Changes in the Aroma of a Strawberry Drink during Storage

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The flavor of a commercially available strawberry drink was investigated with special regard to the changes of the sensory properties during the shelf life of the product. The experiments were performed using gas chromatographic methods after liquid−liquid extraction and after solid-phase microextraction of the headspace. A trained sensory test panel was used to substantiate the results from instrumental analyses. The relative concentrations of several compounds were followed over a storage period of six weeks at elevated temperature (37 °C), which corresponds to about 12 months storage at room temperature. Significant concentration changes of several flavor compounds were determined after a short storage time. These results correlate highly with changes in the aroma observed by the sensory test panel. Further on, changes in the sensorial relevance of aroma active compounds were monitored by comparative aroma extract dilution analysis of extracts of the fresh product and the product at the end of the declared shelf-life time. The results showed a significant decrease in flavor dilution factors of compounds with characteristic fruity attributes.

Keywords: *Strawberry drink; shelf life; aroma; GC−MS; SPME; sensory evaluation*

INTRODUCTION

Juices and nectars from citrus fruits and apples have dominated the fruit juice market for many years. Within the past decade, new types of fruit juice products have come onto the market, including products such as the strawberry drink that was investigated in this study. There are several companies offering strawberry fruit juices and nectars. For the producers of these new products, questions about shelf life and changes in the course of aging have to be answered. It is generally known that the flavor of fruit juices changes drastically during storage. Various compounds have been reported to degrade over time, whereas others are formed. In most cases these degradation and formation reactions lead to a deterioration of the original aroma of the product. Consequently, the shelf life of commercially available fruit juices and nectars is not limited by microbial spoilage but by a drastic decrease of the sensory quality of the products due to chemical reactions (*1*, *2*).

The aroma of strawberries and of strawberry products has been the object of many investigations. Several papers can be found dealing with the aroma of fresh strawberries (*3*−*10*), as well as of frozen fruits (*11*−*13*). Recently, the flavor of high-pressure-treated fruits was the subject of investigations (*16*, *17*). There are mainly two papers dealing with the aroma of thermally processed products such as pasteurized strawberry juice concentrate or strawberry jam *(14, 15)*. Lundahl and colleagues (*15*) investigated changes of strawberry juice concentrate stored at 20 °C with regard to changes of flavor and some other compositional changes. The results of this work indicate that strawberry juice is rather sensitive to the external environment.

In the present study a commercially available and very popular strawberry drink was investigated with special regard to changes in sensory attributes during storage under different conditions (i.e., 4 °C for the reference samples and 37 °C to achieve acceleration of the aging process). Changes in concentration of flavor compounds were followed by the use of gas chromatography−mass spectrometry (GC−MS) using solid-phase micro-extraction (SPME) as the sample preparation technique. SPME, a rather new technique for headspace analysis, was introduced by Janusz Pawliszyn and his group about 10 years ago (*18*−*21*). Meanwhile, SPME equipment has become commercially available and a high number of publications describing the use of SPME as sample preparation technique can be found in the literature. Various types of fibers are commercially available (i.e. different coating materials, as well as different film thicknesses and lengths of the fibers themselves), which makes SPME a very useful tool for a wide range of different analytical problems. It has also been proved in a number of papers that SPME is a potentially useful technique for the extraction of volatiles from fruits or vegetables (*22*−*29*). In this study we used headspace SPME coupled with gas chromatography−mass spectrometry to monitor the relative changes of certain flavor compounds in the course of the aging process of the investigated strawberry drink.

The sensory relevance of the extracted flavor compounds was evaluated by means of GC−olfactometry and comparative aroma extract dilution analysis (cAEDA) (*30*, *31*) of aroma extracts of the fresh product and of the product at the end of its declared shelf life time. Furthermore, to obtain an overall picture of the flavor changes that occur over the course of the aging of the product, the samples were evaluated by a trained sensory test panel throughout the whole storage period.

MATERIAL AND METHODS

Strawberry Drink Samples and Storage Conditions. The investigated strawberry drink is produced on an industrial scale. Main ingredients of the product are, in decreasing

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amounts, water, strawberry pulp, sugar, concentrates from strawberry and lemon juice, a juice concentrate from color-rich berries to improve the color of the product (*Sambuccus nigra, Aronia melanocarpa,* and *Ribes nigrum*), and lactic acid. To compensate for variations caused by the quality and ripeness of the fruits, natural aroma is added. The fruit proportion of the product comes to at least 40%.

The juice samples were hot-filled in green glass bottles with a volume of 200 mL. The samples were taken immediately after the pasteurization and filling process. To achieve accelerated aging, the samples were stored at 37 °C. The reference samples were stored cool at 4 °C; all samples were stored in dark places. Samples were taken in one-week intervals for a period of six weeks.

Sensory Evaluation of the Strawberry Drink. Sensory evaluation of the strawberry drink was performed by a trained sensory test panel of 12 to 15 persons. A duo−trio test (one tailed) was chosen to determine differences between the stored samples (*32, 33*). Sensory test sessions took place weekly over the period of five weeks, and samples stored at 37 °C were compared to those stored at 4 °C. All samples were served in red cups to avoid differentiation based on color differences of the samples.

Solid-Phase Microextraction (SPME) and GC−MS. Headspace SPME was used for the examination of the relative concentration changes of several compounds of all samples. A carboxen/poly(dimethylsiloxane) fiber (film thickness 75 μm) was used (Supelco, Bellefonte, PA). The fiber was conditioned at 280 °C under helium flow for at least 30 min before the exposure of the fiber to the sample headspace. For extraction of the volatiles 10 mL of sample and 5 g of NaCl were put into a 40-mL headspace vial. A 10 μL aliquot of a solution of 1,2,3-trichloropropane (Sigma-Aldrich, Vienna, Austria; 58 mg/L in H_2O/CH_3OH) was added as internal standard. The sample was equilibrated at 30 °C for 10 min while being stirred thoroughly with a magnetic stirrer. The SPME fiber was exposed to the sample headspace for 10 min at 30 °C and was then transferred directly to the injection port of the GC−MS system. The compounds were thermodesorbed from the fiber into the injection liner and cryo-focused at −30 °C on the head of the analytical column using liquid nitrogen as cooling agent. The SPME fiber was left in the injection port for reconditioning during the whole GC run.

