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Odor Thresholds of Microbially Induced Off-Flavor Compounds in Apple Juice

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Microbially derived off-flavor is a major problem in apple juice production as it diminishes the sensory quality of the juice significantly. Fifteen relevant off-flavor compounds that are formed in apple juice, for example, by the strains *Alicyclobacillus acidoterrestris* and *Actinomycetes* (*Streptomyces* ssp.) were investigated with respect to their sensory relevance. The odor threshold values (i.e., detection and recognition values) were determined for all compounds in the matrix apple juice. Odor threshold values for fenchyl alcohol are reported here for the first time. The obtained values were set in relation to the limits of detection and quantification of a previously published GC-MS method. Eight tainted apple juice samples were analyzed for the presence of the 2 strains and the 15 off-flavor compounds. Both strains could be found in the samples; the presence of *Streptomyces* ssp. as spoilage bacteria of apple juice is reported for the first time. In samples with distinct off-flavor, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 2-methylisoborneol, 1-octen-3-ol, fenchyl alcohol, geosmin, and guaiacol as well as 2,6-dibromophenol were determined in concentrations higher than the detection threshold.

KEYWORDS: Apple juice; off-flavor; odor threshold values; *Alicyclobacillus acidoterrestris*; *Actinomycetes*

INTRODUCTION

Off-flavor in apple juice is a frequently observed problem in fruit juice production. As in any other substrate, the reasons for off-flavor formation may be manifold. Microbial contamination and negative injury of the product's sensory properties caused by odor-active metabolites of the involved microorganisms play major roles in fruit juices (*2*, *8*).

Molds, yeasts, and bacteria can cause spoilage as well as sensory defects of fruit juices. Acetic acid and lactic acid bacteria are found most frequently in fruit juices, but spore formers such as *Bacillus*, *Alicyclobacillus*, and *Clostridium* are also found. Among them are species that are very resistant against high temperatures and low pH values. As a result, they may even survive the pasteurization process in the acidic environment of fruit juices (*9*, *10*). Due to their behavior, they are frequently called "thermoacidophilic" bacteria (TAB). In addition, some of them are very modest and do not claim high requirements of the environment to grow. They may regerminate, grow in the shelf-stable product, and cause a distinct off-flavor after a certain storage period of the product on the shelf (*24*).

The best studied among these bacteria is *Alicyclobacillus acidoterrestris*. Its occurrence in apple juice as producer of a medicinal−phenolic off-flavor was described for the first time in 1984 (*1*) and several times afterward (*2*−*7*). 2,6-Dibromophenol and guaiacol are reported to be the compounds responsible for this distinct off-flavor (*4*, *6*). Recent studies of our group indicate that an *Actinomycetes* strain (*Streptomyces griseus griseus*), which was described previously to cause musty and earthy off-flavor in soil and various kinds of foods (*8*−*11*), may also show thermoacidophilic behavior and may consequently be the reason for musty−earthy notes in apple juice (*12*). The list of metabolites responsible for the sensory defects is much longer than that from *Alicyclobacillus*. Representatives thereof are, for example, geosmin and 2-methylisoborneol as well as 1-octen-3-ol. The most important metabolites of both strains can be seen in **Table 1**.

The analytical determination of flavor and off-flavor compounds can be carried out using different sample extraction techniques. The most frequently used method for the detection, identification, and quantification of the compounds is gas chromatography with different detection systems (*13*). Recently, our group published a sensitive and fully validated method for metabolites of *A. acidoterrestris* and *S. griseus griseus* using headspace solid-phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS) (*14*). Nevertheless, as for most off-flavor compounds, the odor thresholds are extremely low, and the efficiency of an analytical method can be judged only when it is set in relation to the respective odor thresholds.

