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ARTICLE

# Characterization of Flavor Modulating Effects in Complex Mixtures via High Temperature Liquid Chromatography

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The identification of flavor modulating compounds, for example, bitter masking or sweet enhancing compounds, in complex mixtures such as botanical extracts or food preparations is difficult and time- and work-intensive. To accelerate this process, an improved screening method was developed on the basis of the separation of complex matrixes by the so-called LC Taste setup and subsequent comparative sensory analysis. The eluent containing only water and ethanol was diluted with a basic tastant solution (500 mg $L^{-1}$caffeine and 5% sucrose, respectively) and evaluated by a trained panel by duo comparison tests. This novel method was applied to the known flavor and taste modulating substances homoeriodictyol (**1**), sterubin (**2**), hesperetin (**3**), and lactisol (**9**) as well as to simple mixtures of homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**). To evaluate the potential of the method for more complex matrixes, the protocol was applied to plant extracts from Yerba Santa (*Eriodictyon californicum*) and honeybush tea (*Cyclopia intermedia*). The flavor modulating activities reported for homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) could be confirmed in these complex mixtures.

**KEYWORDS: LC Taste; high temperature liquid chromatography (HTLC); taste modulation; bitter masking; sweet enhancing; homoeriodictyol; sterubin; hesperetin**

## INTRODUCTION

The identification of flavor modulating compounds, e.g., bitter maskers and sweet or salt enhancers, is important for the general understanding of food flavor as well as for the development of new flavors in the flavor and food industries (*1*−*4*). Approaches to make foods healthier often lead to losses in sensory properties (*4*, *5*). For example, one of the current trends is to reduce the amount of sweetening carbohydrates, especially sucrose or high fructose corn syrup (HFCS), and to replace them by high potency sweeteners. Most of these sweeteners, however, show different sweetness profiles compared to that of sucrose and exhibit bitter, lingering aftertastes (*6*−*9*). Alternatively, sweet taste enhancing compounds showing only weak intrinsic taste can be used, e.g., alapyridaine (*10*), which allows the reduction of the sugar content in a food application and keeps the sweetness at nearly the same level as the that of the full-sugar version. Fortification of foods with healthy ingredients, such as polyphenols, vitamins, and minerals can also lead to bitter off-notes which require the use of bitter masking compounds (*4*) to make such a functional food more pleasant for the consumer.

The identification of flavor modulating compounds is difficult and time-consuming. Optimal flavor modulating compounds show no or only weak intrinsic flavor, as they are not supposed to alter the overall flavor profile of the food matrix except in the desired way. While sweet and bitter compounds can be easily detected by tasting a food, the particular properties of modulating compounds can be identified only in combination with certain other tastants. This increases the number of tests that have to be carried out on different testing bases (e.g., sucrose, bitter compounds such as caffeine, catechins, hydrophobic peptides, etc.) Additionally, relatively large amounts of substances are needed, which in some cases have to be isolated via time-intensive procedures. Standard methods for the identification of taste modulating compounds comprise duo difference tests after fractionation and isolation of the compounds (*11*). There are also more sophisticated techniques such as comparative taste dilution analysis (cTDA), which, for example, led to the isolation of alapyridaine as a general taste enhancer (*12*, *13*).

To overcome the described difficulties, the so-called LC Taste has been developed as a tool for the fast screening of taste compounds (*14*). The system uses the advantages of separation based on high temperature liquid chromatography (*15*) and combines it with an in vivo detection of taste active compounds by a sensory tester or sensory panel. This allows one to correlate analytical and sensory data in a way similar to the combination of gas chromatography and olfactometry (GC-O), which has been widely used since the 1960s for the online evaluation of volatile flavor compounds from complex extracts, such as coffee or bread crust (*16*−*18*). After the preparation of samples, the analyte is

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separated peak-wise into fractions by high temperature liquid chromatography using water as a food grade solvent. Ethanol as well as physiologically compatible acids or buffer substances can be used as modifiers.

The aim of this study was to evaluate the potential of the LC Taste system to identify flavor modulating compounds from complex mixtures without prior isolation of single compounds. In a first step, simple test mixtures of known flavor modifying compounds were used as analytes. This was followed by an investigation of extracts from Yerba Santa (*Eriodictyon angustifolium*) and honeybush tea (*Cyclopia intermedia*). They should serve as more realistic samples, which contain known flavor modulating flavonoids (homoeriodictyol, sterubin, and hesperetin) in a complex matrix (*11*, *19*).

## MATERIALS AND METHODS

The following solvents were purchased from Sigma-Aldrich (Steinheim, Germany): methanol (puriss. p.a.), ethanol (absolute puriss. p.a. min. 99.8%), and DMSO (p.a.). Dried leaves of Yerba Santa (*Eriodictyon angustifolium*) were purchased from JPR Jenaer Pflanzenrohstoffe (Jena, Germany). Fermented honeybush tea (*Cyclopia intermedia*) was purchased from Alfred Galke GmbH (Gittelde/Harz, Germany). Hesperetin (**3**) was obtained from Symrise (Holzminden, Germany). Homoeriodictyol sodium salt and sterubin (**2**) were prepared from dried Yerba Santa leaves according to ref *11*. Free homoeriodictyol (**1**) was obtained by dissolving 4 g of homoeriodictyol sodium salt in 1 L of $H_2O$ and precipitating the free flavanone by adding 2 mL of concentrated acetic acid under continuous stirring. Precipitated homoeriodictyol (**1**) was filtered off, and the off-white residue was dried in vacuo at 40 °C.