For the GC−MS measurements a Hewlett-Packard system (HP G1800A GCD) was used. The capillary column was a HP 5 (cross-linked 5% phenyl methyl siloxane; column length 30 m, i.d. 0.25 mm, film thickness 1 μm). Following are the operating conditions: column-head pressure 0.54 bar, temperature program from −30 °C (holding time 1 min) at 10 °C min^{-1} to 250 °C. Splitless injection mode was used, with the split valve being opened after 2 min. An SPME liner with a 0.75-mm i.d. was used to improve the peak width, especially for compounds with high volatility. The injection temperature was 270 °C, and the detector temperature was 250 °C. Electron impact ionization was used, (70 eV) scanning a mass range from 20−300 atomic mass units.

Preparation of Aroma Extracts. Liquid−liquid extraction of the volatile compounds was performed using fluorotrichlormethane (purity 99+%; Sigma-Aldrich, Vienna, Austria) as organic solvent; choice of the solvent was based on the results published in (*40*). $CFCl_3$ was distilled prior to use to remove residual impurities from the solvent. Because of the low boiling point of $CFCl_3$ (23.7 °C) the samples had to be cooled to 4 °C prior to the extraction. A 200-mL sample of strawberry drink was poured into a 250-mL Erlenmeyer flask. A 50-μL portion of a camphor solution (Sigma-Aldrich, Vienna, Austria; 126 mg/L in $CFCl_3$) was added as internal standard. $CFCl_3$ (20 mL) was used for the extraction. The system was stirred thoroughly using a magnetic stirrer while in an ice bath for 10 min. The mixture was left to stand for a few minutes, and the clear supernatant was poured into a second Erlenmeyer flask. The remaining emulsion was transferred into cooled centrifuge glasses and centrifuged for 5 min at 4,500 rpm. The organic layer was separated. The extraction was performed twice, and the organic layers of both extractions were combined. The whole organic extract was reduced gently to a final volume of 0.5 mL using a Zymark rotary evaporator (Turbo Vap 500, Zymark, Hopkinton, MA) working at atmospheric pressure and a water bath temperature of 20 °C. This extract was used directly for the gas chromatographic−mass spectrometric analyses, as well as for the GC−olfactometry.

Gas Chromatography−Mass Spectrometry of the Aroma Extracts. The GC−MS analyses were performed on a system from Hewlett-Packard (gas chromatograph HP 5892 II plus) with a mass selective detector (HP MSD 5972). The capillary columns used were a HP 5 (Hewlett-Packard; cross-linked 5% phenyl methyl siloxane, column length 30 m, i.d. 0.25 mm, film thickness 0.25 μm) and a DB-WAX (J&W; column length 60 m, i.d. 0.32 mm, film thickness 0.5 μm). Helium was used as carrier gas. The conditions were as follows for the HP 5: column-head pressure 0.81 bar (36 cm sec^{-1} at 143 °C), temperature program from 35 °C at 5.3 °C min^{-1} to 280 °C (5 min). Following are the conditions for the DB-Wax column: column-head pressure 0.82 bar (36 cm sec^{-1} at 143 °C), temperature program from 35 °C at 1.1 °C min^{-1} to 250 °C (10 min). Splitless injection mode was used with an injection volume of 2 μL. Injector temperature was 220 °C, and the detector temperature was 280 °C (HP 5) and 250 °C (DB−Wax). Electron impact ionization (70 eV) was used; the scanned mass range was 35−300 atomic mass units.

Gas Chromatography−Olfactometry (GCO) and Aroma Extract Dilution Analysis (AEDA). GC−olfactometry was carried out on a system from Hewlett-Packard (gas chromatograph HP 5890 II) equipped with a flame ionization detector (FID). The capillary columns used for the sniffing experiments were a HP 5 (cross-linked 5% phenyl methyl siloxane, column length 30 m, i.d. 0.32 mm, film thickness 0.25 μm) and a DB-Wax (J&W, column length 15 m, i.d. 0.25 mm, film thickness 0.5 μm). Helium was used as carrier gas. The experimental conditions were as follows for the HP 5: column-head pressure 1.0 bar (36 cm sec^{-1} at 143 °C), temperature program from 35 °C at 6.6 °C min^{-1} to 280 °C (5 min). Conditions were the following for the DB-Wax: column-head pressure 0.6 bar (36 cm sec^{-1} at 143 °C), temperature program from 35 °C at 5.2 °C min^{-1} to 240 °C (10 min). Splitless injection mode with an injection volume of 2 μL was used.

The effluent of the analytical column was split into two parts (split ratio 1:1). One part was led into the FID, the second part was led into the sniffing port. Humidified air (200 mL) was added into the sniffing port to spare the nasal mucous membrane of the sniffing person. For the determination of the aroma compounds with the highest sensorial relevance, ADEA was performed of the $CFCl_3$ extract. The extract was diluted in steps of 1:2, 1:4, 1:8, 1:16, etc., and each dilution was sniffed at least twice until no odor impressions could be detected (*30, 31*).

Identification of the Investigated Compounds. For the identification of the volatile compounds, all aroma extracts were analyzed by GC−MS on the analytical columns mentioned above. The measured mass spectra were compared to those obtained from reference compounds if available, as well as with data found in the literature and commercially available mass spectra databases (Wiley 275). Additionally, linear temperature programmed retention indices (RI) were calculated according to the equation of Kratz and van den Dool *(34, 35)* for both column types and compared to RI data from a retention index database.

