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Sweetener/Sweetness-Induced Changes in Flavor Perception and Flavor Release of *Fruity* and *Green* Character in Beverages

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Green leaf volatile (GLV) mixtures, commercial orange flavors, and commercial strawberry flavors were applied to beverage bases in which concentrations of citric acid as well as a sweetener (sucrose or aspartame/acesulfame-K) were varied. Sensory profiling showed that flavor-specific fruity character increased as perceptible sweetness increased, independent of whether the sweetness resulted from sucrose (a change from 9 to 12 Brix) or aspartame/acesulfame-K (a change from 0.2 to 0.4 Brix). Sweetness was affected only by the tastants in the base and not by the flavors, although flavor-specific interactions between sweetener type and sweetener level occurred. Flavor release from the sucrose bases was compared to flavor release from bases containing aspartame/acesulfame-K by static headspace measurements and by MS−Nose measurements using an artificial throat. These measurements showed greater flavor volatility from bases having low Brix (fewer soluble solids). This negative Brix effect was also evident in the sensory data for perception of some GLV green notes. The headspace data could not support a positive Brix effect, the typical salting out, which would correspond to the observed perceptual enhancement of fruity notes.

KEYWORDS: **Brix/acidity effects; flavor release; green/fruity flavor perception; APCI-MS; artificial throat**

INTRODUCTION

Flavor−matrix interactions in (model) beverages are well-known (*1*−*3*). The matrix components relevant to this paper are sucrose and artificial sweeteners used in beverage bases. Increasing sucrose concentration in a fruit-flavored aqueous system has been shown to increase perceived retronasal fruitiness, which is often measured as “total flavor”. This fruitiness enhancement has been measured by different sensory methodologies, for example, paired or multiple comparisons (*4*), profiling (*5*), time−intensity (*6*), and MS−Nose, with or without controlled delivery (*7*−*9*). A change of only one degree in the Brix value has been claimed to significantly affect the flavor perception of beverages (*10*). [Brix, or total soluble solids, usually corresponds to the percent (w/w) sucrose in fruit beverages.] Differences in flavor perception from beverages containing different solutes have been reported (*2*, *11*−*13*).

Concentration ranges employed for much of the work on flavor release from aqueous solutions of sucrose or artificial sweeteners extend far beyond Brix 15, which is the maximum normally encountered in beverages (*14*−*16*). Occasionally very high concentrations of sucrose were used because viscosity was actually the effect under investigation (*17*).

The aim of the present study was to compare the flavor release of green leaf volatiles (GLVs) from beverages with their flavor perception, which we reported previously (*18*). Because orthonasal and retronasal odors of these GLVs differed, we measured both their static and dynamic headspaces. The dynamic headspace approximation of retronasal odor was achieved by atmospheric pressure chemical ionization mass spectrometry (APCI-MS) coupled to an artificial throat (*19*, *20*). APCI-MS has proven to be a useful technique for measurement and modeling of static as well as dynamic headspace in studies of flavor release from beverages (*21*−*25*).

Specifically, we investigated by sensory profiling whether the green−fruity shift, that is, the base effect on retronasal odor perception described previously (*18*), was caused by differences in perceived sweetness or by differences in Brix in the beverage bases. Beverages sweetened with either sucrose or an artificial sweetener were flavored with GLVs. Flavor release from the beverages containing sucrose (high Brix) and from those sweetened with an artificial sweetener (low Brix) was contrasted for isoamyl acetate, ethyl butyrate, and the six GLVs studied previously. The same bases were also flavored with commercial orange and strawberry flavors and evaluated sensorially by profiling. The commercial flavors were chosen to represent two distinctly different types of fruit, namely, citrus and berry, respectively.

MATERIALS AND METHODS

Flavor Chemicals. Isoamyl acetate, ethyl butyrate, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-yl acetate, (*Z*)-3-hexen-1-yl formate, (*Z*)-3-hexen-1-yl hexanoate, and (*Z*)-3-hexen-1-yl 3-methylbutyrate were supplied by Quest. Ethanol stock solutions (1% w/w) were prepared

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Table 1. Concentrations of Flavor Compounds in Base (Milligrams per Liter)

flavor compound	mixture 1	mixture 2	mixture 3
isoamyl acetate	0.471		0.727
ethyl butyrate	0.549		0.848
hexanal	0.471	1.200	
(*E*)-2-hexenal	0.157	0.800	
(*Z*)-3-hexen-1-yl acetate	0.157	0.400	
(*Z*)-3-hexen-1-yl formate	0.627	1.600	
(*Z*)-3-hexen-1-yl hexanoate	1.255		1.939
(*Z*)-3-hexen-1-yl 3-methylbutyrate	0.314		0.485

Table 2. Analytical Measurements for Beverage Bases

base	base type	°Brix	acidity as citric acid 0 aq (%)	pH
1	sucrose syrup Brix 9/acidity 0.2	9.0	0.170	2.8
2	sucrose syrup Brix 9/acidity 0.3	9.1	0.265	2.6
3	sucrose syrup Brix 12/acidity 0.2	11.9	0.167	2.8
4	sucrose syrup Brix 12/acidity 0.3	12.1	0.258	2.7
5	Twinsweet 0.19/citric acid 4.2	0.2	0.188	2.8
6	Twinsweet 0.19/citric acid 6.5	0.3	0.295	2.7
7	Twinsweet 0.25/citric acid 4.2	0.2	0.189	2.8
8	Twinsweet 0.25/citric acid 6.5	0.4	0.296	2.7
9	Twinsweet 0.4/citric acid 4.2	0.2	0.192	2.8
10	Twinsweet 0.4/citric acid 6.5	0.4	0.300	2.7

for each of the flavor chemicals. Four commercial flavors (orange 74 and 46, strawberry 52 and 24) were also made available by Quest.