It is well-known that odor thresholds are very much dependent on the matrix in which the compounds occur. Odor thresholds

Table 1. Odor Descriptors and Odor Thresholds from the Literature for the Compounds of Interest

compound	formed by	odor descriptors[a]	previously reported odor threshold (μg L^{-1})
m-anisaldehyde	*Streptomyces* ssp.	musty, moldy, leather, medicinal, sweet, floral	50–200[b]
o-anisaldehyde	*Streptomyces* ssp.	medicinal, pungent, sweet, chemical, floral	50–200[b]
p-anisaldehyde	*Streptomyces* ssp.	marzipan, sweet, pungent,	50–200[b]
α-terpineol	*Streptomyces* ssp.	lilac, fragrant, pungent	4.6–350[b]
2-isobutyl-3-methoxypyrazine	*Streptomyces* ssp.	green pepper, green, acrid, parsley, cut grass	0.002–10[b]
2-isopropyl-3-methoxypyrazine	*Streptomyces* ssp.	earthy, potato, green pepper, pea, acrid, green	0.002–10[b]
2,3-dimethylpyrazine	*Streptomyces* ssp.	peanut, nutty, roasty, fatty, aromatic	400–2500[b]
[(1*S*)-*endo*]-(–)-borneol	*Streptomyces* ssp.	sweet, menthol, pungent	140[b]
2-methylisoborneol	*Streptomyces* ssp.	earthy, humid, moldy, cellar-like, forest-earth	0.002–0.1[b]
1-octen-3-ol	*Streptomyces* ssp.	mushrooms, varnish, earthy	0.005–100[b]
3-octanone	*Streptomyces* ssp.	stale, moldy, old, slightly fruity, sweet, pear-like, candy-like, "cooked"	28–50[b]
fenchyl alcohol	*Streptomyces* ssp.	earthy, humid, pungent, menthol-like, pine tree, detergent	
geosmin	*Streptomyces* ssp.	musty, moldy, cellar-like, sweetish, pungent, red beet	0.01–0.36[b]
guaiacol	*Alicyclobacillus acidoterrestris*	medicinal, sweet, chemical, medical office	0.91–2.0[c]
2,6-dibromophenol	*Alicyclobacillus acidoterrestris*	smoky, pungent, medicinal, dental office	0.0005[d]

[a] The descriptors were acquired with the panel in the training phase (stage 2). Descriptors are listed in decreasing number of entries given by the panelists. [b] Odor threshold in water (*23*). [c] Odor threshold in apple juice (*15*–*17*). [d] Odor threshold in water (*4*).

for microbially induced off-flavor compounds in apple juice can scarcely be found in the literature. The only values described are thresholds for guaiacol [i.e., 2 μg L^{-1} of apple juice (*15*, *16*) and 0.91 μg L^{-1} of apple juice (*17*)]. For all other compounds of interest only odor thresholds in water are available. As a consequence, it was the aim of this study to determine the odor thresholds in apple juice for all compounds and to compare them to the limits of detection and determination obtained by the analytical method. Thus, the efficiency of the analytical method used can be verified and judged in which cases SPME GC-MS analysis is sufficiently sensitive. Furthermore, eight apple juice samples showing distinct off-flavor were analyzed for the presence of the strains of interest and for quantitative data of the described off-flavor compounds to demonstrate the applicability of the methods.

MATERIALS AND METHODS

Chemicals and Solvents Used. 2,3-Dimethylpyrazine (95%+), [(1S)-*endo*]-(−)-borneol (99%), *m*-anisaldehyde (97%), *p*-anisaldehyde (>98% purum), *o*-anisaldehyde (>98% purum), 1-octen-3-ol (98%), 3-octanone (>97% purum), fenchyl alcohol (97%), 3-isopropyl-2-methoxypyrazine (97%), α-terpineol (96%+), guaiacol (98%), and 2,6-dibromophenol (99%) were purchased from Sigma-Aldrich, Steinheim, Germany. Geosmin (98.7%), 2-methylisoborneol (99%), and 2-isobutyl-3-methoxypyrazine (99%) were purchased from Supelco, Bellefonte, PA. Ethanol (p.a. quality) was purchased from Merck, Vienna, Austria. Methanol (picograde, for residue analysis) and sodium sulfate (granular, anhydrous resin for residue analysis) were purchased from Promochem, Wesel, Germany. Stock solutions of the reference compounds (≈200 mg L^{-1}) were checked for the presence of other possibly odor-active compounds by GC-MS (liquid injection). Impurities of >0.5% related to the compound of interest were checked for odor activity by gas chromatography−olfactometry.

Juice Samples. The apple juice for the determination of the threshold values was a commercially available juice from concentrate. For all experiments the same brand was used. pH was controlled (pH 3.6−4.0). The used apple juices were tested by sensory evaluation by three trained panelists prior to the experiments. Only juices without any detectable off-flavor were used.

