Evaluation of Aroma Differences between Hand-Squeezed Juices from Valencia Late and Navel Oranges by Quantitation of Key Odorants and Flavor Reconstitution Experiments

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Twenty-five odor-active compounds were quantified in hand-squeezed juices of Valencia late and Navel oranges using stable isotope dilution assays. Odor activity values (OAVs, ratio of the concentration to odor thresholds) based on odor thresholds in water were calculated for the entire set of aroma compounds in both varieties. It was shown that due to their high OAVs, the fruity-smelling esters ethyl 2-methylpropanoate, ethyl butanoate, (*S*)-ethyl 2-methylbutanoate, and 3a,4,5,-7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (wine lactone), the grassy smelling (*Z*)-hex-3-enal, and the citrus-like decanal were the most potent odorants in both juices. The weaker fruity note in the Navel oranges was clearly correlated with significantly lower OAVs of all fruity-smelling esters but a higher OAV of (*Z*)-3-hexenal compared to Valencia late. Model solutions simulating the odor of both orange varieties confirmed the findings of the quantitation studies.

Keywords: *Stable isotope dilution assay; orange aroma; quantitation; wine lactone; (Z)-3-hexenal; (R)-ethyl 3-hydroxyhexanoate*

INTRODUCTION

Due to its pleasant aroma hand-squeezed, kitchen-made orange juice is very popular all over the world. Industrially processed juices, however, show a quite different aroma, which is mainly caused by the higher pressure used for squeezing and, also, the thermal treatment applied.

The original delicate aroma of fresh orange juice was for a long time thought to be a complex mixture of many volatile constituents blended in the proper proportions (*1*). For this reason, numerous investigations dealt with the identification of many potential contributors, resulting in a considerable number of volatiles identified in orange juice (*2*). On the basis of these data, extensive quantitations of volatiles in the fresh juices from a variety of orange cultivars such as Valencia, Navel, Pera, or Pineapple were performed to gain more accurate information about their contribution to orange flavor (*3*−*5*). Comparison of the quantitative data of some volatiles to odor threshold data suggested limonene, acetaldehyde, ethyl butanoate, and decanal as possible contributors to fresh orange aroma (*4*−*7*). However, a great variance in both the quantitative and sensory data did not allow precise conclusions. Therefore, reconstitution experiments were performed to prove the contribution of several compounds to orange aroma by using pumpout orange juice as the matrix (*8*, *9*). However, the typical aroma of fresh orange juice could not be reconstituted, thereby indicating that important odorants were missing.

By application of aroma extract dilution analysis (AEDA) on an extract prepared from hand-squeezed juice of Valencia late oranges, we recently identified 42 odor-active compounds in the flavor dilution (FD) factor range of 4−1024 (*10*). Among them, ethyl butanoate, ethyl 2-methylpropanoate, (*S*)-ethyl 2-methylbutanoate, and 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (wine lactone) with fruity, sweet odor notes, the grassy smelling (*Z*)-hex-3-enal, the terpene-like (*R*)-limonene and (*R*)-α-pinene, and the metallic-smelling *trans*-4,5-epoxy-(*E*)-dec-2-enal showed the highest FD factors. By static headspace−olfactometry acetaldehyde was established as another important aroma contributor (*10*).

AEDA is a useful screening method for the detection of potent odorants in foods (cf. review in ref *11*). However, this method is based on odor thresholds of the compounds in air. On the other hand, possible losses of odorants during the isolation steps are not fully taken into account. Therefore, the contribution of single odorants to orange juice aroma, depending on odor thresholds in aqueous media, has to be confirmed by accurate quantitations and aroma reconstitution experiments.

In the present study, stable isotope dilution assays (SIDAs) in combination with mass chromatography were used for the determination of the important odorants in two different orange varieties. Beforehand, comparative AEDAs of both hand-squeezed juices were performed to objectify similarities and differences among the aroma compounds of both juices.

EXPERIMENTAL PROCEDURES

Material. Oranges [*Citrus sinensis* (L.) Osbeck cv. Valencia late and cv. Navel, grown in Argentinia and Italy, respectively] were purchased at a local market and used immediately for juice making.

Chemicals. The following compounds were obtained from the suppliers given in parentheses: [$^{13}C_2$]acetaldehyde (Promochem, Wesel, Germany); acetyl chloride, dec-5-en-1-ol, [$^2H_6$]-ethanol, lithium bis(trimethylsilyl)amide, 1.0 M solution in diethyl ether, and pyridinium chlorochromate (Aldrich, Steinheim, Germany); platinum(IV) oxide hydrate (Merck-Schu-

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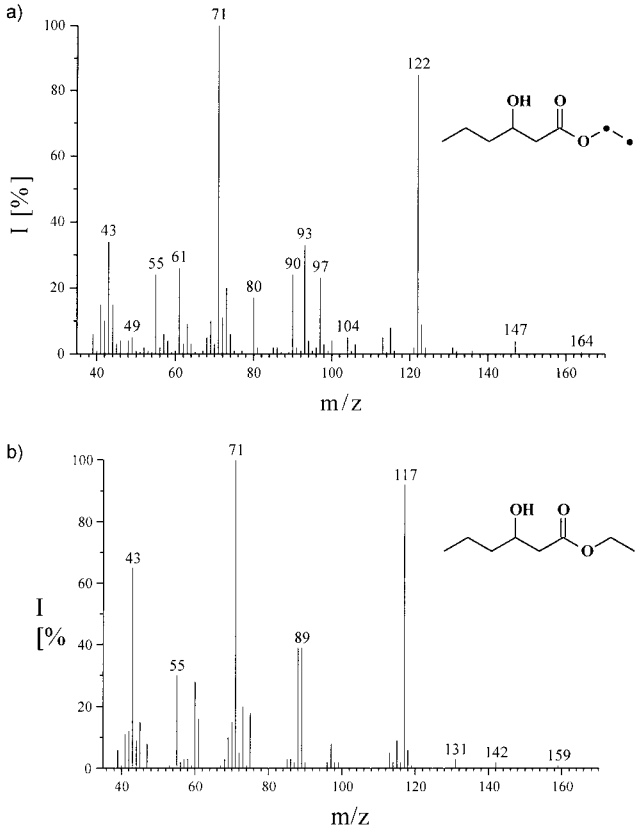