Sample Preparation for Retronasal Evaluation of Flavor Mixtures. The flavor mixtures were prepared by combining either eight or four flavor chemicals, as indicated in **Table 1**. The amount of each flavor chemical in each mixture takes into account the dosage (0.4 g/L) of the 1% (w/w) ethanol solution of this mixture used when the samples were prepared in the appropriate bases. Dosage was constant for all bases and mixtures.

Preparation of Standard Beverage Bases 1−4 (Table 2). Four standard bases for retronasal and gustatory evaluations were made on a pilot-plant scale from sugar syrup (67%) and citric acid (47%, 0 aq), with sodium benzoate and ascorbic acid as preservatives. The full factorial design for these bases had two levels of Brix (9 and 12) and two levels of acidity (0.2 and 0.3).

Preparation of Twinsweet Bases 5−10 (Table 2). In the experiment using artificial sweeteners, bases were made in the laboratory on the day of the experiment. **Table 2** indicates the amounts of Twinsweet LA (34−37% acesulfame-K, 63−66% aspartame, Holland Sweetener Co., Geleen, The Netherlands) and citric acid (47%, 0 aq) that were mixed for each base.

Preparation of Samples for Artificial Throat Experiments. Mixture 1 (**Table 1**) was applied to each of the 10 bases defined in **Table 2**. Preparation was in duplicate; 0.4 g of a 0.1% solution was made up to 100 mL with the appropriate base.

Preparation of Samples for Static Headspace Measurements. For static headspace measurements, hexanal (0.03 g of a 1% EtOH solution made up to 100 mL with each of the 10 bases defined in **Table 2**) was measured separately from a mixture of the 7 other components to avoid an overlapping of retention times for hexanal and ethyl butyrate. The seven-component mixture was made four times (four batches) from 1% EtOH solutions of isoamyl acetate (0.6 g), ethyl butyrate (1.4 g), (*E*)-2-hexenal (2.0 g), (*Z*)-3-hexen-1-yl acetate (2.0 g), (*Z*)-3-hexen-1-yl formate (0.8 g), (*Z*)-3-hexen-1-yl hexanoate (1.6 g), (*Z*)-3-hexen-1-yl 3-methylbutyrate (0.4 g) to give a total weight of 8.8 g. An aliquot of this 7-component mixture (0.44 g) was made up to 100 mL with each of the 10 bases defined in **Table 2**.

Measurement of Total Soluble Solids (Table 2). Brix (in degrees), or total soluble solids, was measured with an RE40 refractometer (Mettler Toledo). Generally speaking, the degree of Brix corresponds to the percent (w/w) sucrose in the base.

Table 3. Experimental Properties of Volatile Flavor Compounds Used in This Study

flavor compound	ion measured (m/z) APCI-MS	retention time (min) GC-HS
(*E*)-2-hexenal	99	14.31
hexanal	101	12.36
ethyl butyrate	117	12.36
(*Z*)-3-hexen-1-yl formate	129	16.65
isoamyl acetate	131	15.07
(*Z*)-3-hexen-1-yl acetate	143	19.63
(*Z*)-3-hexen-1-yl 3-methylbutyrate	185	26.83
(*Z*)-3-hexen-1-yl hexanoate	199	31.01

Measurement of Acidity (Table 2). Total acidity was measured as citric acid 0 aq (%, w/w) by titrating 5 mL of the beverage to pH 8.2 with 0.1 N NaOH using a DL70ES automatic titrator (Mettler Toledo).

pH Measurements (Table 2). The pH of bases was measured with a pH Mettler glass probe (Mettler Toledo). Calibration and measurements were at room temperature.

Dynamic Headspace with Artificial Throat. The MS−Nose atmospheric pressure chemical ionization gas-phase analyzer (APCI-GPA) apparatus and operating procedure for the artificial throat have been described elsewhere (*20*). Samples (5 mL) were released (air flow = 1 L/min) in the artificial throat, which was maintained at 37 °C. Three repetitions of each base were made: two measurements from one 100 mL preparation and the third measurement from the other 100 mL preparation.

Air was sampled for APCI-GPA (50 mL/min) through a capillary tube (0.53 mm i.d., heated to 100 °C). Source and probe temperatures were 80 °C. The compounds were ionized by a 3.0 kV discharge and monitored in selected ion mode (0.08 s dwell on each ion). Ions measured (m/z) are given in **Table 3**. The area below the release curve (AUC) was integrated and multiplied by a correction factor representing the exact weight of the mixture in each 100 mL base preparation.

For statistical analysis, an ANOVA using mean calculated AUC for each of the 8 flavor compounds in each of the 10 bases was determined. Per flavor compound, the following model was used: AUC = Brix + acidity + error. Two levels of Brix were defined: a high level for the sucrose syrup bases 1−4 and a low level for the Twinsweet bases 5−10. The two levels of acidity were high (bases 2, 4, 6, 8, and 10) and low (bases 1, 3, 5, 7, and 9).

Static Headspace. A TraceGC Ultra (Thermo Electron) equipped with a FID, PTV injector (BEST), and a cryogenic interface (Interscience) was used. A Combi-Pal headspace injector (CTC Analytics) with a 2.5 mL syringe was used for sample introduction. Headspace concentrations were expressed as peak areas in arbitrary units (EZ Chrom Elite, Scientific Software).