Eight tainted apple juice samples (juices A−H) were analyzed. The samples were purchased at local markets and analyzed as off-flavor and/or a noticeable turbidity in the juice was observed. A description of the sensory properties of the samples is given in **Table 3**.

Training of the Sensory Test Panel. The sensory test panel consisted of 16 fairly trained panelists (25−46 years, 12 females, 4 males). Their ability to recognize the basic tastes sweet, sour, salty, and bitter were tested as a basic requirement to act as panelist within this project (test procedure according to refs *18*, *19*, and *21*).

The specific training on microbially induced off-flavors was divided into three stages over a period of 6 months with weekly training sessions. In stage 1, strips made of filter paper were dipped into ethanolic solutions (1 vol %) of the off-flavor compounds of interest (15 compounds, 1 compound per solution). The panelists were asked to smell the strips and to describe their olfactory impressions. The obtained descriptors were collected and discussed with the panel. Olfactory training sessions were repeated several times to guarantee that the panelists would recognize and describe the odors reproducibly. In stage 2, off-flavor-free apple juice was spiked with clearly noticeable amounts of the off-flavor compounds. Panelists were asked to smell and to taste the samples to get familiarized with the compounds in the apple juice matrix. Furthermore, they were asked to record their sensory impressions. Differences between the descriptors obtained from the compounds in ethanolic solution presented on strips and in the juice were discussed with the panel. In stage 3, the panelists were asked to perform triangle tests containing two "clean" apple juice samples and one sample that was spiked with one off-flavor compound. The off-flavor compounds were added in the following concentrations: 2-methylisoborneol, 0.1 μg L^{-1}; 2,6-dibromophenol, 0.1 μg L^{-1}; geosmin, 0.1 μg L^{-1}; guaiacol, 5 μg L^{-1}. In addition, ranking tests with one off-flavor compound per ranking test in increasing concentration were performed. The off-flavor compounds were added in the following concentration steps: 1-octen-3-ol, 2-isobutyl-3-methoxypyrazine, and 3-isopropyl-2-methoxypyrazine, 0, 5, 10, 15, and 20 μg L^{-1}; guaiacol, 0.6, 1.8, 5.3, 16, and 48 μg L^{-1} (*15*); 2,3-dimethylpyrazine, 50, 100, 150, 200, and 250 μg L^{-1}. Results from the triangle and ranking tests were used to verify the qualification of the panelists for the described problem. For this purpose sequential analysis was used (*20*).

Odor Threshold Determination. The odor thresholds of the compounds of interest in the matrix apple juice were determined in terms of the so-called "best estimate threshold (BET)" for each panelist as well as the group BET for the whole panel according to refs *22* and *25*. To determine the odor threshold of one compound, a series of five three-alternative forced choice (3-AFC) tests were presented to the panelists at one time. Each triangle test contained two off-flavor-free samples and one apple juice sample spiked with the compound of interest. According to Meilgaard (*22*), the concentration of the off-flavor compound increased stepwise with each triangle test. The panelists were asked to identify the differing sample for each triangle.

Table 2. Odor Thresholds for the Compounds of Interest Given in Terms of the Group BET Compared to the Limits of Detection and Quantification of the Discussed SPME GC-MS Method

compound	detection threshold[a] in apple juice (μg L^{-1})	SD[b] (μg L^{-1})	RSD[c] (%)	recognition threshold[d] in apple juice (μg L^{-1})	SD[b] (μg L^{-1})	RSD[c] (%)	LOD[e] (μg L^{-1})	LOQ[f] (μg L^{-1})
m-anisaldehyde	174	20	11	>250[g]			1.01	3.21
o-anisaldehyde	181	29	16	>250[g]			0.45	1.57
p-anisaldehyde	139	31	22	226	5	2	0.41	1.41
α-terpineol	314	191	60	483	39	8	0.31	1.11
2-isobutyl-3-methoxypyrazine	0.0006	0.0002	33	0.0033	0.0005	15	0.63	2.21
2-isopropyl-3-methoxypyrazine	0.0003	0.00017	55	0.0006	0.0004	66	0.66	2.2
2,3-dimethylpyrazine	123	5	4	>140[g]			7.73	25.4
[(1*S*)-*endo*]-(–)-borneol	48	8	16	67	4	6	1.18	3.73
2-methylisoborneol	0.017	0.01	59	0.033	0.005	15	0.67	2.24
1-octen-3-ol	11	3	27	31	11	35	1.09	3.48
3-octanone	57	2	4	>100[g]			0.93	3.02
fenchyl alcohol	2.1	1.5	71	3.2	0.5	16	0.52	1.78
geosmin	0.023	0.017	74	0.027	0.007	26	0.34	1.21
guaiacol	0.57	0.09	16	2	0.32	16	0.29	1.06
2,6-dibromophenol	0.009	0.003	33	0.085	0.015	17	0.08	0.27

