Technische Universität München
TUM School of Natural Sciences

# New Molecular Insights into Wine Aroma: Studies on the Lychee Note of Muscaris Grapes and the Aroma Difference between Wine and Beer

**Xingjie Wang**

Vollständiger Abdruck der von der TUM School of Natural Sciences der Technischen Universität München zur Erlangung des akademischen Grades eines

**Doktors der Naturwissenschaften (Dr. rer. nat.)**

genehmigten Dissertation.

| | | |
|---|---|---|
| Vorsitz: | | Prof. Dr. Michael Rychlik |
| Prüfende der Dissertation: | 1. | Priv.-Doz. Dr. Martin Steinhaus |
| | 2. | Hon.-Prof. Dr. Martina Gastl |

Die Dissertation wurde am 18.12.2024 bei der Technischen Universität München eingereicht und durch die TUM School of Natural Sciences am 16.01.2025 angenommen.

# ***Acknowledgements***

I want to express my deepest gratitude to my supervisor PD Dr. Martin Steinhaus for accepting me as a PhD student in his working group, for his excellent supervision and for the constructive discussions about scientific work. I am also deeply thankful for his time, patience, and support during my scholarship application, as well as for his guidance in correcting my reports, publications, and dissertation.

I also want to express my deepest gratitude to my mentor Dr. Stephanie Frank for her valuable advice during my practical work, for her suggestions and corrections on my reports and publications, and for her time, patience, and support during my visa extension application.

I would like to thank Hon.-Prof. Dr. Martina Gastl for taking over the role of the second examiner and Prof. Dr. Michael Rychlik for taking over the role of the chairman of the examination.

I gratefully acknowledge funding from the China Scholarship Council (CSC), grant no. 201906300007. Without this scholarship covering my daily living expenses, I would not have had the opportunity to pursue further study in Germany.

I would like to thank the Leibniz-LSB@TUM for providing an excellent working and study environment which ensured I had everything I needed during my stay. I want to thank all the colleagues for the wonderful time we shared. Special thanks go to the administration secretary, whose kindness and patience were invaluable in helping me with letter deliveries and finding what I needed.

I also extend my thanks to Dr. Klaas Reglitz, Dr. Veronika Mall, and Dr. Johanna Kreißl for their warm help and valuable experience concerning equipment, instruments and experiments.

I am also grateful to the experienced workers in our working group for kindly sharing their experiences and skills. I would like to thank all the colleagues in lab E39, especially the ones who have been with me for a long time, for the good work atmosphere and their daily help when I was preparing samples. Special thanks go to Julia Bock and Monika Riedmaier for their help at the early stage of my lab work including sample preparation, column preparation, SAFE operation, column distillation, and concentration. I really appreciated the help from Jörg Stein during the synthetic work. His experience made the work much easier. I also want to thank Inge Kirchmann and Dr. Christine Stübner for their experience of performing aSAFE.

I would like to thank Dr. Christine Stübner, Eva Bauersachs, Philipp Schlumpberger, Carolina Schury, Ines Otte, Charlotte Minig, Ute Weigand, Julia Bock, Julia Schweiger, Jörg Stein, and Dr. Daniela Füllemann for the introduction to the GC–MS instruments and the measurement of my samples. I also want to thank Dr. Johanna Kreißl for the introduction to GC–O/FID, Monika Riedmaier for the introduction to GC–FID, and Dr. Daniela Füllemann for the introduction to the UV-Vis spectrophotometer. Many thanks to Dr. Johanna Kreißl and Daniel Müller for the measurement of NMR samples. Special thanks go to Eva Bauersachs for the introduction and help in using software to process GC–MS data. I also want to thank Robin Maier and Julian Reinhardt for the response to general lab questions.

I really appreciate the instruction and help from Dr. Daniela Füllemann, Dr. Caterina Porcelli, and Franziska Krause on sensory analysis. I want to thank all the colleagues who kindly participated in the sensory tests during my work.

In addition, I owe special thanks to PD Dr. Dietmar Krautwurst for the basic instruction on odorant receptors and to Dr. Franziska Haag, Julia Bauer, Sandra Hoffmann, Ronja Hopf, and Corinna Kammermeier for their careful introduction, guidelines, and help during practical work.

I would like to thank my Chinese colleagues Dr. Liang Dong (梁栋), Dr. Luo Guanjuan (骆广娟), Dr. Zhai Xiaoting (翟小婷), and Shi Jinrui (石金瑞) for their help and tips.

Last but not least, I want to express my deepest gratitude to my parents for their love, trust, endless support, patience, encouragement, and understanding throughout my studies, which enabled me to focus on my work and pursue my dreams.

The experimental part of the present work was performed between October 2019 and September 2024 at the Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany, under the supervision of Priv.-Doz. Dr. rer. nat. habil. Martin Steinhaus.

# Table of contents

# 1 Summary

Wine aroma is a fascinating research subject. There are still many interesting but unanswered questions. The current study investigated the molecular background of the lychee aroma in the berries of Muscaris grapes and the aroma difference between beer and wine.

Lychee aroma is a highly appreciated aroma note in wine, however, it is limited to only a few grape varieties. Muscaris is a relatively new grape variety with a good resistance to fungi and an intensive aroma with a pronounced lychee note. Literature research showed that the odorants in Muscaris grapes have not been systematically evaluated so far. Thus, it was also unclear to which extent the odorants in Muscaris grapes reflect those of its father variety Gelber Muskateller and which odorants constitute the molecular background of the lychee note. As a first step to fill these gaps, a comparative aroma extract dilution analysis (cAEDA) was applied to Muscaris and Muskateller grapes, which revealed 39 and 35 odorants, respectively. Quantitation of the major odorants including all odorants with flavor dilution (FD) factors ≥32 in at least one of the two grape samples resulted in 16 compounds with concentrations beyond their respective odor threshold concentrations (OTCs). These odorants were geraniol, (2*E*,6*Z*)-nona-2,6-dienal, (3*Z*)-hex-3-enal, (3*R*)-linalool, hexanal, (3*E*)-hex-3-enal, (3*S*)-linalool, 3-methylnonane-2,4-dione, oct-1-en-3-one, (2*S*,4*R*)-rose oxide, (3*S*)-*β*-citronellol, (2*E*)-non-2-enal, phenylacetaldehyde, 3-(methylsulfanyl)propanal, (2*E*)-hex-2-enal, and (*E*)-*β*-damascenone. Data showed only minor differences between the two varieties, illustrating their close genetic relation. Odor reconstitution and omission experiments demonstrated that the lychee note in the aroma of Muscaris grapes was generated by the combination of two odorants, namely floral, lychee-like smelling (2*S*,4*R*)-rose oxide and floral, rose-like smelling geraniol. This result may be helpful in the targeted breeding of new grape varieties with distinct aroma properties.

