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Investigation of Potent Odorants and Afterodor Development in Two Chardonnay Wines Using the Buccal Odor Screening System (BOSS)

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Potent odorants of two Chardonnay wines were characterized according to their specific overall aroma profiles and their intraoral release patterns after wine consumption. Therefore, aroma compounds were isolated and analyzed by means of high resolution gas chromatography-olfactometry (HRGC/O), leading to the detection of 36 odor-active compounds in both wines. All compounds were identified. Of the most potent odorants, 25 were quantified in both wines by means of stable isotope dilution assays. For the intraoral investigation of odor compounds at defined times after Chardonnay wine consumption, the recently developed buccal odor screening system was used. Significant differences in the oral persistence of characteristic odor notes were observed for both wines with mainly the characteristic barrique-notes being highly persistent, while fruity notes quickly disappeared from the oral cavity. The obtained analytical data were related to time-resolved retronasal aroma evaluation.

KEYWORDS: Aftersmell; stir bar sorptive extraction; aroma persistence; finish

INTRODUCTION

Wine aroma perception is a complex phenomenon, which is, undoubtly, just about to be understood. Numerous investigations in this field have shown that the chemical elucidation of the key odorants responsible for the fine-tuned nasal sensations can often require heavy analytical tasks. As one example, the characterization of key aroma compounds of different white wine varieties by Guth has to be mentioned (*1*−*3*). About 40 key odorants were characeterized by means of gaschromatographic-olfactometric techniques, stable isotope dilution assays (SIDA) and aroma reconstitution experiments. This study showed in a unique way how a consequent analytical procedure can lead to fundamental understanding of the chemical basics of wine aroma.

However, aroma composition is not the full story when we want to understand each different smell sensation during wine tasting and consumption. Everybody is aware of the fact that not only the matrix composition can alter aroma perception considerably but also the mode of wine consumption. For a characterization of wine taster's evaluation techniques and their impact on aroma perception, please refer to ref 4. Perception of wine odorants can, generally, be divided into different stages: First are the orthonasal sensations, occurring when the headspace over the wine is sniffed for a wines' highly volatile attributes. Second are the retronasally perceived impressions. Here, three key modes have to be distinguished, (a) the immediate aroma impression when wine is present in the oral cavity or (b) has just been swallowed, and (c) the prolonged retronasal aroma perception after swallowing, often called aftertaste or more accurately "afterodor" or "aftersmell" when talking of odorants. With regard to wine evaluation, "finish" might be the best choice. For a detailed explanation of the physiological features influencing aroma transfer from the oral to the nasal cavities, please refer to ref 5. Visualizing "normal" swallowing of liquids using real-time MRI showed that aroma perception does not usually occur prior to swallowing. The reason for this phenomenon is that the nasal cavity is closed off from the pharyngeal and oral parts by the velum either forming a tight velum-tongue connection (swallow preparatory phase) or a velopharyngeal closure (pharyngeal phase of swallowing). Immediately after the swallowing act, the velum returns from these positions, allowing volatiles to be transported with the aid of the "swallow breath" into the nasal cavities. This instinctive swallowing behavior can be influenced deliberately by well-directed opening of the velum-tongue border when wine is present in the oral cavity. This leads to an enormous enhancement of retronasal perception, depending on the evaluater's skills (*6*, *4*).

Apart from these "directly" perceived retronasal perceptions (direct release from the wine matrix), the indirect mode of afterodor is the last, but still important issue for wine taster's evaluation. However, literature on this topic, especially in wines, is very rare and only limited to some general sensory descriptions. Usually, the exact duration of precisely defined aroma impressions after wine consumption is not taken into account. Afterodor perception is influenced by a series of physiological and physicochemical parameters, as shown previously (*7*, *8*). One of these key parameters is the adsorptive potency of odorants to oral mucosa (*5*, *9*). Apart from physicochemical

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parameters such as polarity and volatility, the influence of human salivary enzymes on the differences in persistence between odorants has been proposed (*10*, *11*). It has been assumed that odorants play a major role in prolonged retronasal aroma perception, if they are adsorbed to the oral mucosa to a high extent and not degraded by salivary enzymes. A further premise is that they are not absorbed by the mucosal tissue (leading to removal from perception) but released therefrom.

To verify this process, investigation of afterodor development with time has been performed recently on aroma models using the buccal odor screening system (BOSS) (*12*). This intraoral extraction concept is based on stir bar sorptive extraction (SBSE), a versatile and sensitive extraction technique for gaseous and liquid samples (*13*). In SBSE, a PDMS-coated stir bar is exposed to a sample for a certain extraction time. After trapping of the analytes to the SBSE bars and removal of the matrix system, the analytes are recovered via extraction or thermodesorption and analyzed (e.g., by gaschromatography or liquid chromatography).

For the present investigation, two Chardonnay wines with very different overall aroma profiles were chosen for analysis. The afterodor development in both wines should be studied, that means both the odor qualities and the persistence of aroma impressions. The aim was therefore (i) to identify the key aroma compounds in the Chardonnay wines and (ii) to follow the intraoral wine aroma release after wine consumption by precise analytical and sensory terms. In this context, elucidation of the changes in the retronasally perceived aroma profiles with time was of key interest.

MATERIALS AND METHODS

Chemicals. The following odorants were obtained from the suppliers shown: *p*-cresole, decanal, ethyl butanoate, ethyl cinnamate, ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, ethyl hexanoate, butane-2,3-dione, 2,5-dimethyl-4-hydroxy-2(5*H*)-furanone, 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, indole, 3-(methylthio)-propanal, 3-(methylthio)-propanol, 2/3-methylbutanal, 2/3-methylbutanol, 2/3-methylbutanoic acid, 3-methylbutyl acetate, 3-methylindole, (*E*)-2-nonenal, octanal, phenylacetaldehyde, phenylacetic acid, δ-decalactone, *cis*- and *trans*-whiskeylactone (Aldrich, Steinheim, Germany), acetic acid, 4-allyl-2-methoxyphenol, butanoic acid, 2-methoxyphenol, vanillin (Merck, Darmstadt, Germany), (*E*)-β-Damascenone (Haarmann and Reimer, Holzminden, Germany), β-ionone, geraniol (Roth, Karlsruhe, Germany), (*E*/*Z*)-2,6-nonadienal (Alfa Products, Karlsruhe, Germany), 1-octen-3-one, 4-vinyl-2-methoxyphenol, (Lancaster, Mühlheim, Germany), phenylethanol (Fluka, Buchs, Switzerland). *trans*-4,5-Epoxy-(*E*)-2-decenal and (*Z*)-2-nonenal were synthesized according to refs 14 and 15. The compounds were freshly distilled prior to analysis. Chemical and sensory purity was checked by gaschromatography-olfactometry (GC-O) as well as gaschromatography-mass spectrometry (GC-MS).

