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Volatile Constituents of Uncooked Rhubarb (*Rheum rhabarbarum* L.) Stalks

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Volatiles of rhubarb (*Rheum rhabarbarum* L.) stalks were isolated by means of vacuum headspace method and analyzed by capillary gas chromatography−mass spectrometry. Fifty-nine components were reported for the first time in rhubarb. A striking feature of the extracts obtained is the preponderance (∼65%) of compounds with C_6 skeletons. In addition to unsaturated C_6 aldehydes and alcohols, substantial amounts of the less common (*E*)-2- and (*E*)-3-hexenoic acid were detected. Gas chromatography−olfactometry and determination of odor activity values revealed the sensory importance of the C_6 compounds to the aroma of rhubarb. Comparative experiments involving the inhibition of enzyme activities revealed that the initial spectrum of C_6 components is changed due to subsequent isomerization and reductions. Thus, contributions of (*Z*)-3-hexenal and the unsaturated acids decrease, and (*E*)-2-hexenal/(*E*)-2-hexenol play major sensory roles.

KEYWORDS: Rhubarb; volatiles; vacuum headspace method; C_6 components; GC-O

INTRODUCTION

Rhubarb is a perennial plant of the family Polygonaceae. There are various kinds of rhubarb (*Rheum* species), some of which are known as medicinal rhubarb (e.g., *Rheum officinale* B. and *Rheum palmatum* L.) and others as vegetable rhubarb [*Rheum rhabarbarum* (syn. *undulatum*) L.]. *Rheum rhaponticum* L. is used both as food and as raw material for the pharmaceutical industry.

The original importance of medicinal rhubarb has been based on its pharmaceutical applications. The dried rhizome and the root are still being used as drugs. Some of their constituents, for example, anthraquinone derivates (rheum-emodin, aloe-emodin, rhein, crysophanol, and reocrysidin), tanning agents (rhatannin), and phenylbutanone derivatives (lyndein and isolyndein) are well-known for laxative and stomach-strengthening effects. Depending on the doses they are used as antidiarrheal or purgative drugs.

The extract obtained from the rhubarb rhizome possesses a characteristic bitter taste and is used by the flavor industry for carbonated beverages, syrups, and liqueurs (*1*). The stalks of *R. rhabarbarum* L. and *R. rhaponticum* L. are consumed as vegetables and have a fresh, sour, fruit-like taste and a thirst-quenching effect. They are processed for jam, jelly, compote, and juice and used as ingredients in ice cream, yogurt, candies, and other specialities. The manufacture of jam, compote, juice, and concentrate is the major industrial application (*2*, *3*).

There are numerous reports on the nonvolatile constituents of rhubarb (*4*−*6*). The knowledge of its volatile components had been limited to the essential oils of the rhizomes (*7*−*10*). Rhubarb stalks exhibit a typical pungent aroma, which develops instantly upon peeling. This study aimed at identifying these volatile compounds by applying a vacuum headspace method for isolation. This gentle technique has been reported to produce typical flavor concentrates from various fruits representing their delicate and characteristic notes (*11*, *12*). Aroma extract dilution analysis and gas chromatography−olfactometry were applied to characterize aroma-active constituents. Special emphasis was put on the enzyme-catalyzed formation of C_6 components, which turned out to be major rhubarb stalk volatiles.

MATERIALS AND METHODS

Materials. Rhubarb stalks (*R. rhabarbarum* L.; varieties not specified) were purchased from supermarkets (June 2001) and a local farmers' market (June 2002). The material was stored (for a maximum of 5 days) at 5 °C before analysis.

Chemicals. Authentic reference chemicals were purchased from commercial sources (Aldrich, Steinheim, Germany; Merck, Darmstadt, Germany; and Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). C_6 compounds were obtained as gifts from Frey + Lau, Henstedt-Ulzburg, Germany. 4-Methylhexanoic acid was synthesized via oxidation of 4-methyl-1-hexanol with permanganate (*13*). All solvents were distilled prior to use.