Beer and wine clearly differ in their aroma. However, which substances account for the aroma difference between the two has not been systematically studied yet. It is undoubtful that special raw materials or processing can facilitate recognition, however, we hypothesized that there is a basic olfactory difference between beer and wine that is reflected in the composition of more general odorants. Odorants constituting a basic wine aroma have already been suggested in the literature and included primarily compounds formed during fermentation. However, no quantitative data on these odorants were available and the concept of an aroma base has not been applied to beer yet. To fill these gaps, a comprehensive literature survey focused on general beer odorants and general wine odorants was performed. Odorant concentration data obtained by reliable methodology were extracted from 32 articles on beer covering 160 samples and 252 articles on wine covering 904 samples. Calculation of arithmetic mean concentrations and odor activity values (OAVs) of the 42 basic odorants led to 29 and 32 compounds with mean OAVs ≥1 in beer and wine, respectively. Aroma base models of beer and wine based on these data reflected the basic olfactory difference between beer and wine. Concentration leveling tests finally showed that the fruity smelling odorants played a crucial role in the aroma difference between the beer and wine aroma base models. When the concentrations of fruity odorants in the beer model were adjusted to the levels in the wine model, the odor of the test sample was evaluated as closer to wine than to beer.

# 2 Abbreviations and Nomenclature

*Abbreviations:*

| | |
|---|---|
| AEDA | Aroma extract dilution analysis |
| AF | Alcoholic fermentation |
| 3-AFC | 3-Alternative forced choice |
| aSAFE | Automated solvent-assisted flavor evaporation |
| ASTM | American Society for Testing and Materials |
| AV | Acidic volatiles |
| BDTB | 2-[(1*E*)-Buta-1,3-dien-1-yl]-1,3,4-trimethylbenzene |
| b.p. | Boiling point |
| cAEDA | Comparative aroma extract dilution analysis |
| cAMP | Cyclic adenosine monophosphate |
| CI | Chemical ionization |
| EI | Electron ionization |
| FD | Flavor dilution |
| FFAP | Free fatty acid phase |
| FID | Flame ionization detector |
| GC | Gas chromatography |
| GC×GC–MS | Comprehensive two-dimensional gas chromatography–mass spectrometry |
| GC–GC–HRMS | Two-dimensional heart-cut gas chromatography–high resolution mass spectrometry |
| G protein | Guanine nucleotide-binding protein |
| IBMP | 3-Isobutyl-2-methoxypyrazine = 2-Methoxy-3-(2-methylpropyl)pyrazine |
| i.d. | Inner diameter |
| IPMP | 3-Isopropyl-2-methoxypyrazine = 2-Methoxy-3-(propan-2-yl)pyrazine |
| MLF | Malolactic fermentation |
| MS | Mass spectrometry |
| 4-MSP | 4-Methyl-4-sulfanylpentan-2-one |
| NBV | Neutral and basic volatiles |
| NMR | Nuclear magnetic resonance |
| O | Olfactometry |
| OAV | Odor activity value |
| OTC | Odor threshold concentration |

| | |
|---|---|
| RI | Retention index |
| SBMP | 3-*Sec*-butyl-2-methoxypyrazine = 2-Methoxy-3-(1-methylpropyl)pyrazine |
| 3-SH | 3-Sulfanylhexan-1-ol |
| 3-SHA | 3-Sulfanylhexyl acetate |
| SIDA | Stable isotope dilution assay |
| TDN | 1,1,6-Trimethyl-1,2-dihydronaphthalene |
| TOF | Time-of-flight |
| TPB | (*E*)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene = 2-[(1*E*)-Buta-1,3-dien-1-yl]-1,3,4-trimethylbenzene |

<u>*Nomenclature:*</u>

| | |
|---|---|
| Ascorbic acid | (5*R*)-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one |
| Caffeic acid | (2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoic acid |
| *cis*-Rose oxide | (2*R*,4*S*)-/(2*S*,4*R*)-4-Methyl-2-(2-methylprop-1-en-1-yl)oxane |
| *cis*-Whisky lactone | (4*R*,5*R*)/(4*S*,5*S*)-5-Butyl-4-methyloxolan-2-one |
| Citric acid | 2-Hydroxypropane-1,2,3-tricarboxylic acid |
| *β*-Citronellol | (3*R*)-/(3*S*)-3,7-Dimethyloct-6-en-1-ol |
| (*E*)-*β*-Damascenone | (2*E*)-1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one |
| Dimethyl sulfide | (Methylsulfanyl)methane |
| 4-Ethylguaiacol | 4-Ethyl-2-methoxyphenol |
| Ferulic acid | (2*E*)-3-(4-Hydroxy-3-methoxyphenyl)prop-2-enoic acid |
| Geraniol | (2*E*)-3,7-Dimethylocta-2,6-dien-1-ol |
| Geranyl acetate | (2*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate |
| Glycerol | Propane-1,2,3-triol |
| *β*-Ionone | (3*E*)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-en-2-one |
| *α*-Ketobutyric acid | 2-Oxobutanoic acid |
| Linalool | (3*R*)-/(3*S*)-3,7-Dimethylocta-1,6-dien-3-ol |
| Myrcene | 7-Methyl-3-methylideneocta-1,6-diene |
| Nerol | (2*Z*)-3,7-Dimethylocta-2,6-dien-1-ol |
| *p*-Coumaric acid | (2*E*)-3-(4-Hydroxyphenyl)prop-2-enoic acid |
| Rose oxide | 4-Methyl-2-(2-methylprop-1-en-1-yl)oxane |
| Rotundone | (3*S*,5*R*,8*S*)-3,8-Dimethyl-5-(prop-1-en-2-yl)-3,4,5,6,7,8-hexahydroazulen-1(2*H*)-one |
| Sinapic acid | (2*E*)-3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid |

| | |
|---|---|
| Sotolon | 3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one |
| Tartaric acid | 2,3-Dihydroxybutanedioic acid |
| *α*-Terpineol | 2-(4-Methylcyclohex-3-en-1-yl)propan-2-ol |
| *trans*-Rose oxide | (2*R*,4*R*)-/(2*S*,4*S*)-4-Methyl-2-(2-methylprop-1-en-1-yl)oxane |
| *trans*-Whisky lactone | (4*R*,5*S*)/(4*S*,5*R*)-5-Butyl-4-methyloxolan-2-one |
| Vanillin | 4-Hydroxy-3-methoxybenzaldehyde |
| 4-Vinylguaiacol | 4-Ethenyl-2-methoxyphenol |
| 4-Vinylphenol | 4-Ethenylphenol |
| Whisky lactone | 5-Butyl-4-methyloxolan-2-one |
| Wine lactone | (3*S*,3a*S*,7a*R*)-3,6-Dimethyl-3a,4,5,7a-tetrahydro-3*H*-1-benzofuran-2-one |

# 3 Introduction

## 3.1 Molecular Sensory Science

Consumers' daily selection of food is influenced by numerous factors, such as healthiness, freshness, good appearance, no contaminants, convenience, sustainability, price, seasonality, and regionality. Above all, "to be tasty" is the most important factor.[1-3] That is to say, consumers care the most about the sensory properties of food when making a food selection. Among the sensory properties of food, aroma is considered the most important.[4] When our sense of smell is impaired or lost—such as when we catch a cold or suffer from COVID-19, making it impossible to perceive aroma—the hedonic value of food is drastically reduced.

