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Orthonasal and Retronasal Perception of Some Green Leaf Volatiles Used in Beverage Flavors

BONNIE M. KING,* PAUL ARENTS, C. A. A. DUINEVELD, M. MEYNERS, S. I. SCHROFF, AND S. T. SOEKHAI

Quest International Naarden, P.O. Box 2, 1400 CA Bussum, The Netherlands

Orthonasal perception of six green leaf volatiles (GLVs) classified only hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-yl formate as *green*. (*Z*)-3-Hexen-1-yl hexanoate and (*Z*)-3-hexen-1-yl 3-methylbutyrate were more floral, and (*Z*)-3-hexen-1-yl acetate fell between the two groups. For retronasal perception, classification along a bipolar green−fruity scale is proposed for describing these GLVs. Data from grouping and dissimilarity tests as well as from sensory profiling show green character for these compounds in standard beverage bases having low Brix or high acidity. As the Brix value increases (or acidity decreases) within the limits encountered in commercial beverages, the character becomes fruity. Several tastant-dependent changes in intensity scores for retronasal descriptors were shown to occur for mixtures of GLVs. The GLVs did not affect intensity scores for gustatory descriptors.

KEYWORDS: Brix/acidity effects; green/fruity flavor perception; sensory profiling

INTRODUCTION

Green leaf volatiles (GLVs) is the common name for saturated and monounsaturated six-carbon aldehydes, alcohols, and esters thereof. Mechanical damage to green leaves releases the characteristic odor of these compounds (*1*−*3*), which is then by association referred to as green-leaf odor or, simply, green. GLVs are formed from lipoxygenase pathways during the production of olive oil (*4*). GLVs are notably present in immature fruits (*5*), but have been reported as green odorous components in a number of fruits and flowers (*6*−*10*). Many of these volatile compounds are used in flavoring food to impart either fruity or green notes (*7*, *11*, *12*). The question then arises as to how distinct a fruity or green quality is, sensorially. Can one separate fruity from green notes, or are they part of a sensory continuum, one that mirrors the ripening process?

To investigate the sensory properties of some GLVs, hexanal, (*E*)-2-hexenal, and four (*Z*)-3-hexen-1-yl esters were studied along with two esters, isoamyl acetate and ethyl butyrate, normally classified as fruity (*6*−*8*, *12*−*16*). Of these six GLVs, hexanal was most often described in the literature as having green, (cut) grass notes (*11*, *15*, *17*−*19*) with some additional qualities such as mint (*11*), herbal (*7*), fatty (*8*), apple (*3*, *12*, *20*), unripe green (*14*), macaroon (a bitter almond cookie), and hedge (*21*). However, hexanal was also described as having a characteristic fruity odor and taste (*12*). Moreover, in a regression study on virgin olive oil (*4*), hexanal was positively correlated with the sensory descriptors *banana* and *almond* and negatively correlated with the descriptors *cut green lawn*, *green leaf*, or *twig*.

Some descriptions attributed to (*E*)-2-hexenal in the literature are *leafy* (*3*, *13*, *17*); *green*, *fatty* (*15*); *green*, *grassy*, *vine*, *stale* (*11*); *green apple like* (*14*, *18*); *green*, *fruity* (*8*, *20*); *almond* (*20*); and *rum* (*21*). Hexanal and (*E*)-2-hexenal, both used in a fruit flavor for yogurts, were often confused by panelists, although (*E*)-2-hexenal was defined for these studies with the descriptor *almond* and hexanal with the descriptor *green apple* (*12*). In the regression study on virgin olive oil mentioned above (*4*), (*E*)-2-hexenal correlated positively with the descriptor *cut green lawn* and negatively with descriptor *banana*, which is just the opposite of what was found for correlations with hexanal. In another study, (*E*)-2-hexenal measured by headspace gas chromatography (GC-HS) from tomato cultivars (year 1996) correlated positively with the sensory analysis taste descriptor *raw green* (*22*).

(*Z*)-3-Hexen-1-yl esters are often described in gas chromatography−olfactometry (GC-O) studies as fruity, green, or fruity-green (*8*). Both *green leaves* and *fruity* were descriptors applied to specifically (*Z*)-3-hexen-1-yl acetate (*14*, *20*), sometimes *green* followed by *banana* as a more precise description of the fruity note (*3*). However, (*Z*)-3-hexen-1-yl acetate was often associated only with banana (*18*) or banana candy (*7*, *16*), although green banana (*15*) occurs. In the regression study on virgin olive oil mentioned above (*4*), (*Z*)-3-hexen-1-yl acetate correlated positively with both descriptors *banana* and *walnut husk*. According to another study on olive oil, (*Z*)-3-hexen-1-yl acetate was classified as *green* according to the olive oil statistical sensory wheel classification (*23*). Other authors attribute only a fruity note to (*Z*)-3-hexen-1-yl acetate (*19*). Fenaroli (*13*) describes (*Z*)-3-hexen-1-yl acetate as having a powerful green, floral note reminiscent of banana. The floral

* Corresponding author (telephone +31 35 6992139; fax +31 35 6995697; e-mail Bonnie-van-der.Pers@questintl.com).

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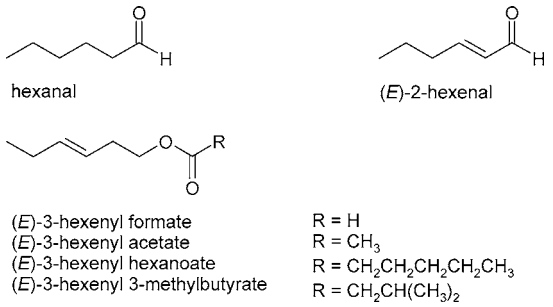

Figure 1. Chemical structures of the six GLVs classified sensorially.

aspect of (Z)-3-hexen-1-yl acetate was also given prominence in another study of virgin olive oil (*24*).

