DOI:10.1021/jf9016866

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Application of the Olfactoscan Method To Study the Ability of Saturated Aldehydes in Masking the Odor of Methional

Kerstin Burseg* and Catrienus de Jong

Flavor Department, NIZO Food Research B.V., Kernhemseweg 2, 6718 ZB Ede, The Netherlands

A novel technology (Olfactoscan screening) was applied to screen for volatiles including saturated aldehydes that mask the perception of methional, an off-flavor commonly found in orange juice. For the screening experiment, methional odor pulses were generated by an olfactometer and, one by one, mixed with alternating odor compounds that were separated by gas chromatography from a model solution. Trained panelists evaluated the odor mixtures so formed for methional intensity. Methional perception was significantly suppressed if it was mixed with octanal ($p < 0.05$). Similar interactions were not observed between methional and other volatiles including hexanal. This observation suggests highly specific interaction between methional and octanal. Octanal−methional interactions were then tested in orange juice (quantitative descriptive analysis with expert panel, $n = 10$). At certain levels, octanal significantly suppressed methional-associated off-notes such as "musty", "decayed orange", and "potato" ($p < 0.01$). The overall flavor quality of the orange juice was significantly improved ($p < 0.01$). As it occurs naturally in orange juice, an enrichment of octanal during juice production is suggested to prevent a methional-induced loss of orange flavor quality.

KEYWORDS: Odor masking; odor mixture quality; off-flavor; olfactory receptor; Olfactoscan

INTRODUCTION

The formation and presence of volatile off-flavors in foods and beverages are widespread and costly problems for the food industry. Common off-flavor sources are microbial metabolites, oxidation of lipids, or endogenous enzymatic decomposition (*1*−*4*). These processes yield a high number of product-specific compounds such as oxidation flavors in products containing polyunsaturated fatty acids (*1*), sulfurous off-flavors in milk products (*5*), or medicinal−phenolic smelling compounds in apple juice (*6*, *7*). Overall, the rejection of foods and beverages due to the presence of off-flavors has become one of the most frequent consumer complaints, causing considerable economic damage to the food industry.

It is often impossible to control or avoid those reactions that lead to off-flavor formation during processing or storage. If the removal of the off-flavor is not feasible, the compound is "tolerated" within the product. Other volatiles may then be added to mask or suppress the perception of the off-flavor. Odor masking is a phenomenon frequently observed in odorant mixtures. Olfactory perception results in a combinatorial code, in which one olfactory receptor (OR) recognizes multiple odorants and different odorants are recognized by different combinations of ORs (*8*, *9*). Signals generated by these ORs are then transmitted to the olfactory bulb (OB) and olfactory cortex (OC) for odor quality (and intensity) processing (*8*, *10*). Studies on olfactory receptor neurons have shown that odorants compete for receptor sites and may act as both receptor agonists and antagonists (*9*, *11*, *12*). Odor masking may occur when an odorant blocks access to a receptor without activating it (antagonist), thereby preventing the detection of an odorant that would be able to activate that receptor (*13*). This competition between odorants for OR binding sites is assumed to be responsible for the observation that, when individual odorants are mixed, the mixture quality is different from that of its individual components (*12*, *14*). Takeuchi et al. furthermore showed a relationship between cyclic nucleotide-gate (CNG) ion channel blockage and olfactory masking (*15*). Blockage was induced by a series of tested odorants, which resulted in suppression of the transduction current through the CNG channel.