### 3.1.1 Odor Perception

A combination of odor-active compounds forms the aroma of food. As soon as these odor-active compounds interact with the olfactory epithelium, the processing of aroma starts in our brain. There are two basic pathways by which odor-active compounds can reach the olfactory epithelium (Figure 1). Before consumption, the odor-active compounds evaporated from the food enter the nasal cavity via inhaling; this is the orthonasal pathway. During food consumption, odor-active compounds released in the oral cavity can enter the nasal cavity via the throat after swallowing and reflexive exhalation; this is the retronasal pathway.[4]

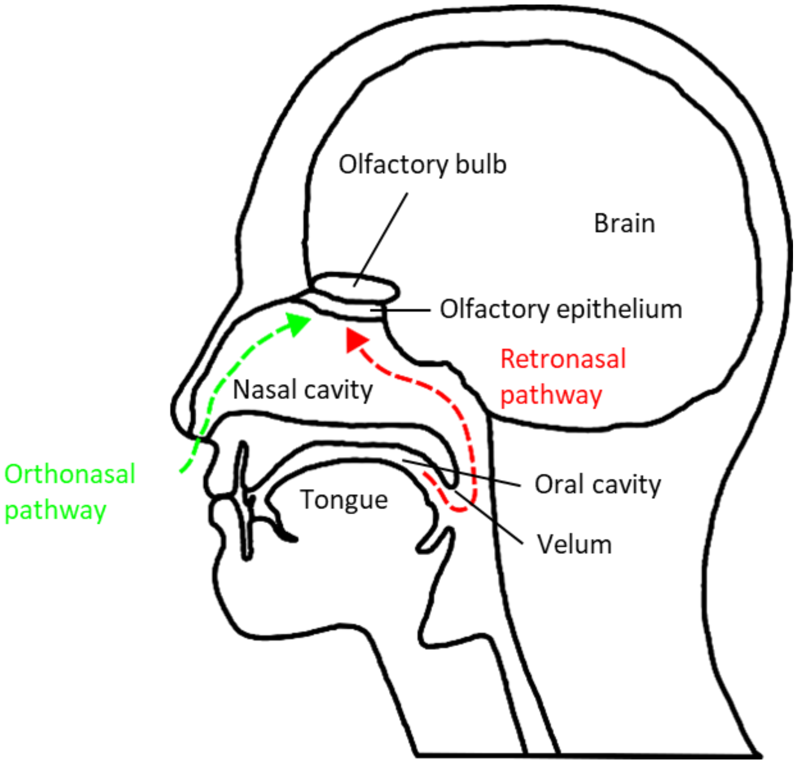


Figure 1: Orthonasal and retronasal pathway in the olfactory system (illustration: Xingjie Wang)

An odor-active compound reaching the olfactory epithelium can bind to an odorant receptor directly or to an odorant-binding protein, which carries it to the odorant receptor.[5] Odorant receptors are located in the cilia membrane of olfactory neurons in the olfactory epithelium. They are seven transmembrane domain G protein-coupled receptors encoded by about 400 functional genes within the human genome.[6-9] As displayed in Figure 2, binding of an odor-active compound to an odorant receptor starts a reaction cascade in the cell. The binding changes the conformation of the odorant receptor, resulting in the increase of cAMP. cAMP-gated ion channels open, leading to the influx of $Ca^{2+}$ and $Na^{+}$ into the cell. Then, the $Ca^{2+}$-gated $Cl^{-}$ ion channel opens, and the efflux of $Cl^{-}$ finally causes the depolarization of the cell, resulting in an action potential. Thus, the chemical signal started by the odor-active compound is converted into an electric signal. The electric signal is further transmitted beyond the cribriform plate to the olfactory bulb. In a glomerulus, the axons of the same type of odorant receptors converge with the primary dendrite of a mitral cell. Thus, each mitral cell only relays information about one type of odorant receptor. The signals from different mitral cells are further forwarded to higher levels of the brain. Finally, the brain generates the aroma impression of the food by combining the information from the different types of odorant receptors.[5,10-14]

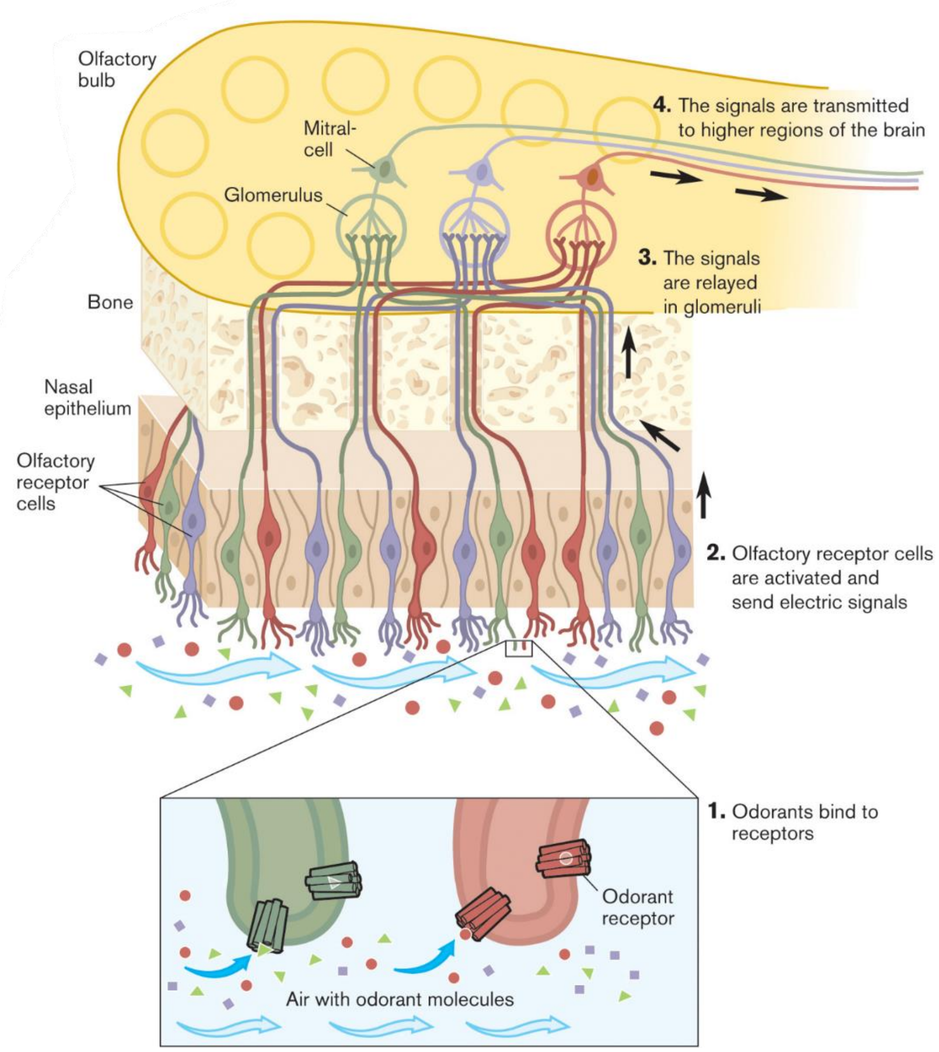


Figure 2: Odorant receptors and the organization of the olfactory system[12]

In many cases, one odor-active compound is able to bind to and activate more than one type of odorant receptor, and one type of odorant receptor can be activated by more than one odorant.[13,15-17] This contributes to the fact that humans are able to perceive and distinguish between a huge number of aromas, the exact number of which is still under discussion.[18,19]

