FLAVOUR AND FRAGRANCE JOURNAL
Flavour Fragr. J. 2004; **19**: 491–498
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ffj.1470

Odour and flavour thresholds for key aroma components in an orange juice matrix: terpenes and aldehydes

Anne Plotto,[1]* Carlos A. Margaría,[2] Kevin L. Goodner,[1] Renée Goodrich[3] and Elizabeth A. Baldwin[1]

[1] USDA—ARS, Citrus and Subtropical Products Laboratory, 600 Avenue S, NW, Winter Haven, FL 33881, USA
[2] United States Distilled Products, 1607 S 12th St., Princeton, MN 55371, USA
[3] University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA

Received 2 September 2003; Revised 13 February 2004; Accepted 16 February 2004

ABSTRACT: Thresholds for flavour volatiles have been traditionally calculated in water or air, but they may vary widely in more complex matrices. Thresholds of key aroma compounds of orange juice (OJ) were determined in a deodorized OJ matrix. The three-alternative-forced-choice (3-AFC) method was used (ASTM: E-679). Untrained panelists, 33–58 in number, were presented with deodorized orange juice samples arranged in five rows of three samples, corresponding to five spiking levels, each separated by a factor of 3, with a 3-AFC presentation at each level. The test was repeated at least three times for experienced panelists. Odour thresholds in the orange juice matrix were 15 times (citral, hexanal) to over 200 times (β-pinene, limonene) higher than published values in water. Retronasal odour thresholds were more consistent with published values, being higher only by 2–60-fold, except for octanal which was higher by 187-fold. These results will provide the industry with more realistic threshold guidelines for use in flavouring citrus juices. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: sensory; orange juice; aroma; flavour; thresholds; aldehydes; terpenes

Introduction

Food flavours are commonly analysed by isolation, qualification and quantification of volatile compounds. The contribution of chemical compounds to food flavour is best understood when their perception threshold is known: compounds in food products present in concentrations higher than their threshold are assumed to contribute to the flavour of the food.[1–4] The major limitation in this approach is that it requires use of published threshold values, mostly established in water or in air.[5,6] Some threshold values are calculated in oil or milk but few, if any, are calculated in other complex matrices. However, there is increasing evidence that volatile molecules interact with the food matrix.[7–9] Lipids, proteins and carbohydrates result in different degrees of volatile compound interaction, depending on the hydrophobicity of the volatile compound, diffusion and partition coefficient, and rheological properties of the aqueous phase.[10,11] Smaller molecules, such as simple sugars and acids, either adsorb or have a 'salting out' effect on some volatile compounds.[12,13] Phenolic compounds present in wine and some fruit juices undergo chemical complexes with flavour volatiles.[14,15] Additionally, to make up the flavoured solutions for threshold determination in water, one encounters the problem of solubility of pure compounds in water. Thresholds of volatile compounds characteristic of tomato aroma were different by as much as 40-fold (2-pentenal) between a deodorized tomato homogenate medium, an ethanol/methanol/water solution, and water.[16] Thresholds were usually higher in the tomato matrix.

There is an inherent variability in the quality of orange juice due to cultivars, cultural practices, season, and juice extraction processes.[17] Blending juices or concentrates from different lots and origins lessens the juice variability. Standardization is also achieved by adding flavour blends, mostly oils from citrus extracts.[18] This is common practice for orange juice from concentrate and not from concentrate.[19] Volatile compounds in orange juice and their individual contributions to aroma and flavour are now well known.[4,20–24] Odour and flavour thresholds of most of those compounds were determined in water by Ahmed and co-workers,[25] and further by Buettner and Schieberle.[20] The combination of compounds added to a model solution that best matched a reference sample, whether a frozen orange juice concentrate[26] or a freshly squeezed orange juice,[20] has also been studied.

The goal of the present study was to establish a database of odour and retronasal odour thresholds for compounds significant to orange juice flavour for use by orange juice manufacturers and flavour companies. Because published threshold values can vary by several-fold due to different methodologies and panel make-up,[27] we chose to use the method approved by the American

* Correspondence to: A. Plotto, USDA—ARS, Citrus and Subtropical Products Laboratory, 600 Avenue S, NW, Winter Haven, FL 33881, USA. E-mail: plotto@citrus.usda.gov

Society for Testing and Materials.[28] Results are presented for selected terpenes and aldehydes.