Isolation of Volatiles by Vacuum Headspace Method (VHS). After the leaves had been trimmed off, the stalks were cut into small pieces (∼2 cm). Five hundred grams of the cut material was homogenized in a laboratory blender (1 min) and placed into a 2 L round-bottom flask. After addition of the internal standard, the flask was connected to the vacuum headspace apparatus described in the literature (*12*). The rhubarb sample was tempered in a water bath (Gerhardt, type SV 24) at ∼35 °C. Vacuum was applied for 3 h (1−10 mbar; Leybold-Heraus vacuum pump, type D4A). The volatiles were condensed in three

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cooling traps, which were cooled with ice−water (I and II) and liquid nitrogen (III), respectively. After 3 h, the aqueous condensates were allowed to thaw, combined, and extracted three times with 50 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v). The pooled extracts were dried over anhydrous sodium sulfate and concentrated at 40−45 °C to a final volume of 300 μL using a Vigreux column (30 cm × 2 cm i.d.).

Inhibition of Enzymes. After the cut stalks (300 g) had been homogenized for 1 min and the crushed material allowed to stand for 2 min, saturated $CaCl_2$ solution (300 mL) was added. The slurry was mixed for 10 s, and then the volatiles were isolated by VHS as described above. For comparison, the procedure was performed in the same way, except that 300 mL of water rather than $CaCl_2$ solution was added to the crushed stalks.

Capillary Gas Chromatography (HRGC). HRGC was performed using a Carlo Erba Mega II 8575 (ThermoFinnigan, C. E. Instruments, Egelsbach) equipped with a flame ionization detector (FID) and a flame photometric detector (FPD). The detector temperatures were set at 260 °C (FID) and at 140 °C (FPD). Parallel detection was achieved by dividing the effluent of the capillary column via a press-fit splitter (BGB-Analytik, Anwill, Switzerland) and short pieces of deactivated fused silica capillaries (BGB-Analytik). Injection into the column DB-Wax (60 m, 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific) was performed in the split mode at 215 °C (split ratio 1:10). Carrier gas used was hydrogen at a constant pressure of 105 kPa. The temperature program started at 40 °C (5 min hold) and was programmed at 4 °C/min to 230 °C (25 min hold). Data acquisition was done via the Chromcard software (ThermoFinnigan).

Quantification. Quantification of compounds was based on 1-octanol as internal standard (75 μg, stock solution in ethanol) taking into consideration extraction efficiences and FID responses. Recoveries by VHS were determined from 800 mL of an aqueous solution (pH 3.5) of authentic compounds (250 μg, stock solution in hexane/ethanol, 1:4, v/v). Eight-five percent of the internal standard was recovered using this procedure. FID response factors were determined with solutions (0.1 μg/μL diethyl ether) of authentic compounds relative to 2-heptanol.

Gas Chromatography—Mass Spectrometry (GC-MS). GC-MS analysis was performed using a gas chromatograph−mass spectrometer (GC 8000^{Top} with a Voyager, ThermoFinnigan). Injection into the column DB-Waxetr (30 m, 0.25 mm i.d., 0.5 μm film thickness; J&W Scientific) was performed in the split mode at 220 °C (split ratio 1:10). The temperature program started at 40 °C (5 min hold) and was programmed at 4 °C/min to 240 °C (25 min hold). Carrier gas used was helium at a constant inlet pressure of 75 kPa. Ionization energy was set at 70 eV, source temperature at 200 °C, and interface temperature at 240 °C. Data acquisition was with the MassLab system (ThermoFinnigan).

HRGC—Olfactometry (GC-O). GC-O analysis was performed using a Carlo Fractovap 4200 equipped with a FID and sniffing port. The FID was set at 235 °C. Injection into the column DB-Wax (55 m, 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific) was performed in the split mode at 215 °C. The effluent of the column was split 1:1 via a press-fit splitter and short pieces of deactivated fused silica capillaries to the detector and to the heated sniffing port. Carrier gas used was hydrogen at a constant pressure of 100 kPa. The temperature program started at 60 °C (5 min hold) and was programmed at 4 °C/min to 230 °C (25 min hold).

Aroma Extract Dilution Analysis (AEDA). Flavor dilution (FD) factors of the odorants were determined by AEDA (*14*). Aliquots of extracts obtained by VHS from 15 rhubarb samples were pooled (corresponding to 3.75 kg of rhubarb) and concentrated to a volume of 150 μL. The extract was stepwise diluted by volume 1:1 with a mixture of *n*-pentane and diethyl ether (1:1, v/v). One microliter of the dilutions was subjected to GC-O.