Quantification of flavonoids was carried out using a C18 column (Grom Saphier 110, 5 $\mu$m, 150 × 2.0 mm, Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) with a gradient of water + 1% glacial acetic acid and acetonitrile, using standard samples for identification. The gradient started with 68% water and 32% acetonitrile, increasing to 50% acetonitrile within 10 min at a flow rate of 0.35 mL $min^{-1}$. Within an additional 5 min, the concentration of acetonitrile was increased to 100%. Detection was carried out using a UV detector at a wavelength of 285 nm; quantification was performed using external calibration. Size exclusion chromatography was carried out using Sephadex LH-20 (Sigma-Aldrich, Steinheim, Germany) in a glass column (Kronlab Eco, 32 × 450 mm, YMC Europe GmbH, Dinslaken, Germany) with methanol as eluent. Flow rate was 2.5 mL $min^{-1}$; detection was carried out using a UV detector at a wavelength of 210 nm.

LC-MS spectra were recorded using a mass spectrometer Bruker microTOF Q II (Bruker, Bremen, Germany, ESI electrospray ionization) in combination with a Waters Aquity UPLC system (Waters, Eschborn, Germany). Chromatographic separation was carried out on a C-18 column (BEH C-18, 1 × 50 mm; Waters, Eschborn, Germany) at a flow rate of 0.2 mL $min^{-1}$ using an acetonitrile/water gradient. The gradient started with 100% water containing 0.01% formic acid, increasing to 95% acetonitrile within 20 min. This concentration was kept for 5 min.

**Preparation of Extracts.** The dried material of *Eriodictyon angustifolium* (500 g) was infused with boiling water and stirred for 1 h. The soaked plant material was filtered off, dried, and extracted twice with 2.0 L of methanol at room temperature while stirring for 1 h each. The extract was filtered, the filtrate evaporated in vacuo at 40 °C, and the residue was dried at 40 °C/0.1 mbar overnight to completely remove the solvent. The extraction resulted in 84.5 g of a dark green solid.

One hundred grams of fermented and cut leaves of *Cyclopia intermedia* were extracted twice with 0.5 L of methanol each at room temperature while stirring for 1 h each. The extract was filtered, the filtrate evaporated in vacuo at 40 °C, and the residue dried at 40 °C/0.1 mbar overnight to completely remove the solvent. The extraction yielded 4.81 g of dried dark brown solid.

**Fractionation via LC Taste.** LC Taste fractionation was performed using high temperature liquid chromatography (HTLC) on a polymer-based PRP-1 column in a semipreparative scale (250 × 10 mm; 10 $\mu$m particle size; Hamilton, Bonaduz, Switzerland) at elevated temperature (120 °C isotherm) and detection on a diode array detector (SunChrom SpectraFlow; wavelength range 200−400 nm, SunChrom, Friedrichsdorf, Germany).

*Fractionation of Single Compounds.* Hesperetin (**3**) (50 mg $mL^{-1}$ hesperetin in DMSO/ethanol 1:9 (v/v), injection volume 100 $\mu$L) was fractionated via LC Taste using HTLC on a polymer-based RP column at 120 °C isotherm conditions employing a water/ethanol gradient at a flow rate of 3 mL $min^{-1}$. The gradient started with 70% water and 30% ethanol, increasing the ethanol concentration to 100% within 50 min. The fraction was collected for 3.33 min to yield 10 mL of eluate.

Homoeriodictyol (**1**) (100 mg $mL^{-1}$ in DMSO, injection volume 25 $\mu$L), sterubin (**2**) (50 mg $mL^{-1}$ in DMSO/ ethanol 1:4; injection volume 100 $\mu$L), and lactisol (**9**) (250 mg $mL^{-1}$ in ethanol/water 1:1 (v/v), injection volume 50 $\mu$L) were fractionated under the same conditions as those described for hesperetin.

*Fractionation of a Mixture of Flavonoids.* A stock solution of 80 mg of homoeriodictyol (**1**), 20 mg of hesperetin (**3**), and 40 mg of sterubin (**2**) per mL DMSO/ethanol 1:4 (v/v) was prepared. The mixture (injection volume 100 $\mu$L) was fractionated by LC Taste using HTLC on a polymer-based RP column under the conditions described for the single flavonoids. Fractions were cut peak-wise; two runs were performed and blended with both sucrose and caffeine solution to yield concentrations of 5% sucrose and 500 mg $L^{-1}$ caffeine, respectively, in the final test solution.

