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Evaluation of Unsaturated Alkanoic Acid Amides as Maskers of Epigallocatechin Gallate Astringency

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Supporting Information

ABSTRACT: Some foods, beverages, and food ingredients show characteristic long-lasting aftertastes. The sweet, lingering taste of high intensity sweeteners or the astringency of tea catechins are typical examples. Epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea, causes a long-lasting astringency and bitterness. These sensations are mostly perceived as aversive and are only accepted in a few foods (e.g., tea and red wine). For the evaluation of the aftertaste of such constituents over a certain period of time, Intensity Variation Descriptive Methodology (IVDM) was used. The approach allows the measurement of different descriptors in parallel in one panel session. IVDM was evaluated concerning the inter- and intraindividual differences of panelists for bitterness and astringency of EGCG. Subsequently, the test method was used as a screening tool for the identification of potential modality-selective masking compounds. In particular, the intensity of the astringency of EGCG (750 mg kg^{-1}) could be significantly lowered by 18−33% during the time course by adding the trigeminal-active compound *trans*-pellitorine (2*E*,4*E*-decadienoic acid *N*-isobutyl amide **1**, 5 mg kg^{-1}) without significantly affecting bitterness perception. Further, structurally related compounds were evaluated on EGCG to gain evidence for possible structure−activity relationships. A more polar derivative of **1**, (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]amino]propanoic acid **9**, was also able to reduce the astringency of EGCG similar to *trans*-pellitorine but without showing the strong tingling effect.

KEYWORDS: *time-intensity, sensory evaluation, taste, catechins, epigallocatechin-3-gallate, green tea, astringency, bitterness, alkamides*

INTRODUCTION

Green tea, brewed from the leaves of *Camellia sinensis*, is one of the most popular beverages worldwide and contains high amounts of catechins, especially epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG).[1−3] The consumption of tea catechins seems to be correlated with a number of health benefits, such as anti-inflammatory,[4] antioxidant,[5] and cardio-protective effects.[6] Beside these beneficial properties, catechins impart the typical astringency and concomitant bitter sensation to green tea. Astringency of EGCG is often described as mouth-drying, puckering, or rough.[7,8] For some exceptions such as green and black teas, red wine, and dark chocolate, astringency is accepted or even considered as a positive quality parameter.[9] In many cases, however, astringency is perceived as aversive and leads to avoidance of ingestion, especially at high levels. Besides catechins, some other polyphenols have been described as elicitors of astringency: tannic acid[10] and flavanol glycosides,[11] as well as some metal salts such as aluminum sulfate[12] or acids, like tartaric acid.[13]

Several solutions to avoid or to reduce off-notes have been developed during the last few years: removal, scavenging, encapsulation, and masking are only a few examples.[14] However, reduction or removal of these compounds in certain food and beverage applications is not always an option due to their potential health benefits. Sometimes, these compounds are even enriched to obtain so-called "functional foods". The use of such potential functional ingredients is getting more and more important, especially concerning their antioxidant activity.[15,16]

The molecular mechanism of astringency perception is still unclear. Currently, two main hypotheses are discussed. The first theory focuses on the fact that numerous astringent molecules are able to complex and/or to precipitate certain proteins in saliva, especially proline-rich basic proteins. This was, for example, shown for (+)-catechin, (−)-epicatechin, (−)-epicatechin *O*-gallate, the procyanidin dimers B1−B8, and trimer C1.[17,18] Because of the precipitation, the viscosity and the lubricant properties of the saliva are drastically changed, which are probably detected by tactile neurons. This is comprehensible, considering the fact that moving the tongue enhances the sensation.[19] Recent studies, however, showed that at least some of the astringency elicitors are sensorially active while having no or only low protein binding activity.[20,21] This indicates that they might interact directly with still unknown receptors. In this case, it should be possible to find antagonists reducing astringency without protection of saliva proteins against precipitation.

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Many tastants such as catechins do not show only a single taste quality but meet a whole dimension of descriptors. For an adequate description of various taste-actives and their aftertaste properties, a time−intensity profile is fundamental. This is especially important for sweeteners with their sweet lingering after-taste[22−24] or catechins,[7] polyphenol glycosides,[25] and tannins,[26] showing lingering bitter and/or astringency sensations. Therefore, the use of time−intensity (TI) measurements dealing with more than one descriptor is necessary. In early TI methods, the panelists were asked to trace the intensity with a pencil on a sheet of paper over time.[27] Guinard et al.[28] developed in 1985 a computerized procedure for time−intensity measurements. Different approaches, such as dual-attribute time−intensity (DATI)[29] and time−intensity profiling (TIP)[30] were described in the following years. DATI allows parallel time−intensity measurements of two descriptors on a two-dimensional plane. The panelists record the changes by moving the mouse on the plane in two directions at the same time, while the data are collected every 3 s. TIP enables the evaluation of even more than two attributes but only in a consecutive way, which is more time-consuming. Usually, only one attribute is described per session, and the different descriptors of one sample or similar attributes of various samples are compared afterward.