Odor Threshold Determinations. Odor thresholds of aroma compounds were determined as described (*15*) using a panel of 12 members. Odor-free Teflon bottles (500 mL) were used as containers for the solutions (250 mL). Each judge was presented with one bottle containing distilled water for control and four bottles of solutions (four concentrations, in descending sequence). Each concentration was repeated at least twice.

RESULTS AND DISCUSSION

Volatile constituents were isolated from freshly cut and crushed rhubarb stalks by means of a vacuum headspace technique (VHS), a procedure based on vacuum steam distillation followed by extraction of the aqueous distillate with organic solvent. Variants of this technique have been applied to isolate volatile compounds from various food materials. In this study a setup analogous to that described for the analysis of fruit flavors was used (*11*, *12*). The technique allows the isolation of volatiles without significant thermal treatment and was considered as an appropriate approach to isolate the aroma compounds of the fresh material.

The extracts obtained were analyzed by means of GC and GC-MS. **Figure 1** shows a typical gas chromatogram. Data obtained by triplicate analyses of three batches are presented in **Table 1**. A total of 78 components were identified (15 tentatively); 59 were reported for the first time in rhubarb.

The spectrum of compounds isolated from the stalks differs significantly from that reported in the essential oils of rhubarb rhizomes. Sesquiterpenes, terpenes, and phenolic derivatives are the most prominent constituents of rhubarb rhizomes from *Rhei sinensis radix* (*7*, *8*) and *Rheum palmatum* L. (*9*). Representatives of these compound classes play only minor roles in the VHS extracts obtained from stalks.

Owing to the VHS technique, the spectrum of components isolated is biased toward volatile constituents. Capillary gas chromatographic analysis of the extracts after trimethylsilylation (data not shown) revealed that major compounds, such as the fruit acids malic acid, citric acid, and oxalic acid (*3*) which may contribute to the sour-astringent flavor of rhubarb (*16*), are not isolated by the method applied. A model experiment demonstrated that oxalic acid is not recovered from an aqueous solution (pH 3.5) by the first step (distillation) of the VHS procedure.

The most striking feature of the extracts obtained by VHS is the preponderance of compounds with C_6 skeletons comprising ∼65% of the volatiles isolated. Unsaturated C_6 aldehydes [e.g., (*E*)-2-hexenal and (*Z*)-3-hexenal] and alcohols [e.g., (*E*)-2-hexenol] are the major representatives. These compounds have long been known to result from the enzyme-catalyzed oxidative degradation of unsaturated fatty acids and occur in a broad spectrum of fruits and vegetables (*17*, *18*).

In addition to the widely spread aldehydes and alcohols, substantial amounts of the less common unsaturated acids (*E*)-2-hexenoic acid and (*E*)-3-hexenoic acid were identified. (*E*)-2-Hexenoic acid has been reported in oolong and black teas (*19*), in pine sprout tea (*20*), in some fruits (*21*−*23*), and in spearmint oil (*24*). (*E*)-3-Hexenoic acid is more rare and has been described in black tea (*25*), breadfruit (*26*), and fish sauce (*27*).

(*E*)-2- and (*E*)-3-hexenoic acid had been observed as breakdown products resulting from the autoxidation of (*E*)-2-hexenal (*28*, *29*). To rule out the chemically induced formation of these acids, model experiments with aqueous solutions (pH 3.5) of authentic (*E*)-2-hexenal under the VHS conditions applied in this study were performed. Neither the unsaturated C_6 acids nor hydroxy-2-alkenals, another class of decomposition products derived from (*E*)-2-hexenal (*30*), were detected.

The spectrum of esters identified is rather limited. Representatives containing (*E*)-2- and (*E*)-3-hexenol, respectively, as alcohol moieties or (*E*)-2-hexenoic acid as acyl donor are preponderant. The origin of the phthalates remains unclear. On the one hand, they are known artifacts (*31*) and contaminants