There are three necessary conditions for a compound to be odor-active: 1) sufficient volatility; 2) basic ability to bind to at least one type of odorant receptor; 3) an amount sufficient to activate the odorant receptor.[4] The lowest concentration of a compound required to perceive an odorant is called the odor threshold concentration (OTC). The OTC varies from compound to compound; for example, the OTC of the beer-like, animalic smelling 3-methylbut-2-ene-1-thiol in water is as low as 0.00076 µg/kg, while the OTC of the sweaty, cheesy smelling 2-methylpropanoic acid is as high as 60000 µg/kg in water. Moreover, the food matrix also has a significant influence on the OTC. For example, the OTCs of the floral, honey-like smelling 2-phenylethan-1-ol are 140 µg/kg in water, 470 µg/kg in starch, and 10000 µg/kg in synthetic wine, respectively. Besides, the conformation could affect the OTC as well; for example, (*R*)-linalool has an OTC in water of 0.82 µg/kg, while (*S*)-linalool has an OTC in water of 8.3 µg/kg.[20]

Approximately 10000 volatile compounds have been determined in food. However, only ~230 of them have been demonstrated to be odor-active compounds in a majority of foods.[21] In general, a specific food typically includes 3–40 odor-active compounds that need to be present in to reconstitute its characteristic aroma.[21] Among these, only a few have a significant influence on the overall aroma impression of the food; these compounds are known as key odorants. For example, only eight of the thirteen odor-active compounds in fresh pink guavas were classified as key odorants.[22] To identify these key odorants in food, molecular sensory science provides a sophisticated approach, which is detailed below.

### 3.1.2 Identification of Key Food Odorants

The concept for the identification of key food odorants was developed by Schieberle[23] and Grosch[24] and is often divided into the seven steps displayed in Figure 3.

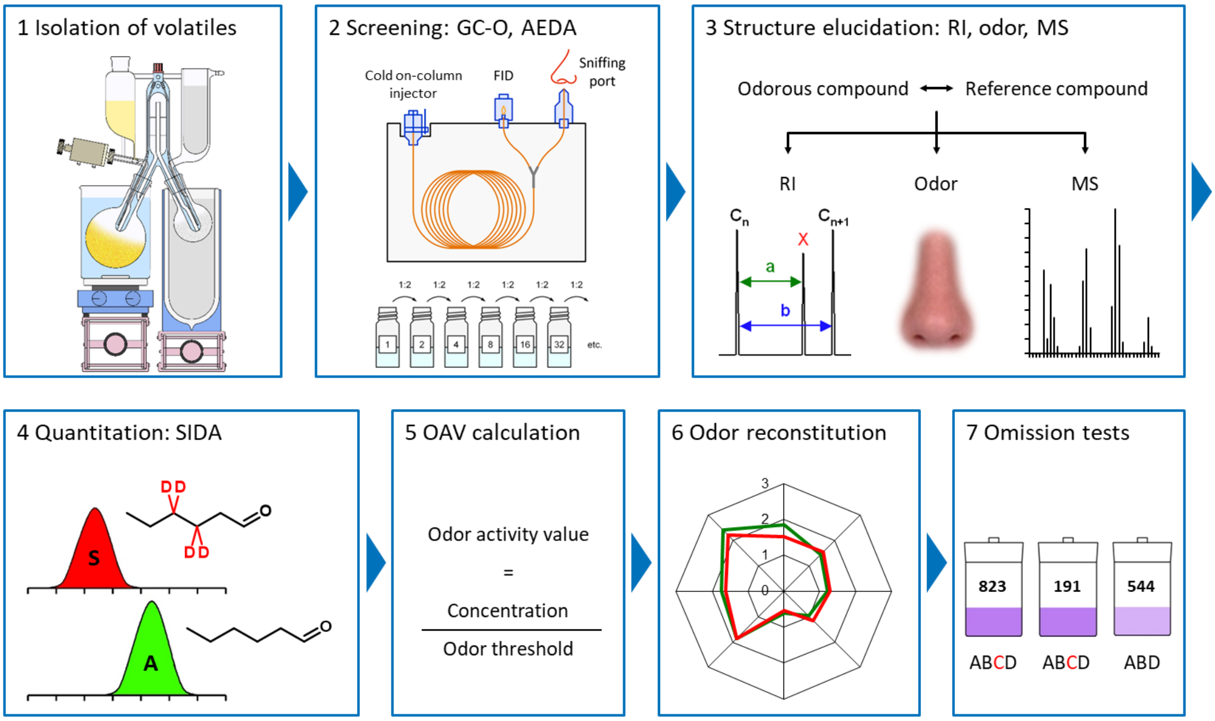


Figure 3: Identification of key odorants (illustration: Martin Steinhaus)

The first step is the isolation of volatiles (Figure 3, Step 1, and Figure 4). The volatiles are typically extracted from food using a rather non-polar organic solvent with a low boiling point, such as diethyl ether (b.p. 35 °C) or dichloromethane (b.p. 40 °C). However, not only organic volatiles but also many nonvolatiles are extracted by the solvent used. Therefore, solvent-assisted flavor evaporation (SAFE) is applied to separate the volatiles from the nonvolatiles at high-vacuum and mild temperature (≤40 °C) and the volatiles are finally re-condensed using liquid nitrogen.[25] This method minimizes the risk of compound degradation and artifact formation. Recently, an automated solvent-assisted flavor evaporation (aSAFE) was developed that saves manpower, improves yields, and reduces the risk of nonvolatiles being transferred to the volatile isolate.[26]

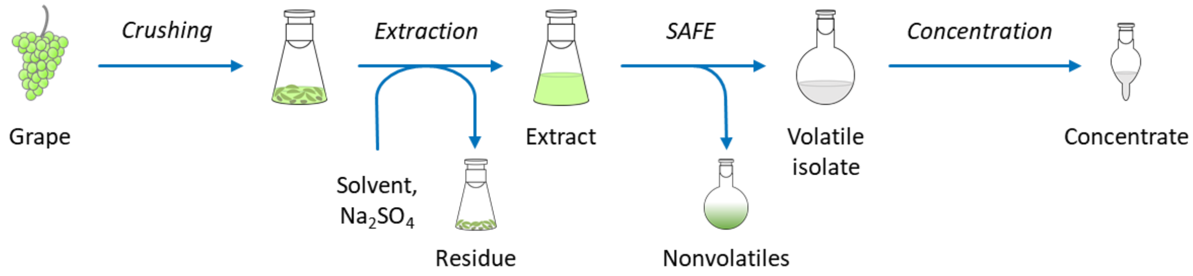


Figure 4: Preparation of volatile isolates from food samples (illustration: Xingjie Wang)

To facilitate further analysis, the volatile isolate can be further fractionated by acid-base extraction.[27] Via preparing a fraction containing acidic volatiles (AV) and a fraction containing neutral and basic volatiles (NBV), the amount of volatiles in each fraction is reduced. In addition, if necessary, the NBV can be further fractionated using silica gel chromatography.[28,29] For the selective isolation of thiols, a method using mercurated agarose gel is available.[28] Subsequently, the volatile isolates (and, in case, the respective fractions thereof) are concentrated to a volume of typically 0.1 to 1 mL using a Vigreux column and a Bemelmans microdistillation device.[30]

The concentrated volatile isolate contains both odor-active compounds and odorless compounds. The second step is thus the screening of the volatiles for odor-active compounds by application of gas chromatography–olfactometry (GC–O).[4] A GC–O system is equipped with a cold on-column injector, a flame ionization detector (FID), and a sniffing port (Figure 3, Step 2, and Figure 5). The FID is connected to a recorder. After introducing a specific volume of the volatile isolate into the GC–O system, the volatiles are separated according to boiling point and polarity on a GC fused silica capillary column using a linear temperature program. A Y-shaped glass splitter connects the end of the column with two uncoated but deactivated fused silica capillaries, which delivers the column effluent in two equal parts to the FID and the sniffing port, respectively. A trained assessor places the nose directly above the sniffing port and evaluates the effluent. Whenever an odor is detected, the position and odor quality are marked in the FID chromatogram. For each odorant, a retention index (RI) is calculated by linear interpolation from its retention time and the retention times of adjacent *n*-alkanes. Odor-active compounds are often minor or trace constituents in the volatile isolate so that no signal is detected by the FID; however, the activity-guided screening using GC–O avoids overlooking important odorants independent of their concentration.[4]