Intensity Variation Descriptive Methodology (IVDM) is another variation of time−intensity scaling allowing the evaluation of multiple attributes in parallel over a certain period of time.[31] It was originally developed as a modification of the descriptive analysis procedure (QDA)[32] that is commonly used in the evaluation of food products. With QDA, trained panelists measure the intensities of key product attributes, and the results are presented in a spider web as a multidimensional model. The combination of multiple attribute ratings at discrete time points is focused on in IVDM. The intensities were rated on line scales, one for each descriptor that occurred at defined time points.[31,33] The method was used for documentation of the time-course, e.g., of astringency sensations of tannic acid, alum, and tartaric acid[33] and of the sweet taste of high intensity sweeteners.[34] Depending on the problem, the number of descriptors and measuring points, the time between the measuring points, and the duration of the test can be varied. With this method, possible relationships between the different descriptors can be observed. By rating them in parallel, we observed that there is a closer correlation between the descriptors compared to that with rating each separately. Especially for complex food samples, this trait can be very important.

The objectives of the present study were to evaluate IVDM regarding panel performance for complex taste profiles of astringent compounds such as EGCG and subsequently to use this method for the screening of compounds having astringency-masking effects. In earlier studies, it was found that certain trigeminal-active compounds, especially tingling compounds such as *trans*-pellitorine (2*E*,4*E*-decadienoic acid *N*-isobutyl amide **1**), can induce salivation.[35] In order to elucidate the hypothesis that weak saliva induction may be correlated with a reduction of astringency sensation, the potential astringency-masking effect of *trans*-pellitorine and other saliva-inducing compounds was evaluated. In a second step, further structurally related compounds without known saliva-inducing effects, such as *cis*-pellitorine **2** and 3*E*-nonenoic acid *N*-isobutyl amide **5**, were tested.

MATERIALS AND METHODS

Chemicals. The following materials were obtained commercially: epigallocatechin gallate (EGCG, 90%, Changsha Sunfull Biotech Co., Ltd., Changsha, China), grape seed extract 848F exGrape, OPC40 (BREKO GmbH, Bremen, Germany), caffeine, ethanol absolute puriss. p.a. min 99.8% (both Sigma-Aldrich, Steinheim, Germany), ascorbic acid (Nutrilo GmbH, Cuxhaven, Germany), and Vittel water (Nestlé Waters Deutschland GmbH, Mainz, Germany).

trans-Pellitorine (2*E*,4*E*-decadienoic acid *N*-isobutyl amide **1**) and *cis*-pellitorine (2*E*,4*Z*-decadienoic acid *N*-isobutyl amide **2**) were synthesized according to Ley et al.,[36] and 2*E*-decenoic acid *N*-isobutyl amide **3**, decanoic acid *N*-isobutyl amide **4**, 3*E*-nonenoic acid *N*-isobutyl amide **5**, and achilleamid (2*E*,4*E*-decadienoic acid *N*-piperidyl amide **9**) were also synthesized according to Ley et al.[37] Spilanthol (2*E*,6*Z*,8*E*-decatrienoic acid *N*-isobutyl amide **8**) was isolated from *Heliopsis longipes* extract (Phytos, Inc., San Anselmo, USA) by preparative HPLC. The amides 2*E*,4*E*-dodecadienoic acid *N*-isobutyl amide **6** and 2*E*,4*E*-tetradecadienoic acid *N*-isobutyl amide **7** were synthesized via NaOH supported amidation of the appropriate acid chlorides with isobutyl amine. The parent acids were prepared by a Wittig−Horner reaction of 2*E*-decenal and 2*E*-dodecenal, respectively, with triethylphosphonoacetate and subsequent saponification in KOH and activation by reaction with thionylchloride. Spectral and physical data of the amides **6** and **7** corresponded to literature data.[38] All compounds had a purity of >95% (GC).

Synthetic Procedure. Reagents and solvents were purchased from commercial suppliers (Acros Organics, Geel, Belgium, Sigma-Aldrich, Steinheim, Germany) and used without further purification or drying. Monitoring of the reaction was accomplished by thin-layer chromatography on silica gel GF254 plates with either UV detection or by use of a staining reagent consisting of ammonium molybdate, cerium sulfate, and sulfuric acid. Melting points are uncorrected and were determined using a Stuart SMP 10. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Varian Mercury Plus spectrometer using tetramethylsilane as internal reference. IR spectra were recorded from a KBr pellet using a Nicolet 6700 GCFT-IR (Thermo Fisher Scientific). LC-MS spectra were obtained by using a coupled system consisting of a Waters Acquity UPLC (column, Kinetex RP-C18, 1.7 μm (100 × 2.1 mm); solvent system, water/acetonitrile/formic acid) and a Bruker micrOTOF II. Optical rotation was determined using a Schmidt+Haensch Unipol L 1000.