Figure 1. MS (EI) of [$^2H_5$]ethyl 3-hydroxyhexanoate (a) and ethyl 3-hydroxyhexanoate (b).

chardt, Hohenbrunn, Germany); butanal (Fluka, Buchs, Switzerland); [^{2}H]methanol (Sigma, Munich, Germany); deuterium gas (Linde, Munich, Germany); and Florisil (Serva, Heidelberg, Germany). Silica gel 60 (0.053−0.2 mm; Merck) was treated with concentrated HCl and deactivated with water (7% w/w) according to the method of ref *12*.

The following reference compounds were used for the reconstitution experiments: acetaldehyde, hexanal, octanal, methional, decanal, (*E*)-non-2-enal, (*E*,*E*)-deca-2,4-dienal, ethyl 2-methylpropanoate, ethyl hexanoate, (*R*)-α-pinene, myrcene, (*R*)-limonene, and 3-methylbutanol (Aldrich, Steinheim, Germany); oct-1-en-3-one (Lancaster, Mühlheim, Germany); nonanal (Roth, Karlsruhe, Germany); (*R*)-linalool and ethyl butanoate (Fluka, Neu-Ulm, Germany); (*R*,*S*)-2-methylbutanol and vanillin (Merck, Darmstadt, Germany); and (*R*,*S*)-linalool (EGA, Steinheim, Germany). Prior to sensory experiments, the compounds were purified according to known procedures such as fractional distillation in vacuo, column chromatography, or HPLC. The chemical purity (>99%) and, also, the sensory purity of the compounds were checked prior to sensory experiments by means of gas chromatography−olfactometry as well as gas chromatography−mass spectrometry. The enantiomerically pure (*S*)-ethyl 2-methylbutanoate was a gift of Dr. Fuhrmann, DFA, Garching, Germany.

Syntheses. *(R)-Ethyl 3-Hydroxyhexanoate.* Following the procedure described previously (*13*), ethyl 3-oxohexanoate (4 g) was added to a solution of sucrose (30 g) and yeast (*Saccharomyces cerevisiae*, 20 g) in tap water (200 mL) and stirred in a flask (500 mL) sealed with a fermentation tube for ∼30 h at room temperature. The aqueous solution was subsequently extracted with diethyl ether (4 × 150 mL), the combined organic phases were concentrated to ∼1 mL, and (*R*)-ethyl 3-hydroxyhexanoate (82% e.e., [α_D^{24}] = −4.75) was isolated by column chromatography (*10*). The mass spectral data of the compound agreed with the data of the racemate (Figure 1).

(R)-Methyl 3-Hydroxyhexanoate. (*R*)-Ethyl 3-hydroxyhexanoate (10 mmol) was added to a solution of sodium (10 mg) in methanol (100 mmol) and stirred for 24 h at room temperature. After the addition of a small amount of water, (*R*)-methyl 3-hydroxyhexanoate (82% e.e., [α_D^{24}] = −4.92) was extracted with diethyl ether (4 × 150 mL). The mass spectral data of the compound agreed with the data of the racemate: MS (EI), 43 (100), 71 (50), 74 (30), 55 (30), 41 (29), 61 (28), 103 (26), 128 (5, $M^+ - H_2O$); MS (CI, isobutane), 147 (100, $M^+ + 1$), 129 (15, $M^+ + 1 - H_2O$), 148 (7).

[$^2H_5$]Ethyl 3-Hydroxyhexanoate. Acetyl chloride (10 mmol) was added dropwise to a solution of [$^2H_6$]ethanol (10 mmol) in pyridine (3 mL) at 0 °C to yield [$^2H_5$]ethyl acetate. The mixture was heated for another 10 min and then cooled to 0 °C. After the addition of ice−water (10 mL) and acidification with concentrated hydrochloric acid, the ester was isolated by extraction with diethyl ether (3 × 20 mL). The combined organic layers were washed with aqueous saturated $NaHCO_3$ solution (40 mL) and then with water (40 mL) and finally dried over anhydrous Na_2SO_4. To obtain [$^2H_5$]ethyl 3-hydroxyhexanoate, a mixture of lithium (bistrimethylsilyl)amide in tetrahydrofuran (10 mL, 1.0 M) was cooled for 15 min in an acetone/dry ice bath. Dropwise addition of the ethereal solution of the labeled ethyl acetate and stirring for a further 15 min followed by dropwise addition of a solution of butanal (10 mmol) in tetrahydrofuran (1 mL) followed by hydrolysis with HCl (20%) gave the title product. After warming to room temperature, the organic layer was removed and the aqueous phase extracted twice with diethyl ether (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to 1 mL by distilling off the solvent using a Vigreux column (50 × 1 cm). The product was purified by column chromatography as described previously for the unlabeled ester (*10*). The mass spectrum (MS-EI) of the title compound is given in Figure 1; MS (CI, isobutane), *m*/*z* (%) 166 (100, $M^+ + 1$), 148 (18, $M^+ + 1 - H_2O$), 167 (16).

