Bitter-Tasting and Kokumi-Enhancing Molecules in Thermally Processed Avocado (*Persea americana* Mill.)

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Sequential application of solvent extraction and RP-HPLC in combination with taste dilution analyses (TDA) and comparative TDA, followed by LC-MS and 1D/2D NMR experiments, led to the discovery of 10 $C_{17}-C_{21}$ oxylipins with 1,2,4-trihydroxy-, 1-acetoxy-2,4-dihydroxy-, and 1-acetoxy-2-hydroxy-4-oxo motifs, respectively, besides 1-*O*-stearoyl-glycerol and 1-*O*-linoleoyl-glycerol as bitter-tasting compounds in thermally processed avocado (*Persea americana* Mill.). On the basis of quantitative data, dose-over-threshold (DoT) factors, and taste re-engineering experiments, these phytochemicals, among which 1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene was found with the highest taste impact, were confirmed to be the key contributors to the bitter off-taste developed upon thermal processing of avocado. For the first time, those $C_{17}-C_{21}$ oxylipins exhibiting a 1-acetoxy-2,4-dihydroxy- and a 1-acetoxy-2-hydroxy-4-oxo motif, respectively, were discovered to induce a mouthfulness (kokumi)-enhancing activity in sub-bitter threshold concentrations.

KEYWORDS: Taste; kokumi; bitterness; taste enhancer; avocado; taste dilution analysis

INTRODUCTION

Avocado (*Persea americana* Mill.), classified in the flowering plant family Lauraceae, is cultivated in tropical climates throughout the world as well as in some temperate zones such as California to produce a green-skinned, egg-shaped or spherical fruit that ripens after harvesting. Depending on the location of the orchard, the oil content in the flesh of these fruits can range from 16% in September to 30% in April. Whereas dozens of cultivars are known, the Hass avocado is today the most common one producing fruit year-round and accounts for 80% of cultivated avocados worldwide. The fat-rich, creamy flesh of avocado is consumed raw as freshly cut slices, for example, in sandwiches, salads, and Maki sushi, as puree in spreads on toasted bread, and as the key base for the Mexican dip "guacamole". In addition, the ripened fruits are used to produce extra virgin avocado oil by minimal cold-press oil extraction as well as bleached and deodorized avocado oil after solvent extraction and refinement, both oils exhibiting a healthful ratio of saturated and unsaturated fatty acids with more than 70% oleic acid and 9% linoleic acid, respectively.

Unfortunately, any thermal treatment or air-drying of avocado and products made thereof has long been known to induce the development of an unpleasant off-taste centering around a slightly pungent mouthfeel and a pronounced lingering bitter after-taste (*1, 2*). Already 80 years ago, a quinine-like bitter off-taste was reported in heated avocado samples, but the chemical structures of the bitter-tasting compounds remained unknown (*3*). A first insight into bitter phytochemicals in avocado was obtained in

1972 as the long-chain C_{17}-aliphatic triols 1,2,4-trihydroxyheptadeca-16-yne (**1**, **Figure 1**) and 1,2,4-trihydroxyheptadeca-16-ene (**2**) were successfully identified in the skin, seed, and pulp of avocado, exhibiting an unpleasant bitter off-flavor (*4*). Moreover, 1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**3**) was reported to be generated as another oxylipin upon heat treatment of avocado and observed to induce a moderately intense bitter taste sensation (*5*). However, these studies focused primarily on the quantitatively predominating phytochemicals, rather than selecting the target compounds to be identified with regard to bitter taste activity. As a consequence, it is still not clear whether the previously reported compounds **1**−**3** and/or unknown plant metabolites contribute to the thermal development of the bitter off-taste of avocado.

Driven by the need to discover the key players imparting the typical taste of foods, the research area "sensomics" has made tremendous efforts in recent years to map the sensometabolome and to identify the most intense taste-active metabolites in fresh and processed foods. Aimed at decoding the typical taste signature of food products on a molecular level, the so-called taste dilution analysis (TDA) was developed as an efficient screening tool enabling the sensory-directed identification of taste-active nonvolatiles in foods (*6*). This approach, combining natural product chemistry and analytical sensory analysis, led to the discovery of various previously unknown taste compounds such as bitter compounds in carrot products (*7, 8*), roasted cocoa (*9*), and red wine (*10*), cooling compounds in dark malt (*11*), taste modulators in beef bouillon (*12,13*), chicken broth (*14*), stewed beef (*15*), common beans (*16*), and Gouda cheese (*17*), and astringent phytochemicals in black tea (*18*), cacao nibs (*19*), red currants (*20*), and spinach (*21*).

The objective of the present investigation was, therefore, to screen extracts of thermally processed avocado pulp for their

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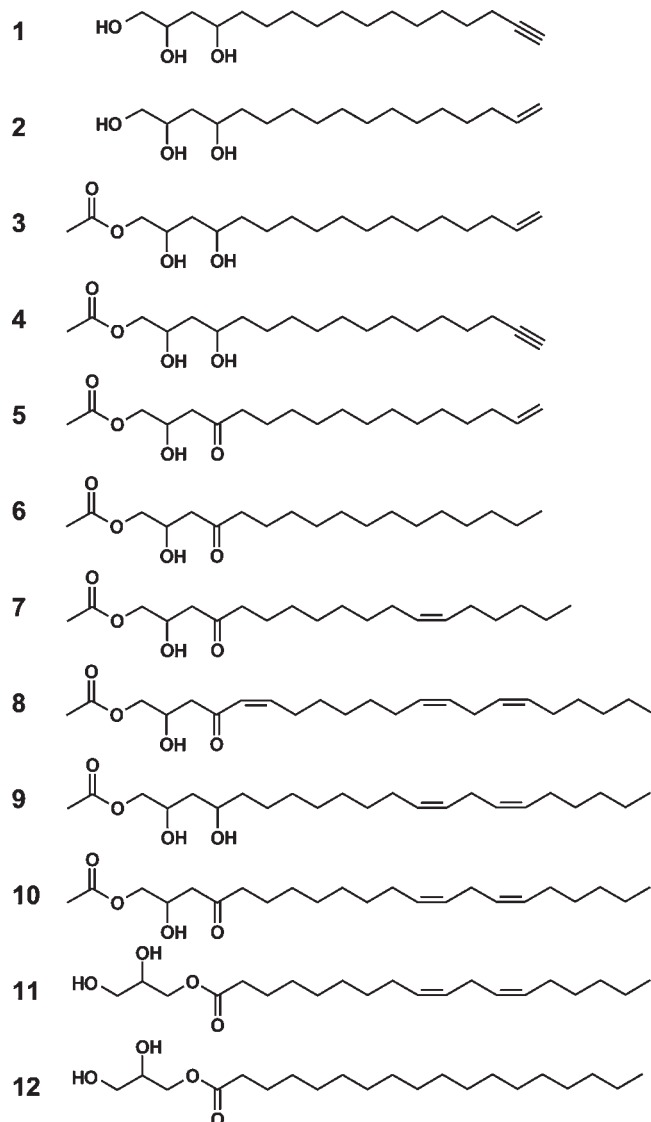
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Figure 1. Chemical structures of 1,2,4-trihydroxyheptadeca-16-yne (**1**), 1,2,4-trihydroxyheptadeca-16-ene (**2**), 1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**3**), 1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**4**), 1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**5**), 1-acetoxy-2-hydroxy-4-oxoheptadecane (**6**), 1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**), (*Z,Z,Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene (**8**), (*Z,Z*)-1-acetoxy-2,4-dihydroxy-heneicosa-12,15-diene (**9**), (*Z,Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene (**10**), 1-*O*-linoleoyl-glycerol (**11**), and 1-*O*-stearoyl-glycerol (**12**).

bitter sensometabolites by application of taste dilution techniques, to isolate and identify the compounds inducing the most intense human taste response, and to evaluate their sensory impact on the basis of their human threshold concentrations and taste re-engineering experiments.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: ethanol, formic acid, monosodium L-glutamate monohydrate, and pentane (Merck KGA, Darmstadt, Germany); 1-*O*-linoleoyl-*rac*-glycerol, 1-*O*-oleoyl-*rac*-glycerol, 2-*O*-oleoyl-*rac*-glycerol, 1-*O*-stearoyl-*rac*-glycerol, and 1-*O*-palmitoyl-*rac*-glycerol, sodium chloride, oleic acid (>99%), maltodextrin, white oil (puriss. paraffin oil), sucrose, caffeine, puriss. gallus tannic acid, reduced L-glutathione, guar, and lactic acid (Sigma-Aldrich, Steinheim, Germany); yeast extract "Gistex XII LS Pulver AGGL" (DSM Food Specialties Savory Ingredients, Delft, Netherlands); and soy lecithin "Emultop" (Degussa, Hamburg, Germany). Water used

for chromatography was prepared by means of a Milli-Q Gradient A 10 system (Millipore, Schwalbach, Germany), and bottled water was used for sensory studies (Evian, Danone, Wiesbaden, Germany). Solvents were of HPLC grade (Fisher Scientific, Schwerte, Germany), and CDCl₃ was supplied by Euriso-Top (Gif-sûr-Yvette, France). Fully ripe avocados (*P. americana* Mill. cv. Hass) were purchased in a local vegetable store. For sensory studies, an aqueous model broth was prepared by dissolving monosodium L-glutamate monohydrate (1.9 g/L), maltodextrin (6.375 g/L), sodium chloride (2.9 g/L), and yeast extract (2.1 g/L) in water and adjusting the pH value to 6.5 with traces of formic acid (0.1% in water) in water.

Preparation of Avocado Pulp and Thermal Processing. After removal of the peel and seed, the pulp (400 g) isolated from three ripened avocados was homogenized for 30 s at 3500 rpm, and aliquots (20 g) of the puree obtained were placed into glass beakers (50 mL), which were closed with aluminum foil and, then, thermally processed for 30, 60, and 120 min at 80, 100, and 120 °C in an laboratory oven. After cooling, the heat-treated pulp as well as a nontreated sample (control) were sensorially evaluated by means of taste profile analysis.

