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Quantitation and taste contribution of sensory active molecules in oat (*Avena sativa* L.)

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ABSTRACT

A total of 59 taste active molecules were quantitated, and then rated for their individual taste impact on the basis of dose-over-threshold factors (DoT) in oat flour (*Avena sativa* L.). A sensitive HPLC-MS/MS-method was developed to quantitate bitter-tasting steroidal and furostanol-saponins as well as avenanthramides. Four monoglycerides, five free fatty acids and four saponins, were confirmed for the first time to be major contributors to the bitter off-taste of oats, among them 1-linoleoyl-*rac*-glycerol, 1-stearoyl-*rac*-glycerol, 1-oleoyl-*rac*-glycerol, 1-palmitoyl-*rac*-glycerol, linoleic acid, linolenic acid, oleic acid, palmitic acid and stearic acid, as well as avenacoside A and B and the recently identified furostanosides 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol and 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol. By means of a Stable Isotope Dilution Assay (SIDA) quantitated avenanthramides 2c, 2p, 2f, 1p, 1c, 1f, and 3f were found in concentrations below their thresholds and, therefore, did not contribute to the bitter sensation of the tested oat flour.

KEYWORDS: oat, *Avena sativa*, saponins, avenanthramides, avenacosides, free fatty acids, monoglycerides, taste, bitter, astringent, SIDA, LC-MS/MS.

INTRODUCTION

In addition to its characteristic attractive aroma, the sensory profile of oats is driven by its typical astringent and bitter off-taste[1]. Whereas, multiple attempts have been made to correlate this off-taste with lipid-derived bitter metabolites, such as free fatty acids[2], 9-hydroxy-10(*E*),12(*Z*)-octadecadienoic acid (9-HODE), 13(*S*)-hydroxy-9(*Z*),11(*E*)-octadecadienoic acid (13-HODE)[3], 9,10,13-trihydroxy-*trans*-11-octadecenoic acid[3] and oxidized monoglycerides[4], few studies have reached beyond these compounds. Although oat saponins, such as avenacosides A and B and their corresponding monodesmosides 26-desglucoavencoside A and B, have been studied for many years[5–10], there is only little knowledge on their sensory power, while other chemical groups in oats are rather poorly investigated.

In order to identify which non-volatile, key taste molecules impart the bitterness and astringency of oats, we recently applied the so-called taste dilution analysis (TDA)[11] on fractions isolated from oat flour after sequential solvent fractionation and separation by means of solid phase extraction followed by high performance liquid chromatography[1]. This activity-guided fractionation led to the identification of four steroidal and furostanol saponins, as well as the three avenanthramides 2c (**5**), 2p (**6**), 2f (**7**) as key bitter and astringent phytochemicals in oats. Sensory evaluation of these saponins and avenanthramides showed potent bitter sensation with taste recognition thresholds between 4 and 170 µmol/L, with the saponin 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**1**) exhibiting by far the lowest bitter threshold[1].

To the best of our knowledge, a comprehensive mapping of key taste molecules of oats linking human sensory recognition thresholds has not been made so far.

Therefore, this study aimed to position and quantitate previously identified taste active metabolites and minerals followed by dose-activity-relationships to positioning these compounds according to their orosensory impact, and validate previous findings by taste re-engineering studies of the non-volatile sensometabolome of oat flour.

MATERIALS AND METHODS

Chemicals and Materials. All chemicals were acquired commercially from Merck (Darmstadt, Germany) and Sigma-Aldrich (Steinheim, Germany), if not stated elsewise. Sucrose, *o*-coumaric acid, *p*-hydroxybenzoic acid and protocatechuic acid were purchased from Carl Roth (Karlsruhe, Germany), *p*-coumaric and 4,5-dimethoxy-2-nitro-benzoic acid from Alfa Aesar (Karlsruhe, Germany), palladium on carbon from EGA Chemie (Steinheim, Germany), and lauric acid from Fluka (Neu-Ulm, Germany). As published recently[1], saponin reference materials were isolated from oat flour and avenanthramide standards were synthesized according to the published synthesis protocol[1]. Stable-isotope labelled amino acids were acquired from Cambridge Isotope Laboratories (Andover, USA), (+)-dihydrorobinetine and ginsenoside Rg_1 and Rb_1 were obtained from Extrasynthese (Genay, France). Deuterated solvents were supplied by Euriso-Top (St. Aubin, France). Solvents for HPLC applications were of HPLC grade (Merck, Darmstadt, Germany), solvents for LC-MS uses were of LC-MS grade (J.T.Baker, Deventer, Netherlands) whereas solvents used for extraction (Merck, Darmstadt, Germany) were distilled prior to use. Water for HPLC separation was purified by means of a Milli-Q water advantage A 10 water system (Millipore, Molsheim, France). Bottled water (Evian, low mineralization: 405 mg/L) was adjusted to pH 6.4 with aqueous formic acid prior to gustatory analysis. Ethanol (absolute, Merck, Darmstadt, Germany) was utilized for sensory analysis. Ground oat flour from heat

treated oat grains was used and stored at −20 °C until use.

Syntheses of Avenanthramide 3f (13) and Labelled Internal Standards [$^2H_3$]-Avenanthramide 1f and 2f (16,17) (c.f. Figure 1). Synthesis of avenanthramide 3f was performed in a stepwise approach following the protocols described in the literature[12–14], with some modifications. In order to generate the educt for the final condensation reaction of the monomalonate with vanilline, 5-hydroxy-4-methoxy-anthranilic acid was synthesized from 4,5-dimethoxy-2-nitro-benzoic acid in a two-step approach. An amount (20 mmol) of 4,5-dimethoxy-2-nitro-benzoic acid was dissolved in 20 mL of NaOH (6 mol) and heated to 100 °C for 3 h[14]. After cooling to room temperature, the solution was dripped into a mixture of concentrated hydrochloric acid and crushed ice (pH 2). To obtain 5-hydroxy-4-methoxy-2-nitro-benzoic acid as an orange powder (18.1 mmol, 91% yield) the icy slurry was extracted with ethylacetate, washed with a saturated saline solution, dried over $MgSO_4$, filtered, freed from solvent under vacuum, and finally dried at 110 °C.

In a second step, reduction of the amino-function was conducted using a catalyst consisting of palladium on activated charcoal[12]. The catalyst (10 wt %) and 33 mL of methanol (dried with molecular sieve 4 Å) were introduced into a three-necked glass with 12.5 mmol of 5-hydroxy-4-methoxy-2-nitro-benzoic acid. Reduction was performed for 4 h with gaseous hydrogen from a reservoir balloon. The solid was filtered off, washed with methanol, and removed from solvent to give 5-hydroxy-4-methoxy-anthranilic acid (5.6 mmol, 45% yield).

As reported recently[13], 5-hydroxy-4-methoxy-anthranilic acid (6 mmol) was mixed in equimolar ratio with meldrums acid, dissolved in dried toluene (15 mL) and refluxed for 4 h. The mixture was allowed to cool down to room temperature, and treated with saturated $NaHCO_3$ (15 mL) solution, followed by a stepwise addition of concentrated

HCl (10 mL). The precipitate was filtered off, washed with water and dried at 100 °C to obtain 5-hydroxy-4-methoxy-2-(2-carboxyacetyl)aminobenzoic acid (2.3 mmol, 36 % yield). In a final step, 5-hydroxy-4-methoxy-2-(2-carboxyacetyl)aminobenzoic acid (0.5 mmol), vanilline (0.5 mmol) and a catalytic amount of *β*-alanine were dissolved in pyridine (3 mL) and refluxed for 110 min at 115 °C[13]. The solution was cooled with ice and acidified with concentrated HCl (3 mL). The resulting solid was filtered, washed with water and dried in a lab oven at 100 °C. Crude synthesis was purified by means of preparative HPLC on a Phenyl-Hexyl Luna column (250 x 21.0 mm, 5 µm, Phenomenex, Aschaffenburg, Germany). Monitoring the effluent at 280 and 340 nm, chromatography was performed using 0.1% formic acid in water (effluent A) and acetonitrile (effluent B) at a flow rate of 19 mL/min and isocratic conditions of 52% of effluent B, respectively. Collected fractions containing the target compounds were combined and separated from solvent under vacuum. After lyophilisation, avenanthramide 3f (**13**) was obtained as white, amorphous powder in purity of more than 98 % (HPLC-DAD, ^{1}H NMR). The structure was verified by means of LC-MS and NMR spectroscopy. Isotope labelled standards were synthesized as described above, using deuterated [$^{2}H_3$]-vanilline for final condensation reaction with the monomalonate.

*N-(4'-Hydroxy-3'-methoxy-(E)-cinnamoyl)-5-hydroxy-4-methoxy-anthranilic acid (avenanthramide **3f**), **13*** (Figure 1): UV/vis (ACN/0.1% HCOOH, 75/25 v/v) λ_{max}= 248 nm, 348 nm; LC-MS (ESI$^-$) *m/z* 358.1 ([$C_{18}H_{18}NO_7$−H]$^-$), 314.1 ([$C_{17}H_{17}NO_5$−H]$^-$), 298.1 ([$C_{17}H_{17}NO_4$−H]$^-$); MS/MS (declustering potential (DP)= −90 V) *m/z* (%) 358.0 (100), 314.0 (13), 298.0 (24), 282.6 (8), 176.0 (36), 149.8 (34); LC-TOF-MS *m/z* 358.0928 ([M−H]$^-$, measured), calculated for [$C_{18}H_{17}NO_7$−H]$^-$ *m/z* 358.0927; ^{1}H-NMR (500 MHz, MeOD-d_4) δ 3.92* [s, 3H, H-C(10')], 3.94* [s, 3H, H-C(8)], 6.57 [d, 1H, *J*=15.7 Hz, H-C(8')], 6.82 [d, 1H, H-C(5')], 7.10 [dd, 1H, *J*=8.2, 1.8 Hz, H-C(6')], 7.23 [d, 1H, *J*=1.7 Hz, H-C(2')], 7.53 [s, 1H, H-C(6)], 7.56 [d, 1H, *J*=15.7 Hz, H-C(7')], 8.42

[s, 1H, H-C(3)]; ^{13}C-NMR [125 MHz, MeOD-d_4) δ 56.3/56.5 [C(10‘/8)], 104.6 [C(3)], 111.6 [C(2‘)], 113.8 [C(1)], 116.5 [C(5‘)], 118.4 [C(6)], 120.2 [C(8‘)], 123.8 [C(6‘)], 128.2 [C(1‘)], 136.3 [C(2)], 142.7/142.8 [C(7‘/5)], 149.4 [C(3‘)], 150.2 [C(4‘)], 152.3 [C(4)], 166.7 [C(9‘)], 172.7 [C(7)]; *maybe interchangeable.

[10‘-$^2H_3$]-*N*-(4‘-hydroxy-3‘-methoxy-(*E*)-cinnamoyl)-anthranilic acid ([$^2H_3$]-avenanthramide 1f), **16** (Figure 1): LC-MS (ESI$^-$): *m/z* 315.0 ([$C_{17}H_{12}{}^2H_3NO_5$-H]$^-$), 271.1 ([$C_{16}H_{12}{}^2H_3NO_3$-H]$^-$), 162.0 ([$C_8H_5NO_3$-H]$^-$), 133.2 ([$C_9H_{10}O$-H]$^-$); MS/MS (DP= −85 V): *m/z* (%) 315.0 (100), 271.1 (32), 252.0 (68), 177.0 (10), 133.2 (37); LC-TOF-MS *m/z* 315.1067 ([M−H]$^-$, measured), calculated for [$C_{17}H_{12}{}^2H_3NO_5$-H]$^-$ *m/z* 315.1107; ^{1}H-NMR (400 MHz, MeOD-d_4) δ/ppm: δ 6.59 [d, 1H, *J*=15.6 Hz, H-C(8‘)], 6.83 [d, 1H, *J*=8.2 Hz, H-C(5‘)], 7,11 [dd, 1H, *J*=8.2, 1.9 Hz, H-C(6‘)], 7.15 [t, 1H, *J*=7.6, 0.9 Hz, H-C(5)], 7.24 [d, 1H, *J*=1.9 Hz, H-C(2‘)], 7.57 [t, 1H, *J*=8.0, 1.6 Hz, H-C(4)], 7.60 [d, 1H, *J*=15.5 Hz, H-C(7‘)], 8.11 [dd, 1H, *J*=8.0, 1.5 Hz, H-C(6)], 8.69 [dd, 1H, *J*=8.4, 0.7 Hz, H-C(3)]; ^{13}C-NMR [100 MHz, MeOD-d_4) δ/ppm: δ 111.63 [C(2‘)], 116.42 [C(5‘)], 117.65 [C(1)], 119.52 [C(8‘)], 121.58 [C(3)], 123.93/123.97 [C(5/6‘)], 127.96 [C(1‘)], 132.64 [C(6)], 135.18 [C(4)], 142.74 [C(2)], 144.03 [C(7‘)], 149.40 [C(3‘)], 150.39 [C(4‘)], 167.16 [C(9‘)], 171.60 [C(7)].