A fruity-green character has been attributed to (*Z*)-3-hexen-1-yl hexanoate (*8*). Fenaroli (*13*) describes the odor of (*Z*)-3-hexen-1-yl hexanoate as powerful, diffusive fruity-green, reminiscent of pear. Other authors suggest only the fruity note, but then reminiscent of prune (*7*). (*Z*)-3-Hexen-1-yl 3-methylbutyrate has been described as having the green odor of apple with a buttery, apple-like taste (*13*). Chitwood and co-workers (*25*) also classified (*Z*)-3-hexen-1-yl 3-methylbutyrate as green and apple-like. (*Z*)-3-Hexen-1-yl formate, on the other hand, has been described as either fruity (*13*) or raw green (*14*).

Few studies have investigated a sucrose effect on retronasal perception of green notes or an effect on both fruity and green notes. In their profiling experiments with mango homogenate suspended in mixtures with sucrose/fructose and citric acid, Malundo and co-workers (*26*) showed an increase in fruity notes (peachy, sweet potato, and banana) and a decrease in grassy notes as Brix (total soluble solids) increased. Retronasal evaluation of green/cut-grass notes in blueberry and cranberry juice has been shown to decrease in intensity with increasing concentrations of sucrose, whereas the intensity for fruity/berry-like notes increases (*27*). These opposing sucrose effects (decreasing green notes, increasing fruity notes) and their counterparts in relation to acidity (increasing green notes, decreasing fruity notes) were found in our own work with a number of different flavors (*28*).

The first goal of the present study was to classify the GLVs, especially the four (*Z*)-3-hexen-1-yl esters, on the basis of their sensory properties. We were curious to see whether this classification would be invariant for orthonasal and retronasal perceptions. Multiple techniques were employed to ensure that GLV classification was not an artifact of the sensory methodology. A second aim was to study the interaction between taste and smell that could occur if beverages were flavored with these GLVs. Specifically, we investigated whether changing the concentrations of tastants in soft drink bases could shift the fruity-green character of GLVs perceived retronasally so that either the fruity or the green note would predominate.

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 for each of the flavor chemicals. Structures for the six GLVs are shown in **Figure 1**.

Sample Preparation for Orthonasal Odor Evaluation. For each flavor chemical, 0.1 g of a 10% (w/w) ethanol solution was applied to the internal wick (30 mm × 20 mm diameter, made from polyester fibers) of a polypropylene fragrance carrier, "Scribrod" (B&S Injection Moulders, Whitstable, Kent, U.K.). Caps were screwed onto the carriers immediately after the solutions had been applied.

Table 1. Concentrations of Flavor Compounds Evaluated as Single Components in Beverage Bases

flavor compound	mg/L
isoamyl acetate	3.0
ethyl butyrate	7.0
hexanal	3.0
(*E*)-2-hexenal	1.0
(*Z*)-3-hexen-1-yl acetate	1.0
(*Z*)-3-hexen-1-yl formate	4.0
(*Z*)-3-hexen-1-yl hexanoate	8.0
(*Z*)-3-hexen-1-yl 3-methylbutyrate	2.0

Table 2. Composition of Flavors Created According to Plackett–Burman Designs

	flavor code			
	PB-1	PB-2	PB-3	PB-4
(*Z*)-3-hexen-1-yl formate (g)	1.0	0.5	1.0	0.5
(*Z*)-3-hexen-1-yl hexanoate (g)	2.0	1.0	1.0	2.0
(*Z*)-3-hexen-1-yl 3-methylbutyrate (g)	0.5	0.5	0.3	0.3
ethanol (g)	0.0	1.5	1.2	0.7
total weight (g)	3.5	3.5	3.5	3.5

	flavor code			
	PB-5	PB-6	PB-7	PB-8
(*Z*)-3-hexen-1-yl acetate (g)	0.5	0.5	0.1	0.1
(*E*)-2-hexenal (g)	0.5	0.1	0.5	0.1
(*Z*)-3-hexen-1-yl hexanoate (g)	3.0	0.5	0.5	3.0
ethanol (g)	0.0	2.9	2.9	0.8
total weight (g)	4.0	4.0	4.0	4.0

Table 3. 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

Sample Preparation for Retronasal Evaluation of Single Components in Aqueous Bases. For evaluation of single flavor components, the amounts given in **Table 1** were weighed from the appropriate stock solution and made up to 1000 mL with the desired base.

Sample Preparation for Retronasal Evaluation of GLV Mixtures Using Two Plackett−Burman Designs. The effect of an individual GLV on the perceived intensity scores of sensory descriptors can be determined efficiently by profiling flavors (mixtures made with one of two concentrations, chosen a priori, for each component) created according to a screening design such as those defined by Plackett and Burman (*29*). Flavors for these experiments (PB1−PB4 and PB5−PB8) are defined by the mixtures given in **Table 2**. These flavors were dosed on the appropriate bases (1−4 in **Table 3**) at 0.4 g/L of a 1% (w/w) ethanol solution of each mixture.

Preparation of Standard Beverage Bases 1−4 (Table 3). 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 Sucrose Bases at Constant Acidity. Three levels (40, 70, and 120 g/L) of sucrose (Kristal suiker extra fijn, Suiker Unie, Dinteloord, The Netherlands) and one level of acidity (4.2 g/L of citric acid 47%, 0 aq) were prepared in the laboratory on the day of the experiment.

Measurement of Total Soluble Solids (Table 3). 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.

Measurement of Acidity (Table 3). 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 3). The pH of bases was measured with a pH Mettler glass probe (Mettler Toledo). Calibration and measurements were at room temperature.

Sensory Panelists. A paid, professional panel consisting of 20 women, who work 2-h sessions 4 days a 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 orthonasal evaluations, the Scribrod caps were unscrewed and the odor was inhaled by normal sniffing. For retronasal evaluation of samples in aqueous bases, 50 mL portions were served in brown plastic cups.

Grouping Tests: Orthonasal Odor. Panelists were presented with eight Scribrods, one for each of the eight flavor compounds. They were asked to separate the Scribrods into at least two, but not more than seven, groups so that the odor similarities were greater within a group than between groups. Subsequently, panelists indicated an odor descriptor label for each group. The data from two replicates (two different days) were pooled and analyzed by correspondence analysis. Additionally, a similarity matrix was constructed on the basis of the number of times two flavor components were indicated as belonging to the same group. Cluster analysis (average linkage) on the similarity matrix defined groups of flavor compounds that were compared visually to the groups indicated by correspondence analysis.