Samples (10 mL) were placed in 20 mL inert vials (Chromacol) and equilibrated for 10 min at 60 °C. Previous experiments in which measurements were made every minute showed that equilibrium was reached after 6 min. The time and temperature chosen for equilibration in these experiments are in keeping with standard procedures that ensure equilibrium and avoid condensation. Headspace injection (1 mL) was performed with a syringe temperature of 70 °C, which was chosen to be slightly higher than the equilibrium temperature to avoid condensation. The headspace was introduced in the cryogenic interface (−100 °C) and injected in the splitless mode with an injector temperature of 280 °C. The column used for analysis was a 50 m × 0.32 mm i.d., 1 μm film HP5 (J&W) with a constant flow of 2 mL of helium/min. Splitless injection was performed under the following conditions: splitless time of 2 min and an oven temperature program of 40 °C for 2 min, 5 °C/min to 250 °C, hold at limit for 16 min. Retention times (**Table 3**) were determined by injecting the pure flavor compounds, the identifcations of which were confirmed by GC-MS. Reproducibility of AUC based on five injections was ±5%.

For statistical analysis, an ANOVA using mean peak areas for each of the 8 flavor compounds in each of the 10 bases was determined. Per flavor compound, with the exception of hexanal, the following

Table 4. Main Effects (Given as Difference in Intensity Scores for High–Low Levels) and Their Associated *p* Values from REML Analysis of Profiling Mixture 1 (**Table 1**) on Sucrose Syrup Bases 1–4 and Twinsweet Bases 7–10, Respectively (**Table 3**)

	sucrose syrup bases 1–4				Twinsweet bases 7–10			
	Brix		acidity		Twinsweet		citric acid	
descriptor	H–L	*p*	H–L	*p*	H–L	*p*	H–L	*p*
sour	**–14.8**	**0.0117**	**30.4**	**<0.0001**	**–14.7**	**0.0063**	**27.6**	**<0.0001**
sweet	**29.6**	**<0.0001**	**–19.3**	**0.0010**	**15.9**	**0.0003**	**–10.1**	**0.0072**
bitter	**–10.6**	**0.0245**	*6.3*	*0.0829*	7.2	0.4084	–6.8	0.3140
apple green	*13.1*	*0.0859*	–7.9	0.3973	8.0	0.3672	–0.7	0.9413
fruity	**15.7**	**0.0500**	4.0	0.5708	6.2	0.2465	3.1	0.5714
green	*–14.9*	*0.0541*	*13.7*	*0.0658*	–7.2	0.3725	0.7	0.9126
pear/candy	**20.9**	**0.0044**	–1.3	0.8504	–3.2	0.6450	–4.2	0.5501

model was used: AUC = block + Brix + acidity + error. Block indicates the batch of sample preparation for the seven-compound mixture. There is no block factor for hexanal; hence, the model used for static headspace is the same as the model used for dynamic headspace. Brix and acidity factors are defined as above for dynamic headspace.

Sensory Panelists. A paid, professional panel consisting of 20 women, who work 2 h sessions 4 days of the week, served as evaluators in all experiments discussed in this paper.

Presentation of Samples for Sensory Evaluation. All samples, coded with randomly chosen three-digit numbers, were served at room temperature (21 ± 1 °C). For retronasal evaluation of samples in aqueous bases, 50 mL portions were served in brown plastic cups.

Design for Profiling Mixture 1 on Bases 1−4 and 7−10. Evaluation of the eight-component flavor mixture can be seen as two full-factorial designs with respect to the bases, one for sucrose syrup and the other for Twinsweet.

Design for Profiling Mixtures 2 and 3 on Bases 1, 3, 5, and 9. Mixtures 2 and 3 were evaluated together in one day on either bases 1 and 3 or bases 5 and 9. The repetitions separated the flavors so that either mixture 2 or mixture 3 was evaluated on all of the four bases.

Design for Profiling Orange and Strawberry Flavors on Bases 1−6, 9, and 10. The experimental design for four samples/day was restricted to only one flavor type in order to use the same descriptor list for all samples. Bases with both sweetener types (sucrose syrup or Twinsweet, same level of acidity/sweetener type) and both levels of sucrose syrup (Brix 9 and 12) or Twinsweet (0.19 and 0.4) were included on each day.

Sensory Profiling. The choice of descriptors and the measurement of intensity have been described in the preceding paper (*18*). For statistical analysis of profiling data, variance components were fitted using REML, where panelists and all interactions with panelists were considered to be random effects. Design variables and all of their two-way interactions were considered to be fixed effects. Full details of these analyses were provided in the preceding paper (*18*).

Equisweet Determination for Bases with Twinsweet. In one experiment panelists scaled (audio method) the sweetness of three concentrations of Twinsweet (0.1, 0.25, and 0.4 g/L) at constant acidity (4.2 g/L citric acid, 47%, 0 aq) against base 1 as reference. The reference sweetness (Brix 9) was defined as the start tone (500 Hz). In a second experiment the reference was base 3 (Brix 12 defined as start tone) and the Twinsweet concentrations were 0.25, 0.4, and 0.6 g/L at constant acidity (4.2 g/L citric acid, 47%, 0 aq). Both experiments were repeated. Equivalent sweetness for each Brix level was determined by linear regression of the unscaled sweetness scores.