Odorant interactions not only occur at the receptor level but have also been observed at higher levels such as the OB and OC. Interactions in the OB may result from interneuron-mediated lateral inhibition among mitral cells (*16*−*19*). In rats, for example, alkylamine responsive glomeruli were mapped, which showed response suppression if surrounding clusters of glomeruli were activated. These surrounding glomeruli were activated upon the inhalation of fennel and clove, spices known to mask the fatty, fishy odor of alkylamines. This suggests that odor masking is mediated, in part, by lateral inhibitory connections in the odor maps of the OB (*20*). Zou and Buck furthermore showed that binary odorant mixes stimulate many cortical neurons in the OC beyond those that respond to their individual component odorants. The activation of these additional neurons, which are not activated by the individual compounds but the mixture only, may be responsible for the generation of unique odor perception of odorant mixtures (*21*). These examples suggest that odor quality alteration in mixtures is a general phenomenon that may be of great interest for the food industry to optimize the flavor quality of foods and beverages. Predicting which odorants masks and/or

*Corresponding author [e-mail kerstin.burseg@NIZO.nl; telephone +31 (0)318 65 96 32; fax +31 (0)318 65 04 00].

 Published on Web 09/17/2009

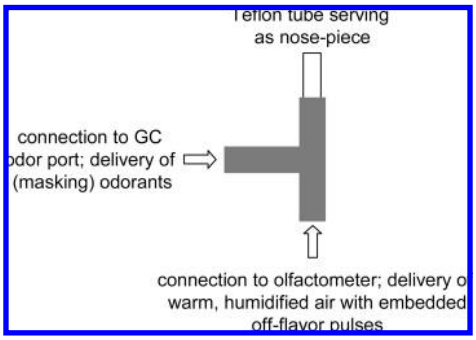

Figure 1. Olfactoscan interface; a T-piece connects the olfactometer outlet with the GC odor port; olfactometer flow (4 L/min) allows dynamic mixing of GC and olfactometer odorants and continuous delivery of mixtures in-nose of subjects.

alters the perception of a certain off-flavor is difficult and often the result of time-consuming trial-and-error methods. Receptor interactions can be studied using in vitro screening assays of (human) olfactory receptors (*22*−*24*). However, these methods do not measure potential odorant interactions in the OB or OC, and interactions found in vitro may be difficult to translate into a sensorial percept.

The aim of the current work was to apply a novel in vivo screening method (Olfactoscan screening) to find an odorant that masks the perception of methional, a common off-flavor in beverages (*25*−*27*). The second objective was to test the effectiveness of the masking compound within a real product (orange juice).

EXPERIMENTAL PROCEDURES

Off-Flavor Screening. The principle of the Olfactoscan screening technique is the automated mixing of a given off-flavor with continuously alternating odor compounds that are separated by a gas chromatograph (GC) from odor mixtures (*28*). This was realized by connecting an olfactometer (generation of off-flavor pulses) and a GC (separation of odorants from mixture). A T-piece was added between the olfactometer outlet and the sniffing port of the GC (**Figure 1**). The olfactometer flow (4 L/min; warm [38 ± 2 °C], humidified air [RH: 80%]) allowed for dynamic mixing of odorants from olfactometer and GC and continuous delivery in-nose of subjects. A 2 cm Teflon tube (inner diameter = 0.4 cm) was connected to the T-piece and served as a nosepiece (**Figure 1**).

Elution of compounds from the GC column was controlled by cryotrapping (liquid CO_2) and enabled a precise mixing of compounds with the off-flavor released by the olfactometer. The odor mixtures so formed (off-flavor and alternating GC compounds; **Figure 2**) were then subjected to sensorial evaluation (monorhinal, orthonasal application).

Methional Off-Flavor Pulses. Methional standards (3-(methylthio)-propionaldehyde; Sigma-Aldrich) in 1,2-propanediol (Sigma-Aldrich) were prepared at three levels (low, 3 mg/L; medium, 6 mg/L; and high, 9 mg/L). Five milliliters of each standard was filled into separate olfactometer odor modules (computer-controlled four-channel air-dilution olfactometer type OM4, Burghart, Wedel, Germany). Methional pulses of three different concentrations (low, medium, and high) were created by sending air (4 L/min; duration, 1 s; interstimulus interval, 20 s) through the standards. Between methional pulses, odorless, humidified air (60% RH, 40 °C) was delivered (4 L/min).