[10‘-$^2H_3$]-*N*-(4‘-hydroxy-3‘-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid ([$^2H_3$]-avenanthramide 2f), **17** (Figure 1): LC-MS (ESI$^-$) *m/z* 331.0 ([$C_{17}H_{12}{}^2H_3NO_6$-H]$^-$), 287.0 ([$C_{16}H_{12}{}^2H_3NO_4$-H]$^-$), 178.0 ([$C_8H_5NO_4$-H]$^-$), 133.2 ([$C_9H_{10}O$-H]$^-$); MS/MS (DP= −85 V): *m/z* (%) 331.0 (100), 287.0 (41), 268.0 (65), 178.0 (7), 160.0 (32), 133.2 (52); LC-TOF-MS *m/z* 331.1018 ([M−H]$^-$, measured), calculated for [$C_{17}H_{12}{}^2H_3NO_6$-H]$^-$ *m/z* 331.1056; ^{1}H-NMR (400 MHz, DMSO-d_6) δ/ppm: δ 6.56 [d, 1H, *J*=15.6 Hz, H-C(8‘)], 6.95 [d, 1H, *J*=8.2 Hz, H-C(5‘)], 7.02 [dd, 1H, *J*=3.0, 9.0 Hz, H-C(4)], 7.10 [dd, 1H, *J*=2.0, 7.9 Hz, H-C(6‘)], 7.11 [d, 1H, *J*=1.9 Hz, H-C(2‘)], 7.37 [d, 1H, *J*=2.9 Hz, H-C(6)], 7.42 [d, 1H, *J*=15.4 Hz, H-C(7‘)], 8.32 [d, 1H, *J*=9.0 Hz, H-C(3)]; ^{13}C-NMR (101 MHz,

DMSO-d_6) δ/ppm: δ 111.19 [C(5')], 113.77 [C(2')], 116.40 [C(6)], 118.72 [C(1)], 119.75 [C(8')], 120.60 [C(6')], 120.71 [C(4)], 122.40 [C(3)], 127.31 [C(1')], 132.65 [C(2)], 140.50 [C(7')], 146.55 [C(3')], 149.44 [C(4')], 152.44 [C(5)], 163.37 [C(9')], 168.97 [C(7)].

Quantitation of Avenanthramides (5−13) by means of Stable Isotope Dilution Assay (SIDA). *Sample Preparation.* A sample weight (7 g) of oat flour was placed into a conical flask, spiked with an internal standard solution (150 µL) of [$^2H_3$]-avenanthramide 1f (**16**, 174 mg/L) and [$^2H_3$]-avenanthramide 2f (**17**, 220 mg/L), dissolved in 70% acetonitrile and equilibrated for 30 min. In a first step, samples were defatted with *n*-hexane (60 mL) and centrifuged. After decantation, the residue was taken up in 140 mL of methanol/water (70/30, v/v, aqueous phase adjusted to pH 4.0) and extracted for 2 h under stirring. The liquid phase was freed from solvent under vacuum and lyophilized to give the methanol/water extract. The extract was taken up in a mixture of acetonitrile/0.1 % formic acid (40/60, v/v), membrane filtered and analyzed by means of HPLC-MS/MS.

LC-MS/MS Analysis. An aliquot (10 µL) of the prepared sample was injected into an LC-MS/MS system combined with a Luna C18 PhenylHexyl (150 × 2.0 mm i.d., 5 µm, Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Eluent A was composed of 0.1 % formic acid in acetonitrile, eluent B of 0.1 % formic acid in water. Using a flow rate of 300 µL/min, the system was operated at 25 °C starting with 40 % A under isocratic conditions for 1 min, then increasing content A to 70 % within 1 min (holding for 2 min), followed by an increase to 100 % A within 2 min and keeping isocratic conditions for 3 min. Eluent was pumped down again to 40 % A within 2 min and held isocratically for further 3 min. Analysis was performed in ESI^- mode using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows:

196 avenanthramide 2c (**5**) (*m/z* 314.2 → 178.0; -65/-16/-11/-10); avenanthramide 2p (**6**)
197 (*m/z* 298.1 → 253.9; -50/-22/-15/-10); avenanthramide 2f (**7**) (*m/z* 328.0 → 283.8;
198 -95/-24/-19/-10); avenanthramide 1p (**8**) (*m/z* 282.0 → 238.0; -85/-24/-7/-10);
199 avenanthramide 1c (**9**) (*m/z* 298.0 → 161.7; -65/-16/-11/-10); avenanthramide 1f (**10**)
200 (*m/z* 312.0 → 251.7; -90/-34/-17/-10); avenanthramide 1s (**11**) (*m/z* 342.0 → 298.1;
201 -85/-24/-9/-10); avenanthramide 2s (**12**) (*m/z* 358.1 → 314.1; -80/-24/-9/-10);
202 avenanthramide 3f (**13**) (*m/z* 358.1 → 175.8; -90/-28/-11/-10); [$^2H_3$]-avenanthramide 2f
203 (**16**) (*m/z* 331.0 → 286.8; -85/-24/-19/-10); [$^2H_3$]-avenanthramide 1f (**17**) (*m/z* 315.0 →
204 271.1; -85/-24/-7/-10).

205 *Calibration.* Internal standards [$^2H_3$]-avenanthramide 2f (**16**) and [$^2H_3$]-avenanthramide
206 1f (**17**) were mixed with analytes **5−13** in molar ratios from 0.03 to 6.8 keeping the
207 concentration of the internal standard constant. Calibration curves were prepared by
208 plotting peak area ratios of analyte/internal standard against concentration ratios of
209 analyte/internal standard. Application of linear regression led to linear responses
210 showing correlation coefficients of >0.99 each.

211 *Method Validation.* To investigate the repeatability (intraday precision) of oat samples,
212 four independent repetitions were analyzed using three replicates each. Precision,
213 given as relative standard deviation (RSD), was confirmed to be between ±3% (**5**) and
214 ±9% (**9** and **10**) according to the individual avenanthramide. Linearity was determined
215 in the range between 0.0025−0.4753 mg/L. To determine recovery, oat flour samples
216 were spiked with methanolic solutions containing known amounts of **5−10** (c.f. Table
217 1) at increasing concentrations and analyzed in duplicates as described before. In
218 order to calculate recovery, baseline samples were defined from a 4-fold determination
219 of unspiked oat flour.

220 **Quantitation of Saponins (1−4).** *Sample Preparation.* A sample weight (7 g) of

oat flour was placed into a conical flask, spiked with a methanolic solution of internal standards ginsenoside Rg_1 (**14**, 400 µL of 577 mg/L) and ginsenoside Rb_1 (**15**, 450 µL of 487 mg/L) and equilibrated for 30 min. Samples were extracted twice under stirring for 10 h using 250 mL of a methanol/water mixture (70/30, v/v; aqueous phase adjusted to pH 4.0) each. Combined liquid phases were separated from solvent under vacuum, freeze-dried and taken up to 10 mL of an acetonitrile/0.1 % formic acid mixture (25/75, v/v). After membrane filtration and, if appropriate dilution, samples were analyzed by means of HPLC-MS/MS.

LC-MS/MS Analysis. An aliquot (5 µL) of the prepared sample was injected into an LC-MS/MS system combined with a 150 × 2.0 mm i.d., 3 µm, Nucleodur C18 Pyramid (Machery-Nagel, Düren, Germany) equipped with a guard column of the same type. Eluent A consisted of 0.1 % formic acid in acetonitrile, eluent B of 0.1 % formic acid in water. Using a flow rate of 300 µL/min, the system was operated at 40 °C starting with 25 % A under isocratic conditions for 1 min, then increasing content A to 65 % within 5 min, followed by an increase to 100 % A within 2 min and keeping isocratic conditions for 2 min. Eluent was pumped down to 25 % A within 2 min and held isocratically for further 4 min. Analysis was performed in ESI^- mode using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows: 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22,26-triol (**1**) (*m/z* 1225.7 → $901.2^{qn=quantifier}$; -225/-74/-55/-10; 1225.7 → $900.9^{ql=qualifier}$; -225/-76/-55/-10); 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[β-D-gluco-pyranosyl(1→4)]-β-D-glucopyranosid)-26-O-β-D-gluco-pyranosyl-(25R)-furost-5-ene-3β,22,26-triol (**2**) (*m/z* 1064.7 → 902.5^{qn}; -185/-62/-51/-10; 1064.7 → 901.9^{ql}; -185/-62/-51/-10) were quantitated using ginsenoside Rg1 (**14**) (*m/z* 799.6 → 89.0^{qn}; -200/-50/-5/-10; 799.5 → 637.5^{ql};

-180/-36/-31/-10); avenacoside B (**3**) (*m/z* 1223.5 → 899.5^{qn}; -200/-72/-41/-10; 1223.5 → 1061.6^{ql}; -200/-72/-53/-10; avenacoside A (**4**) (*m/z* 1062.5 → 899.9^{qn}; -235/-60/-43/-10; 1062.5 → 754.3^{ql}; -235/-64/-35/-10); protodioscin 1047.5 → 901.4^{qn}; -175/-126/-9/-10; 1047.5 → 901.4^{ql}; -175/-126/-9/-10) were quantitated using ginsenoside Rb1 (**15**) (*m/z* 1107.6 → 783.6^{qn}; -210/-64/-37/-10; 1107.6 → 945.4^{ql}; -210/-60/-43/-10) as internal standard (c.f. Figure 2).

Calibration. Internal standards of ginsenoside Rg_1 (**14**, 577 mg/L) and ginsenoside Rb_1 (**15**, 487 mg/L) were diluted 1:10 using acetonitrile/0.1 % formic acid (25/75, v/v) and then added to the analytes **1−4** in molar ratios of 0.3 to 8, keeping the concentration of the internal standard constant. Calibration curves were prepared by plotting peak area ratios of analyte/internal standard against concentration ratios of analyte/internal standard using linear regression ($R^2 > 0.99$).

Method Validation. To investigate the repeatability (intraday precision) of oat samples, three independent repetitions were analyzed using three replicates each. Method precision, given as relative standard deviation (%), was confirmed as follows: **1** (6%), **2** (8%), **3** (5%), **4** (11%). Linearity was determined in the range between 0.2−51.1 mg/L. To determine recovery, oat flour samples were spiked with methanolic solutions containing known amounts of **1−4** (c.f. Table 2) at increasing concentrations and analyzed in duplicates as described before. In order to calculate recovery, baseline samples were defined from a 3-fold determination of unspiked oat flour.