Materials and Methods

Materials

Deodorized orange juice concentrate (termed 'pumpout' by the industry) from Valencia oranges was provided by a Florida juice manufacturer. The pumpout was reconstituted to single-strength orange juice with purified drinking water (Deer Park, Greenwich, CT) to 11.8 ± 0.1°Brix. Specifications of the concentrate were the following: 65.7°Brix, 3.007 g/100 g acid, 0.001% oil. After reconstitution, one batch of juice was analysed for sugars, using a Perkin-Elmer Series 410 HPLC system equipped with a Perkin-Elmer LC-25 refractive index detector (Perkin-Elmer, Norwalk, CT). The sample was prepared as in Baldwin *et al*.;[29] sugars were analysed on a Waters Sugar-Pak column (Waters/Millipore, Milford, MA) with a mobile phase of 100 μM ethylenediaminetetraacetic acid disodium calcium salt (CaEDTA). Titratable acidity was determined by titration with 0.1 N NaOH to a pH = 8.1 endpoint. Pectin content was determined by analysing the total galacturonic acid content, using the Scott assay.[30] Volatiles from 20 ml reconstituted juice were liquid–liquid extracted with 10 ml pentane or diethyl ether, using two 50 ml syringes connected with a Luer-lock connector, and concentrated to 500 μl. Extracts were analysed for volatile and odour-active compounds on an Agilent 6890N (Agilent Technologies, Palo Alto, CA) gas chromatograph (GC) equipped with a 5973N mass selective detector and coupled with a Gerstel ODP2 olfactory detection port (Gerstel, Baltimore, MD). The analytical column was HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness. Headspace of the reconstituted juice was also analysed by direct injection using a Perkin-Elmer 8500 GC equipped with a Model HS-6 headspace sampler and a flame ionization detector.[29] The analytical column was a polar Stabilwax column (Restek, Bellefonte, PA), dimensions 30 m × 0.53 mm i.d., 1.0 μm film thickness. The sensory quality of reconstituted pumpout was assessed by a group of four experienced panellists and was described as bland, sweet, slightly tangy. The gas chromatography–olfactometry (GC–O) of the reconstituted pumpout solvent extract was assessed twice by the experimenter.

All the aroma standards used for spiking were food grade (Sigma-Aldrich, Flavors and Fragrances, Milwaukee, WI), and tested for purity by GC–MS and GC–O (Table 1). When odour impurities were found by smelling the pure compound using GC–O, the compound was diluted by 10-fold until no impurity was detected (Table 1). The highest dilution used was 1000-fold for myrcene. As myrcene was further diluted over 1000 times to determine its threshold, the experimenters estimated that the impurities would be negligible. Additionally, the odour of the impurities were generally perceived with a much lower intensity than the compound of interest. Therefore, it was deemed that the impurities would not affect the threshold significantly at the level used in the test (see Table 1). Compounds that could not be tested due to significant impurities were sinensal, α-phellandrene and α-terpinene.

Sample Preparation

Fresh reconstituted juice was prepared weekly from frozen pumpout and stored at 4 °C for 5 days maximum. Juice (1 l) was spiked with an aroma compound at the

Table 1. Aroma compounds tested, their chemical purity determined by GC–MS, olfactory purity determined by GC–O, and concentration range used for threshold determination in the 3-AFC test

Compound	Chemical purity (%)	Olfactory purity (%)[a]	Concentration range ×/9–9× (μg/l)
α-Pinene	99+	99+	94–7640
β-Pinene	99+	99+	4758–385 413
Myrcene	89+	(10^6 ppb)[a]	26–2112
γ-Terpinene	95+	99+	35–28 602
Limonene	98+	99+	4072–329 836
α-Terpineol	98+	99+	1013–82 026
Linalool	97+	99+	9–753
Vanillin	99+	99+	13–1053
Citral (a and b)	97+	(10^8 ppb)[a]	35–2871
Hexanal	89+	(10^7 ppb)[a]	18–1451
Octanal	85+	99+	11–921
Nonanal	99+	99+	32–2589
Decanal	98+	99+	17–1347
trans-2-Pentenal	98+	(10^7 ppb)[a]	94–7585

[a] Compounds were evaluated pure by GC–O. When impurities were present in pure compounds, the concentration at which the impurity was no longer present is indicated.

highest concentration used in the taste panel (9×), and refrigerated overnight at 4 °C to allow the compound to equilibrate with the juice (Shaw, unpublished data). Four successive three-fold dilutions (3×, ×, ×/3, ×/9) were then prepared immediately before tasting. The amount of aroma compound '×', which represents the putative threshold concentration for most panellists, was predetermined by using published thresholds in water[5] and screened by four panellists, including the experimenter (Table 1). Orange juice (control and spiked, 15 ml aliquots) was poured into 29.5 ml plastic (polystyrene) soufflé cups and capped (Solo® Cup Company, Urbana, IL). Control samples were prepared on the day before the panel and maintained at 4 °C overnight, and spiked samples were prepared immediately before the panel, and placed on the serving trays with the blanks in a cooler at 10–12 °C to equilibrate temperature with the control until served.