Stable-Isotope-Labeled Standards. [$^{13}C_2$] Acetic acid and [$^{13}C_2$] phenylacetic acid were from Aldrich (Steinheim, Germany). The following labeled internal standards were synthesized according to the literature cited: [1,4-$^{13}C_2$] butane-2,3-dione, [$^2H_2$] δ-decalactone (*16*), 3-([$^2H_3$] methylthio)-1-propanol (*17*), [2,2,2-$^2H_3$]-ethyl 2-methylpropanoate, [2,2,2-$^2H_3$]-ethyl 2-methylbutanoate (*18*), 3-methyl [3,4-$^2H_2$] butanoic acid, 3-methyl [3,4-$^2H_{2-5}$]-butanol (*19*), [2,2,2-$^2H_3$]-ethyl butanoate, [3,4-$^2H_2$]-butanoic acid (*20*), [5,6-$^2H_2$]-decanal (*21*), 3-methyl [3,4-$^2H_2$] butyl acetate, [$^2H_3$]-vanillin, [2,2,2-$^2H_3$] ethyl hexanoate, [2,2,2-$^2H_3$] ethyl cinnamate (*2*), [$^{13}C_2$]-furaneol (*22*), 2-[$^2H_3$] methoxy-4-vinylphenol (*23*), 2-phenyl [1,1-$^2H_2$] ethanol (*24*), 2-[$^2H_3$]-methoxyphenol (*25*), [$^2H_2$] *cis*- and *trans*-whiskeylactone (*26*). Eugenol was quantified using 2-[$^2H_3$] methoxy-4-vinylphenol as internal standard.

Chardonnay Wines. The following Chardonnay wines were selected for investigation: 1999 Merryvale Reserve Chardonnay, 14.5% by vol., Napa Valley, Merryvale Vineyards (St. Helena, CA) and 2002 Forest Hill Chardonnay, 13.5% by vol., Jindalee Estate P/L (Moorabool, Victoria, Australia).

Figure 1. Perforated glass capsules for intraoral application of SBSE bars in BOSS analysis.

PDMS-Coated Stir Bars. For the experiments, commercially available Twister-SBSE bars (10-mm long, 0.5-mm PDMS film thickness; Gerstel GmbH, Mühlheim a/d Ruhr, Germany) were used. Prior to analysis, the bars were subjected to a condition procedure according to the suppliers recommendations: the stir bars were first soaked in 100% acetonitrile for at least 2 days then conditioned at 300 °C for 4 h.

Prior to analysis, each SBSE bar was screened for odorants ("background", see Results and Discussion) and then directly applied for analysis. Each stir bar was used for just one single experiment then reconditioned and screened for background again. Each experiment was performed with at least three different SBSE bars to avoid SBSE bar variation.

Encapsulation of the SBSE Bars. For intraoral application, adapted glass capsules were designed (cf. **Figure 1**). For the 10-mm bars, the total length of the capsule was 15 mm. The innner diameter was in both cases 5 mm. The capsules were sealed with a glass stopper. To allow unhindered penetration of air and saliva, the capsules were regularly perforated with pores (1−2-mm diameter) with a distance of about 3 mm between pores.

Panelists. Panelists were nonpregnant volunteers (nonsmokers) of the Technical University of Munich, exhibiting no known illnesses at the time of examination and with normal olfactory and gustatory function. Subjective aroma perception was normal in the past and at the time of examination. The panelists had a normal salivary flow and were selected for their excellent oral hygiene, thereby not suffering from oral diseases and nuisances, such as plaque, caries, tartar, gingivitis, and periodontosis. Intra-oral analyses were performed 2 h after breakfast and thorough cleaning of the teeth and oral cavity with a commercial toothpaste (5 min) and with a commercial alcohol-free, low-aromatized and antimicrobial mouthwash.

Intraoral Sampling of Odorants. Prior to oral application of the sample, the oral cavities of the panelists were screened for odorants ("blank", see Results and Discussion).

Then, 25 mL of the respective sample was taken into the oral cavity, kept for 10 s with closed lips and closed velum and rinsed carefully within the oral cavity then expectorated. At defined time intervals (2-fold increase, "time dilution" approach according to ref 12) after expectoration (15, 30, 60 s, etc.), an extraction capsule containing one SBSE bar was placed into the oral cavity. The lips and velum were kept closed and the capsule was moved carefully within the oral cavity, thereby avoiding swallowing actions. After 5 min of equilibration, the capsule was removed from the oral cavity, the SBSE bar was removed with tweezers, dipped into deodorized water, briefly dried with lint-free tissue and immediately placed into the thermodesorption unit.