RESULTS AND DISCUSSION

Flavor Perception of GLVs from Bases Containing either Sucrose Syrup or Twinsweet. The main effects for Brix and acidity obtained from profiling data for mixture 1 applied as flavor to sucrose syrup bases 1−4 and Twinsweet bases 7−10 are given in **Table 4**. The base effects on gustatory descriptors *sweet* and *sour* were significant for both the sucrose syrup and the Twinsweet bases. **Table 4** indicates comparable perceptual intensity differences for *sour*, independent of the sucrose syrup/Twinsweet base. Intensity differences for *sweet*, on the other hand, were larger between Brix levels 9 and 12 (29.6) than between Twinsweet levels 0.25 and 0.4 g/L (15.9). The Twinsweet concentrations, chosen in keeping with general use, were not equisweet to Brix 9−12 as determined experimentally. Equivalency in scores for *sweet* for these bases was calculated from the experimental data as 0.19 g/L Twinsweet = Brix 9 and as 0.28 g/L Twinsweet = Brix 12. On the basis of dose−response curves for sweetness as a function of aqueous Twinsweet concentration (0.14−0.52 g/L), the range used to profile mixture 1 and the range calculated as equisweet to the Brix were both in the approximately linear portion of the curve. If that had not been the case and a plateau of maximum sweetness had already been attained in the higher concentration range, then one could have anticipated a smaller difference in scores for *sweet* when profiling the Twinsweet beverages.

For sucrose-syrup bases, **Table 4** shows significant positive Brix effects for the retronasal descriptors *pear/candy* and *fruity* and a positive Brix trend for *apple green*. There was a negative Brix trend for *green*. The acidity effect was present as a positive trend only for the descriptor *green*. There were no significant effects for sweetener or acidity on any of the retronasal descriptors when using Twinsweet bases.

Mixture 1 contained GLVs together with isoamyl acetate and ethyl butyrate, which created a rounder flavor. The four-component flavors, mixtures 2 and 3, separated those GLVs with a decided green character (mixture 2) from the more fruity flavor compounds (mixture 3). Mixtures 2 and 3 were profiled on bases that had equivalent acidity but different types and levels of sweetener. The concentrations of Twinsweet for these experiments (0.40−0.19 = 0.21 g/L difference in concentration) were chosen to give a much broader range than would have been required for equisweetness with the sucrose syrup bases (0.28−0.19 = 0.09 g/L difference in Twinsweet concentration). Although REML analyses showed that intensity scores for the gustatory descriptor *sweet* were dependent only on the sweetener level and not on the sweetener type or on flavor, differences in intensity for *sweet* were indeed larger for Twinsweet (36.6) than for sucrose syrup (19.7), as indicated in **Table 5A**.

As sweetener level in the mixtures increased, independent of sweetener type, scores for *apple* increased ($p = 0.0156$) and scores for *cucumber* decreased ($p = 0.0766$). **Table 5A** shows a sweetener dependence for the descriptor *flowers*; increasing sucrose syrup decreases the intensity, whereas a comparable increase in intensity was shown for an increase of Twinsweet. When sucrose syrup was used, the fruity mixture 3 was scored as more sweet than the green mixture 2, whereas with Twinsweet the green mixture was scored as more sweet (**Table 5B**).

All of the retronasal flavor descriptors had significant flavor effects, which means that the two mixtures were indeed different, as could be expected. Mixture 2 (green) scored higher than mixture 3 on *apple*, *apple green*, *banana*, *cucumber*, and *green*. Mixture 3 (fruity) scored higher than mixture 2 on *flowers*, *fruity*, *pear/candy*, and *tin/metallic* ($p = 0.0842$ for *tin/metallic*). Normally, scores for *tin/metallic* decrease with an increase in sweetener level (*13*). **Table 5C** shows that this was true only for mixture 2; there was virtually no effect on this descriptor for mixture 3. Another context-dependent descriptor shown in this table is *apple green*, which increased with sweetener level

Table 5. Differences in Intensity for the Interaction Terms Used To Model Profiling Descriptor Scores in the Experiments with Flavor Mixtures 2 and 3 (**Table 1**); Bases Contained either Sucrose Syrup (SuSy) or Twinsweet (TwSw)

A. Sweetener Level × Sweetener Type:
Change in Intensity per Descriptor When Going from
Low to High Level of Sweetener

descriptor	SuSy	TwSw	*p*
sweet	19.7	36.6	**0.0019**
flowers	–8.5	9.4	**0.0067**

B. Flavor × Sweetener Type:
Change in Intensity per Descriptor When Scorese for
Green Mixture 2 Were Subtracted from Scores for *Fruity* Mixture 3

descriptor	SuSy	TwSw	*p*
sweet	12.5	–5.6	**0.0015**

C. Sweetener Level × Flavor:
Change in Intensity per Descriptor When Going from
Low to High Level of Sweetener

descriptor	green mixture 2	fruity mixture 3	*p*
tin/metallic	–12.4	3.1	*0.0578*
apple green	–2.7	12.2	*0.0603*

only in mixture 3, the mixture that did not contain (*E*)-2-hexenal and therefore had lower absolute scores for this descriptor.

When the commercial orange and strawberry flavors were profiled, descriptors related to the *cooked*, *fruity* (*orange* and *mandarin* but not *lemon* or *grapefruit*), and *flowery* aspects of the orange flavors increased with an increase in the sweetener level, whereas scores for *aldehyde*, *green*, and *terpenes* decreased, as we have reported elsewhere for carbonated orange beverages (*13*). The size of these effects depended on the profile of the particular flavor. Likewise for strawberry flavors, fruit-related descriptors normally increased with higher sweetener level, whereas the descriptors *green* and *tin/metallic* decreased. The effect of acidity level was generally in the opposite direction from that of the sweetener level. These base effects occurred with a difference of approximately 3 Brix for the beverages containing sucrose syrup as well as for the Twinsweet bases having a maximum difference of 0.2 Brix.

