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Quantitative Studies and Sensory Analyses on the Influence of Cultivar, Spatial Tissue Distribution, and Industrial Processing on the Bitter Off-Taste of Carrots (*Daucus carota* L.) and Carrot Products

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Although various reports pointed to 6-methoxymellein (**1**) as a key player imparting the bitter taste in carrots, activity-guided fractionation experiments recently gave evidence that not this isocoumarin but bisacetylenic oxylipins contribute mainly to the off-taste. Among these, (*Z*)-heptadeca-1,9-dien-4,6-diyn-3-ol (**2**), (*Z*)-3-acetoxy-heptadeca-1,9-dien-4,6-diyn-8-ol (**3**), and (*Z*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol (falcarindiol, **4**) have been successfully identified. In the present study, an analytical procedure was developed enabling an accurate quantitation of **1**−**4** in carrots and carrot products. To achieve this, (*E*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol was synthesized as a suitable internal standard for the quantitative analysis of the bisacetylenes. On the basis of taste activity values, calculated as the ratio of the concentration and the human sensory threshold of a compound, a close relationship between the concentration of **4** and the intensity of the bitter off-taste in carrots, carrot puree, and carrot juice was demonstrated, thus showing that compound **4** might offer a new analytical measure for an objective evaluation of the quality of carrot products. Quantitative analysis on the intermediate products in industrial carrot processing revealed that removing the peel as well as green parts successfully decreased the concentrations in the final carrot puree by more than 50%.

KEYWORDS: Bitter taste; carrot; 6-methoxymellein; (*Z*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol; falcarindiol

INTRODUCTION

It has been known for more than half a century that carrots are able to produce a sporadic bitter off-taste (*1*). This bitter taste is often the reason for consumers rejection of carrot products such as carrot puree in the infant diet and is, therefore, a major problem for vegetable processors.

Several compounds such as 6-methoxymellein (**1**) (**Figure 1**) (*2*, *3*), eugenin (*4*), terpenoids (*5*), and water soluble phenolics (*6*) have been proposed in the last 50 years as chemical principles of off-taste development. The data found so far are very contradictory, and for none of the compounds detected in carrots could a correlation be found between the sensory evaluation of the bitter taste and the results obtained by instrumental analysis (*3*, *5*, *7*).

Very recent studies, activity-guided fractionation involving solvent extraction, gel permeation chromatography, and high-performance liquid chromatography (HPLC) in combination with human taste dilution analyses revealed that not a single compound but a multiplicity of bitter tastants contribute to the bitter off-taste of stored carrots and commercial carrot puree, respectively (*8*). Among these bitter compounds, (*Z*)-heptadeca-1,9-dien-4,6-diyn-3-ol (**2**), (*Z*)-3-acetoxy-heptadeca-1,9-dien-4,6-diyn-8-ol (**3**), and (*Z*)-heptadeca-1,9-dien-4,6-diyn-3,8-diol (**4**) (**Figure 1**), called falcarindiol, could be identified for the first time as bitter compounds on the basis of mass spectrometry (MS) as well as one- and two-dimensional nuclear magnetic resonance (NMR) experiments. Preliminary quantitative studies and relating the concentration of these compounds with their individual bitter detection thresholds clearly excluded **1** as the inducer of the bitter off-taste and pinpointed compound **4** as one of the key players in the bitter taste of carrots. The quantitative procedure used for determining the amounts of these taste compounds in carrots was, however, not convenient.

The objectives of the present investigation were, therefore, (i) to develop a suitable analytical method enabling the simultaneous quantification of bisacetylenic oxylipins **2**−**4** and **1** in carrots and carrot puree, (ii) to rate these compounds in their bitter impact on the basis of a dose−activity relationship, (iii) to investigate the spatial distribution of the bitter compounds in the carrot root, (iv) to study the influence of different carrot cultivars on bitter taste, and (v) to investigate the influence of industrial puree manufacturing on bitter taste compounds **1**−**4**.

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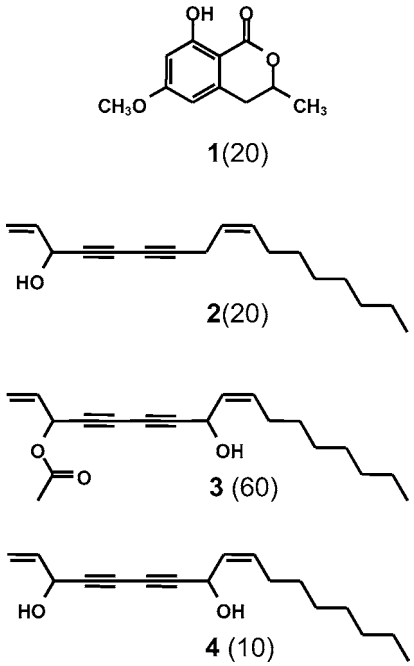

Figure 1. Structures of bitter-tasting **1**–**4**. Recognition threshold concentrations (mg/L) for bitterness are given in brackets.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: (*E*)-2-decenal (**7**) (Acros Organics, Schwerte, Germany), ethinylmagnesiumbromide, 7-methoxycoumarin (Aldrich, Steinheim, Germany), acrolein (**5**) (Fluka, Neu-Ulm, Germany), ammonium chloride, copper(I) chloride, sodium chloride, sodium sulfate, and sodium hydrogen carbonate (Merck, Darmstadt, Germany). The solvents were of HPLC grade (Merck). Fresh carrots were obtained commercially from a local vegetable market and analyzed the same day. Different carrot cultivars were supplied by the companies Bejo (Warmenhuizen, Netherlands), Rijk Zwaan (Welver, Germany), and Hipp (Pfaffenhofen, Germany). The carrot puree exhibiting a significant bitter off-taste was supplied by the German food industry. The reference materials of 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (**1**) and **2**−**4** were isolated from carrots and purified as reported recently (*8*).

