Genetic and Environmental Influences on Chemosensory Perception and Preferences

Outi Törnwall

ACADEMIC DISSERTATION

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“Persistence is the key to solving most mysteries”

Christopher Pike

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ABSTRACT

Chemosensory perceptions have biological relevance in aiding the recognition of nutrients and toxins, and initiating physiological processes that affect the digestion system. Food is first and foremost a source of nutrition affecting our well-being, but it is also characterized by one's culture and is a great source of pleasure and satisfaction. Affective responses to sensory characteristics of foods are the best predictor of human food choice in the absence of economic and availability constraints. The aim of this work was to elucidate the impact of genes and environment on responses and preference for astringent, pungent (sensory 'burn' caused by spicy foods) and sour perceptions. Furthermore, a coherent picture of the chemosensory preferences and their underlying causes in young adulthood was achieved.

Extensive data from 331 Finnish twins (21-25 years, 146 men, 185 women) were collected as part of FinnTwin12-study including pleasantness and intensity responses to samples spiked with sour, pungent, umami, and astringent stimuli (subgroup for astringency n=194, 96 men, 98 women). Liking and use-frequency responses for variety of food and beverage names representing these chemosensory qualities were collected. In addition, eating behavior related traits (e.g. food neophobia) were measured with validated scales. The heritabilities of chemosensory traits and food preferences were estimated. Furthermore, subgroups of respondents were identified based on preferences to sour, pungent and umami foods. Differences in food behavior and sensory responses, and the impact of genes were explored in the obtained subgroups.

Sensory pleasantness and intensity responses to sourness and pungency showed modest heritabilities (12-31%) whereas astringency perception showed no inheritance. However, suggestive evidence that salivary secretion related to astringent stimulation is under genetic control was observed (heritability 43-51%). The preference for foods with sour and spicy flavor qualities showed a clearer and higher impact of genes (heritability 45-50%).

Two subgroups of respondents were identified. The 'basic' group was less dramatic in their flavor preferences, whereas the 'adventurous' favored foods with sour and pungent qualities. The adventurous, were also less food neophobic and exhibited more tolerance for the sensory 'burn' caused by capsaicin compared to the basic subgroup. The influence of genes underlying the subgrouping was discovered (heritability 66%). Being adventurous was suggestively found to be in linkage with umami and sour coding taste receptor genes: TAS1R1 and PKD1L3, respectively.

This is the first work to demonstrate the role of genetics and environment in perceptions and preference for astringent and pungent flavor qualities, and one of the few to study the heritability of sour taste. It was demonstrated that preference for sour and pungent flavors was partially inherited. Furthermore, two subgroups of individuals were found differing in their flavor preferences. Genetic variability together with food neophobic tendency partially explained these differences. Food neophobia and genetic differences may form a barrier through which individual flavor preferences are generated.

TIIVISTELMÄ

Kemiallisilla aistimuksilla on biologinen merkitys syötäväksi kelpaavan ravinnon tunnistamisessa sekä ruoansulatusjärjestelmän fysiologisten prosessien alkuun saattamisessa. Ruoka on kulttuurin ilmentymä, joka ravinnon ja terveysvaikutusten lisäksi on myös mielihyvän lähde. Makumieltymykset selittävätkin pitkälti ruokavalintoja. Tässä tutkimuksessa selvitettiin geenien ja ympäristön osuutta astringoivan, polttavan (mausteiden aiheuttama) ja happaman maun mieltymyksissä. Työssä selvitettiin myös nuorten makumieltymysten kokonaiskuvaa ja mieltymysten taustaa.

Aineisto kerättiin suomalaisilla kaksosilla (21-25 v., 146 miestä, 185 naista) osana Kaksosten kehitys- ja terveystutkimusta (FinnTwin12). Mieltymystä ja aistimuksen voimakkuutta (makumittaus) mitattiin maistamalla erilaisia näytteitä, joihin oli lisätty hapanta, polttavaa, umamia tai astringoivaa yhdistettä (alaryhmä astringoivuusmittauksissa n=194, 96 miestä, 98 naista). Ruokakyselyllä kartoitettiin mieltymyksiä ja käyttöuseutta happamien, polttavien, umami- ja astringoivien ruokien suhteen. Ruokakäyttäytymistä (esim. uusien ruokien pelkoa) mitattiin useilla validoiduilla mittareilla. Aistimusten miellyttävyydelle, voimakkuudelle sekä ruokakyselyn perusteella muodostetuille makumuuttujille määritettiin periytyvyysaste. Lisäksi vastaajat jaettiin alaryhmiin mieltymysten suhteen (happamat, polttavat ja umamia sis. ruoat). Ala-ryhmien välisiä eroja ruokakäyttäytymisen ja aistimusten suhteen sekä geenien vaikutusta tutkittiin.

Makumittauksen happaman ja polttavan maun miellyttävyydestä sekä voimakkuudesta vain pieni osa selittyi perinnöllisillä tekijöillä (12-31%). Astringoivuuden aistiminen ei tämän tutkimuksen mukaan ollut periytyvä ominaisuus, mutta viitteitä saatiin aistimusta osittain selittävän syljentuotannon perinnöllisistä ominaisuuksista (perityvyysaste 43-51%). Ruokakyselyssä lähes puolet mieltymyksistä happamiin ja polttaviin ruokiin selittyi perinnöllisillä tekijöillä (periytyvyysaste 45-50%).

Vastaajat voitiin jakaa kahteen ala-ryhmään. ’Perusryhmä’ oli flavorimieltymysten suhteen maltillisempi verrattuna ’seikkailijat’-ryhmään, joka suosi ruokien happamia ja polttavia ominaisuuksia. Seikkailijoiden keskuudessa uusien ruokien pelko oli vähäisempää ja he osoittivat suurempaa sietokykyä makumittauksissa polttavalle näytteelle. Ryhmittelyn taustalla todettiin geneettinen vaikutus (periytyvyysaste 66%). Viitteitä saatiin umamin ja happaman aistimuksia säätelevien geenien (TAS1R1 ja PKD1L3) ja seikkalijat-ryhmän välisestä kytkennästä.

Tämä työ on ensimmäinen, jolla havainnollistettiin geenien ja ympäristön osuutta astringoivuuden ja polttavuuden aistimuksissa sekä mieltymyksissä. Työ on myös yksi harvoista, jossa on tutkittu happaman maun periytyvyyttä. Voitiin osoittaa, että mieltymys

happamiin ja polttaviin ruokiin on osittain periytyvä ominaisuus. Vastaajat voitiin jakaa kahteen ala-ryhmään flavorimieltymysten suhteen. Eroja selittivät osittain uusien ruokien pelko ja periytyvät ominaisuudet. Yksilölliset flavorimieltymykset voivatkin ilmentyä uusien ruokien pelon ja geneettisten erojen kautta.

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Helsinki, September 2013

Outi Törnwall

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals (I-IV)

I Törnwall O, Dinnella C, Keskitalo-Vuokko K, Silventoinen K, Perola M, Monteleone E, Kaprio J, Tuorila H. 2011. Astringency perception and heritability among young Finnish twins. Chemosens Percept 4:134-144.

II Törnwall O, Silventoinen K, Keskitalo-Vuokko K, Perola M, Kaprio J, Tuorila H. 2012. Genetic contribution to sour taste preference. Appetite 58:687-694.

III Törnwall O, Silventoinen K, Kaprio J, Tuorila H. 2012. Why do some like it hot? Genetic and environmental contributions to the pleasantness of oral pungency. Physiol Behav 107:381-389.

IV Törnwall O, Silventoinen K, Hiekkalinna T, Perola M, Tuorila H, Kaprio J. Identifying flavor preference segments: genetic basis and related eating behavior traits. Submitted.

The author's contribution

The author (O. Törnwall) contributed to the experimental work during the final stage of the data collection. She was responsible for the data analysis, interpreting the results, and she was the main corresponding author of the publications (I-IV). Help was received from the coauthors in all stages of the work: during analysis and writing, discussing of the results and implications, and commenting on the manuscripts at their final stages.

ABBREVIATIONS

A	Additive genetic effects
a^2	Proportion of variance explained by additive genetic effects
A^-	Anion of a weak organic acid
ANOVA	Analysis of variance
α_{pleas}	Cronbach's α for pleasantness
ASIC	Acid-sensing ion channel
α_{use}	Cronbach's α for use-frequency
C	Common (shared) environmental effects
c^2	Proportion of variance explained by common environmental effects
C1	Jelly sample with capsaicin concentration of 0.0001%
C2	Jelly sample with capsaicin concentration of 0.0002%
Ca^{2+}	Calsium ion
CA-juice	Orange juice spiked with citric acid
CO_2	Carbon dioxide
D	Dominant genetic effects
d^2	Proportion of variance explained by dominant genetic effects
DNA	Deoxyribonucleic acid
DZ	Dizygotic twin, non-identical twin
e.g.	Exempli gratia (for example)
EtOH	Ethanol
FNS	Food Neophobia Scale
GxE	Gene-environment interaction
GF	General Food questionnaire
G1	Segment 1, used in study IV to indicate a specific subgroup
G2	Segment 2, used in study IV to indicate a specific subgroup
g/ L	grams per liter
GMP	5'-guanylate, ribonucleotide
GWAS	Genome Wide Association Study
H	Heritability (broad sense)
h^2	Heritability (narrow sense)
H^+	Hydrogen ion
HA	Weak organic acid
HIV	Human Immunodeficiency Virus
HCl	Hydrochloric acid
HCN	Cyclic nucleotide gated channel
HTAS	Health and Taste Attitude Scale
IMP	5'-inosinate, ribonucleotide

LMS	Labeled Magnitude Scale
lod	Logarithm of odds
MSG	Monosodium glutamate
M	Molarity, molar concentration
MZ	Monozygotic twin, identical twin
NaCl	Sodium chloride
-N-C=S	Thiocyanate group
O	Orange juice without added citric acid
pH	A measure of acidity of a solution
PKD1L3/ PKD2L1	Polycystic kidney disease-1 and -2- like proteins
ppm	Parts per million
PROP	6-*n*-prophylthiouracil
PRP	Proline rich protein
PTC	Phenylthiouracil
QSO4	Quinine sulfate
TAS2R38	Taste receptor 2, member 38, gene coding for bitter taste
T1R1+T1R3	G-coupled taste receptor heterodimer, gene coding for umami taste
r	Pearson correlation coefficient
RSE	Rosenberg Self-Esteem Scale
SD	Standard deviation
SF-questionnaire	Specific Food questionnaire
SHU	Scoville Heat Unit
S-jelly	Jelly sample without capsaicin
SNP	Single Nucleotide Polymorphism
T	Marli tomato juice
TFEQ	Three Factor Eating Questionnaire
TI	Time-intensity method
TRP	Transient receptor potential channel
TRPV1	Transient receptor potential channel, subfamily V, member 1
TRPA1	Transient receptor potential channel, subfamily A, member 1
V_A	Additive genetic variance
V_D	Dominant genetic variance
V_E	Environmental variance
V_G	Genetic variance
V_P	Phenotypic (trait) variance
XX	Female sex chromosome pair
XY	Male sex chromosome pair

CONTENTS

1 INTRODUCTION

The factors shaping a person's food choices are complex. Whereas the motivation to eat is vital to the survival of humans and is thus subjected to intense regulation by the homeostatic (hunger / satiety) and hedonic (reward) systems of the brain (Saper et al. 2002), food preferences play a central role in determining food selection and diet quality (Birch 1999; Story et al. 2002). The sensory characteristics of foods stimulating taste, olfactory, tactile and visual sensory systems, form the core of preferences; particularly taste (Drewnowski 1997; Glanz and Basil 1998). Most of what is commonly referred to as 'taste', is in reality flavor, the combined sensory experience of olfaction, gustation and oral somatosensation.

Food preferences, exhibited in a great degree of individual variation, are formed as a result of interactions of environmental factors and genetic predispositions (Birch 1999). Although individual diversity exists in perceptual responses, individuals tend to differ more dramatically in terms of what they like or dislike, than they differ in their sensory perceptions (Moskowitz 1985). Many factors such as culture, learning, food neophobia (fear of new foods), age and gender shape our preferences for tastes and foods. Growing body of evidence from the past decades has also indicated that genes regulate the perceptions of taste and smell (Reed and Knaapila 2010). While the genetics of bitter perception is somewhat understood, gaps in knowledge exist in other tastes such as sour and umami, as well as perceptions of astringency and chemesthesis. Little is known to date of the genetic influences on liking for foods with different flavor qualities.

The following review of literature elucidates the current knowledge of sour taste, and the perceptions of astringency and pungency. Furthermore, the determinants and outcomes of preferences for these perceptual qualities are discussed. Umami and bitter perceptions are described and discussed more briefly. In addition, the methods to investigate the hedonic and intensity responses to stimuli, food and taste preferences, and studying quantitative genetic traits are described. The original four publications focused on revealing the role of genetics affecting the preference for sour taste, astringency and pungency perception, and foods with these chemosensory qualities. Furthermore, a coherent picture of the chemosensory preferences and their underlying causes among the study population was explored based on available data.

2 REVIEW OF THE LITERATURE

2.1 Chemosensory perceptions

Chemosensory perception can be defined as the detection and recognition of chemical stimuli. It is a neurological process in which a chemical stimulus becomes in contact with a sensory organ and causes a molecular signal which is recognized and characterized in the brain. In the current work, *chemosensory perception* is used to represent flavor qualities, including the somatosensory (tactile) perception of astringency. According to Breslin (2013), flavor is defined as the perceptual experience of a food that arises from the integrated sensory signals of several sensory modalities, such as taste, olfaction, oral somatosensation (tactile, temperature, and texture) and oral nociception (pain).

Chemosensory perceptions have biological relevance in aiding the recognition of nutrients and toxins, driving complex ingestion or rejection behaviors and initiating physiological processes in the digestion system (Breslin and Spector 2008; Breslin 2013). From the five tastes of sweet, sour, bitter, salty, and umami, sweet taste permits the identification of energy-rich nutrients whereas sour and bitter warn against potentially harmful foods. Umami in turn, allows the recognition of protein rich foods and salty taste ensures the body's proper electrolyte balance. Other perceptual qualities such as astringency (drying and puckering of the mouth) and pungency affect the overall acceptance of products, and often form an inseparable part of the flavor of a food or beverage.

In the following sections, the perceptions of astringency, sour taste and oral pungency are described in more detail whereas umami and bitter (mainly 6-n-prophylthiouracil, PROP) tastes are discussed only briefly. Salty and sweet tastes are not described in this thesis as they both have been previously studied by Keskitalo (2008) who demonstrated the impact of genes in sweet taste and showed that responses to saltiness were uninfluenced by genetic factors. Similarly, extensive literature exists on PROP and its genetic origin, thus PROP was included in the current study merely as a marker for genetically inherited trait. The focus of the present work was on genetics of astringency, sourness and pungency. Next to nothing is known of the genetics underlying these chemosensory traits and their preference.

2.1.1 The perception and mechanisms of astringency

Astringency is a tactile perception (Breslin 1993), defined as "complex sensation, accompanied by shrinking, drawing or puckering of the skin or mucosal surface in the mouth, produced by substances such as tannins" (ISO 5492, 2008). Astringency both develops and dissipates slowly (Bajec and Pickering 2008; Lesschaeve and Noble 2005) and it is generally perceived as a negative attribute (Childs and Drake 2010; Dinnella et al. 2009). The sensation of astringency can thus lead to the rejection of food products by some consumers (Lesschaeve and Noble 2005). Molecules causing astringent sensation (polyphenols e.g. catechin and tannic acid), are secondary metabolites of plants, essential for their pigmentation, growth, reproduction and resistance to pathogens and predators (Rossi et al. 2008). Other astringency eliciting compounds include aluminum salts, ethanol and organic acids (Bajec and Pickering 2008). A variety of foods and beverages, such as wine, tea, coffee, fruits, nuts and legumes can elicit astringency, frequently alongside bitter or sour tastes (Lee 1991).

Mechanisms underlying astringency are likely to differ from the prototypical tastes, which evoke responses on the taste buds through receptors and ion channels (Chaudhari and Roper 2010). Generally, the loss of oral lubrication which increases the friction within the mucosa, detected by activation of mechanoreceptors, is considered to be the cause of astringency (Breslin 1993). It seems likely though that multiple mechanisms occur simultaneously (Gibbins and Carpenter 2013) as shown in Figure 1. One of the most established mechanisms proposes the involvement of tannins interacting with specific salivary proteins such as proline-rich proteins (PRPs) (Charlton et al. 2002; Kallithraka 1998). The interaction leads to the formation of soluble aggregates in the saliva and eventually, as more tannins become involved, of an insoluble precipitate (Baxter et al. 1997). Protein-tannin interaction may disrupt the lubricating salivary film which covers the oral surfaces and cause either friction or exposure of the oral mucosa allowing the aggregates or tannins directly interact with the receptors (Gibbins and Carpenter 2013). Other, yet unclear mechanisms alongside precipitation of the salivary proteins are presumably involved, since not all astringent compounds bind salivary proteins and not all compounds that do, cause astringency. To date, the physiology of astringency remains partially blurred.

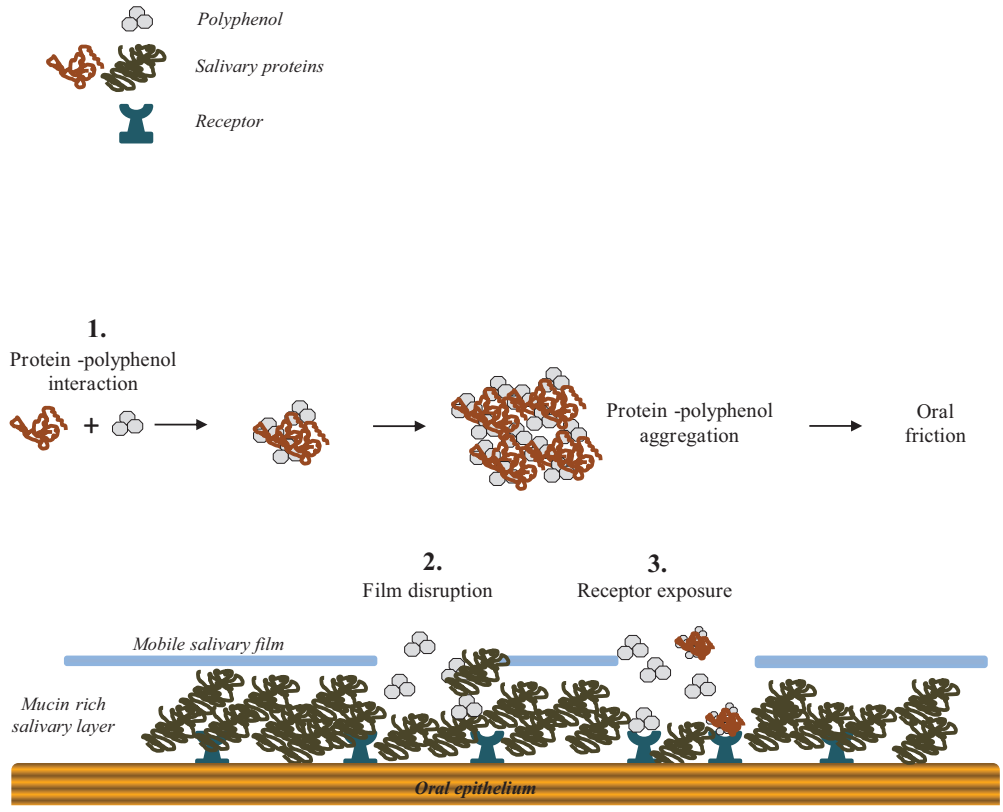

Figure 1. Mechanisms of astringency (modified from Gibbins and Carpenter 2013). Soluble aggregates are formed, eventually leading to an insoluble precipitate (1.) which may cause friction or participate in receptor irritation (3.). Mobile salivary film is disrupted by tannins (2.) exposing the receptors (3.) and leading to receptor irritation or friction. Other yet unclear mechanisms are likely also to exist.

2.1.2 Factors influencing astringency

Both the chemical nature of the polyphenol and the oral physiology are likely to modify astringency perception. It is generally accepted that the greater the degree of polymerization and molecular weight of the astringency eliciting compound, the greater the perceived astringency (Peleg 1999). Increasing the chain length of tannins also increases astringent qualities which are described as *drying, chalkiness, adhesive* and *puckering* (Vidal et al. 2004). The degree of galloylation (the amount of gallate esters in a molecule), causes a rougher astringency emphasizing the qualities of *coarseness, drying* and *chalkiness* (Vidal et al. 2003). Similarly, the 3-dimensional structure affecting the binding ability of the tannin (Cala 2010) can alter the intensity or quality of the astringent sensation. Other modifying

factors of astringency include pH, viscosity (Peleg and Noble 1999) and the presence of sweeteners or acids (Lyman and Green 1990; Siebert and Chassy 2004). Low pH increases astringent sensations, whereas with increasing viscosity or sweetness, the perceived astringency decreases.

Saliva is mainly composed of water (99%), but the presence of various proteins and electrolytes create the physical properties which allow saliva to play a central role in oral lubrication (Nederfors 2000) and health (De Almeida et al. 2008). Salivary proteins such as mucins are essential for lubricating properties of saliva, and are believed to be involved in the adsorbed protein layer on the cell interface (Gibbins and Carpenter 2013). Proline rich proteins (PRPs) are another protein group contributing to oral lubrication (Hatton et al. 1985), but their primary role is thought to be tannin binding, one of the key factors in astringency perception (Jöbstl et al. 2004). Individual variation in the salivary protein content influences the sensitivity to astringency (Dinnella et al. 2009). Individuals capable of maintaining a constant salivary protein concentration while exposed to astringent stimuli, are less sensitive compared to those among whom saliva modifications take place during stimulation. Salivary flow rate and haze forming capacity have also found to be inversely related to the astringency intensity (Condelli et al. 2006), meaning that the greater the saliva flow or/ and the haze forming capacity, the less sensitive the individual is to astringent compounds. By contrast, individuals with low salivary flow rates perceive slower onset and decay of astringent sensations (Ishikawa and Noble 1995).

2.1.3 Nutritional implications of astringent compounds

Originally, astringency was thought to protect mammals from the harmful effects of plant tannins by discouraging the consumption of the plants due to aversive nature of astringency, and by increasing the secretion of proline rich proteins in the saliva (Jansman et al. 1994; Mehansho et al. 1985; Mehansho et al. 1987). The adverse effects of plant tannins in mammals include reduced digestibility, reduced growth and intake of foods, and damage to the intestine, liver or kidneys (Robbins et al. 1991). In humans, polyphenols reportedly decreases protein utilization (Hussein and Abbas 1985) and may inhibit the function of some digestive enzymes (Naz et al. 2011). The research on flavonoids, which constitute the largest family of phenolic compounds, has however revealed a number of positive physiological effects. These include antioxidative, anti-inflammatory and anti-cancer activity, as well as flavonoids' positive effects in prevention of coronary heart diseases (Khan and Mukhtar 2013; Yao et al. 2004). Thus, based on current knowledge, the consumption of flavonoids in moderation is likely to contribute to a healthy lifestyle.

2.1.4 The perception and mechanisms of sour taste

Sour taste is the body's 'nutritional gatekeeper' signaling the presence of dietary acids and helping to guide dietary selection. Mild sourness is acceptable and evokes interest but with increasing intensity, sourness becomes unpleasant (Ganchrow 1983). The generally aversive nature of sour taste is beneficial in avoiding ingestion of potential toxins, spoiled foods, unripe fruits and tissue-damaging dosages of acids. Although diverse compounds can taste sour, the most common are acids (Breslin and Spector 2008). In nature, the combination of acetic, citric, malic or tartaric acids are often found in fruits and berries whereas lactic acid is largely abundant in fermented foods (Roper 2007; Viljakainen et al. 2002). Acids are also widely used as functional ingredients in the food industry affecting for example; pH, preservation, leavening and gel formation, but most importantly, adding and enhancing the flavor and taste of complex foods. Sourness is a shared sensory characteristic of acidulants, however, the degree of sourness, flavor and related non-sour characteristics such as bitterness and astringency can differ (Hartwig and McDaniel 1995).

Taste evokes responses on the taste buds, located on the surface on a tongue through cell membrane-based receptors and ion channels (Chaudhari and Roper 2010). For sour taste, a broad range of cell types, receptors and mechanisms have been proposed, with several ion channels considered as candidates. These include for example cyclic-nucleotide-gated (HCN) channels (Stevens et al. 2001) and acid-sensing ion channels (ASICs) (Ugawa et al. 1998). Recent studies have demonstrated a new group of candidate sour taste-related ion channels (Ishimaru et al. 2006; LopezJimenez et al. 2006; Huque et al. 2009): the polycystic kidney disease-1 and -2- like proteins (PKD1L3 and PKD2L1). These proteins are thought to form acid-sensitive channels involved in sour taste, although the two are not expressed uniformly throughout the oral cavity (Ishimaru et al. 2006). To date, several inorganic (hydrochloric, sulfuric and phosphoric) and organic (malic, succinic, tartaric, citric and acetic) acids have been reported to activate the PKD1L3 and PKD2L1 channel (Ishii et al. 2009; Ishimaru et al. 2006).

It was originally assumed that sour taste receptor cells functioned as extracellular pH detectors evoking sour taste in direct relationship with stimulus pH. Studies, however, indicate otherwise (e.g. Lyall et al. 2001). At the same pH, weak organic acids (e.g. citric acid) are more potent sour stimuli than strong acids (e.g. hydrochloric acid). Two general mechanisms for sour taste have been considered. The first proposes extracellular adsorption of the acidulant (Beidler 1971), while the second assumes the penetration of sour stimuli into the cell causing cytoplasmic acidification (Lyall et al. 2001) and release of transmitters which initiate the excitation of nerve fibers. The latter is the generally accepted mechanism (Figure 2) and indicates that the intracellular pH is the trigger for sour taste transduction (Lyall et al. 2001). The cytoplasmic acidification may affect the receptors and/or sour signal transduction

components, or alternatively, the receptor functions are modified by low pH in the cell (Ishimaru et al. 2006). Evidence supporting intracellular acidification as the proximate sour stimulus is illustrated by results suggesting that nerve responses to weak acids (e.g. acetic acid) are independent of stimulus pH but strongly correlate with the intracellular acidification of polarized taste receptor cells (Lyall et al. 2001).