Taste Panels

Volunteers (n = 33–58) from the University of Florida Citrus Research and Education Center at Lake Alfred, FL, and the USDA Citrus and Subtropical Products Laboratory, Winter Haven, FL, participated in the sensory panels. The panels took place in individual booths, usually from 10:00 a.m. to 12:00 p.m. (noon), with occasional afternoon sessions. Panelists' ages and genders were recorded.

The three-alternative forced choice (3-AFC) test was used for threshold determination[28] (ASTM Designation: E-679, 1997). In this method, the 3-AFC consists in three samples: two are controls, and one is the spiked sample. Panelists were presented with a tray of 15 cups, corresponding to five 3-AFCs with five spiking levels; each level differed from the preceding one by a factor of 3 (×/9; ×/3; ×; 3× and 9×) and were tested in ascending order (most diluted first) and from left to right. All cups were labelled with a randomized three-digit number and the order in which the spiked sample appeared for each level was also random. Panellists were first instructed to uncap the cups near their nose, smell, and choose the sample that had the added compound in each set of three cups. If they could not perceive a difference, they were instructed to guess (forced choice). Panellists then tasted the samples, choosing the odd sample. The probability of choosing the correct sample was one to three. When they could perceive the presence of the added compound, panellists were asked to write an additional comment on the quality of the odour or taste. Sample temperature at serving was 10–12 °C. Each panel was repeated three to five times, but not all panellists could participate in each session. Therefore, data were analysed for all panellists (population threshold), and for panellists who tasted three times or more (experienced panel).

Threshold Determination

The best-estimate criterion was used to calculate individual thresholds: the threshold for each individual at each panel was an interpolated value calculated by taking the geometric mean between the last concentration missed and the first concentration detected. The panellists' individual best estimate of each threshold was the geometric mean of all the sessions thresholds, and the group (population) threshold was obtained by a geometric mean of the individual best estimates for each compound.[28]

Results and Discussion

Thresholds measured in reconstituted deodorized orange juice were at least 10 times higher than published threshold values in water for ortho- and retronasal odour, except for a few compounds (Tables 2 and 3, respectively). Compounds whose odour thresholds were within the same order of magnitude as those in published studies were vanillin[31] and *trans*-2-pentenal.[32] Odour threshold for hexanal was between the values found by Larsen and Poll[33] and Tandon *et al*.[16] In general, population thresholds were higher than experienced panel threshold, and orthonasal were higher than retronasal odour thresholds. Results will be discussed in terms of panel make-up, methodology and matrix effect.

Panel Make-up

The goal of our study was to obtain threshold values that would better reflect the perception of orange juice by consumers than by a trained panel. Therefore, the panels were designed to be similar to consumer panels. Panellists consisted of staff and visitors of two experiment centres: the University of Florida Citrus Research and Experiment Center, and the US Department of Agriculture Citrus and Subtropical Products Laboratory. The proportion of male and female panellists was in the range 33–60% and 40–67%, respectively. The majority of panellists were in the 36–55 year-old age group, with an average 13% below 26, 9% 26–35, 26% 36–45, 30% 46–55, 17% 56–65 and 5% over 65. Panellists were not selected for acuity or reproducibility. Among the experienced panellists (about one-third of all the panellists) who were used to participating in sensory panels, the non-perceivers (people who could not perceive the spiked sample at any concentration) and the over-perceivers (people who could perceive the spiked sample at the most diluted concentrations during the three replicate runs) were also represented. Linalool and citral orthonasal odour thresholds were almost twice for the population than for the experienced panel (Table 2), and

Table 2. Population and experienced panel orthonasal odour thresholds of orange juice compounds in a deodorized orange juice matrix (OJ), and published orthonasal odour thresholds of the same compounds in water

Compound	Population odour threshold in OJ (μg/L) (standard deviation, log ppb)	Number of panelists in population	Experienced panel odour threshold in OJ (μg/L) (standard deviation, log ppb)	Number of experienced panelists	Published threshold in water (μg/l)
α-Pinene	1650 (0.72)	39	2120 (0.57)	17	5;[20] 6;[49] 9.5[25]
β-Pinene	37 200 (0.61)	38	38 700 (0.64)	21	140[49]
Myrcene	773 (0.66)	46	532 (0.59)	18	13;[49] 36[25]
γ-Terpinene	3260 (0.68)	51	2390 (0.64)	15	
Limonene	13 700 (0.59)	40	8500 (0.50)	16	10;[36] 60;[25] 200[5]
α-Terpineol	25 900 (0.52)	33	16 600 (0.51)	18	280;[25] 330;[37] 350[36]
Linalool	113 (0.72)	39	67 (0.78)	20	5;[42] 5.3;[25] 6[49]
Vanillin	764 (0.48)	58	740 (0.49)	16	20;[5] 25;[50] 680[31]
Citral (a and b)	1230 (0.47)	50	656 (0.42)	15	32[36](ger.)–30[32](ner.); 85.3[25]
Hexanal	151 (0.67)	42	135 (0.63)	22	4.5;[35] 5.8;[5] 10.5;[51] 9.2;[25] 50;[33] 479[16]
Octanal	233 (0.72)	45	474 (0.43)	12	0.7;[35] 1.41;[25] 8[5]
Nonanal	312 (0.59)	38	232 (0.52)	21	1;[35] 2.53;[25] 5[5]
Decanal	204 (0.60)	42	197 (0.64)	17	0.1;[35] 1.97;[25] 2;[37] 5[5]
trans-2-Pentenal	2770 (0.46)	33	2330 (0.47)	22	1500;[32] 55[16]