Syntheses. *1-Pentene-4-in-3-ol (6).* A solution of freshly distilled **5** (3 g; **Figure 2**) in anhydrous diethyl ether (15 mL) was added dropwise to a mixture of an ethinylmagnesiumbromide solution (100 mL; 0.5 M in tetrahydrofuran) and anhydrous diethyl ether (40 mL) while stirring. Thereafter, the mixture was refluxed at 60 °C for 2 h. After the mixture was cooled in an ice bath, water (100 mL) and an aqueous saturated NH_4Cl solution (150 mL) were added, and the aqueous layer was washed with diethyl ether (100 mL). The organic phases were combined, were washed with an aqueous saturated $NaHCO_3$ solution (100 mL) as well as a saturated NaCl solution (100 mL), and were then dried over Na_2SO_4. After the solution was filtered and the solvent was removed in vacuo, the title compound **6** (**Figure 2**) was isolated with a purity of more than 99% by distillation (20 mbar, 60 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.17 (1H, s), 2.58 (1H, d, ^3J = 2.3 Hz), 4.89 (1H, m), 5.25 (1H, dt, ^2J = 1.4, ^3J = 10.2 Hz), 5.50 (1H, dt, ^2J = 1.4, ^3J = 17.0 Hz), 5.98 (1H, ddd, ^3J = 5.2 Hz, ^3J = 10.2 Hz, ^3J = 17.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 63.3 (CH), 74.8 (C), 83.0 (CH), 117.2 (CH_2), 136.9 (CH).

(E)-4-Dodecen-1-in-3-ol (8). A solution of **7** (8 g; **Figure 2**) in anhydrous diethyl ether (30 mL) was added dropwise to a mixture of an ethinylmagnesiumbromide solution (100 mL; 0.5 M in tetrahydrofuran) and anhydrous diethyl ether (40 mL) while stirring and was then refluxed at 60 °C for 2 h. After the reaction mixture was worked up following the procedure detailed above, **8** (**Figure 2**) was obtained upon high vacuum distillation (0.5 mbar, 80 °C). HRGC/MS (CI, NH_3): *m*/*z* 180 (100), 181 (14), 163 (9), 172 (4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, 6.8 Hz), 1.35 (10H, m), 1.90 (1H, s), 2.07 (2H, m), 2.56 (1H, d, ^4J = 2.0 Hz), 4.84 (1H, d, ^3J = 5.7 Hz), 5.61 (1H, m), 5.92 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5 (CH), 22.8 (CH_2), 29.2 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 32.2 (CH_2), 32.3 (CH_2), 63.2 (CH), 74.3 (C), 83.8 (CH), 128.8 (CH), 135.0 (CH).

(E)-Heptadeca-1,9-dien-4,6-diyn-3,8-diol (9). A mixture of **6** (2.0 g; 24 mmol), **8** (4.4 g; 24 mmol), copper(I) chloride (1.0 g; 10 mmol), and ammonium chloride (0.75 g; 14 mmol) in methanol (50 mL) was stirred at room temperature in a laboratory autoclave (Roth, Germany) under an atmosphere of oxygen (3 bar) for 18 h. After the suspension was centrifuged and filtered, the reaction mixture was extracted with diethyl ether (100 mL), and the water phase was extracted three times with diethyl ether (100 mL). The combined organic phases were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo. Fractionation of the residue by gel permeation chromatography on a 400 mm × 55 mm Sephadex LH-20 column (Amersham Pharmacia Biotech, Freiburg, Germany) with ethanol as the mobile phase was followed by semipreparative RP-HPLC to yield **9** (**Figure 2**) with a purity of more than 99%. HRGC/MS (EI): *m*/*z* 55 (100), 91 (94), 115 (87), 129 (74), 41 (71), 77 (70), 128 (69), 105 (58), 43 (56), 79 (43). HRGC/MS (CI, NH_3): *m*/*z* 260 (100; $[M]^+$), 154 (68), 225 (58), 172 (50), 242 (43), 243 (31), 204 (26), 229 (23), 261 (22), 205 (19), 245 (19). ^{1}H NMR (360 MHz, $CDCl_3$): δ 0.88 (3H, t, ^3J = 6.8 Hz), 1.27 (10H, m), 1.39 (2H, m), 1.89 (1H, s), 2.06 (2H, q, ^3J = 6.1 Hz), 4.89 (1H, d, ^3J = 5.2 Hz), 4.95 (1H, d, ^3J = 5.2 Hz), 5.27 (1H, m, ^2J = 0.91 Hz, ^3J = 10.0 Hz), 5.48 (1H, m, ^2J = 0.91 Hz, ^3J = 17.0 Hz), 5.57 (1H, m, ^3J = 6.1 Hz, ^3J = 15.2 Hz), 5.91 (1H, m, ^3J = 5.2 Hz, ^3J = 15.2 Hz), 5.95 (1H, ddd, ^3J = 5.2 Hz, ^3J = 10.0 Hz, ^3J = 17.0

