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,*]

[a] Christian Doppler Laboratory for Taste Research, Faculty of Chemistry, University of Vienna, Vienna, Austria
[b] Symrise Distribution GmbH, Vienna, Austria
[c] Symrise AG, Holzminden, Germany
[d] Institute of Food Science, University of Natural Resources and Life Sciences, Vienna, Austria
[e] Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria

ARTICLE INFO

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

---

* Corresponding author at: Christian Doppler Laboratory for Taste Research, Faculty of Chemistry, University of Vienna, Vienna, Austria.
E-mail address: barbara.lieder@univie.ac.at (B. Lieder).

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.
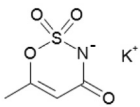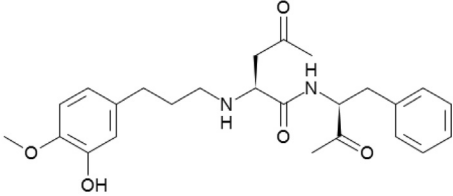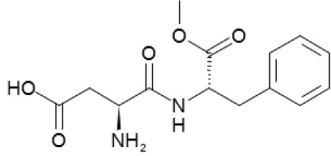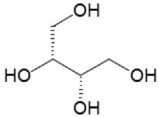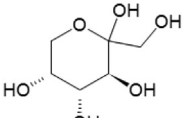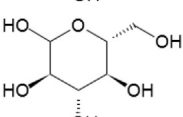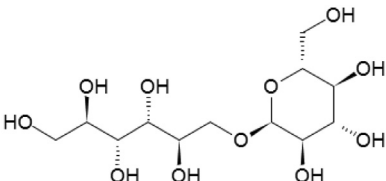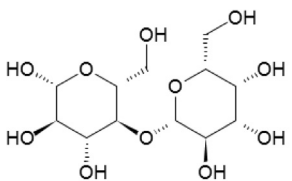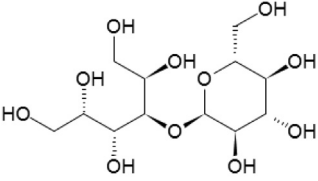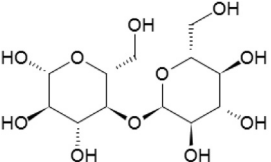
3. Results & discussion

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),
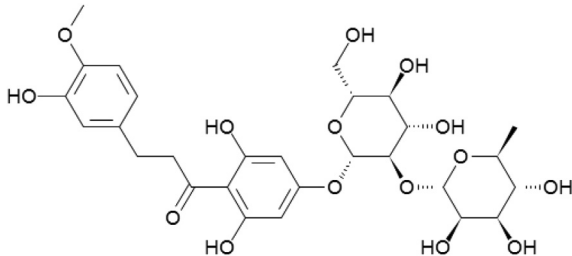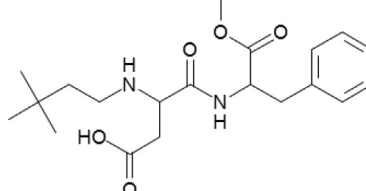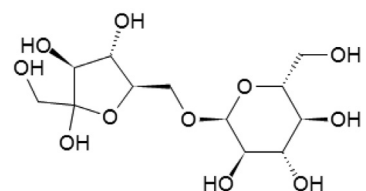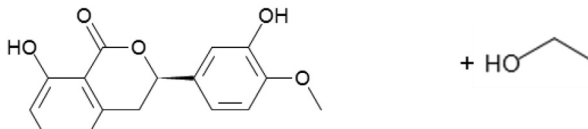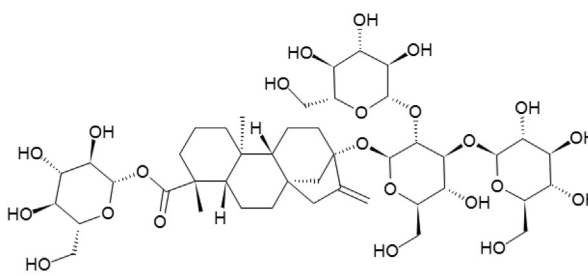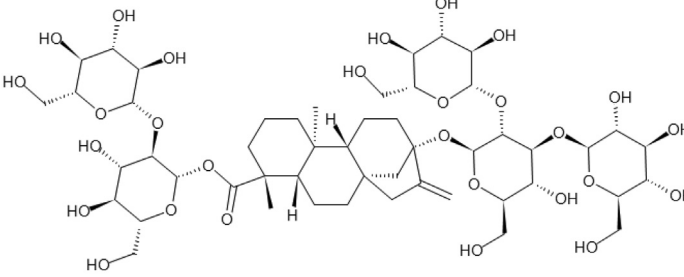
Table 1