Significant interaction terms must be considered to understand the main effects attributed to components in these beverage bases. **Table 6A** shows that interaction term sweetener level × sweetener type was significant for the descriptor *sweet* in both orange flavors (larger difference for Twinsweet than for sugar syrup) but not in the strawberry flavors. This table also shows that Twinsweet reduces bitterness less in both types of flavors than sucrose syrup does. However, the fruity notes—whether in orange or strawberry flavors—are also increased more by higher levels of Twinsweet than by higher levels of sucrose syrup, albeit not consistently in every flavor.

The interaction acidity level × sweetener type is shown in **Table 6B**. Strawberry flavor 52 was more sensitive to an increase in citric acid (larger increase in scores for *sour*) when Twinsweet was used than when sucrose syrup was the sweetener. This effect was not seen for either of the orange flavors. Increased acidity with Twinsweet did increase scores for *orange* with orange flavor 74; sucrose syrup reduced these scores slightly.

The third interaction term, sweetener level × acid level, is shown in **Table 6C**. In orange flavor 46, increasing sweetener level increased the scores for *sweet* to a much lesser extent when the higher acidity level was used. Increasing sweetener level at low acidity generally decreased scores for *bitter* except for strawberry flavor 24. At lower acidity, increasing sweetener level decreased the *aldehyde* scores for both orange flavors and the *grapefruit* scores for one orange flavor.

The significant sweetener/acidity base effects on retronasal odor descriptors have always been accompanied by significant perceptible differences on the descriptors *sweet* and *sour*, independent of the tastants used to make the bases. Practically speaking, these are requisite conditions for considering base effects when flavoring beverages. We have shown (*18*) that the GLVs did not affect the gustatory descriptors, whereas tastants did affect scores on retronasal olfactory descriptors for these flavor compounds. The data in this paper confirm that the measurement of intensity on gustatory descriptors was not affected by the flavor. There are, however, flavor-dependent interactions such as those shown in **Tables** 5 and **6**, and these interactions can be statistically significant without having significant corresponding main effects.

There have been discussions in the literature with regard to fruitiness enhancement. Some authors claimed that orange-flavored beverages (and to a lesser extent strawberry-flavored beverages) showed fruitiness enhancement when sweetened by aspartame but not when sweetened by sucrose (*26*). Our data do not support such a generalization. Within each of these flavor types, fruitiness (or a more specific fruit-dependent descriptor) will increase with a sufficiently large increase in perceived sweetness or a sufficiently large decrease in acidity.

Other authors (*5*, *6*) showed an increase in the fruitiness of an orange flavor when either sugar, aspartame, or citric acid was increased. We have shown that the *sweet*–*sour* descriptions are actually a bipolar scale as far as fruits are concerned (*13*). When descriptors such as *lemon* or *grapefruit* are used, the intensity of these descriptors increases with increased acidity and decreases with increased sweetener. Thus, the base effects we described are directionally different for various aspects of what might be measured under the general term *fruitiness*. When adequate descriptors are employed, base effects for many different fruit flavors can be measured (*2*, *13*).

Flavor Release from Sucrose Syrup and Twinsweet Bases As Measured by Static and Dynamic Headspace. Acidity effects were found for only two flavor compounds: hexanal and (*Z*)-3-hexen-1-yl formate. For hexanal in the static headspace, there was a reduction of 6.13% ($p = 0.0759$) as acidity increased. The static headspace measurements for (*Z*)-3-hexen-1-yl formate showed a significant effect of acidity ($p < 0.0001$), indicating a reduction of 16.34% as acidity increased. In the dynamic headspace there was a reduction of 8.34% ($p = 0.0923$) for (*Z*)-3-hexen-1-yl formate as acidity increased. The negative acidity effects for hexanal and (*Z*)-3-hexen-1-yl formate might be the result of instability of these compounds in aqueous media at low pH. Sensorially, only positive acidity effects were encountered with respect to the green nature of these two compounds.

Table 7 summarizes the Brix effect on flavor release, defined by a comparison of sucrose syrup (high Brix) with Twinsweet (low Brix). Dynamic measurements indicated higher concentrations in the headspace above the Twinsweet bases for hexanal, (*Z*)-3-hexen-1-yl 3-methylbutyrate, (*Z*)-3-hexen-1-yl hexanoate, and, to a lesser extent, (*Z*)-3-hexen-1-yl formate. Static headspace, on the other hand, showed significantly higher concentrations in the headspace above Twinsweet bases for (*Z*)-3-hexen-1-yl formate, ethyl butyrate, and isoamyl acetate and a tendency for (*Z*)-3-hexen-1-yl acetate, (*E*)-2-hexenal, and hexanal as well.