SBSE Thermodesorptive Sample Application. Thermodesorption of the samples was performed by means of a TDS-2 thermodesorption system (Gerstel GmbH) in combination with a CIS-4 PTV injector (Gerstel GmbH) for cryofocusing the analytes prior to transfer onto the analytical column. The following sampling parameters were used:

Table 1. Odorants Detectable by the SBSE-System after Conditioning

odorant[a]	odor quality	retention index[b] on FFAP	retention index[b] on SE–54	OTV [ng/L air] [c]
butane-2,3-dione	buttery	970	<0600	15–30
[octanal][d]	citrus-like	1279	1000	5.8–13.6
oct-1-en-3-one	mushroom-like	1295	0976	0.3–0.6
[acetic acid][d]	acidic	1449	0610	60
[methional][d]	cooked potato	1449	0900	0.1–0.2
(*E*)-2-nonenal	fatty, tallowy	1527	1157	0.1–0.2
2/3-methylbutanoic acid	sweaty	1660	0875	1.5
[β-damascenone][d]	apple-like	1819	1389	0.002–0.004
tr-4,5-Epoxy-(*E*)–2-decenal	metallic	2000	1380	0.0006–0.0025
2,5-dimethyl-4-hydroxy-2 (5*H*)-furanone	caramel-like	2024	1062	1.0–2.0
vanillin	vanilla-like	2567	1397	0.6–1.2

[a] The compound was identified by comparing it with the reference substance on the basis of the folowing criteria: retention index (RI) on two HRGC stationary phases given in the table, mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port. [b] Retention indices were calculated according to (*30*). [c] The odor threshold values in air were determined as described elsewhere (*31*). [d] The compounds given in brackets revealed only very weak olfactory detection (odor intensity 0.5 on a scale from 0 to 3) and were often but not always olfactorally detected in the bars.

Splitless thermal desorption was performed by programming the TDS-2 from 40 to 240 °C (5 min) with a rate of 60 °C. Cryofocusing was performed with liquid nitrogen at −100 °C. Injection was performed with a ramp of 12 °C/s from −100 °C to 240 °C (5 min). The gas chromatographic conditions are given below.

Rating of Odorants using BOSS. Detectability of the odorants was based on their odor intensities. That means that only those substances that were perceived by HRGC/O were rated as detectable by BOSS. Detection by HRGC/MS or HRGC/FID was not taken into account, as this does not necessarily correlate with the sensory impact of the respective compound. For validation of this approach and proof of reproducibility, please refer to ref 12.

High-Resolution GC-O. Application of the samples was either performed as described above (**SBSE Thermodesorptive Sample Application**) or by the cool on-column injection technique at 35 °C (solvent extract samples). The odorants were screened in parallels by five panelists by sniffing the effluent either after one- (for rating via BOSS) or two-dimensional (for identification) gas chromatography. Sniffing analysis was repeated five times by each panelist. Odor intensities were not rated, only olfactory detectability. Only in the case of background evaluation were the perceived intensities rated on a 7-point scale from 0 (no detection) to 3 (high aroma intensity). All detected odorants were identified by comparison with reference substances on the basis of the following criteria: retention index (RI) on two stationary phases of different polarity (FFAP, SE−54), mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing-port.

The one- or two-dimensional (depending on the analytical requirements) gas chromatography system (TD-HRGC) consisted 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. The following fused silica columns were used: DB-FFAP (30-m × 0.32-mm i.d., 0.25-μm FD, J & W Scientific, Folsom, CA) and/or DB-5 (SE-54; 30-m × 0.32-mm i.d., 0.25-μm FD, J & W Scientific). The gas chromatographic conditions were the same as those described previously (*27*).

High-Resolution GC-MS. The odorants were analyzed by two-dimensional gas chromatography (TD-HRGC) as described above. MS analyses were performed with an ITD-800 (Fisons Instruments, Mainz-Kastel, Germany) running in the CI-mode with methanol as the reagent gas. The following fused silica columns were used: DB-FFAP (30-m × 0.32-mm i.d., 0.25-μm FD, J & W Scientific) in combination with DB-5 (SE-54; 30-m × 0.32-mm i.d., 0.25-μm FD, J & W Scientific). The gas chromatographic and mass spectrometric conditions were the same as those described previously (*27*).

Isolation of the Wine Volatiles. A 100-mL aliquot of the respective wine from a freshly opened bottle was extracted with dichloromethane (3 times, 100 mL of solvent and 30 min of extraction time each, total volume of solvent, 300 mL). The combined extracts were dried over Na_2SO_4 overnight, followed by distillation in vacuo (*27*). For analysis by HRGC/O, the sample was concentrated to a total volume of 10 mL.

Quantitation by SIDA. Quantitation using the respective stable isotope labeled standards was performed as described previously (*27*). The mass traces and calibration factors for the labeled and the unlabeled compounds are given in **Table 4**.

Sensory Evaluation. Assessors (five male, five female) were recruited from the Technical University of Munich. In preceding weekly training sessions, the panelists were trained in recognizing orthonasally and retronasally about 150 selected odorants at different odorant concentrations according to their odor qualities. Training in these sessions was at least for one year prior to participation in the actual sensory experiments. Panelists were always asked to score odor intensities from 0.0 (not perceivable) to 3.0 (very intense). Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions. On the basis of reference aroma solutions at defined concentrations, a flavor language was developed, defining the specific smell of a compound for a certain aroma attribute. On the basis of these aroma attributes, both wines were evaluated by the whole panel. Descriptors found to be most often used were selected for further sensory evaluation.

Samples (4 °C) were opened and immediately applied to sensory evaluation. The wines (25 mL each), were singly presented to the sensory panel for retronasal evaluation in covered glass vessels (capacity 45 mL). The total amount of the sample was taken into the oral cavity, kept for 10 s with closed lips and closed velum and rinsed carefully within the oral cavity, then expectorated. At defined time intervals (2-fold increase) after expectoration (15, 30, 60 s, etc.), the intensity of the overall retronasal aroma perception as well as of single predefined odor qualities was rated by the panelists by deliberately opening the velum-tongue border exactly at these times (for precise description of applied tasting techniques, please refer to ref 4). This approach was termed as "time dilution" approach because of the 2-fold increase in time intervals. The results obtained in three different sessions were averaged and plotted in spider-web diagrams. The values obtained in different sessions and for the different assessors differed by not more than 10%. For comparative evaluation of both wines, one was evaluated first, then, after a 15 min break and rinsing of the oral cavity with tap water, evaluation of the second sample was performed. Panelists were also asked to rate the overall difference between both samples from 0.0 to 3.0.

RESULTS AND DISCUSSION

Preliminary investigations showed that the applied SBSE-Bars are applicable intraorally without any toxicological harm and demonstrated a high reproducibility and very close correlation between sensory persistence and detectability of compounds via the BOSS approach (*12*).