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	
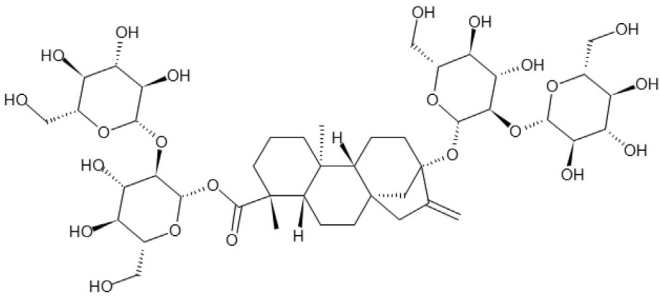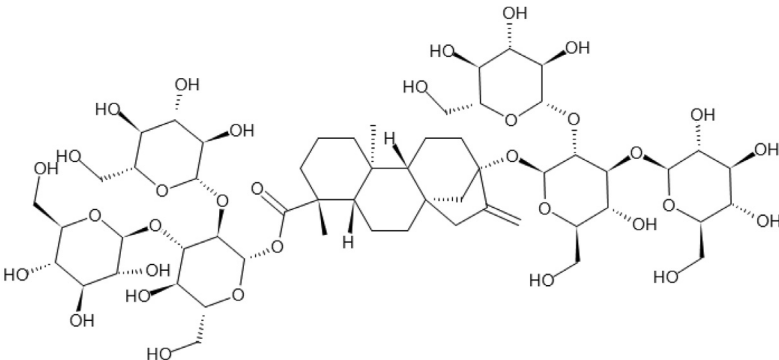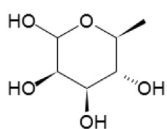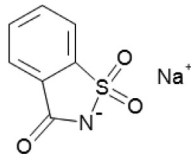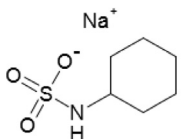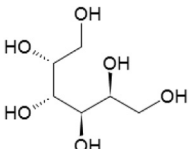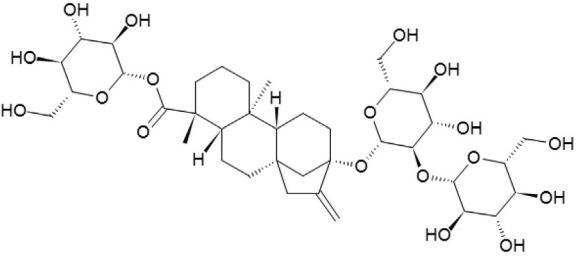
(*continued on next page*)

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	
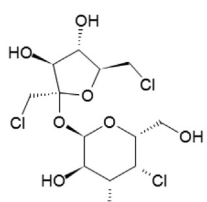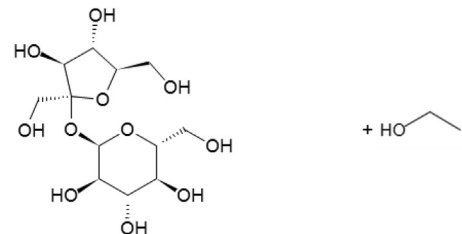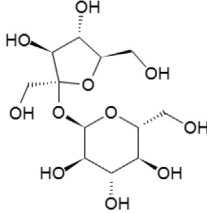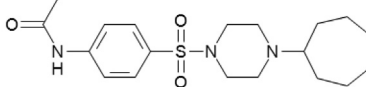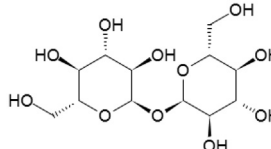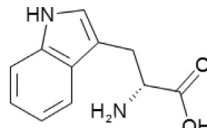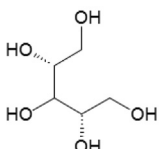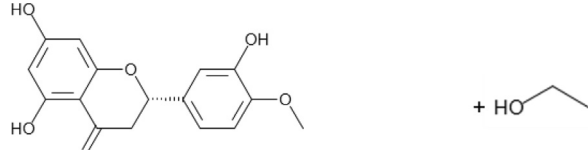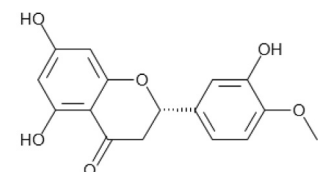
(*continued on next page*)