GC Screening Solution. A solution of eight compounds was prepared in methanol (Sigma): ethyl butyrate (Fluka), 208 mg/L; hexanal (Aldrich), 150 mg/L; (*R*)-(+)-limonene (Fluka), 142 mg/L; heptanal (Fluka), 136 mg/L; *cis*-4-heptenal (Aldrich), 20 mg/L; octanal (Aldrich), 110 mg/L; 2,6-dimethylpyrazine (Aldrich), 82 mg/L; benzaldehyde (Aldrich), 208 mg/L. All compounds were of GC standard quality (purity ≥ 98%; heptanal ≥ 95%). The concentrations were selected to represent equal perceived intensities compared to a methional pulse of medium concentration (evaluated by three trained panelists). Two microliters of the solution was injected in split mode (1:6) into a GC (Finnigan Trace type GCTG4, Interscience B.V., Breda, The Netherlands), which was equipped with a medium-polar fused silica capillary column AT-1000 (30 m × 0.25 mm × 0.25 μm). Compounds were separated using the following oven program: from 60 to 120 °C at 10 °C/min, from 120 to 220 °C at 40 °C/min. Elution of compounds was synchronized with the olfactometer methional pulses to yield mixtures of methional with alternating GC compounds (**Figure 2**).

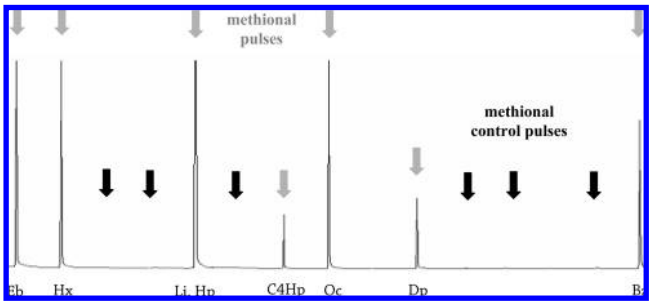

Figure 2. Graphical representation of odor sequence evaluated by panelists during Olfactoscan screening experiment. Compounds of the GC standard solution are shown in the order of elution from the GC from left to right (Eb, ethyl butyrate; Hx, hexanal; Li, (*R*)-(+)-limonene; Hp, heptanal; C4Hp, *cis*-4-heptenal; Oc, octanal; Dp, 2,6-dimethylpyrazine; Bz, benzaldehyde). GC compounds were mixed with methional pulses generated by an olfactometer (gray arrows); black arrows indicate unmixed methional control pulses.

Sensory Evaluation. A total of 16 panelists (ages 23−54, one male) were recruited for the experiment; they had participated in earlier experiments involving gas chromatography−olfactometry. Panelists were trained in a presession on olfactometer methional pulses at all three concentrations (low, medium, and high). For the Olfactoscan screening session, panelists received first a set of methional pulses (warming-up). They were then asked to evaluate a series of odor events that may or may not contain methional. Subjects were instructed to push a button if they recognized methional (either unmixed or in a mixture). Each panelist evaluated each methional odor concentration in a separate Olfactoscan screening run. Each run also contained unmixed methional control pulses (n = 6) to estimate the methional detection probability (**Figure 2**).

Data Analysis. For each methional odor concentration, the number of panel members that correctly identified methional was counted for each odor mixture separately. The expected detection probability of methional (P) was calculated for each methional concentration as the percentage of correctly identified presentations of the 96 presentations (16 subjects; 6 control pulses) of unmixed methional. Data were subjected to one-sided binominal testing to compare for statistical significance (P = 0.8, α = 0.0073). The significance level α is corrected for repeated measures (7), being the seven methional odor mixtures. This results in an overall test significance level of 0.05.