[a] Values are given in terms of group BET. The value for the detection threshold is the level at which the differing sample is selected correctly by the panel without being able to describe the sensory properties of the off-flavor compound. [b] Standard deviation; calculated from all BET, $n = 20–35$. [c] Relative standard deviation. [d] Values are given in terms of group BET. The value for the recognition threshold is the level at which the off-flavor sample is selected correctly by the panel and the sensory defects were described properly. [e] Limit of detection for the discussed SPME GC-MS method (*14*). [f] Limit of determination for the discussed SPME GC-MS method (*14*). [g] The recognition threshold was higher than the highest concentration used in the experiments.

Table 3. Results from Eight Tainted Apple Juices: +, Strain Was Identified in the Juice; –, Strain Was Not Identified in the Juice; Concentrations Given in Micrograms per Liter, $n = 3$

	juice A [a]	juice B [b]	juice C [c]	juice D [d]	juice E [e]	juice F[f]	juice G[g]	juice H[h]
Alicyclobacillus acidoterrestris [a,b]	+	–	–	–	+	+	+	+
Streptomyces griseus griseus [a,b]	–	+	+	+	–	+	+	+
m-anisaldehyde	nd	19.6	25.7	9.4	nd[i]	25.5	2.5	7.5
o-anisaldehyde	nd	12.4	4.4	2.5	nd	7.7	12.3	2.3
p-anisaldehyde	nd	0.9[j]	0.9[j]	1.8	nd	nd	2.1	nd
α-terpineol	nd	24.8	6.1	22.8	nd	27.6	16.6	16.9
2-isobutyl-3-methoxypyrazine	nd	10.5*	14.2*	5.5*	nd	1.4[j]*	1.4[j]*	1.4[j]*
2-isopropyl-3-methoxypyrazine	nd	6.5*	15.9*	22.9*	nd	3.3*	3.3*	4.9*
2,3-dimethylpyrazine	nd	nd	16.6[j]	40.7	n.d	16.6[j]	16.6[j]	16.6[j]
[(1*S*)-*endo*]-(–)-borneol	nd	2.5[j]	nd	2.5[j]	nd	2.5[j]	2.5[j]	2.5[j]
2-methylisoborneol	nd	3.4*	5.4*	8.4*	nd	3.4*	3.3*	3.3*
1-octen-3-ol	nd	26.6*	30.2*	4.9	nd	2.3[j]	23.1*	16.7*
3-octanone	nd	6.3	3.0	5.3	nd	5.4	4.5	2.0[j]
fenchyl alcohol	nd	3.3*	2.3*	3.1*	nd	3.6*	2.8*	3.1*
geosmin	nd	14.7*	18.5*	31.2*	nd	0.8[j]*	0.8[j]*	0.8[j]*
guaiacol	1.5*	nd	nd	nd	5.7*	1.3*	0.7[j]*	0.7[j]*
2,6-dibromophenol	nd	nd	nd	nd	1.2*	nd	nd	nd

[a] Pasteurized juice from concentrate, medicinal pungent flavor, white turbidity, [b] Pasteurized juice from concentrate, slightly musty. [c] Pasteurized juice, musty flavor, turbid. [d] Unpasteurized juice from a rural market, moldy flavor. [e] Pasteurized juice from concentratrate, white turbidity, medicinal off-flavor. [f] Unpasteurized juice from a rural market, strong moldy flavor. [g] Pasteurized juice from concentrate, musty flavor. [h] Unpasteurized juice from a rural market, moldy off-flavor. [i] Not detectable. [j] Compound was identified undoubtedly. The concentration was lower than the LOQ. The concentrations were set to (LOD + LOQ)/2. *Concentrations are higher than the detection threshold.