*Fractionation of Extracts.* The extract of *E. angustifolium* (400 mg $mL^{-1}$ ethanol/water 1:1 (v/v), injection volume 100 $\mu$L) was fractionated and prepared for sensory evaluation in the same way as that described for the mixture of flavonoids. The extract of *C. intermedia* (250 mg $mL^{-1}$ ethanol/water 1:1 (v/v), injection volume 100 $\mu$L) was fractionated under the same conditions as those described for *E. angustifolium* using a gradient starting with 100% water and increasing the ethanol concentration to 50% within 30 min and to 100% after an additional 20 min. The complete fractions obtained from these starting materials were tested in a ratio of 1:10 (v/v) on sucrose and caffeine solution, 5% and 500 ppm, respectively.

**Sensory Studies.** General sensory analysis of extracts and fractions was carried out by a trained panel of 10 healthy adults with no known tasting disorders. Tasting sessions were carried out in the morning hours 1−2 h after breakfast, during which time the panelists were asked not to drink black or green tea or coffee because of adaptation to caffeine. Vittel water was used to prepare the testing solutions. No nose clips were used for the sensory evaluations, as the overall flavor modulating effects of a substance of a fraction were to be observed.

Different combinations of homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) were tested qualitatively in a consensus panel using 5% sucrose and 500 mg $L^{-1}$ caffeine solutions as a testing solution compared to blank reference samples containing sucrose or caffeine only. The bitter masking effects of the single compounds and of the mixture (4 (**1**):2 (**2**):1 (**3**); w/w) were quantitatively determined as described (*5*).

For the determination of taste modulating effects based on LC Taste, the samples were fractionated via HTLC and blended with the corresponding test solutions (sucrose solution for hesperetin (**3**) and caffeine solution for homoeriodictyol (**1**) and sterubin (**2**); both test solutions for the single fractions from the extract). Sensory evaluation was carried out in a blind paired comparison test together with a blank sample. The blank sample contained water, which was fractionated via LC Taste under the same conditions as the test sample so that both samples exhibited the same ethanol concentration and was blended with sucrose and caffeine solution, respectively, in the same ratio. Panelists were asked to compare both samples and to mark the sample, which they perceived as sweeter or as less bitter, depending on the test solution. Sweetness and bitterness were evaluated in separate sessions. A taste modulation probability (*TMP*) value was determined for each pair according to eq 1:

$$TMP = \frac{(n_{\text{higher}} - n_{\text{lower}})}{n_{\text{total}}} \cdot 100 \quad n = \text{number of panelists} \qquad (1)$$

Duo-tests were presented to the testers in randomized order; in the case of discoloration, samples were covered with aluminum foil or tasted under colored light.

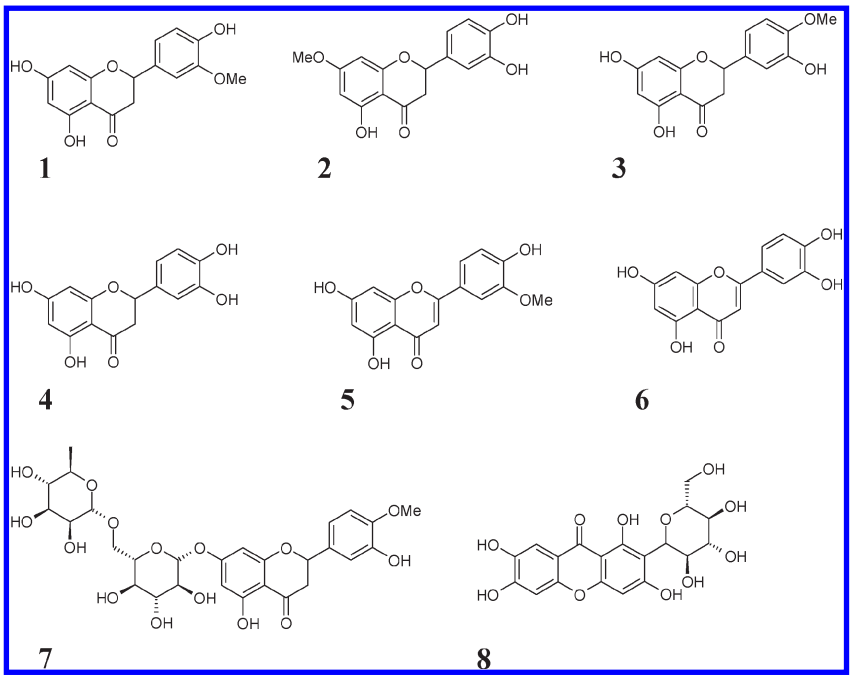


**Figure 1.** Selected compounds from *Eriodictyon* sp. and *Cyclopia* spp.: homoerioctyol (**1**), sterubin (**2**), hesperetin (**3**), eriodicytol (**4**), chrysoeriol (**5**), luteolin (**6**), hesperidin (**7**), and mangiferin (**8**).

## RESULTS AND DISCUSSION

Because of their bitter masking properties, Yerba Santa extracts (*Eriodictyon* sp.) have been recommended by pharmacists as additives to make bitter tasting medicines more pleasant for the patients from as early as the 1890s (*21*). Yerba Santa contains homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) as main flavonoids; eriodictyol (**4**), chrysoeriol (**5**), and luteolin (**6**) have been described as minor compounds (*11*, *20*) (**Figure 1**). The bitter masking principles in *Eriodictyon* extracts were shown to be homoeriodictyol (**1**), eriodictyol (**4**), and to some extent sterubin (**2**) (*11*, *22*). *Eriodictyon angustifolium* was selected for sensory experiments.

