olfactory performance with the Sniffin' Sticks test®. *Eur. Ann. Otorhinolaryngol. Head Neck Dis.* **133**, 203–206 (2016).

116. Doty, R. L., Shaman, P., Kimmelman, C. P. & Dann, M. S. University of pennsylvania smell identification test: A rapid quantitative olfactory function test for the clinic. *Laryngoscope* **94**, 176–178 (1984).

117. Cain, W. S., Gent, J. F., Goodspeed, R. & Leonard, G. Evaluation of olfactory dysfunction in the connecticut chemosensory clinical research center. *Laryngoscope* **98**, 83–88 (1988).

118. Özay, H., Çakır, A. & Ecevit, M. C. Retronasal olfaction test methods: A systematic review. *Balkan Med. J.* **36**, 49–59 (2019).

119. Pierce, J. & Halpern, B. P. Orthonasal and retronasal odorant identification based upon vapor phase input from common substances. *Chem. Senses* **21**, 529–543 (1996).

120. Heilmann, S., Strehle, G., Rosenheim, K., Damm, M. & Hummel, T. Clinical assessment of retronasal olfactory function. *Arch. Otolaryngol. - Head Neck Surg.* **128**, 414–418 (2002).

121. Haxel, B. R. *et al.* The candy smell test in clinical routine. *Am. J. Rhinol. Allergy* **25**, (2011).

122. Renner, B. *et al.* The Candy smell test: A new test for retronasal olfactory performance. *Laryngoscope* **119**, 487–495 (2009).

123. Mestres, M., Moran, N., Jordan, A. & Buettner, A. Aroma release and retronasal perception during and after consumption of flavored whey protein gels with different textures. 1. In vivo release analysis. *J. Agric. Food Chem.* **53**, 403–409 (2005).

124. Mestres, M., Kieffer, R. & Buettner, A. Release and perception of ethyl butanoate during and after consumption of whey protein gels: Relation between textural and physiological parameters. *J. Agric. Food Chem.* **54**, 1814–1821 (2006).

125. Muñoz-González, C., Feron, G. & Canon, F. Physiological and oral parameters contribute prediction of

retronasal aroma release in an elderly cohort. *Food Chem.* **342**, 128355 (2021).

126. Charles, M. *et al.* Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis. *Food Res. Int.* **69**, 9–20 (2015).

127. Romano, A. *et al.* Nosespace analysis by PTR-ToF-MS for the characterization of food and tasters: The case study of coffee. *Int. J. Mass Spectrom.* **365**–**366**, 20–27 (2014).

128. van Eck, A. *et al.* In VivoAroma Release and Dynamic Sensory Perception of Composite Foods. *J. Agric. Food Chem.* **69**, 10260–10271 (2021).

129. Biasioli, F., Gasperi, F., Yeretzian, C. & Märk, T. D. PTR-MS monitoring of VOCs and BVOCs in food science and technology. *TrAC - Trends in Analytical Chemistry* vol. 30 968–977 (2011).

130. Doty, R. L. Psychophysical testing of smell and taste function. *Handb. Clin. Neurol.* **164**, 229–246 (2019).

131. Monteleone, E. *et al.* Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Qual. Prefer.* **59**, 123–140 (2017).

132. Hayes, J. E., Sullivan, B. S. & Duffy, V. B. Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiol. Behav.* **100**, 369–380 (2010).

133. Bartoshuk, L. M. Comparing sensory experiences across individuals: Recent psychophysical advances illuminate genetic variation in taste perception. *Chem. Senses* **25**, 447–460 (2000).

134. Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E. & Keast, R. The Relationships Between Common Measurements of Taste Function. *Chemosens. Percept.* **8**, 11–18 (2015).

135. Puputti, S., Aisala, H., Hoppu, U. & Sandell, M. Multidimensional measurement of individual differences in taste perception. *Food Qual. Prefer.* **65**, 10–17 (2018).

136. Reed, D. R. *et al.* NIH workshop report: Sensory nutrition and disease. *Am. J. Clin. Nutr.* **113**, 232–245 (2021).

137. Dinnella, C. *et al.* Remote testing: Sensory test during Covid-19 pandemic and beyond. *Food Qual. Prefer.* **96**, 104437 (2022).

138. Kourouniotis, S. *et al.* The importance of taste on dietary choice, behaviour and intake in a group of

young adults. *Appetite* **103**, 1–7 (2016).

139. Köster, E. P. Diversity in the determinants of food choice: A psychological perspective. *Food Qual. Prefer.* **20**, 70–82 (2009).

140. Gaillet-Torrent, M., Sulmont-Rossé, C., Issanchou, S., Chabanet, C. & Chambaron, S. Impact of a non-attentively perceived odour on subsequent food choices. *Appetite* **76**, 17–22 (2014).

141. Chambaron, S., Chisin, Q., Chabanet, C., Issanchou, S. & Brand, G. Impact of olfactory and auditory priming on the attraction to foods with high energy density. *Appetite* **95**, 74–80 (2015).

142. Proserpio, C., de Graaf, C., Laureati, M., Pagliarini, E. & Boesveldt, S. Impact of ambient odors on food intake, saliva production and appetite ratings. *Physiol. Behav.* **174**, 35–41 (2017).

143. Proserpio, C. *et al.* Ambient odor exposure affects food intake and sensory specific appetite in obese women. *Front. Psychol.* **10**, 7 (2019).

144. Ruijschop, R. M. A. J., Boelrijk, A. E. M., Burgering, M. J. M., de Graaf, C. & Westerterp-Plantenga, M. S. Acute effects of complexity in aroma composition on satiation and food intake. *Chem. Senses* **35**, 91–100 (2009).

145. Ruijschop, R. M. A. J., Boelrijk, A. E. M., de Ru, J. A., De Graaf, C. & Westerterp-Plantenga, M. S. Effects of retro-nasal aroma release on satiation. *Br. J. Nutr.* **99**, 1140–1148 (2008).

146. Ramaekers, M. G. The appetizing and satiating effects of odours. (2014).

147. Ramaekers, M. G. *et al.* Aroma exposure time and aroma concentration in relation to satiation. *Br. J. Nutr.* **111**, 554–562 (2014).

148. Bolhuis, D. P. *et al.* Slow food: Sustained impact of harder foods on the reduction in energy intake over the course of the day. *PLoS One* **9**, e93370 (2014).

149. Bolhuis, D. P., Lakemond, C. M. M., de Wijk, R. A., Luning, P. A. & de Graaf, C. Consumption with Large Sip Sizes Increases Food Intake and Leads to Underestimation of the Amount Consumed. *PLoS One* **8**, e53288 (2013).

150. Weijzen, P. L. G., Smeets, P. A. M. & De Graaf, C. Sip size of orangeade: Effects on intake and sensory-specific satiation. *Br. J. Nutr.* **102**, 1091–1097 (2009).

151. Li, T. *et al.* The effect of taste and taste perception on satiation/satiety: A review. *Food Funct.* **11**, 2838–2847 (2020).

152. Wang, D. D. *et al.* Fruit and Vegetable Intake and Mortality Results From 2 Prospective Cohort Studies of US Men and Women and a Meta-Analysis of 26 Cohort Studies. *Circulation* **143**, 1642–1654 (2021).

153. Thibodeau, M. & Pickering, G. J. The role of taste in alcohol preference, consumption and risk behavior. *Crit. Rev. Food Sci. Nutr.* **59**, 676–692 (2019).

154. Tan, S. Y. & Tucker, R. M. Sweet taste as a predictor of dietary intake: A systematic review. *Nutrients* **11**, 94 (2019).

155. Tan, S. Y. *et al.* Is dietary intake associated with salt taste function and perception in adults? A systematic review. *Food Qual. Prefer.* **92**, 104174 (2021).

156. Berg, G. *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome* vol. 8 (2020).

157. Sender, R., Fuchs, S. & Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol.* **14**, e1002533 (2016).

158. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **474**, 1823–1836 (2017).

159. Jandhyala, S. M. *et al.* Role of the normal gut microbiota. *World J. Gastroenterol.* **21**, 8836–8847 (2015).

160. Khan, R., Petersen, F. C. & Shekhar, S. Commensal bacteria: An emerging player in defense against respiratory pathogens. *Front. Immunol.* **10**, 1203 (2019).

161. Alcock, J., Maley, C. C. & Aktipis, C. A. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays* vol. 36 940–949 (2014).

162. Han, H. *et al.* From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. *Microbiome* **9**, 1–16 (2021).

163. Herman, A. & Bajaka, A. The role of the intestinal microbiota in eating disorders – bulimia nervosa and binge eating disorder. *Psychiatry Research* vol. 300 113923 (2021).

164. Swartz, T. D., Duca, F. A., De Wouters, T., Sakar, Y. & Covasa, M. 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. *Br. J. Nutr.* **107**, 621–630 (2012).

165. Duca, F. A., Swartz, T. D., Sakar, Y. & Covasa, M. Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota. *PLoS One* **7**, e39748 (2012).

166. Depoortere, I. Taste receptors of the gut: Emerging roles in health and disease. *Gut* **63**, 179–190 (2014).

167. Efeyan, A., Comb, W. C. & Sabatini, D. M. Nutrient-sensing mechanisms and pathways. *Nature* **517**, 302–310 (2015).

168. Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M. & Hummel, T. The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS One* **7**, e36557 (2012).

169. Besnard, P. *et al.* Obese Subjects with Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. *Sci. Rep.* **8**, (2018).

170. Feng, Y. *et al.* The Associations between Biochemical and Microbiological Variables and Taste Differ in Whole Saliva and in the Film Lining the Tongue. *Biomed Res. Int.* **2018**, (2018).

171. Cattaneo, C. *et al.* New insights into the relationship between taste perception and oral microbiota composition. *Sci. Rep.* **9**, (2019).

172. Cattaneo, C., Riso, P., Laureati, M., Gargari, G. & Pagliarini, E. Exploring associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake. *Nutrients* **11**, 1167 (2019).

173. Mameli, C. *et al.* Taste perception and oral microbiota are associated with obesity in children and adolescents. *PLoS One* **14**, (2019).

174. Yousaf, N. Y. *et al.* Daily Exposure to a Cranberry Polyphenol Oral Rinse Alters the Oral Microbiome but Not Taste Perception in PROP Taster Status Classified Individuals. *Nutrients* **14**, 1492 (2022).

175. Gardner, A., So, P. W. & Carpenter, G. H. Intraoral Microbial Metabolism and Association with Host Taste Perception. *J. Dent. Res.* **99**, 739–745 (2020).

176. Neyraud, E. & Morzel, M. Biological films adhering to the oral soft tissues: Structure, composition, and potential impact on taste perception. *J. Texture Stud.* **50**, 19–26 (2019).

177. Fluitman, K. S. *et al.* Associations of the oral microbiota and Candida with taste, smell, appetite and undernutrition in older adults. *Sci. Rep.* **11**, 1–11 (2021).

178. Koskinen, K. *et al.* The nasal microbiome mirrors and potentially shapes olfactory function. *Sci. Rep.* **8**, (2018).

179. Vascellari, S. *et al.* Genetic variants of TAS2R38 bitter taste receptor associate with distinct gut microbiota traits in Parkinson's disease: A pilot study. *Int. J. Biol. Macromol.* **165**, 665–674 (2020).

180. Duarte-Coimbra, S., Forcina, G., Pérez-Pardal, L. & Beja-Pereira, A. Characterization of tongue dorsum microbiome in wine tasters. *Food Res. Int.* **163**, 112259 (2023).

181. Vujkovic-Cvijin, I. *et al.* Host variables confound gut microbiota studies of human disease. *Nature* **587**, 448–454 (2020).

182. Swinburn, B. A. *et al.* The global obesity pandemic: Shaped by global drivers and local environments. *Lancet* **378**, 804–814 (2011).

183. Masala, C. *et al.* An Italian population-based study of the prevalence of olfactory impairment. *Chem. Senses* **44**, e47–e47 (2019).

Section I

Olfaction, food neophobia
and oral microbiota

Chapter 2

Arousal influences olfactory abilities in adults with different degree of food neophobia

Menghi, L., Khomenko, I., Pedrotti, M., Cliceri, D., Aprea, E., Endrizzi, I., Cavazzana, A., Biasioli, F., Giacalone, D. & Gasperi, F.

Scientific Reports, 10, 20538 (2020)
DOI: 10.1038/s41598-020-77428-w

CHAPTER 2:

AROUSAL INFLUENCES OLFACTORY ABILITIES IN ADULTS WITH DIFFERENT DEGREE OF FOOD NEOPHOBIA

Abstract

Food neophobia, i.e., the aversion to novel foods, and olfaction are both factors strongly affecting food choices. Mounting evidence suggests a higher arousal towards food as a key factor underlying the reluctance to eat what is unfamiliar to us. As the role of olfaction behind this phenomenon is poorly understood, we explored the associations between food neophobia and trait anxiety, olfactory functions (odor threshold, discrimination and identification) and retronasal aroma release from a reference food in a healthy cohort of 83 adult volunteers. We grouped participants in Low-Neophobics or neophilics (n = 35), Medium-Neophobics (n = 32) and High-Neophobics (n = 16) according to the widely recognized Food Neophobia Scale. Participants with higher neophobic tendencies were found to have marginally higher trait anxiety levels than neophilics ($p = 0.10$). A lower global olfactory functioning and odor discrimination abilities characterized High-Neophobics, while Medium-Neophobics showed a higher odor sensitiveness than Low-Neophobics. Lastly, High-Neophobics showed a lower extent of retronasal aroma release, likely due to a shorter duration of oral processing and higher anxiety-related physiological responses (such as breathing rate). In summary, this study supports the assumption that the conflicting relationship that neophobics have with food may be led by higher levels of arousal toward foods, rather than different chemosensory functions.

Keywords: Food Neophobia; arousal; trait anxiety, olfactory functioning; aroma release

2.1 | Introduction

Defined as the reluctance to eat unfamiliar foods [1], Food Neophobia (FN) is a multidimensional phenomenon classified as part of the spectrum of feeding difficulties. An adaptive trait shared by all omnivores, FN sits at the core of the so-called "*omnivore's dilemma*" [2,3]: humans are predisposed to exploit a vast and diverse set of nutritional resources to promote chances of survival but, at the same time, need to avoid ingestion of novel foods that might be potentially harmful [4]. FN is a developmentally appropriate behavior at an early age of an individual's lifespan [1,5,6], but it can persist into middle childhood and adulthood if tailored interventions are not provided [7]. Despite this, evidence of how FN may influence eating behaviors in adults, while growing (e.g., [8–15]), is still relatively limited.

In adults, FN is likely to be associated to health-related issues such as higher body weight [14–16] and overall reduced dietary quality and variety [9,16]. Interestingly, the latter association seems to extend beyond rejection of novel foods to encompass items that might be considered commonly consumed [8,9]. This suggests that FN may have a more pervasive influence on food preferences and intake, which is not limited to unfamiliar food. The heritability of FN is well-documented and the contribution of genetic factors on this feeding behavior seems to be still operating in adulthood (especially in women) with an estimated magnitude between 61% and 69% [11,17]. FN is considered to be a stable [18] trait that co-varies with other factors like gender, age [12,19], personality traits [5,11,20], income or educational level [21]. However, the fear related to the unpleasantness of (novel) food's sensory cues still remains a key determinant on its refusal [5]. This may be explained by the way neophobic individuals process sensory experiences as evidenced in several studies focusing on *taste* (e.g., [8,14,15,19,20]) while, quite surprisingly, much less is known about the sense of *smell*, i.e., olfaction.

Olfaction has a prominent role in many domains of our life, from supporting us to process and to encode emotions through odors to social interactions [10,22]. Driving the adoption of behaviors in response to chemosensory stimuli in the environment [10], olfaction has the important evolutionary function of alerting individuals from the ingestion of potentially aversive substances and recognizing foods useful for survival [23]. Moreover, olfaction is extremely influential on food choices and preferences as it is a major contributor to food flavor [24], which is crucial when it comes to the sensory evaluation and appreciation of food.

Neophobic individuals are reportedly different from "neophilics" (i.e., individuals willing to try and accept novel foods) in the way they explore the olfactory environment (see for a review [10]). Raudenbush *et al.* [13] reported that adults less willing to try novel foods rated a series of odors (both familiar and unfamiliar) as less pleasant and less intense, and tended to use smaller sniff magnitudes compared to neophilics, as measured in an odor detection task. These findings suggested an attempt made by neophobics to avoid any potential odor-related experience with foods [25]. Moreover, neophobics are reportedly less accurate at naming odors, possibly reflecting a passive attitude to explore the chemosensory environment [26]. Finally, a recent study measured the spontaneous exploratory behavior that toddlers had towards pleasantly and unpleasantly odorized bottles focusing on mouthing behaviors and reported a positive association in boys between smell reactivity and FN [27]. Similarly, in a study conducted by Farrow and Coulthard [28] involving toddlers aged 5-10, parents' perceptions of their children's taste/smell sensitivity was associated with higher levels of FN and anxiety. Interestingly, the results revealed that children's sensory sensitivity (i.e., individual differences in the detection of, and reaction to, chemosensory stimuli) mediated the relationship between anxiety and children's tendency to selective feeding behavior. These results suggest that a higher sensitivity to chemosensory stimuli may explain why more anxious children are more likely to be selective eaters.

Whether results from these studies would replicate in an adult population is unknown, although it is worth noting that a positive relationship between trait anxiety and FN has also been observed in adults in at least one study [1], where neophobics showed higher arousal responses (i.e., increased heart rate, galvanic skin responses and respiration) compared to neophilics when presented with pictures of food stimuli [29,30]. Similar insights come from large-scale studies, which reported that differences in liking and sensory responses toward food items eliciting *"warning"* (e.g., bitter, sour, astringent) [8] or pungent [20] sensations were not affected by differences in taste sensitivity, operationalized in terms of PROP (6-n-propylthiouracil) responsiveness and/or fungiform papillae density.

Taken collectively, the existing research would suggest that the feeding behavior of adult neophobics is more likely driven by a global higher arousal responsiveness toward foods, rather than by a higher chemosensory functioning. Since neophobics tend to be more alert when confronted with food [30], whether familiar or unfamiliar [8,9], we hypothesized that this behaviour may also be explained by the different way in which neophobics react to the stimulation of their olfactory epithelium from food odorants, which can occur when odorants are inhalated through the nostrils (orthonasal olfaction) or released in the mouth during consumption and then exhalated via the back of the throat (retronasal olfaction). Situated within this context, the present study aimed at identifying differences in olfactory abilities from adult individuals with different selective/restrictive attitudes towards food, and to infer a possible effect of arousal underlying these differences.

To the best of our knowledge, no studies reporting the associations between olfactory functions, arousal responses and FN have been carried out yet. To address this issue, we studied a healthy cohort of adults assessed for orthonasal functioning through the Sniffin' Sticks battery [31]. For each subject, we monitored *in real time* for retronasal aroma release during the consumption of a reference food product (a strawberry flavored candy) through *nose-space* analysis (NS), the analysis of volatile compounds concentration in the nose, with Selected-Ion Flow-Tube Mass Spectrometry

(SIFT-MS) [32]. Participants also completed an online socio-demographic questionnaire and the widely used Food Neophobia Scale (FNS) [1,8] to investigate neophobic traits. Lastly, the trait anxiety subscale of the State-Trait Anxiety Inventory Questionnaire (STAI-T) [33,34] was used to examine the hypothesis that neophobics' responses to olfactory cues may be led by an overall higher arousal state mediated by anxiety rather than by a higher sensitivity to odors.

2.2 | Methods

2.2.1 | Participants

The present study is part of a broader large-scale investigation aiming to evaluate the Italian olfactory function according to biological, psychological, attitudinal and cognitive variables and to analyse the prevalence of olfactory disorders in Italy and its risk factors [35]. We collected data from 83 healthy volunteers (aged 22-68 years old). Participants were recruited from the consumer database of the sensory laboratory of Edmund Mach Foundation through institutional mailing and social network (Facebook) announcements. Pregnant or lactating women, smokers, people with medical conditions or treatments that could modify olfactory functions (e.g., [36]) were not included in the study. Informed, written consent according to the European Data Protection Regulation (UE 679/2016) was obtained from all participants. The present study was performed according to the principles established by the Declaration of Helsinki. The questionnaires and the olfactory protocol (i.e., the Sniffin' Sticks procedure) were approved by the Institutional Ethics Committee of the University of Cagliari (the affiliation of one of the participating research groups) [35]. The NS protocol, not originally part of the broader study design [35], was separately submitted to a local Ethics Committee, which provided an official waiver stating that its approval was not needed.

2.2.2 | Measurements

As part of the broader data collection proposed by Masala *et al.* [35], interested and eligible respondents were given general information about the aim and the workflow of the study and were asked to fill out an online questionnaire collecting socio-demographic (gender, age) and the self-reported weight (kg) and height (m), later used to calculate the Body Mass Index (BMI) in kg/m^2. As additional task from the original procedure described by Masala *et al.* [35], the FNS and the STAI-T questionnaires were also collected remotely. All the respondents were asked to complete the online questionnaires prior to the start of the lab session. Later, participants were invited to the sensory laboratory of Edmund Mach Foundation where measures of olfactory functions and monitoring of retronasal aroma release were performed in a single session lasting approximately 90 minutes. During the lab session, volunteers were firstly given instructions on the olfactory assessment before starting the evaluation. Once the olfactory assessment was concluded, an interval of 10 minutes was enforced, during which participants were introduced to the retronasal aroma release protocol. Participants had been instructed to refrain from smoking, eating, drinking and brushing their teeth for at least 3 hours prior the start of the lab session. Below, we briefly list all the measurements used for this study.

2.2.2.1 | The Italian Food Neophobia Scale (FNS)

FN was measured using an Italian version of the common Food Neophobia Scale (FNS) [1] developed and validated by Laureati *et al.* [8]. The FNS consists of 10 items each measured on a 7-point scale ranging from 1 *'strongly disagree*' to 7 '*strongly agree*'. The scores of five items reflecting neophilic food attitudes were reversed before analyses (see Supplemental Table S2.3). A FNS score, ranged theoretically from 10 to 70, was then computed for each individual as the sum of the scores given to the ten items, with higher scores resulting in higher neophobics tendencies. Cronbach's α of the measure used was 0.88, nearly identical to the one reported by Laureati *et al.* [8] (Cronbach's α = 0.87).

2.2.2.2 | The Italian Trait Anxiety Inventory Questionnaire (STAI-T)

Trait anxiety was measured through a validated Italian version of the trait subscale from the State-Trait Anxiety Inventory Questionnaire [33,34] designed to measure relatively stable aspects of "anxiety proneness" (Cronbach's α= 0.93). The STAI-T subscale consists of 20 statements, of which nine *anxiety-absent* and eleven *anxiety-present* items each measured on 4-point likert scale ranging from 1 "*almost never*" to 4 "*almost always*". The scores from the nine anxiety-absent items were reversed before analyses (see Supplemental Table S2.4). The STAI-T score, ranged theoretically from 20 to 80, was then obtained by the sum of the scores from the 20 statements with higher scores reflecting higher levels of anxiety proneness [34]. STAI-T scores are commonly classified as "no or low anxiety" (20-37), "moderate anxiety" (38-44), and "high anxiety" (45-80) [34].

2.2.2.3 | The Sniffin' Sticks Test

The Sniffin' Sticks battery (Burghart, Wedel, Germany), developed by Hummel *et al.* [31], was applied as psychophysical method to assess the olfactory function of our cohort. It comprises 3 subtests (**OT**= odor threshold; **OD**= odor discrimination; **OI** = odor identification) yielding 4 scores: **T** threshold score, **D** discrimination score, **I** identification score and TDI cumulative olfactory score [31]. The olfactory stimuli were presented using penlike odor dispensing devices placed in front of either nostril of participants for ~3 seconds. Participants were blindfolded in the OT and OD task. An interval of 5 minutes was enforced between each subtest.

2.2.2.3.1 | Odor threshold test (OT)

The odor thresholds were obtained with triplets of odorant pens, each containing one N-butanol-impregnated pen and two odorless blanks, presented in 16 successive 1:2 dilution steps starting from a 4% solution using a single staircase method employing a triple alternative forced

choice paradigm. The correct identification of two consecutive trials triggered a reversal of the staircase, while a wrong answer let the experimenter presenting the step-higher dilution triplet. The OT score, ranged theoretically from 1 to 16, was computed by the geometric mean of the last four staircase reversal points out a total of seven.

2.2.2.3.2 | Odor discrimination test (OD)

In the OD test, participants were asked to sniff 16 triplets of odorant pens, each containing two identical odorant pens and a third with a different (target) odor. Odors were selected to be easily discriminated (more than 75% of times) by healthy individuals (for the name of odors, see [31]). In this forced choice paradigm, participants were asked to identify the "target" odor. One point was assigned for a correct answer while 0 for a wrong one. The OD score, ranged theoretically from 0 to 16, was obtained by computing the sum of the correct responses.

2.2.2.3.3 | Odor identification test (OI)

The OI test was performed using 16 felt pens containing common odorants (for the name of odors see [31]) where participants were asked to identify the odor from a list of four alternative worded pictures, in a forced choice paradigm. As in the OD test, a correct answer was evaluated as 1 point, while an incorrect one as 0 point which resulted in a OI score, ranged theoretically from 0 to 16, computed by the sum of the correct responses.

2.2.2.3.4 | TDI score

The cumulative TDI score represented the sum of the OT, OD and OI scores, ranging theoretically from 1 to 48. This score can be considered as a measure of the individuals' overall olfactory function [31,37] to be used to clinically arrange individuals as normosmic (i.e., normal

olfactory function; TDI ≥ 30.75), hyposmic (impaired olfactory function; TDI≤ 30.75) or functional anosmic (residual or absent olfactory function; TDI≤ 16) [37].

2.2.2.4 | In vivo retronasal aroma release by SIFT-MS

2.2.2.4.1 | Sample

A commercial strawberry flavored candy (Fruittella Caramelle Gelee; Perfetti Van Melle; Italy) was chosen a reference food matrix for our purposes according to the following criteria: a) being widely common in Italy to avoid potentially refusals by neophobics; b) having a flavoring composition easy to trace with SIFT-MS; c) being a reproducible and easy to manage product.

2.2.2.4.2 | SIFT-MS conditions

A commercial SIFT-MS (SYFT VOICE 200 ultra, Syft Ltd, New Zealand) was used to analyze *in vivo* the volatile organic compounds (VOCs) of the candy from the nose space of each participant. Exhaled air from participants' nostrils was sampled through an ergonomic glass nosepiece with a silicone rubber tube. The nosepiece was connected to SIFT-MS with a PEEK tube (at room temperature for 30 cm and then heated at 110 °C). The instrument was calibrated daily with a mixture of standard gases (benzene, ethylene, isobutane, octafluorotoluene, hexaflurotoluene, toluene, p-xylene, and 1,2,3,4-tetrafluorobenzene; SRA Instruments S.p.A). The SIFT-MS was operated in SIM mode scanning 33 nominal m/z values related to eight selected VOCs.

We monitored seven different VOCs, which represented the main aroma compounds present in the candy (2 alcohols, 4 esters and a carboxylic acid; ethyl maltol, 3-hexen-1-ol, ethyl 2-methylbutanoate, (Z)-3-hexenyl acetate, ethyl butanoate, ethyl hexanoate, 2-methylbutanoic acid) plus acetone as a marker of participants' breathing cycle [38]. Acetone is naturally present in human breath and its released intensity is not affected during mastication [38]. Hence, it can be used to monitor

participants' breath rhythm during a nose-space task. Chemical ionization was achieved using H_3O^+, NO^+, O_2^+ precursor ions and the overall scan time was set at 1 second. Data acquisition and processing was performed in LabSyft (Syft Technologies Ltd, version 1.6.2). A complete overview on SIFT-MS technique and on its benefits for real time analyses of odorants can be found in Langford *et al.* [39].

2.2.2.4.3 | Nose-Space analysis

A Nose-Space analysis (NS) through SIFT-MS was implemented to monitor *in-vivo* retronasal aroma release. In brief, the task was carried out under a fixed-rhythm chewing (60 bpm) protocol supported by a video tool designed to train and help participants in following the procedure. The video showed an experimenter's mouth chewing the strawberry flavored candy following a metronome that marked the tempo. Prior to the start of the analysis, laboratory air (environmental background) was sampled for 20 seconds without any interaction with participants. Once the glass nosepiece had been fitted by the experimenter, participants were asked to breathe normally for 30 seconds to sample participants' breath. Later, participants were asked to watch the video tool while simulating a real chewing phase to get familiar with the entire protocol. They were then instructed to put the jelly candy in the mouth and to press a button on the screen to let the experimenters know when the sample has been put in-mouth. The same instruction was given to alert the experimenter once the participant swallowed the sample. After swallowing the sample, participants had to continue breathing for 90 seconds, keeping the mouth closed. In total, the NS measurement lasted around 4 minutes. Taking into account the inter-individual variability on flavor release, (e.g., [40–44]), at least three replicates per participant were performed to get a robust database. Between each replicate, participants were asked to rinse their mouth with mineral water, to have some unsalted bread, to rinse their mouth with mineral water again, and finally to wait at least 15 minutes before the next trial. The entire procedure was conducted in an individual computerized sensory booth [45] under cold white light located in a room

with filtered air at constant temperature. FIZZ v2.50 (Biosystemes, Couternon, France) was used to guide participants along the entire protocol.

2.2.3 | Statistical analysis

Variables of interest were summarized using means ± SD for normally distributed variables or median ± IQR for variables that did not fit normality assumptions, tested using Shapiro and Levene tests. For descriptive purposes, associations between demographic factors (i.e., age, BMI) and the variables of interest (FNS, STAI-T, olfactory performances and extent of retronasal aroma release) were assessed through Pearson or Spearman rank coefficients correlations, while differences in terms of gender were assessed through Welch Two Sample t-test or Mann-Whitney U test.

Participants were grouped according to their FN level using *cut-offs* proposed by [8] and separate 1-*way* ANOVAs were performed for age and BMI to determinate whether a between group effect of such variables occurs as a function of FN level. Similarly, a χ^2 test was used to evaluate differences in gender proportions between FN groups. These analyses were carried out to evaluate the potential confounding effect of variables whose influence on both olfactory performances and retronasal aroma release is known [31,37,43,44,46]. Then, to assess whether FN levels were associated to anxiety proneness, STAI-T scores were submitted to a 1-*way* ANOVA with FN level as "between subject factor". To assess differences between FN levels in olfactory performances, both single scores (OT, OD, OI) and the cumulative one (TDI) were submitted to separate Kruskall-Wallis tests with FN level as "between subject factor". Dunn's test with Bonferroni correction was used as *post-hoc* test when statistically significant differences between FN levels were observed.

For instrumental analyses, a timeslot of 2 minutes starting from the moment when participants put the sample in-mouth was considered for data analysis. Six parameters commonly used to analyze time-intensity (T-I) curves were extracted: the area under the curve (AUC), the maximum (I_{max}), the

median (I_{median}), the average of the last five seconds of the NS session (I_{end}), the time to reach the maximum (TI_{max}) and the slope of the first descending section of the curve (Slope), assuming a linear relationship between time and the logarithm of peak intensity [47,48]. Moreover, duration of oral processing was calculated for each replicate by subtracting the time when participants put the sample in-mouth to the time they swallowed it. Lastly, participants' breathing rate was estimated for each replicate by counting the local minima sites from the acetone curves within the task.

After data pre-processing and treatments following the guidelines suggested by [47,48], a dataset with 262 rows (participants*replicates) and 42 columns, organized in 6 groups (SIFT-MS parameters; AUC, I_{max}, I_{median}, I_{end}, TI_{max}, Slope) of 7 variables each (monitored VOCs), was built and then submitted to a Multiple Factor Analysis (MFA) to visualize global differences between FN levels in terms of aroma release. Each group of variables was log-transformed and scaled to unit of variance prior the analysis. The holistic exploration was followed by a univariate approach where all the parameters investigated plus the duration of oral processing and the breathing rate were analyzed with separate Kruskall Wallis tests with FN level as "between subject factor". Dunn's test with Bonferroni correction was used as *post-hoc* test whenever a statistically significant difference between FN levels was observed. All data analyses were run in R software v3.6.3 [49]. All tests were two-tailed, a $p < 0.05$ was considered as significant while the range $0.05 \leq p \leq 0.10$ was accepted as a trend.

2.3 | Results

2.3.1 | Cohort description

Overall, we collected data from 83 volunteers of which 48 were females (57.8%, mean age = 41.4; SD = 11.9) and 35 were males (42.2 %, mean age = 42.2; SD = 11.4). The mean age of participants was 41.7 ± 11.7 years old. A higher proportion of females were normal-weight, while males were more likely to be overweight or obese ($\chi^2 = 9.98$; p = 0.006).

The mean FN level in the sample was 24.8 ± 11.5, in line with existing data on the Italian population (27.4 ± 11.7) [8] and a mean level of anxiety proneness of 39.9 ± 9.3, indicative of moderate anxiety (38-44) [34]. We did not observe significant correlations between age and FN (R = 0.16, p = 0.14), unlike trait anxiety where a negative correlation was detected (R= -0.28, p = 0.009). BMI (Body Mass Index) was slightly associated with FN (R = 0.19, p = 0.09) but not with STAI-T scores (R = -0.14, p = 0.19). Finally, no gender differences in either FN (t = 1.26, p = 0.21) and STAI-T (t = 1.14, p = 0.25) were observed.

With regard to the olfactory data, we identified 76 normosmic and 7 hyposmic individuals. Their averaged global olfactory abilities, in terms of TDI (odor Threshold, Discrimination, Identification), were mostly higher than the 50th percentiles of the European population [37], thus offering a satisfactory olfactory performance (see Supplemental Table S2.1). In line with previous reports [37,46,50,51], we found considerable negative associations between TDI, age and BMI, but no association between TDI and gender. Similar insights came from correlations between olfactory subtest scores (OT = Odor Threshold, OD = Odor Discrimination, OI = Odor Identification), age and BMI, where OT was inversely correlated with BMI while OD was inversely correlated with both age and BMI. No correlations were found for the OI task and all the olfactory subtests for gender. Lastly, according to the extent of retronasal aroma release (area under the curve: AUC), results revealed no associations with age and BMI or differences in terms of gender for each monitored compound.

The reader is further referred to Supplemental Materials where significant correlations between olfactory performance scores, retronasal aroma release, gender, age and BMI are displayed (Figure S2.1, Figure S2.2 and Table S2.2).

2.3.2 | Participants' segmentation and characterization according to their food neophobia level

Participants were grouped according to their FN level using the *cut-offs* proposed by Laureati *et al.* [8] based on a large Italian sample. The group with neophilic tendencies (Low-Neophobics: LN) fell into the first quartile of the FNS scores distribution (FNS score ≤ 18) and represented the 42.2% of the total sample (48.5% female; mean FNS score: 14.8). The group with medium neophobic tendencies (Medium-Neophobics: MN) included participants that reported FNS scores between 18 and 36 and represented the 38.6% of the sample (62.5% female; mean FNS score: 26.3). Lastly, the group with the highest neophobic traits (High-Neophobics: HN) fell into the highest quartile of the FNS scores distribution (FNS score ≥ 36) and accounted for 19.2% of participants (HN; 68.7% female – mean FNS score: 43.8). The three groups did not differ for age ($F = 1.03$; $p = 0.36$), BMI ($F = 0.81$; $p = 0.44$) or gender proportions ($\chi^2 = 2.29$, $p = 0.31$).

2.3.3 | Association between trait anxiety and food neophobia

STAI-T scores were submitted to a 1-*way* ANOVA to assess possible differences in anxiety proneness between FN groups. Results revealed a trend ($F = 2.32$; $p = 0.10$) by which higher FN levels were associated to a higher anxiety proneness, further corroborated by a weak positive correlation between the two variables ($R = 0.14$; $p = 0.20$). Although substantial differences between FN groups were not observed, higher neophobic traits showed averaged values of STAI-T scores (MN: 42.0; HN: 41.3) commonly indicative of moderate levels of anxiety compared to the LN group, which showed a lower mean anxiety proneness (37.4) [34].

2.3.4 | Association between olfactory performances and food neophobia

To explore whether FN groups dealt differently with olfactory cues, differences on both olfactory subtests (OT, OD, OI) and TDI between FN levels were assessed through separate Kruskall-Wallis tests. Overall, a main effect of neophobic traits on TDI scores was observed ($H = 7.51$, $p = 0.02$), with HN reporting considerably worse global olfactory performances compared to LN and MN. A main effect of FN on OT was also detected ($H = 5.46$, $p = 0.03$) with LN showing higher olfactory thresholds (i.e., lower sensitivity) compared to MN (Dunn's test). In this task, no differences were reported for HN when compared to LN or MN. We found strong differences in the OD task ($H = 21.08$; $p < 0.001$), which indicated that both LN and MN outperformed HN. Conversely, we did not find differences in OI abilities between FN levels ($H = 1.16$; $p = 0.56$) . Raw scores, distributions and significant pairwise comparisons between FN levels for both TDI and olfactory subtests are displayed in Figure 2.1.

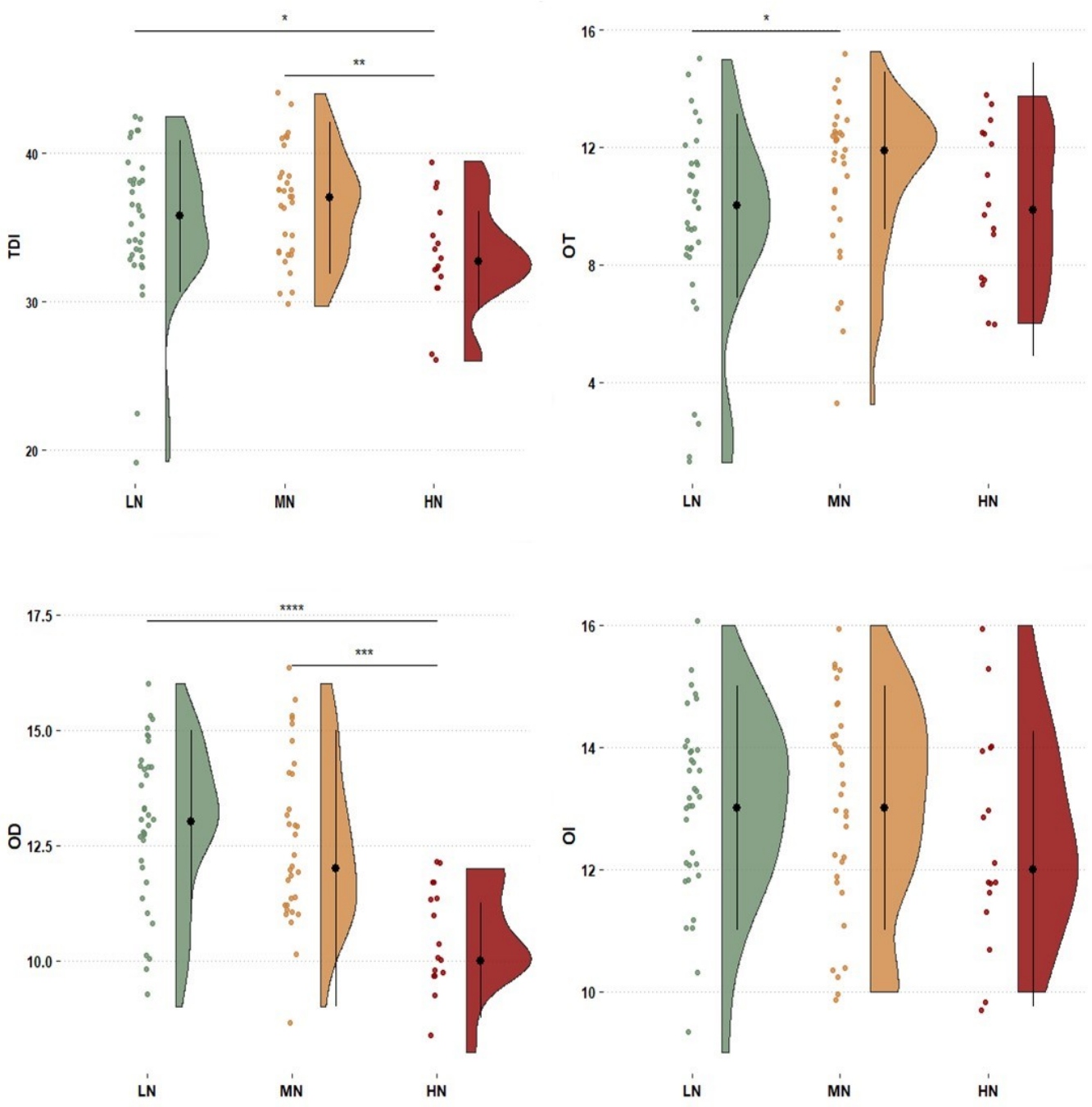

Figure 2.1: Raincloud plot showing the differences on both global (TDI) and relative subtests (OT: Odor Threshold; OI: Odor Identification, OD: Odor Discrimination) olfactory assessments as a function of FN level (LN: Low-Neophobics; MN: Medium-Neophobics; HN: High-Neophobics). The plots provide a representation of data distribution (the 'cloud'), individual raw observations (the 'rain'), the median (black filled circle) ± IQR (perpendicular) within each FN level for both global and subtests olfactory performance. Only statistically significant pairwise differences observed after post hoc Dunn's test with Bonferroni correction are presented (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

2.3.5 | Association between retronasal aroma release and food neophobia

2.3.5.1 | Multivariate exploration

To explore overall differences in retronasal aroma release between FN levels, data from the 7 monitored compounds grouped according to the SIFT-MS extracted parameters (AUC, I_{max}, I_{median}, I_{end}, TI_{max}, Slope) were submitted to a Multiple Factor Analysis (MFA, Figure 2.2). Amount of acetone (endogenous compound) was not considered in this analysis.

Participants were uniformly distributed over the first two dimensions of the MFA score plot, together accounting for 29.5 % of variance (Figure 2.2a). Dim.1 (20.6 % of variance) was positively associated with a higher proportion of replicates from LN and MN while most replicates from HN were located on the opposite end of the plot, suggesting a contrasting behaviour. Dim.2 (8.9 % of variance), further separated FN levels as most of replicates from HN were positively related to this dimension, whereas LN and MN were not clearly separated over Dim.2. Results for additional MFA dimensions (Dim.3 = 8.3 %; Dim. 4 = 5.6% of variance) are given in the Supplemental Materials (Figure S2.3; Figure S2.4). Overall, the first four dimensions of the model accounted for 43.4 % of the total variance.

A deeper characterization of the FN groups can be appreciated in Figure 2.2b, where MFA centroids (LN, MN, HN) and related partial MFA points (one of for each of the six groups of parameters considered) are visible. Starting from MFA centroids, each partial point projects the position of that FN level according to the group of variables considered [52]. We observed the largest differences between FN levels in terms of time-independent parameters (AUC, I_{max}, I_{median}) with LN and MN showing positive partial projections in Dim.1 (which accounted for the most of variation) unlike HN, which showed a contrasting behavior. Associations with Dim. 2, instead, were less helpful to explain differences between FN levels as mostly related to the origin of the MFA model which is indicative

of a lower explanatory power. Overall, these results suggest that HN showed a lower extent of retronasal aroma release than both LN and MN not mediated by different release kinetic patterns.

Partial projections of SIFT-MS extracted parameters within FN levels can be further interpreted by taking into account the variable factor map of the MFA model (Figure 2.2c). Dim. 1 well represents both the increasing direction and the correlations between all time-independent parameters (i.e., AUC, I_{max}, $I_{median,}$ I_{end}). Since both these parameters appear grouped together and are positively correlated to the first dimension, a twofold consideration can be drawn. Firstly, as most of variables within groups were strongly correlated and far from the origin, these groups were well represented by the MFA model [52]. Secondly, the positive correlation with the first dimension of the model gave information on the increasing direction of these variables and confirmed that individuals in the LN and MN groups, with positive coordinates for Dim.1, showed a higher extent of retronasal aroma release compared to the HN group, located on the opposite side of this dimension.

By contrast, the second dimension represented well only the TI_{max} group with most of variables being grouped together and far from the origin. In other words, individuals with positive coordinates on Dim. 2 reached the maximum intensity of most of VOCs later than the ones with negative coordinates on this dimension.

Lastly, most variables related to Slope were close to the origin of axes, meaning that they were not well represented by the MFA model and, therefore, less helpful in explaining variance between FN levels. Overall, results from MFA suggested that HN was associated to a lower extent of retronasal aroma release. Additionally, no clear indication on different release patterns between the compounds monitored were observed, suggesting similar kinetics within individuals.

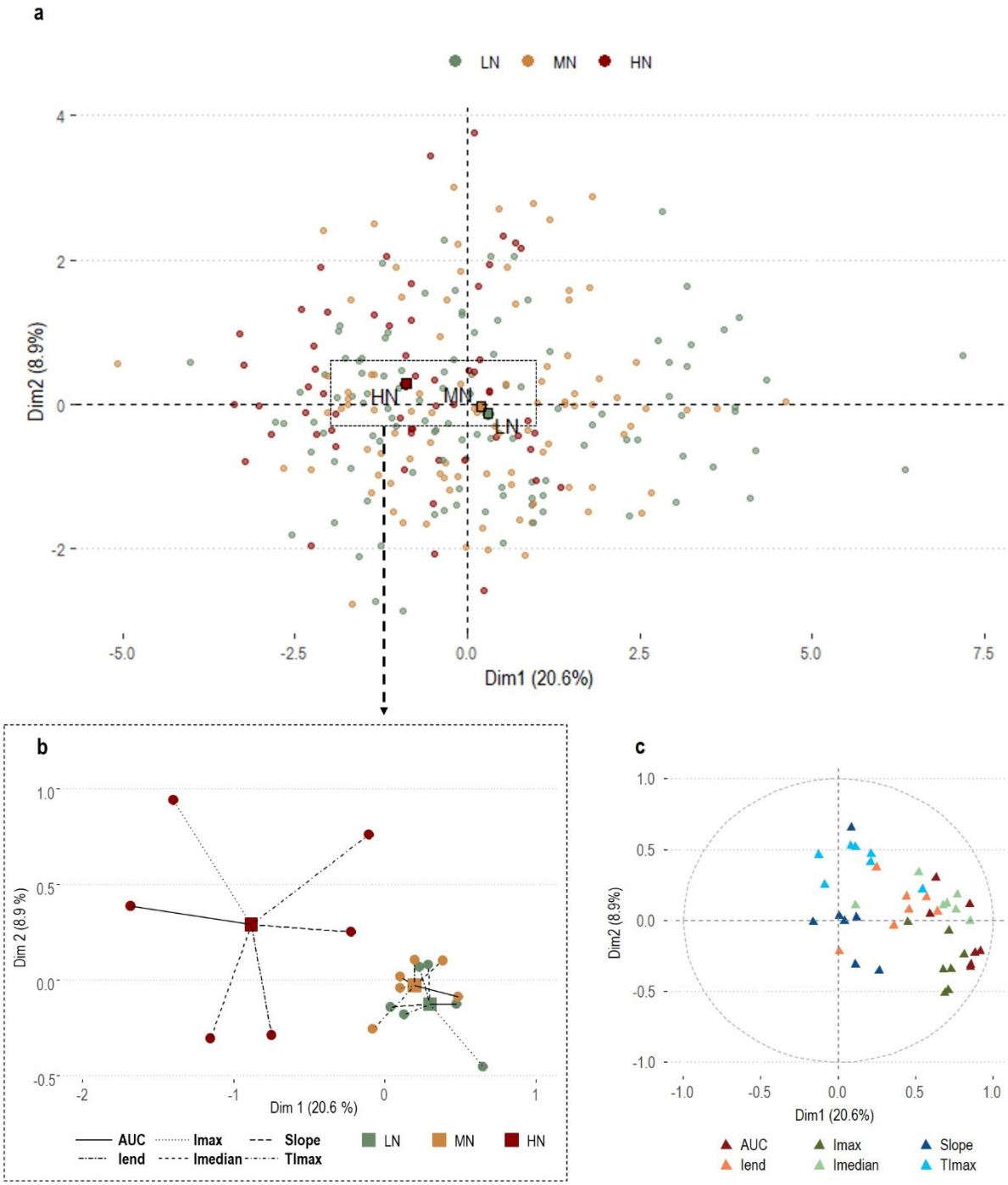

Figure 2.2: First two factors of the individual factor map (a) from the MFA based on matrices of SIFT-MS parameters. Large squares (light green square, light orange square, brown square) are MFA centroids while the circles (light green circle, light orange circle, brown circle) represent participants' position in the bi-dimensional space colored according to their FN level (LN, MN, HN). Each MFA centroid is associated (b) with partial MFA groups points for the six SIFT-MS parameters (small circles with same colour as the corresponding MFA centroid). (c) Variable factor map from the MFA model. Dots represent correlations between SIFT-MS parameters (for the 7 monitored compounds) and the first two significant dimensions of the MFA model.

2.3.5.2 | Univariate exploration

The data on retronasal aroma release were further explored univariately to further assess significant differences between FN levels. Table 2.1 reports all VOCs and T-I curves related parameters coupled with p-values from Kruskall Wallis tests, as well as pairwise comparisons according to Dunn's test with the Bonferroni adjustment. Figure 2.3 displays FN levels' median and smoothed curves for each compound.

Overall, results corroborated the ones from the MFA model (see Figure 2.2). Amongst the six extracted parameters, the ones that showed most of variation between FN levels were those describing aroma release in a time-independent fashion. Accordingly, considerable differences were found in the overall amount of VOCs released (AUC; except for (Z)-3-hexenyl acetate that showed a trend: $p = 0.07$) and in the maximum intensity released (I_{max} , except for ethylmaltol). Fewer differences were observed for the median intensity (for 4 compounds out of 7 with 2-methylbutanoic acid showing a trend: $p = 0.05$) and the averaged released intensity of the last five seconds of the NS task (I_{end}; only for 3-hexen-1-ol). The aforementioned differences always revealed LN and MN outperforming HN in terms of overall retronasal aroma release (Figure 2.3). Conversely, differences in time-related parameters (i.e., Slope, TImax) were lower and compound independent. Slope showed differences only for ethylhexanoate with LN showing a faster decrease in the exhalation of this VOC compared to MN. Only (Z)-3-hexenyl acetate showed differences for TI_{max}, with LN reaching the maximum intensity released faster than the other two groups.

Table 2.1: VOCs monitored by SIFT-MS and related T-I curves related parameters. Median ± IQR are reported for each compound as a function of FN level (LN, MN, HN). Within each parameter, data are presented as log-transformed with the exception of TImax. p values were obtained through separate Kruskall Wallis tests, with significant ones highlighted in bold. Median ± IQR marked with different superscript letters by row indicate statistically significant differences ($p < 0.05$) according to post hoc Dunn's test with Bonferroni adjustment.