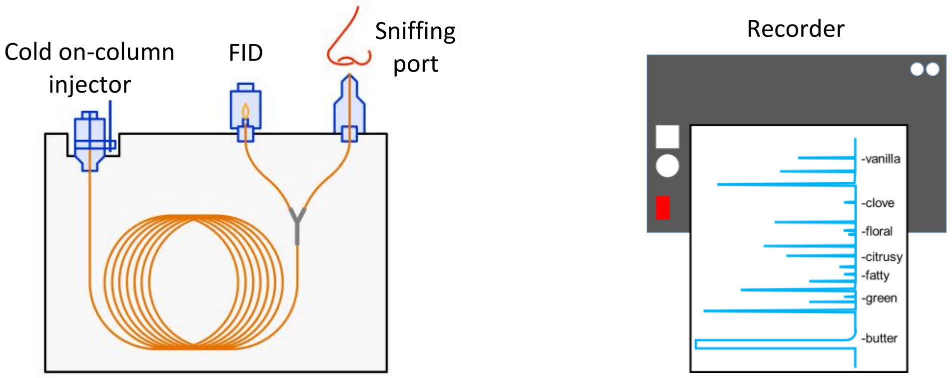
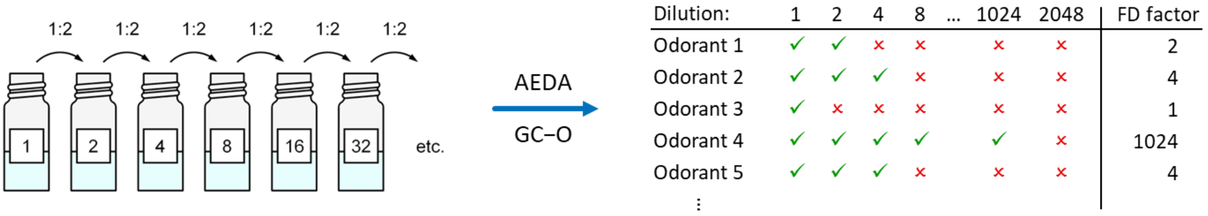


Figure 5: Scheme of a GC–O/FID system (illustration: Martin Steinhaus, modified by Xingjie Wang)

Aroma extract dilution analysis (AEDA)[31] is applied to estimate the odor potency of the odor-active compounds detected during GC–O analysis (Figure 6). The volatile isolate is stepwise diluted 1:2 with the organic solvent used for volatile extraction to obtain dilutions of 1:2, 1:4, 1:8, 1:16, etc. The undiluted sample, as well as each diluted sample, are subjected to GC–O analysis. The sequential analysis starts from the undiluted sample to the most diluted sample. The assessor continues dilution and analysis until no odorous region is detected during the whole GC–O run. Each odorant is then assigned a flavor dilution (FD) factor, representing the dilution factor of the highest diluted sample in which the odorant was detected at the sniffing port during GC–O analysis. Due to the genetic variance of odorant receptors between individuals,[32,33] GC–O analysis must always be conducted by at least two assessors with complementary olfactory abilities, i.e., a specific anosmia present in one assessor must not be present in the other, and vice versa.[4] A comparative AEDA (cAEDA)[23] is useful for comparing the odor-active compounds from two or more samples. It has, for example, been applied to the fruits of six different mango cultivars,[34] infusions made from two different types of roasted barley,[29] fresh and roasted gingers,[35] fermented cocoa seeds with and without an off-flavor, bottom-fermented beers made from three different barley malts,[36] and berries of two different grape varieties with close genetic relationships.[37]



| Dilution: | 1 | 2 | 4 | 8 | … | 1024 | 2048 | FD factor |
|---|---|---|---|---|---|---|---|---|
| Odorant 1 | ✓ | ✓ | × | × |  | × | × | 2 |
| Odorant 2 | ✓ | ✓ | ✓ | × |  | × | × | 4 |
| Odorant 3 | ✓ | × | × | × |  | × | × | 1 |
| Odorant 4 | ✓ | ✓ | ✓ | ✓ |  | ✓ | × | 1024 |
| Odorant 5 | ✓ | ✓ | ✓ | × |  | × | × | 4 |
| ⋮ |  |  |  |  |  |  |  |  |

Figure 6: AEDA: stepwise dilution of the extract and FD factor assignment (illustration: Martin Steinhaus)

The third step is the structure elucidation[4] of the odor-active compounds detected during GC–O analysis (Figure 3, Step 3). Structure elucidation is achieved by comparing several parameters of the target odorants with those obtained from authentic reference compounds under identical conditions. These parameters include retention indices (RIs) on two GC columns with different polarity (often DB-FFAP and DB-5), odor quality and odor intensity perceived during GC–O analysis at similar concentration, and the mass spectra obtained from gas chromatography–mass spectrometry (GC–MS) in both chemical ionization (CI) mode and electron ionization (EI) mode. In case there are co-elution problems in GC–MS analysis, two-dimensional GC approaches, e.g., GC×GC–TOFMS[38] and heart-cut GC–GC–HRMS[39] are helpful options.

The enantiomeric ratio must be determined for chiral odor-active compounds, as the enantiomers may differ significantly in odor quality and/or OTC.[4] If a reference compound is not commercially available, synthesis is required and the structure of the synthesized compound needs to be confirmed by nuclear magnetic resonance (NMR) spectroscopy.

GC–O, in combination with AEDA, is useful to screen for odor-active compounds and assess their odor potency. However, it cannot clarify the contribution of a single odorant to the overall aroma of the analyzed food sample. This is because the odor-active compounds are fully evaporated during GC–O thus not considering their release behavior from the real food, and because interactions between odor-active compounds and matrix components as well as interactions between odor-active compounds during perception are not considered. In addition, losses during workup are not compensated. To substantiate the screening results from GC–O analyses, further experiments are required.

The fourth step is the quantitation of the odor-active compounds, preferentially by using stable isotope dilution assays (SIDA)[40] (Figure 3, Step 4, and Figure 7). In SIDA, stable isotopically substituted analogues of the target analytes, typically deuterated or $^{13}$C-substituted compounds, are used as internal standards. The standards are added to the food sample at the beginning of sample preparation. As the target analytes and their internal standards have almost the same chemical and physical properties, any loss of the target analytes during workup is compensated by the loss of their respective internal standards, leading to a constant concentration ratio of the target analytes and their internal standards. However, sufficient homogenization of the food sample and the internal standards until equilibrium is reached is required before the start of the workup. GC–MS is applied to determine the ratio of target analytes and their internal standards via collecting the peak areas of the analytes and the respective internal standards from the extracted ion chromatograms using appropriate quantifier ions in either CI mode or EI mode. CI mode is usually preferred because of the more intense signals of the molecular ions. Finally, the concentration of each odorant in the food sample is calculated from the peak area counts of the analyte, the peak area counts of the internal standard, the amount of food sample used in the workup, and the amount of internal standard added, by employing a calibration line equation. To obtain the calibration line equation, solutions of the analyte and the respective internal standard are mixed in different concentration ratios and analyzed under the same conditions followed by linear regression.