Determination of Background. After conditioning of the SBSE-bars according to the suppliers recommendations, the bars were screened by HRGC/O for remaining traces of odorants

(cf. **Table 1**). It could be shown that a few odorants were always detectable via HRGC/O at trace concentrations (odor intensity rated 1 on a 7-point scale from 0 to 3) but did not reveal any signal by FI detection due to their very low concentrations. The compounds given in brackets revealed only very weak olfactory detection (odor intensity 0.5) and were often, but not always, olfactorally detected in the bars. When performing the sniffing analysis under the same conditions with an empty sample tube but without application of the SBSE bar, no odorants were detectable. Therefore, it was clearly shown that the detection of odorants was due to adsorption of traces to the SBSE bars even after conditioning. It is assumed that the detection by HRGC/O is on one hand due to the fact that the mentioned odorants exhibit very high odor potency (extremely low odor thresholds). On the other hand, these compounds can be regarded as ubiquituous odorants as they represent aroma products from broadly distributed substances such as linoleic and linolenic acid (e.g., oct-1-en-3-one or *tr*-4,5-epoxy-(*E*)-2-decenal). These substances can be found not only in a broad diversity of food systems but also generally in biological materials such as plants, animals etc. Therefore, "contamination" of the overall environment, including air, with traces of these potent odorants is very likely and is probably unavoidable. For this reason, the presence and the intensity of these substances in the applied SBSE system was always screened prior to the actual analysis and was set as background.

Blank Samples from Oral Cavity. When screening the untreated oral cavities of the participants by means of sensory analysis, all panelists reported a faint buccal smell. It was described as a bit tallowy, slightly acidic, and as the typical buccal smell of healthy people. It was described as only perceivable when directly sniffing the panelists mouth and was not attributed to any increased oral smell as it is induced by, for example, halitosis. Screening of the untreated oral cavities of the participants by means of SBSE/HRGC/O revealed a weak perception of 10 odor active substances which were detectable at each sampling day for each panelist (cf. **Table 2**). Compounds **1**, **2**, **3**, and **5** were detectable with slightly higher intensities compared to the background samples. Therefore, the presence of these compounds in the oral cavity could be verified.

Chardonnay Wines. Two chardonnay wines, which exhibited considerable sensory differences, were profiled by sensory as well as by BOSS analysis. In parallels, quantitation of the potent odorants of the wines were performed by stable isotope dilution assays.

Sensory Evaluation. For comparison, both wines were evaluated retronasally according to selected odor descriptors as described in **Sensory Evaluation**, following the "time dilution" approach. At each evaluation time, the single aroma impression as well as the overall odor intensities were rated. The profiles representing the intensities of the single odor qualities are given as spider web diagrams in **Figure 2**, together with a small bar diagram comparing the respective perceived overall intensities.

When looking at the overall intensities, an interesting phenomenon can be observed: At the beginning of the evaluation, both wines were rated with similarly high intensities (2.6), slightly decreasing during the next 15 s. Then, a shift in intensity was detected, with that of the Forest Hill wine decreasing more rapidly. However, the rating of the sinlge odor qualities shows that these overall intensities cannot be simply related to the same aroma impressions. On the contrary, both aroma profiles differed considerably. From the start of the evaluation, the Forest Hill Chardonnay was described as much more fruity, flowery, pungent, and citrus-like, while the Merryvale wine was dominated by woody, smoky, and vanilla- and clove-like impressions. This general deviation remained more or less the same for the following time dilution evaluations, only with decreasing intensities. It has to be stated that the fruity, flowery, pungent, and citrus notes decreased faster than the presumably barrique-related descriptors of the Merryvale wine. This explains the shift