The aroma active compounds were located in the chromatograms by the odor impression and the FID signal. The compounds were identified by their RI on two columns and their aroma properties in comparison to reference compounds and descriptions found in the literature, as well as by correlation with the GC−MS data.

RESULTS AND DISCUSSION

Methodology. *Sample Preparation.* For the determination of the volatile compounds of the investigated strawberry drink two different sample preparation

techniques (i.e., preparation of aroma extracts and solid-phase microextraction, SPME) were used. Even though SPME has been described in the literature as a suitable sample preparation technique for GC−olfactometry (*45*), we decided to use aroma extracts representing the sensory properties of the investigated product for the performance of comparative aroma extract dilution analysis (cAEDA). This decision was mainly based on the fact that using SPME, discrimination was observed for compounds with very high or low volatility that should not be excluded from the sniffing experiment.

Generally, for the generation of aroma extracts, different sample preparation techniques can be used that are all well described in the literature (*38, 39*) (e.g., liquid−liquid extraction, vacuum-distillation, simultaneous distillation−extraction according to Likens-Nickerson, etc.). In the special case of the strawberry drink, adulteration of the aroma caused by thermal treatment during sample preparation had to be avoided. Consequently, cold liquid−liquid extraction of the volatiles was performed. Aroma extracts were prepared using different organic solvents (i.e., fluorotrichloromethane, diethyl ether, dichloromethane, and pentane) and the obtained aroma extracts were evaluated by the sensory test panel. Extracts prepared with fluorotrichloromethane gave the best sensory qualities in comparison to the original flavor of the beverage. This corresponds well with the results published by the group of Rapp (*40*) who used fluorotrichloromethane for the extraction of aroma compounds from wine grapes.

Solid phase microextraction (SPME) turned out to be a very suitable method to follow the concentration changes of the volatile compounds from the strawberry drink during the storage experiment. For this purpose SPME was chosen, as the method is quick and highly reproducible, and it helps to avoid the use of large amounts of organic solvents in the laboratory. For the whole procedure (including sample preparation, extraction of the volatile compounds from the headspace, and GC−MS measurement) high reproducibility with a maximum standard deviation of ±15% could be achieved when time and temperature were controlled strictly, both for the equilibration of the system, as well as for the exposure of the SPME fiber to the headspace of the sample. Thorough stirring of the sample and saturation of the sample with NaCl showed positive influence on the extraction yield of the headspace compounds. Even compounds with very high volatility, such as dimethyl sulfide, can be determined with high reproducibility when the experimental conditions are followed strictly. Critical steps included selection of the internal standard and introduction of it into the sample. We selected the compound 1,2,3-trichloropropane (TCP) for the following reasons: (1) The compound does not naturally occur in fruit juices. Using the characteristic mass fragments at *m*/*z* 75 and *m*/*z* 110 the compound can be identified easily. (2) At the retention time of TCP, no coelution with any other volatiles originating from the beverage was observed. (3) Because of the polar character of the compound, the standard was prepared in methanol. Further dilutions could be made with double-distilled water and, consequently, the standard solution could be introduced homogeneously into the sample. This was necessary as with the use of an organic solvent the internal standard would not have been dispersed homogeneously in the aqueous matrix. The use of an apolar volatile organic solvent would also have influenced the capacity of the SPME fiber negatively with respect to the flavor compounds.

It is well-known that discrimination of compounds with high or low volatility occurs in SPME. Consequently, quantification cannot be carried out seriously by the use of one single internal standard. We used the internal standard not for quantification but to correct variations of the capacity of the SPME fiber, as well as variations of the sensitivity of the GC−MS system. Therefore, concentrations are noted not as absolute concentration values but as equivalents to the internal standard.

Conditions for the GC Measurements and Calculation of Retention Indices. For the gas chromatographic separation and detection of the volatile compounds, analytical columns of two different polarities were used, and linear temperature programmed retention indices were calculated. Considering the relation between column dimension, flow of the carrier gas, and the heating ramp as proposed by Farkaš et al. (*35*), analytical columns of different dimensions can be used, resulting in the same retention indices for same polarities of the stationary phases. Consequently, the identification of the compounds by comparison of the retention indices with data from the used retention index database was not dependent on the column dimensions and experimental parameters used. For the GC−MS measurements after SPME, slightly modified parameters were used: the use of a column with a higher film thickness of the stationary phase and a very low starting temperature resulted in a better performance, especially for the determination of highly volatile compounds (e.g., dimethyl sulfide). Nevertheless, under these conditions the retention indices did not deviate for more than two retention index units from the data given in the used retention index database.

Storage Conditions. Forced aging of the samples was performed in order to conduct the investigations within a reasonable period of time. On the basis of data that were obtained from sensory evaluation of fruit juices, nectars, and drinks throughout the last years in the course of their production, it was found empirically that one week storage at 37 °C corresponds to about two months storage at room temperature (i.e., 20 °C ± 2 °C). This assumption was reconsidered by comparison of the relative concentrations of several compounds after the appropriate storage times. The data shown in Figure 1 prove the correctness of this assumption in the case of the investigated strawberry drink. On the basis of these findings, this correlation was used for the experiments described in this paper.