Synthesis of (2S)-2-[[(2E,4E)-Deca-2,4-dienoyl]amino]propanoic Acid ***10.*** A suspension of 25.0 g (179.1 mmol) of L-alanine methyl ester hydrochloride in 500 mL dichloromethane was cooled down to −15 °C, and subsequently, 24.0 g (303.4 mmol) of pyridine was added followed by 37.0 g (198.2 mmol) of (2*E*,4*E*)-deca-2,4-dienoyl chloride. The mixture was allowed to warm up to room temperature and was stirred for an additional 12 h. The reaction mixture was washed three times with 5% aqueous hydrogen chloride and twice with water. The organic layer was dried over sodium sulfate, and after evaporation of the solvent, the remaining solid was dissolved in 50 mL of methyl *tert*-butyl ether. After cooling down to −20 °C, the mixture started to solidify and was diluted by the addition of 100 mL of *n*-hexane. After filtration, the product was further purified by recrystallization in 150 mL of a mixture of *n*-hexane and methyl *tert*-butyl ether (2:1, v/v). After drying, 24.1 g (95.1 mmol) of the colorless methyl (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]amino]propanoate was obtained, of which 22.5 g (88.8 mmol) was added to a solution of 3.9 g (97.5 mmol) of sodium hydroxide in 160 mL of water and stirred for 48 h at room temperature. Subsequently, the solution was diluted with 500 mL of water and acidified by addition of 50 g of 10% aqueous hydrogen chloride. After filtration, the obtained solid crude product was further purified by recrystallization from 170 mL of methyl *tert*-butyl ether. After drying, 11.6 g (48.5 mmol, 29%) of the expected (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]amino]propanoic acid was obtained as a colorless solid. mp = 108 °C (under decomposition). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, J = 6.9 Hz; 3H, $CH_2C\underline{H}_3$), 1.25 − 1.46 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 1.49 (d, J = 7.2 Hz, 3H, $NHCHC\underline{H}_3$), 2.15 (m, 2H, $CH_2C\underline{H}_2CH$), 4.64 (*dq*, J = 7.0/7.2 Hz, 1H, $NHC\underline{H}CH_3$),

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5.82 (*d*, *J* = 15.0 Hz, 1H, COCHCH), 6.10 − 6.15 (m, 2H, CH_2CHCH), 6.75 (d, *J* = 7.0 Hz, 1H, CONH), 7.22 (m, 1H, COCHCH), 7.79 (bs, 1H, COOH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0 (CH_2CH_3), 18.0 ($NHCHCH_3$), 22.5 (CH_2CH_3), 28.4 ($CH_3CH_2CH_2CH_2$), 31.4 ($CH_3CH_2CH_2CH_2$), 33.0 (CH_2CH), 48.6 ($NHCHCH_3$), 120.3 (COCH), 128.1 (COCHCHCH), 143.1 (COCHCHCH), 144.8 (CH_2CH), 167.2 (CONH), 175.7 (COOH) ppm. IR (KBr) ν = 3299, 2924, 1705, 1655, 1628, 1616, 1531, 1455, 1419, 1373, 1361, 1333, 1308, 1287, 1248, 1209, 1184, 1163, 1116, 1049, 996, 942, 910, 877, 647, 604 cm^{-1}. LC-MS (ESI$^+$, 4500 V): *m*/*z* (r.I.%) = 263 (5), 262 ($M^{+\bullet}$ + Na, 36), 241 (15), 240 ($M^{+\bullet}$ + H, 100), 151 (37). HRMS (ESI$^+$) calc. for $C_{13}H_{23}NO_3$ *m*/*z* $(M + H)^{+\bullet}$ 240.1594; found, 240.1592; $[\alpha]_D^{22}$ = +22.7° (c = 1.01, C_2H_5OH).

Prior to further evaluation of (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]-amino]propanoic acid, an Ames test (*Salmonella typhimurium* reversed mutation assay) with/without metabolic activation was performed according to OECD guidelines.[39]

Sensory Analysis. *General Conditions and Preparation of the Test Solutions.* Sensory tests were carried out with 15 healthy and trained panelists without known taste disorders. Panelists were fully informed about procedure and intention of the project and had given written consent. They were advised not to swallow the samples, and samples were tested using the sip and spit method. All test sessions were run in the morning hours with a minimum of 1−2 h after breakfast. During this time, the panelists were asked not to drink coffee or black tea. The samples were tested in sensory panel rooms under standardized conditions, and they were given in a blind and randomized manner. For the preparation of the test solutions, Vittel water was used. The test solutions containing pure EGCG were stabilized with 125 mg kg^{-1} ascorbic acid to avoid oxidation of the catechins. This amount of ascorbic acid had no influence on the taste perception (data not shown). Between two samples, the panelists cleaned their palate with Vittel water, white bread, and white chocolate. The fatty matrix of the white chocolate was found to help to compensate the mouth drying and puckering perception of astringent compounds.

Panel Training. Panelists were trained to evaluate the bitterness and astringency, respectively, of different samples and to differentiate between both descriptors. Caffeine was used as a typical reference for bitterness only. Grape seed extract was perceived as astringent with only a slightly bitter taste, while EGCG was described to be astringent as well as bitter. References were offered to the panel in different concentrations (300, 500, 750, and 1000 mg kg^{-1}). A number of testing sessions were carried out to get familiar with both the references and the test method. The panel was trained in group sessions at the beginning where the test procedure was explained in detail. The panelists were instructed to press the start button as soon as they spit out the second sip of the sample. During the actual measurement, we gave instructions on the computer screen before every test. In addition, training sessions were carried out using Compusense FCM Feedback Calibration Method.[40] After rating the intensities of a sample, panelists got immediate feedback on their performance by comparing their ratings with the results of 90% of the participants.

Intensity Variation Descriptive Methodology (IVDM). The panelists took two sips of a sample, 5 mL each. After spitting out the sample, the measurement was started by the panelist, and intensities were rated repeatedly after defined time intervals: right at the start (*t* = 0 s), after 10 s, and afterward repeatedly every 20 s three times. Multiple attributes were rated at the same time by using an unstructured horizontal line scale (12 cm) with anchors at the end points for each descriptor. The left end of the scale was marked with the terms "not bitter/astringent" (0%) and the right end with "extremely bitter/astringent" (100%). Line scale ratings were reset after each measuring point. A maximum of two samples per session and per day were offered to the panelists. Data were collected and analyzed using Compusense five. The arithmetic average of all panelists and replications was represented graphically in a time−intensity curve.