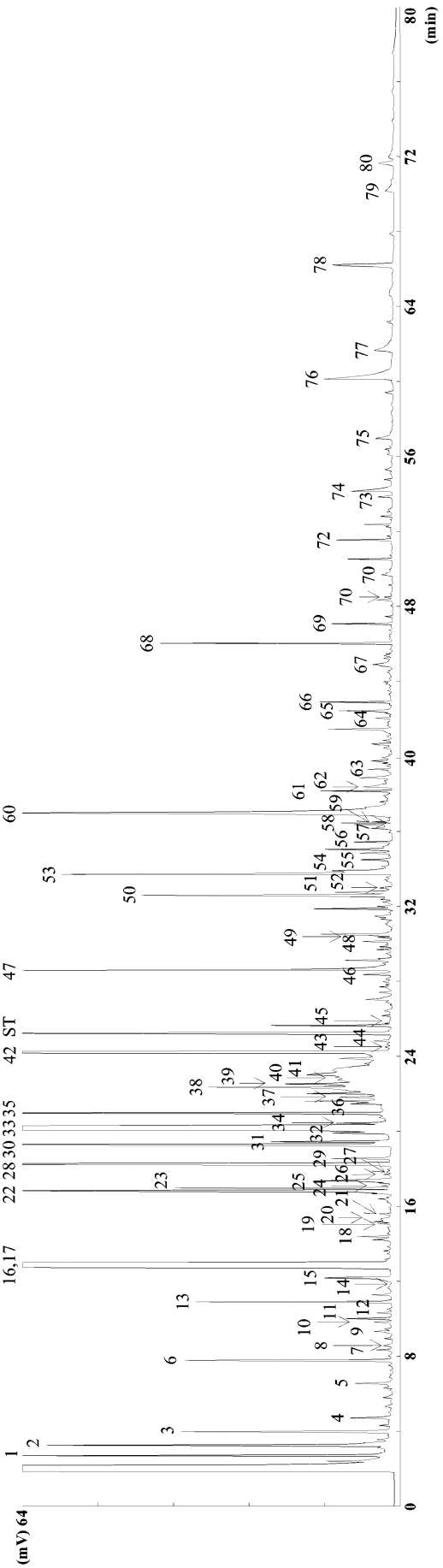

Figure 1. Typical gas chromatographic separation of volatiles isolated from rhubarb stalks by vacuum headspace technique (peak numbers correspond to **Table 1**; for GC conditions see Materials and Methods; ST = internal standard 1-octanol).

reported to result from the contact of foods with plastic materials (*32*). On the other hand, some of them had also been described in rhubarb rhizomes (*7*).

Another interesting class of volatiles identified are methyl-branched compounds. The group comprises common representatives, such as 2-methylbutanol and 2-methylbutyric acid, known to be derived from isoleucine and present in many fruits. In addition, less usual compounds such as 4-methylhexanol and the corresponding 4-methylhexanoic acid were identified. The determination of the enantiomeric compositions of these chiral constituents present in rhubarb will be described in a separate paper (*J. Agric. Food Chem.*, in press).

Sensory Evaluation. The VHS extracts possessed a "green" odor reminiscent of freshly peeled rhubarb stalks. A pooled and concentrated extract obtained from the workup of 15 rhubarb samples was used AEDA as described previously (*14*, *33*). As shown in **Table 2**, the C_6 components were among those exhibiting the highest FD factors, with (*Z*)-3-hexenal (FD = 2048) playing an outstanding role. Considering the high water content of rhubarb (>90%), odor activity values (OAV), that is, the ratios of concentrations and odor thresholds (*34*, *35*), were calculated on the basis of odor thresholds in water. This approach is limited by the fact that odor thresholds available from the literature may vary over a broad range (*36*). (*Z*)-3-Hexenal, (*E*)-2-hexenal, hexanal, and (*E*)-2-hexenol turned out to be compounds present in concentrations well above their odor thresholds and thus potential contributors to the aroma of rhubarb. Due to the low odor thresholds, the calculation of OAVs for (*E,Z*)-2,6-nonadienal and β-ionone also indicates these compounds to be of sensory importance to rhubarb flavors, although they had been detected only at low levels. For this type of trace constituents a more accurate quantification approach, for example, via isotope dilution analysis (*14*, *37*), would be required to get meaningful results.

It is noteworthy that some compounds with low OAVs, for example, hexanol, 4-methylhexanol, and hexanoic acid, exhibited high FD factors in the course of GC-O. This may be due to differences in their odor thresholds in air and water, respectively. According to the concept of aroma activity values (*34*, *35*), compounds with OAVs <1 do not contribute to the aroma. They might act as synergists (or antagonists). However, experiments involving the targeted addition or omission of single compounds or sets of compounds would be required to prove such phenomena.