Sum formula	VOCs	Parameter	LN	MN	HN	H	p.value
$C_7H_8O_3$	ethylmaltol	AUC	3.25 ± 0.42^{ab}	3.35 ± 0.44^{b}	3.18 ± 0.39^{a}	6.34	**0.04**
		Imax	1.24 ± 0.31	1.21 ± 0.34	1.19 ± 0.6	0.94	0.63
		Imedian	0 ± 0	0 ± 0	0 ± 0	0.87	0.65
		Iend	0 ± 1.09	0 ± 1.03	0 ± 1.05	0.47	0.79
		TImax	29 ± 40.5	30 ± 44	38.5 ± 44.25	4.02	0.13
		Slope	0 ± 0	0 ± 0	0 ± 0	0.02	1.00
$C_6H_{12}O$	3-hexen-1-ol	AUC	8.86 ± 0.68^{b}	8.91 ± 0.54^{b}	8.61 ± 0.44^{a}	20.46	**< 0.001**
		Imax	6.05 ± 0.7^{b}	6.04 ± 0.56^{b}	5.84 ± 0.65^{a}	14.21	**< 0.001**
		Imedian	3.49 ± 0.5^{b}	3.56 ± 0.45^{b}	3.33 ± 0.35^{a}	15.25	**< 0.001**
		Iend	2.99 ± 0.56^{b}	2.99 ± 0.38^{b}	2.89 ± 0.38^{a}	7.01	**0.03**
		TImax	17 ± 15	18 ± 14	20 ± 13.5	1.33	0.51
		Slope	0.13 ± 0.1	0.11 ± 0.09	0.12 ± 0.1	3.73	0.16
C7H14O2	ethyl 2-methylbutanoate	AUC	7.01 ± 0.83^{b}	7.13 ± 0.74^{b}	6.65 ± 0.88^{a}	20.1	**< 0.001**
		Imax	4.8 ± 0.66^{b}	4.93 ± 0.78^{b}	4.54 ± 0.9^{a}	16.39	**< 0.001**
		Imedian	0.84 ± 0.97^{b}	0.91 ± 0.99^{b}	0 ± 0.9^{a}	6.72	**0.03**
		Iend	0 ± 0.46	0 ± 0.64	0 ± 0.63	0.16	0.92
		TImax	13 ± 12.5	12 ± 15	13 ± 12.75	0.98	0.61
		Slope	0.38 ± 0.69	0.34 ± 0.7	0 ± 0.56	5.02	0.08
$C_8H_{14}O_2$	(Z)-3-hexenyl acetate	AUC	7.5 ± 0.45	7.56 ± 0.56	7.38 ± 0.56	5.19	0.07
		Imax	4.27 ± 0.46^{b}	4.3 ± 0.54^{b}	4.16 ± 0.4^{a}	8.82	**0.01**
		Imedian	2.66 ± 0.71	2.68 ± 0.88	2.66 ± 0.74	1.16	0.56
		Iend	2.41 ± 0.77	2.46 ± 0.83	2.41 ± 0.85	0.53	0.77
		TImax	20 ± 19.5^{b}	24 ± 22^{a}	27 ± 35^{a}	11.26	**< 0.001**
		Slope	0 ± 0.03	0 ± 0.09	0 ± 0.04	4.7	0.10
$C_6H_{12}O_2$	ethylbutanoate	AUC	9.15 ± 0.73^{b}	9.2 ± 0.53^{b}	8.94 ± 0.48^{a}	18.24	**< 0.001**
		Imax	6.92 ± 0.84^{b}	6.86 ± 0.7^{b}	6.55 ± 0.72^{a}	19.67	**< 0.001**
		Imedian	3.02 ± 0.46^{b}	3.06 ± 0.45^{b}	2.92 ± 0.33^{a}	9.34	**0.01**
		Iend	2.38 ± 0.79	2.49 ± 0.68	2.28 ± 1.05	5.45	0.07
		TImax	0.19 ± 0.14	0.14 ± 0.14	0.16 ± 0.14	1.3	0.52
		Slope	16 ± 12.5	15 ± 15	17.5 ± 15.5	4.05	0.13
$C_8H_{16}O_2$	ethylhexanoate	AUC	9.11 ± 0.82^{b}	9.26 ± 0.69^{b}	8.84 ± 0.74^{a}	19.28	**< 0.001**
		Imax	6.77 ± 0.82^{b}	6.81 ± 0.65^{b}	6.49 ± 0.88^{a}	14.53	**< 0.001**
		Imedian	2.35 ± 0.91	2.46 ± 0.95	2.21 ± 1.18	1.34	0.51
		Iend	0.75 ± 1.43	0.6 ± 1.3	0.48 ± 1.02	1.05	0.59
		TImax	14 ± 13	14 ± 13	16 ± 14.75	1.49	0.47
		Slope	0.21 ± 0.47^{b}	0.14 ± 0.22^{a}	0.21 ± 0.33^{ab}	7.58	**0.02**
$C_5H_{10}O_2$	2-methylbutanoic acid	AUC	6.86 ± 0.37^{b}	6.95 ± 0.42^{b}	6.82 ± 0.25^{a}	11	**< 0.001**
		Imax	3.46 ± 0.51^{b}	3.52 ± 0.5^{b}	3.27 ± 0.46^{a}	17.37	**< 0.001**
		Imedian	2 ± 0.34	2.07 ± 0.34	2 ± 0.24	5.82	0.05
		Iend	1.91 ± 0.47	1.94 ± 0.47	1.9 ± 0.41	0.58	0.75
		TImax	18 ± 14.5	15 ± 17	16.5 ± 15.5	0.03	0.99
		Slope	0 ± 0.14	0 ± 0.12	0 ± 0.06	2.99	0.22

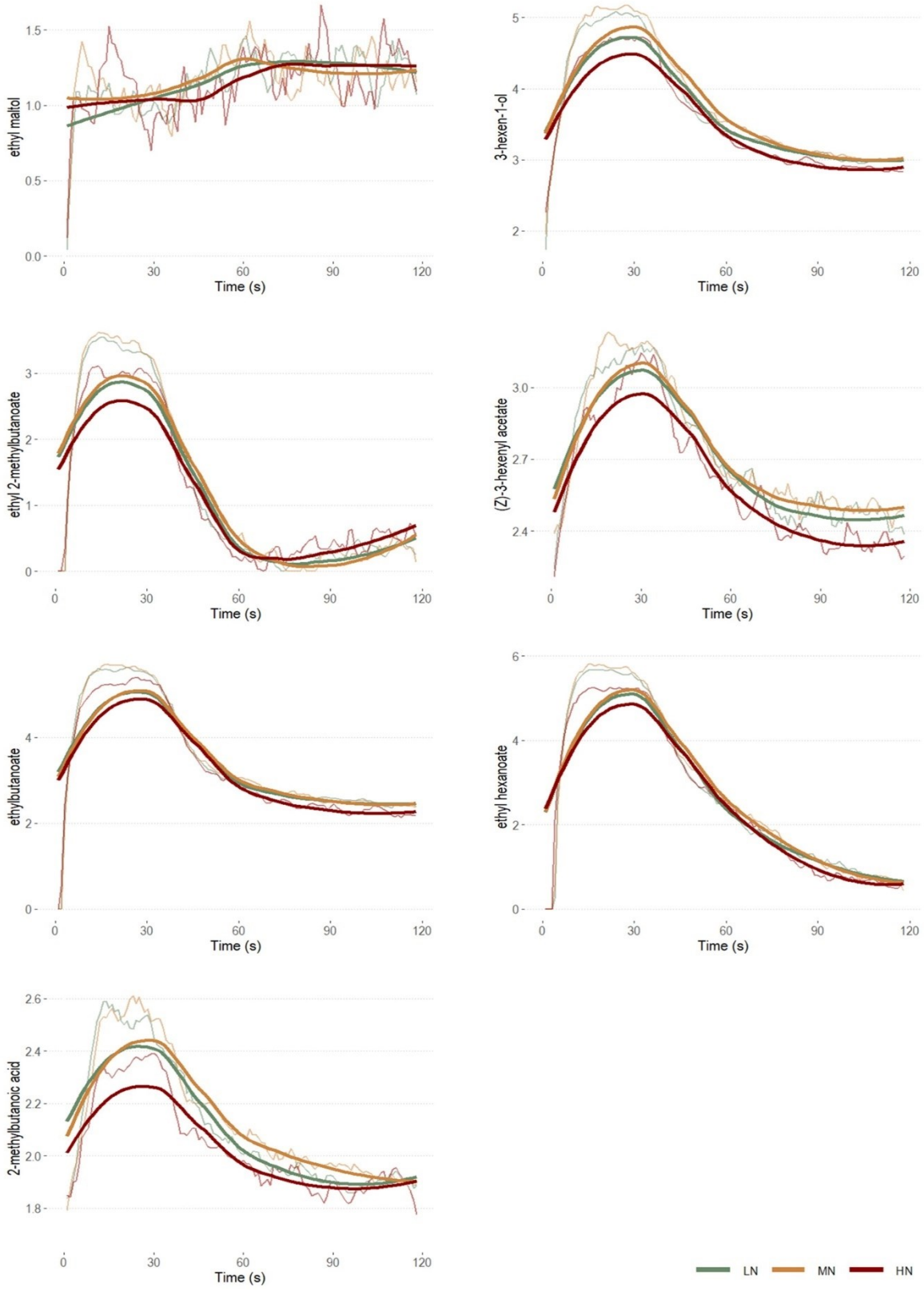

Figure 2.3: Log-transformed median (transparent) and smoothed (bold) release curves for the 7 monitored VOCs by SIFT-MS as a function of FN level.

2.3.6 | Association between food neophobia, retronasal aroma release and behavioral parameters

To corroborate the idea that neophobics may be led by a higher global arousal in their approach to foods, two different parameters were extracted from the NS task: the duration of oral processing and the breathing rate of participants within each replicate. Firstly, we found significant differences on duration of oral processing (i.e., the difference in terms of timing when participants put the sample in-mouth and the time they swallowed the candy) as a function of FN level ($H = 11.93$; $p < 0.0001$), with LN ($Z = 2.38$; $p = 0.008$) and MN ($Z = 3.45$; $p = 0.003$) chewing the sample for a longer time than HN.

Secondly, we estimated the breathing rate of participants for each replicate by counting the local minima sites from the acetone curves within the task. Results revealed a strong significant difference between the groups ($H = 38.68$; $p < 0.0001$), as HN were found to breathe faster than both MN ($Z = 3.92$; $p < 0.0001$) and LN ($Z = 6.21$; $p < 0.0001$). Moreover, a statistically significant difference was also observed between LN and MN, with LN undergoing the task with longer breathing cycles ($Z = 2.49$; $p = 0.006$). Pairwise comparisons according to *post-hoc* Dunn's test with Bonferroni correction between groups (panel a) and examples of breathing behaviors (panels b, c, d) between FN levels are displayed in Figure 2.4.

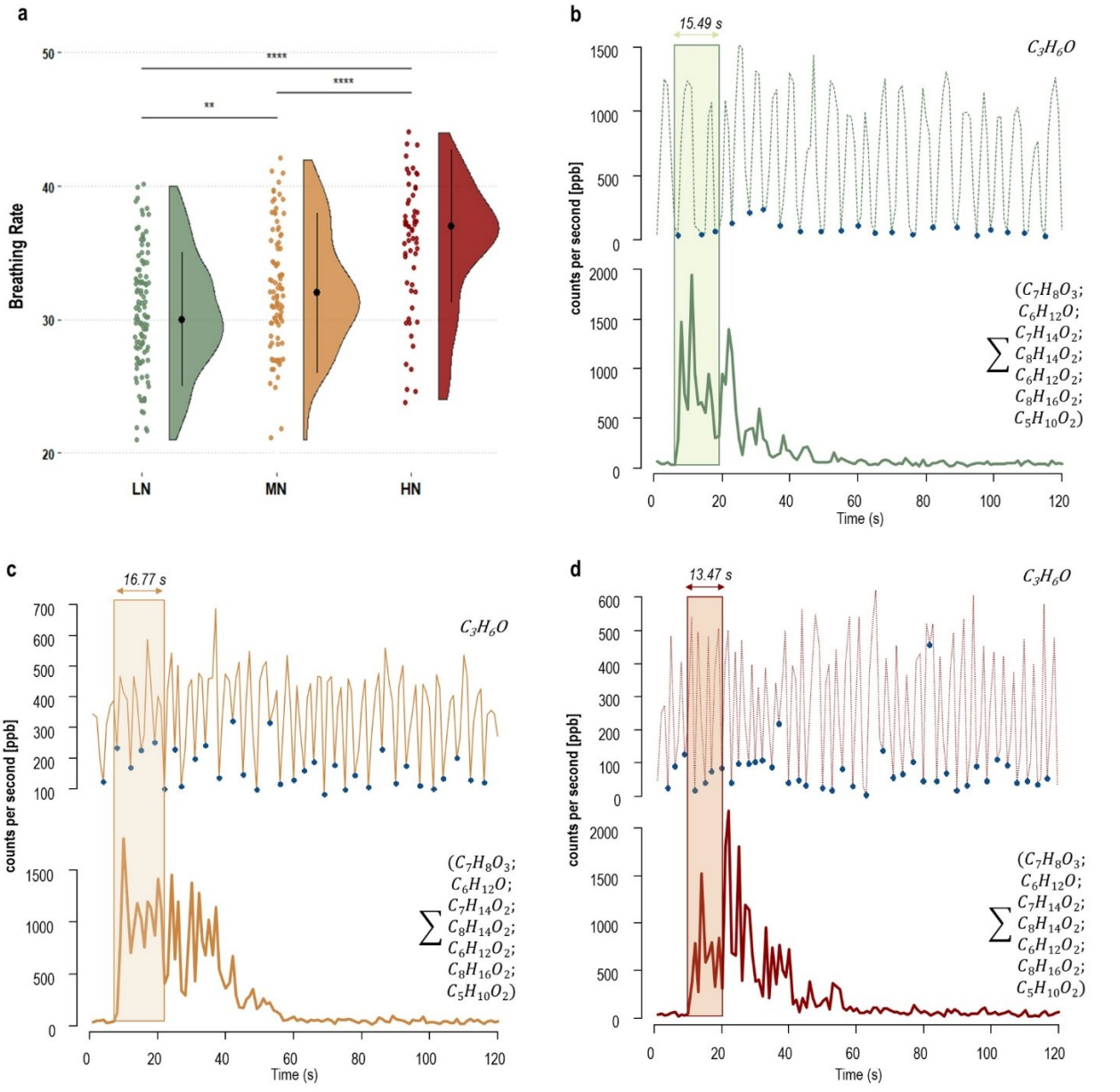

Figure 2.4: Raincloud plot (a) showing the differences in breathing rates between FN levels (LN: Low-Neophobics; MN: Medium-Neophobics; HN: High-Neophobics). The plot provides a representation of data distribution (the 'cloud'), individual raw observations (the 'rain'), the median (black filled circle) ± IQR (perpendicular) within each FN level. Only statistically significant pairwise differences observed after post hoc Dunn's test with Bonferroni correction are presented (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001). b–d: examples of breathing behaviors from a replicate of three participants with similar biological characteristics (b: Female; 51 yo; BMI = 19.27; c: Female; 53 yo; BMI = 25.46; d: Female; 53 yo; BMI = 21.82) but different according to their FN level. The top trace shows the curve of acetone (C3H6O) within the task with relative local minima (blue filled circle). The bottom trace is the sum of the 7 monitored VOCs, which represents the volatilome of the candy. Lastly, the transparent rectangles report the duration of the oral processing (s) for each replicate considered.

2.4 | Discussion

This study assessed the contribution of olfaction (both orthonasal and retronasal) on food neophobia. We hypothesized that this avoidant/selective feeding behaviour may be linked to an adaptive usage of olfaction mediated by a higher arousal responsiveness towards foods.

In our adult cohort, approximately a fifth of individuals showed high neophobic tendencies (FNS ≥ 36) [8], consistent with known distributions for the Italian population (26.2%; Laureati *et al.* [8]). Surprisingly, we did not find differences for age, gender or BMI between FN levels, as previously documented [8,12,16,19–21,51], which is probably due to the smaller size of our cohort than the ones used by the aforementioned studies. However, this finding is advantageous in the present study as it allowed us to draw conclusions on the effects that FN may have on olfactory performances without possible confounders.

The idea that neophobics would respond differently to olfactory stimulation was confirmed by the olfactory assessment, as the HN group offered worse global olfactory performances than LN and MN. Firstly, neophobics differ from neophilics by the way they explore the chemosensory environment. We found strong differences in the OD task between FN levels, with HN performing worse than MN and LN. Our findings are in line with a previous investigation, which assessed olfactory functioning on individuals showing other selective/restrictive feeding behaviors as anorexia nervosa [53], and reported that anorectic patients offered poorer odor discrimination performances compared to a control group. Secondly, participants with medium levels of FN showed a higher odor sensitivity than neophilics. These results seem consistent with Monnery-Patris *et al.* [27], which explored smell and taste reactivity in toddlers and found a modestly higher smell reactivity among young neophobic boys.

A higher sensitivity to chemosensory stimuli has been reported to mediate the well-known relationship between FN and anxiety [1,28]. Also in this study, individuals with higher neophobic tendencies showed a higher anxiety proneness, albeit less strongly (p=0.10) than previously documented [1]. However, according to [34], our neophobic population (MN and HN) showed a mean level of anxiety commonly classified as higher compared to the one observed for neophilics, thus corroborating this hypothesis.

We found some unexpected results from the OI task. Assuming that odor identification is mediated by the degree of exposure one person has with the olfactory world [10], we expected that milder levels of FN would outperformed HN in this task, as previously reported by Dematte *et al.* [26]. In that study, 167 individuals were asked to identify a series of 36 common odors presented in glass vials from a 90-label list (arranged in odor categories), which resulted in neophobic participants performing significantly worse in naming odors than neophilics. In contrast, our findings did not support differences between FN levels in the OI task. This unexpected result might be related to the task being easier than the one proposed by [26], where participants correctly identified on average 38% of the odors provided. In the OI subtest, participants were asked to name highly common odors [31] supported by a four-alternative forced-choice visual-worded paradigm. Cross-modal integrations between vision and olfaction in presence of congruent pictures [54] or relevant sematic information [55] have been proposed to magnify odor identification. Thus, it is possible that the presence of visual-worded cues, combined with the routine clinical purposes for which the Sniffin' Sticks battery was designed [31], made the task less apt to detect differences between FN levels.

Overall, our findings support the idea that a lower willingness to explore the chemosensory world does not have direct relevance once sensory perception of food occurs but, rather, that it would have led neophobics to develop an anxiety-mediated adaptive behaviour [56] that protect them from exaggerated sensations of possible aversive outcomes associated with food items [1,25,28].

The retronasal assessment supported the same interpretation. NS has been widely applied to study the complex network of factors affecting inter-individual differences on retronasal aroma release, such as oral processing [40,41], physiological parameters [40,42], age [43], gender and ethnicity [42] and BMI [43,44]. To the best of our knowledge, our study is the first in which a direct injection mass spectrometry technique was applied to get insights into behavioral traits underlying individuals with different attitudes towards food, thus opening new scenarios for further investigations of eating behaviors. Results from the MFA model revealed that LN and MN showed an overall higher retronasal aroma release compared to HN. These findings were later confirmed by the univariate approach showing lower time-independent parameters (AUC, I_{max}, I_{median}, I_{end}) in individuals with highest neophobic tendencies. By contrast, we did not find clear differences in terms of time-dependent parameters (Slope, TI_{max}), suggesting that all compounds monitored had similar kinetics within individuals. These findings were further corroborated by the shorter duration of oral processing showed by high FN participants, which is consistent with the existing literature. Labouré *et al.* [40] reported that individuals exhaling the highest amount of aroma from a model solid food (i.e., a model cheese) showed the highest chewing activity, while Ruijschop *et al.* [44] reported that a longer duration of oral processing tended to result in a higher retronasal aroma release during consumption of a fixed amount, as also found in our study.

It was therefore not surprising that individuals with higher neophobic tendencies underwent the task by breathing in a faster fashion. This is consistent with the previously reported increase in physiological arousal (in terms of pulse rate, Galvanic skin response and respiration rate) when neophobics are presented with food stimuli [29]. Breathing evaluation is a useful physiological marker of level of anxiety [57], with anxious individuals showing larger respiratory rates in presence of anticipatory anxiety-inducing stimuli [58]. According to a previously proposed theoretical model on anxiety [59], more anxious individuals would address stimuli with an increased arousal response to the

potential of negative consequences, which manifests itself in enhanced anterior insular cortex processing. The same theoretical could be used to explain the way neophobics deal with chemosensory stimulations. Recently, Spinelli *et al.* [20] presented data suggesting that liking and sensory perception of spicy (a "*warning*" sensation) foods is influenced by an increased arousal state associated with neophobic traits, which would modulate sensory and hedonic responses of individuals. Interestingly, this condition seem to be extended beyond the concept of familiarity [8,9]. Our findings supported this conclusion, as they were obtained using a common food item.

However, results have to be taken cautiously. The artificial condition in which individuals underwent the NS task (i.e., the unfamiliar laboratory environment or the oxygen cannula fitted in their nostrils) may have independently affected participants' arousal state. This in turn may have influenced their behavior during the task inducing larger discrepancies between FN levels. Moreover, our study did not provide a hedonic and a perception measurement of the candy. However, it can be reasonably assumed that our sample was in the acceptable range, as it was chosen to elicit a sensory perception (sweetness) which is well accepted as it is commonly related to energy dense foods. Another potential limitation of the study is that the three levels of FN were defined on arbitrary *cut-offs* based on the FNS frequency distribution. While commonplace in the literature (e.g., [8,9,21]), a potential drawback of this approach is that it could downplay the influence of extremely neophobic individuals, given the well-known reluctance of such individuals to participate in similar studies. In future studies, it would be interesting to see whether the magnitude of effects observed between FN levels, for example in terms of oral processing behavior or providing less familiar or "*warning*" sensations (i.e., bitterness, sourness, astringency, pungency) eliciting products, would be even larger with a truly neophobic population (e.g., FNS $\geq$ 55).

To conclude, the present study falls into the research stream acknowledging FN as a behavioral trait leading individuals to be more sensitive to sensory information in their environment. Among our

main outcomes, we observed individuals with highest neophobic tendencies showing lower global olfactory performances. Moreover, we confirmed that selective/restrictive feeding attitudes result in a lower willingness to explore the chemosensory environment. Interestingly, we also observed a higher olfactory sensitiveness in individuals with medium neophobic traits compared to neophilics, and speculated that this effect may be mediated by a higher anxiety proneness. Individuals with neophobic traits also showed a lower extent of retronasal aroma release compared to more neophilic individuals, likely due to a different way they faced the oro-sensory contact with the sample chosen for this experiment. Thus, we can hypothesize that neophobics have developed an adaptive behaviour that protect them from an extended exposure with chemosensory stimuli, which might result in a more pleasant sensory experience less influenced by their higher arousal or anxiety state. Since FN may lead to poorer dietary choices and nutritional deficiencies, based on the reported results we advocate the usage of anxiety-reducing treatment based on desensitization, as a suitable method to reduce these traits in adults with avoidance/restrictive tendencies [7]. As a final remark, this study supports the idea that neophobics' feeding behavior are not driven by a higher sensitivity to sensory stimuli but rather by higher levels of arousal toward foods that would lead them to limit their degree of exposure to the chemosensory world, which is crucial to guarantee the acceptance of a larger variety of food in the diet.

2.5 | Acknowledgements

We would like to thank all participants of the study. The authors greatly appreciated Federica Penza, Jessica Zambanini, and Riccardo Bottiroli for their valuable help in data collection. We would like to thank SRA Instruments S.p.A for providing the SIFT-MS and, in particular, Andrea Caretta for his helpful technical assistance. Last but not least, a special thanks to Prof. Thomas Hummel for his

invaluable support and suggestions during the phases of conceivement of the present study. This work was supported by the ADP 2018-20 funding of Autonomous Province of Trento, Italy.

2.6 | Author contributions

L.M., I.K., E.A., A.C., F.B., F.G. conceived the experiment. L.M., I.K., A.C., I.E. carried out the experiment and collected data. L.M., I.K., M.P., D.C. pre-treated data and built datasets prior statistical analyses. L.M., performed statistical analyses and wrote the original manuscript. L.M., D.G., F.G. interpreted the results. L.M., D.G. prepared the revised manuscript. F.G., provided supervision during the project. All authors provided useful and critical feedback, read and approved the final and revised manuscript.

2.7 | Declaration of interest

None.

2.8 | Supplemental Material

2.8.1 | Figures

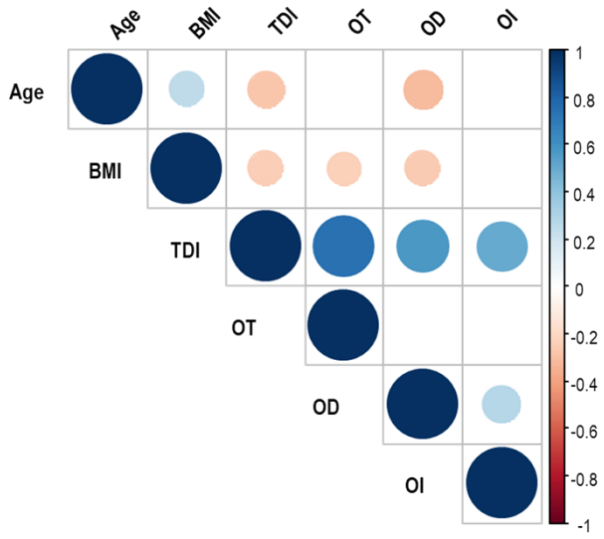

Figure S2.1: Correlations between olfactory performance scores, age and BMI. Only statistically significant differences ($p < 0.05$) according to Spearman's rank correlation coefficients are depicted. Abbreviation: TDI = Odor Threshold, Discrimination, Identification composite score; OT= Odor Threshold; OD= Odor Discrimination; OI= Odor Identification.

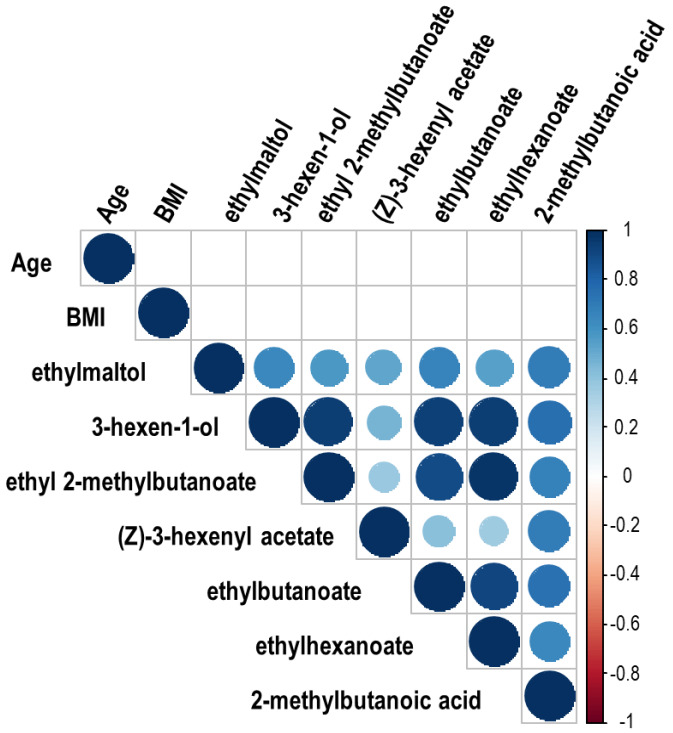

Figure S2.2: Correlations between areas under the curves from the 7 monitored compounds, age and BMI. Only statistically significant differences ($p < 0.05$) according to Spearman's rank correlation coefficients are depicted.

Figure S2.3: First and third principal components of the individual factor map from the MFA model based on matrices of SIFT-MS parameters. Large squares (■ ■ ■) are MFA centroids while the circles (● ● ●) represent participants' position in the bi-dimensional space colored according to their FN level (**LN**, **MN**, **HN**).

Figure S2.4: First and fourth principal components of the individual factor map from the MFA model based on matrices of SIFT-MS parameters. Large squares (■ ■ ■) are MFA centroids while the circles (● ● ●) represent participants' position in the bi-dimensional space colored according to their FN level (**LN**, **MN**, **HN**).

2.8.2 | Tables

Table S2.1: TDI average scores are presented separately for the age groups suggested by normative data [37] comprising our cohort (Participants). Normative data are listed as mean values within age groups-related percentiles ± SD. In bold the age group-related range of performance in which individuals felt in comparison with normative values.

Participants (n=83)		Normative Data [37]							
Age group	**TDI (Mean ± SD)**	**5th**	**10th**	**25th**	**50th**	**75th**	**90th**	**95th**	**SD**
21-30 (n = 22)	36.65 ± 4.04	29.50	30.75	33.06	**35.75**	**38.50**	41.50	43.09	4.20
31-40 (n = 17)	36.04 ± 6.53	28.74	30.50	33.00	**35.50**	**38.50**	40.50	42.01	4.03
41-50 (n = 20)	34.62 ± 3.23	25.50	28.15	**31.50**	**34.75**	37.00	39.50	41.00	4.73
51-60 (n = 20)	34.26 ± 3.7	25.33	27.25	30.34	**33.00**	**36.25**	38.50	40.18	4.69
61-70 (n = 4)	36.12 ± 3.83	22.50	24.88	28.50	31.63	**34.25**	**36.50**	38.25	4.78

Table S2.2: Differences on both olfactory performances and the extent of retronasal aroma release (area under the curve) for all the 7 monitored compounds as a function of gender (F= Females; M= Males) according to Mann Whitney-U test. Values are listed as median values within gender ± IQR. Abbreviation: TDI = Odor Threshold, Discrimination, Identification composite score; OT= Odor Threshold; OD= Odor Discrimination; OI=Odor Identification. Significant rates (p.value) are also tabulated.

Sum formula	Variable	F (n = 48)	M (n = 35)	U	p.value
/	TDI	35.87 ± 5.00	35.25 ± 5.62	809.5	0.78
/	OT	10.75 ± 3.31	10 ± 4.75	716	0.25
/	OD	12 ± 2.00	13 ± 2.50	695	0.17
/	OI	13 ± 2.00	13 ± 2.00	832.5	0.94
$C_7H_8O_3$	ethylmaltol	3.32 ± 0.33	3.29 ± 0.28	775	0.55
$C_6H_{12}O$	3-hexen-1-ol	8.83 ± 0.39	8.82 ± 0.6	802	0.72
C7H14O2	ethyl 2-methylbutanoate	7.04 ± 0.63	6.96 ± 0.89	779	0.57
$C_8H_{14}O_2$	(Z)-3-hexenyl acetate	7.51 ± 0.50	7.43 ± 0.49	701	0.19
$C_6H_{12}O_2$	ethylbutanoate	9.14 ± 0.37	9.09 ± 0.61	772	0.53
$C_8H_{16}O_2$	ethylhexanoate	9.09 ± 0.57	9.17 ± 0.87	797	0.69
$C_5H_{10}O_2$	2-methylbutanoic acid	6.91 ± 0.27	6.82 ± 0.35	689	0.16

Table S2.3: Original (left side of the table) [1] and Italian (right side of the table) [8] version of the Food Neophobia Scale (FNS). (R) indicate the items of FNS reflecting neophilic food attitudes that have to be reversed before analyses.

Food Neophobia Scale – Original version [1]	Italian Version of the Food Neophobia Scale [8]
1. I am constantly sampling new and different foods	1. Provo continuamente cibi nuovi e differenti dal
2. I don't trust new foods	2. Nella scelta del cibo non mi fido delle novità
3. If I don't know what is in a food, I won't try it	3. Se non conosco un alimento, non lo assaggio
4. I like foods from different countries **(R)**	4. Mi piace il cibo di diversi Paesi **(R)**
5. Ethnic food looks too weird to eat	5. Il cibo etnico mi sembra molto strano per poterlo
6. At dinner parties, I will try a new food **(R)**	6. Alle cene con amici mi piace assaggiare cibi
7. I am afraid to eat things I have never had before	7. Ho timore a mangiare cibi mai assaggiati
8. I am very particular about the foods I will eat	8. Sono schizzinoso riguardo al cibo che mangio
9. I will eat almost anything **(R)**	9. Generalmente mangio quasi tutto **(R)**
10. I like to try new ethnic restaurants **(R)**	10. Mi piace provare nuovi ristoranti etnici **(R)**

Table S2.4: Original (left side of the table) [33] and Italian (right side of the table) [34] version of the Trait Anxiety Inventory Questionnaire (STAI-T). (R) indicate the items of the STAI-T reflecting *anxiety-absent* attitudes that have to be reversed before analyses.

The Trait Anxiety Inventory Questionnaire – Original version [33]	Italian Version of the Trait Anxiety Inventory Questionnaire [34]
1. I feel pleasant **(R)**	1. Mi sento bene **(R)**
2. I feel nervous and restless	2. Mi sento teso/a e irrequieto/a
3. I feel satisfied with myself **(R)**	3. Sono soddisfatto/a di me stesso/a **(R)**
4. I wish I could be as happy as others seem to be	4. Vorrei poter essere felice come sembrano essere gli altri
5. I feel like a failure	5. Mi sento un/una fallito/a
6. I feel rested **(R)**	6. Mi sento riposato/a **(R)**
7. I am "calm, cool, and collected" **(R)**	7. Io sono calmo/a, tranquillo/a e padrone/a di me **(R)**
8. I feel that difficulties are piling up so that I cannot	8. Sento che le difficoltà si accumulano tanto da non poterle
9. I worry too much over something that really doesn't	9. Mi preoccupo troppo di cose che in realtà non hanno
10. I am happy **(R)**	10. Sono felice **(R)**
11. I have disturbing thoughts	11. Mi vengono pensieri negativi
12. I lack self-confidence	12. Manco di fiducia in me stesso
13. I feel secure **(R)**	13. Mi sento sicuro **(R)**
14. I make decisions easily **(R)**	14. Prendo decisioni facilmente **(R)**
15. I feel inadeguate	15. Mi sento inadeguato
16. I am content **(R)**	16. Sono contento **(R)**
17. Some unimportant thought runs through my mind and	17. Pensieri di scarsa importanza mi passano per la mente e mi
18. I take disappointments so keenly that I can't put them	18. Vivo le delusioni con tanta partecipazione da non poter
19. I am a steady person **(R)**	19. Sono una persona costante **(R)**
20. I get in a state of tension or turmoil as I think over my	20. Divento teso e turbato quando penso alle mie attuali abitudini

2.9 | References

1. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).
2. Rozin, P. The Selection of Foods by Rats, Humans, and Other Animals. *Adv. Study Behav.* **6**, 21–76 (1976).
3. Fischler, C. *L'homme omnivore*. (1990).
4. Rozin, P. & Vollmecke, T. A. Food likes and dislikes. *Annu. Rev. Nutr.* **6**, 433–456 (1986).
5. Dovey, T. M., Staples, P. A., Gibson, E. L. & Halford, J. C. G. Food neophobia and 'picky/fussy' eating in children: A review. *Appetite* vol. 50 181–193 (2008).
6. Birch, L. L. Development of food acceptance patterns in the first years of life. *Proc. Nutr. Soc.* **57**, 617–624 (1998).
7. Marcontell, D. K., Laster, A. E. & Johnson, J. Cognitive-behavioral treatment of food neophobia in adults. *J. Anxiety Disord.* **16**, 341–349 (2002).
8. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–124 (2018).
9. Jaeger, S. R., Rasmussen, M. A. & Prescott, J. Relationships between food neophobia and food intake and preferences: Findings from a sample of New Zealand adults. *Appetite* **116**, 410–422 (2017).
10. Dematte, M. L., Endrizzi, I. & Gasperi, F. Food neophobia and its relation with olfaction. *Frontiers in Psychology* vol. 5 (2014).
11. Knaapila, A. *et al.* Food neophobia in young adults: Genetic architecture and relation to personality, pleasantness and use frequency of foods, and body mass index-A twin study. *Behav. Genet.* **41**, 512–521 (2011).
12. Tuorila, H., Lähteenmäki, L., Pohjalainen, L. & Lotti, L. Food neophobia among the Finns and related responses to familiar and unfamiliar foods. *Food Qual. Prefer.* **12**, 29–37 (2001).

13. Raudenbush, B., Schroth, F., Reilley, S. & Frank, R. A. Food neophobia, odor evaluation and exploratory sniffing behavior. *Appetite* **31**, 171–183 (1998).

14. Raudenbush, B., Corley, N., Flower, N. R., Kozlowski, A. & Meyer, B. Cephalic phase salivary response differences characterize level of food neophobia. *Appetite* **41**, 211–212 (2003).

15. Proserpio, C., Laureati, M., Invitti, C. & Pagliarini, E. Reduced taste responsiveness and increased food neophobia characterize obese adults. *Food Qual. Prefer.* **63**, 73–79 (2018).

16. Knaapila, A. J. *et al.* Food neophobia associates with lower dietary quality and higher BMI in Finnish adults. *Public Health Nutr.* **18**, 2161–2171 (2015).

17. Knaapila, A. *et al.* Food neophobia shows heritable variation in humans. *Physiol. Behav.* **91**, 573–578 (2007).

18. Alley, T. R. Conceptualization and measurement of human food neophobia. in *Food Neophobia: Behavioral and Biological Influences* 169–192 (2018). doi:10.1016/B978-0-08-101931-3.00009-4.

19. Monteleone, E. *et al.* Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Qual. Prefer.* **59**, 123–140 (2017).

20. Spinelli, S. *et al.* Personality traits and gender influence liking and choice of food pungency. *Food Qual. Prefer.* **66**, 113–126 (2018).

21. Meiselman, H. L., King, S. C. & Gillette, M. The demographics of neophobia in a large commercial US sample. *Food Qual. Prefer.* **21**, 893–897 (2010).

22. Hummel, T. & Nordin, S. Olfactory disorders and their consequences for quality of life. *Acta Oto-Laryngologica* vol. 125 116–121 (2005).

23. Prescott, J. Flavour as a psychological construct: Implications for perceiving and measuring the sensory qualities of foods. *Food Qual. Prefer.* **10**, 349–356 (1999).

24. Small, D. M., Jones-Gotman, M., Zatorre, R. J., Petrides, M. & Evans, A. C. Flavor processing: More than the sum of its parts. *Neuroreport* **8**, 3913–3917 (1997).

25. Prescott, J., Burns, J. & Frank, R. A. Influence of odor hedonics, food-relatedness, and motivational state on human sniffing. *Chemosens. Percept.* **3**, 85–90 (2010).

26. Demattè, M. L. *et al.* Food neophobia and its relation with olfactory ability in common odour identification. *Appetite* **68**, 112–117 (2013).

27. Monnery-Patris, S. *et al.* Smell differential reactivity, but not taste differential reactivity, is related to food neophobia in toddlers. *Appetite* **95**, 303–309 (2015).

28. Farrow, C. V. & Coulthard, H. Relationships between sensory sensitivity, anxiety and selective eating in children. *Appetite* **58**, 842–846 (2012).

29. Raudenbush, B. & Capiola, A. Physiological responses of food neophobics and food neophilics to food and non-food stimuli. *Appetite* **58**, 1106–1108 (2012).

30. Pliner, P. & Melo, N. Food neophobia in humans: Effects of manipulated arousal and individual differences in sensation seeking. *Physiology and Behavior* vol. 61 331–335 (1997).

31. Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E. & Kobal, G. 'Sniffin' sticks'. Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem. Senses* **22**, 39–52 (1997).

32. Smith, D. & Španěl, P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. *Mass Spectrom. Rev.* **24**, 661–700 (2005).

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

34. Pedrabrissi, L., Santinello, M. Verifica della validità dello STAI forma Y di Spielberger. *Giunti Organ. Spec.* **15**, 1–23 (2016).

35. Masala, C. *et al.* An Italian population-based study of the prevalence of olfactory impairment. *Chem. Senses* **44**, e47–e47 (2019).

36. Beecher, K., St John, J. & Chehrehasa, F. Factors that modulate olfactory dysfunction. *Neural Regeneration Research* vol. 13 1151–1155 (2018).

37. Oleszkiewicz, A., Schriever, V. A., Croy, I., Hähner, A. & Hummel, T. Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. *Eur. Arch. Oto-Rhino-Laryngology* **276**, 719–728 (2019).

38. Biasioli, F., Gasperi, F., Yeretzian, C. & Märk, T. D. PTR-MS monitoring of VOCs and BVOCs in food science and technology. *TrAC - Trends in Analytical Chemistry* vol. 30 968–977 (2011).

39. Langford, V. S., Padayachee, D., McEwan, M. J. & Barringer, S. A. Comprehensive odorant analysis for on-line applications using selected ion flow tube mass spectrometry (SIFT-MS). *Flavour and Fragrance Journal* vol. 34 393–410 (2019).

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

41. Repoux, M., Sémon, E., Feron, G., Guichard, E. & Labouré, H. Inter-individual variability in aroma release during sweet mint consumption. *Flavour Fragr. J.* **27**, 40–46 (2012).

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

43. Ruijschop, R. M. A. J., Burgering, M. J. M., Jacobs, M. A. & Boelrijk, A. E. M. Retro-nasal aroma release depends on both subject and product differences: A link to food intake regulation? *Chem. Senses* **34**, 395–403 (2009).

44. Ruijschop, R. M. A. J. *et al.* Effects of bite size and duration of oral processing on retro-nasal aroma release - Features contributing to meal termination. *Br. J. Nutr.* **105**, 307–315 (2011).

45. ISO. Sensory analysis-General guidance for the design of test rooms. *ISO Standard 8589* 9–11 (2007).

46. Schöpf, V., Kollndorfer, K., Pollak, M., Mueller, C. A. & Freiherr, J. Intranasal insulin influences the olfactory performance of patients with smell loss, dependent on the body mass index: A pilot study. *Rhinology* **53**, 371–378 (2015).

47. Normand, V., Avison, S. & Parker, A. Modeling the kinetics of flavour release during drinking. *Chem. Senses* **29**, 235–245 (2004).

48. Charles, M. *et al.* Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis. *Food Res. Int.* **69**, 9–20 (2015).

49. R Core Team. R: A Language and Environment for Statistical Computing (Vienna, 2019). https://www.R-project.org/. Accessed 15 Oct 2020.

50. Hummel, T., Kobal, G., Gudziol, H. & Mackay-Sim, A. Normative data for the 'Sniffin'' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: An upgrade based on a group of more than 3,000 subjects'. *Eur. Arch. Oto-Rhino-Laryngology* **264**, 237–243 (2007).

51. Besser, G. *et al.* Body-mass-index associated differences in ortho-and retronasal olfactory function and the individual significance of olfaction in health and disease. *J. Clin. Med.* **9**, (2020).

52. Kassambara, A. *Practical Guide To Principal Component Methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra. Multivariate Analysis* vol. 2 (2017).

53. Roessner, V., Bleich, S., Banaschewski, T. & Rothenberger, A. Olfactory deficits in anorexia nervosa. *Eur. Arch. Psychiatry Clin. Neurosci.* **255**, 6–9 (2005).

54. Gottfried, J. A. & Dolan, R. J. The nose smells what the eye sees: Crossmodal visual facilitation of human olfactory perception. *Neuron* **39**, 375–386 (2003).

55. Cain, W. S. To know with the nose: Keys to odor identification. *Science (80-.).* **203**, 467–470 (1979).

56. Paulus, M. P. & Stein, M. B. An Insular View of Anxiety. *Biological Psychiatry* vol. 60 383–387 (2006).

57. Paulus, M. P. The breathing conundrum - Interoceptive sensitivity and anxiety. *Depress. Anxiety* **30**, 315–320 (2013).

58. Masaoka, Y. & Homma, I. The effect of anticipatory anxiety on breathing and metabolism in humans. *Respir. Physiol.* **128**, 171–177 (2001).

59. Paulus, M. P. & Stein, M. B. Interoception in anxiety and depression. *Brain structure & function* vol. 214 451–463 (2010).

Chapter 3

Food Neophobia and scarce olfactory performances are linked to oral microbiota.

Valentino, V., De Filippis, F., **Menghi, L.**,
Gasperi, F. & Ercolini, D.
Food Research International, 155, 111092 (2022)
DOI: 10.1016/j.foodres.2022.111092

CHAPTER 3:

FOOD NEOPHOBIA AND SCARCE OLFACTORY PERFORMANCES ARE LINKED TO ORAL MICROBIOTA

Abstract

People suffering from Food Neophobia (FN) tend to follow an unbalanced dietary pattern and show worse olfactory performances. However, scarce data are available on the possible relationships between FN, olfactory performances and the oral microbiota. The purpose of this work was to understand whether FN and its consequences on orthonasal and retronasal olfaction are related to specific signatures in the oral microbiota. We carried out 16S rRNA gene sequencing of salivary specimens from 83 subjects, whose olfactory performances and Food Neophobia were previously estimated. Our results show that the oral microbiota of people showing high neophobic traits and scarce olfactory performances is enriched in several taxa, such as the periodontal pathogen Porphyromonas gingivalis. We hypothesize that these traits are likely attributable to unbalanced dietary patterns, which would need confirmation from dietary records of recruited neophobic subjects.

Keywords: TDI; Olfaction; Oral microbiota; Food choice; Mediterranean Diet

3.1 | Introduction

Food Neophobia (FN) is defined as the reluctance to eat and/or the avoidance of novel or unfamiliar foods [1]. FN is widely recognized as an evolutionary trait since it has protected humans from eating potentially harmful foods for centuries. In addition, it has been proposed to be highly heritable [2–4], with the family environment exerting only a marginal effect on this attitude. FN can narrow an individual's food choice [5,6], thus limiting dietary variety. Several evidence suggests that high neophobic subjects usually have unhealthier dietary habits compared to neophilics (i.e., those prone to try and accept novel foods), in both childhood (e.g., [7,8]) and adulthood [9,10]. This observation is also supported by a recent study by Jezewska-Zychowicz and colleagues, who explored dietary patterns of neophobic adults and showed that they rarely consumed functional foods (e.g., berries, legumes, nuts and superfoods) [6].

Another factor strongly influencing human feeding behavior is orthonasal and retronasal olfaction (hereafter "olfaction"). The role of olfaction on shaping food perception and preferences, appetite and dietary habits has been largely explored [11–13]. As an example, an altered sense of smell can influence the intake of salt, sugars or fats, leading to a potentially unbalanced dietary pattern that might result in weight gain or loss, depending on the subjects' adaptive behavior [14].

Interestingly, olfactory abilities and FN are strongly linked. Indeed, high-neophobics tend to evaluate an odor as less intense and less pleasant than neophilics [15], and they show an overall worse olfactory performance than neophilics as measured by the TDI (odor Threshold, Discrimination and Identification) score obtained with the Sniffin' Sticks battery [16,17]. Moreover, high-neophobic individuals also exhibited increased anxiety-related physiological responses and a lower extent of retronasal aroma release during the consumption of a strawberry jelly candy. These phenomena might be explained by the tendency of neophobics to avoid unpleasant food-related experiences [17].

The impact of dietary choices on shaping the oral microbiome has been highlighted [18,19], as well as its potential link to food aroma perception [20]. However, information on how Food Neophobia is associated to oral microbiota composition is still lacking. Understanding the relationships existing between food-related personality traits, food choices and oral microbiota is important, since alteration in the microbial composition of the oral cavity potentially caused by unhealthy dietary habits might be related to several oral and systematic diseases and may be indicator of potential food preferences [21].

In this study, we investigated the microbial composition of salivary samples from subjects whose olfactory performance and Food Neophobia were previously assessed [17] to understand whether these factors, as well as their influence on dietary habits, might result in an alteration of the oral microbiota.

3.2 | Methods

3.2.1 | Study subjects

The present study is part of a larger national scale project aiming to evaluate the prevalence of olfactory impairments in Italy and its associations with biological and cognitive-related covariates [22].

Out of the 83 participants involved in our previous investigation [17], 2 participants were excluded due to massive DNA sequencing artifacts. Hence, data from 81 participants (56.7 % females, mean age = 41.5 ± 11.7 yo, mean BMI = 23.92 ± 3.73 Kg/m2; self-reported data) were considered in the present study. More information about participants is provided in a previous report [17].

The present study was performed in compliance with the Declaration of Helsinki, and all participants gave their informed and written consent according to the European Data Protection Regulation (UE 679/2016).

3.2.2 | General procedure

Eligible participants were firstly asked to fill out an online questionnaire collecting gender, age, self-reported weight and height, and the Italian version of the FN Scale (IT-FNS; [23]). FN was calculated by summing up the scores given to the ten items of the IT-FNS, with items (n = 5) reflecting neophilic tendencies being reversed prior the computation [23]. To group participants as a function of their FN level, we classified as high-, medium- or low-neophobics those showing pronounced (n = 15; IT-FNS score ≥ 36), mild (n = 32; 18 < IT-FNS score > 36), and low (n = 34; IT-FNS score ≤ 18) neophobic tendencies, respectively. These cut-offs were chosen according to a recent study based on a large Italian sample from Laureati and colleagues [23]. Volunteers were then invited to refrain from eating, drinking nothing but water, smoking, and brushing teeth for at least 3 h prior to testing.

During a single 90 min lasting session, volunteers initially provided an unstimulated saliva sample before being comprehensively assessed for olfactory functioning using the standard Sniffin' Sticks battery [16]. Olfactory subtests (odor Threshold, Discrimination, Identification) scores were individually calculated as suggested by the Sniffin' Sticks' developers [16], and then added to yield a global score of olfactory functioning (TDI).

For the odor Threshold test, 16 triplets of pens were prepared, with each triplet consisting of two odorless pen and one impregnated with a N-butanol solution. The odorant pen with the highest concentration was impregnated with a 4% solution of N-butanol, whereas the others were impregnated with progressive 1:2 dilutions. For each triplet, participants were asked to choose which pen was the odorant one. Two consecutive correct answers for the same triplet represented a turning point, which caused the reversal of the staircase, whereas one wrong answer caused the repetition of the task using the step-higher triplet of pens. For each subject, the odor Threshold score was calculated as the geometric mean of the last four turning points out of a total of seven.

For the odor Discrimination test, 16 triplets of pen were prepared, each with two pens with an identical odor, and one different, defined as “target”. For each triplet, the participants were asked to identify which pen had the target odor. The odor Discrimination score was computed as the sum of the correct answers.

For the odor Identification test, 16 pens with common odorants were presented to the participants. After smelling each pen, the participants were asked to identify the correct odor from a list of four options. The odor Identification score was computed as the sum of the correct answers.

At the end of the olfactory task, participants observed a 10 min break before being tested for retronasal aroma release by *in vivo* nose-space analysis while consuming, at least in triplicate, a commercial strawberry flavored candy (Fruittella Caramelle Gelee; Perfetti Van Melle; Italy). For this latter purpose, we used a Selected-Ion Flow-Tube Mass Spectrometry (SYFT VOICE 200 ultra, Syft Ltd, New Zealand) to monitor in real time the release of 7 key compounds (i.e., ethyl maltol, 3-hexen-1-ol, ethyl 2-methylbutanoate, (Z)-3-hexenyl acetate, ethyl butanoate, ethyl hexanoate, 2-methylbutanoic acid) exhaled by each participant. Raw spectra from the nose space analysis were pre-treated according to [24,25] and used to extract the AUC (Area Under the Curve) of the 7 monitored VOCs. More details are provided in our previous paper [17].

3.2.3 | Saliva collection

Volunteers were invited to accurately rinse their mouth with water prior to providing an unstimulated saliva (5 mL) sample by letting it fall into a 50 mL pre-labelled sterile plastic tube (Thermo Fisher Scientific, Waltham, USA) for 5 min. Once collected, saliva samples were centrifuged at 14,000 g for 15 min at 4° C. The supernatant was immediately stored at −80° C until subsequent down-stream applications.

3.2.4 | DNA extraction, PCR amplification and amplicon sequencing

Four mL of saliva were centrifuged at 13,000 g for 2 min. Supernatant was discarded, then DNA was extracted from the cell pellets using the QIAamp® BiOstic® Bacteremia DNA Kit (Qiagen, Hilden, Germany).

The V3-V4 hypervariable region of the 16S rRNA gene was amplified by PCR (about 460 bp), using universal primers S-D-Bact-0341-b-S-17: 5′-CCTACGGGNGGCWGCAG-3′ and S-D-Bact-0785-a-A-21: 5′-GACTACHVGGGTATCTAATCC-3′ [26]. Amplicons were purified and libraries prepared as previously described [19]. Briefly, PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, USA), then sequences were barcoded using Nextera XT Indexes (Illumina, San Diego, USA) and pooled in an equimolar pool. Sequencing was carried out on Illumina MiSeq platform, leading to 2x250 bp reads.

3.2.5 | Bioinformatic analysis

Forward and reverse raw reads were joined by FLASh [27], then sequences were trimmed at the first instance of a base with a PHRED score < 20, and those that were shorter than 300 bp were discarded using PRINSEQ [28]. The remaining high-quality reads were imported into QIIME 1.9.1 [29] for following analysis. Briefly, OTUs were de-novo picked at 97% of similarity and representative sequences were mapped against the Human Oral Microbiome Database (version 15.1) [30] using the RDP classifier [31]. OTUs represented by a single sequence were discarded, and samples were rarefied at the same number of reads. Alpha-diversity indices were calculated through QIIME.

3.2.6 | Statistical analysis

Statistical analysis was carried out in a R environment (https://www.r-project.org). The function *wilcox.test* (Wilcoxon's rank-sum test) from the *base* package was used to infer statistical

differences between the group's medians, unless otherwise stated, choosing a p-value < 0.05 to assess significance. The function *vegdist* from the *vegan* R package was used to compute pairwise Bray-Curtis distance. The resulting matrix was sent to the command *cmdscale* from the *base* package to produce a PCoA. Only the first two principal coordinates from the PCoA were plotted. The hierarchical complete-linkage clustering based on Canberra distance was computed and plotted with the function *pheatmap* (*pheatmap* R package). Linear regression between the TDI score and the relative abundance of the taxon were computed through the function *lm* from the *base* package. Spearman's correlations were calculated using the function *corr.test* from the package *psych* and were plotted through the function *corrplot* (*corrplot* R package). Bubble plots and boxplots were drawn using the functions *geom_boxplot* and *geom_point* (*ggplot2* R package), whereas the upset plot was produced through the function *upset* (*UpSetR* package).

3.3 | Results

3.3.1 | Taxonomic composition of salivary specimens

We identified a core microbiota (i.e., taxa occurring in at least 99% of subjects) at genus level, including 16 taxa (*Actinomyces, Rothia, Corynebacterium, Porphyromonas, Prevotella, Gemella, Granulicatella,* Streptococcus*, Lachnoanaerobaculum, Veillonella, Fusobacterium, Leptotrichia, Neisseria, Campylobacter, Haemophilus,* and *Saccharibacteria (TM7);* Table 3.1). Among these, *Streptococcus* was the most abundant, with a mean relative abundance of 19.7% (±7.4 %), followed by *Neisseria* (11.4 ± 7.1 %), *Prevotella* (11.0 ± 6.2 %) and *Veillonella* (9.3 ± 4.5 %). These taxa belonged to the phyla of *Firmicutes*, *Proteobacteria*, *Bacteroidetes*. Other abundant genera across all samples were *Porphyromonas* (mean relative abundance of 3.4 ± 2.5 %), *Rothia* (4.9 ± 5.0 %) and *Actinomyces* (4.5 ± 3.2 %).