Dissimilarity Tests: Orthonasal and Retronasal Odor. Orthonasal evaluations made use of the Scribrods, whereas retronasal evaluations were made in bases 1−4 (**Table 3**). Flavor concentrations for the latter are given in **Table 1**. Per medium (air, aqueous base), the 28 possible pairs of different flavor compounds were presented in blocks of four pairs using a cyclic design (*30*). Panelists used a 6-point scale to indicate dissimilarity (1 = no difference, 2 = very small, 3 = small, 4 = average, 5 = large, 6 = very large difference). Separately per pair and per medium, mean scores were obtained over approximately 18 evaluations and used in restricted maximum likelihood (REML) analyses with base as fixed effect. An association matrix was calculated for each medium (also for the grouping data); the similarity of these association matrices was examined by means of STATIS (*31*).

Profiling Experiments Using Retronasal Odor and Gustatory Descriptors. Single flavor compounds and mixtures were evaluated by descriptive profiling. The purpose of profiling experiments varied from providing additional methods for classification of GLVs to investigating smell−taste interaction. Panelists evaluated four samples per day according to a serving design that was balanced for carry-over. All samples were evaluated at least twice by each panelist. The designs for selecting samples/day were balanced and connected to the highest degree possible.

Design for Profiling Flavors PB1−PB4 and PB5−PB8 on Bases 1−4. Interaction between retronasal odor and taste was studied by profiling each of the four flavors (mixtures) in each of the two designs (**Table 2**) on each of the four bases defined in **Table 3**. The 16 samples/design were evaluated as 4 flavors/same base on one day and 1 flavor/4 bases on another day.

Design for Profiling Single Components Evaluated on Three Sucrose Bases at Constant Acidity. These experiments not only clarified the sensory description of each GLV but also facilitated the modeling of a Brix (sucrose concentration) effect on GLV classification as either *fruity* or *green*. A partially balanced incomplete block design expanded in two sessions was employed so that the six samples profiled per day included all three sucrose concentrations for each of two flavor compounds, and all sessions were connected.

Sensory Descriptors. Initially, panelists were given solutions of each of the (*Z*)-3-hexen-1-yl esters [(*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] in base 4 and asked to generate descriptors, because these compounds were new to them. Generally, gustatory and olfactory descriptors were taken from the sensory department's collection of reference-anchored flavor descriptors. Some definitions relevant for this paper include *apple green* [(*E*)-2-hexenal], *cucumber* (2,6-nonadienal), *flowery* (β-ionone, linalool), *fruity* (ethyl butyrate/ester block QL44282), *green* [(*Z*)-3-hexenol/hexanal], and *pear/candy* (isoamyl acetate, "peredrups" candy).

Intensity Scaling. Panelists used the audio method for measuring intensity according to standard profiling procedures described elsewhere (*28*).

Statistical Analysis of Profiling Data. Panel means of intensity per sample/descriptor were obtained by fitting variance components using REML (*32*), where panelists and all interactions with panelists were considered to be random effects. Fixed effects for most experiments were the design variables [Brix or sucrose concentration, acidity, flavor(s)] and their two-way interactions. Note that flavor is used to indicate either a single flavor compound (e.g., one of the GLVs) or a mixture of them. Interactions that were not significant ($p > 0.1$) were dropped from the model. When an interaction was significant, the related fixed effects were no longer interpreted. The Wald statistic (*32*) was used to calculate significance. Effects were considered to be significant when $p \leq 0.05$. For $0.05 < p \leq 0.10$, effects are reported with their exact p values and interpreted, because they may indicate interesting trends. In models with more than one fixed effect, Wald statistics were estimated for a factor by dropping it from the full fixed model.

Data from the experiment examining eight flavor compounds and three concentrations of sucrose at constant acidity were analyzed by two additional methods besides the procedure described above. First, all eight flavor compounds as well as only the six GLVs were examined nonparametrically by the Page test (*33*). Second, the six GLVs were studied by a regression model, which treated log[sucrose] as a continuous variable.

GenStat 7 and R 2.1.0 were used to perform all calculations and statistical analyses discussed in this paper.

RESULTS AND DISCUSSION

Orthonasal Classification. When classified on orthonasal odor, none of the six GLVs was labeled *fruity*, but (*Z*)-3-hexen-1-yl formate, hexanal, and (*E*)-2-hexenal were labeled *green*. The cluster and correspondence analyses (not shown) from the sensory grouping test indicated one cluster for (*Z*)-3-hexen-1-yl 3-methylbutyrate and (*Z*)-3-hexen-1-yl hexanoate (labeled *floral*), one cluster for isoamyl acetate and ethyl butyrate (labeled *fruity* or *fruity-estery*), and a cluster for hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-yl formate (labeled *green*) with (*Z*)-3-hexen-1-yl acetate more or less alone but joining the *green* group at an earlier stage than those three compounds joined the other clusters. These group names have been superimposed on the STATIS analysis shown in **Figure 2**. STATIS allows one to judge the similarity of spatial configurations, in this case for data from the eight flavor compounds as measured by two different sensory methods. In **Figure 2** the distance between solid and open squares (grouping test, respectively, dissimilarity test) for the same flavor compound is smaller than the distance between any two labeled groups. In other words, the same clusters of flavor compounds are formed, independent of the sensory methodology. The *green* and *floral* clusters are separated from the *fruity-estery* cluster, with (*Z*)-3-hexen-1-yl acetate in between.

A recent Japanese study of odors from C-6 alcohols, aldehydes, and esters included all six GLVs except (*Z*)-3-hexen-1-yl hexanoate (*34*). Their data showed that fresh, fruity notes scored higher than green notes for these five GLVs. It should be noted that their study did not include the fruity esters isoamyl acetate and ethyl butyrate, which may have caused our panelists to evaluate the GLVs as more *green* by contrast.