Inhibition of Enzyme-Catalyzed Reactions. Aldehydes and alcohols with C_6 skeletons are typical "secondary" aroma compounds, that is, they are formed upon destroying the cell matrix from nonvolatile precursors via enzyme-catalyzed reactions (*17*, *18*). To follow the influence of enzymatic reactions on the spectrum of C_6 compounds, enzyme activities were inhibited after a total of 3 min by the addition of a saturated $CaCl_2$ solution as described for tomatoes (*38*, *39*). As shown in **Table 3**, the spectra of C_6 compounds isolated with and without inhibition of enzymes differed significantly. In the extracts obtained after inhibition of the enzymes, unsaturated aldehydes [(*Z*)-3-hexenal and (*E*)-2-hexenal] and unsaturated acids [(*E*)-3- and (*E*)-2-hexenoic acid] were the major constituents. Under noninhibiting conditions, reductions and isomerizations result in drastic changes of this initial spectrum of C_6 compounds. The activities of dehydrogenases led to significant decreases in the total amounts of acids and aldehydes and consequently to a higher proportion of alcohols. Due to isomerizations the ratios of the positional and geometric isomers change. Isomers of the

Table 1. Volatile Compounds Isolated from Rhubarb Stalks by Means of Vacuum Headspace Analysis

no.[b]	compound	KI[j] (DBWax)	concn[a] (μg/kg) batch I[c]	batch II[c]	batch III[c]	remark
	alcohols					
5	1-propanol	1040	319 ± 48	214 ± 10	179 ± 48	*d*
7	2-methyl-1-propanol	1093	5 ± 1	5 ± 0.4	4 ± 1	*d*
12	1-butanol	1145	9 ± 2	5 ± 0.1	6 ± 0.3	*d*
16	2-methyl-1-butanol	1213	358 ± 52	328 ± 53	367 ± 57	*d*
24	3-methyl-1-pentanol	1325	<1	<1	1 ± 0.1	*d*
22	4-methyl-1-pentanol	1317	30 ± 3	21 ± 2	43 ± 3	*d*
18	1-pentanol	1253	3 ± 0.2	2 ± <0.1	3 ± 0.3	*d*
9	2-pentanol	1121	3 ± 0.3	2 ± 0.3	5 ± 1	*d*
8	3-pentanol	1101	1 ± 0.4	1 ± 0.2	1 ± 0.3	*d*
13	1-penten-3-ol	1162	35 ± 4	23 ± 1	30 ± 1	*d*
23	(*Z*)-2-penten-1-ol	1322	12 ± 1	7 ± 1	12 ± 0.4	*e*
31	4-methyl-3-penten-1-ol	1390	9 ± 1	7 ± 1	7 ± 1	*f*
28	hexanol	1358	89 ± 14	63 ± 7	92 ± 6	*d*, *g*
33	(*E*)-2-hexenol	1414	1711 ± 170	1194 ± 180	1938 ± 131	*d*
34	(*Z*)-2-hexenol	1418	3 ± 0.4	2 ± 0.2	1 ± 0.3	*d*
29	(*E*)-3-hexen-1-ol	1365	10 ± 1	6 ± 0.4	12 ± 1	*d*
30	(*Z*)-3-hexen-1-ol	1385	79 ± 12	61 ± 6	327 ± 16	*d*
32	cyclohexanol	1403	70 ± 21	3 ± 0.2	8 ± 1	*d*
35	4-methyl-1-hexanol	1434	24 ± 5	18 ± 2	40 ± 3	*d*
40	2-ethyl-1-hexanol	1490	1 ± 0.1	1 ± 0.1	<1	*d*