Table 3. Population and experienced panel retronasal odour thresholds of orange juice compounds in a deodorized orange juice matrix (OJ), and published retronasal odour thresholds of the same compounds in water

Compound	Population retronasal odour threshold in OJ (μg/l) (standard deviation, log ppb)	Number of panelists in population	Experienced panel retronasal odour threshold in OJ (μg/L) (standard deviation, log ppb)	Number of experienced panelists	Published flavour threshold in water (μg/L)
α-Pinene	2010 (0.66)	39	2120 (0.55)	17	33;[20] 1014[25]
β-Pinene	36 100 (0.65)	38	38 700 (0.66)	21	
Myrcene	500 (0.70)	46	399 (0.61)	18	16.6;[5] 42[25]
γ-Terpinene	2140 (0.56)	51	2650 (0.45)	15	
Limonene	13 330 (0.62)	40	8470 (0.46)	16	34;[20] 210[25]
α-Terpineol	9060 (0.50)	33	9020 (0.47)	18	300[25]
Linalool	105 (0.81)	39	66 (0.84)	20	1.5;[52] 3.8[25]
Vanillin	161 (0.72)	58	224 (0.60)	16	30[5]
Citral (a and b)	714 (0.53)	50	580 (0.45)	15	41.4[25]
Hexanal	88 (0.67)	42	79 (0.50)	22	3.66;[25] 10.5;[53] 16;[34] 30;[54] 76[55]
Octanal	97 (0.72)	45	153 (0.60)	12	0.52;[25] 5;[54] 45[55]
Nonanal	165 (0.54)	38	130 (0.51)	21	3.5;[20] 4.25;[25] 12[55]
Decanal	97 (0.51)	42	70 (0.45)	17	3.02;[25] 7[54,55]
trans-2-Pentenal	1970 (0.50)	33	1650 (0.52)	22	

the retronasal odour thresholds were also higher for linalool and limonene (Table 3). Conversely, decanal odour thresholds (orthonasal and retronasal) were higher for the experienced panel (Tables 2 and 3). Such levels of differences were reported between panels made of the same laboratory members.[28] Most studies reporting odour or flavour thresholds use 10–20 panel members. Some panels are selected for their reproducibility,[31,34] others for their sensitivity and reproducibility.[32,35–37] Guadagni and Buttery[38] found that odour threshold for 2,3,6-trichloroanisole was three times higher for untrained than for trained panelists. Thresholds that are determined with panels of less than 10 trained members are most likely to result in very precise values, and are usually used in flavour research.[20,39] However, they do not represent the population average perception, and with a small number of panellists, one cannot determine whether a population is normal, skewed or bi-modal.[27,40] To model consumer taste, a population threshold is preferable to determine the level of a pollutant or contaminant in air or in food, or to be used in product development for the food and beverage industry.[27,28]

Methodology

The high level of odour threshold values determined in this experiment, as compared to published values, is in part due to the presentation method: chilled samples were presented in 30 ml capped plastic soufflé cups, with

15 ml headspace. This is comparable to consumers tasting refrigerated orange juice from a glass. In the initial work performed by the Flavor Research Group at the US Department of Agriculture Western Regional Research Center in Albany, CA, Guadagni, Buttery and co-workers found better reproducibility and lower thresholds when odorants were presented directly to the nostril with a squeeze sniff bottle, as compared to the standard sniffing technique of a glass vial headspace.[35] Thresholds were 1.2 (propanal) to 98 (nonanal) times lower with the sniff bottle as compared to the vial headspace. It is interesting to note that for decanal, the threshold value from the same laboratory, but using different panelists and Teflon bottles instead of polyethylene bottles, resulted in a threshold 20 times higher, 2 ppb vs. 0.1 ppb, respectively.[35,41]

In this study, the ASTM E-679 'rapid' method was used over other methods because it provides a practical value for a group threshold with a minimum of tests (20–40 3-AFC presentations to 5–15 panellists, with a total of 100–600 presentations)[28,40]. We used a minimum of 33 panellists, with up to five replications, for a total of 400 presentations or more, depending on the compound. The ASTM E-1432 method,[40] in which the threshold is the stimulus level detectable by 50% of the population with a 0.5 probability, would be more accurate and bias-free, because it determines individual thresholds by fitting a response curve to the panellist's response to a range of concentrations.[27] But the test requires five times more samples than the rapid method, and it must be repeated by adjusting stimulus levels until the threshold is determined for all panellists.