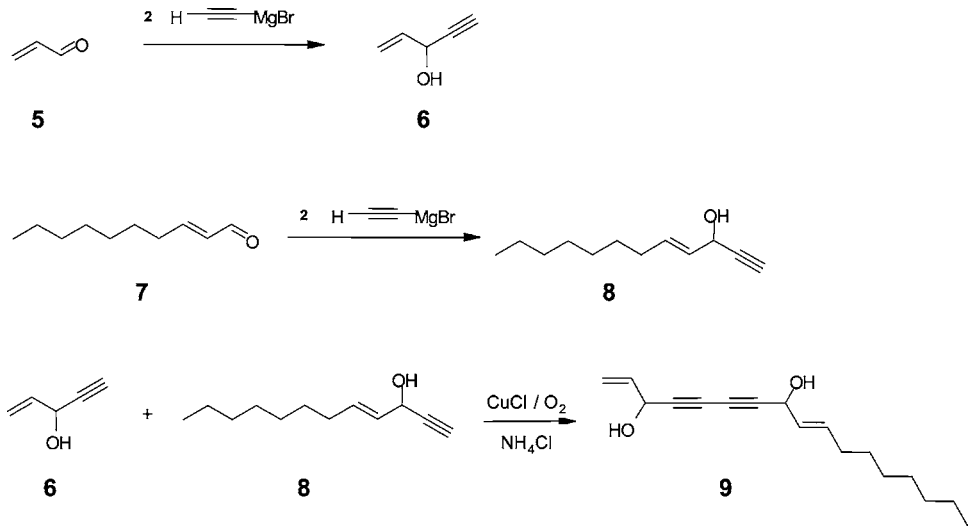

Figure 2. Synthesis of the internal standard **9**.

Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (CH), 22.6 (CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 31.8 (CH_2), 32.0 (CH_2), 63.4 (CH), 63.5 (CH), 69.7 (C), 70.3 (C), 78.3 (C), 79.3 (C), 117.4 (CH_2), 127.7 (CH), 135.3 (CH), 135.8 (CH).

Quantification of Bitter Compounds 1−4 in Carrots and Carrot Puree. Commercial carrot puree (20 g) or fresh puree (20 g) obtained upon mincing fresh carrots using an Ultra-Turrax while cooling, respectively, was spiked with the internal standards (*E*)-falcarindiol (250 μg in 2 mL of MeOH) and 7-methoxycoumarin (50 μg in 2 mL of MeOH) and then intimately mixed with Na_2SO_4 (100 g). The carrot material was then extracted three times with ethyl acetate (100 mL) by stirring for 5 min at room temperature. After the material was filtered, the organic layers were combined and then freed from the solvent in vacuo. The ethyl acetate extractables were dissolved in *n*-pentane (2 mL) and centrifuged (3000 rpm), and the clear supernatant was applied onto the top of a Sep-Pak Classic Silica cartridge (Waters, Ireland) conditioned with *n*-pentane. After the cartridge was flushed with *n*-pentane/diethyl ether (5 mL; 95/5, v/v), polyacetylenic oxylipins as well as **1** were eluted with a mixture (5 mL; 40/60, v/v) of *n*-pentane and diethyl ether. The effluent was collected, the solvent was removed in vacuo, and the residue obtained was dissolved in methanol (2 mL) and membrane filtered. An aliquot (20 μL) of that stock solution was analyzed by HPLC for quantification of **1** using 7-methoxycoumarin as the internal standard. For the quantification of the polyacetylenic compounds, an aliquot (1 mL) of the methanolic stock solution was diluted with methanol (5 mL) and then analyzed by gas chromatography (GC). The results are given as the means of triplicates, and the standard deviation was less than 10%.

Sensory Analyses. *Training of the Sensory Panel.* Twelve assessors were trained to evaluate the taste of aqueous solutions (3 mL each) of the following standard taste compounds by using a triangle test as described in the literature (*9*): saccharose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (12 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, sodium glutamate (8 mmol/L, pH 5.7) for umami taste, and tannin (gallustannic acid; 0.05%) for astringency. The sensory analyses were performed in a sensory panel room at 22−25 °C in three different sessions.

Intensity Ranking Test. Following the procedure reported recently (*9*), the bitter taste of the commercial carrot puree, carrot juice, and chopped, fresh carrots was evaluated by the trained sensory panel using a scale from 0 (no bitter taste detectable) to 3 (strong bitterness).

HPLC. The HPLC apparatus (BIO-TEK Kontron Instruments, Eching, Germany) consisted of two pumps (type 522), a Rheodyne Injector (250 μL loop), and a UV/vis detector (type 535). The HPLC analysis of **1** was performed on a 250 mm × 4.8 mm i.d. analytical scale RP-18, ODS-Hypersil, 5 μm column (ThermoHypersil, Kleinostheim, Germany), monitoring the effluent at 302 nm and using the following methanol/water gradient (flow rate 1.0 mL/min): starting with a mixture (40/60, v/v) of methanol and water, the methanol content was increased to 60% within 10 min and then increased to 80% in 15 min.