Solvent Extraction of Heat-Treated Avocado Puree. Avocado puree (400 g), thermally treated for 120 min at 120 °C as detailed above, was extracted five times with pentane (200 mL each) for 15 min using an ultrasonic bath. After paper filtration, the combined organic layers were extracted three times with ethanol/water (8:2; v/v; 300 mL each), followed by an extraction with ethanol/water (9:1; v/v; 2 × 300 mL) to afford the pentane solubles (fraction A, yield = 45.7%) and the ethanol/water-solubles (fraction B, yield = 6.6%), as well as the nonsoluble residue (fraction C, yield = 47.7%), which were separated from solvents in vacuum and freeze-dried. As fractions A and C showed only marginal taste activity and fraction B imparted an intense bitter taste in aqueous solution and induced pronounced mouthfulness, thickness, complexity, and a long-lasting taste impression, coined kokumi sensation (*16, 17, 22, 23*), when added to the model broth solution, the yellow colored, oily residue of fraction B was dissolved in acetonitrile (300 mL) and kept at −20 °C until used for further analysis.

Isolation of Compounds 1−12 from Avocado Fraction B. To remove residual triglycerides, aliquots of avocado fraction B (1.0 g) dissolved in acetonitrile (75 mL) were applied onto a 100 × 4.6 mm, 57 μm, Strata C18-E cartridge (Phenomenex, Aschaffenburg, Germany), conditioned with acetonitrile, followed by an elution with acetonitrile (150 mL). The effluent was collected and concentrated under vacuum to about 30 mL, and aliquots (1.9 mL) were then separated by means of semipreparative RP-HPLC on a HyperClone ODS C18, 250 × 21.2 mm, 5 μm, RP-18 column (Phenomenex) operated with a flow rate of 20.0 mL/min. Using water (adjusted to pH 5.0 with 1.0% formic acid) as solvent A and acetonitrile as solvent B, chromatography was performed starting with 60% solvent B for 2.5 min and then increasing solvent B to 75% within 60 min and, finally, to 100% within an additional 2.5 min, followed by isocratic elution for 5.0 min. After 70.0 min, solvent B decreased again to 60% and was kept for 3.0 min prior to the next injection. A total of 22 fractions, namely, fractions B1−B22, were collected and separated from solvent under vacuum, and the sensometabolites **1−12** were isolated and identified by means of LC-MS/MS and 1D/2D NMR spectroscopy.

1,2,4-Trihydroxyheptadeca-16-yne (**1**, *Figure 1*): LC-MS (APCI⁺), *m/z* 231 (30, [M − 3H₂O + H]⁺), 249 (100, [M − 2H₂O + H]⁺), 267 (60, [M − -H₂O + H]⁺), 285 (50, [M + H]⁺), 569 (100, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 247 (45, [M − 2H₂O − H]⁻), 265 (25, [M − H₂O − H]⁻), 283 (100, [M − H]⁻); LC-MS/MS (APCI⁻; DP, −120; EP, −11; CE, −25; CXP, −6), *m/z* 247 (100, [M − 2H₂O − H]⁻), 265 (25, [M − H₂O − H]⁻), 283 (45, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ 1.20 [s, 16H, H−C(6−13)], 1.42 [m, 2H, H−C(5)], 1.44 [qu, 2H, *J* = 6.8, 13.8, 21.0 Hz, H−C(14)], 1.53 [m, 2H, H−C(3)], 2.45 [m, 2H, H−C(15)], 2.86 [s, H, H−C(17)], 3.82 [m, 1H, H−C(4)], 3.93 [dd, 1H, *J* = 7.6, 12.1 Hz, H−C(2)], 4.04 [m, 2H, H−C(1)]; ¹³C NMR (400 MHz, CDCl₃), δ 19.8 [C(15)], 25.3 [C(6)], 29.3 [C(7−14)], 38.2 [C(5)], 39.1 [C(3)], 68.4 [C(17)], 68.6 [C(1)], 70.8 [C(2)], 72.5 [C(4)], 77.3 [C(16)].

1,2,4-Trihydroxyheptadeca-16-ene (**2**, *Figure 1*): LC-MS (APCI⁺), *m/z* 233 (30, [M − 3H₂O + H]⁺), 251 (100, [M − 2H₂O + H]⁺), 269 (50, [M − -H₂O + H]⁺), 287 (50, [M + H]⁺), 571 (100, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 249 (45, [M − 2H₂O − H]⁻), 267 (35, [M − H₂O − H]⁻), 285 (100, [M − H]⁻); LC-MS/MS (APCI⁻; DP, −120; EP, −11; CE, −25; CXP, −6), *m/z* 249 (100, [M − 2H₂O − H]⁻), 267 (35, [M − H₂O − H]⁻),

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285 (35, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃, δ 1.20 [(s, 16H, H−C(6−13)), 1.31 [qu, 2H, *J* = 6.8, 13.8, 21.0 Hz, H−C(14)], 1.42 [m, 2H, H−C(5)], 1.53 [m, 2H, H−C(3)], 1.95 [m, 2H, H−C(15)], 3.82 [m, 1H, H−C(4)], 3.93 [dd, 1H, *J* = 7.6, 12.1 Hz, H−C(2)], 4.04 (m, 2H, H−C(1), 4.86 [dd, 1H, *J* = 10.1 Hz, H−C(17β)], 4.92 [d, 1H, *J* = 17.1 Hz, H−C(17α)], 5.74 [m, 1H, H−C(16)]; ¹³C NMR (400 MHz, CDCl₃), δ 25.3 [C(6)], 29.3 [C(7−14)], 33.8 [C(15)], 38.2 [C(5)], 39.1 [C(3)], 68.6 [C(1)], 70.8 [C(2)], 72.5 [C(4)], 114.1 [C(17)], 139.3 [C(16)].

1-Acetoxy-2,4-dihydroxyheptadeca-16-ene (**3**, *Figure 1*): LC-MS (APCI⁺), *m/z* 233 (75, [M − HAc − 2H₂O + H]⁺), 251 (70, [M − HAc − H₂O + H]⁺), 269 (10, [M − HAc + H]⁺), 293 (15, [M − 2H₂O + H]⁺), 311 (70, [M − H₂O + H]⁺), 329 (30, [M + H]⁺), 657 (100, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 655 (100, [2M − H]⁻); EPI (APCI⁺; DP, +120; CE, +25; CES, +10), *m/z* 233 (100, [M − HAc − 2H₂O + H]⁺), 251 (90, [M − HAc − H₂O + H]⁺), 269 (20, [M − HAc + H]⁺), 293 (5, [M − 2H₂O + H]⁺), 311 (75, [M − H₂O + H]⁺), 329 (45, [M + H]⁺); EPI (APCI⁻; DP, −120; CE, −25; CES, −10), *m/z* 285 (10, [M − CH₃O]⁻), 327 (100, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ1.20 [s, 16H, H−C(6−13)], 1.31 [qu, 2H, *J* = 6.8, 13.8, 21.0 Hz, H−C(14)], 1.42 [m, 2H, H−C(5)], 1.53 [m, 2H, H−C(3)], 1.95 [m, 2H, H−C(15)], 2.04 [s, 3H, H−C(2′)], 3.82 [m, 1H, H−C(4)], 3.93 [dd, 1H, *J* = 7.6, 12.1 Hz, H−C(2)], 4.04 [m, 2H, H−C(1)], 4.86 [dd, 1H, *J* = 10.1 Hz, H−C(17β)], 4.92 [d, 1H, *J* = 17.1 Hz, H−C(17α)], 5.74 [m, 1H, H−C(16)]; ¹³C NMR (400 MHz, CDCl₃), δ 20.9 [C(2′)], 25.3 [C(6)], 29.3 [C(7−14)], 33.8 [C(15)], 38.2 [C(5)], 39.1 [C(3)], 68.6 [C(2)], 70.8 [C(4)], 72.5 [C(1)], 114.1 [C(17)], 139.3 [C(16)], 171.2 [C(1′)].

1-Acetoxy-2,4-dihydroxyheptadeca-16-yne (**4**, *Figure 1*): LC-MS (APCI⁺), *m/z* 231 (40, [M − HAc − 2H₂O + H]⁺), 249 (80, [M − HAc − H₂O + H]⁺), 267 (15, [M − HAc + H]⁺), 291 (30, [M − 2H₂O + H]⁺), 309 (45, [M − H₂O + H]⁺), 327 (20, [M + H]⁺), 653 (100, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 247 (35, [M − HAc − H₂O − H]⁻), 265 (30, [M − HAc − H]⁻), 283 (100, [M − CH₃CO]⁻), 325 (25, [M − H]⁻), 651 (100, [2M − H]⁻); EPI (APCI⁺; DP, +120; CE, +25; CES, +10), *m/z* 231 (10, [M − HAc − 2H₂O + H]⁺), 249 (25, [M − HAc − H₂O + H]⁺), 267 (10, [M − HAc + H]⁺), 309 (10, [M − H₂O + H]⁺), 327 (100, [M + H]⁺); EPI (APCI⁻; DP, −120; CE, −25; CES, −10), *m/z* 307 (20, [M − H₂O − H]⁻), 325 (100, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ 1.20 [s, 16H, H−C(6−13)], 1.42 [m, 2H, H−C(5)], 1.44 [qu, 2H, *J* = 6.8, 13.8, 21.0 Hz, H−C(14)], 1.53 [m, 2H, H−C(3)], 2.45 [m, 2H, H−C(15)], 2.04 [s, 3H, H−C(2′)], 2.86 [s, H, H−C(17)], 3.82 [m, 1H, H−C(4)], 3.93 [dd, 1H, *J* = 7.6, 12.1 Hz, H−C(2)], 4.04 [m, 2H, H−C(1)]; ¹³C NMR (400 MHz, CDCl₃), δ 19.8 [C(15)], 20.9 [C(2′)], 25.3 [C(6)], 29.3 [C(7−14)], 38.2 [C(5)], 39.1 [C(3)], 68.6 [C(2)], 68.8 [C(17)], 70.8 [C(4)], 72.5 [C(1)], 77.3 [C(16)], 171.2 [C(1′)].

