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"Characterisation of selected caloric and non-caloric sweeteners and their interaction with oral and extraoral sweet taste receptors."

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Für meine Lieben...

„When nothing is sure, everything is possible"

~ Margaret Drabble ~

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I. Introduction

1.1. The basics of taste physiology

Taste perception is important for survival of living creatures, including humans, because it contributes substantially to the identification and selection of edible foods. The taste perception predominantly happens in the oral cavity, especially on the tongue where the taste buds on the fungiform, foliate and circumvallate papillae are located. The taste buds are onion-shaped accumulations of 50 - 100 cells of the oral mucosa on the tongue and consist of at least four different types of cells (Murray 1973; Witt and Reutter 2015). Currently, five basic tastes are scientifically accepted in mammals, namely sweet, bitter, sour, salty and umami (Chandrashekar et al. 2006; Chen et al. 2011; Avery et al. 2020). In this respect, Roper and Chaudhari (2017) summarised that bitter, sweet and umami are detected by type II taste cells and sour by type III taste cells. Bitter, sweet and umami are detected by G-protein coupled receptors (GPCR) wherein the current knowledge is that bitter is perceived by 25 known different TAS2Rs, umami by the heterodimeric TAS1R1/TAS1R3 and sweet by the heterodimeric TAS1R2/TAS1R3 receptor (Roper and Chaudhari 2017). Although sweet and bitter taste receptors are present in type II cells, it was suggested that both expressions are not overlapping (Nelson et al. 2001), but so far it remains unclear how the type of receptor is selected by the cell. Type I taste cells express epithelial sodium channels (ENaC) and have been associated with the signal transduction of salty taste (Chandrashekar et al. 2010). The proton channel Otop1 on type III cells was recently identified to mediate sour taste in mice (Tu et al. 2018; Teng et al. 2019; Zhang et al. 2019). Type IV cells are discussed to be the undifferentiated precursor for other taste bud cells and are also known as basal cells (Chaudhari and Roper 2010; Behrens et al. 2011). The cells themselves are connected to afferent nerve fibres, which then trigger action potentials and thus transmit the response of the taste bud to the nervous system (Murray 1973). The taste information is conducted via the various cranial nerves to the tractus solitarius nerve in the brain stem and further to the thalamus. The information is then passed on to the primary gustatory cortex and also to the somatosensory cortex in the cerebral cortex and on to the orbitofrontal cortex. Here, the taste information is linked with the other sensory modalities, such as the sense of smell, resulting in the so-called flavour. Finally, the taste information is passed from the orbitofrontal cortex to the amygdala and the lateral hypothalamus, where it is assigned to hedonic properties (Rolls 2006; Behrens et al. 2011; Witt and Reutter 2015). Regarding the hedonic taste preferences, it is typical that bitter, sour and high amounts of salty taste evoke rejections due to the common toxicity of bitter compounds, sourness as a sign for unripe fruits, and the hyperosmolarity of salts (Oka et al. 2013; Beckett et al. 2014; Zhang, Lee, and Macpherson 2021). In contrast, umami, saltiness and

sweet tastes are evaluated as positive and are therefore also preferred (Birch 1999; Prescott 2001; Venditti et al. 2020).

1.2. Different classes of sweet taste affecting molecules and their structures

The sweet taste impression is favoured in all humans from birth, which has been shown by a pleasant face expression in new-borns (Ganchrow, Steiner, and Daher 1983). The preference of sweet taste is not only innate but also universal and therefore the enjoyment of sweetness is present over all cultures and ages (Drewnowski et al. 2012). From an evolutionary point of view, the sweet taste represents foods high in nutrients and energy, especially carbohydrates and therefore was beneficial in the evolution to survive and for development (Ventura and Worobey 2013; Sylvetsky et al. 2017). The typical caloric sweetener and household-sugar sucrose, with the formula $C_{12}H_{22}O_{11}$, is a disaccharide consisting of the monosaccharides β-D-fructose and α-D-glucose. Sucrose in varying amounts is naturally present in many plants and it plays an important role in agriculture and food processing (Huberlant 2003; Imberti et al. 2019). Typical caloric sweeteners beside sucrose are fructose and glucose, often used as high-fructose-corn-syrup for example in soft drinks (Hanover and White 1993). Sugar is also an important basic ingredient of various diets worldwide (Clemens et al. 2016). The sweet taste of sugar provokes a positive impression, which, in addition to the normal taste transmission, also seems to be related to its physical properties. Sensory quality is also related to hedonic preference as well as consumer acceptance, creating the unique taste impression of sucrose as an energy source (Chandrashekar et al. 2006). Food and beverages containing high amounts of sugar are a relevant source of calories and are preferred for that reason from an evolutionary point of view. Nowadays, the availability and intake of calories is not restricted any more in most of the western world and an excessive consumption of calories, especially sugar, can be associated with a range of unfavourable diseases, such as dental caries, obesity, type 2 diabetes, and the metabolic syndrome (Malik et al. 2010; WHO 2015). Hence, a limitation of consumption of caloric sugar is recommended (Malik et al. 2010), more precise, a reduction to below 10 % of total energy intake and below 5% of free sugars in the diet is currently recommend by the World Health Organization (WHO 2015).

Synthetic and natural sweeteners have been proposed as an alternative to caloric sugar, as they have no or only very low physiological calories. For the majority of sweeteners only a small propotion can be broken down and adsorbed by the human metabolism, therefore the glycaemic load and cariogenic effects are less compared to sucrose (Hayes 2001; Edwards et al. 2016; Sylvetsky et al. 2017; Nichol, Holle, and An 2018). Non-caloric sweeteners can reduce the calories

of nourishment, and therefore in diet, leading to a reduction of body weight (Fernstrom 2015). However, although a worldwide increase in sweetener consumption occurred, the prevalence for obesity or type-2 diabetes has not decreased. Without reducing the total calorie intake, the consumption of sweeteners is not sufficient to reduce body weight, which is why a high sugar intake is not the only factor in the development of obesity (Mattes and Popkin 2009). Furthermore, Frank et al. (2008) showed, that the brain response differed between caloric sucrose and non-caloric sucralose, although the subjects were not able to discriminate consciously between the tastes of the two sweet compounds (Frank et al. 2008).

There is an immense variety of alternatives to sucrose, ranging from sugar alcohols to synthetic and natural sweeteners and sweet taste enhancers (Fujimaru, Park, and Lim 2012; O´Brien-Nabors 2016; Mooradian, Smith, and Tokuda 2017; Tan et al. 2019). Sugar alcohols such as xylitol, erythritol, sorbitol or maltitol have a reduced blood glucose response and with that less calories because they are hardly cleaved and less adsorbed in the intestine, but an intestinal fermentation by gut microbiota is possible as well (Payne, Chassard, and Lacroix 2012). The sweetness of sugar alcohols is mostly lower than sucrose and therefore they are often used in combination with other sweeteners to maintain the texture of food (Grembecka 2015). The first synthetic sweetener developed was saccharin, an o-sulphobenzoic imide, in 1879 by Constantin Fahlberg, while commercial production started in the late 19th century (Bart 1968; Cohen 1986; Miller and Frattali 1989). Saccharin was followed by cyclamate, a cyclohexylsulfamic acid, which was discovered in 1937 (Sylvetsky and Rother 2016; O´Brien-Nabors 2016). Since then, several other sweeteners were developed and allowed for usage in the EU, although there are many other sweet tasting compounds from various chemical classes known. In addition to saccharin and cyclamate, these approved sweeteners also include sucralose, a trichlorinated disaccharide of fructose and galactose (Schiffman and Rother 2013), aspartame, consisting of the two amino acids aspartate and phenylalanine, acesulfame K (6-methyl-1,2,3-oxathiazin-4(3H)one-2,2-dioxide), neohesperidin dihydrochalcone, neotame (N-(3,3-dimethylbutyl)-L-aspartic acid + methyl L-phenylalanate), advantame, a N-substituted aspartame derivate, and the protein thaumatin (O´Brien-Nabors 2016; Mooradian, Smith, and Tokuda 2017). These named sweeteners are all permitted in the European Union (Commission Regulation (EU) No 1129/2011) and tested for safety by EFSA and SCF, which was summarized by Mortensen (2016). However, there are regional differences in the authorization of sweeteners, for example cyclamate is not permitted in the USA (FDA 1989). In addition to synthetic sweeteners, the demand for natural-occurring sweeteners has also increased due to increased interest of consumers in green-labelled products (Sylvetsky and Rother 2016). Very popular and in the EU accepted natural sweeteners are steviolglycosides and rebaudiosides from *stevia rebaudiana*, such as rebaudioside A (Reb A) (Prakash et al. 2008;

EFSA 2010; Majchrzak, Ipsen, and Koenig 2015). The alternative sweetener luo han guo extracts, also known as mogroside V from monk fruit, is not allowed in the EU so far, but it is assigned as generally recognised as save ("GRAS") in the USA (Mooradian, Smith, and Tokuda 2017).

Another way to maintain the sweet taste despite a reduction of the sugar content is the use of sweet modulating compounds. These sweet modulating compounds are also known as positive allosteric modulators, PAMs (Servant et al. 2010). In the concentrations used, PAMs taste not or hardly sweet at all, but they enhance the sweet taste of other sweeteners or sugars (Servant et al. 2010; Zhang et al. 2010; Servant et al. 2020). In the concept of synergism by PAMs, two or more molecules do interact simultaneously with the sweet taste receptor and the induced sweet intensity is greater than the simple sum of sweetness of each molecule alone (Schiffman, Booth, Carr, et al. 1995; Schiffman et al. 2000; Reyes, Gravina, and Hayes 2019). For example, neohesperidin dihydrochalcone (NHDC) is a well-known synergist of sweetness and the combination with other sweet compounds as sucrose, sugar alcohols, aspartame, acesulfame K (ace K), saccharin, cyclamate and thaumatin, while both compounds were used in a concentration being equi-sweet to 3 % sucrose led to synergistic sweet intensity. Besides, also steviosides have been described as a good sweet synergists (Schiffman, Booth, Carr, et al. 1995). Hypotheses for this effect are that synergy occurs by binding of sweeteners to different binding sites and further that structurally different sweet compounds act better as PAM due to binding at distal or overlapping binding sites (Reyes, Gravina, and Hayes 2019).

In general, the effects of synthetic and natural sweeteners on consumption, preferences and physiology are controversial and do vary a lot, depending on the type of sweetener. Each sweet compound has its particular chemical structure and therefore own metabolism and other downstream effects. The effects on taste, appetite, BMI, hormone release, microbiota, and many more, are as unique as the their structures are (Hunter et al. 2019). However, the structures of sweet taste affecting compounds and PAMs can be very diverse, and they also belong to very different structural classes. For example, sweet dihydrochalcones such as NHDC, rebaudiosides such as Reb A and Reb M, sweet amino acid derivates such as aspartame and neotame and also sweet proteins such as thaumatin and brazzein. Nevertheless, all these different structures can bind to the canonical human sweet receptor TAS1R2/R3 and thus induce a sweet taste.

1.3. Perception of sweet taste

1.3.1. The sweet taste receptor & signal transduction

In the early 2000´s scientists identified the receptor that is responsible for the perception of sweet taste, the TAS1R2/TAS1R3 (Montmayeur et al. 2001; Max et al. 2001; Nelson et al. 2001; Li

et al. 2002). About five years later, it became known that this receptor is present not only in the papillae of the tongue, but also in other tissues. These extraoral receptors are for example in the hypothalamus (Ren et al. 2009) and in the gastrointestinal tract, where the receptor is supposed to contribute to sugar perception and absorption (Dyer et al. 2005; Sutherland et al. 2007). The human heterodimeric sweet taste receptor belongs to the class-C type G-protein coupled receptors (GPCR) and consists of the two subunits TAS1R2 and TAS1R3. The two subunits of the sweet taste receptor each comprise of three domains, a large extracellular amino terminal domain, also called venus flytrap domain (VFD), linked to a cysteine-rich domain (CRD) and thereafter to a seven helical transmembrane domain (TMD) (Pin, Galvez, and Prézeau 2003; DuBois 2016; Chéron et al. 2016), see Figure 1. The sweet taste receptor was first discovered and described by multiple groups and is located on type II taste bud cells (Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Li et al. 2002). Since then, Nango et al. (2016) were able to purify the recombinant TAS1R2/TAS1R3 VFT domains from medaka fish (*Oryzias latipes*), but to date, the crystallographic structure of the human sweet taste receptor remains unknown. However, it is assumed, that the G-protein is only coupled to one subunit, as it is common for class-C GPCR heterodimeric and homodimeric receptors (Pin and Bettler 2016; Pin et al. 2019).

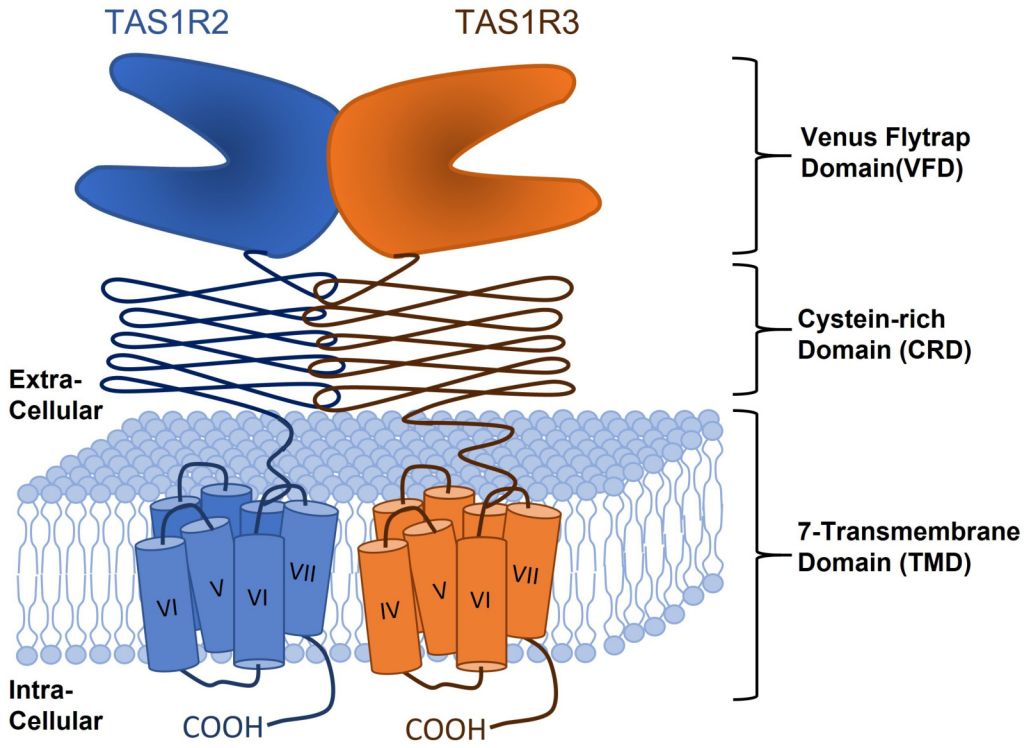

Figure 1: The structure of the sweet taste receptor TAS1R2/R3 created according to DuBois (2016)

After a sweet compound binds to the receptor, a conformational change occurs and the coupled G-protein dissociates in two subunits, α- and β/γ-gustducin (Margolskee 2002; Pérez et al. 2002),

see Figure 2. On the one site, the α-subunit activates the adenylyl cyclase (AC), which facilitates the conversion of adenosine triphosphate (ATP) to cyclic AMP (cAMP) (Striem et al. 1989). The generated cAMP activates protein kinase A (PKA) which causes the closure of the K^+-channels, leading to a stop of K^+-flow out and with that to a depolarisation of the cell (Avenet, Hofmann, and Lindemann 1988; Margolskee 2002). On the other side, the β/γ-gustducin subunit activates the phospholipase Cβ2 (PLC), which enables the hydrolysation of phosphatidylinositol-4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3) (Rössler et al. 1998; Behrens and Meyerhof 2011). DAG activates the protein kinase C (PKC) (Berridge and Irvine 1984), which causes the closure of the K^+-channels, similar to PKA by phosphorylating the channel (Margolskee 2002). Furthermore, IP3 affects the release of Ca^{2+} from the endoplasmic reticulum (Clapp et al. 2001), which leads to the opening of TRPM5, a transient receptor-potential channel, following by an influx of cations into the cell (Pérez et al. 2002).

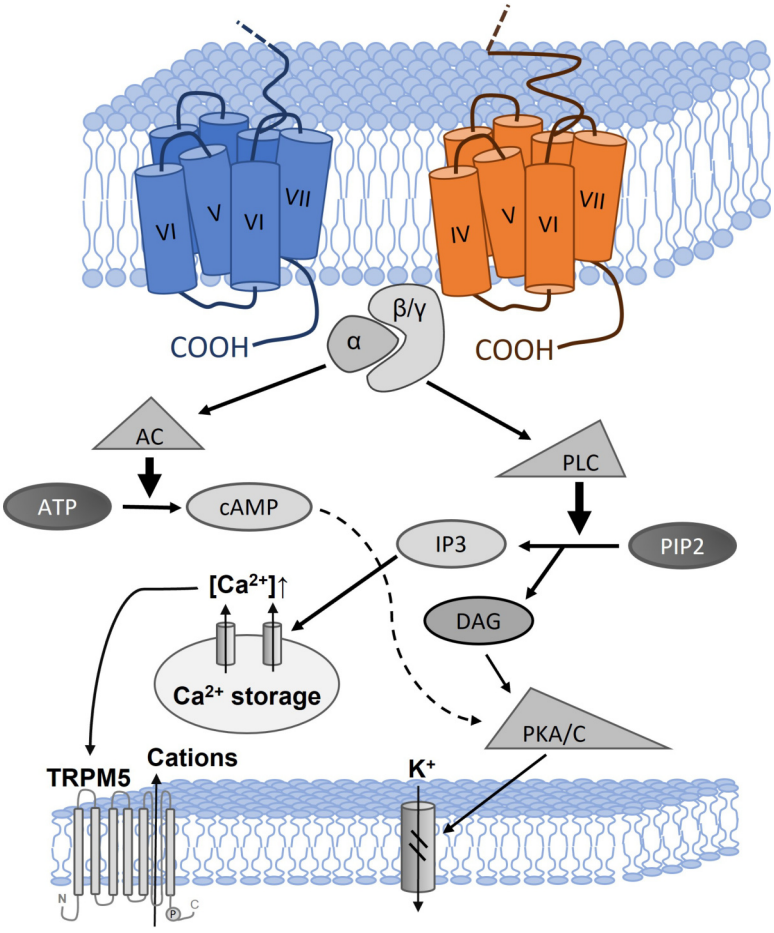

Figure 2: Schematic signal transduction after activation of the G-protein coupled sweet taste receptor TAS1R2/R3

Overall, the increase of cations in the cell leads to the depolarisation and to the signal transduction of the cell (Margolskee 2002). The depolarisation can in turn release ATP via the CALHM1/3 channel (Taruno et al. 2013). Bernhardt et al. (1996) hypothesized that sugars and

artificial sweeteners do involve different parts of the signal transduction and use different Ca^{2+} sources (Bernhardt et al. 1996), however this theory could never be fully proven and was not further considered. The down-signalling pathway including the signalling molecules was also recently summarized by vonMolitor et al. (2021) and they concluded as well the possible involvement of several G-proteins and different pathways in sweet taste transduction. Up to now it remains unclear, how the huge variety of sweeteners and their differences in the sensory properties are recognised by the sweet taste receptor (Masuda et al. 2012; Chéron et al. 2016).

In addition to the known and accredited sweet taste receptor and its signalling pathway, a sweet taste receptor independent pathway has been proposed (Damak et al. 2003; Yee et al. 2011; Sukumaran et al. 2016; Yasumatsu et al. 2020). Damak et al. (2003) was able to show in mice that taste preference for sucrose was reduced but not abolished by TAS1R3 knock-out, by contrast the preference of artificial sweeteners was repealed. These effects were seen in drinking preference and as well in nerve responses (Damak et al. 2003). On that basis, Yee et al. (2011) were able to show that taste cells exhibiting the TAS1R3 subunit express glucose transporters (GLUT) and ATP-sensitive K^+-channels. These markers usually serve as marker for the metabolic status. By applying quantitative RT-PCR, in situ hybridisation and immunohistochemistry, Yee et al. found GLUT8, GLUT9B, SGLT-1, the inward K^+- channel (Kir) 6.1, subunits of the ATP-gated K+ (K_{ATP}) metabolic sensor and the sulfonylurea receptors (SUR) 1 and 2A on taste cells. Furthermore, Glut4, SGLT-1 and SUR 1 were located on the taste cells, which also have the TAS1R3 subunit. These glucose sensors and K_{ATP} could serve as mediators of the sweet taste of monosaccharides independently of the sweet receptor (Yee et al. 2011). In addition, Sukumaran et al. (2016) detected the expression of several α-glycosidases, for example amylase, as well as disaccharide-hydrolysing enzymes, for example maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase by RT-PCR, in situ hybridisation and immunohistochemistry. These enzymes can hydrolyse disaccharides, oligosaccharides, and long-chain sugars to monosaccharides. The monosaccharides can pass through GLUT and SGLT-1 transporters and be metabolised to ATP to close the K_{ATP} -channel and activates the TAS1R-independent sweet perception (Sukumaran et al. 2016). This is confirmed as tongue treatment of wild-type C57BL/6 and TAS1R3-knock-out mice with the α-glucosidases inhibitors miglitol (500 µM) or voglibose (10 µM) significantly reduced the nerve response to disaccharides, but not monosaccharides or sweeteners (Sukumaran et al. 2016). Yasumatsu et al. (2020) confirmed the involvement of SGLT-1 and its sodium dependence in sugar perception by taste nerve recordings in wild-type and TAS1R3-knock-out mice and behavioural response measurements in TAS1R3-KO mice using the SGLT-1 inhibitor phlorizin. Hereby, the nerve-responses to glucose and sucrose were enhanced by natrium chloride (10 mmol) and this effect could be eliminated by phlorizin. Additionally, the total amount and the number of licks of a

glucose-solution was enhanced with natrium chloride and supressed by phlorizin (Yasumatsu et al. 2020). These results were supported by the investigations of Breslin et al. (2021), who compared sensory detection thresholds of glucose, fructose, the non-metabolizable glucose analog α- methyl-D-glucopyranoside and sucralose with and without phlorizin, lactisole or NaCl. They verified that lactisole reduced more the sensibility of sucralose than for glucose. Furthermore, in combination with NaCl glucose showed a reduced threshold, but the sucralose detection was decreased. Phlorizin decreased the detection of glucose more than the detection of sucralose. These results reinforced the hypothesis of a second detection mechanism over glucose transporters for glucose as sweet compound (Breslin et al. 2021).

1.3.2. TAS1R2/TAS1R3 binding sites and compounds

The activation of the sweet taste receptor is not only achieved by the typical mono- and disaccharides, but as well by diverse sweet compounds, for example out of the group of amino acids, proteins, sugar-alcohols and diverse artificial sweeteners (McCaughey 2008). To transmit the sweet taste, substances must first bind to the receptor. There are multiple binding sites on the sweet receptor TAS1R2/R3 to which sweet compounds with diverse structures can bind (Cui et al. 2006; Masuda et al. 2012; DuBois 2016; vonMolitor et al. 2021), see Figure 3.

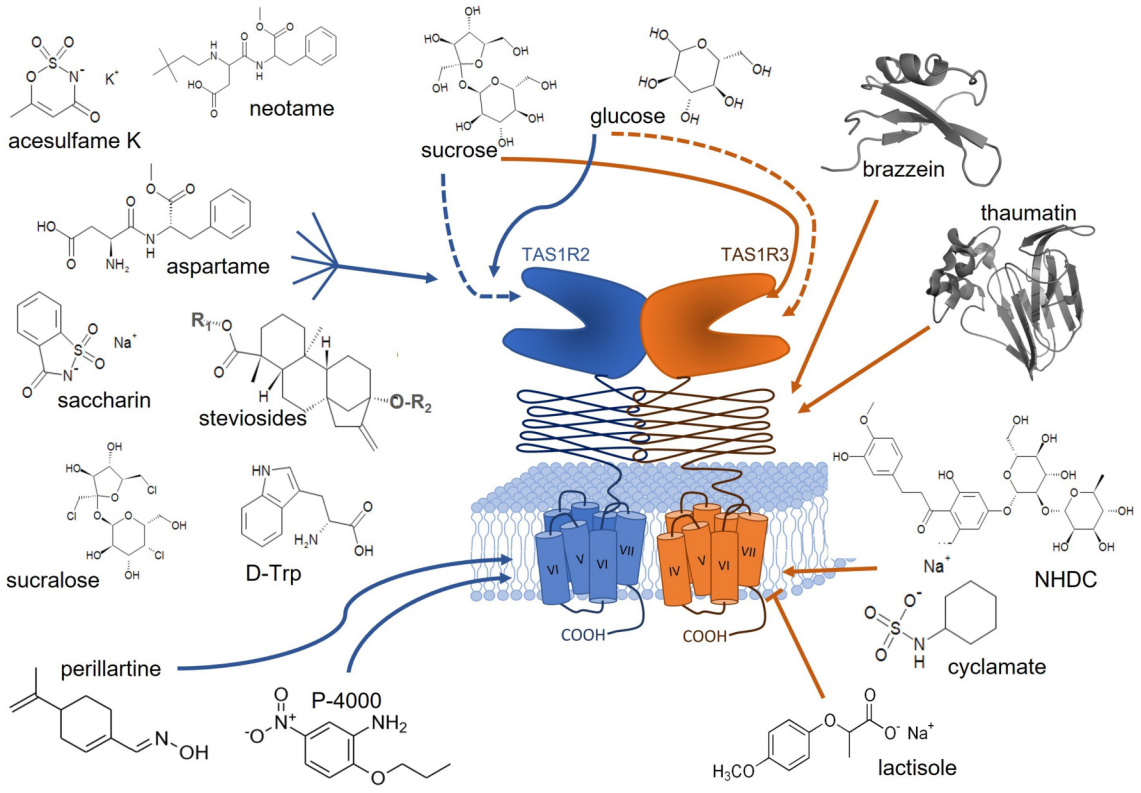

Figure 3: Known binding sites at the TAS1R2/R3 with example compounds, adapted to DuBois (2016)

Sucrose and glucose are known to bind at the VFD of TAS1R2 and TAS1R3, with a higher affinity of sucrose to TAS1R3 and of glucose to TAS1R2. As sucrose is known to have a higher sweetness power than glucose when applied in the same concentration, it is suggested that TAS1R3 causes a more efficient interaction with the compounds, leading to a stronger perception of sweetness (Nie et al. 2005). Beside the sweet carbohydrates, also high potent sweeteners bind to the VFD of TAS1R2, namely aces K, aspartame, neotame, saccharin, steviosides, sucralose and D-tryptophan (Xu et al. 2004; Jiang, Cui, Ji, et al. 2005; Nie et al. 2005; Galindo-Cuspinera et al. 2006; Zhang et al. 2010; Masuda et al. 2012; Maillet et al. 2015; DuBois 2016; Servant et al. 2020). Contrary, the sweeteners cyclamate and neohesperidin-dihydrochalcone (NHDC) were identified to bind at the TMD of TAS1R3 (Jiang, Cui, Zhao, Snyder, et al. 2005; Winnig et al. 2007; Fujiwara et al. 2012; DuBois 2016; Servant et al. 2020). The binding to the TMD of TAS1R2 was discovered for the sweet compounds perillartine and P-4000 (Servant et al. 2020). Furthermore, the larger sweet proteins brazzein and thaumatin were examined to bind at the CRD of TAS1R3 (Jiang et al. 2004; Masuda et al. 2013) and monellin at the VFD of TAS1R2, but outside of the pre-known binding-site of small sweeteners (DuBois 2016).

For the identification of specific binding sites, a combination of different approaches can be used, including chimeric human-mouse receptor combinations, specific point mutations on the receptor, together with measurement of receptor activation via Ca^{2+} release in transfected HEK-293 cells, homology modelling and molecular docking (Winnig et al. 2007; Masuda et al. 2012; Maillet et al. 2015). Beside the activation of the sweet taste receptor by binding of various compounds, several sweet taste inhibitors have also been identified. The most prominent sweet taste inhibitor is lactisole, which targets the TMD of TAS1R3 (Jiang, Cui, Zhao, Liu, et al. 2005; Winnig, Bufe, and Meyerhof 2005). In addition to the sweet inhibitory effects of lactisole, a sweet aftertaste has been identified, also known as “sweet water taste” by Galindo-Cuspinera et al. (2006). This sweet water effect is not only seen for the inhibitor lactisole, but as well for ace K, which was found to have a low affinity binding site at the TMD of TAS1R3 effecting inhibition of sweetness at high concentrations. Furthermore, Galindo-Cuspinera et al. (2006) suggest that sweet taste inhibitors can be identified through their sweet water taste (Galindo-Cuspinera et al. 2006). However, it remains unclear, how the binding site per se and binding activity affects the sensory perception. So far, the latest investigations to combine binding activity in cells and sensory evaluations were conducted by Choi et al. (2021), showing that sweet potency correlated with EC_{50} calculations of an affinity assay with TAS1R2/TAS1R3 transfected HEK-293 cells. Here, sensory potency of sweeteners was only summarised and not investigated and comparisons of more sweet attributes with cell experimental results are still missing.

Since not every individuum is and reacts exactly the same, Waksmonski and Koppel (2016) summarised that the perception of sweeteners can vary individually, as specific binding sites can be altered by different genetic variations in the sweet receptor. By changing the binding site, the sensitivity to a sweetener can be altered. Theoretically, it is possible to have hundreds of single nucleotide polymorphisms (SNP) in TAS1R2/TAS1R3 which may be more or less common depending on the location. However, only a few polymorphisms finally lead to changes of amino acids and only a small part of them do actually alter a potential binding-site. In many cases, the VFT of the TAS1R2 subunit contains variations. Accordingly, a sweetener could taste differently sweet for each person, depending on its binding site in the sweet receptor and genetic make-up (Waksmonski and Koppel 2016).

Relevant characteristics of the compounds for binding to the sweet taste receptor and eliciting sweet taste are, for example, their solubility (Zhong et al. 2013) and hydroxyl groups (Clemens et al. 2016). For steviol glycosides, the glycone chain length and pyranose substitution may have an influence on the sweet taste profile (Hellfritsch et al. 2012). In general, any kind of prediction model needs to be validated by sensory experiments to get insights into the complex sensory system and individual perception, not only in sweetness but also in side-tastes, flavour and overall perception.

1.3.3. Differences in sensory perception

Although all sweet tasting compounds can interact with the canonical sweet taste receptor and convey a sweet taste, the described overall sweet taste impression differs. There are significant differences of the diverse sweet tasting compounds, not only in structure but above all in sensory impression (Masuda et al. 2012; DuBois 2016; Chéron et al. 2016; Reyes, Castura, and Hayes 2017). First, the potency of sweeteners varies a lot. Comparing the sweetness to the obligatory household-sugar sucrose (sweetness= 1) the relative sweetness of fructose is slightly sweeter (1.2 - 1.7) and glucose is less sweet (0.5 - 0.6) (DuBois 2016; Sylvetsky et al. 2017). Sweeteners are often also named as high-potency sweeteners, hence the potency of sweetness is extremely increased compared to sucrose, for example Reb A with a relative sweetness of 300 - 440 times to sucrose, sucralose 220 - 1900 (Fujimaru, Park, and Lim 2012), AceK 130 - 200, Cyclamate 22 - 32, Saccharin 280 - 500 (Ketelsen, Keay, and Wiet 1993; Schiffman, Booth, Losee, et al. 1995) and aspartame 90 - 200 (Gwak et al. 2012). The most potent sweeteners include neotame, which is around 7 000 - 13 000 times sweeter than sucrose and structurally related to aspartame, and advantame with a potency of approximately 20 000, summarized by Mooradian, Smith, and Tokuda (2017). It has to be noticed, that the relative sweetness always depends on the compound and concentration used for comparison, mostly the sweetness that is to be achieved compared to

a specific concentration of sucrose (Schiffman, Booth, Losee, et al. 1995; Fujimaru, Park, and Lim 2012). Thus, the reported relative sweetness may vary a lot in different studies. In general, the sweetness of a compound is often rated on scales (Bartoshuk et al. 2002), while comparison of sweetness is investigated by 2-AFC-tests, to see if differences in sweetness are detectable (Gwak et al. 2012).

The second difference between sweet compounds is the variability in their temporal behaviour (Ketelsen, Keay, and Wiet 1993; Tan et al. 2019). Hereby the terms onset and lingering are commonly used to describe these differences. The onset, also called appearance time, explains the time until a compound reaches its maximum taste sensation. The term lingering is used for the time of the long lasting of the sensation in the mouth and is also called extinction time (DuBois et al. 1977), see Figure *4*. A typical method to evaluate the temporal parameters is the time-intensity measurement. With this method Ott, Edwards, and Palmers (1991) could show that ace K has a faster onset, while Prakash et al. (2008) showed for Reb A a delayed onset, and as well a prolonged lingering effect. Also, NHDC is known to have an enhanced onset and lingering behaviour (DuBois and Lee 1983).

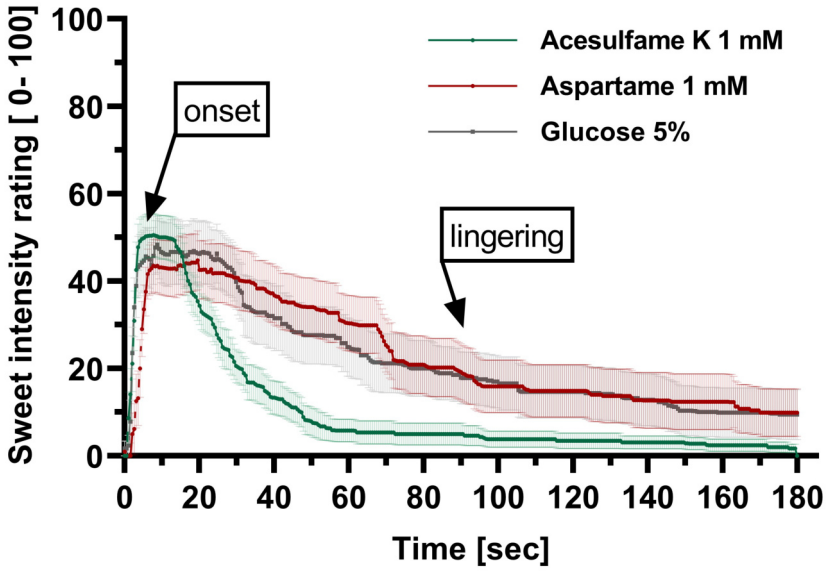

Figure 4: Time-intensity profile of 5% glucose (277.5 mM), 1 mM acesulfame K and 1 mM aspartame, presented as mean ± SEM with onset as first max. sweetness and lingering as long-lasting sweetness (own unpublished data).

Third, not only differences in the sweet taste per se, but as well side-tastes are present, especially in sucrose alternatives. Common side-tastes are bitterness, liquorice, astringency, metallic, or cooling impressions (DuBois 2016; Reyes, Castura, and Hayes 2017; Tan et al. 2019). To compare several tastes and impressions of a compound simultaneously, the temporal check-all-that-apply method (TCATA) is an adequate method (Reyes, Castura, and Hayes 2017; Tan et al. 2019), but as

well the methods temporal dominance of sensation (Pineau et al. 2009) and rating on labelled scales (Bartoshuk et al. 2002; Fujimaru, Park, and Lim 2012) are used to describe more than one sensory attributes of a compound. Reyes, Castura, and Hayes (2017) described that in comparison to sucrose and other carbohydrate sweet compounds, the non-nutritive sweeteners exhibit more side tastes. They investigated the synthetic sweeteners ace K, aspartame, NHDC, sucralose, and saccharin to have more bitter, metallic, drying, and cooling attribute ratings and the natural sweeteners Reb A and thaumatin, and to have an additional higher liquorice attribute rating (Reyes, Castura, and Hayes 2017). Furthermore, Tan et al. (2019) showed an enhanced long-lasting of bitter, metallic and chemical side tastes of ace K and Reb A but with that less sweetness. The most similar taste profiles were shown for nutritive sweeteners and sugar alcohols such as xylitol, maltitol, fructose and glucose (Tan et al. 2019). Compared to Reb A, Reb M has less unpleasantly rated attributes but with similar sweetness. Compared to the sweetener aspartame it showed similar negative side tastes but enhanced sweet lingering (Prakash, Markosyan, and Bunders 2014). It has been hypothesised that an interaction with the umami receptor is one reason for the unpleasant aftertaste of sweet compounds (Acevedo and Temussi 2019). Furthermore, for the astringent sensation, a mechanism of aggregation and dissociation of the salivary protein MUC 1 has been proposed (Canon et al. 2021).

So far, it is still not clarified where all the differences in sweet perception come from. Several variables are known that can be involved in the overall impression of sweeteners. Especially differences in saliva and mouthfeel are currently under investigation and described in more detail in the following chapter 1.3.4 “Effects on saliva and mouthfeel”.

1.3.4. Effects on saliva and mouthfeel

Saliva has various roles in the oral cavity, including the interaction with food ingredients so that substances are dissolved and can be transported to the receptors. The so-called mouthfeel of sensory perception includes the trigeminal sensations and texture beside the perception of aroma and taste (Matuso 2000; Guest et al. 2008; Neyraud 2014; Muñoz-Gonzalez, Feron, and Canon 2018). The secretion of saliva occurs mainly by the submandibular, sublingual and parotid glands, see Figure 5, and some minor glands, including the Ebner glands in the crevice of foliate and circumvallate papillae (Matuso 2000; Neyraud 2014; Varga 2015).

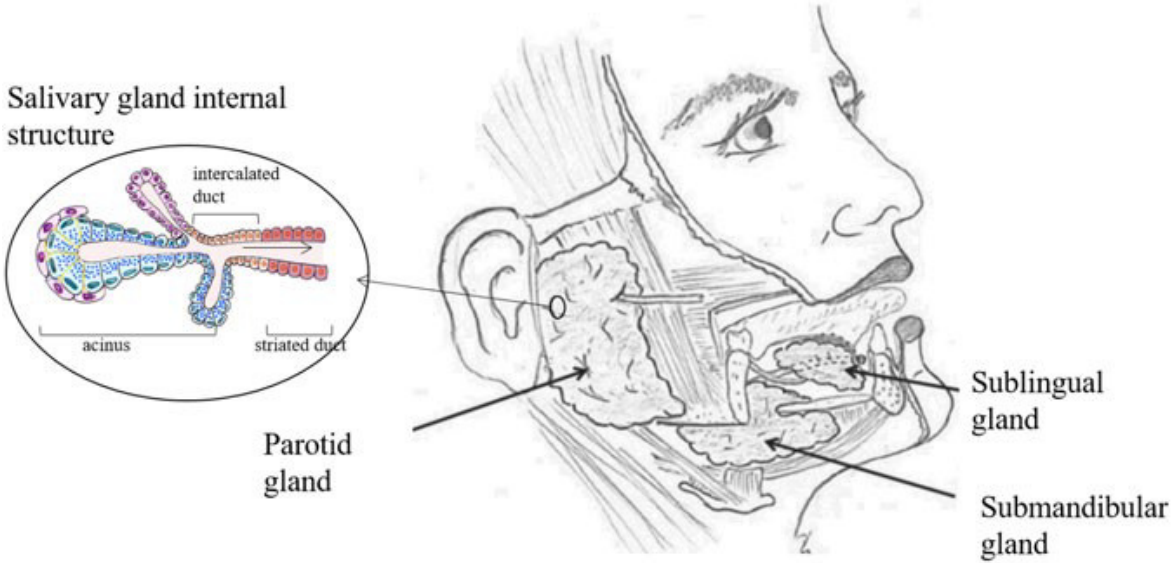

Figure 5: The salivary glands, their locations in human and internal structure. Adapted from Varga (2015) and Xu, Laguna, and Sarkar (2019)

Muñoz-Gonzalez, Feron, and Canon (2018) summarised that salivary composition and flow depend not only on body weight and endogenous factors, such as age, sex, circadian rhythms and some disease states, but also on exogenous factors, for example diet and pharmacological agents. Beside these factors, the salivary flow, as well as protein composition, can be affected by taste (Neyraud et al. 2009). Hence, tastants are able to stimulate salivary flow with citric acid being the most powerful stimulator (Neyraud et al. 2009; Bader et al. 2018; Pedersen et al. 2018). Not only sour taste and chewing is associated with salivary flow, but there are also data for the stimulation by sweet taste (Bonnans and Noble 1995; Neyraud et al. 2009; Satoh-Kuriwada et al. 2018). So far, findings on the effects of salivary flow rate on the perception of food in the mouth and chemosensory perception are diverse (Neyraud 2014). Related to this, the sensory differences of several sweet tasting compounds have not been completely clarified yet. Compared to sugar-sweetened products, sweeteners are often defined to lack a certain mouthfeel (DuBois 1993; Muir et al. 1998; Arazi, Kilcast, and Marrs 2001; Kappes, Schmidt, and Lee 2006). The term mouthfeel is used to describe the entire tactile impression that occurs in the oral cavity in addition to the basic tastes and trigeminal stimuli. The underlying mechanisms that lead to a certain mouthfeel are not fully understood yet, but it is assumed that the formation of the salivary pellicle plays a role, which depends among others on the secretion (flow rate) and composition of the saliva (Guest et al. 2008; Muñoz-Gonzalez, Feron, and Canon 2018; Neyraud and Morzel 2019). Saliva is involved not only in the transportation of flavours, but also in modifying the texture of food. The mechanical chewing process, but also the proteins and enzymes of saliva, which may interact with the ingredients of the food, result in changes in texture (Davies, Wantling, and Stokes 2009). However, the impact of different stimuli on

viscoelastic properties of saliva is not completely understood yet and results are inconsistent here. Davies, Wantling, and Stokes (2009) investigated the rheological properties of saliva after consumption of various drinks and chewing gum. In particular, the consumption of acidic drinks such as cola and iced tea increased the flow of saliva and raised the elasticity of saliva compared to water. This increased salivary elasticity was associated with a negatively associated dry mouth reported by the subjects (Davies, Wantling, and Stokes 2009). Depending on the secreting gland and the used stimulus, more elastic saliva with high-molecular proteins (submandibular and sublingual) or relatively inelastic saliva with a lower protein content (parotid) is secreted (Van der Reijden, Veerman, and Nieuw Amerongen 1993; Stokes and Davies 2007). Chewing thereby excites more the parotid gland and stimulation by tastes excites more the submandibular and sublingual glands (Stokes and Davies 2007). The assumption that higher viscosity of saliva leads to reduced transporting properties was supported by a reduced mixing efficiency in more elastic saliva (Baines and Morris 1987). Thereby a reduced perceived saltiness was determined due to polysaccharides released by salivary amylase and thus reduced mixing efficiency (Ferry et al. 2006). Contrary, this theory was not supported by the findings of Marcinkowska-Gapińska et al. (2018). They investigated thresholds by electro-gustometry and collected stimulated saliva of 27 participants for rotary-oscillating rheometer measurement. Concerning this, no correlation was found between electro-gustometrically determined taste threshold and viscosity values (Marcinkowska-Gapińska et al. 2018). It has to be noted that in the study of Marcinkowska-Gapińska et al. no tastings were performed to determine the thresholds and the effect of rheological properties on taste perception therefore needs further clarification. However, it is known that the failure to build an ideal salivary pellicle is related to less elastic and consequently more viscous saliva (Xu, Laguna, and Sarkar 2019). The oral pellicle has a two-layer structure, with MUC5B being the main protein in the outer layer and other salivary compounds to stabilise the pellicle (Boyd et al. 2021). Beside its importance for protection and lubrication of the oral surface, salivary proteins and other compounds can interact with taste molecules present in mouth (Morzel et al. 2014; Hannig et al. 2017). An interaction of compounds and taste molecules has been suggested for example for astringent sensation, for instance with tannins (Ployon et al. 2018; Canon et al. 2021). Furthermore, the salivary proteome, especially amylase and cystatins, can be related to sweet taste sensitivity and is also partly dependent on gender (Rodrigues et al. 2017). Additionally to the parameters of saliva and oral pellicle itself, the oral microbiota has been suggested to have an influence on the interaction of taste perception, salivary parameters and mouthfeel (Cattaneo, Gargari, et al. 2019; Esberg et al. 2020). The ecology of the oral microbiota is not only related to the consumption of sugar, but has as well an impact on the taste phenotype of the host through allelic variations in the GNAT3 and TAS1R1 (Esberg et al. 2020). A relation of

oral microbiota to taste sensitivity could be shown for PROP-status (Cattaneo, Gargari, et al. 2019) and sweet threshold (Cattaneo, Riso, et al. 2019), but in general the data of oral microbiota related to sweet taste perception is rare.

So far, the findings to the effects of salivary flow rate on taste perception, especially sweet taste, are diverse (Neyraud 2014). Although previous studies suggest an important role of flow rate, viscoelastic properties and proteome, to date, it is not clear how different sensory characteristics of sweet-tasting compounds contribute to salivary flow, salivary viscoelasticity, and related salivary components as part of mouthfeel and overall taste perception.

1.4. Effects of oral sweet taste perception on blood glucose metabolism

Sweet compounds not only act as tastants in our mouth. After swallowing, they can have an impact on nearly all parts of the body, depending on their metabolism, transportation, and interactions, which is strongly specific for the huge variety of compounds. It is known that the sweet taste receptor is, additional to its occurrence in the oral cavity, also present in extraoral tissues such as the brain (Ren et al. 2009), adipose tissue (Masubuchi et al. 2013), the pancreas (Henquin 2012) and gastro-intestinal tract (Bezençon, le Coutre, and Damak 2007; Jang et al. 2007). Therefore, the usage of sweeteners with no or reduced calories does not only elicit a sweet taste perception, but it is assumed that they can also interact with sweet taste receptors present in extraoral tissues such as the stomach (Hass, Schwarzenbacher, and Breer 2010), the small intestine (Dyer et al. 2005), the colon (Rozengurt et al. 2006) or the pancreas (Henquin 2012). Due to this interaction of sweet compounds with the TAS1R2/R3 there is the possibility of interaction with regulation on blood glucose-levels during consumption and digestion, summarised by Renwick and Molinary (2010) and Hunter et al. (2019). In this context, effects of sweet tasting compounds on glucose homeostasis and related hormones have also been shown before (Jang et al. 2007; Margolskee et al. 2007; Just et al. 2008; Kojima et al. 2015; Lee and Owyang 2017), which is described in more detail in the following.

In general, the regulation of blood glucose is a complex interplay involving not only the intestine and pancreas, but also the liver, muscles, adipocyte tissue, and the brain with an impact of various neuropeptides and hormones such as insulin, glucagon and glucagon-like peptide 1 (GLP-1), summarized by Röder et al. (2016). It is known that there are differences between caloric sweet compounds in their effects on blood glucose metabolism. For example, fructose showed different metabolic effects than glucose, inducing lower secretion of insulin (Curry 1989; Teff et al. 2004) and the liver is responsible for metabolising fructose (Mayes 1993). Moreover, the same amount of sucrose and glucose led to different peaks in blood glucose level (Crapo et al. 1982).