Bitterness and astringency were measured for EGCG concentrations ranging from 45 to 3750 mg kg^{-1}. For masking effects, different concentrations of EGCG (750 mg kg^{-1}) and grape seed extract (500 and 750 mg kg^{-1}), were tested in combination with possible masking compounds. As potential maskers *trans*-pellitorine **1**, *cis*-pellitorine **2**, and further structure-related compounds **3**−**10** were evaluated in pretests and via IVDM.

Statistical Analysis. One-way analysis of variance (ANOVA) and Tukey−Kramer HSD test were used to analyze the dose−response curves of EGCG. Data were analyzed using JMP 10 (SAS, UK). Statistical analyses of the masking tests were carried out via Student's *t*-test (one-tailed and heteroscedastic). Error bars indicate the standard deviation of all panelists and all replications, calculated in Microsoft Excel.

RESULTS AND DISCUSSION

In green tea or beverages prepared with green tea extract, various catechins, such as EGCG, epigallocatechin, and epicatechin-3-gallate, play important roles and contribute to the complex flavor sensation.[7] EGCG as the most abundant catechin in green tea[1,2] was used as the reference compound for masking tests. Besides astringency, it also shows a strong bitterness that is also perceived as unpleasant by several people, especially at higher concentrations.

In order to describe these complex flavor profiles and to identify masking effects, a screening tool was necessary to evaluate different candidates. The method should save time, panel work, and the amount of test substances. Paired comparison, duo−trio, or triangle tests are commonly used for the identification of differences between samples.[41] For long-lasting effects, these methods are not sufficient since samples are compared only at one time point and not over a period of time. The other problem is the build-up effect of astringency when comparing different samples. Accordingly, a time−intensity design, based on IVDM, was used as a screening tool for the identification of flavor-modulating effects.

With IVDM, the focus is on consecutively monitoring the intensities of one or more attributes over time. This protocol, in combination with panel training, allows the analysis of several descriptors at the same time. The panelists rate the intensities at defined time points compared to the quasi continuous measurement at TI. This is easier to handle, less exhaustive, and less interference-prone because of the sensitive mouse reaction at TI. Furthermore, the repeated appearance of the line scales makes the panelists aware of the sensations again at every interval.

Differentiation between Descriptors. Tasting sessions were carried out using a combination of typical bitter and astringent compounds to show that the panel was reproducibly able to differentiate between bitterness and astringency. Therefore, EGCG, grape seed extract, and caffeine were used in different combinations and concentrations. In one trial, panelists rated the bitterness and astringency of 500 mg kg^{-1} EGCG without and with 500 mg kg^{-1} caffeine, at which the astringency did not change between both samples, while the bitter taste increased significantly ($p < 0.05$) with added caffeine (Figure F1, Supporting Information). By adding 500 mg kg^{-1} grape seed extract to 500 mg kg^{-1} EGCG, the astringency increased significantly ($p < 0.05$), whereas the bitterness was not affected (Figure F2, Supporting Information). After these training sessions, panelists were able to differentiate between the two attributes.

Dose−Response of Individual Taste Qualities of EGCG. Panel tests included ratings of 12 different EGCG

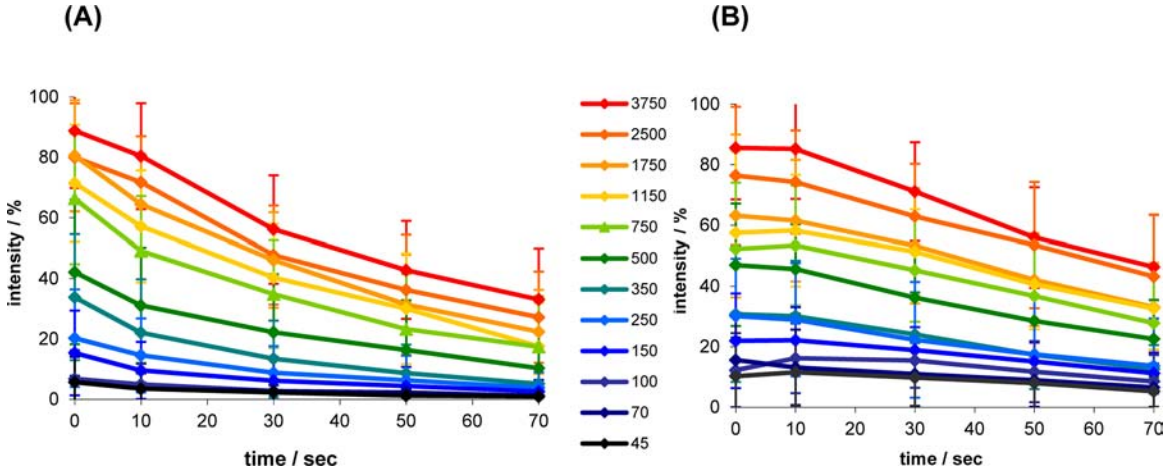

Figure 1. Time−intensity relationship of EGCG for the descriptors bitter (A) and astringent (B) at different concentrations (45 − 3750 mg kg^{-1}) from 0 to 70 s; n = 15.

concentrations, ranging from 45 mg kg^{-1} to 3750 mg kg^{-1}. The results for bitterness and astringency are displayed for every concentration and over the time of 70 s in Figure 1.