Table 6. Differences in Intensity for the Interaction Terms Used To Model Profiling Descriptor Scores in the Experiments with Orange and Strawberry Flavors, Where Bases Contained either Sucrose Syrup (SuSy) or Twinsweet (TwSw)

A. Sweetener Level × Sweetener Type: Change in Intensity per Descriptor When Going from Low to High Level of Sweetener

	orange 74			orange 46				strawberry 52			strawberry 24		
descriptor	SuSy	TwSw	*p*	SuSy	TwSw	*p*	descriptor	SuSy	TwSw	*p*	SuSy	TwSw	*p*
sour	–15.9	–27.6	*0.0913*	–12.2	–11.8	0.9369	*sour*	–15.9	–15.2	0.8924	–10.6	–13.9	0.5936
sweet	33.3	49.5	**0.0445**	18.1	33.0	**0.0051**	*sweet*	24.3	30.0	0.3544	23.4	27.6	0.5068
bitter	–15.8	7.3	**0.0141**	–11.8	–8.0	0.5786	*bitter*	–10.9	2.1	*0.0787*	–5.0	3.5	0.2861
							strawberry	10.7	10.8	0.9953	–5.6	6.2	**0.0486**
terpenes	3.5	–10.4	**0.0160**	0.5	–12.3	0.1227							
mandarin	30.7	27.2	0.7251	10.5	31.5	**0.0096**							
fruity	–1.1	17.7	**0.0241**	11.2	15.4	0.5246							
orange	11.3	15.3	0.5999	4.4	20.3	**0.0070**							

B. Acidity Level × Sweetener Type: Change in Intensity per Descriptor When Going from Low to High Level of Citric Acid

	orange 74			orange 46				strawberry 52			strawberry 24		
descriptor	SuSy	TwSw	*p*	SuSy	TwSw	*p*	descriptor	SuSy	TwSw	*p*	SuSy	TwSw	*p*
sour	20.9	16.2	0.5569	15.5	17.4	0.7568	*sour*	5.7	25.6	**0.0400**	14.5	17.5	0.6835
aldehyde	–16.5	0.3	0.1629	–11.8	4.3	*0.0593*							
orange	–4.2	13.1	**0.0488**	5.3	–1.9	0.3105							
lemon	16.3	11.4	0.6138	8.7	23.5	*0.0654*							

C. Sweetener Level × Acidity Level: Change in Intensity per Descriptor When Going from Low to High Level of Sweetener

	orange 74			orange 46				strawberry 52			strawberry 24		
	acidity			acidity				acidity			acidity		
descriptor	low	high	*p*	low	high	*p*	descriptor	low	high	*p*	low	high	*p*
sour	–21.6	–21.9	0.9575	–15.9	–8.2	0.1661	*sour*	–10.8	–20.3	*0.0931*	–10.2	–14.3	0.5018
sweet	41.0	41.9	0.9057	31.8	19.3	**0.0295**	*sweet*	28.4	25.8	0.6668	28.0	22.9	0.4306
bitter	–11.2	2.7	0.1432	–20.0	0.2	**0.0064**	*bitter*	–7.0	–1.9	0.4890	7.5	–9.1	**0.0383**
aldehyde	–21.0	8.2	**0.0044**	–12.2	11.0	**0.0012**							
grapefruit	–7.0	–14.4	0.3818	–9.2	6.3	**0.0253**							
lemon	–11.8	–29.0	*0.0520*	–4.2	–12.8	0.2586							

Table 7. Changes in Dynamic and Static Headspaces for Flavor Compounds as Brix Decreases, Going from Sucrose Syrup Bases 1–4 to Twinsweet Bases 5–10

	dynamic HS model 1[a]		static HS model 2[b]	
flavor compound	change[c] (%)	*p*	change[c] (%)	*p*
(*E*)-2-hexenal	1.29	0.9055	*8.30*	*0.0800*
hexanal	**15.91**	**0.0010**	*6.07*	*0.0811*[d]
ethyl butyrate	–2.10	0.4816	**11.83**	**0.0028**
(*Z*)-3-hexen-1-yl formate	*9.97*	*0.0519*	**20.01**	**0.0003**
isoamyl acetate	–1.84	0.5709	**10.60**	**0.0140**
(*Z*)-3-hexen-1-yl acetate	*–7.11*	*0.0991*	*9.31*	*0.0632*
(*Z*)-3-hexen-1-yl 3-methylbutyrate	**32.06**	**0.0002**	–0.41	0.9477
(*Z*)-3-hexen-1-yl hexanoate	**24.61**	**0.0028**	1.69	0.6167

[a] Area under curve = Brix + acidity + error. [b] Area under curve = block + Brix + acidity + error. [c] Brix (Twinsweet – Sucrose syrup); therefore, positive values indicate an increase in HS as Brix decreases. [d] Calculated with model 1 because there were no blocks for hexanal sample preparation for static HS.

Higher concentrations above the Twinsweet bases than above the sucrose syrup bases imply a negative Brix effect (greater flavor release at lower Brix). Therefore, negative Brix effects would be the opposite of salting-out, which is the term for an increase in volatility relative to water.

Voilley et al. (*27*) attributed the increase in headspace concentrations of acetone and 1-octanol to an increase in the mole fraction in the liquid phase when sucrose was added to water. Activity coefficients for these odorants remained constant, also for the addition of citric acid, at the solute concentrations relevant for beverages. Relative volatility for selected flavor chemicals in aqueous sucrose solutions was shown to increase as hydrophobicity increased, but there was little change with sucrose concentration in the region (<15%) relevant to beverages (*3*, *25*). Flavor polarity has been postulated as an explanation for volatility from sucrose solutions (*17*). Some flavor molecules have been shown to undergo opposite effects with respect to headspace enrichment/depletion depending on whether the sweetener aspartame or acesulfame-K, the two components of Twinsweet, was used (*24*). Lypophilic impurities do occur in industrial sucrose syrup. These impurities could hold the more lypophilic flavor compounds, such as (*Z*)-3-hexen-1-yl 3-methylbutyrate and (*Z*)-3-hexen-1-yl hexanoate, in solution, although the other effects in **Table 7** are not adequately explained by the presence of such impurities.