A dilution factor of 3 was used as the dilution step.[28] While it would be more accurate to adjust this factor for each compound, because the response power function is different for each compound and subject, this was found to be the best compromise to cover the range of concentrations that might be perceived by all panellists. Grosch and co-workers used 50% dilutions,[20,39] Larsen and Poll presented compounds at 1:10 dilutions.[33,42]

Finally, different levels of error are accounted for in threshold studies. With one spiked and two reference samples,[31,34,39] there is 33% chance of guessing which is the spiked sample, while with a paired comparison test,[35,36] there is a 50% chance of guessing correctly. Larsen and Poll used a modified duo-trio test, with a 1/6 chance of correct guess.[42]

Vanillin odour threshold was similar in the present study to that published by Keith and Powers.[31] These authors presented samples in a decreasing order of concentration, which is known to induce a fatigue or habituation effect in perception. Therefore, this explains why the threshold value found for vanillin in water in that study is higher than other studies (Table 2).

There is a great variation between published odour thresholds for hexanal, one of the most referenced compounds (Table 2). The difference can be accounted for by the panel make-up and size: Guadagni and co-workers[35] selected sensitive panellists, and used 15–20 panellists; Guth and Grosch[39] typically used three to seven trained panellists. The use of the sniff bottles may also explain why the thresholds from Guadagni and Buttery's group were usually lower. The high value published by Tandon *et al.*[16] can be explained by the panel make-up, which was untrained and unexperienced.

Matrix Effect

The thresholds of orange juice aroma-active compounds were previously determined in water in this laboratory, by using a large untrained panel of 55–73 panellists.[25] The study lasted over a period of 2 years, with as many replicates as necessary to obtain a minimum of 100 responses for each concentration. It is interesting to note that 20% of the panellists were smokers in the earlier study, whereas only one regular panellist was a smoker in the present study. Since this is, to our knowledge, the only study that determined both odour and flavour thresholds for many orange juice volatile compounds, the comparison between threshold values in water and in an orange juice matrix will be discussed with these data, unless otherwise specified (Table 4). Additionally, values found by Ahmed and co-workers were generally within a similar range to those previously published from other laboratories.[25]

Orthonasal odour thresholds were 14 and up to 266 times higher in reconstituted orange juice than in water (Table 4). The differences between published thresholds in water and the present thresholds in reconstituted orange juice were not as high for retronasal thresholds. Orange juice samples were presented at 10–12 °C, the temperature at which a consumer would drink juice. This lower temperature would decrease the amount of volatiles in the headspace, and thus increase orthonasal odour thresholds. The temperature difference is decreased once the sample is warmed up in the mouth, theoretically releasing more volatiles into the headspace. This might explain why there is less difference between thresholds in water and reconstituted juice for retronasal than for orthonasal odour thresholds (Table 4). It also explains the lower thresholds retronasally than orthonasally (Table 4), although thresholds lower orthonasally than retronasally were reported with samples (oil, milk or butter) served at 42 °C.[34]

In earlier studies, sample preparation included dissolving the compounds in ethanol to make a stock solution of 100 ppm to increase compound solubility in water.[25,35] As a result, samples spiked with 1 ppb or 10 ppb of compound under testing would contain 10 000 ppb or 100 000 ppb ethanol, respectively. The assumption was that ethanol would not be perceived, because the

Table 4. Comparison between population orthonasal and retronasal odour thresholds of orange juice compounds in a deodorized orange juice matrix (OJ), and published odour thresholds of the same compounds in water

Compound	Orthonasal odour thresholds in OJ	Published thresholds in water[a]	Ratio	Retronasal odour thresholds in OJ	Published thresholds in water[a]	Ratio
α-Pinene	1650	10	174	2010	1014	2
β-Pinene	37 200	140[48]	266	36 100	—	—
Myrcene	773	36	21	500	42	12
γ-Terpinene	3260	—	—	2140	—	—
Limonene	13 700	60	228	13 330	210	63
α-Terpineol	25 900	280	93	9060	300	30
Linalool	113	5.3	21	105	3.8	28
Vanillin	764	20[49]	38	161	30[5]	5
Citral (a and b)	1230	85.3	14	714	41	17
Hexanal	151	9.2	16	88	3.66	24
Octanal	233	1.4	166	97	0.52	187
Nonanal	312	2.5	125	165	4.24	39
Decanal	204	2	102	97	3.02	32
trans-2-Pentenal	2770	150	18	1970	—	—