[5,6-$^2H_2$]Decanal. A mixture of 5-decen-1-ol (10 mmol) and platinum(IV) oxide (50 mg) in [^{2}H]methanol (30 mL) was deuterated in a laboratory autoclave (Roth, Karlsruhe, Germany) at 5 × 10^5 mPa for 90 min at room temperature. After filtration and the addition of water (120 mL), the [5,6-$^2H_2$]-decanol formed was isolated by extraction with diethyl ether (3 × 50 mL). The organic layer was washed with aqueous HCl (1 mol/L, 50 mL), followed by aqueous sodium carbonate (0.5 mol/L, 50 mL) and water (50 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by evaporation in vacuo, the residue taken up in dry dichloromethane (15 mL), and this solution added dropwise to a suspension of pyridinium chlorochromate (15 mmol) and anhydrous sodium acetate (3 mmol) in dry dichloromethane (15 mL). After 2 h of stirring at room temperature under an atmosphere of pure nitrogen, diethyl ether (40 mL) was added, the suspension was filtered through a Florisil column (30 × 2 cm), and the target compound was eluted with diethyl ether (150 mL). The structure was confirmed by MS/EI and MS/CI (Figure 2).

The following compounds were synthesized according to the literature cited: (*Z*)-hex-3-enal (*14*); *trans*-4,5-epoxy-(*E*)-2-decenal (*15*); [2,2,2-$^2H_3$]ethyl 2-methylpropanoate and [2,2,2-$^2H_3$]ethyl 2-methylbutanoate (*16*); [2,2,2-$^2H_3$]ethyl butanoate (*17*); [3,3,4,4-$^2H_4$]hexanal (*18*); [3,4-$^2H_2$]-(*Z*)-hex-3-enal, [4,5-$^2H_2$]oct-1-en-3-one, [2,3-$^2H_2$]-(*E*)-non-2-enal, [7,7,8,8-$^2H_4$]-(*E*,*E*)-deca-2,4-dienal, and *trans*-4,5-epoxy-[7,7,8,8-$^2H_4$]-(*E*)-dec-2-enal (*19*); [$^2H_3$]-3-methylbutanol (*20*); [2,2,2-$^2H_3$]ethyl hexanoate (*21*); [3,3,4,4-$^2H_4$]octanal (*22*); [5,5,6,6-$^2H_4$]nonanal (*23*); 3-([$^2H_3$]-methylthio)-1-propanal (*24*); [3,4-$^2H_2$]butanoic acid (*25*); 3a,4,5,-7a-tetrahydro-3-[$^2H_3$],6-dimethyl-2(3*H*)-benzofuranone and [$^2H_3$]-vanillin (*21*).

The concentrations of the labeled internal standards were determined gas chromatographically using methyl octanoate as the internal standard and using response factors determined in defined mixtures of the respective unlabeled compound and methyl octanoate (*26*). The concentration of [1,2-$^{13}C_2$]acetaldehyde was determined by static headspace in combination with HRGC-MS using the unlabeled aldehyde as the standard (response factor = 1.0).

Isolation and Identification of the Juice Volatiles. The isolation of the juice volatiles was performed by extraction with

Table 1. Selected Ions, Calibration Factors, and Thin Film Capillaries Used in the SIDAs

odorant[a]	ion (*m*/*z*)	internal standard	ion (*m*/*z*)	calibration factor[b]	capillary
acetaldehyde	45	[$^{13}C_2$]acetaldehyde	47	1.00	Rtx
ethyl 2-methylpropanoate	117	[2,2,2-$^2H_3$]ethyl 2-methylpropanoate	120	0.92	DB-5
ethyl butanoate	117	[2,2,2-$^2H_3$]ethyl butanoate	120	1.00	DB-5
(*S*)-ethyl 2-methylbutanoate	131	[2,2,2-$^2H_3$]ethyl 2-methylbutanoate	134	0.95	DB-5
hexanal	83	[3,3,4,4-$^2H_4$]hexanal	86−87	0.73	DB-5
(*Z*)-hex-3-enal	81	[3,4-$^2H_2$]-(*Z*)-hex-3-enal	83	0.74	DB-5
3-methylbutanol	71	[$^2H_3$]-3-methylbutanol	74	0.88	DB-5
2-methylbutanol	71	[$^2H_3$]-3-methylbutanol	74	0.88	DB-5
ethyl hexanoate	145	[2,2,2-$^2H_3$]ethyl hexanoate	148	1.00	DB-5
octanal	111	[3,3,4,4-$^2H_4$]octanal	113−115	0.87	DB-5
oct-1-en-3-one	127	[4,5-$^2H_2$]oct-1-en-3-one	129	0.52	DB-5
nonanal	143	[5,5,6,6-$^2H_4$]nonanal	147	0.87	DB-5
methional	105	3-([$^2H_3$]-methylthio)propanal	108	0.71	DB-5
decanal	157	[5,6-$^2H_2$]decanal	158−160	0.64	DB-5
(*E*)-non-2-enal	141	[2,3-$^2H_2$]-(*E*)-non-2-enal	143	0.83	DB-5
linalool	137	tetrahydrolinalool	141	1.61	DB-FFAP
butanoic acid	89	[3,4-$^2H_2$]butanoic acid	91	0.89	DB-FFAP
ethyl 3-hydroxyhexanoate	161	[1,1,2,2,2-$^2H_5$]ethyl 3-hydroxyhexanoate	166	0.88	DB-5
(*E*,*E*)-deca-2,4-dienal	153	[$^2H_4$]-(*E*,*E*)-deca-2,4-dienal	156−157	0.67	DB-5
trans-4,5-epoxy-(*E*)-dec-2-enal	169	*trans*-4,5-epoxy-[7,7,8,8-$^2H_4$]-(*E*)-dec-2-enal	171−173	0.67	DB-FFAP
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone	167	(3*SR*,3a*SR*,7a*RS*)-3a,4,5,7a-tetrahydro-3[$^2H_3$],6-dimethyl-2(3*H*)-benzofuranone	170	1.00	DB-5
vanillin	153	[$^2H_3$]vanillin	156	1.01	DB-5

[a] Compounds were analyzed by MS/CI using the ion trap detector ITD-800 (Finnigan, Bremen, Germany) and methanol as the reagent gas. [b] The calibration factor was determined as reported previously (*26*).