In addition, they were asked to describe the noticed difference in order to obtain information about differences between detection and recognition threshold levels. In every test session only one series of five 3-AFC tests was presented to the panelists. For each compound the test procedure was repeated at least twice with the whole panel. In the case of significant deviations between the two determinations, a third repetition was performed. This procedure resulted in 20−35 BET values for each compound as calculation basis for the group BET.

The BET for single panelists was calculated as the geometric mean from the highest concentration that was not recognized as differing from the others and the lowest concentration that was identified as differing from the other samples in the triangle. The group BET was calculated as the arithmetic mean from all BET values.

Instrumental Determination of the Compounds. The instrumental determination of the off-flavor compounds was performed by GC-MS after headspace SPME. For the SPME, a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) Stable Flex fiber (length = 2 cm, Supelco) was used. Na_2SO_4 (2.5 g) was added to 0.5 mL of apple juice diluted with 4.5 mL of water. The samples were equilibrated for 5 or 10 min at 60 °C while the sample was thoroughly stirred. The fiber was then exposed into the headspace of the sample at 60 °C for 10 or 30 min, respectively. Afterward, the fiber was transferred immediately into the injection port of the GC for thermal desorption.

For the GC-MS measurements a Hewlett-Packard system (HP G1800A GCD system) was used. SPME sampling was performed using a CombiPAL Multi sampler from CTC Analytics, Zwingen, Switzerland. The capillary column used was an HP5 (cross-linked 5% phenyl methyl siloxane; column length = 30 m, inner diameter = 0.25 mm, film thickness = 1 μm). Helium with a purity of 99.999% (Air Liquide, Schwechat, Austria) was used as carrier gas. The conditions were as follows: column head pressure, 0.54 bar; starting temperature, 10 °C (hold time of 1 min); constant pressure (gas flow at 10 °C, 39.8 cm s^{-1}); different temperature rates, 8 or 10 °C min^{-1}; final temperature,

250 °C. Temperatures below 45 °C were controlled by blowing liquid nitrogen into the GC oven. Splitless injection mode was used, the split valve being opened after 2 min. A special SPME glass liner with a constant inner diameter of 0.75 mm (Supelco) was used. Injector temperature was 270 °C, and detector temperature was 280 °C. Electron impact ionization was used (70 eV); data were acquired in the selected ion mode. Quantification of the compounds was performed using adequate internal standards. Further details on the method and method validation are given in ref *14*.

Identification of the Bacteria Strains. For the identification of the microorganisms, aliquots of the juice samples were plated out on solid culture media. The composition of the media was specifically chosen for the growth of *A. acidoterrestris* (medium 402, DSMZ, Braunschweig, Germany) and *S. griseus griseus* (medium 65, DSMZ). The agar plates were incubated at optimum growing temperatures for the two strains (i.e., 45 °C for *A. acidoterrestris* and 30 °C for *Streptomyces* ssp.). When colonies became visible, liquid culture media were inoculated with these colonies. After accumulation in the liquid media, the appearance and size of bacteria cells were controlled by light optical microscopy.

RESULTS AND DISCUSSION

In most cases, odor thresholds that can be found in the literature show very large variations, up to several orders of magnitude for one compound in one matrix (*23*). This holds also true for the previously reported odor thresholds in water of the investigated compounds (see **Table 1**). To be able to interpret the applicability of the analytical method, we aimed to obtain odor thresholds with deviations as small as possible. As a consequence, in this study special emphasis was put on the training of the sensory panel. Not only was basic training of the panelists performed, but intense training on the off-flavor topic was undertaken. The final sensory tests in stage 3 of the training phase (i.e., triangle and ranking tests) were evaluated by sequential analysis (*20*). Results from sequential analysis showed that all panelists that took part in the training phase could be accepted. For the threshold determination the whole panel was used, as long as the panelists were available.