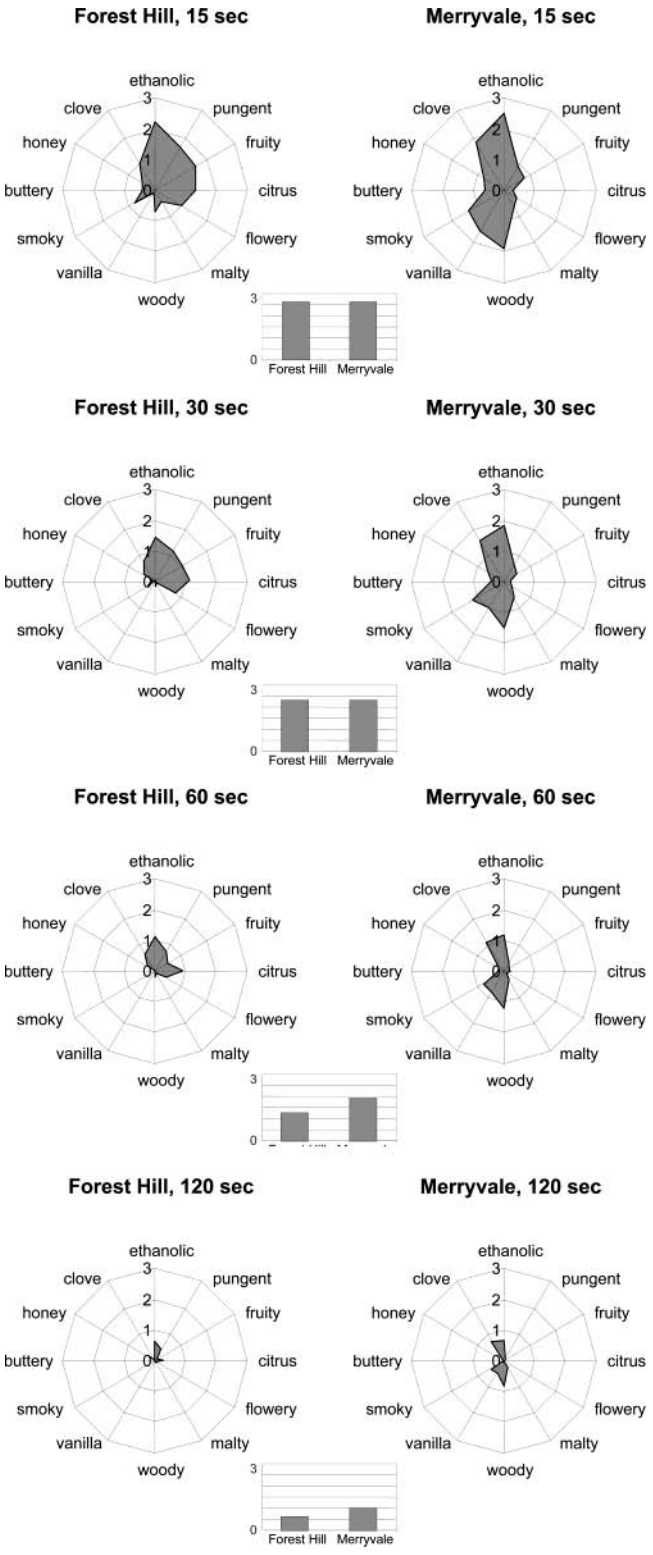

Figure 2. Time-resolved retronasal evaluation of the intensities of odor attributes and their overall odor intensities (middle graph) after intraoral application and expectoration of two Chardonnay wines.

Table 2. Detection of Odorants by Means of BOSS in the Oral Cavity of Healthy Panelists Prior to Food Consumption (Blank)

no.	odorant[a]	odor quality	retention index[b] on FFAP	retention index[b] on SE–54
1	oct-1-en-3-one	mushroom-like	1295	0976
2	acetic acid	acidic	1449	0610
3	methional	cooked potato	1449	0900
4	(*Z*)-2-nonenal	fatty, leaf-like	1502	1143
5	(*E*)-2-nonenal	fatty, tallowy	1527	1157
6	(*E*,*Z*)-2,6-Nonadienal	cucumber-like	1583	1149
7	*p*-cresole	feces-like	2077	1074
8	unknown	green coriander	~2400	nd
9	indole	feces-like	2450	1293
10	3-methylindole	feces-like	2484	1388

[a] The compound was identified by comparing it with the reference substance on the basis of the folowing criteria: retention index (RI) on two HRGC stationary phases given in the table, mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port. [b] Retention indices were calculated according to (*30*).

in the overall aroma intensities of both wines over the time course of evaluation.

Identification of the Potent Wine Odorants in Solvent Extracts. Wine aroma is a complex composition of a diversity of aroma compounds. Therefore, the potent odorants of both wines were first isolated by means of solvent extraction, high vacuum distillation, and concentration procedures and were subsequently analyzed by means of gas chromatography-olfactometry as well as mass spectrometry as, described in Materials and Methods.

This approach led to the detection and identification of a total of 33 potent odorants in both wines. These compounds, together with their odor qualities and retention indices are given in **Table 3**. Generally, most of the odorants were detectable in both wines. Only *cis*- and *trans*-whiskeylactone and eugenol were not perceived in the Forest Hill sample. On the other hand, 3-methylbutyl acetate was not sensorically detectable in the Merryvale wine. The evaluation of the contribution of these compounds to both wine aromas in terms of quantitative composition will be discussed in the following chapters.

Comparative BOSS Analysis. Subsequent screening of both wines by means of comparative BOSS analysis using the "time dilution" approach led to the detection of most of the odorants that were previously found by HRGC/O of the solvent extracts (cf. **Figure 3**). Only the acetic, butanoic, and phenylacetic acid, as well as methionol and abhexone were not perceived. Probable reasons are that these substances are quite polar and that the buffering capacity of the saliva is very high. Therefore, perception of these compounds should be reduced. Odorants obtained by a solvent extraction procedure do not depend on these saliva interaction phenomena and are therefore perceived via HRGC/O. This means the concentrations of these compounds might be sufficient for detection in the concentrated solvent extracts obtained from 100 mL of the respective wines but are not high enough to be of retronasal sensory relevance.

Apart from these compounds, most odorants were detectable in both wines at the starting point of BOSS evaluation (15 s after swallowing). The only exceptions were sotolone, eugenol, 2-methoxyphenol, *cis*- and *trans*-whiskeylactone and methional,

Table 3. Odorant Detection in Solvent Extracts of Two Chardonnay Wines by Means of HRGC/O

	odorant[a]	detection[b] in Forest Hill	detection[b] in Merryvale	odor quality[c]	retention index[d] on FFAP	retention index[d] on SE 54
1	2-/3-methylbutanal	+	+	malty	0913	<0600
2	ethanol	+	+	ethanolic	0930	<0700
3	ethyl methylpropanoate	+	+	fruity	0955	0751
4	butane-2,3-dione	+	+	buttery	0981	<0600
5	ethyl butanoate	+	+	fruity	1028	0802
6	ethyl 2/3-methylbutanoate	+	+	fruity	1041	0845
7	3-methylbutyl acetate	+	-	banana-like	1117	0878
8	2-/3-methylbutanol	+	+	malty	1211	0739
9	ethyl hexanoate	+	+	fruity	1226	1002
10	oct-1-en-3-one	+	+	mushroom-like	1295	0976
11	acetic acid	+	+	acetic	1449	0610
12	methional	+	+	potato-like	1449	0900
13	decanal	+	+	citrus, soapy	1493	1204
14	butanoic acid	+	+	sweaty	1619	0821
15	phenylacetaldehyd	+	+	honey-like	1639	1050
16	2/3-methylbutanoic acid	+	+	sweaty	1661	0875
17	methionol	+	+	potato-like	1705	0978
18	(*E*)-β-damascenone	+	+	cooked apple	1810	1389
19	geraniol	+	+	fresh, fruity	1818	1256
20	trans-whiskeylactone	–	+	coconut-like	1830	1292
21	2-methoxyphenol	(+)	+	smoky	1859	1089
22	2-phenylethanol	+	+	honey-like	1860	1117
23	β-ionone	+	+	violet-like	1920	1496
24	*cis*-whiskeylactone	–	+	coconut-like	1920	1325
25	2,5-dimethyl-4-hydroxy-2(5*H*)-furanone (Furaneol)	+	+	caramel-like	2031	1062
26	*trans*-ethylcinnamat	+	+	flowery, sweet	2123	1469
27	4-allyl-2-methoxyphenol (Eugenol)	–	+	smoky	2159	1460
28	δ-decalactone	+	+	coconut-like	2186	1497
29	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (Sotolone)	+	+	spicy	2192	1110
30	2-methoxy-4-vinylphenol	+	+	clove-like	2196	1317
31	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone (Abhexone)	+	+	spicy	2247	1198
32	phenyl acetic acid	+	+	honey-like	2551	1262
33	vanillin	+	+	vanilla-like	2569	1397