Changes of the Concentrations during Storage. A high number of compounds was identified from the investigated drink, including various esters, carbonyls, alcohols, volatile organic acids, monoterpenes, and furan derivatives. All of them have been described in the literature as components of the aroma of strawberries or strawberry products (*3, 6, 7, 9, 36*). The group of esters dominates the list of identified compounds. As the identification of all compounds was not the main goal of the present study, we abstain from giving a detailed compound list in this paper. Figure 2 shows the SPME−GC−MS chromatograms of a fresh strawberry drink in comparison to a sample at the end of the declared shelf-life time, showing significant differences in concentrations of a number of compounds. Figures 3

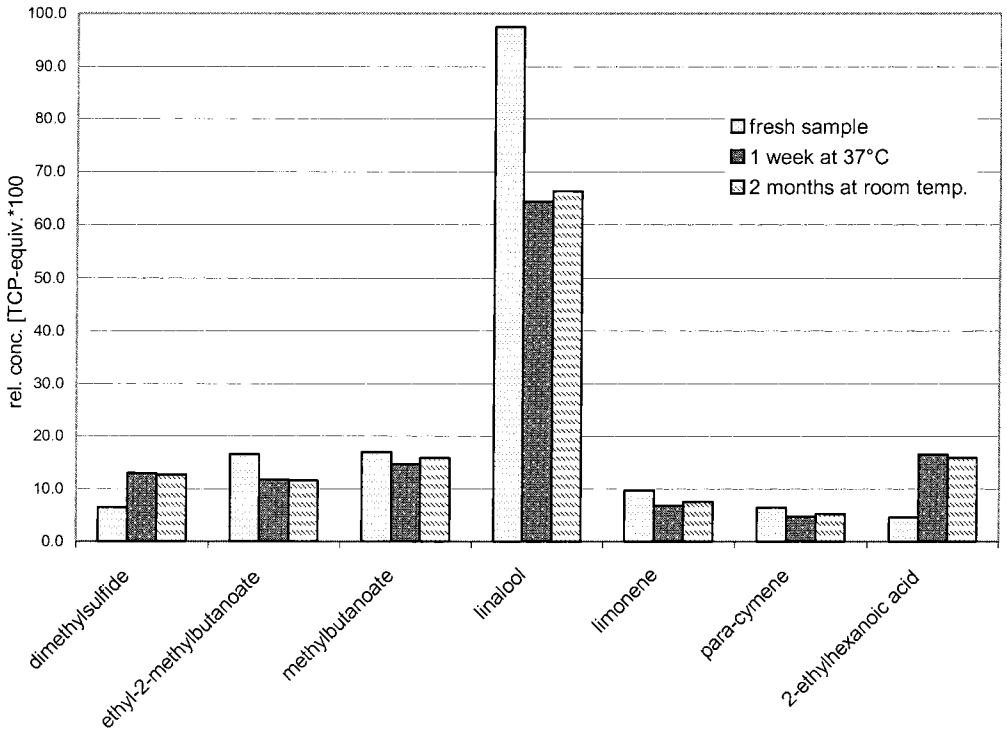

Figure 1. Comparison of concentrations of several compounds after different storage times and temperatures.

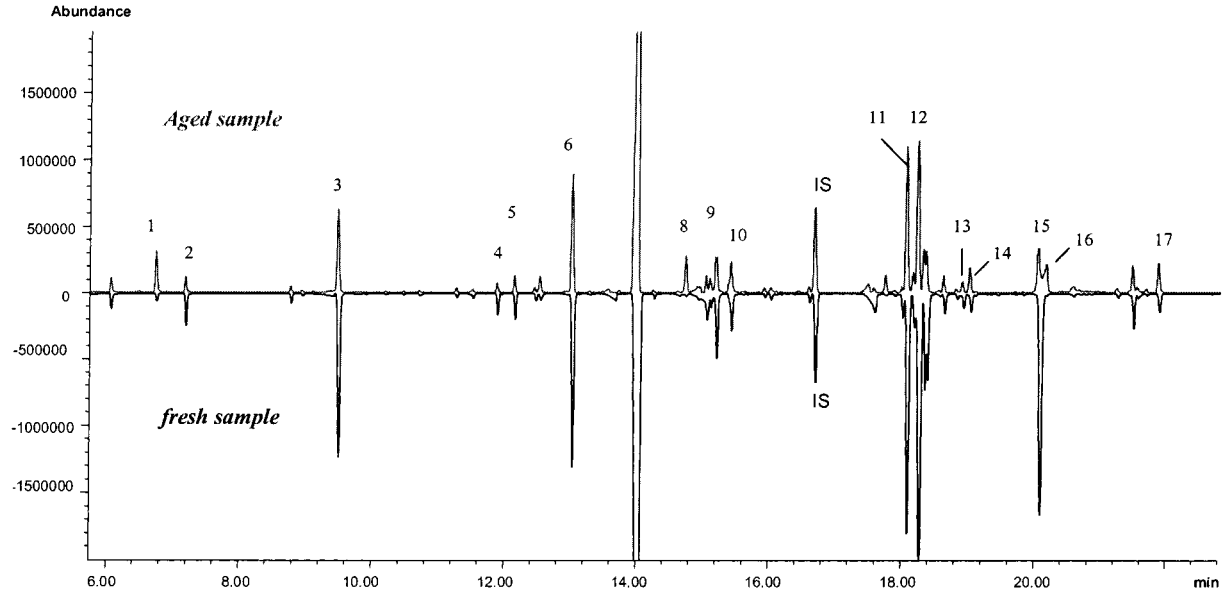

Figure 2. SPME−GC−MS chromatograms of strawberry drink, scan mode; positive mode, aged sample (5 weeks at 37 °C); negative mode, fresh sample; (1) dimethyl sulfide, (2) methyl acetate, (3) ethyl acetate, (4) ethylpropanoate, (5) methylbutanoate, (6) ethyl-2-methylpropanoate, (7) ethylbutanoate, (8) furancarboxaldehyde, (9) 3-hexen-1-ol (Z), (10) 1-hexanol, (IS) internal standard, (11) ethylhexanoate, (12) 3-hexenyl acetate (Z), (13) limonene, (14) 1,8-cineol, (15) linalool, (16) 2-ethylhexanoic acid, and (17) α-terpineol.

through 6 show the time−concentration curves of various selected compounds from different chemical classes.