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	

6
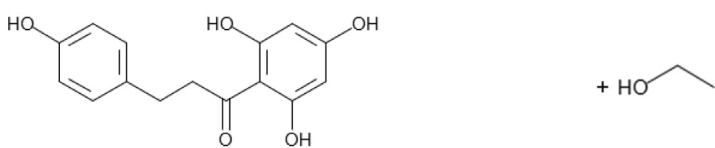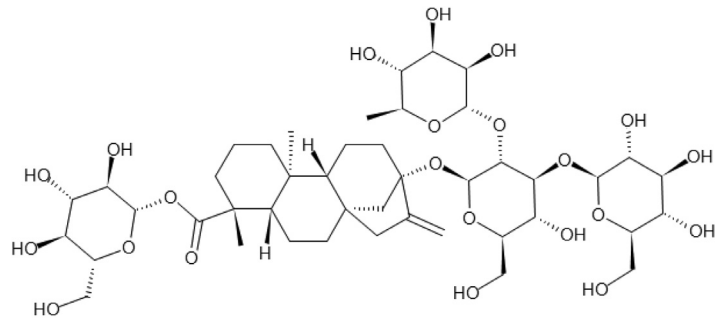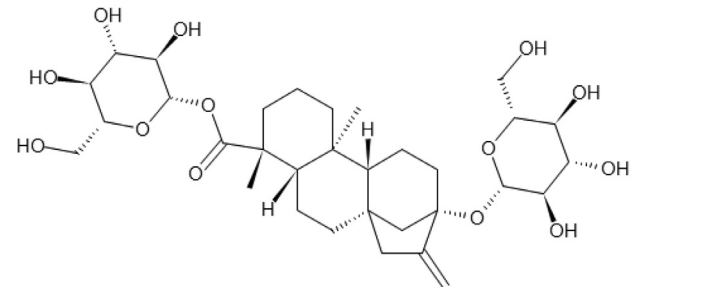
Table 1 (*continued*)

	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	

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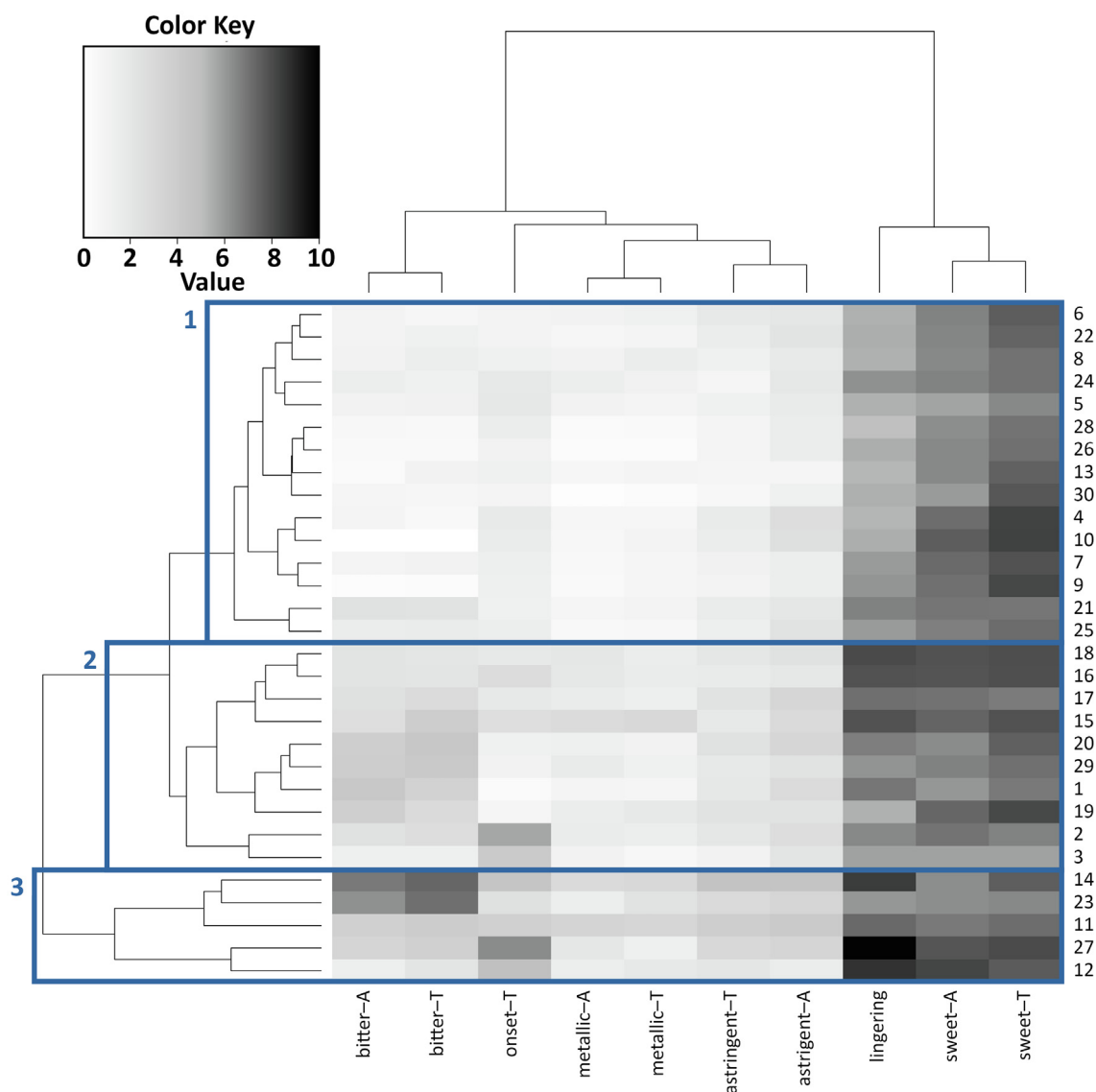
Fig. 1. Heatmap of sensory attributes of sweet and sweet taste affecting compounds with 3 clusters of compounds with rating values 0 – 10. T = taste; A = aftertaste. Numbers refer to compounds given in Table 1.