Quantitation of Free Fatty Acids (18−24) by Means of SIDA. *Sample Preparation.* In order to quantitate free fatty acids, a literature procedure using a stable isotope dilution analysis was followed[15]. An amount of oat flour (12 g) was placed into a conical flask, spiked with 100 µL each of an isopropanolic solution of internal standards [^{13}C]-palmitic acid (**25**, 480 mg/L) and [$^{2}H_2$]-9,10-oleic acid (**26**, 720 mg/L)

and equilibrated for 30 min. Samples were extracted three times with 300 mL of methanol for 2 h under stirring. Liquid phases were combined, freed from solvent under vacuum, and separated from di- and triglycerides by means of solid phase extraction (SPE). The residue was dissolved in 5 mL of methanol and applied onto a RP-18 material (Strata C18-E Giga Tubes, 10 g/60 mL, Phenomenex, Aschaffenburg, Germany). Elution of fatty acids was carried out using 60 mL of methanol. The resulting fraction was narrowed down under vacuum, transferred into a 10 mL volumetric flask and filled up with starting conditions of the HPLC gradient. After membrane filtration and dilution, free fatty acids were determined by means of HPLC-MS/MS using isotope labelled standards.

LC-MS/MS Analysis. An aliquot (5 µL) of the prepared sample was injected into an LC-MS/MS system combined with a 150 × 2.0 mm i.d., 4 µm, Polar-RP ODS C18 (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Eluent A consisted of isopropanol: acetonitrile/ 0.1 % HCOOH (v/v, 60:40), eluent B of 0.1 % formic acid in water. Using a flow rate of 400 µL/min, the system was operated at 40 °C starting with 40 % A under isocratic conditions for 2 min, then increasing content A to 65 % within 8 min, continuing to 100 % A within 2 min and keeping isocratic conditions for 4 min, pumping down to 40 % A within 2 min and keeping constant for further 3 min. Analysis was performed in negative ESI mode using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows: lauric acid (**18**) (*m/z* 199.0 → 199.1; -70/-14/-1/-10); myristic acid (**19**) (*m/z* 227.1 → 227.0; -85/-12/-15/-10); palmitic acid (**20**) (*m/z* 255.2 → 255.2; -80/-12/-7/-10); stearic acid (**21**) (*m/z* 283.2 → 283.2; -80/-14/-17/-10) were quantitated using [^{13}C]-palmitic acid (**25**) (256.1 → 256.2; -95/-16/-7/-10); oleic acid (**22**) (*m/z* 281.2 → 281.4; -100/-14/-7/-10); linoleic acid (**23**) (*m/z* 279.2 → 279.2; -85/-14/-17/-10) and linolenic acid (**24**) (*m/z*

277.2 → 277.1; -80/-14/-7/-10) were quantitated using [$^2H_2$]-9,10-oleic acid (**26**) (*m/z* 283.2 → 283.1; -100/-14/-7/-10) as internal standard (c.f. Figure 3). An ionisation of the fatty acid molecules by negative electrospray ionisation could be achieved during optimisation of MS-parameters, however a fragmentation was neither achieved by ESI nor by APCI ionisation. Nevertheless, the free fatty acids were present in sufficient amounts in the oat flour and separated well given the selectivity of the HPLC method, therefore, the detection of a free fatty acid was obtained by its unfragmented molecular ion.

Calibration. Internal standards [^{13}C]-palmitic acid (**25**) and [$^2H_2$]-9,10-oleic acid (**26**) were mixed with analytes **18−24** in molar ratios of 0.03 to 6.8 keeping the concentration of the internal standard constant. Calibration curves were set up by plotting peak area ratios of analyte to internal standard against concentration ratios of analyte to internal standard. Execution of linear regression led to linear responses showing correlation coefficients of $R^2 > 0.99$ each.

Quantitation of Monoglycerides and oxidized fatty acids by means of LC-MS/MS. *Sample Preparation.* According to a literature protocol[15] monoglycerides and oxidized fatty acids were quantitated as follows with some modifications: 10 g of oat flour was weighed into a conical flask and extracted three times with methanol (3 × 250 mL) for 1 h under stirring. Extracts were combined and freed from solvent under vacuum. To separate from di- and triglycerides, a solid phase extraction (SPE) was carried out on a RP-18 material (Strata C18-E Giga Tubes, 10 g/60 mL, Phenomenex, Aschaffenburg, Germany). Therefore, the residue was taken up in 5 mL of methanol, applied onto the cartridge, and the fatty acids were eluted with 60 mL of methanol. The resulting fraction was concentrated under vacuum, transferred into a 10 mL volumetric flask and filled up with 40% of eluent A. After membrane filtration and dilution, free fatty acids were determined by means of HPLC-MS/MS using external calibration.

LC-MS/MS Analysis. An aliquot (2 µL) of the prepared sample was injected into an LC-MS/MS system combined with a Polar-RP ODS C18 (150 × 2.0 mm i.d., 4 µm, Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Eluent A consisted of isopropanol: acetonitrile/ 0.1 % HCOOH (v/v, 60:40), eluent B of 0.1 % formic acid in water. A flow rate of 400 µL/min was used for both analyte groups.

For analysis of monoglycerides (**29-33**) the system was operated at 40 °C starting at 40 % A increasing to 70 % within 2 min, rising to 100 % A within 5 min and keeping isocratic conditions for a further 5 min, pumping down again to 40 % A within 3 min and holding constant conditions for 3 min. Operation of HPLC gradient for analysis of oxidized fatty acids (**27**/**28**) was run at same temperature starting at 40 % A and kept at constant conditions for 2 min, then increasing to 65 % within 8 min, rising further to 100 % A within 2 min and holding isocratic conditions for 4 min before going back to 40 % A within 2 min and keeping for further 3 min. The oxidized fatty acids 9-HODE (**27**) and 13-HODE (**28**) were not chromatografically separated and therefore integrated together (**27**/**28**). Analysis was performed in positive ESI mode for monoglycerides and negative mode for oxidized fatty acids using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows: 1-myristoyl-*rac*-glycerol (**29**) (*m/z* 303.2 → 284.9; 91/13/8/10); 1-palmitoyl-*rac*-glycerol (**30**) (*m/z* 331.2 → 312.9; 86/13/10/10); 1-stearoyl-*rac*-glycerol (**31**) (*m/z* 359.2 → 341.3; 71/17/10/10); 1-oleoyl-*rac*-glycerol (**32**) (*m/z* 357.2 → 339.0; 51/17/12/10); 1-linoleoyl-*rac*-glycerol (**33**) (*m/z* 355.0 → 262.8; 41/13/8/10); 9-HODE/ 13-HODE (**27**/**28**) (*m/z* 295.2 → 295.0; -100/-12/-19/-10); 13-HpODE (*m/z* 311.3 → 112.9; -95/-22/-1/-10) (c.f. Figure 4).

Calibration. Quantification was performed using external calibration of monoglyceride and oxidized fatty acid reference standards. Monoglyceride calibration standards were

prepared at concentrations of 0.2−335 mg/L using 1-myristoyl-*rac*-glycerol (**29**), 1-palmitoyl-*rac*-glycerol (**30**), 1-stearoyl-*rac*-glycerol (**31**), 1-oleoyl-*rac*-glycerol (**32**) and 1-linoleoyl-*rac*-glycerol (**33**). For oxidized fatty acids calibration standards were made up from 9-HODE/ 13-HODE (**27**/**28**) and 13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HpODE) at concentrations of 0.2−165 mg/L. Calibration curves were prepared by plotting peak areas of the analyte against the analyte concentration. Correlation coefficients of linear regression revealed values greater than 0.99 each.

Quantitation of Free Amino Acids by Means of SIDA. Free amino acids were quantitated by means of HILIC-MS/MS using a stable isotope dilution analysis (SIDA) following a literature protocol[16]. *Sample Preparation.* A sample weight (10 g) of oat flour was placed into a conical flask, spiked with 250 µL of an internal standard solution containing the isotope labelled amino acids in acetonitrile (0.1 mg/L) and equilibrated for 30 min. Samples were defatted with 200 mL of *n*-hexane under stirring followed by centrifugation for 25 min at 4 °C (Avanti J-E, Beckman Coulter, Krefeld, Germany) and decantation of the solvent. Then, samples were extracted two times with 200 mL of methanol/water (50/50, v/v; aqueous phase adjusted to pH 4.0) for 2 h under stirring. Combined extracts were treated on a rotary evaporator at 40 °C to remove solvents and lyophilized afterwards. A weight of 60 mg of the resulting extract was transferred into a volumetric flask (5 mL), filled up with 85% of eluent A, filtered and diluted if required. Quantification of amino acids was done by means of HPLC-MS/MS and stable isotope dilution assay.

LC-MS/MS Analysis. An aliquot (2 µL) of the prepared sample was injected into an LC-MS/MS system combined with a TSK-gel Amide 80 HILIC column (150 × 2.0 mm i.d., 3 µm, Tosoh Bioscience, Stuttgart, Germany) equipped with a guard column of the same type. Eluent A consisted of 5 mmol/L ammonium acetate buffer (pH 3.0, adjusted with acetic acid) in 95 % acetonitrile, eluent B of 5 mmol/L ammonium acetate

buffer (pH 3.0, adjusted with acetic acid) in water. Using a flow rate of 200 µL/min, the system was operated at 40 °C starting with 85 % A under isocratic conditions for 3 min, then increasing content A to 45 % within 7 min, continuing to 100 % A within 4 min and keeping isocratic conditions for 4 min, pumping back to initial 85 % A within 3 min and keeping constant for further 5 min. Analysis was performed in positive ESI mode using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows: L-alanine (*m/z* 90.1 → 90.0; 26/5/6/10); L-alanine-[$^{13}C_3$] (*m/z* 93.0 → 93.0; 41/5/5/10); L-arginine (*m/z* 175.0 → 70.1; 41/35/4/10); L-arginine-[$^{13}C_6$] (*m/z* 181.0 → 74.0; 78/36/5/10); L-asparagine (*m/z* 132.9 → 73.9; 1/19/6/10); L-asparagine-[$^{15}N_2$] (*m/z* 135.0 → 89.0; 39/14/5/10); L-aspartic acid (*m/z* 134.0 → 74.0; 46/19/6/10); L-aspartic acid-[$^{13}C_4$-^{15}N] (*m/z* 139.1 → 92.0; 39/14/10/10); L-glutamine (*m/z* 147.0 → 84.0; 46/23/6/10); L-glutamine-[$^{13}C_5$] (*m/z* 152.0 → 88.0; 43/23/10/10); L-glutamic acid (*m/z* 148.0 → 84.0; 31/23/6/10) was quantitated over L-glutamine-[$^{13}C_5$]; L-histidine (*m/z* 156.1 → 110.0; 41/21/8/10); L-histidine-[$^{13}C_6$] (*m/z* 162.0 → 115.0; 46/21/10/10); L-isoleucine (*m/z* 132.0 → 86.0; 41/15/6/10); L-isoleucine [$^{13}C_6$] (*m/z* 138.0 → 91.0; 47/14/10/10); L-leucine (*m/z* 132.0 → 86.0; 41/15/6/10); L-leucine-[$^{13}C^2$] (*m/z* 134.1 → 87.2; 44/14/10/10); L-lysine (*m/z* 147.0 → 84.0; 46/23/6/10); L-lysine-[^{13}C6-$^{15}N_2$] (*m/z* 155.0 → 90.0; 44/23/10/10); L-methionine (*m/z* 150.0 → 104.0; 31/15/8/10); L-methionine-[$^2H_3$] (*m/z* 153.1 → 107.0; 50/14/10/10); L-phenylalanine (*m/z* 166.0 → 120.0; 51/19/10/10); L-phenylalanine-[$^2H_5$] (*m/z* 171.1 → 125.0; 48/19/10/10); L-proline (*m/z* 116.0 → 70.0; 21/21/4/10); L-proline-[^{13}C5-^{15}N] (*m/z* 122.0 → 75.0; 73/25/5/10); L-serine (*m/z* 106.0 → 60.0; 26/17/4/10); L-serine-[$^{13}C_3$] (*m/z* 109.0 → 62.0; 38/16/5/10); L-threonine (*m/z* 120.0 → 55.9; 36/25/4/10); L-threonine-[^{13}C4-^{15}N] (*m/z* 125.0 → 78.0; 32/14/5/10); L-tryptophan (*m/z* 205.1 → 146.0; 41/25/12/10);

L-tryptophan-[$^2H_5$] (*m/z* 210.0 → 150.0; 40/37/10/10); L-tyrosine (*m/z* 182.1 → 91.0; 26/41/6/10); L-tyrosine-[$^2H_4$] 186.0 → 140.0; 38/19/10/10); L-valine (*m/z* 118.0 → 72.1; 21/15/6/10); L-valine-[$^{13}C_5$-^{15}N] (*m/z* 124.0 → 77.0; 64/31/ 10/10);

Calibration. Internal standards were mixed with analytes in molar ratios of 0.01 to 7, keeping the concentration of the internal standard constant. Calibration curves were set up by plotting peak area ratios of analyte to internal standard against concentration ratios of analyte to internal standard. Execution of linear regression led to linear responses showing correlation coefficients of >0.99 each.