The acidic compounds can penetrate the cells in different ways. Weak organic acids, such as those naturally found in foods (e.g. citric acid), enter the taste cells mainly by diffusion (across the lipid bilayer) as neutral molecules, whereas the strong mineral acids (e.g. HCl) involve proton transporters (channels or exchangers) through which the proton is passed into the cell (DeSimone and Lyall 2006). If acid is present in lower concentrations, the nerve impulse is transduced by the chorda tympani but with higher concentrations an irritation sensation is also elicited, which is transduced via the trigeminal nerve (Da Conceicao Neta et al. 2007).

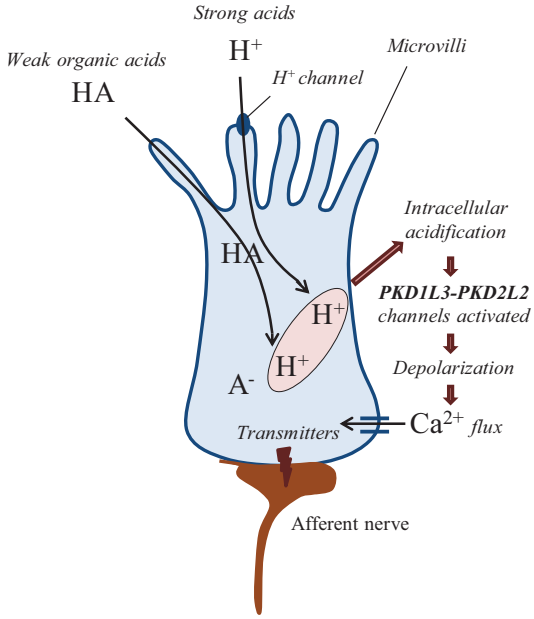

Figure 2. Sour taste transduction model in the taste receptor cells which are located on the taste bud. Intracellular acidification activates the sour taste related ion channels (PKD1L3-PKD2L2) causing cell depolarization, calcium ion (Ca^{2+}) flux into the cell and finally the release of transmitters which activate the afferent nerve. A^-=anion.

2.1.5 Factors influencing sourness

As noted earlier, sour sensation is not a simple measurement of pH in the solution. It is possible that anions and/ or undissociated acid species (acid intact) also play a role in sour taste perception (Da Conceicao Neta et al. 2007). Other factors thought to be important determinants of sour taste are titratable acidity (a measure of both bound and free hydrogen ions in solution) and buffering capacity. However, the taste intensity of acids cannot be entirely explained by these variables. A newer hypothesis by Johanningsmeiner et al. (2005) proposes that sour taste intensity is dependent on the molar concentration of organic acids that have one or more protonated carboxyl groups plus the concentration of free hydrogen ions. This hypothesis was confirmed by Neta et al. (2009) and seems to provide a way to predict sour taste intensity in the formulation of acidified foods.

Saliva acts as a buffering agent modifying the degree to which the sourness is perceived. A higher flow rate of saliva suggests more efficient buffering effects, thus having a diminishing effect on sour taste (Christensen et al. 1987; Spielman 1990). Besides sourness, acids can also elicit non-sour taste qualities such as bitterness, saltiness and astringency (Hartwig and McDaniel 1995) and impair olfactory and tactile sensations by causing irritation by the trigeminal nerve (Settle et al. 1986). Sour taste can be confounded by such non-sour chemosensory factors. Furthermore, the intensity of sourness is altered by the presence of sugars or sweeteners. For example in fruit beverages, the sourness and sweetness are often present simultaneously, and the sourness of acids is suppressed by sweetness from sugars (Lawless and Heymann, 2010).

2.1.6 Nutritional implications of naturally sour foods

Many types of fruits and berries share a common characteristic of sourness, and significant levels of biologically active components that are vital for health (Oomah and Mazza 2000). An optimal mix of antioxidants, fibers and other biotic compounds are thought to underlie their health and nutritional benefits (Kaur and Kapoor 2001). Epidemiological studies have consistently shown that the intake of fruits reduces the rate of ageing, and aids in the prevention of coronary heart diseases, cancer as well as other degenerative diseases (Ames et al. 1993; Gandini et al. 2000; Gerber et al. 2002; Joseph et al. 1999; Ness and Powles 1997; Van Duyn 2000). The consumption of fruits and berries are a positive addition to the diet and as shown by various studies, a substantial contribution to health. Their usage should thus be promoted in all age groups as a part of balanced diet.

2.1.7 Chemesthesis and the mechanisms of oral pungency

Chemesthesis describes a sensation elicited by the chemical stimulation of free nerve endings that can be found throughout the skin and mucosal membranes of the nose, mouth, throat and eyes (Silver 2010). Burning, tingling, pungency and coolness are typical chemesthetic sensations. Various compounds such as capsaicin of chili peppers, piperine of black pepper, cinnamaldehydi from cinnamon and gingerol found in ginger root are responsible for pungent sensations, all differing in their temporal responses and pungent characteristics. Whereas cinnamaldehydi is described to cause burning and tingling sensations and its pungency is experienced quickly, capsaicin and piperine are dominantly eliciting burn with longer lag times and duration (Cliff & Heymann 1993). Lingering effects of pungency or burning are observed for lipophilic compounds which have higher affinity for the mucosa than the saliva. Other everyday sensations of chemesthesis are the fizzy tingle of CO_2 in soda, the cooling of the mint (menthol) and the nasal pungency caused by allyl isothiocyanate in horseradish and mustard.

Chemesthetic nerve fibers, sensitive to pain and temperature (Caterina et al. 1999; Jordt, McKemy and Julius 2003), contain polymodal nociceptors that respond to a variety of noxious and irritative stimuli (mechanical, thermal and chemical). The branches of the trigeminal nerve (Figure 3) are mainly chemesthetically sensitive but other cranial and spinal nerves also respond to this type of chemical stimuli (Alimohammadi and Silver 2002). In the oral cavity, nocireceptors are not present in taste buds but buried within the skin and mucosal tissues. Thus, the compounds causing irritation must penetrate through the tissue to be effective, resulting in delayed onset of irritation, the unique feature of chemesthetic sensations (Galopin 2007). Some irritants penetrate faster than others depending on their chemical structure and thus can act either directly or indirectly via the sodium/calcium ion channels known as transient receptor potential (TRP) channels. The activation of nerve endings through the capsaicin reactive TRPV1 receptor is an example of direct chemical activation. TRPV1 is also activated by agonists such as piperine, eugenol and gingerol (Bandell et al. 2007). Another member of TRP receptor family known to directly react to pungent irritants found in mustard and garlic is TRPA1 (Bautista et al. 2005). Indirect activation occurs when a receptor is activated through an indirect secondary mechanism for example in case of CO_2 in which the molecule is metabolized in order to produce an active irritant (Alimohammadi and Silver, 2002).

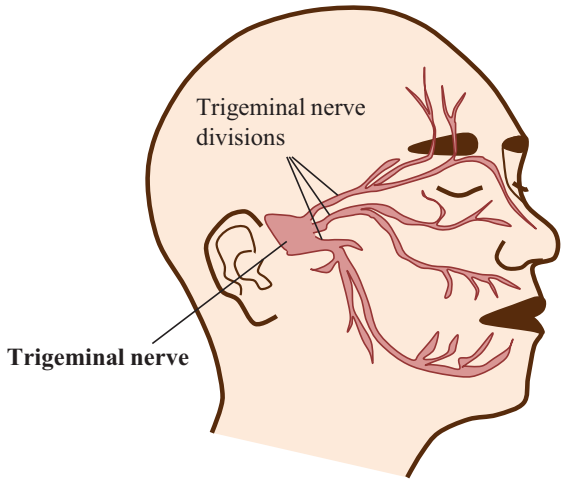

Figure 3. Branches of the trigeminal nerve innervating the areas of eyes, nose and mouth.

TRP-channels such as TRPV1 and TRPA1 are cell-membrane proteins which when activated by the irritant, open up and allow a flow of cations in to the cell that depolarizes the receptor cell and consequently create a neural signal for the sensation of irritation (Galopin 2007). The family of TRPs are also involved in thermal (Voets and Nilius 2003) and pain sensations (Cortright and Szallasi 2009). An overlap between chemical and thermal sensitivity exists while a lesser amount of overlap is apparent between chemical and tactile sensitivity (Alimohammadi and Silver 2002). In addition to the sensory experience, protective reflexes are activated by trigeminal receptors. Secretion of tears and saliva, decreased breathing and increased sweating are reflexes functioning to counteract the effects of the irritant (diluting or preventing more from entering the body).

Chemesthetic irritants causing sensitization and desensitization have created discussion. Evidence indicates that unlike tastes that typically show a reduction in stimulus intensity in repeated exposure, the irritation of capsaicin can produce an increase in rated intensity (sensitization) during subsequent stimulation. However, with a delay in stimulation, a decrease in rated intensity (desensitization) can be observed (e.g. Green 1989). Considerable variability in response patterns to stimulation of capsaicin exists showing that while some individuals are sensitized over a series of irritant stimuli, others show little change, or reduction in rated intensity (desensitization) (Prescott 1999). Desensitization seems to be more consistent a phenomenon compared to sensitization and TRP channels may in part explain it. The current view is that activation of most TRP channels gradually decreases over time even though the chemical stimulus is maintained. Mechanisms as to how the desensitization takes place are complex and found to depend on several extracellular (calcium)

and intracellular components (Bandell et al. 2007). Furthermore, no evidence for sensitization occurring during normal food consumption has been observed (Prescott 1999).

2.1.8 Factors influencing oral pungency

Human responses to chemesthetic irritants show considerable diversity (Cliff and Green 1996) and occasionally more variation is seen than usually observed in taste qualities (Lawless 1984). Studies have shown that frequent users of spicy foods rate the burn caused by chili spice as less intense and more pleasant than the infrequent users of spices (Lawless et al. 1985; Prescott and Stevenson 1995). Thus, a 'chronic' desensitization effect seems to occur in individuals who regularly consume spices allowing them to tolerate more the pungent sensation.

The connection between taste and chemesthesis has been a matter of interest perhaps because the fungiform papillae of the tongue are heavily innervated by the trigeminal nerve (Farbman and Hellekant 1978). Variation in taste sensitivity has been associated with the number of taste buds (Bartoshuk et al. 1994; Miller and Reedy 1990). Sensitivity to bitter 6-n-propylthiouracil (PROP) influencing the responses for chemesthetic irritants has been proposed, meaning that the super-tasters of PROP would perceive the oral pungency from capsaicin more intensely compared to the non-tasters of PROP (Bartoshuk et al. 1994; Karrer et al. 1992). The literature however, shows inconsistent and debatable results on this matter and it is likely that PROP sensitivity does not reliably predict individual responses to chemesthetic stimuli (Green et al. 2005; Lawless and Heymann 2010).

Using a similar idea of tasters vs. non-tasters, Green et al. (2005) tested whether thermal taste (ability to perceive taste from temperature alone) is related to individual differences in responsiveness to oral chemesthesis. The results showed no consistent differences in the sensations of burning from capsaicin between the taster groups. However, when responses to the intensity of sucrose, NaCl, citric acid, and QSO_4 (quinine sulfate) were rated, the thermal tasters did rate all taste stimuli higher compared to the non-tasters. While Green and others (2005) concluded that responses to oral gustatory stimulation did not clearly predict responsiveness to chemesthetic stimulation, they hypothesized that tastes and chemesthetic agents could be co-processed within the flavor system in the same way that tastes and smells can become integrated as flavors. The association between salivary flow and sensory responses to pungency has also been studied as chemical irritants stimulate salivary flow which may dilute the oral irritation. For capsaicin, the perception of pungency however, seems to be independent of parotid salivary flow (Nasrawi and Pangborn 1990).

2.1.9 The benefits of spices and their nutritional implications

The proximate reason for spice use among cultures and countries is to enhance the flavor and palatability of food. Alternative reason is the prevention of food borne illnesses due to antimicrobial properties of spices (Sherman and Billing 1999), and increasing longevity and health among those who find the flavors of spices enjoyable. Some evidence also exists supporting other hypothetical reasons for spice use: the tastes and smells of spoiled foods may be disguised by spices and eating spicy foods may provide evaporative cooling aid by increasing perspiration in warm climates (Billing and Sherman 1998).

Capsium species (hot peppers) are used worldwide as food and spices leading to questions about their potential benefits to health and nutrition. In terms of weight management, combinations of black pepper, coriander, red chili, turmeric, cumina and ginger or anion influenced digestion by stimulating digestive enzyme activities in rats (Platel et al. 2002). In humans, the consumption of spicy foods containing capsaicin increases satiety, energy expenditure, and reduces energy and fat intake (Ludy and Mattes 2011; Westerterp-Plantenga et al. 2005). However, recent meta-analyses evaluating the effects of capsaicin on energy balance showed that although evidence exists that capsaicin promotes weight management, the effects are modest and their long-term sustainability is uncertain (Ludy et al. 2012).

Alongside chili, pungent spices such as garlic have shown positive impact on health with anticarcinogenic and cholesterol lowering effects (Fleischauer and Arab 2001; Yeh and Liu 2001). Spices also have medical properties which have been exploited by traditional medicine for centuries. Capsaicin is used to treat wounds, arthritis, and chronic kidney disease due to its pain relieving properties (Szallasi 2002; Yamamoto and Nawata 2009), cinnamon is known for its antiseptic property (Attokaran 2011) and wasabi can be helpful in treating acute sinus infections (Shanmugam et al. 2011). Hence, spices have many beneficial applications and they may operate as functional agents preventing a positive energy balance and obesity. However, considering their frequency of use at least in the Nordic countries, which is greatly less compared to the 'hotter' climates, their health beneficial influences are likely modest, at least from an individual perspective.

2.2 Other chemosensory perceptions relevant for the study

2.2.1 Bitterness

Bitter taste is initiated by the interaction of bitter tastants with G protein coupled receptors in the membrane of taste receptor cells. Numerous fruits (e.g. citrus, olive, berries), vegetables (beans, zucchini, Brussels sprouts, lettuce) and other foods and beverages (coffee, wine) have been reported to contain bitterness and not surprisingly, the number and diversity of bitter tasting compounds is considerable (Drewnowski 2000; Rouseff 1990). There is an instinctive rejection to bitter taste generally assumed to have evolved as a way to avoid foods that are poisonous (Glendinning 1994). This rejection is justified, as rancid fats, hydrolyzed protein, plant-derived alkaloids, and other toxins generally have an unpleasant bitter taste (Rouseff 1990).

Wide individual differences exist in bitter taste sensitivity. Some individuals may taste bitter intensely, whereas others only weakly or not at all. Examples of compounds creating such bimodal responses are phenylthiouracil (PTC) and PROP (containing functional –N-C=S group). Originally PTC, and more recently PROP have been studied as markers for genetically inherited sensitivity to bitterness although they are not compounds naturally found in foods. Interests have been evoked by their bimodal nature of response distributions which has allowed the separation of individuals into groups of *tasters* and *non-tasters* (Lawless 1980). Subsequent studies have shown that modifications in TAS2R38 gene coding a receptor for bitter taste explained the variability in PTC tasting (Kim et al. 2003) and mostly the sensitivity to PROP (Hayes et al. 2008). Furthermore, sensitivity to PROP is a highly heritable trait (72%) (Hansen et al. 2006).

Despite numerous attempts to demonstrate a predictive relationship of PROP sensitivity relative to other bitter tastants relevant in foods (e.g Bartoshuk 1979), such a relationship has been difficult to prove. The correlations between PROP and other bitter substances (caffeine, urea, quinine) are low, and no evidence supporting a general bitter taste factor regulating perceived intensities of bitter tastants has been found (Delwiche et al. 2001; Hansen et al. 2006). Evidence exists that TAS2R38 genotypes are associated with vegetables that synthesize N-C=S moiety (e.g. broccoli), but this association cannot be generalized to all bitter tasting vegetables (Sandell and Breslin 2006). Furthermore, food preferences in general have not been shown to be related to taste responsiveness to PROP (Drewnowski et al. 2007). Besides TAS2R38, multiple other bitter taste receptor genes of the T2R family have been discovered in humans (Drayna 2005) constituting about 25 functional bitter taste receptor genes with substantial diversity in the coding sequence. Bitter taste appears to be the most

complex taste quality based on both the variety of chemical molecule structures eliciting bitterness and on the large number of genes encoding receptors for this taste perception.

2.2.2 Umami

Umami is the savory, meaty and mouth-filling rich taste elicited by selective G protein coupled receptors in taste buds. These receptors are activated mainly by monosodium glutamate (MSG), 5'-inosinate (IMP) and 5'-guanylate (GMP) compounds found in meat, cheese, tomatoes, fish, and some mushrooms. IMP, found primarily in meats, and GMP which is more abundant in plants, are the two ribonucleotides that contribute most to the umami taste. MSG is well-known as a flavor enhancer and used commonly in Western, and particularly in traditional Eastern cuisines. As opposed to sour and bitter tastes, umami is considered a pleasant taste quality, although it is not preferred in pure water (Roininen et al. 1996). Preference to umami has been detected already in infancy, although it is possible that its presence in breast milk conceivably contributes to its taste acceptability (Yamaguchi and Ninomiya 2000).

MSG has a special interaction with sodium chloride in many food contexts. Multiple experiments have concluded that by adding MSG to the food, the amount of salt can be reduced without altering the palatability (Roininen et al. 1996). It is also generally recognized that umami improves the acceptance of foods in combination with salt (Yamaguchi and Ninomiya 2000). The use of MSG however often raises concerns due to its alleged ability to trigger a series of allergic symptoms such as burning sensations, facial pressure, chest pain and headaches (Schaumburg et al. 1969). While a small number of people may be at risk for developing some of the MSG induced symptoms, decades of research have failed to demonstrate a clear and consistent relationship between them (Williams and Woessner 2009).

Several receptors have been proposed to underlie umami detection, and given the chemical diversity of umami tastants in natural and processed foods, this may be true (Chaudhari et al. 2009). The most potential receptor to date is the T1R1+T1R3 heterodimer (Li et al. 2002) which is activated by a broad range of amino acids. Specific ageusia (loss or impairment of the sense of taste) for MSG have been reported (Lugaz et al. 2002; Singh et al. 2010). It is likely that taste receptor variants or other factors such as exposure to MSG explain the variation in umami taste (Raliou et al. 2009). However, the MSG sensitivity has not yet been confirmed to segregate in families or shown heritable characteristics.

2.3 Perception and hedonic value

Most sensory stimuli, especially food, elicit a hedonic dimension in addition to the basic dimensions of quality, intensity and duration. Liking or disliking however, are not sensory phenomena bur rather affective experiences which can be seen as emotional responses whose bodily effects are accompanied by cognitive experience of the emotion (Cardello 1996).

Cranial nerves transmit the information from taste receptor cells to the brain areas where the stimulus quality is determined and its hedonic value judged (Rolls and Scott 2003). The taste signaling information flow is split between the ventral forebrain and dorsal regions where primary and secondary gustatory cortices give rise to conscious taste sensation. Ventral pathways are involved in affective and emotional processing, and memory and learning, whereas the dorsal pathways participate in process of taste qualities, attention, reward and multimodal sensory integration, higher cognitive functions and decision making (see Breslin 2013). The affective properties are processed in the brain separately from the perceived intensity of a stimulus, but ultimately, the information content of the ventral and dorsal pathways are integrated (Small et al. 2003).

Positive and negative hedonic judgments are largely modified by the environment through exposure and personal as well as cultural experiences. A genetic component may be present as discovered for sweet taste preference (Keskitalo et al. 2007b). Hedonic judgments are also influenced by the time and context in which the food is served, the degree of hunger and mood as well as the time elapsed since the food was last consumed (Lyman 1988).

2.3.1 Flavor-hedonic relationships

Intensity-preference functions have indicated several distinct relationships between the experience of flavor and hedonic responses: linear decreasing, horizontal, linear increasing and inverted U-shape (Figure 4). Although one of these response patterns is usually more popular, the response patterns may vary among individuals (Tuorila 1994). Drewnowski and Greenwood (1983) showed a U-shape function when they measured the perceived intensity of sweetness in dairy products with different fat content. The optimal preference depended upon the particular proportions of sugar and fat in the product. Generally disliked sensations such as bitter and sour tastes are likely to show a decreasing function (Tuorila 1994). Context has a major influence on the shape of the functions. U-shaped curve is usually observed when the flavor is appropriate to its context, in other words, expected.

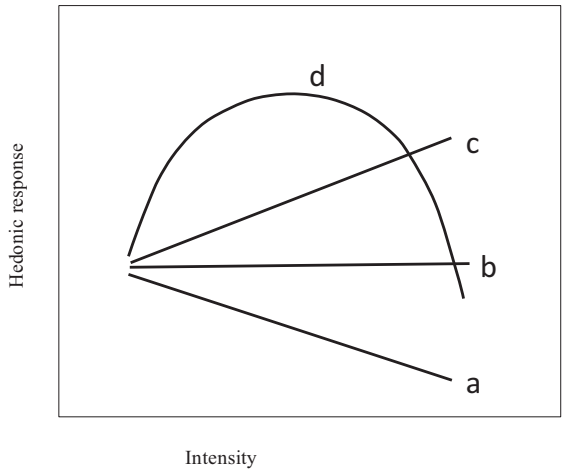

Figure 4. Relationships between hedonic responses and flavor intensity (Tuorila 1996): (a) linear decrease, (b) horizontal, (c) linear increase and (d) inverted U-shape.

2.4 Preference and food choice

Preference is often used to indicate that a person is choosing one item over another, but as often, it can be used as a synonym to liking or affective response to a food. In this work, the preference is used in the sense of liking (unless stated otherwise) and not in the strict sense of 'choice of one object over another'. The term pleasantness is also used in the same sense illustrating liking or affection.

> *"Although foods stimulate the chemosensory, visual, thermal and tactile senses, it is the mental representation invoked by this stimulation that is critical to humans' response: we respond to the mental representation of foods in order to identify particular items as either edible or not. The food itself is at once a source of nutrition, a source of harmful microorganisms or toxins, a great source of pleasure and satisfaction, and a vehicle for the expression of social relations and values"* (Rozin 1986).

As articulated by Rozin (1986), a close relationship between perceptions and preference exists. Taste preferences in particular may have significant impact on food choices (Drewnowski 1997) and the palatability of complex flavors forms the foundation of the eating experience. Affective responses to sensory properties have consistently found as one of the best predictors of human food choice in the absence of economic and availability constraints (Eertmans et al. 2001; Glanz and Basil 1998). Many variables however intervene

between the perceptions (taste, odor, somatosensory) and food choices, and affective responses can be thought as one of the many motives for choosing a food. Figure 5 illustrates the plausible role of genes and environment influencing the chemosensory traits, preferences and the eating behaviors (orientations). These factors together are likely to affect the food choices and the health outcomes of individuals.

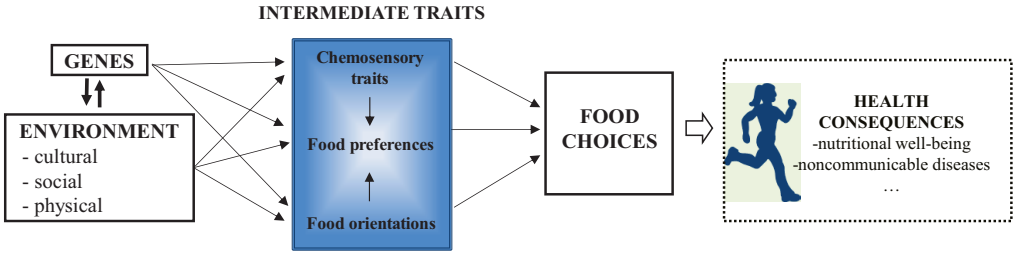

Figure 5. Factors influencing preference, food choices and health consequences (Perola and Tuorila 2009). Chemosensory traits such as taste and odor influence the preference of flavors. Food orientations are a complex ensemble of different factors (e.g. food neophobia, diets etc.).

Food choices have been studied from a wide variety of disciplines and perspectives because of their biological, psychological, economic, social, cultural and epidemiological importance. Different models explaining food choices have been developed since the late 80's (Sobal et al. 2006) signaling the obvious complexity of the matter. Although food choice factors vary according to life stage and from individual or group of people to the next, the determinants can be summarized into six major dimensions (Bellisle 2005).

- Biological (hunger, appetite and taste)
- Economic (cost, income, availability)
- Physical (access, education, cooking skills, meal patterns)
- Social (culture, family, peers, meal patterns)
- Psychological (mood, stress, guilt)
- Attitudinal (beliefs, knowledge about food)

2.5 Factors influencing preference

Food preferences are thought to form in combination with the unlearned, reflexive reactions to basic tastes and the ability to learn based on association with the contexts and eating of various foods (Birch 1999). Interaction of genetic predispositions with environmental factors, such as culture, is thought to work in combination to produce individual preferences of foods and tastes. In other words, food and taste preferences are the phenotypic behaviors that result from genetic and environmental inputs.

Some preferences are stable and unaffected by changes in settings whereas others are transitory and change on a day to day basis (Lyman 1988). A single negative experience may result in a long lasting aversion whereas one positive experience has less pronounced value (Garb and Stunkard 1974). Besides the ability to learn, preferences are modified by various orientations related to foods. These include different diets and eating behavior related traits such as food neophobia (the avoidance of new foods), which may lead into a limited and perhaps unhealthy diet (Falciglia et al. 2000). Sensitivity to odors has also shown influence food acceptability (Jaeger et al. 2012). Other factors influencing preferences are genetic and environmental factors, sex and age.