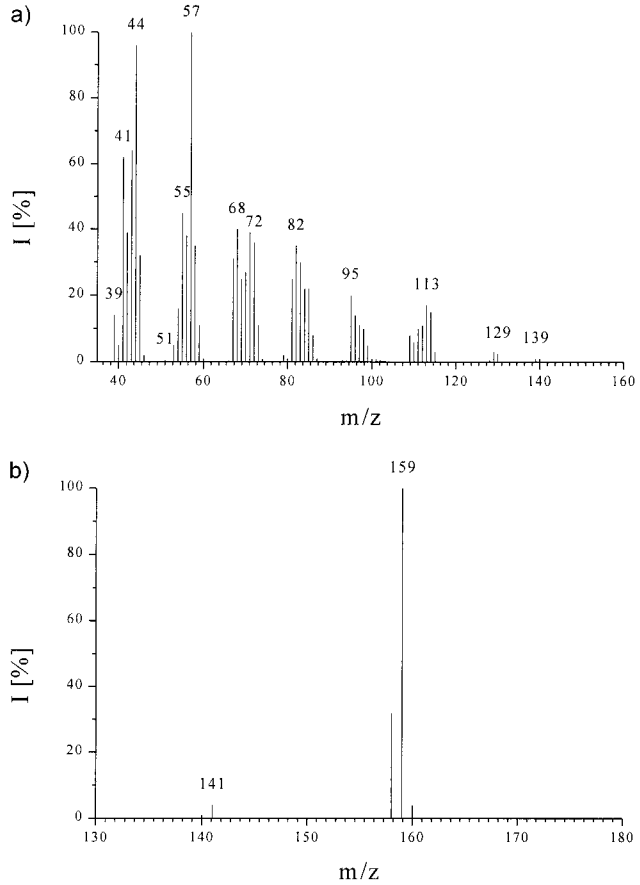

Figure 2. MS (EI) (a) and MS (CI) (b) of [5,6-$^2H_2$]decanal.

diethyl ether followed by distillation in vacuo and subsequent AEDA (*10*). For a comparative AEDA (*11*) of the two orange varieties, exactly the same amounts of juice (600 mL) and solvent (500 mL) were used. The workup procedure was performed for both juices in the same way, yielding 400 μL of extract. The flavor compounds were screened by AEDA and identified by comparison with the reference substances on the basis of the following criteria: retention index (RI) on three stationary phases of different polarities, mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port. Odor intensity was checked by GC−O by comparing the FD factor and the FID signal caused by a defined amount of each reference aroma compound.

Chiral Analysis. The enantiomeric compositions of limonene, α-pinene, ethyl 2-methylbutanoate, and linalool were determined gas chromatographically without derivatization (*10*). Determination of the enantiomeric composition of ethyl 3-hydroxyhexanoate and methyl 3-hydroxyhexanoate was performed on the following capillary and using the GC temperature program: BGB-176 (30 m × 0.25 mm, 2,3-dimethyl-6-*tert*-butyldimethylsilyl-β-cyclodextrine, film thickness = 0.25 μm; BGB Analytik AG, Rothenfluh, Germany). The samples were applied by the cold on-column injection technique at 35 °C. The temperature of the oven was raised at 40 °C/min to 75 °C and then raised at 3 °C/min to 140 °C.

High-Resolution Gas Chromatography—Mass Spectrometry (HRGC-MS). Analysis of the volatiles was performed by two-dimensional gas chromatography (TD-HRGC) by means of a Mega 2 gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany) as the precolumn system in tandem with a Fisons GC 5160 as the main column system (*21*). MS analyses were performed by means of the MS system ITD-800 running in the chemical ionization (CI) mode with methanol as the reagent gas using the following fused silica capillaries: DB-FFAP (30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific, Folsom, CA) in combination with DB-5 (SE-54; 30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific). The samples were applied by the on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (DB-5) or 60 °C (DB-FFAP), held for 2 min isothermally, then raised at 6 °C/min to 180 °C, followed by 15 °C/min to 230 °C, and finally held for 10 min. The flow of the helium carrier gas was 2.5 mL/min. The cut time intervals on the main column were determined by injection of the reference compounds. Details of the mass spectral conditions applied for each odorant are given in Table 1.

Quantitation by Stable Isotope Dilution Assays (HRGC-SIDA) in Solvent Extracts. Three series of experiments using different amounts of orange juice (100 mL or 1 or 5 L, respectively) were performed depending on the amounts of odorants present. The fresh juice was obtained by careful hand-squeezing of the fruits using a kitchen juicer, immediately poured into the same volume of an aqueous saturated $CaCl_2$ solution to inhibit enzymatic reactions, and spiked with known

Table 2. Composition of Matrices Applied in the Sensory Experiments

constituent	composition[a] (%) model I	model II
water	89.9	89.8
sucrose	4.5	4.5
glucose	2.0	2.0
fructose	2.0	2.0
citric acid	1.1	1.1
ascorbic acid	0.5	0.5
sunflower oil + lecithine (10%)	0	0.1

[a] Mean values in orange juices (*28*).

amounts of the labeled internal standards (Table 1). After equilibration (30 min), the juice was extracted with diethyl ether, and the volatiles and the internal standards were isolated as previously described (*10*).

Quantitation of Terpene Hydrocarbons. Quantitation was performed by HRGC-FID using undecane as the internal standard. Analysis was done using a type 8000 gas chromatograph (Fisons, Mainz, Germany) and a capillary DB-FFAP by peak area calculation. Calibration factors were determined using mixtures containing known amounts of undecane and each of the purified terpenes (calibration factors: for limonenee, 0.89; for α-pinene, 0.99; for myrcene, 0.94).