Table 3.1: The core microbiota. The table reports: i) the genus, ii) the *Phylum*, iii) the mean relative abundance of the genus, iv) the relative abundance standard deviation.

Taxon	Phylum	Mean relative abundance	Std. Deviation
Streptococcus	*Firmicutes*	19.72	7.42
Neisseria	*Proteobacteria*	11.37	7.08
Prevotella	*Bacteroidetes*	11.00	6.16
Veillonella	*Firmicutes*	9.32	4.48
Haemophilus	*Proteobacteria*	6.36	3.53
Rothia	*Actinobacteria*	4.89	5.00
Actinomyces	*Actinobacteria*	4.53	3.22
Porphyromonas	*Bacteroidetes*	3.42	2.47
Fusobacterium	*Fusobacteria*	2.94	1.60
Granulicatella	*Firmicutes*	2.54	1.14
Leptotrichia	*Fusobacteria*	2.35	1.36
Gemella	*Firmicutes*	2.23	1.55
Saccharibacteria (TM7)	Candidate phylum (*TM7*)	1.17	1.02
Campylobacter	*Proteobacteria*	0.67	0.58
Corynebacterium	*Actinobacteria*	0.60	0.66
Lachnoanaerobaculum	*Firmicutes*	0.43	0.33

In order to investigate the ecological relationship between microbial taxa, we looked at specific co-occurrence and co-exclusion patterns (Figure 3.1). Co-occurrence analysis highlighted the presence of 2 distinct and co-excluding groups of taxa (Figure 3.1). More specifically, the first group included the genera *Actinomyces*, *Atopobium*, *Veillonella*, *Prevotella*, *Megasphaera* and *Selenomonas*, whereas the other included *Porphyromonas*, *Aggregatibacter*, *Neisseria*, *Capnocytophaga*, *Fusobacterium*, *Gemella* and *Haemophilus*.

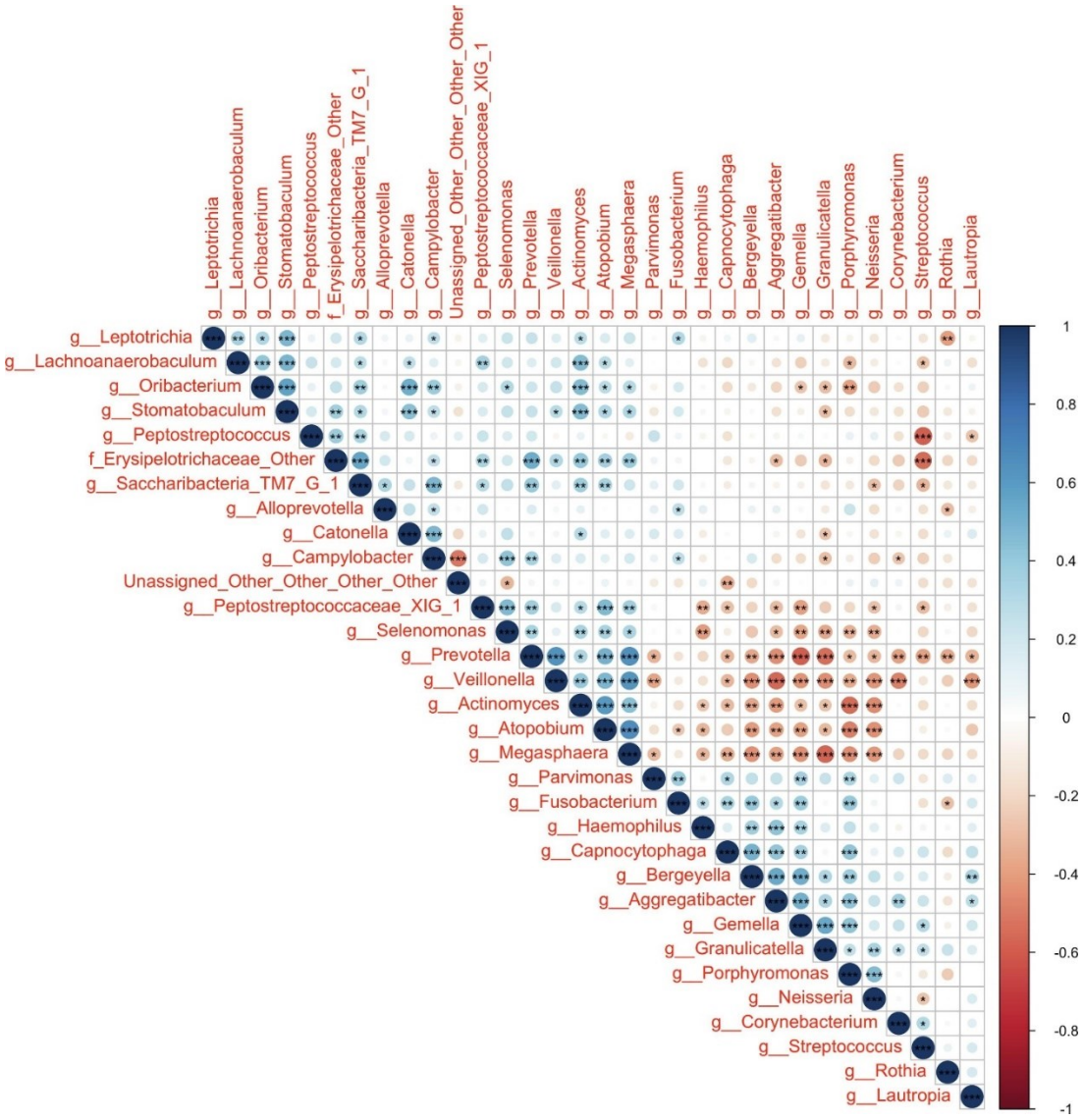

Figure 3.1: Correlation matrix at genera level based on Spearman's rank correlation coefficient. Only taxa present in at least 90% of subjects are shown. *, p-value ≤ 0.05; **, p.value ≤ 0.01, ***, p.value ≤ 0.001.

3.3.2 | Hyposmic subjects show specific traits in their salivary microbiota and higher abundance of potentially pathogenic species

As proposed by [32], a TDI value of 30.75 can differentiate subjects with overall scarce olfactory performances (i.e., "hyposmic") from people with normal olfactory performances (i.e., "normosmic"). According to this cutoff, 7 out of 81 subjects involved in this study were labeled as "hyposmic". Principal Coordinate Analyses (PCoAs) based on Bray-Curtis distance performed at any taxonomic

level did not properly separate hyposmics from normosmics, suggesting that the microbial community structure was not influenced by the TDI score. In addition, none of the alpha-diversity scores showed a significant difference between the two groups.

However, we observed significantly higher abundance of *Granulicatella*, *Parvimonas* and *Peptidiphaga* in hyposmics ($p < 0.05$), while *Pseudopropionibacterium* showed a higher relative abundance in normosmics ($p < 0.05$). Since it has been previously observed that TDI is negatively correlated with BMI in the same cohort [17], we checked if a correlation existed between differentially abundant taxa and BMI. However, none of the previously reported genera were significantly correlated with BMI, thus excluding its influence on the observations.

We also stratified the subjects into quartiles based on TDI score, in order to compare the salivary microbiota between subjects with the highest and lowest values of TDI. In particular, group 1 included 21 subjects, with a TDI score ranging between 19.25 and 33 (1st quartile), while group 2 included 20 subjects, with a TDI score ranging between 38.5 and 44 (4th quartile; Figure 3.2a).

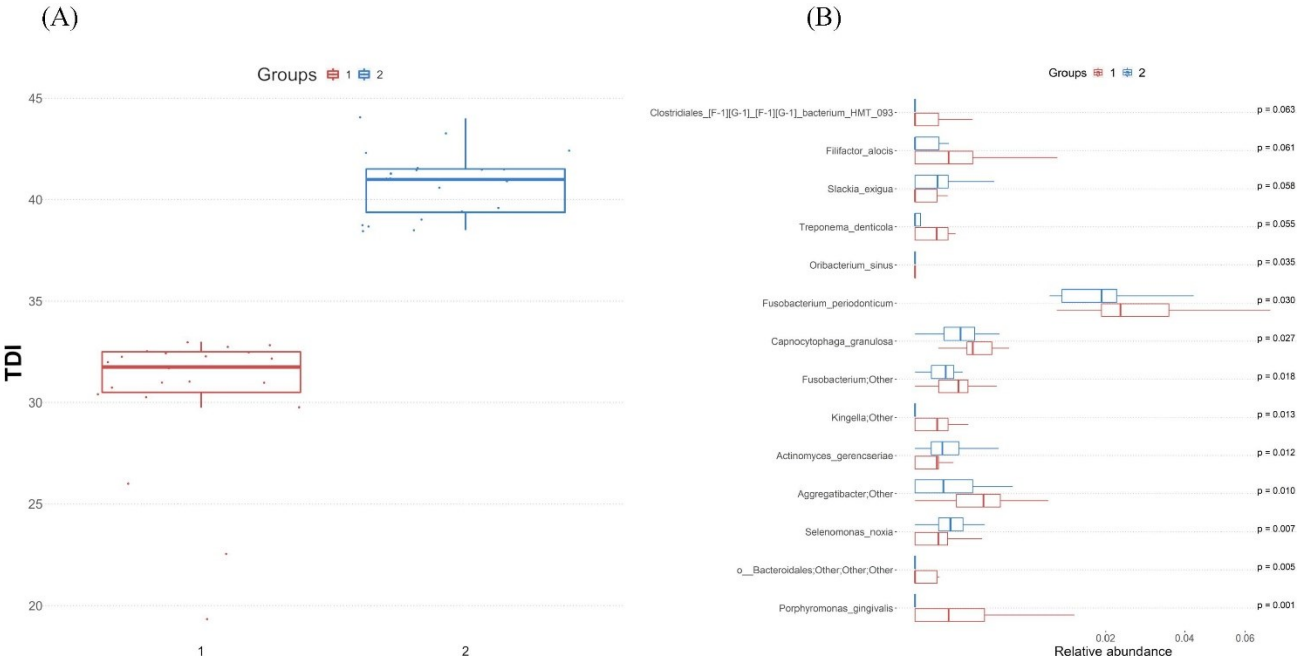

Figure 3.2: A) TDI quartiles. Quartile 1 corresponds to group 1, whereas quartile 4 corresponds to group 2. B) Boxplots showing the relative abundance of significantly different species between group 1 and group 2.

At species level, *Porphyromonas gingivalis*, *Capnocytophaga granulosa*, *Fusobacterium periodonticum* and *Aggregatibacter* sp. were significantly more abundant in subjects belonging to group 1 ($p < 0.05$), together with an unassigned species belonging to the genus *Kingella* ($p = 0.01$). On the other hand, group 2 showed a higher relative abundance of *Selenomonas noxia* ($p = 0.02$) and *Actinomyces gerencseriae* ($p = 0.02$) (Figure 3.2b). Hierarchical complete-linkage clustering based on the relative abundance of these species (Figure 3.3) effectively separated group 1 (i.e., low TDI group) and 2 (i.e., high TDI group). Again, none of these taxa was statistically correlated with BMI.

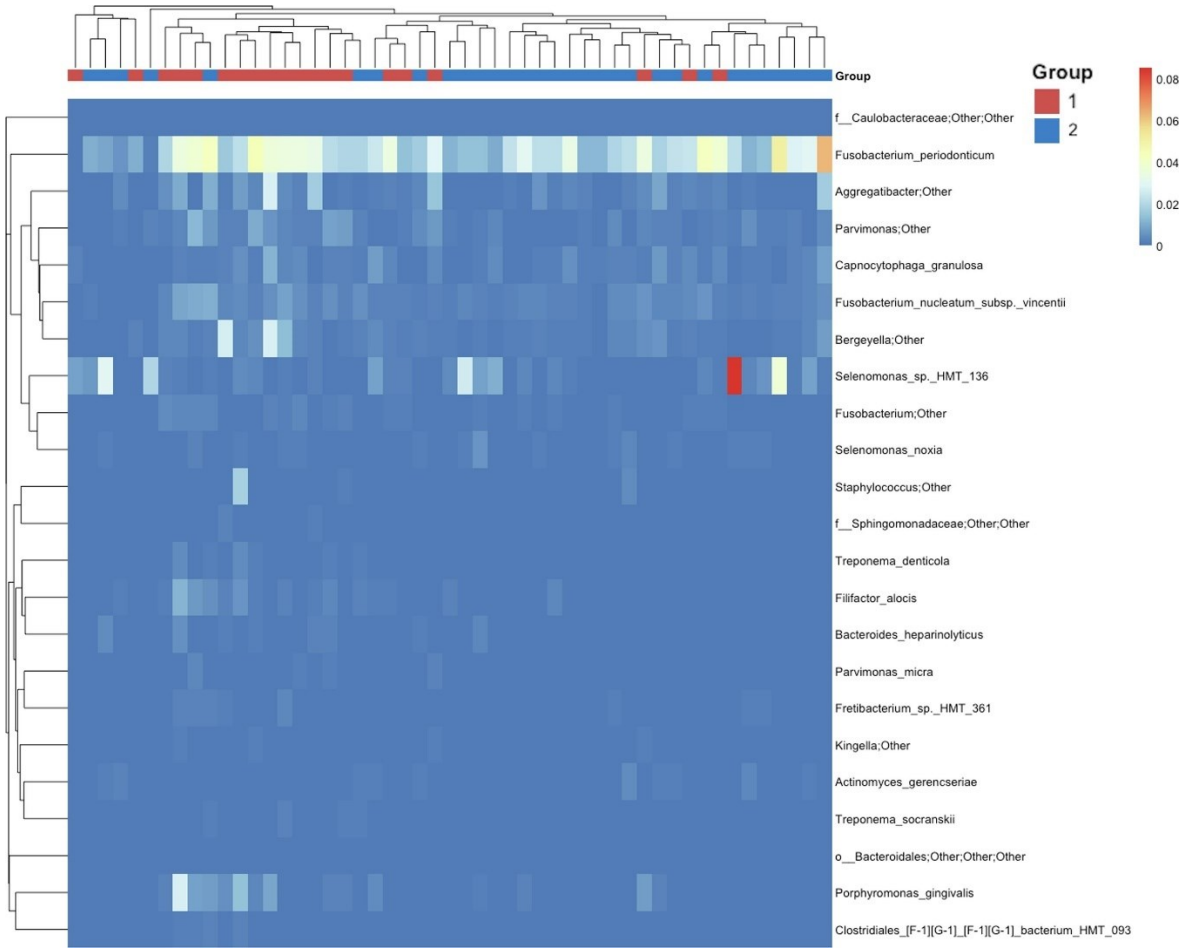

Figure 3.3: Hierarchical complete-linkage clustering of subjects belonging to groups 1 and 2 based on discriminant species. Canberra metric was used to compute pairwise distances. The column bar colors denote the membership of subjects to the groups.

In addition, we observed that *Porphyromonas gingivalis* and *Filifactor alocis* showed a strong negative correlation with the TDI score (adjusted $R^2 = 0.039$, $p = 0.037$; adjusted $R^2 = 0.0459$, $p = 0.031$; Figure 3.4).

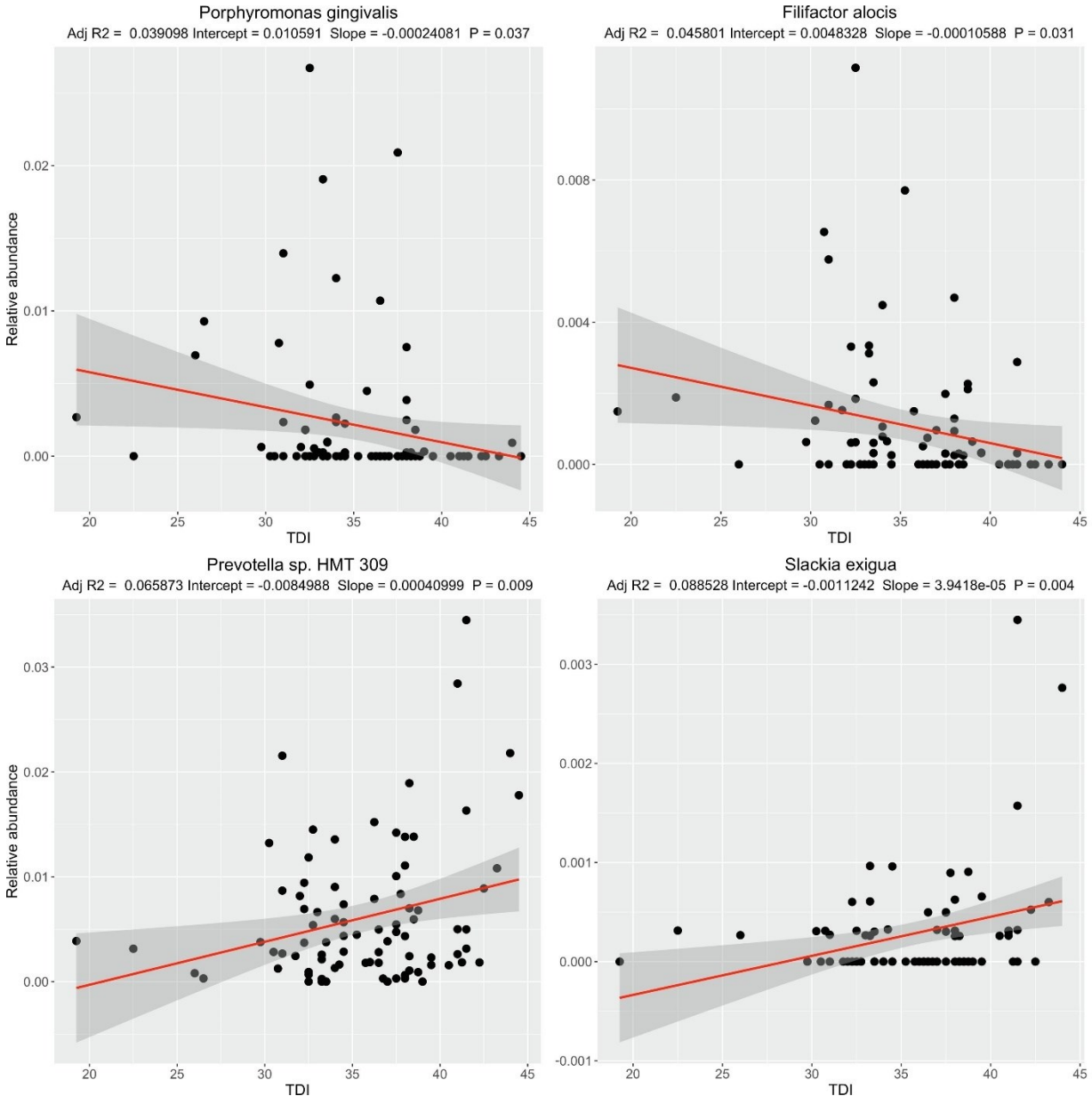

Figure 3.4: Significant linear regressions between oral taxa and the cumulative TDI score.

Interestingly, *Porphyromonas catoniae* and *Fusobacterium nucleatum* subsp. *vincentii* were negatively correlated with the TDI score (adjusted $R^2 = 0.042$, $p = 0.03$; adjusted $R^2 = 0.055$, $p =$

0.02), while no correlation was observed with *Fusobacterium nucleatum* subsp. *animalis* (Figure 3.4). On the contrary, *Prevotella* sp. HMT 309 (adjusted $R^2 = 0.066$, $p = 0.009$) and *Slackia exigua* (adjusted $R^2 = 0.089$, $p = 0.004$) were positively correlated with the TDI score (Figure 3.4).

3.3.3 | Effect of food neophobia on oral microbiota

Menghi and colleagues previously observed that higher levels of neophobia were associated with worse global olfactory performances on the same cohort [17]. Here, we tested whether the salivary microbial community structure or the abundance of specific taxa changed significantly between high-, medium- or low-neophobics and found that the FN groups were not characterized by overall changes in the microbial community, as shown by a PCoA based on the Bray-Curtis distance (Supplemental Figure S3.1). Moreover, the FN score was slightly higher in hyposmics, even though the result was not significant, probably due to the small sample size.

Interestingly, some differences were observed at single taxon level. For instance, *Alloprevotella* sp. HMT 473 and a species belonging to cluster XI of the family *Peptostreptococcaceae* were significantly more abundant in low-neophobic subjects (Kruskall-Wallis test, $p < 0.05$), whereas *Klebsiella pneumoniae* and *Scardovia wiggsiae* prevailed in high-neophobics.

3.3.4 | Correlation analysis between microbiota composition, FN, retronasal aroma release and olfactory scores

A correlation analysis between microbial species abundances, FN, VOCs (Volatile Organic Compounds) [17] release in the retronasal space and both single (Threshold, Discrimination, Identification) and cumulative (TDI) olfactory scores was carried out. Only taxa showing at least 2 significant correlations according to Spearman's correlation index were reported. We observed that the genera *Bifidobacterium*, *Bacteroides*, *Mitsuokella* and *Klebsiella* were negatively correlated to

the release of VOCs in the retronasal space. Of these, *Bifidobacterium* showed the highest number of negative correlations with VOCs release (5 out of 7), while *Klebsiella* was the only taxon also showing a positive association with the FN score (Supplemental Figure S3.2).

Interestingly, *Porphyromonas* and *Fusobacterium* had a negative association with Threshold and Discrimination scores, as well as with the cumulative TDI score, whereas *Slackia* was positively correlated with Identification and TDI. These results were consistent with those observed from the regression analysis.

In addition, subjects were stratified into quartiles based on each of the monitored VOCs, then the abundance of taxa was compared between the extreme quartiles obtained from each VOC. As a result, we observed that *Streptococcus parasanguinis* clade 411 was the only species showing a significant difference between extreme quartiles for 4 VOCs (i.e., ethyl 2-methylbutanoate, ethyl hexanoate, 3-hexen-1-ol and ethyl butanoate), always resulting more abundant in groups with a lower volatile release (Supplemental Figure S3.3).

3.4 | Discussion

The salivary microbial composition from the participants was largely consistent with those reported in previously published studies, which identified a similar "core" microbiota [19,33,34]. Indeed, as reported in literature, we observed a great inter-individual variability.

Focusing on differences between people with different olfactory performances, we observed that subjects with a lower TDI score (i.e., those belonging to group 1) showed a significantly higher abundance of *Porphyromonas gingivalis*, a microorganism belonging to the "red complex" [35], which is associated with periodontal disease progression [36]. In addition, *Fusobacterium nucleatum* subsp. *vincentii* and *Filifactor alocis* showed a negative correlation with the TDI score. The former belongs to a species which is able to co-aggregate with early and late subgingival colonizer, thus enhancing

the dental biofilm formation [36], whereas the latter has been recently suggested as a putative oral pathogen [37].

On the other hand, our results showed that higher values of TDI are associated with taxa normally considered as markers of eubiosis, such as *Actinomyces* spp. For instance, the genus *Actinomyces* was reported as discriminant for caries-free subjects [38], while *A. gerencsiae* was more abundant in healthy control than in patients with aggressive periodontal disease in a previous report [39]. However, studies reporting the influence of *Selenomonas noxia* on the oral health are contrasting, since several authors reported the species as an oral health marker [40,41], whereas others evidenced its potential role in caries formation [38] and periodontitis initiation [42]. These results indicate that oral health can be linked to olfactory performances.

In accordance with a previous study on nasal microbiota in people with olfactory dysfunction [43], we highlighted that the genus *Porphyromonas* was more abundant also in the oral microbiota of people with reduced TDI. Moreover, it is worth noting that the taxa that were enriched in the low-TDI group (e.g., *Porphyromonas gingivalis* and *Treponema denticola*) have been also reported as enriched in subjects consuming an unhealthy diet. For instance, Tennert and colleagues observed the effects of a diet rich in fruit, vegetables and fibre, and low in highly processed sugars on the dental plaque and the salivary microbiota highlighting a significant decrease of *Granulicatella* spp. and *Fusobacterium* spp. [44]. In the same vein, Laiola *et al.* reported a significant reduction of *P. gingivalis* and *T. denticola* in the salivary microbiota of obese and overweight subjects after a 8-week Mediterranean diet-based intervention. These species were significatively and negatively correlated with fiber intake, thus opening new scenarios about the metabolism of periodontopathogenic species [19].

Mediterranean diet (MD) is widely recognized as a healthy dietary pattern [45,46], which provides several antioxidants and anti-inflammatory compounds [47,48]. Interestingly, a recent study investigated the relationship between FN and adherence to the MD, showing that neophobic subjects

had a significantly lower adherence to the MD, and highlighting how they are likely to assume a lower intake of potentially beneficial foods [49].

In addition, although the relative abundance of some taxa such as *T. denticola* and *P. gingivalis* was generally low in our cohort (i.e., mean relative abundance < 1%), evidence in the literature suggests that even low magnitude shifts in the abundance of oral pathogens might exert a significant effect on the community structure [50]. In this sense, the pathogenesis of *P. gingivalis* is emblematic: all the strains of this species produce a protease called gingipain, which not only exerts a cytopathic activity, but also acts as a ligand between *P. gingivalis* and other pathogenic microorganisms [51], thus fostering their growth. Since *P. gingivalis* can exert a pathogenic activity regardless of its abundance in the community, it has been termed a "keystone pathogen" [50,52]. The results from the co-occurrence analysis corroborate this idea: the genus *Porphyromonas* is significantly associated with other potential pathogenic taxa, such as *Capnocytophaga* and *Fusobacterium*, whereas it is inversely correlated with eubiosis-related taxa (e.g., *Prevotella*, *Veillonella* and *Actinomyces*; Figure 3.1).

Although the overall oral microbiota composition was not different between high-, medium- and low-neophobics, some differences were observed at single taxon level, highlighting that FN may be linked with the presence of potential pathogenic and caries-associated species. High-neophobic subjects showed higher abundance of *Scardovia wiggsiae*. This species, which belongs to the family of *Bifidobacteriaceae*, has recently been reported to be aciduric and acid producer [53], thus strongly associated with caries formation in both infants [54] and adolescents [38,55].

In addition, *Klebsiella pneumoniae* was enriched in high-neophobics. This species is recognized as a biofilm producer in the oral cavity [56], as well as an antibiotic resistant opportunistic pathogen, often involved in several infections including, but not limited to, pneumonia [57]. Notably, several researchers showed that strains of *Klebsiella pneumoniae* are able to produce biofilm in acid

environments [58], similarly to *S. wiggsiae*. Although oral biofilms can host a wide range of homeostatic microorganisms, the development of dental plaque in an acid environment is associated with several disease-related species [59].

Among the factors potentially influencing food choice, retronasal smell is one of the most important [60]. Menghi *et al.* [17] observed a significantly lower extent of VOCs released by high-neophobic subjects than neophilics, which was associated with a hostile arousal response and with an anxious behavior towards food. However, the volume of VOCs reaching the receptors is not only dependent on oral processing, but also on physiochemical properties of the food matrix and on its interactions with salivary compounds [61,62]. For instance, a high concentration of proteins in saliva might lead to volatile-protein interactions that in turn limit the perception of retronasal aroma, as we previously observed [20] in obese subjects. In addition, planktonic cells suspended in saliva may influence the release of aroma compounds from food matrices, as it has been demonstrated *in vitro* [63] and *ex vivo* [64]. In fact, oral bacteria are the major source of salivary glycosidase enzymes [65], which are in turn necessary to release several VOCs from glycosylated precursors. Since the ratio between glycosylated and free volatiles is high in strawberries for industrial processing (e.g., strawberries used as ingredients for candies) [66], we explored our data in order to assess whether the release of VOCs is linked not only with the consumer's behaviour (as previously demonstrated for this cohort by Menghi and colleagues), but also with the abundance of specific microbial taxa in the oral microbiota.

From the correlation matrix, *Bifidobacterium* spp. reported the highest number of significant ($p < 0.05$) correlations with the total amount of VOCs released during the experiments (expressed as AUC, Area Under the Curve), followed by the genus *Peptostreptococcus* (Supplemental Figure S3.2). Moreover, *Streptococcus parasanguinis* clade 411 was more abundant in the oral cavity of people with a minor release of 4 out of 7 volatiles during mastication. This species has been recently advised

as associated with a high grade of dental pathology [67] and with smokers' oral microbiome [68]. However, mechanisms underlying these observations are unclear, and a further investigation is needed in order to clarify the metabolic contribution of each taxon to the aroma release.

To the best of our knowledge, this is the first study that attempts to investigate the influence of FN and olfaction on the oral microbiota. Taken collectively, our results suggest the existence of a link between dietary habits, olfactory performances and the composition of the salivary microbiota. Indeed, we hypothesize that FN and scarce olfactory performances might influence the food choice, leading to an alteration of the salivary microbiota composition through the selection of several oral pathogenic taxa.

3.5 | Conclusions

As previously proposed by Menghi and colleagues, FN has a considerable impact on both olfactory performances and VOCs release during mastication, thus probably shaping food choice and indirectly influencing the salivary microbiota composition. We observed that high levels of neophobia and the inclination to perceive odors at a lower extent are associated with a higher abundance of several dysbiosis-related taxa in the salivary microbiota. Since it has been previously observed that high-neophobics show a lower adherence to MD and thus they are likely to follow an imbalanced dietary pattern, we suggest that the influence of FN and arousal toward food on feeding behavior might jeopardize the oral microbiome.

Unfortunately, the lack of detailed dietary records is limiting the confirmation of such hypothesis. In addition, due to the limited sample size and the unbalanced distribution of hyposmics and normosmics, some of the observations might not be representative of the entire population. However, to the best of our knowledge, the relationship that occurs between FN, olfaction and oral microbiota have not been explored previously. Therefore, further investigation involving a larger

cohort and integrated with more detailed information about subjects' FN, olfactory performances and dietary habits might use our result as a starting point, to better explain the influence of these factors on the composition and on the metabolism of the oral microbiome.

3.6 | Acknowledgements

The authors are grateful to all participants of the study and to Annachiara Cavazzana and Federica Penza for their precious help in data collection. Special thanks to Prof. Thomas Hummel (University of Dresden) and Franco Biasioli (Edmund Mach Foundation) for their support in the realization of this study.

3.7 | CRediT authorship contribution statement

Vincenzo Valentino: Investigation, Formal analysis, Writing – original draft, Visualization. **Francesca De Filippis**: Conceptualization, Supervision, Writing – review & editing. **Leonardo Menghi**: Conceptualization, Methodology, Investigation, Writing – review & editing. **Flavia Gasperi**: Conceptualization, Writing – review & editing. **Danilo Ercolini**: Conceptualization, Funding acquisition, Writing – review & editing.

3.8 | Declaration of interest

None.

3.9 | Supplemental material

3.9.1 | Figures

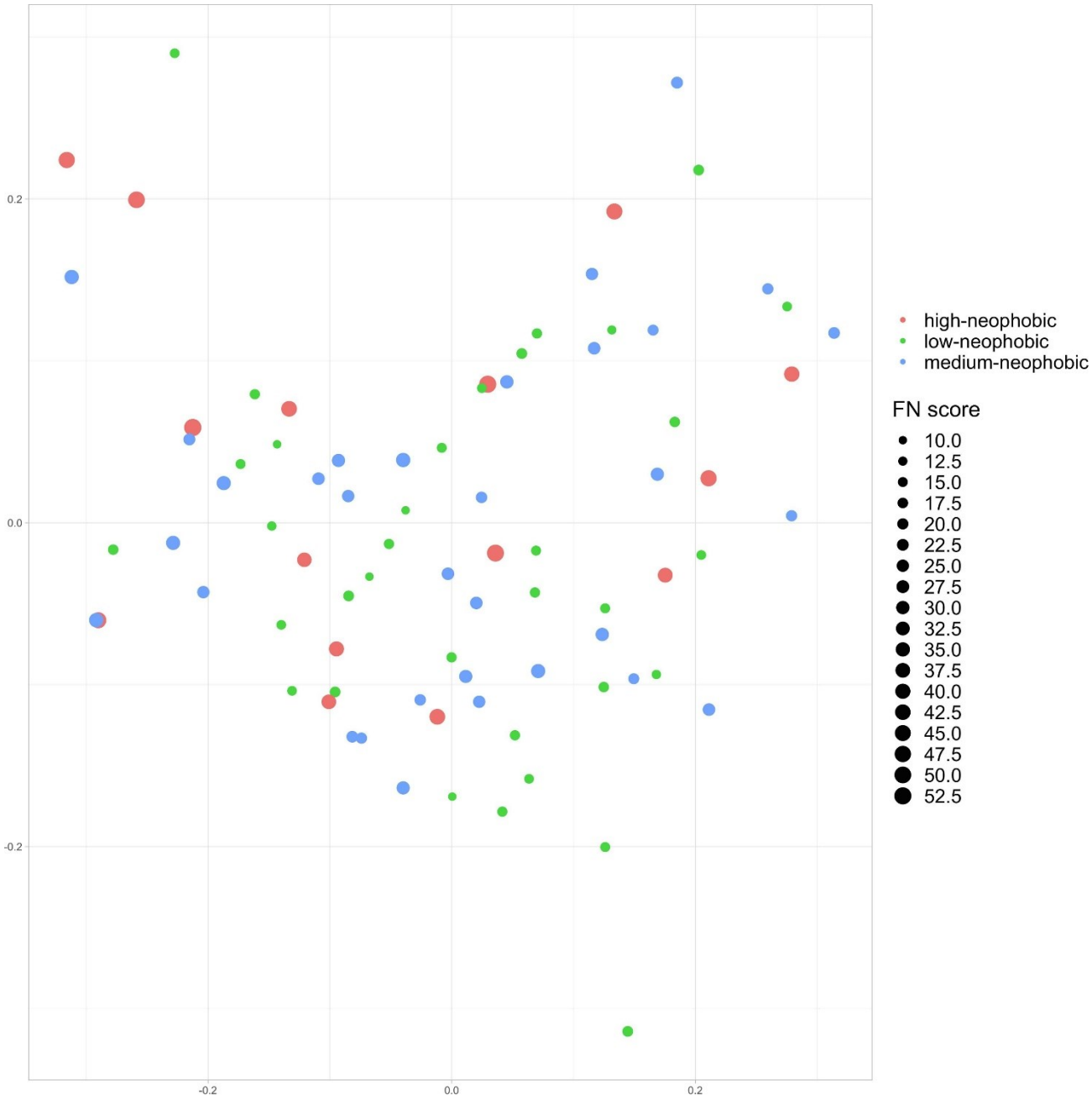

Figure S3.1: PCoA based on Bray-Curtis distance performed at species-level.

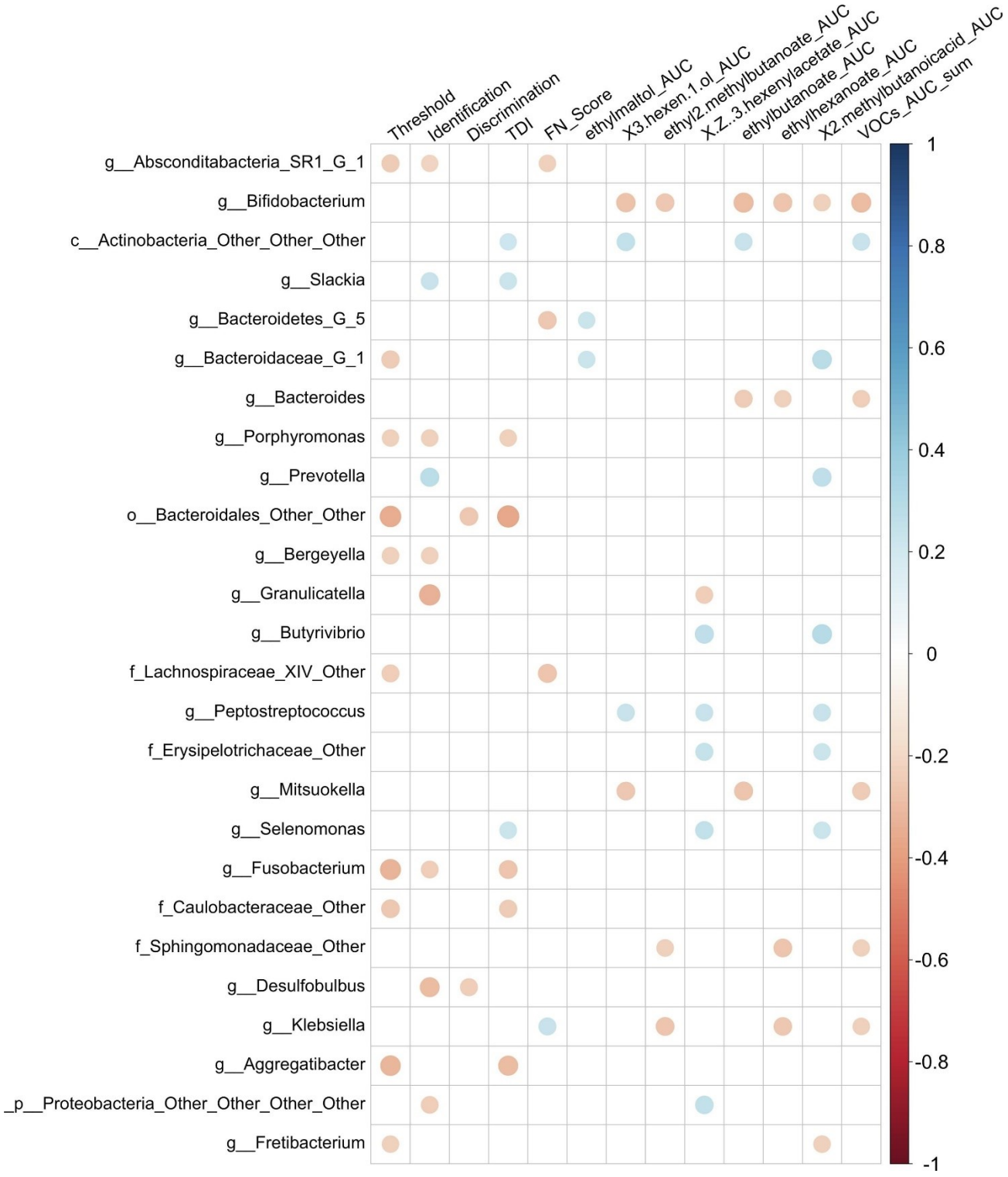

Figure S3.2: Correlation plot showing significant correlations with TDI scores, FN, individual VOCs and VOCs sum (AUC = Area Under the Curve).

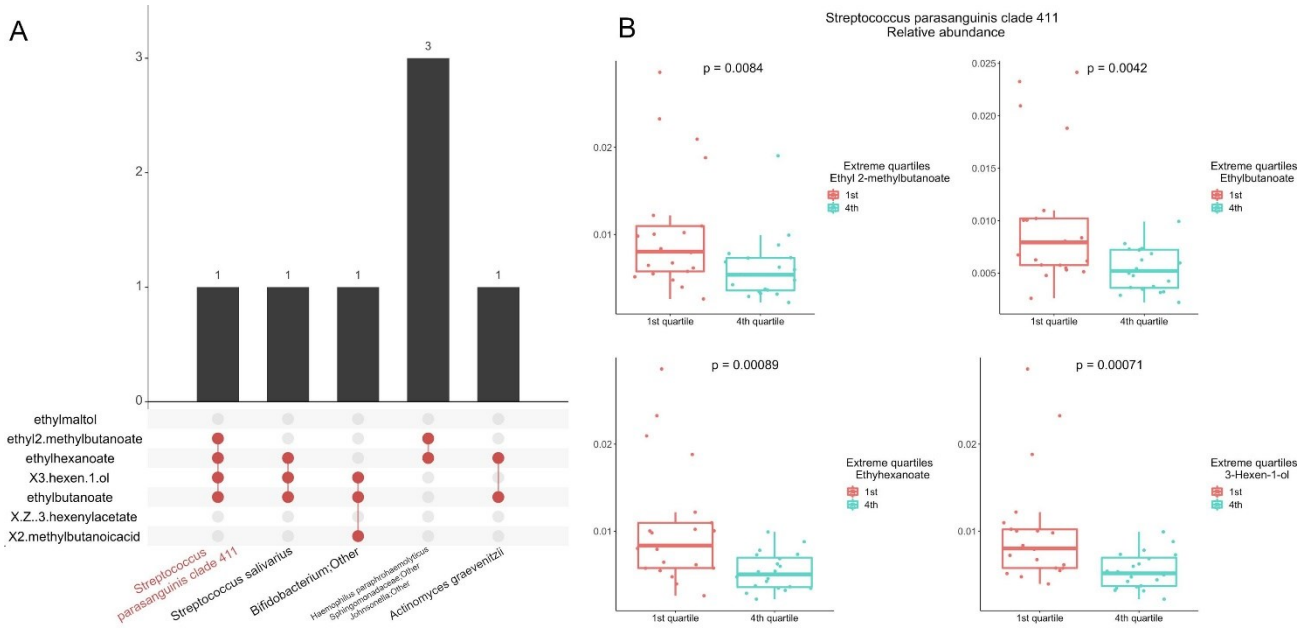

Figure S3.3: A) Upset plot. The bar at top shows the number of species differentially abundant between extreme quartiles shared by multiple VOCs. B) Boxplot showing the relative abundance of *Streptococcus parasanguinis* clade 411 between extreme quartiles for 4 out of 7 VOCs.

3.10 | References

1. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).
2. Knaapila, A. *et al.* Food neophobia shows heritable variation in humans. *Physiol. Behav.* **91**, 573–578 (2007).
3. Faith, M. S., Heo, M., Keller, K. L. & Pietrobelli, A. Child food neophobia is heritable, associated with less compliant eating, and moderates familial resemblance for BMI. *Obesity* **21**, 1650–1655 (2013).
4. Hazley, D., Stack, M., Walton, J., McNulty, B. A. & Kearney, J. M. Food neophobia across the life course: Pooling data from five national cross-sectional surveys in Ireland. *Appetite* **171**, 105941 (2022).
5. Dematte, M. L., Endrizzi, I. & Gasperi, F. Food neophobia and its relation with olfaction. *Frontiers in Psychology* vol. 5 (2014).
6. Jezewska-Zychowicz, M., Plichta, M., Drywień, M. E. & Hamulka, J. Food neophobia among adults: Differences in dietary patterns, food choice motives, and food labels reading in poles. *Nutrients* **13**, 1590 (2021).
7. Perry, R. A. *et al.* Food neophobia and its association with diet quality and weight in children aged 24 months: A cross sectional study. *Int. J. Behav. Nutr. Phys. Act.* **12**, 1–8 (2015).
8. Anjos, L. A. Dos *et al.* Low adherence to traditional dietary pattern and food preferences of low-income preschool children with food neophobia. *Public Health Nutr.* **24**, 2859–2866 (2021).
9. Knaapila, A. J. *et al.* Food neophobia associates with lower dietary quality and higher BMI in Finnish adults. *Public Health Nutr.* **18**, 2161–2171 (2015).
10. Sarin, H. V. *et al.* Food neophobia associates with poorer dietary quality, metabolic risk factors, and increased disease outcome risk in population-based cohorts in a metabolomics study. *Am. J. Clin. Nutr.* **110**, 233–245 (2019).
11. Boesveldt, S. Olfaction and Eating Behavior. *Springer Handbooks* 109–110 (2017) doi:10.1007/978-3-319-26932-0_44.

12. Morquecho-Campos, P., de Graaf, K. & Boesveldt, S. Smelling our appetite? The influence of food odors on congruent appetite, food preferences and intake. *Food Qual. Prefer.* **85**, 103959 (2020).

13. Puleo, S. *et al.* Food neophobia, odor and taste sensitivity, and overall flavor perception in food. *Foods* **10**, 3122 (2021).

14. Kershaw, J. C. & Mattes, R. D. Nutrition and taste and smell dysfunction. *World J. Otorhinolaryngol. - Head Neck Surg.* **4**, 3–10 (2018).

15. Raudenbush, B., Schroth, F., Reilley, S. & Frank, R. A. Food neophobia, odor evaluation and exploratory sniffing behavior. *Appetite* **31**, 171–183 (1998).

16. Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E. & Kobal, G. 'Sniffin' sticks'. Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chem. Senses* **22**, 39–52 (1997).

17. Menghi, L. *et al.* Arousal influences olfactory abilities in adults with different degree of food neophobia. *Sci. Rep.* **10**, 1–15 (2020).

18. De Filippis, F. *et al.* The same microbiota and a potentially discriminant metabolome in the saliva of omnivore, ovo-lacto-vegetarian and vegan individuals. *PLoS One* **9**, e112373 (2014).

19. Laiola, M., De Filippis, F., Vitaglione, P. & Ercolini, D. A mediterranean diet intervention reduces the levels of salivary periodontopathogenic bacteria in overweight and obese subjects. *Appl. Environ. Microbiol.* **86**, e00777-20 (2020).

20. Piombino, P. *et al.* Saliva from obese individuals suppresses the release of aroma compounds from wine. *PLoS One* **9**, e85611 (2014).

21. Lassalle, F. *et al.* Oral microbiomes from hunter-gatherers and traditional farmers reveal shifts in commensal balance and pathogen load linked to diet. *Mol. Ecol.* **27**, 182–195 (2018).

22. Masala, C. *et al.* An Italian population-based study of the prevalence of olfactory impairment. *Chem. Senses* **44**, e47–e47 (2019).

23. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–

124 (2018).

24. Charles, M. *et al.* Understanding flavour perception of espresso coffee by the combination of a dynamic sensory method and in-vivo nosespace analysis. *Food Res. Int.* **69**, 9–20 (2015).

25. Normand, V., Avison, S. & Parker, A. Modeling the kinetics of flavour release during drinking. *Chem. Senses* **29**, 235–245 (2004).

26. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1–e1 (2013).

27. Magoč, T. & Salzberg, S. L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**, 2957–2963 (2011).

28. Schmieder, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* **27**, 863–864 (2011).

29. Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010).

30. Chen, T. *et al.* The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database (Oxford).* **2010**, (2010).

31. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267 (2007).

32. Oleszkiewicz, A., Schriever, V. A., Croy, I., Hähner, A. & Hummel, T. Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. *Eur. Arch. Oto-Rhino-Laryngology* **276**, 719–728 (2019).

33. Bik, E. M. *et al.* Bacterial diversity in the oral cavity of 10 healthy individuals. *ISME J.* **4**, 962–974 (2010).

34. Takeshita, T. *et al.* Bacterial diversity in saliva and oral health-related conditions: The Hisayama Study. *Sci. Rep.* **6**, 1–11 (2016).

35. Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, R. L. Microbial complexes in subgingival plaque. *J. Clin. Periodontol.* **25**, 134–144 (1998).

36. Mohanty, R. *et al.* Red complex: Polymicrobial conglomerate in oral flora: A review. *J. Fam. Med. Prim. Care* **8**, 3480 (2019).

37. Vashishta, A. *et al.* Putative periodontal pathogens, Filifactor alocis and Peptoanaerobacter stomatis, induce differential cytokine and chemokine production by human neutrophils. *Pathogens* **8**, 59 (2019).

38. Eriksson, L., Lif Holgerson, P. & Johansson, I. Saliva and tooth biofilm bacterial microbiota in adolescents in a low caries community. *Sci. Rep.* **7**, 5861 (2017).

39. Gonçalves, L. F. H. *et al.* Levels of Selenomonas species in generalized aggressive periodontitis. *J. Periodontal Res.* **47**, 711–718 (2012).

40. Zhou, M. *et al.* Investigation of the Effect of Type 2 Diabetes Mellitus on Subgingival Plaque Microbiota by High-Throughput 16S rDNA Pyrosequencing. *PLoS One* **8**, e61516 (2013).

41. Panda, M. *et al.* Alterations of salivary microbial community associated with oropharyngeal and hypopharyngeal squamous cell carcinoma patients. *Arch. Microbiol.* **202**, 785–805 (2020).

42. Tanner, A. C. R. Anaerobic culture to detect periodontal and caries pathogens. *J. Oral Biosci.* **57**, 18–26 (2015).

43. Koskinen, K. *et al.* The nasal microbiome mirrors and potentially shapes olfactory function. *Sci. Rep.* **8**, (2018).

44. Tennert, C. *et al.* An oral health optimized diet reduces the load of potential cariogenic and periodontal bacterial species in the supragingival oral plaque: A randomized controlled pilot study. *Microbiologyopen* **9**, e1056 (2020).

45. De Filippis, F. *et al.* High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* **65**, 1812–1821 (2016).

46. Ventriglio, A. *et al.* Mediterranean Diet and its Benefits on Health and Mental Health: A Literature Review. *Clin. Pract. Epidemiol. Ment. Heal.* **17**, 9–9 (2021).

47. Mentella, M. C., Scaldaferri, F., Ricci, C., Gasbarrini, A. & Miggiano, G. A. D. Cancer and mediterranean diet: A review. *Nutrients* **11**, 2059 (2019).

48. Meslier, V. *et al.* Mediterranean diet intervention in overweight and obese subjects lowers plasma

cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* **69**, 1258–1268 (2020).

49. Predieri, S. *et al.* Gender, age, geographical area, food neophobia and their relationships with the adherence to the mediterranean diet: New insights from a large population cross-sectional study. *Nutrients* **12**, 1–14 (2020).

50. Hajishengallis, G. *et al.* Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* **10**, 497–506 (2011).

51. Jia, L. *et al.* Pathogenesis of important virulence factors of Porphyromonas gingivalis via toll-like receptors. *Front. Cell. Infect. Microbiol.* **9**, 262 (2019).

52. Olsen, I., Lambris, J. D. & Hajishengallis, G. Porphyromonas gingivalis disturbs host–commensal homeostasis by changing complement function. *J. Oral Microbiol.* **9**, 1340085 (2017).

53. Kameda, M. *et al.* Sugar Metabolism of Scardovia wiggsiae, a Novel Caries-Associated Bacterium. *Front. Microbiol.* **11**, 479 (2020).

54. Prabhu Matondkar, S., Yavagal, C., Kugaji, M. & Bhat, K. G. Quantitative assessment of Scardovia wiggsiae from dental plaque samples of children suffering from severe early childhood caries and caries free children. *Anaerobe* **62**, 102110 (2020).

55. Kressirer, C. A. *et al.* Scardovia wiggsiae and its potential role as a caries pathogen. *J. Oral Biosci.* **59**, 135–141 (2017).

56. Leoney, A., Karthigeyan, S., Asharaf, A. & Felix, A. J. W. Detection and categorization of biofilm-forming Staphylococcus aureus, Viridans streptococcus, Klebsiella pneumoniae, and Escherichia coli isolated from complete denture patients and visualization using scanning electron microscopy. *J. Int. Soc. Prev. Community Dent.* **10**, 627–633 (2020).

57. Paczosa, M. K. & Mecsas, J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. *Microbiol. Mol. Biol. Rev.* **80**, 629–661 (2016).

58. Nicolau Korres, A. M. *et al.* Comparison of biofilm and attachment mechanisms of a phytopathological and clinical isolate of Klebsiella pneumoniae subsp. Pneumoniae. *Sci. World J.* **2013**, (2013).

59. Marsh, P. D. Dental plaque as a biofilm: the significance of pH in health and caries. *Compend. Contin. Educ. Dent.* **30**, 76–8, 80, 83 (2009).

60. Ployon, S., Morzel, M. & Canon, F. The role of saliva in aroma release and perception. *Food Chem.* **226**, 212–220 (2017).

61. Frank, D. *et al.* Effects of Agar Gel Strength and Fat on Oral Breakdown, Volatile Release, and Sensory Perception Using in Vivo and in Vitro Systems. *J. Agric. Food Chem.* **63**, 9093–9102 (2015).

62. Tarrega, A., Yven, C., Semon, E., Mielle, P. & Salles, C. Effect of oral physiology parameters on in-mouth aroma compound release using lipoprotein matrices: An in vitro approach. *Foods* **8**, 106 (2019).

63. Ly, M. H. *et al.* Retention of aroma compounds by lactic acid bacteria in model food media. *Food Hydrocoll.* **22**, 211–217 (2008).

64. Muñoz-González, C., Cueva, C., Ángeles Pozo-Bayón, M. & Victoria Moreno-Arribas, M. Ability of human oral microbiota to produce wine odorant aglycones from odourless grape glycosidic aroma precursors. *Food Chem.* **187**, 112–119 (2015).

65. Parker, M. *et al.* Factors Contributing to Interindividual Variation in Retronasal Odor Perception from Aroma Glycosides: The Role of Odorant Sensory Detection Threshold, Oral Microbiota, and Hydrolysis in Saliva. *J. Agric. Food Chem.* **68**, 10299–10309 (2020).

66. Gaborieau, S., Cendrès, A., Page, D., Ginies, C. & Renard, C. M. G. C. Variability of free and glycosylated volatiles from strawberries destined for the fresh market and for processing, assessed using direct enzymatic hydrolysis. *Lwt* **98**, 187–196 (2018).