High-Resolution (HR) GC. For HRGC analysis, a Trace GC (Thermo Quest CE Instruments) coupled with either a flame ionization detector or a mass spectrometer was used. Helium was used as the carrier gas with a column pressure of 240 kPa, and nitrogen was used as the makeup gas (30 mL/min). The sample was injected cool on column. For HRGC analysis, a 60 m × 0.25 mm Neutra Bond-1, WCOT fused silica column (GL Sciences Inc., Tokyo, Japan) was used. The injection (1 μL) was performed at an oven temperature of 50 °C. After 1 min, the temperature was increased with a rate of 10 °C/min up to 250 °C and held for 15 min.

HRGC/MS. HRGC was performed with a Type 5890 Series II gas chromatograph (Fisons Instruments, Mainz, Germany) using a 30 m × 0.32 mm DB-5 fused silica capillary, 0.25 mm (J&W Scientific, Fisons) by on-column injection at 40 °C. After 2 min, the temperature of the oven was raised at 10 °C/min to 260 °C and held for 15 min isothermally. The flow of the carrier gas, helium, was 1.8 mL/min. MS analysis was performed with a MAT 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. Mass chromatography in the electron impact mode (MS/EI) was performed at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with ammonia as the reactant gas.

NMR Spectroscopy. ^{1}H, ^{13}C, DEPT-135 NMR spectroscopy, HMQC, and HMBC experiments were performed on a AM-360 spectrometer (Bruker, Rheinstetten, Germany). Deuterochloroform was used as the solvent, and tetramethylsilane was used as the internal standard.

RESULTS AND DISCUSSION

To evaluate the contribution of the bitter compounds **1**−**4** (**Figure 1**) to the bitter off-taste of carrots and carrot products on the basis of a dose−activity relationship, an analytical tool needed to be developed first enabling a rapid and accurate quantitation of the bitter tastants in foods. To achieve this, 7-methoxycoumarin was chosen as a suitable internal standard for the quantification of **1**, but no commercially available compound proved suitable as the internal standard for the analysis of the acetylenic alcohols **2**−**4**. Because the double bonds in these bitter compounds were confirmed in carrots to exist only in the (*Z*)-configuration, the corresponding **9** was synthesized as a suitable internal standard. Following the synthetic sequence given in **Figure 2**, **5** was converted in a Grignard reaction with ethinylmagnesiumbromide to give **6**. In parallel, **7** was ethinylated to **8**. The acetylenic alcohols **6** and **8** were then linked by a Glaser coupling reaction giving rise to **9**, which was purified by gel permeation chromatography and RP HPLC.

Using 7-methoxycoumarin and **9** as the internal standards, the following analytical procedure was developed for a straightforward quantification of the bitter tastants **1**−**4** in carrots and carrot products. Either the carrot tissue minced with an Ultra-Turrax, the carrot puree, or the carrot juice was spiked with the defined amounts of 7-methoxycoumarin and **9** and was then intimately mixed with sodium sulfate until a dry powder was obtained. After the carrot powder was extracted with ethyl acetate and the sample was cleaned up by means of a silica cartridge, **1** was quantified by RP HPLC/diode array detection using 7-methoxycoumarin as the internal standard (**Figure 3**), and the bisacetylenic oxylipins **2**−**4** were analyzed by HRGC using **9** as the standard (**Figure 4**). This straightforward procedure enabled the quantification of the bitter compounds **1**−**4** in several samples in less than 4 h.

Contribution of Compounds 1−4 to the Bitter Taste of Carrots. To elucidate the taste contribution of the compounds **1**−**4** in fresh carrot tissue, the concentrations of these bitter compounds were quantitatively determined in seven carrot samples. In parallel, these samples were presented to a trained sensory panel who was asked to score the intensity of the bitter perception on a scale from 0 (not detectable) to 3 (strong detectable). The results in **Table 1** revealed the highest concentrations for **4** spanning from 21.7 to 84.3 mg/kg, thus confirming the high concentrations of acetylenic alcohol in carrots as reported earlier (*10*−*12*).

The acetylenes **2** and **3** were present in somewhat lower amounts of 8.1−27.5 and 7.7−40.8 mg/kg, respectively. By far, the lowest concentrations were found for **1**, which was present in concentrations below 2 mg/kg, with the exception of sample 5 containing 6.8 mg/kg of compound **1** (**Table 1**).

Because quantitative data alone do not allow any insight into the taste contribution of a compound, compounds **1**−**4** were rated in their taste impact on the basis of a dose−activity relationship. To achieve this, taste activity values (TAVs) were determined as the ratio of the concentration of a compound and its threshold concentration. Calculations of the ratio of the