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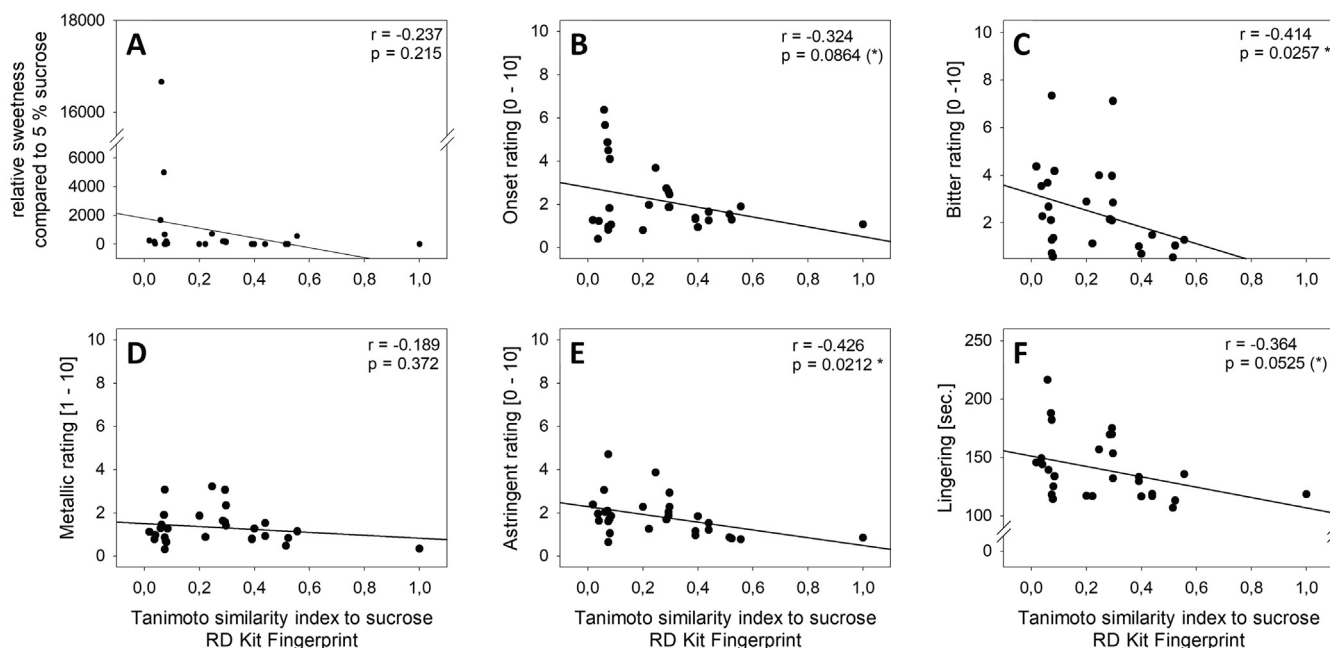
Fig. 2. Pearson correlation of the RD Kit-Fingerprint-Tanimoto similarity index to sucrose with the sensory rating of A: relative sweetness compared to 5% sucrose, B: onset, C: bitter, D: metallic, E: astringent and F: lingering.