67. Relvas, M. *et al.* Relationship between dental and periodontal health status and the salivary microbiome: bacterial diversity, co-occurrence networks and predictive models. *Sci. Rep.* **11**, 1–22 (2021).

68. Sato, N. *et al.* Metagenomic analysis of bacterial species in tongue microbiome of current and never smokers. *npj Biofilms Microbiomes* **6**, 1–9 (2020).

Section II

Taste, gastrointestinal microbiota and dietary habits

Chapter 4

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

Menghi, L., Cliceri, D., Fava, F., Pindo, M., Gaudioso, G., Stefani, E., Giacalone, D. & Gasperi, F.

Food Quality and Preference, 106, 104790 (2023)
DOI: 10.1016/j.foodqual.2022.104790

CHAPTER 4:

VARIATIONS IN ORAL RESPONSIVENESS ASSOCIATE WITH SPECIFIC SIGNATURES IN THE GUT MICROBIOTA AND MODULATE DIETARY HABITS

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. Moreover, CL-1 declared higher intakes of fibers and vegetable proteins, whilst CL-2 habitually consumed more saturated fats. We provide the first empirical evidence that simultaneous variations in sensory acuity

and gut microbial consortia associate with 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

4.1 | 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., [1]). 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 [2]. 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., [3,4]).

Historically, the best-documented sources of interindividual variation in oral responsiveness revolved around genetically-induced bitterness of 6-n-propylthiouracil (PROP) [5] and anatomic phenotypes (i.e., fungiform papillae density) [6]. 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., [7,8]). Nevertheless, recent large scale studies have failed to corroborate this paradigm [6,9,10], though apparently confirming PROP acuity (unlike fungiform papillae density) as a proxy of generalized hypergeusia [9,11]. Thus, as the role of taste phenotypes still remains somewhat 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 [12], a metabolically active reservoir of trillions of microbes that would jointly work with the host chemosensory systems to shape our ingestive behaviors [12–14]. Moreover, gut microbial disruption (or dysbiosis) has been reported in concomitance with unhealthy eating attitudes related to chemosensation, such as craving for high-palatable foods [12] or binge-eating

episodes [15]. Thus, given that nutrient-sensing mechanisms not only operate in the oral cavity but also in the lower gastrointestinal tract [16], research has recently begun to deepen the links between taste and oral or distal gut microbes (e.g., [17,18]).

As an example, Cattaneo, Gargari *et al.* [19] 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 the genus level (*Parvimonas, Peptococcus*, *Peptostreptococcus*, *Prevotella*) to be negatively correlated with salt taste thresholds and carbohydrate daily intake, while the opposite was true for the genus *Rothia* [20]. Nevertheless, although a variety of likely pathways used by oral microbial communities to influence taste/flavor perception has been proposed (see for reviews [13,14]), 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 [13]. However, the afore-mentioned pathways derived evidence from animal studies (e.g., [21]) or were theoretically presumed on the basis of known connections between diet and taste or diet and gut microbial communities (e.g., [22,23]). 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.* [18] 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., [4,24]), 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 interplays between taste perception and gastrointestinal microbes, and opens new research avenues on the aetiology of eating behaviors [12,13].

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 [17,25–27], which are reportedly uncorrelated with measures of taste function more relevant for actual perception of food (i.e., suprathreshold intensity measures) (e.g., [28,29]). Secondly, taste assessments in previous research have exclusively been obtained in response to aqueous solutions (e.g., [17,19,25]) or paper strips [26,27], 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) [8,28,30].

Thirdly, none of the afore-mentioned studies has considered key mediators of sensory responsiveness such as hedonics, attitudes and personality traits (e.g., [2]). Given how both liking and psychological background can mediate variations in oral acuity ultimately shaping food choices (e.g., [31,32]), 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., [20]), and the minority [26,27] has considered sufficiently large cohorts in the light of the numerous confounders (demographic, dietary, environmental) affecting both chemosensation and the gastrointestinal ecosystem (e.g., [33,34]). 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.

4.2 | Methods

4.2.1 | 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 S4.1.

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 S4.2) mostly relies on the protocol used by the Human Microbiome Project [35] to target the core human gut microbiota in health.

4.2.2 | 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 4.1). 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 4.1).

Eligible participants were firstly asked to collect a bag storing all the equipment needed to complete the study (Supplemental Figure S4.1) 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) [36] 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) [37]. 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.

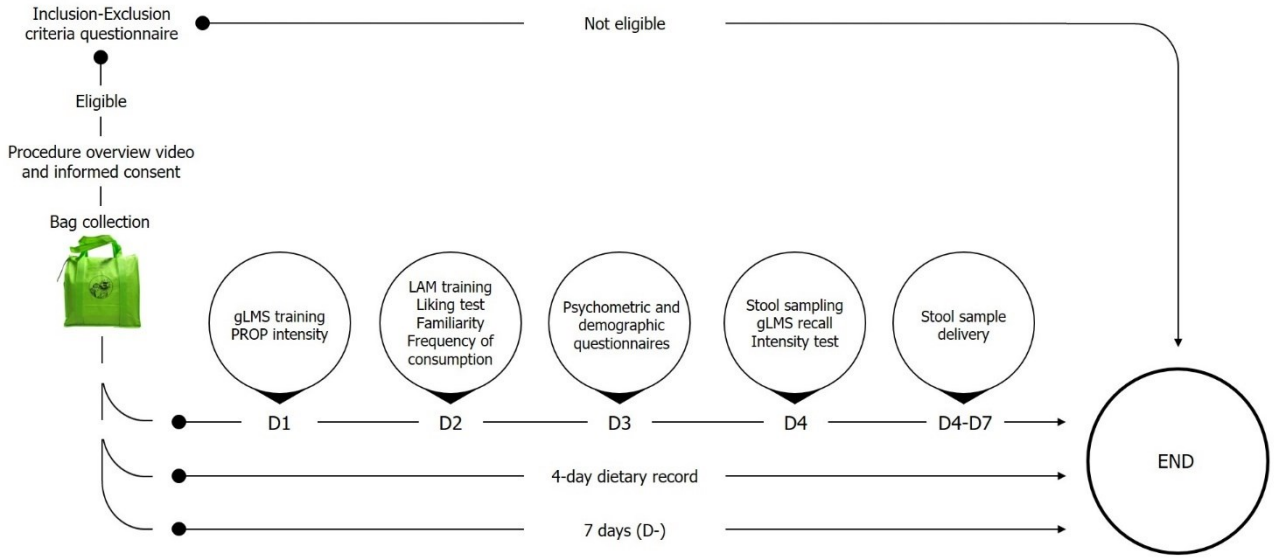

Figure 4.1: Graphical overview of data collection.

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 [38].

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 [38]. 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.

4.2.3 | Sensory stimuli, training and evaluations

4.2.3.1 | 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 S4.2), and the scores given to the sensory ballot were strongly correlated (Supplemental Figure S4.3) thus corroborating the reliability of the remote protocol. Table 4.1 lists relevant information on food matrices and the ballot of sensory attributes here used.

Table 4.1: 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.

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,	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,	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,	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 (Cipster, 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

4.2.3.2 | 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 [29,36,37]. 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 [29,36,39]. 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 [29].

To this end, volunteers rated the intensities of five recalled extraoral stimuli (D1; Figure 4.1), each selected to theoretically represent different rating ranges along the scale [39]. 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 S4.4), 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 S4.5), and by widely-known correlations between the perceived intensity of innately (dis)liked oral sensations and hedonic responses (Supplemental Figure S4.6).

4.2.3.3 | Sensory evaluations

After scales training, volunteers were given access to the tasting sessions. On D1 (Figure 4.1), 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 [40] 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., [9,31]) 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 4.1), instead, food stimuli were evaluated in two independent sets (Table 4.1), 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 4.1) 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 4.1).

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 4.1) 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.

4.2.4 | Psychometric and demographic measures

On D3 (Figure 4.1), 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 [31,41], the trait anxiety subscale of the State-Trait Anxiety Inventory Questionnaire [42,43], the Health and Taste Attitude Scale [44,45], the Dutch Eating Behavior Questionnaire [46,47], and the Big Five Inventory [48,49], 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 [50]. 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 S4.3.

4.2.5 | 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 [51]. 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 [52] and then individually averaged.

4.2.6 | Stool samples

4.2.6.1 | Stool collection and preprocessing

Prior to starting the last session (D4; Figure 4.1), 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.

4.2.6.2 | 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 [53,54]. Amplicons were then purified, and libraries prepared as described by Gaudioso *et al.* [55] prior to being sequenced using the Illumina® MiSeq (PE300) platform (San Diego, CA, USA).

4.2.6.3 | 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 [56]. High-quality sequences were thus resolved into amplicon sequence variants (ASVs) and then mapped against the SILVA database (version 138) [57] 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™) [58], 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* [59].

4.2.7 | Data analysis

4.2.7.1 | 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 4.1) 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 [60]. 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 [61]. 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) [62].

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 [63].

4.2.7.2 | Differences in gut microbial ecology between taste profiles

Given the intrinsic compositional nature of sequencing products [64], 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 [64]. First, to deal with the high sparsity of high-throughput data, zeros were imputed with sensible counts by geometric Bayesian-multiplicative replacement [64,65]. 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 [64]. Differences in α- and β-diversity metrics between taste profiles were then checked via permutational Wilcoxon rank sum test (as previously described in section 4.2.7.1) and permutational multivariate analysis of variance (PERMANOVA; n = 10000), respectively. β-dissimilarities were also graphically represented using Principal Component Analysis [64].

Lastly, raw ASV counts were filtered for taxa present in at least 10 % of participants before differential abundance analysis as previously recommended [66]. 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) [67], a compositionally aware method reportedly reducing the occurrence of false discovery rates [66,67]. 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.

4.2.7.3 | Software

Statistics were calculated using R 4.2.0 [68]. Particularly, MFA model computation and visualization was carried out via *FactoMineR* [69], while the R packages *NbClust* [70] and *clValid* [61] were employed within the data-driven segmentation approach. Lastly, the R packages *zCompositions* [65], *vegan* [71], and *ANCOMBC* [67] were used for zeros replacement, β-dissimilarity and differential abundance analysis, respectively.

4.3 | Results

4.3.1 | Optimal partitioning and taste profiles characterization

Assuming that individuals would show similar patterns of responsiveness across different sensory modalities [8,11,30], 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 S4.7a), and the sensory ballot positively associated with the first component of the model (Supplemental Figure S4.7b). Along Dim. 2, warning sensations (e.g., bitter, pungent) tended to be distributed opposite 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 S4.8), 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 S4.8). Hence, to conclusively define the optimal partitioning, we used the 26 cluster validation indices implemented in the R package *NbClust* [70], and found n = 2 as the cluster number supported by the majority of these indices (Supplemental Figure S4.9).

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) [46] and proneness to use food as a source of reward (Health and Taste Attitudes Scale) [44]. Table 4.2 lists baseline demographics, attitudes and psychological traits, and PROP taste phenotypes distribution across taste profiles.

Table 4.2: 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 = 10,000). Values in bold are intended as statistically significant ($p < 0.05$).

	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	

4.3.2 | 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 4.2) and solid (Figure 4.3) foods. Except for bitterness in PR-08 (Figure 4.3), 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. Similarly, 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. It is noteworthy that 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 S4.10).

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 4.3). 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).

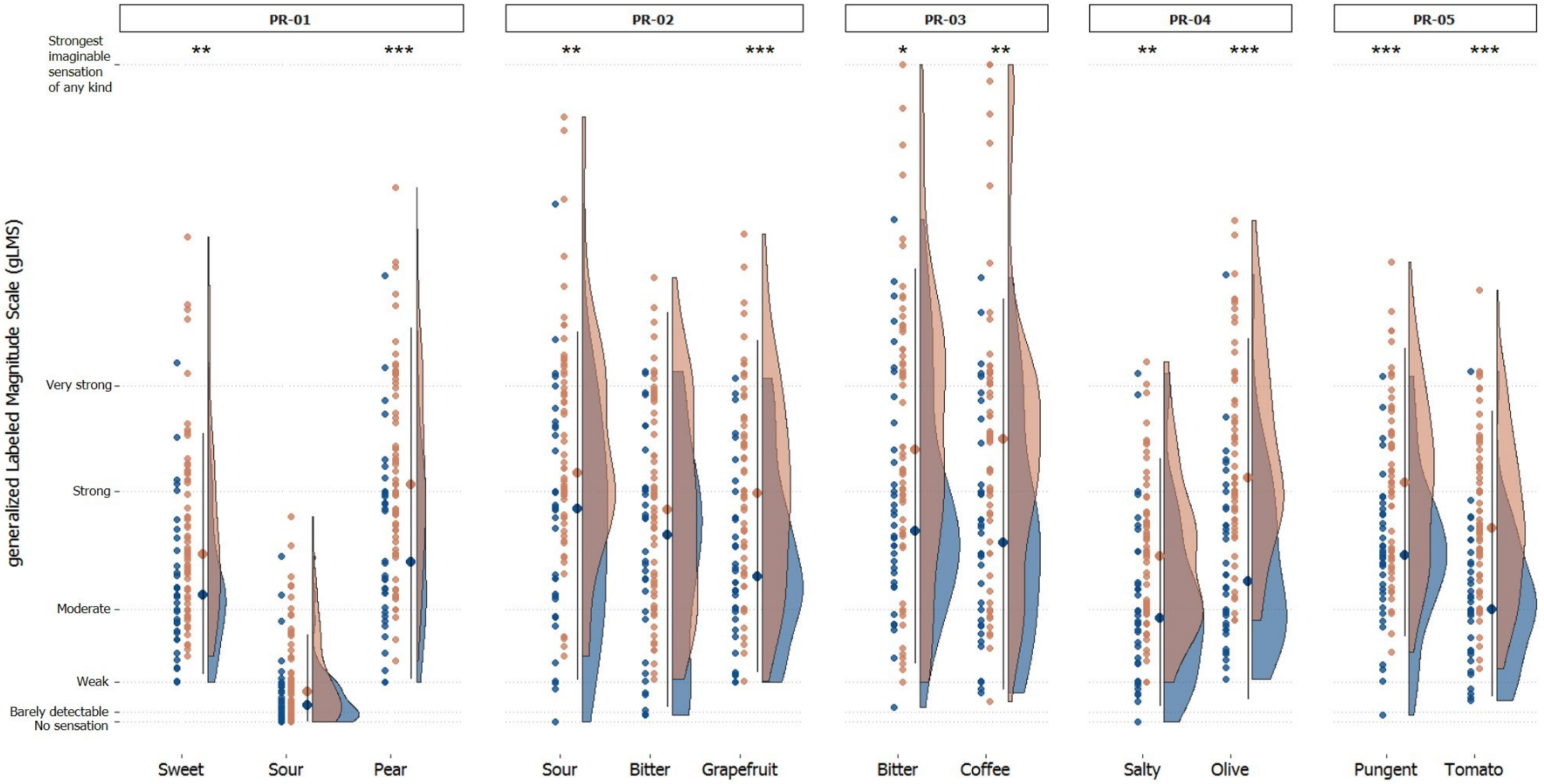

Figure 4.2: Differences in oral responsiveness in liquid foods between CL-1 (dark-blue; n = 36) and CL-2 (orange; n = 64). The raincloud plot graphically represents data distribution (the "cloud"), individual raw observations (the "rain"), and the median (filled circle) ± IQR (perpendicular black line) within each taste profile. Statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10,000) are depicted (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

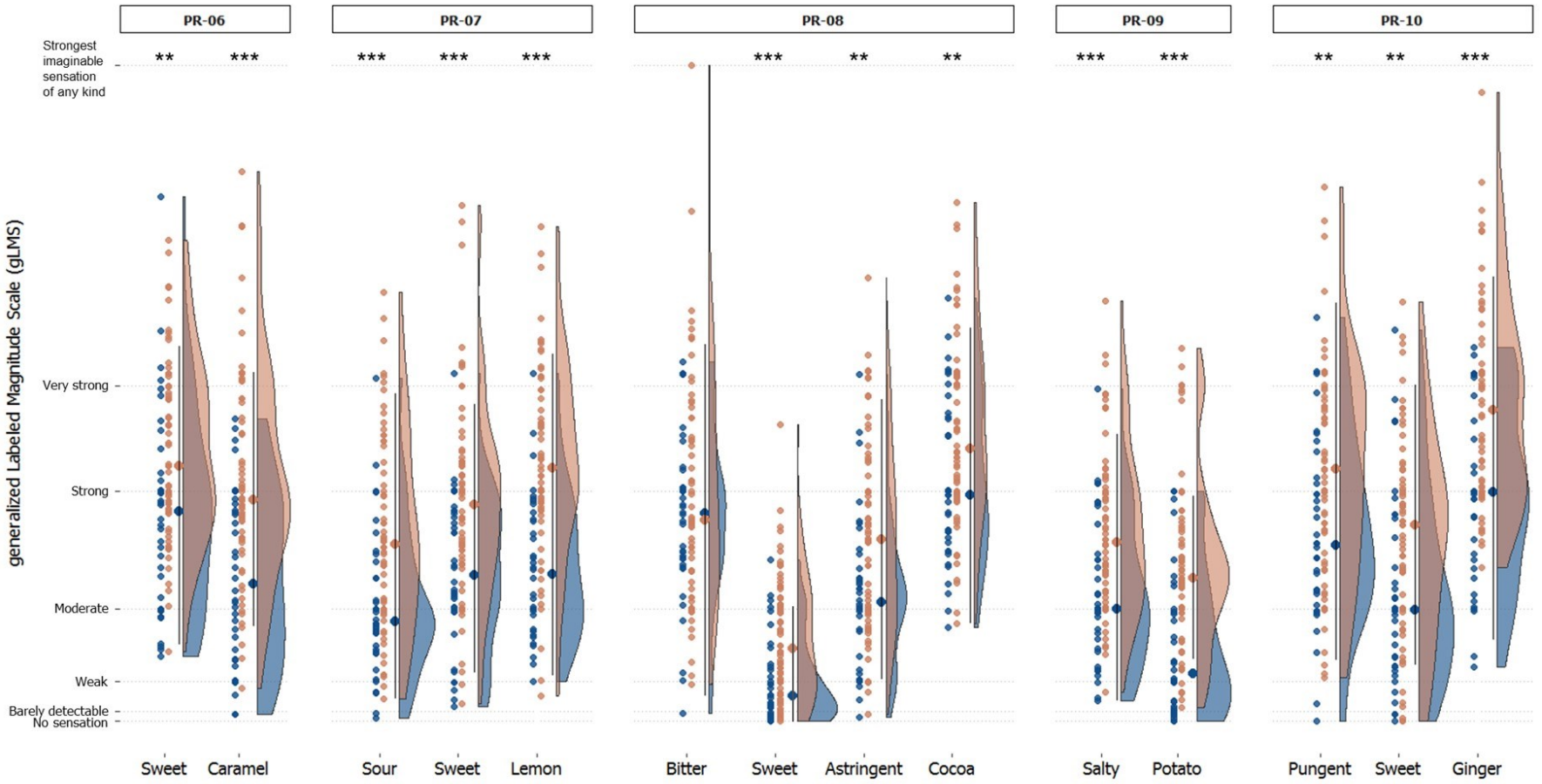

Figure 4.3: Differences in oral responsiveness in solid foods between CL-1 (dark-blue; n = 36) and CL-2 (orange; n = 64). The raincloud plot graphically represents data distribution (the "cloud"), individual raw observations (the "rain"), and the median (filled circle) ± IQR (perpendicular black line) within each taste profile. Statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10,000) are depicted (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Table 4.3: 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 = 10,000) are depicted in bold.

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 ± 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 ± 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

4.3.3 | 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). Conversely, 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 4.4 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 S4.4.

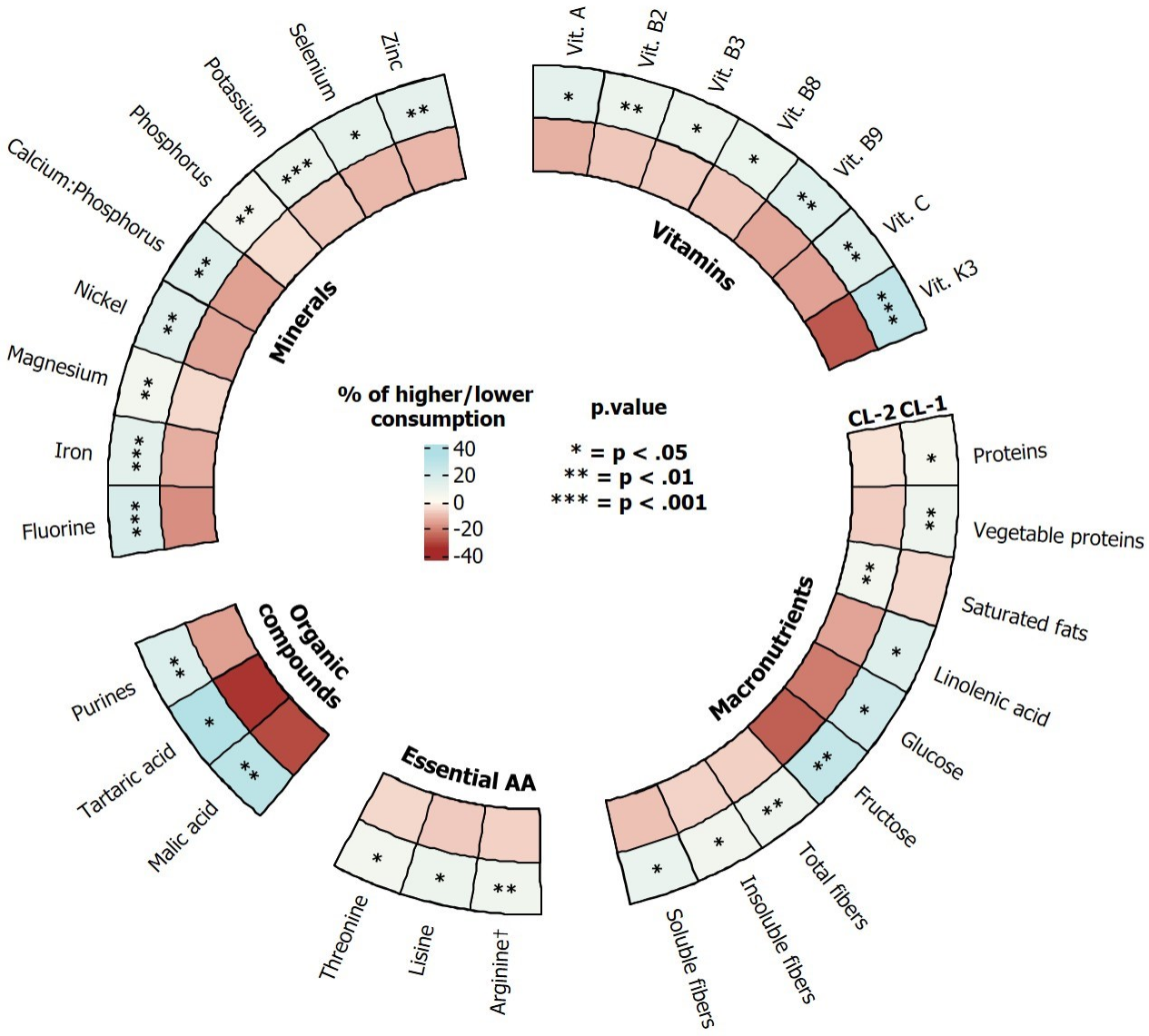

Figure 4.4: Circular heatmap depicting variations (%) in habitual nutrient intakes between CL-1 (n = 36; outer circumference) and CL-2 (n = 64; inner circumference), as calculated by the proportional difference between the medians across taste profiles. Macronutrients, essential amino acids (AA), organic compounds, minerals, and vitamins (Vit.) are plotted. Moreover, statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10,000) are given (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). † to be considered as a semi-essential amino acid.

4.3.4 | 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., [72]), 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 S4.11). Next, we tested the extent of β-dissimilarities between fecal microbial communities of groups using Aitchison distances [64], 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 S4.12).

4.3.5 | 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 [67]. 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 [72].

Top abundant phyla (n = 10) and genera (n = 20) by taste profiles are depicted in Supplemental Figure S4.13.

Nevertheless, several differences emerged when it came to evaluate the differently abundant gut microbial genera between groups. Results (Figure 4.5) 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 S4.5

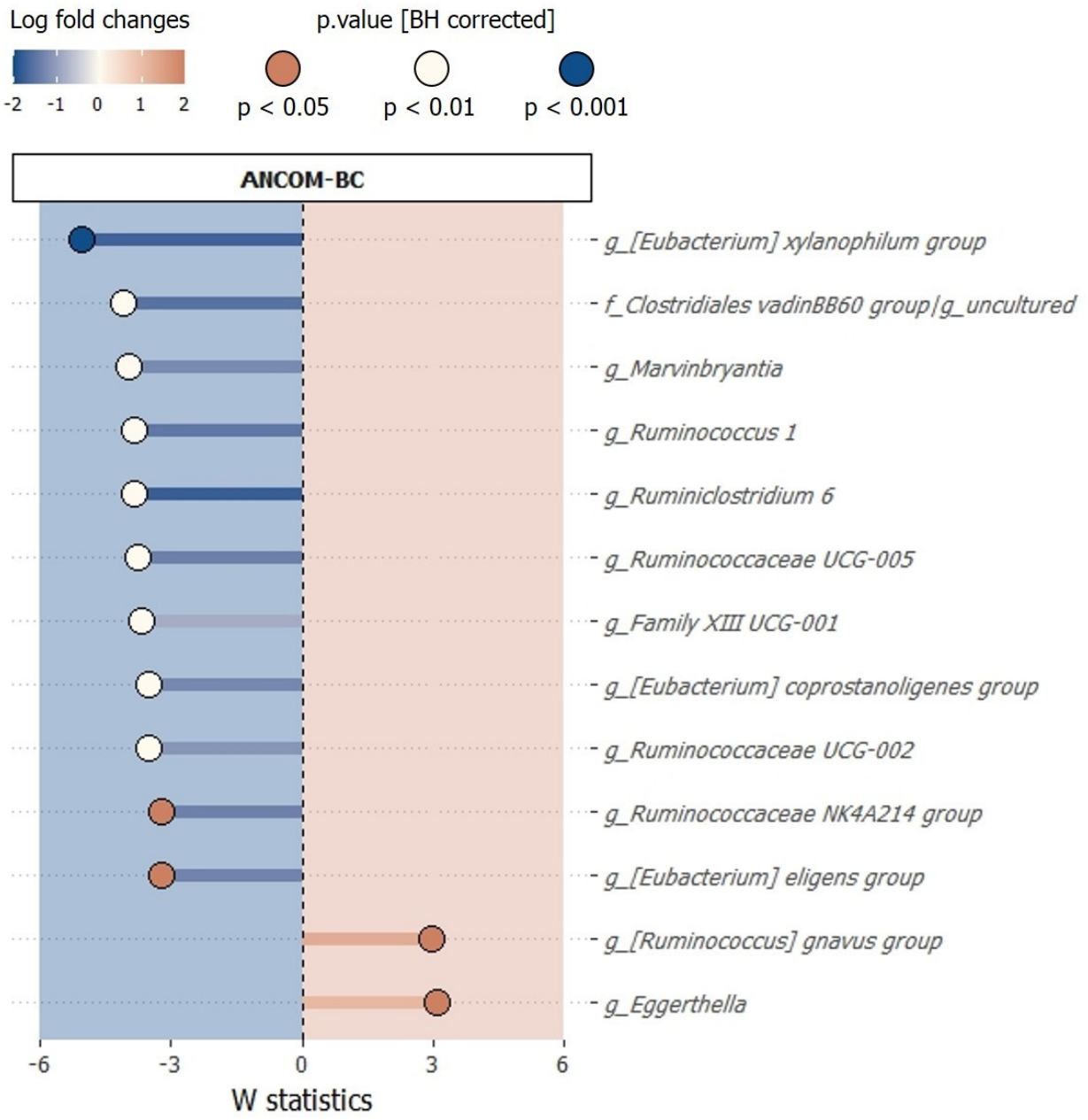

Figure 4.5: Differently abundant taxa between taste profiles. The plot illustrates the main outcome produced by ANCOM-BC (W statistic), which summarizes the ratio between the effect size (log fold change) and the standard error (95 % confidence interval) underlying the differences observed [67]. Genera 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**.** $p < 0.001$).

4.4 | Discussion

4.4.1 | 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., [8,11,30]), 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 [7,73,74] have recently been questioned [75], 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 [8,9,11,28,30].

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 [76], and may not guarantee consistent quantities of PROP across the strip thus inducing biases on phenotypic assignment [77]. 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., [9]).

4.4.2 | 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 4.3) or demographics, dietary styles and psychological traits (Table 4.2), 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 [78]. Furthermore, these results overlap those by Hayes, Sullivan, and Duffy [79] 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 4.2 and 4.3). Moreover, these mostly occurred in palatable (LAM > 50; Table 4.3) 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., [78]). 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.

4.4.3 | 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 [80], and relate to plant-oriented diets [81]. As an example, *[Eubacterium] xylanophilum group* positively associated with long-term consumption of healthful fiber sources such as fruits and vegetables [82], while a resistant starch-supplemented diet promoted increased abundances of *Ruminococcaceae UCG-005* [83]. Similarly, Ma *et al.* [84] 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). Moreover, in the same follow-up study, the pro-inflammatory *[Ruminococcus] gnavus group* was found to exhibit negative correlations with diet quality over time [84], 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 [81,85], 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 [78]. 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., [20]), but motivate future studies to increasingly consider key mediators of taste perception when it comes to evaluate its relationships with dietary patterns.

4.4.4 | 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., [2,47,81,85]). However, an alternative model focused on a putative 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 [12]. A variety of potential mechanisms have been discussed, including the modulation of the host immune system and hormone secretion (see for a review [13]). 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 [13].

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*) [80,81,86,87]. Conversely, the hyperresponsive group showed higher relative abundances of *[Ruminococcus] gnavus group* and *Eggerthella*, two bacterial genera widely associated with

inflammatory bowel disease [88,89]. 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., [90,91]).

It is noteworthy that 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 [13]. 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 [12]. However, mechanisms underlying potential interplays between taste perception and gut microbial ecology are far to be conclusively understood. Indeed, 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 [92,93]. However, such experimental efforts would be poorly informative 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.

4.4.5 | 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 [38], 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., [28,30]) for the sake of clustering reliability and stability. Nevertheless, objective clustering largely outperforms commonly used arbitrary criteria (e.g., [94]), 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 [51], 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 [51,95], 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 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.

4.5 | 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.

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 program 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).

4.6 | CRediT authorship contribution statement

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

4.7 | Declaration of interest

None.

4.8 | Supplemental material

4.8.1 | Figures

Figure S4.1: The bag (a) storing all the equipment needed to complete the study. All material was arranged in separate containers (b), as the expected tasting sessions (n = 3), each labeled with a code (Day 1, Day 2, Day 4). Thus, volunteers easily recognized the equipment needed (e.g., PROP taste strips for the first working session) to handle each single task. The bag was also supplemented with anonymized bottles of water and plain crackers for mouth cleaning, with supports for sensory evaluations (i.e., spoons and graduated plastic cups), with a commercially available kit to autonomously collect one fecal sample (OMNIgene®•GUT - OM-200.100, DNA Genotek Inc., Ottawa, Canada), and with a variety of guideline documents. Particularly, to guarantee the double-blind design, each volunteer was assigned an anonymous e-mail address to whom we sent general instructions to complete the working sessions, and, if needed, to ask for clarifications to an help-desk appositely created for this study.

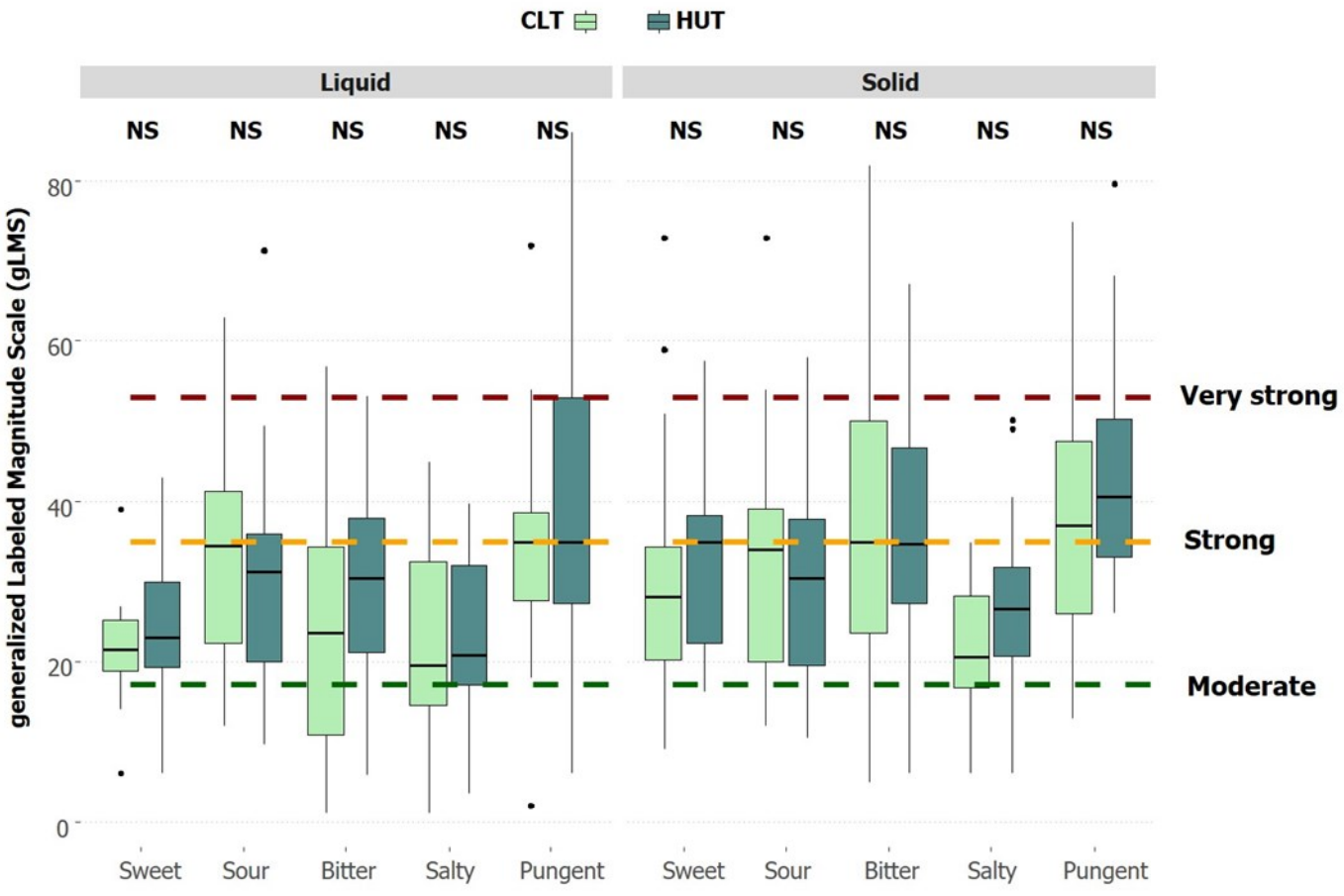

Figure S4.2: Differences (Wilcoxon rank sum test) in orosensory responsiveness (generalized Labeled Magnitude Scale – gLMS) [36] to target tastes (i.e., sweet, sour, bitter, salty) or sensation (i.e., pungent) elicited by the five liquid and five solid food matrices used by the current study in both lab (CLT; Pilot 2) and remote (HUT; Pilot 3) testing conditions (n = 20; 80 % men; 18-30 y/o). Dotted lines (green, orange, red) depict the moderate (gLMS = 17), strong (gLMS = 35) and very strong (gLMS = 51) anchors of the gLMS. Significance rates are also provided (NS = $p > 0.05$).

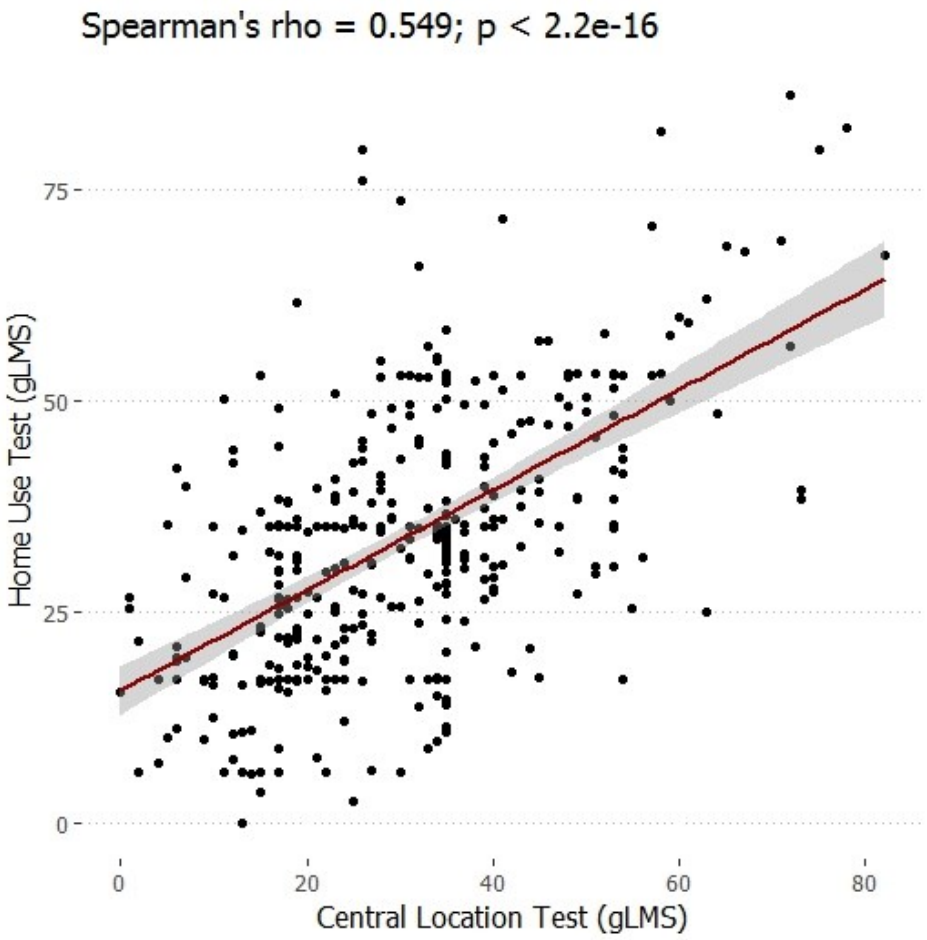

Figure S4.3: Correlation (Spearman's rho correlation) between perceived intensity ratings (generalized Labeled Magnitude Scale – gLMS) [36] given to the sensory ballot in the lab (Central Location Test; Pilot 2) and remote (Home Use Test; Pilot 3) testing condition (n = 20; 80 % men; 18-30 y/o).

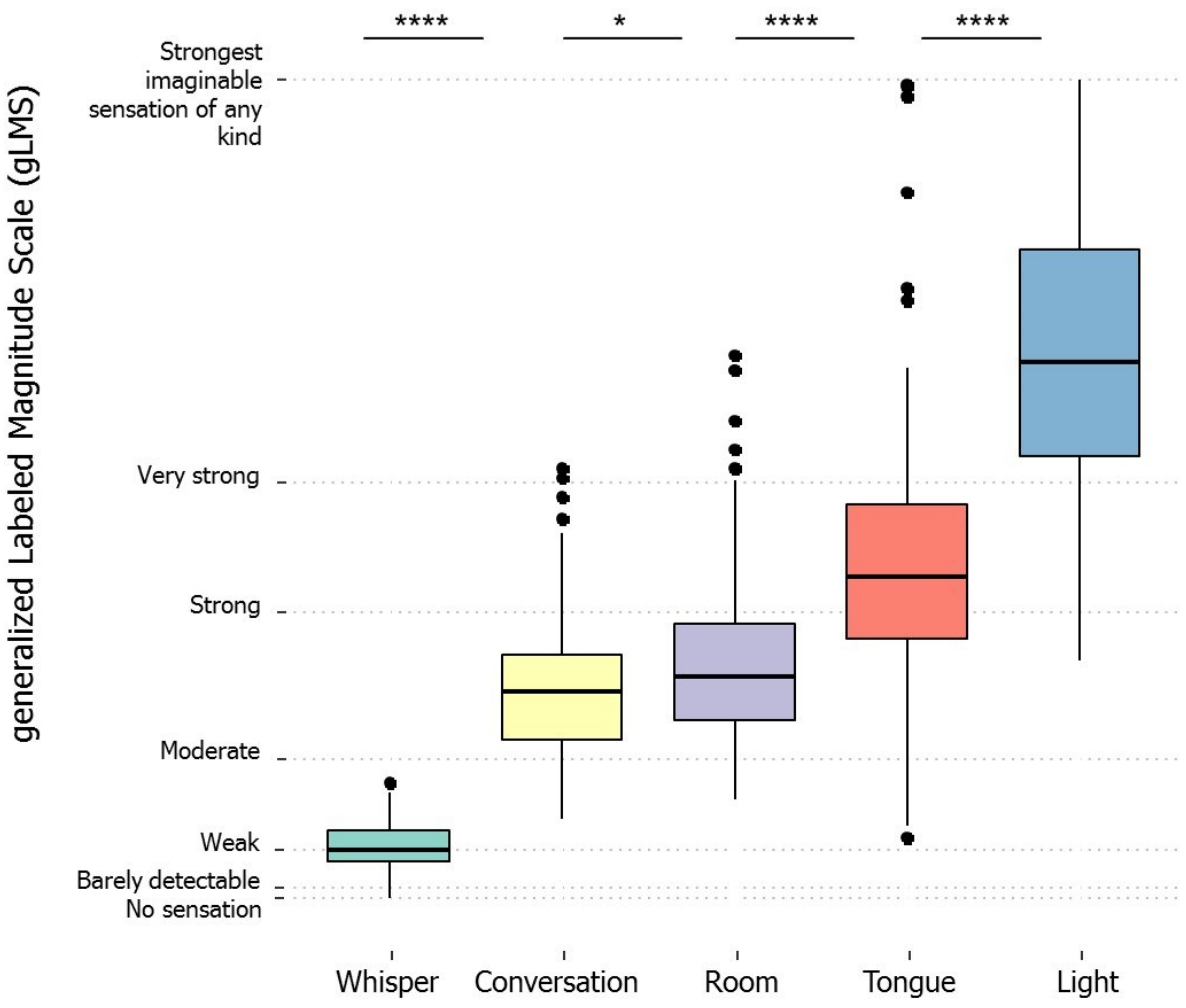

Figure S4.4: Differences (Kruskal Wallis test; n = 100) in recalled intensities (generalized Labeled Magnitude Scale – gLMS) [36] between the extraoral stimuli (Whisper: loudness of a whisper; Conversation: loudness of a conversation; Room: brightness of a well-lit room; Tongue: pain of biting your tongue; Light: brightest light ever seen) used during the gLMS training [39]. Statistically significant pairwise differences ($p < 0.05$) observed after post hoc Dunn's test with Benjamini-Hochberg adjustment are depicted. * = $p < 0.05$; **** = $p < 0.0001$.

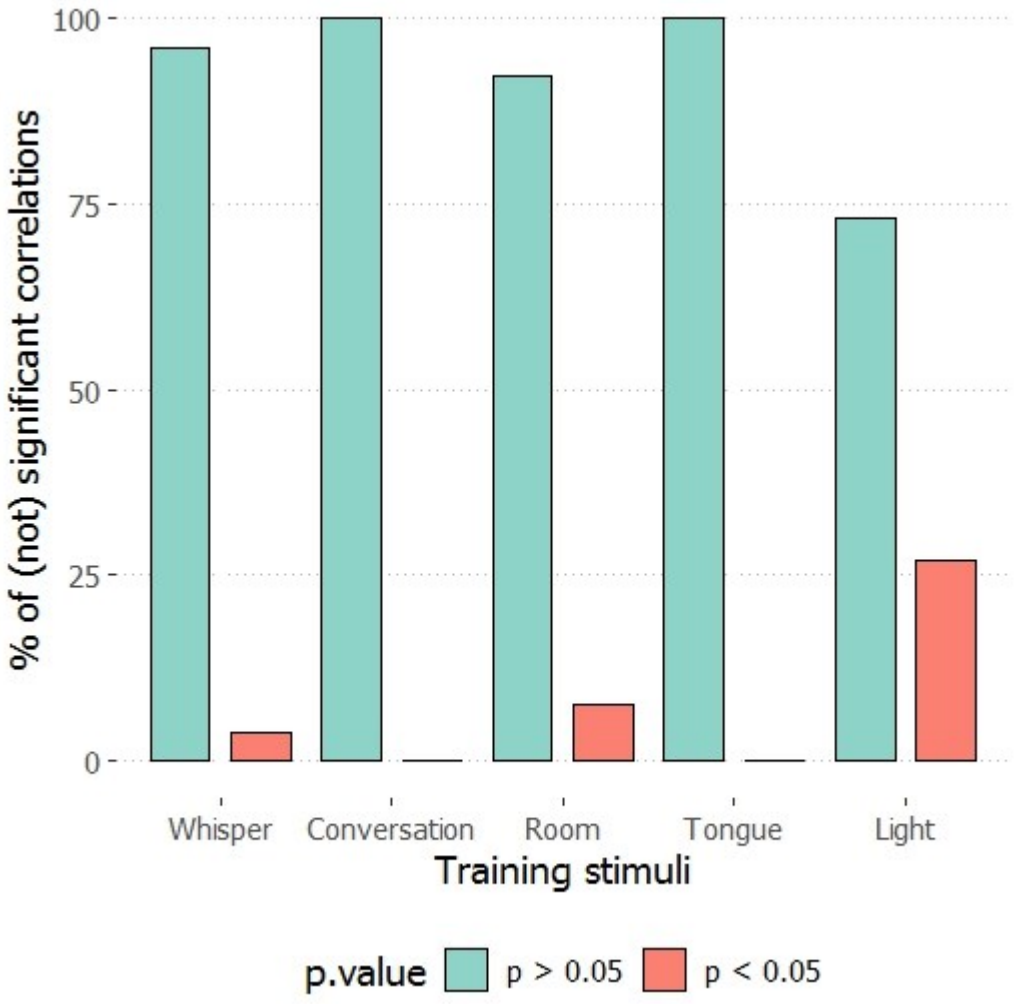

Figure S4.5: Percentage of (not) significant correlations (Spearman's rho) between the five extraoral recalled sensations employed during the gLMS training (Whisper: loudness of a whisper; Conversation: loudness of a conversation; Room: brightness of a well-lit room; Tongue: pain of biting your tongue; Light: brightest light ever seen) [39] and orosensory responsiveness (gLMS; n = 26 attributes) in actual foods. Aquamarine bars depict the percentage of not significant correlations ($p > 0.05$), whilst salmon bars show the percentage of significant ($p < 0.05$) correlations observed.

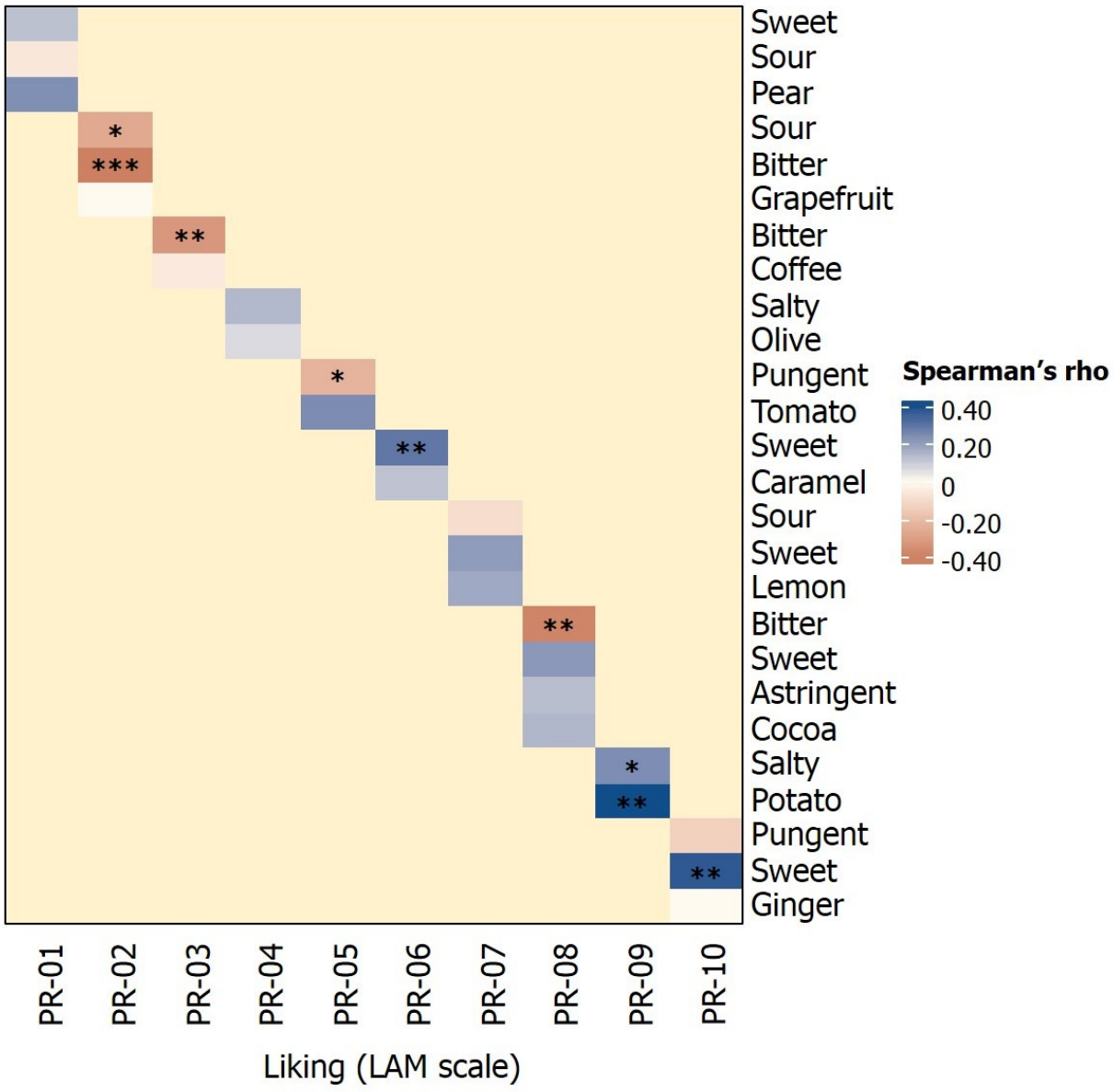

Figure S4.6: Heatmap showing product-specific (PR-) correlations (Spearman's rho) between orosensory responsiveness (generalized Labeled Magnitude Scale – gLMS) [36] and liking responses (Labeled Affective Magnitude Scale) [37]. In each food, hedonic responses were separately correlated with its relevant ballot of sensory attributes (e.g., Sweet, Sour, Pear for PR-01). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

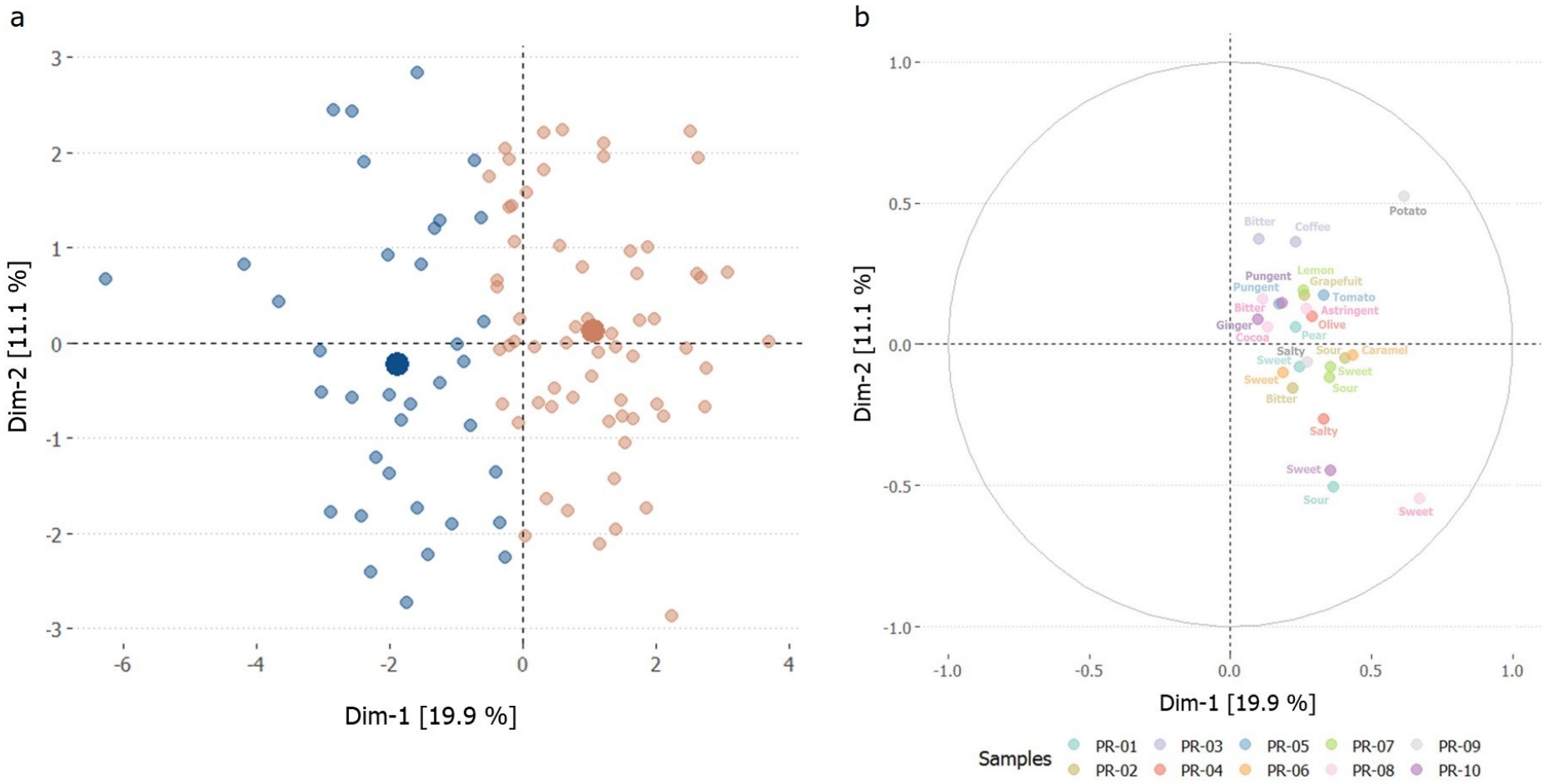

Figure S4.7: First two dimensions of the individual factor map (a) produced by Multiple Factor Analysis (MFA). Large circles (dark-blue and orange) represent the MFA centroids, while transparent circles (CL-1: dark-blue; CL-2: orange) depict participants' configuration in the bi-dimensional space and cluster assignment after K-means partitioning on the first three components of the MFA model (39.7 % of variance). (b) Variable factor map of the MFA model. Circles represent correlations between the ballot of sensory attributes rated within each stimulus and the first two dimensions of the MFA model.