The time−intensity curves showed a clear dose−response relationship. As expected, 3750 mg kg^{-1} was rated with the highest bitter and astringent intensity, and each lower concentration showed a stepwise lower intensity. Differentiability of the tested concentrations was determined. The differences between the consecutive concentrations in the higher and lower concentration range, for bitterness, e.g., 1750 to 3750 mg kg^{-1} and 45 to 150 mg kg^{-1} at 10 s, respectively, are detectable but not significant (Table T1, Supporting Information). The bitterness and astringency could be differentiated significantly ($p < 0.1$) for medium concentrations rated around 50% intensity. The time−intensity curves for the bitter ratings (Figure 1A) declined more rapidly compared to the time−intensity curves for the descriptor astringent (Figure 1B). Especially for the first two measuring points, this effect is distinct. The intensities for astringency in the time slot from 0 to 10 s were nearly constant for all tested concentrations, and the decrease, starting after 20 s is not as steep as that for the bitter ratings. These data confirm the long-lasting effects of bitterness and astringency, with the latter being more pronounced.

Using this protocol, panelists were capable of differentiating between the descriptors bitterness and astringency and their intensities in simple EGCG model solutions (Figure 1). By testing the attributes at the same time, possible interactions can be more easily determined. IVDM permits the evaluation of the aftertaste sensation of complex taste profiles in a very time-saving manner. Therefore, the workload of the panelists and risk of adaptation to the test base can be reduced as well.

On the basis of these results, the optimal dosage of EGCG for masking trials was selected. The optimal dosage should be not too weak for the panelists to detect possible reduction effects and not too strong, as some compounds might also have enhancing effects. Moreover, the variance between the panelists also plays a major role and has to be considered. Therefore, the reference concentration for the following tests was selected to be around the 50% intensity level, in our case at 750 mg kg^{-1}. The test duration was set to be 70 s as the aim was the development of a screening tool for the identification of masking compounds. As soon as a compound shows a masking effect, further tests can be carried out, for example, for a longer period.

Inter- and Intra-Individual Differences of Panelists. The variations of the ratings between different panelists and between different sessions of one panelist were analyzed for the selected reference concentration. The time−intensity curves for the bitterness of 750 mg kg^{-1} EGCG of five panelists were chosen to present the interindividual differences (Figure F3A, Supporting Information). The intensities differ with 20% between the lowest and highest ratings at the selected time points (e.g., 37 and 76% at 0 s; 20 and 42% at 30 s). The arithmetic mean and the standard deviation of these panelists were calculated (Figure F3B, Supporting Information). The standard deviation is in the range of 8 to 17% and is comparable to the standard deviation of the astringency description (Figure F4B, Supporting Information). Between the different panelists, the single time−intensity curves for astringency differ to a certain extent, but the overall shape is comparable (Figure F4A, Supporting Information).

Besides the group performance, the reproducibility of the results of every single panelist is important as well. Therefore, the time−intensity curves of a randomly selected panelist are exemplarily shown for three separated sessions. The single curves for the bitter ratings of 750 mg kg^{-1} EGCG are nearly similar and differ only by maximal 10% over the whole period (Figure F5A, Supporting Information). The standard deviation of the arithmetic mean is 5%, which is very low (Figure F5B, Supporting Information). Nearly the same statement can be made for the astringency description (Figure F6A,B, Supporting Information). Only at the last two measuring points, the distance between the curves and consequently the standard deviation increased to 10% compared to 5% before. The intraindividual difference of the selected panelist is low and thus the reproducibility very high. The same results can be seen for the other panelists (data not shown).

Screening for Masking Effects against Bitterness and Astringency of EGCG. The panel was asked to rate the bitterness and astringency of the EGCG test solution without and with a potential masking compound. The results of both time−intensity curves were compared statistically to determine the reduction of bitterness and astringency after the addition of the compound. Off-notes caused by the intrinsic taste or flavor of the compounds can lead to a bias and have to be avoided.

Therefore, potential masking compounds were tested at concentrations below their detection level of the known sensory attributes on the catechin base. The test was repeated, and the arithmetic mean was determined and analyzed via Student's *t* test.

Among the selected compounds, the known saliva-inducing flavor compound *trans*-pellitorine (**1**, Figure 3) was tested.[35] By adding 5 mg kg^{-1} **1** to 750 mg kg^{-1} EGCG, the absolute bitterness ratings were reduced at the first rating by 6 percentage points (pp) and during the further time course only weakly by about 1 to 3 pp (relative decrease by 3−23%, Figure 2 and Table 1). However, the absolute intensity of

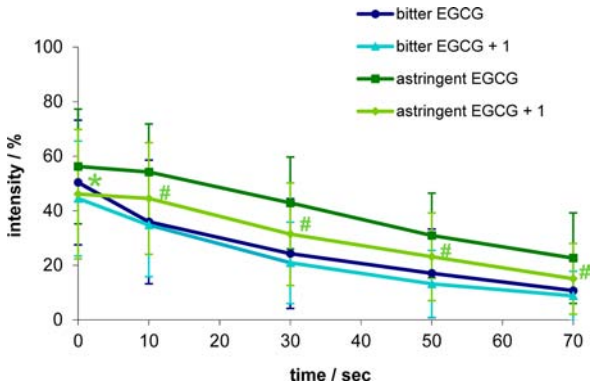

Figure 2. Time−intensity curves of 750 mg kg^{-1} EGCG without *trans*-pellitorine, bitter (dark blue) and astringent (dark green); with 5 mg kg^{-1} *trans*-pellitorine (**1**), bitter (light blue) and astringent (light green); two replications, significant differences $^{*}p \leq 0.1$ and $^{\#}p \leq 0.05$.

astringency could be significantly lowered almost constantly by 7.5 to 11.5 pp over the whole measuring time (relative decrease of 18−33%; Figure 2 and Table 1).