Methional Masking in Orange Juice. The masking interactions between octanal and methional were tested in orange juice with a 10 member expert panel (ages 28−52, two males) using quantitative descriptive analysis (QDA (*29*)). In pretrainings, flavor attributes of a canned, commercially available orange juice with and without methional (12.5 μg/L) were determined. In a second session, attribute anchor points were established on a linear scale (0−100; FIZZ software, Biosystems, Couternon, France) using canned orange juice as a reference. The final list of agreed attributes (taste and smell) is shown in **Table 1**. For the test, a total of five samples (**Table 2**) were analyzed in a randomized order. Assessments were performed in individual booths with controlled temperature and illumination. For each attribute, a computerized linear scale (0−100; FIZZ software, Biosystems) was utilized. Warm-up samples (canned orange juice) were provided for panelists' self-calibration. Samples were evaluated in triplicate. A break of 15 min was given after each set of five samples to avoid fatigue as well as flavor carry-over. Samples were prepared immediately before the test, and about 30 mL was poured into airtight plastic cups closed by a lid and coded with a three-digit random number. The design order was balanced. Filtered water and unsalted crackers were provided for palate cleansing between samples.

Table 1. Descriptors for Orange Juice Samples As Evaluated by an Expert Panel Using Quantitative Descriptive Analysis

compound	quality	descriptor
orange (s)[a]	smell	odor associated with fresh orange juice
musty (s)	smell	odor associated with stale, aged, and nonfresh products
hydrolysate (s)	smell	odor associated with cattle feed and whey
chemical (s)	smell	odor associated with chemicals or vitamin C pills
potato (s)	smell	odor associated with cooked potato and/or water of cooked potato
bitter	taste	taste of quinine hydrochloride
sour	taste	taste of lactic acid or vinegar
sweet	taste	taste of sucrose
orange	taste	taste associated with fresh oranges
musty	taste	taste associated with stale, aged, and nonfresh products
hydrolysate	taste	taste associated with cattle feed and whey
chemical	taste	taste associated with chemicals or vitamin C pills
potato	taste	taste associated with cooked potato and/or water of cooked potato
decayed orange	taste	taste associated with decayed/rotten oranges
metallic	taste	taste associated with iron/metal
grapefruit	taste	taste associated with fresh grapefruits
overall score	taste/smell	overall score representing flavor quality

[a] (s): attribute was only evaluated upon sniffing.

Table 2. Samples Evaluated by Expert Panel Using Quantitative Descriptive Analysis

	orange juice containing	
sample	methional	octanal
reference		
reference + methional	12.5 μg/L	
reference + octanal$_{_low}$		1.25 mg/L
reference + methional + octanal$_{_low}$	12.5 μg/L	1.25 mg/L
reference + methional + octanal$_{_high}$	12.5 μg/L	2.25 mg/L

Data Analysis. Data analysis was carried out using FIZZ software (Biosystems). Data were initially subjected to a two-way analysis of variance (ANOVA) for each attribute and judge, which included a two-way interaction term. When no significant assessor−sample interaction was identified, ANOVA was recalculated without the interaction term. Tukey's HSD tests were performed, at appropriate significance levels ($p = 0.05$), to reveal between which samples differences occurred.

RESULTS AND DISCUSSION

Off-Flavor Screening Experiment. On average, 80% of the methional control pulses were correctly identified by panelists. This value was used to estimate the methional detection probability ($P = 0.8$). In a mixture with a compound from the GC screening solution, detection of methional was strongly dependent on the compound it was mixed with as well as the methional pulse concentration (**Table 3**). A significant proportion of panelists ($n \leq 6, \alpha < 0.0073$) did not perceive methional if it was mixed with limonene and heptanal (low concentration) or with octanal (low and medium concentrations, **Table 3**). At the lowest pulse concentration, methional was no longer detected by panelists if it was mixed with octanal ($n = 0$, **Table 3**).