Dalenberg et al. (2020) concluded that the sweetener sucralose or carbohydrate alone do not compromise the sensitivity for sweetness, but the combination of both (Dalenberg et al. 2020). Furthermore, it has been shown that beside the sodium-dependent transporter of glucose (SGLT-1), also α-gustducin and the sweet taste receptor subunit TAS1R3 are involved in the intestinal glucose sensing. Glucose absorption as well as sucralose consumption did induce a release of GIP and GLP-1 in GLUTag cells (Margolskee et al. 2007). A high carbohydrate diet in wild type mice, but not in TAS1R3-knock-out mice, led to an enhanced SGLT-1 expression in the intestine. In addition, the sweeteners ace K and saccharin, not aspartame, could as well increase mRNA expression of SGLT-1 in the intestine of wild-type mice (Margolskee et al. 2007). This leads to the hypothesis that TAS1R2/R3 ligands could also have an impact on blood glucose metabolism, beside the common sense of today that a chemoreceptor, such as the TAS1R2/R3, may affect physiological responses, although the extent of the contribution remains unclear. Additionally, it has been hypothesised that perceived sweetness induces cephalic phase insulin release (CPIR) due to interaction with the insulin signalling pathway and thus regulating blood glucose levels (Powley and Berthoud 1985; Bruce et al. 1987; Teff, Devine, and Engelman 1995; Abdallah, Chabert, and Louis-Sylvestre 1997). Next to the possibility that the signalling pathway of the sweet taste receptor mediates CPIR, there are further results that indicate an alternative pathway, including the ATP-sensitive K-channel (KATP channel), to be more active in the CPIR (Glendinning et al. 2017). Since the alternative pathway has been only described for caloric carbohydrates, is not known if any or which non-caloric sweeteners can activate it. So far, the effects of consumed sweetness of a food product, especially of non-caloric sweeteners, to the corresponding metabolism including CPIR are controversial and unclear in humans but as well very individual for each type of sweetener (Brown and Rother 2012; Hunter et al. 2019; Han, Bagenna, and Fu 2019). In more detail, Jang et al. (2007) showed that the initial fast increase of insulin after gavage administration of glucose (2 - 5g/kg body weight) in mice was missing in α-gustducin$^{-/-}$ mice and plasma glucose was higher over the time span of 120 minutes. These results therefore confirm the involvement of the sweet taste receptor mechanism in insulin release (Jang et al. 2007). Furthermore, the sweet taste receptors in the intestine had direct effects on glucose metabolism, due to the ability of 450 ppm lactisole to reduce GLP-1 and PYY secretion after intragastric (75 g) and intraduodenal (3 kcal/min) glucose stimulation in healthy human subjects (Gerspach et al. 2011). Due to inhibition of the sweet taste receptor by 500 ppm lactisole before and while conducting an OGTT in ten healthy human subjects, Karimian-Azari et al. (2017) showed an alteration of the insulin response with enhanced insulin in plasma and insulin secretory rate. However, the effects of non-caloric sweeteners on blood glucose and its regulation are controversial. For example, 6 g/ 355 mL sucralose showed similar effects on

short-term glucose homeostasis as water and had no additional effect when added to a glucose solution in eight healthy women (Brown et al. 2011). Compared to 50 g/ 500 mL sucrose, the intragastric infusion of 0.4 mM and 4.0 mM sucralose did not induce a secretion of insulin, GIP or GLP-1 in seven healthy human subjects (Ma et al. 2009). Contrary, GLP-1 was released by sweet sensitive taste cells of mice directly after stimulus with the sweet compounds sucrose (500, 1000 mM), glucose (500, 1000 mM) and saccharin (5, 20, 50 mM) and therefore GLP-1 can possibly activate sweet-sensitive gustatory nerve fibers (Takai et al. 2015).

To summarize, previous studies show that sweet taste signalling has an impact on blood glucose regulation, however, the extent of the effect of sweetness perception in comparison to the structure of the sweet compound remains to be elucidated.

II. Objectives

Humans have an innate preference for sweet taste, and the most favoured sweet compound in food and beverages so far is sucrose. When compared to sucrose, other alternative sweeteners show differences in the sensory profile, most strikingly regarding side-tastes and temporal profile. Some differences are already well explained, such as a bitter side-taste caused by the activation of TAS2R receptors. However, it is not yet fully understood, how the ligand-receptor interaction of sweet taste affecting compounds and the canonical sweet taste receptor TAS1R2/TAS1R3 can cause such differences in the sensorial profile and which further mechanisms contribute to the overall taste impression of sweet compounds. Moreover, following the taste perception in the oral cavity and subsequent swallowing of sweet compounds, the effects of sweet tasting compounds in the gastrointestinal tract are of interest and to date results have often been inconsistent.
The ligand-based molecular basics of sweet taste perception and its metabolic effects are studied in the context of the Christian Doppler Laboratory for Taste Research.

The aim of the present thesis was to investigate sensory, chemical, and physiological properties of various sweet tasting compounds to gain more detailed insights into the reasons for the diversity of the taste profile, including the temporal profile, side-tastes and mouthfeel attributes. Therefore, the main part of this thesis focused on three subsections of sweet taste perception. In the first study, sensory attributes, namely the sweetness and its temporal profile and side-tastes, of 35 sweet tasting compounds were evaluated and these attributes were afterwards related to the structure and physicochemical descriptors of the sweet compounds. For this purpose, a comparison of similarity to sucrose and a multivariate regression analysis were conducted. The second manuscript focused on the binding-site of selected sweet compounds to evaluate the impact of the known binding-sites to temporal and sweet attributes. A sensory time-intensity measurement was implemented in addition to a measurement of sweetener-induced calcium release in TAS1R2/TAS1R3 transfected HEK-293 cells. With the dose-response curves of both experiments, indications of the binding site and its relation to the sweet taste impression were revealed. The third publication investigated another aspect of the differences in the perception of sweet taste, the mouthfeel. Here, the impact of structurally and sensorially diverse selected sweet tasting compounds on the mouthfeel attributes salivation and viscoelasticity of saliva, including several potential influencing factors were studied in 21 healthy adults. These three named studies form the key part of this thesis, focusing on differences in sweet taste perception, especially of the temporal profile, side-tastes, and mouthfeel as part of overall perception of sweet compounds.

As it is known that the sweet taste receptor is not only present in mouth but as well in the gastrointestinal tract, it is consequently of further interest, which effects the oral sweet perception can have here. Therefore, this secondary part of this thesis gives first insights into the effects of sweet taste perception on blood glucose regulation and related hormones and peptide hormones. As the field of metabolic effects of compounds is very broadly, the fourth publication provided primary insights into the impact of oral sweet taste perception of two caloric compounds, but different in their sweet potency, on blood glucose metabolism in healthy adults. Beside evaluating the rating of sweetness, the blood glucose homeostasis, and related hormones and parameters were investigated after consumption of equi-sweet or equi-caloric drinks containing either sucrose, glucose or a combination of those with the sweet taste inhibitor lactisole.

Altogether, the focus of this thesis is to investigate the underlying differences in sweet taste and their effects during and after consumption and on blood glucose metabolism. The overall aim is to get more insights and to contribute to the clarification of differences in sweet taste perception of diverse sweet compounds and further effects of sweetness after consumption.

III. Results

This cumulative dissertation is based on the following research articles and manuscripts:

3.1. "Structure-dependent effects of sweet and sweet taste affecting compounds on their sensorial properties"

Corinna M. Karl, Martin Wendelin, Dariah Lutsch, Gerhard Schleining, Klaus Dürrschmid, Jakob P. Ley, Gerhard E. Krammer, Barbara Lieder

Published in Food Chemistry: X, 7, 100100, 2020

This study compared sweet sensorial attributes of sweet affecting compounds with the physicochemical properties of the underlying molecular structure in order to get more insights into structure-based reasons for the diversity in sweet taste.

I participated in the experimental design, including development of the specific sensory evaluation sheet and building-up the sensory panel. Afterwards I prepared, carried out and evaluated the sensory tastings of over 30 potentially sweet and consumable compounds by the panelists with at least eight panelists per taste session and two repetitions per compound. In addition, I performed the measurement of the viscosity of sweet compounds in solution at the Institute of Food Science, University of Natural Resources and Life Sciences, Vienna, Austria. I determined the physicochemical descriptors of the tested compounds and was contributing to the computational and statistical evaluation. Terminally, I drafted the manuscript.

The Manuscript is located in subchapter 3.5.1 at page 25.

3.2. "Impact of lactisole on the time-intensity profile of selected sweeteners in dependence of the binding site"

Corinna M. Deck, Maik Behrens, Martin Wendelin, Jakob P. Ley, Gerhard E. Krammer, Barbara Lieder

Published in Food Chemistry: X, pre-proof, 100446, 2022

This study investigated cellular and sensorial time-intensity curves of the four sweeteners acesulfame K, aspartame, cyclamate and NHDC including their dose-response curves without and

with the sweet taste inhibitor lactisole to evaluate if the binding-site has an impact on sweet perception, its temporal behaviour and cellular taste receptor activation.

I participated in the experimental design of this study; therefore, I planned, performed, and analysed the sensory experiments. I conducted the statistical analysis of all sensorial parameters for time-intensity and also the resulting dose-dependent parameters. Terminally, I drafted the manuscript.

The Manuscript is located in subchapter 3.5.2 at page 39.

3.3. "Individual sweet taste perception influences salivary characteristics after orosensory stimulation with sucrose and non-caloric sweeteners"

Corinna M. Karl, Ana Vidakovic, Petra Pjevac, Bela Hausmann, Gerhard Schleining, Jakob P. Ley, David Berry, Joachim Hans, Martin Wendelin, Juergen Koenig, Veronika Somoza and Barbara Lieder

Published in Frontiers in Nutrition – Food Chemistry: 9, 831726, 2022

This study investigated the salivary flow and viscoelasticity as part of overall mouthfeel of selected sweet compounds and taste neutral control water to examine potential differences of these compounds here, including potential influencing factors.

For this manuscript, I was involved in the setting up of the experimental design, including contribution to the application for ethical approval of the study. I was responsible for the planning and performing of the study, including the analysis of the study parameters. In addition, I conducted the measurement of salivary rheological properties at the Institute of Food Science, University of Natural Resources and Life Sciences, Vienna, Austria. Furthermore, I was involved in the planning and evaluation of the oral microbiome data, which was analysed in cooperation by the Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, Austria, by the Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science, University of Vienna, Austria and by the Department of Laboratory Medicine, Medical University of Vienna, Austria. Finally, I performed the main statistical analysis, created the graphs, and drafted the manuscript.

The Manuscript is located in subchapter 3.5.3 at page 75.

3.4. "Sweetness Perception is not Involved in the Regulation of Blood Glucose after Oral Application of Sucrose and Glucose Solutions in Healthy Male Subjects"

Verena Grüneis, Kerstin Schweiger, Claudia Galassi, **Corinna M. Karl**, Julia Treml, Jakob P. Ley, Jürgen König, Gerhard E. Krammer, Veronika Somoza, and Barbara Lieder

Published in Molecular Nutrition and Food Research: 65, 2, 2000472, 2020

This study investigates the impact of the sweetness of a dietary concentration of sucrose and isocaloric glucose solution on the regulation of blood glucose, while adjusting the sweetness level with the sweet taste inhibitor lactisole.

I supported this study design with scientific input and did the sensory evaluations on the screening days. Furthermore, I supported the handling of the test persons on the intervention days. Finally, I was involved in the discussion of the data and contributed to the writing of the manuscript.

The Manuscript is located in subchapter 3.5.4 at page 89.

3.5. Presentation of the publications of this thesis

3.5.1. "Structure-dependent effects of sweet and sweet taste affecting compounds on their sensorial properties"

Corinna M. Karl [a], Martin Wendelin [b], Dariah Lutsch [c], Gerhard Schleining [d], Klaus Dürrschmid [d], Jakob P. Ley [c], Gerhard E. Krammer [c], Barbara Lieder [a,e]

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Structure-dependent effects of sweet and sweet taste affecting compounds on their sensorial properties

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ABSTRACT

A reduction in sugar consumption is desirable from a health point of view. However, the sensory profiles of alternative sweet tasting compounds differ from sucrose regarding their temporal profile and undesired side tastes, reducing consumers' acceptance. The present study describes a sensory characterization of a variety of sweet and sweet taste affecting compounds followed by a comparison of similarity to sucrose and a multivariate regression analysis to investigate structural determinants and possible interactions for the temporal profile of the sweetness and side-tastes. The results of the present study suggest a pivotal role for the number of ketones, aromatic rings, double bonds and the M LogP in the temporal profile of sweet and sweet taste affecting compounds. Furthermore, interactions between aggregated physicochemical descriptors demonstrate the complexity of the sensory response, which should be considered in future models to predict a comprehensive sensory profile of sweet and sweet taste affecting compounds.

1. Introduction

During the past decades, the consumption of sugary drinks increased globally (Gakidou et al., 2017). However, an excessive consumption of sugar especially in soft drinks contributes to overweight, obesity and associated diseases like type 2 diabetes, hypertension and hyperlipidemia (Lustig, Schmidt, & Brindis, 2012). In order to reduce sugar consumption, but still sustain the pleasant sweet taste of food, there is a worldwide rising trend for sugar-reduced products using alternative sweeteners with no or a reduced caloric load (Sylvetsky & Rother, 2016). A major challenge when applying alternative sweeteners are the striking differences in the sensory profile of sweeteners in comparison to sucrose, which is the sweet-standard for most consumers. Especially differences in the time-intensity response, the potency, and undesired side-tastes, for example bitterness, metallic, astringency or licorice like taste, limit the application and acceptance of alternative sweeteners (DuBois, 2016; Reyes, Castura, & Hayes, 2017).

The terms onset and lingering are commonly used to describe differences in the sensory time-intensity profile. Onset is used to express the time it takes to reach the maximum of a taste sensation, while lingering is the more or less long lasting time of a sensation in the mouth (DuBois, Crosby, Stephenson, & Wingard Jr., 1977). A large variety of sweet tasting compounds is known, however none of them has exactly the same sensory profile as sucrose. Moreover, the molecular basis for these differences has not been fully elucidated so far. The perception of sweet taste is mediated by activation of the sweet taste receptor, the G protein-coupled heterodimeric receptor T1R2/R3, which has multiple agonist binding sites (Chéron, Golebiowski, Antonczak, & Fiorucci, 2016; Morini, Bassoli, & Temussi, 2005). An alternative pathway for the perception of mono- and disaccharides via glucose transporters has been discussed as well (Sukumaran et al., 2016; Yee, Sukumaran, Kotha, Gilbertson, & Margolskee, 2011). However, it is still not fully understood how the sweet taste receptor recognizes the sensory variety of structures of ligands (Chéron et al., 2016; Masuda et al., 2012). Early, but outdated attempts to provide structure-sweetness relationships without the knowledge of the sweet taste receptor described the hydrophobicity and the logP value, which is the partition coefficient in octanol/water and represents the solubility of a compound, as important characteristics for sweet compounds (Deutsch & Hansch, 1966). This equation was followed by the so called

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AH/B theory, describing the occurrence of a hydrogen bond donor group and a Lewis base (Shallenberger & Acree, 1969). The discovery of the human sweet taste receptor succeeded in the early 2000s (Li, Staszewski, Xu, Durick, Zoller, & Adler, 2002; Montmayeur, Liberles, Matsunami, & Buck, 2001; Nelson, Hoon, Chandrashekar, Zhang, Ryba, & Zuker, 2001), providing a base for advanced prediction models. Lately, there have been several studies describing the prediction of sweetness for example by quantitative structure activity relationship (QSAR) models (Chéron, Casciuc, Golebiowski, Antonczak, & Fiorucci, 2017; Goel, Gajula, Gupta, & Rai, 2018; Yang, Chong, Yan, & Chen, 2011) or by machine learning methods (Zhong, Chong, Nie, Yan, & Yuan, 2013). In addition, *in silico* methods based on one of the binding sites were applied, for example molecular docking and homology models. Those models have shown to be useful tools to provide insights into the mechanism of G-protein coupled taste receptors, including sweet taste, by analyzing selected ligand binding sites (Spaggiari, Di Pizio, & Cozzini, 2020). For example, Ben Shoshan-Galeczki and Niv (2020) recently published homology models for virtual screening to provide novel predictions of sweet tasting molecules. Nevertheless, the crystal structure of the sweet taste receptor remains unknown and the main limitation for structure-based modelling is the availability of closely related proteins, although there have been some important improvements over the last few years (Spaggiari et al., 2020). Furthermore, the available models only describe the sweetness of a compound but are lacking the complete sensory profile including the temporal profile and potential side-tastes which are very important for the consumers' acceptance and preference of a sweet tasting compound. For unpleasant aftertaste, an interaction with the umami receptor has been proposed (Acevedo & Temussi, 2019) and it is known that some sweeteners can also activate one or more bitter taste receptors (Kuhn et al., 2004). In addition, an extended lingering, as well as a delay in the onset of sweet taste is common amongst several non-nutritive sweeteners (DuBois, 2016; DuBois & Prakash, 2012). However, the structural basis for these differences has not been clarified so far.

In order to improve the current understanding of the structural determinants and their interactions for the sensory perception of sweet taste, a ligand-based approach was chosen. In more detail, we performed a comparative sensory characterization of a variety of test compounds at equally sweet levels in one test setup in order to investigate the structural driving forces for onset and lingering, as main parts of the temporal profile of sweet sensation, in addition to selected side-tastes. We hypothesize here that not only single structural characteristics, but also interactions between several characteristics are driving forces for undesired side-tastes and in particular for the onset and lingering of the sweet sensation.

2. Materials and methods

2.1. Chemicals

Acesulfame K, advantame, aspartame, hesperetin sodium salt, iron lactate-II hydrate, maltitol, maltose, neotame, phyllodulcin, phloretin, rebaudioside (reb) A (nat., 99%), reb C, reb D, reb E (contains 20% reb D) and reb M, rubusoside, saccharin sodium salt, sodium cyclamate, sorbitol (D-), stevioside, sucralose, tannic acid (nat.), thaumatin B (pur) and trehalose were kindly provided in food grade (FG) quality by Symrise AG (Holzminden, Germany). Caffeine (anhydrous, 99%, FG), hesperetin (> 95%), neohesperidin dihydrochalcone (> 96%, FG), rhamnose (L-, 99%, FG) and sorbitol (D-; 98%, FCC, FG) were obtained from Sigma-Aldrich (Steinheim, Germany). D-Tryptophan (99%) was obtained from Carbolution Chemicals GmbH (St. Ingbert, Germany), glucose (> 99%, FG) from Dr. Lohmann Diaclean GmbH (Dortmund, Germany), fructose and sucrose were purchased from Wiener Zucker (Vienna, Austria). Citric acid, erythritol, ethanol, isomalt, lactose, monosodium glutamate, palatinose, sodium chloride, sucrose and xylitol were purchased from local supermarkets and pharmacies in the Viennese region in Austria.

2.2. Sensory panel

A total of 23 panelists (19 F, 4 M; 23–34 years) were recruited via notices on billboards at the University of Vienna and the surrounding areas. They confirmed they were in good general health condition, not pregnant and not taking medication. The panelists were asked not to consume intense tasting food or drinks (e.g., chewing gum, garlic, chilli, coffee) or to smoke for at least one hour before testing and to avoid strong odors or perfume, as well as strong abdominal fullness or hunger. All panelists gave their written informed consent.

The panelists were screened in three sessions within three weeks. The first session for basal tastes was performed according to DIN-EN-ISO 8586:2014-05 (2014) with 0.3 g/L citric acid for sour taste, 2.0 g/L sodium chloride for salt taste, 10.0 g/L sucrose for sweet taste, 0.6 g/L monosodium glutamate for umami taste and 0.3 g/L caffeine for bitter taste. To continue with the panel work, at least 80% had to be rated correctly. Additionally, 1.0 g/L iron lactate-II hydrate and 0.5 g/L tannic acid were given to train the panelists for metallic and astringent taste. In a second training session, the stimulus threshold level for sweet taste with sucrose and for bitter taste with caffeine was obtained according to DIN-NORM (1998). Only panelists with a threshold level for sweet below or at 4.0 g/L sucrose and for bitter below or at 0.125 g/L caffeine were allowed to continue. Furthermore, a ranking test for sweet and bitter was conducted according to Busch-Stockfisch (2015), to assess the ability to differentiate between concentrations. After the screening sessions, 20 panelists (17 F, 3 M; 23–34 years) were qualified and willing to continue with the sensory evaluations. At the third session, the panelists were introduced to the test method and the corresponding questionnaire on paper (see description for sensory evaluation) and were provided with the opportunity to train the evaluation-sheet. The attribute onset was separately trained by a guided tasting of sucrose compared to reb A and aspartame, which are known to have a delayed onset (DuBois & Prakash, 2012). This training for onset was repeated several times during overall panel work. The general performance of the panel was assessed by panel check using EyeOpenR with the complete data of sensory evaluation of the test compounds (see Section 2.3). Discrimination performance of the whole panel was good ($p < 0.05$), as was the reproducibility ($p > 0.15$). In particular, the discrimination of onset was excellent with $p < 0.001$, and the reproducibility was good with $p = 0.234$. Because of the overall good performance of the panel, no evaluation or any of the panelists had to be excluded.

2.3. Sensory evaluation

Every compound was tested at least in two sessions on separate test days with a minimum of eight panelists. The panelists were free to choose whether to participate in each of the sessions and the compounds were randomly assigned to the sessions, leading to a randomized order of the compounds to each individual panelist. On average, 30 single evaluations were made per compound on 2–3 separate test days, and 12 panelists evaluated one compound per test day (see Table S1). The reproducibility of the evaluations was tested by repeated rating of several compounds (e.g. sucrose and aspartame). To receive the taste characteristics of the 35 test compounds, an evaluation sheet was created based on a descriptive profile at two time-points, namely taste and aftertaste, in addition to rating of onset and lingering (see Fig. S1). The evaluation-sheet was customized for this study to rate the attributes using unstructured scales (0–10) for taste and aftertaste ("not at all" to "very intensive"), namely the intensity of sweetness, bitterness, astringency and metallic. For onset, panelists were supposed to rate the perceived time until the maximal sweet intensity was reached ("immediately" to "substantially delayed") on an unstructured scale (0–10). Panelists were asked to rinse the mouth with tap water before and in

between the tastings, and white bread was provided for optional additional neutralization. The panelists were instructed to start a new sample after complete neutralization only. A maximum of five test solutions was evaluated at one session and one test day. A total volume of 20 mL of each sample was provided in cups labelled with 3-digit random numbers and presented to the panelists in a randomized order. The panelists rinsed their mouth for 30 s with 20 mL of the test solution, evaluating the onset of sweetness in the first seconds and afterwards the intensity of bitterness, metallic, astringent, and sweet taste on unstructured scales. After spitting out the sample, lingering time was measured using a standard timer while rating the sweet, bitter, metallic and astringent aftertastes on unstructured scales. According to the measured time of sweetness staying in the mouth, the lingering time was recalculated to the range 0–10. The concentrations of the compounds were chosen so that the sweetness was equivalent to 5% (w/v) sucrose. The selected concentrations were determined in preliminary tastings by comparison tests with five selected panelists on structured scales (weak, marked and strong difference) with 5% (w/v) sucrose as reference solution, according to a just-about-right scale.

2.4. Stimuli

The test compounds used in the present study are shown in Table 1, including concentration for a sweetness equivalence of 5% sucrose where applicable, M logP and viscosity (Pa s). All compounds were carefully dissolved in water in a 500 mL graduated flask (± 0.5 mL, DURAN®). Due to the limited solubility in water, phyllodulcin (14), hesperetin (31) and phloretin (33) were dissolved in ethanol (EtOH) to 200× concentrated stock solutions, reaching a final concentration of 0.5% EtOH in the test solution. It must be noticed that these concentrations (see # in Table 1) exceed the common use levels and were only reached using ethanol as solvent. Sucrose with 0.5% EtOH (25) was evaluated as a control for the impact of ethanol on the rated attributes. All solutions were prepared freshly in the morning of the tastings and served at room temperature in 25-mL plastic cups. The test compounds hesperetin + 0.5% EtOH (31), hesperetin sodium salt (32), phloretin + 0.5% EtOH (33), rebaudioside C (reb C) (34) and rubusoside (35) did not reach a sweetness equivalence of 5% sucrose in water soluble concentrations or tolerable bitterness (see * in Table 1) and therefore have been excluded for the statistical analyses.

2.5. Viscosity measurement

As a part of the physicochemical descriptors, the viscosity η [Pa*s] was measured using the rotary viscometer Physica SM (Anton Paar, Graz, Austria) with D/1/s = 20 at 25 °C ± 0,5 °C for 30 s with seven measurement time-points and 2–3 repetitions per sample. Outliers were determined using the Nalimov outlier test. The mean viscosities [Pa*s] of the 35 compounds are listed in Table 1. Each compound was dissolved as described in Section 2.3 and about 100 mL were filled into the test cylinder.

2.6. Computational and statistical analysis

The means and standard errors of the sensory characteristics of all test compounds were calculated with MS-Excel. The heatmap for visualization of the mean ratings of the sensory results with associated dendrogram was created with R studio (R version 3.6.1) using the library “gplots” and the application “heatmap.2” (as.matrix(data_sweet), col = colorRampPalette (c(“white”,“grey”,“black”)) (256), scale = “none”, key = T, keysize = 1.5, density.info = “none”, trace = “none”, cexCol = 0.9). The physicochemical descriptors for each test compound (molecular weight [g/mol], structure, area polar surface [A^2], rotatable bonds, complexity, length glycone, length alkyl chain, as well the numbers of heavy atoms, C-atoms, double bonds, OH-groups, ketones, bonded glucose, aromatic rings, defined atom stereocenters, donors and acceptors) were taken from the open chemistry database PubChem (Aug. 2018). Additionally, the M logP-value was calculated with MedChemDesigner 3.1.0.30 (see Table 1), which estimates the solubility of a compound as octanol/water distribution coefficient (Lipinski, Lombardo, Dominy, & Feeney, 1997). The relative sweetness for each compound was calculated based on the concentrations used in this study to receive a sweetness equivalent to 5% sucrose (relative sweetness = 1).

The calculation of the molecular fingerprints according to Morgan of each test compound, which translates the molecular structure to a binary code, was done with KNIME analytical platform 3.7 using the RD Kit node. Structural similarities to sucrose were then computed by “Tanimoto”-similarity index. To investigate relationships between the sensory attributes and the similarity index or physicochemical descriptors, the Pearson’s product moment correlations were calculated and illustrated with SigmaPlot 13.0. Additionally, a multivariate linear regression analysis with interactions, which includes a factor analysis (FA) with varimax rotation for aggregation of the dependent and independent variables, was carried out using JMP 14.0.0 to consider possible interactions of the physicochemical interactions to explicate the sensory attributes. The explanatory power for the independent factors (IF) of the multivariate linear regression analysis with interactions is explained by the FDR-LogWorth for each IF and their interactions and by the t ratio for each dependent factor separately. The higher the value of FDR-LogWorth or t ratio, the more impact the factor or interaction has for the model. The FA with varimax rotation was performed in order to reduce the number of factors for the multiple regression analysis. A sensory attribute (dependent factor, see Table S2) or physicochemical descriptor (IF, see S3) is represented by the reduced factor with the highest absolute value.

3. Results & discussion

In the present study, a comparative quantitative sensory description of known sweet and sweet taste affecting compounds was performed in order to analyze structural characteristics leading to differences in the sensory temporal profile and undesired side-tastes. A total of 35 compounds previously associated with sweet taste or sweet taste affecting properties was selected based on the availability in food grade from commercial sources. A sensory characterization of the test compounds at a sweetness level equivalent to 5% sucrose was carried out, evaluating the time-intensity response as well as bitter, metallic and astringent side-tastes.

The mean ratings of the attributes (see also Table S1) are displayed as a heat map, showing the sensory mean values for each of the 30 test compounds that reached a sweetness equivalent to 5% sucrose (Fig. 1 with related numbers of the compounds in Table 1). The color of each field represents the mean value of an attribute for each of the compounds with light to dark indicates a value from 0 to 10 (see color key in Fig. 1). Furthermore, the compounds are sorted vertically and attributes horizontally by similarity. A dendrogram demonstrates the clustering of the sensory attributes, as well as the clustering of the test compounds by similarity. The clustering of the attributes shows that taste and aftertaste for each attribute are associated. The attribute onset pertained to the cluster of the attributes “metallic” and “astringent”. In contrast, lingering pertained to the cluster of sweet sensation. Although the concentration of each compound was adjusted to be as similar as possible to 5% sucrose, the perception of sweetness may vary based on the individual rating of each panelist. In addition, the intensity of sweet aftertaste after spitting out, but not the taste ratings within the first 30 s, correlated positively ($r = 0.56$, $p < 0.01$ by Pearson correlation) with the lingering time. Hence, the more intensive the sweet aftertaste, the longer the lingering of the tested compounds. Moreover, we found a significant enhanced onset ($p < 0.05$ by ANOVA on ranks with Dunńs test as post-hoc, compared to sucrose) for advantame (2), aspartame (3), neotame (12), phyllodulcin + 0.5% EtOH (14) and thaumatin (27),

Table 1
Test compounds and the concentrations (conc.) used [g/L] in the present study, M logP, viscosity and molecular structure. Compounds tested in concentrations exceeding the common or realistic use levels are labelled with #; compounds, which did not reach the required sweetness level of a 5% sucrose solution are labelled with *.

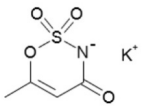
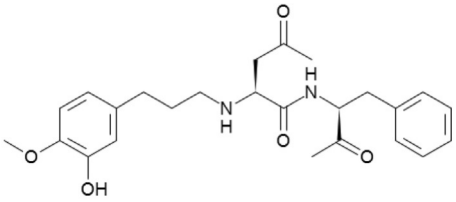
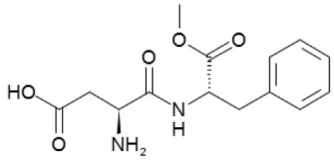
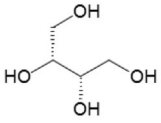
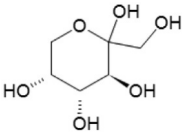
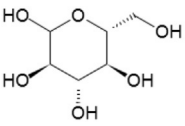
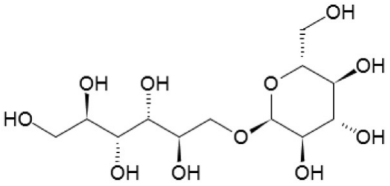
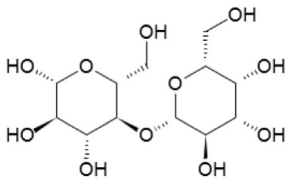
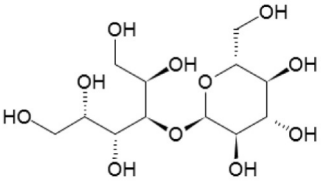
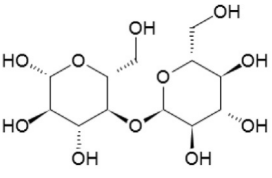
	Substance	Conc. [g/L]	M logP	Viscosity [Pa s]	Structure
1	Acesulfame K	0.3	−0.908	0.041	
2	Advantame	0.003	2.293	0.042	
3	Aspartame	0.25	−0.231	0.046	
4	Erythritol	100	−1.724	0.048	
5	Fructose	42	−2.483	0.042	
6	Glucose	100	−2.483	0.050	
7	Isomalt	167	−4.304	0.051	
8	Lactose	175	−3.898	0.053	
9	Maltitol (Maltit)	100	−4.304	0.053	
10	Maltose	180	−3.898	0.049	

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Table 1 (*continued*)

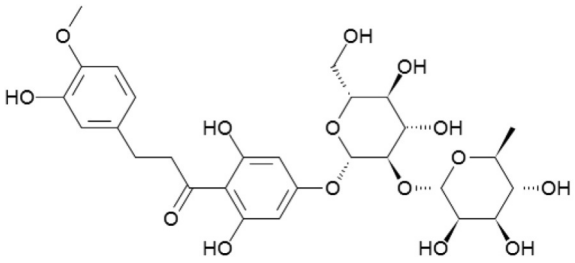
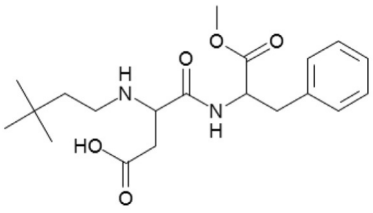
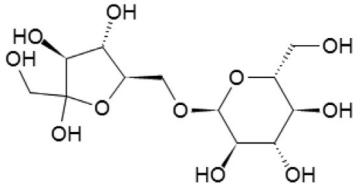
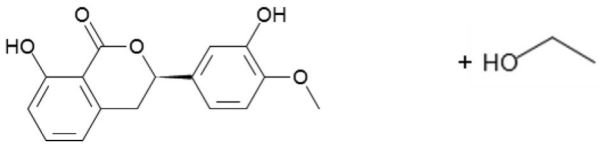
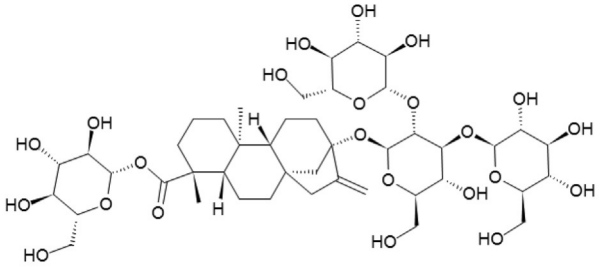
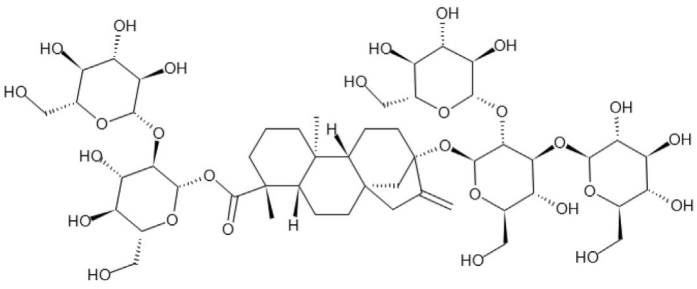
	Substance	Conc. [g/L]	M logP	Viscosity [Pa s]	Structure
11	Neohesperidin dihydrochalcone	0.07	−2.176	0.053	
12	Neotame	0.01	1.215	0.047	
13	Palatinose	130	−3.898	0.053	
14	Phyllodulcin + 0.5% EtOH	0.075#	2.105	0.058	
15	Reb A	0.3	−4.703	0.052	
16	Reb D	0.25	−6.648	0.059	

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Table 1 (*continued*)

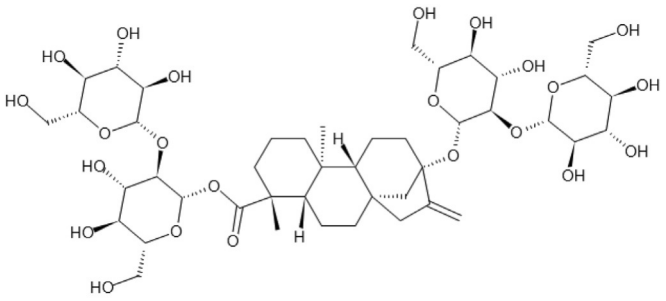
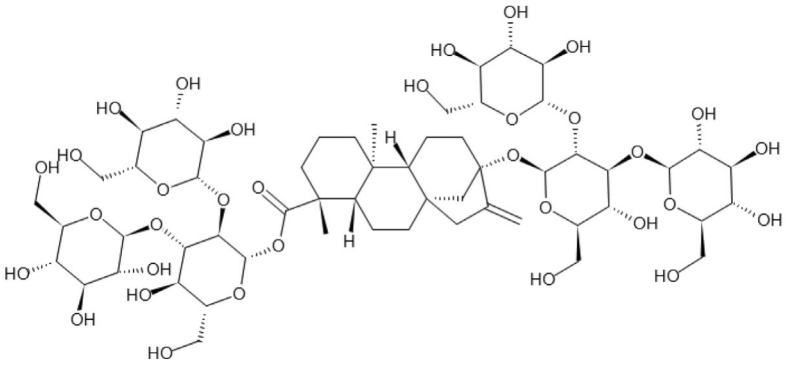
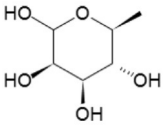
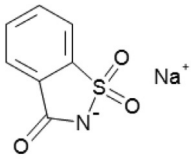
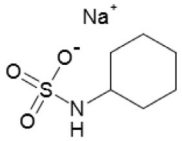
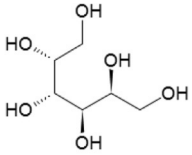
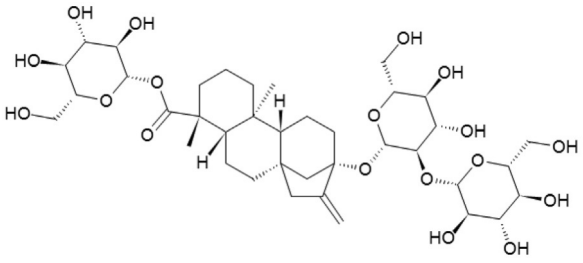
	Substance	Conc. [g/L]	M logP	Viscosity [Pa s]	Structure
17	Reb E	0.3	−4.703	0.036	
18	Reb M	0.25	−8.579	0.039	
19	Rhamnose (L-)	100	−1.711	0.051	
20	Saccharin sodium salt	0.2	0.17	0.048	
21	Sodium cyclamate	2.1	0.448	0.046	
22	Sorbitol (D-)	100	−2.497	0.049	
23	Stevioside	0.4	−2.739	0.051	

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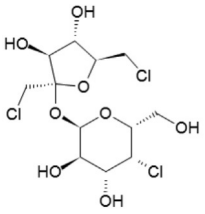
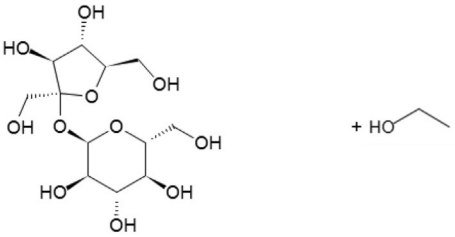
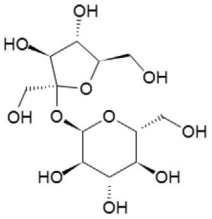
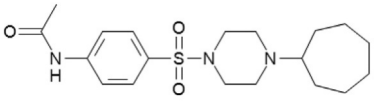
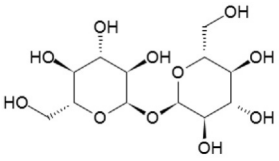
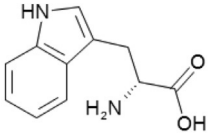
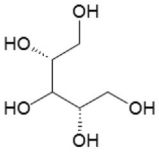
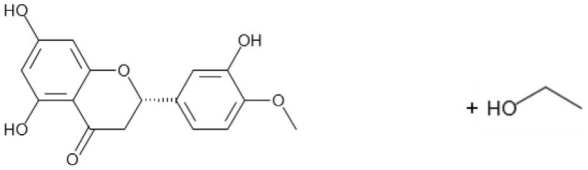
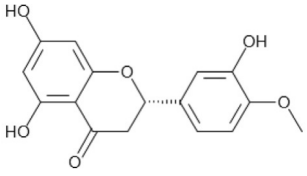
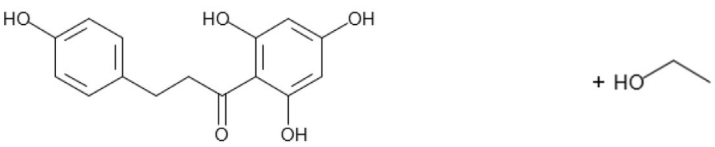
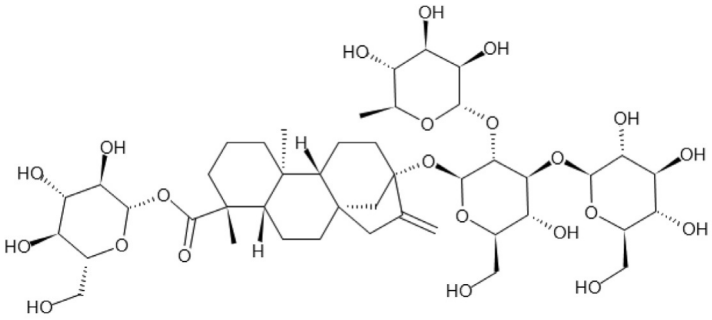
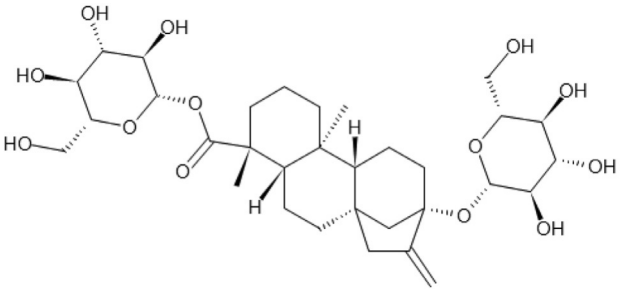
Table 1 (*continued*)

	Substance	Conc. [g/L]	M logP	Viscosity [Pa s]	Structure
24	Sucralose	0.09	−0.913	0.046	
25	Sucrose + 0.5% EtOH	50	−3.898	0.043	
26	Sucrose	50	−3.898	0.038	
27	Thaumatin B	0.03	1.891	0.048	
28	Trehalose	150	−3.898	0.045	
29	Tryptophan (D-)	1.0	−2.147	0.053	
30	Xylitol	65	−2.103	0.047	
31	Hesperetin + 0.5% EtOH	0.07[#*]	0.927	0.048	
32	Hesperetin sodium salt	0.07[#*]	0.927	0.058	

(*continued on next page*)

Table 1 (*continued*)

	Substance	Conc. [g/L]	M logP	Viscosity [Pa s]	Structure
33	Phloretin + 0.5% EtOH	0.25#*	1.842	0.047	
34	Reb C	1.2#*	−4.033	0.040	
35	Rubusoside	0.7#*	−0.745	0.039	

when tested at a sweetness level according to 5% sucrose.

The grouping of the test compounds resulted in three main clusters according to their tastes, side-tastes and aftertastes. In the first cluster, mainly caloric sweeteners and polyols were assigned, namely erythritol (4), fructose (5), glucose (6), isomalt (7), lactose (8), maltitol (9), maltose (10), palatinose (13), sodium cyclamate (21), D-sorbitol (22), sucralose (24), sucrose + 0.5% EtOH (25), sucrose (26), trehalose (28) and xylitol (30). Thus, in the first cluster the sweet taste and aftertaste is nearly exclusively present. To the second cluster belonged almost all steviol glycosides (15–18), the amino acid D-tryptophan (29), the 6-deoxy-monosaccharide L-rhamnose (19), and the sweeteners saccharin sodium salt (20), acesulfame K (1), advantame (2), and aspartame (3). This cluster comprises compounds that had, in addition to the sweet taste and aftertaste, also some negative side-tastes and as well a slightly enhanced lingering effect. In the third cluster, five compounds, noteworthy the isocoumarin phyllodulcin (14), and the non-nutritive sweeteners stevioside (23), neohesperidin dihydrochalcone (NHDC, 11), thaumatin (27) and neotame (12) were assigned. Thus, in the third cluster, the negative side-tastes are the most intense, supplemented by a strongly enhanced lingering of sweetness. These clustering results are consistent with the results of Tan, Wee, Tomic, and Forde (2019), who showed by using the Temporal Check-all-that-Apply (TCATA) method that the side taste profiles, sweetness onset and lingering of compounds like fructose and maltitol, are most similar to 10% sucrose. Furthermore, Tan et al. (2019) showed that aspartame, acesulfame K, reb A, sucralose, as well as allulose and sorbitol had higher bitterness than sucrose which was mostly accompanied by higher metallic taste and chemical taste compared to sucrose. Also Reyes et al. (2017) described that non-nutritive sweeteners showed more side tastes compared to carbohydrate based sweet compounds when evaluating sucrose, aspartame, acesulfame K, sucralose, reb A, fructose, NHDC, thaumatin, glucose and saccharin with weak and moderate sweetening concentrations by TCATA (Reyes et al., 2017).

As a next step, structural characteristics that are associated with sweetness, onset, lingering and undesired side-tastes were analyzed. Firstly, the overall structure of the compounds was characterized using Morgan's Fingerprints, followed by calculation of the Tanimoto similarity index to sucrose, which was correlated with the taste attributes. The results are displayed in Fig. 2, demonstrating a negative correlation for bitter and astringent taste ($p < 0.05$) to the similarity index, meaning that the higher the similarity to sugar, the lower was the rating of bitterness and astringency. In addition, there was a trend ($p < 0.1$) to a negative correlation with onset and lingering and the Tanimoto similarity index. Thus, also here, we found that the higher the similarity to sugar was, the lower was the rating for both attributes of the temporal profile. Since the relative sweetness did not correlate with the similarity index to sucrose, it can be assumed that compounds can taste sweet independently of the structural similarity to sucrose. However, they are more likely to have undesired bitter and astringent side tastes, as well as an increased onset and lingering. Moreover, the results suggest that structural characteristics are important for the taste attributes. Thus, in a second step, a variety of physicochemical descriptors was evaluated which are commonly used to differentiate the overall shape, size, degree of branching and flexibility of molecules as numerical values (Zhong et al., 2013). The calculation of the physicochemical descriptors is based on the 2D structure of a compound and additionally the physicochemical descriptors are supplemented with values for relative sweetness and viscosity. In the present study, we focused on the structural driving forces for onset, lingering and the relative sweetness compared to a 5% sucrose solution. For this purpose, the chemical information of each test compound was transformed into various numerical quantities within a symbolic representation of a molecule for the IF. Such conformation-independent methods have been validated as an efficient alternative strategy to evolve models based on

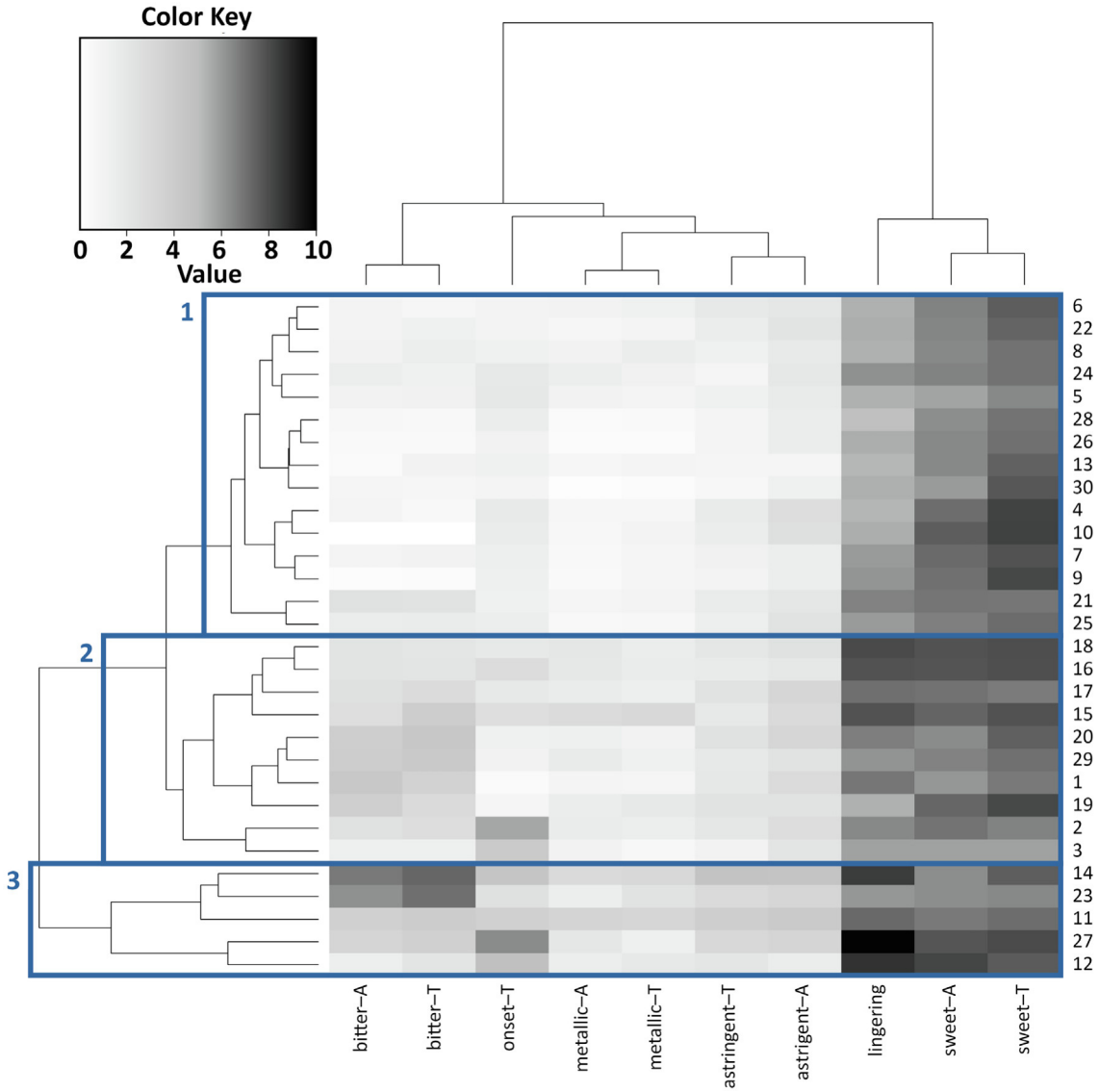


Fig. 1. Heatmap of sensory attributes of sweet and sweet taste affecting compounds with 3 clusters of compounds with rating values 0 – 10. T = taste; A = aftertaste. Numbers refer to compounds given in Table 1.

constitutional and topological molecular characteristics of chemical compounds (Chéron et al., 2017; Ojha & Roy, 2018; Rojas, Ballabio, Consonni, Tripaldi, Mauri, & Todeschini, 2016; Rojas et al., 2017), avoiding that differences between the 3D conformers manipulate the descriptor values due to geometrical optimization. However, this is at the same time one limitation of this study, since the physicochemical descriptors based on the 2D structure ignore the conformation of the test compounds, which might affect a compound's binding to the receptor. To gain more insight into the role of the 3D structure of a compound, e.g. homology modelling based on the structure of the receptor is needed in further studies.