Quantitation of Acetaldehyde. For the determination of acetaldehyde, 10 g of the fresh juice and 10 mL of an aqueous $CaCl_2$ solution were poured into a glass vessel (100 mL), sealed with a septum, and spiked with defined amounts of [1,2-$^{13}C_2$]-acetaldehyde. After 30min of stirring to reach equilibration (checked in preliminary experiments), aliquots of the headspace were withdrawn with a gastight syringe and analyzed by means of an MS Incos XL (Finnigan) connected to a capillary Rtx-5 (30 m × 0.53 mm i.d., 1.5 μm FD, Amchro, Sulzbach/Taunus, Germany) and running in the CI mode at 115 eV. Methane was used as the as reagent gas.

Determination of Odor Thresholds. Odor thresholds in air were determined by HRGC-O using (*E*)-dec-2-enal (2.7 ng/L of air) as the reference (*27*). Odor thresholds (orthonasal and retronasal) were determined in water by using the triangle test (*17*). The samples were presented in order of increasing concentrations in 1:1 dilution steps. Determinations were performed in three separate sessions and values were averaged.

Sensory Evaluation. Assessors were recruited from the German Research Center of Food Chemistry. In preceding sessions the panelists were asked to evaluate five solutions containing acetaldehyde (pungent), (*Z*)-hex-3-enal (grassy), ethyl 2-methylbutanoate (fruity), (*R*)-limonene (terpene-like, peel-like), and octanal (citrus-like), respectively, at suprathreshold concentrations. The odor intensities of the samples were scored from 0.0 to 3.0. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions.

Orange Juice. Hand-squeezed orange juice was judged orthonasally as described recently (*16*). Ten assessors (five males and five females) were asked to rate six odor qualities (fruity, sweet, grassy, terpene-like, pungent, and citrus-like) using a seven-point intensity scale from 0.0 to 3.0. These odor qualities had been selected for the descriptive analysis in previous evaluations of fresh orange juice aroma to be the most intense and characteristic. Flavor profile analyses (FPA) were performed for the juices of Valencia late and Navel oranges and their corresponding model mixtures. The concentration levels of the odorants in the models were equal to those determined in the fresh juices.

Flavor Models. Two model mixtures in tap water were prepared containing the matrix compounds summarized in Table 2 (*28*), one with 0.1% of odorless sunflower oil added (containing 10% of odorless lecithine as emulsifier) and one without. Sunflower oil was used because it showed the weakest overall aroma compared to other oils.

The following 23 compounds dissolved in 500 μL of ethanol (corresponding to the average natural amount of ethanol per liter in a fresh orange juice) were added to 1 L of the matrix solution: acetaldehyde, hexanal, (*Z*)-hex-3-enal, octanal, oct-1-en-3-one, nonanal, methional, decanal, (*E*)-non-2-enal, (*E*,*E*)-deca-2,4-dienal, *trans*-4,5-epoxy-(*E*)-dec-2-enal, ethyl 2-methylpropanoate, ethyl butanoate, (*S*)-ethyl 2-methylbutanoate, ethyl hexanoate, (*R*)-ethyl 3-hydroxyhexanoate, (*R*)-α-pinene, myrcene, (*R*)-limonene, 2- and 3-methylbutanol, linalool, and vanillin.

After 30 min of stirring, the juices from Valencia late oranges and the corresponding model mixtures with and without fat (15 mL each) were presented to the panel for comparative orthonasal evaluation in covered glass beakers (capacity = 45 mL; i.d. = 40 mm). The results obtained in three different sessions were averaged and plotted in a spider web diagram. The values obtained in the different sessions and for the different assessors differed by not more than 10%.

Omission Experiments. On the basis of the quantitative data obtained, 23 model solutions were prepared by omitting one odorant in each mixture. Each mixture was presented in a triangle test for sensory evaluation in comparison to the complete model mixture. Panelists were asked whether dif-

Table 3. Most Odor-Active Volatiles (FD ≥ 8) in Hand-Squeezed Juices of Valencia Late and Navel Oranges

odorant[a]	odor quality[b]	FD factor[c] Valencia late[d]	Navel
ethyl acetate	fruity, solvent-like	32	16
2/3-methylbutanal	malty	32	<1
ethyl propionate	fruity	32	8
ethyl 2-methylpropanoate	fruity	128	16
butane-2,3-dione	buttery	16	8
(*R*)-α-pinene	pine tree	64	16
pent-1-en-3-one	ethereal, pungent	16	8
ethyl butanoate	fruity	1024	128
(*S*)-ethyl 2-methylbutanoate	fruity	128	32
hexanal	green, grassy	32	8
(*Z*)-hex-3-enal	green, grassy	512	256
myrcene	mossy	32	32
(*R*)-limonene	citrus-like	64	32
2/3-methylbutanol	malty	64	8
ethyl hexanoate	fruity	32	32
octanal	green, citrus-like	64	128
1-octen-3-one	mushroom-like	64	32
(*Z*)-octa-1,5-dien-3-one	geranium-like	32	16
nonanal	soapy, citrus-like	16	32
2-isopropyl-3-methoxy-pyrazine	earthy, beany	32	32
methional	cooked potato	64	32
acetic acid	sour, pungent	16	8
decanal	green, soapy	16	8
(*Z*)-non-2-enal	fatty, green	32	32
(*E*)-non-2-enal	fatty, tallowy	32	16
(*S*)-linalool	flowery	16	8
(*E*,*Z*)-nona-2,6-dienal	cucumber-like	8	<1
p-menth-1-ene-8-thiol	grapefruit-like	8	<1
butanoic acid	sweaty, rancid	8	4
(*R*)-ethyl 3-hydroxyhexanoate	sweet, fruity	64	32
(*E*,*E*)-2,4-decadienal	fatty, waxy	8	8
β-ionone	violet-like	16	<1
trans-4,5-epoxy-(*E*)-2-decenal	metallic	128	64
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	8	<1
unknown	metallic	64	8
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone	sweet, spicy	256	128
vanillin	vanilla-like	32	16

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on three stationary phases of different polarity, mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port as reported previously (*10*). [b] Odor quality as perceived at the sniffing port. [c] FD factor determined in extracts containing the juice volatiles. Analyses were performed by two assessors in duplicates. The data differed to not more than 2 FD factors. [d] Data from ref *10*.