The ability to detect a compound in a mixture depends, among other factors, on the number of compounds in the mixture (*12*). Limonene and heptanal coeluted to yield a ternary odor mixture with methional (**Figure 2**). The higher complexity of this mixture may explain why it was more difficult for panelists to identify methional compared to the binary mixtures (e.g., methional mixed with hexanal, **Table 3**). The methional−octanal mixture was the only binary mixture that yielded a significant reduction in correct methional identifications at medium and low odor pulse concentrations. As the GC compound concentrations were set to be of equal perceived intensities, intensity-induced methional suppression in the presence of octanal alone may not explain this observation. Odor masking may be the result of odorants interfering with the binding of other odorants to olfactory receptor sites (*30*). To date, the structural principles that determine whether a compound is an agonist or an antagonist or does not bind to a given receptor at all are not fully understood. Araneda et al. studied the receptive range of the rat OR I7. They found that, although receptors were capable of a high degree of discrimination, in many cases, compounds that were very closely related by chemical structure did not share similar activity (e.g., hexanal did not activate OR I7, but heptanal did). Overall, a high specificity for certain molecular features (e.g., functional group), but a high tolerance for others (degree of unsaturation), was detected. The selection of compounds used to screen against methional was made on the basis of chemical structure and odor quality to cover a range of compounds naturally occurring in beverages (e.g., octanal, citrus; hexanal, grassy-green; limonene, orange; ethyl butyrate, strawberry, fruity; benzaldehyde, almond, cherry). The perceptual masking of methional by octanal may be the result of competition for binding sites of at least one type of OR. Equally, odorant-induced CNG channel blockage may be involved (*15*).

Table 3. Number of Panel Members from n= 16 Who Correctly Identified Methional in Odor Mixtures[a]

	methional concentration		
compound methional was mixed with	9 mg/L	6 mg/L	3 mg/L
ethyl butyrate	15	14	10
hexanal	14	8	11
limonene; heptanal	13	11	6
cis-4-heptenal	15	11	11
octanal	9	6	0
2,6-dimethylpyrazine	13	8	13
benzaldehyde	11	10	13

[a] Methional pulses were produced by an olfactometer from three different concentrations (3, 6, and 9 mg/L in 1,2-propanediol).

Interactions between methional and hexanal were less pronounced. This is in line with findings that olfactory receptors are capable of discriminating between homologue compounds (*24*). Also, interactions between compounds of different chemical classes such as ethyl butyrate and 2,6-dimethylpyrazine were not observed. Interactions between methional and heptanal were not tested separately as heptanal coeluted with limonene (**Figure 2**). For a better understanding of the nature of the octanal−methional interactions, as well as possible interactions with heptanal or nonanal, an in vitro screening study as described by Araneda et al. using human OR is therefore suggested. This may also indicate to what extent odorant mixture suppression at higher levels such as the OB or the OC is involved in the observed suppression of methional perception (*16*−*20*).

Masking Methional Perception in Orange Juice. In the second part of the study the masking effect of octanal on methional perception in orange juice was tested. The aim was to measure the robustness of the masking interactions with respect to food matrix effects, in-mouth processes, and the presence of other volatiles. Of special interest was also to test whether the route of aroma delivery (orthonasal versus retronasal) influenced the perception of methional in the presence of octanal (*31*). The results of the sensory study are summarized in **Table 4** and **Figure 3**. The amount of methional used to spike the orange juice

Table 4. Mean Attribute Scores of Orange Juice Samples Evaluated by Expert Panel Using Quantitative Descriptive Analysis[a]

attribute	reference	reference + methional	reference + octanal$_{low}$	reference + methional + octanal$_{low}$	reference + methional + octanal$_{high}$
orange (s)[b]	53.6AB	42.1C	56.1A	50.2B	48.5B
musty (s)	15.1C	26.8A	15.6C	19.8BC	23.3AB
hydrolysate (s)	0.01B	0.0A	0.3B	0.05B	0.05B
chemical (s)	24.3A	23.3A	21.5A	22.5A	24.0A
potato (s)	4.5B	16.3A	2.1B	2.9B	4.9B
bitter	35.9B	40.0AB	38.0B	37.2B	43.0A
sour	55.7A	55.5A	53.2A	56.9A	57.9A
sweet	20.0A	19.8A	19.8A	17.9A	18.2A
orange	54.8A	44.0BC	52.8A	47.2B	41.4C
musty	15.0B	25.7A	17.5B	17.3B	25.1A
hydrolysate	0.25B	2.85A	0.0B	0.1B	1.05B
chemical	31.8AB	34.8A	30.5B	35.9A	35.5A
potato	2.9B	9.5A	2.8B	1.6B	4.1B
decayed orange	8.8B	17.5A	11.1B	11.0B	20.0A
metallic	12.4A	16.0A	12.2A	11.1A	14.6A
grapefruit	25.7A	23.1AB	20.4BC	17.8C	21.8B
overall score	65.8AB	51.8C	66.8A	60.5B	54.0C

[a] Samples denoted with the same letter are not significantly different ($p = 0.01$). [b] (s): attribute was only evaluated upon sniffing.