69	hexadecanol	2383	11 ± 2	20 ± 4	7 ± 2	*d*
55	benzyl alcohol	1874	10 ± 1	8 ± 1	8 ± 0.4	*d*, *g*
56	2-phenylethanol	1910	10 ± 1	8 ± 1	7 ± 1	*d*, *g*
	aldehydes					
10	(*E*)-2-pentenal	1134	2 ± 0.1	2 ± 0.2	3 ± 0.4	*d*
6	hexanal	1077	132 ± 25	122 ± 36	163 ± 40	*d*, *g*
17	(*E*)-2-hexenal	1216	1077 ± 158	989 ± 160	1092 ± 171	*d*
15	(*Z*)-2-hexenal	1196	7 ± 2	4 ± 0.3	4 ± 1	*e*
11	(*Z*)-3-hexenal	1138	27 ± 8	29 ± 11	26 ± 6	*d*
43	(*E*)-2-nonenal	1533	<1	<1	<1	*d*
45	(*E*,*Z*)-2,6-nonadienal	1592	<1	<1	<1	*e*
41	decanal	1496	17 ± 0.4	11 ± 1	10 ± 1	*d*, *g*
	esters					
1	ethyl formate	814	18 ± 1	16 ± 1	19 ± 2	*d*, *g*
2	ethyl acetate	856	20 ± 1	17 ± 2	22 ± 2	*d*
19	hexyl acetate	1271	1 ± 0.1	<1	2 ± 0.3	*d*
20	2-methylbutyl 2-methylbutanoate	1276	<1	<1	<1	*e*
21	methyl (*E*)-2-hexenoate	1284	2 ± 0.4	1 ± 0.1	1 ± 0.1	*d*
27	ethyl (*E*)-2-hexenoate	1345	<1	<1	<1	*d*
25	(*E*)-2-hexenyl acetate	1333	4 ± 1	3 ± 0.4	13 ± 0.3	*d*
38	(*E*)-2-hexenyl butanoate	1475	17 ± 2	16 ± 3	9 ± 1	*d*
46	(*E*)-2-hexenyl hexanoate	1663	3 ± 0.2	3 ± 1	1 ± 0.3	*d*
37	(*E*)-3-hexenyl butanoate	1452	2 ± 0.3	1 ± 0.1	3 ± 0.2	*e*
50	(*E*)-2-hexenyl (*E*)-2-hexenoate	1805	9 ± 5	4 ± 2	6 ± 5	*e*
62	isopropyl myristate	2040	<1	<1	3 ± 1	*d*
72	diisobutyl phthalate	2592	4 ± 0.4	3 ± 1	2 ± 0.3	*f*, *h*
73	dibutyl phthalate	2693	4 ± 1	2 ± 0.2	1 ± 0.3	*d*, *h*
80	di(2-ethylhexyl) phthalate	3160	56 ± 5	119 ± 5	74 ± 4	*d*
	ketones					
4	3-pentanone	956	6 ± 1	3 ± 0.4	6 ± 0.2	*e*
27	6-methyl-5-hepten-2-one	1341	<1	<1	<1	*e*
54	(*E*)-geranylacetone	1853	1 ± 0.4	2 ± <0.1	2 ± 0.1	*d*
39	2,6,6-trimethyl-2-vinyltetrahydropyran-3-one	1478	4 ± 0.3	3 ± 0.1	4 ± 0.3	*f*
	acids					
36	acetic acid	1449	14 ± 3	9 ± 1	18 ± 3	*d*
47	2-methylbutanoic acid	1670	43 ± 19	96 ± 26	250 ± 29	*d*
51	4-methylpentanoic acid	1813	15 ± 1	14 ± 1	9 ± 0.3	*d*
53	hexanoic acid	1846	33 ± 13	54 ± 1	77 ± 3	*d*, *g*
57	4-methylhexanoic acid	1936	1 ± 0.2	1 ± 0.1	2 ± 0.2	*d*
60	(*E*)-2-hexenoic acid	1967	142 ± 69	351 ± 48	574 ± 73	*d*
59	(*E*)-3-hexenoic acid	1948	29 ± 4	17 ± 2	31 ± 8	*d*
63	octanoic acid	2056	3 ± 0.2	1 ± 0.3	3 ± 0.4	*d*, *g*
64	nonanoic acid	2165	2 ± 0.2	2 ± 1	1 ± 0.1	*d*, *g*, *h*
67	decanoic acid	2286	2 ± 0.4	2 ± 0.4	1 ± 0.1	*d*, *g*, *h*
71	dodecanoic acid	2502	2 ± 0.2	2 ± 0.1	2 ± 0.1	*d*, *g*
74	tetradecanoic acid	2706	19 ± 5	38 ± 9	26 ± 1	*d*, *g*
75	pentadecanoic acid	2819	38 ± 8	52 ± 7	42 ± 2	*d*, *g*