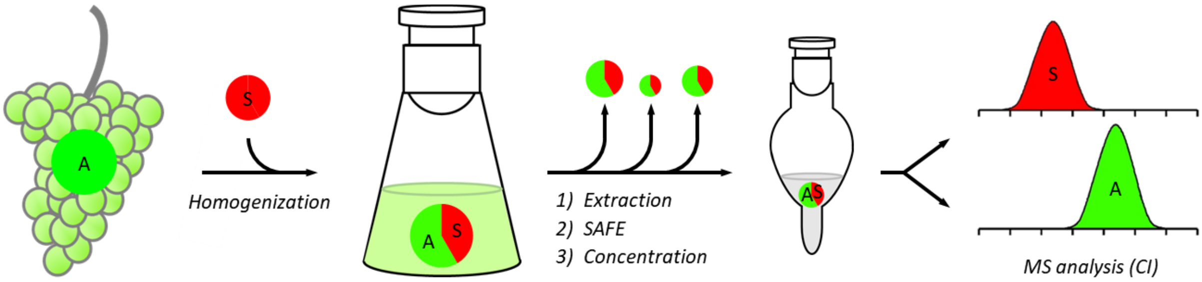


Figure 7: SIDA (illustration: Martin Steinhaus, modified by Xingjie Wang)

The fifth step is the calculation of the odor activity value (OAV)[41] (Figure 3, Step 5). For each odorant, an OAV is calculated as the ratio of the determined concentration in the food sample to the OTC determined in a matrix close to the food sample. OTCs are typically determined according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[42] In general, odorants with an OAV ≥1 are potential contributors to the overall aroma of the food, while odorants with an OAV <1 normally do not contribute.

The sixth step is aroma reconstitution (Figure 3, Step 6, and Figure 8). This experiment is conducted to verify that all odorants crucial for the overall aroma of the food sample are correctly identified and quantitated. The odor reconstitution model is prepared by adding all the odorants with an OAV ≥1 at their determined concentrations to an appropriate matrix. To adjust the model to the food sample, matrix components like water, oil, ethanol, and starch, as well as pH are typically considered. The odor reconstitution model is then orthonasally compared with the authentic food sample by trained assessors in a quantitative olfactory profile analysis. The odor reconstitution is considered successful if the odor profiles of the reconstitution model and the authentic food are in good agreement.

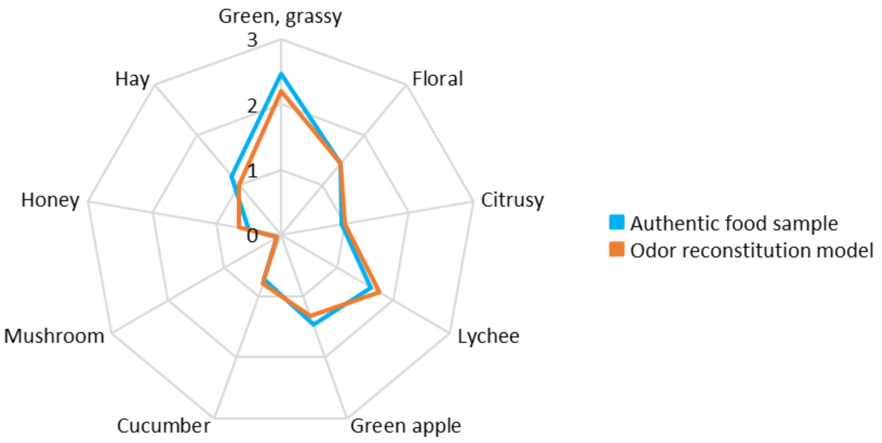


Figure 8: Odor reconstitution (illustration: Xingjie Wang)

The seventh and last step is the omission tests[24] (Figure 3, Step 7). After successful odor reconstitution, this experiment is performed to eventually identify the key odorants in food. An incomplete odor reconstitution model with a single odorant omitted,[43,44] or alternatively a group of odorants within a specific OAV range,[29,43] or a group of odorants with close odor qualities,[29,43] is compared to the complete odor reconstitution model in a 3-alternative forced choice (3-AFC) test. If there is a significant difference between the incomplete model and the complete model, which is mathematically derived from a *p*-value <5%, the importance of the omitted odorant to the overall aroma is considered proven and the compound is regarded a key odorant in this food.

## 3.2 Wine Odorants

Wine is a popular alcoholic beverage made from grape berries. According to the data from the International Organisation of Vine and Wine, the annual production volume of wine ranged from 248 to 295 million hectoliters between 2000 and 2022 (Figure 9), with an average of ~270 million hectoliters. France, Italy, and Spain are the top three wine-producing countries in the world.[45] Wines can be classified based on various factors such as color, sweetness (residual sugar content), carbonation level, and grape variety.

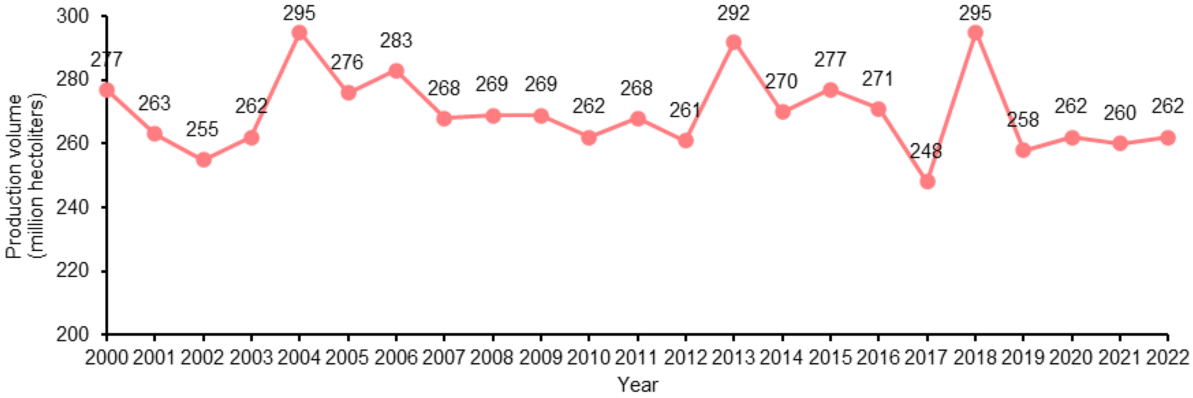


Figure 9: The world production volume of wine from 2000 to 2022[45]

Aroma is one of the major drivers for the popularity of wine. Wine aroma is caused by volatile wine components. Over 1000 volatiles have been identified in wine;[46-48] however, only a minority of them are odor-active and thus potentially important for wine aroma. The odorant composition can vary widely in different wines and additionally show substantial changes during storage and aging. Sometimes, even a minor change in an odorant's concentration can substantially alter the wine's aroma quality.[49] The odorant composition of a wine depends on factors such as grape variety, grapevine age, ripeness of the grape berries, weather, vineyard condition, viticulture management, winemaking strategy as well as storage and aging conditions.[50-53] The primary sources of wine odorants are the grape berries, the microbial metabolism during fermentation, and the aging processes.