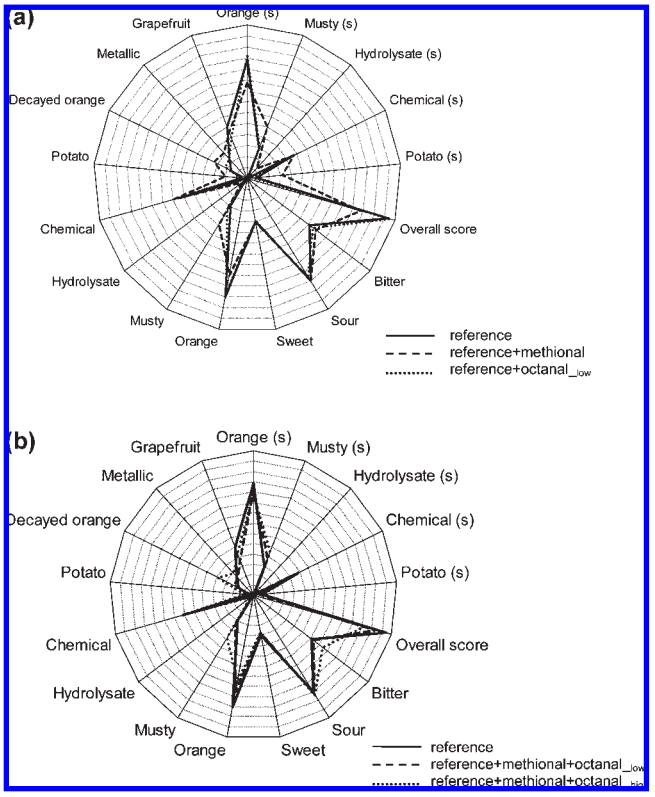

Figure 3. Sensory panel descriptive analysis average scores for orange juice samples (= reference) containing methional (12.5 μg/L) and/or octanal (1.25 mg/L = low, 2.25 mg/L = high); attributes denoted "s" were evaluated upon smelling.

was 12.5 μg/L and represented a methional level that can be found in stored orange juice (*25*). Methional-spiked orange juice samples were perceived as significantly different from the reference (overall score), and attributes such as "musty, decayed orange, hydrolysate, and potato" were significantly enhanced ($p = 0.05$). Attributes associated with fresh orange juice such as "orange flavor, grapefruit" were reduced (**Table 4**; **Figure 3a**). With the exception of the attribute "grapefruit", the addition of octanal did not significantly alter the quality of the orange juice. This was an important requirement as the addition of octanal to mask methional should not result in an off-flavor itself. Adding about 1 mg/L of octanal to orange juice spiked with methional did not significantly improve the flavor quality (data not shown). At about 1.25 mg/L, however, typical methional-associated attributes (musty, decayed orange, hydrolysate, and potato) were suppressed and the overall score significantly improved. At higher octanal levels (≥2.25 mg/L), however, a negative impact on the orange juice flavor quality was introduced. Attribute scores for "musty, bitter, and decayed orange" were increased, whereas attributes associated with fresh orange juice such as "orange and grapefruit" as well as the overall score were reduced (**Table 4**; **Figure 3b**). Overall, these results proved that the masking interactions between octanal and methional as initially identified during the Olfactoscan screening experiment could also be observed within a real food matrix. The route of delivery (orthonasal versus retronasal) did not seem to influence these interactions. The scores for orange juice attributes did not differ if evaluated upon sniffing (orthonasal aroma delivery) or after swallowing (retronasal aroma delivery; **Table 4**). In orange juice, optimum methional masking interactions were observed at octanal levels of 1.25 mg/L. Octanal occurs naturally in orange juice and is an important volatile for the orange flavor quality (*32*). An enrichment of octanal during the production of orange juice may therefore overcome a loss in flavor quality induced by methional.