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.

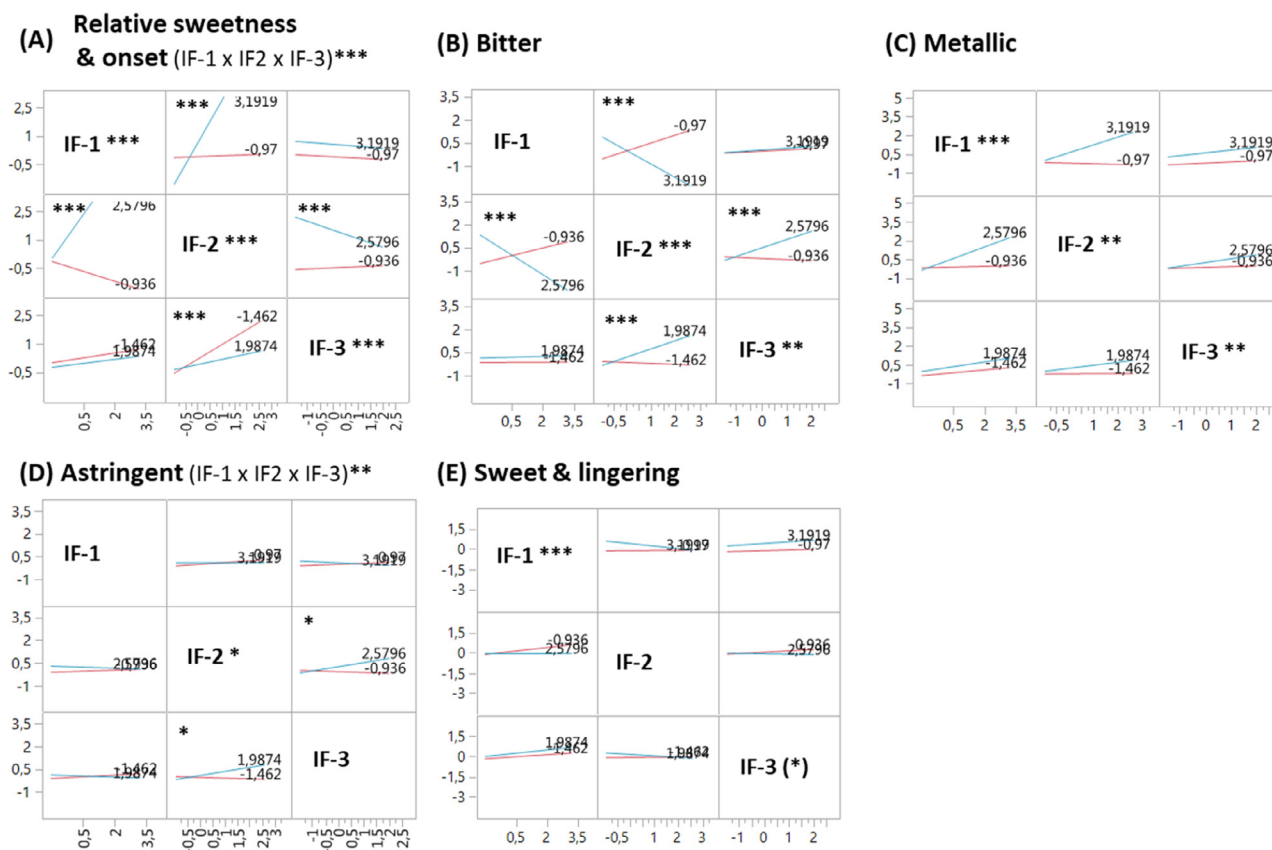| dependent factors / indep. factors	FDR LogWorth	relative sweetness & onset	bitter	metallic	astringent	sweet & lingering
		t ratio				
Intercept		2.24 *	−0.02	0.00	−0.92	0.19
IF-1	12.63 ***	7.45 ***	0.55	5.39 ***	0.71	3.88 ***
IF-2	91.71 ***	23.08 ***	4.83 ***	2.59 **	2.35 *	−0.87
IF-3	3.48 ***	−3.6 ***	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

Acknowledgments

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.