The concentration of dimethyl sulfide (Figure 3) was increasing significantly. Fresh products showed very low concentrations, whereas the concentration increased almost linearly and was 7−8-fold higher at the end of the storage period. The Strecker degradation of methionine is the source of dimethyl sulfide (*37*). Furancarboxaldehyde (Figure 3) shows a similar behavior; very low concentrations are present in the fresh product, whereas the concentration at the end of the storage time is almost 9 times higher than at the beginning. Furancarboxaldehyde is supposed to be formed via two different pathways, which are both well described in the literature (*1*): (i) degradation of ascorbic acid and (ii) reaction of various sugars under acid conditions under the formation of furancarboxaldehyde. Both compounds − dimethyl sulfide and furancarboxaldehyde − seem to be suitable to be used as indicator compounds for stored strawberry products.

Figures 4 and 5 show the concentration curves of various selected esters. The number of esters in the observed strawberry drink was very high. Most of the identified esters show pleasant and fruity sensory

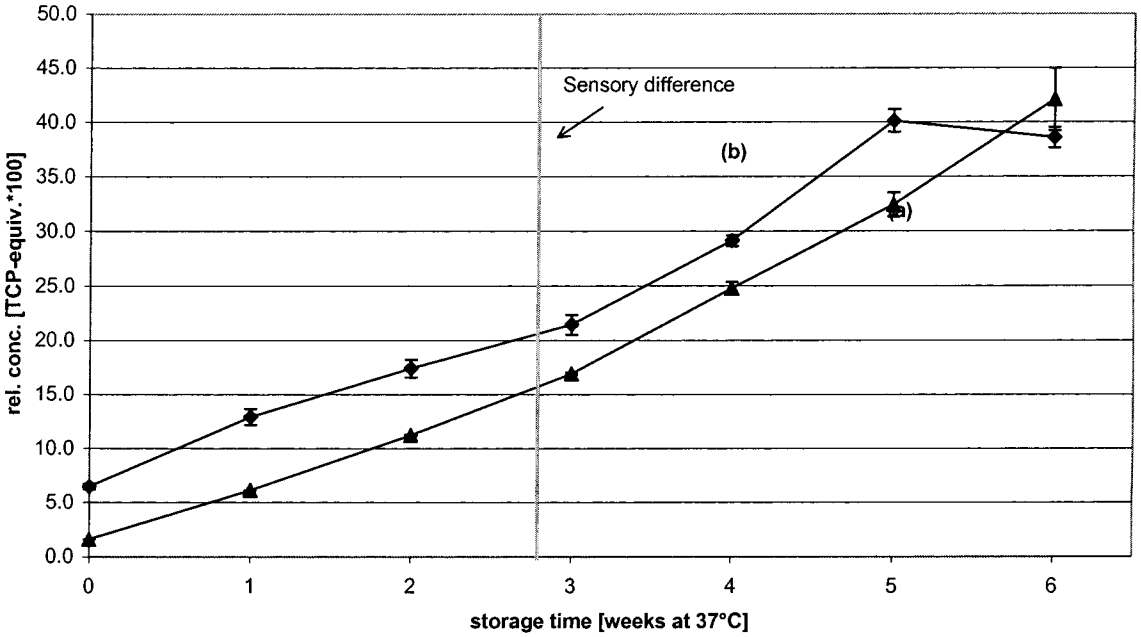

Figure 3. Concentration of (a) dimethyl sulfide and (b) furancarboxaldehyde during storage; the grey line indicates the time when a significant sensory difference was observed.

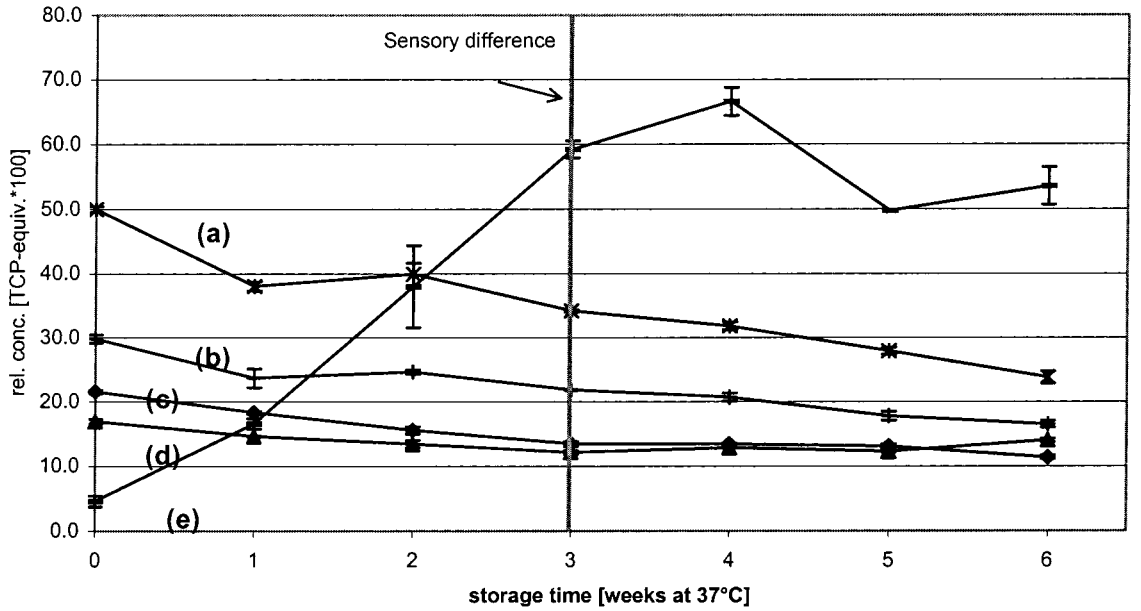

Figure 4. Concentration curves of (a) hexyl acetate, (b) (E)-2-hexenyl acetate, (c) methylbutanoate, (d) ethylpropanoate, and (e) 2-ethylhexanoic acid during storage; the grey line indicates the time when a significant sensory difference was observed.

properties and, consequently, have a rather high impact on the fruity aroma of the product. All esters observed in the investigated drink showed decreasing concentrations resulting in a loss of the corresponding fruity aroma notes. Contrary to the concentration changes of the esters, the concentration of 2-ethylhexanoic acid increased significantly (Figure 4) Other acids such as propanoic acid, butanoic acid, and 2-methyl-butanoic acid were identified in the product, but showed only slight concentration increases. The rather unpleasant flavor attributes of free acids (showing unpleasant, sweaty aroma notes) have a negative influence on the overall aroma of the strawberry drink.