Overall, combining olfactometry and GC proved to be a successful approach to study odor mixture interactions. Similar approaches have been described earlier (*33*, *34*). In this study, the Olfactoscan technique was applied to screen for masking interactions. Other applications such as screening for synergistic mixture effects to enhance sweet or savory notes are also possible.

ACKNOWLEDGMENT

We thank Margreet Rippen and Harold Bult for their advice on sensory and statistic related topics.

LITERATURE CITED

(1) Reineccius, G. Off-flavors in foods. *Crit. Rev. Food Sci. Nutr.* **1991**, *29* (6), 381–402.
(2) Ashie, I. N.; Simpson, B. K.; Smith, J. P. Mechanisms for controlling enzymatic reactions in foods. *Crit. Rev. Food Sci. Nutr.* **1996**, *36* (1−2), 1–30.
(3) Ashie, I. N.; Smith, J. P.; Simpson, B. K. Spoilage and shelf-life extension of fresh fish and shellfish. *Crit. Rev. Food Sci. Nutr.* **1996**, *36* (1−2), 87–121.
(4) Silla Santos, M. H. Biogenic amines: their importance in foods. *Int. J. Food Microbiol.* **1996**, *29* (2−3), 213–231.
(5) Vazquez-Landaverde, P. A.; Velazquez, G.; Torres, J. A.; Qian, M. C. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. *J. Dairy Sci.* **2005**, *88* (11), 3764–3772.
(6) Siegmund, B.; Pollinger-Zierler, B. Growth behavior of off-flavor-forming microorganisms in apple juice. *J. Agric. Food Chem.* **2007**, *55*, 6692–6699.
(7) Siegmund, B.; Pollinger-Zierler, B. Odor thresholds of microbially induced off-flavor compounds in apple juice. *J. Agric. Food Chem.* **2006**, *54*, 5984–5989.
(8) Buck, L. B. Unraveling smell. *Harvey Lect.* **2005**, *101*, 117–134.
(9) Sanz, G.; Schlegel, C.; Pernollet, J. C.; Briand, L. Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. *Chem. Senses* **2005**, *30* (1), 69–80.
(10) Zou, Z.; Li, F.; Buck, L. B. Odor maps in the olfactory cortex. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (21), 7724–7729.