Before understanding the driving forces of onset and lingering by a multivariate linear regression analysis with interactions, multiple dependent and various independent variables were aggregated to fewer factors by factor analysis (FA) with varimax rotation to reduce the number of factors. The relative sweetness and ten sensory attributes were aggregated into five factors serving as dependent variables (Table S2) according to the strongest interaction of an attribute with one factor. Each of the five dependent factors had an eigenvalue above 1.0 and together 67.05 cumulative percent of variance. The taste and aftertaste of each side-taste metallic, bitter and astringent were aggregated to the first three factors. Sweet taste, aftertaste and lingering are allocated to the fourth factor. The fifth factor combines the relative sweetness and onset. This reduction confirms the results of the clustering of the sensory attributes in Fig. 1, in which the taste and aftertaste of each attribute appear to be highly correlated. The reduction of the independent variables, the 18 physicochemical descriptors, resulted in three independent factors (IF) (Table S3) according to the strongest interaction of a descriptor with one factor. Each of the three IF had an eigenvalue above 1.0 and a predictive power of 90.15 cumulative percent of variance. Here, IF-1 consolidated the most physicochemical descriptors, namely heavy atom count, molecular weight [g/mol], complexity, C-atoms, acceptors, bounded glucose, area polar surface [A^2], defined atom stereocenter count, donors, length glycone, rotatable bonds and OH-groups. IF-2 aggregates double bonds, ketones, aromatic rings and M logP and IF-3 combines the length of the alkyl chain and viscosity.

The explanatory power of the multivariate linear regression analysis with interactions is shown in Table 2 and is defined by the FDR-LogWorth for each IF and their interactions. The explanatory power by IF and interactions for each of the dependent factors is shown with the t ratio. FDR-LogWorth and t ratio were calculated within the multivariate

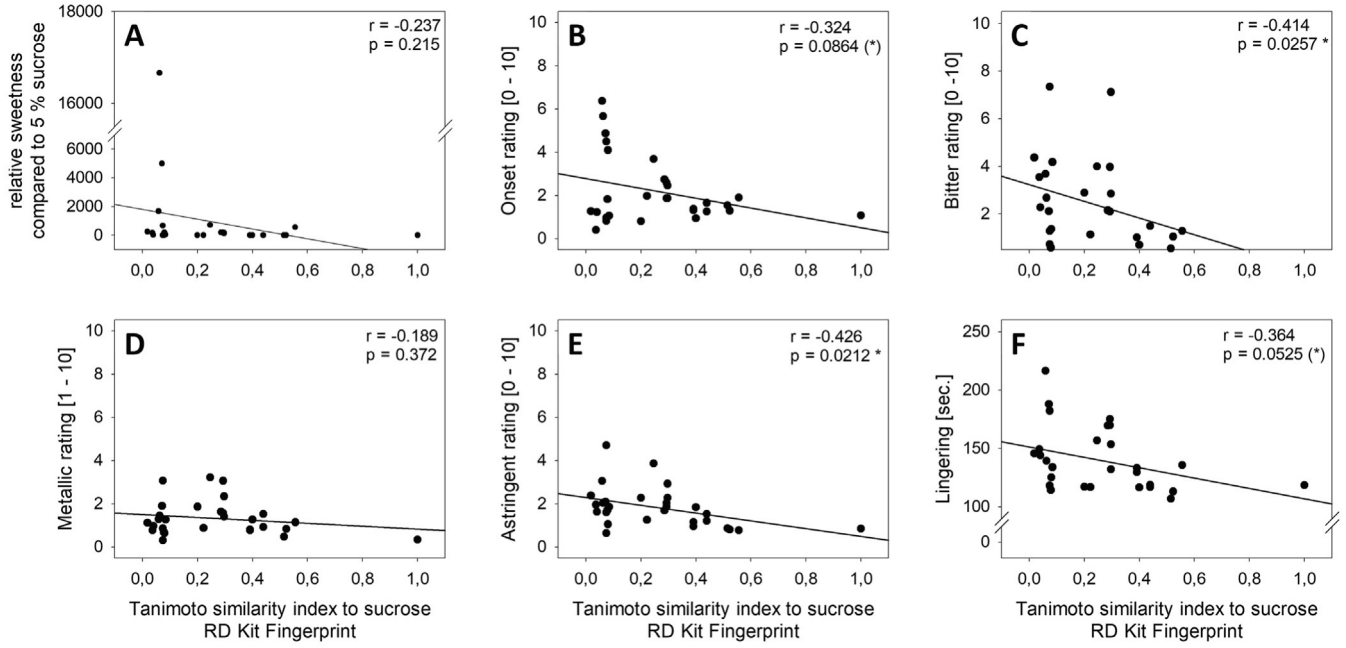


Fig. 2. Pearson correlation of the RD Kit-Fingerprint-Tanimoto similarity index to sucrose with the sensory rating of A: relative sweetness compared to 5% sucrose, B: onset, C: bitter, D: metallic, E: astringent and F: lingering.

linear regression analysis using JMP 14.0.0 and represent the power of the influence on the model. The darker the background color of a value, the stronger the effect, whereas red colors indicate positive and blue colors indicate negative associations. The multivariate linear regression analysis with interactions revealed that IF-2 with its descriptors double bonds, ketone, aromatic rings and M logP had the strongest explanatory power on the whole regression model with a FDR LogWorth of 91.7, followed by interaction of IF-1 and IF-2 with 16.4, IF-1 with 12.6, the interaction of IF-1, IF-2 and IF-3 with 11.1 and the interaction of IF-2 and IF-3 with 6.7 as FDR LogWorth, see Table 2. Except the interaction of IF-1 and IF-3, all interactions were significant (FDR p-value < 0.001) and with a value of 3.5, IF-3 had the lowest FDR LogWorth. This clearly shows that interactions among physicochemical descriptors may influence the sensory attributes and thus the perception of the tested compounds, particularly the temporal profile of the sweet sensation. After having profiled the 30 test compounds with a sweet-equivalence to 5% sucrose for selected sensory taste attributes, the influence of aggregated IF-1, IF-2 and IF-3 on the aggregated taste attributes as dependent variables were explored. Therefore, a multivariate regression analysis with preceding aggregation of dependent and independent variables to five dependent and three independent factors (IF) was carried out. The influence of the independent factors on dependent sensory factors are depicted for each dependent factor separately in Fig. 3 and related *t*-ratios are shown in Table 2. The t ratio reflects the strength of a factor

Table 2
LogWorth of independent factors (IF) on the whole model and t ratios of main and interaction effects of IF-1, IF-2 and IF-3 on the aggregated sensory attributes bitter, metallic, astringent, sweet & lingering and SF & onset in sweet tasting compounds. Depending on a positive (red) or negative (blue) t ratio, the interaction effect on a dependent factor is positive or negative. Calculated by a multiple regression analysis after aggregation of dependent to 5 factors and independent variables to 3 factors. Significant p values are labelled with * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

dependent factors / indep. factors	FDR LogWorth	relative sweetness & onset	bitter	metallic	astringent	sweet & lingering
		t ratio				
Intercept		2.24 *	–0.02	0.00	–0.92	0.19
IF-1	12.63 ***	7.45 ***	0.55	5.39 ***	0.71	3.88 ***
IF-2	91.71 ***	23.08 ***	4.83 ***	2.59 **	2.35 *	–0.87
IF-3	3.48 ***	–3.6 ***	2.61 **	3.27 **	0.95	1.94
IF-1 × IF-2	16.37 ***	8.58 ***	–3.34 ***	1.53	–0.27	–0.48
IF-1 × IF-3	0.52	–0.38	0.24	0.82	–1.04	0.52
IF-2 × IF-3	6.67 ***	–5.23 ***	3.89 ***	1.45	2.19 *	–0.86
IF-1 × IF-2 × IF-3	11.09 ***	–6.93 ***	0.06	–0.01	2.84 **	–0.59

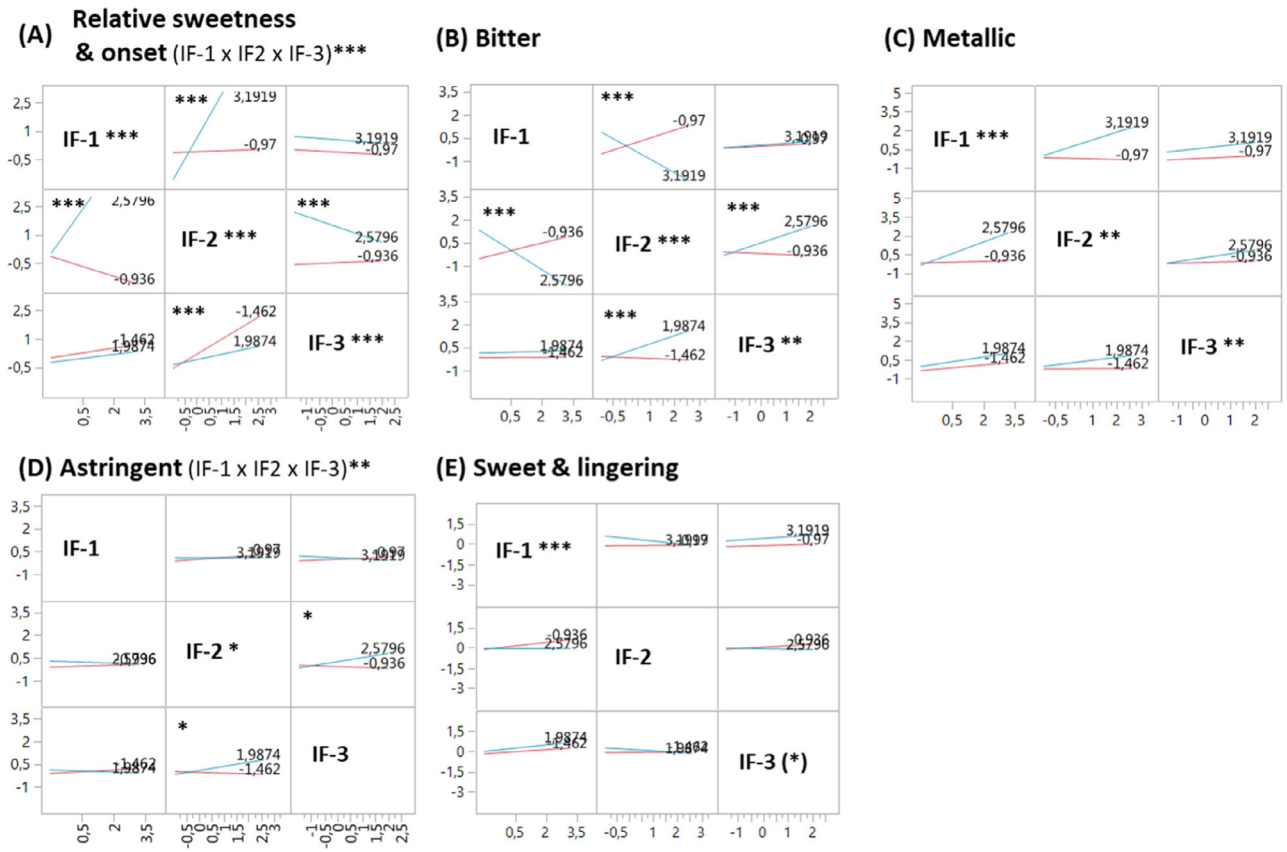


Fig. 3. Regression plots visualizing the interactions between the independent factors (IF–1, IF–2, and IF–3) for each dependent factor (relative sweetness & onset, bitter, metallic, astringent and sweet & lingering). Each interaction plot in a matrix shows the interaction of the row effect with the column effect for the dependent factor. This was calculated by a multiple regression analysis after aggregation of dependent and independent variables to 5 and 3 factors with JMP. Significant interactions between the IF are labelled with (*) for $p < 0.1$, * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

or of an interaction of several factors. Each interaction plot in a matrix shows the interaction of the row effect with the column effect for a dependent factor, demonstrating whether the impact of one factor depends on the value of another one. The analysis demonstrated that the impact of IF-1 on relative sweetness & onset changes as IF-2 increases, but is independent of the value of IF-3. Besides, the effect of IF-2 on sweetness & onset depends on the value of IF-3 and the other way around (see Fig. 3A). The analysis for relative sweetness & onset showed that IF-2 and IF-3 alone and as well the interaction of IF-1 and IF-2 are positively associated with the sweetness & onset, whereas IF-3 alone and the interaction of IF-2 and IF-3, as well as the interaction of all three IF had a negative association (see Table 2). In addition, the analysis showed that IF-2 and IF-3 alone and as well the interaction of IF-2 and IF-3 enhanced, whereas the interaction of IF-1 and IF-2 suppressed the intensity of bitterness (see Table 2). In contrast, the rating of metallic was only influenced by IF-1, IF-2 and IF-3 alone, but not by interactions (see Fig. 3C and Table 2). An increased astringency was associated with IF-2, in addition to the interaction of IF-2 and IF-3 besides the interaction of IF-1, IF-2 and IF-3 (see Table 2 and Fig. 3D). Sweet taste and sweet lingering were enhanced with increasing values for IF-1 and IF-3, but not by any interactions (see Fig. 3E and Table 2). This seems to be reasonable, because the sweet taste was adjusted to 5% sucrose, and lingering was correlated with the relative sweetness. Overall, the analysis demonstrated a pivotal role for the number of double bonds, ketones, aromatic rings and the M LogP. This is also demonstrated by the t ratio, reflecting the strength of a factor or interaction, which is largest for IF-2 for relative sweetness & onset (see Table 2).

Zhong et al. (2013) found a correlation between the aqueous solubility, which is related to the M logP value, and the sweetness, which is supported by the findings of a higher relative sweetness correlating with M logP in the present analysis. Sweet taste chemoreceptors in the oral cavity are covered mainly by water based saliva, hence solubility is thought to play an important role in sweet taste perception (Behrens, Meyerhof, Hellfritsch, & Hofmann, 2011; Meyerhof, 2015). The logP value was discovered quite early as an important descriptor for structure-sweet relationships (Deutsch & Hansch, 1966), but so far has not been associated with a delay in the onset of the sweet sensation. Clemens et al. (2016) summarized that the relative sweetness of sugars was associated with attached groups, especially hydroxyl groups as part of stereochemical configuration. In our analysis, no correlations between the relative sweetness and attached glucose, the length of alkyl chains or hydroxyl groups were detected. When focusing on the relationship of structure and sweetness of steviol glycosides, the C_{16}-C_{17} part was identified to be essential for the sweetness (Hellfritsch, Brockhoff, Stahler, Meyerhof, & Hofmann, 2012; Upreti, Dubois, & Prakash, 2012). Furthermore, it has also been discovered by Hellfritsch et al. (2012) that the glycone chain length and the pyranose substitution are responsible for the differences in the taste profile of steviol glycosides, too. However, when comparing several structurally strikingly different sweeteners and not only steviol glycosides, the similarities rather than the structural differences of the steviol glycosides are predominant. It can be assumed that due to their structural similarity, all steviol glycosides interact with the same binding site. This is supported by the fact that most of the steviol glycosides tested in the present study (substances 15–18 in Table 1) were joined together in one sensory-based cluster (see Fig. 1). This gives rise to the hypothesis that for the steviol glycosides tested here, the undesired

side-tastes, as well as onset and lingering of the sweet sensation, are based on the core structure of steviol glycosides rather than on the variable side chains.

Based on the results of the present study, we hypothesize that a longer lasting lingering is associated with more complex and heavier molecules, which might be based on the receptor binding. Also Tan et al. (2019) concluded that an enhanced lingering is the result of higher affinities of the non-nutritive sweeteners to the binding sites of the taste receptor. Similarly, a delay in onset could be due to an inferior fit of a compound to the respective binding site. This would explain the finding that more rigid double bonds, ketones and aromatic rings were associated with a high onset of the sweet sensation. Acevedo and Temussi (2019) suggested that one of the main reasons for unpleasant aftertaste is an interaction of sweeteners with the umami receptor. Furthermore, they reviewed that some sweeteners can of course also be recognized by other receptors, e.g. bitter and umami, which can contribute to an unpleasant side-taste (Acevedo & Temussi, 2019). Hence, we hypothesize that there are some similarities of compounds binding to the same receptor, as shown with the correlation and interaction analysis of the present study, also to the umami receptor. Moreover, Acevedo, Ramirez-Sarmiento, and Agosin (2018) could show that the electrostatic potential is important for the interaction of sweet proteins with the sweet taste receptor, as well the stabilization of the receptor by formation of hydrogen bonds, for example by the occurrence of sugars in the structures (Acevedo et al., 2018), which is represented by the IF-1 in this work. However, the actual sweetening potency cannot necessarily be inferred from the binding affinity. By analyzing the sweetness of isovanillyl derivates, Bassoli, Merlini, and Morini (2002) associated a 6-membered ring with two oxygen atoms in position 1,3 with a more intense sweetness. In the analysis of the present study, IF-1, to which the bonded OH-groups belong to, is positively correlated with the relative sweetness and the onset of a compound. Additionally IF-2, to which the aromatic rings belong to, is as well positively correlated to relative sweetness and onset, but here there was no interaction, as Bassoli et al. (2002) could show it for the group of isovanillic sweeteners. Thus, a group-specific structure–activity relationship, depending also on the different binding sites of the receptor, is supported by the results of the present study.

4. Conclusions

In the present study, a variety of sweet taste or sweet taste affecting compounds was used in a comparative sensory evaluation in order to analyze structural characteristics leading to differences in the time-intensity profile and undesired side-tastes. Our results show that the taste is highly correlated with the aftertaste, and that less structural similarity to sucrose results in enhanced bitterness, astringency and as well as a trend for onset and lingering. In addition, we demonstrate here for the first time that interactions between several physicochemical descriptors explain the relative sweetness and onset, providing an enhanced understanding of the molecular base for temporal sensory perception. The prediction of time intensity profiles and of undesired side-tastes of sweet and sweet taste affecting compounds has not been considered in previous models and the present study provides a starting point for improving those models in future studies in order to get a more detailed prediction and suggest the consideration of interactions.

CRediT authorship contribution statement

Corinna M. Karl: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Martin Wendelin:** Conceptualization, Methodology, Software, Validation, Resources. **Dariah Lutsch:** Software, Validation, Formal analysis, Visualization. **Gerhard Schleining:** Methodology, Resources. **Klaus Dürrschmid:** Conceptualization, Methodology. **Jakob P. Ley:** Conceptualization, Methodology, Resources, Supervision, Funding acquisition. **Gerhard E. Krammer:** Resources, Supervision, Funding acquisition. **Barbara Lieder:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors M. Wendelin, D. Lutsch, J. P. Ley and G. E. Krammer are employees of the company Symrise AG.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2020.100100.

References

Acevedo, W., Ramirez-Sarmiento, C. A., & Agosin, E. (2018). Identifying the interactions between natural, non-caloric sweeteners and the human sweet receptor by molecular docking. *Food Chemistry, 264*, 164–171. https://doi.org/10.1016/j.foodchem.2018.04.113.

Acevedo, W., & Temussi, P. A. (2019). The origin of unpleasant aftertastes in synthetic sweetener: A hypothesis. *Frontiers in Molecular BioScience, 5*(119), https://doi.org/10.3389/fmolb.2018.00119.

Bassoli, A., Merlini, L., & Morini, G. (2002). Isovanillyl sweeteners. From molecules to receptors. *Pure and Applied Chemistry, 74*(7), 1181–1187. https://doi.org/10.1351/pac200274071181.

Behrens, M., Meyerhof, W., Hellfritsch, C., & Hofmann, T. (2011). Sweet and umami taste: Natural products, their chemosensory targets, and beyond. *Angewandte Chemie International Edition, 50*(10), 2220–2242. https://doi.org/10.1002/anie.201002094.

Ben Shoshan-Galeczki, Y., & Niv, M. Y. (2020). Structure-based screening for discovery of sweet compounds. *Food Chemistry, 315*, Article 126286. https://doi.org/10.1016/j.foodchem.2020.126286.

Busch-Stockfisch, M. (2015). Prüferauswahl und Prüferschulung. In M. Busch-Stockfisch (Ed.). *Sensorik kompakt in der Produktentwicklung und Qualitätssicherung*. Hamburg, Germany: B. Behr's Verlag GmbH & Co. KG.

Chéron, J. B., Casciuc, I., Golebiowski, J., Antonczak, S., & Fiorucci, S. (2017). Sweetness prediction of natural compounds. *Food Chemistry, 221*, 1421–1425. https://doi.org/10.1016/j.foodchem.2016.10.145.

Chéron, J. B., Golebiowski, J., Antonczak, S., & Fiorucci, S. (2016). The anatomy of mammalian sweet taste receptors. *Proteins, 85*(2), 332–341. https://doi.org/10.1002/prot.25228.

Clemens, R. A., Jones, J. M., Kern, M., Lee, S.-Y., Mayhew, E. J., Slavin, J. L., & Zivanovic, S. (2016). Functionality of sugars in foods and health. *Comprehensive Reviews in Food Science and Food Safety, 15*(3), 433–470. https://doi.org/10.1111/1541-4337.12194.

Deutsch, E. W., & Hansch, C. (1966). Dependence of relative sweetness on hydrophobic bonding. *Nature, 211*, 75. https://doi.org/10.1038/211075a0.

DIN-EN-ISO. (2014). Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. German version, DIN EN ISO 8586:2014-05. Geneva, Switzerland.

DIN-NORM (1998). *Bestimmung der Geschmacksempfindlichkeit.* Berlin, Germany: Beuth.

DuBois, G. E. (2016). Molecular mechanism of sweetness sensation. *Physiology & Behavior, 164*, 453–463. https://doi.org/10.1016/j.physbeh.2016.03.015.

DuBois, G. E., Crosby, G. A., Stephenson, R. A., & Wingard, R. E., Jr. (1977). Dihydrochalcone sweeteners. Synthesis and sensory evaluation of sulfonate derivatives. *Journal of Agricultural and Food Chemistry, 25*(4), 763–772. https://doi.org/10.1021/jf60212a056.

DuBois, G. E., & Prakash, I. (2012). Non-caloric sweeteners, sweetness modulators, and sweetener enhancers. *Annual Review of Food Science and Technology, 3*, 353–380. https://doi.org/10.1146/annurev-food-022811-101236.

Gakidou, E., Afshin, A., Abajobir, A. A., Abate, K. H., Abbafati, C., Abbas, K. M., ... Murray, C. J. L. (2017). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of

risks, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet, 390*(10100), 1345–1422.

Goel, A., Gajula, K., Gupta, R., & Rai, B. (2018). In-silico prediction of sweetness using structure-activity relationship models. *Food Chemistry, 253*, 127–131. https://doi.org/10.1016/j.foodchem.2018.01.111.

Hellfritsch, C., Brockhoff, A., Stahler, F., Meyerhof, W., & Hofmann, T. (2012). Human psychometric and taste receptor responses to steviol glycosides. *Journal of Agricultural and Food Chemistry, 60*(27), 6782–6793. https://doi.org/10.1021/jf301297n.

Kuhn, C., Bufe, B., Winnig, M., Hofmann, T., Frank, O., Behrens, M., ... Meyerhof, W. (2004). Bitter taste receptors for saccharin and acesulfame K. *Journal of Neuroscience, 24*(45), 10260–10265. https://doi.org/10.1523/JNEUROSCI.1225-04.2004.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. *PNAS, 99*(7), 4692–4696. https://doi.org/10.1073/pnas.072090199.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery, 23*, 3–25. https://doi.org/10.1016/S0169-409X(96)00423-1.

Lustig, R. H., Schmidt, L. A., & Brindis, C. D. (2012). Public health: The toxic truth about sugar. *Nature, 482*(7383), 27–29. https://doi.org/10.1038/482027a.

Masuda, K., Koizumi, A., Nakajima, K., Tanaka, T., Abe, K., Misaka, T., & Ishiguro, M. (2012). Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds. *PLoS ONE, 7*(4), Article e35380. https://doi.org/10.1371/journal.pone.0035380.

Meyerhof, W. (2015). Sensorische Grundlagen - Geschmack. In M. Busch-Stockfisch (Ed.). *Sensorik kompakt in der Produktentwicklung und Qualitätssicherung*. Hanburg, Germany: B. Behr's Verlag GmbH & Co. KG.

Montmayeur, J. P., Liberles, S. D., Matsunami, H., & Buck, L. B. (2001). A candidate taste receptor gene near a sweet taste locus. *Nature Neuroscience, 4*(5), 492–498. https://doi.org/10.1038/87440.

Morini, G., Bassoli, A., & Temussi, P. A. (2005). From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. *Journal of Medicinal Chemistry, 48*, 5520–5529. https://doi.org/10.1021/jm0503345.

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J. P., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell, 106*(3), 381–390. https://doi.org/10.1016/S0092-8674(01)00451-2.

Ojha, P. K., & Roy, K. (2018). Development of a robust and validated 2D-QSPR model for sweetness potency of diverse functional organic molecules. *Food and Chemical Toxicology, 112*, 551–562. https://doi.org/10.1016/j.fct.2017.03.043.

Reyes, M. M., Castura, J. C., & Hayes, J. E. (2017). Characterizing dynamic sensory properties of nutritive and nonnutritive sweeteners with temporal check-all-that-apply. *Journal of Sensory Studies, 32*(3), https://doi.org/10.1111/joss.12270.

Rojas, C., Ballabio, D., Consonni, V., Tripaldi, P., Mauri, A., & Todeschini, R. (2016). Quantitative structure–activity relationships to predict sweet and non-sweet tastes. *Theoretical Chemistry Accounts, 135*(66), 13. https://doi.org/10.1007/s00214-016-1812-1.

Rojas, C., Todeschini, R., Ballabio, D., Mauri, A., Consonni, V., Tripaldi, P., & Grisoni, F. (2017). A QSTR-based expert system to predict sweetness of molecules. *Frontiers in Chemistry, 5*, 53. https://doi.org/10.3389/fchem.2017.00053.

Shallenberger, R. S., & Acree, T. E. (1969). Molecular structure and sweet taste. *Journal of Agricultural and Food Chemistry, 17*(4), 701–703. https://doi.org/10.1021/jf60164a032.

Spaggiari, G., Di Pizio, A., & Cozzini, P. (2020). Sweet, umami and bitter taste receptors: State of the art of in silico molecular modeling approaches. *Trends in Food Science & Technology, 96*, 21–29. https://doi.org/10.1016/j.tifs.2019.12.002.

Sukumaran, S. K., Yee, K. K., Iwata, S., Kotha, R., Quezada-Calvillo, R., Nichols, B. L., ... Margolskee, R. F. (2016). Taste cell-expressed alpha-glucosidase enzymes contribute to gustatory responses to disaccharides. *PNAS, 113*(21), 6035–6040. https://doi.org/10.1073/pnas.1520843113.

Sylvetsky, A. C., & Rother, K. I. (2016). Trends in the consumption of low-calorie sweeteners. *Physiology & Behavior, 164*, 446–450. https://doi.org/10.1016/j.physbeh.2016.03.030.

Tan, V. W. K., Wee, M. S. M., Tomic, O., & Forde, C. G. (2019). Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA). *Food Research International, 121*, 39–47. https://doi.org/10.1016/j.foodres.2019.03.019.

Upreti, M., Dubois, G., & Prakash, I. (2012). Synthetic study on the relationship between structure and sweet taste properties of steviol glycosides. *Molecules, 17*(4), 4186–4196. https://doi.org/10.3390/molecules17044186.

Yang, X., Chong, Y., Yan, A., & Chen, J. (2011). In-silico prediction of sweetness of sugars and sweeteners. *Food Chemistry, 128*(3), 653–658. https://doi.org/10.1016/j.foodchem.2011.03.081.

Yee, K. K., Sukumaran, S. K., Kotha, R., Gilbertson, T. A., & Margolskee, R. F. (2011). Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells. *PNAS, 108*(13), 5431–5436. https://doi.org/10.1073/pnas.1100495108.

Zhong, M., Chong, Y., Nie, X., Yan, A., & Yuan, Q. (2013). Prediction of sweetness by multilinear regression analysis and support vector machine. *Journal of Food Science, 78*(9), S1445–1450. https://doi.org/10.1111/1750-3841.12199.

3.5.2. "Impact of lactisole on the time-intensity profile of selected sweeteners in dependence of the binding site"

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Impact of lactisole on the time-intensity profile of selected sweeteners in dependence of the binding site

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Abstract (Food Chemistry: 150 words)

Currently, there is limited insight into the influence of the different binding sites of agonists and antagonists of the sweet taste receptor TAS1R2/TAS1R3 on temporal sensory properties of sweet tasting compounds. We investigated whether the binding site and a competitive or allosteric inhibition of TAS1R2/TAS1R3 influence the time-dependent sensory perception and *in vitro* TAS1R2/TAS1R3-activation profiles. We compared time-intensity ratings of cyclamate, NHDC, acesulfame K, and aspartame with and without lactisole with the corresponding TAS1R2/TAS1R3-activation in transfected HEK293 cells. In combination with lactisole, cyclamate and NHDC demonstrated a shift of the dose-response curve corresponding to a competitive inhibition by lactisole in the sensory and the cell experiments. Allosteric inhibition by lactisole for aspartame and acesulfame K was seen in the cell experiments, but not the sensory ratings. In conclusion, the data do not support a major impact of the binding site on the time-intensity profile of the tested sweeteners.

Keywords: sweet taste receptor, time-intensity, cyclamate, NHDC, aspartame, acesulfame K

1. Introduction

The human preference for sweet taste is innate, probably because sweetness signals a caloric benefit of food (Ganchrow et al., 1983; Nelson et al., 2001; Ventura & Worobey, 2013). Beside the classical house-hold sugar sucrose, a large variety of structurally diverse compounds is known to lead to a sweet taste perception. However, the sensorial sweetness impression can differ largely between the different compounds. Especially differences in the sweet temporal profile are well-described in literature and mainly refer to differences in the onset and lingering of the sweetness (DuBois, 2016; DuBois & Prakash, 2012; Karl et al., 2020; Tan et al., 2019). The onset describes the time of appearance until the taste reaches its first maximum intensity, whereas lingering refers to the extinction time a taste remains in the mouth (DuBois et al., 1977; Karl et al., 2020). To date, the molecular basis of those differences in the temporal profile of sweet perception is not yet fully understood. In general, sweet tasting compounds are known to activate the canonical sweet taste receptor TAS1R2/TAS1R3, a class C G-protein-coupled and heterodimeric receptor. The two receptor subunits TAS1R2 and TAS1R3 are each composed of a large extracellular amino terminal domain, also known as Venus Flytrap Domain (VFD), which is linked to a Cysteine-Rich Domain (CRD) and further to a Trans-Membrane Domain (TMD) with seven helices (Pin et al., 2003). For multiple sweet tasting compounds, at least one binding site at the TAS1R2/TAS1R3 has been identified. While sucrose and glucose can bind to the VFD of both receptor subunits, with different affinities for the two subunits (Nie et al., 2005), cyclamate and neohesperidin dihydrochalcone (NHDC) have been demonstrated to bind to the TMD of TAS1R3 only (Jiang, Cui, Zhao, Snyder, et al., 2005; Winnig et al., 2007; Xu et al., 2004). However, the cleft formed by two lobes of the VFD at TAS1R2 subunit is regarded as the predominant binding site for many sweet tasting compounds. For example, the carbohydrate fructose, as well as the classical sweeteners sucralose, aspartame, neotame, saccharin and acesulfame K (ace K) have been shown to bind to the VFD of TAS1R2 (Masuda et al., 2012; Xu et al., 2004; Zhang et al., 2010). However, saccharin and ace K have been reported to bind with lower affinity also to the TMD of TAS1R3, which inhibits the sweet taste signaling by shifting the receptor to

an inactive confirmation (Galindo-Cuspinera et al., 2006). Zhao et al. additionally proposed inhibitory residues for saccharin at the TAS1R2 by using chimeric human/mouse receptors (Zhao et al., 2022). Another compound that was previously shown to suppress the sweetness of several common sweeteners and sugars is lactisole, which binds to the TMD of the TAS1R3 subunit (Jiang, Cui, Zhao, Liu, et al., 2005; Jiang, Cui, Zhao, Snyder, et al., 2005; Winnig et al., 2007; Xu et al., 2004).

Since the sweet taste receptor has multiple binding sites, different compounds can act as positive or negative allosteric modulators, or competitive inhibitors depending on the specific binding sites of the agonists and antagonists, respectively. Lactisole can thus act as an allosteric or competitive inhibitor for certain compounds based on their binding site (Servant et al., 2020; Winnig et al., 2007). Competitive inhibition by lactisole via binding to the same binding-site, for example with cyclamate and NHDC, is then characterized by a right-shift of the dose-response relationship with an enhanced EC_{50}-value, but a similar E_{max} and hillslope value. In contrast, allosteric inhibition through binding to two different binding-sites, for example with aspartame and ace K, is characterized by a similar EC_{50} value in addition to a decreased E_{max} and hillslope value (May et al., 2007; Winnig et al., 2007). This concept has been successfully applied by Winnig et al. (2007), who, in addition to experiments using receptor chimera, targeted point mutations, and docking studies, used the analysis of dose-response relationships obtained from TAS1R2/TAS1R3-transfected HEK293 cells of the above-named sweeteners in combination with lactisole for the confirmation of the binding site of NHDC (Winnig et al., 2007). In addition to its sweet taste inhibiting effect, lactisole is also known to induce a delayed sweet taste, called “water taste”, after rinsing with water (Galindo-Cuspinera et al., 2006).

Despite the increasing knowledge regarding the interaction of sweeteners and the TAS1R2/TAS1R3, there is only limited insight into the influence of the different binding sites of an agonist and antagonist on temporal sensory properties. Thus, we here aimed to investigate whether the binding site and a competitive or allosteric inhibition of the sweet taste receptor influence the time-dependent sensory perception and sweet taste receptor activation profiles and will translate into the corresponding changes in the time-dependent dose-response relationships. A well-established

method to measure the temporal sensory properties of one specific attribute is the time-intensity (TI) measurement (Guinard et al., 1995; Ott et al., 1991). To analyze the activation of a particular GPCR as for example the sweet taste receptor, Ca^{2+}-mobilization in transfected HEK293 cells is the standard method (Behrens et al., 2017; Ben Abu et al., 2021). Thus, in the present study, two sweeteners that have been proposed to bind to the TAS1R2-VFTD (ace K, aspartame) and two sweeteners that target the TAS1R3-TMD (cyclamate and NHDC) have been selected and their TI-profiles for sweet taste have been recorded in a broad range of concentrations alone or in combination with the sweet taste receptor antagonist lactisole. In parallel, dose-dependencies of the Ca^{2+}-responses of TAS1R2/TAS1R3 transfected HEK293 cells were measured after stimulation with the same test compounds with or without lactisole and compared to sensory results. We hypothesized that the determined parameters for the temporal sensory properties of ace K and aspartame, will demonstrate an allosteric inhibition mode, whereas for cyclamate and NHDC, a competitive inhibition in the respective dose-response curves will be seen when applied in combination with lactisole in the sensory and cell studies.

2. Materials & Methods

2.1 Materials

All compounds used for sensory evaluations were obtained in food grade (FG) quality. Citric acid, ethanol, monosodium glutamate, sodium chloride and sucrose were purchased from local supermarkets and pharmacies (Vienna, AT). Caffeine (anhydrous, 99 %, FG, W222402) and NHDC (≥96 %, FG, W381101) was obtained from Merck KGaA (Darmstadt, DE). Acesulfame K (> 98 %), aspartame (> 99 %), cyclamate (> 99 %), lactisole (> 99 %), iron lactate-IIhydrate, and tannic acid (nat.) were kindly provided in FG quality by the Symrise AG (Holzminden, DE). Compounds used in cell experiments were aspartame, cyclamate (sodium salt, ≥ 99 %), neohesperidin dihydrochalcone (≥ 95 %), acesulfame K (≥ 99 %) from Sigma-Aldrich; lactisole (sodium salt, ≥ 98 %) from Cayman Chemical.

116 2.2 Sensory evaluation

117 For performing sensory analysis, a panel of 37 test persons (f: 26, m: 11, age: 28.6 ± 6.3 years, BMI:
118 22.7 ± 2.7) rated the test solutions. All panelists gave their written informed consent for participating
119 in the panel and had to complete a sensory training. First, a training of the basic tastes sweet by
120 10.0 g/L sucrose, bitter by 0.3 g/L caffeine, salty by 2.0 g/L sodium chloride, sour by 0.3 g/L citric acid
121 and umami by 0.6 g/L monosodium glutamate (DIN-EN-ISO, 2014) was conducted. For sweet and
122 bitter thresholds ascending concentrations of sucrose and caffeine were tested (DIN-EN-ISO, 2011;
123 Höhl & Busch-Stockfisch, 2015). Secondly, the panelists had to rank four concentrations each of a
124 bitter, sweet (Busch-Stockfisch, 2015), metallic (iron lactate-II hydrate), and also two astringent
125 (tannic acid, nat.), solutions in order of intensity (Karl et al., 2020). On a last training day, the
126 evaluation on unstructured scales and the time-dependent evaluation on the computer were trained.
127 All panelists reported to be in good general health condition, and not being under medication or
128 pregnant. At least one hour before every training or tasting, panelists were instructed not to smoke
129 and not to consume intense tasting food or beverage (e.g. coffee, chili, garlic, chewing gum) and to
130 avoid in general strong hunger or fullness as well as strong odors or perfume on test days.

131 For evaluation of the time-dependent parameters of the selected sweet test compounds, a TI-
132 measurement was applied, using the software EyeQuestion® 4.11.74 (Logic8 BV, NL) online. The 7 - 8
133 ascending concentrations of the test compounds were pre-tested by selected panelists (n= 3 - 4) to
134 be in a sensory consumable range, namely 0.01 - 50 mM ace K, 0.01 - 20 mM aspartame,
135 0.1 - 100 mM cyclamate and 0.001 - 1.0 mM NHDC. Each test compound and concentration was
136 tested alone and in combination with 0.46 mM (100 ppm) and 0.92 mM (200 ppm) lactisole. The
137 concentrations were chosen because lactisole (sodium 2-(4-methoxy phenoxy) propanoate) is
138 commonly used up to 150 ppm in food (Burdock et al., 1990) and typical cell experiments were
139 conducted with 1.0 mM (Winnig et al., 2005). Every test solution was assessed randomly at two
140 different test days with at least 8 panelists participating per replicate. The panelists were free to
141 choose to participate at the different test days. A maximum of five test compounds were assessed in

one session. All samples were coded with three-digit random code and presented to the panelists in randomized order. On each test day, panelists were presented five sweet solutions with 0, 25, 50, 100 and 200 g/L sucrose as a scale training for the ratings from “not at all” to “very intensive” sweetness. The panelists had to rate the intensity of the samples on an unstructured scale from “not at all” to “very intensive” sweetness for 180 seconds (sec) by moving the slider control in the software according to their perceived sweet intensity. The three minutes of evaluation were chosen to capture as much of the lingering as possible without wearing out the panelists during the evaluation. A total of 350 measuring time points was recorded within the pre-set time frame, especially to see differences in the first seconds of evaluation, which are important for the onset. The panelists were asked to take the sample (20 mL) into their mouth, while starting the timer and simultaneously the evaluation of sweet intensity over the time, and to spit out the sample after ten seconds while continuing the evaluation of the sweetness intensity until the end of the three minutes.

For comparison of the time-dependent properties, the following descriptors were used: the maximum intensity at the first 30 sec of evaluation as “max. intensity”, the time-point of the first maximum intensity as “onset” in sec, the intensity at 90 sec as “lingering” effect, and the area under the curve from the time-intensity plots as “AUC” as a marker for the overall intensity and duration.

All sensory experiments were conducted at the Christian Doppler Laboratory for Taste Research at the Institute for Physiological Chemistry, University of Vienna, Austria, at room temperature (21 - 23 °C).

2.3 Calcium mobilization assay

For the functional characterization of the human sweet taste receptor, we used HEK293 Flp-In T-Rex cells stably transfected with the G protein chimera $G\alpha15_{Gi3}$, and the two subunits of the human sweet taste receptor, TAS1R2 and TAS1R3 (Galindo-Cuspinera et al., 2006). The G protein chimera

and TAS1R2 subunit are constitutively expressed, whereas the expression of the TAS1R3 subunit is inducible through addition of 0.5 µg/mL tetracycline. The experiments were done exactly as before (Behrens et al., 2017; Ben Abu et al., 2021). Briefly, cells were seeded onto 96-well plates (black, flat clear bottom) treated with 10 µg/mL poly-D-lysine and grown in low-glucose DMEM supplemented with 10 % fetal bovine serum, 100 U Penicillin/mL, 0.1 mg/mL Streptomycin, 2 mM L-glutamine, at 37°C, 5 % CO_2, saturated air-humidity overnight. About 24 hours before the experiment tetracycline was added to induce TAS1R3 expression. Next, cells were loaded for 1 h with Fluo-4 am in the presence of 2.5 mM probenecid and washed twice with C1-buffer (130 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM sodium pyruvate, and 2 mM $CaCl_2$, pH 7.4). Then, plates were placed in a fluorometric imaging plate reader (FLIPR^{tetra}, Molecular Devices, San Jose, CA, United States) for automated compound application and measurement of fluorescence changes. Fluorescence changes of control wells not induced with tetracycline were subtracted from the data. Next, measurements were corrected for background fluorescence. Dose-response relationships of three independent experiments each performed in duplicates were calculated with SigmaPlot 14.0 software using the function $f(x)= min+(max-min)/(1+(x/EC_{50})^{nH})$.

2.4 Computational and statistical analysis

TI-ratings were recorded and analyzed using the software EyeQuestion® 4.11.74 (Logic8 BV, NL) online and MS Excel 16.0 (Microsoft Corporation, USA). The statistical analysis and graphical representation were performed with GraphPad Prism 9.0 for sensory results and with SigmaPlot 14 for cell experiments. To evaluate the sensory dose-response effects of ascending sweetener concentration w/o lactisole, an asymmetrical five-parameter curve (Richard´s five-parameter dose-response curve) was fitted. The data are presented as means ± SEM from at least 16 single evaluations. Statistical significance between the different test compounds and concentration-dependent effects was assessed by two-way ANOVAs with Tukey post hoc test using Graph Pad Prism. The normal distribution of the data sets was checked by evaluation of the kurtosis (between -7

to +7) and the skewness (between -3 to +3). Equal variance was tested using Levene´s test. In case the assumptions of a normal distribution and equal variance were not met, the non-parametric ANOVA on ranks (Kruskal-Wallis Test) with Dunn´s post-hoc test without the calculation of interaction was applied. A Pearson product moment correlation was conducted to investigate relationships between sensory results and cell responses of the tested sweeteners.

3. Results

In the present study, the impact of selected sweet tasting compounds, namely acesulfame K (ace K), aspartame, cyclamate and NHDC on the sweet taste receptor TAS1R2/TAS1R3 was evaluated. First, TI-measurements for sweetness of the test compounds were conducted in order to obtain time-dependent parameters in dependence of concentration with and without the combined application of the sweet taste inhibitor lactisole. As lactisole acts either as a competitive or allosteric inhibitor for sweeteners based on their binding site at the sweet taste receptor, the aim of this experiment was to detect whether a different binding site at the sweet taste receptor also translates into differences on the temporal sensory profile.

A broad for panelists acceptable concentration range of each test compound was used, in absence or presence of 0.46 mM and 0.92 mM lactisole (100 ppm and 200 ppm). Fig. 1 shows the time-intensity profiles of the tested concentrations of cyclamate (A-C), NHDC (D-F), ace K (G-I) and aspartame (J-L) w/o lactisole. Higher concentrations of the sweeteners led to a higher intensity curve of all test compounds with more pronounced lingering (two-way-ANOVA, $p<0.0001$), whereas the lowest test concentrations were hardly perceived as sweet. Cyclamate (Fig. 1 A-C) showed a strong dose-dependency of the maximum intensity in the time-intensity curves without major discontinuities in the perceived intensity in the tested concentrations. The inhibition of cyclamate's sweetness by lactisole was effective for all concentrations and dose-dependent with effective inhibition for up to 5.0 mM and 10.0 mM cyclamate for 0.46mM and 0.92 mM of lactisole (Fig. 1 B&C). Lactisole had a

dose-dependent effect on the sweetness of NHDC (two-way-ANOVA, $p < 0.001$). A proper inhibition was detected up to 0.2 mM NHDC with 0.46 mM lactisole (Fig. 1 E) and up to 0.5 mM NHDC with 0.92 mM lactisole (Fig. 1 F). In case of ace K and aspartame, a saturation of the perceived maximum intensity was present for the three highest concentrations. Interestingly, the intensity of ace K (Fig. 1 G-I) and aspartame (Fig. 1 J-L) rose sharply by increasing the concentration from 2 mM to 5 mM, regardless of whether lactisole was added or not. The addition of 0.46 mM and 0.92 mM lactisole to up to 1 mM ace K effectively inhibited the sweetness over the time (Fig. 1 H & I). Aspartame revealed similar TI-curves with a strong lingering effect in all test concentrations compared to ace K. The addition of 0.46 mM lactisole (Fig. 1 K) inhibited the sweetness of aspartame up to a concentration of 1.0 mM of aspartame, and up to 2.0 mM when 0.92 mM of lactisole was used (Fig. 1 L).

Notably, an increased sweetness in the combinations with lactisole was reported by the panelists after around 30 sec, which is similar to the temporal sweetness recordings of lactisole alone (Fig. 1 M). This increased sweetness after approximately 30 sec was seen for all combinations at which lactisole was able to completely inhibit the sweetness of the test compounds and was higher for 0.92 mM lactisole compared to 0.46 mM lactisole.

Next, the effect of lactisole in dependence of the different binding sites of the sweeteners on the temporal markers maximum intensity, AUC, onset, and lingering was analyzed. In more detail, we hypothesized that the dose-response relationships for the selected markers will show a competitive inhibition in combination with lactisole for cyclamate and NHDC, and an allosteric inhibition in combination with ace K and aspartame. First, the max. intensity was plotted against all tested concentrations. The dose-response curves for ace K, aspartame, NHDC and cyclamate obtained from the maximum intensities of the respective time-intensity curves showed a sigmoidal pattern and are displayed in Fig. 2. The applied two-way ANOVA models revealed that the inhibitory effect of lactisole was dependent on the sweetener concentrations, except for NHDC.