[a] From ref. 25, unless otherwise specified.

ethanol threshold in water is 100 000 ppb.[5,42] However, Lopetcharat[43] observed suppression effects on the intensities of odorants at supra-thresholds when an odorant was present at sub-threshold. Specifically, ethanol at sub-threshold (different for each panellist, therefore solutions made accordingly) suppressed the perceived intensity of acetaldehyde at supra-threshold; but when ethanol and acetic acid were presented together at sub-threshold concentrations, they enhanced the perception of overall intensity, sweetness and sourness of acetaldehyde.[43] By using the deodorized orange juice as the matrix, the aroma compounds were first dispersed in the juice to make a stock solution and immediately diluted to the appropriate concentration to prepare samples. Therefore, no solvent carrier was introduced that might interfere with the aroma perception.

In our study, there were no volatiles extracted by diethyl ether or pentane from reconstituted juice. Likewise, there were no perceived odours in the pumpout by GC–O. The direct headspace GC analysis revealed traces of limonene, valencene and decanal. Those results suggest that interactions that would occur between orange juice compounds and the compound under study would be mostly with the juice solutes.

The sugar and acids analysis results were 1.42% sucrose, 1.52% glucose, 1.82% fructose and 0.61% citric acid equivalents for the reconstituted juice. Pectin content was 0.215%, as galacturonic acid (GA) units. Ahmed and co-workers showed that sugars and acids (1% malic and 7% citric), alone or in combination with each other or with pectin, increased the retronasal odour threshold perception of D-limonene in water, while pectin did not have any effect.[44] While the research approach in the latter study is interesting, it only evaluated the effect of soluble solids on the threshold of the compound present in the largest amount in orange juice, i.e. limonene.[4,23,24] However, other compounds with a lower threshold may be as important as limonene.[20] Studies with model solutions also do not reproduce exactly all the compounds that are present in the pumpout. For instance, Buettner and Schieberle[20] showed that the addition of 0.1% fat to the model solution decreased the terpene-like perception and increased the fruity perception retronasally. This result seems logical, due to the fact that terpene compounds are very lipophilic. But the same authors did not include pectin or phenolic compounds in the model solution, despite their presence in orange juice.

By using threshold values determined in the orange juice matrix, the odour units[3] or odour activity values (OAVs),[2] which are the ratios of compound concentration to their thresholds, will be different than when calculated from threshold values obtained in water. OAVs are widely used to determine which compounds in a product are important for its aroma.[20,45–47] For example, Moshonas and Shaw[4] infer that limonene, myrcene, α-pinene, decanal, octanal, linalool, and ethyl butanoate (not presented here) are often the most important contributors to orange juice flavour. Likewise, Buettner and Shieberle[20] found 19 compounds out of 25 with an OAV above 1 for fresh squeezed orange juice. However, by using the present threshold values, limonene, myrcene, linalool and hexanal would be the only terpenes and saturated aliphatic aldehyde with an OAV above 1 in the latter study (Table 5). Table 5 presents the concentrations of the compounds under study found in the headspace (Juice A[4]), or extracted with diethyl ether (Juice B[20]) from fresh squeezed 'Valencia' orange juice. OAVs are calculated by using published threshold values in water (column 3), or the present thresholds in orange juice (column 4). Similar to odour activity values, we present retronasal odour activity values (r-OAV) by using published retronasal odour thresholds obtained in water and the

Table 5. Orthonasal and retronasal odour activity values for selected terpenes and aldehydes in 'Valencia' hand-squeezed orange juice, using published threshold values in water or threshold values from deodorized orange juice (OJ)

Compound	Conc. in juice A[4]	Conc. in juice B[20]	Odour threshold in water[a]	Odour threshold in OJ[a]	OAV in water	OAV in OJ	Retronasal threshold in water[a]	Retronasal threshold in OJ[a]	r-OAV in water	r-OAV in OJ
α-Pinene	100	308	10	1650	10–31	<1	1014	2010	<1	<1
β-Pinene	—	—	140	37 200	<1	—	—	36 100	—	—
Myrcene	340	594	36	773	9–16	≈1	42	500	8.1–14.1	0.7–1.2
γ-Terpinene	10	—	—	3260	—	<1	—	2140	—	<1
Limonene	18 000	85 598	60	13 700	30–1426	1.3–6	210	13 330	85–407	1.4–6.4
α-Terpineol	tr	—	93	25 900	<1	<1	300	9060	<1	<1
Linalool	130	81	5.3	113	15–25	≈1	3.8	105	34–21	1.2–0.8
Vanillin	—	67	20	764	3.3	<1	30	161	2.2	<1
Citral	tr	—	14	1230	<1	<1	41	714	<1	<1
Hexanal	—	197	9.2	151	21	1.3	3.66	88	53.8	2.2
Octanal	4	25	1.4	233	3–18	<1	0.52	97	7.7–48	<1
Nonanal	1	13	2.5	312	<1–5.2	<1	4.24	165	<1–3.0	<1
Decanal	16	45	2	204	8–22	<1	3.02	97	5.3–15	<1
trans-2-Pentenal	—	—	150	2770	—	—	—	1970	—	—