Figure 6 shows the concentration changes over time of some selected monoterpenes. Because of the different structures of monoterpenes, different reaction pathways are possible in acidic media like fruit juices. Therefore, no general trend can be given for the time−concentration curves of the monoterpenes. In the investigated strawberry drink, the highest monoterpene concentration was found for linalool. The linalool concentration decreased significantly to about 20% of the original concentration within 6 weeks at 37 °C (Figure 6). Different possible reaction products of the linalool degradation have been described; one is α-terpineol, which was found with increasing concentrations in the strawberry drink. The compounds limonene and myrcene were found in relatively high concentrations; both compounds degraded during the storage period.

Comparative Aroma Extract Dilution Analysis (cAEDA). GC−olfactometry (GC−sniffing) was used to determine the sensory relevance of the volatile compounds of the investigated product. Comparative aroma extract dilution analysis (cAEDA) was performed on

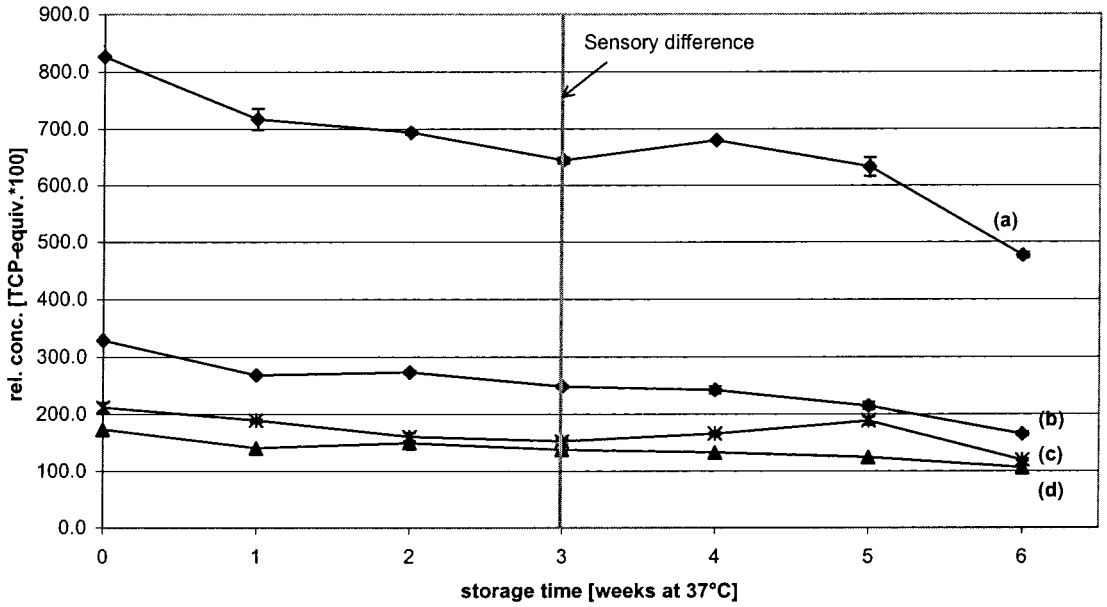

Figure 5. Concentrations of (a) ethylbutanoate, (b) 3-hexenyl acetate (Z), (c) ethyl-2-methyl-propanoate, and (d) ethylhexanoate during storage; the grey line indicates the time when a significant sensory difference was observed.

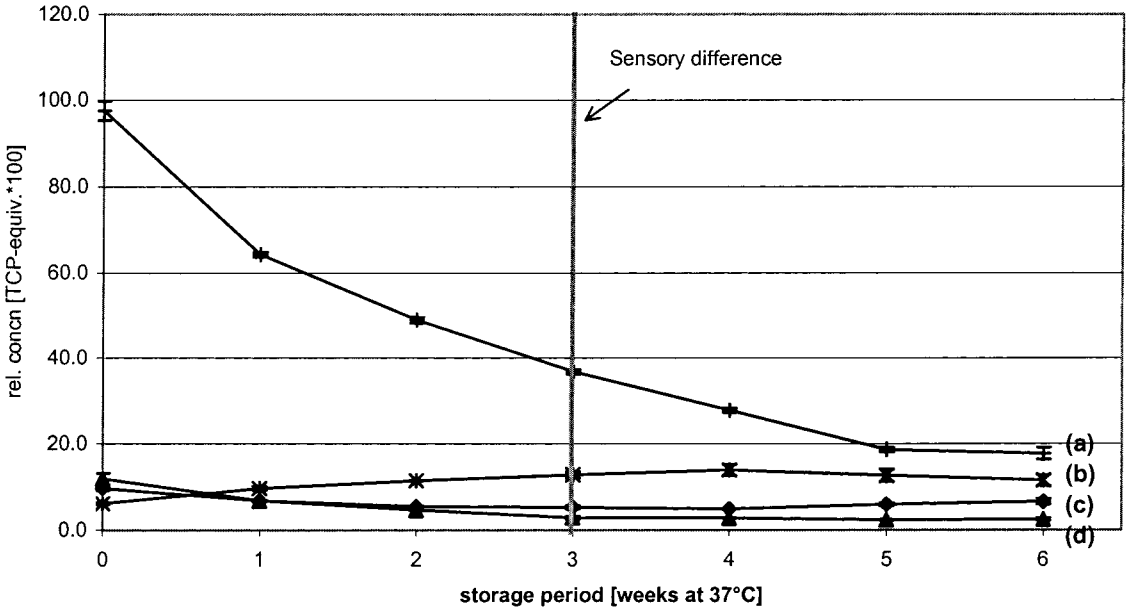

Figure 6. Concentrations of (a) linalool, (b) α-terpineol, (c) limonene, and (d) myrcene during storage; the grey line indicates the time when a significant sensory difference was observed.