(11) Oka, Y.; Nakamura, A.; Watanabe, H.; Touhara, K. An odorant derivative as an antagonist for an olfactory receptor. *Chem. Senses* **2004**, *29* (9), 815–822.
(12) Kay, L. M.; Lowry, C. A.; Jacobs, H. A. Receptor contributions to configural and elemental odor mixture perception. *Behav. Neurosci.* **2003**, *117* (5), 1108–1114.
(13) Kurahashi, T.; Lowe, G.; Gold, G. H. Suppression of odorant responses by odorants in olfactory receptor cells. *Science* **1994**, *265* (5168), 118–120.
(14) Oka, Y.; Omura, M.; Kataoka, H.; Touhara, K. Olfactory receptor antagonism between odorants. *EMBO J.* **2004**, *23* (1), 120–126.
(15) Takeuchi, H.; Ishida, H.; Hikichi, S.; Kurahashi, T. Mechanism of olfactory masking in the sensory cilia. *J. Gen. Physiol.* **2009**, *133* (6), 583–601.
(16) Wilson, D. A. Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. *J. Neurophysiol.* **2003**, *90* (1), 65–72.
(17) Tabor, R.; Yaksi, E.; Weislogel, J. M.; Friedrich, R. W. Processing of odor mixtures in the zebrafish olfactory bulb. *J. Neurosci.* **2004**, *24* (29), 6611–6620.
(18) Giraudet, P.; Berthommier, F.; Chaput, M. Mitral cell temporal response patterns evoked by odor mixtures in the rat olfactory bulb. *J. Neurophysiol.* **2002**, *88* (2), 829–838.
(19) Wilson, D. A.; Stevenson, R. J. The fundamental role of memory in olfactory perception. *Trends Neurosci.* **2003**, *26* (5), 243–247.
(20) Takahashi, Y. K.; Nagayama, S.; Mori, K. Detection and masking of spoiled food smells by odor maps in the olfactory bulb. *J. Neurosci.* **2004**, *24* (40), 8690–8694.
(21) Zou, Z.; Buck, L. B. Combinatorial effects of odorant mixes in olfactory cortex. *Science* **2006**, *311* (5766), 1477–1481.
(22) Shirokova, E.; Schmiedeberg, K.; Bedner, P.; Niessen, H.; Willecke, K.; Raguse, J. D.; Meyerhof, W.; Krautwurst, D. Identification of specific ligands for orphan olfactory receptors. G protein-dependent agonism and antagonism of odorants. *J. Biol. Chem.* **2005**, *280* (12), 11807–11815.
(23) Krautwurst, D.; Yau, K. W.; Reed, R. R. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* **1998**, *95* (7), 917–926.
(24) Araneda, R. C.; Kini, A. D.; Firestein, S. The molecular receptive range of an odorant receptor. *Nat. Neurosci.* **2000**, *3* (12), 1248–1255.
(25) Bezman, Y.; Rouseff, R. L.; Naim, M. 2-Methyl-3-furanthiol and methional are possible off-flavors in stored orange juice: aroma-similarity, NIF/SNIF GC-O, and GC analyses. *J. Agric. Food Chem.* **2001**, *49*, 5425–5432.
(26) Perpete, P.; Collin, S. Contribution of 3-methylthiopropionaldehyde to the worty flavor of alcohol-free beers. *J. Agric. Food Chem.* **1999**, *47*, 2374–2378.
(27) Escudero, A.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Clues about the role of methional as character impact odorant of some oxidized wines. *J. Agric. Food Chem.* **2000**, *48*, 4268–4272.
(28) Burseg, K. M. M.; deJong, C. In *The Olfactoscan: In-Vivo Screening for Off-Flavour Solutions*; Weurman Symposium, Interlaken, Switzerland; Yertzian, C., Ed.; Zurich University: Zurich, 2008.
(29) Meilgaard, M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: Boca Raton, FL, 1999.
(30) Rawson, N. E.; Yee, K. K. Transduction and coding. In *Taste and Smell. An Update*; Hummel, T., Welge-Lussen, A., Eds.; Karger: Basel, Switzerland, 2006; Vol. *63*, pp 23−43.
(31) Ishii, A.; Roudnitzky, N.; Beno, N.; Bensafi, M.; Hummel, T.; Rouby, C.; Thomas-Danguin, T. Synergy and masking in odor mixtures: an electrophysiological study of orthonasal vs. retronasal perception. *Chem. Senses* **2008**, *33* (6), 553–561.
(32) Komthong, P.; Katoh, T.; Igura, N.; Shimoda, M.; Hayakawa, I. Ascending bubble extraction of terpenes from freshly squeezed orange juice. *Food Res. Int.* **2005**, *39* (1), 53–58.
(33) Hattori, S.; Takagaki, H.; Fujimori, T. Identification of volatile compounds which enhance odor notes in Japanese green tea using the OASIS (Original aroma simultaneously input to the sniffing port) method. *Food Sci. Technol. Res.* **2005**, *11* (2), 171–174.
(34) Acree, T. E.; Williams, R. C.; Kurtz, A. J.; Parisot, F. A.; Satre, E. In *A Gas Chromatography−Pedestal Olfactometer for the Study of Odor Mixtures*; Weurman Symposium, Interlaken, Switzerland; Yeretzian, C., Ed.; Zurich University: Zurich, 2008.

Received May 25, 2009. Revised manuscript received August 28, 2009. Accepted August 29, 2009.