A 20% change, such as that shown in **Table 7** for (*Z*)-3-hexen-1-yl formate, could have sensory significance, and (*Z*)-3-hexen-1-yl formate did show consistently high *green* intensity scores that increased as Brix decreased. A similar case could be made for ethyl butyrate, but not for isoamyl acetate, on the basis of the Page test described previously (*18*). Note that the Page test used the opposite formulation, namely, that there was a significant decrease in *green* scores when progressing from the lowest to the highest of the three sucrose concentrations (i.e., increasing Brix). A 20% increase in ethyl butyrate headspace from fruit punch when going from 11 to 14 Brix, but no change in the headspace of ethyl butyrate from a citrus base, has been reported (*10*). According to these authors, ethyl butyrate from breath measurements of both beverages was unchanged as Brix increased.

The static headspace data showed a tendency for hexanal and (*E*)-2-hexenal to release more from the Twinsweet bases (lower Brix) than from the sucrose syrup bases. These results are contrary to those of Nahon and co-workers (*14*), who found no significant difference in flavor release for hexanal and (*E*)-2-hexenal in an orange aroma when 10% sucrose and its equisweet concentration of sodium cyclamate were compared. Both hexanal and (*E*)-2-hexenal were classified by these authors as belonging to a medium retention time group for which increasing sucrose concentration did not change the flavor release. Our Page test (*18*), on the other hand, showed that increasing sucrose (4, 7, and 12%) significantly affected flavor perception of both hexanal and (*E*)-2-hexenal. The headspace data could support only the negative Brix effect for green notes and not the positive Brix effect for fruity notes perceived.

In **Table 7** there is an indication of a trend toward higher (*Z*)-3-hexen-1-yl acetate release with higher Brix (sucrose syrup as opposed to Twinsweet) in the dynamic headspace but not in the static measurements. Both Rabe et al. (*15*) and Hansson et al. (*16*) showed a significant increase in the flavor release of (*Z*)-3-hexen-1-yl acetate from aqueous sucrose solutions when going from 20 to 50%, respectively, 20 to 60%, sucrose. These concentrations are far above the range used in our experiments. Neither paper showed significant change in the 5−20% range, which would be more representative of sucrose concentrations used in soft drinks.

Our dynamic headspace measurements showed a significant increase when going from sucrose syrup to Twinsweet bases for hexanal but not for isoamyl acetate and (*E*)-2-hexenal. These measurements support the decreased greenness of hexanal and (*E*)-2-hexenal with higher Brix but not the increased fruitiness also found for (*E*)-2-hexenal. In MS−Nose experiments using model dairy desserts flavored with a mixture of ethyl pentanoate, isoamyl acetate, hexanal, and (*E*)-2-hexenal, perceived flavor intensity, but not flavor release, increased with an increase in sucrose level (*9*). In fact, for desserts containing ι-carrageenan, $I_{\max}$ for (*E*)-2-hexenal decreased as the sucrose level increased. Flavor was evaluated by these authors with just one descriptor, *flavor*, and not profiled, which means that differences between the *green* and *fruity* characters of these compounds cannot be assessed.

The dynamic headspace showed sufficiently large changes for (*Z*)-3-hexen-1-yl 3-methylbutyrate and (*Z*)-3-hexen-1-yl hexanoate that these should be perceptible. These data imply a negative Brix effect, but the sensory data support only a positive Brix effect for these compounds, namely, a significant increase in fruitiness as Brix increased.

Variations in acidity and Brix within the range of normal beverage compositions were claimed to be insufficient to affect flavor release in the headspace from orange drinks as measured by MS−Nose (*28*). It is difficult to explain—in terms of flavor release—both increased and decreased flavor perception for components of the same mixture when a solute such as sucrose or Twinsweet is added. The fact that both Twinsweet and sucrose syrup can increase cooked and fruity (or fruit-related) notes while decreasing green, tin/metallic, aldehydic, and terpenic notes shows that the effect is not related to soluble solids in the solution (Brix) but probably to the sweetness created by these solutes.

LITERATURE CITED

(1) Deibler, K. D.; Acree, T. E. Effect of beverage base conditions on flavor release. In *Flavor Release*; Roberts, D. D., Taylor, A. J., Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 333−341.

(2) Malundo, T. M. M.; Shewfelt, R. L.; Ware, G. O.; Baldwin, E. A. Sugars and acids influence flavor properties of mango (*Mangifera indica*). *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 115−121.

(3) Godshall, M. A. How carbohydrates influence food flavor. *Food Technol.* **1997**, *51* (1), 63−67.

(4) Valdés, R. M.; Simone, M. J.; Hinreiner, E. H. Effect of sucrose and organic acids on apparent flavor intensity II. Fruit nectars. *Food Technol.* **1956**, *10*, 387−390.

(5) Stampanoni, C. R. Influence of acid and sugar content on sweetness, sourness and the flavor profile of beverages and sherbets. *Food Qual. Pref.* **1993**, *4*, 169−176.

(6) Bonnans, S.; Noble, A. C. Effect of sweetener type and of sweetener and acid levels on temporal perception of sweetness, sourness and fruitiness. *Chem. Senses* **1993**, *18*, 273−283.

(7) Hort, J.; Hollowood, T. A. Controlled continuous flow delivery system for investigating taste−aroma interactions. *J. Agric. Food Chem.* **2004**, *52*, 4834−4843.