In this Japanese study, hexanal was quite different from the other four compounds. Odors of hexanal and (*E*)-2-hexenal were

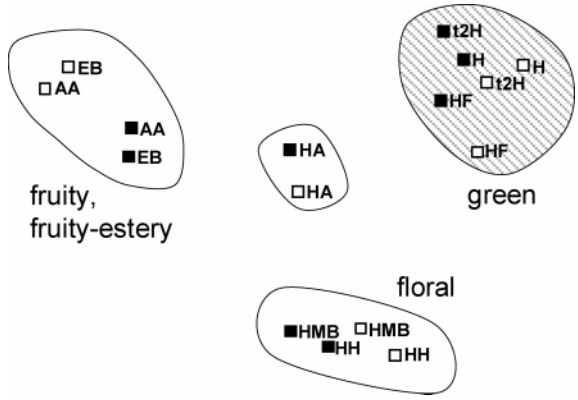

Figure 2. Comparison of GLV classifications by STATIS analysis of **orthonasal odor** evaluations: dimensions 1 (horizontal, 33% explained variation) and 2 (vertical, 27% explained variation). Symbols indicate classification based on grouping tests (■) and dissimilarity tests (□). Flavor compounds from **Table 1** are indicated by the following abbreviations: isoamyl acetate, AA; ethyl butyrate, EB; hexanal, H; (*E*)-2-hexenal, t2H; (*Z*)-3-hexen-1-yl acetate, HA; (*Z*)-3-hexen-1-yl formate, HF; (*Z*)-3-hexen-1-yl hexanoate, HH; (*Z*)-3-hexen-1-yl 3-methylbutyrate, HMB.

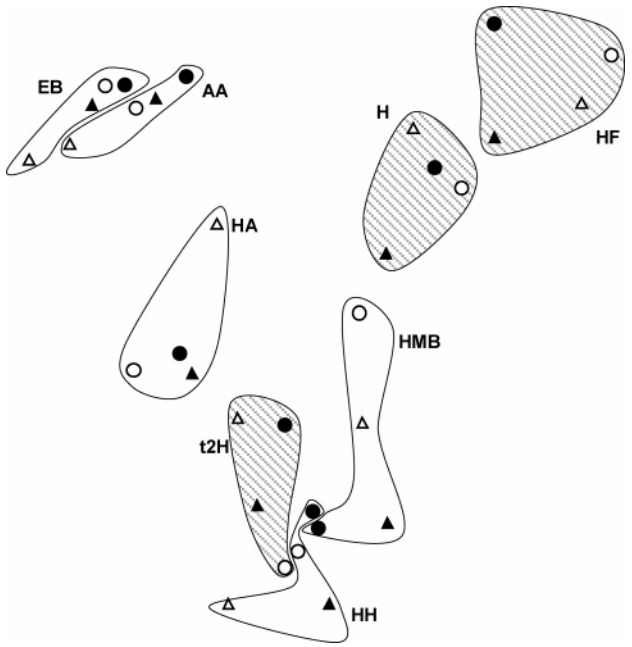

Figure 3. Comparison of GLV classifications by STATIS analysis of **retronasal odor** evaluations. Dissimilarity tests of flavor compounds were conducted in four bases (**Table 3**). Symbols correspond to base 1 (△), base 2 (○), base 3 (▲), and base 4 (●). Flavor compounds from **Table 1** are indicated by the following abbreviations: isoamyl acetate, AA; ethyl butyrate, EB; hexanal, H; (*E*)-2-hexenal t2H; (*Z*)-3-hexen-1-yl acetate, HA; (*Z*)-3-hexen-1-yl formate, HF; (*Z*)-3-hexen-1-yl hexanoate, HH; (*Z*)-3-hexen-1-yl 3-methylbutyrate, HMB.

both characterized as *fruity* and *sweet*, but they differed greatly from each other in that hexanal was *oily fatty* and *spicy*, whereas (*E*)-2-hexenal was *fresh*. According to these authors, (*Z*)-3-hexen-1-yl formate was *fresh*, *fruity*, *grassy-leafy green*, *sweet*, and *vegetable green*. In our experiments, hexanal was the most *green* GLV and (*Z*)-3-hexen-1-yl formate the most *cucumber* GLV.

Retronasal Classification. In the STATIS analysis shown in **Figure 3**, we are examining similarity among four bases for the eight flavor compounds evaluated in the sensory dissimilarity Tests. This analysis shows that the bases do affect the relationships among flavor compounds. Moreover, we can compare similarity of the orthonasal and retronasal configurations of the flavor compounds by comparing **Figures 2** and **3**. The GLVs are spread out along a diagonal in **Figure 3**, with isoamyl acetate and ethyl butyrate separated from them in the upper left corner, as was also seen in **Figure 2**. Likewise, as in the orthonasal evaluations, (*Z*)-3-hexen-1-yl acetate occupies a middle position between isoamyl acetate/ethyl butyrate and the other GLVs.

On the other hand, in **Figure 3** (*E*)-2-hexenal has shifted completely away from (*Z*)-3-hexen-1-yl formate and hexanal. This compound had been defined for the panel as *apple green*, which makes it difficult to decide—linguistically or experimentally—whether its classification should be *fruity* or *green*. Not only differences in Brix but also differences in acidity changed the character of (*E*)-2-hexenal, as verified by paired comparison tests at constant Brix: (*E*)-2-hexenal at acidity 0.3 was more *green* than at acidity 0.2 ($p = 0.0581$, $N = 55$).

The *green* label given to both (*Z*)-3-hexen-1-yl formate and hexanal in the orthonasal tests was maintained in all retronasal evaluations, especially at lower Brix and higher acidity. In fact, (*Z*)-3-hexen-1-yl formate and hexanal were most similar in base 2 (the least sweet and most acidic base).

Examination of the residuals in these statistical analyses indicates that in base 1 (△), isoamyl acetate, (*Z*)-3-hexen-1-yl acetate, ethyl butyrate, and (*Z*)-3-hexen-1-yl hexanoate deviate from the consensus position determined for all four bases. (*Z*)-3-Hexen-1-yl 3-methylbutyrate and (*E*)-2-hexenal deviate in base 2 (○), and hexanal deviates in base 3 (▲).