For cyclamate, the applied model gave similar E_{max} values without or with 0.46 mM lactisole, and due to the higher variation, no E_{max} could be calculated for 0.92 mM lactisole. The corresponding EC_{50}

values for cyclamate increased with higher lactisole concentration. The hillslope remained at a similar level for the effects with or without lactisole, see Fig. 2 A. The model calculated based on the max. intensities of NHDC showed that the E_{max} values remained similar with increasing lactisole concentrations (Fig. 2 B), and the calculated EC_{50} values for NHDC rose from 0.056 mM without lactisole to 0.14 mM with 0.46 mM lactisole, and 0.18 mM with 0.92 mM lactisole. Similar to cyclamate, the hillslopes of the NHDC dose-response curves remained similar (Fig. 2 B, $p > 0.05$). To summarize, the curve shifts for cyclamate and NHDC mostly follow the expectation for a competitive binding mode with lactisole. For ace K, the E_{max} was reduced by 19.34 % and 22.38 % ($p < 0.01$), respectively, for 0.46 and 0.92 mM lactisole (Fig. 2 C). The EC_{50} values of ace K curves increased with increased lactisole concentrations without dose-dependent changes in the hillslopes (Fig. 2 C). In case of aspartame (Fig. 2 D) the EC_{50}-values increased with increasing lactisole concentrations, but the hillslope of the aspartame dose-response curves decreased from 2.56 without lactisole to 1.28 at 0.46 mM and 1.32 with 0.92 mM lactisole. Thus, the curve shift for ace K mainly followed the expected allosteric inhibition mode with lactisole, which was not consistently the case with aspartame.

As a second parameter, the AUC of the TI-curves in the tested range of concentrations was compared, to assess the summated overall sweetness impression exerted by the different sweeteners w/o lactisole as a function of duration and intensity. The concentration dependency of the AUC for the four sweeteners w/o lactisole is displayed in Fig. 3. In contrast to the dose-dependent curves of the max. intensities, no saturation for the AUC was reached with the highest concentrations of sweeteners, although a dose-dependency was seen for the sweeteners without lactisole (Fig. 3, red lines). Similar to the max. intensity, the AUC of the time-intensity curves showed that the effect of lactisole was dependent on the sweetener concentration, demonstrated by the significant interaction of the sweetener concentration and the inhibitory effect of lactisole in the two-way ANOVA ($p < 0.05$), except for NHDC. In lower concentrations of the sweeteners, the sweet aftertaste of lactisole after 30 sec, as described above, led to increased AUCs. Similar E_{max} values

were obtained for the combination with 0.46 mM or 0.92 mM lactisole, although in the combinations with lactisole, the rise of the curves was present only from a concentration of 10 mM or 20 mM cyclamate respectively (right shift).

In addition, the onset, the time until the max. intensity is first reached, (see Supplemental Fig. S1) and the lingering, analyzed as intensity at t= 90 sec (Supplemental Fig. S2) were evaluated for a dose-dependent effect. Regarding the onset (Supplemental Fig. S1), the sweet aftertaste of lactisole after 30 sec in the lower sweetener concentration dominated and overruled the intrinsic sweetness of the test compounds, as a sweet perception for the sweet taste inhibitor lactisole was reported by the panelists after around 30 sec. This can be seen from the dose-response plots of the onset, at which the onset was largely increased within the lower test concentrations at which the sweeteners did not show significant sweetness on their own. There was no clear difference between the test compounds based on their binding site detectable. Similarly, there were no major differences in the dose-response curves of the different sweeteners for the lingering (Supplemental Fig. S2), however, all compounds showed an increased lingering with higher concentration of the test compound (sweetener concentration effect $p< 0.0001$) and only for cyclamate the effect of lactisole was dependent on its concentration ($p< 0.001$). Furthermore, similarly to the onset, there was an increased lingering for all tested sweeteners in combination with lactisole which was independent of the binding site.

The onset and lingering were additionally analyzed by comparing the first onset and decay time for a concentration at the sweet intensity saturation level (Fig. 4). The onset was in a similar range for all test compounds, namely 4.6 sec for ace K, followed by cyclamate and NHDC with 5.1 sec and aspartame with 5.7 sec. The lingering effect was analyzed by comparing the time point at which a sweet compound reached less than 50 % of its maximum sweetness. The fastest sweetness decay was recorded for ace K with less than 50 % of maximum intensity after 44.7 sec, followed by cyclamate with 60.1 sec and aspartame with 71.4 sec. NHDC showed the most prolonged lingering

aftertaste with 100.7 sec, see Fig. 4. In summary, the temporal parameters were not consistently different between the orthosteric (TAS1R2-VFD) or allosteric (TAS1R3-TMD) binding site.

The results of the sensorial time-intensity measurements were compared to activation of the sweet taste receptor in transfected HEK293 cells. The functional analyses of a sweet taste receptor expressing mammalian cell line confirmed previous results on the differential lactisole sensitivities of the sweet taste receptor responses to sweeteners (Winnig et al. 2007). Sweeteners binding at the VFD of the TAS1R2 subunit showed pronounced depressions of their maximal signal amplitudes at elevated lactisole concentrations (Fig. 5, ace K (A) and aspartame (B)). Sweeteners binding to the TMD of the TAS1R3 subunit overlapping with the binding site for lactisole exhibited mostly a right shift in the dose-response relationships without comparable pronounced depressions of the maximum signal amplitudes (Fig. 5, cyclamate (C) and NHDC (D)). The exemplarily fluorescence traces shown in Fig. 5, (E-F) demonstrated that the onset and the decay of sweet taste receptor signal deviate among different sweeteners also *in vitro*. Whereas sweet taste receptor expressing cells stimulated with 10 mM of cyclamate (Fig. 5 G) reaches peak activity already after 39 sec (19 sec after agonist application at 20 sec), Ace K (Fig. 5 E) and NHDC (Fig. 5 H) stimulated cells required 47 sec (27 sec). Aspartame responses (Fig. 5 F) with 43 sec (23 sec) fell between these extremes. Thus, the activation of the sweet taste receptor via the orthosteric (TAS1R2-VFD) or allosteric (TAS1R3-TMD) binding site seems to have no consistent effect on the speed of signal onset. Also signal decays showed considerable differences. The only sweetener-induced receptor activation showing a signal decay back to baseline or even below was documented for Ace K (Fig. 5 E) after about 3 min, whereas aspartame (Fig. 5 F), cyclamate (Fig. 5 G) and NHDC (Fig. 5 H) stimulated cells maintained signals above the initial baseline for 6 min and beyond. Therefore, the times where signals decreased just below 50 % of the maximum signal amplitudes were monitored as well. Here, the fastest signal decrease was seen for Ace K (85 sec, 65 sec after agonist application at 20 sec) and the slowest for NHDC (107 sec and 87 sec, respectively). Aspartame and cyclamate exhibited identical signal decay times with 93 sec (73 sec). Again, signal decay seems to be independent on the interaction site.

A direct comparison of the obtained EC_{50} values for the sensory data and derived from the transfected HEK293 cells revealed a strong correlation (Fig. 6), with r= 0.88 (p< 0.001) for the EC_{50} of max. intensity of sensory to EC_{50} of response of HEK296 cells.

4. Discussion

The present study examined the impact of the binding site of the high-impact sweeteners ace K, aspartame, cyclamate, and NHDC when applied in combination with the sweet taste inhibitor lactisole on TI- sweetness profiles in order to investigate a potential connection between the binding site and the impact on the temporal profile. We hypothesized that lactisole, which binds to the TMD of the TAS1R3 subunit of the sweet taste receptor, will lead to either allosteric (ace K, aspartame) or competitive (cyclamate, NHDC) inhibition with typical shift in the dose-response relationships.

As markers for the temporal characteristics of the sweetness perception, the maximum intensity, the AUC from the respective TI-plots, as well as onset and lingering were investigated. As expected, with rising concentration of the sweeteners, also the sweetness rating, represented by the maximum intensities as well as the AUC of the corresponding TI- plots were increased. Two, respectively three for the cell experiments, different concentrations of lactisole were included in the present study which are in a relevant range for food applications and cell experiments (Burdock et al., 1990; Winnig et al., 2007). A sweet perception for the sweet taste inhibitor lactisole was reported by the panelists after around 30 sec. This sweet aftertaste of lactisole was dose-dependent and also present at lower sweetener concentrations at which lactisole inhibited the sweetness of the test compounds completely. A certain sweet aftertaste of lactisole and other sweet taste inhibiting compounds is well-known and was previously described as “sweet water taste” as it can be induced by rinsing with water after the inhibitor stimulus (DuBois, 2016; Galindo-Cuspinera et al., 2006; Sigoillot et al., 2012). To elucidate the molecular bases of this effect, Galindo-Cuspinera et al. (2006) investigated the signal of TAS1R2/TAS1R3 transfected HEK293 cells and as well sensory approaches after stimulation with

several sweeteners and sweet inhibitory compounds before and after rinsing with water. They concluded from their experiments that the sweet water effect is based on a lactisole-induced shift of the TAS1R2/TAS1R3 to its inactive conformation. Rinsing with water then dissociates lactisole from the receptor and changes the balance from the inhibited receptor state towards a constitutively active state, inducing stimulus transmission with the following sweet impression. Furthermore, they suggest the sweet water effect as an identifying feature for sweet taste inhibitors (Galindo-Cuspinera et al., 2006). The results of the present study lead to the assumption that after approximately 30 sec, also saliva secretion, without the necessity of rinsing with water, can started to induce a sweet aftertaste impression, which supports the idea of the dissociation from the receptor to be important for the sweet water taste of lactisole. We propose that not only water can induce the sweet lactisole effect, but also salivary secretion during tasting. Because of the clear noticeable sweet-water effect of lactisole, we only used the values up to 30 sec to identify the maximum sweetness for the sweeteners.

Focusing on the TI-parameters and the effect of lactisole on the different sweeteners, the shift of the dose-response relationships of the maximum intensity for cyclamate and NHDC fit into the model of competitive inhibition in combination with lactisole. Similarly, the shifts in the dose-response relationships of the AUC can be best explained by a competitive inhibition model. As the binding site of cyclamate, NHDC, and lactisole has been previously shown to be located at the TMD of TAS1R3 (Jiang, Cui, Zhao, Liu, et al., 2005; Jiang, Cui, Zhao, Snyder, et al., 2005; Winnig et al., 2007; Xu et al., 2004), these results are as expected and are consistent with the results by Winnig et al. obtained with receptor cell models *in vitro* (Winnig et al., 2007) and additionally confirmed by the here presented cell experiments. Nevertheless, for all analyzed sweet sensory parameters, the concentrations of NHDC did not influence the effect of lactisole, as it was seen for the other sweeteners by interaction of the two-way-ANOVA factors. A reason for these sensory differences and independency of lactisole could be the high affinity of NHDC for the sweet taste receptor, which is reflected by a sensory sweetness factor of approximately 900 compared to around 30 for cyclamate,

both compared to 5 % sucrose (Schiffman et al., 1995). This high potency of NHDC suggests a strong affinity of NHDC for its binding site. An effective displacement of lactisole even at low concentrations of NHDC is consequently observed, since the binding site of NHDC and lactisole are overlapping.

The inhibitory effect of lactisole on ace K and aspartame was expected to reflect an allosteric inhibition mode, as these two sweeteners have been described to bind with high affinity to the VFD of TAS1R2 (Maillet et al., 2015; Masuda et al., 2012). The dose-response relationships of the maximum intensities of aspartame are similar to the cell-based single receptor assay, however, the E_{max} in the sensory experiments was less reduced than expected. This was not the case in the *in vitro* experiments, where pronounced reductions in the maximal signal amplitudes already at 100 µM of lactisole were evident. A reason for the discrepancy between the results obtained from the cell model and the sensory study regarding the Emax might be that some of the test compounds such as ace K are also activating other taste receptors, e.g. bitter taste receptors (Kuhn et al., 2004), that might interfere with the reporting of the sweet perception in the sensory studies. However, the present study focused on sweetness only, leaving out attributes such as bitterness, astringency or metallic impressions. In addition, the negatively charged lactisole could exhibit a lower bioavailability due to its interaction with basic and proline-rich salivary proteins. This concept has been previously suggested by Canon et al. for tannins and polyphenols (Canon et al., 2021; Canon et al., 2013). The interaction of lactisole with salivary proteins would thus lead to a higher required amount of lactisole when used in oral application. As a further limitation it must be noted that in the cell-based assay of the present work, sweeteners with or without lactisole remained in the wells, while the sensory experiments were done in sip-and-spit mode. In addition, the cell assay does not mimic increasing dilution by salivary flow. This could influence especially the lingering due to a longer presence of the compounds at the receptor sites in the cell experiments. Thus, one would have to assume that part of the signal decay in vivo is due to dilution and not to receptor desensitization. Accordingly, it can be concluded that the in vitro experiments might tend to underestimate while the in vivo experiments tend to overestimate the receptor kinetics. An even more realistic picture of the in vitro receptor

kinetics might be obtained by using a superfusion approach to dynamically modulate stimulus concentrations and hence, mimic saliva dilution and swallowing better.

For ace K, a significant reduction of the E_{max} was shown, although the increased EC_{50} values in combination with lactisole do not fit into an allosteric inhibition model. For typical allosteric inhibition induced by lactisole, a similar EC_{50} and reduced hillslope would have been expected (Bindslev, 2013; Winnig et al., 2007) and was also shown by the here presented cell results. However, not only simple allosteric or competitive inhibition is possible, but as well mixed effects, such as allo-mixed-competitive inhibition (Bindslev, 2013), which could be also suitable for ace K and aspartame here. No such effect that would argue for a second binding site to fit in a two-state allosteric model was shown for aspartame in this study (Galindo-Cuspinera et al., 2006), although it has to be noted that aspartame was tested in lower concentrations only that may not have been high enough to detect a low-affinity binding site. Looking at the TI-curves of ace K and aspartame, a steep increase in the mean maximum sweet intensity was recorded between 2 mM and 5 mM for aspartame, and 0.5 mM and 1 mM for ace K. Beside the binding site, also specific binding residues may play a role for further taste transduction. For example, Masuda et al. (2012) suggested by using a combined approach of molecular modelling, concentration-dependent Ca^{2+}-release of heterologous transfected cells and point mutations, that the binding sites for ace K and aspartame, although located both at the VFD of TAS1R2, have strikingly different and specific binding residues (Masuda et al., 2012). Since the results of the present sensory study reflect the results of the single-receptor model very well, we exclude the involvement of an unknown alternative sweet-signaling pathway for the here tested compounds cyclamate, NHDC, aspartame and ace K. The present study focused on the time-dependent attributes onset and lingering for the four test compounds with or without lactisole addition as well. However, a relationship to the binding site could not be concluded for all four sweet tasting compounds based on the present data. This suggests that the binding site does not play a major role for the temporal profile or that the effect is overruled by other taste signals that occur simultaneously at oral applications. More compounds would be needed in following studies to

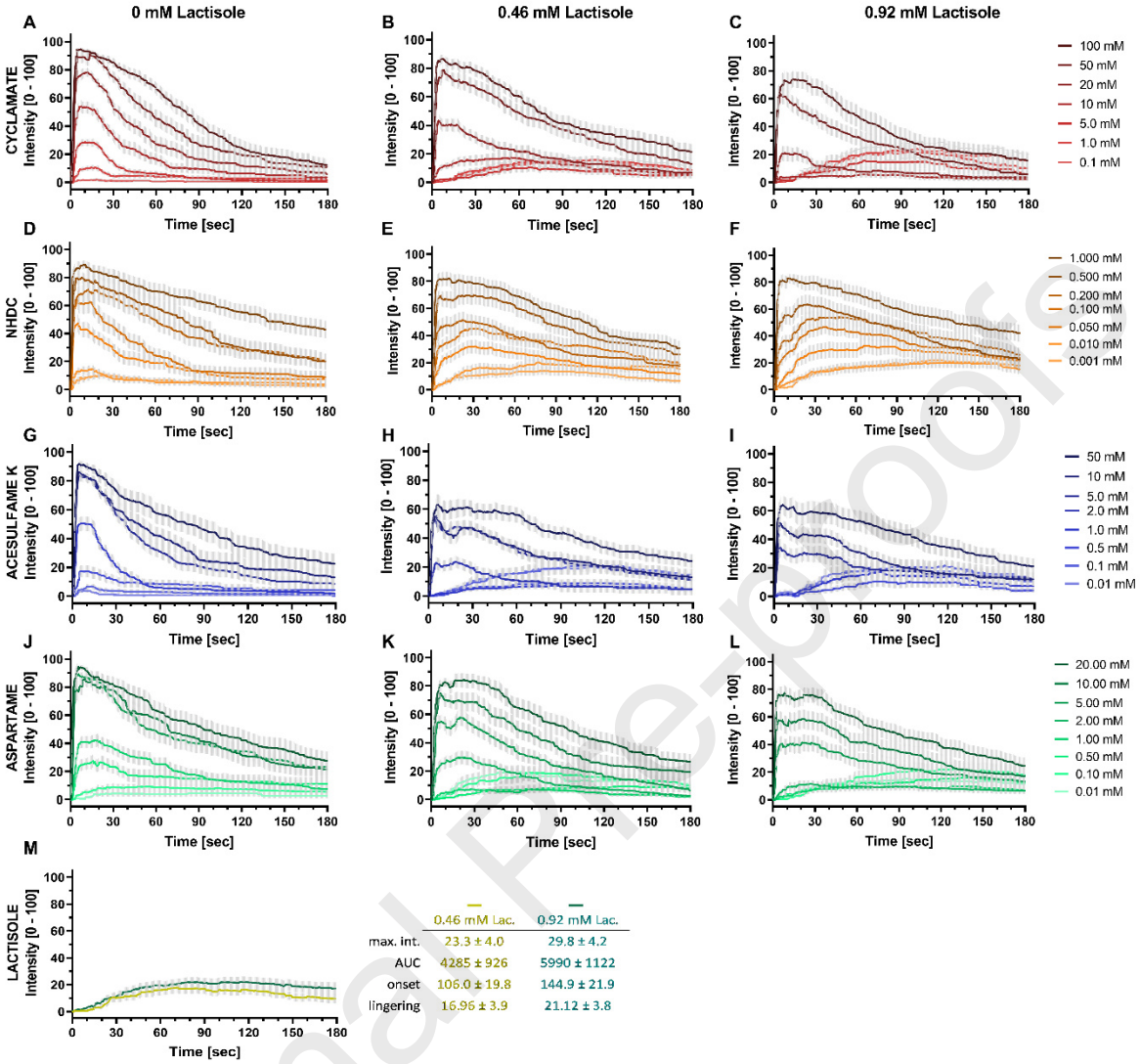
confirm this effect. There are some hypotheses about the origin of onset and lingering. Schiffman et al. (2007) hypothesized that the size and complexity of a molecule impact onset and lingering either by reducing the diffusion to the receptor or by enhanced time to orient properly to the receptor or by the demand of a multistep progress of several binding interactions (Schiffman et al., 2007). Supporting this hypothesis, Karl et al. (2020) demonstrated that an increased onset is primarily related to an increasing number of aromatic rings, double bonds, ketones and higher MlogP (Karl et al., 2020), indicating more complex structures. Furthermore, the study showed that an increased lingering, together with sweetness rating, is related to the physicochemical descriptors molecular weight [g/mol], complexity, heavy atom count, rotatable bonds, C-atoms, bound glucose, glycone length, area polar surface [A2], defined atom stereocenter count, acceptors, donors, and OH-groups (Karl et al., 2020). Also, an earlier hypothesis described by DuBois (2011) suggested that large molecules such as rebaudioside A will non-specifically bind to cell membranes in the oral cavity, leading to a long-lasting lingering effect by enabling re-binding to the receptor (DuBois, 2011). The recorded onset values were all in a similar range in the sensory recordings, the cell experiments showed a greater variance with a more pronounced delay in onset of Ace K and NHDC at the cell experiments. In addition, the kinetic responses obtained from the single receptor cell model in the present study show that decay of the fluorescent signal resembles the lingering in the sensory study. It can be concluded that the onset and lingering directly depend on the activation of TAS1R2/TAS1R3, which supports previous assumptions by DuBois and colleagues (DuBois, 2011). However, the results of the present study thus exclude a role for a mucus membrane which is lacking in the cell model. Tan et al. (2019) summarized that a prolonged sweet intensity is induced by higher affinities of non-nutritive sweeteners to the binding site of taste receptor (Tan et al., 2019). However, to date, a specific binding-site was not shown to be important for a prolonged onset and lingering of sweet compounds. Also in the present study, the effect of lactisole on the onset and lingering time of the test compounds was not associated with a specific binding site and does not support the idea that the binding site is the major driving force for the temporal sensory profile of sweeteners. However,

the detailed mechanism leading to an extended or shortened onset and lingering remains unknown and further research is required.

To summarize, in combination with lactisole, cyclamate and NHDC demonstrate a shift of the dose-response curve corresponding to a competitive inhibition by lactisole in the sensory and the single receptor cell experiments. In contrast to the expectations, aspartame was able to overrule the lactisole inhibition in higher concentrations in the sensory experiments, which could argue for a second, low-affinity binding site at the TAS1R3-TMD. Moreover, the effect of lactisole on the temporal markers of the sensory profile AUC, onset, and lingering for the sweeteners was independent of the major binding site of the sweeteners. In conclusion, the data do not support a major impact of the binding site on the time-intensity profile of the tested sweeteners. Future studies are needed to assess the effect of lactisole and further compounds to confirm their intensity and lingering effects in cell experiments related to sensory properties.

Figures & Figure legends

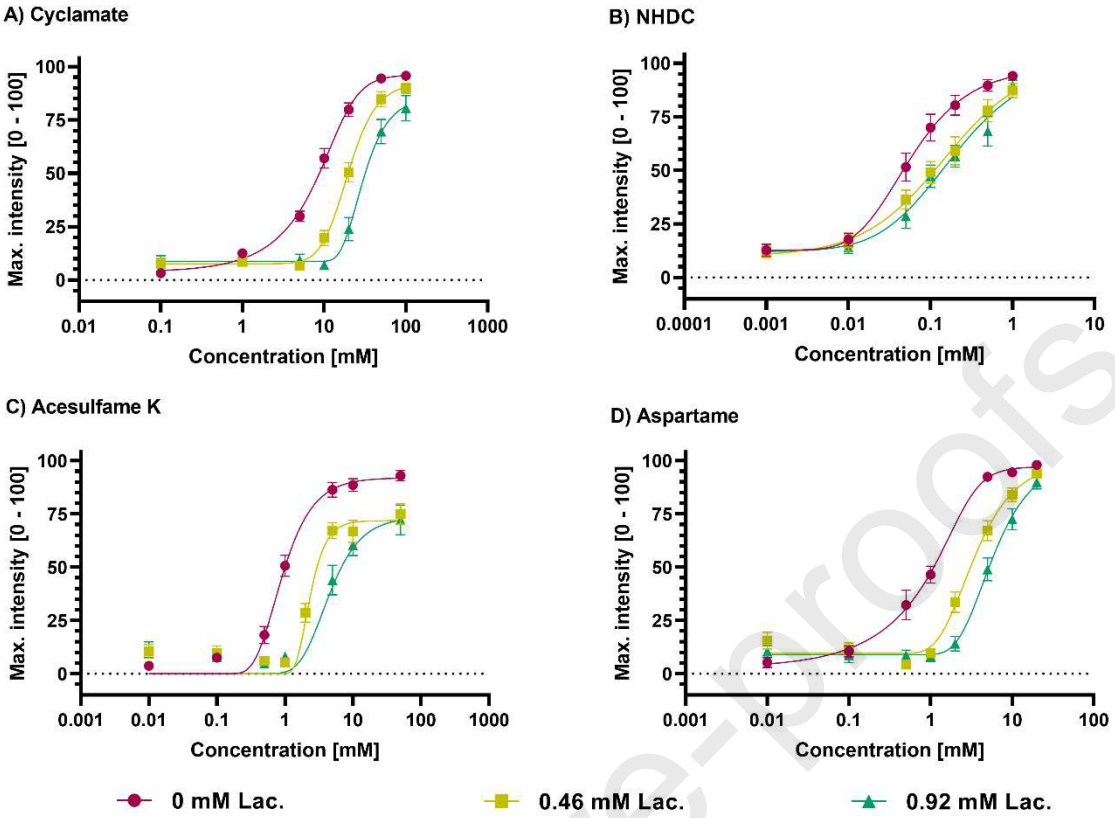
464 Figure 1



465

466 Fig. 1: Time-intensity curves of 0.01 - 50 mM cyclamate (A-C), 0.001 - 1.0 mM neohesperidin
467 dihydrochalcone (NHDC) (D-F), 0.01 - 50 mM acesulfame K (G-I), 0.01 - 20 mM aspartame (J-L), each
468 without and in combination with 0.46 mM and 0.92 mM. Time-intensity curves of 0.46 mM and
469 0.92 mM lactisole (Lac.) separately and the temporal markers maximum intensity (max.int.), AUC,
470 onset and lingering (M). 2 rep. with n= 18 - 27 single evaluations, presented as mean ± SEM.

471 Figure 2



E)

Cyclamate	EC50 [mM]	Emax	Hillslope
0 mM Lac.	8.72	96.11	2.60
0.46 mM Lac.	19.60	92.37	2.18
0.92 mM Lac.	30.15	unstable	2.10

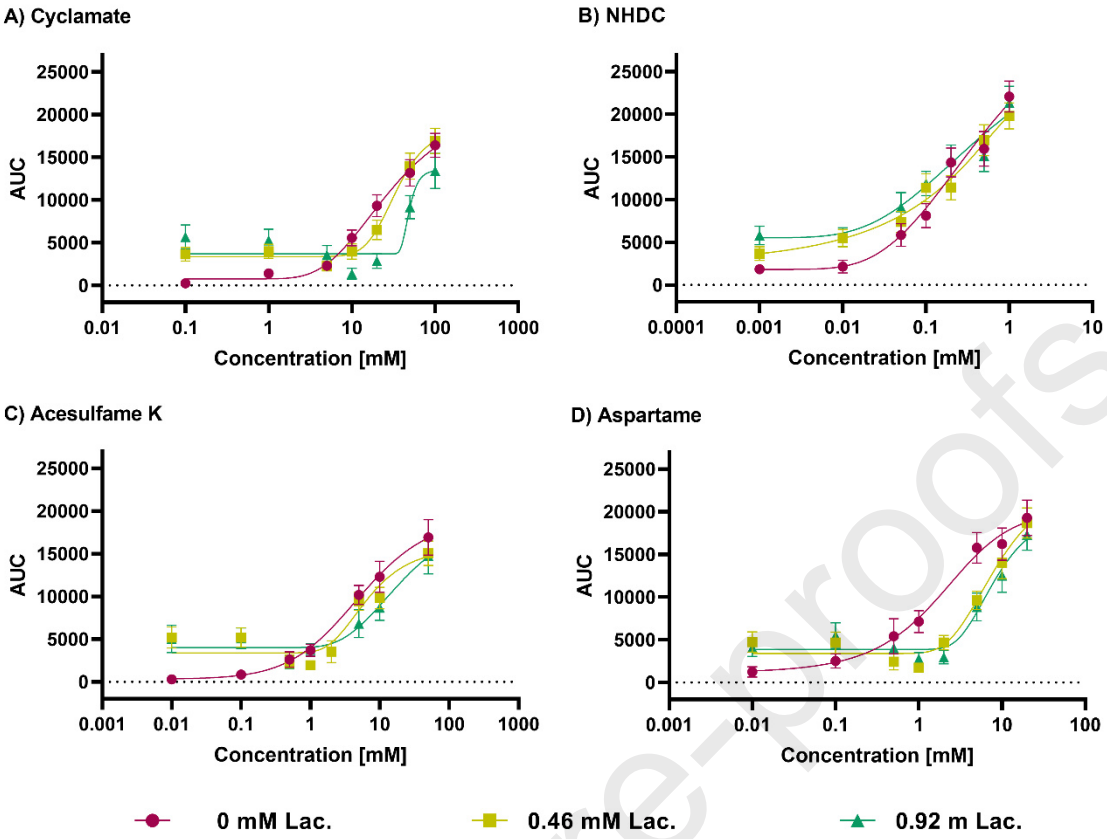
NHDC	EC50 [mM]	Emax	Hillslope
0 mM Lac.	0.056	97.73	0.94
0.46 mM Lac.	0.144	100.00	0.89
0.92 mM Lac.	0.184	100.00	0.82

Acesulfame K	EC50 [mM]	Emax	Hillslope
0 mM Lac.	0.92	unstable	1.43
0.46 mM Lac.	2.39	71.83	2.75
0.92 mM Lac.	4.46	73.60	1.45

Aspartame	EC50 [mM]	Emax	Hillslope
0 mM Lac.	1.09	97.05	2.56
0.46 mM Lac.	3.46	99.22	1.28
0.92 mM Lac.	5.80	100.00	1.32

Fig. 2: Max. intensity [0 - 100 s] of (A) cyclamate (0.1 - 100mM), (B) NHDC (0.001 - 1.0mM), (C) acesulfame K (0.01 - 50mM) and (D) aspartame (0.01 - 20mM); presented as mean ± SEM; 2 rep. with n= 18 - 27 single evaluations for combination with 0 mM, 0.46 mM and 0.92 mM lactisole (Lac.); (E) EC50 [mM], Emax and hillslope calculated with GraphPad Prism 9 curve fitting with an asymmetric five parameter curve, top< 100 and bottom >0.

Figure 3



479

480 Fig. 3: Area under the curve (AUC) of time-intensity curves of (A) cyclamate (0.1 - 100mM), (B) NHDC

481 (0.001 - 1.0mM), (C) acesulfame K (0.01 - 50mM) and (D) aspartame (0.01 - 20mM); presented as

482 mean ± SEM; 2 rep. with n= 18 - 27 single evaluations for combination with 0 mM, 0.46 mM and

483 0.92 mM lactisole (Lac.).

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491 Figure 4

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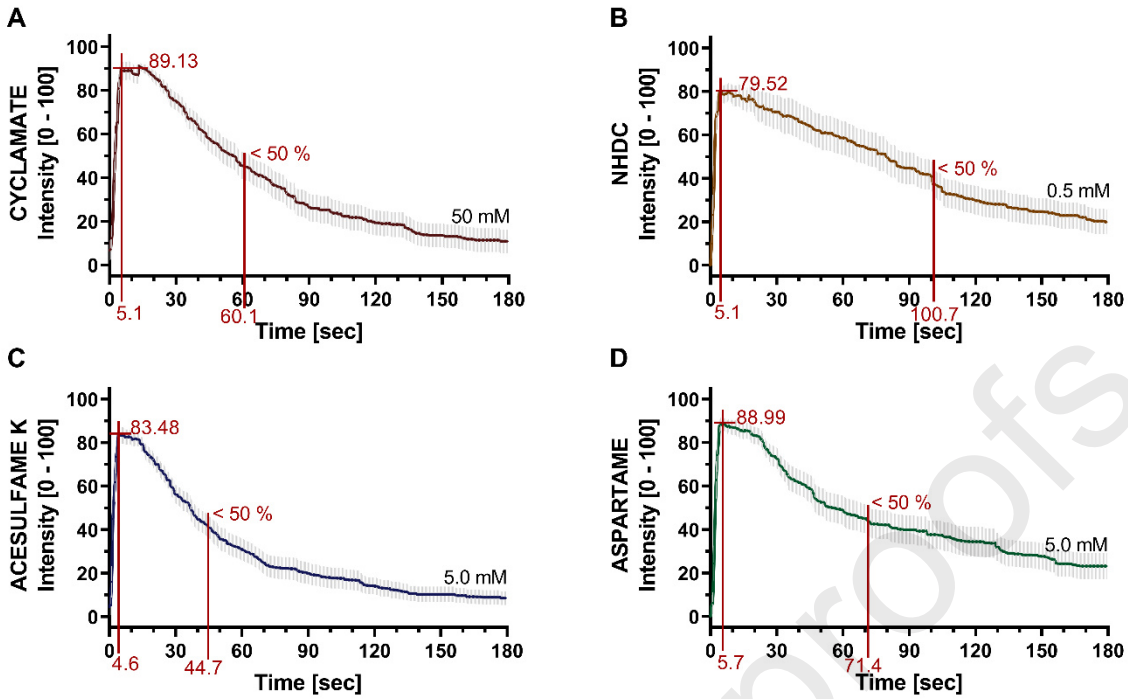

493

494 Fig. 4: Time-intensity curves of (A) 50 mM cyclamate, (B) 0.5 mM NHDC, (C) 5.0 mM acesulfame K,

495 and (D) 5.0 mM aspartame. All curves are marked in red for maximum sweetness, half maximum

496 intensity and their temporal occurrences (onset and lingering time). Data presented as mean ± SEM;

497 2 rep. with n= 19 - 24 single evaluations.

499 Figure 5

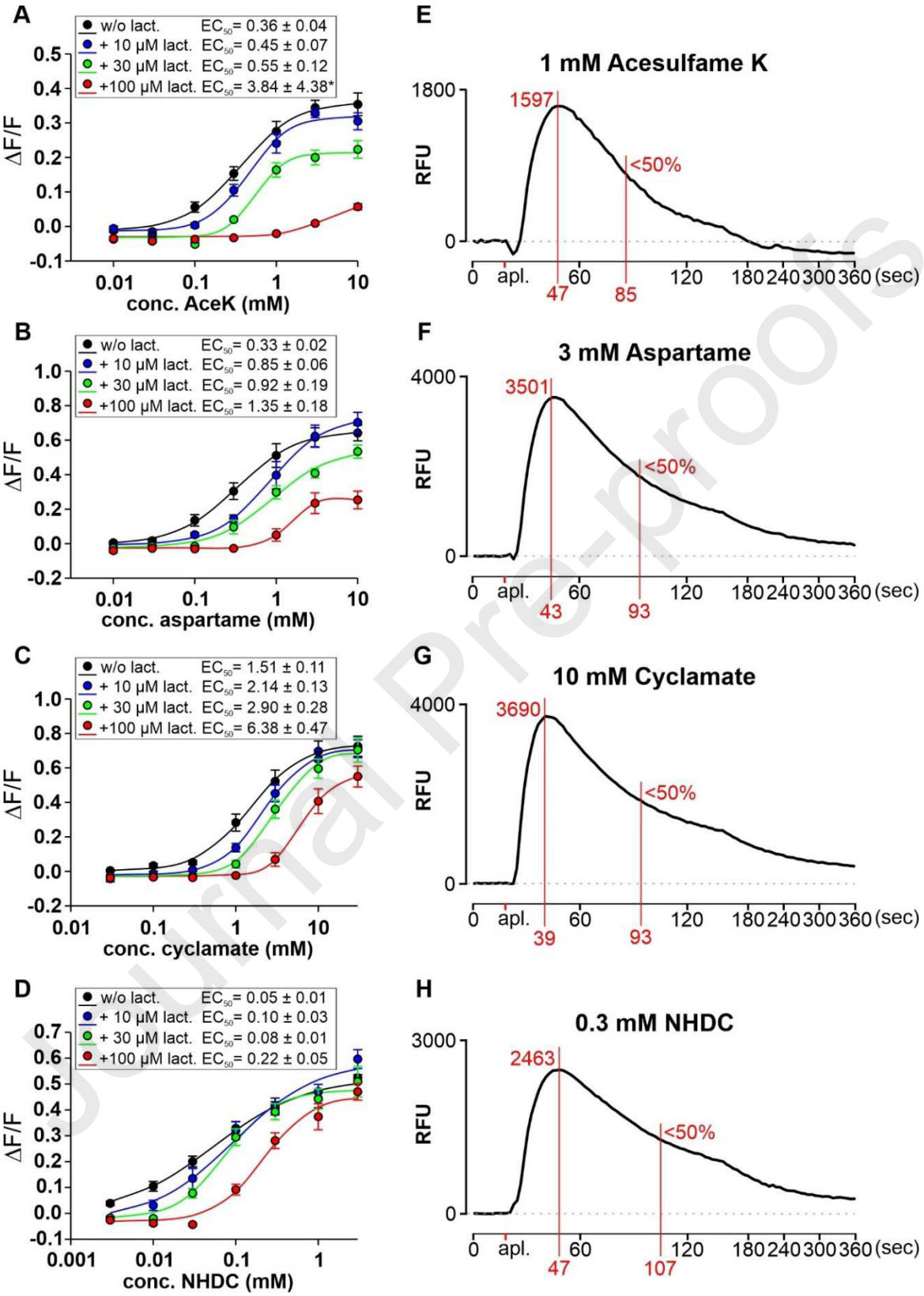

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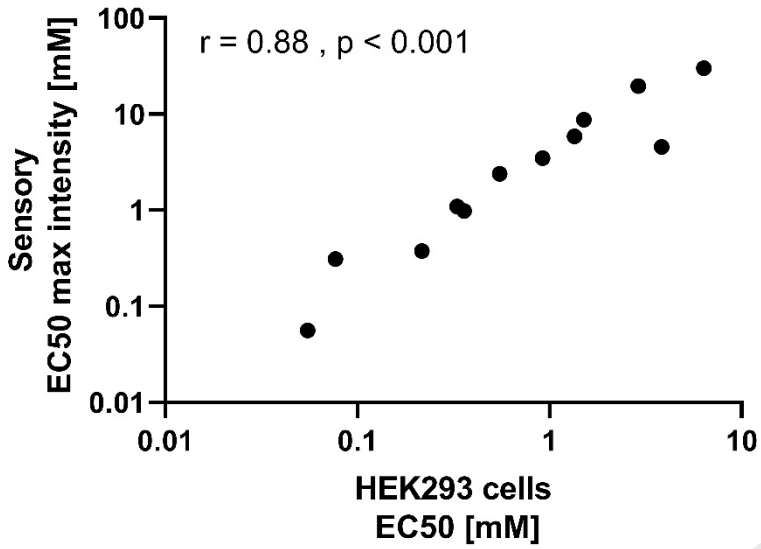
Fig. 5: *In vitro* analyses of cells expressing the human sweet taste receptor. Left panels (A-D): Dose-response relationships of HEK293 Flp-In T-Rex-Gα15Gi3-TAS1R2/TAS1R3 cells stimulated with increasing concentrations of the sweeteners acesulfame K (Ace K) (A), aspartame (B), cyclamate (C), and neohesperidin dihydrochalcone (NHDC) (D) in the presence or absence of the sweet taste inhibitor lactisole (lact.). The agonist concentrations in millimolar (mM) are labeled on the logarithmically scaled x-axes, the relative fluorescence changes (ΔF/F) on the y-axes. The curves are color coded according to the fixed lactisole concentrations indicated in the insets. Determined EC50-concentrations are provided in the insets. Right panels (E-H): Raw traces of fluorescence changes of cells expressing TAS1R2/TAS1R3 at selected concentrations of sweeteners (E, Ace K; F, aspartame; G, cyclamate; H, NHDC). The traces of 3 independent experiments performed in duplicates were averaged and thus, reflect 6 wells receiving the identical treatment. The fluorescence changes (RFU) are plotted on the y-axes and the measurement time in seconds (sec) is shown on the x-axes. The times until reaching the peak amplitudes (including 20 sec of baseline monitoring prior to agonist application) as well as the corresponding peak amplitudes are indicated with red lines and printing. As only one trace (Ace K) crossed the baseline upon prolonged monitoring, the time points where the traces fell below 50 % of the respective peak fluorescence are indicated with red lines and printing.

519 Figure 6



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521 Fig. 6: Comparison of EC50 values calculated from sensory time-intensity (TI) curves n ≥ 16) and
522 transfected HEK293 cells (n ≥ 3) for NHDC, cyclamate, acesulfame K and aspartame w/o lactisole
523 (sensory 0.46 and 0.92 mM, cells 30 and 100 µM). Statistics: Pearson product moment correlation.

524

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors M. Wendelin, J. P. Ley, and G. E. Krammer are employees of the Symrise Distribution GmbH or Symrise AG, respectively.

References

Behrens, M., Blank, K., & Meyerhof, W. (2017). Blends of Non-caloric Sweeteners Saccharin and Cyclamate Show Reduced Off-Taste due to TAS2R Bitter Receptor Inhibition. *Cell Chemical Biology, 24*(10), 1199-1204 e1192. https://doi.org/10.1016/j.chembiol.2017.08.004

Ben Abu, N., Mason, P. E., Klein, H., Dubovski, N., Ben Shoshan-Galeczki, Y., Malach, E., Prazienkova, V., Maletinska, L., Tempra, C., Chamorro, V. C., Cvacka, J., Behrens, M., Niv, M. Y., & Jungwirth, P. (2021). Sweet taste of heavy water. *Communications Biology, 4*(1), 440. https://doi.org/10.1038/s42003-021-01964-y

Bindslev, N. (2013). Allosteric transition: a comparison of two models. *BMC Pharmacology and Toxicology, 14*(4), 1-12. https://doi.org/doi.org/10.1186/2050-6511-14-4

Burdock, G. A., Wagner, B. M., Smith, R. L., Munro, I. C., & Newberne, P. M. (1990). 15. GRAS Substances - A list of flavoring ingredient substances considered generally recognized as safe by the Flavor & Extract Manufacturers' Association Expert Panel. *Food Technology, 44*(2), 78-86.

Busch-Stockfisch, M. (2015). Prüferauswahl und Prüferschulung. In M. Busch-Stockfisch (Ed.), *Sensorik kompakt in der Produktentwicklung und Qualitätssicherung.* (Vol. 1). B. Behr's Verlag GmbH & Co. KG.

Canon, F., Belloir, C., Bourillot, E., Brignot, H., Briand, L., Feron, G., Lesniewska, E., Nivet, C., Septier, C., Schwartz, M., Tournier, C., Vargiolu, R., Wang, M., Zahouani, H., & Neiers, F. (2021).

Perspectives on Astringency Sensation: An Alternative Hypothesis on the Molecular Origin of Astringency. *Journal of Agricultural and Food Chemistry, 69*(13), 3822-3826. https://doi.org/10.1021/acs.jafc.0c07474

Canon, F., Pate, F., Cheynier, V., Sarni-Manchado, P., Giuliani, A., Perez, J., Durand, D., Li, J., & Cabane, B. (2013). Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. *Langmuir, 29*(6), 1926-1937. https://doi.org/10.1021/la3041715

DIN-EN-ISO. (2011). Sensory analysis - Methodology - Method of investigating sensitivity of taste (ISO 3972:2011 + Cor. 1:2012). *DIN-EN-ISO-3972:2013-12*.

DIN-EN-ISO. (2014). Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. *German version, DIN EN ISO 8586:2014-05*.

DuBois, G. E. (2011). Validity of early indirect models of taste active sites and advances in new taste technologies enabled by improved models. *Flavour and Fragrance Journal, 26*(4), 239-253. https://doi.org/10.1002/ffj.2042

DuBois, G. E. (2016). Molecular mechanism of sweetness sensation. *Physiology & Behavior, 164*, 453-463. https://doi.org/10.1016/j.physbeh.2016.03.015

DuBois, G. E., Crosby, G. A., Stephenson, R. A., & Wingard Jr., R. E. (1977). Dihydrochalcone sweeteners. Synthesis and sensory evaluation of sulfonate derivatives. *Journal of Agricultural and Food Chemistry, 25*(4), 763-772. https://doi.org/10.1021/jf60212a056,

DuBois, G. E., & Prakash, I. (2012). Non-caloric sweeteners, sweetness modulators, and sweetener enhancers. *Annual Review of Food Science and Technology 3*, 353-380. https://doi.org/10.1146/annurev-food-022811-101236

Galindo-Cuspinera, V., Winnig, M., Bufe, B., Meyerhof, W., & Breslin, P. A. (2006). A TAS1R receptor-based explanation of sweet 'water-taste'. *Nature, 441*(7091), 354-357. https://doi.org/10.1038/nature04765

584 Ganchrow, J. R., Steiner, J. E., & Daher, M. (1983). Neonatal facial expressions in response to
585 different qualities and intensities of gustatory stimuli. *Infant Behavior and Development, 6*,
586 473-484. https://doi.org/10.1016/S0163-6383(83)90301-6

587 Guinard, J.-X., Hong, D. Y., & Budwig, C. (1995). Time-intensity properties of sweet and bitter stimuli:
588 implications for sweet and bitter taste chemoreception. *Journal of sensory studies, 10*(1), 45-
589 71. https://doi.org/10.1111/j.1745-459X.1995.tb00004.x

590 Höhl, K., & Busch-Stockfisch, M. (2015). The influence of sensory training on taste sensitivity. Effects
591 on sweet and bitter perception over a half-year period. *Ernährungs Umschau, 62*(12), 208-
592 215. https://doi.org/10.4455/eu.2015.035

593 Jiang, P., Cui, M., Zhao, B., Liu, Z., Snyder, L. A., Benard, L. M., Osman, R., Margolskee, R. F., & Max,
594 M. (2005). Lactisole interacts with the transmembrane domains of human T1R3 to inhibit
595 sweet taste. *Journal of Biological Chemistry, 280*(15), 15238-15246.
596 https://doi.org/10.1074/jbc.M414287200

597 Jiang, P., Cui, M., Zhao, B., Snyder, L. A., Benard, L. M., Osman, R., Max, M., & Margolskee, R. F.
598 (2005). Identification of the cyclamate interaction site within the transmembrane domain of
599 the human sweet taste receptor subunit T1R3. *Journal of Biological Chemistry 280*(40),
600 34296-34305. https://doi.org/10.1074/jbc.M505255200

601 Karl, C. M., Wendelin, M., Lutsch, D., Schleining, G., Dürrschmied, K., Ley, J. P., Krammer, G. E., &
602 Lieder, B. (2020). Structure-dependent effects of sweet and sweet taste affecting compounds
603 on their sensorial properties. *Food Chemistry, X*(7).
604 https://doi.org/10.1016/j.fochx.2020.100100

605 Kuhn, C., Bufe, B., Winnig, M., Hofmann, T., Frank, O., Behrens, M., Lewtschenko, T., Slack, J. P.,
606 Ward, C. D., & Meyerhof, W. (20040). Bitter taste receptors for saccharin and acesulfame K.
607 *Journal of Neuroscience, 24*(45), 10260-10265. https://doi.org/10.1523/JNEUROSCI.1225-
608 04.2004

Maillet, E. L., Cui, M., Jiang, P., Mezei, M., Hecht, E., Quijada, J., Margolskee, R. F., Osman, R., & Max, M. (2015). Characterization of the Binding Site of Aspartame in the Human Sweet Taste Receptor. *Chemical Senses, 40*(8), 577-586. https://doi.org/10.1093/chemse/bjv045

Masuda, K., Koizumi, A., Nakajima, K., Tanaka, T., Abe, K., Misaka, T., & Ishiguro, M. (2012). Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds. *PLoS One, 7*(4), e35380. https://doi.org/10.1371/journal.pone.0035380

May, L. T., Leach, K., Sexton, P. M., & Christopoulos, A. (2007). Allosteric modulation of G protein-coupled receptors. *Annual Review of Pharmacology and Toxicology, 47*, 1-51. https://doi.org/10.1146/annurev.pharmtox.47.120505.105159

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J. P., & Zuker, C. S. (2001). Mammalian Sweet Taste Receptors. *Cell, 106*(3), 381-390. https://doi.org/10.1016/S0092-8674(01)00451-2

Nie, Y., Vigues, S., Hobbs, J. R., Conn, G. L., & Munger, S. D. (2005). Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Current Biology, 15*(21), 1948-1952. https://doi.org/10.1016/j.cub.2005.09.037

Ott, D. B., Edwards, C. L., & Palmer, S. J. (1991). Perceived taste intensity and duration of nutritive and non-nutritive sweeteners in water using time-intensity (T-I) evaluations. . *Journal of Food Science, 56*(2), 535-542. https://doi.org/10.1111/j.1365-2621.1991.tb05319.x

Pin, J.-P., Galvez, T., & Prézeau, L. (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacology & Therapeutics, 98*(3), 325-354. https://doi.org/10.1016/s0163-7258(03)00038-x

Schiffman, S. S., Booth, B. J., Losee, M. L., Pecore, S. D., & Warwick, Z. S. (1995). Bitterness of Sweeteners as a Function of Concentration. *Brain Research Bulletin, 36*(5), 505-5013. https://doi.org/doi.org/10.1016/0361-9230(94)00225-P

Schiffman, S. S., Sattely-Miller, E. A., & Bishay, I. E. (2007). Time to maximum sweetness intensity of binary and ternary blends of sweeteners. *Food Quality and Preference, 18*(2), 405-415. https://doi.org/10.1016/j.foodqual.2006.04.007

Servant, G., Kenakin, T., Zhang, L., Williams, M., & Servant, N. (2020). The function and allosteric control of the human sweet taste receptor. *Adv Pharmacol, 88*, 59-82. https://doi.org/10.1016/bs.apha.2020.01.002

Sigoillot, M., Brockhoff, A., Meyerhof, W., & Briand, L. (2012, Nov). Sweet-taste-suppressing compounds: current knowledge and perspectives of application. *Applied Microbiology and Biotechnology 96*(3), 619-630. https://doi.org/10.1007/s00253-012-4387-3

Tan, V. W. K., Wee, M. S. M., Tomic, O., & Forde, C. G. (2019). Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA). *Food Research International, 121*, 39-47. https://doi.org/10.1016/j.foodres.2019.03.019

Ventura, A. K., & Worobey, J. (2013). Early influences on the development of food preferences. *Current Biology, 23*(9), R401-408. https://doi.org/10.1016/j.cub.2013.02.037

Winnig, M., Bufe, B., Kratochwil, N. A., Slack, J. P., & Meyerhof, W. (2007). The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor. *BMC Structual Biology, 7*(1), 12. https://doi.org/10.1186/1472-6807-7-66

Winnig, M., Bufe, B., & Meyerhof, W. (2005). Valine 738 and lysine 735 in the fifth transmembrane domain of rTas1r3 mediate insensitivity towards lactisole of the rat sweet taste receptor. *BMC Neuroscience, 6*, 22. https://doi.org/10.1186/1471-2202-6-22

Xu, H., Staszewski, L., Tang, H., Adler, E., Zoller, M., & Li, X. (2004). Different functional roles of T1R subunits in the heteromeric taste receptors. *PNAS, 101*(39), 14258–14263. https://doi.org/10.1073/pnas.0404384101

Zhang, F., Klebansky, B., Fine, R. M., Liu, H., Xu, H., Servant, G., Zoller, M., Tachdjian, C., & Li, X. (2010,). Molecular mechanism of the sweet taste enhancers. *PNAS, 107*(10), 4752-4757. https://doi.org/10.1073/pnas.0911660107

Zhao, X., Liu, M., Cui, M., & Liu, B. (2022, Feb). Multiple interaction modes between saccharin and sweet taste receptors determine a species-dependent response to saccharin. *FEBS Open Bio, 12*(2), 494-499. https://doi.org/10.1002/2211-5463.13355

Impact of lactisole on the time-intensity profile of selected sweeteners in dependence of the binding site

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Corinna M. Deck: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - original draft, Writing - review & editing, Visualization. **Maik Behrens:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - original draft, Writing - review & editing, Visualization. **Martin Wendelin:** Conceptualization, Methodology, Writing - review & editing. **Jakob P. Ley:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Gerhard E. Krammer:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition **Barbara Lieder:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Highlights:

- The effect of lactisole on time-intensity profiles of selected sweeteners is shown
- Allosteric vs. competitive inhibition of TAS1R2/TAS1R3 was used in a sensory study
- A comparison of sensory experiments with in vitro TAS1R2/TAS1R3 activation is shown
- Effect of lactisole on onset & lingering was not related to a specific binding site
- The EC50 of the max. intensity correlated between in vitro and sensory experiments

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors M. Wendelin, J. P. Ley, and G.E. Krammer are employees of the Symrise Distribution GmbH or Symrise AG, respectively.