Rozin (1982) presented a scheme of flavor principles (Figure 6) which illustrates how biological behavior may be transmitted through culture to individuals, ultimately leading to preference of flavors. The principle presents food neophobia as a steady barrier through which the opposing desires for ‘wanting variety’ and ‘holding on to the familiar’ is manifested.

2.5.1 Food neophobia

Food neophobia is a personality trait expressed as a fear of new foods. It is mostly studied in children but for some people it persists into adulthood and forms one dimension influencing the diversity of foods consumed. Koivisto and Sjoden (1996) observed that the higher the measured food neophobia in mothers and children, the more restricted was the list of uncommon foods that had been served in the family. Similarly, Falciglia and others (2000) found that neophobic children had a lower quality of overall diet than less neophobic children. Although food neophobic individuals perceive the sensory characteristics similarly to the less neophobic, they show a different degree of liking for the food (Henriques et al. 2009), particularly in regards to novel foods. Food neophobics also rate expected and actual taste pleasantness of a product lower than less neophobic individuals as demonstrated by Arvola

and others (1999) who also concluded that neophobics both avoid and dislike novel foods. Several factors such as age, education and degree of urbanization indicate that food neophobia is not a static condition and varies widely in populations. Tuorila and others (2001) found that men were more neophobic than women, elderly expressed higher degree of food neophobia, and the higher the education or degree of urbanization the lower the food neophobia.

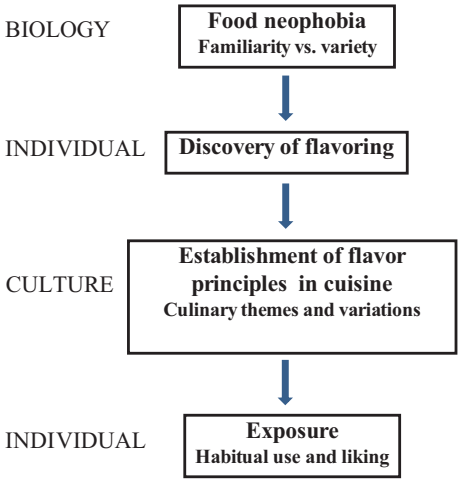

Figure 6. Scheme of flavor principles showing the biological behavior transmitting through culture to individuals (Rozin 1982).

2.5.2 Culture and experience

Food preferences have been closely linked to culture from ancient days to the modern times. Within the range of available foods, culture acts as a filter. The ingredients, culinary techniques and the flavor evoke certain ethnicity. Flavor seems to be the most crucial factor in providing the sensory and cultural label for the food (Rozin 2000). Whereas Nordic dishes are traditionally bland and lightly seasoned, the recipes in tropic cuisines such as in Mexico, call for variety of spices, above all, those with pungent qualities (Sherman and Billing 1999). Nowadays, ethnicity in food is perceived as a positive attribute and the high seasoning associated with esteem and fashion (Rozin 2000). Acquisition of cultural aspects of a cuisine is thought to occur in two stages: first initiated by 'forced' exposures to foods based on

traditional cooking maintaining individual behavior, and second followed a stage during which food preference is internalized, in other words, learned (Rozin 1982).

Cross-cultural differences in chemosensory perception have been observed but they are not pronounced (Prescott and Bell 1995). Previous experiences of foods are likely to influence the discrimination of the sensory properties as well as the sensitivity to tastes in foods. Cross-cultural differences in preference for some tastes have however emerged. When a population of Indian laborers was tested for taste preferences (sweet, sour, bitter and salty) anomalous liking, particularly for sour taste, was observed (Moskowitz et al. 1975). While Western populations find the taste of citric acid unpleasant, the laborers reported it increasingly pleasant as the concentration was increased. It was noted that the laborers' diet emphasized sour tastes e.g. tamrind fruit, which was eaten frequently. Thus, dietary history was the likely explanation for the preference for sourness, or alternatively, genetic inbreeding. Later, research on Japanese and Australians, who rated their liking for seven tastant solutions (sucrose, NaCl, citric acid, caffeine and three umami solutions; MSG, IMP, GMP) showed that differences in liking were evident in higher concentrations of sour and umami stimuli, however, the researchers concluded that the groups were more similar than different in their responses to the stimuli (Prescott et al. 1992). It is important to note that taste intensity does not necessarily predict the level of affective responses. The power of familiarity is true for stimuli of different tastes as well as for entire foods (Prescott and Bell 1995; Tuorila 2007).

2.5.3 Learning

The majority of human food preferences are learned via experience with food and eating (Birch 1999; Yeomans 2009). Neophobic reactions function together with learning mechanisms which in turn serve to diminish initial avoidance of the new foods. In other words, with experience, the original neophobic rejection can transform into a preference (Birch 1999). The association between foods and flavors are also learned with the consequences that follow eating. Two models how humans acquire preferences for new foods and drinks have been proposed. Flavor-flavor learning refers to a situation in which a familiar flavor element (liked or disliked) reinforces a second novel flavor element. This can result in acquired liking or disking for the novel flavor depending on the reaction to the familiar one. Another type of reinforcement (flavor-consequence learning) arises when a food or drink ingested has biological consequences. Negative consequences such as nausea can lead to aversion that can persist for decades, while positive consequences such as satiety or 'drug' related effects, caused by e.g. caffeine, can lead to preference due to positive post-ingestive experience (Yeomans 2009).

Aversions can be formed even for highly preferred foods, although they are more readily formed with novel foods (Schafe and Bernstein 1996). Mattes (1991) who studied the prevalence and features of food aversions concluded that nearly 1/3 of the population have current aversions. A stable and higher level of aversions was observed in individuals 11–40 years of age compared to the younger or older, and the majority of aversive foods were rated previously as familiar and pleasant (mainly protein origin: eggs, meats, seafood). Moreover, some families were more prone to learned aversions than others (family resemblance). Preferences, on the other hand, form more slowly, are a result of normal eating, and may have more subtle but pervasive effects (Birch 1999). Preferences are also much harder to establish than aversions (Rozin 1986).

Learning continues throughout life, though some of the preferences are formed during childhood. Exposure to flavors in infancy bears particular importance in the formation of taste preferences due to existence of sensitive periods (Beauchamp and Mennella 1998). These preferences also seem to be relatively stable over time (Liem and Mennella 2002). Short term exposure effects may be less effective, at least in adulthood. Liem and others (2004) showed that adults did not change their preferences for sweet or sour taste during short repeated exposure, but exposures did affect children's preference for sweet taste. They hypothesized that some taste preferences, such as sour taste, may be more resistant to changes and thus need prolonged exposure times. It may be easier to modify children's liking responses with exposure because unlike adults, they have a limited idea of what foods should be like, and thus perhaps less rigid expectations (Tuorila 1996). What seems evident is that exposure is more likely to enhance liking when it occurs at a moderate frequency, and when the stimuli are novel, relatively complex, or both (Rozin 1986). In line with this notion, Rozin and Schiller (1980) concluded that exposure to chili over a longer period of time, with increasing the amounts consumed little by little, were the major factors producing a gradual increase in preference for the 'burn' of chili peppers. It is important to note thought that a taste or chemosensory quality in a certain context does not necessarily generalize to a wider preference for that taste in other contexts.

2.5.4 Age and gender

Taste perception is relatively stable across the life span. It is the most durable and well-defended of all of the sensory systems. One evidence of this is shown by the observation that humans truly lacking the ability to taste are extremely rare (Breslin 2013). A heightened preference for sour taste has been shown with children of 5-9 years (Liem and Mennella 2003) relating to intense stimuli and novelty seeking behavior (Liem et al. 2004). It is not known whether the heightened preference ceases in adulthood. It has been reported that citric acid level is perceived as equally sour between two age groups (20-29 vs. 70-79 y.) but the elderly

rated the sourness as more pleasant, indicating their capability to tolerate higher sourness (Chauhan and Hawrysh 1988).

Although taste system is highly resistant to senescence (old age) and damage, some age-related declines in taste sensitivity have been observed. These alterations are often modest and quality- (e.g. sour taste) or compound-specific (e.g. NaCl) (Cowart 2010). Age-associated loss of taste, and particularly smell (affected more by age), are likely to lead to altered flavor perception. This does not however necessarily cause a difference in preference (Murphy 1993). The overall flavor combination may be differentially perceived between the elderly and the young, but when a flavor component is falling below the elderly's threshold, the altered flavor may consequently drive preference.

The reasons underlying taste alterations among the elderly may be due to anatomical changes such as alterations in taste cell membranes (Misretta 1984) that occur during normal ageing. Certain diseases and medications can also change the perception of tastes. Sjögren's syndrome for example is an autoimmune disease commonly affecting peri-menopausal women which can lead to impaired salivary gland function, decreased taste threshold sensitivity and loss of an enjoyment of foods (Weiffenbach et al. 1995). Drug-induced taste disorders are reported frequently when treating cancer, HIV, cardiovascular or psychological conditions (e.g. depression) (Doty et al. 2008). Decreased or increased taste acuity and phantom tastes (bitter/metallic side tastes) are the common causes of numerous drugs. Eventually, the changes in taste and smell systems can lead to poor appetite, inappropriate food choices and lower nutrient intake (Schiffman and Graham 2000).

In regards to taste preferences in general, age seems to induce change. The change may be seen as wider set of foods liked that were not preferred in childhood. When preferences of French adolescents (10-20 years old) were explored it was shown that while the younger preferred bland and familiar foods, the older were more likely, after puberty, to begin to like 'adult' food items such as vegetables, spices, fish, coffee and tea (Nu et al. 1996). Mojet and others (2005) studied age related sensitivity and pleasantness to stimuli in different foods (sweetness in ice tea, saltiness in tomato soup, umami in broth, bitterness in a chocolate drink and sourness in mayonnaise) and found no evidence that age-related loss of taste sensitivity would lead to preference for taste-enhanced foods. They did show however, that age has an effect on liking. In general, elderly (60-75 y.) preferred the products more compared to the young (19-33 y.).

Conflicting results have been reported for the existence of gender differences in chemosensory abilities. Guinard and others (1997) illustrated differing results for men and women in taste intensities (sweet and umami) but none in the perception of astringency. The direction of the difference was however stimulus dependent; the maximum intensity for umami was higher with men, whereas sweetness was higher with women. They also found

men to salivate significantly more than women in response to all stimuli. James and others (1997) in turn, found detection thresholds for sweet, salty, sour and bitter tastes similar for men and women. They concluded that if differences exist between genders, they are small and favor women. Differences in food perception sensitivity between genders were studied by Michon and others (2009) who measured sensitivities to taste (sweet, sour, salty and bitter) and perceptions of pungency (piperine) and astringency (tannic acid). Gender did not influence the ability to identify tastes (except bitter) nor did it impact the perceived intensity of other chemosensory perceptions. Women were observed to give higher intensity scores for sour and sweet taste than men, and to identify bitter taste better.

Men and women have different preferences of foods with different sensory characteristics. Foods with rich and strong taste are generally more attractive to men. Men favor red meat and high-fat products, while women are more likely to choose lighter colored meats and healthier foods such as fresh fruits and vegetables (Frewer and Trijp 2006). Logue and Smith (1986) reported women to prefer low-calorie foods, wine and candy and as having lower preference for spicy foods, meat, beer and milk. Women also reported to have more food aversions, and higher tendency to reject certain foods compared to men. However the reasons for rejection were the same for men and women, and mainly due to sensory cues (Nordin et al. 2004). It should be noted that women tend to attach greater importance to health belief and weight control than men which may partially explain the differences in preferred foods (Westenhoefer 2005).

2.5.5 Genetic variation

Evidence for genetic influences on food and taste preferences has emerged in family and twin studies throughout the years. Falciglia and others (1994) showed based on intrapair correlations (identical twins vs non-identical twins), that genetic factors influence preferences for orange juice, broccoli, cottage cheese, chicken, sweetened cereal and hamburger. However, no heritability estimates were provided and as only few food items were used and treated as individual items, it is not necessarily clear what property of the items (sweetness, sourness etc.) produced the results. Rozin (1991) suggested that the heritable component for individual foods is low, and later on, Reed and others (1997) concluded that the preference for carbohydrates and fat are partially genetically determined, more so than that of individual food items. More extensive list of foods in a preference questionnaire was used by Breen and others (2006) who examined heritability (h^2) of preferences in children for food groups of desserts (h^2=20%), vegetables (h^2=37%), fruits (h^2=51%) and protein foods (h^2=78%). They concluded that the preference traits were moderately heritable characteristics. Similarly, 54% heritability was shown for preference of sweet foods (Keskitalo et al. 2007a). Recently, a population-based study investigating a broad spectrum of food preferences and their relationship with 37 different taste and olfaction related genes revealed significant

associations between several variants in the taste receptor coding genes (Pirastu et al. 2012). For example, TAS1R3 and liking of vodka, white wine and lamb meat, as well as TPRV1 and beet liking.

Genetic factors are also implicated in components influencing food preferences such as taste. Recent advances in molecular genetics have allowed the identification of candidate genes encoding the taste receptors (e.g. bitter and sweet taste), and demonstrated that genetic variants contribute to individual differences in taste perceptions (Kim et al. 2003; Knaapila et al. 2012). These results have raised interests to unravel what implications the variants have on food choices. Recent reviews (Negri et al. 2012; Dotson et al. 2012; Feeney 2011; Garcia-Bailo et al. 2009) have aimed to elucidate the impact of genetic variability in food preference, selection, intake and healthy eating with similar conclusions. Genetic variation is likely to affect taste perception predicting preferences and influencing dietary habits, but they are only one of the many factors involved, and the extent of these influences are modest or still largely unknown. Apart from bitter and sweet taste, little is known of the genetic variability or heritability of other chemosensory qualities. While the genetics of umami, pungency and astringency remain poorly understood, it has been shown that the sensitivity to sourness is attributable to genetic effects by 53% (Wise 2007). In order to better understand the chemosensory gene variants in relation to food preferences, more research needs to be conducted in this area. It is also important to note that a significant proportion of variation observed in taste and food preferences may depend on genes other than those involved directly in taste processing e.g. genes regulating reward or motivation (Dotson et al. 2012).

Another component with a genetic basis that influences food preferences is food neophobia. Evolutionary, this tendency has given humans an advantage by protecting us from harmful foods, but on the other hand, a disadvantage by narrowing the variety of a diet. Ultimately, the most food neophobic individuals may lose the health benefits and hedonic advantages of foods. Food neophobia has shown heritable variation in humans with heritability estimation as high as 69% (Knaapila et al. 2007) although the genes underlying this trait are not known to date. Interestingly, women and men seem to differ in the importance of genetic and environmental influence on food neophobia. It is possible that in women, a stronger genetic control in variation of food neophobia exists due to the biological functions of child bearing and nurturing, while men may be more influenced by social pressure from the environment. In other words, while women are given the freedom to express their individuality concerning foods, men may not have the same freedom, hindering them from following their genetic tendencies (Knaapila et al. 2011).

2.5.6 Olfaction

Part of the pleasure derived from foods is attributed to odors that constitute one of the major determinants of foods' flavor. Smell is also one of the key first defenses against spoiled foods. Thus, the sense of smell has implications on food intake and eating habits but also on the quality of life (Hummel and Nording 2005). Humans have nearly 400 functional receptor genes and it is obvious that far more genes are responsible for the receptors for smell than for taste. The great number of olfactory receptors and the large individual variation in perception to odors have inspired researchers to unravel the genetics of olfaction. The research has shown that the ability to smell some odorants is heritable. For instance, the differences among people in their ability to smell androstenone (sweaty, urine like smell) is of heritable nature (Knaapila et al. 2008). Similarly, the detection of cis-3-hexen-1-ol (green leaf odor) has been shown to be under genetic control (Jaeger et al. 2010).

2.6 Measuring chemosensory traits

Human sensory data provides the best model to estimate how individuals are likely to perceive taste and flavors, and react to food products. Sensory tests are often conducted in a laboratory, which may not always be representative of natural conditions. Multiple study and scaling methods are used in sensory laboratories to describe the perceived intensity of a sensory quality or the degree of liking. In this section a few examples of methods and scales how responses to astringent, sour and pungent stimuli have been measured are presented. Measuring the bitterness of PROP and the umami perception are briefly discussed.

2.6.1 Astringency

As opposed to taste perceptions, astringency builds slowly in intensity after ingestion and may persist for a long time (Bajec and Pickering 2008; Lesschaeve and Noble 2005). Former studies have used time-intensity (TI) methods with trained panels to fully describe astringent responses in which repeated sipping of astringent solutions, at definite intervals, while continuously rating the astringency intensity have taken place (Ishikawa and Noble 1995; Peleg 1999). These type of studies have demonstrated the importance of experimental design when conducting sensory tests with astringent compounds by reporting maximum intensity (linear increase regardless of concentration of stimulus), the time to maximum (13-15 s post ingestion), the tendency of astringency to increase with repeated sipping-, and the time at which astringency is extinguished (70-120s) (Bajec and Pickering 2008). Alongside TI-

methods, the intensity of astringency has been measured using various scales. Peleg and Noble (1999); Siebert and Chassy (2004) used unstructured line-scales. Nayak (2008); Condelli et al. (2006) utilized structured point scales, while Dinnella et al. (2009); (2010); (2011) used the Labeled Magnitude Scale developed by Green et al. (1996). Most of the studies used tannic acid as a stimulant for astringency (0,5-3.0g/L) also expressed as phenols per gram (catechin or epicatechin 0.75-0.9 g/L).

Hedonic evaluation of astringency has received less attention but with more consistent use of scaling (the same hedonic 9-point scale used). Tang and others (2001) measured hedonic ratings (n=40) using a 9-point scale developed by Peryam and Pilgrim (1957) in evaluation of sets of sweetened and un-sweetened sea buckthorn samples. The data was combined later on with intensity ratings produced by a sensory panel. Dinnella et al. (2011) used the same hedonic scale with untainted juices, and tannin acid spiked apple, grape and carrot juices, and trained the respondents (n=77) to recognize the perception before evaluating the intensities. Consumer perceptions of astringency in varying whey protein content beverages were also evaluated using a 9-point hedonic scale in a consumer test (n=120) combined with focus group interviews (n=19) (Childs and Drake 2010).

2.6.2 Sourness

Multiple scales and techniques have been used to measure responses to sourness. A line matching method was utilized with a population of Indian laborers to evaluate sourness intensity of citric acid with increasing molar concentrations (Moskowitz et al. 1975). Using a sensory panel, flavor characteristics of lactic, malic, citric and acetic acids (each at varying pH=3.5, 4.5 and 6.5) were determined with a 16-point intensity scale (0=none, 7=moderate, 15=extreme) (Hartwig and McDaniel 1995). A study examining sour taste characteristics and risk of alcoholism used 112 respondents who evaluated the pleasantness and intensity of citric acid in different concentrations (0.02-0.10%) with a 200 mm anchored visual analog scale (Sandstrom et al. 2003).

Responses to sour taste measured from children and the elderly have received a fair amount of attention (Liem and Mennella 2003; Liem and de Graaf 2004; Pelletier et al. 2004; Zandstra et al. 2001). Mostly, citric acid has been used (0-0.25M) as a stimulant and often with particular methods and scaling suitable for children. Liem and others (2003) used a game-like rank-by-elimination task (children n=61) when determining the preference rankings (in this context preference was not used as a synonym to liking) for lemon flavored gelatins with varying citric acid concentrations. The same methodology was used to distinguish the different sourness intensities of the samples. In 2004, Liem used a rank-ordering procedure for sour preference testing for yoghurt and orangeades, again with

children (n=59), to establish *most preferred*, *neutral* and *least preferred* sample categories. Pelletier and others (2004) used a box scale (15 boxes, end and middle points labeled: not at all, moderately, extremely) in determining the intensity of sourness (different concentrations of citric acid in water solution) in the elderly (n=22). Zandstra and de Graaf (1998) in turn, used orange beverages with varying sourness and a 5-point category scale for intensity, and facial hedonic scale to indicate different degrees of like and dislike for both children (n=31), and elderly (n=30).

Detection and recognition thresholds with twins have been used when examining the heritability of sour taste. Kaplan (1967) used a series of dilutions ($7.32 \times 10^{-7} – 6.0 \times 10^{-3}$M) of inorganic acid (HCl) to measure detection thresholds (n=368), whereas Wise (2007) used citric acid ($6.10 \times 10^{-4} – 5.0 \times 10^{0}$ mM) to determine recognition thresholds (n=218). Both studies used a method developed by Harris and Kalmus (1949) in which the respondents separate 8 samples into two groups; 4 of these samples include water and the other 4 are spiked with a certain concentration of stimuli. If the samples are correctly grouped, the task is repeated with the next lower concentration and so on until the respondent can no longer discriminate the groups correctly.

2.6.3 Oral pungency

The first laboratory test described in the literature to measure chili pungency was the Scoville Organoleptic Test (Scoville 1912) in which a taste panel of five respondents evaluated the pungency level of a chili sample (capsaicin oil extracted from dried pepper). The sample was then diluted and evaluated until pungency could no longer be detected. A single unit of dilution is called a Scoville Heat Unit (SHU). The SHU scale ranges from zero (most sweet peppers) to over two million, as shown for *Trinidad Moruga Scorpion* (Bosland et al. 2012), one of the world's 'hottest' chili pepper variety originating from the southernmost island in the Caribbean. The organoleptic test is cheaper than more accurate chromatographic methods (Collins et al. 1995) but it has its limitations. Measuring pungency with Scoville units doesn't measure the amount of capsaicinoids present, nor does it provide objective values, or values independent of tasters sensitivity to pungency. Earlier studies have used Scoville units to describe the level of pungency in the samples (Lawless 1984; Rozin et al. 1982) but nowadays it is less common.

Measuring pungency poses similar concerns as when measuring astringency (duration and lag time), thus temporal measurements have been popular in determining the properties of oral pungency, even for hedonic evaluations (Rozin et al. 1982). Lawless (1984) used intensity magnitude estimation to determine the psychophysical properties of capsaicin samples (7.2-900 000 SHU) in which numbers were assigned to the intensities relative to reference (e.g. if

twice as strong, 20 assigned). Multiple evaluations were made within a time interval of 600s. When many samples with varying concentrations of capsaicin have been used, the order of presentation has been either increasing in terms of the burn (not randomized) or in roughly increasing series where the first sample has been the weakest and the last the strongest, leaving room for randomization in the middle (Lawless 1984; Rozin et al. 1982). Sometimes several sessions on separate days have taken place, apparently to minimize the carry-over effects and respondent's fatigue (Lawless et al. 1985).

The samples used in the studies of oral pungency have varied from capsaicin in 0.2% EtOH solutions (Cliff and Green 1996; Nasrawi and Pangborn 1990) to real typically pungent foods, such as potato crackers, tomato soup and ratatouille (Lawless 1984; Stevenson and Yeomans 1993; Stevenson and Yeomans 1995) with levels of capsaicin between 1-256 ppm. The use of unfamiliar flavor combinations were demonstrated by (Prescott and Stevenson 1995) who used sweet/sour/pungent flavored test solutions to avoid the familiarity effect in the intensity evaluations.

Line scales and LMS have been used by several studies. Stevenson and Yeomans 1993) studied both intensity and pleasantness of the capsaicin burn by utilizing visual analogue scales for both measures. Similar to PROP intensity measurements, pungency have also been determined by a capsaicin-impregnated filter paper method with LMS for the magnitude estimation (McBurney et al. 1997; Prescott 1999).

2.6.4 PROP and umami

The studies on PROP and umami have both received abundant attention but partially from different perspectives. While PROP has been investigated as a marker for genetically inherited sensitivity to bitterness, umami taste has been mainly described on the receptor level, mostly in animals. When potential ageusia for MSG was discovered by Lugaz and others (2002), genetic variation explaining the phenomena became an issue of interest (Raliou et al. 2009). A rough screening for sensitivity to umami has been performed using quality discrimination testing or difference testing where respondents were asked to discriminate two samples from each other (water and NaCl, water and 29mM of MSG), or report if they perceive a difference between the two samples (Lugaz et al. 2002; Raliou et al. 2009; Singh et al. 2010). Raliou and others (2009) also completed further testing to measure intensity, quality and preference for umami using paired comparisons (five pairs of samples including water, 29nM NaCl, 29nM MSG, and 14.5nM GMP). The hedonic judgments were assessed on a -10 to 10 scale. Hedonic ratings using a 9-point scale was used by Roininen and others (1996) to rate the pleasantness of different soups without and with added umami (0.2% MSG, 0.05% 5'ribonucleotides).

The bitterness of PROP has been measured in multiple studies with a variety of scales and measuring techniques including threshold and suprathreshold tests. In need of a simple and more reliable screening of PROP tasters, Zhao and others (2003) developed a PROP-impregnated filter paper test and examined its validity relative to formerly introduced three-solution test (Tepper et al. 2001). The results were convincing. Both studies used LMS scale for evaluating the samples as suggested by Green and others (1993).

2.7 Responses to food names

Alongside hedonic evaluation of stimuli or food, affective judgments can also be given to food names. Extensive lists of foods (stimuli presented as a list of food names) can be useful when trying to survey the respondent's preferences, particularly the underlying sensory dimensions of foods that affect liking. It is important to note thought, that a tendency to use more extreme ratings for food names than for the actual stimuli has been shown (Cardello and Maller 1982). Respondents tend to rate the food names based on the best or worst example. Furthermore, ratings for food names may be somewhat biased with attitudes to the food in question more, than they would be for the actual food tasted (Tuorila 2007). In addition to liking, the consumption of a food might be of interest. Instruments collecting food consumption data are similar to those used in nutrition research e.g. food frequency questionnaires. A common estimate of the relationship between the affective judgments to a food and the consumption is that affection predicts 25-50% of consumption (Cardello and Maller 1982).