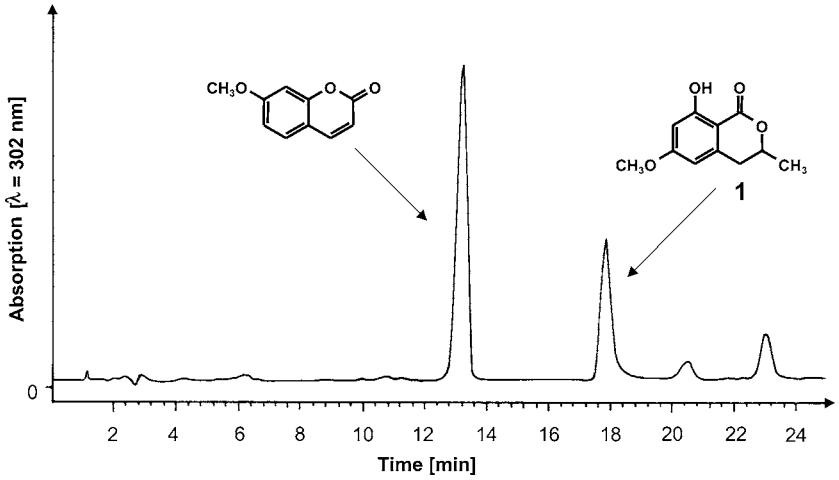

Figure 3. HPLC chromatogram of the quantification of **1** with the internal standard 7-methoxycoumarin.

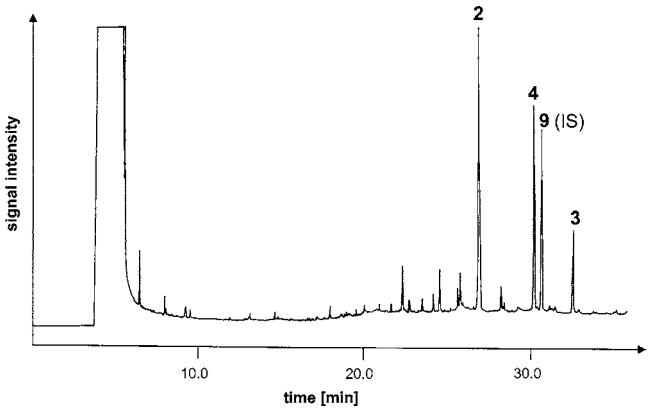

Figure 4. HRGC chromatogram of the quantification of the bisacetylenic oxylipins **2**–**4** with the internal standard **9**.

Table 1. Concentrations of **1**–**4** and the Bitter Intensity of Fresh Carrots

	concentration (mg/kg) of				
sample	**1**	**2**	**3**	**4**	bitter intensity[a]
1	0.1	8.1	8.0	21.7	0.1
2	<0.1	11.1	17.1	31.7	0.2
3	0.6	27.5	9.2	42.3	0.9
4	<0.1	24.1	29.7	38.5	1.0
5	6.8	20.0	7.7	42.7	1.0
6	2.0	18.5	8.6	46.8	1.5
7	0.1	10.0	40.8	84.3	1.7

[a] The bitter intensity was evaluated by the trained sensory panel using a scale from 0 (no bitter taste detectable) to 3 (strong bitterness).

concentration (**Table 1**) and the threshold concentrations reported recently for **1** (20 mg/kg), **2** (20 mg/kg), **3** (60 mg/kg), and **4** (10 mg/kg) (*8*) revealed the highest TAVs for compound **4** in all of the samples investigated, e.g., the concentration of compound **4** in sample 7 was 8.4 times above its threshold concentration (**Figure 5**). In contrast, the concentration of compound **2** was above its detection threshold only in the samples 3−5 (**Figure 5**). In contrast, the TAVs calculated for compounds **1** and **3** were below 1, thus indicting that these compounds do not contribute to the bitter off-taste of the carrots under investigation. Taking these analytical as well as the sensory data into consideration, it is obvious that the TAV of compound **4** increased parallel to the intensity of the bitterness perceived. These data indicate a close relationship between the concentration of **4** and the intensity of the bitter off-taste in carrots, whereas, in contrast, no correlation could be observed for the other taste compounds. Quantitative as well as sensory analysis of an additional 28 commercial carrot batches further strengthened this relationship. The data displayed in **Figure 6** clearly show that the analytical data run in parallel to the sensory data, thus confirming compound **4** as a reasonable indicator substance for the analytical monitoring of bitterness in carrot tissue.

Influence of the Carrot Cultivar and Soil on Bitter Compounds. To investigate the influence of the carrot cultivar on the concentration of bitter compounds, compounds **1**−**4** were quantified in freshly harvested carrots from 11 different cultivars grown in the same soil, and in addition, the intensity of the carrot bitter taste was rated by a trained sensory panel on a scale from 0 (no bitterness detectable) to 3 (strong bitterness detectable). As given in **Table 2**, these cultivars differed significantly in their bitter taste intensity being evaluated with scores spanning from 0.1 to 1.7. The cultivars Bersky, Florida, Fayette, Infinity, Kingston, and Kazan were evaluated with bitter intensities of 0.1−0.3, whereas the bitterness of the cultivars Bangor, Nandrin, Kamaran, Kathmandu, and Sunset was generally rated with higher bitterness scores; for example, the cultivar Sunset showed the most pronounced bitter off-taste rated with an intensity of 1.7 (**Table 2**). Quantitative analysis of the bitter tastants revealed that the concentrations of compound **4** in these carrots increased with increasing bitter taste intensity. The cultivars evaluated with bitter intensities between 0.1 and 0.3 contained compound **4** in concentrations of 16.2−38.5 mg/kg, whereas the carrots scored with bitter intensities of 0.9−1.7 contained the tastant in amounts of 45.0−84.3 mg/kg (**Table 2**). Relating these concentrations to the threshold concentration of 10 mg/kg revealed TAVs ranging from 1.6 to 8.4, thus demonstrating that compound **4** contributes to the bitter off-taste of all of the carrot cultivars investigated. In contrast, neither for the isocoumarin derivative **1** nor for the oxylipins **2** and **3** could any correlation between the concentration of these compounds and the data of the sensory analysis be found. On the basis of these findings, it can be concluded that bitter off-taste is strongly dependent on the carrot cultivar and that compound **4** can be used as an analytical measure for off-taste development.