1-Acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**5**, *Figure 1*): LC-MS (APCI⁺), *m/z* 231 (65, [M − HAc − 2H₂O + H]⁺), 249 (85, [M − HAc − H₂O + H]⁺), 267 (20, [M − HAc + H]⁺), 291 (70, [M − 2H₂O + H]⁺), 309 (100, [M − H₂O + H]⁺), 327 (70, [M + H]⁺); LC-MS (APCI⁻), *m/z* 283 (45, [M − CH₃CO]⁻), 325 (40, [M − H]⁻), 651 (100, [2M − H]⁻); EPI (APCI⁺; DP, +120; CE, +25; CES, +10), *m/z* 231 (15, [M − HAc − 2H₂O + H]⁺), 249 (35, [M − HAc − H₂O + H]⁺), 267 (45, [M − HAc + H]⁺), 309 (10, [M − H₂O + H]⁺), 327 (100, [M − H]⁺); EPI (APCI⁻; DP, −120; CE, −25; CES, −28), *m/z* 247 (10, [M − HAc − H₂O − H]⁻), 307 (10, [M − H₂O − H]⁻), 325 (100, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ 1.20 [s, 16H, H−C(7−14)], 1.53 [m, H−C(6)], 1.98 [m, 2H, H−C(15)], 2.04 [s, 3H, H−C(1′)], 2.48 [t, 2H, H−C(5)], 2.79 [m, 2H, H−C(3)], 4.12 [m, 2H, H−C(1)], 4.32 [m, 1H, H−C(2)], 4.86 [dd, 1H, *J* = 10.1 Hz, H−C(17β)], 4.92 [d, 1H, *J* = 17.1 Hz, H−C(17α)], 5.74 [m, 1H, H−C(16)]; ¹³C NMR (400 MHz, CDCl₃), δ 20.9 [C(2′)], 23.5 [C(6)], 29.3 [C(7−14)], 33.8 [C(15)], 43.6 [C(5)], 45.8 [C(3)], 66.6 [C(2)], 72.5 [C(1)], 114.1 [C(17)], 139.3 [C(16)], 171.0 [C(1′)], 210.9 [C(4)].

1-Acetoxy-2-hydroxy-4-oxoheptadecane (**6**, *Figure 1*): LC-MS (APCI⁺), *m/z* 233 (100, [M − HAc − 2H₂O + H]⁺), 251 (55, [M − HAc − H₂O + H]⁺), 269 (10, [M − HAc + H]⁺), 293 (35, [M − 2H₂O + H]⁺), 311 (80, [M − H₂O + H]⁺), 329 (40, [M + H]⁺); LC-MS (APCI⁻), *m/z* 285 (45, [M − CH₃CO]⁻), 327 (25, [M − H]⁻), 655 (100, [2M − H]⁻); EPI (APCI⁺; DP, +120; CE, +25; CES, +10), *m/z* 233 (60, [M − HAc − 2H₂O + H]⁺), 251 (40, [M − HAc − H₂O + H]⁺), 269 (10, [M − HAc + H]⁺), 311 (5, [M − H₂O + H]⁺), 329 (10, [M + H]⁺); EPI (APCI⁻; DP, −120; CE, −25; CES, −10), *m/z* 327 (100, [M − H]⁻); LC-TOF-MS, *m/z* 329.4954 ([M + H]⁺, measured), *m/z* 329.4947 ([M + H]⁺, calcd for C₁₉H₃₇O₄); ¹H NMR (400 MHz, CDCl₃), δ 0.89 [t, 3H, *J* = 6.5, 13.1 Hz, H−C(17)], 1.20 [s, 18H, H−C(7−16)], 1.53 [m, H−C(6)], 2.04 [s, 3H, H−C(1′)], 2.48 [t, 2H,

H−C(5)], 2.79 [m, 2H, H−C(3)], 4.12 [m, 2H, H−C(1)], 4.32 [m, 1H, H−C(2)]; ¹³C NMR (400 MHz, CDCl₃), δ 14.1 [C(17)], 20.9 [C(2′)], 23.5 [C(16)], 29.3 [C(7−14)], 33.8 [C(15)], 43.6 [C(5)], 45.8 [C(3)], 66.6 [C(2)], 72.5 [C(1)], 171.0 [C(1′)], 210.9 [C(4)].

1-Acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**, *Figure 1*): LC-MS (APCI⁺), *m/z* 287 (75, [M − 3H₂O + H]⁺), 305 (80, [M − -2H₂O + H]⁺), 323 (100, [M − H₂O + H]⁺), 341 (25, [M + H]⁺), 663 (95, [2M − H₂O + H]⁺), 681 (40, [2M + H]⁺); LC-MS (APCI⁻):, *m/z* 339 (40, [M − H]⁻), 385 (95, [M + HCOO]⁻), 679 (100, [2M − H]⁻); EPI (APCI⁺; DP, +50; CE, +30; CES, +25), *m/z* 287 (45, [M − 3H₂O + H]⁺), 305 (50, [M − 2H₂O + H]⁺), 323 (100, [M − H₂O + H]⁺), 341 (15, [M + H]⁺); EPI (APCI⁻; DP, −50; CE, −30; CES, −25), *m/z* 303 (100, [M − 2H₂O − H]⁻), 321 (80, [M − H₂O − H]⁻), 339 (35, [M − H]⁻); LC-TOF-MS, *m/z* 341.5060 ([M + H]⁺, measured), *m/z* 341.5054 ([M + H]⁺, calcd for C₂₀H₃₇O₄); ¹H NMR (400 MHz, CDCl₃), δ 0.89 [t, 3H, *J* = 6.5, 13.1 Hz, H−C(18)], 1.30 [m, 14H, H−C(7−10), H−C(15−17)], 1.49 [m, 2H, H−C(6)], 2.03 [dd, 4H, *J* = 6.6, 13.3 Hz, H−C(11), H−C(14)], 2.11 [s, 3H, H−C(2′)], 2.52 [t, 2H, *J* = 5.8, 12.8 Hz, H−C(5)], 2.79 [m, 2H, H−C(3)], 4.12 [m, 2H, H−C(1)], 4.32 [m, 1H, H−C(2)], 5.37 [m, 4H, *J* = 10.8 Hz, H−C(12), H−C(13)]; ¹³C NMR (400 MHz, CDCl₃), δ 14.1 [C(18)], 20.9 [C(2′)], 22.6 [C(17)], 25.3 [C(6)], 27.2 [C(11), C(14)], 29.1 [C(7)], 29.3 [C(8)], 29.4 [C(15)], 29.6 [C(9)], 29.7 [C(10)], 31.5 [C(16)], 41.2 [C(5)], 43.0 [C(3)], 68.6 [C(2)], 70.8 [C(1)], 130.2 [C(12), C(13)], 171.3 [C(1′)], 210.9 [C(4)].

(Z,Z,Z)-1-Acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene (**8**, *Figure 1*): LC-MS (APCI⁺), *m/z* 283 (10, [M − HAc − 2H₂O + H]⁺), 301 (100, [M − HAc − H₂O + H]⁺), 319 (75, [M − HAc + H]⁺), 361 (10, [M − H₂O + H]⁺), 379 (20, [M + H]⁺); LC-MS (APCI⁻), *m/z* 361 (10, [M − H₂O − H]⁻), 377 (40, [M − H]⁻), 755 (50, [2M − H]⁻); EPI (APCI⁺; DP, +90; CE, +35; CES, +30), *m/z* 283 (30, [M − HAc − 2H₂O + H]⁺), 301 (100, [M − HAc − H₂O + H]⁺), 319 (15, [M − HAc + H]⁺), 343 (5, [M − 2H₂O + H]⁺), 361 (50, [M − H₂O + H]⁺), 379 (50, [M + H]⁺); EPI (APCI⁻; DP, −60; CE, −15; CES, −9), *m/z* 299 (5, [M − HAc − H₂O − H]⁻), 317 (25, [M − HAc − H]⁻), 377 (10, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ 0.89 [m, 3H, H−C(21)], 1.28 [m, 12H, H−C(8−10), H−C(18−20)], 2.06 [m, 6H, H−C(7), H−C(11), H−C(17)], 2.11 [s, 3H, H−C(2′)], 2.32 [qu, 2H, *J* = 6.9, 13.9 Hz, H−C(14)], 2.77 [d, 2H, *J* = 5.7 Hz, H−C(3)], 4.12 [m, 2H, H−C(2)], 4.35 [m, 1H, H−C(1)], 5.37 [m, 4H, H−C(12), H−C(13), H−C(15), H−C(16)], 6.11 [d, 1H, *J* = 15.9 Hz, H−C(5)], 6.89 [m, 1H, H−C(6)]; ¹³C NMR (400 MHz, CDCl₃), δ 14.1 [C(21)], 20.9 [C(2′)], 22.6 [C(20)], 25.6 [C(14)], 27.2 [C(11), C(17)], 29.6 [C(18)], 27.9 [C(8)], 28.8 [C(9)], 29.4 [C(10)], 31.5 [C(19)], 32.5 [C(7)], 42.3 [C(3)], 66.2 [C(2)], 67.3 [C(1)], 127.8 [C(13), C(15)], 129.7 [C(12), C(16)], 130.3 [C(5)], 149.4 [C(6)], 171.1 [C(1′)], 199.7 [C(4)].