### 3.2.1 Odorants Originating from Grape Berries

Grape berries do not have such a strong and complex aroma as wine. Nonetheless, many wine odorants and/or their precursors are already present in the grape berries and in the winemaking process are transferred to the must during crushing and maceration. Their concentrations are usually rather low; however, their impact on the overall aroma of wine must not be underestimated. Many of them contribute to the characteristic olfactory profile. Grape odorants important in wine include terpenoids, C13-norisoprenoids, thiols, and methoxypyrazines[54] (Table 1, Figure 10).

Terpenoids are biosynthesized in grapes from dimethyl allyl diphosphate and isopentenyl diphosphate, both of which originate in the mevalonic acid pathway and/or the methylerythritol phosphate pathway.[55,56] Terpenoids are present in grapes in both, the free form and the glycosidically bound form.[56,57] Terpenoid glycosides are common in grapes and usually show higher concentrations than the corresponding free forms.[54,57,58] During winemaking, an additional portion of free terpenes are released from the glycosidically bound precursors by glycosidases of grape, yeast, or bacterial origin,[49] or by acid-catalysis.[54,59]

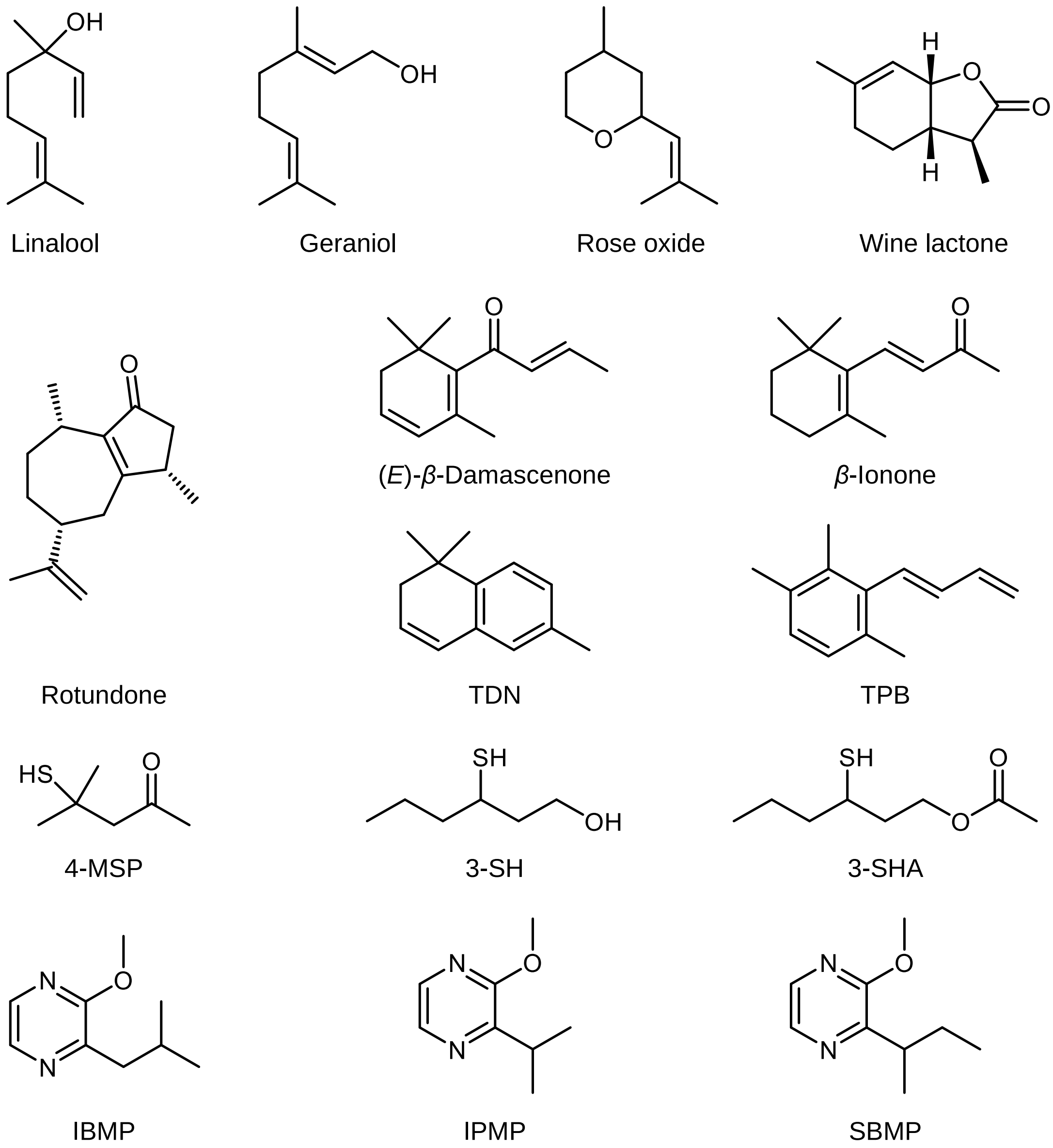
Terpenoids crucial to wine aroma include monoterpenoids[49,50,59,60] and sesquiterpenoids.[50,61,62] The most common monoterpenoid odorants in wine are the monoterpene alcohols linalool and geraniol (Table 1, Figure 10). The concentrations of linalool and geraniol in wines, especially in wines made from Muscat[63,64] and other aromatic grapes,[65-67] often exceed their OTCs of 25.2 µg/L[68] and 36 µg/L[69], respectively. *Cis*-rose oxide is another important monoterpenoid odorant often present in wines made from aromatic grape varieties.[70] Particularly in Gewürztraminer wine, it significantly contributes to the overall aroma.[65,66] Wine lactone is an odorant reported in both Gewürztraminer and Scheurebe wines with a concentration of 0.10 mg/L,[65] and in Riesling wine with a concentration of 4.6 µg/L[71]; however, quantitative data in other wines are scarcely available.[60] Other typical monoterpenoids like nerol (OTC 500 µg/L)[72], *β*-citronellol (OTC 100 µg/L)[65], and *α*-terpineol (OTC 250 µg/L)[68] are also frequently detected in wine; however, their concentrations are often below their respective OTCs,[63,64,67] indicating minor importance to wine aroma. Likewise, sesquiterpenes usually appear below the OTC.[56] Nearly 100 sesquiterpenes have been reported in grapes and wines. However, only rotundone has clearly demonstrated to contribute to wine aroma.[73] This compound was first identified in Australian Shiraz wines to which it imparts the characteristic peppery aroma.[61,62]

C13-norisoprenoids are degradation products of tetraterpenoid carotenoids.[50,55,74] C13-norisoprenoids have a megastigmane skeleton. They can be divided into two groups according to the presence or absence of an oxygen function in the lateral chain.[74,75] Like with terpenoids, both free and glycosidically bound forms of C13-norisoprenoids have been found in grapes.[54,76] During fermentation, the glycosides are hydrolyzed by enzymes and acids, thus releasing the free C13-norisoprenoids.[54,74] C13-norisoprenoid odorants appear in wine only in trace amounts, however, some of them show a substantial impact on wine aroma due to their comparably low OTCs[54] (Table 1, Figure 10). *β*-Damascenone has been reported in wines of most grape varieties with concentrations exceeding its OTC.[49,60,77] Even though its concentration substantially differs between different wines, there is no particular correlation with specific grape varieties.[60,77] *β*-Ionone is an outstanding odorant given that nearly half of the population is unable to smell it.[78,79] This compound seems to be more important to the aroma of red wine than to the aroma of white wine.[60] Compared to *β*-damascenone and *β*-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) is not a widely detected odorant in wines. High concentrations are limited to Riesling wines and evoke the characteristic petrol-like note of such wines.[80] TDN concentrations are usually higher in aged wines than in young wines.[81,82] 2-[(1*E*)-Buta-1,3-dien-1-yl]-1,3,4-trimethylbenzene (TPB) is another odorant rather rarely detected in wine. It was first reported in several aged white wines made from Semillon, Chardonnay, and Riesling grapes. In these wines, it showed concentrations beyond its OTC of 40 ng/L as determined in a neutral white wine.[83] A later study showed that it is characteristic for white wines, especially Semillon wines, and formed during aging.[84] Its absence in red wine can be attributed to the presence of polyphenols.[83,84] More C13-norisoprenoids have been reported in wine, however, their impact on wine aroma is yet unclear due to their low detection frequency and the lack of OTC data.