2.8 Biases and considerations of sensory measurements

Multiple challenges exist in sensory measurements and all judgements are a function of the observing conditions. To put it bluntly, few if any ratings have absolute meaning. However, whereas humans fail to operate as absolute measuring instruments, they are very good at comparing things (Lawless and Heymann 2010). Frames of references based on experiences may vary, which leads to noise in the data. For example trouble in understanding what is high and low on the response scale, or understanding concepts without explicit references. The sensory data can be 'fuzzy' because of other reasons too. For example, consumers tend to react to tested products as a whole. This means difficulties in accurately focusing on individual attributes of a sample. A good example of this phenomenon is that intensity ratings are likely to be influenced by the persons liking of the product (Lawless and Heymann 2010; Cardello and Maller 1982).

Simple contrast effects are often present in chemosensory measurements. There can be a shift in ratings depending on other samples present which are evaluated in the same testing session. Hedonic shift is an example of this type of event where good stimuli can reduce the pleasantness of less good stimuli (Zellner 2006). Another classical issue of sensory measurement is the stimulus error. This happens when the rating is biased due to expectations about stimulus identity.

Many approaches in dealing with context effects and biases (judgment changing as a function of the context) exist such as randomization, stabilization, calibration and interpretation (Lawless and Heymann 2010). Randomizing samples is part of good practice in sensory testing. Stabilization refers to holding the experimental context constant across all testing situations and calibration to using reference samples in the experimental session. Lastly, when interpreting the results, it is important to note the context and bias effects that may be operating and draw conclusions bearing the restrictions in mind.

2.9 Genome and inheritance

2.9.1 Human genome

The human genome, which is a term describing the entire hereditary material of an organism, consists of 23 chromosome pairs (diploid) including 22 autosome pairs and one sex chromosome pair (XX females, XY males). Each cell nucleus carries approximately 3 billion base pairs that are packed in these 23 chromosomes (Figure 7). Over 20 000 protein coding genes are known to date. While heredity was previously thought primarily to include only protein coding genes, it is now viewed more widely. Genes are not compact or uniquely important but rather spread out with protein coding and regulatory regions that overlap with other genes. Furthermore, the distribution of exons, introns, promoters, gene start sites, and other DNA features suggest that gene expression is regulated by multidimensional network (Pennisi 2007).

2.9.2 Genetic variability

Genetic variability can be translated to a measure of how much the trait or the genome tends to vary in a given population. It is based on the inheritance of genetic material from both parents. One homologue of each chromosome is inherited from the mother and one from the

father. Thus the diploid cell from, which all cells in a human are derived from, is formed from two gametes (egg cell from mother and sperm cell from father). The gametes contain only one copy of each chromosome (haploid).

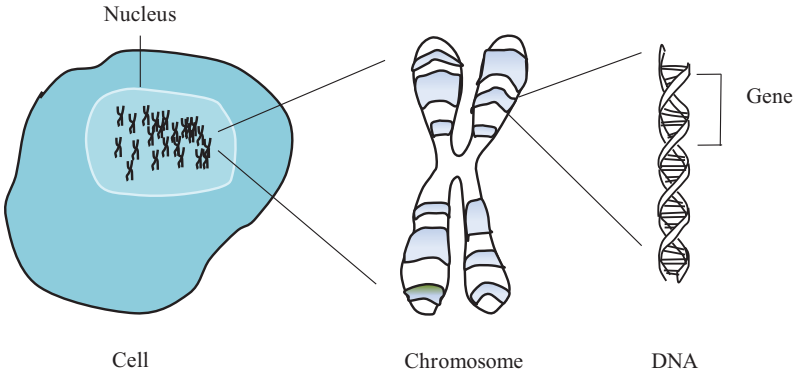

Figure 7. DNA organized into a chromosome.

Meiosis is a special form of cell division that takes place in the testis and ovary to produce haploid sperm and egg cells. The homologues of each chromosome are assorted to gametes independently, which allows 2^{23} combinations of parental chromosomes in one person. Genetic material is also reorganized by recombination or crossing over producing a recombinant which contains genetic material from both homologs (Figure 8). An egg after fertilization can have any possible (4^{23}) combinations of the parental chromosomes. Considering the independently inherited homologous chromosomes, and the shuffling of genetic information during meiosis and fertilization, the number of different gametes is virtually unlimited (Strachan and Read 2004).

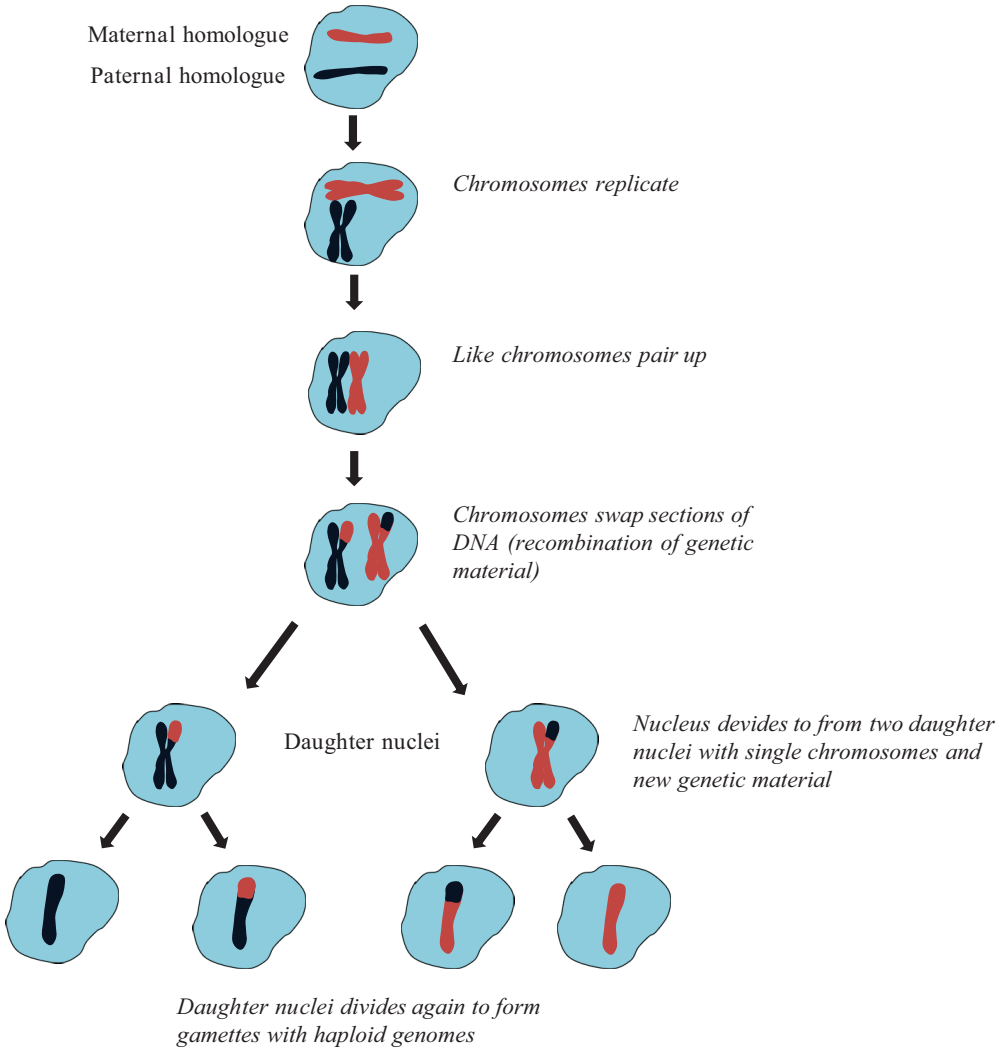

Figure 8. Meiosis and recombination presented for one chromosome.

2.10 Methods for studying genetic background of traits

Exploring the genetic background of a trait is usually started with observing the variation of the trait in question. A genetic component is then determined using heritability analysis which reveals the relative genetic and environmental influences on the trait. If an underlying genetic component is found, the search for the genetic loci, candidate genes or genetic segments linked to the trait can be achieved by genetic linkage analysis. Subsequently, identifying gene variants (alleles) and establishing the association between a particular genetic polymorphism and a trait can be investigated with association analysis. Heritability and linkage analysis requires a sample where familial relationships are known (family studies) whereas association analysis can be conducted in a population sample (case control setting). Linkage and association analyses are often seen as complementary. Linkage analysis is used to scan the genome for localization of the candidate regions linked to the trait, and association analysis is used to narrow down the area in identifying the susceptibility genes (Strachan and Read 2004). In other words, linkage analysis answers the question 'is there an association between segments of DNA and the trait in question?' whereas association analysis is saying 'what are the differences in the genome'.

There are two approaches for genetic dissection of complex traits (Zhu and Zhao 2007): candidate gene approach and genome-wide scanning, known as GWAS (Genome Wide Association Study). Candidate gene approach relies on existing knowledge about the genes with biological function relevant to the investigated traits. While it enables the scientists to discover the mutations directly influencing the gene in question, it is limited by the required knowledge or presumptions about the trait biology, which in many cases is unknown. By contrast, the genome-wide scanning is proceeded without assumptions or previous knowledge of the relevant genes and the whole genome is scanned for common genetic variation. This way new regions or gene variants of interest may be discovered. Detection of new loci however calls for statistical power. Variants need to be common enough, occurring in 1-5% of population.

2.10.1 Heritability

Heritability is the proportion of a trait variation explained by genetic effects. It is a measure of inheritance of a trait (phenotype, P) and its estimation requires data from relatives (e.g. family data). Heritability analysis aims to separate the variance (V) of the trait into genetic (G) and environmental (E) components as shown by the equation:

$V_P = V_G + V_E$

V_G can be further decomposed into

- additive genetic variance (V_A) which consists of the sum of the allelic effects over all relevant loci
- dominant genetic variance (V_D) which includes interaction of alleles in the same locus

Dominance refers to a situation where the other allele of the gene (two gene variants: one from mother and one from father) dominates the second in a way that heterozygous (with different gene variants) individuals deviate from homozygous (with same gene variants) individuals.

There are two interpretations of heritability: *broad-sense* heritability and *narrow-sense* heritability. Broad sense heritability refers to all the genetic effects including additive, epistasis (one gene masks the effect of another or several genes work together) and dominance effects. Narrow-sense heritability includes only additive genetic effects.

Broad-sense heritability $H^2 = V_G / V_P$
Narrow-sense heritability $h^2 = V_A / V_P$

Narrow-sense heritability is widely used as a parameter that describes the magnitude of genetic influence on a trait, and it is often expressed as percentage value. For example 40% heritability means that 40% of the trait variance can be explained by genetic differences between the individuals in that population. Hence, heritability is a population-specific parameter, applicable only to the population in question and it does not apply to an individual. High heritability estimates are generally obtained for physiological traits such as height (68-93%), body mass index (64-84%) and diabetes (88%) (van Dongen et al. 2012), whereas personality traits tend to be moderately heritable e.g. thrill and adventure seeking ~63%, neuroticism 60%, and experience seeking ~58% (Boomsma 2002).

When using family data to calculate heritability, the estimate includes all the variation making the family members similar to each other, in other words, shared environmental effects raises the value of heritability because shared environmental effects tend to mimic genetic similarity among the relatives. This problem can be overcome by using twin data and quantitative genetic modeling for estimating heritability.

2.10.2 Classical twin design

Twins have captured the curiosity of researchers for years with first systematic analysis results emerging in 1924. Siemens, a dermatologist and one of the pioneering twin scientists, studied the similarity between monozygotic (MZ, identical) and dizygotic (DZ, non-identical) twins and formulated the twin rule of pathology. That is; any heritable disease will be more concordant in identical twins than non-identical twins, and concordance will be even lower in non-siblings (Siemens 1924). Siemens combined correlation analysis and twin data when studying skin moles. He correlated the mole counts in one twin with the mole counts in the other, and compared this correlation in MZ and DZ pairs of twins. Comparing the resemblance of MZ twins for a trait with the resemblance of DZ twins forms the basis of classical twin studies. Twins provide a special group widely used in epidemiologic and behavioral research when estimating the genetic and environmental effects on a trait. Twin comparisons rely on several assumptions:

1) MZ twins share all genes (in sequence level) and DZ twins, on average, share half of their segregating genes.
2) The environment shared by a twin pair influences twins similarly regardless of their zygosity (equal environment assumption).
3) No gene environment interactions, epistasis, or assortative mating (correlation of phenotypic values of spouses) exists.

Thus, as MZ twins are assumed to share their genes and partially their environment (common, shared environmental factors), any resemblance between them is thought to stem from these, while any difference between them is thought to arise from non-shared (specific) environmental factors. Resemblance of DZ twins is also due to shared genes and environment but because DZ twins only share 50% of the genes, any resemblance between them due to genetic effects is smaller than that of the MZ twins (Boomsma, 2002). Thus, when higher correlation within MZ twins is observed compared to that of the DZ twins, genetic causes (heritability) are the likely explanation.

To obtain reliable results, the 2nd assumption can be tested by examining that the means and variances of the trait are similar when comparing twin 1 to twin 2, MZs to DZs. If this is true (equal means and variances) the only source of difference between the within-pair correlations of MZ and DZ is the genetic correlation.

The validity of the equal environment assumption has been questioned by a suggestion that parents treat MZ twins differently to the DZ twins. This is likely to be true to some extent, however as indicated by Kendler and others (1994), the approach of the mother and father to

raising twins had no significant influence on twin resemblance. Emerging evidence has also cast a shadow on the 1st assumption (MZ share 100% of their genes). In fact, MZ twins can differ in chromosomal and DNA level (Gringras and Chen 2001). The number of morphology of chromosomes may vary, DNA mutations occur, or epigenetic modifications exist such as methylation by which the expression of a sequence of DNA can be modified by becoming silenced (switched off). Differences in copy number variations (gains and losses of large chucks of DNA sequence) in MZ twins have also been shown (Bruder et al. 2008).

2.10.3 Quantitative genetic modeling of twin data

In twin studies, the variance of a trait can be decomposed to genetic and environmental factors. Genetic factors further divide to additive (A) and dominant (D) effects. When the effects are additive, each allele has an additive effect, whereas interaction of alleles in the same locus is occurring when genetic factors are dominant. The environmental factors are either common (C) or specific/ non-shared (E). Common factors are those that make the twins similar while specific factors make the twins different from each other. Based on the known genetic similarity of the twins and equal environmental assumption, the differences between the MZ twins are due to E, thus the correlation between the MZ (r_{MZ}) twins is A + C, and E equals 1- r_{MZ}. Respectively, the correlation between the DZ (r_{DZ}) twins is 1/2A + C (DZ twins share on average half of their genes). The following equations can be thus derived to provide crude estimations of the proportional sources of variation:

$a^2 = 2(r_{MZ} - r_{DZ})$	proportion of additive genetic effects
$d^2 = 2r_{MZ} - 4r_{DZ}$	proportion of dominant genetic effects
$c^2 = 2r_{DZ} – r_{MZ}$	proportion of common environmental effects
$e^2 = 1 - r_{MZ}$	proportion of non-shared environmental effects

Computerized decomposition of the variance of a trait is however a more general approach as opposed to crude hand calculations. Structural equation modeling provides a way to model genetic and environmental effects as unmeasured latent variables causing the differences in the phenotype (Figure 8). The latent factors represent the effects of many unidentified influences. Genetic factors for example, may be due to potentially large, but unknown number of genes. In a study including only twins reared together (e.g. not adopted and living in separate families), only three (A, C/D, E) of the four latent variance components can be estimated due to the confounding nature of C and D components.

The starting point of the modeling is either ACE or ADE (so called full models). The choice between the starting models is made according to the within-pair correlation patterns: if the

MZ within-pair correlation is more than twice as high than the correlation within DZ twins, this indicates the presence of dominant genetic effects (ADE-model as starting point), if less than, the influence of common environment is assumed (ACE). If the correlations are similar, the resemblance of the twins is likely to be due to common environment. May the analysis start with either ACE or ADE model; the significance of each component (A, C and D) can then be tested leading to additional model options: AE, CE or E. The best model is chosen according to the goodness-of-fit statistics (χ^2, p-value). The DE model without the influence of A is not biologically plausible because traits are likely to be influenced by many genes (Neale and Cardon 1992). The E component cannot be left out from any model because it, by definition, includes the measurement error. The benefits of computerized modeling are that it enables the estimation of confidence intervals (CI) for each parameter (A, C/D, E) and furthermore provides a statistical way to compare the different models.

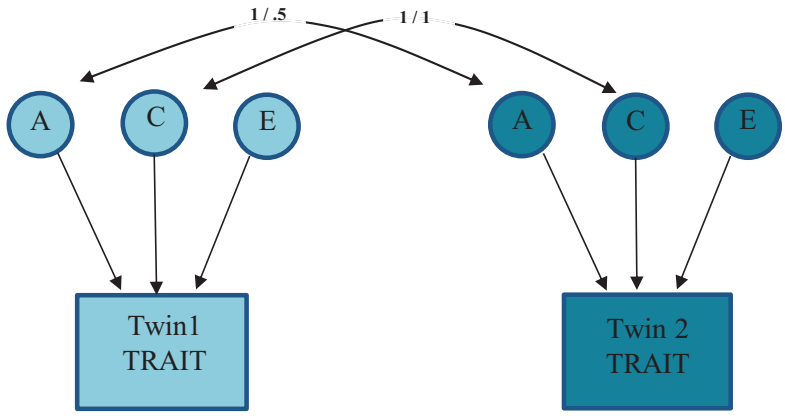

Figure 9. A Pathway diagram for a trait of the twins influenced by latent variables: A additive genetic effects, C common environmental effects and E non-shared environmental effects. Above numbers refer to genetic and environmental correlations. As identical twins (MZ) share all their genes, the correlation between twin1 and twin2 is **1**, while this is **0.5** between the non-identical (DZ) twins. Twin1 and 2 share their common environment, thus the correlation is **1** for both MZ and DZ twins. No correlation exists between the twins for E as this component refers to the non-shared environment and makes the twins different from each other.

The univariate model (Figure 9) can be used to calculate the heritability of one trait, and most twins studies, unless they are very large, considers narrow-sense heritability (van Dongen et al. 2012). Multivariate designs, e.g. bivariate model, in which two traits per person are analyzed, can provide answers to the causes of association and co-variance. Multivariate twin studies make it possible to ask questions such as 'does the variation in one trait cause variation in the other?' In other words, if the traits cluster, is this because they are influenced by the same set of genes or same environmental factors. The basic twin modeling, whether uni- or multivariate, does not consider epistatic effects, gene-environment interactions or random mating with respect to the trait in question nor does it implicate which genes or environmental factors influence the trait.

2.10.4 Linkage analysis

After the heritability of a trait has been determined, the next step is to locate the genes influencing the trait. The linkage, as the name applies, is based on the phenomenon of genetic linkage: the tendency of alleles to be inherited together during meiosis (Figure 8). Alleles located near each other in the same chromosome are more likely to be transmitted together to the offspring than the ones located further apart on separate chromosomes.

Linkage analysis aims to identify genetic loci where an allele segregates in a family along with a locus affecting a trait of interest. Linkage utilizes microsatellite markers (repeating sequences of 2-6 base pairs of DNA) or SNPs (Single Nucleotide Polymorphisms) as genetic markers. The analysis can be performed either by testing one marker (singlepoint) relative to the trait or several markers (multipoint) in small regions. If linkage is found, it provides evidence that a locus associated with the trait resides near this marker. Generally, the significance of a linkage is evaluated by using the logarithm of odds (lod) score and by convention, a score greater than 3.0 (1000 to 1 odds) is used to indicate the statistical significance of the result, at least in genome-wide studies. The threshold for suggestive results is proposed with a lod score of 1.9 (Lander and Kryglak 1995).

2.11 Missing heritability

Moving to the era of population-based GWAS has been an important step beyond family-based linkage studies. The original rationale for GWAS was the 'common diseases – common variant' hypothesis implying that association mapping would be a powerful tool for detecting common, complex trait loci of small effects. Alternatively, substantial allelic heterogeneity exists making the mapping task much more difficult due to the tendency of

different mutations to cancel out each other's signals (Slager et al. 2000). Although hundreds of common variants have been found since the early days of GWAS, they explain only a small proportion of heritability for most traits leading to the question of how the remaining, 'missing' heritability can be explained. Many examples of these come from research of complex traits, e.g. human height, with an estimated heritability of approximately 80%. At least 40 loci have been found, yet they explain together only 5% of the trait variation (Visscher 2008). Considering the complexity of food preferences, it is no surprise that studies in this field show similar results. Breen and others (2006) found 78% heritability for liking meat and fish products (beef, lamb, pork, chicken, bacon, fried fish, white fish and oily fish), while Pirastu and others (2012) showed that three SNP variants in chromosome 1 and 6 are associated with liking of lamb meat each explaining 0.03% of the variance. These studies are not directly comparable since they are from different populations but taken together they do indicate similar problem of the 'missing heritability' as demonstrated with height.

Many explanations have been proposed as to why the screening for common variants has delivered less than was hoped for. One obvious explanation would be that the accuracy of narrow sense heritability estimates are not sufficient, as they can be inflated if family resemblance is influenced by non-additive genetic effects (dominance, epistasis, or gene-gene interaction), shared familial environments, or by interactions among genotypes and environment (Manolio et al. 2009). Some researchers are claiming that the heritability estimates are sound, and studies suggest against the inflated values (Maher 2008; Manolio et al. 2009). However, in light of current research views, there seems to be many truths to the matter. The complexity of the genome, its allelic architecture (number, type, effect, size and frequency of susceptibility variants) and complex inheritance forms (e.g. epigenetic effects such as DNA methylation) may provide an explanation for the missing heritability (Eichler et al. 2010; Manolio et al. 2009), as may the occurrence of genetic interaction creating 'phantom heritability'. Van Dangen and others (2012) concluded in their review covering 'The value of twin studies in the omics era', that it is possible the heritability of phenotypes that are epigenetically regulated may be overestimated. This matter however, requires further studies to fully understand the prenatal developmental processes of twinning which may influence the epigenetic resemblance of twins. Be as it may, it should be kept in mind that the goal is not to explain heritability, but to understand traits and their underlying pathways, and to use that knowledge in developing strategies to tackle the traits with harmful effects (Zuk et al. 2012).

3 AIMS OF THE STUDY

The aims of the study were the following:

- To examine the perception of astringency among young Finnish adult twins by exploring the genetic background of the astringency and its implications to liking and use-frequency of phenol-rich foods and beverages (I).

- To evaluate the proportions of genetic and environmental effects underlying sour taste preferences in young adulthood (II).

- To evaluate the proportions of genetic and environmental effects underlying the pleasantness of oral pungency in young adulthood (III).

- To obtain a coherent picture of chemosensory preferences among young adults and to study their underlying causes. Subgroups were identified based on available preference data (sour, umami and pungency), and subsequently, the groups' genetic sensory and behavior related characteristics were explored (IV).

4 MATERIALS AND METHODS

2.12 Developing the chemosensory tests

Since 2006, the FinnTwin12 project had included sensory testing as part of the study protocol to investigate the heritability of sweet taste (Keskitalo 2008) and human responses to odors (Knaapila 2008). In the beginning of 2008, these phenotypes were replaced by three other traits: sourness, umami and pungency. The test for astringency perception was added to the protocol but much later in the end of 2008. The testing procedures for the new traits (sourness, umami and pungency) were developed by the sensory research group in the University of Helsinki (Kuumola 2008) aiming to generate 1) tests which were fast to perform and administer, 2) samples that were easily prepared even outside laboratory conditions, and 3) measures with sufficient individual variation for the heritability estimations. In addition, questionnaires were developed for each new trait to increase the validity and multidimensionality of the measures.

The search for suitable chemosensory tests for twin studies I-IV was completed in three phases. *Pilot testing* during which several sample media and concentrations of stimuli were considered and narrowed down to 2 options per chemosensory perception, *final testing* during which the samples for twin studies were selected and *repeatability testing* where the developed protocols were tested and the repeatability of the results evaluated. A brief description of the *pilot, final* and *repeatability testing* are provided below. The development of measures was external to the published articles (I-IV) but this section is included to this work to provide some background of the selected methodologies.

2.12.1 Pilot tests

SOURNESS: Orange juice was considered as a suitable medium for pilot testing. Untainted orange juice (reference O) was tasted and compared to an orange juice sample spiked with three concentrations of citric acid (CA, 0.273 %, 0.336% and 0.420, Fluka, Busch, Switzerland). Based on comments from few respondents the two most concentrated CA-juices were chosen for the final testing as they were clearly distinguishable from the reference sample O.

UMAMI: Two different media types were considered: tomato juice and 'mouthfeel flavor' (Givaudan, Switzerland) in water solution. Four tomato juices from different manufacturers were tasted by few respondents. "Marli tomato juice" (reference T) was selected for further

testing based on the availability and the pleasantness of the flavor. Two different concentrations of monosodium glutamate (MSG) and ribotide (sample 1: 0.45% MSG + 0.02% ribotide; sample 2: 0.6% MSG + 0.04% ribotide) were then chosen by a small panel to be suitable concentrations for further testing.

Thirty-one respondents tasted the tomato juice samples in two pairs: 1) T vs. sample 1, and 2) T vs. sample 2. The pleasantness and intensity of MSG (Merck, Dramstad, Germany) + ribotide (Ribotide ®) spiked samples were evaluated relative to T on a 9-point scale (-4 → 4, -4 being less pleasant/intense than the reference, 0=as the reference, 4=more pleasant/intense than the reference). Based on the results it was concluded unlikely that respondents noticed any difference between T and the spiked juices. Discrimination tests were then conducted and tomato juice found as an unsuitable media for umami testing (samples spiked with MSG + ribotide could not be distinguished from the reference sample).

The 'mouthfeel flavor' in a water solution was pilot tested using the manufacturers' recommended concentration range of 0.1-0.5%. Based on comments from a small panel, concentrations of 0.1% and 0.2% were chosen for the final testing.