Although *Eriodicyton* extracts are reported to act as bitter masking preparations against certain bitter compounds (e.g., quinine) (*21*), it is very difficult to detect the active bitter masking principles in this complex mixture, when, e.g., caffeine is used as a testing base. Sensory evaluation of a dried methanolic extract of *E. angustifolium*, containing 19.5% of homoeriodictyol, on 5% sucrose and 500 mg $L^{-1}$ caffeine solution, respectively, revealed a number of unpleasant tastes that were described as bitter or herbal, among others. In a pretest, five trained experts rated the bitterness of the test solution containing caffeine and the dried methanolic extract in a concentration of 500 mg $L^{-1}$ as clearly more bitter than the blank caffeine solution. Even though the active compounds homoeriodictyol (**1**) and sterubin (**2**) are contained in the extracts in large quantities, their effect seemed to be covered by the bitter and intensely herbal taste of the extract.

Cleanup of a raw extract of Yerba Santa was carried out using Sephadex LH-20 as the stationary phase and methanol as the mobile phase to separate the flavonoids from a number of unidentified compounds, which were suspected to cover the bitter masking activities of homoeriodictyol (**1**) and hesperetin (**3**). This fractionation procedure resulted in two clearly separated peaks. One of them contained a number of so far unknown compounds; the detailed structural elucidation of these substances, named erionic acids A−F, has been reported in a separate paper (*23*). The other peak contained a mixture of various flavanones, predominantly homoeriodictyol (**1**), hesperetin (**3**), and sterubin (**2**). Their composition was determined by LC-MS; quantification by HPLC revealed a ratio of 4 (**1**):2 (**2**):1 (**3**), w/w. Tasting of this particular flavonoid-containing Sephadex LH-20 fraction of the Yerba Santa extract after evaporation of methanol showed an unclear picture of bitter masking activity when tested on 500 mg $L^{-1}$ caffeine solution at a concentration of 170 mg $L^{-1}$ (corresponding to 100 ppm pure homoeriodictyol), although high amounts of the active compounds were contained. To verify this effect, a mixture containing the three flavonoids in the same ratios as those determined in the native extract was prepared, and its masking ability was compared qualitatively and quantitatively to the activity of the neat compounds each at 100 mg $L^{-1}$ (**Table 1**). The variability in the bitterness perceived for the flavanoid mixture by 15 testers (relative standard deviation: 31%) was comparable to the experiments previously performed with the single substances (*11*). However, the sensory assessment demonstrated that the mixture containing the same absolute amount of homoeriodictyol (**1**) did not show bitter masking activities but even seemed to increase the perceived bitterness of the 500 mg $L^{-1}$ caffeine solution.

To clarify these effects, different mixtures containing homoeriodictyol (**1**)/sterubin (**2**) and homoeriodictyol (**1**)/hesperetin (**3**) in varying ratios were qualitatively evaluated sensorially on 5% sucrose and 500 mg $L^{-1}$ caffeine solution. Whereas both sterubin (**2**) and homoeriodictyol (**1**) show masking activities for

**Table 1.** Masking Effects of Homoeriodictyol (**1**), Sterubin (**2**), Hesperetin (**3**) and a Mixture of These Three Compounds Based on Their Typical Ratios in a Methanolic Extract from Yerba Santa[a]

| compound | quantitative modulation of bitter taste in a 500 mg $L^{-1}$ caffeine solution |
|---|---|
| **1** | −35% |
| **2** | −20% |
| **3** | −12% |
| 4 (**1**): 2 (**2**): 1 (**3**) *m*/*m* | +6% |

[a] Total concentration of neat flavanons $c$ = 100 mg $L^{-1}$ and of the mixture $c$ = 170 mg $L^{-1}$ (15 panelists). Data for single compounds are taken from ref *11*.

**Table 2.** Masking Effects of Varying Mixtures of Homoeriodictyol (**1**), Sterubin (**2**), and Hesperetin (**3**)[a]

| 1 | 2 | 3 | taste description in 5% sucrose solution | qualitative masking effect in 500 mg $L^{-1}$ caffeine solution |
|---|---|---|---|---|
| 1 | 1 | 0 | slightly woody, bitter, herbal | yes (very weak) |
| 1 | 2 | 0 | more bitter compared to 1:1 | no (more bitter than reference) |
| 1 | 3 | 0 | even more bitter compared to 1:2, disgusting | no (more bitter than reference) |
| 2 | 1 | 0 | less bitter compared to 1:1, more pleasant | yes (weak) |
| 3 | 1 | 0 | sweetish | yes |
| 1 | 0 | 1 | sweet enhancing, balsamic | yes |
| 1 | 0 | 2 | sweet, syrup-like, balsamic | yes (weak) |
| 1 | 0 | 3 | very sweet | yes (weak) |
| 2 | 0 | 1 | sweet enhancing | yes |
| 3 | 0 | 1 | slightly sweet enhancing | yes |

[a] Total concentration of flavanons $c$ = 100 mg $L^{-1}$ (8 panelists).