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3.5.3. "Individual sweet taste perception influences salivary characteristics after orosensory stimulation with sucrose and non-caloric sweeteners"

Corinna M. Karl [a,b], Ana Vidakovic [a], Petra Pjevac [c,d], Bela Hausmann [c,e], Gerhard Schleining [f], Jakob P. Ley [g], David Berry [c,d], Joachim Hans [g], Martin Wendelin [h], Jürgen König [i], Veronika Somoza [b,j,k], Barbara Lieder [a,b]

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Individual Sweet Taste Perception Influences Salivary Characteristics After Orosensory Stimulation With Sucrose and Noncaloric Sweeteners

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Emerging evidence points to a major role of salivary flow and viscoelastic properties in taste perception and mouthfeel. It has been proposed that sweet-tasting compounds influence salivary characteristics. However, whether perceived differences in the sensory properties of structurally diverse sweet-tasting compounds contribute to salivary flow and saliva viscoelasticity as part of mouthfeel and overall sweet taste perception remains to be clarified. In this study, we hypothesized that the sensory diversity of sweeteners would differentially change salivary characteristics in response to oral sweet taste stimulation. Therefore, we investigated salivary flow and saliva viscoelasticity from 21 healthy test subjects after orosensory stimulation with sucrose, rebaudioside M (RebM), sucralose, and neohesperidin dihydrochalcone (NHDC) in a crossover design and considered the basal level of selected influencing factors, including the basal oral microbiome. All test compounds enhanced the salivary flow rate by up to 1.51 ± 0.12 g/min for RebM compared to 1.10 ± 0.09 g/min for water within the 1st min after stimulation. The increase in flow rate was moderately correlated with the individually perceived sweet taste ($r = 0.3$, $p < 0.01$) but did not differ between the test compounds. The complex viscosity of saliva was not affected by the test compounds, but the analysis of covariance showed that it was associated ($p < 0.05$) with mucin 5B (Muc5B) concentration. The oral microbiome was of typical composition and diversity but was strongly individual-dependent (permutational analysis of variance (PERMANOVA): $R^2 = 0.76$, $p < 0.001$) and was not associated with changes in salivary characteristics. In conclusion, this study indicates an impact of individual sweet taste impressions on the flow rate without measurable changes in the complex viscosity of saliva, which may contribute to the overall taste perception and mouthfeel of sweet-tasting compounds.

Keywords: sweet taste, saliva, mouthfeel, sucrose, rebaudioside M, neohesperidin dihydrochalcone, sucralose, oral microbiome

INTRODUCTION

The flavor perception of sweeteners involves not only gustation and olfaction but also the overall tactile impression called mouthfeel. The origin of the multidimensional sensation of mouthfeel has not yet been fully characterized, but several contributing factors have been identified. In addition to the organoleptic, textural, and surface properties of foods or beverages [reviewed by Guinard and Mazzucchelli (1)], oral physiology, especially saliva with its lubricating and transporting properties, plays a major role in the overall flavor, mouthfeel, and the so-called afterfeel (2, 3). In particular, the flow rate and rheological properties of saliva have been reported to be related to the difference in the flavor impression of a compound (4). Salivary flow, which may act against oral surface irrigation (5), can be stimulated mechanically by chewing and through various taste stimuli depending on the concentration of the stimulus. Although the most potent activation of salivary flow has been shown with sour-tasting citric acid (6–8), sweet taste has also been associated with the stimulation of salivary flow (9, 10), but the interaction of salivary characteristics, including flow rate, and sweet taste perception remains to be elucidated. In addition, a complex interplay of mechanisms affects salivary flow, depending, for example, on the nature of the molecules, food matrices, or interactions with salivary compounds, making it difficult to generalize the effect of taste stimuli on salivary flow (11).

The lubricating effects of saliva are determined not only by the flow rate but also by the viscous and elastic components; however, the effects of different stimuli on the viscoelastic properties of saliva have not yet been completely clarified. A previous study by Stokes and Davies (12) compared the viscoelasticity after stimulation with citric acid, water, and chewing gum. They showed that the viscoelasticity of whole oral saliva was associated with the type of stimulus independent of the induced flow rate, while the viscosity of saliva after different stimuli was similar (12). Later, Davies et al. (4) compared the rheology of saliva after stimulation with ice tea, fizzy cola, sparkling water, chewing gum, mint, or water. The results showed that ice tea and cola induced the highest salivary flow rate and higher elasticity compared to chewing gum or water but had similar overall viscosity. The authors concluded that the elasticity of saliva is independent of the flow rate and that the rheology of saliva can affect the sensory properties, including mouthfeel, of beverages (4). Other compositional factors that determine the rheological properties and flow of saliva are the enzymes α-amylase (8, 11, 13, 14) and cystatin S. The latter one is mainly secreted from submandibular and sublingual glands (15, 16). Also, mucin 5B (Muc5B), a major mucin protein in saliva (17), and the pH of saliva, which normally ranges from 6.7 to 7.4 (18), influence the viscoelasticity of saliva. If saliva has higher viscosity and lower elasticity, it cannot form an optimal salivary pellicle (19). The formation of a salivary pellicle is important for lubrication and protection of the oral surface and involves salivary proteins. Thus, the interaction of sweet compounds with salivary proteins may influence chemosensation (3). The mucin Muc5B is an important protein involved in the formation of a salivary pellicle and has been associated with taste perception and astringency (20, 21). Recently, the aggregation of mucosal pellicles by polyhydroxyphenols (tannins), leading to the dissociation of the protein Muc1, has been proposed as the underlying mechanism to sense astringency (22). A similar disruption of the mucosal pellicle by polyphenolic sweeteners such as neohesperidin dihydrochalcone (NHDC) is conceivable but has not been proven yet. While mechanical stimulation leads to reduced elasticity of the saliva, stimulation with citric acid leads to secretion of more elastic saliva (12), leading to the conclusion that different types of stimuli induce secretion from distinct types of glands, which affect the elasticity of saliva (12, 23). In the case of sucrose, enhanced concentration was shown to increase the viscosity rating of aqueous solutions (24). At this point, it is not clear whether this perceived viscosity is based mainly on cross-modal effects in the brain that associate increased sweet taste with higher viscosity or on actual changes in perceived mouthfeel.

In addition, the influence of the oral microbiota on the interplay of mouthfeel, taste perception, and salivary parameters has been proposed (25, 26). For example, the ecological effect of the oral microbiota not only depends on sugar intake but also is influenced by the taste phenotype of the host through allelic variation in the *TAS1R1* and *GNAT3* genes or by the salivary flow rate (25). Previous studies also provided evidence that the oral microbiota is associated with the PROP status of individuals, reflecting their ability and sensibility to taste bitter 6-*n*-propylthiouracil based on their genetic variation in *TAS2R38* (26, 27). However, to date, only limited data exist on the association between taste perception and the oral microbiota.

In summary, emerging evidence suggests that salivary flow and viscoelastic properties play a major role in taste perception and mouthfeel. However, it is not clear whether and how differences in the sensory properties of structurally diverse sweet-tasting compounds contribute to salivary flow and saliva viscoelasticity as part of mouthfeel and overall taste perception. In addition, the role of the oral microbiome in the interplay of saliva, taste, and mouthfeel remains largely unknown. Thus, we hypothesized in this study that the structurally and sensorially diverse sweet tasting compounds sucrose and the noncaloric rebaudioside M (RebM), sucralose, and NHDC compounds differentially affect salivary flow and the complex viscosity of saliva. Several factors that might influence salivary characteristics were considered, namely, body mass index (BMI), age and sweet threshold of test subjects, individual sweet taste perception of the test compounds, pH, α-amylase activity, cystatin S, Muc5B, storage modulus (*G'*), and phase angle (δ) of basal saliva. Moreover, we investigated whether there was a relationship between the composition of the basal oral microbiota, salivary properties, and sweet taste and overall flavor perception.

MATERIALS AND METHODS

Test Compounds and Test Subjects

Four structurally and sensorially diverse sweet-tasting compounds, namely, NHDC (>96%, FG; Sigma-Aldrich, Steinheim, Germany), RebM (90%; Symrise AG, Holzminden,

Germany), sucralose (>98%; Symris AG, Holzminden, Germany), and sucrose (AGRANA Zucker GmbH, Vienna, Austria) were selected as test compounds (refer to **Table 1** for the corresponding IUPAC nomenclature and structures). The compounds and their concentrations were selected based on a previous sensory study by Karl et al. (28), in which sweet taste affecting compounds were sorted into three main clusters based on their sensory properties (28). For the present study, a representative compound was selected from each cluster in addition to the sweet reference compound, sucrose. The concentration of the compounds was chosen to be equivalent to the sweet taste of 5% (w/v) sucrose, with 0.07 g/L NHDC, 0.25 g/L RebM, 0.09 g/L sucralose, and 50.0 g/L sucrose according to Karl et al. (28) (see also **Figure 1**). All compounds were dissolved in Viennese tap water (pH = 7.88 ± 0.02) and thus an equivalent volume of water was applied as a taste-neutral volume control. Viennese tap water was chosen because the local test subjects are accustomed to its taste and it provides a stable quality.

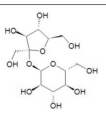
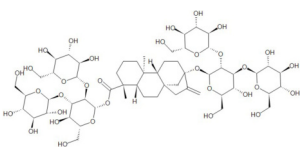
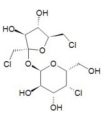
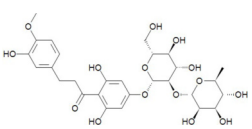
The number of test subjects was estimated using a power analysis with the software G*Power 3.1 based on the study by Neyraud et al. (6). The study showed an increase in salivary flow after stimulation with sweet-tasting carbohydrates, from which an effect size of 0.94 was calculated, leading to the total number of at least 17 subjects with $\alpha = 0.05$ and 95% power.

The ethics committee of the University of Vienna (reference no. 00421; 2019) approved this study, and all test subjects gave written informed consent. The test subjects were recruited from the University of Vienna and the surrounding area. In total, 29 test subjects participated in the screening. Participants had to be between 18 and 60 years of age and in good general health. The exclusion criteria included smoking, pregnancy, or breastfeeding, chronic conditions with teeth or gingivitis, permanent medication, antibiotics treatment within the last 2 months, diagnosed anosmia or ageusia, viral or bacterial infections within the last 3 weeks, alcohol or drug addiction, known allergies to any of the test substances, and a basal salivary flow rate of less than 0.3 g/min. Age, body weight [Soehnle Industrial Solutions GmbH, Backnang, Germany (61,350), max: 150 kg; accuracy: 0.1 kg], and body height [stadiometer from Seca, Hamburg, Germany, max: 2.10 m, accuracy: 0.01 m] were recorded, and individual BMI [BMI = weight [kg]/height^2 [m^2]] was calculated. Of the 29 test subjects enrolled, six were excluded after screening for not meeting the inclusion criteria and two withdrew their consent to participate. A total of 21 test subjects completed all five treatments (see also **Figure 1**).

Study Design

The study design was an open, single-centered, randomized, crossover, single blinded, and controlled study. Each test substance was tested on a separate study day and by each test subject (see **Figure 1**) to prevent carryover effects of the test substances, at least 3 days apart. Test subjects completed all test days within 3–6 weeks between May and October 2019. On each study day, saliva samples were collected at 9 a.m. at three time points (**Figure 2**) in 5 ml tubes (Carl Roth, Karlsruhe, Germany). Because salivation and salivary parameters can be easily affected, for example by time of day, stimulation, and diet (29, 30), the sampling procedure was standardized and training of saliva collection during screening was performed. Test subjects with less than 0.3 g/min of unstimulated saliva were excluded from the study as this volume is described as the threshold of the normal range of salivation (31). A flow rate of 0.7 g/min of stimulated saliva was required to determine all parameters. On each study day, test subjects were asked to arrive in a fasting state and without brushing their teeth at 8 a.m. at the research facility. First, approximately 1 cm^2 of one side of the tongue dorsum was brushed with a sterile swab (ESwab 480C, Copan Diagnostics, Inc., Murrieta, CA, United States) according to the

TABLE 1 | IUPAC names and chemical structures of the test compounds.

Test compounds	IUPAC computed by Lexichem TK 2.7.0 (PubChem release 2021.05.07)	Structure
Sucrose	(2R,3R,4S,5S,6R) -2-[(2S,3S,4S,5R) -3,4-dihydroxy -2,5-bis(hydroxymethyl)oxolan -2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	
Reb M	[(2S,3R,4S,5R,6R) -5-hydroxy -6-(hydroxymethyl) -3,4-bis [[(2S,3R,4S,5S,6R) -3,4,5-trihydroxy -6-(hydroxymethyl)oxan -2-yl]oxy]oxan-2-yl] (1R,4S,5R,9S,10R,13S) -13-[(2S,3R,4S,5R,6R) -5-hydroxy -6-(hydroxymethyl) -3,4-bis [[(2S,3R,4S,5S,6R) -3,4,5-trihydroxy -6-(hydroxymethyl)oxan -2-yl]oxy]oxan-2-yl]oxy -5,9-dimethyl -14-methylidenetetracyclo [11.2.1.01,10.04,9] hexadecane -5-carboxylate	
Sucralose	(2R,3R,4R,5R,6R) -2-[(2R,3S,4S,5S) -2,5-bis(chloromethyl) -3,4-dihydroxyoxolan-2-yl] oxy -5-chloro -6-(hydroxymethyl)oxane -3,4-diol	
NHDC	1-[4-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4R,5R,6S) -3,4,5-trihydroxy -6-methyloxan -2-yl] oxyoxan -2-yl] oxy-2,6-dihydroxyphenyl] -3-(3-hydroxy -4-methoxyphenyl) propan-1-one	

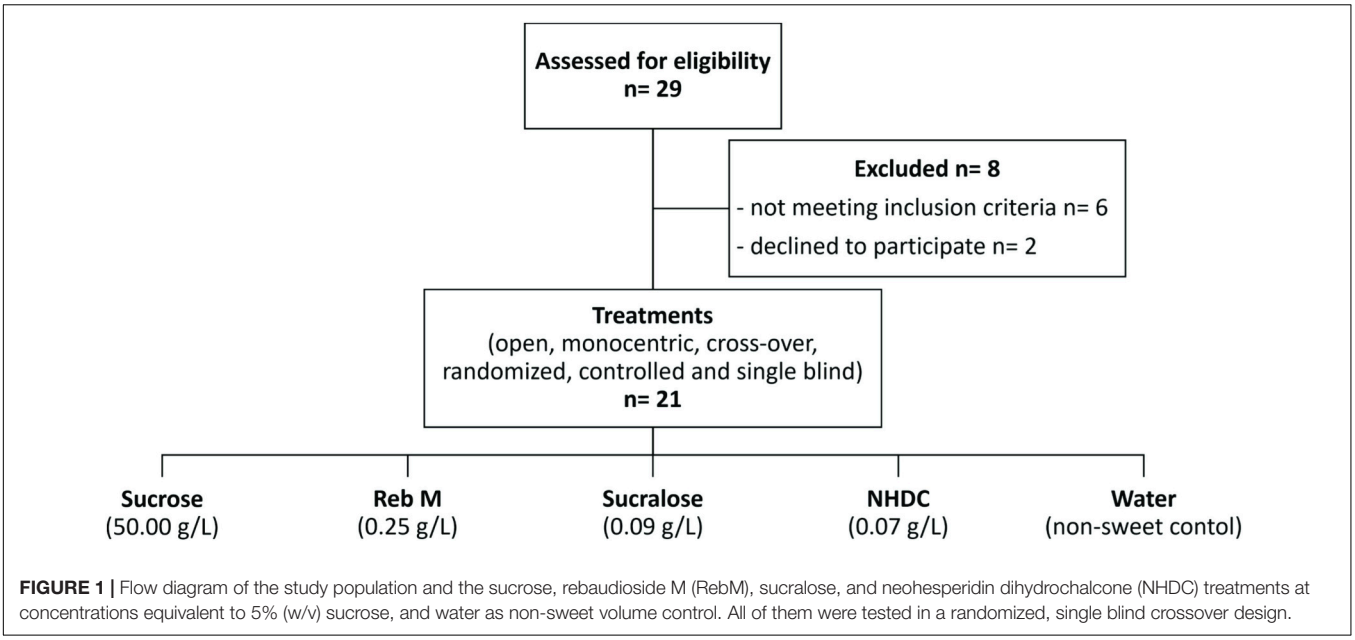


FIGURE 1 | Flow diagram of the study population and the sucrose, rebaudioside M (RebM), sucralose, and neohesperidin dihydrochalcone (NHDC) treatments at concentrations equivalent to 5% (w/v) sucrose, and water as non-sweet volume control. All of them were tested in a randomized, single blind crossover design.

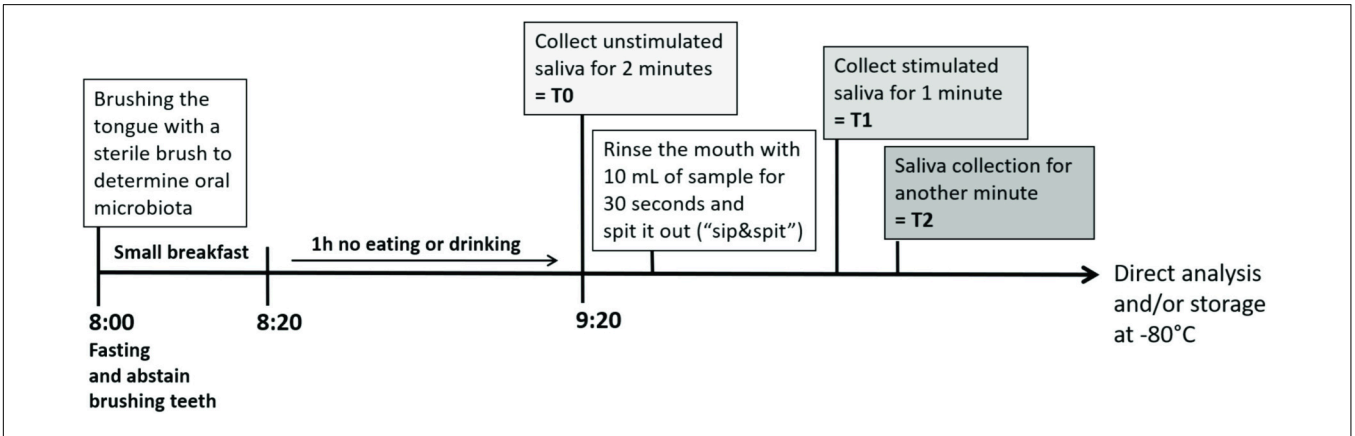


FIGURE 2 | Study design—timeline and overview of a study day with sampling of oral microbiome, unstimulated (T0) and stimulated saliva in the first (T1) and second (T2) min after stimulation with one of the test compounds. The procedure was repeated for each test compound on a separate day with at least 3 days apart.

manual of procedures for the human microbiome project, version 12.0[1] to determine the basal composition of the oral microbiome in the area of the fungiform papillae to see if the individual oral microbiota is constant over the study time and might influence taste perception or mouthfeel attributes. Samples were frozen at −80°C until analysis. After swabbing, the test subjects consumed the standardized breakfast provided (one pretzel with 10 g of butter and up to 300 ml of water), followed by brushing the teeth with a flavor-neutral toothpaste composed solely of calcium carbonate (obtained from a local pharmacy) and tap water. Test subjects had to abstain from eating and drinking for 1 h before starting saliva collection. Unstimulated resting saliva was collected for 2 min (T0). After the collection of unstimulated saliva, test subjects rinsed their mouth with 10 ml of the sample for 30 s and spat out the entire sample. Stimulated saliva was then collected after spitting out the sample separately for the first min (T1) and the second min (T2). All saliva samples were kept on ice immediately after collection. Aliquots of saliva samples were frozen at −80°C for subsequent analysis of protein content, α-amylase activity, cystatin S, and Muc5B. The flow rate, pH, and viscoelastic parameters of saliva were analyzed directly after collection.

Sensory Evaluation

The screening included the determination of the individual sweet taste threshold and the sweet intensity rating of the test compounds in a fully equipped sensory laboratory. The sweet threshold level of the test subjects was determined with increasing sucrose concentrations from 0.34 to 12.00 g/L according to DIN EN ISO 3972:2013-12 (32) described in detail by Höhl and Busch-Stockfisch (33). Moreover, the sweet taste intensity for each test compound was rated on an unstructured continuous scale [0–10]

[1]https://www.hmpdacc.org/hmp/doc/HMP_MOP_Version12_0_072910.pdf

after pre-tasting five sweet solutions with 0, 6, 20, 50, and 100 g/L of sucrose as "not at all" to "very intensive" sweet taste.

Determination of Salivary Parameters

Salivary Flow

Salivary flow was determined gravimetrically on an analytical scale (Satorius AG, Göttingen, Germany 224i-1S, with a reproducibility of 0.1 mg) immediately after sample collection. For this purpose, the test subjects were asked to salivate in individually pre-weighed 5 ml tubes for exactly 2 (T0) or 1 min (T1 and T2). Data are presented as salivary flow in g/min.

pH

Salivary pH was measured using 20 μl of fresh saliva samples with a pocket pH meter (PH60F, Apera Instruments GmbH, Wuppertal, Germany; pH ± 0.01, measuring range pH -2.0–16.0).

Total Protein Content

Total protein content in saliva was measured according to Bradford (34). Samples were centrifuged at 3 000 × *g* for 15 min at 4°C, and the supernatant was mixed with an equal volume of RIPA lysis buffer (50 mM tris(hydroxymethyl)-aminomethane, 25 mM sodium chloride, 1 mM ethylenediaminetetraacetic acid, 1 mM sodium fluoride). Samples, or 0.025–1.0 mg/ml bovine serum albumin (Thermo Scientific, Rockford, United States) as standards, were mixed with Bradford color reagent (2.5 mg Coomassie Blue G-250 with 150 ml methanol and 50 ml phosphoric acid, filled with ddH_2O to 1 L) (1:100) and incubated for 15 min. The absorbance of each sample or standard was measured in triplicate using a multimode plate reader (Tecan Infinite M200, Tecan Group Ltd., Männedorf, Switzerland) at 595 nm and a reference wavelength of 850 nm and the protein concentration was presented as mg/ml.

α-Amylase Activity

The α-amylase activity in saliva was determined using an enzymatic hydrolysis assay (35, 36) with three technical replicates for each saliva sample. The saliva samples were centrifuged at 3,000 × *g* for 15 min at 4°C, and the supernatant was used for further analysis. An equal volume of 1% (w/v) starch solution was added to the saliva samples and, after exactly 3 min, one volume of color reagent solution was added. The color reagent solution consisted of 1.0 g of 3, 5-dinitrosalicylic acid with 30.0 g of sodium potassium tartrate tetrahydrate and 20 ml of 2 M NaOH solution in 100 ml water. The reaction was stopped by adding five volumes of ddH_2O. The α-amylase activity was determined by the detection of maltose cleaved from the starch. Maltose reduces 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, causing a shift in the absorbance at 540 nm, which was analyzed using a multimode plate reader (Tecan Infinite M200, Tecan Group Ltd., Männedorf, Switzerland). Quantification of enzymatic starch cleavage was accomplished using an external standard curve (maltose 0.125–5.0 μmol/ml), and the amount of maltose produced in the presence of salivary α-amylase was determined by extrapolation after subtraction of the blank (ddH_20 instead of maltose solution) from the standard and sample values. The results were normalized to total protein content and presented as μmol of maltose per mg protein released per minute (μmol/mg protein/min).

Cystatin S

The amount of cystatin S in saliva was determined using a quantitative colorimetric sandwich-ELISA kit (abx 151234, Abbexa Ltd, Cambridge, United Kingdom, range: 0.156–10 ng/ml, sensitivity: <0.066 ng/ml). The saliva samples were centrifuged at 3 000 × g for 15 min at 4°C, and the supernatant was analyzed in duplicates according to the manufacturer's protocol. Absorbance values were detected at 450 and 650 nm as the reference wavelength using a multimode plate reader (Tecan Infinite M200, Tecan Group Ltd., Männedorf, Switzerland). The results were normalized to the amount of protein and were presented as μg per mg protein (μg/mg protein).

Rheological Properties

Rheological properties, such as complex viscosity (η), storage modulus (G') representing the elastic part, loss modulus (G'') representing the viscous part, and the phase angle (δ) representing the relative saliva viscoelasticity, were measured with a regularly calibrated oscillating rheometer (Kinexus, Malvern Panalytical GmbH, Kassel, Germany). A 20-mm diameter plate–plate probe (plate PU-20) was used with a gap of 0.5 mm. The saliva samples were kept on ice and measured directly after collection. Measurements were carried out using a frequency sweep at 36°C in the linear region of an amplitude sweep at a strain of 0.5% and a frequency range from 0.6 to 0.1 with six linear measuring points. SDS was not added to the samples as it is known that this can cause unfolding and dissociation of salivary proteins, affecting the aggregation state of mucins (37) and thereby influencing the structure and viscosity of saliva. Prior to performing the experiments, the method was established and data were validated using an amplitude sweep at a frequency of 0.5 with linear moduli up to 1% (see **Supplementary Table 1**). A strain of 0.5% was chosen as the software determined that this was the optimum strain for the selected frequency sweep, and the frequency range was chosen according to the low-interference area of the device. As the measurement time was less than 3 min, it was not necessary to cover the samples to avoid evaporation. The mean of two to three measurements of each salivary sample [n = 21 for each test compound (see **Figure 1**) and the three time points of saliva collection (see **Figure 2**)] was used as the value for each of the rheological parameters. The results are presented as Pa s for complex viscosity η, Pa for storage modulus G', Pa for loss modulus G'', and ° for phase angle δ.

Determination of Muc5B

A quantitative colorimetric sandwich-ELISA kit (abx 250243, Abbexa Ltd., Cambrige, United Kingdom, range: 0.625–40 ng/ml, sensitivity: 0.38 ng/ml) was used to determine the amount of the glycoprotein Muc5B in saliva. After centrifuging saliva samples at 3,000 × g for 15 min at 4°C to remove debris, the supernatant was analyzed in duplicate according to the manufacturer's manual. Absorbance values were detected at 450 and at 650 nm as the

reference wavelength using a multimode plate reader (Tecan Infinite M200, Tecan Group Ltd., Männedorf, Switzerland). The results were normalized to the amount of protein and presented as ng per mg protein (ng/mg protein).

Oral Microbiome Composition

The oral microbiome was analyzed by the 16S rRNA gene amplicon sequencing analysis performed at the Joint Microbiome Facility (project ID JMF-1908-4) using a previously described two-step PCR approach (38). Briefly, DNA from tongue swabs and control swabs was extracted using the QIAamp DNA Microbiome kit (Qiagen) following the manufacturer's instructions. In the first PCR step, the V4 region of bacterial and archaeal 16S rRNA genes was amplified (35 cycles) with the 515F and 806R primers (39, 40), which were modified with linker sequences [UDB-H12 barcoding approach (38)]. In the second step, the amplicons were barcoded (eight cycles) in a unique dual (UDD-H12) setup. After the first step PCR and after barcoding, the samples were purified and normalized over the SequalPrep^{TM} Normalization Plate kit (Invitrogen) using the Biomek® NXP Span-8 pipetting robot (Beckman Coulter). Barcoded samples were pooled and concentrated on columns (Analytik Jena), and the indexed sequencing libraries were prepared from these amplicon pools with the Illumina TruSeq Nano kit, as described in a previous study (38). Amplicon pools were sequenced in a paired-end mode (2 × 300 nt; v3 chemistry) on an Illumina MiSeq following the manufacturer's instructions. The workflow systematically included four negative controls (PCR blanks, i.e., PCR-grade water as template) for all 90 samples sequenced. Amplicon pools were extracted from the raw sequencing data using the FASTQ workflow in BaseSpace (Illumina) with default parameters. Further, demultiplexing was performed with the python package demultiplex (Laros JFJ[2]), allowing one mismatch for barcodes and two mismatches for linkers and primers each. Amplicon sequence variants (ASVs) were inferred using the DADA2 R package [3] applying the recommended workflow (41). The resulting FASTQ reads were trimmed at 145 nt with the allowed expected error of 2. ASVs were classified using SINA version 1.2.11 (42) and the SILVA database SSU Ref NR 99 release 132 (43) using default parameters. All generated amplicon sequencing data were deposited to the Sequence Read Archive (SRA) and can be found under the BioProject ID PRJNA726851.

Statistical Analysis

Data calculation and statistical analysis of the salivary characteristics were performed using MS Excel 16.0, GraphPad Prism 8.0, and IBM SPSS Statistics 26. All data sets were tested for normality with the Shapiro–Wilk test. To assess compound- and time-dependent effects, a repeated measures two-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test for dependent data was performed and checked for normally distributed residuals. To evaluate differences between the treatments, the data were normalized to the respective baseline value at T0 (ΔT1, ΔT2) and to the volume control water [$\Delta\Delta Tx = (Tx–T0)–(Tx—T0)_{H2O}$]. To evaluate the impact of the test compounds and selected influencing factors on the salivary flow and the complex viscosity (N* complex), a repeated measures analysis of covariance (ANCOVA) was carried out using SPSS. The ANCOVA was used to see if there were effects of selected and potentially influencing metric covariates on salivary flow and complex viscosity either dependent (intrasubject factor) or independent (intermediate subject effects) of the test compounds. Several factors that may influence salivary characteristics were included as covariates, namely, the BMI, age, and sweet threshold of the test subjects, individual sweet perception of the test compounds, pH, α-amylase activity, cystatin S, Muc5B, storage modulus (G'), and phase angle (δ) of basal saliva. The Pearson's product moment correlation was applied for a correlation analysis. To compare differences in the perceived sweet taste of the test compounds, a one-way ANOVA with Tukey's *post-hoc* test was performed in GraphPad Prism. Unless otherwise indicated, data are presented as mean ± standard error of the mean (SEM). Differences were considered as significant at $p < 0.05$ and with $p < 0.1$ as a trend. In all figures and tables, significant differences were marked with either asterisk or different letters.

To test for associations between various recorded descriptive and physiological parameters and the tongue dorsum microbiome composition, the ASV table was rarefied to the minimum sample depth (3,650 sequences) using the "rrarefy" function from the R package vegan[3] (44). PERMANOVA was performed with the "adonis" function (45) of the R package vegan. Bray–Curtis dissimilarity was used as a dissimilarity metric. Otherwise, default parameters were used.

RESULTS

Salivary Flow

The characteristics of the test subjects, including the sweet taste threshold [g/L sucrose], are summarized in **Table 2**. The distribution of the sweet sensitivity threshold is shown in **Supplementary Figure 1**.

First, the impact of oral stimulation with the selection of sensorially and structurally different sweet-tasting compounds adjusted for sweet taste, namely, sucrose, RebM, sucralose, and NHDC, on salivary flow with water as taste-neutral volume

[2]github.com/jfjlaros/demultiplex

[3]https://cran.r-project.org/web/packages/vegan/vegan.pdf

TABLE 2 | Characteristics of the study participants.

Test subjects	Total *n* = 21	
	Mean	±SD
Age [y]	26.57	±5.07
Female/Male	10/11	
Weight [kg]	71.89	±11.84
Height [m]	1.77	±0.09
BMI [kg/m^2]	22.74	±2.18
Threshold sweet taste [g/L sucrose]	2.48	±1.54

control was investigated. The basal unstimulated salivary flow rate (T0) between the test days did not differ significantly ($p > 0.05$). All tested stimuli, including water, enhanced the salivary flow during the 1st min after stimulation (T1): sucrose 1.33 ± 0.11 g/min, RebM 1.51 ± 0.12 g/min, sucralose 1.43 ± 0.10 g/min, NHDC 1.38 ± 0.12 g/min, and water 1.10 ± 0.09 g/min (**Figure 3A**). In comparison to water, salivary flow was significantly enhanced after stimulation with RebM and sucralose at T1. In the 2nd min after stimulation (T2), salivary flow was decreased compared to T1 after stimulation with each treatment (T2: sucrose 0.93 ± 0.08 g/min, RebM 1.02 ± 0.07 g/min, sucralose 0.92 ± 0.09 g/min, NHDC 0.99 ± 0.09 g/min, and water 0.84 ± 0.07 g/L) but was still increased in comparison to the basal flow rate except for sucralose and the water control (**Figure 3A**). There was no difference in salivary flow stimulation at T2 between the compounds.

The concentration of the test compounds was selected to reach the sweet taste level equivalent to 5% sucrose, based on the rating of trained panelists, according to Karl et al. (28). However, the sensorially naïve test subjects in this study rated NHDC and RebM to be sweeter than sucralose and sucrose (**Figure 3B**). As individually perceived sweet taste between the compounds differed, a more detailed look was taken at the relationship between individually perceived sweet taste and the salivary flow rate. As expected, sucrose, RebM, sucralose, and NHDC were rated to be significantly sweeter than the volume control water. In addition, there was a moderate positive correlation between individually perceived sweet taste and the salivary flow rate within the 1st min ($r = 0.3$, $p < 0.01$, **Figure 3C**).

To investigate the hypothesis that sensorially and structurally distinct sweet-tasting compounds will lead to differences in the flow rate, the stimulated salivary flow was normalized to the unstimulated flow rate on each test day and to the effect of the

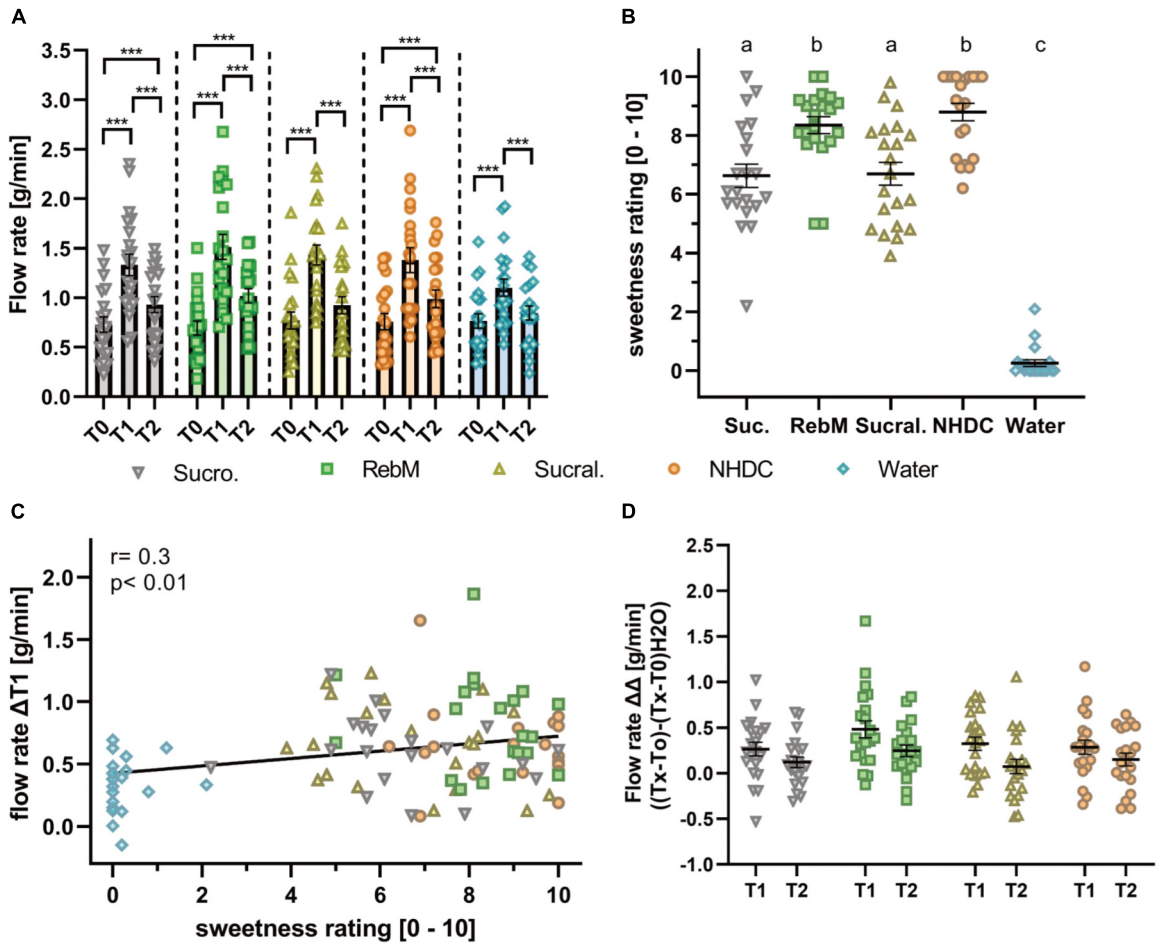


FIGURE 3 | (A) Flow rate (g/min) of unstimulated (T0) and stimulated saliva in the 1st (T1) and 2nd (T2) min after stimulation with the test solutions sucrose (suc.), RebM, sucralose (sucral.), NHDC, and water as control; presented as mean ± standard error of the mean (SEM); tested for difference with a two-way ANOVA and Tukey's *post-hoc* test; significant differences between measurement time points (T0-1-2) are marked with ***($p < 0.001$). **(B)** Individually, perceived sweet taste rating (0-10) of the test solutions. Significant differences were tested with a one-way ANOVA with Tukey's *post-hoc* test and are labeled with different letters. **(C)** Pearson product moment correlation of sweet taste rating and ΔT1 flow rate (g/min). **(D)** Normalized flow rate, calculated as [g/min] $[(Tx\text{-}T0)\text{-}(Tx\text{-}T0)_{H2O}]$ after stimulation with the test solutions, presented as mean ± SEM. All figures include single values ($n = 21$) for each test compound.

volume control water ($\Delta\Delta T_x$) (see **Figure 3D**). Using a repeated measures ANCOVA, which passed the Mauchly test for sphericity ($p > 0.05$), the effect of the test compounds and several potentially influencing factors as covariates, namely, the basal values (T0) of pH, α-amylase activity, cystatin S content, the sweet threshold, body height, BMI, and age of test subjects on the salivary flow rate ($\Delta\Delta T1$) was evaluated. The sweet taste evaluation was excluded as we determined differences in the mean sweet taste evaluation of the test compounds by our untrained panelists. None of the covariates influenced the salivary flow rate in dependence of the test compounds (intrasubject factor, see **Supplementary Table 2**). However, independent of the treatment (intermediate subject effects, see **Supplementary Table 2**), the body height and the interaction of α-amylase activity with the sweet taste threshold showed a trend ($p < 0.1$) to affect the flow rate. **Table 3** shows the unadjusted and covariate-adjusted means of the salivary flow rate ($\Delta\Delta T1$). No significant difference was found between the test substances for unadjusted or adjusted salivary flow values.

Viscoelastic Properties of Saliva

Next, we investigated the impact of oral stimulation with sucrose, RebM, sucralose, and NHDC on the viscoelastic properties of saliva. A representative measurement of G', G'', and phase angle (δ) against the frequency of one saliva sample with two repetitions is shown in **Supplementary Figure 2**. Furthermore, **Figure 4A** shows the mean complex viscosity (η) of saliva before (T0) and at the 1st (T1) and 2nd (T2) min after stimulation with the test solutions. The complex viscosity of basal, unstimulated saliva samples did not differ throughout the different study days. In contrast to our hypothesis, oral stimulation with none of the test compounds resulted in differences in η (**Figure 4A**). Also, no differences were found for the storage and loss modulus (G' and G'') of unstimulated and stimulated saliva (see **Supplementary Table 3** for raw data). The sweet taste threshold of the test subjects correlated with G' ($\Delta T1$) of saliva after stimulation with sucrose ($r = 0.6$, $p < 0.01$, **Figure 4B**).

The consideration of potentially influencing covariates was evaluated using a repeated measures ANCOVA for the values of η normalized to the water control ($\Delta\Delta T1$) (see **Figure 4C**). The ANCOVA included as covariates the basal values (T0) of Muc5B, pH, α-amylase, storage modulus (G') and phase angle (δ) of saliva as well as sweet threshold and age of test subjects and passed the Mauchly test for sphericity ($p > 0.05$). The results (**Supplementary Table 4**) of the intrasubject factor showed that, depending on the test compounds, the interaction of G' with the sweet threshold influenced complex viscosity η of the saliva ($p < 0.05$). The intermediate subject effects showed that, independent of the test compound, the basal amount of Muc5B significantly influenced the complex viscosity ($p < 0.05$). There was no effect of the pH of saliva samples or age of test subjects on complex viscosity ($p > 0.1$). Overall, no significant differences between the test compounds were found for either the unadjusted or the covariate-adjusted means of the complex viscosity (**Table 4**).

TABLE 3 | Unadjusted values and covariate-adjusted means (±SD/SE) of the normalized salivary flow rate $\Delta\Delta T1$ [g/min] = $[(Tx\ T0)-(Tx-T0)_{H2O}]$ after stimulation with each test solution analyzed by means of an repeated measures ANCOVA with the basal level of α-amylase activity, cystatin S, pH, threshold, body height, body mass index (BMI), and age of participants as covariates.

ΔΔT1 flow rate [g/min]		Unadjusted		Adjusted	
	N	Mean	±SD	Mean	±SE
Sucrose	21	0.26	0.35	0.32	0.06
RebM	21	0.48	0.43	0.53	0.11
Sucralose	21	0.32	0.33	0.37	0.08
NHDC	21	0.28	0.36	0.32	0.11
p-value		0.532		0.215	

Oral Microbiome

Finally, we addressed the question of whether the individual basal oral microbiome is associated with the sweet taste perception and the analyzed salivary characteristics of unstimulated and stimulated saliva as well as their related parameters, including age, BMI, and sweet taste threshold. Tongue dorsum microbiome composition was neither influenced by the sampling day and tongue side nor by age, sex, and BMI of the test subjects, the sweet recognition thresholds, or basal salivary parameters (PERMANOVA, $p > 0.05$), except for the pH of unstimulated saliva (T0), which displayed a weak but statistically significant correlation with the observed microbiome composition ($R^2 = 0.03$, $p < 0.01$; data not shown). All analyzed samples, regardless of the sampled individuals and the sample collection time point, displayed a composition and a diversity typical of human oral microbiome samples previously obtained from tongues of healthy individuals (46–48). The microbiomes of tongue samples from the 21 subjects investigated here were colonized by bacteria phylogenetically affiliated with eight different phyla, with *Proteobacteria* being the most abundant, followed by *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, *Saccharimonadia*, *Gracilibacteria*, and *Epsilonbacteraeota*. Specifically, samples were dominated by ASVs affiliated with the genera *Haemophilus, Neisseria, Streptococcus, Veillonella, Gemella, Prevotella, Rothia*, and *Leptotrichia* (see **Figure 5**). Oral microbiota composition was strongly individual-dependent (PERMANOVA: $R^2 = 0.76$, $p < 0.001$) and stable over the testing period, which lasted at least 14 days, by comparing the samples obtained on test days 1 and 5 of each test subject (see **Supplementary Figure 3**). All analyses of the association between microbiome composition and physiological parameters determined in saliva samples of the test subjects were constrained (strata) to the test subjects.

DISCUSSION

In this study, we investigated the influence of the stimulation with three structurally diverse sweeteners, namely, sucralose, RebM, and NHDC, compared to the most abundant sweet compound sucrose, on salivary flow and the viscoelastic properties of saliva in 21 healthy, adult test persons. Furthermore, we investigated whether there is an association between the

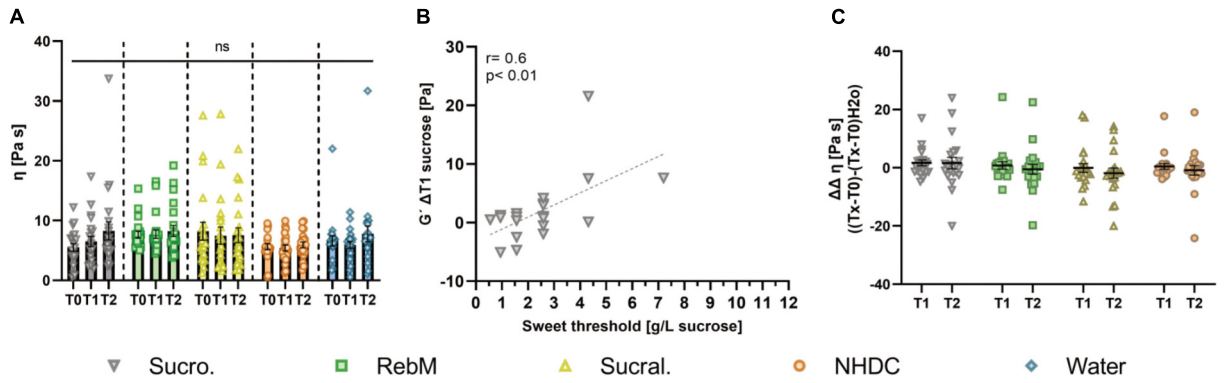


FIGURE 4 | (A) Complex viscosity η (Pa s) of unstimulated (T0) and stimulated saliva in the 1st (T1) and 2nd (T2) min after stimulation with test solutions sucrose (suc.), RebM, sucralose (sucral.), NHDC, and water as control; presented as mean ± SEM; tested for difference with two-way ANOVA and Tukey's *post-hoc* test; (ns) no significant results were detected. **(B)** Normalized complex viscosity η, calculated as (Pa s) $[(Tx\text{-}T0)\text{-}(Tx\text{-}T0)_{H2O}]$ of saliva samples after stimulation with each test solution; presented as mean ± SEM, $n = 21$. **(C)** Pearson product moment correlation for storage modulus G' (Pa) ΔT1 after stimulation with sucrose and the sweet taste threshold (g/L sucrose).

individual oral microbiome and the salivary characteristics or sweet taste perception.