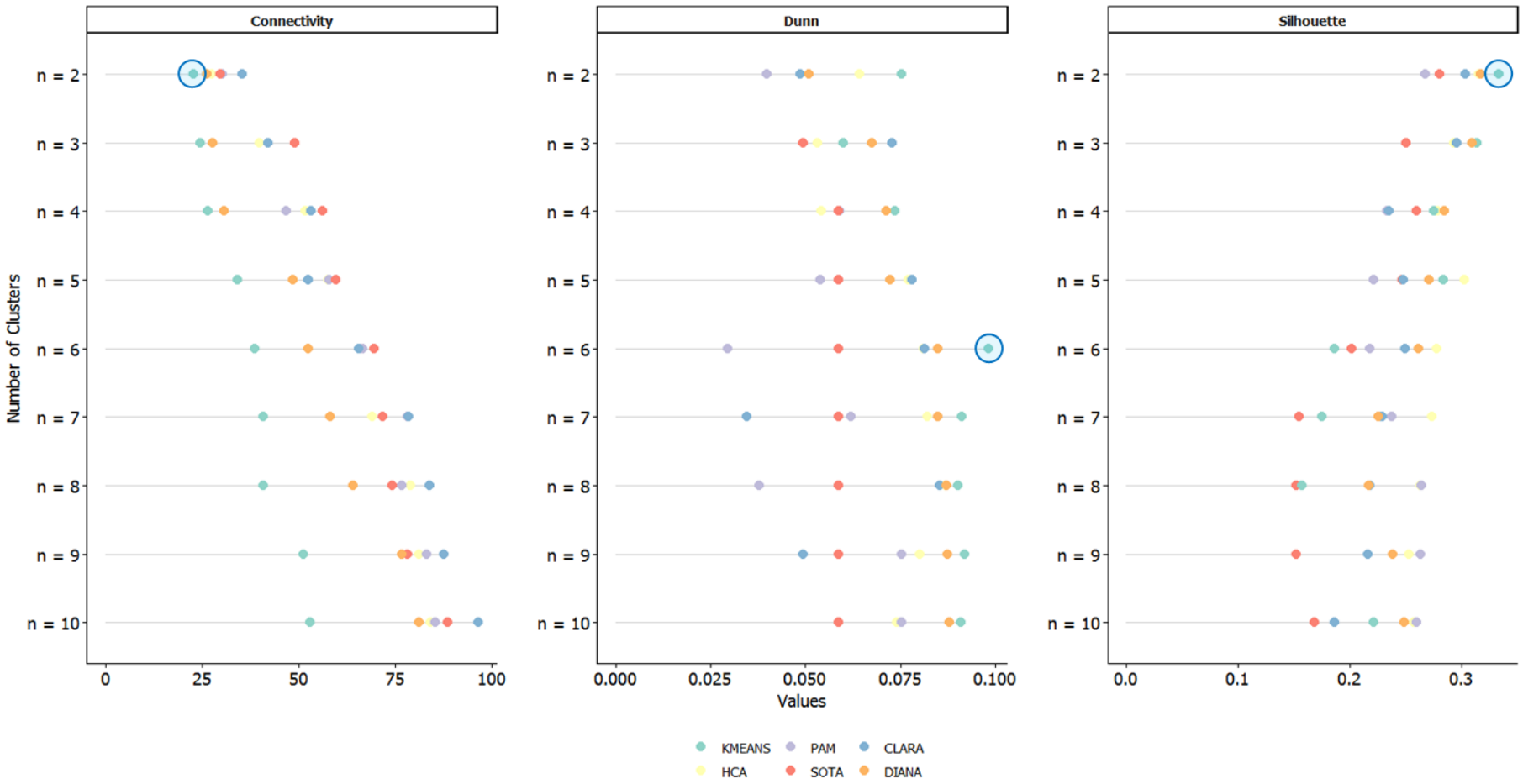

Figure S4.8: Results from the data-driven segmentation approach. Indices of connectivity, Dunn and silhouette within six algorithms (KMEANS, HCA, PAM, SOTA, CLARA, DIANA) at an increasing number of cluster solutions (n = 2 to n = 10) were tested. Optimal partitioning was defined in the light of the lowest cluster connectivity and the highest silhouette width and Dunn index observed [61]. Best algorithm and cluster solution across the three indices is highlighted by the light-blue transparent circles.

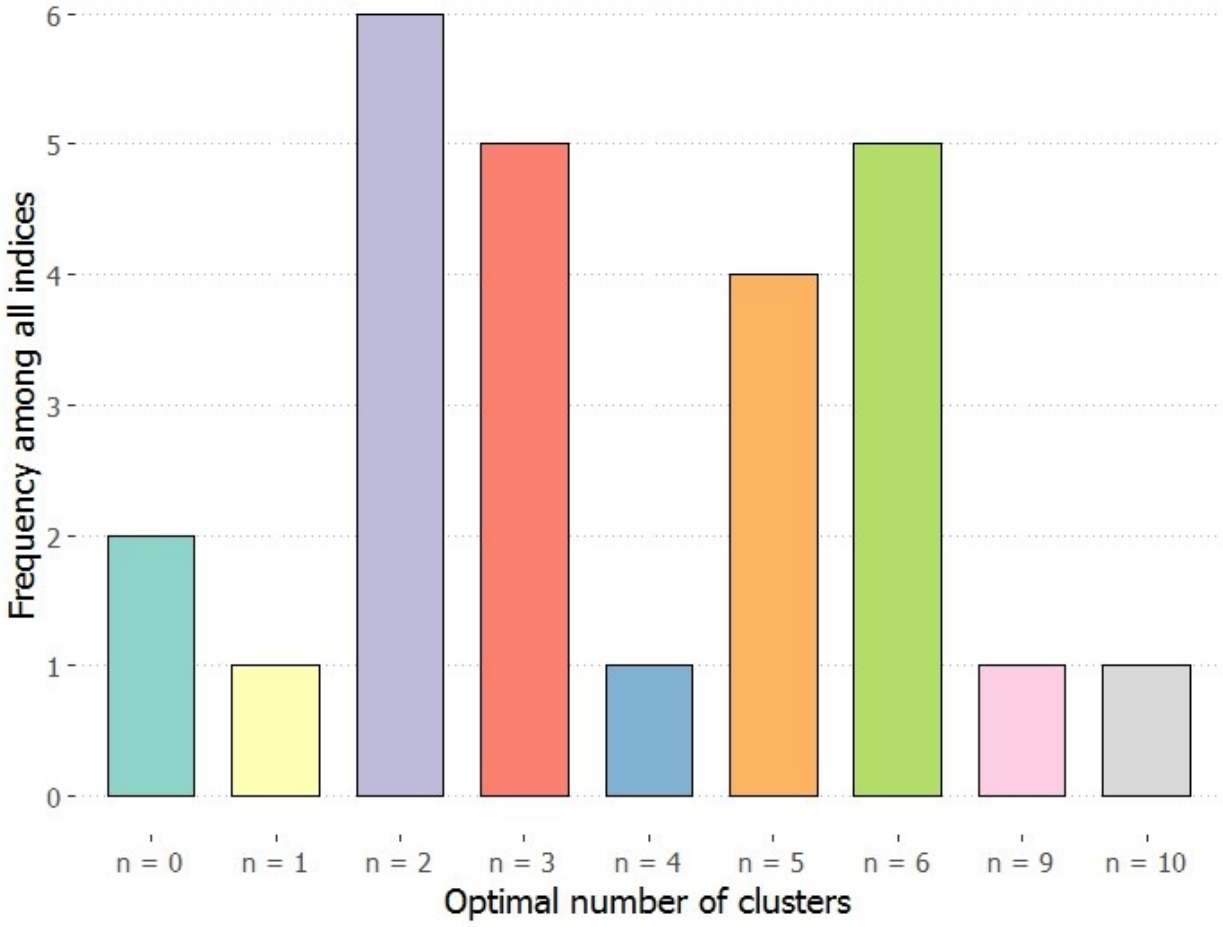

Figure S4.9: Optimal number of clusters. Best partition was determined according to the 26 cluster validation indices implemented into the *NbClust* R package [70], and the number of clusters indicated by the majority of these indices is intended as the best fitting.

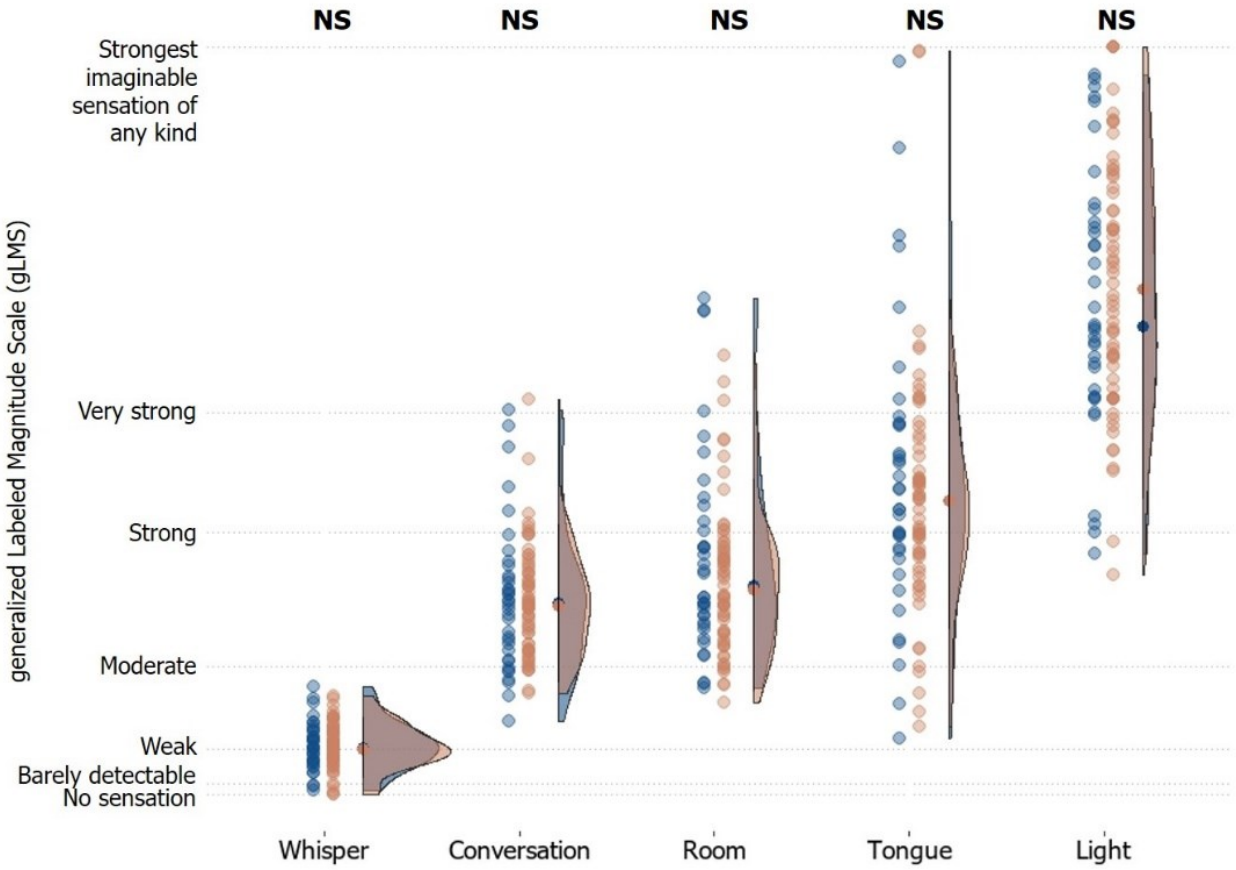

Figure S4.10: Differences between CL-1 (dark-blue; n = 36) and CL-2 (orange; n = 64) as a function of recalled intensities elicited by the five extra-oral stimuli (Whisper: loudness of a whisper; Conversation: loudness of a conversation; Room: brightness of a well-lit room; Tongue: pain of biting your tongue; Light: brightest light ever seen) used during the gLMS training [39]. 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. Significance rates observed after permutational Wilcoxon rank sum test (n = 10000) are depicted (NS = $p > 0.05$).

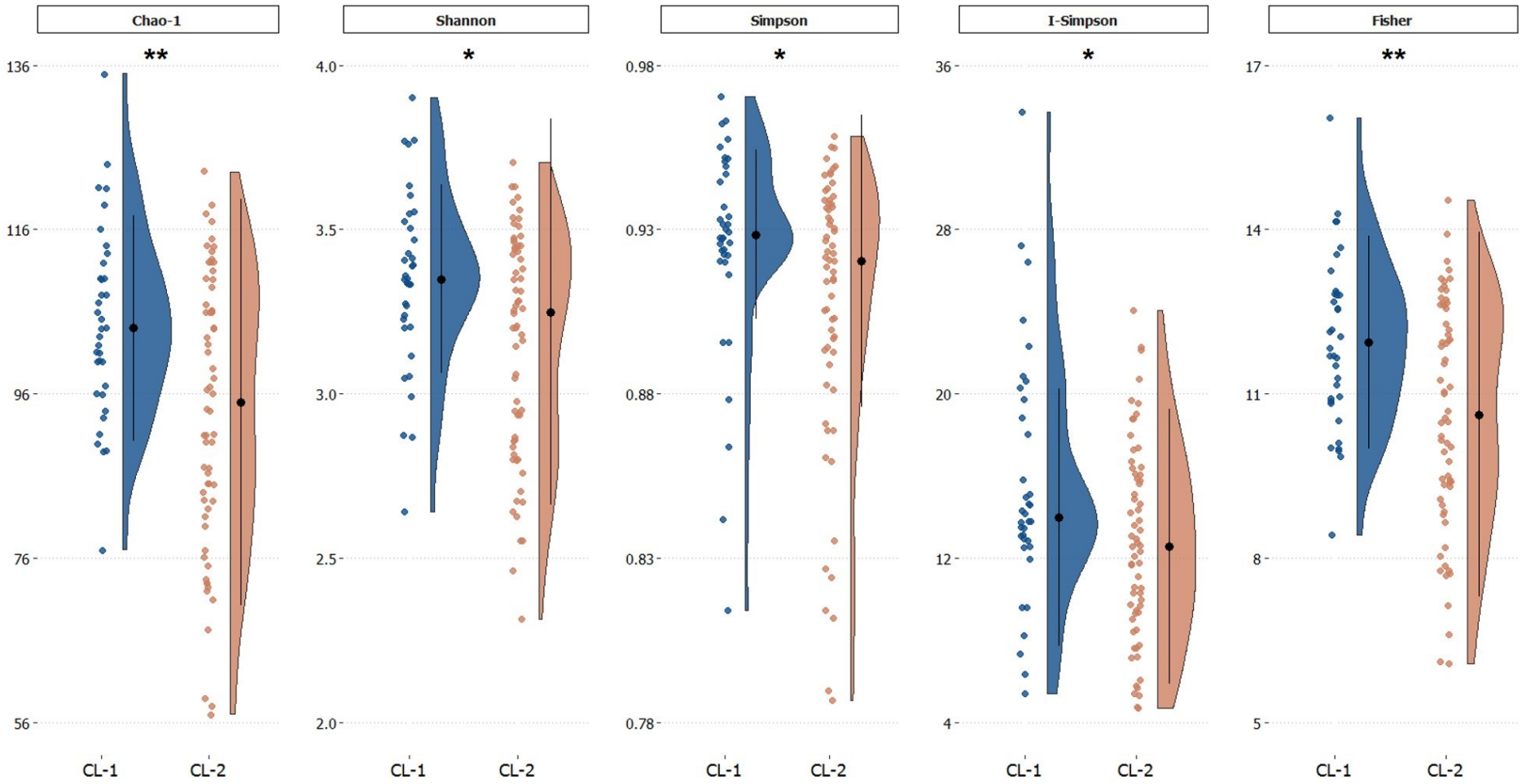

Figure S4.11: Differences in intra-sample (α-) diversity between taste profiles. Metrics underlying both taxonomic richness and/or evenness (i.e., Chao-1, Shannon, Simpson, Inverse Simpson, Fisher indices) are presented. The raincloud plot graphically represents data distribution (the "cloud"), individual raw observations (the "rain"), and the median (filled circle) ± IQR (perpendicular black line) within each taste profile (CL-1, CL-2). Statistically significant differences observed after permutational Wilcoxon rank sum test (n = 10000) are depicted (* = $p < 0.05$; ** = $p < 0.01$).

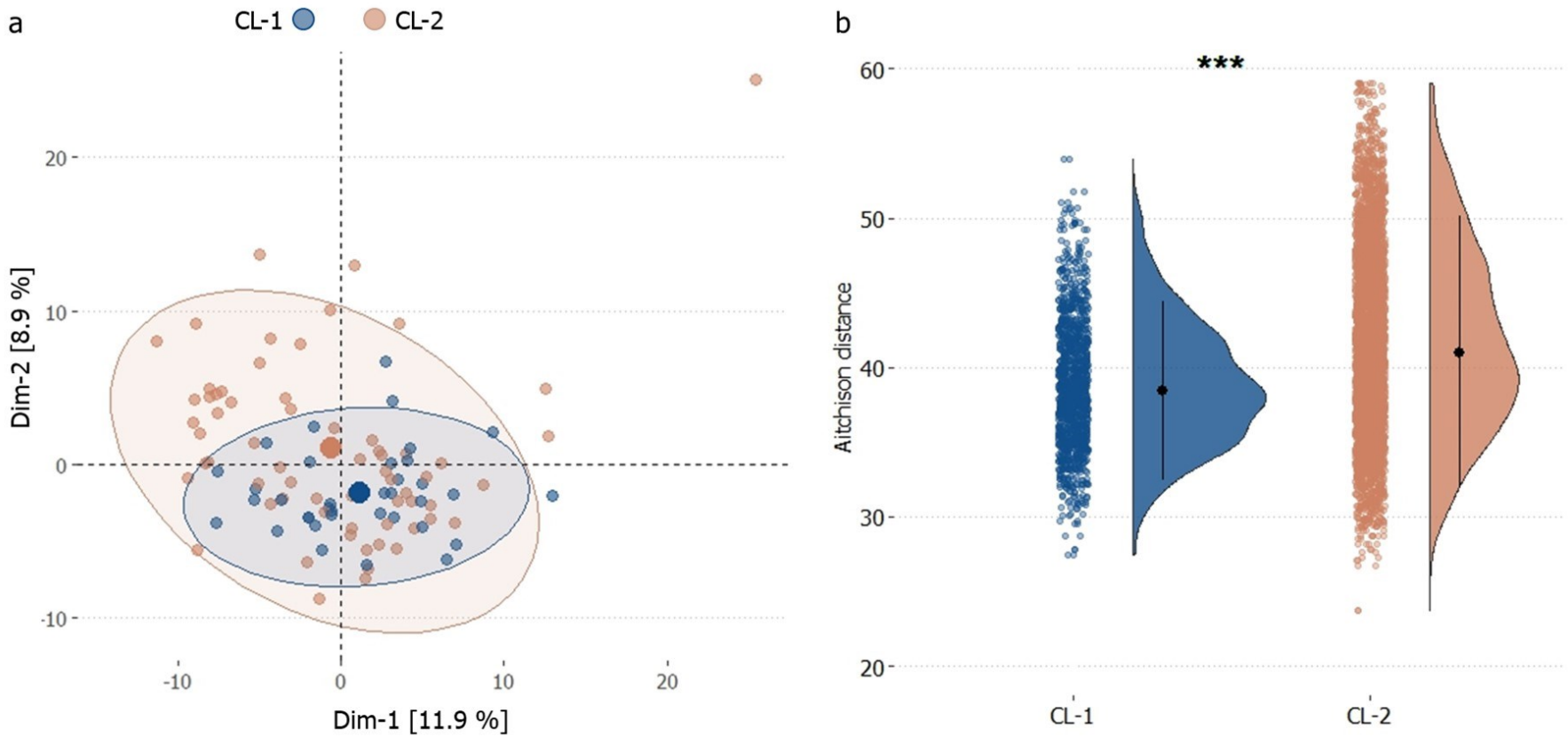

Figure S4.12: Compositional Principal Component Analysis (PCA, a) on fecal (β-) dissimilarities (i.e., Aitchison distances) between taste profiles [64]. Transparent colored circles represent members of taste profiles (CL-1 = dark-blue; CL-2 = orange), while the largest circles (n = 2) indicate PCA centroids. b) Differences in Aitchison distances within groups (CL-1 = dark-blue; CL-2 = orange). The 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 also represented (*** = $p < 0.001$).

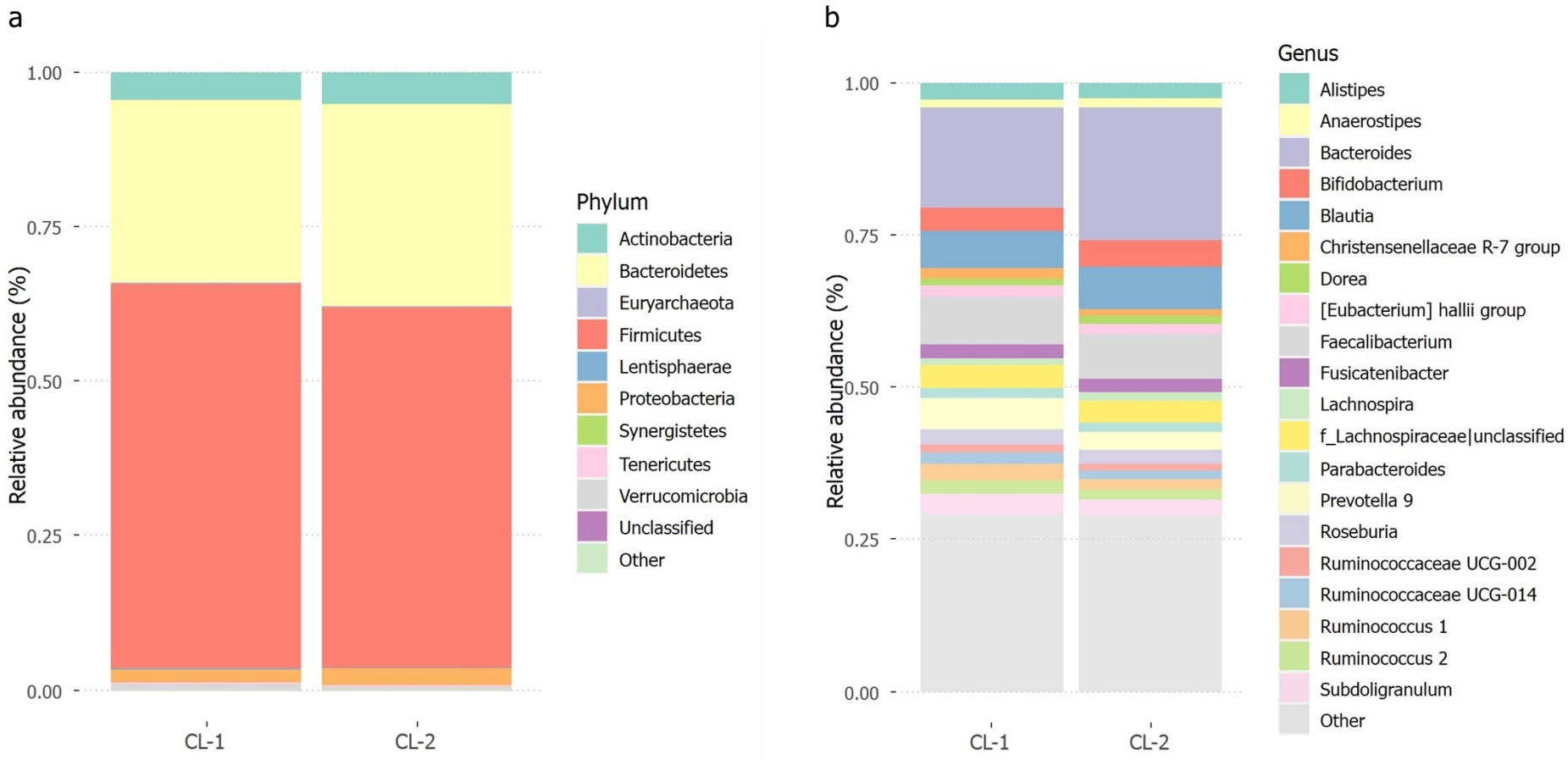

Figure S4.13: Relative abundances (%) of phyla (a) and genera (b) housed by the gut microbiota of CL-1 (n = 36) and CL-2 (n = 64). The top 10 most abundant phyla (a) and the top 20 most abundant genera (b) observed are illustrated. Proportions of phyla and genera are graphically represented as ordered from top to bottom in their respective legends (i.e., Phylum and Genus).

4.8.2 | Tables

Table S4.1: Socio-demographic characteristics of participants. Differences in socio-demographic variables between women (n = 52) and men (n = 48) are listed (p.value). Values in bold are intended as statistically significant ($p < 0.05$).

	Total	Women	Men	p.value
N	100	52	48	/
Age (mean ± SD)	23.7 ± 3.9	24.2 ± 3.9	23.1 ± 4.0	0.178†
BMI (mean ± SD)	22.5 ± 2.6	21.8 ± 2.5	23.2 ± 2.6	**0.007**†
Diet				
Omnivores	62	31	31	0.575††
Flexitarians	28	15	13	
Vegetarians	9	6	3	
Vegans	1	0	1	
Educational level				
Lower secondary school	1	1	0	0.085††
Upper secondary school	44	18	26	
Bachelor's degree	17	9	8	
Master's degree	32	22	10	
Post-degree (e.g., PhD)	5	1	4	
Other	1	1	0	
Job Occupation				
Student	65	32	33	0.454††
Employee	33	19	14	
Merchant / Craftsman	1	1	0	
Freelancer	1	0	1	
Income				
0 - 4.999 €	55	25	30	0.514††
5.000 - 5.999 €	6	4	2	
10.000 - 14.999 €	14	10	4	
15.000 - 19.999 €	13	8	5	
20.000 - 24.999 €	6	3	3	
25.000 - 29.999 €	4	1	3	
30.000 - 39.999 €	2	1	1	

†p.value calculated via unpaired t-test.
††p.value calculated via chi-squared test

Table S4.2: Full list of the inclusion and exclusion criteria used by the current study. The list most entirely relies on the protocol used by the Human Microbiome Project [35] to identify the core human gut microbiota in health. Volunteers who simultaneously met the inclusion criteria and none of the exclusion criteria were declared eligible.

Inclusion criteria	Exclusion criteria
• Both genders • Age between 18 and 30	• Body Mass Index greater than or equal to 30 or less than or equal to 18 • Habitual smokers • Pregnancy and lactation • Use of pre- and probiotics, and antibiotics in the 6 months prior to the start of the study • Use of drugs that could alter taste functions and/or gut microbiota homeostasis in the 6 months prior to the start of the study (e.g., proton pump inhibitors, psychotropic drugs, laxatives, antihistamines, immunomodulators) • Evidence of chronic alcohol consumption in the 6 months prior to start of the study (or ongoing), defined as more than 5 servings (14 gr/serving) per day • Food allergies and/or intolerances to: eggs, gluten, lactose, nuts, peanuts, sesame, soy • Major surgery of the gastrointestinal tract, with the exception of cholecystectomy and appendectomy, in the past 5 years. Any major bowel resection at any time • Historical and/or ongoing diagnosis of: 1- COVID-19 disease 2- Taste and smell disorders (ageusia, hypogeusia, anosmia, hyposmia) 3- Inflammatory bowel diseases (e.g., Crohn's disease, coeliac disease) 4- Oral diseases and infections (e.g., periodontitis, chronic xerostomia, oral candidiasis) 5- Type I and Type II diabetes 6- Psychiatric diseases (e.g., anorexia nervosa, bulimia, major depressive disorder) 7- Neurodegenerative diseases (e.g., Parkinson's diseases, multiple sclerosis) 8- Chronic, viral, alcoholic and nonalcoholic liver diseases (e.g., cirrhosis, viral hepatitis, hepatic steatosis) 9- *Clostridium difficile, Helicobacter pylori,* or *HIV* infection 10- Oncological diseases

Table S4.3: Battery of psychometric measures collected in the present study. Questionnaires, number of items, domains, internal reliability (ordinal Cronbach's α) [96,97], rating scales, score (s) computation strategy, and references are tabulated.

Questionnaire	Items (Domains)	Ordinal Cronbach's α	Scale	Score (s) computation	References
Food Neophobia Scale	10 (1)	0.902	7-point Likert Scale (1 = "Strongly disagree"; 7 = "Strongly agree")	Negatively-keyed items (n = 5) were reversed prior to sum up the 10 items	[31,41]
Trait Anxiety Inventory	20 (1)	0.941	4-point Likert Scale (1 = "Almost never"; 4 = "Almost always)	Anxiety-absent items (n = 9) were reversed prior to sum up the 20 items	[42,43]
lth and Taste Attitude Scales	38 (6)		7-point Likert Scale (1 = "Strongly disagree"; 7 = "Strongly agree")	Subscale scores (n = 6) were computed by reversing all negatively-keyed items (half of the items) in each domain prior to average individual ratings	[44,45]
General Health Interest	8	0.842			
Natural Product Interest	6	0.885			
Light Product Interest	6	0.811			
Craving for sweet foods	6	0.930			
Using food as reward	6	0.908			
Pleasure	6	0.658			
ting Behaviour Questionnaire	33 (3)		5-point Likert Scale (1 = "Never"; 7 = "Very often")	Subscale scores (n = 3) were computed by summing up individual ratings in each domain	[46,47]
Restrained eating	10	0.904			
Emotional eating	13	0.930			
External eating	10	0.823			
Big Five Inventory	44 (5)		5-point Likert Scale (1 = "Strongly disagree"; 7 = "Strongly agree")	Subscale scores (n = 5) were computed by reversing all negatively-keyed items (n = 19) in each domain before averaging individual	[48,49]
Extraversion	8	0.898			
Agreeableness	9	0.767			
Conscientiousness	9	0.871			
Neuroticism	8	0.869			
Openness	10	0.856			

Table S4.4: Significantly different ($p < 0.05$) habitual nutrient intakes between taste profiles (CL-1, CL-2). Data are expressed as median ± IQR. Differences between taste profiles are also tabulated (p.value), and calculated via permutational Wilcoxon rank sum test (n = 10000).

	CL-1 (n = 36)	CL-2 (n = 64)	p.value
Macronutrients (g)			
Proteins	76.7 ± 15	70.4 ± 13.6	0.038
Vegetable proteins	31.8 ± 10.6	27.4 ± 12.9	0.002
Saturated fats	18.5 ± 4.6	20.8 ± 5.9	0.005
Linolenic acid	0.6 ± 0.4	0.5 ± 0.4	0.030
Glucose	8.5 ± 4.7	5.4 ± 6.3	0.011
Fructose	11.7 ± 9.5	6.8 ± 7	0.003
Fibers	20.2 ± 9.7	17.5 ± 9.3	0.001
Insoluble fibers	9.3 ± 7.7	8.2 ± 6.8	0.021
Soluble fibers	3.4 ± 1.9	2.8 ± 1.5	0.021
Essential AA (mg)			
Arginine†	3017.2 ± 930.0	2625.3 ± 835.2	0.007
Lisine	3878.8 ± 1155.0	3294.5 ± 1109.0	0.038
Threonine	2345.7 ± 642.0	2091.1 ± 710.9	0.042
Organic compounds (mg)			
Malic acid	985.6 ± 797.4	529.6 ± 784.1	0.005
Tartaric acid	294.4 ± 387.9	146 ± 280.2	0.015
Purines	160.5 ± 134.7	117.6 ± 87.5	0.006
Minerals (mg)			
Fluorine	0.2 ± 0.1	0.1 ± 0.1	0.001
Iron	12.5 ± 4.4	9.6 ± 4.9	< 0.001
Magnesium	313.6 ± 114	280.3 ± 89.4	0.008
Nickel (mcg)	129.2 ± 81	96.7 ± 76	0.003
Calcium:Phosphorus	10.5 ± 5.5	7.7 ± 3.9	0.002
Phosphorus	1192.8 ± 286.2	1073.5 ± 282.7	0.005
Potassium	3075.5 ± 770.4	2606.6 ± 1018.8	0.001
Selenium (mcg)	40.7 ± 24.4	32.7 ± 27.2	0.028
Zinc	11.1 ± 4	8.8 ± 3.4	0.003
Vitamins			
Vitamin A (mcg)	1170.5 ± 697.7	908.6 ± 755.8	0.039
Vitamin B2 (mg)	1.6 ± 0.5	1.3 ± 0.5	0.006
Vitamin B3 (mg)	17.2 ± 4.8	14.8 ± 6.4	0.018
Vitamin B8 (mcg)	37.5 ± 12.8	31.5 ± 17.6	0.018
Vitamin B9 (mcg)	336.3 ± 177.9	253.5 ± 165.9	0.003
Vitamin C (mg)	160 ± 66.4	118.3 ± 87.2	0.005
Vitamin K3 (mcg)	179.2 ± 185.8	100.9 ± 119.5	0.001

†to be considered as a semi-essential amino acid.

Table S4.5: Relative abundances (%) of significantly different ($p < 0.05$) gut microbial genera between taste profiles. Phylum-, family- and genus-level taxonomic annotations are provided. The main statistic produced by ANCOM-BC (W), and the significance rates after Benjamini-Hochberg adjustment (p.value) are also listed.

Phylum	Family	Genus	CL-1 (n = 36)	CL-2 (n = 64)	W	p.value
Actinobacteriota	*Eggerthellaceae*	*Eggerthella*	0.00 ± 0.01	0.03 ± 0.07	3.09	0.029
Firmicutes	*Anaerovoracaceae*	*Family XIII UCG-001*	0.04 ± 0.03	0.02 ± 0.03	3.66	0.006
Firmicutes	*Clostridiales vadinBB60 group*	*uncultured bacterium*	0.31 ± 0.61	0.20 ± 0.40	4.08	0.004
Firmicutes	*Lachnospiraceae*	*Marvinbryantia*	0.17 ± 0.19	0.08 ± 0.08	3.95	0.004
Firmicutes	*Lachnospiraceae*	*[Eubacterium] eligens group*	0.63 ± 0.69	0.37 ± 0.52	3.22	0.020
Firmicutes	*Lachnospiraceae*	*[Eubacterium] xylanophilum group*	0.28 ± 0.22	0.20 ± 0.27	5.06	< 0.001
Firmicutes	*Lachnospiraceae*	*[Ruminococcus] gnavus group*	0.00 ± 0.01	0.24 ± 0.88	2.97	0.039
Firmicutes	*Ruminococcaceae*	*Ruminiclostridium 6*	0.44 ± 0.69	0.15 ± 0.37	3.85	0.004
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae NK4A214 group*	0.44 ± 0.68	0.27 ± 0.46	3.23	0.020
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae UCG-002*	1.55 ± 1.33	1.22 ± 1.36	3.51	0.009
Firmicutes	*Ruminococcaceae*	*Ruminococcaceae UCG-005*	0.57 ± 0.49	0.46 ± 0.58	3.77	0.005
Firmicutes	*Ruminococcaceae*	*Ruminococcus 1*	2.79 ± 2.15	1.84 ± 1.80	3.86	0.004
Firmicutes	*Ruminococcaceae*	*[Eubacterium] coprostanoligenes group*	1.02 ± 1.24	0.79 ± 1.02	3.51	0.009

4.9 | References

1. Swinburn, B. A. *et al.* The global obesity pandemic: Shaped by global drivers and local environments. *Lancet* **378**, 804–814 (2011).
2. Köster, E. P. Diversity in the determinants of food choice: A psychological perspective. *Food Qual. Prefer.* **20**, 70–82 (2009).
3. Cox, D. N., Hendrie, G. A. & Carty, D. Sensitivity, hedonics and preferences for basic tastes and fat amongst adults and children of differing weight status: A comprehensive review. *Food Quality and Preference* vol. 48 359–367 (2016).
4. Duffy, V. B. Variation in oral sensation: Implications for diet and health. *Curr. Opin. Gastroenterol.* **23**, 171–177 (2007).
5. Bartoshuk, L. M. Comparing sensory experiences across individuals: Recent psychophysical advances illuminate genetic variation in taste perception. *Chem. Senses* **25**, 447–460 (2000).
6. Fischer, M. E. *et al.* Factors related to fungiform papillae density: The beaver dam offspring study. *Chem. Senses* **38**, 669–677 (2013).
7. Essick, G. K., Chopra, A., Guest, S. & McGlone, F. Lingual tactile acuity, taste perception, and the density and diameter of fungiform papillae in female subjects. *Physiol. Behav.* **80**, 289–302 (2003).
8. Hayes, J. E. & Keast, R. S. J. Two decades of supertasting: Where do we stand? *Physiol. Behav.* **104**, 1072–1074 (2011).
9. Dinnella, C. *et al.* Individual variation in PROP status, fungiform papillae density, and responsiveness to taste stimuli in a large population sample. *Chem. Senses* **43**, 697–710 (2018).
10. Garneau, N. L. *et al.* Crowdsourcing taste research: Genetic and phenotypic predictors of bitter taste perception as a model. *Front. Integr. Neurosci.* **8**, 33 (2014).
11. Nolden, A. A., McGeary, J. E. & Hayes, J. E. Predominant Qualities Evoked by Quinine, Sucrose, and Capsaicin Associate with PROP Bitterness, but not TAS2R38 Genotype. *Chem. Senses* **45**, 383–390 (2020).

12. Alcock, J., Maley, C. C. & Aktipis, C. A. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays* vol. 36 940–949 (2014).

13. Leung, R. & Covasa, M. Do gut microbes taste? *Nutrients* **13**, 2581 (2021).

14. Schwartz, M., Canon, F., Feron, G., Neiers, F. & Gamero, A. Impact of oral microbiota on flavor perception: From food processing to in-mouth metabolization. *Foods* vol. 10 (2021).

15. Herman, A. & Bajaka, A. The role of the intestinal microbiota in eating disorders – bulimia nervosa and binge eating disorder. *Psychiatry Research* vol. 300 113923 (2021).

16. Efeyan, A., Comb, W. C. & Sabatini, D. M. Nutrient-sensing mechanisms and pathways. *Nature* **517**, 302–310 (2015).

17. Feng, Y. *et al.* The Associations between Biochemical and Microbiological Variables and Taste Differ in Whole Saliva and in the Film Lining the Tongue. *Biomed Res. Int.* **2018**, (2018).

18. Vascellari, S. *et al.* Genetic variants of TAS2R38 bitter taste receptor associate with distinct gut microbiota traits in Parkinson's disease: A pilot study. *Int. J. Biol. Macromol.* **165**, 665–674 (2020).

19. Cattaneo, C. *et al.* New insights into the relationship between taste perception and oral microbiota composition. *Sci. Rep.* **9**, (2019).

20. Cattaneo, C., Riso, P., Laureati, M., Gargari, G. & Pagliarini, E. Exploring associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake. *Nutrients* **11**, 1167 (2019).

21. Swartz, T. D., Duca, F. A., De Wouters, T., Sakar, Y. & Covasa, M. 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. *Br. J. Nutr.* **107**, 621–630 (2012).

22. Turner, A. *et al.* Interactions between bitter taste, diet and dysbiosis: Consequences for appetite and obesity. *Nutrients* **10**, 1336 (2018).

23. Turner, A. *et al.* Interactions between taste receptors and the gastrointestinal microbiome in inflammatory bowel disease. *Journal of Nutrition and Intermediary Metabolism* vol. 18 (2019).

24. Guo, P., Zhang, K., Ma, X. & He, P. Clostridium species as probiotics: Potentials and challenges. *J.*

Anim. Sci. Biotechnol. **11**, 1–10 (2020).

25. Besnard, P. *et al.* Obese Subjects with Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. *Sci. Rep.* **8**, (2018).
26. Fluitman, K. S. *et al.* Associations of the oral microbiota and Candida with taste, smell, appetite and undernutrition in older adults. *Sci. Rep.* **11**, 1–11 (2021).
27. Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M. & Hummel, T. The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS One* **7**, e36557 (2012).
28. Puputti, S., Aisala, H., Hoppu, U. & Sandell, M. Multidimensional measurement of individual differences in taste perception. *Food Qual. Prefer.* **65**, 10–17 (2018).
29. Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E. & Keast, R. The Relationships Between Common Measurements of Taste Function. *Chemosens. Percept.* **8**, 11–18 (2015).
30. Piochi, M., Dinnella, C., Spinelli, S., Monteleone, E. & Torri, L. Individual differences in responsiveness to oral sensations and odours with chemesthetic activity: Relationships between sensory modalities and impact on the hedonic response. *Food Qual. Prefer.* **88**, (2021).
31. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–124 (2018).
32. Spinelli, S. *et al.* Personality traits and gender influence liking and choice of food pungency. *Food Qual. Prefer.* **66**, 113–126 (2018).
33. Vujkovic-Cvijin, I. *et al.* Host variables confound gut microbiota studies of human disease. *Nature* **587**, 448–454 (2020).
34. Diószegi, J., Llanaj, E. & Ádány, R. Genetic Background of Taste Perception, Taste Preferences, and Its Nutritional Implications: A Systematic Review. *Front. Genet.* **10**, 1272 (2019).
35. Turnbaugh, P. J. *et al.* The Human Microbiome Project. *Nature* vol. 449 804–810 (2007).
36. Bartoshuk, L. M. *et al.* Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. *Physiol. Behav.* **82**, 109–114 (2004).

37. Schutz, H. G. & Cardello, A. V. A labeled affective magnitude (LAM) scale for assessing food liking/disliking. *J. Sens. Stud.* **16**, 117–159 (2001).

38. Dinnella, C. *et al.* Remote testing: Sensory test during Covid-19 pandemic and beyond. *Food Qual. Prefer.* **96**, 104437 (2022).

39. Hayes, J. E., Allen, A. L. & Bennett, S. M. Direct comparison of the generalized visual analog scale (gVAS) and general labeled magnitude scale (gLMS). *Food Qual. Prefer.* **28**, 36–44 (2013).

40. Smutzer, G., Desai, H., Coldwell, S. E. & Griffith, J. W. Validation of edible taste strips for assessing PROP taste perception. *Chem. Senses* **38**, 529–539 (2013).

41. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).

42. Pedrabrissi, L., Santinello, M. Verifica della validità dello STAI forma Y di Spielberger. *Giunti Organ. Spec.* **15**, 1–23 (2016).

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

44. Roininen, K. & Tuorila, H. Health and taste attitudes in the prediction of use frequency and choice between less healthy and more healthy snacks. *Food Qual. Prefer.* **10**, 357–365 (1999).

45. Saba, A. *et al.* Measuring consumers attitudes towards health and taste and their association with food-related life-styles and preferences. *Food Qual. Prefer.* **73**, 25–37 (2019).

46. van Strien, T., Frijters, J. E. R., Bergers, G. P. A. & Defares, P. B. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *Int. J. Eat. Disord.* **5**, 295–315 (1986).

47. Monteleone, E. *et al.* Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Qual. Prefer.* **59**, 123–140 (2017).

48. Corr, P. J. *Handbook of Personality Psychology*. *Personality and Individual Differences* vol. 24 (Guilford Press, 1998).

49. Fossati, A., Borroni, S., Marchione, D. & Maffei, C. Reliability and validity of its italian translation in

three independent nonclinical samples. *Eur. J. Psychol. Assess.* **27**, 50–58 (2011).

50. De Backer, C. J. S. & Hudders, L. Meat morals: Relationship between meat consumption consumer attitudes towards human and animal welfare and moral behavior. *Meat Sci.* **99**, 68–74 (2015).

51. Thompson, F. E. & Subar, A. F. Dietary assessment methodology. *Nutr. Prev. Treat. Dis.* 5–48 (2017) doi:10.1016/B978-0-12-802928-2.00001-1.

52. Poslusna, K., Ruprich, J., De Vries, J. H. M., Jakubikova, M. & Van'T Veer, P. Misreporting of energy and micronutrient intake estimated by food records and 24hour recalls, control and adjustment methods in practice. *Br. J. Nutr.* **101**, S73–S85 (2009).

53. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1–e1 (2013).

54. Apprill, A., Mcnally, S., Parsons, R. & Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75**, 129–137 (2015).

55. Gaudioso, G. *et al.* 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. *Int. J. Mol. Sci.* **22**, 5454 (2021).

56. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).

57. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **41**, (2013).

58. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019).

59. McMurdie, P. J. & Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**, e61217 (2013).

60. Kassambara, A. *Practical guide to cluster analysis in R: unsupervised machine learning*. *Journal of Computational and Graphical Statistics* vol. 1 (Sthda, 2017).

61. Brock, G., Pihur, V., Datta, S. & Datta, S. ClValid: An R package for cluster validation. *J. Stat. Softw.* **25**, 1–22 (2008).

62. Kaiser, H. F. The Application of Electronic Computers to Factor Analysis. *Educ. Psychol. Meas.* **20**, 141–151 (1960).

63. Endrizzi, I. *et al.* Relationships between intensity and liking for chemosensory stimuli in food models: A large-scale consumer segmentation. *Foods* **11**, 5 (2022).

64. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. & Egozcue, J. J. Microbiome datasets are compositional: And this is not optional. *Front. Microbiol.* **8**, 2224 (2017).

65. Palarea-Albaladejo, J. & Martín-Fernández, J. A. ZCompositions - R package for multivariate imputation of left-censored data under a compositional approach. *Chemom. Intell. Lab. Syst.* **143**, 85–96 (2015).

66. Nearing, J. T. *et al.* Microbiome differential abundance methods produce different results across 38 datasets. *Nat. Commun.* **13**, 1–16 (2022).

67. Lin, H. & Peddada, S. Das. Analysis of compositions of microbiomes with bias correction. *Nat. Commun.* **11**, 1–11 (2020).

68. Andy Bunn, M. K. A language and environment for statistical computing. *R Found. Stat. Comput.* **10**, 11–18 (2017).

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

70. Charrad, M., Ghazzali, N., Boiteau, V. & Niknafs, A. Nbclust: An R package for determining the relevant number of clusters in a data set. *J. Stat. Softw.* **61**, 1–36 (2014).

71. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**, 927–930 (2003).

72. Rinninella, E. *et al.* What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* **7**, 14 (2019).

73. Breen, S. P., Etter, N. M., Ziegler, G. R. & Hayes, J. E. Oral somatosensatory acuity is related to particle size perception in chocolate. *Sci. Rep.* **9**, 1–10 (2019).

74. Linne, B. & Simons, C. T. Quantification of oral roughness perception and comparison with mechanism of astringency perception. *Chem. Senses* **42**, 525–535 (2017).

75. Mani, E. *et al.* Exploring the association between oral tactile sensitivity measures and phenotypic markers of oral responsiveness. *J. Texture Stud.* **53**, 345–356 (2022).

76. Lawless, H. A comparison of different methods used to assess sensitivity to the taste of phenylthiocarbamide (PTC). *Chem. Senses* **5**, 247–256 (1980).

77. Zhao, L., Kirkmeyer, S. V. & Tepper, B. J. A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. *Physiol. Behav.* **78**, 625–633 (2003).

78. Burton, P., J. Smit, H. & J. Lightowler, H. The influence of restrained and external eating patterns on overeating. *Appetite* **49**, 191–197 (2007).

79. Hayes, J. E., Sullivan, B. S. & Duffy, V. B. Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiol. Behav.* **100**, 369–380 (2010).

80. Vacca, M. *et al.* The controversial role of human gut lachnospiraceae. *Microorganisms* **8**, (2020).

81. Cronin, P., Joyce, S. A., O'toole, P. W. & O'connor, E. M. Dietary fibre modulates the gut microbiota. *Nutrients* **13**, (2021).

82. Miao, Z. *et al.* Gut microbiota signatures of long-term and short-term plant-based dietary pattern and cardiometabolic health: a prospective cohort study. *BMC Med.* **20**, 1–15 (2022).

83. Zhang, L. *et al.* Metabolic phenotypes and the gut microbiota in response to dietary resistant starch type 2 in normal-weight subjects: a randomized crossover trial. *Sci. Rep.* **9**, 1–11 (2019).

84. Ma, E. *et al.* Long-term association between diet quality and characteristics of the gut microbiome in the multiethnic cohort study. *Br. J. Nutr.* **128**, 93–102 (2022).

85. Wolters, M. *et al.* Dietary fat, the gut microbiota, and metabolic health – A systematic review conducted within the MyNewGut project. *Clin. Nutr.* **38**, 2504–2520 (2019).

86. Kenny, D. J. *et al.* Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level. *Cell Host Microbe* **28**, 245-257.e6 (2020).

87. Ohira, H., Tsutsui, W. & Fujioka, Y. Are short chain fatty acids in gut microbiota defensive players for

inflammation and atherosclerosis? *J. Atheroscler. Thromb.* **24**, 660–672 (2017).

88. Henke, M. T. *et al.* Ruminococcus gnavus, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 12672–12677 (2019).

89. Pascal, V. *et al.* A microbial signature for Crohn's disease. *Gut* **66**, 813–822 (2017).

90. Zouiouich, S. *et al.* Markers of metabolic health and gut microbiome diversity: findings from two population-based cohort studies. *Diabetologia* **64**, 1749–1759 (2021).

91. Le Chatelier, E. *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).

92. Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. & Willerslev, E. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* **21**, 2045–2050 (2012).

93. Ranjan, R., Rani, A., Metwally, A., McGee, H. S. & Perkins, D. L. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem. Biophys. Res. Commun.* **469**, 967–977 (2016).

94. Sauvageot, N. *et al.* Stability-based validation of dietary patterns obtained by cluster analysis. *Nutr. J.* **16**, 1–13 (2017).

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

96. Gadermann, A. M., Guhn, M. & Zumbo, B. D. Estimating ordinal reliability for likert-type and ordinal item response data: A conceptual, empirical, and practical guide. *Pract. Assessment, Res. Eval.* **17**, 1–13 (2012).

97. Zumbo, B. D., Gadermann, A. M. & Zeisser, C. Ordinal versions of coefficients alpha and theta for likert rating scales. *J. Mod. Appl. Stat. Methods* **6**, 21–29 (2007).

Chapter 5

Salivary microbial profiles associate with responsiveness to warning oral sensations and dietary intakes

Menghi, L., Cliceri, D., Fava, F., Pindo, M., Gaudioso, G., Giacalone, D. & Gasperi, F.