Table 1 gives the complete list of investigated compounds formed by the two strains *A. acidoterrestris* and *Streptomyces* ssp. The listings of odor descriptors are results from the training phase. All descriptors that were given by the panelists were collected and discussed in group sessions. In **Table 1** the descriptors are listed in decreasing frequency of nominations by the panel members. At the end of the training phase the panelists were familiar and satisfied with the descriptors. When the off-flavor compounds were presented to the panel either on filter strips or in apple juice in clearly noticeable concentrations, they were able to recognize the odors and to describe them properly. Great importance was assigned to this fact in order to be able not only to determine precise data concerning the detection threshold but also to receive information about the recognition threshold. **Table 1** also shows odor threshold values of the compounds found in the literature. Odor thresholds in apple juice could be found only for guaiacol [0.91−2.0 μg L^{-1} (*15*−*17*)]. For all other compounds thresholds are given in the water matrix (*23*). For fenchyl alcohol—in apple juice described by the panel as earthy, humid, and pungent (see **Table 1** for all given descriptors)—no odor threshold value in any liquid matrix could be found in the literature.

Table 2 gives the odor thresholds for the investigated compounds. For most compounds both values—the detection threshold as well as the recognition threshold—could be determined. For some compounds (i.e., *m*-anisaldehyde, *o*-anisaldehyde, 2,3-dimethylpyrazine, and 3-octanone) the recognition threshold could not be determined. For these compounds the panelists were able to select the differing sample in the 3-AFC tests reproducibly, but they were not able to recognize and describe the related off-flavor within the investigated concentration range.

As mentioned earlier, guaiacol is the only compound of which odor thresholds for the apple juice matrix were reported in previous papers. With our sensory test panel, we determined a detection threshold in the juice of 0.57 μg L^{-1}. This value correlates well with the data reported by other groups [0.91−2.0 μg L^{-1} apple juice) (*15*−*17*)] and can consequently be regarded as a tool to cross-check the performance of the sensory test panel and the validity of the obtained results.

Approaching the experiments of this study, we expected that due to the rather intense genuine flavor of apple juice, the odor thresholds in the juice would be higher than those reported for the water matrix. After a closer look at the detection thresholds, this holds true for 2,6-dibromophenol only. The detection threshold in apple juice determined in our study is ≈1 order of magnitude higher than the threshold in water reported previously. For the compounds *o*-, *m*-, and *p*-anisaldehyde, α-terpineol, 2-methylisoborneol, 1-octen-3-ol, 3-octanone, and geosmin, our detection threshold values in apple juice are in the same concentration range as the previously reported values for the water marix. For the compounds 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, and 2,3-dimethylpyrazine as well as for [(1*S*)-*endo*]-(−)-borneol, the odor thresholds in apple juice are even significantly lower than those reported for the water matrix previously. We suppose that the long training phase and the intense discussion of the sensory properties of the compounds with the panelists resulted in the high sensitivity to the compounds. For fenchyl alcohol, we determined a detection threshold in apple juice of 2.11 μg L^{-1}. Odor thresholds (detection and recognition threshold) for fenchyl alcohol are reported here for the first time.

Regarding the limits of variation of the reported odor thresholds, it can be seen that for 10 compounds [*o*-, *m*-, and *p*-anisaldehyde, 2-isobutyl-3-methoxypyrazine, 2,3-dimethylpyrazine, [(1*S*)-*endo*]-(−)-borneol, 1-octen-3-ol, 3-octanone, guaiacol, and 2,6-dibromophenol] the standard deviations are rather low (i.e., relative standard deviations from ±4% for 2,3-dimethylpyrazine to ±33% for 2-isobutyl-3-methoxypyrazine, which can be regarded as sufficiently accurate for results from sensory analysis). For the remaining five compounds, the relative standard deviations for the detection threshold values are much higher (from ±59% for 2-methylisoborneol to ±74% for geosmin). After a closer look at the raw data (i.e., BETs for single panelists from single sessions; data not shown), it seems obvious that there are two different reasons for such large deviations. For the compounds geosmin, 2-isopropyl-3-methoxypyrazine, and 2-methylisoborneol different sensitivities for the respective compound from panelist to panelist seem to be the reason for the large deviations in the group BET. On the contrary, for the compounds α-terpineol and fenchyl alcohol the BET values for single panelists from manifold determinations already show rather large deviations. For those two compounds the panelists gave the additional comments that these compounds are very hard to identify as off-flavor in the apple juice matrix. We suppose that the genuine apple flavor may somehow mask these off-flavor compounds.