Quantitation of Polyphenols by means of SIDA. *Sample Preparation.* For the quantification of polyphenols a modified literature protocol was used[17]. Oat flour (15 g) was placed into a conical flask, spiked with an internal standard solution (250 µL, 0.015 mg/L) and equilibrated for 30 min. Samples were defatted with *n*-hexane (300 mL) for 30 min in an ultrasonic bath and centrifuged. After decantation, the residue was taken up two times in 250 mL of methanol/water (70/30, v/v, aqueous phase adjusted to pH 4.0) and extracted for 30 min in an ultrasonic bath. The solvent was removed from the liquid phase under vacuum and lyophilized to give the methanol/water extract. The extract was taken up in starting conditions of the HPLC gradient, filtered and analyzed by means of HPLC-MS/MS. Quantification was performed by unlabelled and labelled internal standards.

LC-MS/MS Analysis. An aliquot (5 µL) of the prepared sample was injected into an LC-MS/MS system combined with a 150 × 2.0 mm i.d., 3 µm, Luna C18 (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Eluent A was composed of 0.1 % formic acid in acetonitrile, eluent B of 0.1 % formic acid in water. Using a flow rate of 300 µL/min, the system was operated at 40 °C starting from 40 % A to 70 % A within 2 min. Effluent was increased to 100 % within 5 min (holding

for 5 min), followed by a pump down to 40 % A within 3 min and holding conditions for a further 3 min. Analysis was performed in negative ESI mode using the mass transition and declustering potential (DP in V), collision energy (CE in V), cell exit potential (CXP in V), and entrance potential (EP in V) as follows: *p*-coumaric acid (*m/z* 162.98 → 119.0; -55/-20/-1/-10); caffeic acid (*m/z* 178.96 → 135.0; -50/-22/-9/-10) and ferulic acid (*m/z* 193.01 → 133.8; -55/-22/-7/-10) were quantitated using *o*-coumaric acid (*m/z* 162.98 → 119.1; -55/-20/-1/-10); protocatechuic acid (*m/z* 152.96 → 108.8; -20/-20/-5/-10) and gallic acid (*m/z* 168.96 → 124.8; -50/-22/-7/-10) were quantitated using [$^2H_5$]-gallic acid ethyl ester (*m/z* 201.92 → 123.9; -85/-32/-7/-10); vanilline (*m/z* 151.03 → 135.8; -50/-18/-9/-10); *p*-hydroxybenzoic acid (*m/z* 136.94 → 92.8; -50/-22/-7/-10); *p*-hydroxybenzaldehyde (*m/z* 121.00 → 91.80; -90/-34/-5/-10); dihydrorobinetine (*m/z* 302.97 → 284.8; -60/-14/-17/-10) and quercetine (*m/z* 301.02 → 150.8; -80/-30/-9/-10) were quantitated using [$^2H_3$]-methoxybenzoic acid (*m/z* 154.04 → 91.8; -65/-30/-5/-10) while sinapinic acid (*m/z* 223.02 → 163.9; -60/-20/-9/-10) quantification was done using [$^2H_5$]-caffeic acid ethyl ester (*m/z* 211.98 → 133.9; -80/-28/-7/-10).

Calibration. Internal standards were mixed with analytes in molar ratios from 0.01 to 2.3 keeping the concentration of the internal standard constant. Calibration curves were prepared by plotting peak area ratios of analyte/internal standard against concentration ratios of analyte/internal standard. Application of linear regression led to linear responses showing correlation coefficients of >0.99 each.

Quantitation of Minerals by means of HPIC. *Sample Preparation.* A defined amount of oat flour (1.5 g) was placed into a conical tube (50 mL, Carl Roth, Karlsruhe, Germany), filled with 15 mL deionized water and homogenized for 75 sec at 9000 rpm by means of an Ultra-Turrax T25 digital (Ika Labortechnik, Staufen, Germany).

Samples were centrifuged for 30 min at 4 °C and 4700 rpm using a Heraeus Multifuge X3 FR Zentrifuge (Thermo Fisher Scientific, Waltham, USA). The aqueous supernatant was transferred into a volumetric flask, filled up to the mark with deionized water and, finally, filtered. Analysis of the samples was performed by means of high performance ion chromatography (HPIC), following a modified literature procedure[18], using an Dionex ICS-2000 Ion Chromatography System (Thermo Fisher Scientific, Waltham, USA) consisting of a DS 6 heated conductivity cell detector, a dual piston pump, a CSRS 300 suppressor cell, an eluent generator RFIC EluGen Cartridge; EGC II MSA and an AS-AP autosampler. Using 5 mM methanesulfonic acid as mobile phase and a flow rate of 300 µL/min the system was operated at 40 °C under isocratic conditions for 20 min (injection volume 10 µL). Quantitative data were generated by means of external calibration at concentration levels between 0.8 and 250 mg/L. To quantitate cations, reference standards of potassium chloride, sodium chloride, magnesium chloride, and calcium chloride were used.

Quantitation of Carbohydrates by means of Enzymatic Assay. *Sample Preparation.* Quantification of D-(+)-glucose, D-(+)-fructose und sucrose was performed using an enzymatic assay kit for glucose/fructose/sucrose (R-Biopharm, Darmstadt, Germany). An amount of 10 g oat flour was placed into a volumetric flask (100 mL), and filled up with 60 mL deionized water. After the addition of 5 mL of Carrez I and II solutions for protein precipitation, the flask was filled up to the mark with deionized water and then filtered. Samples were treated according to the protocol of the assay kit and measurements were recorded on a UV-2401 PC UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) at 340 nm. The quantitative data are given as the mean of three replicates (RSD for each data point <4.1 %).

Sensory Experiments. *Panel Training.* According to literature[19] and in order to familiarize the panelists (six female, six male; 22-35 years in age) with the taste language, assessors were trained on different qualities of oral sensation. The panelists, who had no history of known taste disorders and who gave informed consent to participate in the sensory studies, took part in weekly sessions for at least two years. Aqueous solutions (2 mL; pH 6.4) of the following standard taste components were used for training: sucrose (50 mM) for sweet taste, L-lactic acid (20 mM) for sour taste, NaCl (20 mM) for salty taste, caffeine (1 mM) for bitter taste, and sodium glutamate (3 mM) for umami taste. For the astringent oral sensation, tannic acid (0.05 %) was used to train the panelists using the so-called half-tongue test.[19,20] All sensory studies were performed in a sensory panel room at 22−25 °C in three different sessions and using nose clips to avoid cross-modal interactions with odorants.

Pretreatment of Food Fractions and Taste Compounds for Sensory Analysis. Prior to sensory analysis, fractions or compounds, either commercially available or isolated from synthesis, were suspended in water and freeze dried twice, after removing the volatiles in high vacuum (<5 mPa). ^{1}H NMR and ion chromatography revealed that fractions treated by that procedure are essentially free of solvents and buffer compounds.

Taste Profile Analysis. An amount of methanol/water fraction II (2.5 g) isolated from oat flour as reported recently[1], was suspended in water (25 mL) containing 3% ethanol and adjusted to pH 6.4. After a dilution of 1:10, the aqueous solution was presented to the trained sensory panel to evaluate the taste sensations sweet, sour, umami, salty, bitter, and astringent on a linear intensity scale from 0 (not detectable) up to 5 (strongly detectable).

Taste Recognition Threshold Concentrations. Following the protocol reported recently[21], commercial or synthesized standard compounds were first proven to be

pure by means of ^{1}H-NMR spectroscopy and HPLC-MS and, then, taken up in bottled water (pH 6.4, 2% ethanol) to determine threshold concentrations for sweet, sour, salty, bitter and umami, as well as astringent taste sensation. Taste recognition thresholds were recorded by means of the half-tongue[19,22] test in order of increasing concentration. Taste thresholds concentrations were determined as the geometric mean across all panelists in three individual sessions. The values between individual and separate sessions differed by not more than plus or minus one dilution step, meaning that a threshold concentration of, for example, 81 µmol/L for avenanthramide 3f (**13**) represents a range of 40.5−162 µmol/L.

Preparation of Taste Recombinants in Aqueous Solution for Comparative Taste Analysis. A basic taste recombinant OR was prepared by dissolving the tastants from group I−V (see Table 3) in their "natural" concentrations (as determined in oat flour). Tastants were taken up in bottled water with a pH adjusted to 6.4 using 1 % formic acid and an ethanol content of 3 %. A 1:10 dilution of this stock solution was rated against a reference of the methanol-water fraction II (pH 6.4, 3 % ethanol, dilution 1:10) isolated from oat flour. Taste sensations sweet, sour, umami, salty, bitter, and astringent were then evaluated on a linear scale from 0 (not detectable) up to 5 (strongly detectable).

In the same way, a second recombinant ($OR_{\geq 1}$) was prepared consisting of the tastants from group I−V showing DoT-factors solely greater than 1.0 (Table 3) in their "natural" concentrations (as determined in oat flour). This recombinant was then rated against a reference of the basic taste recombinant OR (pH 6.4, 3 % ethanol, dilution 1:10) as described before. The overall comparative taste profiles of the recombinants were evaluated by means of a taste profile analysis using nose-clips and ranked on a linear scale from 0 to 5. Data are based on the average of three individual panel sessions.

High Performance Liquid Chromatography (HPLC). Preparative analysis of crude synthesis was performed on a HPLC apparatus (Jasco, Groß-Umstadt, Germany) consisting of two PU-2087 Plus pumps, a MD 2010 Plus photodiode array detector and a Rh 7725i type Rheodyne injection valve (Rheodyne, Bensheim, Germany). Data acquisition was executed by means of Chrompass 1.9. (Jasco, Groß-Umstadt, Germany).

High-Performance Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). Mass spectral analysis was performed on an API 4000 Q-Trap LC-MS/MS (AB Sciex. Darmstadt, Germany) connected to a Dionex UHPLC UltiMate-system 3000 (Dionex, Idstein, Germany). For electrospray ionization, the ion spray voltage was set at −4500 V in the negative mode and at 5500 V in the positive mode. Both quadrupoles operated at unit mass resolution, and nitrogen served as both curtain gas (25 psi) and turbo gas (425 °C). Fragmentation of the pseudo molecular ions $[M+H]^+$ or $[M-H]^-$ into specific product ions was induced by collision with nitrogen (4.5×10^{-5} Torr). Data acquisition and instrumental control were performed with Analyst 1.5.1 (AB Sciex, Darmstadt, Germany).

Nuclear Magnetic Resonance Spectroscopy (NMR). One- and two-dimensional ^1H and ^{13}C NMR spectra were acquired on a 500 MHz Bruker Avance III equipped with a triple resolution cryo probe (TCI) (Bruker, Rheinstetten, Germany). Samples were dissolved in methanol-d_4 containing 0.03 % trimethylsilane (TMS) or pyridine-d_5. The chemical shifts are referenced to the TMS or the solvent signal (pyridine-d_5: ^1H: 7.22 ppm; ^{13}C: 123.87 ppm). TOPSPIN version 2.1 (Bruker, Rheinstetten, Germany) was used for data processing. ^1H-, ^{13}C-, COSY-, HSQC- and HMBC-spectroscopy were recorded using standard pulse sequences of the Bruker library. Interpretation of the obtained spectra was performed with MestReNova 8.1.0-11315. (Mestrelab Research, Santiago de Compostela, Spain).