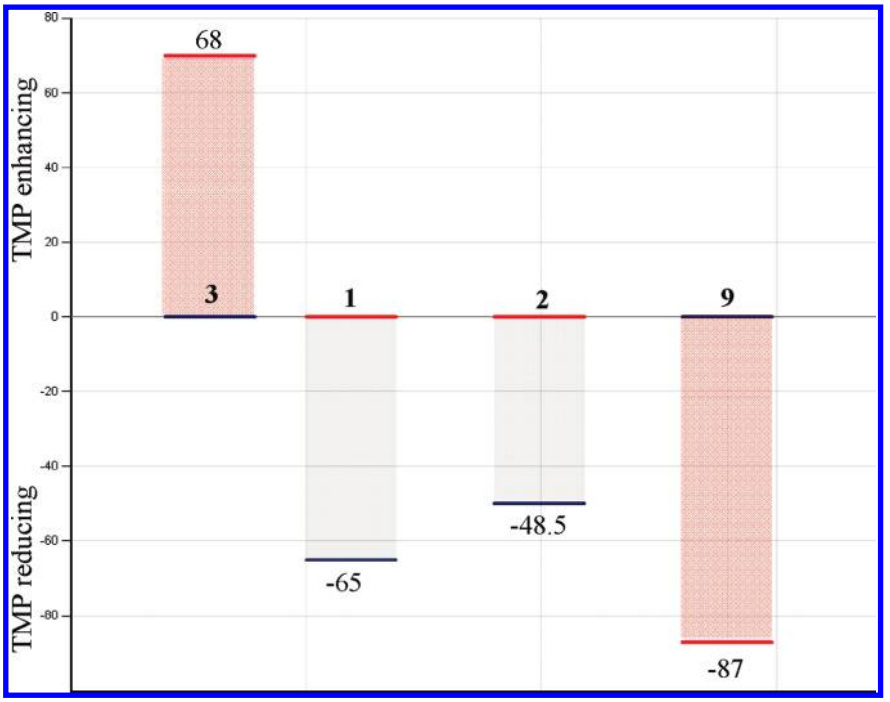


**Figure 2.** TMP values for taste modulating compounds hesperetin (**3**), homoeriodictyol (**1**), sterubin (**2**), and lactisol (**9**) after fractionation via LC Taste. For each fractionation, the fractions were diluted 1:10 with a 5% sucrose (red) and/or a 500 mg $L^{-1}$ caffeine solution (blue), respectively, and compared to a blind HTLC fractionation (same conditions as those for the compounds) blended 1:10 with a 5% sucrose or a 500 mg $L^{-1}$ caffeine solution, respectively, by a paired comparison test ($n$ = 10). TMP was calculated as given in eq 1.

themselves (*5*), the masking effect disappeared in those mixtures where the content of sterubin exceeded that of homoeriodictyol (**2**) (**Table 2**). Hesperetin (**3**) seems to exhibit no or only minor effects on the activity of homoeriodicytol. The reduced bitter masking activity of homoeriodictyol (**1**) in combination is not caused by simple dilution in the testing solution; in previous studies, it was also shown that **1** is active at concentrations lower than 100 ppm (*11*). The single flavanones show a relatively broad masking activity against several bitter components (*11*). They may modulate a promiscuous bitter receptor activated by more than one bitter compound class (*24*). Therefore, the observed phenomena may be a result of an allosteric effect on one bitter receptor with different binding sites, but it cannot be excluded that the flavanones also bind as agonists or partial agonists to other bitter receptors. At present, the physiological basis to explain the observed effects is not available. However, the data from the sensory evaluation clearly demonstrate the high importance of separating taste modulating compounds because of their unpredictable nonlinear combinatorial effects.

Before the evaluation of taste modulating compounds using LC Taste was carried out with complex plant extracts, pretests were accomplished using homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**). Lactisol (**9**) was tested as a negative control at a concentration of 100 mg $L^{-1}$ because of its sweet inhibiting effects (*25*). Compounds were fractionated via LC Taste, blended with testing solution, and tested in paired comparison tests together with a blank sample containing the standard tastants sucrose and caffeine only (**Figure 2**).