Food Research International (2023)
Accepted

CHAPTER 5:

SALIVARY MICROBIAL PROFILES ASSOCIATE WITH RESPONSIVENESS TO WARNING ORAL SENSATIONS AND DIETARY INTAKES

Abstract

Oral microbiota-host interactions are gaining recognition as potential factors contributing to interindividual variations in taste perception. However, whether such possible links imply specific bacterial co-occurrence networks remains unknown. To address this issue, we used 16s rRNA gene sequencing to profile the salivary microbiota of 100 healthy individuals (52 % women; 18-30 y/o), who provided hedonic and psychophysical responses to 5 liquid and 5 solid commercially-available foods, each chosen to elicit a target sensation (sweet, sour, bitter, salty, pungent). The same cohort also completed several psychometric measures and a 4-day food diary. Unsupervised data-driven clustering of genus-level Aitchison distances supported the existence of two salivary microbial profiles (CL-1, CL-2). While CL-1 (n = 57; 49.1 % women) exhibited higher α-diversity metrics and was enriched in microbial genera assigned to the class *Clostridia* (e.g., *Lachnospiraceae_[G-3]*), CL-2 (n = 43; 55.8 % women) harbored greater amounts of taxa with potential cariogenic effects (e.g., genus *Lactobacillus*) and significantly lower abundances of inferred MetaCyc pathways related to the metabolic fate of acetate. Intriguingly, CL-2 showed enhanced responsiveness to warning oral sensations (bitter, sour, astringent) and a higher propensity to crave sweet foods or engage in prosocial behaviors. Further, the same cluster reported habitually consuming more simple carbohydrates and fewer beneficial nutrients (vegetable proteins, monounsaturated fatty acids). In summary, while the mediating role of participants' baseline diet on findings can not be definitively excluded, this work provides evidence suggesting that microbe-microbe and microbe-taste interactions may exert an

influence on dietary habits and motivates further research to uncover a potential “core” taste-related salivary microbiota.

Keywords: Oral microbiota; Saliva; Taste; Diet; Liking; Psychological traits

5.1 | Introduction

The human oral cavity constitutes a flourishing habitat for a plethora of microbial taxa. Bacterial colonization targets both mucosal and dental surfaces, resulting in the construction of unique ecological niches that harbor distinct groups of microorganisms [1]. Nevertheless, this large site-dependent microbial heterogeneity is largely represented by the ensemble of microbes suspended in saliva. Indeed, saliva bathes the entire oral cavity and is enriched with numerous bacterial residents shed from all oral surfaces [2], which are closely associated with health status and dietary patterns [3,4].

In addition to the salivary microbiota, differences in eating habits have also been linked to a myriad of host biological and attitudinal factors [5], of which the sense of taste is among the most influential [6]. Taste perception varies widely between individuals, and several anatomical, demographic, psychological or genetic sources of variation have been extensively discussed (e.g., [7,8]). In particular, humans differ significantly in their genetically mediated responsiveness to bitter-tasting compounds, such as phenylthiocarbamide or 6-n-propylthiouracil (PROP) [9].

Phenotypic responses to PROP bitterness range from null (Non Tasters: NTs) to moderate (Medium Tasters: MTs) or extreme (Super Tasters: STs), and these are mainly ascribed to variations in the haplotypes of the TAS2R38 gene [10,11]. Increasingly, ample evidence suggests that PROP perception is associated with acuity for a variety of oral stimuli (e.g., [12–14]), which has motivated its frequent use as a marker of generalized hypergeusia. However, recent large-scale studies noted that weak PROP tasting does not necessarily correspond to weak perceived intensities of other taste qualities [15], the relationship between PROP and taste perception follows a linear pattern only in individuals with low density of fungiform papillae [12]. Hence, while PROP acuity remains a valid indicator of interindividual differences in chemoperception, its actual predictive value needs to be reconsidered [12,14,15].

In attempting to further elucidate the sources of individual taste variation, it is worth noting that taste perception is intimately related to saliva. The salivary milieu (e.g., flow rate, protein content, ionic composition) plays a key role in taste and flavor perception, as it serves as a solubilizer of tastants and flavor-active stimuli that facilitates their transport near chemosensory receptors [16]. Interestingly, chemoreceptors can also be triggered by a variety of salivary metabolites produced by microbial enzymes from endogenous and exogenous sources (nutrients), whose activity would increase the peri-receptor concentration of such molecules and ultimately induce sensory adaptation [17–20]. Alternatively, salivary microbial communities may convert taste-active compounds into tasteless molecules, thereby promoting a reduced stimulation of chemosensory systems [18]. Moreover, two recent reviews [18,19] have examined a possible manipulative effect of oral microbes on taste receptor expression, although its direct consequences on taste are still debated.

Against this backdrop, the oral microbiota is emerging as an additional candidate to explain interindividual differences in taste perception [17,20–27]. For instance, acutely hospitalized elderly with poor sour sensitivity were found to harbor greater amounts of salivary *Lactobacilli* [23], whilst the abundances of the phylum *Actinobacteria* were inversely related to salt detection abilities in a small cohort of 21 adults [17]. Furthermore, children and adolescents with higher proportions of salivary members of the phylum *Bacteroidetes* exhibited a generalized lower sensitivity to tastes (especially bitter), regardless of BMI [24]. Lastly, differences in salivary microbial composition have also been discussed in relation to PROP taster status, with STs housing more bacterial taxa of the genera *Prevotella, Veillonella*, *Alloprevotella*, and *Actinomyces* compared to NTs at baseline of an 11-day oral rinsing intervention [25]. However, the mechanistic links underlying such cross-sectional associations remain to be demonstrated empirically.

It is noteworthy that the polymicrobial salivary ecosystem is governed by ecological relationships among its residents [28]. Indeed, microbes benefit from salivary environmental features

(e.g., pH, nutrient availability, oxygen) and favorable inter-species interactions to establish opportunistic patterns of co-existence [28]. These have recently referred to as "*stomatotypes*" [29], and have been linked to various lifestyle and diet-related factors, including oral health [2], drinking water composition [29], and sugar intakes [30]. Given how oral health and diet may relate to the sense of taste [31,32], this raises the question on whether specific co-occurring guilds of salivary bacteria can efficiently distinguish individuals with varying taste acuity and dietary habits. Importantly, unlike canonical bioinformatic pipelines aimed at detecting single bacterial markers related to taste phenotypes, identifying groups of co-abundant microbes offers the key advantage of capturing ecological relationships between taxa that are likely to share the same nutritional needs and exert similar functionalities to interact with the host [25,33].

However, this would not be very informative without filling in a few additional gaps in the newly-born taste-oriented microbiome research field. First is the widespread use of detection thresholds as exclusive measure of taste functioning in response to artificial stimuli such as single taste aqueous solutions [20,22,24,27] or paper strips [17,23]. Although common in the taste literature, detection thresholds suffer from poor predictive power with respect to everyday food perceptions, which are mostly allocated at suprathreshold level [34,35]. Hence, favoring complex food stimuli to water or paper-based single tastant delivers would tremendously increase the real-life (ecological) power of the results.

Second, conscious taste perception never arises as a standalone phenomenon. Indeed, hedonics, attitudes or psychological traits (among others) act as important confounders of how food tastes to different individuals (e.g., [36,37]), and can promote dissimilarities in food choices [5] ultimately shaping the salivary microbiota [26]. Despite this, such factors have only been sparsely operationalized in previous reports [24,26]. Lastly, research examining the interactions between taste, oral microbiota and dietary outcomes is still surprisingly little [27], thus raising the need to expand current knowledge.

Taken collectively, these limitations highlight the importance of: a) investigating the associations between taste and the salivary microbiota in light of the ecological links among its inhabitants; b) assessing taste function using more ecologically valid stimuli and psychophysical tools; c) considering key mediators of taste perception; d) collecting measures of dietary behavior.

To address these gaps, this double-blind cross-sectional study represents the first attempt to examine whether distinct salivary microbial networks co-occur with variations in taste and flavor perception of real foods in healthy individuals, and to explore how these associations may reflect self-reported habitual dietary patterns. This work builds on a previous investigation [38], in which the same individuals (n = 100) were assessed for associations between taste perception, diet, and distal gut microbiota, controlling for a variety of hedonic, attitudinal and psychometric covariates.

5.2 | Methods

5.2.1 | Participants

Data were collected from a cohort of 100 healthy young Italian adults (52 % women; 18-30 y/o; mean age = 23.7 ± 3.9; mean BMI = 22.5 ± 2.6) as part of a project focusing on the complex crosstalk between taste and the oral or gut microbiota [38]. Attendance was contingent upon meeting a long list of inclusion criteria designed to limit the influence of factors altering taste and/or oral microbial homeostasis on outcomes. Eligibility criteria included, but were not limited to: no evidence of a historical or current diagnosis of COVID-19; oral (e.g., periodontitis, chronic xerostomia) and gastrointestinal (e.g., coeliac or Crohn's disease) diseases; taste disorders (e.g., dysgeusia, anosmia); habitual smoking; pregnancy or breastfeeding; BMI ≥ 30 or ≤ 18.5 Kg/m^2; use of medications that may affect taste function (e.g., proton pump inhibitors) and use of (pre-) probiotics and antibiotics within the last 6 months prior to study entry. Full details on recruitment strategy, exclusion criteria and demographics can be found in our previous work [38].

Informed consent was obtained electronically from each participant. Additionally, the study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the University of Trento (n° prot. 2020-040, approved on 08/02/2021).

5.2.2 | General procedure

In brief, eligible participants attended remotely a double-blind, 7-day (D-) lasting design. Data collection took place during four separate daily sessions (D1, D2, D3, D4), which volunteers were asked to complete within one week, along with a not-consecutive 4-day food diary (D1-D7). Remote attendance was subject to the autonomous collection of a bag containing all the materials needed to complete the study from various pick-up points located in the Autonomous Province of Trento (Italy). Also, bag collection was preparatory to accessing the online platforms used for data collection.

On D1, participants rated the bitterness evoked by two PROP impregnated strips using the generalized Labeled Magnitude Scale (gLMS) [9], whilst D2 was designed to collect hedonics (Labeled Affective Magnitude scale; LAM) [39] in response to 5 liquid and 5 solid commercially-available foods (Table 1), each evocative of a target taste (sweet, sour, bitter, salty) or sensation (pungent). Immediately after, familiarity (5-point Likert scale; 1 = Not at all familiar, 5 = Extremely familiar), and weekly frequency of consumption (5-point Likert scale; 1 = Never, 5 = Five or more times/week) of the evaluated food product categories were tested.

D3 was instead planned to gather a detailed demographic, attitudinal and psychosocial profile of our cohort, while the final working session (D4) was devoted to the collection of an unstimulated saliva sample just before asking volunteers to rate the intensity (gLMS) of oral sensations elicited by the ten products previously evaluated on D2. Completion of D4 marked the end of the protocol, and participants were allowed to return their salivary sample (D4-D7) upon confirming to have fulfilled all the expected tasks.

Both sensory (D1, D4) and hedonic (D2) work sessions were preceded by extensive text and video training to avoid idiosyncratic use of the gLMS [9] and LAM [39] scales, respectively. In addition, access to the online data collection platforms was only granted if volunteers confirmed to have refrained from eating, drinking (except water) and brushing their teeth for at least 3 h prior to the test, as well as to have respected common practices in sensory testing [38]. Lastly, data were collected using Eye Question (Elst, The Netherlands) and Alchemer (Louisville, CO, USA) software, with the exception of food diaries, which were collected and processed via Dietosystem® (DS Medica, Milan, Italy). The reader is referred to Menghi *et al.* [38] for a detailed overview of the data collection.

5.2.3 | Salivary microbial samples

5.2.3.1 | Sampling and processing

Salivary sampling was performed as the initial task planned by the last work session (D4) using OMNIgene®•ORAL (OM-501, DNA Genotek Inc., Ottawa, Canada), a self-administered commercial kit that allows long-term (up to 1 year) storage of microbial DNA at room temperature. Volunteers were given text and video instructions to self-collect an unstimulated salivary sample by dropping it into the funnel attached to the OM-501 tube until it reached a marked fill line (~ 1 mL). Participants then mixed the DNA stabilization buffer (~ 1 mL; stored in the funnel lid) with their salivary specimen before closing and shaking the tube for 30 s to ensure homogenization. Overall, samples were delivered within 1 day (mean = 1.1 ± 2.3 days) upon collection and were immediately incubated in a water bath at 50° C for 1 hour, mixed by inversion for 30 s, and aliquoted into 1 mL vials before storage at ambient temperature until ready to use.

Genomic DNA was extracted from 1 mL of stabilized saliva using the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany) in line with manufacturer's recommendations. Total bacterial DNA was then quantified using a NanoDrop™ Lite spectrophotometer (Thermo Fisher

Scientific, Waltham, MA, USA), and stored at -80 °C prior to amplification. Upon ice thawing, the V3-V4 hypervariable regions of the 16s rRNA gene were PCR-amplified using the bacterial primers 341 F (5' CCTACGGGNGGCWGCAG 3') and 806 R (5' GACTACNVGGGTWTCTAATCC 3') complemented by Illumina overhang adapters [40,41]. Lastly, amplicon libraries were prepared and purified according to Gaudioso *et al.* [42], and subsequently sequenced using 300bp paired-end reads on an Illumina® MiSeq platform (Control Software 2.6.2.1 and Real-Time Analysis software 1.18.54; San Diego, CA, USA).

5.2.3.2 | Bioinformatics

Demultiplexed and primer free paired-end sequences were analyzed following the standard DADA2 microbiome pipeline [43]. In brief, amplicon products were first filtered and truncated (F: 283 and R: 213 bp) to retain sequences with a median PHRED > 30. Later, filtered reads were dereplicated and denoised before being inferred as amplicon sequence variants (ASVs). Complete denoised sequences were then derived by merging forward and reverse ASVs before removing chimeras as well as *Cyanobacteria* and mitochondrial reads. Lastly, ASVs were blasted against the expanded Human Oral Microbiome Database (version 15.22) [44] for taxonomic assignment up to the genus level.

5.2.4 | Sensory assessments

5.2.4.1 | Food products

Five liquid and five solid commercially-available, ready-to-use, easily portionable, and widely distributed food products in the Italian market were selected for our scopes (Table 5.1). Most importantly, each matrix was expected to clearly evoke a recognizable target taste (sweet, sour, bitter, salty) or sensation (pungent) falling within an expected moderate/very strong range of intensity within

the gLMS [38]. Adherence to with the above criteria was corroborated by the results of three pilot tests. For details, please refer to our previous work [38] (Chapter 4; section 4.2.3).

Table 5.1: Sets and evaluation order of food stimuli (Product) presented to participants within the liking (D2) and intensity (D4) tasks. Food-related information (Brand, Amount, Consistency) plus the full list of sensory descriptors (Target sensation, Other sensations, Flavor) here used are also tabulated. * In PR-08, participants rated bitterness before sweetness, astringency, and cocoa flavor. Adapted from "*Variations in oral responsiveness associate with specific signatures in the gut microbiota and modulate dietary habits*" by Menghi, L. *et al.* (2023), Food Quality and Preference, 106, 104790, p. 4.

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	Spicy tomato sauce "Arrabbiata" (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 (Cipster, 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

5.2.4.2 | Scaling and sensory testing

Scale training and sensory assessments were conducted as previously reported [38]. Briefly, to avoid artifacts in the use of the gLMS (0 = no sensation, 100 = the strongest imaginable sensation of any kind; D1 and D4) and LAM (0 = greatest imaginable dislike, 100 = greatest imaginable like; D2) scales, volunteers were given extensive text and video instructions following standard guidelines [9,34,39]. Particular emphasis was placed on the gLMS training. In this vein, volunteers were oriented to the scale by being asked to rate the intensity of five recalled extraoral stimuli that were assumed to be representative of the full length of the scale [9,34,38,45]. Phenotypic responses to PROP bitterness were operationalized using two commercial paper strips (3-5 μg, MediSens, Groningen, The Netherlands). To this end, participants were instructed to place each strip on the tongue and spread the stimulus over the mucosal surfaces of the mouth for 10 s [46] before expectorating and waiting a further 5 s to rate the intensity of bitterness (gLMS). The ratings were then averaged and volunteers classed as Non Tasters (NTs) or Super Tasters (STs) when their scores fell below the 25th (gLMS < 9.5) and above the 75th (gLMS > 31.3) percentiles of the distribution, respectively. All others were assigned to the Medium Tasters (MTs) group (9.5 ≥ gLMS ≤ 31.3).

Instead, two separate sets of five foods each were instead presented in the liking (D2) and intensity (D4) tasks (Table 5.1). Liquid products (Set 1) were always evaluated first, followed by the five solid foods (Set 2) after a 5 min break. Both series of stimuli (Set 1; Set 2) were presented in a fixed order and rated for relevant sensory attributes (Table 5.1). Particularly, pungent foods (spicy tomato sauce and ginger candy) were always assessed as last to control for potential carry-over effects driven by irritants, while psychophysical responses to target sensations were constantly collected before other product-related taste qualities and flavors (Table 5.1). Additionally, prior to beginning

the evaluation of each sample, volunteers were provided with video instructions to facilitate sample preparation and portioning.

All food products were stripped of brand identifiers and stored in paper-based packaging with a 3-digit code and a label, the color of which had to be indicated by the participants (at the end of each evaluation) to ascertain the correctness of the tasting protocol [38]. Lastly, mineral water and unsalted crackers were used as palate cleaners during a 90 s break between all tastings (D1, D2, D4).

5.2.5 | Psychometrics and demographics

On D3, volunteers were administered a series of psychometric and demographic measures to capture salient background information that could potentially act as confounders within the links examined here [38]. In detail, our cohort was first screened for food neophobia (i.e., unwillingness to try novel foods) and trait-anxiety levels using the common Food Neophobia Scale [36,47] and the State-Trait Anxiety Inventory Questionnaire (trait anxiety subscale) [48,49]. Next, attitudes towards health- or taste-guided food choices and eating behaviors were operationalized using the Health and Taste Attitude Scale [50,51] and the Dutch Eating Behaviour Questionnaire [8,52], respectively. Lastly, volunteers were tested for facets of personality by the Big Five Inventory [53,54]. All measures employed were back-translated and validated into Italian, and were found to be internally consistent [38]. Full details on the psychometric tools used by the current study can be found in Menghi *et al.* [38].

Participants then completed the D3 tasks by providing demographic information, i.e., age, gender, weight and height (later used to calculate the BMI as Kg/m^2), education level, occupation and annual income, as well as self-reported habitual diet type [38]. For this latter purpose, we classified participants as omnivores, flexitarians, vegetarians or vegans as described in [55].

5.2.6 | Food diaries

Each participant was also invited to complete a food diary, listing all foods and beverages consumed on four (3 weekdays and 1 weekend day) of the seven days foreseen by the protocol. The food record was preceded by a practical video tutorial, which was designed to train the volunteers: a) to be meticulous in recording recipes and grammages; b) to use a photographic food atlas (Istituto Scotti Bassani, Milan, Italy), based on the Italian Food Composition Database (https://www.ieo.it/bda), as a landmark to detail portion sizes [38].

Dietary intakes were tracked using a smartphone app (Dietosystem®, DS Medica, Milan, Italy), while Terapia Alimentare Dietosystem® (version 295 17.00.02, DS Medica, Milan, Italy) was employed to extract energy intakes (as Kcal) and exact quantities of a lengthy list (n = 93) of macro- and micronutrients. Lastly, the data were energy-adjusted by residual method [56] to unpack nutrient density from variations in total energy intake attributable to known covariates (gender, BMI, physical activity) and averaged.

5.2.7 | Data analysis

Homogeneous groups of individuals with patterns of similarly co-occurring microbial consortia were derived using a compositionally coherent data analysis approach [57], designed to capture the co-dependent nature of high-throughput sequencing products. First, the unfiltered ASV table was collapsed at the genus level before treating zero counts with geometric Bayesian-multiplicative replacement [58], and centering log-ratio transforming the data [57]. Second, we calculated the Euclidean (or Aitchison) distances between samples [57], which served as input for the subsequent derivation of salivary microbial patterns. For the latter, we replicated a previously reported [38] unsupervised data-driven clustering method to objectively determine the algorithm and the number of clusters that best fit the data. According to previous tutorials [59], six different algorithms (HCA, K-means, PAM, SOTA, CLARA, and DIANA clustering) were tested within various clustering solutions

(from 2 to 10), and evidence of optimal partitioning was certified by the lowest cluster connectivity and the highest silhouette width and Dunn index obtained [60]. Salivary microbial profiles were then checked for differences in α-diversity metrics (Chao-1, Fischer, Shannon, Inverse Simpson indices) by Wilcoxon Rank Sum Test, whilst multivariate analysis of variance (PERMANOVA, n = 10000) was used to test for dissimilarities in Aitchison distances (inter-sample β-diversity) between groups.

Next, to deal with rarely occurring bacteria and reduce the likelihood of potential false positives, the differential abundance analysis was preceded by an unsupervised permutation (n = 10000) filtering of taxa with a null contribution to the total covariance of the data according to Smirnova *et al*. [61]. To this end, the functions *PERFect_sim* and the *PERFect_perm* from the R package *PERFect* were subsequently applied to the original ASV table with default parameters [61]. Clusters were thus checked for differentially abundant microbial taxa at different taxonomic levels (phylum, class, order, family, genus) using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) with default parameters [62]. The filtered ASV table was also used as input to infer the abundances of functional microbial pathways via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) [63], later mapped against the MetaCyc database for subsequent annotation. As recommended by the original authors [63], PICRUSt2 data should be treated as compositional. Therefore, we computed the Aitchison distances (as for bacterial counts) on the imputed bacterial functionalities and tested omnibus differences between clusters by PERMANOVA (n = 10000). Variations in MetaCyc pathway proportions between salivary microbial profiles were then tested using ANCOM-BC [62] as described above.

Lastly, differences between salivary microbial profiles as a function of hedonics, psychophysics in response to the relevant sensory ballot of the ten food stimuli, attitudes and psychological traits, and dietary habits were calculated using the Wilcoxon rank sum test. All data are summarized as median ± interquartile range (IQR) unless otherwise stated. A p value < 0.05 was

considered as statistically significant, and multiple inferences were adjusted with the Holm method [64].

5.2.8 | Software

Bioinformatics and statistics were run in R 4.2.2 [65], with the exception of the PICRUSt2 analysis, which was launched on a Python 3.8.0 machine. Among others, α-diversity metrics were calculated via *phyloseq* [66], while imputation of zeros and subsequent β-diversity analyses were performed using the packages *zCompositions* [58] and *vegan* [67] packages, respectively. The package *clValid* package [60] was instead used for cluster derivation and validation, while the package *PERFect* [61] was used to filter the ASV table before differential abundance analyses. Bacterial metabolic activities were predicted using the full PICRUSt2 pipeline [63] with default parameters (https://github.com/picrust/picrust2/wiki/Full-pipeline-script), and the *ANCOMBC* R package [62] was used to test differentially abundant taxa and inferred MetaCyc pathways between salivary microbial profiles.

5.3 | Results

5.3.1 | Overall salivary microbial ecology

16s rRNA gene amplicon sequencing of salivary specimens conclusively recovered 1717 unique ASVs from a total of 7,898,164 (mean = 78,981.6 ± 14,591.5 per sample) reads, which were later assigned to 10 phyla, 23 classes, 35 orders, 64 families, and 124 genera. Overall, the salivary microbiota of our cohort was governed by the phylum *Firmicutes* (74.1 ± 6.5 %), which has been reported to be the most abundant consortium inhabiting the healthy salivary microbial environment [68]. The phylum-level salivary bacterial composition of our cohort then included bacteria from the phyla *Actinobacteria* (24.3 ± 6.6 %), *Saccharibacteria_TM7* (1.2 ± 1.2 %), *Fusobacteria* (0.2 ± 0.8

%), *Proteobacteria* (0.1 ± 0.2 %), and *Bacteroidetes* (0.1 ± 0.1 %), which together accounted for over 99 % of the total amount of sequences generated.

Large overlaps were also observed at the genus-level with the 68 core residents of the healthy human salivary microbiota [68], with members of the genera *Streptococcus* (48.1 ± 6.5 %), *Rothia* (15.2 ± 7.1 %), *Veillonella* (7.2 ± 3.6 %), *Gemella* (6.6 ± 3.5 %), *Granulicatella* (5.1 ± 2.2 %), *Scaalia* (4.1 ± 2.4 %), *Actinomyces* (2.4 ± 2.4 %), *Atopobium* (1.5 ± 2.4 %), *Saccharibacteria_(TM7)_[G1]* (0.9 ± 1.0 %), *Oribacterium* (0.4 ± 0.4 %), *Lachnoanaerobaculum* (0.4 ± 0.4 %) and *Solobacterium* (0.4 ± 0.3 %), detected in at least 99 % of the samples.

5.3.2 | Derivation and description of salivary microbial profiles

To derive homogeneous salivary microbial profiles capturing ecological relationships among microbial taxa, a compositional data paradigm was employed [57]. To this end, the unfiltered ASV table (n = 1717) was collapsed to the genus level, zeros imputed and centered-log transformed prior to computing the Aitchison metric as a compositionally-aware pairwise distance between samples [57]. Such input was then employed to objectively determine the best clustering algorithm and grouping solution underlying the data. Results indicated that n = 2 clusters derived via Hierarchical Agglomerative Clustering (Figure 5.1) represented the optimal partition (Supplemental Figure S5.1), as provided the lowest connectivity and the highest silhouette and Dunn indices among all different combinations of algorithms (n = 6) and groupings (from 2 to 10) probed here [60]. As expected, the salivary microbial consortia of the two clusters largely mirrored those previously reported (section 5.3.1), with *Firmicutes* and *Streptococcus* dominating the phylum- and genus-level bacterial composition of both groups, respectively. Relative abundances of phyla (n = 10) and genera (top 20) as a function of salivary microbial profiles are shown in Supplemental Figure S5.2.

In line with the strict selection criteria of our protocol (section 4.2.2; Menghi *et al.*, [38]), both groups were homogeneous ($p > 0.05$) with respect to gender, age, BMI, habitual type of diet, food neophobia, trait anxiety, eating behaviors, and health-related attitudes towards foods (Table 5.2). In addition, both groups showed similar distributions of PROP taste phenotypes, though CL-2 tended to be populated by a higher number of PROP MTs (and fewer PROP NTs) than CL-1 (Table 5.2; $p = 0.084$). Nevertheless, some cluster-dependent differences in food-related attitudes and personality traits were observed (Table 5.2), with CL-2 exhibiting higher tendencies to crave sweet foods (Health and Taste Attitude Scale) [50] and to endorse prosocial behaviors (Agreeableness; Big Five Inventory) [54].

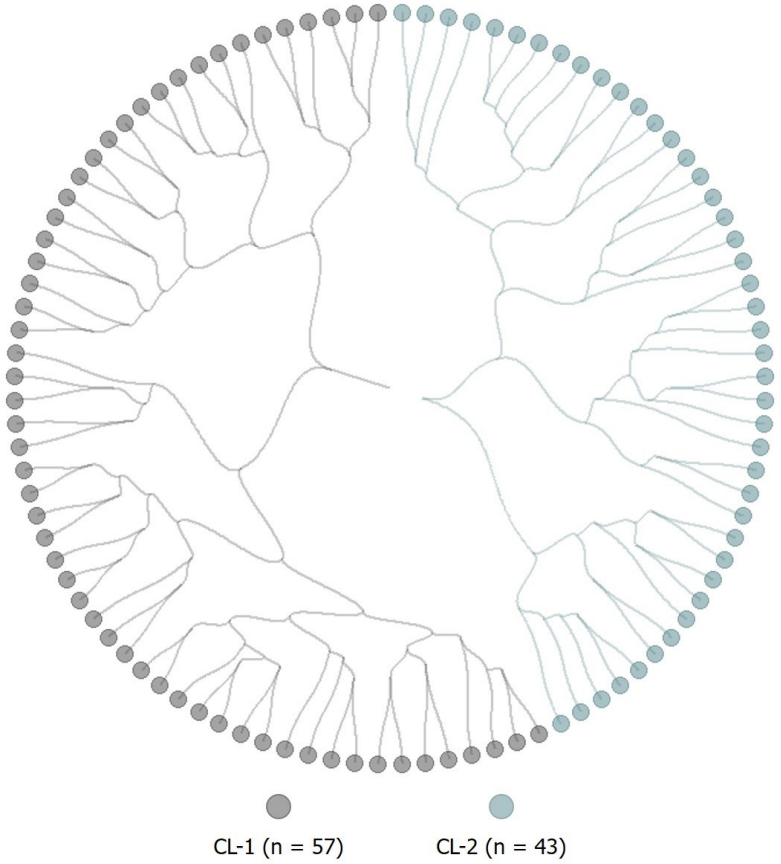

Figure 5.1: Circular dendrogram depicting the Hierarchical Agglomerative clustering (Ward D2) of Aitchison distances between members (transparent circles) of CL-1 (n = 57; light gray) and CL-2 (n = 43; cadet blue).

Table 5.2: Demographic, psychological, and food-related attitudinal background of salivary microbial profiles (CL-1 = 57; CL-2 = 43). Data are tabulated as raw observations (n), mean ± SD or median ± IQR whenever stated. Statistically significant (p.value; $p < 0.05$) differences between clusters are depicted in bold and computed via chi-squared test (†), unpaired t-test (††) or Wilcoxon rank sum test.

	CL-1 (n = 57)	CL-2 (n = 43)	p.value
Gender (n)			
Women	28	24	0.507†
Men	29	19	
Age (mean ± SD)	23.8 ± 3.8	23.6 ± 4.2	0.876††
BMI (mean ± SD)	22.4 ± 2.6	22.5 ± 2.7	0.859††
Diet (n)			
Omnivores	38	24	
Flexitarians	14	14	0.501†
Vegetarians	4	5	
Vegans	1	0	
Food Neophobia Scale (median ± IQR)	22 ± 10	24 ± 12.5	0.587
Trait Anxiety Inventory	44 ± 14	46 ± 12	0.524
Health and Taste Attitude Scale			
General health interest	4.5 ± 1.0	4.4 ± 1.4	0.302
Light product interest	3.8 ± 1.3	4.2 ± 1.3	0.113
Natural product interest	4.0 ± 1.8	3.8 ± 1.2	0.823
Craving for sweet foods	4.8 ± 1.8	5.7 ± 1.6	**0.005**
Using food as reward	4.7 ± 1.5	4.8 ± 1.8	0.900
Pleasure	4.8 ± 1.0	4.7 ± 1.2	0.613
Dutch Eating Behaviour Questionnaire			
Restrained Eating	2.6 ± 1.0	2.7 ± 1.3	0.805
Emotional Eating	2.5 ± 0.8	2.5 ± 0.9	0.269
External Eating	3.3 ± 0.4	3.2 ± 0.9	0.933
Big Five Inventory			
Extraversion	3.3 ± 1.1	3.3 ± 1.4	0.569
Agreeableness	3.6 ± 0.7	3.9 ± 0.7	**0.029**
Conscientiousness	3.7 ± 0.9	3.4 ± 0.8	0.329
Neuroticism	3.0 ± 1.1	3.1 ± 1.1	0.839
Openness	3.7 ± 0.9	3.9 ± 1.0	0.246
PROP Taster Status (n)			
Non Tasters	19	6	
Medium Tasters	25	25	0.084†
Super Tasters	13	12	

5.3.3 | Salivary microbial profiles showed differences in α- and β-diversities

First, we examined the differences in bacterial richness and evenness between salivary microbial profiles. While both groups did not differ ($p > 0.05$) in both Chao-1 and Inverse Simpson indices, CL-1 showed higher Shannon (CL-1: 3.7 ± 0.8; CL-2: 3.2 ± 0.7; $p = 0.006$) and Fisher (CL-1: 3.7 ± 0.8; CL-2: 3.2 ± 0.7; $p = 0.006$) metrics than CL-2 (Supplemental Figure S5.3). Clusters were later tested for β-dissimilarities, and PERMANOVA highlighted statistically significant differences ($R^2 = 0.081$; $p < 0.001$) between the groups (Supplemental Figure S5.4a). Additionally, we tested whether such differences translated into variations in the degree of intra-cluster compositional homogeneity (Supplemental Figure S5.4b), and found Aitchison distances among members of CL-1 (13.8 ± 3.6) to be significantly shorter ($p < 0.001$) than those observed in CL-2 (15.4 ± 3.5). Overall, results of the ecological analysis indicated that CL-1 harbored a more complex and homogenous salivary microbiota.

5.3.4 | Salivary microbial profiles differed in relative abundances of a large panel of taxa

Second, we uncovered the differentially abundant bacteria housed by the two clusters. Prior to performing the analysis, contaminant taxa were removed in accordance with the standard two-step procedure outlined by Smirnova *et al.* [61]. This enabled us to objectively retain all those ASVs with informative power in regard to the total covariance of the dataset, thus preventing inflated results driven by rarely occurring microbial communities and controlling the occurrence of false discovery rates [61]. After permutation filtering (n = 10000), 585 out of 1717 ASVs (34.1 %) were considered important for estimating the underlying covariance of the data and were retained for downstream applications. Despite the massive filtering loss, the majority (98.2 %; 7,757,607; mean = 77,576.1 ± 14,293.5 per sample) of all generated sequences (7,898,164) were retained.

The filtered ASV table was thus collapsed at each taxonomic level (phylum, class, order, family, genus) to perform separate differential abundance analyses via ANCOM-BC [62]. While no compositional dissimilarities ($p_{adj} > 0.05$) were observed between clusters at the phylum-level, a panel of salivary bacterial signatures significantly ($p_{adj} < 0.05$) distinguished CL-1 from CL-2. These included the proportions of 5 classes, 8 orders, and 12 families of microbes (Supplemental Table S5.1). More interestingly, we also found that the relative abundances (Supplemental Table S5.1) of 13 genera were differentially enriched in the two groups (Figure 5.2a).

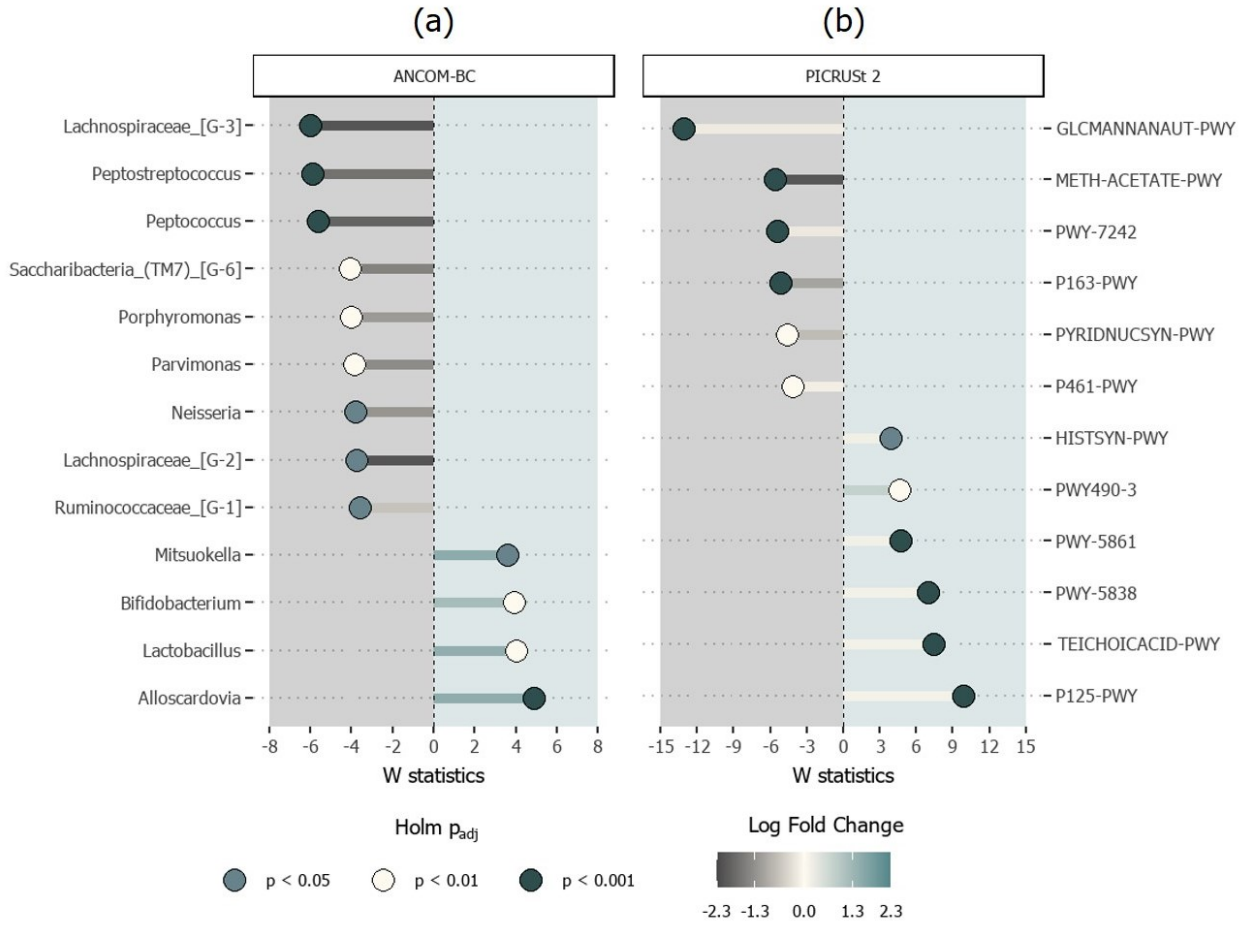

Figure 5.2: Pool of bacterial genera (a) and imputed MetaCyc (b) pathways housed in significantly different ($p_{adj} < 0.05$) proportions by salivary microbial profiles. In each panel (a; b), the colored vertical bands comprise the taxa (a) or the inferred bacterial functions (b) found to be differently abundant between CL-1 (left; light gray) and CL-2 (right; cadet blue). The ANCOM-BC main statistic (W statistics) and its relative effect sizes (log fold change) are represented by the length and the color of the horizontal bars, respectively. Holm's adjusted p. values ($p < 0.05$; $p < 0.01$; $p < 0.001$) are also provided, and illustrated by the dark cyan- ($p < 0.05$), white- ($p < 0.01$), and dark slate grey-filled ($p < 0.001$) circles.

Compared to CL-2, CL-1 harbored greater amounts of genera *Lachnospiraceae_[G-2]* (W = 3.7; $p_{adj} = 0.014$), *Lachnospiraceae_[G-3]* (W = 6.0; $p_{adj} < 0.001$), *Neisseria* (W = 3.8; $p_{adj} = 0.012$), *Parvimonas* (W = 3.9; $p_{adj} = 0.009$), *Peptococcus* (W = 5.6; $p_{adj} < 0.001$), *Peptostreptococcus* (W = 5.9; $p_{adj} < 0.001$), *Porphyromonas* (W = 4.0; $p_{adj} = 0.005$), *Ruminococcaceae_[G-1]* (W = 3.5; $p_{adj} = 0.028$), and *Saccharibacteria_(TM7)_[G-6]* (W = 4.1; $p_{adj} = 0.007$). Conversely, CL-2 was enriched in genera *Alloscardovia* (W = 4.9; $p_{adj} < 0.001$), *Bifidobacterium* (W = 3.9; $p_{adj} = 0.006$), *Lactobacillus* (W = 4.0; $p_{adj} = 0.004$) and *Mitsuokella* (W = 3.6; $p_{adj} = 0.025$).

5.3.5 | Variations in MetaCyc modules between salivary microbial profiles

Third, the full PICRUSt2 pipeline was launched to infer functional pathways from the 585 ASVs (mean NSTI = 0.2 ± 0.4) retained after permutation filtering. Overall, 315 MetaCyc pathways were imputed and annotated before testing the groups for β-dissimilarities by PERMANOVA and differential abundance analysis via ANCOM-BC. Pathway analysis revealed statistically significant omnibus differences (PERMANOVA; $R^2 = 0.055$; $p < 0.001$) in bacterial metabolic functions between salivary microbial profiles (Supplemental Figure S5.5), which were then resolved into 12 differently abundant ($p_{adj} < 0.05$) MetaCyc pathways (Figure 5.2b).

Of particular interest, we observed prominent deviations between clusters with respect to the metabolism of a sour-eliciting compound, with CL-1 showing higher methanogenesis from acetate (METH-ACETATE-PWY; W = 5.6; $p_{adj} < 0.001$) or its production from L-lysine (P163-PWY; W = 5.1; $p_{adj} < 0.001$) and hexitol (P461-PWY; W = 4.1; $p_{adj} = 0.009$) fermentation. Conversely, CL-2 was enriched in pathways involved in the formation of vitamin K2, namely the superpathways of menaquinol-8 (PWY-5838; W = 6.9; $p_{adj} < 0.001$) and demethylmenaquinol-8 (PWY-5861; W = 4.7; $p_{adj} = 0.001$) biosynthesis.

5.3.6 | Differences in oral responsiveness, hedonics, familiarity, and frequency of consumption of actual foods between salivary microbial profiles

Salivary microbial profiles were then evaluated for differences in acuity for oral stimulations evoked by the five liquid and five solid foods used in the current study. As a result, clusters differed ($p < 0.05$) in their responsiveness to oral sensations elicited by 7 out of the 10 foods. In liquid products (Figure 5.3), CL-2 perceived innately disliked tastes (sour in PR-02; bitter in PR-03) at a higher extent ($p < 0.05$) compared to CL-1, and this effect went along with enhanced perceptions of sour- (grapefruit in PR-02) and bitter-evoking (olive in PR-04) flavors. Similar trends were observed in solid foods (Figure 5.4), with CL-2 showing heightened acuity ($p < 0.05$) for astringency (PR-08) and for sour- or pungent-evoking flavors such as lemon (PR-07) and ginger (PR-10), respectively. In addition, CL-2 gave higher intensity ratings when experiencing a sweet-evoking flavor such as caramel (PR-06).

To exclude the effect of potential confounders underlying such findings, salivary microbial profiles were examined for differences in liking, familiarity and frequency of consumption. Further, variations in psychophysical responses to the five extraoral stimuli employed within the gLMS training (section 5.2.4.2) were tested to confute possible idiosyncratic uses of the scale. Overall, while the clusters showed no differences ($p > 0.05$) in liking and familiarity scores (Table 5.3), CL-2 declared to consume more frequently ($p < 0.05$; Table 5.3) a few energy-dense food products (PR-07: lemon candy; PR-08: dark chocolate). Importantly, all recalled intensities evoked by the gLMS orienteering extraoral stimuli were equally rated ($p > 0.05$) by both groups (Supplemental Figure S5.6), thus conclusively corroborating the reliability of variations in sensory perception observed.

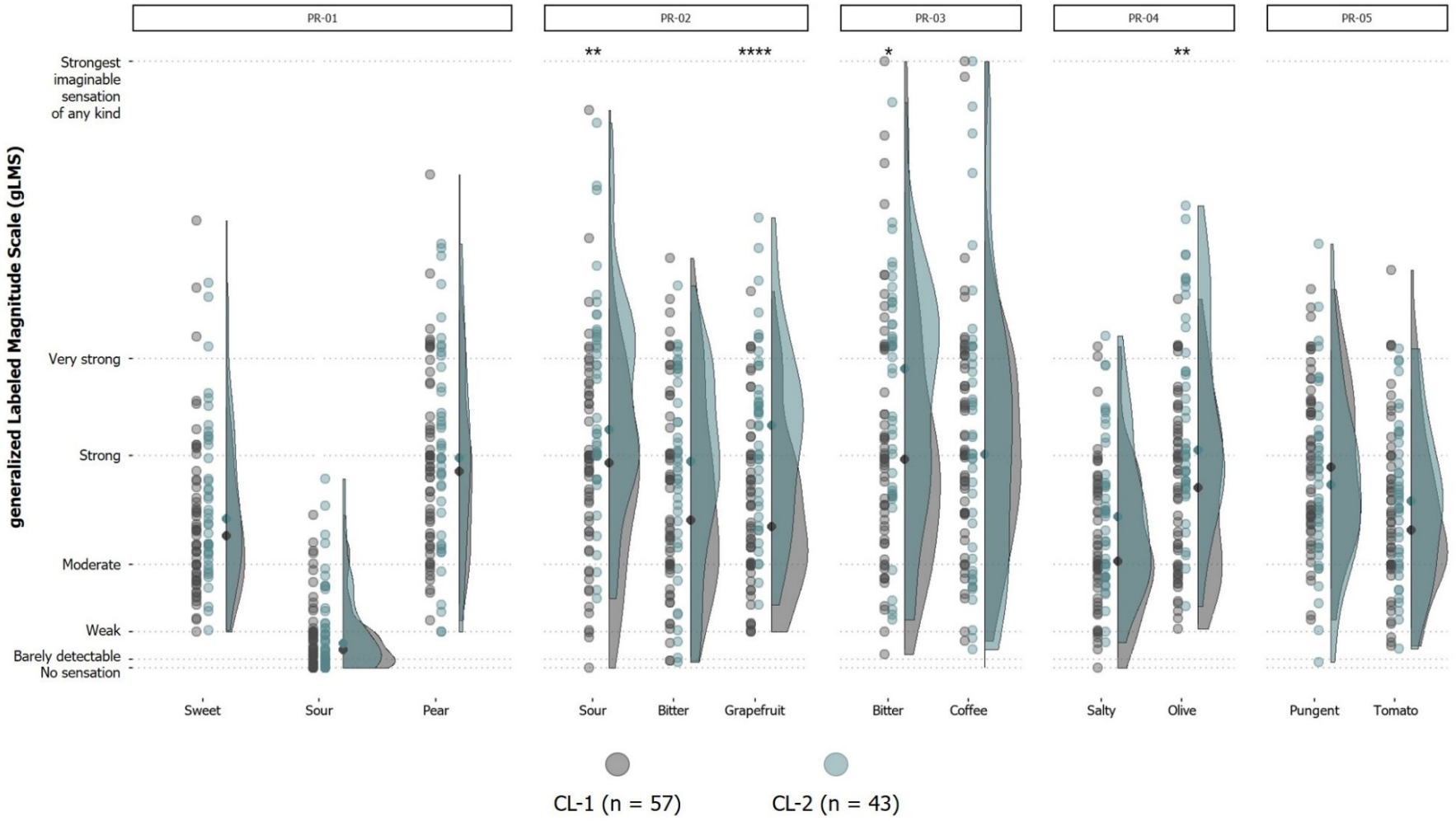

Figure 5.3: Variations in acuity for oral sensations evoked by the five liquid foods as a function of salivary microbial profiles (CL-1: light gray; CL-2: cadet blue). Distribution (the "cloud") of raw observations (the "rain") plus the median (filled circle) ± IQR (perpendicular black line) are provided. * = $p < 0.05$; ** = $p < 0.01$; **** = $p < 0.0001$.

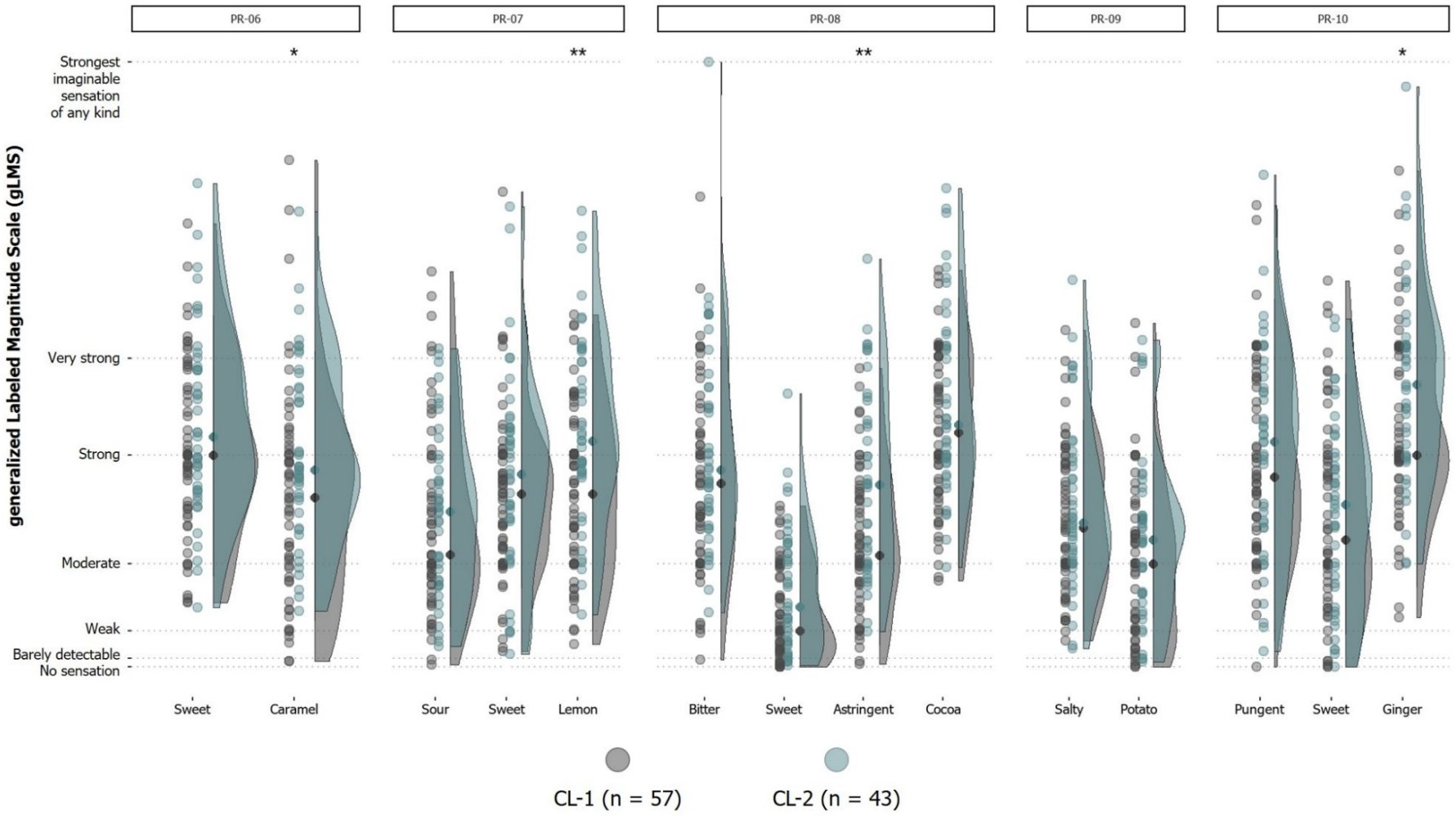

Figure 5.4: Variations in acuity for oral sensations evoked by the five solid foods as a function of salivary microbial profiles (CL-1: light gray; CL-2: cadet blue). Distribution (the "cloud") of raw observations (the "rain") plus the median (filled circle) ± IQR (perpendicular black line) are provided. * = $p < 0.05$; ** = $p < 0.01$.

Table 5.3: Liking, familiarity and frequency of consumption ratings (median ± IQR) given by salivary microbial profiles (CL-1; CL-2) to the ten food matrices (Sample). Values in bold are considered 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	68.2 ± 11.7	67.6 ± 19.5	0.883	4 ± 2	4 ± 1	0.533	2 ± 0	2 ± 0	0.749
PR-02	41.4 ± 20.6	45.3 ± 28.2	0.633	2 ± 2	2 ± 1.5	0.130	2 ± 1	2 ± 1	0.659
PR-03	39.2 ± 24.7	28.1 ± 28.9	0.093	4 ± 2	5 ± 2	0.611	5 ± 2	5 ± 2	0.949
PR-04	64.7 ± 27.9	68.2 ± 22.9	0.633	3 ± 2	2 ± 1	0.233	2 ± 1	2 ± 1	0.843
PR-05	64.7 ± 19.1	68.2 ± 21.9	0.362	5 ± 1	5 ± 1	0.694	3 ± 1	3 ± 1.5	0.566
PR-06	77.5 ± 17.5	78.4 ± 11.9	0.423	5 ± 1	5 ± 1	0.274	4 ± 3	4 ± 2	0.369
PR-07	67.9 ± 16.8	72.1 ± 15.4	0.052	3 ± 2	4 ± 2	0.122	2 ± 1	2 ± 1	**0.011**
PR-08	63.6 ± 27.1	63.5 ± 23.5	0.471	4 ± 1	5 ± 1	0.158	3 ± 2	4 ± 1	**0.038**
PR-09	74.4 ± 11.4	74.9 ± 13.4	0.337	4 ± 1	4 ± 2	0.359	2 ± 1	2 ± 0.5	0.921
PR-10	43.9 ± 42.2	47.8 ± 38.3	0.089	2 ± 2	2 ± 2	0.376	1 ± 1	1 ± 1	0.681

5.3.7 | Differences in habitual dietary intakes by salivary microbial profiles

Lastly, salivary microbial profiles were assessed for variations in habitual dietary intake, taking into account the large number of nutrients (n = 93) plus total energy (Kcal) retrieved from the food diaries (Table 5.4). Although the dietary habits of both clusters largely overlapped ($p > 0.05$), a few differences emerged. Specifically, CL-1 reported habitually consuming higher amounts of several beneficial nutrients either from plant- or animal-based sources. These included vegetable proteins ($p = 0.029$), monounsaturated fatty acids ($p = 0.043$), and vitamins such as folic acid ($p = 0.049$), menadione ($p = 0.014$) and pantothenic acid ($p = 0.014$). Moreover, CL-1 also showed an almost significant ($p = 0.050$) higher habitual intake of ascorbic acid. Conversely, CL-2 was found to habitually consume larger quantities of simple carbohydrates ($p = 0.010$).