RESULTS AND DISCUSSION

As reported recently[1], sequential application of solvent extraction, solid phase extraction (SPE), and isolation by means of RP-HPLC followed by a taste dilution analysis disclosed that, besides steroidal saponins avenacoside A and B, two novel furostanol saponins as well as, unique oat constituents of avenanthramides were identified as bitter and astringent taste active molecules in oat flour[1]. In order to evaluate the sensory impact of previous findings, LC-MS/MS methods were developed to quantitate saponins and avenanthramides in oat flour samples. Furthermore, additional known taste active compounds were screened and analysed by means of LC-MS/MS, such as free fatty acids[15], monoglycerides[15], free amino acids[16], polyphenols[17], whereas soluble carbohydrates[17] were determined by means of an enzymatic assay, and minerals[16] were quantitated by means of HPIC.

HPLC-MS/MS Method Development and Quantification of Saponins 1−4 in Oats. A *Selected Ion Monitoring* method by means of LC-MS/MS was established with the aim of determining the concentrations in oats of recently[1] identified bitter and astringent tasting saponins, namely, 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**1**), 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**2**), avenacoside A (**4**) and avenacoside B (**3**).

To quantitate exact concentrations, commercially available ginsenosides Rb_1 (**14**) and Rb_1 (**15**) were utilized as internal calibration standards. For method validation, oat flour measurements were run in three independent analyses, each injected into the

LC-MS/MS system in triplicate. Resulting measurement precision, expressed as relative standard deviation, ranged between 5% (**3**) and 11% (**4**). Recovery studies were performed by spiking known concentrations of saponins **1**−**4** into oat flour at two different concentrations. A comparison to those amounts found in unspiked oat flour samples resulted in average recovery rates of 101.0% (**4**), 104.0% (**3**), 126.0% (**2**), and 140.5% (**1**).

Quantitative analysis revealed avenacoside A (**4**, 231.8 µmol/kg) and avenacoside B (**3**, 178.9 µmol/kg) as to be the most abundant saponins in oats, which is well in line with literature reported earlier[10]. The so far unknown furostanosides **1** and **2** were quantitated for the first time in oats and were found at 40-fold lower concentrations, with 6.0 and 12.3 µmol/kg, respectively.

HPLC-MS/MS Method Development and Quantification of Avenanthramides 5−10 and 13 in Oat by Means of SIDA. With the aim to investigate the recently[1] identified bitter and astringent tasting avenanthramides, namely, 2c (**5**), 2p (**6**), 2f (**7**), 1p (**8**), 1c (**9**), 1f (**10**), 1s (**11**), 2s (**12**), and the newly synthesized 3f (**13**), a LC-MS/MS method using *Selected Ion Monitoring* mode was set up to screen their presence in oats. As reported recently[1], the presence of 1s (**11**) and 2s (**12**) has not been confirmed in oat flour so far, whereas bismethoxylated avenanthramide 3f (**13**) was screened and successfully confirmed[23] to be present in oats within this study after tuning and optimisation of LC-MS/MS parameters using synthesized avenanthramide compounds in combination with a screening of oat flour samples and co-chromatography experiments.

To quantitate exact concentrations by means of SIDA, isotope labelled standards of [$^2H_3$]-avenanthramide 2f (**16**) and [$^2H_3$]-avenanthramide 1f (**17**) were synthesized by condensation reaction with labelled [$^2H_3$]-vanillin. Incorporation of the labelled OCD_3-function was confirmed by means of NMR and LC-MS/MS spectra. ^{1}H-NMR spectra of

unlabelled avenanthramide 1f (**10**) revealed a singlet at δ 3.93 ppm, demonstrating an intensity of three protons, for the methoxylation at position C-H(10') whereas for the labelled [$^2H_3$]-avenanthramide 1f (**17**) no proton signal could be detected at C-H(10'). In addition, mass spectrometry measurements of **10** revealed an exact mass of *m/z* 312.0868 for $[M-H]^-$, while the labelled [$^2H_3$]-avenanthramide 1f **17** gave a mass of *m/z* 315.1067 for $[M-H]^-$, showing an increase of three daltons for the incorporated three deuterium molecules.

To validate the method, oat flour measurements were run in four independent analysis, each injected into LC-MS/MS in three replicates. Resulting measurement precision ranged between 3% (**5**) and 9% (**9** and **10**). Recovery studies were performed by spiking known concentrations of avenanthramides **5−10** to oat flour samples at three different concentrations. A comparison to those amounts found in unspiked oat flour samples resulted in average recovery of 53.7% (**9**), 56.3% (**8**), 78.5% (**10**), 90.3% (**5**), 95.5% (**6**), and 98.3% (**7**).

Quantitative analysis revealed avenanthramide 3f (**13**, 35.9 µmol/L), 2f (**7**, 33.5 µmol/L), 2p (**6**, 23.0 µmol/L), 2c (**5**, 14.8 µmol/L) as the major abundant avenanthramides, whereas 1p (**8**), 1c (**9**), 1f (**10**), were found in lower quantities, between 0.3 (**10**) and 1.3 µmol/L (**9**). These results are well in line with data reported in previous studies[23-25].

Concentration and DoT Factors of Taste Compounds. With the aim of evaluating the relative contribution of individual taste molecules to the overall off-taste in oats, a total of 59 compounds were quantitated in oat flour, among them the saponins **1−4**, avenanthramides **5−10** and **13**, free fatty acids **18−24** and **27**/**28**, monoglycerides **29−33**, and in addition, ten polyphenols, eighteen amino acids, four minerals, and three soluble carbohydrates. Taking their individual taste recognition thresholds into consideration, quantitated molecules of oat taste were grouped into five

clusters according to their different taste sensations (see Table 3). In order to map out the key molecules driving the overall oat taste sensation, Dose-over-Threshold (DoT) factors were calculated for each individual compound.

Representing compounds of bitter and astringent oro-sensations, saponins **1**−**4**, avenanthramides **5**−**10** and **13**, free fatty acids **18**−**24** and **27**/**28,** monoglycerides **29**−**33** as well as magnesium, calcium, L-tryptophan, L-arginine, L-lysine, L-valine, L-histidine, L-phenylalanine, L-isoleucine, L-tyrosine, L-leucine were classified into group I (c.f. Table 3).

Calculation of the DoT factors for bitter taste showed an extremely high value of 305.1 for 1-linoleoyl-*rac*-glycerol (**33**). Furthermore, 1-stearoyl-*rac*-glycerol (**31**), 1-oleoyl-*rac*-glycerol (**32**), linoleic acid (**23**), avenacoside A (**4**), avenacoside B (**3**), 1-palmitoyl-*rac*-glycerol (**30**), linolenic acid (**24**), oleic acid (**22**) and palmitic acid (**20**) were rated with slightly lower DoT factors between 4.6 and 42.9 for bitterness, while 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**1**) and 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**2**) and stearic acid (**21**) only slightly exceeded their bitter thresholds showing DoT factors between 1 and 1.5. Although lipid derived compounds are well known to induce a bitter off-taste[3,26-29] this study positions monoglycerides, free fatty acids and the furostanol saponins (**1**) and (**2**) for the first time as to be the major bitter taste stimuli in oats.

In contrast, twenty-one out of the 34 bitter and astringent compounds where found at concentrations below their respective taste threshold among them the bitter amino acids, 1-myristoyl-*rac*-glycerol (**29**), the bivalent cations magnesium and calcium, the fatty acids 9/13-hydroxy-9*Z*,11*E*-octadecadienoic acid (**27**/**28**) myristic

660 acid (**19**), as well as the avenanthramides **5−10** and **13** showing DoT factors below 0.1
661 up to 0.6. Avenanthramides 1s (**11**)**,** 2s (**12**) and lauric acid (**18**) were not detected by
662 means of LC-MS/MS in oat flour samples and considered not to be present in oat flour.
663 Among the astringent taste actives of group 2, *p*-hydroxybenzaldehyde was
664 found to be the most abundant polyphenol with a DoT factor of 104.0, followed by
665 ferulic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid and sinapinic acid
666 showing DoT values between 2.1 and 27.3 (see Table 3). Polyphenols of
667 protocatechuic acid, vanillin, and gallic acid were detected at concentrations below
668 their taste thresholds (DoT <1.0).
669 Group 3, consisting of sweet-tasting soluble carbohydrates and sweet amino
670 acids (Table 3), revealed sucrose as to be the key sweet parameter in oat flour with a
671 DoT factor of 1.9. In contrast, fructose, L-serine, glucose, L-proline, L-alanine,
672 L-threonine, and L-methionine did not reach DoT values greater than 0.2.
673 Potassium and sodium, both analyzed at concentrations above their recognition
674 thresholds (DoT 3.4 and 2.0), were classified into salty-tasting compounds of group 4.
675 Finally, the umami-tasting compounds of group 5 revealed L-aspartic acid as to
676 be the major umami active component, showing a DoT greater than 1, while for
677 L-glutamic acid, L-asparagine and L-glutamine the calculated DoT values were ≤0.1.
678 With the exception of sucrose and L-aspartic acid, we conclude that sweet- and umami-
679 tasting compounds are of minor importance to the characteristic taste of oats, taking
680 into consideration that the overall concentrations of group 3 and 5 were below their
681 taste recognition thresholds.
682 **Taste Reconstitution Experiments.** In order to confirm the results of
683 quantitation studies and to determine whether the compounds already identified can
684 create the characteristic taste of oats, we compiled an aqueous taste recombinant of
685 59 substances in “natural” concentrations as given in Table 3 and compared it against

686 a freshly prepared aqueous solution of the methanol water-extract isolated from oat
687 flour. The taste compounds were dissolved in bottled water and the pH value was
688 adjusted to those of the methanol-water fraction by addition of trace amounts of formic
689 acid. After dilution (1:10) of both aqueous solutions, trained panelists were asked to
690 judge taste quality intensity of the taste recombinant against the methanol-water
691 extract on a linear scale from 0 (not detectable) to 5 (strongly detectable). Averaged
692 intensities for bitter (3.4/2.7 ± 1.2), astringent (2.2/1.8 ± 0.6), sour (1.1/1.0 ± 0.3), sweet
693 (0.8/0.7 ± 0.3), scratchy (0.4/0.5 ± 0.4), given in Table 4, were close to those of the
694 reference. Descriptors for umami and salty-taste were rated below 0.5 and were
695 therefore excluded from further discussions. Assuming a symmetric distribution, results
696 were tested for significance using a Wilcoxon rank test[30,31]. Calculated test values for
697 bitterness (PG_{calc} 29; PG_{tab} 21) and astringency (PG_{calc} 24; PG_{tab} 21) exceeded
698 tabulated values, which confirmed the null hypothesis H_0 to be true, resulting in a non-
699 significant difference between the taste recombinant and the methanol-water fraction.
700 Based on these findings it was concluded, that the compounds in group 1 and 2 are
701 sufficient to create the typical taste of an extract prepared from oat flour. For sweet
702 (PG_{calc} 19; PG_{tab} 21), sour (PG_{calc} 21; PG_{tab} 21) and scratchy (PG_{calc} 19; PG_{tab} 21)
703 attributes, the alternative hypothesis H_1 was accepted, leading to the conclusion, that
704 the extract and the recombinant are significantly different from each other. Due to the
705 fact that we focused, in our previous SENSOMICS study, on astringent and bitter
706 notes, further not-quantitated sweet, sour and scratchy taste stimuli may be present in
707 oats. While the major sweet tasting molecules are more or less screened, the presence
708 of oat organic acids e.g. such as free anthranilic acid or 5-hydroxyanthranilic acid could
709 be clarified in a further study. Furthermore, it could be tested if previously reported fatty
710 acids derivatives, such as 9,12,13-trihydroxy-*trans*-10-octadecenoic acid[3], 9,10,13-
711 trihydroxy-*trans*-11-octadecenoic acid[3], 9-hydroxy-*trans,cis*-10,12-octadecadienoic-1'-

monoglyceride[4] and 13-hydroxy-*cis,trans*-9,11-octadecadienoic-1'-monoglyceride[4] impact not only the bitter but also the scratchy impression described by the sensory panel.