PUNGENCY: Unusual flavors such as green apple and strawberry in a sweet and jelly-like media were chosen for pilot testing based on previous literature (Prescott and Stevenson 1995). Four pilot tests were completed in search of a suitable gelling agent (agar vs. jelly sugar) and six different recipes were tested. Based on discussions from few panellists, a suitable recipe for the jelly sample was found containing: 2.5 dl of water, 85 g jelly sugar, 1.55 g of strawberry aroma and 0.0055g of red food dye (S-jelly, the reference).

Based on previous literature (Prescott and Stevenson 1995; Sizer and Harris 1985), two capsaicin (Fluka, Busch, Switzerland) concentrations, 0.0001% (C1) and 0.0002% (C2) were pilot tested by eight respondents. C1 and C2 were evaluated for their pleasantness and intensity relative to S-jelly on a 9-point scale (-4 → 4). Based on these results, both concentrations were chosen for the final testing.

2.12.2 Final tests

SOURNESS: The final testing was organized in the National Institute for Health and Welfare, Helsinki, in an undisturbed room where the respondents (n=31, 24 females, aged 22-58 years) arrived in groups of maximum seven people at a time. The samples: reference O, 0.336% CA-juice and 0.420% CA-juice, were prepared in advance during the morning of the testing day and presented in two pairs: 1) O vs. 0.336% CA-juice and 2) O vs. 0.420% CA-

juice. The pleasantness and intensity relative to reference were evaluated relative to O using a 9-point scale (-4 → 4).

UMAMI: The final testing was organized at the Viikki campus in the University of Helsinki, in an undisturbed room with 26 respondents (19 females, aged 19-44 years). The samples with two different 'mouthfeel flavor' concentrations (0.1% and 0.2%) were prepared in advance during the morning of the test day. The pleasantness and intensity were evaluated for each concentration using a 9-point scale (1 → 9, 1=very unpleasant/ no taste, 5=not pleasant nor unpleasant, 9=very pleasant/intense). No reference sample was used.

PUNGENCY: The final testing was organized at the Viikki campus in the University of Helsinki, in an undisturbed room with 26 respondents (19 females, aged 19-44 years). The samples: S, C1 and C2 were prepared the previous day of testing and served in two pairs: 1) S vs. C1 2) S vs. C2. The pleasantness and intensity were evaluated relative to S using a 9-point scale (-4 → 4).

2.12.3 Sample selection and repeatability

Fulfilling the aims of 1-3 (originally set for the tests, see section 2.12.) and using the results of the *final testing*, the samples were chosen to be used in the twin study. The protocol and the repeatability of the sensory tests was explored. This was organized at the Viikki campus in the University of Helsinki, with 25 respondents (22 females, aged 18-44 years) in two separate ocations (5 days between the tests). All three chemosensory tests were conducted mimicking the protcol that was planned to be used with the twins. Umami was tested first (due to the different evaluation scale), then sourness and last pungency (due to the lingering perception). The results of the repeated testing are shown in Table 1. t-test showed no significant differences between the first and the second measurement. What is evident though when comparing the correlations of the pleasantness and intensity tests is, that they were clearly lower with the intensity responses. This could indicate that the concept of intensity was unclear or that the respondents had troubles in understanding what is high and low on the response scale.

Food preference and use-frequency questionnaires were prepared including 23 more or less sour items, 17 more or less savory items, 10 more or less pungent items. The items were selected during the testing by the sensory group and the list was modified during the different testing phases according to the feed back from the participants . Pleasantness responses to food names in test 1 and 2 correlated between 0.61-0.94 for sour items, 0.61-0.95 for umami and 0.57-0.89. Use-frequency response correlations were in between 0.66-0.98, 0.73-0.93, and 0.55-0.58, respectively (Kuumola 2008). Apart from few exceptions, all correlations

were statistically significant ($p<0.001$) indicating acceptable level of measurement repeatability.

Table 1. Pleasantness and intensity evaluations from repeated testing, and the correlation between the responses from test 1 and test 2 (r). Kuumola (2008).

Chemosensory test	Mean (SD)		
	Test 1	Test 2	r
Sour			
Pleasantness	-1.2 (1.8)	-1.1 (1.4)	0.67
Intensity	1.9 (1.4)	2.0 (1.3)	0.45
Umami			
Pleasantness	3.7 (1.7)	4.0 (1.7)	0.76
Intensity	5.0 (1.3)	4.7 (1.4)	0.29
Pungency			
Pleasantness	-1.3 (2.2)	-1.3 (2.0)	0.78
Intensity	3.2 (1.0)	2.6 (1.8)	0.34

2.13 Twin studies (I-IV)

The approach to understand the genetics of chemosensory perceptions and preference was comprehensive (behavior to biology) aiming to estimate the importance of genetic and environmental influences on complex trait variation. Twin methodology was chosen due to its competence of teasing apart these two influences. Further, twin methodology enabled the optimal use of genetic data with linkage analysis in DZ twins, which in contrary to the analysis of non-twin siblings, is not affected by age differences (van Dongen et al. 2012).

2.13.1 Respondents

The respondents were 331 Finnish adult twins, aged from 21 to 25 years (146 males and 185 females). The data were collected during 2008-2009 as part of longitudinal FinnTwin12-study (Kaprio et al. 2002; Kaprio 2006). Testing of chemosensory traits was included in the fourth wave of data collection as part of already established infrastructure of twin studies in Finland (fourth wave data collected during 2006-2009). Power calculations as to how many twins were needed for the sensory study were speculative and based on presumption of adequate number of twins that participating the study. The statistical power of twin studies to

detect genetic variation is dependent on the ratio of MZ and DZ twin full pairs and the number of the total twin pairs (Visscher 2004) and the assumed degree of heritability. However, at the time of the testing, little was known of the expected heritability of the traits that were under the investigation. Furthermore, as the fourth wave of the data collection was already ongoing, it was difficult to estimate the final number of full pairs participating the study.

The respondents were MZ and DZ pairs, as well as twin individuals without their co-twin. Number of the twins and pairs used in the studies (I-IV) are presented in Table 2. Zygosity was confirmed at the Paternity Laboratory in the National institute for Health and Welfare, Helsinki, Finland. The study protocol was approved by the Ethics Committee of Helsinki University Hospital District. The respondents gave their written informed consent upon the arrival to the twin research unit.

Table 2. Number, gender and zygosity of the respondents.

Original publication	I	II	III	IV
N				
Total	194	328	331	331
Women	96	143	146	146
Men	98	185	185	185
Zygosity				
Monozygotic full pairs	24	46	47	47
Dizygotic full pairs	57	92	93	93
Twin individuals without co-twin	32	52	51	51

2.13.2 Data collection procedure

The respondents were invited to the twin research unit located in Helsinki, Finland for a one day assessment including sensory tests and other extensive data collection procedures (e.g. neuropsychological tests and an interview) as part of the FinTwin12-study. The sensory measurements used in studies I-IV were conducted in the morning after 12 h fasting. Respondents were given both written and oral instructions prior to testing. Five sensory tests were conducted, two saliva collections were completed, weight and height were measured, blood samples were taken and several questionnaires were filled in. The schematic representation of the sensory procedure and related data collection is presented in Figure 10.

2.13.3 Measures

2.13.3.1 Astringency perception and saliva collection (I)

The astringency sensory test protocol was developed in Italy as described by Dinnella et al. (2009) and used with slight modifications (I). The astringency test was added to the testing repertoire later on and thus conducted on a sub group of the twins (n=194). Untainted apple juice (the reference) and apple juice spiked with 0.15% tannic acid (TA) were used as test samples for astringency. The respondents rated the pleasantness and intensity of the TA-juice relative to the reference using a 9-point scale (Figure 11). This scale was chosen as suitable to measure the difference between the samples with 'consumer-type' respondents. The scale was also suitable for both, hedonic and intensity measurements, making the tasks easier for the participants. For hedonic testing, the use of bipolar scale is common, with a zero or neutral point of opinion at the center (Lawless and Heymann 2010). This enables the recognition of the fact that neutral response may occur and that there are two modes of reaction (disliking – liking). The downside of the scale is that people may have favorite numbers or they may use some numbers more often than others. Numbers in the scale however allow to signify ratios or proportions of both less pleasant/intense and more pleasant/intense responses.

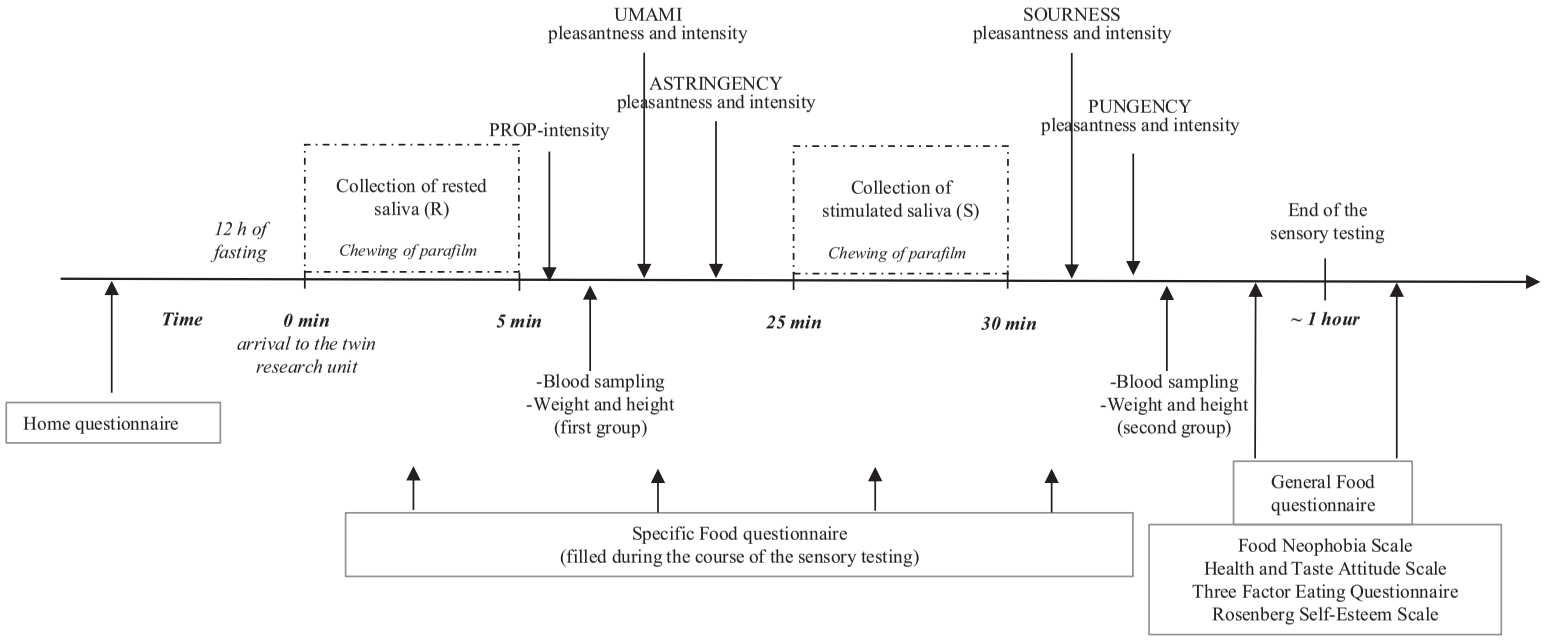

Figure 10. Schematic representation of the data collection procedure.

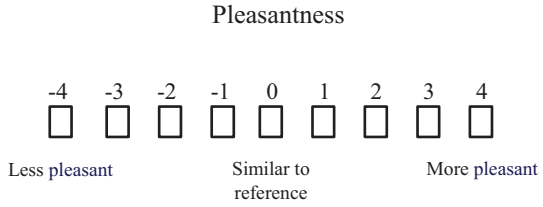

Figure 11. The simple check-box scale for difference in pleasantness from a reference sample. The same scale was used for the intensity measurement by replacing the word *pleasant* with the word *intense*.

Two saliva samples were collected and analyzed for protein content. After 12 h fasting, the first saliva sample was collected (rested saliva). The second saliva collection was completed at a minimum of 20 min from the first saliva collection, after the astringency perception test (stimulated saliva). Total salivary content and saliva protein profile (proline rich proteins, mucins, amylases, histatins and cystatins) were determined in the University of Padova, Italy (I).

2.13.3.2 PROP intensity rating (I, III-IV)

The intensity rating of PROP (6-n-propylthiouracil) was included in the study protocol as a positive control for heritability (I) and due to its alleged relationship with other chemosensory perceptions (III-IV). The sensitivity for PROP was screened using a filter paper method developed by Zhao et al. (2003) with LMS (Figure 12) as the rating scale (Green et al. 1996). This method and scaling technique was chosen as together they have shown a reliable screening tool for assessing sensitivity to PROP (Zhao et al. 2003). In addition, LMS is particularly suitable for sensations with broadly defined perceptual qualities (Green et al. 1996). The drawback of this type of scale is that respondents may choose to make markings near the verbal labels, rather than distributing them across the scale (Lawless and Heymann 2010). This bias was minimized by instructing the respondents that their evaluation need not be anchored to the verbal labels but can be located anywhere in the scale.

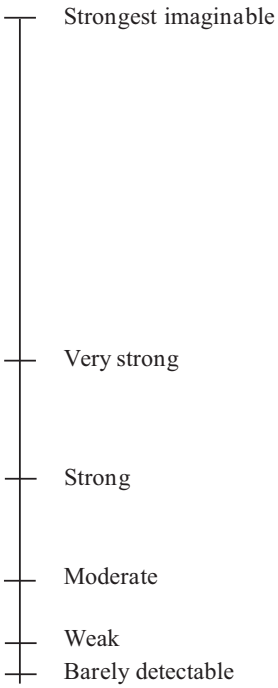

Figure 12. The labeled magnitude scale (LMS) of Green et al. (1993) used in the present study to measure the intensity of PROP (6-n-propylthiouracil).

2.13.3.3 Umami (IV), sourness (II-IV) and oral pungency (III-IV) evaluations

The respondents rated the pleasantness and intensity of umami, sour and pungent samples using a 9-point scale. For umami the scale from 1 → 9 was used (Fig 13) without a reference sample, whereas for sourness and oral pungency, the ratings were given relative to the reference using a scale from -4 to 4 (Figure 11).

For Umami measurement, the scales were modified slightly as no reference sample was available. The hedonic scale (Figure 13 A) was changed to unipolar but a neutral response option was maintained at the center. The rationale behind this was to make the task more sensible for the respondents by recognizing the possibility of neutral response. The intensity was measured with a simple scale for sensation strength (Figure 13 B). This type of scale has been previously demonstrated applicable when dealing with a heterogeneous population of consumers (Lawless and Malone 1986). The lack of the reference was the weakness in this measurement. The different scaling technique compared to the sourness and pungency measurements may also have been somewhat confusing to the respondents.

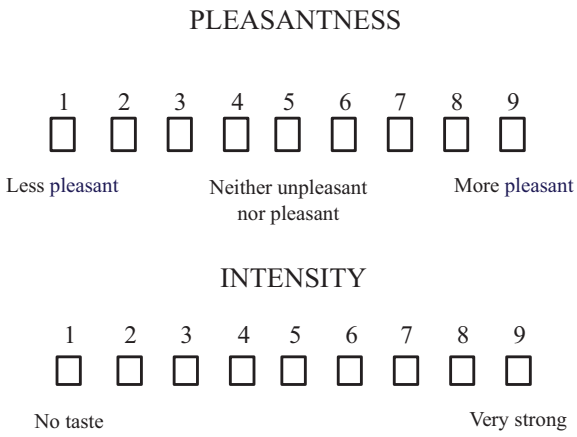

Figure 13. Hedonic 9-poin scale (A), and a simple integer scale for sensation strength (B) after (Lawless and Malone 1986).

2.13.3.4 Specific Food questionnaires (I-IV)

Four Specific Food questionnaires (SF-questionnaire) were prepared to collect the liking and use-frequency responses (7-point scales) to food and drink names (items) representing astringent, umami, sour and spicy qualities. In the first study (I), the pleasantness and use-frequency responses to eight astringent and less astringent item pairs were used. In the second study (II) the pleasantness responses to 21 sour and less sour items were utilized. The third paper (III) included the pleasantness responses to 10 spicy and less spicy items, and liking responses to spiciness in foods (mild, strong and extremely strong spiciness). The last study (IV) utilized pleasantness ratings for sour, spicy and umami qualities (13 items).

The 7-point pleasantness scale was selected for affective rating task because Finnish language suffers from the lack of the word “dislike”. As opposed to the most commonly used 9-point (dislike extremely – like extremely) scale by Peryam and Pilgrim (1957) the categories were truncated into 7- points due to the rare use of word “extreme” in Finnish language. Anchoring the scale ends with “extreme” would undoubtedly have led to the avoidance of the end categories (Tuorila et al. 2008). The original 9-point scale suffers critique such as that the categories are not equally spaced and the consumer’s tend to avoid the end points (Moskowitz 1980). A balanced liking scale is however a recommended scaling method to obtain a person’s overall opinion of a product (Lawless and Heymann 2010).
The items were categorized using factor analysis (maximum likelihood, Varimax rotation) and named based on their essential content. The scores for the groups were calculated as

factor loadings (I) or the mean of ratings (II-IV). Studies I-II also showed mean ratings of the individual items. All the items in each of the questionnaires are presented in Table 3, including the coefficients for the internal consistency (Cronbach's α) of the composite measures. The higher the α-score, the more reliable the generated measure is. The level of 0.7 has been indicated as acceptable reliability coefficient but lower thresholds are as well have been used in the literature (Bland and Altman 1997).

2.13.3.5 General Food questionnaire (II, IV)

Pleasantness ratings of general foods were used in studies II and IV. The General Food questionnaire (GF-questionnaire) included 41 food and drink items (e.g. pasta, French fries, vegetarian food etc.) and covered the main food groups: cereals, rice, pasta meat, poultry, fish, eggs, fresh and cooked vegetables, fruits, berries, milk products, yoghurt, cheese, fats, oils, sweets and fast food). The individual items were categorized based on the work of Keskitalo et al. (2007b); I) and Knaapila et al. (2011); IV) and the mean of ratings given to food items in each category used as scores. In the second study (II), the liking of *sweet foods* was used and the fourth (IV) study utilized categories of *salty-and-fatty foods, sweet-and-fatty foods, fruits and vegetables* and *fish*.

2.13.3.6 Home questionnaire (IV)

A home questionnaire was sent to the respondents prior to the test day to be filled at home and returned at the twin research unit on the testing day. *Education*, *smoking, alcohol consumption* and *drunkenness frequency* data was used from this questionnaire in the last study (IV).

Table 3. Specific food questionnaire items, composite measures and their reliabilities (α_{pleas}= pleasantness, α_{use}= use-frequency). Items which are not part of composite measures (Other items) are printed in lighter font.

Food and beverage items	α_{pleas}	α_{use}
UMAMI		
Cheese	0.79	0.58
Cheddar cheese		
Parmesan cheese		
Emmental cheese		
Cheese spread		
Unripened cheese		
Salad	0.67	0.76
Cucumber		
Tomato		
Lettuce		
Pulse and corn	0.69	0.80
Corn		
Peas		
Other items		
Mushrooms		
Stock tubes		
Soy sause		
SOUR		
Sour fruits and berries	0.82	0.72
Redcurrant		
Cranberry		
Currant juice		
Lingonberry		
Rhubarb		
Lemon		
Sour dairy	0.78	0.65
Natural sour milk		
Natural yogurt		
Buttermilk		
Less sour fruits and berries	0.63	0.66
Strawberry		
Peach		
Orange		
Banana		
Blueberry		
Other items		
Pineapple		
Pineapple juice		
Pickeled fish		
Pickeled cucumber		
Sour fruit candy		
Flavoured yoghurt		
Flavoured sourmilk		

Food and beverage items	α_{pleas}	α_{use}
ASTRINGENT		
Astringent drinks	0.83	0.69
Coffee without milk		
Coffee without sweetener		
Dark beer		
Lager beer		
Red wines	0.83	0.97
Matured red wine		
Young red wine		
Ripe fruits	0.69	0.68
Fruits (very ripe)		
Banana (very ripe)		
Other items		
Dark chocolate		
Milk chocolate		
Coffee with sweetener		
Tea without sweetener		
Tea with sweetener		
Coffee with milk		
Fruits (not very ripe)		
Banan (not very ripe)		
PUNGENT		
Spicy foods and spices	0.92	0.83
Chili spice		
Spicy food		
Tabasco		
Spicy mustard		
Mexican food		
Indian food		
Chili con carne		
Other items		
Non spicy food		
Typical finnish food		
Mild sicy food		

2.13.3.7 Other scales (II, IV)

In the last paper (IV), four other scales were used: the Food Neophobia Scale (FNS) by Pliner and Hobden (1992), the Health and Taste Attitude Scale (HTAS) by Roininen et al. (1999), the Three Factor Eating Questionnaire (TFEQ) by Karlsson et al. (2000) and the Rosenberg Self-Esteem Scale (RSE) by Rosenberg (1989). Originally, TFEQ-R18 measures three dimensions of human eating behavior (*Restraint, Disinhibition* and *Hunger*) but following the work of Karlsson and others (2000), a revised version of the questionnaire was used with three following dimensions: *Cognitive Restraint, Uncontrolled Eating* and *Emotional Eating.* HTAS consisted of two subscales: *General Health Interest* and *Craving for Sweet Foods*. FNS and the subscales of HTAS were rated on a 7-point scale whereas RSE and the subscales of TFEQ on a 4-point scale. The *Craving for Sweet Foods* was used in study II.

2.13.3.8 DNA collection and genotyping

Blood samples were extracted for DNA using an automated Autopure LS instrument (Qiagen). The DNA samples were genotyped using single nucleotide polymorphism markers (SNP) by Illumina platform to obtain GWAS data. Candidate gene approach was utilized in the last work (IV) where GWAS data was used on 27 gene regions previously known to function in the regulation of taste, chemesthesis and food palatability (379 SNPs).

2.14Data analysis (I-IV)

The questionnaire items were categorized by factor analysis (I-IV) and named according their essential content (e.g. 'sour fruits and berries'). Heritability analysis (quantitative genetic modeling) was performed in all studies (I-IV) using Mx program version 1.7. The PSEUDOMARKER software (Hiekkalinna et al. 2011) performing likelihood-based linkage and /or linkage disequilibrium analysis was utilized in the last work (IV). Other analysis including factor analysis, ANOVA, t-test, hierarchical clustering, K-means clustering and logistic regression were performed by PASW (SPSS) statistics 17 (I) and 18 (II-IV), and corrected for the paired structure of the twin data with complex samples procedures, general linear model (II-IV).

5 RESULTS

5.1 Pleasantness and intensity ratings of chemosensory traits

Pleasantness and intensity ratings of astringency, sourness and pungency (I, II, III) showed that the spiked sample was rated as less pleasant and more intense than the reference in all sensory tests (Table 4). However, sourness along with pungency produced the most 'neutral' pleasantness responses (similar to the reference). Individual variation was apparent in all measures, observed highest with astringency intensity and pungency pleasantness. The astringency pleasantness and pungency intensity distributions were both somewhat skewed, astringency to the right and pungency to the left (1.5 and -1.5, respectively). The pleasantness and intensity ratings correlated negatively (Table 4) suggesting that as the intensity of stimuli increases, the pleasantness decreases. In addition, correlation indicates the possibility of partially common factors influencing these traits.

Table 4. Pleasantness and intensity ratings and their correlation (r_{pleas}, r_{int}, respectively) of the chemosensory stimuli. * $p<0.001$

Chemosensory test	Mean (SD)	$r_{pleas\ vs.int}$	n
ASTRINGENCY[a]			189
Pleasantness	-2.1 (1.5)		
Intensity	1.0 (1.9)	-0.21*	
SOURNESS[a]			322
Pleasantness	-0.2 (1.5)		
Intensity	1.2 (1.3)	-0.20*	
PUNGENCY[a]			323
Pleasantness	-0.9 (1.9)		
Intensity	2.3 (1.5)	-0.16*	
UMAMI[b]			329
Pleasantness	-3.7 (1.8)		
Intensity	5.4 (1.6)	-0.19*	
PROP[c]			330
Intensity	4.9 (3.6)		

[a] Rating scale from -4 to 4, relative to reference
[b] Rating scale 1-9, no reference
[c] Labelled Magnitude scale, no reference, PROP = 6-n-propylthiouracil

5.2 Pleasantness and use-frequency profiles of foods

Pleasantness and use-frequency profiles (Figure 14), sorted by pleasantness scores from low to high, showed that all food item groups were generally either mostly liked or indifferent in pleasantness (results from the Specific Food questionnaires). The variations of the use-frequency ratings were similar whereas more variation could be observed with the liking responses. The less liked items were mostly sour (II) or astringent (I) with higher deviations compared to the rest of the groups. Generally, the decrease or increase in pleasantness paralleled similar direction of ratings in use-frequency, and the correlations between pleasantness and use-frequency ranged from 0.37-0.77 (R^2=0.14-0.59). These results indicated a moderate to strong relationship between liking and food use. Some exceptions such as *red wines, less sour fruits and berries* and *salad items*, even when well liked, indicated the influence of other factors (e.g. availability, context related use) that lead to seasonal or sporadic consumption of the items.

5.3 The association between PROP and other chemosensory traits

The heritability of intensity responses to PROP was evaluated as a positive control in study I. PROP intensity ratings did not correlate significantly with any of the sensory traits: pleasantness and intensity of astringency (r=-0.08, r=0.11), sourness (r=0.02, r=0.03), pungency (r=-0.03, r=0) and umami (r=0.01, r=0.06). This indicates that no specific relationship existed between PROP intensity and the studied flavor qualities.

In study III, the relationship of oral pungency responses and PROP sensitivity was explored by dividing respondents: 1) into likers and non-likers of pungency, and 2) into super-tasters and non-tasters of PROP subsequently exploring the differences between these groups. No association was found between pungency liker status and PROP tasting, nor was an association observed between PROP sensitivity and responses to oral pungency. Thus, PROP sensitivity was not unambiguously predicting responses to chemesthesis.