Our results show that the stimulation with all test compounds, as well as the water control, enhanced saliva flow compared to unstimulated saliva, with RebM leading to the most long-lasting effect. However, we not only focused on salivary flow itself, but also considered several influencing factors as covariates. Thus, by applying a repeated measures ANCOVA, we excluded the impact of several potentially influencing factors, namely, the basal cystatin S content, pH of saliva, and BMI and age of the test subjects. Those factors were selected as previous studies showed associations to salivary flow and overall taste perception (15, 49–52) although complex relationships with several factors have not been addressed so far. Our results show that, independent of the test compounds, salivation tended to be associated with body size and basal α-amylase activity. In addition, individually perceived sweet taste of the compounds showed a moderate but significant correlation to the salivary flow rate, which supports the fact that sweet stimulus increases salivary flow stronger than the taste-neutral water control. It is hypothesized that the caloric load and concentration of a compound do not have a major impact on salivary flow, but the perception of sweet taste is the main driving force, independent of the type of sweetener. Bonnans and Noble (9) concluded in their study, with regard to salivary flow and the perception of sweet and sour, that the salivary flow response is not only based on the concentration of the stimulus but is also influenced by individual cognitive taste perception (9), which includes processed perception in the central nervous system. Because the stimulation was the strongest in the 1st min after stimulation (ΔT1), it reflects the fast adaptation previously described to a sweet stimulus (53, 54). A fast adaption process of salivary flow to food-derived stimuli was later also confirmed by the results in Criado et al. (55), demonstrating a stronger immediate effect of wine aroma on salivary flow than the long-lasting effect of the aroma. It has to be noted that, in this study, the sweet taste level of the test solutions was adjusted to be equivalent to 5% sucrose, which was previously demonstrated with trained subjects (28). Nevertheless, the test subjects rated the sweet taste of the test solutions to be different. One explanation could be the fact that the test subjects were sensorially naïve and were not specifically trained to differentiate the onset, maximum sweet taste, and lingering of a compound, whereas untrained panelists do present taste impressions from everyday society. Hence, it cannot be excluded that the test subjects confounded the well-known long-lasting lingering of NHDC and RebM (28) with enhanced maximum sweet taste, and trained panelists would have been able to distinguish this. It should be noted that RebM and NHDC showed longer lasting stimulation of salivary flow than sucralose, which is reflected by the increase in the flow rate in the 2nd min of stimulation (T2). This also argues for an effect of perceived sweet taste as NHDC and RebM are known for their long-lasting sweet taste, as described above. A reason could be that those compounds stick longer to the chemosensory surface, but this remains speculative. Further studies with trained panelists are needed to focus on the interaction of lingering and a long-lasting salivary flow, including a complete time-intensity profile and concentration dependence of the test compounds.

TABLE 4 | Unadjusted values and covariate-adjusted means (±SD/SE) of normalized complex viscosity η values ($\Delta\Delta$T1 [Pa s] = $[(Tx\ T0)\text{-}(Tx\text{-}T0)_{H2O}]$) of saliva samples after stimulation with each test solution analyzed by means of an repeated measures ANCOVA with the basal level of mucin 5B (Muc5B), storage modulus G', phase angle δ, pH, α-amylase activity, threshold, and age of participants as covariates.

ΔΔT1 η [Pa s]		Unadjusted		Adjusted	
	N	Mean	±SD	Mean	±SE
Sucrose	21	1.65	4.70	0.72	1.13
RebM	21	0.75	5.92	−0.41	1.56
Sucralose	21	−0.08	6.99	−0.17	1.35
NHDC	21	0.44	4.38	−0.44	1.25
p-value		0.699		0.565	

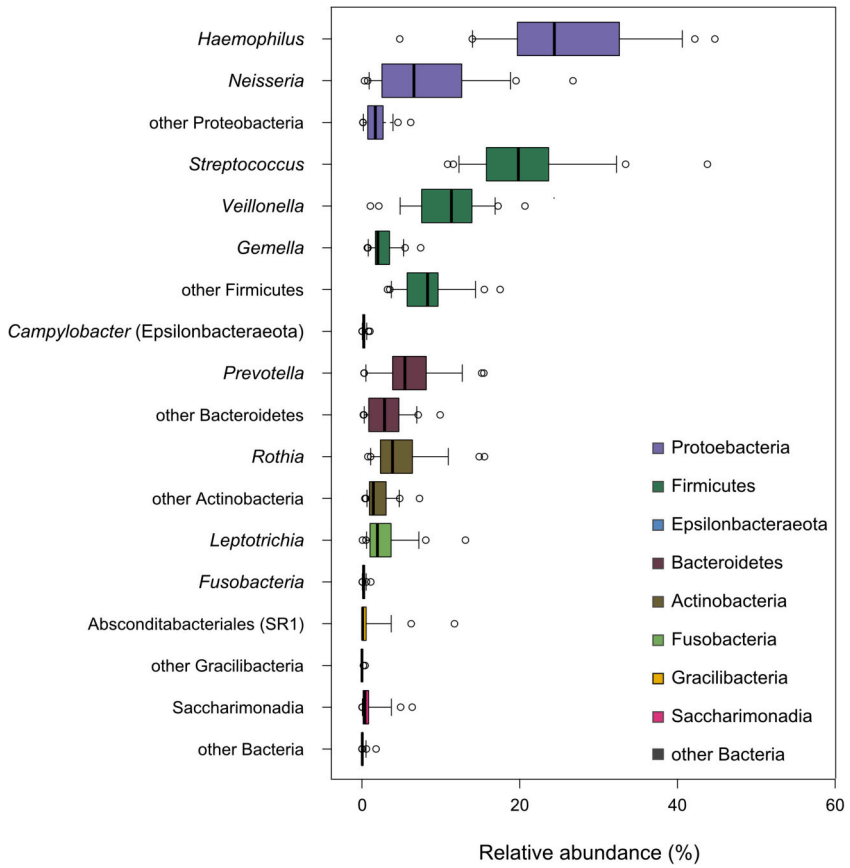


FIGURE 5 | Tongue dorsum microbiome composition across all study participants. The cumulative relative abundance per individual of amplicon sequence variants (ASVs) classified as select, dominant bacterial genera is shown in the box plots. The phylum-level taxonomic classification of these genera is indicated by the box color.

The second aim of our study was to explore a possible change of the salivary complex viscosity η after stimulation with sweet-tasting compounds. In general, saliva with low elasticity can lead to a moister mouthfeel, as demonstrated after the consumption of plain water (4). However, knowledge on the interactions of salivary rheology, sweet taste perception, and its mouthfeel remains scarce. Schipper et al. (56) summarized a wide range of studies investigating whole saliva viscosities: apparent viscosity η_a can range from 1.1 up to 10 mPas. The variation of values is based on the different methods applied (types of rheometer, shear rate, and temperature), collection and handling of saliva, circadian rhythm, type of glands, and individual variation (56). Therefore, especially the raw data can variate due to different study protocols and a comparison with raw data of other studies is difficult. However, the crossover design of our study allows us to compare the responses of the test persons to different stimuli. In contrast to our hypothesis, no differences between unstimulated and stimulated saliva or differences between treatments were found. As there were no differences in viscosity markers for unstimulated saliva on each taste day, we assume differences after stimulation with the test compounds—if any—to be below the limit of detection. The repeated measures ANCOVA excluded the influence of basal salivary pH and age of the test subjects. The statistical model revealed that, depending on the test compound, the complex viscosity is influenced by the interaction of elasticity and sweet taste threshold. Moreover, Muc5B had an impact on complex viscosity independent of the test compounds. Thus, the viscoelastic properties might affect the determination of the sweet taste threshold as well. After stimulation with sucrose, a higher storage modulus (G'), representing the elastic component of the saliva sample, was positively correlated with a higher sweet taste threshold. We hypothesize that this may be due to impaired transport of tastants to the taste pores as it has been shown for lower mixing efficiency in more elastic saliva (57). In this context, Ferry et al. (58) also showed that lowering the mixing efficiency by salivary amylase-released polysaccharides reduced the perceived saltiness. Those results support the importance of the interaction of the tastants with components in saliva (58). The protein precipitating properties of the sweeteners, especially polyphenolic structures like NHDC, could also contribute to the mouthfeel of sweeteners by following a similar mechanism as suggested for astringent sensations. Polyhydroxyphenols like tannins are proposed to aggregate the salivary pellicle, leading to the dissociation of the two subunits of the transmembrane protein MUC1, which causes pull out of the pellicle and

neurotransmitter release (22). Karl et al. (28) have shown that NHDC and RebM show low but detectable astringent properties at the concentrations applied here. In addition, a potential direct interaction, especially of more complex sweeteners like NHDC and RebM, with salivary proteins present in mucosal pellicles, such as proline-rich proteins (PRPs), Muc5B, amylase, and cystatin, needs to be investigated in future studies. Muc5B was previously described to determine the viscosity of saliva (17, 59, 60) and this relation of Muc5B to η was confirmed here with a repeated measures ANCOVA, independent of the test compounds. Furthermore, the rheology of saliva depends on many inter- and intra-individual factors such as gender and hormonal balance (61), health status (62), and age (60). The rheological properties of food and saliva are constantly changing during the dynamic process of oral perception. Although we standardized sample collection and measurements and used a crossover design for the study, this dynamic process may be difficult to capture *in vivo* (5).

Another possible player in the taste perception and mouthfeel of sweet compounds and associated salivary parameters is the oral microbiome (25, 26). Especially, for the salivary pH, the role of oral microbiota is crucial because there are associations between sugar intake and oral microbiota ecology, and a variable microbiota response to sugar (25). The composition and diversity of oral microbiota shown here are typical of healthy human tongue samples (46–48). In line with previous studies of a healthy mouth environment (63), the composition of microbial communities was observed to be quite stable over time within an individual. However, we did not find an association between the oral microbiota and the analyzed salivary parameters. The impact of the oral microbiome is also discussed discordantly in the literature. On the one hand, Cattaneo et al. (64) associated one taxon with a negative correlation to the sweet taste threshold. A reason could be that less-sensitive individuals more frequently consume sweets and desserts. On the other hand, Feng et al. (65) did not find a correlation between sweet taste sensitivity and bacterial count in saliva and tongue salivary film, which corresponds to the findings of the present study with the same number of test subjects. This aspect needs further investigations with larger study populations to clarify the role of microbiota in sweet taste perception.

In this study, there are limitations related to sensory tasting and mouthfeel. First, we focused on the pure sweet taste impression of the test compounds and did not consider secondary tastes and temporal attributes, which led to an increased rating of sweet taste intensity for NHDC and RebM by sensorially naïve test subjects. In addition, future studies with trained panelists are needed to determine the impact of structure vs. sweet taste. The second limitation was a narrow range of characteristics such as BMI, basal salivary flow rate, age, and the limited number of test subjects. Furthermore, a moderate correlation between individually perceived sweet taste and the salivary flow rate in the 1st min ($r = 0.3$, $p < 0.01$) may be due to differences in water and sweet compounds. Thus, the association between the perception of sweet taste and flow rate, as well as possible associations of taste impression with the oral microbiome, needs to be verified in a larger study population. In addition, the pH effect of the tap water used in our study as a solvent for the tested sweet-tasting compounds remains unclear and needs to be specifically tested in future studies. Contrarily, the strengths of this study were that we included a wide variety of different influencing factors to ensure a broad overview on salivary characteristics and associated mouthfeel and, for the first time, included complex interactions between the different factors in the statistical analysis.

CONCLUSION

The results presented in this study demonstrate that individual sweet taste perception after oral stimulation with sucrose, sucralose, NHDC, and RebM is associated with salivary flow, which indicates an impact of predominantly cognitive sweet taste impression on the salivary flow rate without measurable changes in the rheological properties of saliva. Nonetheless, the complex viscosity of saliva was influenced by Muc5B, as well as by an interaction of the test compounds with elasticity and sweet taste threshold. The results indicate that salivary flow and saliva viscoelasticity may contribute to the overall taste perception and mouthfeel and may affect the sensory profile of sweet-tasting compounds. This study provides a basis for further studies to understand the complex interaction of saliva and the sensory properties of sweet-tasting compounds.

DATA AVAILABILITY STATEMENT

The amplicon sequencing datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra, BioProject ID PRJNA726851.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Vienna (reference no. 00421; 2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CK: conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, and writing—original draft, reviewing, and editing. AV: data curation, formal analysis, investigation, and writing—reviewing and editing. PP: conceptualization, formal analysis, investigation, methodology, resources, software, validation, visualization, and writing—original draft, reviewing, and editing. BH: data curation, formal analysis, software, validation, and writing—reviewing and editing. GS: methodology, resources, software, and writing—reviewing and editing. JL: conceptualization, funding acquisition, resources, supervision, and writing—reviewing and editing. DB: conceptualization,

methodology, resources, and writing—reviewing and editing. JH: conceptualization and writing—reviewing and editing. MW: conceptualization, methodology, and writing—reviewing and editing. JK: resources and writing—review and editing. VS: conceptualization, resources, and writing—reviewing and editing. BL: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, and writing—original draft, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.831726/full#supplementary-material

REFERENCES

1. Guinard JX, Mazzucchelli R. The sensory perception of texture and mouthfeel. *Trends Food Sci Technol.* (1996) 7:213–9. doi: 10.1016/0924-2244(96)10025-X
2. Guest S, Essick G, Young M, Phillips N, McGlone F. The effect of oral drying and astringent liquids on the perception of mouth wetness. *Physiol Behav.* (2008) 93:889–96. doi: 10.1016/j.physbeh.2007.12.006
3. Muñoz-Gonzalez C, Feron G, Canon F. Main effects of human saliva on flavour perception and the potential contribution to food consumption. *Proc Nutr Soc.* (2018) 77:423–31. doi: 10.1017/S0029665118000113
4. Davies GA, Wantling E, Stokes JR. The influence of beverages on the stimulation and viscoelasticity of saliva: relationship to mouthfeel? *Food Hydrocoll.* (2009) 23:2261–9. doi: 10.1016/j.foodhyd.2009.06.002
5. Stokes JR, Boehm MW, Baier SK. Oral processing, texture and mouthfeel: from rheology to tribology and beyond. *Curr Opin Colloid Interface Sci.* (2013) 18:349–59. doi: 10.1016/j.cocis.2013.04.010
6. Neyraud E, Sayd T, Morzel M, Dransfield E. Proteomic analysis of human whole and parotid salivas following stimulation by different tastes. *J Proteome Res.* (2006) 5:2474–80. doi: 10.1021/pr060189z
7. Bader M, Dunkel A, Wenning M, Kohler B, Medard G, Del Castillo E, et al. Dynamic proteome alteration and functional modulation of human saliva induced by dietary chemosensory stimuli. *J Agric Food Chem.* (2018) 66:5621–34. doi: 10.1021/acs.jafc.8b02092
8. Pedersen A, Sorensen CE, Proctor GB, Carpenter GH. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. *Oral Dis.* (2018) 24:1399–416. doi: 10.1111/odi.12867
9. Bonnans SR, Noble AC. Interaction of salivary flow with temporal perception of sweetness, sourness, and fruitiness. *Physiol Behav.* (1995) 57:569–74. doi: 10.1016/0031-9384(94)00367-E
10. Neyraud E, Heinzerling CI, Bult JHF, Mesmin C, Dransfield E. Effects of different tastants on parotid saliva flow and composition. *Chemosens Percept.* (2009) 2:108–16. doi: 10.1007/s12078-009-9041-9
11. Neyraud E. Role of saliva in oral food perception. In: Ligtenberg AJM, Veerman ECI editors. *Saliva: Secretion and Functions.* (Basel: Monographs in Oral Science) (2014). p. 61–70. doi: 10.1159/000358789
12. Stokes JR, Davies GA. Viscoelasticity of human whole saliva collected after acid and mechanical stimulation. *Biorheology.* (2007) 44:141–60.
13. Harthoorn LF, Brattinga C, Van Kekem K, Neyraud E, Dransfield E. Effects of sucrose on salivary flow and composition: differences between real and sham intake. *Int J Food Sci Nutr.* (2009) 60:637–46. doi: 10.3109/09637480802039814
14. Sharma M, Pico J, Martinez MM, Duizer L. The dynamics of starch hydrolysis and thickness perception during oral processing. *Food Res Int.* (2020) 134:109275. doi: 10.1016/j.foodres.2020.109275
15. Henskens YC, Veerman ECI, Mantel MS, Van der Velden U, Nieuw Amerongen A. Cystatins S and C in human whole saliva and in glandular saliva in periodontal health and disease. *J Dent Res.* (1994) 73:1606–14. doi: 10.1177/00220345940730100501
16. Dickinson DP. Salivary (SD-type) cystatins: over one billion years in the making—but to what purpose? *Crit Rev Oral Biol Med.* (2002) 13:485–508. doi: 10.1177/154411130201300606
17. Thornton DJ, Khan N, Mehrotra R, Howard M, Sheehan JK, Veerman E, et al. Salivary mucin MG1 is comprised almost entirely of different glycosylated forms of the MUC5B gene product. *Glycobiology.* (1999) 9:293–302. doi: 10.1093/glycob/9.3.293
18. Hans R, Thomas S, Garla B, Dagli RJ, Hans MK. Effect of various sugary beverages on salivary PH. flow rate, and oral clearance rate amongst adults. *Scientifica (Cairo).* (2016) 2016:5027283. doi: 10.1155/2016/5027283
19. Xu F, Laguna L, Sarkar A. Aging-related changes in quantity and quality of saliva: where do we stand in our understanding? *J Texture Stud.* (2019) 50:27–35. doi: 10.1111/jtxs.12356
20. Morzel M, Siying T, Brignot H, Lherminier J. Immunocytological detection of salivary mucins (MUC5B) on the mucosal pellicle lining human epithelial buccal cells. *Microsc Res Tech.* (2014) 77:453–7. doi: 10.1002/jemt.22366
21. Hannig C, Hannig M, Kensche A, Carpenter G. The mucosal pellicle – an underestimated factor in oral physiology. *Arch Oral Biol.* (2017) 80:144–52. doi: 10.1016/j.archoralbio.2017.04.001
22. Canon F, Belloir C, Bourillot E, Brignot H, Briand L, Feron G, et al. Perspectives on astringency sensation: an alternative hypothesis on the molecular origin of astringency. *J Agric Food Chem.* (2021) 69:3822–6. doi: 10.1021/acs.jafc.0c07474
23. Van der Reijden WA, Veerman ECI, Nieuw Amerongen AV. Shear rate dependent viscoelastic behavior of human glandular salivas. *Biorheology.* (1993) 32:141–52. doi: 10.3233/BIR-1993-30205
24. Christensen CM. Effects of taste quality and intensity on oral perception of viscosity. *Percept Psychophys.* (1980) 28:315–20. doi: 10.3758/BF03204390
25. Esberg A, Haworth S, Hasslof P, Lif Holgerson P, Johansson I. Oral microbiota profile associates with sugar intake and taste preference genes. *Nutrients.* (2020) 12:681. doi: 10.3390/nu12030681
26. Cattaneo C, Gargari G, Koirala R, Laureati M, Riso P, Guglielmetti S, et al. New insights into the relationship between taste perception and oral microbiota composition. *Sci Rep.* (2019) 9:3549. doi: 10.1038/s41598-019-40374-3
27. Hayes JE, Bartoshuk LM, Kidd JR, Duffy VB. Supertasting and PROP bitterness depends on more than the TAS2R38 gene. *Chem Senses.* (2008) 33:255–65. doi: 10.1093/chemse/bjm084
28. Karl CM, Wendelin M, Lutsch D, Schleining G, Dürrschmied K, Ley JP, et al. Structure-dependent effects of sweet and sweet taste affecting compounds on their sensorial properties. *Food Chem.* (2020) 7:100100. doi: 10.1016/j.fochx.2020.100100
29. Mandel ID. Relation of saliva and plaque to caries. *J Dent Res.* (1974) 53:246–66. doi: 10.1177/00220345740530021201

30. Brandao E, Soares S, Mateus N, de Freitas V. Human saliva protein profile: influence of food ingestion. *Food Res Int.* (2014) 64:508–13. doi: 10.1016/j.foodres.2014.07.022
31. Jager DHJ, Bots CP, Forouzanfar T, Brand HS. Clinical oral dryness score: evaluation of a new screening method for oral dryness. *Odontology.* (2018) 106:439–44. doi: 10.1007/s10266-018-0339-4
32. DIN-EN-ISO-3972:2013-12. *Sensory Analysis – Methodology – Method of Investigating Sensitivity of Taste (ISO 3972:2011 + Cor. 1:2012)*. Genev: DIN (2011).
33. Höhl K, Busch-Stockfisch M. The influence of sensory training on taste sensitivity. Effects on sweet and bitter perception over a half-year period. *Ernährungs Umschau.* (2015) 62:208–15. doi: 10.4455/eu.2015.035
34. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* (1976) 72:248–54. doi: 10.1016/0003-2697(76)90527-3
35. Dasso M, Pagotto R, Pignataro OP, Diez RA, Sales ME. Effect of bitter compounds on amylase secretion in murine submandibular glands: signaling pathway mechanisms. *Biochim Biophys Acta.* (2011) 1810:1212–9. doi: 10.1016/j.bbagen.2011.08.009
36. Bernfeld P. Amylases, α and β. *Methods Enzymol.* (1955) 1:149–58. doi: 10.1016/0076-6879(55)01021-5
37. Bastardo L, Claesson P, Brown W. Interactions between mucin and alkyl sodium sulfates in solution. A light scattering study. *Langmuir.* (2002) 18:3848–53. doi: 10.1021/la015717u
38. Pjevac P, Hausmann B, Schwarz J, Kohl G, Herbold CW, Loy A, et al. An economical and flexible dual barcoding, two-step PCR approach for highly multiplexed amplicon sequencing. *Front Microbiol.* (2021) 12:669776. doi: 10.3389/fmicb.2021.669776
39. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat Microb Ecol.* (2015) 75:129–37. doi: 10.3354/ame01753
40. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ Microbiol.* (2016) 18:1403–14. doi: 10.1111/1462-2920.13023
41. Callahan BJ, Sankaran K, Fukuyama JA, McMurdie PJ, Holmes SP. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses. *F1000Res.* (2016) 5:1492. doi: 10.12688/f1000research.8986.1
42. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics.* (2012) 28:1823–9. doi: 10.1093/bioinformatics/bts252
43. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* (2013) 41:D590–6. doi: 10.1093/nar/gks1219
44. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. *Community Ecology Package 'vegan'. R Package Version 2.5-7*. (2020).
45. Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* (2001) 26:32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x
46. Tribble GD, Angelov N, Weltman R, Wang BY, Eswaran SV, Gay IC, et al. Frequency of tongue cleaning impacts the human tongue microbiome composition and enterosalivary circulation of nitrate. *Front Cell Infect Microbiol.* (2019) 9:39. doi: 10.3389/fcimb.2019.00039
47. Eren AM, Borisy GG, Huse SM, Mark Welch JL. Oligotyping analysis of the human oral microbiome. *PNAS.* (2014) 111:E2875–84. doi: 10.1073/pnas.1409644111
48. Zaura E, Keijser BJF, Huse SM, Crielaard W. Defining the healthy "core microbiome" of oral microbial communities. *BMC Microbiol.* (2009) 9:259. doi: 10.1186/1471-2180-9-259
49. Santos JL, Saus E, Smalley SV, Cataldo LR, Alberti G, Parada J, et al. Copy number polymorphism of the salivary amylase gene: implications in human nutrition research. *J Nutrigenet Nutrigenomics.* (2012) 5:117–31. doi: 10.1159/000339951
50. Martini D, Gallo A, Vella S, Sernissi F, Cecchettini A, Luciano N, et al. Cystatin S-a candidate biomarker for severity of submandibular gland involvement in sjogren's syndrome. *Rheumatology (Oxford).* (2017) 56:1031–8. doi: 10.1093/rheumatology/kew501
51. Carpenter GH. The secretion, components, and properties of saliva. *Ann Rev Food Sci Technol.* (2013) 4:267–76. doi: 10.1146/annurev-food-030212-182700
52. Walliczek-Dworschak U, Schops F, Feron G, Brignot H, Hahner A, Hummel T. Differences in the density of fungiform papillae and composition of saliva in patients with taste disorders compared to healthy controls. *Chem Senses.* (2017) 42:699–708. doi: 10.1093/chemse/bjx054
53. Bornstein BL, Wiet SG, Pombo M. Sweetness adaptation of some carbohydrate and high potency sweeteners. *J Food Sci.* (1993) 58:595–8. doi: 10.1111/j.1365-2621.1993.tb04332.x
54. Matuso R. Role of saliva in the maintenance of taste sensitivity. *Crit Rev Oral Biol Med.* (2000) 11:216–29. doi: 10.1177/10454411000110020501
55. Criado C, Chaya C, Fernandez-Ruiz V, Alvarez MD, Herranz B, Pozo-Bayon MA. Effect of saliva composition and flow on inter-individual differences in the temporal perception of retronasal aroma during wine tasting. *Food Res Int.* (2019) 126:108677. doi: 10.1016/j.foodres.2019.108677
56. Schipper RG, Silletti E, Vingerhoeds MH. Saliva as research material: biochemical, physicochemical and practical aspects. *Arch Oral Biol.* (2007) 52:1114–35. doi: 10.1016/j.archoralbio.2007.06.009
57. Baines ZV, Morris ER. Flavour/taste perception in thickened systems: the effect of guar gum above and below c*. *Food Hydrocoll.* (1987) 1:197–205. doi: 10.1016/S0268-005X(87)80003-6
58. Ferry ALS, Mitchell JR, Hort J, Hill SE, Taylor AJ, Lagarrigue S, et al. In-mouth amylase activity can reduce perception of saltiness in starch-thickened foods. *J Agric Food Chem.* (2006) 54:8869–73. doi: 10.1021/jf061607n
59. Vijay A, Inui T, Dodds M, Proctor G, Carpenter G. Factors that influence the extensional rheological property of saliva. *PLoS One.* (2015) 10:e0135792. doi: 10.1371/journal.pone.0135792
60. Inoue H, Ono K, Masuda W, Inagaki T, Yokota M, Inenaga K. Rheological properties of human saliva and salivary mucins. *J Oral Biosci.* (2008) 50:134–41. doi: 10.1016/S1349-0079(08)80027-8
61. Davis RH, Kramer D, Sackman J, Balin H. Saliva viscosity reflects the time of ovulation. *Experientia.* (1974) 30:911–2. doi: 10.1007/BF01938356
62. Dawes C, Pedersen AML, Villa A, Ekström J, Proctor GB, Vissink A, et al. The functions of human saliva: a review sponsored by the world workshop on oral medicine VI. *Arch Oral Biol.* (2015) 60:863–74. doi: 10.1016/j.archoralbio.2015.03.004
63. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, et al. The oral microbiome – an update for oral healthcare professionals. *Br Dent J.* (2016) 221:657–66. doi: 10.1038/sj.bdj.2016.865
64. Cattaneo C, Riso P, Laureati M, Gargari G, Pagliarini E. Exploring associations between interindividual differences in taste perception, oral microbiota composition, and reported food intake. *Nutrients.* (2019) 11:1167. doi: 10.3390/nu11051167
65. Feng Y, Licandro H, Martin C, Septier C, Zhao M, Neyraud E, et al. The associations between biochemical and microbiological variables and taste differ in whole saliva and in the film lining the tongue. *Bio Med Res Int.* (2018) 2018:2838052. doi: 10.1155/2018/2838052

Conflict of Interest: JL and JH were employed by Symrise AG, Holzminden, Germany. MW were employed by Symrise Distribution GmbH, Vienna, Austria.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.5.4. "Sweetness Perception is not Involved in the Regulation of Blood Glucose after Oral Application of Sucrose and Glucose Solutions in Healthy Male Subjects"

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Sweetness Perception is not Involved in the Regulation of Blood Glucose after Oral Application of Sucrose and Glucose Solutions in Healthy Male Subjects

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Scope: This study investigates the effect of the sweetness of a sucrose versus an isocaloric glucose solution in dietary concentrations on blood glucose regulation by adjusting the sweetness level using the sweet taste inhibitor lactisole.

Methods and Results: A total of 27 healthy males participated in this randomized, crossover study with four treatments: 10% glucose, 10% sucrose, 10% sucrose + 60 ppm lactisole, and 10% glucose + 60 ppm lactisole. Plasma glucose, insulin, glucagon-like peptide 1, and glucagon levels are measured at baseline and 15, 30, 60, 90, and 120 min after beverage consumption. Test subjects rated the sucrose solution to be sweeter than the isocaloric glucose solution, whereas no difference in sweetness is reported after addition of lactisole to the sucrose solution. Administration of the less sweet glucose solution versus sucrose led to higher blood glucose levels after 30 min, as reflected by a lower ΔAUC for sucrose (1072 ± 136) than for glucose (1567 ± 231). Application of lactisole leads to no differences in glucose, insulin, or glucagon responses induced by sucrose or glucose.

Conclusion: The results indicate that the structure of the carbohydrate has a stronger impact on the regulation of blood glucose levels than the perceived sweetness.

1. Introduction

Sweet taste is innately highly preferred by humans, but the global excessive consumption of sweet tasting carbohydrates largely contributes to the overall energy intake,[1] leading to an increased risk for obesity and comorbidities like type 2 diabetes.[2] One of the main sources for dietary sugars are sugar-sweetened beverages like fruit drinks, lemonades, and ice tea.[3] Beside the caloric load, the consumption of such sugar-sweetened beverages is associated with the exposure to a high level of sweetness. The perceived sweetness has been hypothesized to interact with signaling pathways of insulin secretion, also known as cephalic phase insulin release (CPIR), in the regulation of blood glucose levels.[4–7] The finding that chemosensory signaling pathways of sweet taste receptors are not only present in the oral cavity, but also in non-gustatory-tissues like the

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gastrointestinal tract and the pancreas, fueled the debate about the impact of an activation of hT1R2/hT1R3 on the regulation of blood glucose levels, which is also affecting the usage of non-caloric sweeteners.[8] However, results are conflicting, and the contribution of the perceived sweetness to its metabolic effect of a beverage remains unclear. For example, sweet taste receptors expressed in the gut directly affected glucose metabolism, independently of sweetness signaling in the mouth.[9] But also the activation of sweet taste receptors in the oral cavity influenced the blood glucose regulation by a higher secretion of insulin.[10] In contrast, the acute consumption of sweeteners without caloric load did not have an impact on the regulation of blood glucose,[11,12] arguing against the regulatory role of sweetness. A more recent study concluded that only the combination of the non-caloric sweetener sucralose and a carbohydrate, but neither sucralose nor the carbohydrate alone, impairs insulin sensitivity.[13] The regulatory process of blood glucose concentrations is characterized by a complex interaction of various hormones like insulin, glucagon-like peptide 1 (GLP-1), and glucagon and neuropeptides derived from brain, pancreas, liver, intestine, muscle, and adipocyte tissue.[14] A dysregulation of this complex interactions can lead to serious diseases like type 2 diabetes and associated comorbidities. Thus, a precise understanding of the blood glucose regulation affecting parameters is of high social and scientific relevance.

A sucrose solution is rated significantly sweeter than an iso-caloric glucose solution by trained panelists (own unpublished data, also described by Carocho et al.[15]) and a previous study by Crapo et al. showed that an iso-caloric administration of 100 g glucose or 100 g sucrose led to different peaks in blood glucose levels.[16] However, it is not yet known whether the different level of sweetness of glucose and sucrose contributes to the above-named differences in the blood glucose peaks, or whether these differences are only based on their different structures, namely a monosaccharide versus a disaccharide. The disaccharide sucrose consists of one molecule of glucose and fructose. Fructose has different metabolic effects than glucose, which can also contribute to differences in blood glucose metabolism. For example, fructose does not stimulate insulin secretion,[17] leading to reduced plasma glucose and insulin responses when fructose- opposed to glucose-sweetened beverages are consumed.[18] Another characteristic of fructose is that fructose metabolism occurs primarily in the liver.[19]

To determine the effect of sugars on blood glucose levels, most of the conducted studies used high amounts (up to 100 g of the tested sugars),[16,20–22] exceeding the quantities typically consumed at one time, leading to exalted effects regarding blood glucose levels, associated hormones, and neuropeptides. Thus, in the present study, test solutions were chosen to imitate a typical amount of sugar in sugar-sweetened soft drinks or juices. To test iso-caloric sucrose and glucose solutions with a similar sweetness level, the sweetness of the test solutions was modulated using lactisole. Lactisole, the sodium salt of 2-(4-methoxyphenol)propionic acid, is a selective competitive inhibitor of the T1R3 subunit of the human sweet taste receptor.[23] To the best of our knowledge, previous studies applied lactisole in high concentrations solely, completely eliminating the stimulatory effect of sugars and sweeteners.[20] However, no studies are currently available that applied lactisole for adjusting the sweetness of different sugars in a human intervention trial to obtain equally sweet tasting solutions using the same concentrations of sugars.

In summary, in the context of the prevention of nutrition-dependent diseases, it is of special interest to understand whether the blood glucose response is modulated by the sweetness perception of the test solution, or mainly based on the structure of the carbohydrate. Thus, in the present study, we aimed to investigate the impact of a sweetness modulation of a sucrose solution in comparison to a glucose solution on blood glucose metabolism of male healthy subjects. We hypothesized here that the structure (monosaccharide vs disaccharide) as well as the sweetness may have an impact on the regulation of blood glucose levels in healthy subjects.

2. Experimental Section

2.0.0.1. Participants: Thirty-nine male subjects were recruited for a medical screening by advertisements in web forums and billboards at Universities in Vienna. The study inclusion criteria were metabolic healthy males aged between 18 and 45 years with a body mass index between 18.5 and 30 kg m^{-2} and no taste disorders. Fasting blood glucose <120 mg dl^{-1} was mandatory for registration. The exclusion criteria were major chronic diseases, metabolic diseases such as type 2 diabetes or lipometabolic disorders, tobacco consumption, medical treatment, alcohol or drug abuse, as well as intolerances or allergies to test products.

Female test persons were excluded from the study, because of fluctuations concerning blood glucose levels during menstrual cycle, which can distort the results of this study.[24]

The present study procedures were approved by the ethical committee of the University of Vienna (approval no. 00432). All study participants provided written informed consent prior to the interventions.

2.0.0.2. Design: This study was a single blinded, cross-over human intervention study with four different interventions and all measurements were conducted using coded samples. Participants received four different interventions on four study days. The four visits were carried out at least five days apart. Participants were blinded to the treatment allocation. All participants were randomly assigned to the treatments using the online tool "randomizer.org", and the sequence of the treatments was balanced.

2.0.0.3. Test Solutions: According to amounts commonly found in soft drinks or juices, a concentration of 10% (w/v) sucrose in 300 mL water was chosen. Hence, a solution using the same concentration of glucose was selected. A sucrose solution is rated to be sweeter than an iso-caloric glucose solution.[15] The sweetness of the iso-caloric glucose and sucrose solution (10% w/v) was adjusted to a similarly rated sweetness by the addition of 60 ppm lactisole to the sucrose solution in preliminary tests ($n = 5$, data not shown). The applied test solutions were thus as follows: 1) 10% (w/v) glucose in 300 mL water, 2) 10% (w/v) sucrose in 300 mL water, 3) 10% (w/v) sucrose in 300 mL water with 60 ppm lactisole, and 4) 10% (w/v) glucose in 300 mL water with 60 ppm lactisole as an additional control for the effect of lactisole.

2.0.0.4. Dosage Information: Participants ingested 30 g of glucose or 30 g of sucrose with or without 60 ppm lactisole

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respectively. Glucose, sucrose, as well as lactisole were dissolved in tap water. All four test solutions were ingested by every participant once, on different days, at least five days apart. Participants were instructed to drink the test solutions within five minutes after the first sip. The dose of glucose and sucrose corresponds to those typically found in soft drinks or juices. Lactisole has been used in a previous study with a dosage of 500 ppm.[20]

2.0.0.5. Procedure: All participants were asked to attend five sessions consisting of one medical screening session and four consecutive test sessions. Metabolic disorders were excluded during the medical screening session. Fasting hematological parameters, plasma lipids, as well as glucose concentrations in plasma and urine samples 60 and 120 min after an oral glucose tolerance test (oGTT) were analyzed by "Ihr Labor 1220" (Medical diagnostics laboratory, Dr. Gabriele Greiner, Vienna, Austria). The compliant elevation of blood glucose levels during the oGTT was additionally monitored after 15, 30, 60, 90, and 120 min with a blood glucose meter in the capillary blood of the fingertip (Accu-Chek Performa, Roche, Switzerland). Also, blood pressure measurements were conducted in triplicates. Basic anthropometric measurements were recorded, namely body height with a precision of 0.01 m by means of a stadiometer (Seca, Germany) and body weight to the nearest of 0.1 kg using a body scale (Soehnle, Germany). Participants were asked to fill out 1) a SCOFF questionnaire, to identify and exclude eating disorders,[25] and 2) a screening questionnaire including questions such as food allergies or intolerances, chronic diseases, and basic health information.

In addition, the sweet threshold level of the test persons was determined according to DIN EN ISO 3972:2013–12 in Höhl and Busch–Stockfisch (2015).[26] The sweet intensity was rated for each test solution on an unstructured scale [0–10] after pre-tasting five sweet solutions with 0 to 100 g L^{-1} sucrose as "not at all" to "very intensive" sweetness. The test compounds were dissolved in tap water. All sensory tests were conducted in a sensory laboratory. The test solutions were additionally rated on every test day as described above, but without pre-testing of different sucrose solutions, to ensure no different sweet perception between screening day and study day and between sensory laboratory and study room.

Power analysis by means of the software GPower 3.1 resulted in an estimated number of 26 test subjects based on a study of Pepino et al.,[27] with an effect size of 0.55 (power of 0.85, $\alpha = 0.05$). A total of 39 subjects was recruited, out of which 29 volunteers passed the medical screening. One volunteer did not finish the study due to personal reasons, and one participant was excluded due to obvious violation against the study protocol. Accordingly, 27 participants completed all four treatments and were included in the study. The mean characteristics of the participants are given in **Table 1**.

An overview of the study protocol is shown in **Figure 1**. On each study day, baseline blood collection (t0) after 12 h overnight fast was carried out. Further blood samples were collected 15, 30, 60, 90, and 120 min after administration of the test solution. The participants were asked to rate the sweetness of the respective test solution on a 10 cm unstructured scale (0 cm = not at all and 10 cm = very intensive). After the last blood collection, a standard continental breakfast was served as described in previous studies.[28,29]

Table 1. Study subjects´ characteristics.

n	27
Gender	male
Age [years]	27.6 ± 0.88
Body Weight [kg]	77.8 ± 2.29
Height [m]	1.81 ± 0.01
BMI [kg m^{-2}]	23.7 ± 0.07

Data are depicted as mean ± SEM.

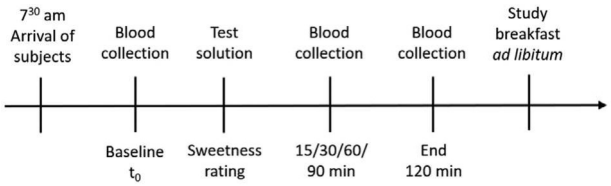

Figure 1. Flow chart showing the procedure of the study day. A total of 27 volunteers underwent the following four interventions as test solutions in a randomized order: 1) 10% sucrose in 300 mL water, 2) 10% sucrose in 300 mL water with 60 ppm lactisole, 3) 10% glucose in 300 mL water, and 4) 10% glucose in 300 mL water with 60 ppm lactisole.

2.0.0.6. Blood Sample Collection: Venous blood samples were collected in EDTA-coated monovettes (Sarstedt, Germany), centrifuged immediately at 1800 x g at 4 °C for 15 min and the plasma was stored at −80 °C until analysis for concentrations of GLP-1 and glucagon. In addition, blood was collected in fluoride-coated monovettes to determine plasma glucose and heparin-coated monovettes (both Sarstedt, Germany) were used for plasma insulin as described previously.[30]

2.0.0.7. Plasma Concentrations of total GLP-1, Glucagon, Glucose, and Insulin: Total GLP-1 (LOD: 2 pM, inter-assay CV 8 ± 4.8%, intra-assay CV 7.4 ± 1.1%) and glucagon (LOD: 2.5 pg mL^{-1}, inter-assay CV < 12%, intra-assay CV < 10%) plasma concentrations were determined by means of a sandwich ELISA (Merck Millipore, Darmstadt, Germany, and Thermo–Fisher Scientific, Waltham, USA, respectively). Plasma glucose concentrations were quantitated by a colorimetric assay with an LOD of 0.23 mg dl^{-1} (inter-assay CV 1.7%, intra-assay CV 4.6%) (Cayman Europe, Tallinn, Estonia). Insulin concentrations in the plasma were assessed using sandwich ELISA (LOD: 50 pg mL^{-1}, inter-assay CV 2.6%, intra-assay CV 5.99%) obtained from IASON (Graz, Austria).

2.0.0.8. Statistical Analyses: Statistical analyses were performed using GraphPad Prism 8. Normally distributed data sets, assessed by a Shapiro–Wilk test, are presented as means ± standard errors of the mean (SEM) unless stated otherwise. In case of no normal distribution, a non-parametric test was applied as indicated in the figure legends. Statistically significant differences were assumed at $P < 0.05$. Time dependent effects were determined by a mixed effect analysis with Tukey´s multiple comparison. To test for differences between two treatments, a two-tailed, paired *t*-test was conducted. Δ values were calculated by subtracting the baseline values (t0). Area under the curve (AUC) was calculated according to the trapezoidal rule. For glucose and insulin, the positive ΔAUC over time, and for GLP-1 and glucagon total ΔAUC over time was calculated. Correlation was assessed by

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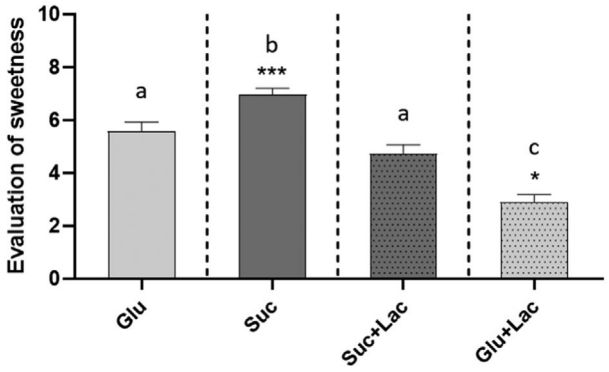

Figure 2. Sensory evaluation of test solutions. Participants rated the sweetness of the four different test solutions on an unstructured scale [0–10]. Values are shown as mean ± SEM. Statistically significant differences were tested by one-way ANOVA on Ranks ($P < 0.05$). *** indicates statistically significant difference compared to glucose solution ($P < 0.001$) and distinct letters are used to mark statistically significant differences in the sweetness level ($P < 0.05$).

Pearson Correlation between glucose and threshold, sweet perception, BMI as well as for insulin and insulin/glucose ratio, respectively.

3. Results

3.1. Sensory Evaluation–Rating of Sweetness

As described above, unpublished data from sensory studies showed that sweetness of a 10% glucose and 10% sucrose can be adjusted by adding 60 ppm lactisole to the sucrose solution. This resulted in a similar sweetness, also called equi-sweetness, of 10% glucose and 10% sucrose with lactisole. Sensory evaluation of the test solutions by the sensorially untrained test subjects in the present study was carried out by rating the sweetness on the screening day in a sensory laboratory, and on each study day directly after application of the test solution. As displayed in **Figure 2**, the sensorially untrained test subjects rated the glucose solution to be less sweet than the sucrose solution ($P < 0.001$). After addition of 60 ppm lactisole to the 10% sucrose solution, there was no difference in the rating between glucose and the sucrose with lactisole solution in sweet sensation ($P = 0.85$). As expected, the glucose solution with 60 ppm lactisole was rated less sweet then the glucose solution ($P = 0.01$). The above presented sensory evaluation results originate from the screening day in the sensory laboratory. There was no significant different rating for sweetness level of the test solutions at the screening day or the study day.

3.2. Plasma Concentrations of Glucose and Insulin

The plasma glucose level was lower after the administration of 10% sucrose solution compared to 10% glucose solution after 30 min ($P = 0.01$, **Figure 3**A), which is mirrored by a reduced ΔAUC ($P = 0.023$, Figure 3B). The application of the glucose solution elicited a 31.56% ± 6.04% higher plasma glucose level

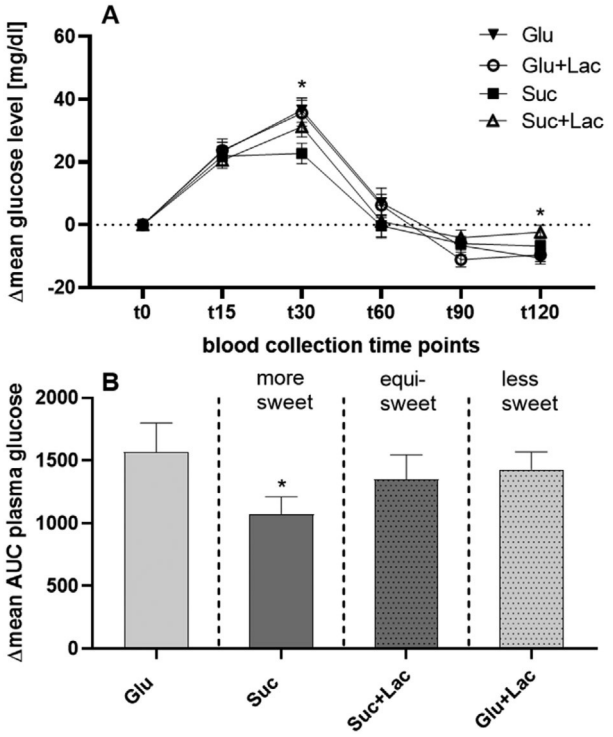

Figure 3. A) Mean change in plasma glucose level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc), and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. * Indicates significant difference after 30 min (Glu vs Suc) and significant difference after 120 min (Glu vs Suc+Lac). B) Effect of glucose versus sucrose administration on plasma glucose; effect of glucose versus sucrose with lactisole administration on plasma glucose, and effect of glucose versus glucose with lactisole on plasma glucose (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's *t*-test ($P < 0.05$). * indicates significant differences.

over time compared to sucrose (ΔAUC for plasma glucose 1567± 231 vs sucrose 1072 ± 136; $P = 0.02$). In contrast, the application of the equi-sweet test solutions (Figure 3B), 10% glucose versus 10% sucrose with 60 ppm lactisole, led to no significant difference in blood glucose levels over time (ΔAUC glucose 1567 ± 231 and ΔAUC sucrose with lactisole 1351 ± 193; $P = 0.29$). However, there was no effect of lactisole administration on 10% sucrose solution on blood glucose peaks ($P = 0.14$). In addition, there was no difference in blood glucose levels after application of the glucose solutions with or without the addition of lactisole (Figure 3B) (ΔAUC glucose 1567 ± 231 and ΔAUC glucose with lactisole 1427 ± 139; $P = 0.60$).

The regulation of insulin over time showed no differences in the time-dependent effect after administration of the test solutions (**Figure 4**A). However, the ΔAUC of insulin (Figure 4B) showed a trend ($P = 0.053$) towards a lower ΔAUC after administration of sucrose compared to the glucose solution (−21.4% ± 2.3%, ΔAUC glucose 2577 ± 278 and ΔAUC sucrose 2024 ± 219). Moreover, there was a significant difference ($P = 0.02$) in the

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Table 2. ΔPeak values of blood glucose, inlsun, GLP-1, and glucagon concentrations.