Aimed at getting first insight into the influence of the soil on bitter tastant formation, the concentrations of bitter compounds **1**−**4** were quantified in the less bitter carrot cultivar Kazan and the bitter carrot cultivar Kamaran, respectively, each grown on three different fields, and in addition, the intensity of

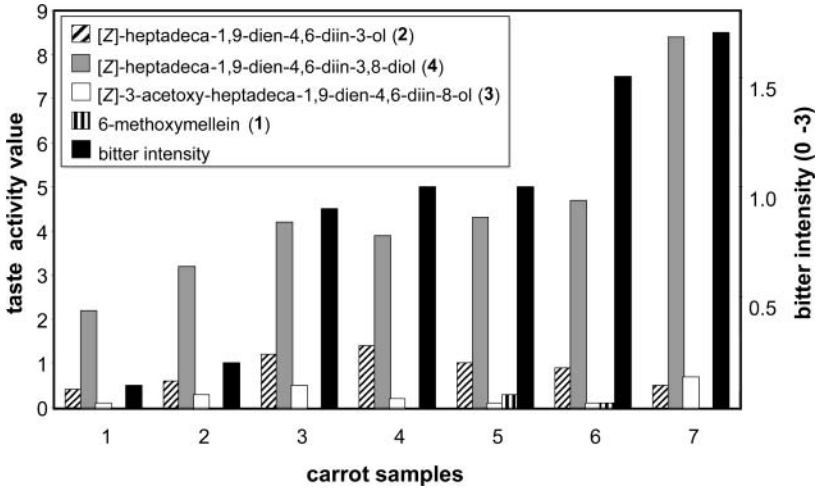

Figure 5. Bitter activity values of **1** and bisacetylenic oxylipins (**2**–**4**) and the bitter intensity of fresh carrot tissue.

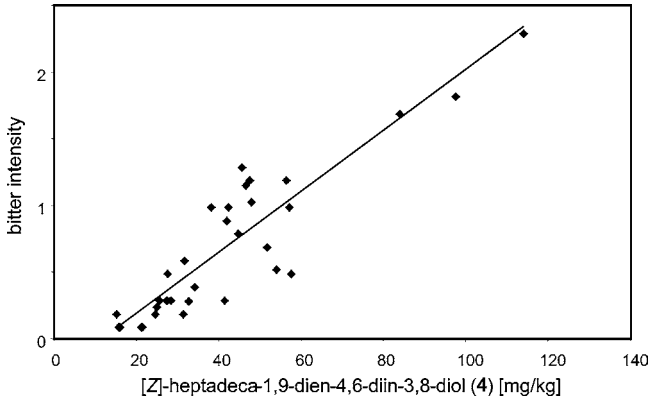

Figure 6. Correlation between the concentration of oxylipin **1** and the bitter taste of 28 carrot samples.

Table 2. Influence of the Carrot Cultivar on the Concentrations of **1**–**4** and the Bitter Intensity of Fresh Carrots

	concentration[b] (mg/kg) of					
cultivar[a]	**1**	**2**	**3**	**4**	TAV of **4**	bitter intensity[c]
Bersky	<0.1	5.2	8.9	16.2	1.6	0.1
Florida	0.3	30.9	7.0	16.0	1.6	0.1
Fayette	6.1	10.5	11.0	24.9	2.5	0.2
Infinity	<0.1	11.1	17.1	31.7	3.2	0.2
Kingston	0.8	15.5	16.2	32.4	3.2	0.3
Kazan	0.6	27.5	9.2	38.5	3.9	0.3
Bangor	<0.1	16.0	18.7	45.0	4.5	0.9
Nandrin	0.4	23.6	12.1	43.5	4.4	0.9
Kamaran	0.1	12.0	8.8	46.4	4.6	1.2
Kathmandu	<0.1	6.1	28.3	45.7	4.6	1.3
Sunset	0.1	10.0	40.8	84.3	8.4	1.7

[a] Carrots were grown on the same field. [b] Concentrations are given as the mean of triplicates. [c] Bitter intensity was rated on a score from 0 (not detectable) to 3 (strongly detectable).

the carrot bitter taste was rated by a trained sensory panel on a scale from 0 (no bitterness detectable) to 3 (strong bitterness detectable). Independent from the soil, the cultivar Kazan imparted only a weak bitter off-taste evaluated with intensities below 0.3, whereas the samples of the cultivar Kamaran were evaluated with significantly higher bitter scores spanning from 1.2 to 2.0 (**Table 3**). Quantitative analysis of the bitter compounds and calculation of TAVs revealed that the concentrations of compound **4** were again above the threshold concentrations in all of the samples, but in the less bitter-tasting cultivar Kazan, the taste acitivity values (TAVs) reached 3.9 at the maximum, whereas the more intensely bitter cultivar Kamaran reached a maximum value of 11.4 (**Table 3**). Confirming the data in **Table 2**, there was no correlation between the sensorially perceived bitterness and the quantitative data of compounds **1**−**3**. Taking all of these data into account, it can be concluded that the carrot cultivar rather than the soil conditions is more the key to bitter off-taste development.