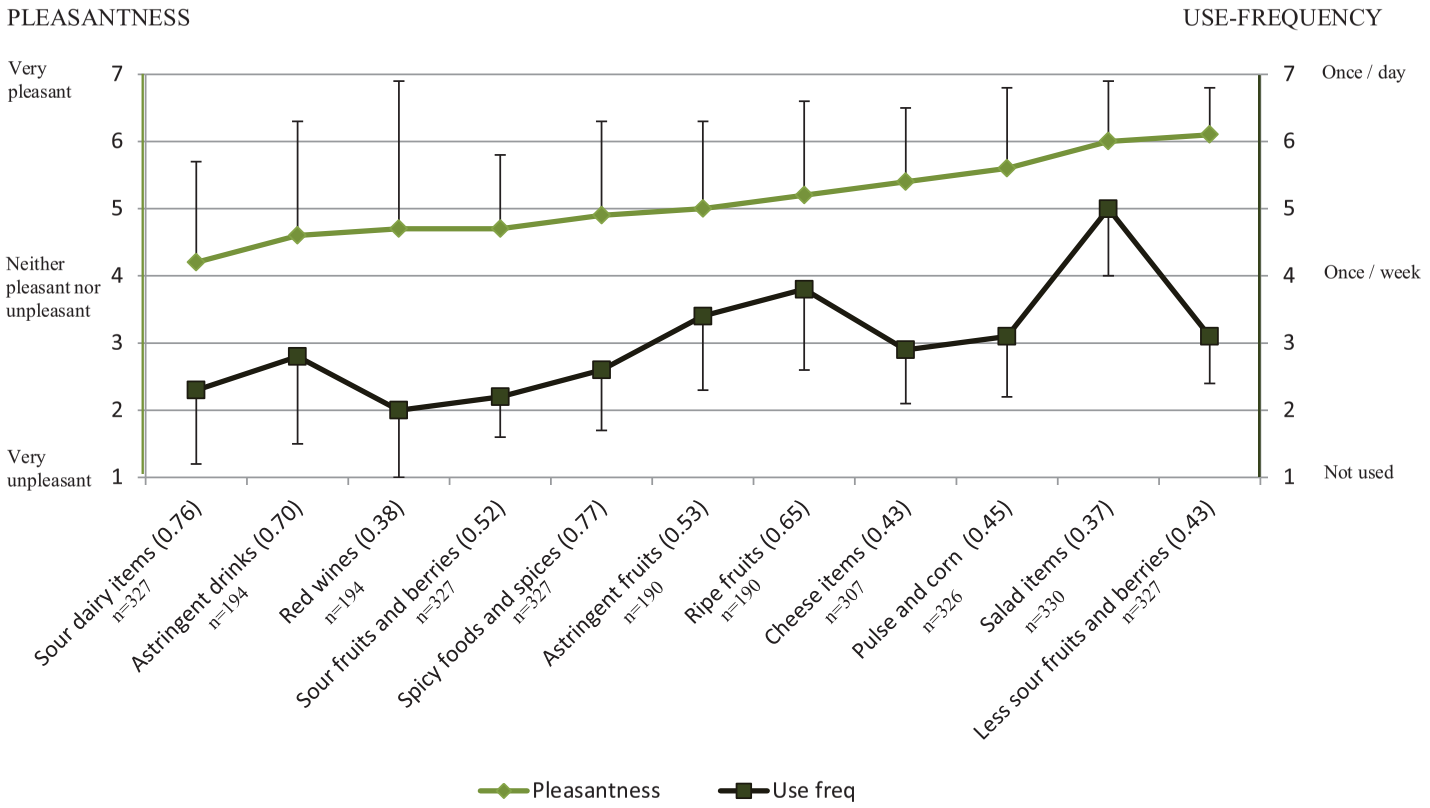

Figure 14. Pleasantness and use-frequency profiles (mean, SD) for 11 composite measures from Specific Food questionnaires and their correlations (Pearson, in brackets), number of respondents (n). Standard deviations presented as bars. For all the correlations, $p<0.001$.

5.4 The heritability of liking astringency (I), sourness (II) and oral pungency (III)

Sensory responses to astringent stimuli showed no evidence of heritability while salivary protein production and liking for *astringent drinks* indicated familiar aggregation and the existence of a genetic component (Table 5). However, due to statistical power issues, the single, statistically significant best-fitting structural model could not be established, thus these results were interpreted as suggestive (I). The power issues can also be seen in the large confidence intervals for all the heritability estimates for astringency.

Sensory responses to sourness showed modest heritability estimates (II), intensity with a slightly higher estimated value (31%) compared to that of the pleasantness of the stimulant (14%). Liking scores for the sour food items showed higher and more reliable heritabilities as approximately half of the variation in these traits were shown to be attributed to genetic effects and the rest to the specific environmental effects. Similarly to sourness, the sensory responses to oral pungency showed moderate heritability estimates, 18% for pleasantness and 12% for intensity. Liking of spicy foods produced the highest heritability, 50%, while the rest of the variation in this trait was explained by specific environmental effects.

The estimated heritability for PROP was 32% (CI:0-0.67). Simiilar to the astringency measures, the limited number of respondents (24 MZ pairs, 45 DZ pairs) influenced the confidence intervals. Umami heritability was not determined. In all traits studied (Table 5), the environmental effects were large. For astringency 36-57%, sourness 50-86%, and pungency 42-88% of the variation was explained by environmental factors.

Table 5. Heritability estimates (% of the variation, AE-models with additive genetic effects and specific environmental effects) of chemosensory traits, salivary protein profiles (expressed as the change in concentration of proteins before and after astringent stimulation), and pleasantness of astringent, sour and pungent foods in the three studies (I-III). CI= confidence interval, PRP=proline rich proteins, tot=total amount.

Trait	Heritability % (CI)	Publication
ASTRINGENCY		I
Pleasantness (stimulant)	-	
Intensity (stimulant)	-	
Salivary proteins (tot)[a]	46 (0-69)	
Salivary PRPs[a]	51 (0-76)	
Salivary mucins[a]	43 (0-69)	
Pleasantness		
Astringent drinks[b]	64 (0-83)	
Red wines	-	
Ripe fruits	-	
SOURNESS		II
Pleasantness (stimulant)	14 (0-33)	
Intensity (stimulant)	31 (1-47)	
Pleasantness		
Sour fruits and berries	50 (23-70)	
Sour dairy items	45 (25-61)	
Less sour fruits and berries	47 (23-65)	
PUNGENCY		III
Pleasantness (stimulant)	18 (0-39)	
Intensity (stimulant)[a]	12 (0-61)	
Pleasantness		
Spicy foods and spices	50 (20-72)	
Mild pungency	25 (0-46)	
Strong pungency	58 (38-71)	
Extremely strong pungency	34 (0-55)	

[a] heritability from full ADE model, statistically significant best model could not be established

[b] heritability from full ACE model, statistically significant best model could not be established

5.5 Preference based respondent subgroups (IV)

The last study (IV) aimed to obtain a coherent picture of the chemosensory preferences and their underlying causes among the study population. Based on liking responses to food names representing sour, umami and spicy qualities, hierarchical and K-means clustering analysis was applied to obtain respondent groups. The differences in liking responses that lead to these distinct groups were then explored, as well as subgroups' differences in other measures (sensory hedonic and intensity measures, food behavior related traits, liking of general foods). The genetics was explored by calculating the heritability underlying grouping, and conducting a linkage analysis for 27 candidate gene regions previously known to function in taste (umami, sour and bitter), chemesthesis (pungency) and food reward (palatable food).

Two subgroups were identified among the 331 respondents (G1 n=140, G2 n=152, non-grouped n=39) particularly differing in sour and pungent measures. G2, named as 'adventurous' showed higher liking for sour and spicy items and had more tolerance for capsaicin burn in the sensory-hedonic test compared to G1, named as 'basic'. In addition, the adventurous were less food neophobic than the basic group (FNS_{G2} = 25.9 ± 9.1, FNS_{G1} = 32.5 ± 10.6) and the general population (FNS = 31-35). It was discovered that division of individuals into these two subgroups was explained by the genetic differences between the respondents (h^2=66%, CI 3-95%). Food neophobic tendency, sensory ratings to oral pungency and liking responses to *fruits and vegetables* predicted the subgroups (IV).

It was postulated that being adventurous may be linked to genes known to regulate chemosensory traits. Suggestive linkage peaks with lod scores of 1.86 in TAS1R1, and 1.88 in PKD1L3 gene areas, controlling for umami and sourness perception were discovered (IV).

6 DISCUSSION

5.6 Association among study traits

5.6.1 Stimuli: pleasantness vs. intensity

The sensory pleasantness and intensity ratings were found to correlate negatively and to a similar extent in all three traits (-0.16 to -0.21): astringency (I), sour taste (II), and oral pungency. This indicates that some common factors underlie the sensory pleasantness and intensity. Thus, even though distinct brain areas are responsible for the stimulus intensity and affective responses to stimuli (Small et al. 2003), the signals interact. It was shown with sourness (II) that the co-variance of pleasantness and intensity was fully explained by genetic correlation. This may indicate that there are a set of genes that regulate the two brain areas. It is also possible a set of genes regulate the intensity and affective ratings indirectly through a common factor, for example learning. The correlations also indicate that with increasing intensity, the pleasantness of the stimuli is decreased. It has been shown that astringency is generally perceived as a negative product attribute and can thus lead to rejection of foods rich in polyphenols (Bajec and Pickering 2008; Dinnella et al. 2009). Similarly, sourness can be rejected (Ganchrow 1983) consequently narrowing the diet quality by possible avoidance of many health beneficial, naturally sour foods. With pungency, the association of pleasantness and intensity ratings of the stimulus is largely related to regular use of spicy foods which can result in tolerance of the sensation (Lawless et al. 1985).

5.6.2 PROP vs. chemesthesis

PROP sensitivity has been suggested to act as a marker for individual difference in taste perception that influences food preferences (Tepper 2008). Particular interest has been given to the relationship of PROP sensitivity and chemesthesis due to anatomically associated trigeminal nerve fibres and fungiform papillae (Whitehead et al. 1985). Although some evidence indicates that super tasters of PROP perceive capsaicin burn more intensely (Bartoshuk et al. 1994; Karrer et al. 1992), others have failed to find a either clear or statistically significant association between them (Prescott and Swain-Campbell 2000; Tepper and Nurse 1997). In line with this, no such association was found in study III. Super tasters of PROP did not rate the perceived oral burn from capsaicin spiked jelly sample higher than the non-taster group. Methodological issues of how PROP taster status is established could explain the discrepancy. Whereas some studies use the psychophysical function for NaCl as a standard against which the PROP function can be compared to (taster

status does not influence intensity judgments for NaCl), we used the PROP filter paper test as the screening method for tasters and non-tasters of PROP. However, even if the filter paper method may provide a crude measure for screening of the tasters, it has produced reliable basis for heritability estimates in the earlier studies (Keskitalo et al. 2007b). Either way, our results (III) corroborate the current view which is that taste and chemesthesis are mostly independent systems and PROP sensitivity should not be used as unambiguous predictor of individual responses (Lawless and Heymann 2010).

5.6.3 Pleasantness: stimuli vs. food names

The association between sensory pleasantness and liking of different food groups showed varying results. With sour taste (II), the sensory pleasantness was minimally associated with liking of *sour fruits and berries*, (r=0.16) *sour dairy products* (r=-0.03) and *less sour fruits and berries* (r=-0.06). The pleasantness of oral pungency (III) was clearly more associated with spiciness liking: with *spicy foods and spices* (r=0.43), and with *mild*, (r=0.36) *strong* (r=0.52) and *extremely strong spiciness* (r=0.44). This illustrated that chemosensory qualities may be divided into different dimensions and the level of liking highly dependent on the product. Alternatively, the measures were unable to capture the true variation leading to low correlations between the sensory and food questionnaire variables.

The situation with oral pungency seems relatively simple. The correlations between pungency measures indicate that it is a sensation that is easy to recognize, and restricted in dimensions. It is also possible that the participants were able to truly concentrate to rate the sensory 'burn' from capsaicin without familiarity effect since the sample was unusual in the context of spicy foods (strawberry jelly spiked with capsaicin). With sourness, the situation is more complicated. Sourness in foods conveys mixed signals. Multiple sensory qualities and food specific reactions may become more highlighted. Sourness can be very unpleasant at high concentrations, disliked in some food contexts but liked in others.

While the sensory liking of sourness in study II is probably related to orange juice, the responses to food names refer to several types of items. Furthermore, they represent an image of foods (Cardello and Maller 1982) which is likely to differ between the respondents relative to context. The *dairy items* (natural sour milk, natural yogurt, and buttermilk) showed large individual variation possibly due to the different ways these foods are eaten (with berries and sugar, müsli or honey etc.). Lastly, it is possible that the low correlation among the sourness measures (sensory pleasantness and liking of foods) is an implication of different dimensions of the phenomenon. While *sour fruits and berries* (red currant, lingonberry etc.) may reflect sourness *per se*, the responses to *less sour fruits and berries* (strawberry, peach etc.) could be influenced by the combination of sourness and sweetness in them. The interaction of sour and

sweet taste (mixture suppression) is particularly important in sour foods and they are closely linked to the overall appeal of the flavor (Lawless and Heymann 2010).

5.6.4 Food names: pleasantness vs. use-frequency

Mean responses to food names showed that the food categories were mostly pleasant or indifferent in pleasantness. This could be explained by the tendency of respondents to use more extreme ratings towards food names than they would give to the same foods if tasted. Preference responses to food names thus may provide an index of relative acceptance of a food as suggested by Cardello and Maller (1982) rather than 'real' level of liking. The variation in the use-frequency measures was clearly smaller compared to the pleasantness ratings. The reason for this may be that the evaluation of use-frequency is easier for respondents than the evaluation of liking. Correlations between the pleasantness ratings of foods vs. the use-frequency indicated that 14-59% of the variability in use-frequency measures could be attributed to preference. This is in line with previous studies which showed that the affection predicts 25-50% of the consumption (Cardello and Maller 1982; Tuorila et al. 2008).

5.7 Heritability of the study traits

5.7.1 Heritability of sensory responses to stimuli

Studies I-III showed that the degree of sensory pleasantness and intensity of astringency, sourness, and oral pungency are weakly, if at all, heritable. These were the first studies to date to investigate the heritability of perceptions of astringency and oral pungency. Sensory responses to astringent stimuli showed no inheritance, whereas with pungency, both pleasantness and intensity appeared to be weakly inherited (18% and 12%, respectively). More convincing evidence of genetic regulation underlying astringency was obtained from salivary protein profiles, which indicated that the protein secretion (total amount, PRPs and mucins) related to astringent stimulation is under genetic control. Previously, Rudney and others (1994) studied the heritability of saliva protein concentration in twins and concluded that a significant genetic contribution to total protein concentration in saliva exists. This fortifies our suggestive results, even though, Rudney and others (1994) did not calculate heritability, and the study suffered, similarly to ours, from issues related to a small number of twins. In conclusion, it seems likely that a physiologic trait such as salivary secretion would

be genetically regulated. Furthermore, it seems reasonable that genetic contribution rather than common environmental factors affect salivary secretion of twins living apart in their adulthood (Rudney et al. 1994).

Although it is commonly known that individuals differ in their perception of sensory 'burn' caused by spices such as capsaicin (Cliff and Green 1996), very little is known about whether the differences are heritable. Pungency sensitive receptors TRPV1 and TRPA1 have been previously identified by Caterina et al. (1997). Polymorphisms in the genes coding these receptors have also been observed and their role in pain susceptibility suggested (Hayes et al. 2000; Kremeyer et al. 2010), however, nothing is known of the degree to which genetic variation explain responses to pungent stimuli. Study III is the first of its kind to show that sensory responses to oral pungency are heritable but to a small extent. Environment seems to play a large role in both, the pleasantness and intensity responses to sensory 'burn' caused by capsaicin. As no heritability was detected for the liking or intensity of astringency perception, it seems reasonable to conclude that responses to pungent and astringent stimuli are largely affected by the environmental factors, which lead to individual responses in liking and perceived intensity of the stimulus. It is possible that particularly with astringency representing a complex tactile sensation, learning and familiarity plays a much larger role compared to oral pungency or taste perceptions.

Sensory pleasantness of sourness was weakly heritable (14%) while intensity responses showed moderate inheritance (31%) (II). Latter result is in line with a previous twins study showing that recognition threshold of sourness is inherited (50%) (Ishimaru et al. 2006). Candidate genes coding the PKD1L3 and PKD2L1 receptor proteins, which form the acid sensitive channels involved in sour taste are known (Ishii et al. 2009) but it is unclear yet if the allelic variation in these genes is responsible for differences in perceptions. Keskitalo et al. (2007b) used comparable methodology with sucrose solutions as stimuli and observed that liking of sweet solutions was partially explained by genetic effects (3.0% sucrose h^2=19.5%, 7.5% sucrose h^2=29.2, 18.75% sucrose h^2=40.9%) but the intensity responses showed no inheritance. It is plausible that the measurement of intensity is difficult with 'consumer' type of respondents who are not trained sensory panelists leading to 'fuzzy' data and thus making it difficult to teas a part the genetic influence. It is known that consumers tend to react to tested products as a whole and intensity ratings may be inflated by liking of the product (Lawless and Heymann 2010). While PROP intensity has shown high heritability (72%), the perceived intensity of other bitter compounds showed moderate genetic input: sucrose octate-acetate 28%, quinine 34%, and caffeine 30% (Hansen et al. 2006). Furthermore, the polymorphisms in umami receptor gene TAS1R3 and variations in the perception imply that umami perception could be at least partially influenced by genetic effects (Chen et al. 2009; M. Raliou et al. 2011). This comparison of taste genetics shows that taste perceptions indeed are modestly heritable traits but the contribution of genes is both, taste and stimulus dependent.

5.7.2 Heritability of responses to food names

Heritability estimates of responses to food names showed higher values compared to sensory traits, and similar estimates regardless of the stimuli presented by the food group (47-64%). The reason why the questionnaire traits exhibited higher heritabilities in relation to sensory traits may be because the questionnaire traits are composite measures. The heritable component for individual food is usually lower (Rozin 1991). It is possible that combining individual food items for which liking vary greatly, brings us closer to the 'true' preference, and may provide a better and more general tool for predicting food acceptance. This is supported by the fact that relatively narrow confidence intervals were obtained for the food groups compared to the heritability of the sensory measures. The conclusion of food names' superiority to tasting is however speculative and would require replication as it is known that depending on the way stimulus is presented, the liking varies (Cardello and Maller 1982; Cardello et al. 2000). When comparing liking ratings given to food names vs. tasted foods, Cardello and Maller (1982) showed that highly liked foods are rated lower when actually tasted. Similarly, less liked foods are rated higher in the actual tasting situation.

Previously foods grouped as desserts, vegetables, fruits and protein foods have shown heritabilities varying between 20-78% (Keskitalo et al. 2007b). A Swedish study comparing the intrapair correlations of MZ and DZ twins also indicated that preference of fruits has a genetic basis (Heitmann 1999). Similarly, a questionnaire was used by Faust (1974) who studied personal preferences of identical and non-identical twins for food and other traits, and found that the liking for spicy food was highly heritable. Furthermore, heritability of 54% was shown for preference of sweet foods by Keskitalo et al. 2007a) who used the same methodology (responses to food names) to measure liking as used in studies (I-IV). In light of the results presented in studies I-III and according to the existing literature, approximately up to half of the variation in taste preferences may indeed be attributed to genetic factors. Genes other than those involved directly in taste processing e.g. those regulating reward or motivation could explain a significant proportion of the variation (Dotson et al. 2012).

5.7.3 The role of environment

The role of environment in the formation of taste preferences is both convincing and undeniable. Studies I-III showed that a large proportion of the variation in taste preferences, regardless of the measuring technique (sensory or questionnaire), was explained by environmental effects: astringency 36-100%, sourness 50-86% and pungency 42-88%. Sourness and pungency traits for which statistically superior structural models could be established, showed that the specific/ unique (E) rather than common environment (C) explained the variance (II-III).

E refers to environmental factors (or events) which make the twins from the same family *different from each other*. The interpretation of E is based on the concept: they can be events occurring to only one twin (e.g. living alone, different friends and partners), but they can as well be common events which affect each twin in a different way (e.g. food served in the family). It has been shown that culture, prior exposure and learning are particularly important modifiers of food preferences (Beauchamp and Mennella 1998), and it is possible that food neophobia provides an ultimate 'filter' through which diet is selected (Rozin 1982). Furthermore, the formation of preferences has shown to start already in early infancy (Liem and de Graaf 2004) and to require longer exposure times (Liem and de Graaf, 2004) consequently leading to relatively stable and pervasive traits (Rozin and Schiller 1980). Hence, it is possible that many types of exposures to foods are seen as part of E, also those environmental cues we commonly like to interpret as part of the common environment (the food habits of the family). It should be noted however, that the content E contains also the measurement error. The respondents were equivalent to regular 'consumers' in the sense that they were not trained beforehand to recognize any perceptions that were tested, nor did they necessarily have experience in sensory testing in general. This may inflate the estimated role of specific environment thus the 'true' impact of environmental cues are blurred.

5.7.4 Taste preference patterns among twins and underlying genetics

Study IV showed that two distinctive adult subgroup segments exist among the twins differing in their taste preferences particularly with sour and spicy foods, and their responses to the food neophobia questionnaire. Adventurous preferred sourness and spiciness in foods more and were clearly less food neophobic compared to the basic group and the general population. Genetic differences among the twins (explained 66% of the variation) and differences in sensory responses to capsaicin and respondent's neophobic reactions were the likely causes for the subgroups. This rationale seems plausible since food neophobia is highly heritable (67-78%) (Knaapila et al. 2012; Pirastu et al. 2012), and the studies II-III show that preference for sourness and spiciness in foods is also partially genetically determined. Furthermore, our results corroborate what Rozin (1982) suggested. Food neophobia may act as a general barrier preventing or encouraging the exposures to novel stimuli thus influencing the respondent's tolerance for flavors.

Study IV also showed suggestive linkage in umami (TAS1R1) and sour taste (PKD1L3) genes among the adventurous. Variants in genotypes could not be identified as the association test results were non-significant (IV). Thus, it seems as these genes may contribute to the differences between the subgroups but the effects of the different variants are too small to be detected.

5.8 Study limitations

5.8.1 Respondents

The Finnish respondents, originating from different parts of the country were participants of the longitudinal Finntwin12 study and were not selected for sensory or food preference study purposes. They were a group of young twins from which not only information on lifestyle but also substance use, psychiatric condition, health, personality and cognitive functions were collected. Therefore, no selection biases resulting from recruitment should exist. In terms of sensory studies, the respondents represented a fairly large group of naïve young 'consumers' but in terms of genetic research, the sample size was at times inadequate and lacking of statistical power. This was particularly pronounced in study I in which the number of twins was restricted due to the later implementation of astringency measurements to the test protocol.

5.8.2 Analysis

Most measures were treated as continuous variables and analyzed utilizing parametric methods as they contained at least 7-points from which all had been used by the respondents. Some of the variables did show non-optimal features (skewed distributions) but most variables were roughly normally distributed and the underlying phenomena were assumed to be as such.

We observed DZ twin correlations to be higher than MZ correlations in some of the variables in study I. That may be an artifact due to small number of pairs. The attempts to find a single statistically significant structural model was also challenging, thus the results were reported as suggestive (I). Similar difficulties were experienced in studies II-IV but the use of multivariate modeling of twin data in which the co-variation of the traits was utilized provided a more powerful means of analysis, and the differences between the structural models were more pronounced. Furthermore, due to the small samples size, the data did not allow sex comparisons in any of the heritable traits.

The corner stones of classical twin studies are the assumptions of: equal environment, random mating and the absence of gene-environment interactions (see section 2.10.2). The assumption of equal environment was tested (equal means and variances between the twins, and MZ and DZ twin groups) in all studies and they were not violated. Different treatment from parents (MZ twins treated more similarly than DZ twins) that would increase the trait similarity observed in MZ twins was not possible for us to test, nor was it possible to observe

the random mating effect because no data from parents were available. It is plausible that similar treatment of MZ twins by their parents increased the estimated heritability. However, it is stated that parents treat the MZ twins more similarly than the DZ twins because they in fact are more similar making the parents respond accordingly. Furthermore, the approach of parents to raising twins seems to have no significant influence on twin resemblance (Neale and Cardon 1992). Non-random mating is unlikely to occur in case of food preferences. It is more probable with other traits such as height.

Gene-environment interaction (GxE) is a complex matter. A person with a certain genotype can react differently to environmental factors. For example one can be more sensitive to tastes and act accordingly, but on the other hand, environmental factors can strengthen or quench a genetic outcome or alter a gene function. Twin modeling does not take into account these phenomena separately, thus distortion may be observed either with additive genetic or specific environmental variance. In other words, if the environmental factors are shared by the twin pair, the effect of GxE becomes a part of the additive genetic variance. If they are not shared, GxE is included in the specific environmental variation (Neale and Cardon 1992). GxE may be important in food preferences, thus it may have influenced the modeling results. To detect it, environmental measurements have to be included on the basis which the twin sample can be stratified.

Twin analysis provides a way to estimate the magnitude of genetic and environmental effects causing the differences seen in the phenotypes. Thus, we can explain the variance with twin modeling but it does not answer the question to which genes or environmental factors underlie the differences or similarities between the twins. We can only speculate on such answers. In study IV, umami and sour gene regions provided suggestive linkage results in relation to being adventurous. This fortifies the common view that genes regulating the sensory perceptions are associated with hedonic judgments but how these genes affect the actual preference of foods remains still unclear. Furthermore, genes that affect other traits (learning, metabolism, satiety etc.) than taste may be more powerful determinants of food liking than the current research is aware of. Genes directly regulating preferences may also exist. These questions should be tackled in the future studies by using larger sample sizes and more comprehensive battery of measures.