Table 4. Odor Thresholds of Orange Juice Odorants

odorant	odor threshold in air[a] (ng/L)	odor threshold in water[b] (μg/kg) orthonasal	odor threshold in water[b] (μg/kg) retronasal
acetaldehyde	41[c]	25[c]	10[c]
ethyl 2-methylpropanoate	nd[e]	0.02[c]	0.03[c]
(*R*)-α-pinene	5.3	5	33
ethyl butanoate	2.7	1[c]	0.1[c]
(*S*)-ethyl 2-methylbutanoate	nd	0.006[c]	0.004
hexanal	30[c]	10.5[c]	10.5[c]
(*Z*)-3-hexenal	0.09−0.36[c]	0.25[c]	0.03[c]
myrcene	44.5	14[c]	16.6[c]
(*R*)-limonene	424	200[c]	34
3-methylbutanol	nd	1000[c]	250[c]
2-methylbutanol	nd	320[c]	nd
ethyl hexanoate	3.0	5[c]	0.5
octanal	5.8−13.6[c]	8[c]	45[c]
1-octen-3-one	0.3−0.6[c]	1[c]	0.01[c]
nonanal	5.2−12.1[c]	5[c]	3.5
methional	0.1−0.2[c]	1.8[c]	0.04[c]
decanal	1[c]	5[c]	7[c]
(*E*)-2-nonenal	0.1[c]	0.8[c]	0.08[c]
linalool[d]	0.4−0.8[c]	6[c]	1.5[c]
butanoic acid	nd	1000[c]	1000[c]
(*R*)-methyl 3-hydroxyhexanoate	2.1	3760	nd
(*S*)-methyl 3-hydroxyhexanoate	4380	nd	nd
(*R*)-ethyl 3-hydroxyhexanoate	2.1	270	63
(*S*)-ethyl 3-hydroxyhexanoate	264.5	nd	nd
(*E*,*E*)-2,4-decadienal	0.13[c]	0.2[c]	0.05[c]
trans-4,5-epoxy-(*E*)-2-decenal	0.0006−0.0025[c]	0.12[c]	0.015[c]
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone	0.00001−0.00004[c]	nd	0.008[c]
vanillin	0.6−1.2[c]	25[c]	30[c]

[a] The odor thresholds in air were determined as described previously (*27*). [b] Odor thresholds in water were determined using the triangle test (*16*). Average values of triplicates are given. [c] Odor thresholds reported in the literature (*30*). [d] The odor threshold of the racemic compound is given. [e] nd, not determined.

ferences were detectable. The significance α of the detected differences was calculated (*29*), with the highest significance level corresponding to 0.1 and the lowest to 5.

RESULTS AND DISCUSSION

Comparative AEDA of the Hand-Squeezed Juices of Valencia Late and Navel Oranges. In preliminary hedonic sensory evaluations (25 students), the juice made from Valencia late oranges was characterized by the most fruity-sweet aroma, whereas the juice from Navel oranges was judged to be less fruity but more citrus-like and pungent.

Flavor extracts of both juices were prepared by solvent extraction and high-vacuum transfer, and the odor-active volatiles were detected by AEDA (*10*). Of the 42 odorants found in the extract of the Valencia late oranges, all compounds with higher FD factors were also present in Navel (Table 3). In both extracts, ethyl butanoate, (*Z*)-hex-3-enal, *trans*-4,5-epoxy-(*E*)-dec-2-enal, and 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone were among the most potent odorants as judged from high FD factors between 64 and 1024. In particular, the FD factors of the fruity odorants ethyl 2-methylpropanoate and ethyl butanoate and those of the malty-smelling methylbutanols were significantly lower in the Navel juice.

Enantiomeric analysis showed that limonene and α-pinene were present in both juices as pure (*R*)-enantiomers (100% e.e.), whereas ethyl 2-methylbutanoate and linalool were predominantly the (*S*)-enantiomers (98 or 90% e.e., respectively). In ethyl 3-hydroxyhexanoate, the (*R*)-enantiomer predominated in both juices (48% e.e. in Valencia late and 66% e.e. in Navel), whereas in methyl 3-hydroxyhexanoate (FD < 4, data not shown), the excess of the (*R*)-compound was lower (14% e.e. in Valencia late and 16% e.e. in Navel). It is interesting to note that the odor qualities of both, the enantiomers of ethyl 3-hydroxyhexanoate and of methyl 3-hydroxyhexanoate, differed significantly. The more odor-active (*R*)-enantiomers (Table 4) elicited a very intense sweet, woody odor note at the sniffing port, whereas the smell of the (*S*)-compounds was weak and aldehyde-like.

In general, the results reported here for Valencia late were in good agreement with those given earlier by us (*10*).

Quantitative Analysis. To objectify the flavor differences observed, 25 odorants were quantified in both juices. (*R*)-Limonene and acetaldehyde were the most abundant aroma compounds in both orange juices, showing concentrations in the milligrams per kilogram range (Table 5). In addition, quite high amounts of ethyl butanoate and (*R*)-ethyl 3-hydroxyhexanoate were found in Valencia late juice. Odorants present in extremely low concentrations were 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone and (*E*)-non-2-enal.