tr, trace.
[a] From Table 4.

present retronasal odour thresholds in orange juice. Only four compounds have a r-OAV above 1; myrcene, limonene, linalool and hexanal. It is clear that the use of orthonasal or retronasal odour activity values only gives an indication of the ranking of the importance of a compound in a food, and not its perception among the mixture of all the other compounds. Also, because perceived intensity increases at different rates for different compounds, the OAVs cannot be used to indicate potency of a compound when it is present at a level resulting in high OAV in a mixture.[48] Finally, compounds that are below threshold concentration are as important to the overall aroma and flavour perception, acting by enhancing or suppressing each other.[43]

Because of the reduction of compound concentration in the headspace of complex food matrices, Bezman *et al.*[9] suggested that odour thresholds be determined in air, such as by GC–O, and headspace concentration of aroma volatiles measured to deduce OAV values in the food product. Similarly, because of complex interactions between volatile and non-volatile compounds resulting in high threshold values, we suggest that flavour determination be done by using the deodorized food as the complex matrix whenever possible, especially when product development is the objective.

References

1. Patton S, Josephson DV. *Food Res.* 1957; **22**: 316–318.
2. Grosch W. *Flavour Fragr. J.* 1994; **9**: 147–158.
3. Teranishi R, Buttery RG, Stern DJ, Takeoka G. *Lebensm.-Wiss. Technol.* 1991; **24**: 1–5.
4. Moshonas MG, Shaw PE. *J. Agric. Food Chem.* 1994; **42**: 1525–1528.
5. Rychlik M, Schieberle P, Grosch W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants.* Deutsche Forschungsanstalt für Lebensmittelchemie and Institut für Lebensmittelchemie der Technischen Universität München: Garching, Germany, 1998.
6. Fazzalari FA. In *ASTM Data Series DS 48A*, Fazzalari FA (ed.). American Society for Testing and Materials, Philadelphia, PA, 1978.
7. Bakker, J. In *Ingredient Interactions: Effects on Food Quality*, Gaonkar AG (ed.). Marcel Dekker: New York, 1995; 411–439.
8. Leland JV. *Food Technol.* 1997; **51**(1): 75–80.
9. Bezman Y, Mayer F, Takeoka GR *et al. J. Agric. Food Chem.* 2003; **51**: 722–726.
10. Rogacheva S, Espinosa-Diaz MA, Voilley A. *J. Agric. Food Chem.* 1999; **47**: 259–263.
11. Hansson A, Leufvén A, Pehrson K, Stenlöf B. *J. Agric. Food Chem.* 2002; **50**(13): 3803–3809.
12. Godshall MA. *Food Technol.* 1997; **51**(1): 63–67.
13. Nahon DF, Navarro PA, Roozen JP, Posthumus MA. *J. Agric. Food Chem.* 1998; **46**: 4963–4968.
14. Jung D-M, de Ropp JS, Ebeler SE. *J. Agric. Food Chem.* 2000; **48**: 407–412.
15. Dufour C, Bayonove CL. *J. Food Agric. Chem.* 1999; **47**: 678–684.
16. Tandon KS, Baldwin EA, Shewfelt RL. *Postharv. Biol. Technol.* 2000; **20**: 261–268.
17. Nagy S. In *Quality Control Manual for Citrus Processing Plants*, vol III, Redd JB, Shaw PE, Hendrix CM, Hendrix DL (eds). Agscience: Auburndale, FL, 1996; 102–133.
18. Hendrix CM, Hendrix DL. In *Quality Control Manual for Citrus Processing Plants*, vol III, Redd JB, Shaw PE, Hendrix CM, Hendrix DL (eds). Agscience: Auburndale, FL, 1996; 3–61.
19. Dominguez-Lopez A. PhD Thesis, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Québec, Canada, 2002.
20. Buettner A, Schieberle P. *J. Agric. Food Chem.* 2001; **49**: 2387–2394.
21. Moshonas MG, Shaw PE. *Food Technol.* 1986; November: 100–103.
22. Moshonas MG, Shaw PE. *Lebensm. Wiss. Technol.* 1992; **25**: 236–239.
23. Nisperos-Carriedo MO, Shaw PE. *J. Agric. Food Chem.* 1990; **38**: 1048–1052.
24. Shaw PE. In *Volatile Compounds in Foods and Beverages*, Maarse H (ed.). Marcel Dekker: New York, 1991; 305–327.
25. Ahmed EM, Dennison RA, Dougherty RH, Shaw PE. *J. Agric. Food Chem.* 1978; **26**: 187–191.
26. Ahmed EM, Dennison RA, Shaw PE. *J. Agric. Food Chem.* 1978; **26**: 368–371.