(8) Cook, D. J.; Davidson, J. M.; Linforth, R. S. T.; Taylor, A. J. Measuring the sensory impact of flavor mixtures using controlled delivery. In *Handbook of Flavor Characterization*; Deiler, K. D., Delwiche, J., Eds.; Dekker: New York, 2003; pp 135−149.

(9) Lethuaut, L.; Weel, K. G. C.; Boelrijk, A. E. M.; Brossard, C. D. Flavor perception and aroma release from model dairy desserts. *J. Agric. Food Chem.* **2004**, *52*, 3478−3485.

(10) Asquith, T. N.; Swaine, R. L., Jr. Effects of high-fructose corn syrup on perception and release of flavors in soft drinks. In *Challenges in Taste Chemistry and Biology*; Hofmann, T., Ho, C.-T., Pickenhagen, W., Eds.; ACS Symposium Series 867; American Chemical Society: Washington, DC, 2004; pp 254−262.

(11) Nahon, D. F.; Roozen, J. P.; De Graaf, C. Sensory evaluation of mixtures of sodium cyclamate, sucrose, and an orange aroma. *J. Agric. Food Chem.* **1998**, *46*, 3426−3430.

(12) von Sydow, E.; Moskowitz, H.; Jacobs, H.; Meiselman, H. Odor−taste interactions in fruit juices. *Lebensm.-Wiss. Technol.* **1974**, *7*, 18−24.

(13) King, B. M.; Duineveld, C. A. A.; Arents, P.; Meyners, M.; Schroff, S. I.; Soekhai, S. T. Retronasal odor dependence on tastants in profiling studies of beverages. *Food Qual. Pref.* **2006**, *17*, in press.

(14) Nahon, D. F.; Navarro y Koren, P. A.; Roozen, J. P.; Posthumus, M. A. Flavor release from mixtures of sodium cyclamate, sucrose, and an orange aroma. *J. Agric. Food Chem.* **1998**, *46*, 4963−4968.

(15) Rabe, S.; Krings, U.; Berger, R. G. Dynamic flavor release from sucrose solutions. *J. Agric. Food Chem.* **2003**, *51*, 5058−5066.

(16) Hansson, A.; Andersson, J.; Leufvén, A. The effect of sugars and pectin on flavour release from a soft drink-related model system. *Food Chem.* **2001**, *72*, 363−368.

(17) Roberts, D. D.; Elmore, J. S.; Langley, K. R.; Bakker, J. Effects of sucrose, guar gum, and carboxymethylcellulose on the release of volatile flavor compounds under dynamic conditions. *J. Agric. Food Chem.* **1996**, *44*, 1321−1326.

(18) King, B. M.; Arents, P.; Duineveld, C. A. A.; Meyners, M.; Schroff, S. I.; Soekhai, S. T. Orthonasal and retronasal perception of some green leaf volatiles used in beverage flavors. *J. Agric. Food Chem.* **2006**, *54*, 2664−2670.

(19) European Patent Application EP 1 494 027 A1, Artificial Throat; Quest International B.V., 2003.

(20) Weel, K. G. C.; Boelrijk, A. E. M.; Burger, J. J.; Jacobs, M. A.; Gruppen, H.; Voragen, A. G. J.; Smit, G. Effect of emulsion properties on release of esters under static headspace, in vivo, and artificial throat conditions in relation to sensory intensity. *J. Agric. Food Chem.* **2004**, *52*, 6572−6577.

(21) Hodgson, M. D.; Langridge, J. P.; Linforth, R. S. T.; Taylor, A. J. Aroma release and delivery following the consumption of beverages. *J. Agric. Food Chem.* **2005**, *53*, 1700−1706.

(22) Rabe, S.; Linforth, R. S. T.; Krings, U.; Taylor, A. J.; Berger, R. G. Volatile release from liquids: a comparison of *in vivo* APCI-MS, in-mouth headspace trapping and *in vitro* mouth model data. *Chem. Senses* **2004**, *29*, 163−173.

(23) Banavara, D. S.; Rabe, S.; Krings, U.; Berger, R. G. Modeling dynamic flavor release from water. *J. Agric. Food Chem.* **2002**, *50*, 6448−6452.

(24) Deibler, K. D.; Lavin, E. H.; Linforth, R. S. T.; Taylor, A. J.; Acree, T. E. Verification of a mouth simulator by *in vivo* measurements. *J. Agric. Food Chem*. **2001**, *49*, 1388−1393.

(25) Friel, E. N.; Linforth, R. S. T.; Taylor, A. J. An empirical model to predict the headspace concentration of volatile compounds above solutions containing sucrose. *Food Chem*. **2000**, *71*, 309−317.

(26) Wiseman, J. J.; McDaniel, M. R. Modification of fruit flavors by aspartame and sucrose. *J. Food Sci*. **1991**, *56*, 1668−1670.

(27) Voilley, A.; Simatos, D.; Loncin, M. Gas-phase concentration of volatiles in equilibrium with a liquid aqueous phase. *Lebensm. Wiss. Technol*. **1977**, *10*, 45−49.

(28) Zehentbauer, G.; Asquith, T.; Li, J.-J. The use of real-time gas-phase analysis as a tool to optimize aroma composition in beverages. In *Flavour Research at the Dawn of the Twenty-First Century*; Le Quéré, J. L., Etiévant, P. X., Eds.; Intercept: London, U.K., 2003; pp 159−163.

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