Differences between the varieties were established mainly in ethyl butanoate and (*S*)-ethyl 2-methylbutanoate, being lower by a factor of ∼10−20 in the Navel juice, and in ethyl 2-methylpropanoate and ethyl 3-hydroxyhexanoate, being lower by a factor of 3 compared to the Valencia late juice. The amounts of the methylbutanols in Valencia late significantly exceeded that in Navel. On the other hand, octanal and nonanal were higher in Navel.

OAVs. To estimate their respective odor contributions, the OAVs of the odorants were calculated on the basis of their nasal and retronasal odor thresholds in water (cf. Table 4). In Valencia late, the highest OAVs were calculated for (*S*)-ethyl 2-methylbutanoate, ethyl butanoate, and (*Z*)-3-hexenal, followed by ethyl 2-methylpropanoate, acetaldehyde, and (*R*)-limonene (Table

Table 5. Concentrations of Potent Odorants in Hand-Squeezed Juice of Valencia Late and Navel Oranges

odorant	concn[a] (μg/kg)	
	Valencia late	Navel
acetaldehyde	8305	6400
ethyl 2-methylpropanoate	8.8	2.7
(*R*)-α-pinene	308	133
ethyl butanoate	1192	50
(*S*)-ethyl 2-methylbutanoate	48	4.2
hexanal	197	65
(*Z*)-hex-3-enal	187	399
myrcene	594	230
(*R*)-limonene	85598	26452
3-methylbutanol	639	16
2-methylbutanol	270	4.5
ethyl hexanoate	63	51
octanal	25	88
oct-1-en-3-one	4.1	5.7
nonanal	13	32
methional	0.4	0.3
decanal	45	149
(*E*)-non-2-enal	0.6	1.5
(*S*)-linalool	81	73
butanoic acid	74	43
(*R*)-ethyl 3-hydroxyhexanoate	1136	361
(*E*,*E*)-deca-2,4-dienal	1.2	1.2
trans-4,5-epoxy-(*E*)-dec-2-enal	4.3	5.8
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone	0.8	2.1
vanillin	67	212

[a] Data are mean values of at least duplicates.

Table 6. OAV of Potent Odorants in Hand-Squeezed Juices of Valencia Late (VL) and Navel (NV) Oranges

odorant	OAV (n)[a]		OAV (rn)[a]	
	VL	NV	VL	NV
acetaldehyde	332	256	831	640
ethyl 2-methylpropanoate	440	135	293	90
(*R*)-α-pinene[b]	62	27	9	4
ethyl butanoate	1192	50	11920	504
(*S*)-ethyl 2-methylbutanoate[b]	8000	700	12000	1050
hexanal	19	6	19	6
(*Z*)-hex-3-enal	747	1598	6227	13313
myrcene	42	16	36	14
(*R*)-limonene[b]	228	35	1339	205
3-methylbutanol	<1	<1	3	<1
2-methylbutanol[c]	<1	<1	nd[d]	nd
ethyl hexanoate	13	10	125	102
octanal	3	11	<1	2
oct-1-en-3-one	4	5	410	570
nonanal	3	6	4	9
methional	<1	<1	10	8
decanal	9	30	6	21
(*E*)-non-2-enal	<1	2	8	19
(*S*)-linalool[c]	13	12	54	49
butanoic acid	<1	<1	<1	<1
(*R*)-ethyl 3-hydroxyhexanoate[b]	4	1	18	6
(*E*,*E*)-deca-2,4-dienal	6	6	24	24
trans-4,5-epoxy-(*E*)-dec-2-enal	36	48	287	387
3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone[b]	nd	nd	94	269
vanillin	3	9	2	7

[a] The OAV (n = nasally; rn = retronasally) were calculated by dividing the concentrations of the odorants by their nasally or retronasally determined detection thresholds in water (cf. Table 4). [b] The OAVs were calculated on the basis of the detection thresholds of the enantiomerically pure compounds. [c] The OAVs were calculated on the basis of the odor threshold determined for the racemic compound. [d] nd, not determined.

6), thus establishing a major contribution of the fruity esters to the overall aroma of the Valencia juice. In Navel orange juice, the highest OAVs were found for

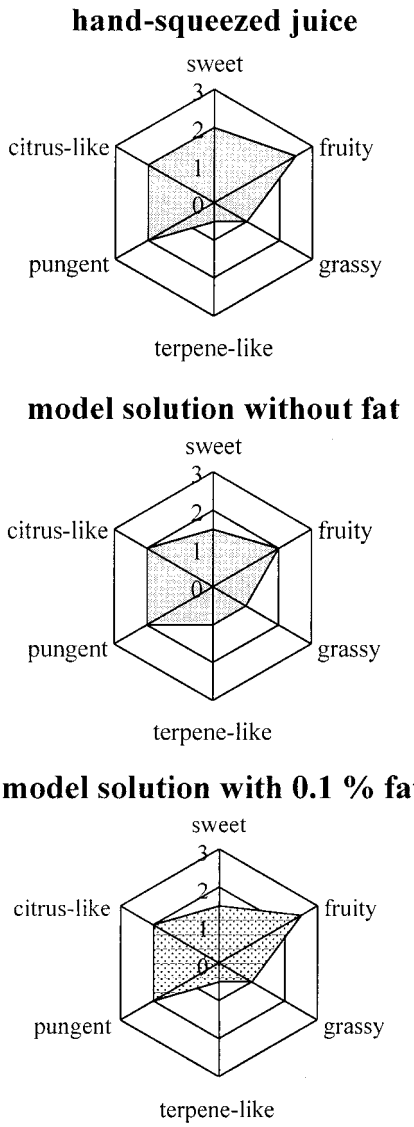

Figure 3. Comparative flavor profile analysis of hand-squeezed juice of Valencia late oranges and of two model solutions with or without added fat.

the green-smelling (*Z*)-3-hexenal, followed by (*S*)-ethyl 2-methylbutanoate, acetaldehyde, ethyl 2-methylpropanoate, and ethyl butanoate. Moreover, higher retronasal OAVs compared to the orthonasal ones were determined for ethyl hexanoate, oct-1-en-3-one, *trans*-4,5-epoxy-(*E*)-dec-2-enal, and 3a,4,5,7a-tetrahydro-3,6-dimethyl-2(3*H*)-benzofuranone (Table 6).