Table 5.4: Variations in habitual dietary intakes between salivary microbial profiles (CL-1; CL-2). Data are tabulated as median ± IQR, and p.values observed after Wilcoxon rank sum test are highlighted in bold whether statistically significant ($p < 0.05$).

	CL-1 (n = 57)	**CL-2 (n = 43)**	**p.value**
Carbohydrates (g)			
Simple carbohydrates	69.8 ± 20.3	80.6 ± 28.1	**0.010**
Proteins (g)			
Vegetable proteins	31.4 ± 11.1	27.1 ± 11.8	**0.029**
Fats (g)			
Monoinsaturated fatty acids	21.2 ± 8.3	19.1 ± 5.0	**0.043**
Vitamins			
Ascorbic acid (mg)	152.5 ± 116.5	121.6 ± 74.5	0.050
Folic acid (mg)	306.6 ± 163.6	264.8 ± 142.5	**0.049**
Menadione (mcg)	154.9 ± 112.1	102.6 ± 98.5	**0.014**
Pantothenic acid (mg)	1.8 ± 1.0	1.5 ± 0.9	**0.014**

5.4 | Discussion

Here, we probed whether homogeneous patterns of bacterial co-habitation in the salivary microbiota could reflect variations in orosensory acuity and habitual eating habits. Overall, unsupervised data-driven clustering of the genus-level Aitchison distances objectively resolved into two distinct salivary microbial profiles, distinguished by α- and β-diversity metrics and by a spectrum of differentially abundant bacterial members and predicted metabolic functionalities. Intriguingly, the clusters further differed in their responsiveness to oral sensations or flavors that elicit alarming chemosensory properties, pleasure-oriented tendencies and endorsement of prosocial behaviors, and habitual consumption of beneficial dietary components or simple carbohydrates.

5.4.1 | Confirming previously observed networks of salivary bacteria

At first, we observed compositional commonalities with the healthy salivary microbiota and with previously reported networks of microbes suspended in saliva. As common in healthy individuals (e.g., [2,68]), we found that the phylum- and genus-level salivary microbiota of our cohort were dominated by *Firmicutes* and *Streptococcus*, respectively. Furthermore, although the depth of our sequencing approach did not permit taxonomically annotating the majority of ASVs at the species level, we detected multiple similarities with the genera assigned to the 68 core residents of the salivary microbiota [68].

Notably, we also identified known networks of taxa governing the genus-level microbial consortia of the clusters. Indeed, salivary microbial profiles showed both *Streptococcus* and *Rothia* as dominant genera (Supplemental Figure S5.2), thus falling into one of the five "*stomatotypes*" [29] observed by Zaura *et al.* [69] in a large cohort (n = 268) of similarly aged (18-32 y/o) adults. Similarly, our findings are consistent with previously reported patterns of co-occurrence and co-exclusion of bacterial genera in health [26,70]. As a result, *Streptococcus* positively related to *Gemella* and

Granulicatella, whereas *Atopobium* and *Megasphaera* exhibited contrasting behavior and co-occurred with *Actinomyces*, *Stomatobaculum,* and *Lachnoanaerobaculum* (Supplemental Figure S5.7). Taken collectively, our findings reinforce ample evidence pointing out the existence of core salivary bacteria whose intimate relationships operate in the safeguarding of host's homeostasis [2,28,68–70].

5.4.2 | Differences in chemosensation between salivary microbial profiles

Interestingly, we found that salivary microbial profiles differed in terms of oral sensations (astringent, bitter, sour) or flavors (grapefruit, olive, lemon, ginger) linkable to warning chemosensory signals. Notably, CL-1 (hereafter hyporesponsive cluster) perceived alarming taste qualities, trigeminal sensations, and flavors to a lesser extent than CL-2 (hereafter hyperresponsive cluster), and this was true for both liquid (Figure 5.3) and solid foods (Figure 5.4). Importantly, such differences did not correspond to differences in demographics, dietary styles, nearly all the psychological traits, and food-related attitudes considered other than liking and familiarity for the ten stimuli between groups. Moreover, we excluded systematic use of the gLMS, as both clusters rated the five extraoral stimuli used for scale orientation as equally intense. Thus, it appears plausible that physiological rather than external cues explained how salivary microbial profiles behaved differently in response to oral stimulation.

Consistent with a previous report on the same cohort [38], enhanced acuity for warning sensations did not translate into higher phenotypic responses to PROP. As we have previously argued [38], such an unexpected finding is likely related to two potential drawbacks on using paper strips (rather than water solutions) to assess PROP Taster Status: a) the tendency of impregnated strips to overestimate the percentage of individuals with enhanced acuity for PROP, though poorly responsive [71]; and b) discrepancies on the amount of PROP tasted by each participant due to potential inconsistencies in its amount throughout the strip [72] and/or by difficulties in adhering to the artificial

tasting procedure [38]. Nevertheless, the hyperresponsive cluster (CL-2) tended to be populated by fewer PROP NTs (and more MTs as a percentage) than the hyporesponsive cluster (CL-1; Table 5.2; $p = 0.084$) and to systematically rate (Figure 5.3; Figure 5.4) all sensations as more intense (although not always statistically significant).

5.4.3 | Habitual dietary intakes might be affected by mutualisms between salivary microbiota, oral responsiveness and psychological traits

We showcased a complex crosstalk between host related non-genetic (microbiota), biological (sensory perception) and psychosocial factors underlying the differences in habitual dietary habits between members of the hypo- and hyperresponsive clusters. Specifically, hyporesponsive individuals (CL-1) harbored a richer and more complex salivary microbiota and reported higher intakes of beneficial nutrients (vegetable proteins, monounsaturated fatty acids, vitamins) compared to the hyperresponsive group, who was found to habitually consume higher quantities of simple carbohydrates. Also, hyperresponsiveness to warning sensations was parallel to higher craving for sweet foods and levels of agreeableness.

Our findings are broadly consistent with previous reports linking oral bacterial diversity, chemoperception, and psychological traits to dietary habits, albeit never together in a single study. As in the current study, salivary microbial richness and evenness have been associated with higher habitual intake of nutrients from plant-based sources [73] and lower daily sugar consumption [30], whereas higher acuity for bitterness and sourness has recently been shown to hinder the choice of sour- and bitter-eliciting phenol-rich foods [74]. Further, individuals with high craving for sweet foods were found to be prone to selecting more frequently chocolate bars over apples [50] and to consume more high-fat sweet snacks [75].

Conversely, results from agreeableness appear to be less consistent with previous knowledge, as prosocial personalities usually tend to be associated with healthier food choices (see for a review [76]). Nevertheless, such personality traits also co-occurred with high sweet [77] and low bitter [78] taste preferences, which are proxies for the observed dietary patterns. Thus, as the role of agreeableness on eating habits remains controversial and inconsistent across studies [79], further studies are motivated to conclusively elucidate its influence on diet.

In an attempt to further explain the differences in dietary habits between salivary microbial profiles, potential interplays between oral taxa, inferred bacterial functions, and chemosensory abilities can be deduced. Firstly, we associated hyporesponsiveness to warning sensations and healthier dietary habits with a number of bacterial genera, mostly belonging to the class *Clostridia*. Remarkably, these results are consistent with a recent study on the same cohort [38], in which gut commensal *Clostridia* (families *Lachnospiraceae* and *Ruminococcaceae*) were more abundant in individuals with generalized hypogeusia to oral sensations elicited by the same range of foods used here. Moreover, salivary members of the family *Lachnospiraceae* were previously found to be inversely correlated with sour acuity [80], whereas taxa from the family *Peptostreptococcaceae* [81] and 2 ASVs from the genus *Porphyromonas* (class *Bacteroidia* [25]) were found to be enriched in PROP-insensitive individuals, who are typically unresponsive to chemosensory warning signals [12–14]. Similar salivary bacterial consortia have also been evidenced with respect to olfactory performances [26], with an unclassified genus of the family *Lachnospiraceae_XIV* and the genus *Porphyromonas* being inversely related to orthonasal olfactory acuity. Thus, in line with the notion that individuals would be similarly responsive across various (taste, olfaction, chemesthesis) sensory modalities [13,35], we suggest that a *Clostridia*-enriched salivary microbiota might relate to lower chemosensory abilities.

This can be further speculated based on both past research and our findings. Indeed, *Clostridia* are known to be producers of free catecholamines [82], whose pharmacological reuptake inhibition acutely blunts bitter sensitivity [83], and this could act as a suppressor of taste function [84]. Moreover, hyporesponsive (relative to hyperresponsive) individuals (CL-1) showed higher proportions of MetaCyc modules attributable to the biosynthesis of a sour-eliciting compound (acetate), whose increased concentration nearby the taste buds could promote sensory adaptation [19]. Given that hyporesponsiveness to a taste quality usually exacerbates its intake (e.g., [27,38]) and that dietary outcomes associated with salivary *Clostridia* members are consistent with a previous report [27], we urge further research to elucidate the links between *Clostridia*, sensory perception, and dietary habits.

Secondly, interesting host-microbe interactions that might have influenced the dietary habits of the hyperresponsive group (CL-2) also emerged. We found that this cluster simultaneously housed more cariogenic bacterial genera (*Bifidobacterium*, *Lactobacillus*) and habitually consumed larger amounts of simple carbohydrates than the hyporesponsive group (CL-1). These results overlap with those of Esberg *et al.* [30], who noted the same bacteria to be enriched in individuals with high sugar consumption, and reinforce previous evidence suggesting synergisms between oral microbes sharing the same nutritional requirements for survival in the salivary milieu [28].

More interestingly, hyperresponsive individuals (CL-2) also harbored greater amounts of some taxa from the *Actinobacteria* phylum (families *Bifidobacteriaceae* and *Eggerthellaceae*; genera *Alloscardovia* and *Bifidobacterium*). While the salivary members of this phylum have previously been shown to be negatively correlated with taste (especially saltiness) perception [17], some authors have observed opposite trends in the tongue *dorsum* microbiota [21,80]. Moreover, higher proportions of the genus *Actinomyces* were detected in the salivary microbiota of PROP STs compared to NTs [25], and we recently documented that the abundances of a pro-inflammatory gut taxon of the *Actinobacteria* phylum (genus *Eggerthella*) were associated with hypergeusia to chemosensory stimuli in the same

individuals involved here [38]. Thus, our results suggest that an enrichment of microbes belonging to this phylum might relate to enhanced orosensory acuity.

Nevertheless, the direction of this association remains largely unclear, as it has been inferred so far from small sample sizes, from oral niches known to house distinct microbial communities [17], and from poorly consistent bioinformatics and taste assessment procedures across studies. Thus, to conclusively address this issue, future research should devote additional effort to: a) include larger sample sizes; b) collect microbial samples from multiple oral niches potentially communicating with taste transduction systems (saliva and tongue *dorsum*); c) ensure homogeneous (higher) sequencing depth and downstream (compositionally-aware) data treatments; d) test taste function via real foods to increase the ecological validity of outcomes; and e) use methods capturing suprathreshold intensities, as advocated to best relate to actual food perception [34,35].

5.4.4 | Strengths, limitations and conclusions

For the first time, homogeneous patterns of salivary bacterial co-habitation have been linked to systematic variations in orosensory responsiveness to liquid and solid foods, psychological traits, and habitual eating habits. The strengths of the current study revolve around the high external validity of outcomes, which was ensured by a large data collection protocol, a wide range of oral sensations and food products tested, and by a substantial background homogeneity among individuals preventing our conclusions from being strongly biased by underlying confounders.

Nevertheless, the results should also be interpreted in light of some limitations. First, this study provides a limited picture of the potential bacterial networks inhabiting the salivary environment, albeit those found have been proven to be consistent with the literature [69]. Second, the homogeneity and relatively small size of our cohort make our findings poorly generalizable to other age groups and/or ethnically diverse populations. Third, while corroborated by acceptable NSTI mean values (0.2

± 0.4), results from PICRUSt2 should still be taken with caution, as the soundness of functional prediction is limited by its inability to infer taxa-specific pathways [63]. However, these data was still pivotal to speculate on potential mechanistic links between taste function, salivary microbiota and eating habits. Fourth, our dietary outcomes may be partly influenced by psychological biases associated with self-reporting [85], although significant overlap with current knowledge was evidenced. Lastly, given the cross-sectional nature of this report, it is not possible to infer a causal relationship underlying our findings. Thus, we can not exclude the possibility that diet itself may be an active contributor to the associations found here. In this sense, future longitudinal intervention studies will prove critical in clarifying this open question.

In conclusion, this work depicts a complex scenario in which microbe-microbe and microbe-taste interactions play in tandem with host psychology to shape dietary behavior, and derives putative underlying mechanisms that require empirical confirmation. Specifically, we observed that a *Clostridia*-enriched microbiota corresponded to lower responsiveness to warning oral sensations and higher habitual intake of beneficial nutrients, and we speculated the ability of such taxa to produce free catecholamines [82] and/or an increased microbial biosynthesis of acetate to be foundational to this link. Conversely, a salivary microbiota harboring more cariogenic bacteria and members of the *Actinobacteria* phylum led to opposite sensory- and diet-related outcomes. Taken together, given that peculiar co-occurring salivary bacterial networks were associated with specific patterns of orosensory acuity and dietary habits, the current study also motivates future investigations to test the hypothesis of the existence of a "core" taste-related salivary microbiota.

5.5 | Acknowledgements

Special thanks to all those who volunteered and completed this study. The authors greatly appreciate Elisa Facen, Katia Franzoso, Carola Pedrinolla, Giulia Pirrello, Michele Ricci, Elena

Santoro and Jessica Zambanini for their help throughout the project. Many thanks to Istituto Scotti Bassani (Milan, Italy) and Perfetti Van Melle S.p.A (Italy) for permission to use the food atlas and providing one of the food products used, respectively. In this regard, we sincerely thank Dr. Francesca Passannanti (Istituto Scotti Bassani) and Dr. Maria Laura Corollaro (Perfetti Van Melle S.p.A) who technically supported us along the study. Lastly, we thank Giract (Geneve, Switzerland) for awarding Leonardo Menghi with the 1st year PhD Student European Flavour Research Award for the scientific purposes of this project.

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).

5.6 | CRediT authorship contribution statement

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. **Davide Giacalone:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Flavia Gasperi:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

5.7 | Declaration of interest

None.

5.8 | Supplemental material

5.8.1 | Figures

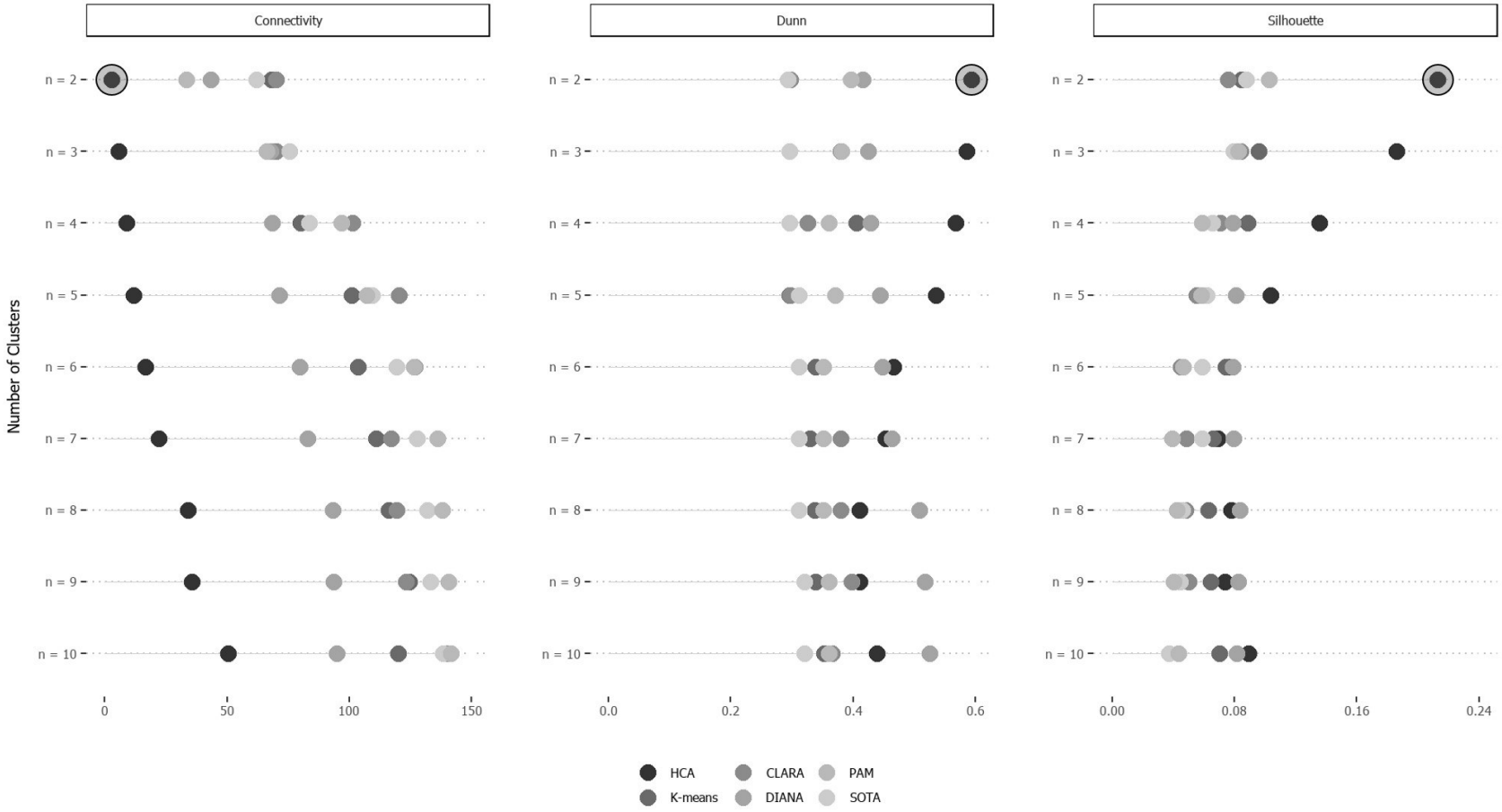

Figure S5.1: Results from the data-driven segmentation approach. Indices of connectivity, Dunn and silhouette within six algorithms (KMEANS, HCA, PAM, SOTA, CLARA, DIANA) at an increasing number of cluster solutions (from 2 to 10) were tested. Optimal partitioning was defined in the light of the lowest cluster connectivity and the highest silhouette width and Dunn index observed [59]. Best algorithm and cluster solution across the three indices is highlighted by the light-gray transparent circles.

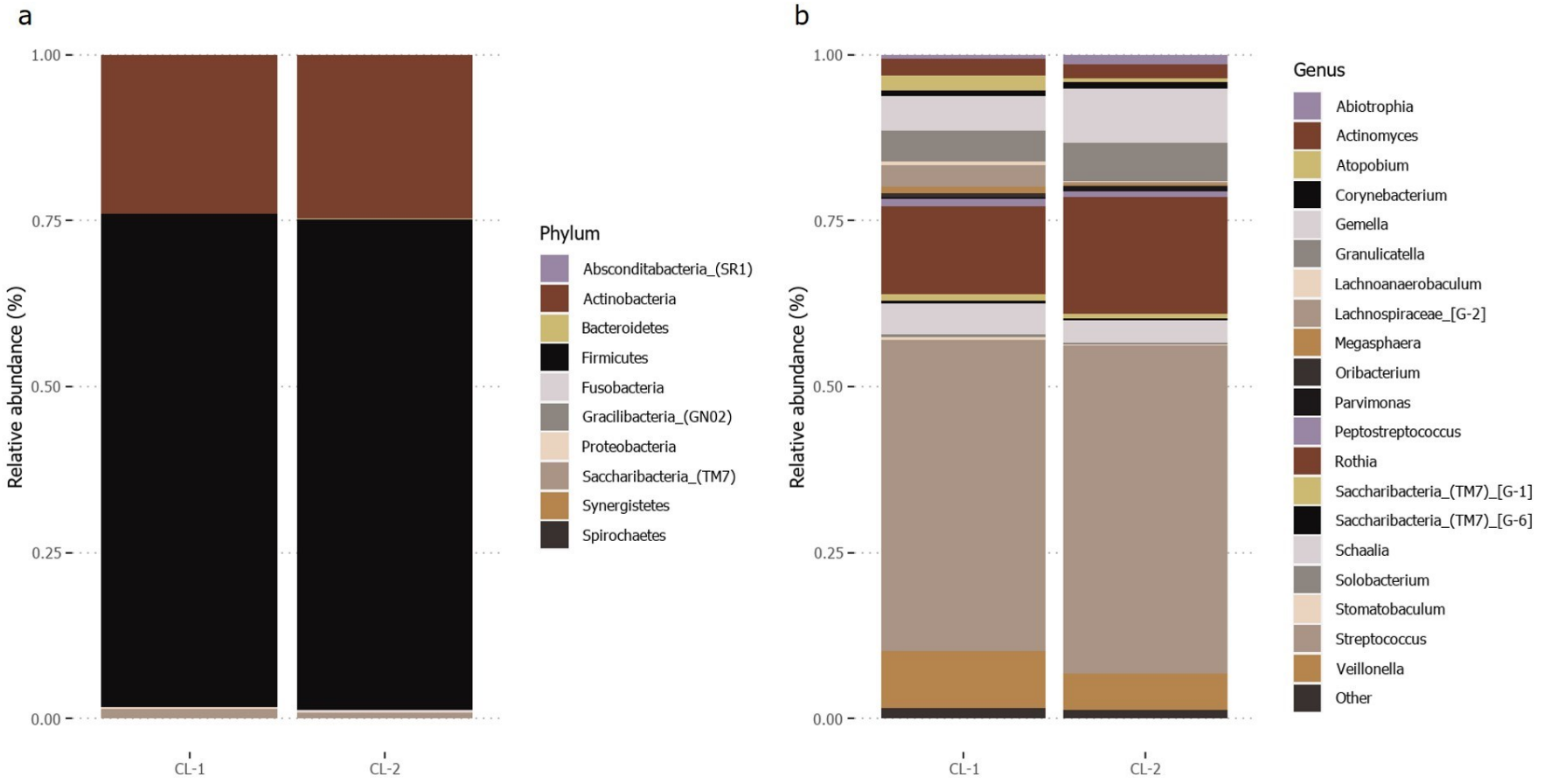

Figure S5.2: Phylum- (a) and genus-level (b) salivary microbial composition of CL-1 (n = 57) and CL-2 (n = 43). Relative abundances (%) of the 10 phyla and the 20 genera detected as predominant are graphically illustrated in alphabetic order (from top to bottom).

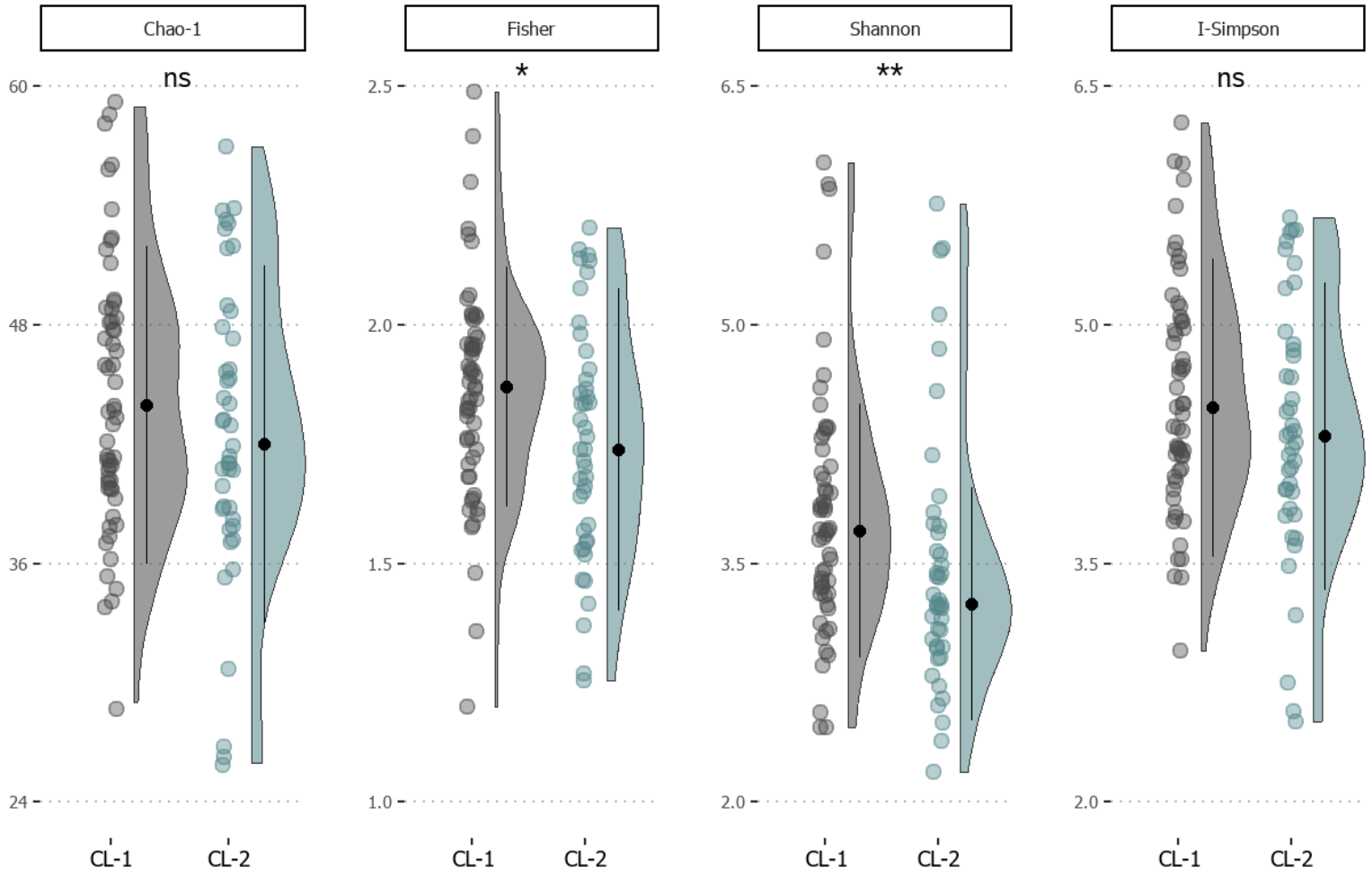

Figure S5.3: Variations in α-diversity metrics (Chao-1, Fisher, Shannon, I-Simpson indices) by salivary microbiota profiles (CL-1: n = 57; CL-2: n = 43). For each group, data distribution (the "cloud"), individual raw observations (the "rain"), the median (black filled circle) ± IQR (perpendicular black line), are provided. * = $p < 0.05$; ** = $p < 0.01$; ns = $p > 0.05$.

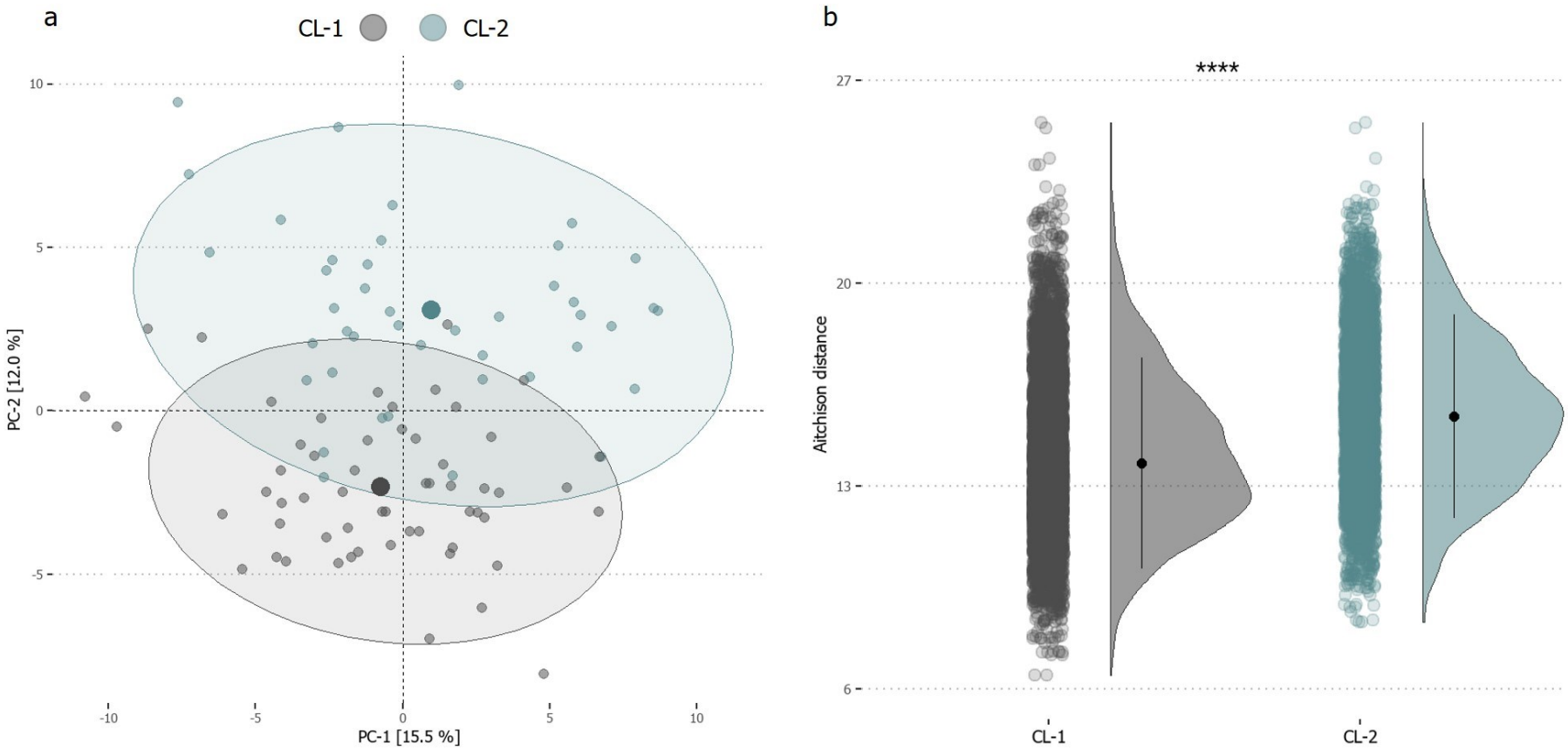

Figure S5.4: Compositional Principal Component Analysis (a) on Aitchison distances [56] between CL-1 (light gray) and CL-2 (cadet blue). Members of each group (transparent circles), relative centroids (largest circles) and 95 % confidence ellipses are depicted. b) Variations in salivary microbiota homogeneity between clusters (CL-1 = light gray; CL-2 = cadet blue). For each salivary microbial profile, the raincloud plot illustrates the distribution of data (the "cloud"), individual raw observations (the "rain"), and the median (black filled circle) ± IQR (perpendicular black line). **** = $p < 0.0001$.

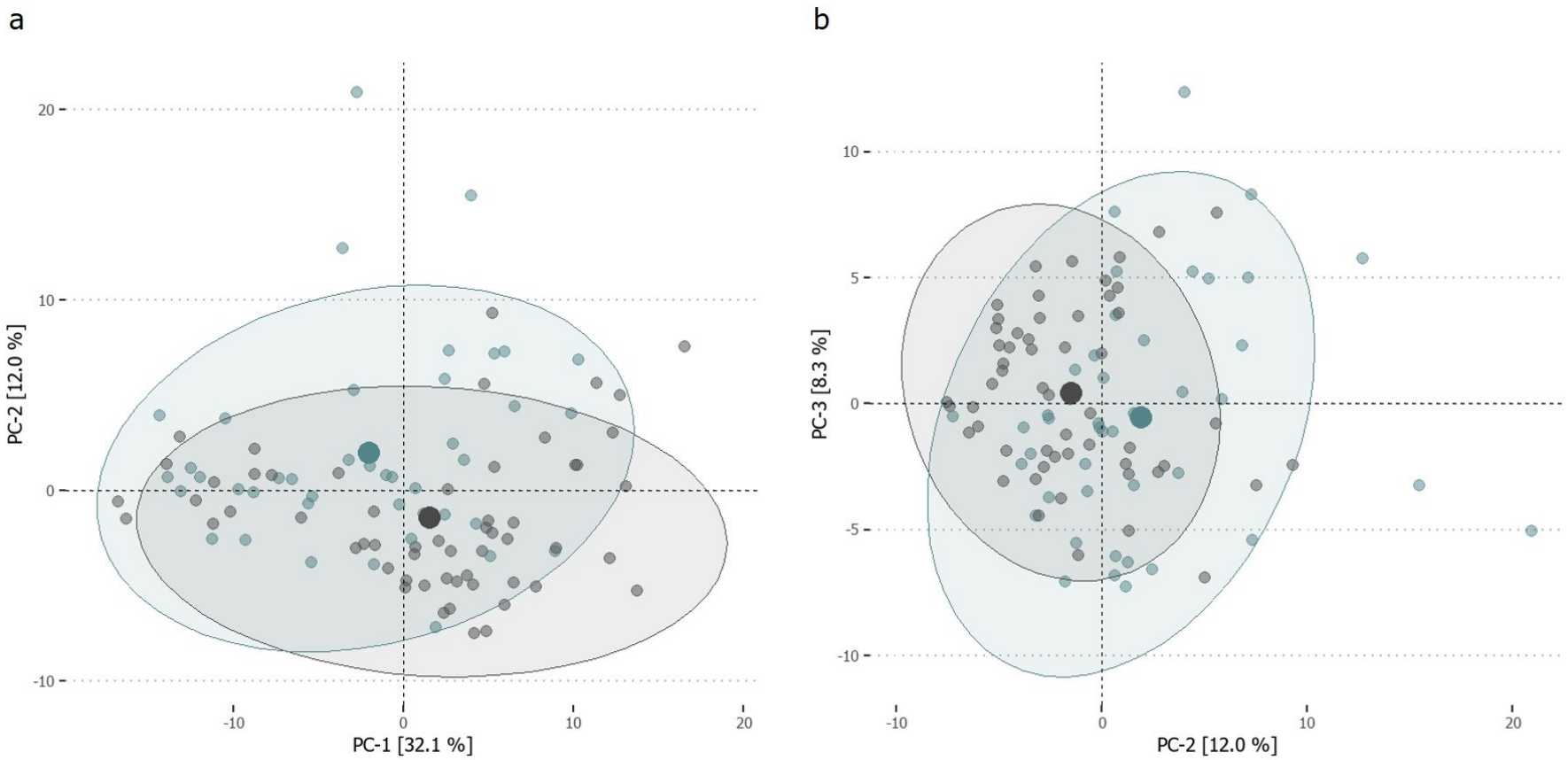

Figure S5.5: β-dissimilarities in inferred MetaCyc pathways between CL-1 (light gray) and CL-2 (cadet blue). The first three significant components derived from the Compositional Principal Component Analysis [56] are plotted (a: first vs second; b: second vs third). Transparent light gray- and cadet blue-filled circles represent the members of CL-1 (n = 57) and CL-2 (n = 43), respectively. Additionally, the largest circles represent clusters' centroids (CL-1: light gray; CL-2: cadet blue), whereas ellipses show the 95 % confidence interval.

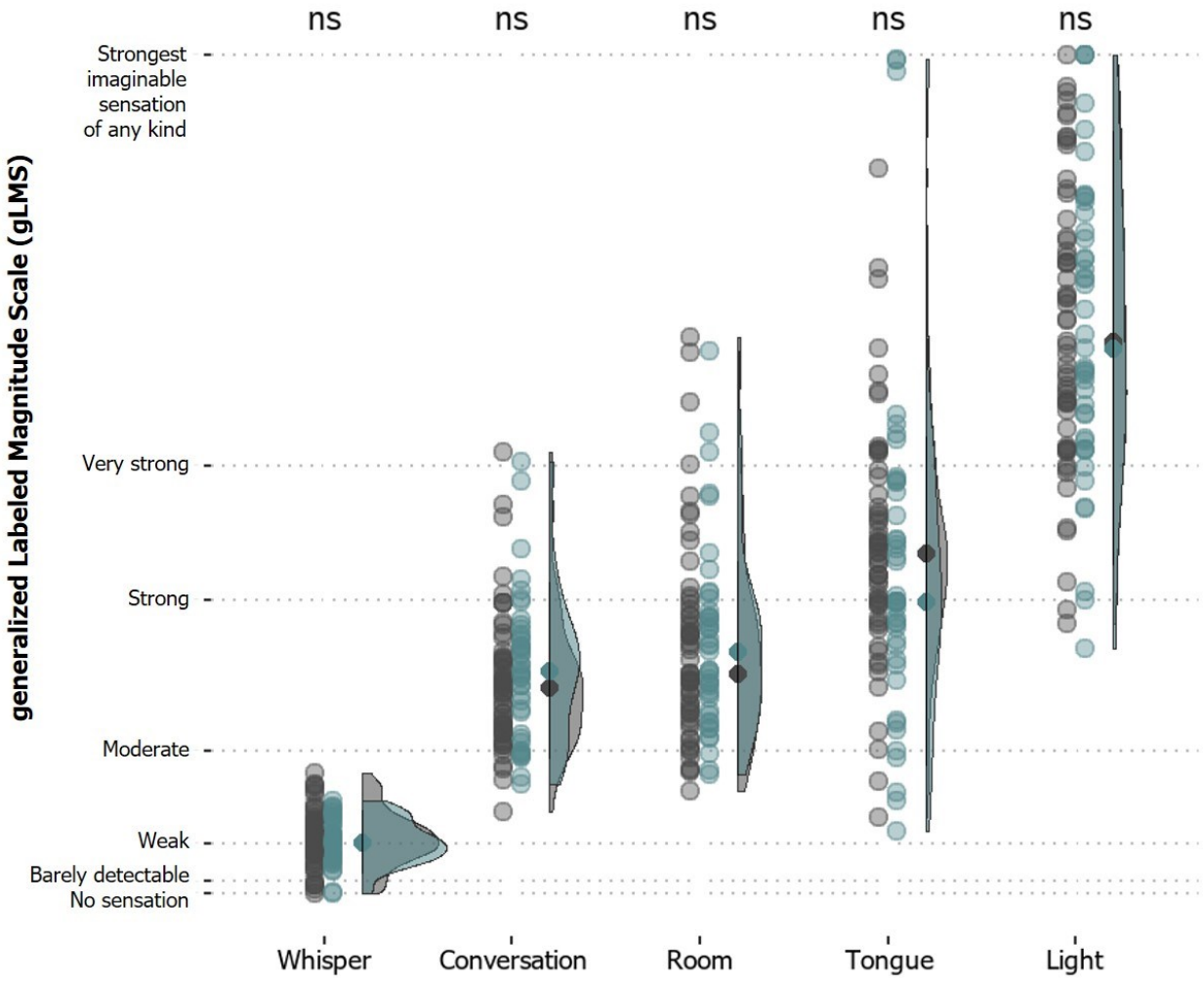

Figure S5.6: Salivary microbial profiles (CL-1: light gray; CL-2: cadet blue) equally rated the recalled intensities of the five extraoral stimuli employed during the gLMS training [44]. For each stimulus (Whisper: loudness of a whisper; Conversation: loudness of a conversation; Room: brightness of a well-lit room; Tongue: pain of biting your tongue; Light: brightest light ever seen), the distribution (the "cloud") of raw observations (the "rain"), the median (black circle), and the IQR (perpendicular black line) are depicted. ns = $p > 0.05$.

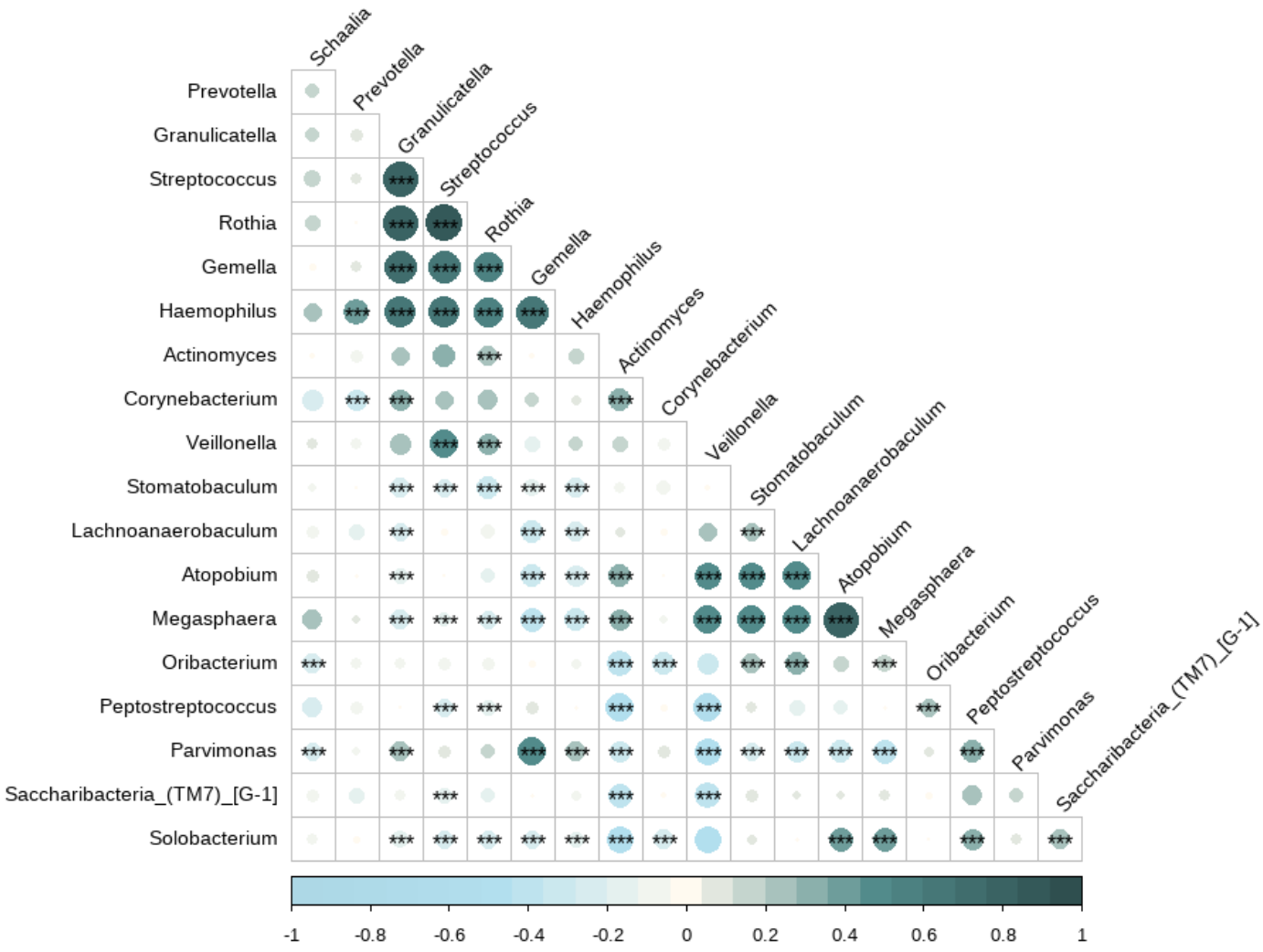

Figure S5.7: Ecological relationships between microbial genera detected in at least 90 % of participants. The correlogram depicts both correlation coefficients (colored circles) and bootstrapped (n = 10000) p values (*** = $p < 0.001$) computed via SparCC [85].

5.8.2 | Tables

Table S5.1: Mean relative proportions (%) ± SD of differently abundant (ANCOM-BC) [61] classes, orders, families and genera of bacterial taxa between salivary microbial profiles (CL-1: n = 57; CL-2: n = 43). W statistics produced by ANCOM-BC plus Holm's adjusted statistically significant p.values ($p < 0.05$) are also tabulated.

Taxonomic level	Taxa	CL-1 (n = 57)	CL-2 (n = 43)	W	p.value
Class	*Betaproteobacteria*	0.1 ± 0.1	0.0 ± 0.0	3.5	0.008
	Clostridia	5.8 ± 4.6	3.3 ± 2.7	3.5	0.007
	Erysipelotrichia	0.4 ± 0.4	0.3 ± 0.2	3.0	0.036
	Negativicutes	6.5 ± 3.4	9.5 ± 4.5	3.4	0.009
	Saccharibacteria_(TM7)_[C-1]	1.5 ± 1.3	0.9 ± 0.9	3.6	0.005
Order	*Bifidobacteriales*	0.0 ± 0.0	0.1 ± 0.2	5.0	< 0.001
	Clostridiales	5.8 ± 4.6	3.3 ± 2.7	3.5	0.010
	Eggerthellales	0.0 ± 0.0	0.0 ± 0.1	3.4	0.014
	Erysipelotrichales	0.4 ± 0.4	0.3 ± 0.2	3.0	0.049
	Neisseriales	0.1 ± 0.1	0.0 ± 0.0	3.8	0.004
	Saccharibacteria_(TM7)_[O-1]	1.5 ± 1.3	0.9 ± 0.9	3.6	0.007
	Selenomonadales	0.0 ± 0.1	0.2 ± 0.3	3.7	0.006
	Veillonellales	6.5 ± 3.4	9.4 ± 4.3	3.3	0.017
Family	*Bifidobacteriaceae*	0.0 ± 0.0	0.1 ± 0.2	5.0	< 0.001
	Eggerthellaceae	0.0 ± 0.0	0.0 ± 0.1	3.4	0.024
	Lactobacillaceae	0.0 ± 0.0	0.1 ± 0.2	4.0	0.002
	Neisseriaceae	0.1 ± 0.1	0.0 ± 0.0	3.8	0.006
	Peptococcaceae	0.1 ± 0.1	0.0 ± 0.1	5.6	< 0.001
	Peptoniphilaceae	0.6 ± 0.6	0.4 ± 0.7	3.8	0.005
	Peptostreptococcaceae_[XI]	1.6 ± 1.1	0.7 ± 1.0	5.3	< 0.001
	Porphyromonadaceae	0.0 ± 0.0	0.0 ± 0.0	4.0	0.003
	Ruminococcaceae	0.0 ± 0.0	0.0 ± 0.0	3.4	0.022
	Saccharibacteria_(TM7)_[F-1]	1.5 ± 1.3	0.9 ± 0.8	3.7	0.009
	Selenomonadaceae	0.0 ± 0.1	0.2 ± 0.3	3.7	0.009
	Veillonellaceae	6.5 ± 3.4	9.4 ± 4.3	3.3	0.030
Genus	*Alloscardovia*	0.0 ± 0.0	0.0 ± 0.1	4.9	< 0.001
	Bifidobacterium	0.0 ± 0.0	0.1 ± 0.2	3.9	0.007
	Lachnospiraceae_[G-2]	2.3 ± 3.6	1.1 ± 2.2	3.7	0.014
	Lachnospiraceae_[G-3]	0.1 ± 0.2	0.0 ± 0.0	6.0	< 0.001
	Lactobacillus	0.0 ± 0.0	0.1 ± 0.2	4.0	0.005
	Mitsuokella	0.0 ± 0.1	0.2 ± 0.3	3.6	0.026
	Neisseria	0.1 ± 0.1	0.0 ± 0.0	3.8	0.013
	Parvimonas	0.6 ± 0.6	0.4 ± 0.7	3.9	0.009
	Peptococcus	0.1 ± 0.1	0.0 ± 0.1	5.6	< 0.001
	Peptostreptococcus	1.3 ± 1.0	0.5 ± 0.8	5.9	< 0.001
	Porphyromonas	0.0 ± 0.0	0.0 ± 0.0	4.0	0.005
	Ruminococcaceae_[G-1]	0.0 ± 0.0	0.0 ± 0.0	3.6	0.029
	Saccharibacteria_(TM7)_[G-6]	0.4 ± 0.4	0.2 ± 0.4	4.1	0.004

5.9 | References

1. Mark Welch, J. L., Ramírez-Puebla, S. T. & Borisy, G. G. Oral Microbiome Geography: Micron-Scale Habitat and Niche. *Cell Host Microbe* **28**, 160–168 (2020).
2. Takeshita, T. *et al.* Bacterial diversity in saliva and oral health-related conditions: The Hisayama Study. *Sci. Rep.* **6**, 1–11 (2016).
3. Belstrøm, D. The salivary microbiota in health and disease. *J. Oral Microbiol.* **12**, (2020).
4. Lu, M., Xuan, S. & Wang, Z. Oral microbiota: A new view of body health. *Food Sci. Hum. Wellness* **8**, 8–15 (2019).
5. Köster, E. P. Diversity in the determinants of food choice: A psychological perspective. *Food Qual. Prefer.* **20**, 70–82 (2009).
6. Kourouniotis, S. *et al.* The importance of taste on dietary choice, behaviour and intake in a group of young adults. *Appetite* **103**, 1–7 (2016).
7. Fischer, M. E. *et al.* Factors related to fungiform papillae density: The beaver dam offspring study. *Chem. Senses* **38**, 669–677 (2013).
8. Monteleone, E. *et al.* Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Qual. Prefer.* **59**, 123–140 (2017).
9. Bartoshuk, L. M. *et al.* Valid across-group comparisons with labeled scales: The gLMS versus magnitude matching. *Physiol. Behav.* **82**, 109–114 (2004).
10. Robino, A. *et al.* Combined influence of TAS2R38 genotype and PROP phenotype on the intensity of basic tastes, astringency and pungency in the Italian taste project. *Food Qual. Prefer.* **95**, 104361 (2022).
11. Risso, D. S. *et al.* Global diversity in the TAS2R38 bitter taste receptor: Revisiting a classic evolutionary PROPosal. *Sci. Rep.* **6**, 1–9 (2016).
12. Dinnella, C. *et al.* Individual variation in PROP status, fungiform papillae density, and responsiveness to taste stimuli in a large population sample. *Chem. Senses* **43**, 697–710 (2018).
13. Piochi, M., Dinnella, C., Spinelli, S., Monteleone, E. & Torri, L. Individual differences in

responsiveness to oral sensations and odours with chemesthetic activity: Relationships between sensory modalities and impact on the hedonic response. *Food Qual. Prefer.* **88**, (2021).

14. Nolden, A. A., McGeary, J. E. & Hayes, J. E. Predominant Qualities Evoked by Quinine, Sucrose, and Capsaicin Associate with PROP Bitterness, but not TAS2R38 Genotype. *Chem. Senses* **45**, 383–390 (2020).
15. Fischer, M. E. *et al.* The associations between 6-n-propylthiouracil (PROP) intensity and taste intensities differ by TAS2R38 haplotype. *J. Nutrigenet. Nutrigenomics* **7**, 143–152 (2014).
16. Canon, F., Neiers, F. & Guichard, E. Saliva and Flavor Perception: Perspectives. *J. Agric. Food Chem.* **66**, 7873–7879 (2018).
17. Feng, Y. *et al.* The Associations between Biochemical and Microbiological Variables and Taste Differ in Whole Saliva and in the Film Lining the Tongue. *Biomed Res. Int.* **2018**, (2018).
18. Schwartz, M., Canon, F., Feron, G., Neiers, F. & Gamero, A. Impact of oral microbiota on flavor perception: From food processing to in-mouth metabolization. *Foods* vol. 10 (2021).
19. Leung, R. & Covasa, M. Do gut microbes taste? *Nutrients* **13**, 2581 (2021).
20. Gardner, A., So, P. W. & Carpenter, G. H. Intraoral Microbial Metabolism and Association with Host Taste Perception. *J. Dent. Res.* **99**, 739–745 (2020).
21. Cattaneo, C. *et al.* New insights into the relationship between taste perception and oral microbiota composition. *Sci. Rep.* **9**, (2019).
22. Besnard, P. *et al.* Obese Subjects with Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. *Sci. Rep.* **8**, (2018).
23. Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M. & Hummel, T. The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS One* **7**, e36557 (2012).
24. Mameli, C. *et al.* Taste perception and oral microbiota are associated with obesity in children and adolescents. *PLoS One* **14**, (2019).
25. Yousaf, N. Y. *et al.* Daily Exposure to a Cranberry Polyphenol Oral Rinse Alters the Oral Microbiome but Not Taste Perception in PROP Taster Status Classified Individuals. *Nutrients* **14**, 1492 (2022).

26. Valentino, V., De Filippis, F., Menghi, L., Gasperi, F. & Ercolini, D. Food Neophobia and scarce olfactory performances are linked to oral microbiota. *Food Res. Int.* **155**, 111092 (2022).

27. Cattaneo, C., Riso, P., Laureati, M., Gargari, G. & Pagliarini, E. Exploring associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake. *Nutrients* **11**, 1167 (2019).