(Z,Z)-1-Acetoxy-2,4-dihydroxyheneicosa-12,15-diene (**9**, *Figure 1*): LC-MS (APCI⁺), *m/z* 287 (100, [M − HAc − 2H₂O + H]⁺), 305 (90, [M − HAc − H₂O + H]⁺), 323 (30, [M − HAc + H]⁺), 347 (55, [M − 2H₂O + H]⁺), 365 (85, [M − H₂O + H]⁺), 383 (75, [M + H]⁺), 765 (100, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 381 (15, [M − H]⁻), 763 (10, [2M − H]⁻), 809 (35, [2M + HCOO]⁻); EPI (APCI⁺; DP, +60; CE, +20; CES, +15), *m/z* 287 (40, [M − HAc − 2H₂O + H]⁺), 305 (100, [M − HAc − H₂O + H]⁺), 323 (10, [M − HAc + H]⁺), 347 (5, [M − 2H₂O + H]⁺), 365 (20, [M − H₂O + H]⁺), 383 (5, [M + H]⁺); EPI (APCI⁻; DP, −60; CE, −15; CES, −9), *m/z* 303 (10, [M − HAc − H₂O − H]⁻), 321 (30, [M − HAc − H]⁻), 363 (50, [M − H₂O − H]⁻), 381 (100, [M − H]⁻); ¹H NMR (400 MHz, CDCl₃), δ 0.89 [t, 3H, *J* = 6.5, 13.1 Hz, H−C(21)], 1.30 [m, 14H, H−C(6−10), H−C(18−20)], 1.49 [m, 2H, H−C(5)], 1.59 [m, 2H, H−C(3)], 2.03 [qu, 4H, *J* = 6.6, 13.3 Hz, H−C(11), H−C(17)], 2.11 [s, 3H, H−C(2′)], 2.62 [t, 2H, *J* = 5.8, 12.8 Hz, H−C(14)], 3.90 [m, 1H, H−C(4)], 4.06 [qu, 1H, *J* = 7.9, 12.1 Hz, H−C(2)], 4.30 [m, 2H, H−C(1)], 5.36 [m, 4H, H−C(12−16)]; ¹³C NMR (400 MHz, CDCl₃), δ 14.1 [C(21)], 20.9 [C(2′)], 22.6 [C(20)], 25.3 [C(6)], 25.6 [C(14)], 27.2 [C(11), C(17)], 29.1 [C(7)], 29.3 [C(8)], 29.4 [C(18)], 29.6 [C(9)], 29.7 [C(10)], 31.5 [C(19)], 38.2 [C(5)], 39.0 [C(3)], 68.6 [C(2)], 70.8 [C(1)], 72.6 [C(4)], 128.0 [C(13), C(15)], 130.2 [C(12), C(16)], 171.3 [C(1′)].

(Z,Z)-1-Acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene (persin) (**10**, *Figure 1*): LC-MS (APCI⁺), *m/z* 285 (20, [M − HAc − 2H₂O + H]⁺), 303 (100, [M − HAc − H₂O + H]⁺), 321 (65, [M − HAc + H]⁺), 363 (10, [M − H₂O + H]⁺), 381 (50, [M + H]⁺), 643 (20, [2M − H₂O + H]⁺), 761 (20, [2M + H]⁺); LC-MS (APCI⁻), *m/z* 319 (45, [M − HAc − H]⁻), 379 (10, [M − H]⁻), 805 (15, [2M + HCOO]⁻); EPI (APCI⁺; DP, +60; CE, +45; CES, +50), *m/z* 285 (20, [M − HAc − 2H₂O + H]⁺),

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303 (35, [M − HAc − H_2O + H]$^+$), 321 (20, [M − HAc + H]$^+$), 363 (5, [M − H_2O + H]$^+$), 381 (100, [M + H]$^+$); EPI (APCI$^−$; DP, −90; CE, −30; CES, −40), *m/z* 301 (90, [M − HAc − H_2O − H]$^−$), 319 (80, [M − HAc − H]$^−$), 361 (15, [M − H_2O − H]$^−$), 379 (100, [M − H]$^−$); ^{1}H NMR (500 MHz, CDCl$_3$), δ 0.87 [t, 3H, *J* = 6.7, 14.0 Hz, H−C(21)], 1.28 [m, 14H, H−C(7−10), H−C(18−20)], 1.56 [q, 2H, *J* = 7.3, 14.7 Hz, H−C(6)], 2.03 [m, 4H, H−C(11), H−C(17)], 2.09 [s, 3H, H−C(2′)], 2.48 [t, 2H, *J* = 7.1, 7.6 Hz, H−C(5)], 2.62 [m, 2H, H−C(14)], 2.79 [t, 2H, *J* = 6.6, 6.7 Hz, H−C(3)], 4.06 [m, 2H, H−C(1)], 4.30 [m, 1H, H−C(2)], 5.37 [m, 4H, H−C(12), H−C(13), H−C(15), H−C(16)]; ^{13}C NMR (500 MHz, CDCl$_3$), δ 14.1 [C(21)], 20.9 [C(2′)], 22.6 [C(20)], 23.5 [C(6)], 25.6 [C(14)], 27.2 [C(11), C(17)], 29.1 [C(7)], 29.3 [C(8)], 29.4 [C(18)], 29.6 [C(9)], 29.7 [C(10)], 31.5 [C(19)], 43.6 [C(5)], 45.2 [C(3)], 66.6 [C(2)], 77.2 [C(1)], 127.9 [C(13), C(15)], 130.1 [C(12), C(16)], 171.0 [C(1′)], 210.9 [C(4)].

1-O-Linoleoyl-glycerol (**11**, *Figure 1*): LC-MS (APCI$^+$), *m/z* 277 (100, [M − HAc − H_2O + H]$^+$), 295 (95, [M − HAc + H]$^+$), 355 (25, [M + H]$^+$), 691 (100, [2M − H_2O + H]$^+$); LC-MS (APCI$^−$), *m/z* 293 (40, [M − HAc − H]$^−$), 353 (35, [M − H]$^−$), 399 (15, [M + HCOO]$^−$), 707 (55, [2M − H]$^−$); EPI (APCI$^+$; DP, +120; CE, +20; CES, +30), *m/z* 277 (100, [M − HAc − H_2O + H]$^+$), 295 (25, [M − HAc + H]$^+$), 337 (5, [M − H_2O + H]$^+$), 355 (15, [M + H]$^+$); EPI (APCI$^−$; DP, −120; CE, −60; CES, −40), *m/z* 335 (5, [M − H_2O − H]$^−$), 353 (50, [M − H]$^−$); ^{1}H NMR (400 MHz, CDCl$_3$), δ 0.87 [t, 3H, *J* = 5.4, 12.5 Hz, H−C(4−7), H−C(15−17)], 1.26 [m, 12H, H−C(8), H−C(14)], 2.23 [qu, 2H, *J* = 7.2, 14.4 Hz, H−C(2)], 2.77 [d, 2H, *J* = 5.18 Hz, H−C(11)], 3.65 [m, 2H, H−C(3′)], 3.93 [q, 1H, *J* = 6.1, 3.8 Hz, H−C(2′)], 4.17 [m, 2H, H−C(1′)], 5.57 [m, 2H, H−C(9), H−C(13)], 5.74 [m, 2H, H−C(10), H−C(12)]; ^{13}C NMR (400 MHz, CDCl$_3$), δ 14.0 [C(18)], 22.6 [C(17)], 24.9 [C(3)], 25.3 [C(11)], 27.5 [C(8), C(14)], 28.9 [C(4), C(5)], 29.4 [C(6), C(7), C(15)], 30.6 [C(16)], 34.1 [C(2)], 63.42 [C(3′)], 65.1 [C(1′)], 70.3 [C(2′)], 127.1 [C(10), C(12)], 129.9 [C(9), C(13)], 174.4 [C(1)].

1-O-Stearoyl-glycerol (**12**, *Figure 1*): LC-MS (APCI$^+$), *m/z* 263 (35, [M − HAc − $2H_2O$ + H]$^+$), 281 (50, [M − HAc − H_2O + H]$^+$), 305 (25, [M − $3H_2O$ + H]$^+$), 323 (35, [M − $2H_2O$ + H]$^+$), 341 (60, [M − H_2O + H]$^+$), 359 (35, [M + H]$^+$), 717 (25, [2M + H]$^+$); EPI (APCI$^+$; DP, +90; CE, +10; CES, +20), *m/z* 263 (100, [M − HAc − $2H_2O$ + H]$^+$), 281 (40, [M − HAc − H_2O + H]$^+$), 305 (5, [M − $3H_2O$ + H]$^+$), 323 (35, [M − $2H_2O$ + H]$^+$), 341 (40, [M − H_2O + H]$^+$), 359 (35, [M + H]$^+$); EPI (APCI$^−$; DP, −60; CE, −10; CES, −20), *m/z* 357 (100, [M − H]$^−$); ^{1}H NMR (400 MHz, CDCl$_3$), δ 0.82 [t, 3H, *J* = 6.9 Hz, H−C(18)], 1.92 [m, 28H, H−C(4−17)], 1.63 [q, 2H, *J* = 7.6, 14.9 Hz, H−C(3)], 2.25 [t, 2H, *J* = 7.4 Hz, H−C(2)], 3.60 [qu, 1H, *J* = 5.8, 11.4 Hz, H−C(3′α)], 3.69 [qu, 1H, *J* = 3.8, 7.6 Hz, H−C(3′β)], 3.93 [q, 1H, *J* = 6.1, 3.8 Hz, H−C(2′)], 4.17 [m, 2H, H−C(1′)]; ^{13}C NMR (400 MHz, CDCl$_3$), δ 14.0 [C(18)], 22.6 [C(17)], 24.9 [C(3)], 28.9 [C(4), C(5), C(15)], 29.1 [C(6−14)], 31.6 [C(16)], 34.1 [C(2)], 63.42 [C(3′)], 65.1 [C(1′)], 70.3 [C(2′)], 174.4 [C(1)].

Sensory Analysis. *General Conditions, Panel Training.* To familiarize the subjects with the taste language used by our sensory group and to get them trained in recognizing and distinguishing different qualities of oral sensations in analytical sensory experiments, 15 subjects (8 women and 7 men, ages 23−40 years), who gave consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated for at least two years in weekly training sessions. For the training of the individual orosensory modalities, aqueous solutions (3 mL each) of the following reference taste compounds dissolved in bottled water (pH 6.5) were used by means of the sip-and-spit method: sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (20 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium L-glutamate (3 mmol/L) for umami taste, gallus tannic acid (0.05%) for astringency, and an aqueous emulsion of oleic acid (5 mmol/L), white oil (5%) and guar gum (5%) for creaminess. For the training of the activity of mouthfulness enhancement and complexity increase, termed kokumi activity (*16*), the panel was asked to compare the gustatory impact of the aqueous model broth (control) with a solution of reduced L-glutathione (0.5, 1.0, or 2.5 mmol/L) in same model solution (pH 6.5). The sensory experiments were performed at 20−22 °C in three independent sessions. To prevent cross-modal interactions with olfactory inputs, the panelists used nose clips.