Sensory Experiments. To verify the analytical data, sensory experiments were performed to mimick the overall aromas in model solutions. In model $M \geq 1$ all odorants showing OAVs ≥1 in the fresh juice of Valencia late oranges were used in the same concentrations as determined as "natural" amounts in the juices (cf. Table 5).

In a preliminary experiment, the influence of lipids (which are reported to occur between 0.1 and 0.2% in fresh orange juice) on the aroma of the model solutions was studied. Addition of 0.1% oil to the entire model solution ($M \geq 1$) resulted in a decrease of the terpene-like odor quality, whereas the fruity note was increased (Figure 3). In general, the model containing fat was described as more pleasant with respect to the typical fresh, orange-like aroma. A comparative evaluation done for both the model solutions of Valencia late and Navel

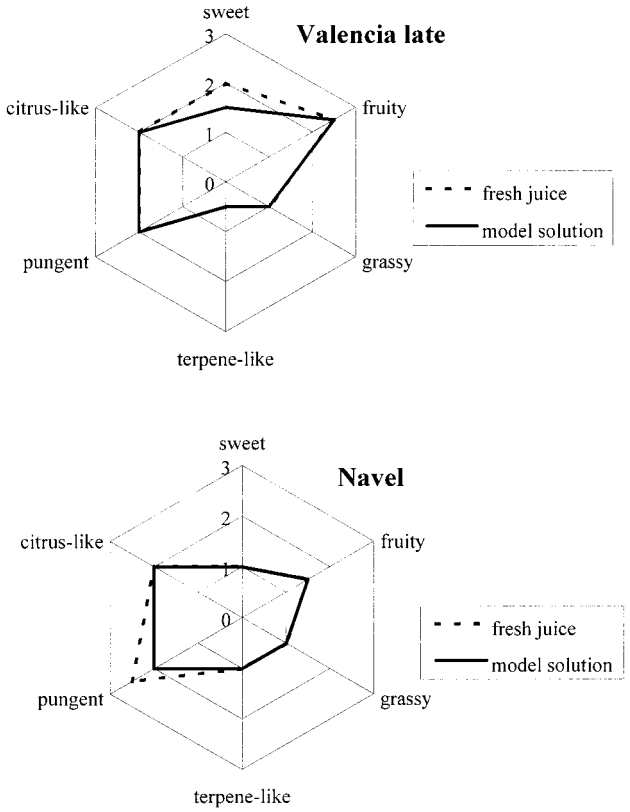

Figure 4. Comparative flavor profile analysis of the fresh juices of Valencia late and Navel oranges and the corresponding model solutions.

Table 7. Odor Differences between the Complete Model Solution ($M \geq 1$) and Model Solutions Containing One Odorant Less

odorant omitted[a]	significance α^b (%) orthonasal	retronasal
acetaldehyde	1	0.1
(*Z*)-hex-3-enal	nd[c]	1
oct-1-en-3-one	nd	5
decanal	5	nd
trans-4,5-epoxy-(*E*)-dec-2-enal	nd	1
(*R*)-limonene	0.1	1

[a] Model $M \geq 1$: All odorants present with OAVs ≥1 in the juice of Valencia late oranges (cf. Table 6) were used in the sensory experiments. [b] Determination of the differences by triangle test and calculation of the significance α according to the literature (*29*). 0.1 = highest significance; 5 =lowest significance. [c] nd, not detectable.

revealed high similarity to the natural aromas of both juices (Figure 4).

Omission Experiments. In another series of sensory experiments, the aroma contribution of single odorants to the model mixture was tested in triangle tests comparing the odor of the complete mixture of 25 odorants to a mixture containing one odorant less.

Only the absence of acetaldehyde and (*R*)-limonene, respectively, was detectable with high significance ($\alpha = 0.1$) by either orthonasal or retronasal evaluation (Table 7). Retronasally, a difference was detectable when (*Z*)-hex-3-enal, oct-1-en-3-one, or *trans*-4,5-epoxy-(*E*)-dec-2-enal was omitted, whereas the absence of decanal was detectable with a low significance of $\alpha = 5$.

Further experiments were performed to clarify whether additive effects exist for odorants with similar odor quality. Three groups of odorants (terpene hydrocarbons, esters, or aldehydes) were omitted from the complete mixture. For all groups, a significant difference from the original model was observed (Table 8). The omission of ethyl 2-methylpropanoate, (*S*)-ethyl 2-methylbutanoate, and ethyl hexanoate with ethyl butanoate still present was clearly detected by the panelists, thereby indicating additive effects for the fruity aroma note. In contrast, no difference was perceivable when myrcene and (*R*)-α-pinene were omitted.

Table 8. Odor Differences between Model $M \geq 1$ and Model Solutions Lacking in an Odorant Group

odorant group omitted	significance α^b (%) orthonasal	retronasal
terpene hydrocarbons	0.1	0.1
esters	0.1	0.1
aldehydes	0.1	0.1
ethyl isobutanoate, (*S*)-ethyl-2-methylbutanoate, ethyl hexanoate	5	5
hexanal, octanal, nonanal, decanal	1	1
myrcene, (*R*)-α-pinene	nd[c]	nd

[a] Model $M \geq 1$: All odorants with OAVs ≥1 in fresh juice of Valencia late oranges (cf. Table 6) were used for the sensory experiments. [b] Calculation of the significance α according to ref *29*. [c] nd, not detectable.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; FD, flavor dilution factor; OAV, odor activity value; SIDA, stable isotope dilution assay; e.e., enantiomeric excess.

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