From REMLs for the four dissimilarity evaluations of retronasal perception, only three pairs of flavor compounds had significant base effects. (*Z*)-3-Hexen-1-yl 3-methylbutyrate and hexanal ($p = 0.0037$) were significantly more similar at Brix 9/acidity 0.3 than at Brix 9/acidity 0.2. (*Z*)-3-Hexen-1-yl 3-methylbutyrate and (*Z*)-3-hexen-1-yl hexanoate ($p = 0.0135$) were significantly more similar at Brix 12 independent of acidity than at Brix 9/acidity 0.3. (*Z*)-3-Hexen-1-yl formate and isoamyl acetate ($p = 0.0118$) were significantly more similar at Brix 12/acidity 0.3 than on any of the other bases. Two other pairs with (*Z*)-3-hexen-1-yl formate had base effects worthy of mention: (*Z*)-3-hexen-1-yl formate and (*Z*)-3-hexen-1-yl acetate ($p = 0.0810$) were more similar at Brix 9/acidity 0.2 than at Brix 9/acidity 0.3; (*Z*)-3-hexen-1-yl formate and hexanal ($p = 0.1069$) were more similar at Brix 9/acidity 0.3 than at Brix 12/acidity 0.2.

During the panel's initial encounter with the four (*Z*)-3-hexen-1-yl esters, they generated the descriptor *green* for (*Z*)-3-hexen-1-yl acetate, although this compound falls in an undefined middle position in both **Figures 2** and **3**. The Japanese authors (*34*) described (*Z*)-3-hexen-1-yl acetate odor as *fruity*, *fresh*, *sweet*, and *grassy-leafy green*. In our retronasal evaluations, both these fruity and green aspects became apparent, and one or the other could be given prominence by changing the base.

Initially, (*Z*)-3-hexen-1-yl 3-methylbutyrate and (*Z*)-3-hexen-1-yl hexanoate were more difficult for the panel to describe than (*Z*)-3-hexen-1-yl acetate and (*Z*)-3-hexen-1-yl formate. There was no suggestion of any green character in the odor description given in ref *34*: (*Z*)-3-hexen-1-yl 3-methylbutyrate was *fruity*, *sweet*, *fresh*, and *spicy*. (*Z*)-3-Hexen-1-yl 3-methylbutyrate has been described as *sweet* by other authors as well (*13*, *25*). For our panel, the orthonasal floral character of (*Z*)-3-hexen-1-yl 3-methylbutyrate was also perceived retronasally. Fruity characteristics of both (*Z*)-3-hexen-1-yl hexanoate and (*Z*)-3-hexen-1-yl 3-methylbutyrate were increased in the bases with higher Brix.

Table 4. Main Effects (Given as Differences in Intensity Scores for High–Low Levels) and Their Associated *p* Values from REML Analysis of Profiling Plackett–Burman Flavors PB1–PB4 (**Table 2**)

	Brix		acidity		(*Z*)-3-hexen-1-yl 3-methylbutyrate		(*Z*)-3-hexen-1-yl formate		(*Z*)-3-hexen-1-yl hexanoate	
descriptor	H–L	*p* value	H–L	*p* value	H–L	*p* value	H–L	*p* value	H–L	*p* value
sour	**–15.9**	**<0.0001**	**21.9**	**<0.0001**	3.0	0.2693	3.2	0.2318	4.2	0.1712
sweet	**25.4**	**<0.0001**	**–11.3**	**<0.0001**	–3.2	0.1041	–0.8	0.6667	0.2	0.9466
bitter	–0.6	0.8505	3.4	0.1679	–1.5	0.5779	4.6	*0.0858*	–2.5	0.4320
apple	1.4	0.6045	2.3	0.3815	2.8	0.2462	**6.4**	**0.0096**	–4.3	0.1180
apple green	3.5	0.3443	–5.9	0.1071	3.5	0.2888	**7.8**	**0.0179**	–3.6	0.3404
banana	4.2	0.2389	*–5.9*	*0.0749*	5.0	0.1181	**6.3**	**0.0490**	2.7	0.4482
cucumber	**–7.3**	**0.0437**	*–5.7*	*0.0507*	*5.9*	*0.0662*	**11.6**	**0.0003**	–3.2	0.3757
flowers	1.6	0.5539	2.8	0.2909	0.5	0.8526	–0.3	0.9175	–1.9	0.4849
fruity	4.0	0.2076	–0.9	0.7409	*5.5*	*0.0558*	2.9	0.3151	2.8	0.3849
garden herbs	1.4	0.6140	–2.2	0.3418	0.8	0.7590	–1.0	0.7014	1.9	0.5022
green	**–17.9**	**0.0002**	–0.5	0.9051	1.1	0.7636	**10.8**	**0.0028**	2.9	0.4708
pear/candy	**13.0**	**0.0014**	–1.0	0.7499	**9.2**	**0.0035**	**7.0**	**0.0264**	1.2	0.7345
tin/metallic	0.6	0.8387	*5.0*	*0.0834*	–0.3	0.9023	4.2	0.1205	2.3	0.4587

Table 5. Main Effects (Given as Differences in Intensity Scores for High–Low Levels) and Their Associated *p* Values from REML Analysis of Profiling Plackett–Burman Flavors PB5–PB8 (**Table 2**)

	Brix		acidity		(*Z*)-3-hexen-1-yl acetate		(*E*)-2-hexenal		(*Z*)-3-hexen-1-yl hexanoate	
descriptor	H–L	*p* value	H–L	*p* value	H–L	*p* value	H–L	*p* value	H–L	*p* value
sour	**–16.3**	**0.0001**	**19.5**	**<0.0001**	1.3	0.6314	4.4	0.1047	2.2	0.4129
sweet	**38.0**	**<0.0001**	**–13.5**	**<0.0001**	1.6	0.5401	–2.9	0.2555	1.4	0.5874
bitter	**–6.5**	**0.0208**	2.7	0.5677	0.8	0.7885	–0.1	0.9786	–0.4	0.8980
apple green	2.5	0.6492	–2.3	0.5222	2.4	0.4643	2.4	0.4528	–4.3	0.1861
fruity	8.5	0.1162	0.5	0.8896	**9.2**	**0.0070**	0.7	0.8241	0.1	0.9871
green	**–10.6**	**0.0130**	*6.8*	*0.0762*	4.2	0.2519	*6.1*	*0.0913*	4.3	0.2352
pear/candy	**10.9**	**0.0045**	–0.1	0.9823	**12.4**	**0.0001**	2.4	0.4112	–2.9	0.3317

Profiling the Flavors Created According to Plackett–Burman Designs. The two Plackett–Burman experiments provide additional data on the perception of five GLVs and show how perception can be influenced by changes in Brix or acidity. Results from profiling flavors PB1–PB4 are given in **Table 4**. This table shows the change induced by each of the five main effects, accompanied by the *p* value associated with each effect.