[a] The compound was identified by comparing it with the reference substance on the basis of the folowing criteria: retention index (RI) on two HRGC stationary phases given in the table, mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port. [b] Detection via HRGC/O; +, intense detection; (+), weak detection; –, odorant was not detectable. [c] Odor quality perceived at the sniffing port. [d] Retention indices were calculated according to (*30*).

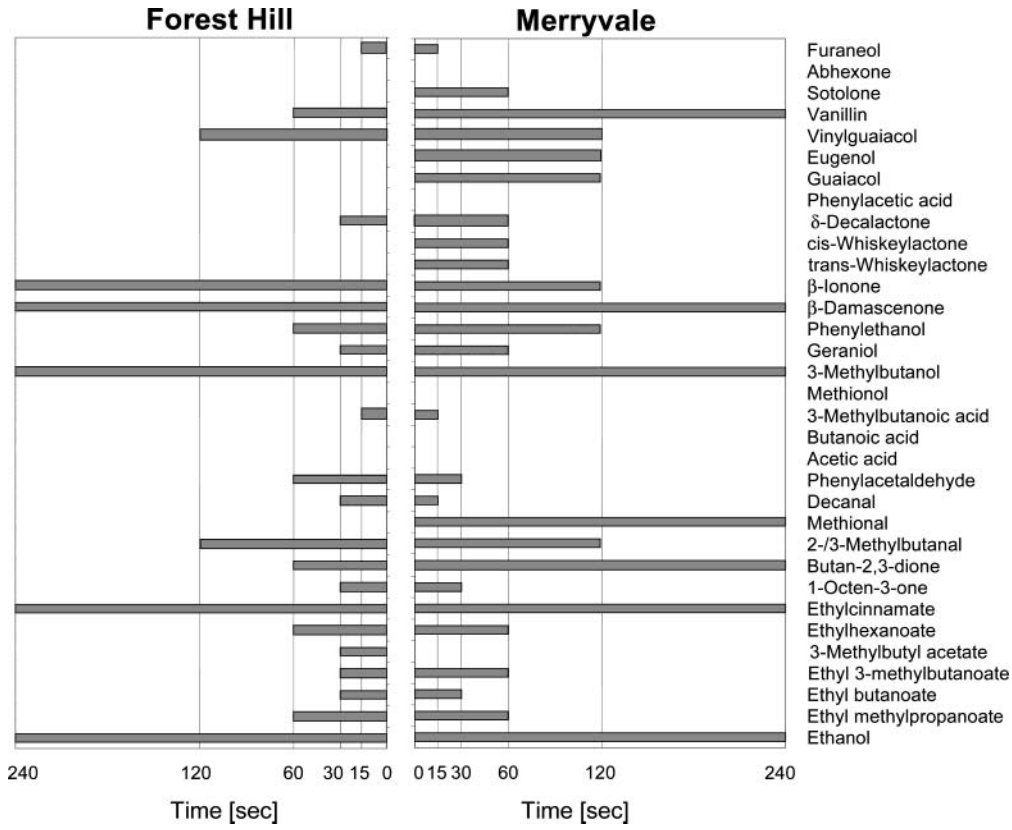

Figure 3. Comparative BOSS Analysis of two Chardonnay wines.

which were only detectable after consumption of the Merryvale wine, while 3-methylbutyl acetate was missing.

When looking at the total durations of detection of the odorants remaining in the oral cavity, some significant differences become evident. First of all, vanillin, sotolone, eugenol, 2-methoxyphenol, *cis*- and *trans*-whiskeylactone, methional, and butan-2,3-dione were detectable much longer after intraoral application of the Merryvale wine and also (just by one "time dilution" step) phenylethanol, geraniol, and ethyl 3-methylbutanoate, as compared to Forest Hill. In contrast to this, the persistence of β-ionone, phenylacetaldehyde, and decanal was a bit reduced for Merryvale, but always just by one "time dilution" step.

The correlation of these observations to the sensory evaluation is striking. Both BOSS profiles mirror the higher persistence of woody, smoky, and vanilla- and clove-like odor notes from Merryvale wine (mainly represented by vanillin, eugenol, 2-methoxyphenol, and the whiskeylactones). Persistence of up to 2 min detected via BOSS correlated directly with sensory perceivability of the woody, clove-like and vanilla-like notes. It can also be assumed that the more fruity, flowery, citrus-like impressions of Forest Hill can not only be explained by the higher persistence of β-ionone, phenylacetaldehyde and decanal, and the additional detection of 3-methylbutyl acetate. An additional reason might be the lower intensities of the above-mentioned woody, smoky, vanilla- and clove-like qualities. For these detection differences, several explanations are possible. First of all, both wines might simply contain different amounts of the respective odorants, resulting in higher intensities and persistences. This will be discussed in the following chapter. Also, there might be differences in the matrix composition of the wines so that the intraoral release parameters might be changed. In this context, it also has to be mentioned that the ethanol content of both wines was not identical (Merryvale 14.5% by vol., Forest Hill 13.5% by vol.). Whether this difference has an effect (e.g., on odorant−mucosa interactions, and as a consequence, on retronasal perceptability with time) needs to be further investigated.