	Glu	Suc	Suc+Lac	Glu+Lac
ΔPeak glucose [mg dl^{-1}]	38.28 ± 3.68	30.98 ± 2.72$^{\#}$	37.61 ± 3.16	40.61 ± 3.49
ΔPeak insulin [μIU mL^{-1}]	61.98 ± 6.94	50.30 ± 4.70$^{\#}$	53.80 ± 6.61$^{\#}$	57.58 ± 6.02
ΔPeak GLP-1 [pM]	6.05 ± 0.93	5.49 ± 1.04	4.02 ± 0.80*	5.79 ± 0.96
ΔPeak glucagon [pg mL^{-1}]	0.56 ± 0.21	1.56 ± 0.77	1.69 ± 1.01	1.18 ± 0.65

Data are depicted as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. *indicates statistically significant difference compared to glucose Treatment. $^{\#}$ indicates a p for trend ($P < 0.1$).

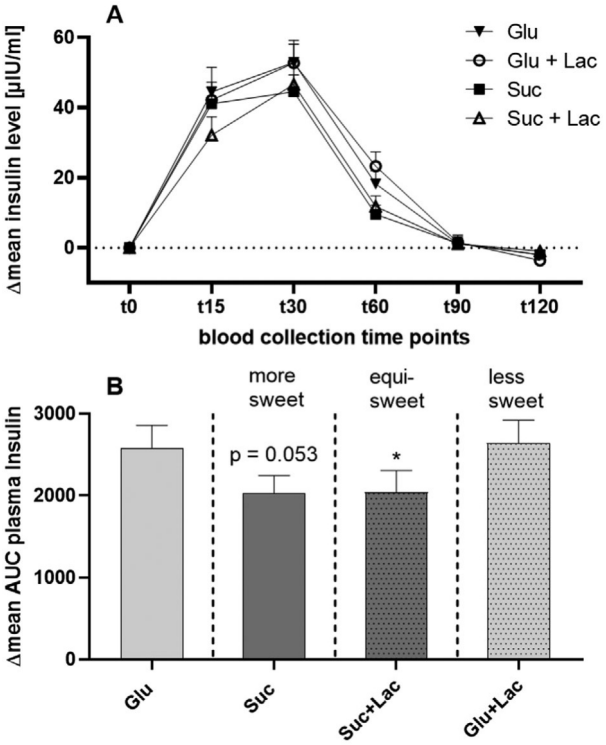

Figure 4. A) Mean change in plasma insulin level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference was determined by mixed effect analysis with multiple comparisons. B) Effect of glucose versus sucrose administration on plasma insulin, effect of glucose versus sucrose with lactisole administration on plasma insulin, and effect of glucose versus glucose with lactisole on plasma insulin (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's-test ($P < 0.05$). * indicates significant differences.

ΔAUC for insulin after application of sucrose with lactisole compared to application of the glucose solution with a 21.08% ± 0.3% lower ΔAUC after the application of sucrose with lactisole (ΔAUC glucose 2577 ± 278 and ΔAUC sucrose with lactisole 2034 ± 271). Further, there was no difference in insulin levels neither between glucose without or with lactisole ($P = 0.76$), nor between sucrose and sucrose with lactisole ($P = 0.97$).

The Δpeak values for glucose and insulin reflect the results of the ΔAUC calculation, showing the lowest peak after administration of sucrose (**Table 2**). Also, the Δinsulin/Δglucose ratio (**Table 3**) was calculated for all treatments over time, there was no difference between the treatments and time points.

3.3. Plasma Concentrations of GLP-1 and Glucagon

For blood glucose regulation parameters, GLP-1 and glucagon concentrations were assessed at fasting and after administration over time for 120 min (t15, t30, t60, t90, t120). The administration of 10% glucose led to an increase in GLP-1 level compared to the equi-sweet solution 10% sucrose with 60 ppm lactisole at timepoint 30 min ($P = 0.01$, **Figure 5**A). This is also reflected in the ΔAUC values: the application of sucrose in combination with lactisole elicited a 102.66% decrease in plasma GLP-1 compared to the glucose solution ($P = 0.02$; ΔAUC glucose −219 ± 77 and ΔAUC sucrose with lactisole −446 ± 104). The application of the more sweet 10% sucrose solution compared to 10% glucose, as well as the less sweet solution 10% glucose with 60 ppm lactisole compared to glucose led to no difference ($P = 0.7$ and $P = 0.5$, respectively; Figure 5B). Lactisole had no influence on plasma GLP-1 concentrations after glucose administration ($P = 0.5$; glucose compared with glucose + lactisole). In contrast, GLP-1 levels were lower after the administration of sucrose in combination with lactisole compared to the administration of sucrose ($P = 0.04$).

The application of the different solutions led to no difference in the glucagon plasma levels neither at the time-response- curve, nor at the ΔAUC as depicted in **Figure 6**A,B ($P > 0.05$; Δ AUC glucose −51.42 ± 19.85, ΔAUC sucrose 69.71 ± 74.37, ΔAUC sucrose with lactisole 88.73 ± 103.48, ΔAUC glucose with lactisole 43.38 ± 71.73). Concomitant application of lactisole with either sucrose or glucose did not lead to differences in plasma glucagon concentrations. Notably, eight out of 27 participants were under the limit of detection, resulting in a number of 19 subjects for glucagon.

The Δpeak values for GLP-1 and glucagon are in accordance with the calculated ΔAUCs (Table 2). Further, the calculated Δglucagon/Δinsulin ratio showed no difference between treatments and time points (Table 3).

3.4. Correlation Analysis

To answer the question, if there is an association between the glucose regulation and their hormones with the sweet thresh-

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Table 3. ΔRatios for insulin/glucose and glucagon/insulin over time.

	Treatment	t15	t30	t60	t90	t120	*Fixed-effect P* value
ΔInsulin/ΔGlucose	*Glu*	2.27 ± 0.29	2.48 ± 0.67	0.56 ± 0.69	−0.21 ± 0.40	0.50 ± 0.25	0.61
	Suc	4.69 ± 2.55	1.40 ± 0.60	−28.28 ± 29.36	2.53 ± 2.09	0.24 ± 0.32	
	Suc+Lac	2.24 ± 0.62	1.45 ± 0.57	1.93 ± 1.11	−0.03 ± 0.41	−0.81 ± 0.91	
	Glu+Lac	0.29 ± 1.86	1.37 ± 0.50	−5.94 ± 6.87	0.41 ± 0.56	0.37 ± 0.22	
ΔGlucagon/ΔInsulin	*Glu*	0.005 ± 0.008	−0.007 ± 0.003	0.280 ± 0.298	−0.412 ± 0.238	0.051 ± 0.233	0.56
	Suc	0.017 ± 0.011	0.008 ± 0.013	0.235 ± 0.278	−0.592 ± 0.587	−0.010 ± 0.149	
	Suc+Lac	−0.191 ± 0.214	0.053 ± 0.058	−0.230 ± 0.166	−0.275 ± 0.157	−0.118 ± 0.142	
	Glu+Lac	0.016 ± 0.016	0.006 ± 0.011	0.007 ± 0.020	0.150 ± 0.094	0.002 ± 0.174	

Data are depicted as mean ± SEM. Statistically significant differences ($P < 0.05$) were excluded by mixed effect analysis with multiple comparisons.

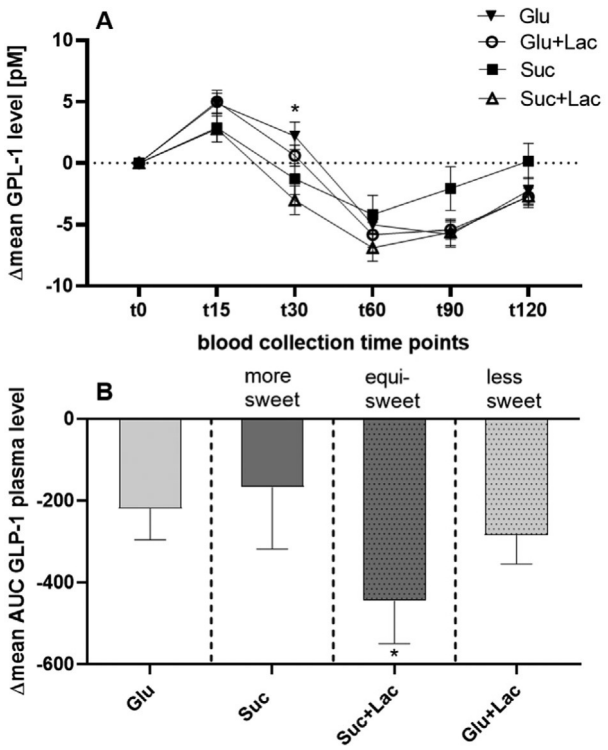

Figure 5. A) Mean change in plasma GLP-1 level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 27$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was determined by mixed effect analysis with multiple comparisons. * Indicates significant difference after 30 min (Glu vs Suc+Lac). B) Effect of glucose versus sucrose administration on plasma GLP-1, effect of glucose versus sucrose with lactisole administration on plasma GLP-1 and effect of glucose versus glucose with lactisole on plasma GLP-1 (expressed as AUC [mg dl^{-1} × min] respectively) in 27 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose-treatment were tested by Student's *t*-test ($P < 0.05$). * indicates significant differences.

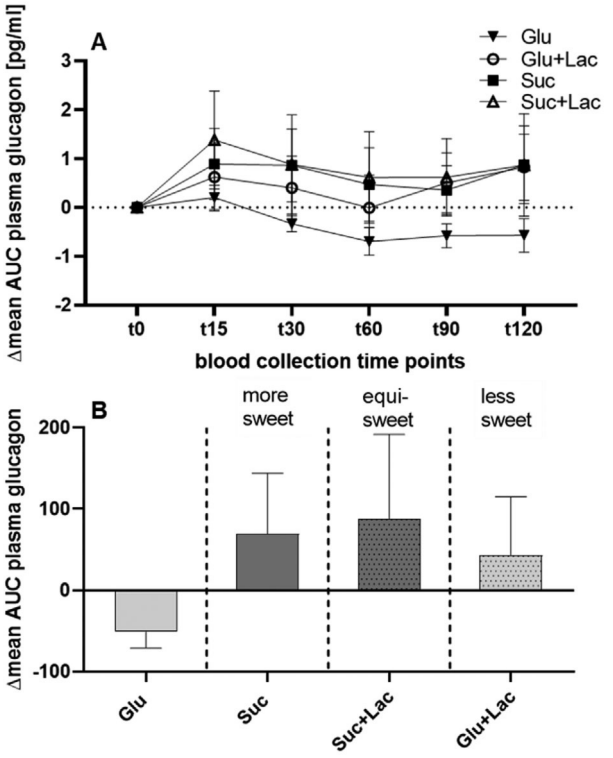

Figure 6. A) Mean change in plasma glucagon level normalized to the level at fasting and 15, 30, 60, 90, and 120 min after administration of glucose (Glu), glucose with lactisole (Lac), sucrose (Suc) and sucrose with lactisole ($n = 19$ respectively). Values are shown as mean ± SEM. Statistical difference ($P < 0.05$) was excluded by mixed effect analysis with multiple comparisons. B) Effect of glucose versus sucrose administration on plasma glucagon, effect of glucose versus sucrose with lactisole administration on plasma glucagon and effect of glucose versus glucose with lactisole on plasma glucagon (expressed as AUC [mg dl^{-1} × min] respectively) in 19 healthy volunteers. Values are shown as mean ± SEM. Statistically significant differences against glucose treatment were excluded by Student's *t*-test ($P > 0.05$).

old and sweet perception, a correlation analysis using Pearson's product moment correlation was carried out. However, neither a correlation between the regulation of glucose, GLP-1, and insulin with the individual sweetness rating, nor an association the threshold for sweet taste was found (data not shown). Furthermore, the results of correlation analysis of sweet perception and sweet threshold with BMI showed no association (data not shown).

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4. Discussion

Sweetness perception has been postulated to interact with hormones to regulate blood glucose levels via targeting sweet taste receptor signaling in the oral cavity and in non-gustatory tissues.[8] However, the impact of the sweetness level of sugar-sweetened beverages on blood glucose levels in typically consumed amounts is not well understood.

In the present study, we investigated the role of the different sweetness of isocaloric glucose and sucrose solutions via adjusting the sweetness level using the sweet taste inhibitor lactisole in amounts typically used in soft drinks. We hypothesized here that if the sweetness drives reinforcement on blood glucose levels, then equi-sweet solutions of the two different carbohydrates should induce a similar response on blood glucose levels.

Being the essential base for investigating this hypothesis, we confirmed that the sensorially untrained test persons were able to distinguish the different levels of sweetness of sucrose and glucose with or without the addition of lactisole. This was of importance, since other studies showed that untrained test persons not always rank the sweetness of sucrose as sweeter than an equicaloric glucose solution.[31] In addition, the participants of the present study rated the solutions containing glucose and sucrose in combination with lactisole with the same sweetness level. This result given, the participants underwent the four consecutive interventions in a cross-over design using 10% sucrose and 10% glucose without or with the addition of 60 ppm lactisole, analyzing plasma concentrations of glucose, insulin, glucagon, and GLP-1.

Results of the plasma glucose levels after application of the different test solutions showed a time-dependent difference after application of the glucose and the sucrose solution, due to the significant higher blood glucose plasma level at time point 30 min after glucose administration compared to the sucrose administration. This result confirmed the initial hypothesis of a differential blood glucose level after glucose and sucrose consumption and is in accordance with the results by Crapo et al., who showed a time-dependent difference in the increase of the plasma glucose levels when comparing a glucose or sucrose load.[16] This indicates a time-dependent component in the absorption of different sugars which may be attributed to the fact that glucose and sucrose vary in their structure, monosaccharide and disaccharide, leading to a delay in the absorption of glucose from the disaccharide sucrose. It has to be noticed that fructose, which is a part of the disaccharide sucrose, is differently metabolized as glucose.[17–19] After ingestion, glucose is taken up by enterocytes mainly via the sodium-dependent glucose transporter 1 (SGLT-1), whereas fructose is predominantly absorbed passively from the intestinal lumen via GLUT-5.[32] Moreover, in contrast to glucose, fructose is not an insulin secretagogue and is mainly metabolized by the liver.[33] Fructose sweetened beverages lead to a reduced plasma glucose and insulin response compared to glucose sweetened beverages,[18] which is likely to contribute to the lower glucose peak after 30 min. This result was also reflected by the incremental ΔAUC, at which glucose application resulted in higher plasma glucose levels over total time compared to sucrose application. In contrast, after application of the sucrose solution supplemented with lactisole as equi-sweet solution to the glucose solution this effect was abolished, there were no differences in the glucose plasma levels over time. However, there was neither a direct effect of lactisole on sucrose-mediated plasma glucose levels, nor did the supplementation of the glucose solution with the same amount of lactisole, 60 ppm, change plasma glucose levels over time. This result suggests that the sweetness of the glucose and sucrose solutions had no impact on blood glucose regulation in the present study. Although it should be finally clarified, why the difference between glucose and sucrose was abolished when applied as equi-sweet solutions, the missing direct effect of lactisole on either sucrose- or glucose–induced blood glucose peaks strongly suggest that the structure plays a predominant role on blood glucose regulation. Notably, these are the results of healthy participants, in patients with type 2 diabetes, the regulation of the sweet taste receptors in response to glucose exposure is disordered,[34] which could modify short-term responses as well.

In the next step, we analyzed if and how hormones involved in the regulation of blood glucose levels are affected by the test solutions. First, the plasma insulin concentration was analyzed as one of the most important hormones regarding the regulation of the glucose homeostasis with blood glucose-lowering effects.[35] The time curve of plasma insulin showed no significant time-dependent effect following the different treatments. Comparison of the ΔAUCs after application of sucrose versus glucose indicated a trend toward a lower ΔAUC after oral ingestion of sucrose, which is in accordance with the data obtained for the blood glucose levels. Moreover, the application of the sucrose solution supplemented with lactisole resulted in a significant lower ΔAUC compared to the equi-sweet glucose solution, indicating that the structure of the carbohydrate has more impact than the sweetness perception. This is further supported by the comparison of the ΔAUC for insulin after ingestion of glucose with or without lactisole at which no difference was detected, although the glucose with lactisole was the least sweet solution tested in the present study. If the sweetness would have a regulatory impact on plasma insulin levels, no difference in the equi-sweet solutions, but a difference comparing glucose and glucose with lactisole would have been expected. Additionally, a direct effect of lactisole on sucrose-mediated insulin secretion was also not detected. To summarize the data obtained for insulin, the regulation of insulin levels was not associated with the sweetness of the test solution. However, Karimian Azari et al. reported that the application of 500 ppm lactisole prior to a 12.5% glucose solution increased plasma responses to insulin in ten healthy subjects.[20] The higher amount of lactisole used in the study by Karimain Azari et al., blocking the sweet sensation completely, in addition to the time-dependent effect caused by the prior application of lactisole may explain the difference to the results of our study. Moreover, it has to be noticed, that a study by Renwick et al. did not find an effect of the sweetness of a solution on insulin regulation.[8] In the 1990s, a number of studies hypothesized that the stimulation of the sweet taste receptor on the tongue can act as signaling for insulin release, known as CPIR.[36] There was no evidence of an increase in insulin after tasting various low-energy sweeteners, which led to the conclusion that the sweetness level has no effect on insulin release.[36] This issue has been raised again in a more recent study,[37] in which blood insulin levels were measured in volunteers who tasted different solutions for 45 s. The authors reported that both, sucrose and saccharin led to an increase in insulin, but the increases in insulin for starch and

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water were larger than those for the sweeteners. Further, a recent study showed that the taste of sugars which contain glucose, but not high-intensive sweeteners, elicited CPIR in mice.[38] The authors hypothesized that carbohydrates were hydrolyzed in the mouth, and that the released glucose triggered CPIR. Our results on insulin are in accordance with the described studies and provide evidence that the sweetness perception does not have a major effect on the insulin release, it is rather the carbohydrate itself. Also, a recently published study concluded that short-term consumption of sucralose decreased insulin sensitivity only when applied in the combination with a carbohydrate.[13] Also, the calculated peak values are in agreement with the ΔAUCs. The Δinsulin/Δglucose ratio shows no difference between the treatments, which does not support that the sweetness induces an exaggerated insulin response. Also, the Δglucagon/Δinsulin ratio shows no difference, indicating that the glucagon response fits to the insulin response and the sweetness does not influence the need for endogenous hepatic glucose production.

Blood glucose is not only regulated by insulin, but by a complex interaction of several hormones, amongst others GLP-1, which is known to enhance pancreatic insulin secretion, and to suppress pancreatic glucagon secretion.[39] In contrast to the results obtained for plasma glucose and insulin levels, lactisole administration reduced GLP-1 plasma levels applied in combination with sucrose, but not glucose. This result does not support a general impact of the sweetness, but points to the involvement of the different structure between sucrose and glucose and requires further investigation. We hypothesize that a different interaction with the sweet taste receptor may have an impact on GLP-1 secretion: lactisole targets the T1R3 subunit,[40] and while glucose and sucrose both target the venus flytrap domains of T1R2 and T1R3, a higher binding affinity of glucose for T1R2, and of sucrose for T1R3 is described.[41] This may explain that sucrose-mediated effects are more sensitive to lactisole-effects, but this requires further mechanistic studies. Due to the selected concentrations as typically found in soft drinks, 10% sucrose or glucose in 300 mL water corresponding to 30 g of sugar, lower GLP-1 peaks were expected compared to studies that applied a regular oGTT with 75 g of glucose.[42,43] The GLP-1 curve shows the peak value at time point 15 min which is in agreement with the insulin response with a peak value at time point 30 min, as insulin release is described to be controlled by GLP-1. In more detail, GLP-1 is secreted directly in response to glucose present in the chymus and subsequently promotes insulin secretion via GLP-1 receptors expressed in pancreatic beta cells.[44] Overall, the here presented results from plasma GLP-1 concentrations are in accordance with the insulin data and suggest as well that the sweetness plays a minor role on the regulation of GLP-1 release after sugar-sweetened beverages consumption. If the sweetness would have an impact on GLP-1 release, the equisweet solutions are expected to lead to no difference in plasma GLP-1 concentrations, whereas the different sweet tasting solutions, sucrose versus glucose, as well as glucose with lactisole versus glucose alone, would be expected to lead to differences in the time-dependent regulation of plasma GLP-1. The artificial sweetener sucralose has been reported to induce release of GLP-1 and GIP from the murine endocrine cell line GLUTag by activation of the sweet taste receptor,[45] which suggests that the sweetness plays a major role for GLP-1 release. However, although mechanistically interesting, the tested concentration of 50 mM sucralose is very high and raises the question of the physiological relevance. Moreover, a study from Fujita et al. does not support the notion that sweeteners acutely induce the release of meaningful quantities of incretin hormones in rodents.[46] Further, Wu et al. conclude that the prior exposure to sweetness in form of the artificial sweeteners sucralose and acesulfame K did not influence GLP-1 concentration as well as blood glucose and plasma insulin after ingestion of 75 g glucose.[47] These results are in agreement with a clinical study that reported no effect of oral stevioside consumption on release of GIP and GLP-1 in subjects with type 2 diabetes,[48] supporting the assumption that the sweetness would have rather only little or even no impact on GLP-1 release.

The results of glucagon concentrations revealed no differences in the time-dependent regulation after application of the test solutions. The amount of carbohydrates administered in the present study was lower than the concentrations commonly used for an oGTT, which may have influenced the glucagon regulation. Since no differences in the regulation were found, no statement regarding a different impact of mono- or disaccharide or the sweetness can be made. However, since there was no effect of lactisole on sucrose- or glucose-mediated glucagon responses, a major impact of the sweetness can be excluded. We hypothesized that an influence of the individual sweetness perception on blood glucose parameters, especially insulin and GLP-1, would be mirrored in a correlation to the subject's sweet taste threshold and the sweet perception. More precisely, a higher sweet threshold and a low insulin release should then correlate with each other as well as a high sweet perception with a low insulin release. This should be also reflected in correlation analysis with GLP-1 release, at which also a high sweet threshold and a low GLP-1 release as well as a high sweet perception and a low GLP-1 release would be expected to correlate, if the sweetness is involved. However, the Pearson's product moment correlation analysis showed no effect between sensory parameters and blood glucose parameters insulin and GLP-1. This supports our results from insulin and GLP-1, that the sweetness has no or only a minor impact on blood glucose hormones.

This study has potential limitations. First, no female subjects were included in the study due to interaction of blood glucose regulation with female sex hormones. Second, the blood samples to analyze glucose and regulating hormones were drawn at six different time points, a higher resolution could have refined the data, especially at the earlier time points. Moreover, future studies should consider the incretin hormone GIP beside the here analyzed GLP-1 to analyze the lactisole-effect on sucrose and glucose. Also, long-term intervention studies are needed to investigate if a prolonged activation of sweet taste receptors would enhance glucose absorption via upregulation of intestinal glucose transporters. In addition, further studies are needed to clarify if lactisole mixed with glucose and sucrose maintained the same level of suppression on the sweet taste receptor signal throughout the observation window and to test the effects of higher concentrations of lactisole for complete inhibition of the sweet taste receptor.[23] Having that said, a major strength of the current study includes its robust and clear cross-over study design using the sweet taste inhibitor lactisole for adjusting the sweetness of a glucose and sucrose solution in typically consumed concentrations. Moreover,

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the achieved power with the 27 test subjects is 82% (1-β) based on the reached effect size of 0.5 for the differences in the plasma glucose levels after glucose and sucrose consumption and an error probability of 0.05 (G-Power 3.1).

To summarize, the present study investigated for the first time the influence of the sweetness compared to the structure of glucose and sucrose on blood glucose regulation in dietary-relevant concentrations. The results obtained for insulin, GLP-1, and glucagon levels argue against a major role for the sweetness of the test solutions in the regulation of hormone levels. In addition, there was no association between the sweetness perception and the plasma glucose or the hormone levels. In conclusion, sweetness perception plays no major role in the differences in the time-dependent regulation of blood glucose following oral ingestion of a sucrose versus a glucose solution. Future studies with modulated sweetness are needed to analyze the differences in the impact of lactisole on glucose and sucrose in GLP-1 regulation. Moreover, the results provide a solid basis for future studies to unravel the role of sweetness perception in blood glucose levels after carbohydrate consumption in females, and to study the long-term impact of sweet carbohydrates.

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Conflict of Interest

The authors G. Krammer and J. P. Ley are employees at Symrise AG, Holzminden, Germany.

Author Contributions

The authors ′ responsibilities were as follows – B.L., V.S., J.P.L., and G.E.K. designed the research. V.G. and K.S. conducted this study. K.S. and C.G. did blood sample preparations. C.G. and J.T. analyzed the blood samples. C.M.K. and C.G. performed the sensory study. V.G. performed data and statistical analysis. J.K. provided access to a fully equipped sensory laboratory. V.G. and B.L. wrote the manuscript. B.L. had primary responsibility for the final content. All authors edited the manuscript, provided comments, and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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blood glucose, glucose, lactisole, sucrose, sweetness

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[1] M. Y. Pepino, J. A. Mennella, *Chem. Senses* **2005**, *30*, i319.
[2] L. Te Morenga, S. Mallard, J. Mann, *BMJ* **2012**, *346*, e7492.
[3] V. Azaïs-Braesco, D. Sluik, M. Maillot, F. Kok, L. A. Moreno, *Nutr. J.* **2017**, *16*, 6.
[4] T. L. Powley, H. R. Berthoud, *Am. J. Clin. Nutr.* **1985**, *42*, 991.
[5] D. G. Bruce, L. H. Storlien, S. M. Furler, D. J. Chisholm, *Metabolism* **1987**, *36*, 721.
[6] K. L. Teff, J. Devine, K. Engelman, *Physiol. Behav.* **1995**, *57*, 1089.
[7] L. Abdallah, M. Chabert, J. Louis-Sylvestre, *Am. J. Clin. Nutr.* **1997**, *65*, 737.
[8] A. G. Renwick, S. V. Molinary, *Br. J. Nutr.* **2010**, *104*, 1415.
[9] A. C. Gerspach, R. E. Steinert, L. Schoenenberger, A. Graber-Maier, C. Beglinger, *Am. J. Physiol. Endocrinol. Metab.* **2011**, *301*, E317.
[10] A. Secchi, R. Caldara, A. Caumo, L. D. Monti, D. Bonfatti, V. Di Carlo, G. Pozza, *Metabolism* **1995**, *44*, 1153.
[11] J. Ma, M. Bellon, J. M. Wishart, R. Young, L. A. Blackshaw, K. L. Jones, M. Horowitz, C. K. Rayner, *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2009**, *296*, G735.
[12] A. W. Brown, M. M. Bohan Brown, K. L. Onken, D. C. Beitz, *Nutr. Res.* **2011**, *31*, 882.
[13] J. R. Dalenberg, B. P. Patel, R. Denis, M. G. Veldhuizen, Y. Nakamura, P. C. Vinke, S. Luquet, D. M. Small, *Cell Metab.* **2020**, *31*, 493.
[14] P. V. Röder, B. Wu, Y. Liu, W. Han, *Exp. Mol. Med.* **2016**, *48*, e219.
[15] M. Carocho, P. Morales, I. Ferreira, *Food Chem. Toxicol.* **2017**, *107*, 302.
[16] P. A. Crapo, J. A. Scarlett, O. G. Kolterman, L. R. Sanders, F. D. Hofeldt, J. M. Olefsky, *Diabetes Care* **1982**, *5*, 512.
[17] D. L. Curry, *Pancreas* **1989**, *4*, 2.
[18] K. L. Teff, S. S. Elliott, M. Tschop, T. J. Kieffer, D. Rader, M. Heiman, R. R. Townsend, N. L. Keim, D. D'Alessio, P. J. Havel, *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2963.
[19] P. A. Mayes, *Am. J. Clin. Nutr.* **1993**, *58*, 754S.
[20] E. Karimian Azari, K. R. Smith, F. Yi, T. F. Osborne, R. Bizzotto, A. Mari, R. E. Pratley, G. A. Kyriazis, *Am. J. Clin. Nutr.* **2017**, *105*, 1001.
[21] S. L. Tey, N. B. Salleh, J. Henry, C. G. Forde, *Int. J. Obes.* **2017**, *41*, 450.
[22] G. H. Anderson, N. L. Catherine, D. M. Woodend, T. M. Wolever, *Am. J. Clin. Nutr.* **2002**, *76*, 1023.
[23] S. S. Schiffman, B. J. Booth, E. A. Sattely-Miller, B. G. Graham, K. M. Gibes, *Chem. Senses* **1999**, *24*, 439.
[24] S. Zarei, L. Mosalanejad, M. A. Ghobadifar, *Clin. Exp. Reprod. Med.* **2013**, *40*, 76.
[25] J. F. Morgan, F. Reid, J. H. Lacey, *BMJ* **1999**, *319*, 1467.
[26] K. Höhl, M. Busch-Stockfisch, *Ernahrungs Umschau* **2015**, *62*, 208.
[27] M. Y. Pepino, C. D. Tiemann, B. W. Patterson, B. M. Wice, S. Klein, *Diabetes Care* **2013**, *36*, 2530.
[28] C. M. Hochkogler, B. Lieder, P. Rust, D. Berry, S. M. Meier, M. Pignitter, A. Riva, A. Leitinger, A. Bruk, S. Wagner, J. Hans, S. Widder, J. P. Ley, G. E. Krammer, V. Somoza, *Mol. Nutr. Food Res.* **2016**, *61*, 1600731.
[29] V. Stoeger, B. Lieder, J. Riedel, K. Schweiger, J. Hoi, V. Ruzsanyi, M. Klieber, P. Rust, J. Hans, J. P. Ley, G. E. Krammer, V. Somoza, *Mol. Nutr. Food Res.* **2019**, *63*, 1900133.
[30] C. M. Hochkogler, B. Rohm, K. Hojdar, M. Pignitter, S. Widder, J. P. Ley, G. E. Krammer, V. Somoza, *Mol. Nutr. Food Res.* **2014**, *58*, 1282.
[31] N. S. Stamataki, C. Scott, R. Elliott, S. McKie, D. Bosscher, J. T. McLaughlin, *J. Nutr.* **2020**, *150*, 1126.
[32] V. Gorboulev, A. Schurmann, V. Vallon, H. Kipp, A. Jaschke, D. Klessen, A. Friedrich, S. Scherneck, T. Rieg, R. Cunard, M. Veyhl-Wichmann, A. Srinivasan, D. Balen, D. Breljak, R. Rexhepaj, H. E. Parker, F. M. Gribble, F. Reimann, F. Lang, S. Wiese, I. Sabolic, M. Sendtner, H. Koepsell, *Diabetes* **2012**, *61*, 187.

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[33] C. Caliceti, D. Calabria, A. Roda, A. F. G. Cicero, *Nutrients* **2017**, *9*, 395.
[34] R. L. Young, B. Chia, N. J. Isaacs, J. Ma, J. Khoo, T. Wu, M. Horowitz, C. K. Rayner, *Diabetes* **2013**, *62*, 3532.
[35] J. Gromada, I. Franklin, C. B. Wollheim, *Endocr. Rev.* **2007**, *28*, 84.
[36] A. G. Renwick, *Physiol. Behav.* **1994**, *55*, 139.
[37] T. Just, H. W. Pau, U. Engel, T. Hummel, *Appetite* **2008**, *51*, 622.
[38] J. I. Glendinning, Y. G. Frim, A. Hochman, G. S. Lubitz, A. J. Basile, A. Sclafani, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2017**, *312*, R597.
[39] P. Nadkarni, O. G. Chepurny, G. G. Holz, *Prog. Mol. Biol. Transl. Sci.* **2014**, *121*, 23.
[40] P. Jiang, M. Cui, B. Zhao, Z. Liu, L. A. Snyder, L. M. Benard, R. Osman, R. F. Margolskee, M. Max, *J. Biol. Chem.* **2005**, *280*, 15238.
[41] Y. Nie, S. Vigues, J. R. Hobbs, G. L. Conn, S. D. Munger, *Curr. Biol.* **2005**, *15*, 1948.
[42] K. Faerch, S. S. Torekov, D. Vistisen, N. B. Johansen, D. R. Witte, A. Jonsson, O. Pedersen, T. Hansen, T. Lauritzen, A. Sandbaek, J. J. Holst, M. E. Jorgensen, *Diabetes* **2015**, *64*, 2513.
[43] A. Lund, T. Vilsboll, J. I. Bagger, J. J. Holst, F. K. Knop, *Am. J. Physiol. Endocrinol. Metab.* **2011**, *300*, E1038.
[44] B. Jones, S. R. Bloom, T. Buenaventura, A. Tomas, G. A. Rutter, *Peptides* **2018**, *100*, 75.
[45] R. F. Margolskee, J. Dyer, Z. Kokrashvili, K. S. Salmon, E. Ilegems, K. Daly, E. L. Maillet, Y. Ninomiya, B. Mosinger, S. P. Shirazi-Beechey, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15075.
[46] Y. Fujita, R. D. Wideman, M. Speck, A. Asadi, D. S. King, T. D. Webber, M. Haneda, T. J. Kieffer, *Am. J. Physiol. Endocrinol. Metab.* **2009**, *296*, E473.
[47] T. Wu, M. J. Bound, S. D. Standfield, M. Bellon, R. L. Young, K. L. Jones, M. Horowitz, C. K. Rayner, *Diabetes Care* **2013**, *36*, e202.
[48] S. Gregersen, P. B. Jeppesen, J. J. Holst, K. Hermansen, *Metabolism* **2004**, *53*, 73.

IV. Conclusion and Perspectives

The preference for sweet taste is innate for all humans as sweet carbohydrates provide an adequate resource of calories (Ganchrow, Steiner, and Daher 1983; Nelson et al. 2001; Ventura and Worobey 2013). The sweet taste receptor TAS1R2/R3, mainly responsible for the perception of the sweet taste, was discovered in the early 2000´s (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Li et al. 2002). The involvement of further signalling pathways especially for sweet carbohydrates is currently under discussion in the scientific community (Damak et al. 2003; Yee et al. 2011; Sukumaran et al. 2016; Yasumatsu et al. 2020). Sucrose is the prototypical sweet tasting compound, but an immense variety of other sweet tasting compounds is known. The detrimental metabolic effects, either from excessive sugar consumption or as well from sweeteners is a widely debated area and scientific results are inconsistent here. Before coming to digestion and metabolic effects of sweet tasting compounds, they are perceived with a multitude of oral receptors, including the sweet taste receptor TAS1R2/R3. Just as metabolic effects can vary, it is known for sweet carbohydrates and alternative sweeteners that their overall sensory impression of sweet taste shows a huge variety. However, the differences in the sweet taste, temporal effects, side-tastes, mouthfeel, and also metabolic effects of the huge variety of sweet tasting compounds are not fully understood and detailed insights and comparisons are missing so far.

The here presented research, which was implemented at the Christian Doppler Laboratory for Taste Research (Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Austria), focused on the differences of sweet taste perception and metabolic effects of sweet tasting compounds due to the presence of oral and extraoral sweet taste receptors. In depth contemplation of sensorial differences, including temporal and mouthfeel attributes form the main part of the here presented results. In summary, the present cumulative thesis aimed to

(1) compare for the first time more than 30 sweet taste affecting compounds by their complex sensory profile and physicochemical characteristics and additional to get more detailed insights about sweet temporal profile, the corresponding binding-sites to sweet taste receptor and mouthfeel attributes of selected diverse sweet compounds, and

(2) get first insights into the impact of oral sweet taste perception of two caloric compounds, but different in sweet potency, to blood glucose metabolism and regulation.

The main part of this thesis focused on the oral perception of diverse sweet tasting compounds. The variety of sweet taste affecting compounds and their structures is diverse, and so far, no

direct comparison of this broad variety of sweet tasting compounds exists in equi-sweet concentrations with respect to temporal, side-taste and structural differences, besides the pure sweet taste perception. For this reason, the first publication presented here (3.1 “Structure-dependent effects of sweet and sweet taste affecting compounds on their sensorial properties.”) focused on the comparison of physicochemical and structural properties of sweet tasting compounds and the association to differences in sensory attributes including sweetness, its onset and lingering and the side-tastes bitterness, metallic and astringency. It was demonstrated that the here tested 35 sweet compounds could be split into three clusters due to their sensory taste and aftertaste attributes. Additionally, for the first time a comparison of the sensory sweet temporal and side-taste profile was evaluated with one consistent method at equi-sweet concentrations for the selected 35 compounds, connecting the diverse sensory attributes to the physicochemical characteristics of the compounds. Overall, it was shown by evaluating the “Tanimoto”-similarity index in comparison to sucrose based on the molecular fingerprints of each test compound according to Morgan, that the less similar the structural fingerprint of a compound was to sucrose, the more negative side-tastes of bitterness and astringency as well as a tendency for prolonged onset and lingering were identified. Following on from previous findings that identified individual properties such as solubility as drivers of sweetness, in the here presented publication, the influence of a variety of descriptors was investigated. It was demonstrated by means of a factor analysis followed by a multivariate regression analysis, that the number of double bonds, ketones, aromatic rings and MlogP had the strongest impact on the sensory attributes. The grouped structural characteristics showed therefore different importance for the sweet taste attributes and as well interactions between the structural groups do impact specific sweet taste attributes. In contrast to previous studies, the here presented research focused not only on sweetness per se, but also on its temporal and side-taste attributes. In more detail, an increased relative sweetness and onset of a compound was mainly driven by the independent factor condensing high amounts of double bonds, ketones, aromatic rings and MlogP, but as well by interactions of physicochemical descriptors. Contrary, the sweet and lingering factor was reinforced by the independent factor that combines the following structural features, namely heavy atom count, molecular weight [g/mol], complexity, C-atoms, acceptors, bonded glucose, area polar surface [A2], defined atom stereocenter count, donors, glycone length, rotatable bonds and OH-groups. In general, the sensory attribute groups relative sweetness and onset, bitterness and astringency were not only driven by exclusive impact of some structural characteristics but also by multiple interactions of these characteristics. Thus, this study showed that the explanation of differences in sweet taste by physicochemical differences in structure of compounds is highly complex. It must be noticed that the selection of compounds

was limited to availability in food grade and safety for human consumption. Still, further studies with more sweet and also non-sweet compounds would be needed to get more detailed prediction parameters.

Besides structure-based approaches, there is the hypothesis that the binding-sites of the receptor can have an important contribution to perception of sweetness, as the receptor exhibits at least six potentially binding sites. Furthermore, a closer look at the differences in temporal parameters and their elucidation is of interest, as in-depth explanations of these differences are missing so far. Therefore, the second study (3.2 “Impact of lactisole on the time-intensity profile of selected sweeteners in dependence of the binding site”) addressed for the first time the impact of binding-sites of the four selected sweeteners acesulfame K (ace K), aspartame, cyclamate and neohesperidin dihydrochalcone (NHDC) to their time-dependent attributes of sweet taste. The aim was to examine whether the binding-site and an allosteric or competitive inhibition of the sweet taste receptor by lactisole does affect the temporal profile of sweeteners. This was investigated based on a direct comparison of time-intensity (TI) and dose-response sensory with the sweet taste receptor activation profiles in cells. Furthermore, it was of interest whether the receptor activation of the TAS1R2/R3 transfected HEK293 cells can reflect the complex sweet sensory temporal behaviour. In general, confirming previous studies, a typical sweet aftertaste induced by lactisole, especially in combination with low sweetener concentrations was observed in all sensory TI experiments of the here presented research. Further, it has been examined for the first time that competitive inhibition of the sweet taste receptor can be seen in sensory and single receptor cell experiments by the combinations of lactisole with cyclamate or NHDC as the EC_{50} of max. intensity dose-dependency curves increased while hillslope and the E_{max} remained similar. In the case of ace K and aspartame, only the cell experiments showed an effective allosteric inhibition by lactisole, indicating the presence of one or more low-affinity binding sites which influences especially the more complex sensory perception of this research. On the other hand, and contrary to the expectations, no influence of the binding site could be reported here for the temporal markers onset and lingering, as lactisole did not induce clear allosteric or competitive inhibition and the behaviour of cyclamate and NHDC was not different from ace K and aspartame. The EC_{50} of cell experiments in general, reflected by Ca^{2+} release after receptor activation by ascending sweeteners and did correlate with the EC_{50} of sensory max. intensity of tested sweeteners, confirming the HEK-cell model and activation induced Ca^{2+} release as adequate measurement for activation potency of a sweet compound. In addition, the results showed for the first time that the tested sweet compounds have a similar order in the time of lingering in sensory and cell experiments. Hence, the Ca^{2+} release by activation of TAS1R2/R3 transfected HEK cells is not only a good indicator for intensity of sweet perception in human, but

also for lingering effects. Nevertheless, this study does not support an impact of the major binding-site on the differences of temporal sweet attributes. Further research with more compounds would be needed to substantiate the predictability of the here used cell experiments for lingering and intensity of sweet perception. A clear assumption or even justification of the temporal differences between sweet compounds remains outstanding so far. Nevertheless, the here presented results show clear evidence that the differences in sweet attributes, including temporal differences, lie more in the complexity of the sweet compound than in the exact binding site at the sweet receptor. Of course, more research is needed to get detailed information and justifications for the differences in the taste, especially temporal attributes, of the diverse sweeteners.

Since the sweet tasting compounds are not only perceived on the tongue and at the specific receptors, but in the entire oral cavity, many other perceptual mechanisms also play a role here. Beside the differences in sweet taste that are most likely mediated through the sweet taste receptor, the so-called mouthfeel is always present during consumption, including salivation and viscosity of saliva. However, information about the impact of different sweet tasting compounds on mouthfeel attributes has not been considered so far. Therefore, the here presented third publication (3.3 “Individual sweet taste perception influences salivary characteristics after orosensory stimulation with sucrose and non-caloric sweeteners”) compared four different sweet compounds regarding their effect on salivary flow, viscoelasticity of saliva and the impact of selected influencing factors including the basal oral microbiome of 21 healthy test subjects as part of their mouthfeel and overall sweet taste perception. The sweet compounds were chosen because of their strongly different sensory profile in the first publication (3.5.1), sucrose as most preferred sweet compound, sucralose as sweetener with low negative side-tastes out of cluster one, rebaudioside M (Reb M) with more negative side-tastes and a delayed temporal profile out of cluster two, NHDC with a high occurrence of negative side-tastes out of cluster three, and water as taste neutral volume control. As the sweet taste by sucrose was shown before to enhance salivary flow, now we know that all tested compounds can enhance the salivary flow, but no differences were observed between the compounds. This increase in the flow rate was correlated moderately with individually perceived sweetness, indicating a more dominant role of the sweet taste of the compounds in general than of the different type, caloric load, or concentration of sweetener. This hypothesis was supported by the enhanced salivary flow of NHDC and RebM, knowing that these sweeteners are previously described to have a prolonged lingering. More test compounds and the examination of further sensory attributes would be needed to confirm this hypothesis. Nevertheless, the fact that mouthfeel in the context of sweet taste perception is even more complex as single enhancement of salivary flow was demonstrated

for the first time by the repeated measurement ANCOVA by which the influencing factors body height and the interaction of α-amylase with the sweet taste threshold tended to influence the salivary flow independent of the test compounds. The complex viscosity η of saliva was not influenced by the selected compounds, but after the stimulation with sucrose, a correlation between storage modulus G´ and the sweet taste threshold was seen. Concerning this, it is conceivable that the amount of a compound needed to taste sweet, here sucrose, depends on the elasticity of saliva. In other words, an enhanced elasticity of saliva could therefore increase the threshold for caloric compounds with low sweetening power, here sucrose, possibly because of the impaired transport of tastants to the taste pores. To confirm these effects, more sweet compounds with low sweetening power should be tested. If the effect is only short-term, or if long-term effects exist, such as that a regularly high sugar consumption will lead to generally more elastic saliva and therefore increase thresholds, is not clarified so far. However, the complex effects of the viscoelasticity of saliva and sweet taste perception was emphasized by the repeated measurement ANCOVA by which the interaction of storage modulus G´ with the sweet threshold influenced complex viscosity η of the saliva in dependence of the test compounds. Furthermore, the basal amount of MUC5B influenced the complex viscosity independent of the test compounds. Hence, this study confirms the complexity and interactions of influencing factors to mouthfeel parameters of sweet taste perception. Next to this shown complex interactions, the interaction of tastants with components of saliva cannot be excluded with the present results. However, the oral microbiome, which could additionally influence mouthfeel and perception and has not been associated with sweet perception and mouthfeel attributes previously, did not show an impact to the mouthfeel attributes of sweet tasting compounds in the here presented study (3.5.3) of this dissertation. To clarify the role of oral microbiota in sweet taste perception, further research with a larger study population is needed.

To summarise, the first three presented publications demonstrate the importance of the individual structure of sweet compounds, especially double bonds, ketones, aromatic rings and MlogP, for differences in sweet taste perception and taste attributes. Hence, the physicochemical characteristics and their interactions contribute to sweet attributes and as well to the temporal parameters onset and lingering. In contrast, the binding-site was not indicated to influence the differences in the temporal attributes onset and lingering here. However, an enhanced reported lingering of sweet compounds showed a prolonged salivary flow. In general, the relationship of sweet taste in the whole oral cavity and mouthfeel-attributes showed a complex relationship, with sweetness and taste per se playing a more important role than the structure or calories of the substance. In brief, this research indicates an important role of structural characteristics but not of the binding-site for the temporal profile of sweet tasting compounds. Furthermore, the

interplay of mouthfeel attributes and salivary properties can have an impact on the sweet taste perception and its attributes. Overall, the perception of sweetness with all oral receptors and senses is as complex as the structures of the different sweeteners are and the here presented research gives important insights into the contribution of structural characteristics, mouthfeel attributes and interactions of characteristics to sweet taste perception. Many parameters play a role in the oral perception, whereby certainly not all are elucidated yet. Considering the knowledge of the complexity in sweet perception, it might be valuable to perform more mechanistic and *in vitro* studies, investigating the molecular background to exclude some of the many interactions between the mechanisms.

It is known that the structures of sweet compounds show great diversity, each exhibiting its own effects, also during glucose metabolism. As the sweet taste receptor is not only present in the oral cavity but as well in the gastrointestinal tract, differences in orally perceived sweetness could also have an impact here, especially due to the varying differences in relative sweetness of sweet compounds. So far it is not clarified, if differences in sweet perception of sweet compounds have an impact on blood glucose metabolism. Therefore, the secondary part of this dissertation provided initial insights into the metabolic effects of oral sweet taste perception in general, focusing on the contribution of sweet taste perception on glucose metabolism, independent of the compound's characteristics. The consumption of carbohydrates inevitably leads to an increase followed by regulation of blood sugar under contribution of corresponding hormones and peptide hormones. So far, it has not been clarified, if the perceived sweetness or the structure of a caloric sweet compound has more influence on blood glucose regulation. Therefore, the fourth study of this thesis (3.4 "Sweetness Perception is not Involved in the Regulation of Blood Glucose after Oral Application of Sucrose and Glucose Solutions in Healthy Male Subjects") examined for the first time the effects on blood glucose regulation of sweetness of sucrose solution compared to an isocaloric but less sweet glucose solution and adjustment of sweetness by the sweet taste inhibitor lactisole in 27 healthy males. As intended, the participants rated the 10 % glucose solution less sweet than the isocaloric 10 % sucrose solution and this difference in sweetness was eliminated by the addition of 60 mg/L lactisole. This confirmation of sweetness was relevant to validate differences in the perception of the sensorially naïve participants. Therefore, same sweetness levels were hypothesized to induce similar blood glucose responses. Contrary to the differences in sweet perception induced by lactisole, lactisole did not lead to differences in glucose, insulin or glucagon responses of sucrose or glucose. These results suggested that the different sweetness of two sweet caloric compounds does not impact the parameters of blood glucose regulation. However, while comparing the blood glucose induced by consumption of sucrose and glucose, sucrose caused lower blood glucose levels after 30 min and lower Δ AUC of

blood glucose over the time of 120 min after sucrose compared to glucose consumption. Matching this, the ΔAUC of insulin was tended to be lower after sucrose compared to glucose consumption, whereby also after the equi-sweet sucrose + lactisole consumption the Δ AUC was reduced. Lactisole had no influence on GLP-1 level in plasma for the glucose consumption, but indeed for sucrose. Compared to glucose, consumption with equi-sweet sucrose + lactisole also showed a reduction in GLP-1 levels. These results of the present study therefore suggest a stronger impact of the structure of the carbohydrate than perceived sweetness has on the blood glucose regulation, which argues against a role of oral sweetness perception on blood glucose regulation. Further research is required with modulated sweetness, for example with combination of non-caloric sweeteners to enhance the sweetness to confirm these results.