Pretreatment of Fractions. Prior to sensory analysis, the fractions or isolated compounds were suspended in water, and, after removal of the volatiles under high vacuum (<5 hPa), were freeze-dried twice. GC-MS

Table 1. TD Factors Determined and Sensory Active Compounds Identified in Avocado Fraction B by Means of TDA and cTDA, Respectively

	TD factor for		
fraction[a]	bitter taste[b]	kokumi enhancement[c]	compd identified (no.)[d]
B1	1	16	ni
B2	1	1	ni
B3	8	1	1,2,4-trihydroxyheptadeca-16-yne (**1**)
B4	8	1	1,2,4-trihydroxyheptadeca-16-ene (**2**)
B5	8	16	1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**4**)
B6	8	32	1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**5**)
B7	2	8	ni
B8	2	4	ni
B9	2	4	ni
B10	8	8	1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**3**)
B11	8	64	1-acetoxy-2-hydroxy-4-oxoheptadecane (**6**)
B12		8	ni
B13	8	32	1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**)
B14	2	8	ni
B15	1	2	ni
B16	1	8	ni
B17	8	32	1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene (**8**)
B18	2	16	ni
B19	8	32	1-acetoxy-2,4-dihydroxy-*n*-heneicosa-12,15-diene (**9**)
B20	16	256	1-acetoxy-2-hydroxy-4-oxo-*n*-heneicosa-12,15-diene (**10**)
B21	2	<1	1-linoleyl-glycerol (**11**)
B22	1	<1	1-stearoyl-glycerol (**12**)

[a] Fraction numbering refers to **Figure 4**. [b] Fractions were dissolved in water (pH 6.5), and TD factors were determined by means of TDA using the half-tongue test. [c] Fractions were dissolved in model broth (pH 6.5), and TD factors were determined by means of cTDA. [d] Chemical structures of identified compounds are given in **Figure 1**; ni, not identified.

and ion chromatographic analysis revealed that food fractions treated by that procedure are essentially free of solvents and buffer compounds used. The pH value of all samples was adjusted to 6.5 with trace amounts of either aqueous formic acid (1 g/100 g) or potassium hydroxide (0.1 mol/L). Formic acid, which is GRAS listed as a flavoring agent for food and feed applications, was used to adjust the pH value of solutions to be sensorially evaluated, because trace amounts of this acid do not influence the sensory profile of the test solution. To minimize the uptake of any toxic compound to the best of our knowledge, all of the sensory analyses were performed by using the sip-and-spit method, which means the test materials were not swallowed but expectorated.

Taste Profile Analysis. Aliquots (2.5 g) of the fresh and thermally treated samples of avocado puree, respectively, were randomly presented to the trained panelists, who were asked to evaluate the intensities of the descriptors astringency, bitterness, umami taste, sourness, sweetness, saltiness, creaminess, and mouthfulness (kokumi) using a linear scale from 0 (not detectable) to 5.0 (very strong). The geometric means of the individual values obtained for each descriptor by triplicate analysis were calculated and used to describe the taste profile. The values between panelists and three independent sessions differed by not more than ±0.8.

Taste Dilution Analysis (TDA) and Comparative Taste Dilution Analysis (cTDA). The individual fractions B1−B22 obtained by RP-HPLC were dissolved in ethanol (1.0 mL) and, then, diluted with water (20.0 mL; pH 6.5) to screen for bitter taste compounds by means of TDA or with a model broth solution (20 mL; pH 6.5) to study the kokumi-enhancing activity by means of cTDA (*6*, *24*). The parent solutions were stepwise diluted 1+1 either with water (pH 6.5) in case of the TDA or with the model broth solution (pH 6.5) in case of the cTDA. The dilutions of each fraction were presented to the sensory panel in order of increasing concentrations by means of a duo test using water (pH 6.5) or the model broth solution (pH 6.5) as blank, respectively. For the evaluation of bitterness by means of the TDA, a randomized half-tongue test was used following exactly the protocol reported earlier (*18−21*). The participants

Table 2. Monitored Mass Transitions, MS/MS Parameters, and Retention Times of Sensory Active Compounds **1**−**12**

compd	MW (Da)	mass transition	DP[a]	CE[b]	CXP[c]	RT[d] (min)
1,2,4-trihydroxyheptadeca-16-yne (**1**)	284	*m/z* 285 → 67	+31	+55	+4	4.6
1,2,4-trihydroxyheptadeca-16-ene (**2**)	286	*m/z* 287 → 69	+31	+23	+6	4.9
1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**3**)	328	*m/z* 329 → 67	+36	+57	+4	17.9
1,2,4-trihydroxyheptadeca-16-yne (**4**)	326	*m/z* 327 → 67	+31	+59	+6	8.1
1,2,4-trihydroxyheptadeca-16-ene (**5**)	326	*m/z* 327 → 67	+41	+59	+4	8.6
1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**6**)	328	*m/z* 329 → 81	+46	+55	+6	18.6
1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**7**)	340	*m/z* 341 → 67	+36	+65	+4	21.0
1-acetoxy-2-hydroxy-4-oxoheptadecane (**8**)	378	*m/z* 379 → 301	+41	+13	+14	30.5
1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**9**)	380	*m/z* 381 → 81	+36	+49	+6	34.7
1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene (**10**)	380	*m/z* 381 → 303	+36	+13	+8	35.0
1-*O*-linoleoyl-glycerol (**11**)	354	*m/z* 355 → 113	+41	+33	+8	37.8
1-*O*-stearoyl-glycerol (**12**)	358	*m/z* 359 → 95	+31	+27	+8	40.6
1-*O*-palmitoyl-glycerol (IS)	316	*m/z* 317 → 57	+61	+43	+0	28.4

[a] Declustering potential. [b] Collision energy. [c] Cell exit potential. [d] Retention time.

were instructed to determine the dilution step at which a taste difference between sample and blank solution could be detected. This so-called taste dilution (TD) factor determined by the sensory subjects in three separate sessions was averaged (**Table 1**). The TD factors between individuals and separate sessions did not differ by more than plus/minus one dilution step for the TDA and plus/minus two dilution steps for the cTDA.

Bitter Taste Recognition Thresholds by Means of the Half-Tongue Test. TD factors for the bitter taste of HPLC fractions B1−B22 (for TDA) as well as human bitter recognition thresholds for purified compounds **1**−**12** were determined by means of the half-tongue test following exactly the protocol reported earlier (*18*−*21*). Using bottled water as the solvent and an interstimulus interval length of 15 min, serial 1+1 dilutions of the samples were presented in order of increasing concentrations to a trained panel of 12 persons in three different sessions using the sip-and-spit method. In the case of a correct selection by the panelist, the same concentration was presented again aside one blank as a proof for the correctness of the data. The geometric mean of the last and the second to last concentrations was calculated and taken as the individual recognition threshold. The threshold values between bitter-trained individuals and between three separate sessions differed by not more than plus/minus one dilution step; that is, a threshold value of 42.8 μmol/L for 1,2,4-trihydroxyheptadeca-16-yne (**1**) represents a range from 21.4 to 85.6 μmol/L.

Recognition Threshold Concentrations for Mouthfulness Enhancement (Kokumi) Activity. TD factors for the kokumi impression of the HPLC fractions B1−B22 (for cTDA) as well as human kokumi recognition thresholds for compounds **1**−**12** were determined by means of a three-alternative forced-choice test as reported recently (*14*). The threshold value of the sensory group was approximated by averaging the threshold values of the individuals in three independent sessions. Values between kokumi-trained individuals and separate sessions differed by not more than plus/minus two dilution steps; as a result, a threshold value of 4.6 mmol/L for 1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**3**) represents a range of 1.15−18.4 μmol/L.

Taste Re-engineering Experiment. A sample of fresh avocado pulp was spiked with appropriate amounts of the sensory active compounds **1**−**12** to match their concentrations determined in an avocado puree sample heated for 120 min 120 °C and compared in the bitter taste and kokumi intensity with the nonspiked sample as well as the heat-treated avocado pulp on a 5-point linear scale.

Quantitative Analysis of Sensometabolites 1−12. Aliquots (200 g each) of freshly prepared avocado puree, before and after thermal treatment for 120 min at 120 °C, were spiked with a methanolic solution (1.1 mL) of the internal standard 1-*O*-palmitoyl-glycerol (10.1 mg/L), homogenized, and extracted five times with pentane (200 mL each) for 15 min followed by ultrasonication. After paper filtration, the combined organic layers were extracted three times with ethanol/water (8:2; v/v; 200 mL each), followed by an extraction with ethanol/water (9:1; v/v; 2 × 200 mL). The pentane layer was discarded, whereas the combined ethanol/water fractions were freed from solvent under vacuum and taken up in acetonitrile (100 mL). To remove residual triglycerides, aliquots of the avocado extract in acetonitrile were applied onto a Strata C18-E, 100 × 4.6 mm, 57 μm, cartridge (Phenomenex), conditioned with acetonitrile,

followed by an elution with acetonitrile (100 mL). The effluent was collected and concentrated under vacuum to about 10 mL, and aliquots (10 μL) were then analyzed for compounds **1**−**12** as well as the internal standard 1-*O*-palmitoyl-glycerol by means of HPLC-MS/MS.