Quantitation of the Potent Wine Odorants. As discussed above, the question arose whether both wines differed in the contents of some key odorants. To clarify this, 23 of the identified odorants were selected according to their detection during gaschromatography-olfactometry and according to the differences observed in Comparative BOSS-analysis. As sensory key differences were mainly related to the characteristic barrique-notes, the following experiments focused on typical barrique-related substances (*28*, *29*). Quantitation was performed by means of stable isotope dilution assays as described in Materials and Methods. **Table 4** shows the concentrations of the selected odorants in both wines together with the respective mass traces analyzed in SIDA.

A direct correlation was found for a series of substances between the quantitative data and the BOSS detection (cf. **Table 5** and **Figure 3**). First of all, considerably higher amounts of the whiskeylactones were found in the Merryvale wine with about 13 and 19-fold higher concentrations, respectively, than in Forest Hill. Also vanillin, eugenol, furaneol, and 2-methoxyphenol were increased by a factor of about five. Apart from this, about 2- to 3-fold higher concentrations were detected for ethyl 3-methylbutanoate, phenylethanol, ethyl cinnamate, and phenylacetic acid, while the amounts of 3-methylbutyl acetate were lower by a factor of about five than in Forest Hill. All these differences might have been expected from the BOSS results, only for furaneol, no increase in persistence was found. The reasons for this are not yet fully clear. However, the concentrations in both wines were very low (for a comparison see the data of strawberry aroma with considerably higher amounts of furaneol (*12*)), only resulting in a detection at 15 s and were probably not high enough to cause any significant effect in terms of persistence. There might have been just enough

Table 4. Mass Traces and Calibration Factors of Potent Odorants in Two Chardonnay White Wines Used for Quantitation by SIDA

	odorant	mass traces		calibration factor
		unlabeled	labeled	
1	2-/3-methylbutanal			
2	ethanol			
3	ethyl methylpropanoate	117	120	0.92
4	butane-2,3-dione	87	91	1.00
5	ethyl butanoate	117	120	1.00
6	ethyl 3-methylbutanoate	131	134	0.95
7	3-methylbutyl acetate	131	133	0.79
8	3-methylbutanol	71	73	1.08
9	ethyl hexanoate	145	148	1.00
10	oct-1-en-3-one			
11	acetic acid	61	63	1.00
12	methional			
13	decanal	157	158–160	0.64
14	butanoic acid	89	91	0.89
15	phenylacetaldehyd			
16	3-methylbutanoic acid	103	105	0.59
17	methionol	107	110	1.05
18	(*E*)-β-damascenone			
19	geraniol			
20	trans-whiskeylactone	157	159	0.95
21	2-methoxyphenol	125	128	1.00
22	2-phenylethanol	105	107	1.02
23	β-ionone			
24	*cis*-whiskeylactone	157	159	0.95
25	2,5-dimethyl-4-hydroxy-2(5*H*)-furanone	129	131	1.00
26	*trans*-Ethylcinnamat	177	182	1.00
27	Eugenol	165	169–171	0.40
28	δ-decalactone	171	173	0.82
29	Sotolone			
30	2-methoxy-4-vinylphenol	151	154	1.00
31	Abhexon			
32	phenyl acetic acid	137	139	1.00
33	vanillin	153	156	1.01

[a] Compounds were determined using the respective stable isotope labeled standards by means of the ion trap detector ITD-800 (Finnigan, Bremen, Germany) running in the CI-mode with methanol as reagent gas. [b] The calibration factor was determined as reported previously (*32*).

traces of wine back in the oral cavity to lead to detection at 15 s but possibly there was not yet a real release from oral mucosa. It has to be stated that from the quantitative data it cannot be excluded that additional matrix effects occur, as discussed above. This will be elucidated by further model experiments, where wine solids will be isolated, recomposed according to the respective requirements, and exposed to changing aroma compositions. This approach will lead to a fundamental understanding of aroma release under in vivo conditions and which is much more important in real-food concentrations. The data presented in this study set the analytical starting point to continue into this direction.

Summarizing the results of the quantitation experiments, a striking correlation between the most potent odorants of both wines, the detectability via BOSS analysis, and the perception of retronasal intensities of odor qualities with time has been achieved.

It has been shown that the aroma changes perceived with time did not result from a release of some compounds with later on-set (starting point) but that all odorants were detectable right from the starting point of the analysis of afterodor. Changes were induced by the faster removal of some odorants from the oral cavity while others persisted for longer time. As a consequence, the perception of these compounds became more dominant as the short-lasting odorants were removed (at later times).

Table 5. Concentrations of Potent Odorants in Two Chardonnay White Wines

	odorant	concentration [μg/L]		factor of difference (related to Forest Hill)
		Forest Hill	Merryvale	
1	2-/3-methylbutanal	nd	nd	nd
2	ethanol	nd	nd	nd
3	ethyl methylpropanoate	72.2	99.9	1.4
4	butane-2,3-dione	nd	172.7	nd
5	ethyl butanoate	263.0	341.5	1.3
6	ethyl 3-methylbutanoate	9.2	19.9	2.2
7	3-methylbutyl acetate	943.7	163.5	0.2
8	3-methylbutanol	253591	356725	1.4
9	ethyl hexanoate	757.2	737.5	1.0
10	oct-1-en-3-one	nd	nd	nd
11	acetic acid	434232	489370	1.1
12	methional	nd	nd	nd
13	decanal	20.0	15.3	0.8
14	butanoic acid	1839	1611	0.9
15	phenylacetaldehyd	nd	nd	nd
16	3-methylbutanoic acid	588.0	561.6	1.0
17	methionol	563.4	795.6	1.4
18	(*E*)-β-damascenone	nd	nd	nd
19	geraniol	nd	nd	nd
20	*trans*-whiskeylactone	7.1	131.1	18.5
21	2-methoxyphenol	2.7	9.9	3.7
22	2-phenylethanol	12415	24971	2.0
23	β-ionone	nd	nd	nd
24	*cis*-whiskeylactone	17.0	214.8	12.6
25	2,5-dimethyl-4-hydroxy-2(5*H*)-furanone	2.1	13.7	6.5
26	*trans*-ethylcinnamat	1.5	3.1	2.1
27	Eugenol	1.6	8.9	5.6
28	δ-decalactone	30.4	32.4	1.1
29	Sotolone	nd	nd	nd
30	2-methoxy-4-vinylphenol	50.5	49.3	1.0
31	Abhexon	nd	nd	nd
32	phenyl acetic acid	34.6	90.0	2.6
33	vanillin	48.5	241.6	5.0

Generally, this correlation between sensory perception and analytical data was a convincing proof of the validity of the BOSS approach to profile retronasal aroma perception as a function of the composition of food aroma and food matrix.