To further investigate the importance of compounds showing a DoT factor below 1 to the overall taste profile, a partial taste recombinant $OR_{\geq 1}$ was prepared from 23 analytes showing a DoT factor ≥ 1 (c.f. Table 4) and compared to the taste recombinant OR containing all of the 59 analytes quantitated in oat flour. The resulting averaged descriptors bitter (2.4/2.1 ± 0.4), astringent (1.8/1.6 ± 0.4), sour (0.8/0.9 ± 0.3), sweet (0.5/0.6 ± 0.1), scratchy (0.9/0.6 ± 0.4) were very close to those of the completed recombinant. A significance test, using a Wilcoxon rank test as discussed before, revealed higher test values compared to tabulated values ($PG_{calc} > PG_{tab}$), leading to a non-significant difference between the two recombinants: bitter (PG_{calc} 20; PG_{tab} 8), astringent (PG_{calc} 21; PG_{tab} 8), sweet (PG_{calc} 25; PG_{tab} 8), sour (PG_{calc} 10; PG_{tab} 8) and scratchy (PG_{calc} 24; PG_{tab} 8). Therefore, it can be concluded that the overall sensory profile of oat flour can be achieved solely from the 23 compounds used within the partial taste recombinant $OR_{\geq 1}$. Consequently, it can be derived that neither avenanthramides **5**−**7** and **13** nor bitter amino acids, bivalent cations such as magnesium and calcium or oxidized fatty acids **27**/**28** play an important role in oat bitterness.

In summary, quantification of non-volatile taste active compounds in oat flour and recombination experiments led to the overall identification of 59 taste active components. Out of these, 23 compounds were identified as key taste contributors showing a DoT factor above a threshold of 1.0. Lipid-derived taste molecules such as monoglycerides and free fatty acids, namely, 1-linoleoyl-*rac*-glycerol (**33**), 1-stearoyl-*rac*-glycerol (**31**), 1-oleoyl-*rac*-glycerol (**32**), 1-palmitoyl-*rac*-glycerol (**30**), linolenic acid

738 (**24**), oleic acid (**22**), palmitic acid (**20**), linoleic acid (**23**), and stearic acid (**21**) were
739 validated for the first time to be the most abundant bitter substances in oat followed by
740 the saponins avenacoside A (**4**) and B (**3**) and 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-
741 glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-
742 glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**1**) and 3-(*O*-*α*-L-
743 rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-
744 glucopyranosyl-(25*R*)-furost-5-ene-3*β*,22,26-triol (**2**). However, recently identified
745 avenanthramides, imparting a bitter and astringent oro-sensation, did not exceed their
746 recognitions thresholds, and thus, were not confirmed to play a key role in overall bitter
747 off-taste of the analyzed oats.
748

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REFERENCES

(1) Günther-Jordanland, K.; Dawid, C.; Dietz, M.; Hofmann, T. Key Phytochemicals Contributing to the Bitter Off-Taste of Oat (*Avena sativa* L.). *J. Agric. Food Chem.* **2016**, *64*, 9639–9652.

(2) Molteberg, E. L.; Magnus, E. M.; Bjorge, J. M.; Nilsson, A. Sensory and chemical studies of lipid oxidation in raw and heat-treated oat flours. *Cereal Chem.* **1996**, *73*, 579–587.

(3) Biermann, U.; Wittmann, A.; Grosch, W. Vorkommen bitterer Hydroxyfettsäuren in Hafer und Weizen. *Fette, Seifen, Anstrichm.* **1980**, *82*, 236–240.

(4) Biermann, U.; Grosch, W. Bitter-tasting monoglycerides from stored oat flour. *Z. Lebensm.-Unters. Forsch.* **1979**, *169*, 22–26.

(5) Tschesche, R.; Tauscher, M.; Fehlhaber, H.-W.; Wulff, G. Steroidsaponine mit mehr als einer Zuckerkette, IV. Avenacosid A, ein bisdesmosidisches Steroidsaponin aus Avena sativa. *Chem. Ber.* **1969**, *102*, 2072–2082.

(6) Tschesche, R.; Lauven, P. Steroidsaponine mit mehr als einer Zuckerkette, V. Avenacosid B, ein zweites bisdesmosidisches Steroidsaponin aus *Avena sativa*. *Chem. Ber.* **1971**, *104*, 3549–3555.

(7) Tschesche, R.; Wiemann, W. Steroidsaponine mit mehr als einer Zuckerkette, XI. Desgluco-avenacosid-A und -B, biologisch aktive Nuatigeninglycoside. *Chem. Ber.* **1977**, *110*, 2416–2423.

(8) Önning, G.; Asp, N.-G. Analysis of saponins in oat kernels. *Food Chemistry* **1993**, *48*, 301–305.

(9) Pecio, L.; Jedrejek, D.; Masullo, M.; Piacente, S.; Oleszek, W.; Stochmal, A. Revised structures of avenacosides A and B and a new sulfated saponin from *Avena sativa* L. *Magn. Reson. Chem.* **2012**, *50*, 755–758.

780 (10) Pecio, L.; Wawrzyniak-Szolkowska, A.; Oleszek, W.; Stochmal, A. Rapid
781 analysis of avenacosides in grain and husks of oats by UPLC-TQ-MS. *Food Chem*
782 **2013**, *141*, 2300–2304.
783 (11) Schieberle, P.; Hofmann, T. 18 Mapping the Combinatorial Code of Food
784 Flavors by Means of Molecular Sensory. *in Food Flavors: Chemical, Sensory and*
785 *Technological Properties. (H. Jelen, ed.); CRC Press, Taylor and Francis Group*
786 **2011**, 411–437.
787 (12) Wéber, C.; Bielik, A.; Demeter, Á.; Borza, I.; Szendrei, G. I.; Keserű, G. M.;
788 Greiner, I. Solid-phase synthesis of 6-hydroxy-2,4-diaminoquinazolines.
789 *Tetrahedron* **2005**, *61*, 9375–9380.
790 (13) Kamat, S. P.; Parab, S. J. A Simple Two-Step Synthesis of Avenanthramides,
791 Constituents of Oats (*Avena sativa* L). *Indian Journal of chemistry, Sektion B-*
792 *Organic Chemistry including Medicinal Chemistry* **2007**, *46B*, 2074–2078.
793 (14) Helal, C. J.; Kang, Z.; Hou, X.; Pandit, J.; Chappie, T. A.; Humphrey, J. M.; Marr,
794 E. S.; Fennell, K. F.; Chenard, L. K.; Fox, C.; Schmidt, C. J.; Williams, R. D.; Chapin,
795 D. S.; Siuciak, J.; Lebel, L.; Menniti, F.; Cianfrogna, J.; Fonseca, K. R.; Nelson, F.
796 R.; O'Connor, R.; MacDougall, M.; McDowell, L.; Liras, S. Use of Structure-Based
797 Design to Discover a Potent, Selective, In Vivo Active Phosphodiesterase 10A
798 Inhibitor Lead Series for the Treatment of Schizophrenia. *J. Med. Chem.* **2011**, *54*,
799 4536–4547.
800 (15) Lainer, J. E. Chemosensory characterization of a bitter off-taste in poppyseeds
801 (*Papaver somniferum* L.) (in German). Ph.D. thesis, TU Munich, Germany, 2019.
802 (16) Hillmann, H.; Hofmann, T. Quantitation of Key Tastants and Re-engineering the
803 Taste of Parmesan Cheese. *J. Agric. Food Chem.* **2016**, *64*, 1794–1805.
804 (17) Hillmann, H.; Mattes, J.; Brockhoff, A.; Dunkel, A.; Meyerhof, W.; Hofmann, T.
805 Sensomics Analysis of Taste Compounds in Balsamic Vinegar and Discovery of 5-

806 Acetoxymethyl-2-furaldehyde as a Novel Sweet Taste Modulator. *J. Agric. Food*
807 *Chem.* **2012**, *60*, 9974–9990.
808 (18) Meyer, S.; Dunkel, A.; Hofmann, T. Sensomics-Assisted Elucidation of the
809 Tastant Code of Cooked Crustaceans and Taste Reconstruction Experiments. *J.*
810 *Agric. Food Chem.* **2016**, *64*, 1164–1175.
811 (19) Scharbert, S.; Holzmann, N.; Hofmann, T. Identification of the Astringent Taste
812 Compounds in Black Tea Infusions by Combining Instrumental Analysis and
813 Human Bioresponse. *J. Agric. Food Chem.* **2004a**, *52*, 3498–3508.
814 (20) Hufnagel, J. C.; Hofmann, T. Orosensory-Directed Identification of Astringent
815 Mouthfeel and Bitter-Tasting Compounds in Red Wine. *J. Agric. Food Chem.*
816 **2008a**, *56*, 1376–1386.
817 (21) Dawid, C.; Hofmann, T. Identification of Sensory-Active Phytochemicals in
818 Asparagus (*Asparagus officinalis* L.). *J Agric Food Chem* **2012b**, *60*, 11877–11888.
819 (22) Stark, T.; Hofmann, T. Isolation, Structure Determination, Synthesis, and
820 Sensory Activity of N-Phenylpropenoyl-L-amino Acids from Cocoa (*Theobroma*
821 *cacao*). *J. Agric. Food Chem.* **2005b**, *53*, 5419–5428.
822 (23) Dimberg, L. H.; Theander, O.; Lingnert, H. Avenanthramides - A Group of
823 Phenolic Antioxidants in Oats. *Cereal Chem.* **1993**, *70*, 637–641.
824 (24) Bratt, K. & Sunnerheim, K., et al. Avenanthramides in Oats (Avena sativa L.)
825 and Structure−Antioxidant Activity Relationships. *J. Agric. Food Chem* **2003**, 51,
826 594–600.
827 (25) Mattila, P., Pihlava, J.-m. & Hellstrom, J. Contents of Phenolic Acids, Alkyl- and
828 Alkenylresorcinols, and Avenanthramides in Commercial Grain Products. *J. Agric.*
829 *Food Chem* **2005**, 53, 8290–8295

(26) Baur, C., Grosch, W., Wieser, H. & Jugel, H. Enzymatic oxydation of linoleic acid: Formation of bittertasting fatty acids. *Z Lebensm Unters Forch* **1977**, 164, 171–176.

(27) Grosch, W. & Laskawy, G. Untersuchungen über die Beteifigung von Linolsäure am Bittergeschmack von Mohnsamen (Papaver somniferum). *Z Lebensm Unters Forch* **1984**, 178, 257–265.

(28) Stephan, A. & Steinhart, H. Bitter taste of unsaturated free fatty acids in emulsions: contribution to the off-flavour of soybean lecithins. *European Food Research and Technology* **2000**, 212, 17–25.

(29) Degenhardt, A. G. & Hofmann, T. Bitter-Tasting and Kokumi-Enhancing Molecules in Thermally Processed Avocado (Persea americana Mill.). *J. Agric. Food Chem.* **2010**, 58, 12906–12915.

(30) Kromidas, S. *Validierung in der Analytik*; WILEY-VCH Verlag: Weinheim, 2011.

(31) Imam, A., Usman, M. & Abanyam Chiawa, M. On Consistency and Limitation of paired t-test, Sign and Wilcoxon Sign Rank Test. *IOSR Journal of Mathematics* **2014**, 10.

(32) Toelstede, S.; Hofmann, T. Quantitative Studies and Taste Re-engineering Experiments toward the Decoding of the Nonvolatile Sensometabolome of Gouda Cheese. *J. Agric. Food Chem.* **2008b**, *56*, 5299–5307.

(33) Hufnagel, J. C.; Hofmann, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008b**, *56*, 9190–9199.