Calibration and Recovery. A stock solution of compounds **1**−**12** (1.0 mg each) in 50 mL of acetonitrile was diluted 1:1, 1:5, 1:10, 1:20, and 1:40 with acetonitrile; each dilution was spiked with a methanolic solution (1.1 mL) of the internal standard 1-*O*-palmitoyl-glycerol (10.1 mg/L) and was, then, analyzed by means of HPLC-MS/MS. Calibration curves were prepared by plotting peak area ratios of analyte to internal standard against concentration ratios of each analyte to the internal standard using linear regression (see the Supporting Information). To determine the recovery, freshly prepared avocado puree (100 mg) was spiked with defined amounts of compounds **1**−**12** dissolved in acetonitrile (10 mL). The mixture was stirred for 20 min for equilibration and, after sample workup as detailed above, was analyzed by means of HPLC-MS/MS. The initial concentration of the analytes in unspiked, fresh avocado pulp (control; *n* = 5) was used to calculate the recovery rate showing an average value of 92.6% (±5.2%).

Semipreparative High-Performance Liquid Chromatography (HPLC). The preparative HPLC system (Varian, Darmstadt, Germany) consisted of two pumps (ProStar 210), a gradient mixer (1 mL), a Rheodyne injector with a 1.9 mL loop, and a ProStar 325 UV−vis detector operating at 220 nm as well as a Sedex 85-type evaporative light scattering detector (Sedere, Alfortville Cedex, France). The system was controlled with a Star Chromatography Workstation, version 6.2 software (Varian).

Liquid Chromatography−Mass Spectrometry (LC-MS/MS). Atmospheric pressure chemical ionization (APCI) mass spectra, MS/MS product ion spectra, and enhanced product ion (EPI) scans were acquired on an API 4000 QTRAP LC-MS/MS system (AB Sciex Instruments, Darmstadt, Germany) with direct flow infusion. For APCI, the ion spray voltage was set at −4500 V in the negative mode and at +5500 V in the positive mode (350 °C). The mass spectrometer was operated in the full-scan mode detecting positive and negative ions. For MS/MS experiments, nitrogen was used as collision gas (4×10^{-5} Torr).

For HPLC-MS/MS analysis, the mass spectrometer, operated in the APCI+ mode, was coupled to an Agilent 1100 pump, an Agilent 1100 degasser, and an Agilent 1200 autosampler (Waldbronn, Germany). The nebulizer gas was zero-grade air (45 psi), whereas the curtain gas was nitrogen (35 psi). By means of the multiple reaction monitoring (MRM) mode, the transition from the pseudomolecular ion [M + H]+ to a main fragment was recorded after collision-induced dissociation. The declustering potential (DP), cell exit potential (CXP), and collision energy (CE) were optimized to the values given in **Table 2** for each substance by a special tuning process. The dwell time for each mass transition was 80 ms. The quadrupoles operated at unit mass resolution. For instrumentation control and data collection Sciex Analyst software (v 1.4.2) was used. After sample injection (10.0 μL), chromatography was performed on a Hyperclone C18, 150 × 2.0 mm i.d., 5 μm, RP-18 column (Phenomenex) with gradient elution at a flow rate of 0.2 mL/min. Using 0.1% formic acid in water as solvent A and acetonitrile as solvent B, chromatography started with 60% solvent B for 3 min, increasing linearly to 100% within 60 min, followed by isocratic elution for 7.5 min.
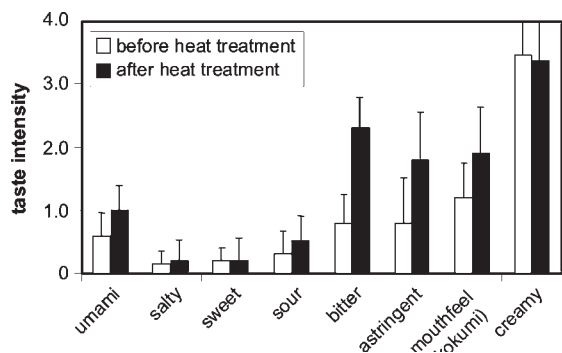
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Figure 2. Taste profile analysis of avocado puree before and after thermal treatment (30 min, 120 °C).

Nuclear Magnetic Resonance Spectroscopy (NMR). The ^{1}H, ^{13}C, COSY, DEPT, HMQC, and HMBC spectroscopic experiments were performed on either a 400 MHz DRX 400 or a 500 MHz Avance III NMR spectrometer from Bruker (Rheinstetten, Germany). CDCl$_3$ was used as solvent, and chemical shifts were referenced to the solvent signal. Data interpretation was done using XWIN-NMR software (version 3.5; Bruker) as well as MestReNova 5.1.0-2940 (Mestrelab Research S.L., Santiago de Compostela, Spain).

RESULTS AND DISCUSSION

As heat treatment of avocado is well-known in the literature to induce the evolution of an unpleasant, lingering bitter aftertaste (*1–3*), first the influence of thermal processing on the taste profile of avocado was studied.

Influence of Thermal Treatment on the Taste Profile of Avocado Puree. Samples of freshly minced avocado pulp, before and after thermal treatment for 30 min at 120 °C, were evaluated by means of a taste profile analysis. To achieve this, a trained sensory panel was asked to evaluate the intensities of the descriptors astringency, bitterness, umami taste, sourness, sweetness, saltiness, creaminess, and mouthfulness (kokumi) on a 5-point linear scale (**Figure 2**). Prior to heating, creaminess was judged with the highest intensity of 3.5, followed by mouthfulness (1.2), astringency (0.8), and bitterness (0.8) evaluated with somewhat lower intensities. In comparison, the other orosensations were perceived only with low intensities of < 0.3. After thermal treatment, the most pronounced changes were found for bitterness and astringency, the perceived intensity of which increased from 0.8 to 2.3 and 1.8, respectively (**Figure 2**). The sensory panel described the bitter taste quality as a long-lasting unpleasant bitterness perceived at the back of the tongue and throat. The intensity of creaminess, sourness, sweetness, and saltiness was not significantly influenced by heating. However, the umami sensation (1.0) and the mouthfulness (1.9) were perceived as seemingly more intense, although the standard deviation for the perceived mouthfullness (kokumi) impression was rather high (±0.8). Most interestingly, the entire panel described this kokumi impression induced by the avocado puree to be more longlasting after thermal treatment. On the basis of these sensory studies, it was suggested that thermal treatment of the avocado pulp induces the generation of bitter as well as mouthfulness-enhancing (kokumi) molecules.

To facilitate the isolation of the taste compounds formed upon heating, first, additional model experiments were done aimed at maximizing their amounts formed. To achieve this, fresh avocado pulp was thermally treated at 80, 100, and 120 °C for up to 120 min and, after cooling, the bitter taste intensity of the puree was evaluated by the trained sensory panel. As displayed in **Figure 3**, the bitterness was perceived as more intense with increasing
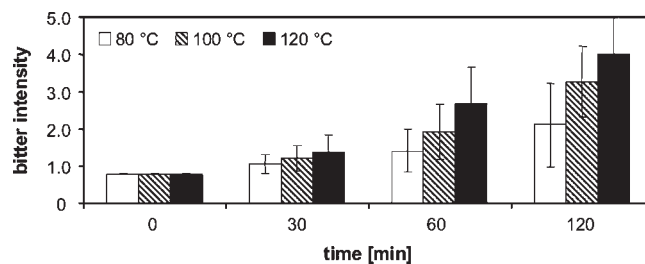

Figure 3. Influence of temperature and heating time on bitter taste evolution in avocado pulp.

heating time and temperature. By far, the highest bitter intensity of 4.0 was observed for the sample heated for 120 min at 120 °C, whereas the sample treated for 30 min at 80 °C was judged only with a taste intensity of 1.1. As the bitter taste was most pronounced in the avocado puree heated for 120 min at 120 °C, this sample was used in the following fractionation experiments for the identification of taste compounds.

Solvent Fractionation of Heat-Treated Avocado Puree. Aimed at identifying the orosensory active molecules in the heated avocado puree, the puree was extracted with pentane, followed by an extraction of the organic layer by an ethanol/water mixture to afford the hydrophobic pentane solubles (fraction A), the ethanol/water-solubles (fraction B), and the nonsoluble residue (fraction C).

After removal of the solvents under vacuum and freeze-drying, an aliquot of these residues was taken up in water or in a savory-tasting model broth solution in "natural" concentration ratios to sensorially evaluate the intrinsic taste quality and the umami- and/or kokumi-enhancing activity, respectively. Whereas fractions A and C showed only marginal taste activity, fraction B imparted an intense bitter taste in water judged with an intensity of 3.8 (±0.5) as well as a pronounced mouthfullness-enhancing (kokumi) impression when evaluated in the model broth; for example, the intensity of the kokumi impression of the model broth was increased from 0.5 (±0.3) to 2.5 (±0.8) when fraction B was present. To remove residual triglycerides, the sensory active fraction B was applied onto the top of an RP-18 cartridge, the effluent obtained upon flushing with acetonitrile was collected, and the solvent was removed under vacuum. After repeated freeze-drying, the defatted fraction B was confirmed to exhibit bitterness in aqueous solution and to enhance the kokumi impression in the model broth (data not shown) and was, therefore, used in the following fractionation experiments for the identification of the orosensory active compounds.