Because the concentration of each compound in a certain fraction may differ and the effect therefore cannot be based on the absolute activity of a single compound, a probability factor is introduced as a possibility to characterize the taste modulation effect of a particular fraction. The taste modulation probability (TMP) describes the number of panelists experiencing a modulation effect of a fraction compared to chance. Therefore, the higher the TMP, the higher the probability that there is a detectable effect. The TMP does not directly describe the maximum activity of single compound, but fractions with a high TMP show hints for strong modulators. Negative TMP values indicate reducing or

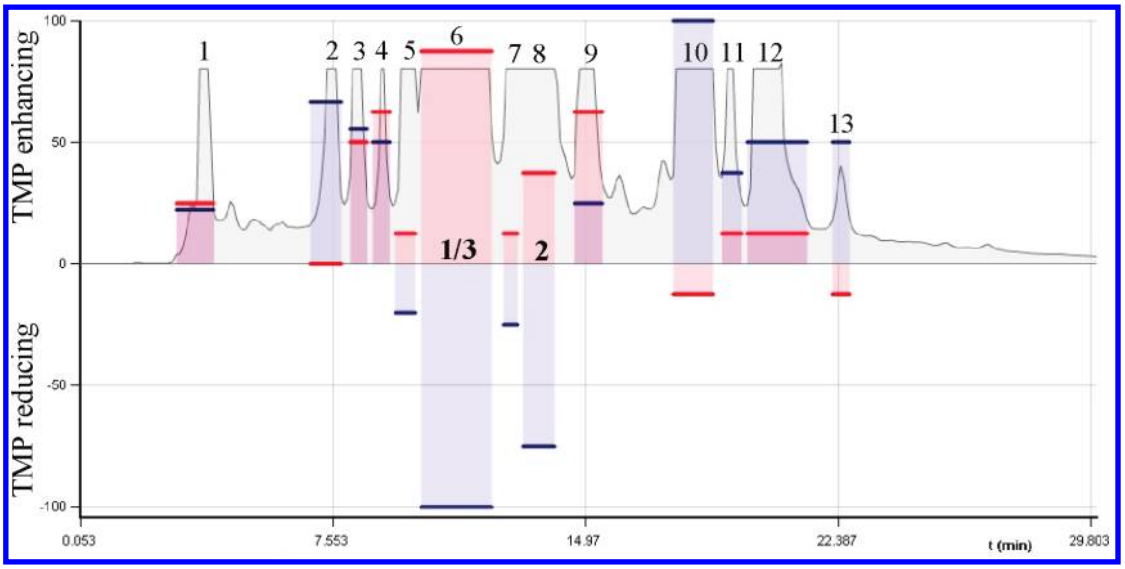


**Figure 3.** TMP values for a mixture of hesperetin (**3**), homoeriodictyol (**1**), and sterubin (**2**) dissolved in DMSO/ethanol 1:4 (*v*/*v*) after fractionation via LC Taste. The fractions were diluted 1:10 with a 5% sucrose (red) and 500 mg $L^{-1}$ caffeine solution (blue), respectively, and compared to a blind HTLC fractionation (same conditions as those for the compounds) blended 1:10 with a 5% sucrose or a 500 mg $L^{-1}$ caffeine solution, respectively, by a paired comparison test ($n$ = 10). TMP was calculated as given in eq 1.

masking effects, while positive TMP values stand for enhancing effects. Consequently, the TMP value reflects the probability that a compound or a fraction has taste modulating effects but only gives indicative information about the intensity of the effect. To gain quantitative data, further tests have to be carried out, in which the panelists are asked to quote the intensity of the taste compared to the blank sample. The TMP values showed that the taste modulating effects of the four compounds were detected by most of the panelists (**Figure 2**).

As a next step, the above-described mixture containing homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) in the naturally occurring ratio of 4:2:1 was evaluated via LC Taste, as described for the single flavonoids. Because of the high amounts injected, the chromatogram was overloaded so that hesperetin (**3**) and homoeriodictyol (**1**) could not be fractionated separately as the two peaks converged to one. Therefore, the whole peak was fractionated by LC Taste and tested on sucrose solution as well as on caffeine solution (**Figure 3**). Bitter masking effects for fractions 1, containing homoeriodictyol (**1**) and hesperetin (**3**), and 2, containing sterubin (**2**), are reflected by the TMP values of −68 and −50. The lower modulation probability observed for the sterubin-containing fraction (**2**) is in agreement with the lower concentration of this compound in the mixture compared to that of homoeriodictyol (**1**) as well as with the reported lower activity of sterubin (**2**) compared to that of homoeriodictyol (**1**) (*5*). Because of the lower concentration of hesperetin (**3**) in the flavonoid mixture compared to that of the pretest, the TMP value of fraction 1, containing homoeriodictyol (**1**) and hesperetin (**3**) (50), was lower in the extract than in the flavonoid mixture used in the pretest. The sensory evaluation of the pretest, where a coelution for homoeriodictyol and hesperetin was observed, reflects the sensory results of the combination trials; whereas sweet modulating and bitter modulating effects in this case seem not to influence each other, for masking effects a separation is necessary.

After the pretest with single compounds and the flavonoid mixture, the method was applied to a complex Yerba Santa methanolic extract, containing high amounts of homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) as well as a number of minor flavonoids (**Figure 4**). Sensory evaluation of selected fractions on sucrose and caffeine solution showed high TMP values for bitter masking for the fractions containing homoeriodictyol (**1**) and sterubin (**2**). Fraction 6 containing homoeriodictyol (**1**) and high amounts of hesperetin (**3**) also revealed a high TMP value for sweet enhancing. As both compounds are among the main constituents of the extract, the elution of the corresponding fraction required several minutes because of overloading of the column. At lower concentrations, a clear separation of both compounds could be observed. The data obtained from the combination experiments with homoeriodictyol (**1**), sterubin (**2**), and hesperetin (**3**) (**Table 2**) showed that no obvious interactions between HED and hesperetin could be detected, which might have negatively influenced the sensory results of the HED/hesperetin fraction because of the unsatisfactory separation of both compounds under the used experimental conditions. Although the TMP values for these fractions are not identical to the TMP values obtained from the tests using the isolated compounds (**Figure 2**), because of different concentrations present in the testing solutions and extract, the ratio between the TMP values of the single compounds (HED, sterubin, and hesperetin) and corresponding fractions from the extract is the same in both tests.