28. Marsh, P. D. & Zaura, E. Dental biofilm: ecological interactions in health and disease. *J. Clin. Periodontol.* **44**, S12–S22 (2017).

29. Willis, J. R. *et al.* Citizen science charts two major 'stomatotypes' in the oral microbiome of adolescents and reveals links with habits and drinking water composition. *Microbiome* **6**, (2018).

30. Esberg, A., Haworth, S., Hasslöf, P., Holgerson, P. L. & Johansson, I. Oral microbiota profile associates with sugar intake and taste preference genes. *Nutrients* **12**, (2020).

31. Kaur, K. *et al.* Bitter and sweet taste perception: relationships to self-reported oral hygiene habits and oral health status in a survey of Australian adults. *BMC Oral Health* **21**, 1–11 (2021).

32. Diószegi, J., Llanaj, E. & Ádány, R. Genetic Background of Taste Perception, Taste Preferences, and Its Nutritional Implications: A Systematic Review. *Front. Genet.* **10**, 1272 (2019).

33. Wu, G., Zhao, N., Zhang, C., Lam, Y. Y. & Zhao, L. Guild-based analysis for understanding gut microbiome in human health and diseases. *Genome Med.* **13**, 1–12 (2021).

34. Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E. & Keast, R. The Relationships Between Common Measurements of Taste Function. *Chemosens. Percept.* **8**, 11–18 (2015).

35. Puputti, S., Aisala, H., Hoppu, U. & Sandell, M. Multidimensional measurement of individual differences in taste perception. *Food Qual. Prefer.* **65**, 10–17 (2018).

36. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–124 (2018).

37. Spinelli, S. *et al.* Personality traits and gender influence liking and choice of food pungency. *Food Qual. Prefer.* **66**, 113–126 (2018).

38. Menghi, L. *et al.* Variations in oral responsiveness associate with specific signatures in the gut microbiota and modulate dietary habits. *Food Qual. Prefer.* **106**, 104790 (2023).

39. Schutz, H. G. & Cardello, A. V. A labeled affective magnitude (LAM) scale for assessing food liking/disliking. *J. Sens. Stud.* **16**, 117–159 (2001).

40. Apprill, A., Mcnally, S., Parsons, R. & Weber, L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75**, 129–137 (2015).

41. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1–e1 (2013).

42. Gaudioso, G. *et al.* 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. *Int. J. Mol. Sci.* **22**, 5454 (2021).

43. Callahan, B. J. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).

44. Chen, T. *et al.* The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database (Oxford).* **2010**, (2010).

45. Hayes, J. E., Sullivan, B. S. & Duffy, V. B. Explaining variability in sodium intake through oral sensory phenotype, salt sensation and liking. *Physiol. Behav.* **100**, 369–380 (2010).

46. Smutzer, G., Desai, H., Coldwell, S. E. & Griffith, J. W. Validation of edible taste strips for assessing PROP taste perception. *Chem. Senses* **38**, 529–539 (2013).

47. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).

48. Pedrabrissi, L., Santinello, M. Verifica della validità dello STAI forma Y di Spielberger. *Giunti Organ. Spec.* **15**, 1–23 (2016).

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

50. Roininen, K. & Tuorila, H. Health and taste attitudes in the prediction of use frequency and choice between less healthy and more healthy snacks. *Food Qual. Prefer.* **10**, 357–365 (1999).

51. Saba, A. *et al.* Measuring consumers attitudes towards health and taste and their association with food-related life-styles and preferences. *Food Qual. Prefer.* **73**, 25–37 (2019).

52. van Strien, T., Frijters, J. E. R., Bergers, G. P. A. & Defares, P. B. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *Int. J. Eat. Disord.* **5**, 295–315 (1986).

53. Fossati, A., Borroni, S., Marchione, D. & Maffei, C. Reliability and validity of its italian translation in three independent nonclinical samples. *Eur. J. Psychol. Assess.* **27**, 50–58 (2011).

54. Corr, P. J. *Handbook of Personality Psychology*. *Personality and Individual Differences* vol. 24 (Guilford Press, 1998).

55. De Backer, C. J. S. & Hudders, L. Meat morals: Relationship between meat consumption consumer attitudes towards human and animal welfare and moral behavior. *Meat Sci.* **99**, 68–74 (2015).

56. Poslusna, K., Ruprich, J., De Vries, J. H. M., Jakubikova, M. & Van'T Veer, P. Misreporting of energy and micronutrient intake estimated by food records and 24hour recalls, control and adjustment methods in practice. *Br. J. Nutr.* **101**, S73–S85 (2009).

57. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. & Egozcue, J. J. Microbiome datasets are compositional: And this is not optional. *Front. Microbiol.* **8**, 2224 (2017).

58. Palarea-Albaladejo, J. & Martín-Fernández, J. A. ZCompositions - R package for multivariate imputation of left-censored data under a compositional approach. *Chemom. Intell. Lab. Syst.* **143**, 85–96 (2015).

59. Kassambara, A. *Practical Guide To Cluster Analysis in R: Unsupervised machine learning*. *Sthda* vol. 1 (Sthda, 2017).

60. Brock, G., Pihur, V., Datta, S. & Datta, S. ClValid: An R package for cluster validation. *J. Stat. Softw.* **25**, 1–22 (2008).

61. Smirnova, E., Huzurbazar, S. & Jafari, F. PERFect: PERmutation filtering test for microbiome data.

Biostatistics **20**, 615–631 (2019).

62. Lin, H. & Peddada, S. Das. Analysis of compositions of microbiomes with bias correction. *Nat. Commun.* **11**, 1–11 (2020).

63. Douglas, G. M. *et al.* PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* **38**, 685–688 (2020).

64. Sture Holm. A Simple Sequentially Rejective Multiple Test Procedure. *Scand. J. Stat.* **6**, 65–70 (1979).

65. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *http//www. R-project. org/* (2022).

66. McMurdie, P. J. & Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**, e61217 (2013).

67. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **14**, 927–930 (2003).

68. Ruan, X., Luo, J., Zhang, P. & Howell, K. The salivary microbiome shows a high prevalence of core bacterial members yet variability across human populations. *npj Biofilms Microbiomes* **8**, 1–14 (2022).

69. Zaura, E. *et al.* On the ecosystemic network of saliva in healthy young adults. *ISME J.* **11**, 1218–1231 (2017).

70. De Filippis, F. *et al.* The same microbiota and a potentially discriminant metabolome in the saliva of omnivore, ovo-lacto-vegetarian and vegan individuals. *PLoS One* **9**, e112373 (2014).

71. Lawless, H. A comparison of different methods used to assess sensitivity to the taste of phenylthiocarbamide (PTC). *Chem. Senses* **5**, 247–256 (1980).

72. Zhao, L., Kirkmeyer, S. V. & Tepper, B. J. A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. *Physiol. Behav.* **78**, 625–633 (2003).

73. Hansen, T. H. *et al.* Impact of a vegan diet on the human salivary microbiota. *Sci. Rep.* **8**, (2018).

74. Pagliarini, E. *et al.* The role of sour and bitter perception in liking, familiarity and choice for phenol-rich plant-based foods. *Food Qual. Prefer.* **93**, 104250 (2021).

75. Zandstra, E. H., De Graaf, C. & Van Staveren, W. A. Influence of health and taste attitudes on consumption of low- and high-fat foods. *Food Qual. Prefer.* **12**, 75–82 (2001).

76. Esposito, C. M., Ceresa, A. & Buoli, M. The Association between Personality Traits and Dietary Choices: A Systematic Review. *Advances in Nutrition* vol. 12 1149–1159 (2021).

77. Meier, B. P., Moeller, S. K., Riemer-Peltz, M. & Robinson, M. D. Sweet taste preferences and experiences predict prosocial inferences, personalities, and behaviors. *J. Pers. Soc. Psychol.* **102**, 163–174 (2012).

78. Sagioglou, C. & Greitemeyer, T. Individual differences in bitter taste preferences are associated with antisocial personality traits. *Appetite* **96**, 299–308 (2016).

79. Pfeiler, T. M. & Egloff, B. Personality and eating habits revisited: Associations between the big five, food choices, and Body Mass Index in a representative Australian sample. *Appetite* **149**, 104607 (2020).

80. Duarte-Coimbra, S., Forcina, G., Pérez-Pardal, L. & Beja-Pereira, A. Characterization of tongue dorsum microbiome in wine tasters. *Food Res. Int.* **163**, 112259 (2023).

81. Sandell, M. A. & Collado, M. C. Genetic variation in the TAS2R38 taste receptor contributes to the oral microbiota in North and South European locations: A pilot study. *Genes Nutr.* **13**, (2018).

82. Asano, Y. *et al.* Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **303**, 1288–1295 (2012).

83. O'Driscoll., S. *et al.* Serotonin-specific reuptake inhibitors (SSRI) blunt bitter taste acutely (minutes), but enhance it chronically (hours) in normal healthy humans. in *Proceedings of The Physiological Society* vol. 11 11, PC102 (2006).

84. Huang, Y. A., Dando, R. & Roper, S. D. Autocrine and paracrine roles for ATP and serotonin in mouse taste buds. *J. Neurosci.* **29**, 13909–13918 (2009).

85. Thompson, F. E. & Subar, A. F. Dietary assessment methodology. *Nutr. Prev. Treat. Dis.* 5–48 (2017) doi:10.1016/B978-0-12-802928-2.00001-1.

Chapter 6

General discussion

CHAPTER 6:

GENERAL DISCUSSION

6.1 | General discussion and future perspectives

While the benefits of healthy eating have never been more emphasized than nowadays, the epidemic spread of diet-related non-communicable diseases seems unabated [1,2]. Globally, suboptimal diets high in sodium or poor in whole grains or fruits have been labelled as the three leading dietary risk factors for a staggering number of deaths (~ 11 million) and years of life (~ 255 million) lost prematurely [3]. Furthermore, such dramatic estimates are reportedly not confounded by age, sex, or development status of a nation [3]. It is therefore apparent that improving our knowledge about aetiology of eating behaviors turns out to be essential for the sake of public health.

Decades of research on this topic has illuminated the multidimensional nature of food choices and distilled its foundational elements [4]. Among these, sensory perception is one of the most influential, as eliciting unequivocal messages to discern beneficial nutrients from toxicants, as well as making explicit how palatable the food is [5]. While the evolutionary purposes of chemical senses are shared among humans, the same is not true for chemosensory abilities. Indeed, odorants and tastants are perceived differently from person to person, and this clue has been acknowledged as amongst the major determinants of individual food choices (e.g., [4,6,7]). Interestingly, emerging evidence suggests that the cornucopia of microorganisms inhabiting the GI tract may play in concert with chemosensation to modulate eating behaviors [8–10]. Nonetheless, the youth of the sensory-related microbiome field makes the subject largely unexplored and affected by a number of gaps. Thus, the current thesis was motivated by the clear need to expand the current knowledge supporting the existence of synergistic interplays between domains of sensory perception and the GI microbiota that

would act as additional mediators of dietary habits. To this end, we employed a multidisciplinary approach aimed at gathering a variety of sensory-related measures as well as dietary habits and samples of salivary and gut microbiota in both controlled (**Chapter 2-3**) and natural (**Chapter 4-5**) settings to maximize the ecological value of results.

Specifically, **Chapter 2** and **3** were devoted to probing the associations between food neophobia (FN), olfactory performances and the composition of the salivary microbiota. A twofold purpose encouraged these experimental efforts. First, it was still unclear whether the endorsement of neophobic behaviors was more likely attributable to superior olfactory abilities or higher levels of negative arousal triggered by chemosensory stimuli (**Chapter 2**). Second, little research existed on the links between ortho- and retronasal olfaction and salivary microbial communities, and we also took advantage of the large data collection protocol to test whether the negative effects of FN on health spilled over to the oral microbial consortium (**Chapter 3**).

In both **Chapter 2** and **3**, a gender-balanced cohort of 83 healthy Italian adults (57.8 % women; 22-68 y/o) were tested for FN and trait anxiety levels before being asked to provide an unstimulated salivary sample. Next, volunteers underwent a full assessment of orthonasal olfactory functioning and monitoring of retronasally exhaled VOCs during consumption of a commercial strawberry jelly candy. Altogether, pronounced FN traits inversely associated with global olfactory functioning or the extent of retronasal aroma release, and we speculated this effect to be mediated by a marginally higher anxiety proneness and (especially) by physiologically clues of heightened levels of negative arousal in response to chemosensation (**Chapter 2**). Thus, these findings extend to olfaction the notion that FN tendencies would be fostered by unpleasant emotional states aroused by exogenous stimuli rather than by enhanced chemosensory functions [11,12]. Importantly, further support to such paradigm has subsequently been provided by two large-scale reports involving 749 [13] and 8906 [14] adults, respectively. It turned out that neophobics rated a few common odorants (banana, pine) as less intense

and pleasant relative to neophilics [13], and that FN was more negatively correlated with hedonic responses to elicitors of high arousal (e.g., names of novel, complex and high flavored foods) than to elicitors of mild arousal (e.g., names of familiar or sweet foods) [14]. Hence, while further studies coupling actual food tasting with physiological measures of arousal are needed to confirm the arousal hypothesis [14], the existing literature is seemingly solid enough to be translated in real-life contexts. As already argued [15], the development of therapeutic strategies targeting the arousal-promoting psychological correlates of FN (e.g., anxiety [16]) may be a viable new research frontier to reduce the prevalence of a health-damaging behavioral trait now documented in ~ 25 % of the global adult population [11,17–23].

The call for interventional studies aimed at alleviating FN tendencies is further warranted by results of **Chapter 3**. Here, we provided the first empirical evidence extending the deleterious health outcomes caused by FN to a disruption of the salivary microbiota. Notably, high neophobics were found to harbor greater proportions of two dysbiotic salivary microbial species (*Scardovia wiggsiae* and *Klebsiella pneumoniae*) than neophilics, and we tentatively attributed these findings to the variations in dietary patterns commonly observed between FN levels. More interestingly, the same study also highlighted a spectrum of oral microbial signatures correlated with both ortho- and retronasal olfactory performances. First, normosmia paralleled an eubiotic status of the salivary microbiota, whereas those who were classed as hyposmic (TDI < 30.75) [24] housed higher abundances of oral pathogens (e.g., *Porphyromonas gingivalis*). Second, a number of salivary microbial genera were negatively correlated with olfactory thresholds (e.g., *Porphyromonas*, *Fusobacterium*), or with the retronasal exhalation of most of the VOCs monitored (e.g., *Bifidobacterium, Bacteroides*). Of note, our findings on hyposmics were consistent with a previous study [25], which noted an enrichment of similar microbes in the nasal microbiota of people with poor olfactory abilities. Nevertheless, how these taxa might be allocated to diminished chemosensory abilities is even hard to speculate. Indeed,

the lack of dietary and/or supra-threshold measures unfortunately gives our results a merely observational connotation. However, it is worth mentioning that both **Chapter 2** and **3** were carried out within the framework of a broader project aimed at clarifying the prevalence of smell disorders in Italy by collecting a large panel of psychological and cognitive correlates of the olfactory function [26,27]. Therefore, we preferred rapid or simple to lengthy or demanding tasks (e.g., *nose-space* analysis coupled with temporal sensory methods) to minimal participant load and ensure data accuracy.

As legitimately pointed out by an anonymous reviewer of **Chapter 2** [28], one might questions the appropriateness of our conclusions, as potentially ascribable to domains of cognition we gathered. These included the Italian validated versions of the Cognitive Reserve Index questionnaire (CRIq) [29] and the Montreal Cognitive Assessment (MoCA) [30]. The former captures variations in interpersonal abilities to cope with cognitive decline [29], whilst the latter serves as a rapid detecting method of mild cognitive defects [30]. Nonetheless, a cognitive-related confounding effect on results was reasonably excluded, as FN levels were proven to show similar cognitive abilities (CRIq Total Score: $H = 0.18$; $p = 0.914$; MoCA Score: $H = 0.09$; $p = 0.952$), and both CRIq and MoCA total scores exhibited no significant correlations either with olfactory performances or amount of VOCs exhaled (Table 6.1).

Table 6.1: Spearman's rho coefficients between olfactory performances (TDI, OT, OD, OI) or amounts of the 7 VOCs (area under the curve) exhaled during the *nose-space* task and cognitive reserve (CRIq) or the extent of mild cognitive impairment (MoCA). Significance rates (p.value) are also tabulated.

Sum formula	Test	CRIq	p.value	MoCA	p.value
/	TDI	-0.127	0.251	0.130	0.239
/	OT	-0.101	0.360	-0.088	0.428
/	OD	-0.176	0.119	0.165	0.135
/	OI	0.067	0.544	0.153	0.164
$C_7H_8O_3$	ethylmaltol	-0.032	0.772	0.124	0.259
$C_6H_{12}O$	3-hexen-1-ol	-0.092	0.407	0.128	0.245
$C_7H_{14}O_2$	ethyl 2-methylbutanoate	-0.079	0.474	0.090	0.415
$C_8H_{14}O_2$	(Z)-3-hexenyl acetate	-0.154	0.163	-0.023	0.835
$C_6H_{12}O_2$	ethylbutanoate	-0.076	0.491	0.150	0.173
$C_8H_{16}O_2$	ethylhexanoate	-0.081	0.465	0.119	0.280
$C_5H_{10}O_2$	2-methylbutanoic acid	-0.086	0.437	0.052	0.639

Taken collectively, **Chapter 2** and **3** describe a scenario wherein neophobics would adopt protective behaviors to deal with unpleasant levels of arousal in response to chemosensation [14,28], which would cascade to promote unhealthy dietary habits and alterations in the oral microbiota [31]. Moreover, our results represent an important contribution in support of the existence of crosstalks between olfactory function and salivary microbial consortia, which deserve more attention in future research to unravel the underlying mechanisms.

In addition to expanding current knowledge on the associations between olfactory abilities and the GI microbiota, this thesis has succeeded in filling a few of the existing gaps within the taste-oriented microbiome research field. Undoubtedly, the lengthy list of exclusion criteria combined with the comprehensive experimental design used in both **Chapter 4** and **5** were the key to success. Hitherto, there were no studies simultaneously linking responsiveness to PROP, hedonic and psychophysical responses to actual food products, and food-related attitudinal and psychological measures with salivary or gut microbial ecology. We addressed the issue by asking a gender-balanced cohort of 100 young Italian adults (52 % women; 18-30 y/o) to remotely attend a 7-day double-blind protocol, whose tasks were designed to: a) minimize participant burden; b) ensure respect of good practices in sensory evaluations in remote settings; c) facilitate understanding of psychophysical scales; d) maximize compliance with guidelines for accurate food diary completion; and e) enable independent collection of biological sample. Moreover, special emphasis was placed on selecting food stimuli, and more importantly, on devising countermeasures to safeguard participants from the health risks caused by the ongoing COVID-19 pandemic.

In **Chapter 4**, we provided the first empirical evidence supporting the notion that variations in orosensory acuity can simultaneously mirror both gut microbial ecology and dietary patterns, as well as confirming known interactions between diet and the gut microbiota. Overall, hyporesponsiveness to nearly all oral sensations corresponded to greater proportions of a panel of

commensal gut microbial genera, mainly from families *Lachnospiraceae* and *Ruminococcaceae*, and higher consumption of beneficial nutrients (e.g., fibers, vegetable proteins, vitamins). Conversely, being hyperresponsive to oral stimulations translated into a more pro-inflammatory gut microbiota and intake of saturated fats. Hence, we reasonably concluded that sensory perception plus its psychometric correlates and dietary habits would play in concert to ultimately shape the gut microbial ecosystem (e.g., [4,32–34]). Nonetheless, **Chapter 4** also speculated an alternative pathway by which gut microbes might actively contribute to such paradigm via the systemic release of inflammatory products that would reach the taste buds and modulate taste receptor expression (see for a review [9]). Whether this might be plausible is yet to be unveiled, but it could be an additional research frontier motivated by our findings.

Similarly, **Chapter 5** substantially evidenced that a *Clostridia*-enriched salivary microbiota positively associated with hyporesponsiveness to warning oral sensations (e.g., bitter, sour, astringent) in both solid and liquid foods, habitual intakes of beneficial nutrients, and less pronounced pleasure-oriented attitudes towards foods. Conversely, the opposite emerged in concomitance with greater abundances of oral cariogenic bacterial genera (*Lactobacillus*, *Bifidobacterium*), which were also linked to consumption of simple carbohydrates. Intriguingly, the extensive data collection protocol together with the outputs from a metagenome prediction tool (PICRUSt2 [35]) enabled us to speculate putative biological pathways underlying these results. In detail, we observed three MetaCyc modules related to the metabolic fate of a sour-eliciting compound to be enriched in those exhibiting poor responsiveness to alarming chemosensory stimuli. While PICRUSt2 inferences can not be directly allocated to specific microbial consortia [35], our findings would agree with the assumption that increased concentrations of taste-active secondary metabolites in proximity of taste buds may induce taste receptor adaptation and downregulate signaling cascade (see for a review [9]). Moreover, a few salivary microbial signatures here associated with patterns of oral hyporesponsiveness have recently

been reported with poor PROP acuity [36,37], sour sensitivity [38] or olfactory thresholds (**Chapter 3**) [31], thus leading us to encourage new studies to clarify the role of *Clostridia* on chemosensation.

On the other hand, we also detected a few salivary microbial taxa that may serve as signatures of enhanced responsiveness to warning sensations, which were taxonomically assigned to the phylum *Actinobacteria*. Our findings are apparently consistent with previous reports, which found members of this phylum to be enriched in salivary specimens of PROP STs [36] or in the tongue *dorsum* of hyperresponsive individuals to a range of oral sensations [39]. However, prominent compositional deviations between salivary and lingual bacterial consortia have been highlighted [40], and higher abundances of *Actinobacteria* suspended in saliva not necessarily translated into higher chemosensory abilities [40]. While such discrepancies may partly be explained by methodological differences across studies in both taste assessments and/or microbial sequencing methods, new research involving the simultaneous collection of samples from various oral sites is needed to resolve the debate.

Interestingly, **Chapter 5** also represents a striking example of how the individual psychological sphere may putatively contribute to shape the salivary microbiota. Indeed, we noted that high craving for sweet foods went along with increased intake of simple carbohydrates and enrichment of known cariogenic taxa. Hence, given that caries progression closely tied to such bacteria and exposure to sweet foods (e.g., [41]), which in turn is promoted by pleasure-oriented tendencies [42,43], these findings seem to corroborate the mediating role of individual psychological background on oral health previously highlighted in **Chapter 3**. Notably, similar conclusions can be drawn from results of **Chapter 4**, wherein higher external eating behaviors and proneness to use food as a source of reward co-occurred with greater consumption of saturated fats and abundances of pro-inflammatory gut microbial consortia. Moreover, further commonalities arose between **Chapter 4** and **Chapter 5**, which revolved around the extent to which bacterial α-diversity metrics and patterns of oral responsiveness associated with psychological traits. Indeed, pleasure-oriented tendencies were

systematically linked to lower gut (**Chapter 4**) or salivary (**Chapter 5**) microbial evenness and richness, heightened oral acuity, and unhealthy dietary habits. As a result, besides strengthening a broad literature aware of the links between individual psychology, chemosensory abilities and food choices, this thesis motivates the scientific community to increasingly consider the variety of food-related psychometric constructs as important covariates when relationships between GI microbial consortia and dietary outcomes are of research interest.

To conclude, this thesis further consolidated several existing knowledge within the sensory and consumer science field. Specifically, we confirmed that: a) a segment of population can exhibit hypergeusia in response to chemosensory stimuli across various sensory modalities (e.g., [44–46]); b) supra-threshold intensities collected from real food matrices are effective in predicting variations in food choices and dietary habits (e.g, [34,47]); c) hyporesponsiveness to a certain taste quality generally amplifies the consumption of foods that evoke it (e.g., [48]). Conversely, we failed to provide strong evidence supporting PROP acuity as an index of enhanced orosensory abilities, though more responsive individuals to oral stimuli tended to be classed more as PROP MTs rather than PROP NTs (**Chapter 4-5**). Moreover, some concerns have arisen about the accuracy of paper strips [49,50] in reliably assessing associations between PROP taste phenotypes and chemosensation, which may explain much of this surprising finding. Lastly, this work also offered a few methodological clues for optimal planning of sensory remote testing, which converge with the guidelines recently outlined by Dinnella *et al.* [51]. Remarkably, an element of novelty of current thesis lies in the logic-basic tool designed to avoid idiosyncratic uses of the gLMS scale, which proved its efficacy in disentangling the intensity ratings given to extraoral training stimuli from those given in response to oral stimulation.

6.2 | Methodological considerations

The above discussion has anticipated several potential new avenues that can be interesting to pursue in the near future to further expand a burgeoning research field. However, any experimental effort would be poorly informative if a number of recommendations were not taken into account. First, one should always consider that eating behaviors are affected by a large series of factors [4], whose impact can vary from person to person. As a result, the individuality of food choices can only be reliably investigated within the framework of multidisciplinary designs. And this especially applies when links between domains of sensory perception and the GI microbiota are supposed to be functional in describing interpersonal variations in dietary habits. Nevertheless, such approach can pose several methodological and organizational issues, which can severely affect the duration of planning due to the multitude of skills needed to plan a robust experiment. In this vein, the sensory-oriented microbiome field would profit from the formation of research consortia, which should be made up of specialists from pertinent disciplines to improve the efficiency of logistical aspects and aid in raising public awareness of the topic. Importantly, this would likely solve another major issue of the exiting literature, which has so far precluded the generalization of outcomes. Indeed, the majority of the studies has employed low sample sizes [25,36,39,40,48,52–54] in view of the myriad covariates of both sensory perception and GI microbial ecology. While our cohorts were also relatively small, we sought to overcome this drawback by applying a long list of exclusion criteria that enabled us draw inferences minimally affected by known confounding factors. Nonetheless, we can not conclude that our findings are transferable to broader populations. To this end, multicenter studies may be a viable and cost-effective solution to ease the recruitment of a large number of individuals and maximize the external validity of outcomes.

Second, there is a clear need to move towards more ecologically valid measures of both taste and smell functioning. As previously discussed, the existing literature operationalized chemosensory functions almost exclusively via thresholds in response to stimuli elicited by artificial model systems (aqueous solutions or paper strips) [25,39,40,48,52–56]. Nevertheless, such measures poorly approximate what people actually sense from foods, and their usefulness as valuable predictors of dietary outcomes has been questioned [45,57,58]. On the other hand, collecting supra-threshold intensities from real food matrices resolves these limitations (e.g., [34,47,59]), and our findings add further support to the claim that this approach ought to be regarded as the gold standard method for examining the associations between chemosensation and dietary habits [59]. Obviously, this recommendation should also be increasingly shared by the sensory-oriented microbiome research field.

Third, a number of inconsistent results will keep to typify the existing literature if the whole treatment and subsequent sequencing of microbial samples are not standardized. Therefore, additional effort should be spent on providing the scientific community with detailed guidelines encompassing all stages of the workflow, from sample collection to data processing. Numerous valuable reviews are now available for this latter purposes (e.g., [60–63]), which would offer the major advantage of enabling fairer comparisons between studies and future meta-analysis studies. Further, this would also aid in the resolution of several open questions within the field, most notably the extent to which microbes inhabiting distinct oral niches (saliva, tongue *dorsum*) relate to orosensory abilities. Notwithstanding, this would only be achievable if future studies devote attention to the simultaneous collection of samples from various sites in the oral cavity, and this clue can be extended to the nasal and gut microbiota if a holistic approach linking sensory perception with the microbial consortia probably linked to chemosensation is the research question at hand.

Lastly, the biological mechanisms underlying the crosstalks between chemosensation and the GI microbial communities are far from being conclusively understood due to the common use of low-

resolution sequencing strategies and the lack of metabolomic or genetic data. In this vein, the sensory-oriented microbiome field could initially invest its resources by favoring metagenomic (shotgut sequencing) over canonical metataxonomic (16s rRNA gene sequencing) sequencing techniques, as ensuring a deeper taxonomic depth (up to the strain level) and coverage (all microorganisms plus viruses) [64]. Beyond detecting a robust array of putative sensory-related microbial signatures, such tools enable to reliably estimate the functional profiling of microbial residents [64], which is instrumental to unravel the potential metabolic pathways underlying the microbe-taste interactions. Ideally, combining these results with metabolic and/or genetic data would be the optimal scenario to pave the way for the ultimate understanding of a research subject of relevant importance.

6.3 | Conclusions

Taken collectively, this work clearly proofed how complex the dynamics underlying eating behaviors are, and how a comprehensive and multidisciplinary protocol is critical to drawing not inflated inferences. The idea that microbes could play in tandem with chemosensation to ultimately modulate eating behaviors is as fascinating as it is challenging to investigate. Nevertheless, this thesis constitutes an initial experimental effort to elucidate an extremely complex scenario, whose elements are intimately interconnected. Hence, in view of the cross-sectional nature of our studies, it would be misleading to infer causality over the associations here observed. Throughout this thesis, we provided evidence on the existence of putative interactions between chemosensation, psychological traits, and the GI microbiota that would operate as mediators of dietary habits. However, like a classic "*chicken and egg*" situation, whether diet itself fosters the above mentioned associations can only be ruled out by longitudinal studies, in which causation can more reasonably be deduced.

To conclude, this work represents a prominent contribution to the fundamentals of sensory perception and eating behaviors, and turns the spotlight on the possibility that the GI microbiota may

serve as a further candidate to explain interindividual variations in chemosensory abilities. Moreover, it also offers methodological cues to robustly assess the links between chemosensation and host-related non genetic factors, and paves the way for future interventional studies targeting the efficacy of sensory-related microbial taxa as potential modulators of dietary habits.

6.4 | References

1. Chooi, Y. C., Ding, C. & Magkos, F. The epidemiology of obesity. *Metabolism.* **92**, 6–10 (2019).
2. Swinburn, B. A. *et al.* The global obesity pandemic: Shaped by global drivers and local environments. *Lancet* **378**, 804–814 (2011).
3. Afshin, A. *et al.* Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **393**, 1958–1972 (2019).
4. Köster, E. P. Diversity in the determinants of food choice: A psychological perspective. *Food Qual. Prefer.* **20**, 70–82 (2009).
5. Breslin, P. A. S. An evolutionary perspective on food and human taste. *Curr. Biol.* **23**, R409–R418 (2013).
6. Boesveldt, S. & de Graaf, K. The differential role of smell and taste for eating behavior. *Perception* **46**, 307–319 (2017).
7. Duffy, V. B. Variation in oral sensation: Implications for diet and health. *Curr. Opin. Gastroenterol.* **23**, 171–177 (2007).
8. Alcock, J., Maley, C. C. & Aktipis, C. A. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays* vol. 36 940–949 (2014).
9. Leung, R. & Covasa, M. Do gut microbes taste? *Nutrients* **13**, 2581 (2021).
10. Schwartz, M., Canon, F., Feron, G., Neiers, F. & Gamero, A. Impact of oral microbiota on flavor perception: From food processing to in-mouth metabolization. *Foods* vol. 10 (2021).
11. Laureati, M. *et al.* Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample. *Food Qual. Prefer.* **68**, 113–124 (2018).
12. Spinelli, S. *et al.* Personality traits and gender influence liking and choice of food pungency. *Food Qual. Prefer.* **66**, 113–126 (2018).
13. Puleo, S. *et al.* Food neophobia, odor and taste sensitivity, and overall flavor perception in food. *Foods*

10, 3122 (2021).

14. Jaeger, S. R., Chheang, S. L. & Prescott, J. Variations in the strength of association between food neophobia and food and beverage acceptability: A data-driven exploratory study of an arousal hypothesis. *Nutrients* **13**, 3657 (2021).

15. Marcontell, D. K., Laster, A. E. & Johnson, J. Cognitive-behavioral treatment of food neophobia in adults. *J. Anxiety Disord.* **16**, 341–349 (2002).

16. Pliner, P. & Hobden, K. Development of a scale to measure the trait of food neophobia in humans. *Appetite* **19**, 105–120 (1992).

17. Meiselman, H. L., King, S. C. & Gillette, M. The demographics of neophobia in a large commercial US sample. *Food Qual. Prefer.* **21**, 893–897 (2010).

18. de Barcellos, M. D., Aguiar, L. K., Ferreira, G. C. & Vieira, L. M. Willingness to try innovative food products: A comparison between British and Brazilian consumers. *BAR - Brazilian Adm. Rev.* **6**, 50–61 (2009).

19. Choe, J. Y. & Cho, M. S. Food neophobia and willingness to try non-traditional foods for Koreans. *Food Qual. Prefer.* **22**, 671–677 (2011).

20. Jaeger, S. R., Rasmussen, M. A. & Prescott, J. Relationships between food neophobia and food intake and preferences: Findings from a sample of New Zealand adults. *Appetite* **116**, 410–422 (2017).

21. Olabi, A., Najm, N. E. O., Baghdadi, O. K. & Morton, J. M. Food neophobia levels of Lebanese and American college students. *Food Qual. Prefer.* **20**, 353–362 (2009).

22. Idowu-Adebayo, F., Fogliano, V., Oluwamukomi, M. O., Oladimeji, S. & Linnemann, A. R. Food neophobia among Nigerian consumers: a study on attitudes towards novel turmeric-fortified drinks. *J. Sci. Food Agric.* **101**, 3246–3256 (2021).

23. Sarin, H. V. *et al.* Food neophobia associates with poorer dietary quality, metabolic risk factors, and increased disease outcome risk in population-based cohorts in a metabolomics study. *Am. J. Clin. Nutr.* **110**, 233–245 (2019).

24. Oleszkiewicz, A., Schriever, V. A., Croy, I., Hähner, A. & Hummel, T. Updated Sniffin' Sticks

normative data based on an extended sample of 9139 subjects. *Eur. Arch. Oto-Rhino-Laryngology* **276**, 719–728 (2019).

25. Koskinen, K. *et al.* The nasal microbiome mirrors and potentially shapes olfactory function. *Sci. Rep.* **8**, (2018).
26. Masala, C. *et al.* An Italian population-based study of the prevalence of olfactory impairment. *Chem. Senses* **44**, e47–e47 (2019).
27. Masala, C. *et al.* Correlation between olfactory function, age, sex, and cognitive reserve index in the Italian population. *Eur. Arch. Oto-Rhino-Laryngology* **279**, 4943–4952 (2022).
28. Menghi, L. *et al.* Arousal influences olfactory abilities in adults with different degree of food neophobia. *Sci. Rep.* **10**, 1–15 (2020).
29. Nucci, M., Mapelli, D. & Mondini, S. Cognitive Reserve Index questionnaire (CRIq): A new instrument for measuring cognitive reserve. *Aging Clin. Exp. Res.* **24**, 218–226 (2012).
30. Conti, S., Bonazzi, S., Laiacona, M., Masina, M. & Coralli, M. V. Montreal Cognitive Assessment (MoCA)-Italian version: regression based norms and equivalent scores. *Neurol. Sci.* **36**, 209–214 (2015).
31. Valentino, V., De Filippis, F., Menghi, L., Gasperi, F. & Ercolini, D. Food Neophobia and scarce olfactory performances are linked to oral microbiota. *Food Res. Int.* **155**, 111092 (2022).
32. Cronin, P., Joyce, S. A., O'toole, P. W. & O'connor, E. M. Dietary fibre modulates the gut microbiota. *Nutrients* **13**, (2021).
33. Wolters, M. *et al.* Dietary fat, the gut microbiota, and metabolic health – A systematic review conducted within the MyNewGut project. *Clin. Nutr.* **38**, 2504–2520 (2019).
34. Monteleone, E. *et al.* Exploring influences on food choice in a large population sample: The Italian Taste project. *Food Qual. Prefer.* **59**, 123–140 (2017).
35. Douglas, G. M. *et al.* PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* **38**, 685–688 (2020).
36. Yousaf, N. Y. *et al.* Daily Exposure to a Cranberry Polyphenol Oral Rinse Alters the Oral Microbiome

but Not Taste Perception in PROP Taster Status Classified Individuals. *Nutrients* **14**, 1492 (2022).

37. Sandell, M. A. & Collado, M. C. Genetic variation in the TAS2R38 taste receptor contributes to the oral microbiota in North and South European locations: A pilot study. *Genes Nutr.* **13**, (2018).

38. Duarte-Coimbra, S., Forcina, G., Pérez-Pardal, L. & Beja-Pereira, A. Characterization of tongue dorsum microbiome in wine tasters. *Food Res. Int.* **163**, 112259 (2023).

39. Cattaneo, C. *et al.* New insights into the relationship between taste perception and oral microbiota composition. *Sci. Rep.* **9**, (2019).

40. Feng, Y. *et al.* The Associations between Biochemical and Microbiological Variables and Taste Differ in Whole Saliva and in the Film Lining the Tongue. *Biomed Res. Int.* **2018**, (2018).

41. Esberg, A., Haworth, S., Hasslöf, P., Holgerson, P. L. & Johansson, I. Oral microbiota profile associates with sugar intake and taste preference genes. *Nutrients* **12**, (2020).

42. Roininen, K. & Tuorila, H. Health and taste attitudes in the prediction of use frequency and choice between less healthy and more healthy snacks. *Food Qual. Prefer.* **10**, 357–365 (1999).

43. Zandstra, E. H., De Graaf, C. & Van Staveren, W. A. Influence of health and taste attitudes on consumption of low- and high-fat foods. *Food Qual. Prefer.* **12**, 75–82 (2001).

44. Hayes, J. E. & Keast, R. S. J. Two decades of supertasting: Where do we stand? *Physiol. Behav.* **104**, 1072–1074 (2011).

45. Puputti, S., Aisala, H., Hoppu, U. & Sandell, M. Multidimensional measurement of individual differences in taste perception. *Food Qual. Prefer.* **65**, 10–17 (2018).

46. Piochi, M., Dinnella, C., Spinelli, S., Monteleone, E. & Torri, L. Individual differences in responsiveness to oral sensations and odours with chemesthetic activity: Relationships between sensory modalities and impact on the hedonic response. *Food Qual. Prefer.* **88**, (2021).

47. Pagliarini, E. *et al.* The role of sour and bitter perception in liking, familiarity and choice for phenol-rich plant-based foods. *Food Qual. Prefer.* **93**, 104250 (2021).

48. Cattaneo, C., Riso, P., Laureati, M., Gargari, G. & Pagliarini, E. Exploring associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake.

Nutrients **11**, 1167 (2019).

49. Lawless, H. A comparison of different methods used to assess sensitivity to the taste of phenylthiocarbamide (PTC). *Chem. Senses* **5**, 247–256 (1980).
50. Zhao, L., Kirkmeyer, S. V. & Tepper, B. J. A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. *Physiol. Behav.* **78**, 625–633 (2003).
51. Dinnella, C. *et al.* Remote testing: Sensory test during Covid-19 pandemic and beyond. *Food Qual. Prefer.* **96**, 104437 (2022).
52. Besnard, P. *et al.* Obese Subjects with Specific Gustatory Papillae Microbiota and Salivary Cues Display an Impairment to Sense Lipids. *Sci. Rep.* **8**, (2018).
53. Mameli, C. *et al.* Taste perception and oral microbiota are associated with obesity in children and adolescents. *PLoS One* **14**, (2019).
54. Gardner, A., So, P. W. & Carpenter, G. H. Intraoral Microbial Metabolism and Association with Host Taste Perception. *J. Dent. Res.* **99**, 739–745 (2020).
55. Solemdal, K., Sandvik, L., Willumsen, T., Mowe, M. & Hummel, T. The impact of oral health on taste ability in acutely hospitalized elderly. *PLoS One* **7**, e36557 (2012).
56. Fluitman, K. S. *et al.* Associations of the oral microbiota and Candida with taste, smell, appetite and undernutrition in older adults. *Sci. Rep.* **11**, 1–11 (2021).
57. Duffy, V. B., Rawal, S. & Hayes, J. E. Measurement of Gustation: From Clinical to Population-Based Methods. *Sens. Sci. Chronic Dis.* 65–102 (2021) doi:10.1007/978-3-030-86282-4_4.
58. Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E. & Keast, R. The Relationships Between Common Measurements of Taste Function. *Chemosens. Percept.* **8**, 11–18 (2015).
59. Reed, D. R. *et al.* NIH workshop report: Sensory nutrition and disease. *Am. J. Clin. Nutr.* **113**, 232–245 (2021).
60. Knight, R. *et al.* Best practices for analysing microbiomes. *Nat. Rev. Microbiol.* **16**, 410–422 (2018).
61. Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. & Egozcue, J. J. Microbiome datasets are compositional: And this is not optional. *Front. Microbiol.* **8**, 2224 (2017).

62. Bellagambi, F. G. *et al.* Saliva sampling: Methods and devices. An overview. *TrAC - Trends Anal. Chem.* **124**, 115781 (2020).

63. Tang, Q. *et al.* Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. *Front. Cell. Infect. Microbiol.* **10**, 151 (2020).

64. Ranjan, R., Rani, A., Metwally, A., McGee, H. S. & Perkins, D. L. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem. Biophys. Res. Commun.* **469**, 967–977 (2016).

Appendix

APPENDIX

About the author

Leonardo Menghi was born in San Severino Marche (MC – Italy) in 1990. He began his university career in 2009 at the University of Urbino (Italy) with a Bachelor in "*Nutritional Sciences*". There he developed a particular interest in the human microbiota, which prompted him to focus his dissertation on the physio-pathological role of the gut microbiota in the onset of the coeliac disease. He then moved to the University of Florence in 2013 for a master's degree in "*Food Science"*. During his MSc, he did a 9-month internship at the sensory lab of the University of Florence, where he was involved on a project designed to test the effectiveness of categorization techniques to investigate consumer preferences toward vegetables. In 2016, he completed his coursework by defending a thesis on the role of hedonic and sensory expectations in the acceptability of vegetable-based dishes. After graduating with a MSc degree, in 2017 he was hired as a Technologist at the sensory lab of the Edmund Mach Foundation (San Michele All'Adige, Italy) to be part of a project focusing on monitoring and improving the sensory quality of local cheeses.

In November 2019, he started his PhD project "*Understanding the role of human microbiota on sensory perception*", as part of a double degree PhD program run by the University of Trento and the University of Southern Denmark. His project aimed at expanding the current knowledge on the links between domains of sensory perception of food and residents of the gastrointestinal microbiota, and how these might be related to habitual dietary habits.

List of publications

This thesis

1 | **Menghi, L.**, Cliceri, D., Fava, F., Pindo, M., Gaudioso, G., Giacalone, D. & Gasperi, F. (2023). *Salivary microbial profiles associate with oral responsiveness to warning oral sensations and dietary intakes*. Food Research International (In preparation)

2 | **Menghi, L.**, Cliceri, D., Fava, F., Pindo, M., Gaudioso, G., Stefani, E., Giacalone, D. & Gasperi, F. (2023). *Variations in oral responsiveness associate with specific signatures in the gut microbiota and modulate dietary habits*. Food Quality and Preference, 106, 104790. https://doi.org/10.1016/j.foodqual.2022.104790

3 | Valentino, V., De Filippis, F., **Menghi, L.**, Gasperi, F. & Ercolini, D. (2022). *Food Neophobia and scarce olfactory performances are linked to oral microbiota*. Food Research International, 155, 111092. https://doi.org/10.1016/j.foodres.2022.111092

4 | **Menghi, L.**, Khomenko, I., Pedrotti, M., Cliceri, D., Aprea, E., Endrizzi, I., Cavazzana, A., Biasioli, F., Giacalone, D. & Gasperi, F. (2020). *Arousal influences olfactory abilities in adults with different degree of food neophobia*. Scientific Reports, 10(1), 1–15. https://doi.org/10.1038/s41598-020-77428-w

Other publications in peer-reviewed journals

1 | Ricci, M., Gasperi, F., Betta, E., **Menghi, L.**, Endrizzi, I., Cliceri, D., Franceschi, P., & Aprea, E. (2023). *Multivariate data analysis strategy to monitor Trentingrana cheese real-scale production through volatile organic compounds profiling*. LWT, 173, 114364. https://doi.org/10.1016/j.lwt.2022.114364

2 | **Menghi, L.**, Endrizzi, I., Cliceri, D., Zampini, M., Giacalone, D., & Gasperi, F. (2022). *Validating the Italian version of the Adult Picky Eating Questionnaire*. Food Quality and Preference, 101, 104647. https://doi.org/10.1016/j.foodqual.2022.104647

3 | Ricci, M., Gasperi, F., Endrizzi, I., **Menghi, L.**, Cliceri, D., Franceschi, P., & Aprea, E. (2022). *Effect of*

Dairy, Season, and Sampling Position on Physical Properties of Trentingrana Cheese: Application of an LMM-ASCA Model. Foods, 11(1), 127. https://doi.org/10.3390/foods11010127

4 | Masala, C., Cavazzana, A., Sanna, F., Cecchini, M. P., Zanini, A., Gasperi, F., **Menghi, L.**, Endrizzi, I., Borgogno, M., Drago, S., Cantone, E., Ciofalo, A., Macchi, A., Monti, G., Parma, V., Piochi, M., Pinna, I., Torri, L., Cabrino, G., … Hummel, T. (2022). *Correlation between olfactory function, age, sex, and cognitive reserve index in the Italian population*. European Archives of Oto-Rhino-Laryngology, 279(10), 4943–4952. https://doi.org/10.1007/s00405-022-07311-z

5 | 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

6 | Pagliarini, E., Proserpio, C., Spinelli, S., Lavelli, V., Laureati, M., Arena, E., Di Monaco, R., **Menghi, L.**, Gallina Toschi, T., Braghieri, A., Torri, L., Monteleone, E. & Dinnella, C. (2021). *The role of sour and bitter perception in liking, familiarity and choice for phenol-rich plant-based foods*. Food Quality and Preference, 93, 104250. https://doi.org/10.1016/j.foodqual.2021.104250

7 | Endrizzi, I., Cliceri, D., **Menghi, L.**, Aprea, E. & Gasperi, F. (2021). *Does the 'mountain pasture product' claim affect local cheese acceptability?* Foods, 10(3), 682. https://doi.org/10.3390/foods10030682

8 | Cliceri, D., Aprea, E., **Menghi, L.**, Endrizzi, I. & Gasperi, F. (2021). *Variability in the temporal perception of polyphenol-related sensations in extra virgin olive oil and impact on flavor perception*. Food Quality and Preference, 93, 104249. https://doi.org/10.1016/j.foodqual.2021.104249

9 | Mosca, A. C., **Menghi, L.**, Aprea, E., Mazzucotelli, M., Benedito, J., Zambon, A., Spilimbergo, S. & Gasperi, F. (2020). *Effect of CO2 preservation treatments on the sensory quality of pomegranate juice*. Molecules, 25(23), 5598. https://doi.org/10.3390/molecules25235598

Oral communications

1 | **Menghi, L.**, Cliceri, D., Giacalone, D. & Gasperi, F. (2022). *Understanding the role of human microbiota on sensory perception: the MicroSens project*. D2S PhD Seminar in Sensory and Consumer Science. Copenhagen (Denmark). 10 October 2022.

2 | **Menghi, L.**, Cliceri, D., Gaudioso, G., Stefani, E., Fava, F., Pindo, M., Giacalone, D. & Gasperi, F. (2022). *What's behind the differences in sensory responsiveness to oral stimuli in real foods?: a possible link with oral and gut microbiot*a. 10th European Conference on Sensory and Consumer Research. Turku (Finland). 13-16 September 2022.

3 | **Menghi, L.**, Khomenko, J., Valentino, V., Biasioli, F., Ercolini, D., Giacalone, D. & Gasperi, F. (2022). *Food neophobia is associated with scarce olfactory performances and specific signatures on oral microbiota*. E3S Next Generation Webinar: "*Take a Seat at the Table of the Future*". Online Conference. 13 April 2022.

4 | **Menghi, L.**, Khomenko, I., Pedrotti, M., Cliceri, D:, Aprea, E., Endrizzi, I., Cavazzana, A., Hummel, T., Biasioli, F., Giacalone, D. & Gasperi, F. (2021). *Inter-individual variability in flavor release is explained by both biological and behavioral factors*. 6th International Conference on Food Oral Processing Physics, Physiology and Psychology of Eating (FOP 2020). Online Conference. 12-14 July 2021.

5 | **Menghi, L.,** Khomenko, I., Pedrotti, M., Cliceri, D:, Aprea, E., Endrizzi, I., Cavazzana, A., Hummel, T., Biasioli, F., Giacalone, D. & Gasperi, F. (2021). *Real time nose space monitoring by SIFT-MS allows to get insights into biological and behavioral factors affecting the inter-individual variability on flavor release*. American Chemical Society (ACS) Meeting 2021. Online Conference. 05-30 April 2021.

6 | **Menghi, L.,** Khomenko, I., Pedrotti, M., Cliceri, D:, Aprea, E., Caretta, A., Cavazzana, A., Hummel, T., Biasioli, F., Giacalone, D. & Gasperi, F. (2020). *Beyond food neophobia: the link with orthonasal and retronasal olfaction*. 9th European Conference on Sensory and Consumer Research. Online Conference. 13-16 December 2020.

Posters

1 | **Menghi, L.**, Khomenko, I., Valentino, V., Biasioli, F., Ercolini, D., Giacalone, D. & Gasperi, F. (2022). *Food neophobia is associated with scarce olfactory performances and specific signatures on oral microbiota*. 10th European Conference on Sensory and Consumer Research. Turku (FI). 13-16 September 2022.

2 | **Menghi, L.**, Franceschi, P., Giacalone, D. & Gasperi, F. (2022). *A bootstrap-based approach for sample size calculation when traditional estimations are not possible*. 10th European Conference on Sensory and Consumer Research. Turku (FI). 13-16 September 2022.

3 | **Menghi, L.**, Endrizzi, I., Cliceri, D., Zampini, M., Giacalone, D. & Gasperi, F. (2021). *Adult picky eating is negatively associated with the adherence to the Mediterranean Diet*. 14th Pangborn Sensory Science Symposium. Online Conference. 09-12 August 2021.

4 | **Menghi, L.**, Khomenko, J., Pedrotti, M., Cliceri, D., Aprea, E., Caretta, A., Cavazzana, A., Hummel, T., Biasioli, F. & Gasperi, F. (2019). *Exploring the inter-individual variability in flavor release: preliminary results.* 13th Pangborn Sensory Science Symposium. Edinburgh (UK). 26 July - 01 August 2019.

5 | **Menghi, L.**, Penza, F., Zanbanini, J., Endrizzi, I., Cavazzana, A., Hummel, T. & Gasperi, F. (2019) *How is the smell of a picky eater?.* 13th Pangborn Sensory Science Symposium. Edinburgh (UK). 26 July - 01 August 2019.

6 | **Menghi, L.**, Endrizzi, I., Aprea, E., Zanbanini, J., Conterno, F. & Gasperi, F. (2018). *Acceptability of a new olive pomace enriched biscuit (PREBIÒ®) in a dietary intervention with mildly hypercholesterolemic volunteers.* 8th European Conference on Sensory and Consumer Research. Verona (IT). 02-05 September 2018.

Awards

1 | **E3S EuroSense Student Awards 2022 dedicated to Pieter Punter** (€1500; European Sensory Science Society). Awarded contribution: *What's behind the differences in sensory responsiveness to oral stimuli in real foods?: a possible link with oral and gut microbiota*.

2 | **1st year PhD Student European Flavour Research Award** (€3000; GIRACT - Switzerland) for the academic year 2019-2020. Awarded project: *Understanding the role of human microbiota on sensory perception*.

Overview of completed training activities

Courses

1 | How to write a research project – advanced, UniTN, Online, 2022

2 | Academic Writing for the Sciences and Engineering, UniTN, Online, 2021

3 | Data Exploration, UniTN, Online, 2020

4 | Research to business - a technology transfer approach, UniTN, Online, 2020

5 | Introduction to metagenomics, UniTN, Online, 2020

6 | Data visualization, UniTN, Online, 2020

7 | Figures and Posters, UniTN, Online, 2020

8 | Responsible Conduct of Research Course, SDU, Online, 2020

9 | Computational microbial genomics, UniTN, Trento (IT), 2020

10 | Ethics of research in Neuroscience, UniTN, Rovereto (IT), 2020

11 | Introduction to Nonparametric Analysis, UniTN, San Michele A/A (IT), 2020

Conferences

1 | Rethinking eating 2022, AIAS, Aarhus (DK), 2022

2 | PhD seminar in Sensory and Consumer Science, D2S, Copenhagen (DK), 2022

3 | 10th European Conference on Sensory and Consumer Research, Elsevier, Turku (FI), 2022

4 | VII Convegno Nazionale SISS, SISS, Matera (IT), 2022

5 | E3S Next Generation Webinar: "*Take a Seat at the Table of the Future*", E3S, Online, 2022

6 | ACS Meetings 2021, ACS, Online, 2021

7 | FOP 2020, CSIC, Online, 2021

8 | 14th Pangborn Sensory Science Symposium, Elsevier, Online, 2021

9 | 9th European Conference on Sensory and Consumer Research, Elsevier, Online, 2020

10 | Sensometrics 2020, Nofima AS, Online, 2020