(34) Stark, T.; Bareuther, S.; Hofmann, T. Molecular definition of the taste of roasted cocoa nibs (*Theobroma cacao*) by means of quantitative studies and sensory experiments. *J. Agric. Food Chem.* **2006**, *54*, 5530–5539.

856 **FIGURE LEGEND**

Figure 1. Chemical structures of taste-active compounds identified in oat flour.

Figure 2. HPLC-MS/MS chromatograms (MRM, ESI^-) of 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-*ene*-3*β*,22,26-triol (**1**), 3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-*ene*-3*β*,22,26-triol (**2**), avenacoside B (**3**) and A (**4**) and the internal standards ginsenoside Rg_1 (**14**) and Rb_1 (**15**) in fraction II extracted from oat flour.

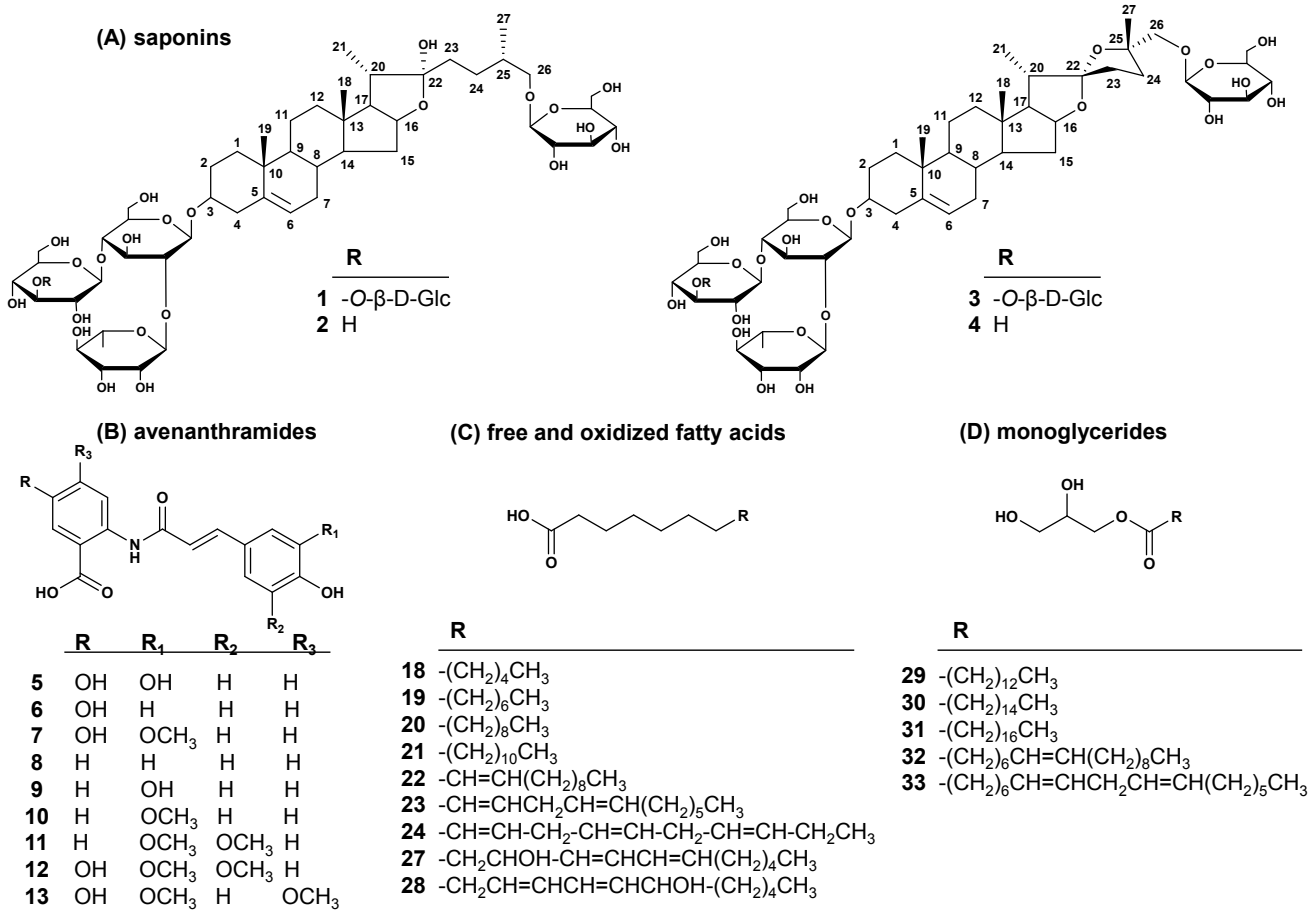
Figure 3. HPLC-MS/MS analysis (MRM, ESI^-) of oat flour showing mass transitions of taste active myristic acid (**19**), palmitic acid (**20**), stearic acid (**21),** oleic acid (**22**), linoleic acid (**23**), linolenic acid (**24**), 9-hydroxy-10*E*,12*Z*-octadecadienoic acid/13-hydroxy-9*Z*,11*E*- octadecadienoic acid (9/13-HODE) (**27**/**28**). Labeled internal standards [^{13}C]-palmitic acid (**25**) und [$^2H_2$]-9,10-oleic acid (**26**) were used for quantification of **19**−**24**.

Figure 4. HPLC-MS/MS chromatograms (MRM, ESI^-) of 1-Myristoyl-*rac*-glycerol (**29**), 1-Palmitoyl-*rac*-glycerol (**30**), 1-Stearoyl-*rac*-glycerol (**31**), 1-Oleoyl-*rac*-glycerol (**32**), and 1-Linoleoyl-*rac*-glycerol (**33**) extracted from oat flour.

857

858

859 **Figure 1.** (Günther-Jordanland et al.)



	R	R_1	R_2	R_3
5	OH	OH	H	H
6	OH	H	H	H
7	OH	OCH_3	H	H
8	H	H	H	H
9	H	OH	H	H
10	H	OCH_3	H	H
11	H	OCH_3	OCH_3	H
12	OH	OCH_3	OCH_3	H
13	OH	OCH_3	H	OCH_3

	R
18	$-(CH_2)_4CH_3$
19	$-(CH_2)_6CH_3$
20	$-(CH_2)_8CH_3$
21	$-(CH_2)_{10}CH_3$
22	$-CH=CH(CH_2)_8CH_3$
23	$-CH=CHCH_2CH=CH(CH_2)_5CH_3$
24	$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2CH_3$
27	$-CH_2CHOH-CH=CHCH=CH(CH_2)_4CH_3$
28	$-CH_2CH=CHCH=CHCHOH-(CH_2)_4CH_3$

	R
29	$-(CH_2)_{12}CH_3$
30	$-(CH_2)_{14}CH_3$
31	$-(CH_2)_{16}CH_3$
32	$-(CH_2)_6CH=CH(CH_2)_8CH_3$
33	$-(CH_2)_6CH=CHCH_2CH=CH(CH_2)_5CH_3$

860

Table 1. Determination of Recovery Rates for Avenanthramides 5−10 in Oat Flour.

	amount added[a] (nmol/kg) and recovery (%) at spiking level					
experiment	**5**	**6**	**7**	**8**	**9**	**10**
1	2.41	3.41	2.81	2.20	1.82	2.48
	100.1 %	99.4 %	99.1 %	61.6 %	60.9 %	76.2 %
2	5.36	7.57	6.25	4.89	4.04	5.52
	91.5 %	98.3 %	99.2 %	56.1 %	53.5 %	81.0 %
3	11.48	16.22	13.39	10.48	8.65	11.82
	79.3 %	88.8 %	96.8 %	51.3 %	46.8 %	78.3 %
mean value (%)	90.3 %	95.5 %	98.3 %	56.3 %	53.7 %	78.5 %

[a] avenanthramides **5−10**; Mean out of two independent repetitions at three different concentration levels (experiment 1−3). Values were compared to those of non-spiked oat flour.

Table 2. Determination of Recovery Rates for Saponins 1−4 in Oat Flour.

	amount added[a] (nmol/kg) and recovery (%) at spiking level			
experiment	**1**	**2**	**3**	**4**
1	17.2	51.9	84.1	193.3
	143.0 %	125.0 %	97.0 %	95,0 %
2	116.6	117.5	79.2	89.3
	138.0 %	127.0 %	111.0 %	107.0 %
mean value (%)	140.5	126.0 %	104.0 %	101.0 %

[a] saponins **1−4**; Mean out of two independent repetitions at two different concentration levels (experiment 1−2). Values were compared to those of non-spiked oat flour.

Table 3. Taste Qualities. Taste Thresholds (TC), Concentrations, and Dose-over-Threshold (DoT) Factors of Taste-Active Compounds in Oat Flour.

taste compound	**conc. (µM/kg)[a]**	**TC (µM/L)[b]**	**DoT[c] factor**
group 1: bitter, scratchy and astringent-tasting compounds			
1-linoleoyl-*rac*-glycerol (**33**)[q]	74448	152[d]/244[e]	489.8/305.1
1-stearoyl-*rac*-glycerol (**31**)[q]	9579	91[d]/223[e]	105.3/42.9
1-oleoyl-*rac*-glycerol (**32**)[q]	47699	172[d]/1147[e]	277.3/41.6
linoleic acid (**23**)[q]	60209	270[d]/1810[e]	223.0/33.3
avenacoside A (**4**)[o]	232	3[f]/7[e]	77.3/33.1
avenacoside B (**3**)[o]	179	4[f]/6[e]	44.7/29.8
1-palmitoyl-*rac*-glycerol (**30**)[q]	13225	118[d]/888[e]	112.1/14.9
linolenic acid (**24**)[q]	3620	189[d]/277[e]	19.2/13.1
oleic acid (**22**)[q]	15676	203[d]/2180[e]	77.2/7.2
palmitic acid (**20**)[q]	7036	1002[d]/1546[e]	7.0/4.6

3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-gluco-pyranosyl(1→4)]-*β*-D-glucopyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-*ene*-3*β*,22,26-triol (**1**)[o]	6.0	3[f]/4[e]	2.0/1.5
3-(*O*-*α*-L-rhamnopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→4)]-*β*-D-gluco-pyranosid)-26-*O*-*β*-D-glucopyranosyl-(25*R*)-furost-5-*ene*-3*β*,22,26-triol (**2**)[o]	12.3	3[f]/9[e]	4.1/1.4
stearic acid (**21**)[q]	697.8	645[d]/726[e]	1.1/1.0
1-myristoyl-*rac*-glycerol (**29**)[q]	179.9	277[d]/359[e]	0.6/0.5
avenanthramide 3f (**13**)[p]	35.9	49[f]/81[e]	0.7/0.4
avenanthramide 2p (**6**)[p]	23.0	55[f]/60[e]	0.4/0.4
avenanthramide 2f (**7**)[p]	33.5	135[f]/170[e]	0.2/0.2
avenanthramide 2c (**5**)[p]	14.8	45[f]/78[e]	0.3/0.2
magnesium	1238.4	6400[g,h]	0.2
9/13-hydroxy-9*Z*,11*E*-octadecadienoic acid (**27**/**28**)[q]	139.8	300[d]/750[e]	0.5/0.2
calcium	776.6	6200[g,h]	0.1
L-tryptophan	359.5	5000[i]	0.1
myristic acid (**19**)[q]	189.5	1620[d]/1703[e]	0.1/0.1
L-arginine	837	75000[i]	<0.1
L-lysine	528	80000[i]	<0.1
L-valine	410	30000[i]	<0.1
L-histidine	355	45000[i]	<0.1
L-phenylalanine	333	45000[i]	<0.1
L-isoleucine	217	10000[i]	<0.1
L-tyrosine	172	4000[i]	<0.1
L-leucine	126	11000[i]	<0.1
avenanthramide 1c (**9**)[p]	1.3	56[f]/130[e]	<0.1
avenanthramide 1p (**8**)[p]	0.7	70[f]/96[e]	<0.1
avenanthramide 1f (**10**)[p]	0.3	98[f]/113[e]	<0.1
group 2: astringent-tasting compounds			
p-hydroxybenzaldehyde	69247	665	104.0
ferulic acid	1828	67[j]	27.3
p-hydroxybenzoic acid	17123	665[j]	25.7
caffeic acid	1136	72[j]	15.8
p-coumaric acid	1255	139[j]	9.0
sinapinic acid[p]	1459	693	2.1
protocatechuic acid	105	206[j]	0.5
vanilline	43	829[k]	0.1

gallic acid	24	292[j]	0.1
group 3: sweet-tasting compounds			
sucrose	23591	12500[m]	1.9
fructose	1739	10200[k]	0.2
L-serine	960	25000[i]	<0.1
glucose	855	18000[k]	<0.1
L-proline	800	25000[i]	<0.1
L-alanine	338	12000[i]	<0.1
L-threonine	67	35000[i]	<0.1
L-methionine	49	5000[i]	<0.1
group 4: salty-tasting compounds			
potassium	43812	13000[g,h]	3.4
sodium	7690	3900[g,h]	2.0
group 5: umami-tasting compounds			
L-aspartic acid	1577	600[i]	2.6
L-glutamic acid	129	1100[i]	0.1
L-asparagine	2422	50000[i]	<0.1
L-glutamine	24	50000[i]	<0.1