aroma extracts of a fresh sample as well as those of an aged sample (i.e., a sample at the end of the declared shelf-life time, which corresponds to an age of 9 months). Table 1 shows the results of the cAEDA of the fresh and the aged strawberry drink. The flavor dilution (FD) factors describe the sensory relevance of the aroma active compounds: the higher the observed FD factor, the higher the influence of the compound on the overall aroma of the investigated sample (*30*, *31*). The results of the AEDA and the comparison of the flavor dilution factors (FD factors) show significant differences for several compounds that correlate very well with changes of the corresponding concentrations.

In summary, FD factors of compounds with pleasant and fruity attributes decreased, whereas other, more unpleasant, aroma notes increased. It was demonstrated before that the concentrations of several esters decreased significantly over the observed storage period. Using GC−olfactometry five of the identified esters were found to have high influence on the overall aroma of the strawberry drink. Ethyl-2-methylpropanoate (compound no. 1 in Table 1) showed an FD factor of 512, which was the highest FD factor observed in the aroma extracts. The influence of this compound decreased slightly over the storage period. The compounds ethylbutanoate (no. 2) and ethylhexanoate (no. 11), both with high FD factors in the fresh beverage (FD 512 for ethylbutanoate and FD 256 for ethylhexanoate), showed lower FD factors and consequently decreasing influence on the sensory attributes over the course of the shelf-life time. A decline of the FD-factor was also observed for the compound 3-hexenyl acetate (Z) (no. 12). The interpretation of the FD factors for compound no. 4, ethyl-2-methylbutanoate, which showed a strongly increasing FD factor, is inconsistent with all other results. We suppose that the odor impression that was detected

Table 1. Results from cAEDA of Fresh and Aged Strawberry Drink

					fresh sample		aged sample (9 months)	
no.	compound	RI (HP5)	RI (wax)	odor description	FD factor[a]	conc. [IS equiv][b]	FD factor[a]	conc. [IS equiv][b]
1	ethyl-2-methylpropanoate[e] (*m*/*z* 71)[c]	755.7	968.8	fruity	512	4.0	256	1.6
2	ethylbutanoate[e] (*m*/*z* 71)[c]	802.2	1040.4	fruity, strawberries, raspberries	512	49.7	256	22.1
3	unknown[f]	820.0	1117.0	sulfury	64		8	
4	ethyl-2-methylbutanoate[e] (*m*/*z* 102)[c]	850.0	1055.2	fruity, sweet	(32)[g]	0.4	(256)[g]	0.3
5	3-hexen-1-ol (Z)[d] (*m*/*z* 67)[c]	855.0	1397.8	green, grassy	4	5.8		7.9
6	2-methylbutanoic acid[e] (*m*/*z* 74)[c]	856.2	1680.6	sweet, sweaty, unpleasant	16	6.0	32	8.6
7	unknown[f]	875.0		yeast hydrolysate	64		64	
8	3-(methylthio)-propanal[d]	905.1	1456.0	cooked potatoes	256	[h]	16	[h]
9	1-octen-3-one[d]	978.1	1306.5	mushrooms	64	[h]	64	[h]
10	β-myrcene[d] (*m*/*z* 93)[c]	991.3	1167.3	fresh, aromatic, spicy	128	[h]	64	[h]
11	ethylhexanoate[d] (*m*/*z* 88)[c]	1000.0	1240.0	fruity, sweet	256	8.7	128	2.6
12	3-hexenyl acetate (Z)[d] (*m*/*z* 67)[c]	1005.0	1324.2	banana	8	25.0	2	10.2
13	1,8-cineole[d] (*m*/*z* 108)[c]	1030.8	1209.7	fresh, spicy, eucalyptol	4	0.5	8	0.5
14	(E)-β-ocimen[e]	1040.0	1240.0	putrid, unpleasant, grassy	16	[h]	2	[h]
15	2,5-dimethyl-4-methoxy-3[2H]-furanone[d] (*m*/*z* 142)[c]	1057.0	1600.0	sweet, caramel, candy-floss	32	2.1	8	1.9
16	2,5-dimethyl-4-hydroxy-3[2H]-furanone[d] (*m*/z128)[c]	1060.0	2043.3	cotton candy, melted-hot butter	256	0.6	128	0.7
17	linalool[d] (*m*/*z* 93)[c]	1100.0	1559.9	fruity, citrus, bergamotte-oil	256	32.8	128	7.1
18	2-ethylhexanoic acid[d] (*m*/*z* 88)[c]	1128.0	1962.5	unpleasant, slightly sweet, putrid	8	4.8	32	8.0
19	unknown[f]	1387.0	1889.1	cooked apples	128		256	
20	unknown[f]	1470.4	2103.0	coconut	512		0	

[a] AEDA were carried out on a HP 5. [b] Integration of the peak area was performed by using characteristic ions for the respective compounds to avoid possible interference by other compounds. As no response ratios between the different ions were taken into account, the concentrations are given in terms of equivalents to the internal standard (IS equiv.). The internal standard (camphor) was added in a concentration of 31.5 μg/L strawberry drink. [c] Characteristic mass charge ratio that was used for integration of the peaks. [d] The identification was based on RI on both columns and comparison with data from the RI database and the reference compounds. Mass spectra were compared with data from reference compounds and commercially available MS libraries. Odor impressions were compared with those of reference compounds. [e] The identification was based on RI on both columns and comparison with data from the RI database. Mass spectra were compared to data from literature and commercially available MS libraries. Odor impressions were compared with descriptions found in the literature. [f] The given retention indices refer to the respective odor impressions. [g] See text for the interpretation of the FD factors. [h] GC−MS signal too weak for integration.