In addition to homoeriodictyol (**1**)/hesperetin (**3**), three other fractions showed increased TMP values for sweet enhancement. But unlike the hesperetin (**3**) fraction, these fractions showed positive TMP values for bitter taste. Other fractions exhibited only minor sweet enhancing effects but strong bitter or bitter increasing effects. Fractions 4 and 9 showed increased TMP values for sweet and also for bitter taste. Analysis by LC-MS and structural elucidation via NMR (*23*) revealed the identities of the previously unknown benzoic acid derivatives 4-hydroxy-3-((*E*)-7-hydroxy-3,7-dimethyl-4-oxo-oct-5-enyl)-5-((*E*)-4-hydroxy-3-methyl-but-2-enyl)-benzoic acid (erionic acid A; fraction 4) and 3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-((*E*)-4-hydroxy-3-methyl-but-2-enyl)-benzoic acid (erionic acid C; fraction 9). Fractions 2 and 13 also exhibited increased TMP values for bitter flavor; in these fractions, 3-hydroxy-8-((*E*)-7-hydroxy-3,7-dimethyl-4-oxo-oct-5-enyl)-2,2-dimethyl-chroman-6-carbonic acid (erionic acid B, fraction 2) and 3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-(3-methyl-but-2-enyl)-benzoic acid (erionic acid F, fraction 13) were identified (*23*).

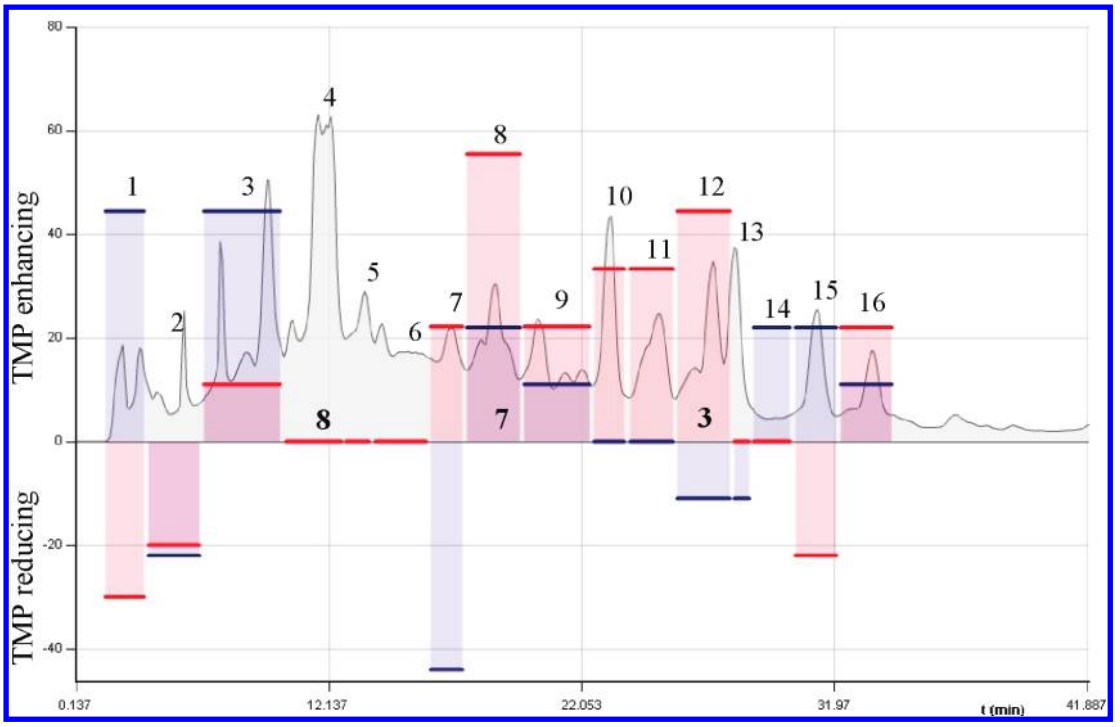


**Figure 4.** TMP values for Yerba Santa (*Eriodictyon angustifolium*) extract for taste modulation trials on sucrose (red) and caffeine solution (blue) after fractionation via HTLC. Conditions are the same as those given in **Figure 3**.