Looking first at the effect of each of the three GLVs on the retronasal odor descriptors, we see that (*Z*)-3-hexen-1-yl 3-methylbutyrate significantly drove *pear/candy* and had a positive contribution to *cucumber* and *fruity*. (*Z*)-3-Hexen-1-yl formate significantly drove *apple*, *apple green*, *banana*, *cucumber*, *green*, and *pear/candy*. There were no significant effects for (*Z*)-3-hexen-1-yl hexanoate.

There were no significant effects of these three GLVs on the gustatory descriptors, which were driven only by the Brix and acidity. On the other hand, tastants in the base did have significant effects on the scores for retronasal odorants. Brix had a significant negative effect on both *green* and *cucumber* and a significant positive effect on *pear/candy*. For *cucumber*, however, the interaction Brix × (*Z*)-3-hexen-1-yl formate ($p = 0.0405$) indicated a larger Brix effect at the higher level of (*Z*)-3-hexen-1-yl formate (−13.2 vs −1.3 at the lower level). The negative acidity trend on *cucumber* is contrary to what is usually seen when the Brix effect is negative. This negative acidity trend needs to take into consideration the interaction acidity × (*Z*)-3-hexen-1-yl formate ($p = 0.0984$), which showed a change of −10.6 for the higher level of (*Z*)-3-hexen-1-yl formate versus −0.9 for the lower level. There was also a tendency for *banana* intensity to decrease and for *tin/metallic* intensity to increase as acidity increased. The interaction Brix × (*Z*)-3-hexen-1-yl 3-methylbutyrate ($p = 0.0253$) for the descriptor *tin/metallic* showed that the negative Brix effect usually found for this descriptor occurred only at the higher level of (*Z*)-3-hexen-1-yl 3-methylbutyrate.

The changes induced by each of the five main effects in the second Plackett–Burman experiment, flavors PB5–PB8, are shown in **Table 5**, accompanied by the associated *p* values. Looking first at the effect of each of the three GLVs on the retronasal odor descriptors, we see that (*Z*)-3-hexen-1-yl acetate significantly drove *fruity* and *pear/candy*. The panel was not able to recognize (*E*)-2-hexenal in these mixtures as a significant driver of *apple green* despite the fact that (*E*)-2-hexenal served as the reference for this descriptor. On the other hand, (*E*)-2-hexenal contributed positively to *green*. There were again no significant effects for (*Z*)-3-hexen-1-yl hexanoate.

As in the first Plackett–Burman design, there were no significant effects of these three GLVs on the gustatory descriptors, which were driven only by the Brix and acidity. There were, however, significant interactions Brix × (*E*)-2-hexenal for two of the gustatory descriptors. In the first case, the negative Brix effect for *sour* was much more evident at the lower concentration of (*E*)-2-hexenal (a change of −23.5 vs −8.8 at the higher level, $p = 0.0011$). In the second case, the negative Brix effect for *bitter* was more evident at the higher concentration of (*E*)-2-hexenal (a change of −11.3 at the higher level vs −1.9 at the lower level, $p = 0.0472$). As in the first Plackett–Burman design, tastants in the base had significant effects on the scores for retronasal odorants. The Brix effect was significantly negative for *green* and positive for *pear/candy*. A positive acidity trend was seen for *green*.

Figure 4 summarizes the main effects for the three GLVs that significantly drove one or more of the retronasal odor descriptors. In this figure, the factors used to establish the differences between high and low concentration levels of each GLV (see **Table 2**) have been taken into consideration, that is,

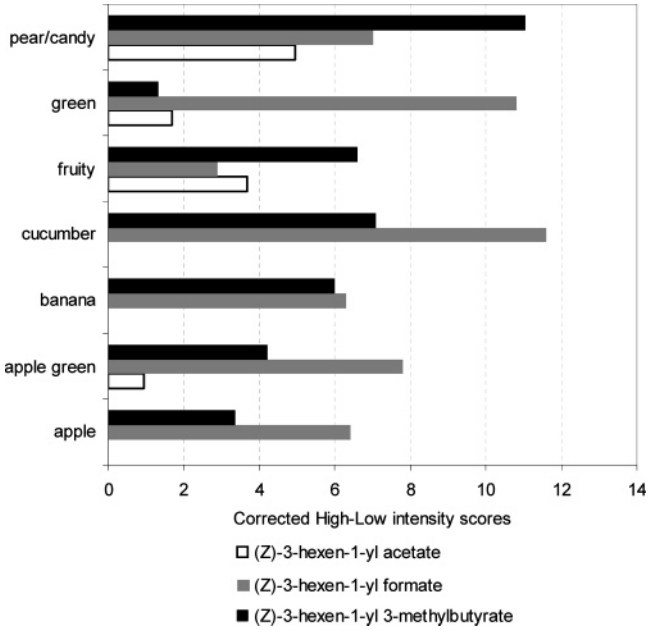

Figure 4. Main effects (given as differences in intensity scores for high–low levels) for the three GLVs that significantly drove one or more retronasal odor descriptors when flavors PB1–PB8 were profiled. Note that (*Z*)-3-hexen-1-yl acetate was not evaluated on the descriptors *cucumber*, *banana*, and *apple*.