After this distinct astringency masking effect, compounds structurally related to **1** were evaluated. First, the isomer *cis*-pellitorine (**2**, Figure 3) was tested regarding its activity against EGCG off-tastes. The addition of 2 mg kg^{-1} **2** showed the best effect among the tested concentrations and resulted in a masking effect of up to 10 pp (Table 1).

Further, amides with certain structural alterations compared to *trans*-pellitorine (Figure 3) were tested on 750 mg kg^{-1} EGCG. The monosaturated 2*E*-decenoic acid *N*-isobutyl amide

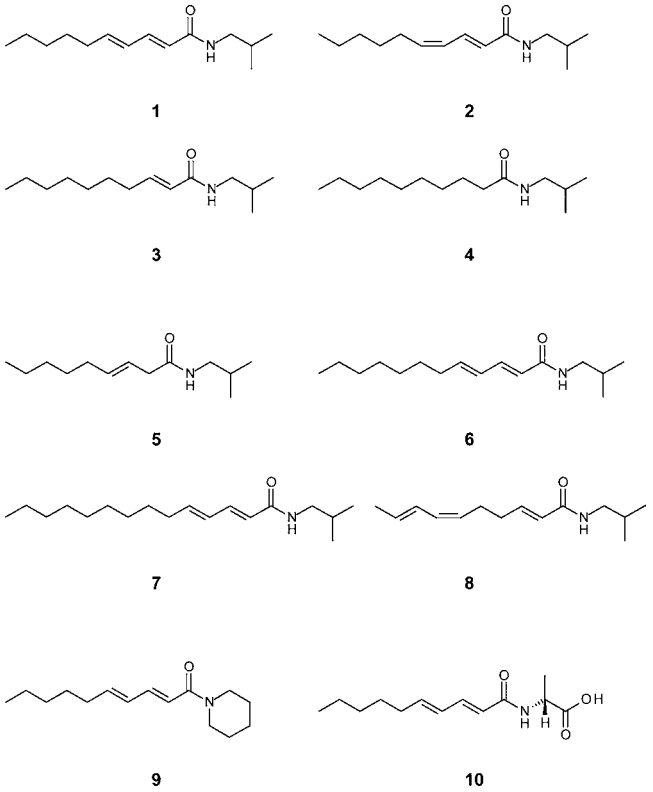

Figure 3. Structures of compounds **1**−**10**: *trans*-pellitorine (2*E*,4*E*-decadienoic acid *N*-isobutyl amide **1**), *cis*-pellitorine (2*E*,4*Z*-decadienoic acid *N*-isobutyl amide **2**), 2*E*-decenoic acid *N*-isobutyl amide **3**, decanoic acid *N*-isobutyl amide **4**, 3*E*-nonenoic acid *N*-isobutyl amide **5**, 2*E*,4*E*-dodecadienoic acid *N*-isobutyl amide **6**, 2*E*,4*E*-tetradecadienoic acid *N*-isobutyl amide **7**, spilanthol (2*E*,6*Z*,8*E*-decatrienoic acid *N*-isobutyl amide **8**), achilleamid (2*E*,4*E*-decadienoic acid *N*-piperidyl amide **9**), and (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]-amino]propanoic acid **10**.

3 and the saturated decanoic acid *N*-isobutyl amide **4** showed no masking effect at 10 and 20 mg kg^{-1} (data not shown). The same result was achieved for 3*E*-nonenoic acid *N*-isobutyl amide **5** at 2, 10, and 20 mg kg^{-1}. At the tested concentrations (10, 50, and 100 mg kg^{-1}), for the two *trans*-pellitorine homologues 2*E*,4*E*-dodecadienoic acid *N*-isobutyl amide **6** and 2*E*,4*E*-tetradecadienoic acid *N*-isobutyl amide **7** with chain

Table 1. Dose−Response Relationship of *trans*-Pellitorine 1, *cis*-Pellitorine 2, and (2*S*)-2-[[(2*E*,4*E*)-Deca-2,4-dienoyl]amino]propanoic Acid 10 at Different Concentrations on 750 mg kg^{-1} EGCG over 70 s and Their Masking Effects for Bitterness and Astringency in Percentage Points (pp; *n* = 15)

time (s)	bitter masking/pp					astringency masking (pp)				
c(EGCG)	0	10	30	50	70	0	10	30	50	70
750 mg kg^{-1}	**1** (5 mg kg^{-1})[a]									
	−5.9	−1.2	−3.4	−3.9	−1.9	−10.1[c]	−9.7[d]	−11.5[d]	−7.8[d]	−7.6[d]
	2 (2 mg kg^{-1})[a]									
	−1.0	1.7	−4.9	−5.4	−4.4	−9.6	−10.0[c]	−9.7	−8.3[c]	−5.2
	10 (50 mg kg^{-1})[b]									
	−2.5	−4.4	−2.6	−2.6	−4.1	−3.8	−5.5[c]	−9.5[d]	−6.7[d]	−5.8[c]
	10 (100 mg kg^{-1})[a]									
	3.1	−0.2	−3.3	−2.0	−3.4	−6.3	−7.8[c]	−8.1[c]	−5.5	−3.1
	10 (250 mg kg^{-1})[a]									
	1.3	0.9	0.2	0.4	−0.5	0.2	−3.4	−1.0	−2.4	−4.7

[a]Two replications. [b]Three replications. Significant differences are shown. [c]$p < 0.1$. [d]$p < 0.05$.