To evaluate apple juice samples with instrumental−analytical techniques with respect to the presence of off-flavor compounds, we recently described a method using headspace SPME as extraction technique with subsequent separation and determination via GC-MS (*14*). The limits of detection and determi-

nation for the investigated compounds are given in **Table 2**. The need for an adequate analytical method follows from the necessity for quantitative data for the compounds of interest. When the growth behavior of the strains of interest is evaluated in the apple juice matrix, growth rates should be obtained in terms of colony-forming units. Quantitative information about the off-flavor compounds, the odor activity values (i.e., quotient from concentration to odor threshold value), must be determined to be able to judge the sensory relevance of the off-flavor compounds on the flavor of the product.

When the described limits of detection are set in relation to the odor threshold values, it can be seen that the limits of detection are lower than the odor threshold values for the compounds *o*-, *m*-, and *p*-anisaldehyde, α-terpineol, 2,3-dimethylpyrazine, [(1*S*)-*endo*]-(−)-borneol, 1-octen-3-ol, 3-octanone, fenchyl alcohol, and guaiacol. As a consequence, the determination of odor activity values does not represent any problem for these compounds. On the contrary, for the compounds 2-methylisoborneol, geosmin, and 2,6-dibromophenol the odor thresholds are ≈1 order of magnitude lower than the limits of detection. The two pyrazines, 2-isobutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine, are very potent odorants. The respective limits of detection are ≈3 orders of magnitude higher than the odor threshold values. These values demonstrate that at the present stage the described GC-MS method is not sensitive enough to determine the influence of the compounds 2-methylisoborneol, geosmin, 2,6-dibromophenol, 2-isobutyl-3-methoxypyrazine, and 2-isopropyl-3-methoxypyrazine on a possibly pronounced off-flavor of apple juice when the compounds are present at concentration levels of the detection threshold.

Eight tainted apple juice samples, which were purchased at local markets by chance, were analyzed for the presence of *A. acidoterrestris* and *S. griseus griseus* as well as for the investigated off-flavor compounds. Results are given in **Table 3**. The role of *A. acidoterrestris* as a common fruit juice spoiling bacteria, as described previously (*1*−*7*), was confirmed. The strain was present in five of the investigated samples. *S. griseus griseus* was identified in six samples. This is the first time that the presence of *Streptomyces* ssp. is shown in tainted apple juice. In two samples, only *A. acidoterrestris* was identified; the juices were described as medicinal and pungent. In all other juices, either *Streptomyces* spp. or both strains were found. The sensory properties of the samples were mainly described as musty and/or moldy. All investigated off-odor compounds could be identified in the samples (**Table 3**). In summary, the concentrations of the off-odor compounds are rather high. In those samples where *Streptomyces* ssp. was identified, the compounds 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-methylisoborneol, 1-octen-3-ol, and fenchyl alcohol as well as geosmin were found in concentrations higher than the detection threshold. The other compounds were identified in most cases, but in concentrations lower than the detection threshold. Synergistic effects of the compounds were not investigated. Guaiacol as a metabolite of *A. acidoterrestris* was found in all samples where the strain was present, whereas 2,6-dibromophenol was identified in only one sample. This sample also shows very high concentrations of guaiacol.

The results obtained from the tainted samples indicate that the strains find very good growth conditions in the apple juice medium. When samples are contaminated with *A. acidoterrestris* and/or *Streptomyces* ssp. and a pronounced off-odor can be perceived, the sensitivity of the used analytical method is high enough to determine the compounds of interest. Nevertheless, to be able to determine the potent odorants also in concentrations of the detection threshold values, further investigations will be performed to lower the sensitivity of the instrumental−analytical method for the respective compounds. Alternative extraction techniques such as stir bar sorptive extraction (SBSE) and solid-phase dynamic extraction (SPDE), using different sorption mechanisms and different ratios between fiber and sample in comparison to headspace SPME will be used. Until this aim can be reached, sensory evaluation is indispensable for the judgment of suspicious juice samples.

ABBREVIATIONS USED

3-AFC test, three-alternative forced test; BET, best estimate threshold; GC-MS, gas chromatography−mass spectrometry; LOD, limit of detection; LOQ, limit of determination; RSD, relative standard deviation; SD, standard deviation; SPME, solid-phase microextraction; ssp., subspecies; TAB, thermoacidophilic bacteria.

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