Screening for Bitter and Kokumi Enhancing Molecules in Defatted Fraction B. To identify the molecules responsible for the bitter taste and the kokumi-enhancing activity, respectively, the defatted fraction B was separated by means of RP-HPLC/ELSD to give the 22 subfractions B1–B22 (**Figure 4**). After lyophilization, one aliquot of each fraction was taken up in water (pH 6.5) and evaluated for the intrinsic taste impact by means of a TDA, whereas another aliquot was taken up in a model broth solution (pH 6.5) to study taste-modulating activity by means of a cTDA. Application of the TDA revealed an intrinsic bitter taste for all 22 fractions, among which fraction B20 was found with the highest TD factor of 16, closely followed by fractions B3–B6, B10–B13, B17, and B19 judged with a TD factor of 8. All other fractions showed TD factors of 2 or below (**Table 1**). Determination of the TD factor for the kokumi sensation recorded by means of the cTDA again revealed the highest value of 256 for fraction B20, followed by B21 and B11 judged with TD factors of 128 and 64, respectively (**Table 1**). In addition, a kokumi sensation was detectable in fractions B1, B5, B6, B13, and B17–B19 even when
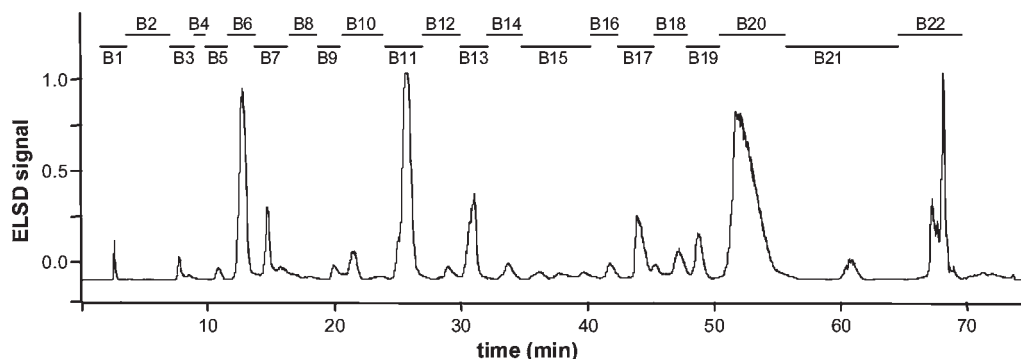
Figure 4. RP-HPLC/ELSD-TDA chromatogram of fraction B isolated from avocado puree thermally treated for 120 min at 120 °C.

diluted by factors of 32 and 16, respectively. To identify the bitter and/or kokumi-enhancing molecules in these HPLC fractions, each compound was purified by rechromatography and the chemical structure determined by means of LC-MS/MS and 1D/2D NMR spectroscopy.

The sensory active compound isolated from fraction B20, judged with the highest TD factors of 16 and 256 for the bitter taste and kokumi sensation, respectively, was identified as (*Z,Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene (**10**; **Figure 1**). This compound, known as persin, has been earlier isolated as an antifungal compound from avocado leaves (*25–27*), but its distinct kokumi-enhancing activity has not been previously reported. Optical rotation studies using the synthetic (*R*)- and (*S*)-isomers of **10** led to an unequivocal identification of the 2(*R*)-configuration for the naturally occurring isomer (*28, 29*).

Structure determination of the key compounds present in fractions B3–B5 and B10 revealed 1,2,4-trihydroxyheptadeca-16-yne (**1**) and 1,2,4-trihydroxyheptadeca-16-ene (**2**) as well as the corresponding acetyl derivatives 1-acetoxy-2,4-dihydroxyhepta-deca-16-yne (**4**) and 1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**3**) as the sensory active molecules (**Figure 1**). The derivatives 1,2,4-trihydroxyheptadeca-16-yne (**1**), named avocadyne, and 1,2,4-trihydroxyheptadeca-16-ene (**2**), named avocadene, were earlier reported as antifungal compounds from the peel of immature avocado fruits as well as bitter tastants (*4, 30*). Inter-estingly, none of these molecules exhibited any kokumi activity. Both compounds were found to exhibit an (*R*)-configuration at C(2) and C(4) (*31*). In addition, 1-acetoxy-2,4-dihydroxyhepta-deca-16-ene (**3**) and 1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**4**), both identified in the present study as bitter as well as kokumi-enhancing molecules, were earlier reported in fruits and seeds of different avocado cultivars (*30–36*). The 1-acetoxy-2,4-dihydroxy-heptadeca-16-ene (**3**) from avocado seeds were confirmed to show the (*R*)-configuration at C(2) and C(4) (*37*). However, neither the bitter taste of **1–4** nor the kokumi-enhancing activity of **3** and **4** has been reported earlier.

Additional bitter and kokumi-enhancing oxylipins were iso-lated from HPLC fractions B6, B11, B13, B17, and B19 and were identified as 1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**5**), 1-acetoxy-2-hydroxy-4-oxoheptadecane (**6**), 1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**), 1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene (**8**), and 1-acetoxy-2,4-dihydroxyheneicosa-12,15-diene (**9**; **Figure 1**). Whereas compounds **5**, **8**, and **9** were isolated from fruits and seeds of avocado (*34, 38*), the structures of compounds **6** and **7** have, to the best of our knowledge, not been previously reported in the literature.

In eye-catching difference from all of the other HPLC fractions, fractions B21 and B22 exhibited a bitter taste without showing any kokumi-enhancing activity (**Table 1**). By means of LC-MS/MS and 1D/2D NMR experiments, followed by cochromatography

Table 3. Recognition Threshold Concentrations for Bitter Taste and Kokumi-Enhancing Activity of Avocado Compounds

	threshold concn (μmol/L) for	
compd (no.)[a]	bitter taste[b]	kokumi enhancement[c]
1,2,4-trihydroxy-heptadeca-16-yne (**1**)	43	nd
1,2,4-trihydroxyheptadeca-16-ene (**2**)	52	nd
1-acetoxy-2,4-dihydroxyheptadeca-16-ene (**3**)	34	9
1-acetoxy -2,4-dihydroxyheptadeca-16-yne (**4**)	27	5
1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene (**5**)	88	11
1-acetoxy-2-hydroxy-4-oxoheptadecane (**6**)	313	17
1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**)	70	5
1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12, 15-triene (**8**)	70	2
1-acetoxy-2,4-dihydroxyheneicosa-12,15-diene (**9**)	92	2
1-acetoxy-2-hydroxy-4-oxoheneicosa-12, 15-diene (**10**)	121	8
1-*O*-palmitoyl-glycerol	161	nd
1-*O*-stearoyl-glycerol (**12**)	174	nd
1-*O*-oleoyl-glycerol	138	nd
2-*O*-oleoyl-glycerol	57	nd
1-*O*-linoleoyl-glycerol (**11**)	72	nd

[a] Chemical structures are given in **Figure 1**. [b] Bitter taste recognition thresholds were determined in water (pH 6.5). [c] Recognition threshold concentrations for the kokumi sensation were determined in a model broth (pH 6.5); nd, not detectable.

with the corresponding reference compounds, not an addi-tional oxylipin, but the monoglycerides 1-*O*-linoleoyl-glycerol (**11**) and 1-*O*-stearoyl-glycerol (**12**) were identified as the key bitter substances in fractions B21 and B22, respectively (**Figure 1**; **Table 1**).

Sensory Activity of Compounds 1–11. Prior to sensory analysis, the purity and identity of the oxylipins **1–10** as well as the 1-*O*-acyl-glycerols **11** and **12** were checked by HPLC-MS and ^{1}H NMR spectroscopy.

To gain insight into the intrinsic bitter taste of these molecules, in a first set of experiments bitter recognition threshold concen-trations were determined in aqueous solution (pH 6.5) by means of the half-tongue test (*18–21*). The lowest bitter threshold concentrations of 28 and 34 μmol/L were found for 1-acetoxy-2,4-dihydroxyheptadeca-16-yne (**4**) and 1-acetoxy-2,4-dihydroxy-heptadeca-16-ene (**3**), respectively, followed by the corresponding deacetylated triols **1** and **2**, exhibiting bitter taste above threshold concentrations of 43 and 53 μmol/L, respectively (**Table 3**). In comparison to the triols **1** and **2** and the 1-acetoxy-2,4-dihydroxy derivatives **3** and **4**, all of the compounds sharing a 1-acetoxy-2-hydroxy-4-oxo structure (**5–8**, **10**) showed higher bitter thresh-olds; for example, compound **3** showed a 3 times lower threshold as the corresponding 4-oxo derivative **5** (**Table 3**). Also, the *O*-acyl-glycerols were evaluated with rather low bitter thresholds

ranging from 57 to 174 μmol/L; for example, 1-*O*-linoleoyl-glycerol (**11**) and 1-*O*-stearoyl-glycerol (**12**) exhibited values of 72 and 174 μmol/L.

Determination of the threshold concentration for the kokumi sensation perceived in the model broth revealed by far the lowest value of 2 μmol/L for compounds **8** and **9**, whereas the highest threshold of 17 μmol/L was found for compound **6** (**Table 3**). For all of these compounds, the threshold found for kokumi enhancement was far below the bitter taste threshold concentration. Most interestingly, among the avocado oxylipins identified, exclusively the 1-acetoxy derivatives induced a kokumi sensation, whereas the deacetylated triols **1** and **2** as well as the 1-*O*-acyl-glycerols did not show any influence on kokumi perception. Whereas previously reported kokumi compounds are rather polar molecules sharing an *S*-alkyl-L-cysteine sulfoxide and/or a γ-glutamyl moiety (*16*, *17*, *22*, *23*), this is the first report on kokumi-enhancing, lipophilic C_{17}–C_{21} oxylipins exhibiting a 1-acetoxy-2,4-dihydroxy- and
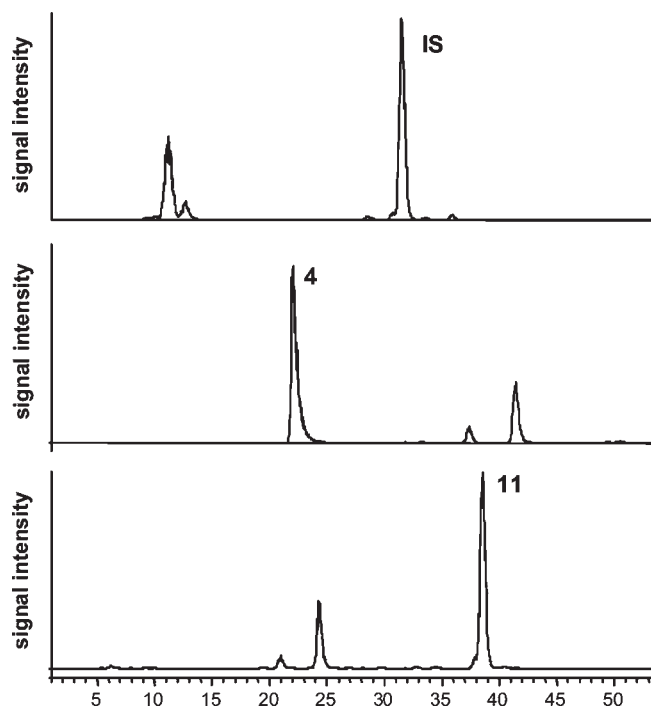

Figure 5. LC-MS/MS quantitation of compounds **1**–**12** in a thermally treated sample of avocado using 1-*O*-palmitoyl-glycerol (IS) as the internal standard.

a 1-acetoxy-2-hydroxy-4-oxo motif, respectively, as the common structural feature required for their taste-modulating activity.