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elongations by two and four carbon atoms, respectively, also no masking effect could be detected on 750 mg kg^{-1} EGCG. As an example, the time−intensity measurements of **7** are presented in Figure 4. The rated astringency is nearly similar with and without **7**, while the bitterness even slightly increased by adding the testing molecule.

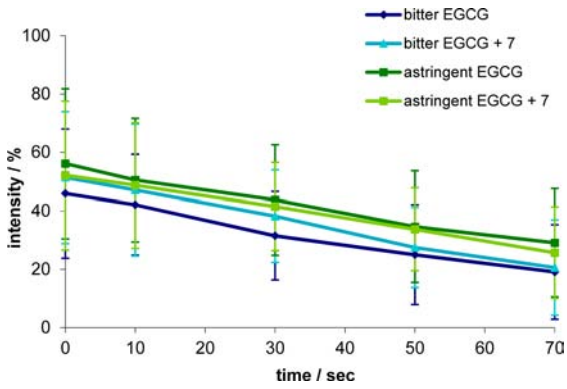

Figure 4. Time−intensity curves of 750 mg kg^{-1} EGCG without **7**, bitter (dark blue) and astringent (dark green); with 10 mg kg^{-1} **7**, bitter (light blue) and astringent (light green); one replication.

The results of the known salivation causing alkamide spilanthol[35,37] **8** as a potential masking compound are not as obvious as in the previous experiments. After three replications, no significant masking effect occurred, except for one measuring point (30 s, Figure 5). Two mg kg^{-1} **8** showed

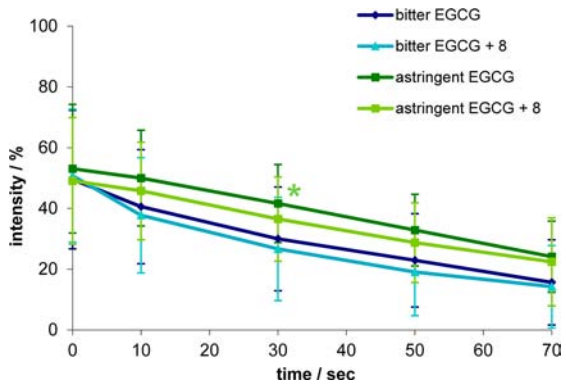

Figure 5. Time−intensity curves of 750 mg kg^{-1} EGCG without **8**, bitter (dark blue) and astringent (dark green); with 2 mg kg^{-1} **8**, bitter (light blue) and astringent (light green); three replications, significant differences $^{*}p \leq 0.1$.

only a hint concerning bitter and astringency masking. Two more concentrations, 1 and 5 mg kg^{-1} were tested as well but without any effect (data not shown).

A further structural variant of *trans*-pellitorine, achilleamid **9**, did not show any effects when added in concentrations of 5 and 20 mg kg^{-1} to 750 mg kg^{-1} EGCG. Additionally, (2*S*)-2-[[(2*E*,4*E*)-deca-2,4-dienoyl]amino]propanoic acid **10**, which is more hydrophilic and better water-soluble due to its amino acid moiety, was synthesized and evaluated. The concentration of **10** was varied (50, 100, and 250 mg kg^{-1}), while the concentration of EGCG was fixed at 750 mg kg^{-1}. The results are presented in Table 1 and exemplarily depicted for 50 mg kg^{-1} in Figure 6. Whereas **10** did not show pronounced tingling effects at these concentrations (data not shown), a significant masking effect

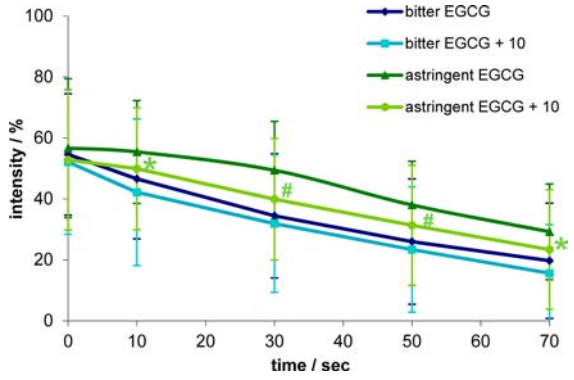

Figure 6. Time−intensity curves of 750 mg kg^{-1} EGCG without **10**, bitter (dark blue) and astringent (dark green); with 50 mg kg^{-1} **10**, bitter (light blue) and astringent (light green); three replications, significant differences $^{*}p \leq 0.1$ and $^{\#}p \leq 0.05$.

was determined for 50 mg kg^{-1} with up to 10 pp. An astringency reduction was also shown on 100 mg kg^{-1} with up to 8 pp and was significant ($p < 0.1$) at 10 and 30 s. Higher concentrations of the test compound did not result in better masking effects at the tested EGCG concentrations.