Table 1. (Continued)

no.[b]	compound	KI[i] (DBWax)	concn[a] (μg/kg) batch I[c]	batch II[c]	batch III[c]	remark
	acids (continued)					
76	hexadecanoic acid	2913	196 ± 41	340 ± 62	241 ± 19	*d*, *g*
77	9-hexadecenoic acid	2957	1 ± 0.2	3 ± 1	2 ± 0.1	*f*
76	octadecanoic acid	3132	2 ± 0.3	3 ± 1	3 ± 0.4	*d*
	others					
14	limonene	1192	2 ± 0.4	1 ± 0.4	6 ± 1	*d*
44	linalool	1548	<1	<1	2 ± 0.3	*d*
65	sesquiterpene (MW 204)	2180	3 ± 1	5 ± 1	1 ± 0.1	*f*
66	sesquiterpene (MW 204)	2201	2 ± 0.4	7 ± 3	1 ± 0.1	*f*
78	squalene	3058	136 ± 28	276 ± 128	141 ± 63	*d*
58	β-ionone	1943	<1	<1	<1	*d*
52	anethole	1820	1 ± <0.1	1 ± 0.1	1 ± 0.2	*d*, *g*
68	*p*-allylphenol	2338	14 ± 0.2	12 ± 1	6 ± 1	*e*
61	methyleugenol	2013	7 ± 1	3 ± 1	2 ± 0.4	*d*, *g*
48	1,2-dimethoxybenzene	1721	1 ± <0.1	2 ± 0.1	2 ± 0.1	*d*
49	1,4-dimethoxybenzene	1728	2 ± 0.2	1 ± <0.1	<1	*d*
70	indole	2336	2 ± 0.2	2 ± 0.3	3 ± 1	*d*

[a] Data from triplicate experiments for each batch; mean ± standard error. [b] Numbers correspond to chromatogram shown in **Figure 1**. Peaks 3 (ethanol used as solvent for the internal standard) and 42 (an unidentified and nonreproducible artifact) are not listed in this table. [c] Material from 2001. [d] Identification based on comparison of mass spectral and GC data with those of authentic reference compounds. [e] Tentatively identified on the basis of comparison of mass spectral and GC data with those from the literature. [f] Tentatively identified on the basis of comparison of mass spectral data with those from the literature. [g,h] Compounds reported in rhubarb rhizomes in refs *9* and *7*, respectively. [i] Kovats retention indices.

Table 2. Concentrations and Odor Activity Values (OAV) of Key Odorants of Rhubarb Stalks[a]

no.[b]	odorant	odor quality	FD factor	odor threshold (μg/L in water)	concn (μg/kg)	OAV[c]
6	hexanal	green, grassy	256	4.5 [d]	76	17
11	(*Z*)-3-hexenal	green, grassy	2048	0.25 [d]	11	44
13	1-penten-3-ol	green	4	400 [d]	24	0.1
16	2-methyl-1-butanol	bitter, medicinal, musty	16	320 [e]	304	1
17	(*E*)-2-hexenal	green, grassy, apple, bitter almond	256	17 [d]	916	54
28	hexanol	fruity, citrus-like, green	256	2500 [f]	74	0.03
30	(*Z*)-3-hexen-1-ol	green, grassy	512	70 [d]	139	2
33	(*E*)-2-hexen-1-ol	fruity, green	16	100 [g]	1241	12
35	4-methyl-1-hexanol	green, nutty, roasty, oily, carrot-like	128	2000	30	0.02
41	decanal	carrot-like, citrus-like	16	2 [f]	6	3
43	(*E*)-2-nonenal	carrot-like, fatty	256	0.08 [f]	0.4	5
44	linalool	citrus-like, lemon, sweety	128	6 [d]	0.6	0.1
45	(*E*,*Z*)-2,6-nonadienal	cucumber, floral	256	0.01 [e]	0.4	40
47	2-methylbutanoic acid	pungent, cheese, acidic	64	540 [e]	187	0.3
53	hexanoic acid	pungent, acidic	32	3000 [d]	56	0.02
56	2-phenylethanol	bitter floral, chamomile-like	32	1100 [d]	11	0.01
58	β-ionone	sweet, floral, violet	256	0.007 [d]	0.3	43
60	(*E*)-2-hexenoic acid	fruity, sour	8	300	516	1.7

[a] Data relate to material from 2001; GC-O and AEDA were performed using a concentrated VHS extract corresponding to 3.75 kg of rhubarb (see Materials and Methods). [b] Numbers correspond to the chromatogram shown in **Figure 1**. [c] OAVs were calculated by dividing the concentrations by the odor thresholds. Odor thresholds were taken from the literature. [d] Reference *40*. [e] Reference *36*. [f] Reference *41*. [g] Reference *42*.

remaining unsaturated acids are shifted in favor of (*E*)-2-hexenoic acid, (*E*)-2 hexenal as intermediate, and (*E*)-2-hexenol as final reduction product.