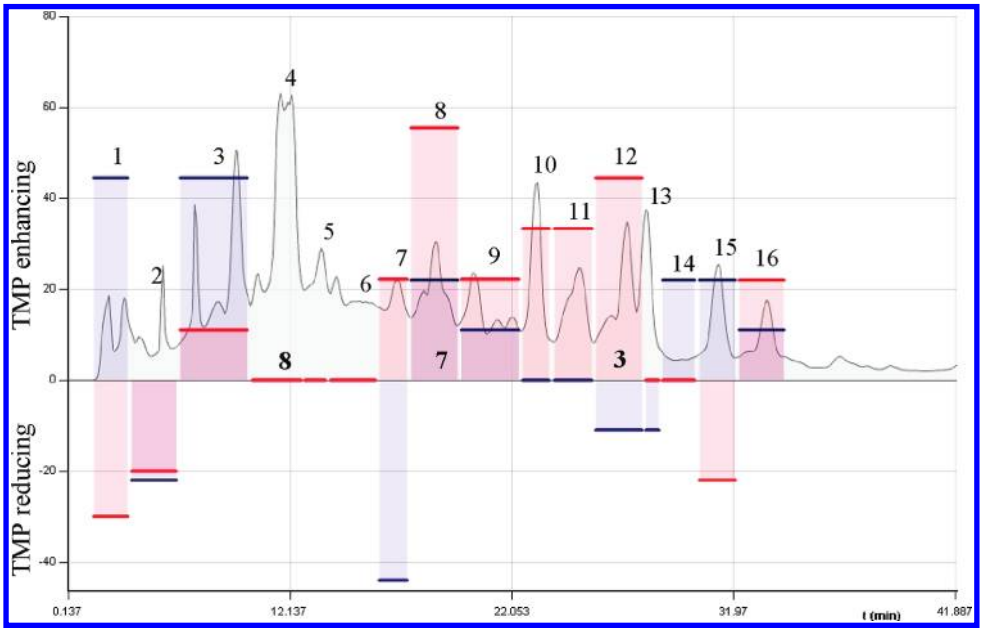


**Figure 5.** TMP values for honeybush (*Cyclopia intermedia*) extract for taste modulation trials on sucrose (red) and caffeine solution (blue) after fractionation via HTLC. Conditions are the same as those given in **Figure 3**.

Honeybush tea, produced from fermented *Cyclopia* spp., especially *C. intermedia*, *C. genistoides*, and *C. subternata*, is traditionally used by the indigenous people of South Africa, but because of its pleasant honey-like flavor and its reported health benefits (low tannin content, lack of caffeine, and high antioxidative capacity), it is becoming more and more popular in the western world (*26*). Among the most important compounds from *Cyclopia* spp. are xanthones (**Figure 1**, mangiferin, **8**), flavanones, and flavanone glycosides (e.g., hesperetin, **3**, hesperidin, **7**), some minor flavones, isoflavones, and flavan-3-ols. As hesperetin (**3**) has already been described to have sweet enhancing properties (*19*), honeybush tea (*C. intermedia*) was chosen as a second testing system to evaluate the suitability of the developed LC Taste protocol.

A methanolic extract from *C. intermedia* was fractionated via LC Taste under the same conditions as those described for *E. angustifolium* and evaluated sensorially on a sucrose and caffeine solution. The results are shown in **Figure 5**. Because of the poor solubility of the extract, overall the observed modulating effects were not as significant as those for the *E. angustifolium* extract. Nevertheless, several fractions showed slightly enhanced TMP values compared to those of the other fractions. The fractions with the highest TMP values contained the compounds hesperidin (**7**) and the aglycone hesperetin (**3**) as confirmed by

LC-MS. Considering the toxicological data reported for mangiferin (8) (*27*), fraction 4, containing this xanthone, was not subjected to sensory evaluation by the panel.

Previously, it was reported that LC Taste can be employed for a fast screening of taste active substances from complex matrixes, such as plant or food extracts, without prior isolation of the relevant compound. The results presented in this study indicate that the LC Taste protocol is not only suitable for the sensory evaluation of single compounds regarding their mere taste activity but also suitable for the challenging topic of identifying taste modulating compounds, e.g., from raw or prefractionated extracts without complex evaporation and redilution steps. For identifying flavor modulating compounds, it is mandatory to have a fairly good peak separation because of possible nonlinear effects of compositions, as shown for homoeriodictyol (**1**) and sterubin (**2**). The HTLC protocol of the presented method is still not optimal, e.g., in the partial coelution of hesperetin (**3**) and homoeriodictyol (**1**), and will have to be improved in further studies by using preparative scale systems instead of semipreparative scale columns. This is expected to clearly improve the peak separation without overloading of the column. Nevertheless, the new approach allows for an accelerated first evaluation of single fractions and preselection of interesting active fractions for additional sensory analysis steps. As compounds can be tested at their naturally occurring concentrations, it also gives an indication on the actual contribution of a flavor modulating fraction or compound to the overall flavor of a sample. This makes LC Taste a valuable expansion of the already existing arsenal of methodologies for screening interesting flavor and taste compounds.

## ABBREVIATIONS USED

cTDA, comparative taste dilution analysis; DMSO, dimethyl sulfoxide; HTLC, high temperature liquid chromatography; TMP, taste modulation probability; GC-O, gas chromatography and olfactometry; GPC, gel permeation chromatography.

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