5.8.3 Measurements

The chemosensory stimuli used in the studies (I-IV) were simple compared to the variety of foods and beverages in normal diet. The goal of developing the sensory tests however was to find samples that were easily prepared outside laboratory circumstances and provide responses with sufficient variation. The time reserved for the sensory tests was restricted by

the protocol of Finntwin12-study, thus the sensory tests needed to be fast and easy, and the sample preparation facilities were suitable only for simple sample preparation protocols. Furthermore, the respondents were not trained but only instructed prior to each measurement so it was crucial that the tasks were simple to understand. With these restrictions in mind, the sensory stimulus and tasks were selected, thus more complex foods were not used nor were the respondents provided with more than one stimulus per trait.

As noted before, it is likely that the responses to sensory pleasantness and intensity were product specific and thus do not reflect the wide range of astringent, sour and pungent foods. In this respect the test stimuli were inadequate to capture the natural or 'true' variation of chemosensory traits, thus resulting in measures with high confidence intervals in heritability analysis. Using simple stimuli may have been the best approach as a first step but in the future studies a larger range of real foods may be in place to better understand the phenomena. As concluded by Cardello and others (2000), single measures are inadequate when predicting consumption of foods eaten in real life situations. Multiple sample approach however, will present logistical set-up challenges and respondent fatigue issues. As this kind of testing is similar to consumer acceptance testing, it is important to also consider the serving conditions because they are likely to influence the ratings. King et al. (2007) showed that testing foods as items rather than as part of a meal can lead to diminished liking scores.

Some form of training might be in place to familiarize the respondents of the testing procedure. We partly tried to compensate the lack of real foods by Specific Food questionnaires where the stimulus was presented as a list of food names and the responses used in the analysis as composite measures. The weakness of the composite measure, however, resides in their abstract nature. Using pictures instead of food names could have been beneficial in terms of task realism. Photographic images are likely to provide as reliable results as responses to actual stimuli (Jaeger et al. 2000; Olsen et al. 2012).

5.8.4 Astringency (I)

Using naïve 'consumers' as respondents to evaluate intensity of a stimulus also presents challenges. Respondents may have experienced difficulty providing perceptual ratings of intensity independently from affective ratings (Cardello and Maller 1982) but it seems unlikely as the measurements were performed relative to a reference (apart from umami). Astringency measurement (I) were most challenging. It can be speculated whether the respondents were able to concentrate on the slowly developing perception during the ratings. This might have resulted in responses that did not truly reflect astringency per se and thus made the influence of genes non-existing in these traits. Another challenge with the astringency measures was provided by the food names. We could not extract composite

measures (by factor analysis) that would clearly reflect high or low astringency levels. The composite measure best illustrating astringent items was *astringent drinks*, but since the items in that group contained caffeine and alcohol, which are known for their addictive and physiological effects, it can be speculated whether or not this group of items actually related to the astringency alone.

5.8.5 Food names (I-IV)

Responses to food names seemed to provide an index of relative acceptance of a food. If the food is preferred, the rating reflects the best opinion of the food and vice versa if the food is not preferred the respond reflects the worst preparation of the food (Cardello and Maller, 1982). This could be beneficial for several reasons. Firstly, we are likely to obtain data on general food likings and secondly, we are to observe enough variation to indicate individual patterns of preference. Larger variations were indeed observed with pleasantness ratings compared to use-frequency scores but they were not deviated from the sensory pleasantness rating variations. On the other hand, the responses to food names do not completely reflect reality, and individuals are likely to differ in their frame of references. In order to predict liking or consumption in real life situations, adding questions about 'expected liking' and 'appropriateness' for the food in its intended use situation may have been beneficial (Cardello et al. 2000) .

5.8.6 Preference subgroups (IV)

Subgroups of the population (IV) were discovered based on liking responses to food names representing sour, pungent and umami qualities. This setting was not balanced since we did not have data on foods or beverages representing sweetness, bitterness or saltiness. With all tastes represented, we could have ended up with different preference patterns or yielded a larger number of respondent groups. However, evidence of the validity of the subgrouping result emerged as the sensory responses were in alignment with the sensory responses: pungent and sour food likers rated pungency and sourness more pleasant. Furthermore, genetic resemblance was discovered to underlie the subgroups. This indicates that the clustering result was not coincidental. The heritability estimate explaining the preference subgroups however did show large confidence intervals which illustrate again the lack of statistical power.

5.9 Implications of preferences for sour, astringent and pungent flavor qualities

Taste system is a 'guardian' of the human body offering signals for food's nutrient and toxic content and aiding complex ingestion related physiological processes (Breslin 2013). Flavor in turn, is crucial for palatability and paramount in the enjoyment of foods. In pursuit of promoting the consumption of foods with nutritional benefits, their sensory qualities alongside individual preferences should be highlighted in dietary counseling. New tools to influence preference for a taste may be developed through which inadequate diets could be tackled. This is particularly important in case of fruits and berries which all share the common sensory characteristic of sourness. Fruits and berries are well known for their health benefits providing a number of positive physiological effects in prevention of chronic conditions such as hear diseases and cancer (Gandini et al. 2000; Gerber et al. 2002; Van Duyn 2000).

Whereas avoiding naturally sour tasting foods can lead to narrowed diet selection influencing our health, the sensory based rejection of astringent foods and beverages can be both advantageous and disadvantageous to nutrition. The rejection may be advantageous as polyphenols reportedly decrease the utilization of food proteins and inhibit the function of digestive enzymes (Khan and Mukhtar, 2013; Yao et al., 2004). Disadvantages of rejection are evident because polyphenols such as flavonoids have antioxidative and anti-cancer activity (Van Duyn 2000). Spices in turn seem to offer more sensory pleasure than nutritional value. However, their use in various cuisines have been attributed to combat foodborne micro-organisms and to prevent food poisonings (Sherman and Billing 1999). Furthermore, the pungent component in chili peppers has been indicated to generate modest weight loss, offering health benefits at least on a population scale (Ludy et al. 2012). The implications of the consumption of astringent foods and the use of spices with pungent qualities thus seem to contribute to a healthy lifestyle. However, their importance in dietary counseling may be less pronounced.

7 CONCLUSIONS AND FUTURE PROSPECTS

It was demonstrates that preferences for sour and pungent foods are moderately heritable traits. Furthermore, suggestive evidence was found that salivary protein secretion related to astringent stimulation is under genetic control. This thesis was the first to demonstrate the role of genetics in astringency and pungency perceptions, and one of the few to study the heritability of sour taste perception. Most importantly, the existence of a genetic component in preference of foods with astringent, pungent and sour qualities was illustrated.

Sensory pleasantness and intensity of astringency did not show any inheritance while approximately one fifth of the variation in sensory traits of sourness, and pungency was attributed to genetic factors. By contrast, up to one half of the variation in preference for *sour foods* and *spicy foods and spices* (responses to food names used as composite measures) were explained by genetic influences. The results indicated that sensory responses to stimulus provides a product specific measure whereas the responses to food names used as composite measures may provide a better tool for predicting food acceptance in a wider scope.

Findings also revealed that in all of studied traits (sourness, astringency and pungency), the environment plays a crucial role and that the preference for foods is at least half determined by environmental factors. Environmental cues exhibited as exposures to different chemosensory qualities should be highlighted in dietary counseling, for example in the form of 'taste learning' aiming to change disliked tastes or foods into liked ones when relevant or desired in terms of the diet goals. This could provide new tools to alter the dieting patterns, for example the consumption of nutritiously beneficial sour tasting fruits and berries, provide ways to balance the diet with flavonoid rich astringent foods or influence the consumption of foods with spicy qualities which may induce weight management on a population scale.

By contrast to environmental influences, findings of this thesis also highlight the role of genetics and food neophobia in individual flavor preferences. The flavor preferences of this population indicated the existence of two subgroups differing in their liking for foods with sour, pungent and umami flavor qualities. While the adventurous seemed to find pleasure in foods with more dramatic flavor sensations and to better tolerate the sensory 'burn' of capsaicin, the basic group preferred foods with bland flavors. Underlying causes of the grouping were attributed to inherited differences and food neophobia. Thus food neophobia and genetic differences may form a barrier through which individual flavor preferences are generated. In future studies, exploring flavor preference subgroups with a wider set of taste and odor variables could reveal a spectrum of groups, and combined with the genetic data, new found links between preference patterns and genes.

Genetic differences in taste perception modify preferences in a complicated manner. Besides bitter taste, it is largely unknown which alleles affect the receptor function for sour, astringent or pungent perceptions, and more importantly, to what extent these differences influence the actual preference of foods. Genetics is a fast moving area where new tools are continuously developed to better understand complex traits. Sensory scientists may soon face an ever-growing list of receptor gene variants with only minor effects to the trait in question. The common trait, common variant theory, could fail like it has in other complex human traits. However, to unravel the genetic basis in food choices, the receptor variants need to be found and their impact on perceptions explored. Perceptions are, after all, the first stepping stone towards liking.

It may be too simple to draw straight links between preferences and food choices due to the complexity of the matter, yet it is clear that a modern human with seemingly endless supply of foods ultimately eat what he/she prefers. Therefore, it would be worthwhile to further pursue the genes underlying preferences to widen the scope of traditional taste genetics. A large part of the variation in individual likes and dislikes may also be explained by other genes than those directly related to chemosensory perceptions (hunger, satiation, metabolism, learning, food reward etc.). Novel genetic techniques, larger data sets and establishing novel links between genes and preferences may reveal such associations in the future. Lastly, next to nothing is known of gene environment interactions influencing chemosensory perceptions or preferences although this interaction may have significant impact on the development of taste preferences and the etiology of food choices. In order to unravel this, careful exposure assessment and large sample sizes are needed. Longitudinal studies are also likely to provide insight into the fundamental biological mechanisms of taste and food preferences.

REFERENCES

Alimohammadi H, Silver W. 2002. Chemesthesis: Hot and cold mechanisms. ChemoSense 4:1-2.

Ames B, Shigenaga M, Hagen T. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci 90:7915-7922.

Arvola A, Lähteenmäki L, Tuorila H. 1999. Predicting the intent to purchase unfamiliar and familiar cheeses: The effects of attitudes, expected liking and food neophobia. Appetite 32:113-126.

Attokaran M. 2011. Cinnamon leaf. In: Natural food flavors and colorants. Wiley –Blackwell, Oxford, UK, pp. 141-143.

Bajec M, Pickering G. 2008. Astringency: Mechanisms and perception. Crit Rev Food Sci Nutr 48:858-875.

Bandell M, Macpherson L, Patapoutian A. 2007. From chills to chilis: Mechanisms for thermosensation and chemesthesis via thermo TRPs. Curr Opin Neurobiol 17: 490-497.

Bartoshuk L. 1979. Bitter taste of saccharin related to the genetic ability to taste the bitter substance 6-n-propylthiouracil. Science 205:934-935.

Bartoshuk L, Duffy V, Miller I. 1994. PTC/PROP tasting: Anatomy, psychophysics, and sex effects. Physiol Behav 56:1165-1171.

Bautista D, Movahed P, Hinman A, Axelsson H, Sterner O, Högestätt E, Julius D, Jordt S, Zygmunt P. 2005. Pungent products from garlic activate the sensory ion channel TRPA1. Proc Nat Acad Sci USA. 102:12248-12252.

Baxter N, Lilley T, Haslam E, Williamson M. 1997. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 36:5566-5577.

Beauchamp G, Mennella J. 1998. Sensitive periods in the development of human flavor perception and preference. Ann Nestle 56:19-31.

Beidler L. 1971. Taste receptor stimulation with salts and acids. In: Beidler L, Ed. Handbook of Sensory Physiology. Chem Senses 2. Taste. Springer-Verlag, Berlin, Heidelberg, New York, pp.200-220.

Bellisle F. 2005. The determinants of food choice. EUFIC Rev 17:1-8.

Billing J, Sherman P. 1998. Antimicrobial functions of spices: Why some like it hot. Q Rev Biol 73:3-49.

Birch L. 1999. Development of food preferences. Annu Rev Nutr 19:41-62.

Bland M, Altman D. 1997. Statistics notes: Cronbach's alpha. BMJ 314:572.

Boomsma D. 2002. Classical twin studies and beyond. Nat Rev Genet 3:872-882.

Bosland P, Coon D, Reeves G. 2012. *Trinidad moruga Scorpion* pepper is the world's hottest measured chile pepper at more than two million Scoville heat units. HortTechnology 22:534-538.

Breen F, Plomin R, Wardle J. 2006. Heritability of food preferences in young children. Physiol Behav 88: 443-447.

Breslin P. 2013. An evolutionary perspective on food and human taste. Curr Biol 23:R409-R418.

Breslin P, Spector A. 2008. Mammalian taste perception. Curr Biol 18:R148.

Breslin P. 1993. Psychophysical evidence that oral astringency is a tactile sensation. Chem Senses 18:405-417.

Bruder C, Piotrowski A, Gijsbers A, Andersson R, Erickson S, Diaz de Ståhl T, Menzel U, Sandgren J, von Tell D, Poplawski A, Crowley M, Crasto C, Partridge E, Tiwari H, Allison D, Komorowski J, van Ommen G, Boomsma D, Pedersen N, den Dunnen J, Wirdefeldt K, Dumanski J. 2008. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. Am J Hum Genet 82:763-771.

Cala O. 2010. NMR and molecular modeling of wine tannins binding to saliva proteins: Revisiting astringency from molecular and colloidal prospects. FASEB J 24:4281-4290.

Cardello A, Maller O. 1982. Relationships between food preferences and food acceptance ratings. J Food Sci 47: 1553-1557.

Cardello A, Schutz H, Snow C. Lesher L. 2000. Predictors of food acceptance, consumption and satisfaction in specific eating situations. Food Qual Prefer11: 201-216.

Cardello A. 1996. The role of the human senses in food acceptance. In: Meiselman H, MacFie H, Eds. Food choice acceptance and consumption. pp. 1-64.

Caterina M, Rosen T, Tominaga M, Brake A, Julius D. 1999. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436-441.

Caterina M, Schumacher M, Tominaga M, Rosen T, Levine J, Julius D. 1997. The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 389: 816-824.

Charlton A, Baxter N, Khan M, Moir A, Haslam E, Davies A, Williamson M. 2002. Polyphenol/peptide binding and precipitation. J Agric Food Chem 50:1593-1601.

Chaudhari N, Pereira E, Roper S. 2009. Taste receptors for umami: The case for multiple receptors. Am J Clin Nutr 90:738S-742S.

Chaudhari N, Roper S. 2010. The cell biology of taste. J Cell Biol 190:285-296.

Chauhan J, Hawrysh Z. 1988. Suprathreshold sour taste intensity and pleasantness perception with age. Physiol Behav 43:601-607.

Chen Q, Alarcon S, Tharp A, Ahmed O, Estrella N, Greene T, Rucker J, Breslin P. 2009. Perceptual variation in umami taste and polymorphisms in TAS1R taste receptor genes. Am J Clin Nutr 90:770S-779S.

Childs J, Drake M. 2010. Consumer perception of astringency in clear acidic whey protein beverages. J Food Sci 75:S513.

Christensen C, Brand J, Malamud D. 1987. Salivary changes in solution pH: A source of individual differences in sour taste perception. Physiol Behav 40:221-227.

Cliff M, Heymann H. 1993. Descriptive analysis of oral pungency. Food Qual Prefer 4:279-290.

Cliff M, Green B. 1996. Sensitization and desensitization to capsaicin and menthol in the oral cavity: Interactions and individual differences. Physiol Behav 59:487-494.

Collins M, Wasmund L, Bosland P. 1995. Improved method for quantifying capsaicinoids in capsicum using high-performance liquid chromatography. HortScience 30:137-139.

Condelli N, Dinnella C, Cerone A, Monteleone E, Bertuccioli M. 2006. Prediction of perceived astringency induced by phenolic compounds II: Criteria for panel selection and preliminary application on wine samples. Food Qual Prefer 17:96-107.

Cortright D, Szallasi A. 2009. TRP channels and pain. Curr Pharm Des 15:1736-1749.

Cowart B. 2010. Ageing and Chemical Senses. In: Goldstein E, Ed. Encyclopedia of perception SAGE Publications, California, USA pp. 17-19

Da Conceicao Neta E, Johanningsmeier S, McFeeters R. 2007. The chemistry and physiology of sour taste--A review. J Food Sci 72:R33.

De Almeida Pdel V, Gregio A, Machado M, De Lima A, Azevedo L. 2008. Saliva composition and functions: A comprehensive review. J Contemp Dent Pract 9:72-80.

Delwiche J, Buletic Z, Breslin P. 2001. Covariation in individuals' sensitivities to bitter compounds: Evidence supporting multiple receptor/transduction mechanisms. Percept Psychophys 63:761-776.

DeSimone J, Lyall V. 2006. Taste receptors in the gastrointestinal tract III. Salty and sour taste: Sensing of sodium and protons by the tongue. Am J Physiol Gastrointest Liver Physiol 291:G1005-G1010.

Dinnella C, Recchia A, Tuorila H, Monteleone E. 2011. Individual astringency responsiveness affects the acceptance of phenol-rich foods. Appetite 56: 633-642.

Dinnella C, Recchia A, Fia G, Bertuccioli M, Monteleone E. 2009. Saliva characteristics and individual sensitivity to phenolic astringent stimuli. Chem Senses 34:295-304.

Dinnella C, Recchia A, Vincenzi S, Tuorila H, Monteleone E. 2010. Temporary modification of salivary protein profile and individual responses to repeated phenolic astringent stimuli. Chem Senses 35:75-85.

Dotson C, Babich J, Steinle N. 2012. Genetic predisposition and taste preference: Impact on food intake and risk of chronic disease. Cur Nutr Rep 1:175-183.

Doty R, Shah M, Bromley S. 2008. Drug-induced taste disorders. Drug Saf 31:199-215.

Drayna D. 2005. Human taste genetics. Annu Rev Genomics Hum Genet 6:217-235.

Drewnowski A. 1997. Taste preferences and food intake. Annu Rev Nutr 17:237-253.

Drewnowski A, Henderson S, Cockroft J. 2007. Genetic sensitivity to 6-n-propylthiouracil has no influence on dietary patterns, body mass indexes, or plasma lipid profiles of women. J Am Diet Assoc 107:1340-1348.

Drewnowski A. 2000. Bitter taste, phytonutrients, and the consumer: A review. Am J Clin Nutr 72:1424-1435.

Eertmans A, Baeyens F, Van Den Bergh O. 2001. Food likes and their relative importance in human eating behavior: Review and preliminary suggestions for health promotion. Health Educ Res 16:443-456.

Eichler E, Flint J, Gibson G, Kong A, Leal S, Moore J, Nadeau J. 2010. Missing heritability and strategies for finding the underlying causes of complex disease. Nat Rev Genet 11: 446-450.

Falciglia G, Couch S, Gribble L, Pabst S, Frank R. 2000. Food neophobia in childhood affects dietary variety. J Am Diet Assoc 100:1474-1481.

Farbman A, Hellekant G. 1978. Quantitative analyses of the fiber population in rat chorda tympani nerves and fungiform papillae. Am J Anat 153:509-521.

Faust J. 1974. A twin study of personal preferences. J Biosoc Sci 6:75-91.

Feeney E. 2011. Genetic variation in taste perception: Does it have a role in healthy eating? Proc Nutr Soc, 70(1), 135-143.

Fleischauer A, Arab L. 2001. Garlic and cancer: A critical review of the epidemiologic literature. J Nutr 131:1032S-1040S.

Matforsk Ø. 2006. Gender differences in food choice. In: Frewer L, Trijp H, Eds. Understanding consumers of food products. Woodhead Publishing Ltd, Cambridge, UK. pp.316-328.

Galopin C. 2007. New pungent and cooling compounds for use in foods. In: Taylor A, Hort J, Eds. Modifying flavour in food. Woodhead Publishing Ltd. Cambridge, UK pp. 146-168.

Ganchrow J. 1983. Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli. Infant Behav Dev 6:473-484.

Gandin S, Merzenich H, Robertson C, Boyle P. 2000. Meta-analysis of studies on breast cancer risk and diet: The role of fruit and vegetable consumption and the intake of associated micronutrients. Eur J Cancer 36: 636-646.

Garb J, Stunkard A. 1974. Taste aversions in man. Am J Psychol 131:1204-1207.

Garcia-Bailo B, Toguri C, Eny K, El-Sohemy A. 2009. Genetic variation in taste and its influence on food selection. OMICS 13: 69-80.

Gerber M, Boutron-Ruault M, Hercberg S, Riboli E, Scalbert A, Siess M. 2002. Food and cancer: State of the art about the protective effect of fruits and vegetables. Bull Cancer 89: 293-312.

Gibbins H, Carpenter G. 2013. Alternative mechanisms of Astringency–What is the role of saliva? J Texture Stud ISSN 1745-4603.

Glanz K, Basil M. 1998. Why americans eat what they do: Taste, nutrition, cost, convenience, and weight control concerns. J Am Diet Assoc 98: 1118-1126.

Glendinning J. 1994. Is the bitter rejection response always adaptive? Physiol Behav 56:1217-1227.

Green B. 1989. Capsaicin sensitization and desensitization on the tongue produced by brief exposures to a low concentration. Neurosci Letters 107:173-178.

Green B, Alvarez-Reeves M, George P, Akirav C. 2005. Chemesthesis and taste: Evidence of independent processing of sensation intensity. Physiol Behav 86:526-537.

Green B, Dalton P, Cowart B, Shaffer G, Rankin K, Higgins J. 1996. Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell. Chem Senses 21:323-334.

Green B, Shaffer G, Gilmore M. 1993. Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. Chem Senses 18:683-702.

Gringras P, Chen W. 2001. Mechanisms for differences in monozygous twins. Early Hum Dev 64:105-117.

Guinard J, Zoumas-Morse C, Walchak C. 1997. Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods. Physiol Behav 63:109-118.

Hansen J, Reed D, Wright M, Martin N, Breslin P. 2006. Heritability and genetic covariation of sensitivity to PROP, SOA, quinine HCl, and caffeine. Chem Senses 31:403-413.

Harris H. Kalmus H. 1949. The measurement of taste sensitivity to phenylthiourea (PTC). Ann Eugen 15: 24-31.

Hartwig P, McDaniel M. 1995. Flavor characteristics of lactic, malic, citric, and acetic acids at various pH-levels. J Food Sci 60:384-388.

Hatton M, Loomis R, Levine M, Tabak L. 1985. Masticatory lubrication. the role of carbohydrate in the lubricating property of a salivary glycoprotein-albumin complex. Biochem J 230:817-820.

Hayes J, Bartoshuk L, Kidd J, Duffy V. 2008. Supertasting and PROP bitterness depends on more than the TAS2R38 gene. Chem Senses 33:255-265.

Hayes P, Meadows H, Gunthorpe M, Harries M, Duckworth D, Cairns, Harrison D, Clarke C, Ellington K, Prinjha R, Barton A, Medhurst A, Smith G, Topp S, Murdock P, Sanger G, Terrett J, Jenkins O, Benham C, Randall A, Gloger I, Davis J. 2000. Cloning and functional expression of a human orthologue of rat vanilloid receptor-1. Pain 88:205-215.

Heitmann B. 1999. Genetic effects on weight change and food intake in Swedish adult twins. Am J Clin Nutr 69:597-602.

Henriques A, King S, Meiselman H. 2009. Consumer segmentation based on food neophobia and its application to product development. Food Qual Prefer 20:83-91.

Hiekkalinna T, Schäffer A, Lambert B, Norrgrann P, Göring H, Terwilliger J. 2011. PSEUDOMARKER: A powerful program for joint linkage and/or linkage disequilibrium analysis on mixtures of singletons and related individuals. Hum Her 71:256-266.

Huque T, Cowart B, Dankulich-Nagrudny L, Pribitkin E, Bayley D, Spielman A, Feldman R, Mackler S, Brand J. 2009. Sour ageusia in two individuals implicates ion channels of the ASIC and PKD families in human sour taste perception at the anterior tongue. PLoS One 4:e7347.

Hussein L, Abbas H 1985. Nitrogen balance studies among boys fed combinations of faba beans and wheat differing in polyphenolic contents. Nutr Rep Int 31:67-81.

International standard ISO 5492. 2008. Definition of terms in sensory analysis. Suomen standardisoimisliitto.

Ishii S, Misaka T, Kishi M, Kaga T, Ishimaru Y, Abe K. 2009. Acetic acid activates PKD1L3–PKD2L1 channel—A candidate sour taste receptor. Biochem Biophys Res Commun 385:346-350.

Ishikawa T, Noble A. 1995. Temporal perception of astringency and sweetness in red wine. Food Qual Prefer 6: 27-33.

Ishimaru Y, Inada H, Kubota M, Zhuang H, Tominaga M, Matsunami H. 2006. Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci USA 103:12569-1274.

Jaeger S, Hedderley D, MacFie H. 2000. Methodological issues in conjoint analysis: a case study. Eur J Marketing 35:1217-1237.

Jaeger S, McRae J, Salzman Y, Williams L, Newcomb R. 2010. A preliminary investigation into a genetic basis for cis-3-hexen-1-ol odour perception: A genome-wide association approach. Food Qual Prefer 21:121-131.

Jaeger S, Pineau B, Bava C, Atkinson K, McRae J, Axten L, Chheang S, Peresford M, Peng M, Paisley A. 2012. Investigation of the impact of sensitivity to cis-3-hexen-1-ol (green/grassy) on food acceptability and selection. Food Qual Prefer 24: 230-242.

James C, Laing D, Oram N. 1997. A comparison of the ability of 8–9-year-old children and adults to detect taste stimuli. Physiol Behav 62:193-197.