To summarise, the presented research of the secondary part of this dissertation showed that sweet taste during consumption of caloric sweeteners did not affect blood glucose metabolism, but the type of sweet compound did matter, as the plasma glucose was lower after consumption of sucrose compared to the same caloric amount of glucose. The plasma insulin and GLP-1 were as well reduced by sucrose with lactisole although it was equi-sweet to the glucose solution. More studies are indeed needed for an improved understanding of the biomolecular mechanism following the consumption of sweet tasting compounds and their interaction with the sweet taste receptor in the oral and gastrointestinal tract. This could be implemented for example with enhancing the sweetness with non-caloric sweeteners, which additionally could target different binding-sites of the sweet taste receptor to gain more insights into these effects.

Overall, this dissertation shows that the sweetness, temporal behaviour, and side-tastes of sweet compounds reveal great variety due to differences in structure. An involvement of sweet taste receptor binding-site in temporal differences of sweet compounds could not been shown here. The mouthfeel associated with sweet taste perception is driven more by the individual perception of sweetness but is likely to contribute to the perception of sweet taste. Furthermore, blood glucose metabolism depends on the type of consumed carbohydrate and are not altered similar by sweet taste in general. In total, the perception of sweetness, the compounds that induce sweet taste, and their metabolism, are as diverse as the structures are.

Further research is required to better understand the diversity of sweet taste to improving the applicability of sweet compounds and also for development of more acceptable and healthy alternative sweeteners. Considering the gained knowledge of this dissertation, the individual structure of a compound should play the dominant role in further research and application fields, especially for the temporal characteristics of sweet tasting compounds.

V. References

Abdallah, L. , M. Chabert, and J. Louis-Sylvestre. 1997. 'Cephalic phase responses to sweet taste.', *The American journal of clinical nutrition*, 65: 737-43.

Acevedo, W., and P. A. Temussi. 2019. 'The Origin of Unpleasant Aftertastes in Synthetic Sweetener: A Hypothesis', *Frontiers in Molecular Bioscience*, 5.

Arazi, S. , D. Kilcast, and W. M. Marrs. 2001. 'Hydrocolloid/sweetener systems: a multiple ingredient approach to improving the mouthfeel of low-calorie drinks.', *Leatherhead Research Reports* 774: 1-30.

Avenet, P. , F. Hofmann, and B. Lindemann. 1988. 'Transduction in taste receptor cells requires cAMP-dependent protein kinase_nature.pdf>', *Nature*, 331: 351–54.

Avery, J. A., A. G. Liu, J. E. Ingeholm, C. D. Riddell, S. J. Gotts, and A. Martin. 2020. 'Taste Quality Representation in the Human Brain', *The Journal of Neuroscience*, 40: 1042-52.

Bader, M., A. Dunkel, M. Wenning, B. Kohler, G. Medard, E. Del Castillo, A. Gholami, B. Kuster, S. Scherer, and T. Hofmann. 2018. 'Dynamic Proteome Alteration and Functional Modulation of Human Saliva Induced by Dietary Chemosensory Stimuli', *Journal of Agricultural and Food Chemistry*, 66: 5621-34.

Baines, Z. V., and E. R. Morris. 1987. 'Flavour/taste perception in thickened systems: the effect of guar gum above and below c*', *Food Hydrocolloids*, 1: 197-205.

Bart, J. C. J. . 1968. 'The crystal and molecular structure of saccharin (o-sulphobenzoic imide).', *ournal of the Chemical Society B: Physical Organic*: 376-82.

Bartoshuk, L. M. , V. B. Duffy, K. Fast, B. G. Green, J. Prutkin, and D. J. Snyder. 2002. 'Labeled scales (e.g., category, Likert, VAS) and invalid across-group comparisons - what we have learned from genetic variation in taste.', *Food Quality and Preference*, 14: 125-38.

Beckett, E. L., C. Martin, Z. Yates, M. Veysey, K. Duesing, and M. Lucock. 2014. 'Bitter taste genetics--the relationship to tasting, liking, consumption and health', *Food and Function*, 5: 3040-54.

Behrens, M., and W. Meyerhof. 2011. 'Gustatory and extragustatory functions of mammalian taste receptors', *Physiology & Behavior*, 105: 4-13.

Behrens, M., W. Meyerhof, C. Hellfritsch, and T. Hofmann. 2011. 'Sweet and umami taste: natural products, their chemosensory targets, and beyond', *Angewandte Chemie International Edition*, 50: 2220-42.

Bernhardt, S.. J., M. Naim, U. Zehavi, and B. Lindemann. 1996. 'Changes in IP3 and cytosolic Ca2+ in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat', *Journal of Physiology*, 490: 325-36.

Berridge, M. J. , and R. F. Irvine. 1984. 'Inositol trisphosphate, a novel second messenger in cellular signal transduction', *Nature*, 312: 315-21.

Bezençon, C., J. le Coutre, and S. Damak. 2007. 'Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells', *Chemical Senses*, 32: 41-9.

Birch, L. L. 1999. 'Development of food preferences', *Annual Reviews of Nutrition*, 19: 41-62.

Bonnans, S. R., and A. C. Noble. 1995. 'Interaction of salivary flow with temporal perception of sweetness, sourness, and fruitiness.', *Physiology & Behavior*, 57: 569-74.

Boyd, H., J. F. Gonzalez-Martinez, R. J. L. Welbourn, P. Gutfreund, A. Klechikov, C. Robertsson, C. Wickstrom, T. Arnebrant, R. Barker, and J. Sotres. 2021. 'A comparison between the structures of reconstituted salivary pellicles and oral mucin (MUC5B) films', *Journal of Colloid and Interface Science*, 584: 660-68.

Breslin, P. A. S., A. Izumi, A. Tharp, T. Ohkuri, Y. Yokoo, L. J. Flammer, N. E. Rawson, and R. F. Margolskee. 2021. 'Evidence that human oral glucose detection involves a sweet taste pathway and a glucose transporter pathway', *PLoS One*, 16: e0256989.

Brown, A. W., M. M. Bohan Brown, K. L. Onken, and D. C. Beitz. 2011. 'Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women', *Nutrition Research*, 31: 882-8.

Brown, R. J., and K. I. Rother. 2012. 'Non-nutritive sweeteners and their role in the gastrointestinal tract', *The Journal of Clinical Endocrinology and Metabolism*, 97: 2597-605.

Bruce, D. G. , L. H. Storlien, S. M. Furler, and D. J. Chisholm. 1987. 'Cephalic phase metabolic responses in normal weight adults.', *Metabolism* 36: 721-25.

Canon, F., C. Belloir, E. Bourillot, H. Brignot, L. Briand, G. Feron, E. Lesniewska, C. Nivet, C. Septier, M. Schwartz, C. Tournier, R. Vargiolu, M. Wang, H. Zahouani, and F. Neiers. 2021. 'Perspectives on Astringency Sensation: An Alternative Hypothesis on the Molecular Origin of Astringency', *Journal of Agricultural and Food Chemistry*, 69: 3822-26.

Cattaneo, C., G. Gargari, R. Koirala, M. Laureati, P. Riso, S. Guglielmetti, and E. Pagliarini. 2019. 'New insights into the relationship between taste perception and oral microbiota composition', *Scientific Reports*, 9: 3549.

Cattaneo, C., P. Riso, M. Laureati, G. Gargari, and E. Pagliarini. 2019. 'Exploring Associations between Interindividual Differences in Taste Perception, Oral Microbiota Composition, and Reported Food Intake', *Nutrients*, 11.

Chandrashekar, J., M. A. Hoon, N. J. Ryba, and C. S. Zuker. 2006. 'The receptors and cells for mammalian taste', *Nature*, 444: 288-94.

Chandrashekar, J., C. Kuhn, Y. Oka, D. A. Yarmolinsky, E. Hummler, N. J. Ryba, and C. S. Zuker. 2010. 'The cells and peripheral representation of sodium taste in mice', *Nature*, 464: 297-301.

Chaudhari, N. , and S. D. Roper. 2010. 'The cell biology of taste. ', *Journal of Cell Biology* 190: 285-96.

Chen, X. , M. Gabitto, Y. Peng, N. J. Ryba, and C. S. Zuker. 2011. 'A Gustotopic Map of Taste Qualities in the Mammalian Brain', *Science*, 333: 1262-66.

Chéron, J. B., J. Golebiowski, S. Antonczak, and S. Fiorucci. 2016. 'The anatomy of mammalian sweet taste receptors', *Proteins*, 85: 332-41.

Choi, Y., J. A. Manthey, T. H. Park, Y. K. Cha, Y. Kim, and Y. Kim. 2021. 'Correlation between in vitro binding activity of sweeteners to cloned human sweet taste receptor and sensory evaluation', *Food Science and Biotechnology*, 30: 675-82.

Clapp, T. R. , L. M. Stone, R. F. Margolskee, and S. C. Kinnamin. 2001. 'Immunocytochemical evidence for co-expression of Type III IP3receptor with signaling components of bitter taste transduction.pdf>', *BMC Neuroscience*, 2.

Clemens, R. A., J. M. Jones, M. Kern, S.-Y. Lee, E. J. Mayhew, J. L. Slavin, and S. Zivanovic. 2016. 'Functionality of Sugars in Foods and Health.', *Comprehensive Reviews in Food Science and Food Safety*, 15: 433-70.

Cohen, S.M. 1986. 'Saccharin: Past, present, and future', *Journal of the American Dietetic Association*, 86: 929-31.

Crapo, P. A. , J. A. Scarlett, O. G. Kolterman, L. R. Sanders, F. D. Hofeldt, and J. M. Olefsky. 1982. 'The effects of oral fructose, sucrose, and glucose in subjects with reactive hypoglycemia.', *Diabetes Care*, 5: 512-17.

Cui, M., P. Jiang, E. Maillet, M Max, R. F. Margolskee, and R. Osman. 2006. 'The Heterodimeric Sweet Taste Receptor has Multiple Potential Ligand Binding Sites', *Current Pharmaceutical Design*, 12: 4591-600.

Curry, D. L. . 1989. 'Effects of mannose and fructose on the synthesis and secretion of insulin.', *Pancreas*, 4: 2-9.

Dalenberg, J. R., B. P. Patel, R. Denis, M. G. Veldhuizen, Y. Nakamura, P. C. Vinke, S. Luquet, and D. M. Small. 2020. 'Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs Neural and Metabolic Sensitivity to Sugar in Humans', *Cell Metabolism*, 31: 493-502 e7.

Damak, S. , M. Rong, K. Yasumatsu, Z. Kokrashvili, V. Varadarajan, S. Zou, P. Jiang, Y. Ninomiya, and R. F. Margolskee. 2003. 'Detection of sweet and umami taste in the absence of taste receptor T1r3.', *Science* 301: 850-53.

Davies, G. A. , E. Wantling, and J. R. Stokes. 2009. 'The influence of beverages on the stimulation and viscoelasticity of saliva: Relationship to mouthfeel?', *Food Hydrocolloids*, 23: 2261-69.

Drewnowski, A., J. A. Mennella, S. L. Johnson, and F. Bellisle. 2012. 'Sweetness and food preference', *J Nutr*, 142: 1142S-8S.

DuBois, G. E. 1993. 'Challenges in the reproduction of sucrose taste with highpotency sweeteners.' in Yalpani M (ed.), *Science for the food industry of the 21st century:biotechnology, supercritical fluids, membranes and other advanced technologiesfor low calorie, healthy food alternatives.* (ALT Press, ScientificPublishers, Inc: Mount Prospect (IL)).

DuBois, G. E. . 2016. 'Molecular mechanism of sweetness sensation', *Physiology & Behavior*, 164: 453-63.

DuBois, G. E., G. A. Crosby, R. A. Stephenson, and R. E. Wingard Jr. 1977. 'Dihydrochalcone sweeteners. Synthesis and sensory evaluation of sulfonate derivatives', *Journal of Agricultural and Food Chemistry*, 25: 763-72.

DuBois, G. E., and J. F. Lee. 1983. 'A simple technique for the evaluation of temporal taste properties', *Chemical Senses*, 7: 237-47.

Dyer, J. , K.S.H. Salmon, L. Zibrik, and S. P. Shirazi-Beechey. 2005. 'Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells', *Biochemical Society Transactions*, 33: 302-05.

Edwards, Cathrina H., Megan Rossi, Christopher P. Corpe, Peter J. Butterworth, and Peter R. Ellis. 2016. 'The role of sugars and sweeteners in food, diet and health: Alternatives for the future', *Trends in Food Science & Technology*, 56: 158-66.

EFSA. 2010. 'Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive. European Food Safety Authority. ', *EFSA Journal*, 8.

Esberg, A., S. Haworth, P. Hasslof, P. Lif Holgerson, and I. Johansson. 2020. 'Oral Microbiota Profile Associates with Sugar Intake and Taste Preference Genes', *Nutrients*, 12.

FDA. 1989. "21 Food and Drug Administration. Subchapter B - Food for Human Consumption. Part 189 - Substances Prohibited from Unse in Human Food. Subpart C - Substances Generally Prohibited From Direct Addition or Use as Human Food. 21CFR189.135 Cyclamate and its derivatives." In *(21CFR189.135)*. Code of Federal Regulations - U.S. Food & Drug Administration.

Fernstrom, J. D. 2015. 'Non-nutritive sweeteners and obesity', *Annual Review Food Science and Technology*, 6: 119-36.

Ferry, A. L. S. , J. R. Mitchell, J. Hort, S. E. Hill, A. J. Taylor, S. Lagarrigue, and B. Vallès-Pàmies. 2006. 'In-mouth amylase activity can reduce perception of saltiness in starch-thickened foods.', *Journal of Agricultural and Food Chemistry*, 54: 8869-73.

Frank, G. K., T. A. Oberndorfer, A. N. Simmons, M. P. Paulus, J. L. Fudge, T. T. Yang, and W. H. Kaye. 2008. 'Sucrose activates human taste pathways differently from artificial sweetener', *Neuroimage*, 39: 1559-69.

Fujimaru, T., J. H. Park, and J. Lim. 2012. 'Sensory characteristics and relative sweetness of tagatose and other sweeteners', *Journal of Food Science*, 77: S323-8.

Fujiwara, S. , T. Imada, T. Nakagita, S. Okada, T. Nammoku, K. Abe, and T. Misaka. 2012. 'Sweeteners interacting with the transmembrane domain of the human sweet-taste receptor induce sweet-taste synergisms in binary mixtures', *Food Chemistry*, 130: 561-68.

Galindo-Cuspinera, V. , M. Winnig, B. Bufe, W. Meyerhof, and P. A. Breslin. 2006. 'A TAS1R receptor-based explanation of sweet 'water-taste'', *Nature*, 441: 354-7.

Ganchrow, J. R. , J. E. Steiner, and M. Daher. 1983. 'Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli', *Iinfant Behavior and Development*, 6: 473-84.

Gerspach, A. C., R. E. Steinert, L. Schonenberger, A. Graber-Maier, and C. Beglinger. 2011. 'The role of the gut sweet taste receptor in regulating GLP-1, PYY, and CCK release in humans', *American Journal Of Physiology-Endocrinology And Metabolism*, 301: E317-25.

Glendinning, J. I., Y. G. Frim, A. Hochman, G. S. Lubitz, A. J. Basile, and A. Sclafani. 2017. 'Glucose elicits cephalic-phase insulin release in mice by activating KATP channels in taste cells', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 312: R597-R610.

Grembecka, Małgorzata. 2015. 'Sugar alcohols - their role in the modern world of sweeteners: a review', *European Food Research and Technology*, 241: 1-14.

Guest, S., G. Essick, M. Young, N. Phillips, and F. McGlone. 2008. 'The effect of oral drying and astringent liquids on the perception of mouth wetness', *Physiology & Behavior*, 93: 889-96.

Gwak, Mi-Jin, Seo-Jin Chung, Young Jae Kim, and Chun Sun Lim. 2012. 'Relative sweetness and sensory characteristics of bulk and intense sweeteners', *Food Science and Biotechnology*, 21: 889-94.

Han, P., B. Bagenna, and M. Fu. 2019. 'The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: a review', *International Journal of Food Sciences and Nutrition*, 70: 125-35.

Hannig, C., M. Hannig, A. Kensche, and G. Carpenter. 2017. 'The mucosal pellicle - An underestimated factor in oral physiology', *Archives of Oral Biology*, 80: 144-52.

Hanover, L. M. , and J. S. White. 1993. 'Manufacturing, composition, and applications of fructose. ', *The American journal of clinical nutrition*, 58: 724S-32S.

Hass, N., K. Schwarzenbacher, and H. Breer. 2010. 'T1R3 is expressed in brush cells and ghrelin-producing cells of murine stomach', *Cell and Tissue Research*, 339: 493-504.

Hayes, C. . 2001. 'The Effect of Non-Cariogenic Sweeteners on the Prevention of Dental Caries: A Review of the Evidence', *Journal of dental education* 65: 1106-09.

Hellfritsch, C., A. Brockhoff, F. Stahler, W. Meyerhof, and T. Hofmann. 2012. 'Human psychometric and taste receptor responses to steviol glycosides', *Journal of Agricultural and Food Chemistry*, 60: 6782-93.

Henquin, J. C. 2012. 'Do pancreatic β cells “taste” nutrients to secrete insulin?', *Science signaling*, 5: pe36 1-4.

Huberlant, J. . 2003. 'Sucrose.' in, *Encyclopedia of Food Sciences and Nutrition* (Academic Press).

Hunter, S. R., E. J. Reister, E. Cheon, and R. D. Mattes. 2019. 'Low Calorie Sweeteners Differ in Their Physiological Effects in Humans', *Nutrients*, 11.

Imberti, S. , S. E. McLain, N. H. Rhys, F. Bruni, and M. A. Ricci. 2019. 'Role of Water in Sucrose, Lactose, and Sucralose Taste: The Sweeter, The Wetter?', *ACS Omega*, 4: 22392-98.

Jang, H.-J., Z. Kokrashvili, M. J. Theodorakis, O.D. Carlson, B.-J. Kim, J. Zhou, H. H. Kim, X. Xu, S. L. Chan, M. Juhaszova, M. Bernier, B. Mosinger, R. F. Margolskee, and J. M. Egan. 2007. 'Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1', *PNAS*, 104: 15069-74.

Jiang, P., M. Cui, B. Zhao, Z. Liu, L. A. Snyder, L. M. Benard, R. Osman, R. F. Margolskee, and M. Max. 2005. 'Lactisole interacts with the transmembrane domains of human T1R3 to inhibit sweet taste', *Journal of Biological Chemistry*, 280: 15238-46.

Jiang, P., M. Cui, Q. Ji, L. Snyder, Z. Liu, L. Benard, R. F. Margolskee, R. Osman, and M. Max. 2005. 'Molecular mechanisms of sweet receptor function', *Chemical Senses*, 30: i17-i18.

Jiang, P., M. Cui, B. Zhao, L. A. Snyder, L. M. Benard, R. Osman, M. Max, and R. F. Margolskee. 2005. 'Identification of the cyclamate interaction site within the transmembrane domain of the human sweet taste receptor subunit T1R3', *Journal of Biological Chemistry* 280: 34296-305.

Jiang, P., Q. Ji, Z. Liu, L. A. Snyder, L. M. Benard, R. F. Margolskee, and M. Max. 2004. 'The cysteine-rich region of T1R3 determines responses to intensely sweet proteins', *J Biol Chem*, 279: 45068-75.

Just, T., H. W. Pau, U. Engel, and T. Hummel. 2008. 'Cephalic phase insulin release in healthy humans after taste stimulation?', *Appetite*, 51: 622-7.

Kappes, S. M., S. J. Schmidt, and S. Y. Lee. 2006. 'Mouthfeel Detection Threshold and Instrumental Viscosity of Sucrose and High Fructose Corn Syrup Solutions', *Journal of Food Science*, 71: S597-S602.

Karimian-Azari, E., K. R. Smith, F. Yi, T. F. Osborne, R. Bizzotto, A. Mari, R. E. Pratley, and G. A. Kyriazis. 2017. 'Inhibition of sweet chemosensory receptors alters insulin responses during glucose ingestion in healthy adults: a randomized crossover interventional study', *The American journal of clinical nutrition*, 105: 1001-09.

Ketelsen, S. M. , C. L. Keay, and S. G. Wiet. 1993. 'Time-Intensity Parameters of Selected Carbohydrate and High Potency Sweeteners.', *Journal of Food Science*, 58: 1418-21.

Kitagawa, M., Y. Kusakabe, H. Miura, Y. Ninomiya, and A. Hino. 2001. 'Molecular genetic identification of a candidate receptor gene for sweet taste', *Biochemical and Biophysical Research Communications*, 283: 236-42.

Kojima, I., Y. Nakagawa, K. Hamano, J. Medina, L. Li, and M. Nagasawa. 2015. 'Glucose-Sensing Receptor T1R3: A New Signaling Receptor Activated by Glucose in Pancreatic β-Cells', *Biological and Pharmaceutical Bulletin*, 38: 674-79.

Lee, A. A., and C. Owyang. 2017. 'Sugars, Sweet Taste Receptors, and Brain Responses', *Nutrients*, 9.

Li, X., L. Staszewski, H. Xu, K. Durick, M. Zoller, and E. Adler. 2002. 'Human receptors for sweet and umami taste', *PNAS*, 99: 4692-96.

Ma, J., M. Bellon, J. M. Wishart, R. Young, L. A. Blackshaw, K. L. Jones, M. Horowitz, and C. K. Rayner. 2009. 'Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects', *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 296: G735-9.

Maillet, E. L., M. Cui, P. Jiang, M. Mezei, E. Hecht, J. Quijada, R. F. Margolskee, R. Osman, and M. Max. 2015. 'Characterization of the Binding Site of Aspartame in the Human Sweet Taste Receptor', *Chem Senses*, 40: 577-86.

Majchrzak, Dorota, Annika Ipsen, and Juergen Koenig. 2015. 'Sucrose-replacement by rebaudioside a in a model beverage', *Journal of Food Science and Technology*, 52: 6031-36.

Malik, V. S., B. M. Popkin, G. A. Bray, J. P. Despres, W. C. Willett, and F. B. Hu. 2010. 'Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis', *Diabetes Care*, 33: 2477-83.

Marcinkowska-Gapińska, A. , K. Linkowska-Świdzińska, T. Świdziński, and A. Surdacka. 2018. 'Rheological parameters of saliva in comparison with taste examination', *Biorheology*, 55: 51-60.

Margolskee, R. F. 2002. 'Molecular mechanisms of bitter and sweet taste transduction', *Journal of Biological Chemistry* 277: 1-4.

Margolskee, R. F., J. Dyer, Z. Kokrashvili, K. S. Salmon, E. Ilegems, K. Daly, E. L. Maillet, Y. Ninomiya, B. Mosinger, and S. P. Shirazi-Beechey. 2007. 'T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1', *PNAS*, 104: 15075-80.

Masubuchi, Y., Y. Nakagawa, J. Ma, T. Sasaki, T. Kitamura, Y. Yamamoto, H. Kurose, I. Kojima, and H. Shibata. 2013. 'A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells', *PLoS One*, 8: e54500.

Masuda, K., A. Koizumi, K. Nakajima, T. Tanaka, K. Abe, T. Misaka, and M. Ishiguro. 2012. 'Characterization of the modes of binding between human sweet taste receptor and low-molecular-weight sweet compounds', *PLoS One*, 7: e35380.

Masuda, T., W. Taguchi, A. Sano, K. Ohta, N. Kitabatake, and F. Tani. 2013. 'Five amino acid residues in cysteine-rich domain of human T1R3 were involved in the response for sweet-tasting protein, thaumatin', *Biochimie*, 95: 1502-05.

Mattes, R. D. , and B. M. Popkin. 2009. 'Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms', *American Journal of Clinical Nutrition*, 89: 1-14.

Matuso, R. 2000. 'Role of saliva in the maintenance of taste sensitivity', *Critical Reviews in Oral Biology & Medicine*, 11: 216-29.

Max, M. , Y. G. Shanker, L. Huang, M. Rong, Z. Liu, F. Campagne, H. Weinstein, S. Damak, and R. F. Margolskee. 2001. 'Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac.', *Nature*, 28: 58-63.

Mayes, P. A. 1993. 'Intermediary metabolism of fructose. ', *The American journal of clinical nutrition*, 58: 754S-65S.

McCaughey, S. A. 2008. 'The taste of sugars', *Neuroscience and Biobehavioral Reviews*, 32: 1024-43.

Miller, S. A. , and V. P. Frattali. 1989. 'Saccharin', *Diabetes Care*, 12: 75-80.

Montmayeur, J. P. , S. D. Liberles, H. Matsunami, and L. B. Buck. 2001. 'A candidate taste receptor gene near a sweet taste locus.', *Nature neuroscience*, 4: 492-98.

Mooradian, A. D., M. Smith, and M. Tokuda. 2017. 'The role of artificial and natural sweeteners in reducing the consumption of table sugar: A narrative review', *Clin Nutr ESPEN*, 18: 1-8.

Mortensen, Alicja. 2016. 'Sweeteners permitted in the European Union: safety aspects', *Scandinavian Journal of Food and Nutrition*, 50: 104-16.

Morzel, M., T. Siying, H. Brignot, and J. Lherminier. 2014. 'Immunocytological detection of salivary mucins (MUC5B) on the mucosal pellicle lining human epithelial buccal cells', *Microscopy Research and Technique*, 77: 453-7.

Muir, D. D. , E. A. Hunter, S. A. R. Williams, and R Brennan, M. . 1998. 'Sensory profiles of commercial fruit juice drinks - influence of sweetener type.', *Journal of the Science of Food and Agriculture*, 77: 559-65.

Muñoz-Gonzalez, C., G. Feron, and F. Canon. 2018. 'Main effects of human saliva on flavour perception and the potential contribution to food consumption', *Proceedings of the Nutrition Society*, 77: 423-31.

Murray, R. G. 1973. 'The ultrastructure of taste buds.' in Friedmann I. (ed.), *The Ultrastructure of Sensory Organs* (North-Holland Publ. Co.: Amsterdam).

Nango, E., S. Akiyama, S. Maki-Yonekura, Y. Ashikawa, Y. Kusakabe, E. Krayukhina, T. Maruno, S. Uchiyama, N. Nuemket, K. Yonekura, M. Shimizu, N. Atsumi, N. Yasui, T. Hikima, M. Yamamoto, Y. Kobayashi, and A. Yamashita. 2016. 'Taste substance binding elicits conformational change of taste receptor T1r heterodimer extracellular domains', *Sci Rep*, 6: 25745.

Nelson, G. , M. A. Hoon, J. Chandrashekar, Y. Zhang, N. J. P. Ryba, and C. S. Zuker. 2001. 'Mammalian Sweet Taste Receptors.', *Cell*, 106: 381-90.

Neyraud, E. 2014. 'Role of saliva in oral food perception.' in A.J.M. Ligtenberg and E.C.I. Veerman (eds.), *Saliva: Secretion and Functions* (Monographs in Oral Science: Basel, Karger).

Neyraud, E., C. I. Heinzerling, J. H. F. Bult, C. Mesmin, and E. Dransfield. 2009. 'Effects of Different Tastants on Parotid Saliva Flow and Composition', *Chemosensory Perception*, 2: 108-16.

Neyraud, E., and M. Morzel. 2019. 'Biological films adhering to the oral soft tissues: Structure, composition, and potential impact on taste perception', *Journal of Texture Studies*, 50: 19-26.

Nichol, A.D. , M.J. Holle, and R. An. 2018. 'Glycemic impact of non-nutritive sweeteners: a systematic review and meta-analysis of randomized controlled trials', *European Journal of Clinical Nutrition*, 72: 796-804.

Nie, Y., S. Vigues, J. R. Hobbs, G. L. Conn, and S. D. Munger. 2005. 'Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli', *Current Biology*, 15: 1948-52.

O´Brien-Nabors, L. (ed.)^(eds.). 2016. *Alternatives sweeteners.* (CRC Press, Taylor & Francis Group).

Oka, Y., M. Butnaru, L. von Buchholtz, N. J. Ryba, and C. S. Zuker. 2013. 'High salt recruits aversive taste pathways', *Nature*, 494: 472-5.

Ott, D. B. , C. L. Edwards, and S. J. Palmers. 1991. 'Perceived Taste Intensity and Duration of Nutritive and Non-nutritive Sweeteners in Water using Time-intensity (T-I) Evaluations', *Journal of Food Science*, 56: 535-42.

Payne, A. N., C. Chassard, and C. Lacroix. 2012. 'Gut microbial adaptation to dietary consumption of fructose, artificial sweeteners and sugar alcohols: implications for host-microbe interactions contributing to obesity', *Obes Rev*, 13: 799-809.

Pedersen, A., C. E. Sorensen, G. B. Proctor, and G. H. Carpenter. 2018. 'Salivary functions in mastication, taste and textural perception, swallowing and initial digestion', *Oral Diseases*, 24: 1399-416.

Pérez, C. A., L. Huang, M. Rong, J. A. Kozak, A. K. Preuss, H. Zhang, M. Max, and R. F. Margolskee. 2002. 'A transient receptor potential channel expressed in taste receptor cells', *Nat Neurosci*, 5: 1169-76.

Pin, J.-P., and B. Bettler. 2016. 'Organization and functions of mGlu and GABAB receptor complexes', *Nature*, 540: 60-68.

Pin, J.-P., T. Galvez, and L. Prézeau. 2003. 'Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors', *Pharmacology & Therapeutics*, 98: 325-54.

Pin, J.-P., J. Kniazeff, L. Prezeau, J. F. Liu, and P. Rondard. 2019. 'GPCR interaction as a possible way for allosteric control between receptors', *Mol Cell Endocrinol*, 486: 89-95.

Pineau, N., P. Schlich, S. Cordelle, C. Mathonnière, S. Issanchou, A. Imbert, M. Rogeaux, P. Etiévant, and E. Köster. 2009. 'Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity', *Food Quality and Preference*, 20: 450-55.

Ployon, S., M. Morzel, C. Belloir, A. Bonnotte, E. Bourillot, L. Briand, E. Lesniewska, J. Lherminier, E. Aybeke, and F. Canon. 2018. 'Mechanisms of astringency: Structural alteration of the oral mucosal pellicle by dietary tannins and protective effect of bPRPs', *Food Chemistry*, 253: 79-87.

Powley, T. L. , and H. R. Berthoud. 1985. 'Diet and cephalic phase insulin responses. ', *The American journal of clinical nutrition*, 42: 991-1002.

Prakash, I., G. E. Dubois, J. F. Clos, K. L. Wilkens, and L. E. Fosdick. 2008. 'Development of rebiana, a natural, non-caloric sweetener', *Food and Chemical Toxicology*, 46 Suppl 7: S75-82.

Prakash, I., A. Markosyan, and C. Bunders. 2014. 'Development of Next Generation Stevia Sweetener: Rebaudioside M', *Foods*, 3: 162-75.

Prescott, J. 2001. 'Taste hedonics and the role of umami', *Food Australia*, 53: 550-54.

Ren, X., L. Zhou, R. Terwilliger, S. S. Newton, and I. E. de Araujo. 2009. 'Sweet taste signaling functions as a hypothalamic glucose sensor', *Frontiers in Frontiers in Integrative Neuroscience*, 3: 15.

Renwick, A. G., and S. V. Molinary. 2010. 'Sweet-taste receptors, low-energy sweeteners, glucose absorption and insulin release', *British Journal of Nutrition*, 104: 1415-20.

Reyes, M. M., J. C. Castura, and J. E. Hayes. 2017. 'Characterizing Dynamic Sensory Properties of Nutritive and Nonnutritive Sweeteners with Temporal Check-All-That-Apply', *Journal of Sensory Studies*, 32.

Reyes, M. M., S. A. Gravina, and J. E. Hayes. 2019. 'Evaluation of Sweetener Synergy in Humans by Isobole Analyses', *Chemical Senses*, 44: 571-82.

Röder, P. V., B. Wu, Y. Liu, and W. Han. 2016. 'Pancreatic regulation of glucose homeostasis', *Experimental & Molecular Medicin*, 48: e219.

Rodrigues, L., G. Costa, C. Cordeiro, C. Pinheiro, F. Amado, and E. Lamy. 2017. 'Salivary proteome and glucose levels are related with sweet taste sensitivity in young adults', *Food & Nutrition Research*, 61: 1389208.

Rolls. 2006. 'Brain mechanisms underlying flavour and appetite', *Philosophical Transactions of the Royal Society*, 361: 1123–36.

Roper, S. D., and N. Chaudhari. 2017. 'Taste buds: cells, signals and synapses', *Nature Reviews Neuroscience*, 18: 485-97.

Rössler, P. , C. Kroner, J. Freitag, J. Noè, and H. Breer. 1998. 'Identification of a phospholipase C β subtype in rat taste cells.', *European Journal of Cell Biology*, 77: 253-61.

Rozengurt, N., S. V. Wu, M. C. Chen, C. Huang, C. Sternini, and E. Rozengurt. 2006. 'Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon', *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 291: G792-802.

Satoh-Kuriwada, S., N. Shoji, H. Miyake, C. Watanabe, and T. Sasano. 2018. 'Effects and Mechanisms of Tastants on the Gustatory-Salivary Reflex in Human Minor Salivary Glands', *Biomedical Research International*, 2018: 3847075.

Schiffman, S. S. , B. J. Booth, B. T. Carr, M. L. Losee, E. A. Sattely-Miller, and B. G. Graham. 1995. 'Investigation of synergism in binary mixtures of sweeteners.', *Brain research bulletin*, 38: 105-20.

Schiffman, S. S. , B. J. Booth, M. L. Losee, S. D. Pecore, and Z. S. Warwick. 1995. 'Bitterness of Sweeteners as a Function of Concentration', *Brain research bulletin*, 36: 505-5013.

Schiffman, S. S. , and K. I. Rother. 2013. 'Sucralose, a synthetic organochlorine sweetener: overview of biological issues', *Journal of Toxicology and Environmental Health, Part B*, 16: 399-451.

Schiffman, S. S. , E. A. Sattely-Miller, B. G. Graham, B. J. Booth, and K. M. Gibes. 2000. 'Synergism among Ternary Mixtures of Fourteen Sweeteners.', *Chemical Senses*, 25: 131-40.

Servant, G., T. Kenakin, L. Zhang, M. Williams, and N. Servant. 2020. 'The function and allosteric control of the human sweet taste receptor', *Adv Pharmacol*, 88: 59-82.

Servant, G., C. Tachdjian, X. Q. Tang, S. Werner, F. Zhang, X. Li, P. Kamdar, G. Petrovic, T. Ditschun, A. Java, P. Brust, N. Brune, G. E. DuBois, M. Zoller, and D. S. Karanewsky. 2010. 'Positive allosteric modulators of the human sweet taste receptor enhance sweet taste', *PNAS*, 107: 4746-51.

Stokes, J. R. , and G. A. Davies. 2007. 'Viscoelasticity of human whole saliva collected after acid and mechanical stimulation.', *Biorheology*, 44: 141-60.

Striem, B. J. , U. Pace, U. Zehavi, M. Naim, and D. Lancet. 1989. 'Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes', *The Biochemical Journal* 260: 121-26.

Sukumaran, S. K., K. K. Yee, S. Iwata, R. Kotha, R. Quezada-Calvillo, B. L. Nichols, S. Mohan, B. M. Pinto, N. Shigemura, Y. Ninomiya, and R. F. Margolskee. 2016. 'Taste cell-expressed alpha-glucosidase enzymes contribute to gustatory responses to disaccharides', *PNAS*, 113: 6035-40.

Sutherland, K. , R. L. Young, N. J. Cooper, M. Horowitz, and L. A. Blackshaw. 2007. 'Phenotypic characterization of taste cells of the mouse small intestine.', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 292: 1420-28.

Sylvetsky, A. C., E. M. Conway, S. Malhotra, and K. I. Rother. 2017. 'Development of Sweet Taste Perception: Implications for Artificial Sweetener Use', *Developmental Biology of Gastrointestinal Hormones*, 32: 87-99.

Sylvetsky, A. C., and K. I. Rother. 2016. 'Trends in the consumption of low-calorie sweeteners', *Physiology & Behavior*, 164: 446-50.

Takai, S., K. Yasumatsu, M. Inoue, S. Iwata, R. Yoshida, N. Shigemura, Y. Yanagawa, D. J. Drucker, R. F. Margolskee, and Y. Ninomiya. 2015. 'Glucagon-like peptide-1 is specifically involved in sweet taste transmission', *FASEB Journal*, 29: 2268-80.

Tan, V. W. K., M. S. M. Wee, O. Tomic, and C. G. Forde. 2019. 'Temporal sweetness and side tastes profiles of 16 sweeteners using temporal check-all-that-apply (TCATA)', *Food Research International*, 121: 39-47.

Taruno, A., V. Vingtdeux, M. Ohmoto, Z. Ma, G. Dvoryanchikov, A. Li, L. Adrien, H. Zhao, S. Leung, M. Abernethy, J. Koppel, P. Davies, M. M. Civan, N. Chaudhari, I. Matsumoto, G. Hellekant, M. G. Tordoff, P. Marambaud, and J. K. Foskett. 2013. 'CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes', *Nature*, 495: 223-6.

Teff, K. L. , J. Devine, and K. Engelman. 1995. 'Sweet taste: Effect on cephalic phase insulin release in men.', *Physiology & Behavior*, 57: 1089-95.

Teff, K. L. , S. S. Elliott, M. Tschop, T. J. Kieffer, D. Rader, M. Heiman, R. R. Townsend, N. L. Keim, D. D'Alessio, and P. J. Havel. 2004. 'Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women', *The Journal of Clinical Endocrinology & Metabolism*, 89: 2963-72.

Teng, B., C. E. Wilson, Y. H. Tu, N. R. Joshi, S. C. Kinnamon, and E. R. Liman. 2019. 'Cellular and Neural Responses to Sour Stimuli Require the Proton Channel Otop1', *Current Biology*, 29: 3647-56 e5.

Tu, Y. H., A. J. Cooper, B. Teng, R. B. Chang, D. J. Artiga, H. N. Turner, E. M. Mulhall , W. Ye, A. D. Smith, and E. R. Liman. 2018. 'An evolutionarily conserved gene family encodes proton-selective ion channels', *Science*, 359: 1047-50.

Van der Reijden, W. A. , E. C. I. Veerman, and A. V. Nieuw Amerongen. 1993. 'Shear rate dependent viscoelastic behavior of human glandular salivas. ', *Biorheology*, 32: 141-52.

Varga, Gábor. 2015. 'Physiology of the salivary glands', *Surgery (Oxford)*, 33: 581-86.

Venditti, C., K. Musa-Veloso, H. Y. Lee, T. Poon, A. Mak, M. Darch, J. Juana, D. Fronda, D. Noori, E. Pateman, and M. Jack. 2020. 'Determinants of Sweetness Preference: A Scoping Review of Human Studies', *Nutrients*, 12.

Ventura, A. K. , and J. Worobey. 2013. 'Early influences on the development of food preferences', *Current Biology*, 23: R401-8.

vonMolitor, E., K. Riedel, M. Krohn, M. Hafner, R. Rudolf, and T. Cesetti. 2021. 'Sweet Taste Is Complex: Signaling Cascades and Circuits Involved in Sweet Sensation', *Frontiers in Human Neuroscience*, 15: 667709.

Waksmonski, J. C. , and K. Koppel. 2016. 'Variation in human sweet taste receptor may result in different levels of sweet intensity variability between sweet stimuli', *International Journal of Food Science & Technology*, 51: 1958-66.

WHO. 2015. 'Guideline: sugar intake for adults and children.pdf>', *Geneva: World Health Organization*.

Winnig, M., B. Bufe, N. A. Kratochwil, J. P. Slack, and W. Meyerhof. 2007. 'The binding site for neohesperidin dihydrochalcone at the human sweet taste receptor', *BMC Struct Biol*, 7: 66.

Winnig, M., B. Bufe, and W. Meyerhof. 2005. 'Valine 738 and lysine 735 in the fifth transmembrane domain of rTas1r3 mediate insensitivity towards lactisole of the rat sweet taste receptor', *BMC Neuroscience*, 6: 22.

Witt, M. , and K. Reutter. 2015. 'Anatomy of the Tongue and Taste Buds.' in R. L. Doty (ed.), *Handbook of olfaction and gustation* (John Wiley & Sons: Hoboken, N.J.).

Xu, F., L. Laguna, and A. Sarkar. 2019. 'Aging-related changes in quantity and quality of saliva: Where do we stand in our understanding?', *Journal of Texture Studies*, 50: 27-35.

Xu, H., L. Staszewski, H. Tang, E. Adler, M. Zoller, and X. Li. 2004. 'Different functional roles of T1R subunits in the heteromeric taste receptors', *PNAS*, 101: 14258–63.

Yasumatsu, K., T. Ohkuri, R. Yoshida, S. Iwata, R. F. Margolskee, and Y. Ninomiya. 2020. 'Sodium-glucose cotransporter 1 as a sugar taste sensor in mouse tongue', *Acta Physiol (Oxf)*, 230: e13529.

Yee, K. K., S. K. Sukumaran, R. Kotha, T. A. Gilbertson, and R. F. Margolskee. 2011. 'Glucose transporters and ATP-gated K+ (KATP) metabolic sensors are present in type 1 taste receptor 3 (T1r3)-expressing taste cells', *PNAS*, 108: 5431-6.

Zhang, F., B. Klebansky, R. M. Fine, H. Liu, H. Xu, G. Servant, M. Zoller, C. Tachdjian, and X. Li. 2010. 'Molecular mechanism of the sweet taste enhancers', *Proc Natl Acad Sci U S A*, 107: 4752-7.

Zhang, J. , H. Lee, and L. J. Macpherson. 2021. 'Mechanisms for the sour taste.' in R.K. Palmer and G. Servant (eds.), *The Pharmacology of Taste . Handbook of Experimental Pharmacology* (Springer, Cham).

Zhang, J., H. Jin, W. Zhang, C. Ding, S. O'Keeffe, M. Ye, and C. S. Zuker. 2019. 'Sour Sensing from the Tongue to the Brain', *Cell*, 179: 392-402 e15.

Zhong, M., Y. Chong, X. Nie, A. Yan, and Q. Yuan. 2013. 'Prediction of sweetness by multilinear regression analysis and support vector machine', *Journal of Food Science*, 78: S1445-50.

VI. Abstract

Humans have an innate preference for sweet taste because it indicates a high energy content in food. Although the sweet taste is known to be mediated by the G-protein coupled receptor TAS1R2/R3, many aspects remain unclear. There is a wide variety of sweet-tasting compounds, but they show great differences in the sensory profile, especially regarding the temporal profile and side tastes, but knowledge regarding the molecular origin of these differences is scarce. Furthermore, their effects on glucose metabolism can be very diverse. This thesis investigated the interaction of the structural properties and the temporal sensory profile of sweet tasting compounds and its consequences for mouthfeel and metabolic effects.

The main part of this thesis focused on the differences in the temporal taste profile of diverse sweet compounds in relation to their structures and binding-sites. Additionally of interest were the flow rate and viscoelasticity of saliva as part of overall mouthfeel while consuming selected diverse sweet compounds. The results show that the number of ketones, aromatic rings, double bonds and the MLogP had an essential role on the temporal profile of sweet tasting compounds. Looking more closely at the temporal parameters of selected sweeteners, no relationship was found to their corresponding binding-site studied by competitive or allosteric inhibition due to lactisole. The flow rate and viscoelasticity of saliva, which contribute to the overall mouthfeel, indicated that individual sweet taste perception by the test persons was more relevant for salivary flow than differences between compounds, while viscoelasticity was related to the MUC5B content of saliva. Hence, the sweet taste is individually dependent on structure of a compound, while salivary attributes are mainly influenced by cognitively perceived sweetness.

The secondary part of this thesis gives first insights into the contribution of differences in the perceived sweetness level of two carbohydrates to blood glucose metabolism. The adjustment of the sweetness of sucrose with lactisole to the sweetness level of glucose had no effect on plasma glucose and insulin levels. Blood glucose metabolism is rather regulated by the type of carbohydrate then by the perceived sweetness.

Taken together, the results of the present thesis suggest that more complex structures tend to have a longer lasting lingering and induce an increased salivary flow, independent of the binding site to the sweet taste receptor. The individual structure of a compound should play the dominant role in further research and application fields, especially for the temporal characteristics of sweet tasting compounds and metabolic consequences.

VII. Zusammenfassung

Der Mensch hat eine angeborene Vorliebe für den süßen Geschmack, da dieser auf einen hohen Energiegehalt der Nahrung hinweist. Obwohl bekannt ist, dass der süße Geschmack durch den G-Protein-gekoppelten Rezeptor TAS1R2/R3 vermittelt wird, bleiben viele Aspekte unklar. Es gibt eine Vielzahl von süß schmeckenden Verbindungen, die jedoch große Unterschiede im sensorischen Profil aufweisen, insbesondere hinsichtlich des zeitlichen Profils und der Nebengeschmäcker, wobei der molekulare Ursprung dieser Unterschiede derzeit nicht gut verstanden ist. Außerdem können ihre Auswirkungen auf den Glukosestoffwechsel sehr unterschiedlich sein. In der vorliegenden Arbeit wurde die Wechselwirkung zwischen den strukturellen Eigenschaften und dem zeitlichen sensorischen Profil von süß schmeckenden Verbindungen und deren Folgen für das Mundgefühl und die metabolischen Auswirkungen untersucht.

Der Hauptteil dieser Arbeit konzentrierte sich auf Unterschiede im zeitlichen Geschmacksprofil verschiedener süßer Verbindungen in Abhängigkeit von ihren Strukturen und Bindungsstellen. Darüber hinaus wurden die Flussrate und die Viskoelastizität des Speichels als Teil des gesamten Mundgefühls beim Verzehr ausgewählter süßer Verbindungen untersucht. Die Ergebnisse zeigen, dass die Anzahl der Ketone, der aromatischen Ringe, der Doppelbindungen und des MLogP eine wesentliche Rolle für das zeitliche Profil der untersuchten Verbindungen spielten. Bei näherer Betrachtung der zeitlichen Parameter ausgewählter Süßstoffe wurde keine Beziehung zu ihrer entsprechenden Bindungsstelle gefunden, die durch kompetitive oder allosterische Hemmung durch Lactisol untersucht wurde. Die Sekretionssrate und die Viskoelastizität des Speichels, die zum gesamten Mundgefühl beitragen, zeigten, dass die individuellen Süßwahrnehmung für die erhöhte Speichelsekretion relevanter waren als die Unterschiede zwischen den Testsubstanzen, während die Viskoelastizität mit dem MUC5B-Gehalt des Speichels zusammenhing. Der süße Geschmack ist also individuell von der Struktur einer Verbindung abhängig, während die Speicheleigenschaften hauptsächlich von der kognitiv wahrgenommenen Süße beeinflusst werden.

Der zweite Teil dieser Arbeit gibt erste Einblicke in den Beitrag von Unterschieden im wahrgenommenen Süßgeschmack von zwei Kohlenhydraten zum Blutzuckerstoffwechsel. Die Anpassung der Süße von Saccharose mit Lactisol an den Süßegrad von Glukose hatte keine Auswirkungen auf den Plasmaglukose- und Insulinspiegel. Der Blutzuckerstoffwechsel wird eher durch die Art des Kohlenhydrats als durch die wahrgenommene Süße reguliert.

Insgesamt deuten die Ergebnisse der vorliegenden Arbeit darauf hin, dass komplexere Strukturen tendenziell ein längeres Lingering und einen erhöhten Speichelfluss induzieren, unabhängig von der Bindungsstelle an den Süßgeschmacksrezeptor. Die individuelle Struktur einer süßen Verbindung sollte in weiteren Forschungs- und Anwendungsfeldern die dominierende Rolle spielen, insbesondere für das temporale sensorische Profil und deren metabolischen Konsequenzen.