Concentrations and Dose-over-Threshold (DoT) Factors of Compounds 1−12 in Fresh and Thermally Treated Avocado. To gain a first insight into the influence of heat processing on the formation of the taste compounds **1**–**12**, an aliquot of a sample of freshly prepared avocado puree was thermally treated for 120 min at 120 °C and, after cooling, this heat-treated sample as well as the nonprocessed sample was spiked with a defined amount of the internal standard 1-*O*-palmitoyl-glycerol, which was shown to be absent in both samples by means of preliminary RP-HPLC-MS/MS(APCI) screening. After optimization of chromatographic conditions as well as MS parameters, all of the analytes **1**–**12** as well as the internal standard were separated within 45 min as exemplified for the oxylipin **4**, the 1-*O*-acylglycerol **11**, and the internal standard (IS) (**Figure 5**). Quantitative analysis revealed rather low levels between 0.5 mg/100 g (**7**) and 5.6 mg/100 g (**12**) for compounds **1**–**12** in the fresh sample and a drastic increase of the concentrations of all taste molecules upon heat treatment, for example, the concentrations of 1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**) and 1-*O*-linoleoyl-glycerol (**11**) raised by factors of 870 and 90 upon avocado processing (**Table 4**).

To evaluate the potential contribution of these compounds to the bitter off-taste of avocado, DoT factors were calculated from the ratio of the concentration of a compound in the avocado sample and its bitter taste threshold given in **Table 4** (*39*). With the exception of 1-*O*-linoleoyl-glycerol (**11**), none of the bitter compounds exceeded its taste threshold in the native avocado pulp, thus being well in line with the low bitter taste intensity observed for fresh avocado (**Figure 2**). After thermal treatment for 120 min at 120 °C, all of the target compounds (**1**–**12**) far exceeded their bitter thresholds to reach DoT factors between 14, found for 1-*O*-stearyl-glycerol (**12**), and 162, found for 1-acetoxy-2-hydroxy-4-oxooctadeca-12-ene (**7**). These data clearly indicate the contribution of the compounds **1**–**12** to the bitter off-taste of processed avocado.

Taste Re-engineering Experiment. To confirm the proposed taste impact of compounds **1**–**12**, a sample of fresh avocado pulp was spiked with purified material of the sensory active compounds **1**–**12** to match the concentrations determined in the avocado sample thermally treated for 120 min at 120 °C. Thereafter, the nonspiked fresh sample and the spiked fresh samples as well as the heat-treated avocado sample were evaluated in the bitter taste and kokumi intensity with on a 5-point linear scale (**Figure 6**). In accordance with the results of **Figure 2**, the fresh and heat-treated avocado samples, respectively, were judged with

Table 4. Concentrations and Dose-over-Threshold (DoT) Factors for Oxylipins in Native and Heated Avocado Pulp

| | | concn (mg/100 g) (DoT factor)[a] | |
compd no.[b]	identified compd	native pulp	heated pulp[c]
1	1,2,4-trihydroxyheptadeca-16-yne	0.7 ± 0.1 (0.6)	151.8 ± 2.0 (124.4)
2	1,2,4-trihydroxyheptadeca-16-ene	0.7 ± 0.1 (0.6)	126.5 ± 7.8 (103.7)
3	1-acetoxy-2,4-dihydroxyheptadeca-16-ene	0.8 ± 0.1 (0.7)	136.2 ± 2.5 (122.7)
4	1-acetoxy-2,4-dihydroxyheptadeca-16-yne	0.7 ± 0.1 (0.8)	74.8 ± 4.1 (84.1)
5	1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene	2.0 ± 0.4 (0.7)	303.8 ± 1.4 (105.5)
6	1-acetoxy-2-hydroxy-4-oxoheptadecane	4.8 ± 0.1 (0.5)	363.6 ± 10.2 (35.3)
7	1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene	0.5 ± 0.1 (0.2)	435.4 ± 16.9 (161.8)
8	1-acetoxy-2-hydroxy-4-oxoheneicosa-5,12,15-triene	2.3 ± 0.8 (0.9)	340.3 ± 28.7 (128.4)
9	1-acetoxy-2,4-dihydroxyheneicosa-12,15-diene	1.6 ± 0.5 (0.6)	301.4 ± 20.9 (116.4)
10	1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene	3.6 ± 0.3 (0.8)	117.9 ± 10.5 (25.6)
11	1-*O*-linoleoyl-glycerol	3.3 ± 1.2 (1.3)	296.7 ± 39.4 (115.6)
12	1-*O*-stearoyl-glycerol	5.6 ± 3.1 (0.4)	191.1 ± 23.2 (14.2)

[a] Concentrations are given as the mean of three independent repetitions (± RSD); DoT factors are calculated using the bitter taste threshold (**Table 3**) of the corresponding compound. [b] Chemical structures of oxylipins are given in **Figure 1**. [c] Freshly prepared avocado pulp was thermally treated for 120 min at 120 °C.
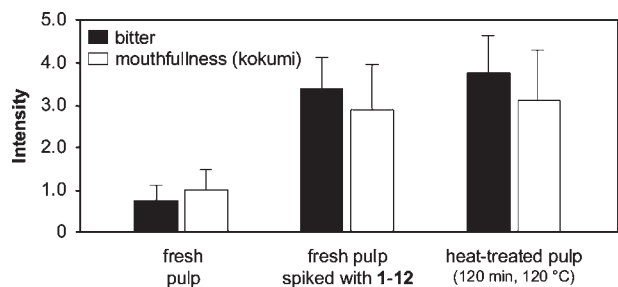
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Figure 6. Influence of spiking fresh avocado puree with natural amounts of compounds **1**–**12** on the perceived bitter taste and kokumi intensity.
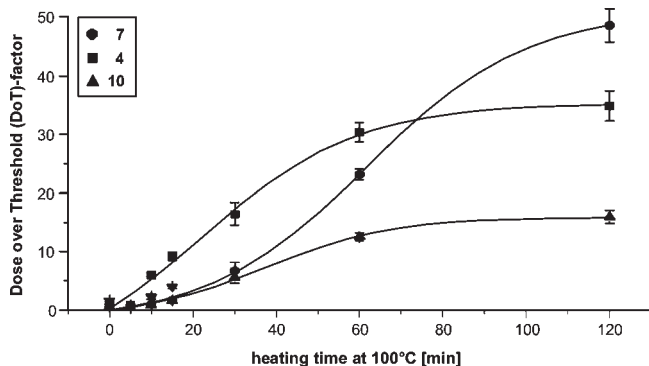

Figure 7. Influence of heating time on the evolution of the dose-over-threshold (DoT) factors of selected bitter compounds in avocado puree.

values of 0.8 and 2.7 for bitterness and with values of 1.1 and 2.0 for kokumi activity. Most interestingly, spiking the fresh sample with compounds **1**–**12** to mimic their concentrations in the heat-treated sample revealed a bitter taste intensity of 3.4 and a kokumi impact of 2.9, closely matching the corresponding taste intensities found in the heat-treated sample (**Figure 6**).

These data demonstrate for the first time that the 1-*O*-acyl-glycerols (**11**, **12**) and C_{17}–C_{21} oxylipins (**1**–**10**), among which 1-acetoxy-2-hydroxy-4-oxooctadeca-12-ene (**7**) was found with the highest taste impact on the basis of dose/activity considerations, are the key contributors to the bitter off-taste of processed avocado. Among the compounds identified, the oxylipins **3**–**10** exhibiting a 1-acetoxy-2,4-dihydroxy- and a 1-acetoxy-2-hydroxy-4-oxo motif, respectively, were discovered to induce a pronounced kokumi-enhancing activity.

Time-Dependent Evolution of DoT Factors of Selected Senso-metabolites during Thermal Avocado Processing. To investigate the time course of their thermal generation, 1-acetoxy-2,4-dihydroxy-heptadeca-16-yne (**4**), found with the lowest bitter taste threshold (**Table 3**), 1-acetoxy-2-hydroxy-4-oxo-octadeca-12-ene (**7**), evaluated with the highest DoT factor (**Table 4**), and the literature reported 1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene (**10**) were quantified by HPLC-MS/MS in avocado samples before and after thermal treatment for up to 100 min at 120 °C. Calculation of DoT factors revealed that the concentrations of compounds **4** or **7** and **10** exceeded their threshold concentrations already after 10 or 15 min of heating, respectively (**Figure 7**). Prolongation of the heating time led to a substantial increase of the DoT values of the selected bitter compounds, reaching high values of 30.4 for compound **4**, followed by 23.2 and 12.5 for **7** and **10** after 60 min. Interestingly, a further increase of heating time did not lead to a strong additional increase of **4** and **10**, but favored the generation of compound **7**, reaching the highest DoT factor of 48.7 after 120 min.

These data obtained suggest the structure-dependent release of these oxylipins from unknown precursors present in the avocado pulp. Precursor fractions, liberating compounds **1** and **3** upon heating, have been located in the fat-soluble fraction of canned avocado juice, and the formation of the target molecules has been suggested to be due to lipid oxidation (*40*). As classical lipid oxidation mechanisms are expected to affect the double bonds in the target molecules such as the 1,4-pentadiene system in compounds **8**–**11**, another formation pathway seems to be more likely. Future investigations are therefore needed to locate and identify one or the other precursor leading to the sensory-active C_{17}–C_{21} oxylipins.

Supporting Information Available: Linear regression line of calibration points of **1**–**11** and IS generated by LC-MS/MS quantitation. This material is available free of charge via the Internet at http://pubs.acs.org.

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