To have comparable conditions in the experiments performed to investigate the influence of enzyme activities, equal amounts of $CaCl_2$ solution and water, respectively, were added to the rhubarb material before isolation via VHS. As shown in **Table 3**, the ratios of (*E*)-2-hexenal to (*E*)-2-hexenol detected in the noninhibited samples (addition of water) turned out to be different from those obtained under noninhibiting conditions without addition of water (comparable to the conditions for **Table 1**). Apparently, the activities of dehydrogenases are influenced by the presence/absence of water. This confirms the importance of having identical external conditions if spectra of "secondary" volatiles are to be compared.

The effects of enzyme-catalyzed changes of the initial spectrum of C_6 components on OAVs are demonstrated in **Table 4**. Owing to the high concentration of (*Z*)-3-hexenal and its low odor threshold, the sensory properties of the extract obtained after crushing of the rhubarb stalks and allowing enzymatic reactions for 3 min are dominated by this aldehyde. If subsequent enzymatic reactions are not inhibited, the importance of this component decreases and the reduction/isomerization products (*E*)-2-hexenal and (*E*)-2-hexenol eventually play major sensory roles.

In conclusion, the investigations revealed rhubarb to be another example of a plant system the aroma of which is strongly influenced by C_6 compounds. The initially formed spectrum of C_6 aldehydes, alcohols, and unsaturated acids is subject to substantial changes due to ongoing enzyme-catalyzed reactions.

Table 3. Concentrations and Percentage Distributions of C_6 Alcohols, Aldehydes, and Acids Determined in Rhubarb Stalks by VHS with and without Inhibition of Enzymes

	batch IV[a]					
			noninhibited			
	inhibited		with water		without water	
compound	μg/kg	%	μg/kg	%	μg/kg	%
aldehydes						
hexanal	217 ± 9	4.8	323 ± 27	8.6	131 ± 16	4.4
(*Z*)-3-hexenal	1785 ± 457	39.8	31 ± 3	0.8	38 ± 9	1.3
(*Z*)-2-hexenal	18 ± 2	0.4	8 ± 3	0.2	5 ± 2	0.2
(*E*)-2-hexenal	1484 ± 50	33.1	2077 ± 74	55.0	922 ± 106	31.0
alcohols						
hexanol	8 ± 1	0.2	39 ± 2	1.0	78 ± 9	2.6
(*Z*)-3-hexenol	24 ± 2	0.5	32 ± 1	0.8	59 ± 1	2.0
(*E*)-3-hexenol	<1	0.02	3 ± 0.1	0.1	6 ± 0.1	0.2
(*E*)-2-hexenol	215 ± 31	4.8	890 ± 21	23.6	1412 ± 46	47.5
acids						
hexanoic acid	39 ± 5	0.9	53 ± 8	1.4	53 ± 8	1.8
(*E*)-3-hexenoic acid	225 ± 13	5.0	36 ± 7	1.0	28 ± 7	0.9
(*E*)-2-hexenoic acid	466 ± 49	10.4	282 ± 47	7.5	239 ± 53	8.0

[a] Material from 2002. [b] Data from triplicate experiments; mean ± standard error.

Table 4. Odor Activity Values (OAV) of Selected C_6 Compounds in a VHS Extract Obtained with and without Inhibition of Enzyme Activities

	OAV[a]	
compound	inhibited (after 3 min)	noninhibited
hexanal	48	72
(*Z*)-3-hexenal	7140	124
(*E*)-2-hexenal	87	122
hexanol	0.003	0.02
(*E*)-2-hexenol	0.3	0.6
(*Z*)-3-hexenol	2.2	8.9
(*E*)-3-hexenoic acid[b]	0.4	0.06
(*E*)-2-hexenoic acid	1.6	0.9

[a] OAVs were calculated by dividing the concentrations determined in batch IV (**Table 3**) by the odor thresholds (**Table 2**). [b] Odor threshold in water = 600 μg/L.

This may explain the fact that the very distinct aroma impression perceivable immediately upon slicing and cutting of rhubarb stalks deteriorates rather quickly. The sensory roles of the C_6 compounds indicated by this study will have to be evaluated in reconstitution experiments using aqueous solutions of nonvolatile rhubarb constituents (especially the fruit acids) as base.

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