Table 6. Estimated Parameters for GLVs from Two-Way REML Model Intensity = Flavor Compound + log[Sucrose] for Descriptors Green and Fruity

effect	*green*	*fruity*
intercept	502.3	409.6
flavor compound		
hexanal	0	0
(*Z*)-3-hexen-1-yl formate	−2.6	5.7
(*Z*)-3-hexen-1-yl 3-methylbutyrate	−44.6	2.1
(*E*)-2-hexenal	−48.5	6.4
(*Z*)-3-hexen-1-yl acetate	−51.1	6.3
(*Z*)-3-hexen-1-yl hexanoate	−55.3	8.0
log[sucrose]	−31.1	40.8

a factor of 2 for (*Z*)-3-hexen-1-yl formate, a factor of 5 for (*Z*)-3-hexen-1-yl acetate, a factor of 1.67 for (*Z*)-3-hexen-1-yl 3-methylbutyrate. (*Z*)-3-Hexen-1-yl formate was taken as a reference. The intensity score differences (H−L) in **Table 5** for (*Z*)-3-hexen-1-yl acetate were multiplied by 0.4; H−L differences for (*Z*)-3-hexen-1-yl 3-methylbutyrate in **Table 4** were multiplied by 1.2. **Figure 4** shows that (*Z*)-3-hexen-1-yl formate has a major effect on most of the descriptors but a proportionally larger effect on the green-related descriptors. (*Z*)-3-Hexen-1-yl 3-methylbutyrate, on the other hand, contributes more to the fruit-related descriptors.

Profiling Single Components: Three Sucrose Concentrations at Constant Acidity. Multiple comparison of means (least significant difference, LSD) for the flavor effect per descriptor confirmed panel training for those four flavor compounds used as descriptor references: ethyl butyrate scored highest on the descriptor *fruity* ($p < 0.0001$), (*E*)-2-hexenal scored highest on *apple green* ($p < 0.0001$), isoamyl acetate scored highest on *pear/candy* ($p < 0.0001$), and hexanal scored highest on *green* ($p < 0.0001$). The order of scores for the other GLVs on the descriptor *green* can be found in **Table 6**. Two of the (*Z*)-3-hexen-1-yl esters had significantly higher scores ($p < 0.0001$) on a specific descriptor than all other flavor compounds: (*Z*)-3-hexen-1-yl formate scored highest on *cucumber*, and (*Z*)-3-hexen-1-yl 3-methylbutyrate scored highest on *flowers*. (*Z*)-3-Hexen-1-yl formate also scored highest on *apple* ($p = 0.0089$) and *banana* ($p = 0.0439$), albeit not significantly higher than all other flavor compounds. The flavor effect for *tin/metallic* ($p = 0.0001$) showed highest scores for (*Z*)-3-hexen-1-yl hexanoate followed by (*Z*)-3-hexen-1-yl 3-methylbutyrate, both of which were significantly higher than isoamyl acetate, (*E*)-2-hexenal, ethyl butyrate, and (*Z*)-3-hexen-1-yl formate.

To compare these data with other data for Brix effects when using bases 1−4, a sucrose effect was calculated for each descriptor by treating sucrose concentration as a discrete variable with three levels. This effect was significant for all descriptors except *cucumber*. Scores for *apple*, *apple green*, *banana*, *flowers*, *fruity*, and *pear/candy* increased as the sucrose concentration increased; scores for *green* and *tin/metallic* decreased.

For the six GLVs, regression coefficients for the *green* and *fruity* models using log[sucrose] as a continuous variable are given in **Table 6**. Sucrose had a larger effect on the scores for *fruity* ($p < 0.0001$) than for *green* ($p = 0.0279$). The flavor effect for the six GLVs was significant only for the descriptor *green* ($p < 0.0001$).

Independently of whether all eight flavor compounds or only the six GLVs were analyzed nonparametrically by the Page test, there was a significant trend shown for increasing sucrose concentration: scores for *fruity* increase and scores for *green* decrease. Analyzing the trend for each flavor compound separately shows increases on *fruity* for (*Z*)-3-hexen-1-yl acetate ($p = 0.0122$), (*Z*)-3-hexen-1-yl hexanoate ($p = 0.0331$), (*Z*)-3-hexen-1-yl 3-methylbutyrate ($p = 0.0576$), and (*E*)-2-hexenal ($p = 0.0031$). Decreases on *green* were shown for ethyl butyrate ($p = 0.0169$), hexanal ($p = 0.0263$), (*Z*)-3-hexen-1-yl acetate ($p = 0.0846$), and (*E*)-2-hexenal ($p = 0.0046$).

Some of these sucrose-induced shifts were confirmed by paired comparison tests conducted at constant acidity: (*Z*)-3-hexen-1-yl formate, (*Z*)-3-hexen-1-yl 3-methylbutyrate, ethyl butyrate, and isoamyl acetate were significantly more *fruity* at Brix 12 than at Brix 9, whereas (*Z*)-3-hexen-1-yl acetate was more *green* ($p = 0.0576$, $N = 34$) at Brix 9 than at Brix 12.

Correlations for some GLVs with sensory gustatory descriptors have been reported, although these are conflicting. *Sweet* was correlated with (*E*)-2-hexenal (*22*) and with hexanal (*4*, *23*); *sour* was correlated with hexanal (*12*, *15*, *22*); *bitter* was correlated with (*E*)-2-hexenal (*3*, *20*, *23*); *bittersweet* was correlated with (*E*)-2-hexenal (*21*). The results obtained from the varying sensory methodologies presented in this paper would support a base-dependent bipolar green-fruity scale for classifying the retronasal odor of GLVs. The shift from *green* to *fruity* corresponds to the natural ripening process of fruits and vegetables along a sour-to-sweet scale.

LITERATURE CITED

(1) Ruther, J. Retention index database for identification of general green leaf volatiles in plants by coupled capillary gas chromatography−mass spectrometry. *J. Chromatogr. A* **2000**, *890*, 313−319.

(2) Mayland, H. F.; Flath, R. A.; Shewmaker, G. E. Volatiles from fresh and air-dried vegetative tissues of tall fescue (*Festuca arundinacea* Schreb.): relationship to cattle preference. *J. Agric. Food Chem.* **1997**, *45*, 2204−2210.

(3) Salas, J. J.; Sánchez, C.; Garcia-González, D. L.; Aparicio, R. Impact of the suppression of lipoxygenase and hydroperoxide lyase on the quality of the green odor in green leaves. *J. Agric. Food Chem.* **2005**, *53*, 1648−1655.