Table 3. Influence of the Soil on the Concentrations of **1**–**4** and the Bitter Intensity of Fresh Carrots

	concentration[d] (mg/kg) of					
cultivar	**1**	**2**	**3**	**4**	TAV of **4**	bitter intensity[e]
Kazan[a]	0.3	20.8	16.9	15.5	1.6	0.2
Kazan[b]	0.1	7.9	15.3	33.0	3.3	0.2
Kazan[c]	0.6	27.5	9.2	38.5	3.9	0.3
Kamaran[a]	0.1	12.0	8.8	46.4	4.6	1.2
Kamaran[b]	2.0	18.5	8.6	46.8	4.7	1.3
Kamaran[c]	7.8	7.0	32.4	114.3	11.4	2.0

[a–c] Carrots were grown in the same field. [d] Concentrations are given as the mean of triplicates. [e] Bitter intensity was rated on a score from 0 (not detectable) to 3 (strongly detectable).

Table 4. Distribution of **1**–**4** in the Carrot Root

	concentration (mg/kg) of					
tissue[a]	**1**	**2**	**3**	**4**	TAV of **4**	bitter intensity[a]
upper end	0.2	24.0	15.2	33.5	3.4	0.5
lower end	0.2	20.4	8.0	18.7	1.8	<0.1
phloem	0.3	28.6	11.5	32.2	3.2	0.6
xylem	<0.1	24.7	13.3	14.9	1.5	0.2

Spatial Distribution of Bitter Compounds in the Carrot Root. To investigate the spatial distribution of bitter compounds in the carrot root, compounds **1**−**4** were quantified in the outer phloem, the inner xylem, and the lower as well as the upper end of the root, and in addition, the intensity of the carrot bitter taste was rated by a trained sensory panel on a scale from 0 (no bitterness detectable) to 3 (strong bitterness detectable). The sensory panel detected an intense bitter taste in the phloem (0.6) as well as the upper end (0.5) of the root, whereas the bitterness of the xylem as well as the lower end was comparatively low (**Table 4**). Relating the sensory data to the quantitative analytical data again demonstrated that the more bitter the sample, the higher the concentrations of compound **4**. The more bitter upper end and the phloem contained 33.5 and 32.3 mg/kg of compound **4**, whereas in the less bitter lower end and the xylem lower

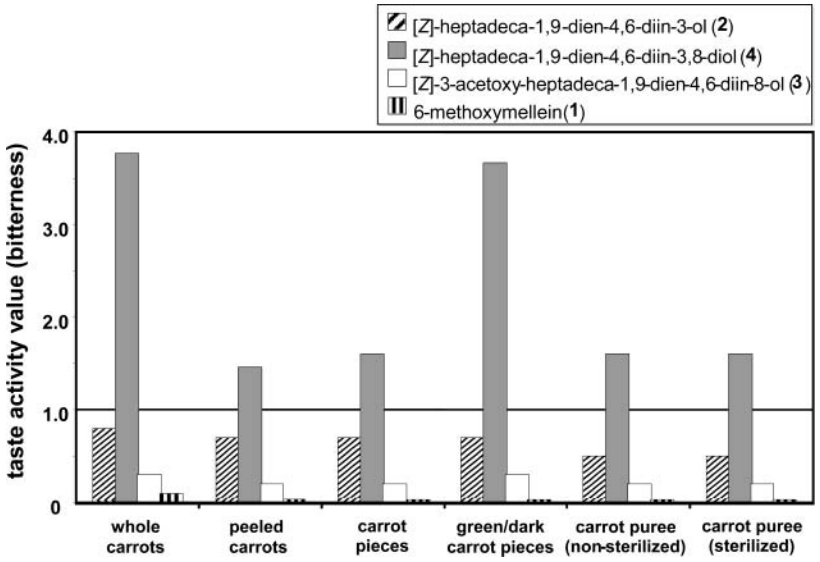

Figure 7. TAVs of **1** and bisacetylenic oxylipins (**2**–**4**) in intermediate products of industrial carrot puree manufacturing.

concentrations of 1.8 and 1.5 mg/kg were found. As already found for the entire carrot tissue (e.g., **Tables 1**−**3**), no correlation was found between the concentration of compounds **1**−**3** and the bitter off-taste intensity. However, it is interesting to notice that among the group of bisacetylenic oxylipins, compounds **2** and **3** have a different spatial distribution than the key bitter tastant **4**; for example, the concentrations of compounds **2** and **3** in the phloem equal those found in the xylem, whereas the content of compound **4** in the phloem is double the amount determined in the xylem (**Table 4**).