Effect of trans-Pellitorine on Grape Seed Extract. Besides EGCG, **1** was also tested on a commercial GSE to explore the general use of *trans*-pellitorine as a masker for astringent compounds. Phenolic substances in grape seeds comprise among others the oligomeric proanthocyanidins (OPCs), which are mostly di- or trimers of catechins. These OPCs are known for their antioxidant activity,[42,43] as well as for their astringent sensation.[44,45] The intensity of astringency at 0 s was rated at around 50% and thus was quite similar to the rating of EGCG at the same time point and the same concentration. As shown in Figure 7, the addition of **1** (5 mg kg^{-1}) did not result

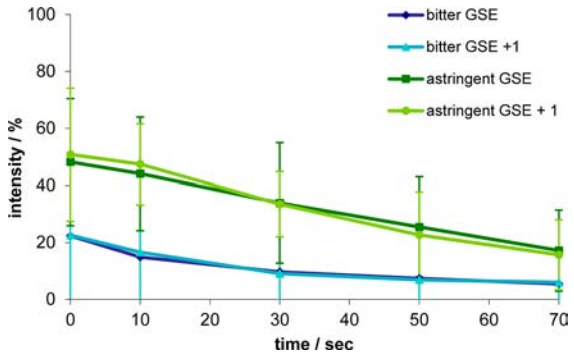

Figure 7. Time−intensity curves of 750 mg kg^{-1} grape seed extract (GSE) without **1**, bitter (dark blue line) and astringent (dark green line); with **1** (5 mg kg^{-1}), bitter (light blue line) and astringent (light green line), $n = 15$; one replication.

in modulations of the bitterness and astringency ratings. In order to confirm this unexpected result and to investigate the possible role of the relative ratio rather than the absolute concentrations, another test was carried out using 500 mg kg^{-1} grape seed extract and 5 mg kg^{-1} *trans*-pellitorine **1**. Again, no masking effect was detected (data not shown).

In conclusion, IVDM can be employed as a screening tool for potential flavor modifiers. This was shown for *trans*-pellitorine **1**, which had significant astringency masking effects at 5 mg kg^{-1} on 750 mg kg^{-1} EGCG (Figure 2 and Table 1). The upper use level in this application is marked by a tingling effect, which

is perceived by the panelists at concentrations of 8 mg kg^{-1} or higher. The geometrical isomer *cis*-pellitorine **2** already showed significant astringency masking effects at 2 mg kg^{-1} against 750 mg kg^{-1} EGCG (Table 1), but similar to *trans*-pellitorine, the intrinsic taste and trigeminal effect increased at higher concentrations. Compound **2** exhibited some pungency at these concentrations, which may disturb the panelists' ratings for astringency and bitterness.

Additionally, structurally related compounds were tested to see whether a structure−activity relationship can be found. Amides **3**, **4**, **5**, **6**, **7**, and **9** (Figure 3) were tested at different concentrations on 750 mg kg^{-1} EGCG without any masking effect (Figure 4). In comparison, a minor bitter and astringency-masking effect was described for spilanthol **8** at 2 mg kg^{-1} (Figure 5). Of the pellitorine derivative **10**, 50 and 100 mg kg^{-1} concentrations were necessary to obtain a similar effect on astringency (Table 1 and Figure 6) compared to that of **1** and **2**. In contrast to *trans*-pellitorine **1**, however, its derivative **10** does not show a trigeminal effect or off-notes at this concentration.

The effect of **1** was also tested on a different base, grape seed extract (GSE). In this case, **1** was not able to reduce the astringency of grape seed polyphenols (Figure 7). Possible different molecular mechanisms of astringency perception for EGCG and OPCs could be an explanation.

No obvious structure−activity relationship can be found with the tested compounds and astringents at the tested concentrations. The results indicate an apparently specific base-to-compound interaction, but the molecular mechanism of these effects still remains unclear. There are no reports that *trans*-pellitorine, for example, can prevent proteins from precipitation, but early reports that tingling alkamides can directly activate trigeminal neurons are present.[46]

The starting question for this study was whether an enhanced salivation effect causes the clearance of neurons from EGCG. Some of the compounds that were described to have a strong tingling and mouth-watering effect showed an astringency-masking effect in a defined concentration range (*trans*-pellitorine **1**), while others, e.g., **3**, did not. Therefore, no definite conclusion can be drawn if a compound has masking activity on astringency based on its salivating effect only (activity−activity relationship). Moreover, the tingling effect may not be directly correlated with masking effects as shown for compound **10**.

■ ASSOCIATED CONTENT

Supporting Information

Time−intensity curves of EGCG with/without caffeine and with/without grape seed extract; time−intensity curves of five panelists in one session for the bitterness ratings of EGCG, for the astringency ratings of EGCG; bitter time−intensity curves of EGCG, evaluated in three sessions by a selected panelist; astringency time-intensity curves of EGCG, evaluated in three sessions by a selected panelist; and comparison for all pairs of the tested EGCG concentrations by using Tukey−Kramer HSD. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

EGCG, epigallocatechin-3-gallate; GSE, grape seed extract; TI, time−intensity method; DATI, dual-attribute time-intensity; TIP, time−intensity profiling; IVDM, Intensity Variation Descriptive Methodology; pp, percentage points

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