It has to be stated that the investigation of trace key aroma compounds involved in afterodor development under unchanged in vivo conditions has been achieved for the first time using the BOSS approach. The technique was successfully applied in real-food odorant concentrations and under realistic matrix composition conditions.

The feasability of BOSS to screen intraorally even traces of key odorants with regard to their retronasal aroma contribution and involvement in the phenomenon of "afterodor" or "after-smell" has been demonstrated. Sensory differences in the afterodors of two different Chardonnay wines were followed by time-resolved sensory profiling. High correlation was found to the intraoral persistence of key aroma compounds. Further studies will be performed on the analysis of model systems based on the data presented here.

ABBREVIATIONS USED

ADA, aroma dilution analysis; AEDA, aroma extract dilution analysis; BOSS, buccal odor screening system; SBSE, stir bar sorptive extraction; SIDA, stable isotope dilution assay

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LITERATURE CITED

(1) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022−3026.

(2) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022−3026.

(3) Guth, H. Determination of the configuration of wine lactone. *Helv. Chim. Acta* **1996**, *79*, 1559−1571.

(4) Buettner, A. Retronasale Geruchswahrnehmung − Die Freude am Essen und Trinken. *Nachrichten aus der Chemie* **2004**, accepted for publication.

(5) Buettner, A.; Beer, A.; Hannig, C.; Settles, M.; Schieberle, P. Quantitation of the in-mouth release of heteroatomic odorants. In *Flavor Chemistry of Heteroatomic Compounds*; Reineccius, T., Reineccius, G., Eds.; ACS Symposium Series 826; American Chemical Society: Washington, DC, 2002; pp 296−311.

(6) Buettner, A. Physiology and chemistry behind retronasal aroma perception during winetasting. *Proceedings of the VIIth International Symposium d'Oenology*, 2003, Bordeaux, in press.

(7) Buettner, A.; Schieberle, P. Influence of mastication on the concentrations of aroma volatiles − some aspects of flavour release and flavour perception. *Food Chem.* **2000**, *71*, 347−354.

(8) Buettner, A.; Beer, A.; Hannig, C.; Settles, M. Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging − consequences for aroma perception. *Chem. Senses* **2001**, *26* (9), 1211−1219.

(9) Buettner, A.; Schieberle, P. Changes in the concentrations of key fruit odorants induced by mastication. In *Flavor Release*; Roberts, D. D., Taylor, A. J., Eds.ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 87−98.

(10) Buettner, A. The influence of human salivary enzymes on odorant concentration changes occurring in vivo; Part I: Esters and thiols. *J. Agric. Food Chem.* **2002**, *50* (11), 3283−3289.

(11) Buettner, A. Influence of human saliva on odorant concentrations; Part II: Aldehydes, alcohols, 3-alkyl-2-methoxypyrazines, methoxyphenols, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone. *J. Agric. Food Chem.* **2002**, *50*, 7105−7110.

(12) Buettner, A.; Welle, F. Intraoral detection of potent odorants using a modified stir-bar sorptive extraction system in combination with HRGC/O called buccal odor screening system (BOSS). *Flavours Fragr. J.* **2004**, in press.

(13) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. Stir Bar Sorptive Extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcolumn Sep.* **1999**, *11* (10), 737−747.

(14) Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb − differences to the crust and effect of a longer dough fermentation. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 130−135.

(15) Ullrich, F.; Grosch, W. Flavour deterioration of soya-bean oil: identification of intense odour compounds formed during flavour reversion. *Fat Sci. Technol.* **1988**, *90*, 332−336.

(16) Schieberle, P.; Gassenmeier, K.; Guth, H.; Sen, A.; Grosch, W. Character impact odour compounds of different kinds of butter. *Lebensm. Wiss. Technol.* **1993**, *26*, 347−356.

(17) Sen, A.; Grosch, W. Synthesis of six deuterated sulfur containing odorants to be used as internal standards in quantification assays. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 541−547.

(18) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513−518.

(19) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.

(20) Schieberle, P.; Hofmann, T. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.* **1997**, *45*, 227−232.

(21) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387−2394.

(22) Sen, A.; Schieberle, P.; Grosch, W. Quantitative determination of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and its methyl ether using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1991**, *24*, 364−369.

(23) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution analysis. *Flavour Fragr. J.* **1995**, *10*, 1−7.

(24) Schieberle, P. Primary odorants of pale lager beer. Differences to other beers and changes during storage. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 558−565.

(25) Guth, H.; Grosch, W. Odorants of extrusion products of oat meal. Changes during storage. *Z. Lebensm.-Unters. Forsch.* **1993**, *196*, 22−28.

(26) Poisson, L.; Schieberle, P. *J. Agric. Food Chem.*, 2004, in preparation.

(27) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (Citrus paradisi Macfayden) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 1358−1363.

(28) Díaz-Plaza, E. M.; Reyero, J. R.; Pardo, F.; Alonso, G. L.; Salinas, M. R. Influence of oak wood on the aromatic composition and quality of wines with different tannin contents. *J. Agric. Food Chem.* **2002**, *50*, 2622−2626.

(29) Díaz-Plaza, E. M.; Reyero, J. R.; Pardo, F.; Salinas, M. R. Comparison of wine aromas with different tannic content aged in French oak barrels. *Anal. Chim. Acta* **2002**, *458*, 139−145.

(30) Halang, W. A.; Langlais, R.; Kugler, E. Cubic spline interpolation for the calculation of retention indices in temperature-programmed gas−liquid chromatography. *Anal. Chem.* **1978**, *50*, 1809−1832.

(31) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm.-Unters. Forsch.* **1987**, *184*, 277−282.

(32) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.

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