Contribution of Compounds 1−**4 to Bitter Off-Taste of Carrot Products.** To investigate the contribution of the compounds **1**−**4** to the bitter off-taste of carrot products, the compounds were quantified in eight commercial carrot puree batches as well as in six batches of carrot juice, and the bitter taste intensity of these samples was evaluated by a trained sensory panel on a scale from 0 (no bitterness detectable) to 3 (strong bitterness detectable). The quantitative data showed that compound **4** was present in the highest concentrations spanning from 8.1 to 21.6 mg/kg in carrot puree and from 6.6 to 20.6 mg/kg in carrot juice (**Table 5**). The polyacetylenes **2** and **3** were present in lower concentrations, and also, the concentration differences between the individual samples were much smaller, e.g., compounds **2** and **3** were present in concentrations from 6.9 to 11.4 and from 4.7 to 8.9 mg/kg. The lowest concentrations, however, found for **1**, e.g., 0.3−2.3 and 0.9−3.8 mg/kg, respectively, were quantified in carrot puree and carrot juice. Relating the concentrations of these compounds to their individual bitter taste thresholds demonstrated that the TAV of compound **4** was close to or above 1.0, thus indicating this compound as a contributor to the bitter off-taste of the carrot products. Well in line with this suggestion, the overall bitter taste intensity of the individual puree and juice samples ran in parallel with the TAVs of compound **4**, e.g., in puree sample 8, which was evaluated with an overall bitter intensity of 2.0, a TAV of 2.2 was determined for compound **4** (**Table 5**). As the sensory panel described all of the carrot puree samples evaluated with a bitter intensity of ≥1.5 with the term "intolerably bitter", compound **4** might be used as a suitable indicator substance for the analytical monitoring of bitter off-taste development in carrot products. In contrast, the concentrations of compounds **1**−**3** were significantly below their taste thresholds (data not shown), demonstrating that these compounds do not contribute to the bitter taste of carrot puree and juice, respectively.

Table 5. Concentrations of 1–4 and the Bitter Intensity of Commercial Carrot Puree and Carrot Juice

	concentration (mg/kg) of					
sample	**1**	**2**	**3**	**4**	TAV of **4**	bitter intensity[a]
			Carrot Puree			
1	0.3	7.1	5.2	8.1	0.8	0.5
2	0.3	7.9	4.7	10.9	1.1	1.0
3	0.8	9.8	5.8	13.2	1.3	1.0
4	0.3	6.9	6.9	13.8	1.4	1.0
5	0.5	10.3	6.3	14.0	1.4	1.0
6	1.2	11.4	8.9	16.9	1.7	1.5
7	0.2	7.4	5.0	21.0	2.1	2.0
8	2.3	7.9	7.2	21.6	2.2	2.0
			Carrot Juice			
1	2.1	5.5	3.5	6.6	0.7	0.3
2	2.2	6.2	3.9	8.1	0.8	0.3
3	0.9	4.9	3.8	11.4	1.1	0.5
4	3.8	7.5	5.7	14.3	1.4	0.5
5	2.7	12.7	5.9	17.6	1.8	0.5
6	1.6	7.2	6.9	20.6	2.1	1.0

[a] The bitter intensity was evaluated by the trained sensory panel using a scale from 0 (no bitter taste detectable) to 3 (strong bitterness).

Influence of Industrial Carrot Processing on Compounds 1−**4.** One of the major products for the infant food industry is carrot puree. For the industrial manufacturing of carrot puree, whole carrot roots are steam-peeled, and the peeled carrots are then cut into cubic pieces (10 mm i.d.). Using an optoelectronic device, green and dark parts are detected and sorted out by means of an air pressure jet; the high-quality carrot pieces are then minced and cooked, filled into jars, and finally heat-sterilized.

To investigate the influence of the carrot puree manufacturing process on the taste activity of individual bitter compounds, compounds **1**−**4** were quantified in samples drawn from each step of the industrial process, and TAVs were calculated as the ratio of their concentration to their individual bitter threshold concentrations (**Figure 7**). As expected, the analysis of the whole carrot root showed a high TAV of 3.7 for compound **4**, whereas the concentrations of compounds **1**−**3** did not reach the taste threshold. After steam peeling, the TAV of compound **4** dropped from 3.7 to 1.4, whereas the low taste impact of compounds

1−**3** was not strongly influenced. These data indicate that during the peeling process a major amount of compound **4** is selectively removed with the peel and clearly confirm the data found for the spatial distribution of bitter compounds in the carrot root (**Table 4**). After the peeled carrot was cut into cubic pieces and sorting out the green and dark pieces, compound **4** was present in the premium pieces with a TAV of 1.6, whereas in the colored particles the concentration of the bitter compound exceeded its sensory threshold by a factor of 3.6. These data indicate that not only the peel but also the green and dark parts of the carrot root contained the highest concentrations of compound **4**. After the samples were minced and cooked as well as after the heat sterilization process of the puree, the taste activity of compound **4** equaled the value found in the carrot pieces, thus indicating that heat processing is not an influence on taste activity of compound **4**.

Taking all of these data into consideration, compound **4** could be used to analytically objectify the bitterness of carrot products and might offer a new standard for an objective evaluation of the quality of carrot products. Analysis of various cultivars demonstrated that some cultivars seem to produce higher amounts of that bitter compound than others, whereas the soil seems not to be a key driver for bitter taste development. In the course of industrial carrot processing, removing the peel as well as green and dark parts was demonstrated to successfully remove more than 50% of the bitter tastant from the carrot material. Such information might be helpful for carrot breeders and producers as well as the carrot-processing industry to find possibilities as to how to reduce the bitter off-taste in carrots and carrot products in the future.

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