(4) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **2000**, *68*, 283−287.

(5) Ménager, I.; Jost, M.; Aubert, C. Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation. *J. Agric. Food Chem.* **2004**, *52*, 1248−1254.

(6) Binder, R. G.; Flath, R. A.; Mon, T. R. Volatile components of bittermelon. *J. Agric. Food Chem.* **1989**, *37*, 418−420.

(7) Jordán, M. J.; Margaría, C. A.; Shaw, P. E.; Goodner, K. L. Volatile components and aroma active compounds in aqueous essence and fresh pink guava fruit puree (*Psidium guajava* L.) by GC-MS and multidimensional GC/GC-O. *J. Agric. Food Chem.* **2003**, *51*, 1421−1426.

(8) Pino, J. A.; Marbot, R.; Vázquez, C. Characterization of volatiles in strawberry guava (*Psidium cattleianum* Sabine) fruit. *J. Agric. Food Chem.* **2001**, *49*, 5883−5887.

(9) Takeoka, G. R.; Flath, R. A.; Güntert, M.; Jennings, W. Nectarine volatiles: vacuum steam distillation versus headspace sampling. *J. Agric. Food Chem.* **1988**, *36*, 553−560.

(10) Lavilla, T.; Recasens, I.; Lopez, M. L.; Puy, J. Multivariate analysis of maturity stages, including quality and aroma, in 'Royal Glory' peaches and 'Big Top' nectarines. *J. Sci. Food Agric.* **2002**, *82*, 1842−1849.

(11) Baldwin, E. A.; Goodner, K.; Plotto, A.; Pritchett, K.; Einstein, M. Effect of volatiles and their concentration on perception of tomato descriptors. *J. Food Sci.* **2004**, *69* (8), S310−S318.

(12) Paçi Kora, E.; Latrille, E.; Souchon, I.; Martin, N. Texture-flavor interactions in low fat stirred yogurt: how mechanical treatment, thickener concentration and aroma concentration affect perceived texture and flavor. *J. Sensory Stud.* **2003**, *18*, 367−390.

(13) *Fenaroli's Handbook of Flavor Ingredients*, 2nd ed.; Furia, T. E., Bellanca, N., Eds.; CRC Press: Cleveland, OH, 1975; Vol. 2.

(14) Wright, J. *Flavor Creation*; Allured: Carol Stream, IL, 2004.

(15) Azodanlou, R.; Darbellay, C.; Luisier, J.-L.; Villettaz, J.-C.; Amadò, R. Quality assessment of strawberries (*Fragaria* species). *J. Agric. Food Chem.* **2003**, *51*, 715−721.

(16) Jordán, M. J.; Goodner, K. L.; Shaw, P. E. Characterization of the aromatic profile in aqueous essence and fruit juice of yellow passion fruit (*Passiflora edulis* Sims F. *Flavicarpa degner*) by GC-MS and GC/O. *J. Agric. Food Chem.* **2002**, *50*, 1523−1528.

(17) Krumbein, A.; Auerswald, H. Characterization of aroma volatiles in tomatoes by sensory analyses. *Nahrung* **1998**, *42*, 395−399.

(18) Reiners, J.; Grosch, W. Odorants of virgin olive oils with different flavor profiles. *J. Agric. Food Chem.* **1998**, *46*, 2754−2763.

(19) Buettner, A.; Mestres, M. Investigation of the retronasal perception of strawberry aroma aftersmell depending on matrix composition. *J. Agric. Food Chem.* **2005**, *53*, 1661−1669.

(20) Morales, M. T.; Aparicio, R. Effect of extraction conditions on sensory quality of virgin olive oil. *J. Am. Oil Chem. Soc.* **1999**, *76*, 295−300.

(21) Bult, J. H. F.; Schifferstein, H. N. J.; Roozen, J. P.; Boronat, E. D.; Voragen, A. G. J.; Kroeze, J. H. A. Sensory evaluation of character impact components in an apple model mixture. *Chem. Senses* **2002,** *27*, 485−494.

(22) Baldwin, E. A.; Scott, J. W.; Einstein, M. A.; Malundo, T. M. M.; Carr, B. T.; Shewfelt, R. L.; Tandon, K. S. Relationship between sensory and instrumental analysis for tomato flavor. *J. Am. Soc. Hortic. Sci.* **1998**, *123*, 906−915.

(23) Aparicio, R.; Morales, M. T.; Alonso, V. Authentication of European virgin olive oils by their chemical compounds, sensory attributes, and consumers' attitudes. *J. Agric. Food Chem.* **1997**, *45*, 1076−1083.

(24) Servili, M.; Piacquadio, P.; De Stefano, G.; Taticchi, A.; Sciancalepore, V. Influence of a new crushing technique on the composition of the volatile compounds and related sensory quality of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 483−489.

(25) Chitwood, R. L.; Pangborn, R. M.; Jennings, W. GC/MS and sensory analysis of volatiles from three cultivars of capsicum. *Food Chem.* **1983**, *11*, 201−216.

(26) 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.

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

(28) 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**, in press.

(29) Plackett, R. L.; Burman, J. P. The design of optimum multifactorial experiments. *Biometrika* **1946**, *33*, 305−325.

(30) David, H. A. *The Method of Paired Comparisons*; Charles Griffin: London, U.K., 1988.

(31) Schlich, P. Defining and validating assessor compromises about product distances and attribute correlations. In *Multivariate Analysis of Data in Sensory Science*; Naes, T., Risvik, E., Eds.; Elsevier Sciences: Amsterdam, The Netherlands, 1996; pp 259−306.

(32) Genstat 5 release 3. *Reference Manual*; Oxford University Press: Oxford, U.K., 1993; pp 539−583.

(33) Hollander, M.; Wolfe, D. A. *Nonparametric Statistical Methods*, 2nd ed.; Wiley: New York, 1999; pp 284−287.

(34) Sano, K.; Suzuki, J.; Hatanaka, A. Chemical structure-odor relationships of "green odor" affected by terminal functional groups. *Nippon Aji to Nioi Gakkaishi* **2001**, *8*, 419−422.

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