[a] concentration (µM/kg dry matter) of oat flour. [b] Taste threshold concentrations (µM/L) were recorded in water at pH 6.4 or taken from literature. [c] Dose-over-threshold (DoT) factor is calculated as the ratio of concentration and taste threshold. [d] TC for scratchy perception. [e] TC for bitter taste. [f] TC for astringent mouthfeel. [g] TC for the corresponding chloride ion. [h] value taken from [32]. [i] value taken from [16]. [j] value taken from [33]. [k] value taken from [17]. [l] value taken from [18]. [m] value taken from [34]. [o] recorded in 1 % ethanol at pH 6.4. [p] recorded in 2 % ethanol at pH 6.4. [q] recorded in 3 % ethanol.

Figure 2. (Günther-Jordanland et al.)

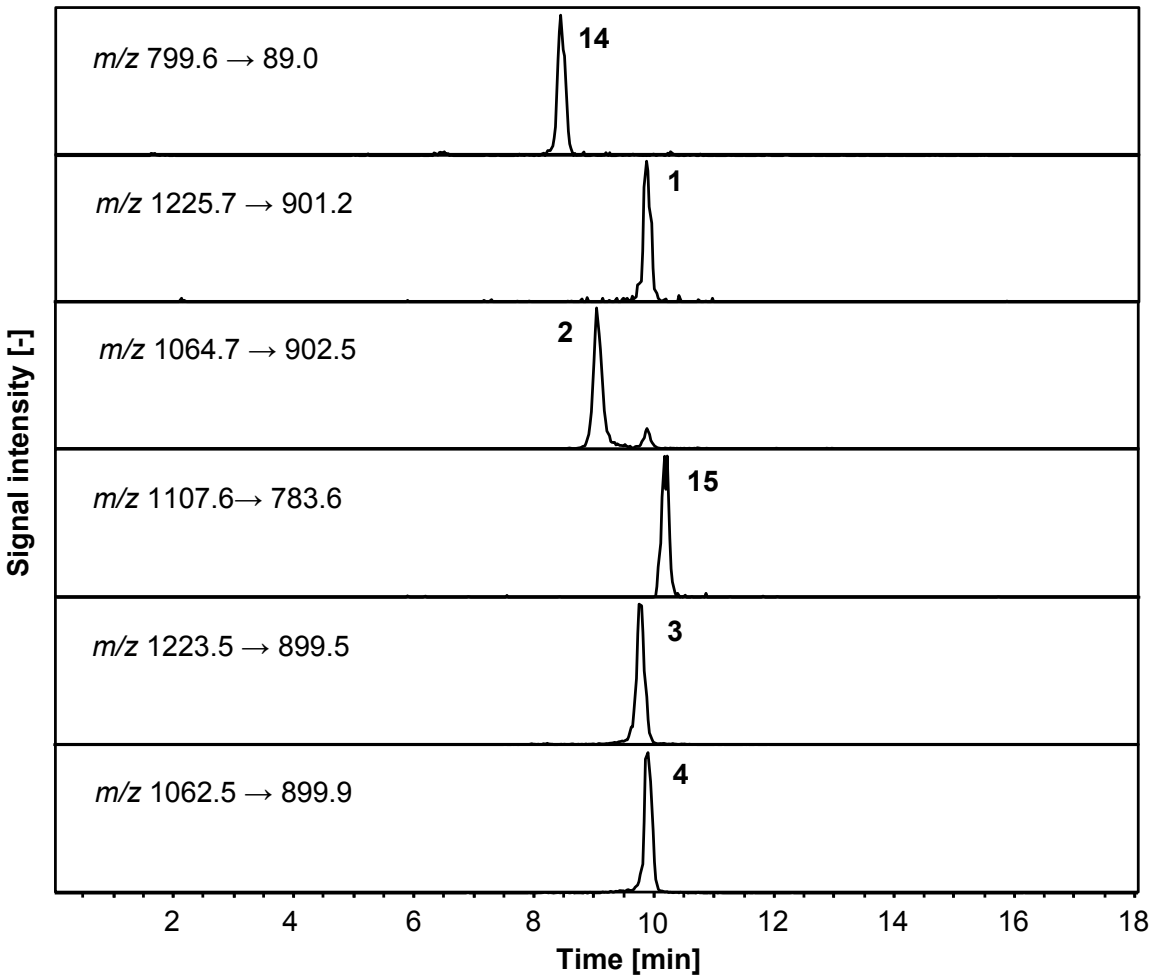


Figure 3. (Günther-Jordanland et al.)

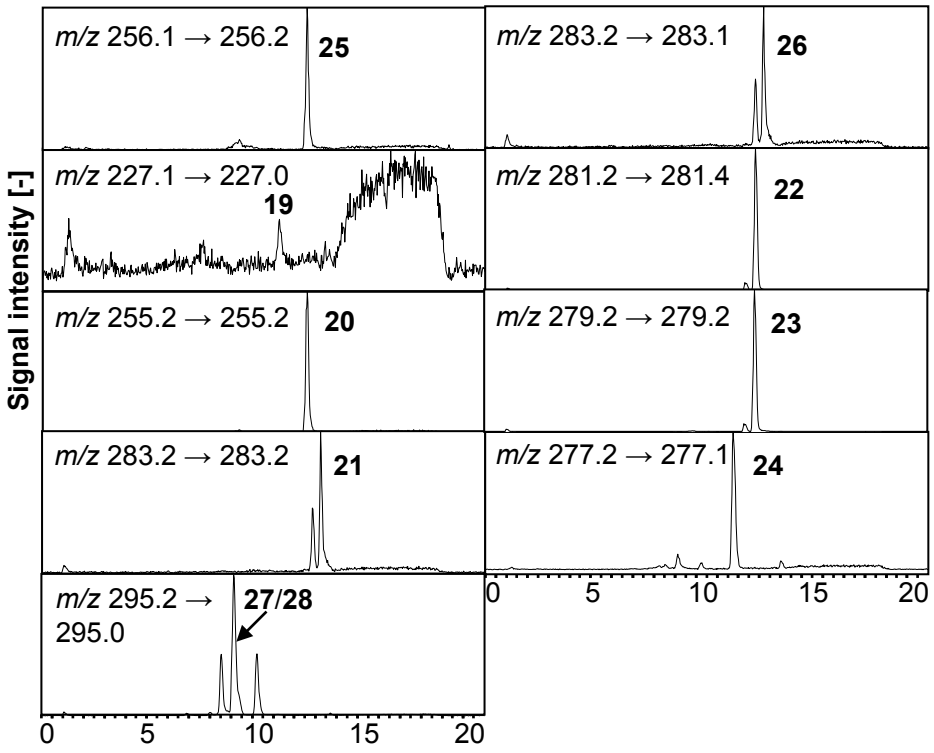


Figure 4. (Günther-Jordanland et al.)

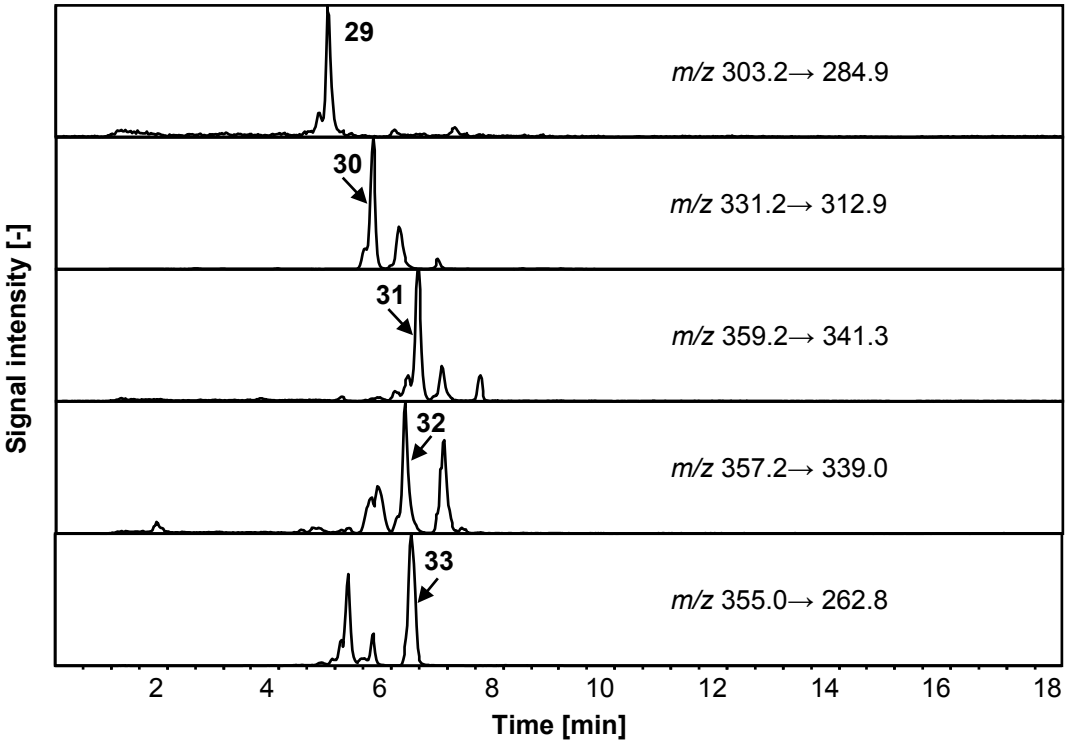


Table 4. Comparative Sensory Evaluation of Methanol-Water Extract versus Taste Recombinant (OR) Containing the Tastants of Groups 1−5 and of Taste Recombinant (OR) versus Partial Taste Recombinant ($OR_{\geq 1}$) Containing Compounds Showing a DoT Factor ≥1.

	Intensity		
Taste quality	methanol-water[a]	OR[b]	OR≥1[c]
bitter	3.4	2.7 ± 1.2[nsd]	2.1 ± 0.4[nsd]
astringent	2.2	1.8 ± 0.6[nsd]	1.6 ± 0.4[nsd]
sour	1.1	1.0 ± 0.3	0.9 ± 0.3[nsd]
sweet	0.8	0.7 ± 0.3	0.6 ± 0.1[nsd]
scratchy	0.4	0.5 ± 0.4	0.6 ± 0.4[nsd]
umami	<0.1	<0.1	<0.1
salty	<0.1	<0.1	<0.1

[a] equal to fraction II isolated from oat flour as reported recently[1], [b] Total recombinant (pH adjusted to 6.4, 3 % ethanol) containing of 59 substances (group 1 to 5; Table 3); rated against a reference of methanol water extract, [c] Total recombinant (pH adjusted to 6.4, 3 % ethanol) containing of 23 substances showing a DoT≥1 (group 1 to 5; Table 3) rated against a reference of OR, [nsd] not significantly different versus the reference.

TOC figure. (Günther-Jordanland et al.)