in the GC−sniffing experiments was not provoked by the compound ethyl-2-methylbutanoate alone, but that it was influenced by 2-methylbutanoic acid that elutes from the used column immediately after the ester. As organic acids do not form symmetrical peaks on the used GC column, but a triangular peak shape, coelution of ethyl-2-methylbutanoate and 2-methylbutanoic acid occurs. This fact might lead to a synergistic effect, which could cause the observed increase of the FD factor of ester no. 4. The FD factors of the two free organic acids − 2-methylbutanoic acid and 2-ethylhexanoic acid − increase significantly, and because of their rather unpleasant aroma properties they influence the aroma of the aged drink negatively.

The behaviors of the unknown compound (no. 3) with sulfury aroma notes and of 3-(methylthio)-propanal (methional, no. 8) are very interesting. Both show significantly lower FD factors in the aged sample than in the fresh one. Methional is a degradation product of the amino acid methionine and can be found in almost all types of aromas of different origin. The continuation of the Strecker degradation of methionine forming methional, and the subsequent degradation of methional under the formation of methylmercaptane and dimethyl disulfide, seem to be the reason for the decrease of the FD factor of methional in the aged sample. This correlates well with the finding of the highly increasing dimethyl sulfide concentration (Figure 3) that is usually formed from dimethyl disulfide (diproportioning to dimethyltrisulfide and dimethyl sulfide). Considering the sensory properties of the unknown compound (no. 3) with sulfury attributes we assume that this compound is another sulfur-containing compound that is possibly degraded in a comparable way.

Linalool (no. 17) with its fresh, citrus-like flavor notes influences the aroma of the strawberry drink very strongly. Linalool showed a slight decline of its FD factor, which means a loss of fresh flavor notes. As mentioned above, linalool is known to be a quite reactive compound in acid media, and it degrades rapidly by formation of compounds such as α-terpineol (*1*). A pH value of 2.5 for the investigated strawberry drink and

Table 2. Results of the Sensory Evaluation of the Storage Experiment; Duo-Trio-Test (One-Tailed, $p = q = 1/2$)

storage period [weeks]	hypothetical age[a] [months]	significance[b]	probability for H_0 [%][c]
1	2	ns	15.1
2	4	ns	60.5
3	6	*	3.3
4	8	**	0.3
5	10	**	0.2

[a] Hypothetical age refers to the samples stored at 37 °C under the assumption that one week at 37 °C corresponds to two months at room temperature. [b] Significance: ns, not significant; *, significant ($\alpha = 95\%$); **, high significance ($\alpha = 99\%$); ***, very high significance ($\alpha = 99.9\%$). [c] Probability for the null hypothesis H_0 in %.

a significant increase of α-terpineol (Figure 6) are indicators that the proposed reaction pathway takes place during the shelf life of the product.

The compounds 2,5-dimethyl-4-methoxy-3[2H]-furanone (no. 15) and 2,5-dimethyl-4-hydroxy-3[2H]-furanone (no. 16) are both described in the literature as main contributors to strawberry aroma. These compounds, with aroma notes such as sweet, caramel, or cotton candy, were detected by GC−olfactometry in the fresh sample as well as in the aged sample. Both compounds showed a slight decrease of their FD factors, which also indicates a reduction of the typical strawberry notes for the investigated product.

Three compounds that could not be identified showed different influences on the overall aroma; compound no. 7 with a flavor note of yeast hydrolysate did not change its influence, but compound no. 19 and especially compound no. 20 showed large differences in their FD factors in the fresh and in the aged drink. As those compounds could not be identified unambiguously, no interpretation can be given for their behavior.

Sensory Evaluation. Sensory evaluation of the strawberry drink samples was performed by a trained sensory test panel. The aim of the sensory evaluation was to find the time at which the aged sample was significantly different from the fresh sample. As this is a classical application of a difference test, a duo-trio test was chosen for this experimental series. As the posed question was "*Which sample is the same as the standard sample?*" the one-tailed binomial test ($p = q = 1/2$) was used (*32*). Table 2 gives the results from the sensory evaluation of the storage experiment. The results show that the investigated strawberry drink is a product that changes very quickly. After three weeks of storage at 37 °C in the dark, which corresponds to a storage time of about six months at room temperature, a significant difference was observed between the aged sample and the sample stored at 4 °C. The changes in the aroma of the strawberry drink were described as a decline of the fresh, fruity, and typical strawberry-like aroma notes with a significant increase of stale and musty attributes.

Drastic changes of the color were noticed; a significant decline of the powerful red color with a significant increase of brown pigments was observed. The browning of the product was not investigated in detail in this study, as a number of papers dealing with browning reactions of strawberry products has been published previously (*15, 41*−*44*). Nevertheless, for the sensory evaluation of the aroma of the beverage it was extremely necessary to exclude color impressions, which was performed by serving all samples in dark red cups as described above.

CONCLUSIONS

The results of the presented study show very clearly that the aroma properties of strawberry drinks are very sensitive to the external environment which results in a short shelf life of these products. Over the investigated storage period the strawberry drink lost its pleasant fruity and typical strawberry-like aroma notes, whereas unpleasant stale and musty attributes increased. These results found by sensory evaluation correlate well with results obtained from instrumental analyses. As a consequence, it must be highly recommended to store strawberry drinks, juices, and nectars at low temperatures (i.e., best at 4 to 6 °C, but not higher than 20 °C) and to shorten the storage time as much as possible to maintain the quality of these products.

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