Jansman A, Frohlich A, Marquardt R. 1994. Production of proline-rich proteins by the parotid glands of rats is enhanced by feeding diets containing tannins from faba beans (*vicia faba L.*). J Nutr 124:249-258.

Jöbstl E, O'Connell J, Fairclough J, Williamson M. 2004. Molecular model for astringency produced by polyphenol/protein interactions. Biomacromolecules 5:942-949.

Johanningsmeiner S, McFeeters R, Drake M. 2005. A hypothesis for the chemical basis for perception of sour taste. J Food Sci 70:R44-R48.

Jordt S, McKemy D, Julius D. 2003. Lessons from peppers and peppermint: The molecular logic of thermosensation. Curr Opin Neurobiol 13:487-492.

Joseph J, Shukitt-Hale B, Denisova N, Bielinski D, Martin A, McEwen J, Bickford P. 1999. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 19:8114-8121.

Kallithraka S. 1998. Evidence that salivary proteins are involved in astringency. J Sens Stud 13:29-43.

Kaplan A. 1967. Taste thresholds in twins and siblings. Acta Genet Med Gemellol (Roma) 16: 229-243.

Kaprio J. 2006. Twin studies in Finland 2006. Twin Res Hum Genet 9:772-777.

Kaprio J, Pulkkinen L, Rose R. 2002. Genetic and environmental factors in health-related behaviors: Studies on Finnish twins and twin families. Twin Res 5:366-371.

Karlsson J, Persson L, Sjöström L, Sullivan M. 2000. Psychometric properties and factor structure of the three-factor eating questionnaire (TFEQ) in obese men and women. Results from the Swedish obese subjects (SOS) study. Int J Obes Relat Metab Disord 24:1715-1725.

Karrer T, Bartoshuk L, Conner E, Fehrenbaker S, Grubin D, Snow D. 1992. PROP status and its relationship to the perceived burn intensity of capsaicin at different tongue loci. Chem Senses 17:649.

Kaur C, Kapoor H. 2001. Antioxidants in fruits and vegetables–the millennium's health. Int J Food Sci Technol 36:703-725.

Kendler K, Neale M, Kessler R, Heath A, Eaves L. 1994. Parental treatment and the equal environment assumption in twin studies of psychiatric illness. Psychol Med 24:579-590.

Keskitalo K. 2008. A matter of taste: Genetic and environmental influences on responses to sweetness. Academic dissertation. National Public Health Institute, Finland. 77 pages ISSN 0359-3584; 1458-6290.

Keskitalo K, Tuorila H, Spector T, Cherkas L, Knaapila A, Silventoinen K, Perola M. 2007a. Same genetic components underlie different measures of sweet taste preference. Am J Clin Nutr 86:1663-1669.

Keskitalo K, Knaapila A, Kallela M, Palotie A, Wessman M, Sammalisto S, Peltonen L, Tuorila H, Perola M. 2007b. Sweet taste preferences are partly genetically determined: Identification of a trait locus on chromosome 16. Am J Clin Nutr 86:55-63.

Khan N, Mukhtar H. 2013. Tea and health: Studies in humans. Curr Pharm Des.

Kim U, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299: 1221-1225.

King S, Meiselman H, Hottenstein A, Work T, Cronk V. 2007. The effects of contextual variables on food acceptability: a confirmatory study. Food Qual Prefer 18:58-65.

Knaapila A. 2008. Genetic and environmental influences on human responses to odors. Academic dissertation. National Public Health Institute, Finland. 80 pages ISSN 0359-3584; 1458-6290.

Knaapila A, Hwang L, Lysenko A, Duke F, Fesi B, Khoshnevisan A, James R, Wysocki C, Rhyu M, Tordoff M. 2012. Genetic analysis of chemosensory traits in human twins. Chem Senses 37:869-881.

Knaapila A, Silventoinen K, Broms U, Rose R, Perola M, Kaprio J, Tuorila H. 2011. 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.

Knaapila A, Tuorila H, Silventoinen K, Keskitalo K, Kallela M, Wessman M, Peltonen L, Cherkas L, Perola M. 2007. Food neophobia shows heritable variation in humans. Physiol Behav 91:573-578.

Knaapila A, Tuorila H, Silventoinen K, Wright, M, Kyvik K, Cherkas L, Keskitalo K, Hansen J, Martin N, Spector T, Kaprio J, Perola M. 2008. Genetic and environmental contributions to perceived intensity and pleasantness of androstenone odor: an international twin study. Chemosens Percept 1:34-42.

Koivisto U, Sjoden P. 1996. Food and general neophobia in Swedish families: Parent-child comparisons and relationships with serving specific foods. Appetite 26:107-118.

Kremeyer B, Lopera F, Cox J, Momin A, Rugiero F, Marsh S, Woods C, Jones N, Paterson K, Fricker F. 2010. A gain-of-function mutation in TRPA1 causes familial episodic pain syndrome. Neuron 66:671-680.

Kuumola J. 2008. The development of indicators for the heritability of sensory perception. M.Sc. Thesis, EKT-Series, No. 1410 [Abstract in English]. University of Helsinki.

Lander E, Kryglak K. 1995. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat Genet 11:241-247.

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

Lawless H. 1984. Oral chemical irritation: Psychophysical properties. Chem Senses 9:143-155.

Lawless H, Heymann H. 2010. Sensory evaluation of food: Principles and practices. NY Springer.

Lawless H, Malone G. 1986. A comparison of scaling methods: Sensitivity, replicates and relative measurement. J Sens Stud 1:155-174.

Lawless H, Rozin P, Shenker J. 1985. Effects of oral capsaicin on gustatory, olfactory and irritant sensations and flavor identification in humans who regularly or rarely consume chili pepper. Chem Senses 10:579-589.

Lee C. 1991. Time-course of astringent sensations. Chem Senses 16:225-238.

Lesschaeve I, Noble A. 2005. Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. Am J Clin Nutr 81:330s-335s.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. 2002. Human receptors for sweet and umami taste. Proc Nat Acad Sci 99:4692-4696.

Liem D, de Graaf C. 2004. Sweet and sour preferences in young children and adults: Role of repeated exposure. Physiol Behav 83:421-8.

Liem D, Mennella J. 2002. Sweet and sour preferences during childhood: Role of early experiences. Dev Psychobiol 41:388-395.

Liem D, Mennella J. 2003. Heightened sour preferences during childhood. Chem Senses 28:173-180.

Liem D, Westerbeek A, Wolterink S, Kok F, de Graaf C. 2004. Sour taste preferences of children relate to preference for novel and intense stimuli. Chem Senses 29:713-720.

Logue A, Smith M. 1986. Predictors of food preferences in adult humans. Appetite 7:109-125.

LopezJimenez N, Cavenagh M, Sainz E, Cruz-Ithier M, Battey J, Sullivan S. 2006. Two members of the TRPP family of ion channels, Pkd1l3 and Pkd2l1, are co-expressed in a subset of taste receptor cells. J Neurochem 98:68-77.

Ludy M, Mattes R. 2011. The effects of hedonically acceptable red pepper doses on thermogenesis and appetite. Physiol Behav 102:251-258.

Ludy M, Moore G, Mattes R. 2012. The effects of capsaicin and capsiate on energy balance: Critical review and meta-analyses of studies in humans. Chem Senses 37:103-121.

Lugaz O, Pillias A, Faurion A. 2002. A new specific ageusia some humans cannot taste L-glutamate. Chem Senses 27:105-115.

Lyall V. Alam R, Phan D, Ereso G, Phan T, Malik S, Montrose M, Xhu S, Heck G, Feldman G. 2001. Decrease in rat taste receptor cell intracellular pH is the proximate stimulus in sour taste transduction. Am J Physiol Cell Physiol 281:C1005-C1013.

Lyman B. 1988. A sychology of food. Long-term food preferences.Van Nostrand Reinhold, New York, USA.pp 13-33

Lyman B, Green B. 1990. Oral astringency: Effects of repeated exposure and interactions with sweeteners. Chem Senses 15:151-164.

Maher B. 2008. The case of the missing heritability. Nature 456:18-21.

Manolio T, Collins F, Cox N, Goldstein D, Hindorff, L, Hunter D, McCarthy M, Ramos E, Cardon L, Chakravarti A, 2009. Finding the missing heritability of complex diseases. Nature 461:747-753.

Mattes R. 1991. Learned food aversions: A family study. Physiol Behav 50:499-504.

McBurney D, Balaban C, Christopher D, Harvey C. 1997. Adaptation to capsaicin within and across days. Physiol Behav 61:181-190.

Mehansho H, Butler L, Carlson D. 1987. Dietary tannins and salivary proline-rich proteins - interactions, induction, and defense-mechanisms. Annu Rev Nutr 7:423-440.

Mehansho H, Clements S, Sheares B, Smith S, Carlson D. 1985. Induction of proline-rich glycoprotein synthesis in mouse salivary glands by isoproterenol and by tannins. J Biol Chem 260:4418-4423.

Michon C, O'Sullivan M, Delahunty C, Kerry J. 2009. The investigation of gender-ralated sensitivity differences in food perception. J Sens Stud 24:922-937.

Miller I, Reedy F. 1990. Variations in human taste bud density and taste intensity perception. Physiol Behav 47:1213-1219.

Misretta C. 1984. Aging effects on anatomy and neurophysiology of taste and smell. Gerontology 3:131-136.

Mojet J, Christ-Hazelhof E, Heidema J. 2005. Taste perception with age: Pleasantness and its relationships with threshold sensitivity and supra-threshold intensity of five taste qualities. Food Qual Prefer 16:413-423.

Moskowitz H. 1985. New directions for product testing and sensory analysis of food. Food &Nutrition Press.

Moskowitz H. 1980. Psychometric evaluation of food preferences. J Foodservice Syst 1:149-167.

Moskowitz H, Kumaraiah V, Sharma K, Jacobs H, Sharma S. 1975. Cross-cultural differences in simple taste preferences. Science 190:1217-1218.

Murphy C. 1993. Nutrition and chemosensory perception in the elderly. Crit Rev Food Sci Nutr 33:3-15.

Nasrawi C, Pangborn R. 1990. Temporal gustatory and salivary responses to capsaicin upon repeated stimulation. Physiol Behav 47:611-615.

Nayak A. 2008. A physiological model of tea-induced astringency. Physiol Behav 95:290-294.

Naz S, Siddiqi R, Dew T, Williamson G. 2011. Epigallocatechin-3-gallate inhibits lactase but is alleviated by salivary proline-rich proteins. J Agric Food Chem 59:2734-2738.

Neale M, Cardon L. 1992. Methodology for genetic studies of twins and families. Kluwer Academic Publishers, Dordrecht, Germany.

Nederfors T. 2000. Xerostomia and hyposalivation. Adv Dent Res 14:48-56.

Negri R, Di Feola M, Di Domenico S, Scala M, Artesi G, Valente S, Smarrazzo A, Turco F, Morini G, Greco L. 2012. Taste perception and food choices. J Pediatr Gastroenterol Nutr 54:624-629.

Ness A, Powles J. 1997. Fruit and vegetables, and cardiovascular disease: A review. Int J Epidemiol 26:1-13.

Neta E, Johanningsmeier S, Drake M, McFeeters R. 2009. Effects of pH adjustment and sodium ions on sour taste intensity of organic acids. J Food Sci, 74(4), S165-S169.

Nordin, S., Broman, D., Garvill, J., & Nyroos, M. (2004). Gender differences in factors affecting rejection of food in healthy young Swedish adults. Appetite 43:295-301.

Nu C, MacLeod P, Barthelemy J. 1996. Effects of age and gender on adolescents' food habits and preferences. Food Qual Prefer 7:251-262.

Olsen A, Kildegaard H, Gabrielsen G, Thybo A, Møller P. 2012. Measuring children's food preferences: using pictures in a computerized conjoint analysis. J Sens Stud 27:264-276.

Oomah B, Mazza G. 2000. Functional foods. In: Francis J, Ed. Wiley encyclopedia of food science and technology. Wiley, New York, USA. pp. 1176-1182

Peleg H, Noble A. 1999. Effect of viscosity, temperature and pH on astringency in cranberry juice. Food Qual Prefer 10:343-347.

Peleg H. 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. J Sci Food Agric, 79: 1123-1128.

Pelletier C, Lawless H, Horne J. 2004. Sweet–sour mixture suppression in older and young adults. Food Qual Prefer 15:105-116.

Perola M, Tuorila H, 2009. Unpublished.

Peryam D, Pilgrim F. 1957. Hedonic scale method of measuring food preferences. Food Tech11:9-14.

Pennisi E. 2007. DNA forces rethink of what it means to be a gene. Science 316:1556-1557.

Pirastu N, Robino A, Lanzara C, Athanasakis E, Esposito L, Tepper B, Gasparini P. 2012. Genetics of food preferences: A first view from silk road populations. J Food Sci 77:S413-S418.

Platel K, Rao A, Saraswathi G, Srinivasan K. 2002. Digestive stimulant action of three Indian spice mixes in experimental rats. Nahrung 46: 394-398.

Pliner P, Hobden K. 1992. Development of a scale to measure the trait of food neophobia in humans. Appetite 19:105-120.

Prescott J. 1999. The generalizability of capsaicin sensitization and desensitization. Physiol Behav 66:741-749.

Prescott J, Bell G. 1995. Cross-cultural determinants of food acceptability: Recent research on sensory perceptions and preferences. Trends Food Sci Tech 6:201-205.

Prescott J, Laing D, Bell G, Yoshida M, Gillmore R, Allen S, Yamazaki K, Ishii R. 1992. Hedonic responses to taste solutions: A cross-cultural study of Japanese and Australians. Chem Senses 17:801-809.

Prescott J, Stevenson R. 1995. Effects of oral chemical irritation on tastes and flavors in frequent and infrequent users of chili. Physiol Behav 58:1117-1127.

Prescott J, Swain-Campbell N. 2000. Responses to repeated oral irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters. Chem Senses 25:239-246.

Raliou M, Wiencis A, Pillias A, Planchais A, Eloit C, Boucher Y, Faurion A. 2009. Nonsynonymous single nucleotide polymorphisms in human tas1r1, tas1r3, and mGluR1 and individual taste sensitivity to glutamate. Am J Clin Nutr 90:789S-799S.

Raliou M, Grauso M, Hoffmann B, Schlegel–Le-Poupon C, Nespoulous C, Débat H, Belloir C, Wiencis A, Siqoillot M, Bano S. 2011. Human genetic polymorphisms in T1R1 and T1R3 taste receptor subunits affect their function. Chem Senses 36:527-537.

Reed D, Knaapila A. 2010. Genetics of taste and smell: Poisons and pleasures. Prog Mol Biol Transl Sci 94:213-240.

Reed D, Bachmanov A, Beauchamp G, Tordoff M, Price R. 1997. Heritable variation in food preferences and their contribution to obesity. Behav Genet 27:373-387.

Robbins C, Hagerman A, Austin P, McArthur C, Hanley T. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. J Mammal 72:480-486.

Roininen K, Lähteenmäki L, Tuorilla H. 1996. Effect of umami taste on pleasantness of low-salt soups during repeated testing. Physiol Behav 60:953-958.

Roininen K, Lähteenmäki L, Tuorila H. 1999. Quantification of consumer attitudes to health and hedonic characteristics of foods. Appetite 33:71-88.

Rolls E, Scott T. 2003. Central taste anatomy and neurophysiology. In Doty R, Ed. Handbook of olfaction and gustation. Marcel Dekker, New York, USA. pp 679-705.

Roper S. 2007. Signal transduction and information processing in mammalian taste buds. Pflugers Arch 454: 759-776.

Rosenberg M. 1989. Society and the adolescent self-image. Wesleyan University Press, Middletown, UK.

Rossi L, Mazzitelli S, Arciello M, Capo C, Rotilio G. 2008. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. Neurochem Res 33:2390-2400.

Rouseff R. 1990. Bitterness in foods and beverages. In: Rouseff R. Ed. Developments in food science. Bitterness in Foods. Elsevier, Amsterdam, The Netherlands. pp 1-14.

Rozin E. 2000. The role of flavor in the meal and the culture. In: Meiselman H, Ed. Dimensions of the meal. the science, culture, business, and art of eating. pp. 134-142.

Rozin P. 1982. Human food selection: The interaction of biology, culture and individual experience. In: Barker L, Ed. The psychobiology of human food selection. Ellis Horwood Ltd. UK. pp. 225-254

Rozin P. 1991. Family resemblance in food and other domains: The family paradox and the role of parental congruence. Appetite 16:93-102.

Rozin P, Ebert L, Schull J. 1982. Some like it hot: A temporal analysis of hedonic responses to chili pepper. Appetite 3:13-22.

Rozin P, Schiller D. 1980. The nature and acquisition of a preference for chili pepper by humans. Motiv Emotion 4:77-101.

Rozin P. 1986. Food likes and dislikes. Annu Rev Nutr 6:433-456.

Rudney J, Michalowicz B, Krig M, Kane P, Pihlstrom B. 1994. Genetic contributions to saliva protein concentrations in adult human twins. Arch Oral Biol 39:513-517.

Sandell M, Breslin P. 2006. Variability in a taste-receptor gene determines whether we taste toxins in food. Curr Biol 16: R792-R794.

Sandstrom K, Rajan T, Feinn R, Kranzler H. 2003. Salty and sour taste characteristics and risk of alcoholism. Alcohol Clin Exp Res 27:955-961.

Saper C, Chou T, Elmquist J. 2002. The need to feed: Homeostatic and hedonic control of eating. Neuron 36:199-211.

Schafe G, Bernstein I. 1996. Taste aversion learning. In: Capaldi E, Bernstein I, Eds. Why we eat what we eat. The psychology of eating. American Psychological Association, Washington, USA. pp. 31-35.

Schaumburg H, Byck R, Gerstl R, Mashman J. 1969. Monosodium L-glutamate: Its pharmacology and role in the Chinese restaurant syndrome. Science 163:826-828.

Schiffman S, Graham B. 2000. Taste and smell perception affect appetite and immunity in the elderly. Eur J Clin Nutr 54:54-63.

Scoville W. 1912. Note on capsicums. J Am Pharm Assoc 1:453-454.

Settle R, Meehan K, Williams G, Doty R, Sisley A. 1986. Chemosensory properties of sour tastants. Physiol Behav 36: 619-623.

Shanmugam N, Grotton J, Huang D, Jacob J. 2011. Food additives. In: Got food? Recent Advances in Food Science and Technology. An e-book, Massachusetts Academy of Math and Science.pp.148-163.

Sherman P. Billing J. 1999. Darwinian gastronomy: Why we use spices. Bioscience 49:453-463.

Siebert K, Chassy A. 2004. An alternate mechanism for the astringent sensation of acids. Food Qual Prefer 15:13-18.

Siemens H. 1924. Die zwillingspathologie: Ihre bedeutung, ihre methodik, ihre bisherigen. Springer, Berlin, Germany.

Silver W. 2010. Common chemical sense (chemesthesis). In: Goldstein E, Ed. Encyclopedia of perception. SAGE Publications, California, USA. pp. 275-278.

Singh P, Schuster B, Seo H. 2010. Variation in umami taste perception in the German and Norwegian population. Eur J Clin Nutr 64:1248-1250.

Sizer F, Harris N. 1985. The influence of common food additives and temperature on threshold perception of capsaicin. Chem Senses 10:279-286.

Slager S, Huang J, Vieland V. 2000. Effect of allelic heterogeneity on the power of the transmission disequilibrium test. Genet Epidem 18:143-156.

Small D, Gregory M, Mak Y, Gitelman D, Mesulam M, Parrish T. 2003. Dissociation of neural representation of intensity and affective valuation in human gustation. Neuron 39: 701-711.

Sobal J, Bisogni C, Devine C, Jastran M. 2006. A conceptual model of the food choice process over the life course. In Sepherd R, Raats M, Eds. The Psychology of Food Choice. Biddles Ltd. Kin's Lynn, UK. pp. 1-18.

Spielman A. 1990. Interaction of saliva and taste. J Dent Res 69:838-843.

Stevens D, Seifert R, Bufe B, Müller F, Kremmer E, Gauss R, Meyerhof W, Kaupp U, Lindemann B. 2001. Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature 413:631-635.

Stevenson R, Yeomans M. 1993. Differences in ratings of intensity and pleasantness for the capsaicin burn between chili likers and non-likers; implications for liking development. Chem Senses 18:471-482.

Stevenson R, Yeomans M. 1995. Does exposure enhance liking for the chilli burn? Appetite 24:107-120.

StoryM, Neumark-Sztainer D, French S. 2002. Individual and environmental influences on adolescent eating behaviors. J Am Diet Assoc 102:S40-S51.

Strachan T, Read A. 2004. Human molecular genetics. Garland Press, New York, USA.

Szallasi A. 2002. Vanilloid (capsaicin) receptors in health and disease. Am J Clin Pathol 118:110-121.

Tang X, Kälviäinen N, Tuorila H. 2001. Sensory and hedonic characteristics of juice of sea buckthorn (*Hippophae rhamnoides L.*) origins and hybrids. LWT-Food Sci Technol 34:102-110.

Tepper B. 2008. Nutritional implications of genetic taste variation: The role of PROP sensitivity and other taste phenotypes. Annu Rev Nutr 28:367-388.

Tepper B, Christensen C, Cao J. 2001. Development of brief methods to classify individuals by PROP taster status. Physiol Behav 73: 571-577.

Tepper B, Nurse R. 1997. Fat perception is related to PROP taster status. Physiol Behav 61:949-954.

Tuorila H. 1996. Hedonic responses to flavor and their implications for food acceptance. Trends Food Sci Tech 7:453-456.

Tuorila H. 2007. Sensory perception as a basis of food acceptance and consumption. In: McFie H, Ed. Consumer-led food product development. Woodhead Publishing Ltd, UK. pp. 34-65.

Tuorila H, Huotilainen A, Lähteenmäki L, Ollila S, Tuomi-Nurmi S, Urala N. 2008. Comparison of affective rating scales and their relationship to variables reflecting food consumption. Food Qual Prefer 19:51-61.

Tuorila H, Lähteenmäki L, Pohjalainen L, Lotti L. 2001. Food neophobia among the Finns and related responses to familiar and unfamiliar foods. Food Qual Prefer 12:29-37.

Ugawa S, Minami Y, Guo W, Saishin Y, Takatsuji K, Yamamoto T, Tohyama M, Shimada S. 1998. Receptor that leaves a sour taste in the mouth. Nature 395:555-556.

van Dongen P, Draisma H, Martin N, Boomsma D. 2012. The continuing value of twin studies in the omics era. Nat Rev Genet 13:640-653.

van Dongen J, Slagboom P, Draisma, H, Martin N, Boomsma D. 2012. The continuing value of twin studies in the omics era. Nat Rev Genet 13:640-653.

van Duyn, M. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. J Am Diet Assoc 100:1511-1521.

Vidal S, Francis L, Guyot S, Marnet N, Kwiatkowski M, Gawel R, Cheynier V, Waters E. 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J Sci Food Agric 83:564-573.

Vidal S, Francis L, Noble A, Kwiatkowski M, Cheynier V, Waters E. 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal Chim Acta 513:57-65.

Viljakainen S, Visti A, Laakso S. 2002. Concentrations of organic acids and soluble sugars in juices from Nordic berries. Acta Agric Scand Sect B: Soil Plant Sci 52:101-109.

Visscher P. 2004. Power of the classical twin design revisited. Twin Res 7:505-512.

Visscher P. 2008. Sizing up human height variation. Nat Genet 40:489-490.

Voets T, Nilius B. 2003. TRPs make sense. J Membr Biol 192:1-8.

Weiffenbach J, Schwartz L, Atkinson J, Fox P. 1995. Taste performance in Sjögren's syndrome. Physiol Behav 57:89-96.

Westenhoefer J. 2005. Age and gender dependent profile of food choice. Forum Nutr 57:44-51.

Westerterp-Plantenga M, Smeets A, Lejeune M. 2005. Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int J Obes (Lond) 29:682-688.

Whitehead M, Beeman C, Kinsella B. 1985. Distribution of taste and general sensory nerve endings in fungiform papillae of the hamster. Am J Anat 173:185-201.

Williams A, Woessner K. 2009. Monosodium glutamate ‘allergy’: Menace or myth? Clin Exp Allergy 39:640-646.

Wise P. 2007. Twin study of the heritability of recognition thresholds for sour and salty taste. Chem Senses 32:749-754.

Yamaguchi S, Ninomiya K. 2000. Umami and food palatability. J Nutr 130:921S-926S.

Yamamoto S, Nawata E. 2009. Use of *capsicum frutescens L*. by the indigenous peoples of Taiwan and the Batanes islands. Econ Bot 63:43-59.

Yao L, Jiang Y, Shi J, Tomas-Barberan F, Datta N, Singanusong R, Chen S. 2004. Flavonoids in food and their health benefits. Plant Food Hum Nutr 59:113-122.

Yeh Y, Liu L. 2001. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. J Nutr 131:989S-993S.

Yeomans M. 2009. Understanding individual differences in acquired flavour liking in humans. Chemosens Percept 3:34-41.

Zandstra E, de Graaf C. 1998. Sensory perception and pleasantness of orange beverages from childhood to old age. Food Qual Prefer 9:5-12.

Zandstra E, de Graaf C, Van Staveren W. 2001. Influence of health and taste attitudes on consumption of low-and high-fat foods. Food Qual Prefer 12:75-82.

Zellner D, Allen D, Henley M, Parker S. 2006. Hedonic contrast and condensation: Good stimuli make mediocre stimuli less good and less different. Psychonomic Bull Rev13:235-239.

Zhao L, Kirkmeyer S, Tepper B. 2003. A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. Physiol Behav 78:625-633.

Zhu M, Zhao S. 2007. Candidate gene identification approach: progress and challenges. International Journal of Biological Sciences 3:420-427.

Zuk O, Hechter E, Sunyaev S, Lander E, 2012. The mystery of missing heritability: genetic interactions create phantom heritability. Proc Natl Acad Sci 109:1193-1198.