27. Meilgaard M, Civille GV, Carr BT. In *Sensory Evaluation Techniques*, 3rd edn. CRC Press: Boca Raton, FL, 1999; 123–132.
28. ASTM. Standard Practice Designation E 679-91. American Society for Testing and Materials: Philadelphia, PA, 1997; 34–38.
29. Baldwin EA, Nisperos-Carriedo MO, Baker R, Scott JW. *J. Agric. Food Chem.* 1991; **39**: 1135–1140.
30. Scott RW. *Anal. Chem.* 1979; **51**(7): 936–941.
31. Keith ES, Powers JJ. *J. Food Sci.* 1968; **33**: 213–218.
32. Buttery RG, Teranishi R, Flath RA, Ling LC. In *Flavor Chemistry: Trends and Developments*, Teranishi R, Buttery RG, Shahidi F (eds). ACS Symposium Series, Washington, DC, 1989; 213–222.
33. Larsen M, Poll L. *Z. Lebensm. Unters. Forsch.* 1992; **195**: 120–123.
34. Siek TJ, Albin IA, Sather LA, Lindsay RC. *J. Food Sci.* 1969; **34**: 265–267.
35. Guadagni DG, Buttery RG, Okano S. *J. Sci. Food Agric.* 1963; **14**: 761–765.
36. Buttery RG, Seifert RM, Guadagni DG, Ling LC. *J. Agric. Food Chem.* 1971; **19**: 524–529.
37. Takeoka GR, Flath RA, Mon RM, Teranishi R, Guentert M. *J. Agric. Food Chem.* 1990; **38**: 471–477.
38. Guadagni DG, Buttery RG. *J. Food Sci.* 1978; **43**: 1346–1347.
39. Guth H, Grosch W. *J. Am. Oil Chem. Soc.* 1993; **70**: 513–518.
40. ASTM. Standard Practice Designation E 1432-91. American Society for Testing and Materials, Philadelphia, PA, 1997; 74–81.
41. Buttery RG, Turnbaugh JG, Ling LC. *J. Agric. Food Chem.* 1988; **36**: 1006–1009.
42. Larsen M, Poll L. *Z. Lebensm. Unters. Forsch.* 1990; **191**: 129–131.
43. Lopetcharat K. PhD Thesis, Oregon State University, Corvallis, OR, 2002.
44. Ahmed EM, Dennison RA, Dougherty RH, Shaw PE. *J. Agric. Food Chem.* 1978; **26**: 192–194.
45. Czerny M, Grosch W. *J. Agric. Food Chem.* 2000; **48**: 868–872.
46. Schieberle P, Hofmann T. *J. Agric. Food Chem.* 1997; **45**: 227–232.
47. Stephan A, Steinhart H. *J. Agric. Food Chem.* 1999; **47**: 4357–4364.
48. Audoin V, Bonnet F, Vickers ZM, Reineccius GA. In *Gas Chromatography–Olfactometry. The State of the Art*, Leland JV, Schieberle P, Buettner A, Acree TE (eds). ACS Symposium Series (782): Washington, DC, 2001; 156–171.
49. Guadagni DG, Buttery RG, Harris J. *J. Sci. Food Agric.* 1966; **17**: 142–144.
50. Semmelroch P, Laskawy G, Blank I, Grosch W. *Flavour Fragr. J.* 1995; **10**: 1–7.
51. Grosch W, Zeiler-Hilgart G, Cerny C, Guth H. In *Progress in Flavour Precursor Studies*, Schreier P, Winterhalter P (eds). Allured: Carol Stream, IL, 1993; 329–342.
52. Guth H, Grosch W. In *Progress in Flavour Precursor Studies*, Schreier P, Winterhalter P (eds). Allured, Carol Stream, IL, 1993; 401–407.
53. Milo C, Grosch W. *J. Agric. Food Chem.* 1993; **41**: 2076–2081.
54. Lea CH, Swoboda PAT. *Chem. Indust. Lond.* 1958; **1958**: 1289–1290.
55. Boelens MH, van Gemert LJ. 1987. *Perfum. Flavor.* 1987; **12**: 31–43.