Table 1: Odor and OTC of important wine odorants originating from grape berries

| odorant | CAS no. | odor[a] | OTC[b] (µg/L) | reference[c] |
|---|---|---|---|---|
| linalool | 78-70-6 | citrusy, floral | 25.2 | 68 |
| geraniol | 106-24-1 | rose-like, citrusy | 36 | 69 |
| *cis*-rose oxide | 16409-43-1 | floral, rose-like | 0.2 | 65 |
| wine lactone | 182699-77-0 | coconut-like, dill-like | 0.01 | 65 |
| rotundone | 18374-76-0 | pepper-like, musty | 16 | 61 |
| *β*-damascenone | 23726-93-4 | cooked apple-like | 0.05 | 65 |
| *β*-ionone | 79-77-6 | floral, violet-like | 0.09 | 68 |
| TDN | 30364-38-6 | petrol-like | 1.5 (model wine) | 85 |
| | | | 2.3 (Riesling wine) | 85 |
| TPB | 5732-00-3 | green, cut-grass-like | 0.430 (model wine) | 83 |
| | | | 0.040 (white wine) | 83 |
| 4-methyl-4-sulfanylpentan-2-one (4-MSP) | 19872-52-7 | blackcurrant-like | 0.0008 | 86 |
| 3-sulfanylhexan-1-ol (3-SH) | 51755-83-0 | grapefruit-like | 0.060 | 86 |
| 3-sulfanylhexyl acetate (3-SHA) | 136954-20-6 | blackcurrant-like | 0.0042 | 86 |
| 2-methoxy-3-(2-methylpropyl)pyrazine (IBMP) | 24683-00-9 | earthy,<br>bell pepper-like | 0.002 | 87 |
| 2-methoxy-3-(propan-2-yl)pyrazine (IPMP) | 25773-40-4 | earthy, pea-like | 0.000318 (white wine) | 88 |
| | | | 0.001563 (aromatic wine) | 88 |
| | | | 0.001031 (red wine) | 88 |
| 2-methoxy-3-(1-methylpropyl)pyrazine (SBMP) | 24168-70-5 | bell pepper-like, earthy | not found | |

[a]The odor of each odorant is taken from the Leibniz-LSB@TUM Odorant Database,[20] except that of TPB, which was taken from reference (83). [b]Orthonasal odor threshold concentration. An OTC of SBMP determined in wine or model wine was not found in the literature. [c]Reference from which the OTC value has been taken. The OTCs from the references were determined in different matrices, including synthetic wine (11% ethanol, v/v, 7 g/L glycerol, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH),[68] synthetic wine (10% ethanol, v/v, 5 g/L tartaric acid, pH 3.2),[69] water/ethanol (90 + 10, w/w),[65] a young (<12 months old) red wine,[61] model wine (12.0% ethanol, v/v, 10 g/L tartaric acid, pH adjusted to 3.0 using 1 N NaOH) and one-year-old Riesling wine (12.0% ethanol, 1.6 g/L residual sugar, 7.8 g/L titratable acidity, pH 3.1),[85] model wine (unbuffered 10% aqueous ethanol) and a neutral dry white wine (Chenin Blanc, 11.5% ethanol, pH 3.5),[83] synthetic wine (12% ethanol, v/v, 5 g/L tartaric acid, pH adjusted to 3.5),[86] Australian white blended wine,[87] and neutral white wine (Chardonnary wine), aromatic white wine (Gewürztraminar wine), and red wine blend.[88]



Linalool

Geraniol

Rose oxide

Wine lactone

Rotundone

(*E*)-*β*-Damascenone

*β*-Ionone

TDN

TPB

4-MSP

3-SH

3-SHA

IBMP

IPMP

SBMP

Figure 10: Chemical structures of important wine odorants originating from grape berries

Thiols are another group of varietal volatiles originating from grape berries. Although many thiols have been detected in grapes and wines, the most widely reported and most potent odorants in this group are 4-MSP, 3-SH, and 3-SHA[49,56,89-93] (Table 1, Figure 10). Their OTCs determined in synthetic wine amount to 0.8 ng/L, 60 ng/L, and 4.2 ng/L, respectively.[86] 4-MSP, 3-SH, and 3-SHA play a vital role in shaping the aroma of wine from many grape varieties, particularly Sauvignon Blanc[49,91] and Scheurebe wines.[65] All three odorants were first reported in Sauvignon Blanc wines.[94-96] Although the free form of 3-SH has been found in Sauvignon Blanc grape juices,[97] 4-MSP and 3-SH appear predominantly in the form of odorless precursors in grapes.[49,54,91] In the winemaking process, free 4-MSP and 3-SH are released from their precursors, mainly by the yeast.[49,56,91,93] The odorless precursors of 4-MSP and 3-SH are glutathione conjugates and cysteine conjugates, but also cysteinyl-glycine and γ-glutamyl-cysteine conjugates.[54,76,91,98,99] No such precursor has been reported for 3-SHA; it may thus be formed from 3-SH during fermentation by yeast.[90]

Methoxypyrazines also form an important group of odorants in grapes and wines. They are present in the free form in grape berries.[100] Among the identified methoxypyrazines, IBMP, IPMP and SBMP are of particular importance to wine aroma[54,56,92,100-102] (Table 1, Figure 10). For example, they are responsible for the green, vegetative, bell pepper-like notes of some Cabernet Sauvignon wines.[50,103,104] Their OTCs determined in synthetic wine or real wine are very low. Even though present only in trace amounts in wine, their concentrations are frequently above the OTCs. Leucine, valine, and isoleucine are supposed to be the precursors of IBMP, IPMP, and SBMP, respectively. However, the related biosynthetic pathways in grapes have not been fully clarified. During winemaking, they directly enter into the must and wine.[105] It has been reported that the concentrations of IBMP and IPMP in the final wines were roughly at the same level as in the grapes and the grape juice.[106,107] IBMP is the most abundant and thus shows the highest impact on wine aromas.[88,93,102,104,108] Because its concentration decreases during grape ripening,[103] its quantitation is used in the wine industry to decide the harvest time.[109] As shown in Table 1, IPMP exhibits an even lower OTC in wine than IBMP. Even though its concentration is often lower than that of IBMP, it can play an important role in wine aroma. SBMP is less investigated, particularly no OTC in wine matrix is currently available in the literature.

### 3.2.2 Odorants Originating from Microbial Metabolism during Fermentation

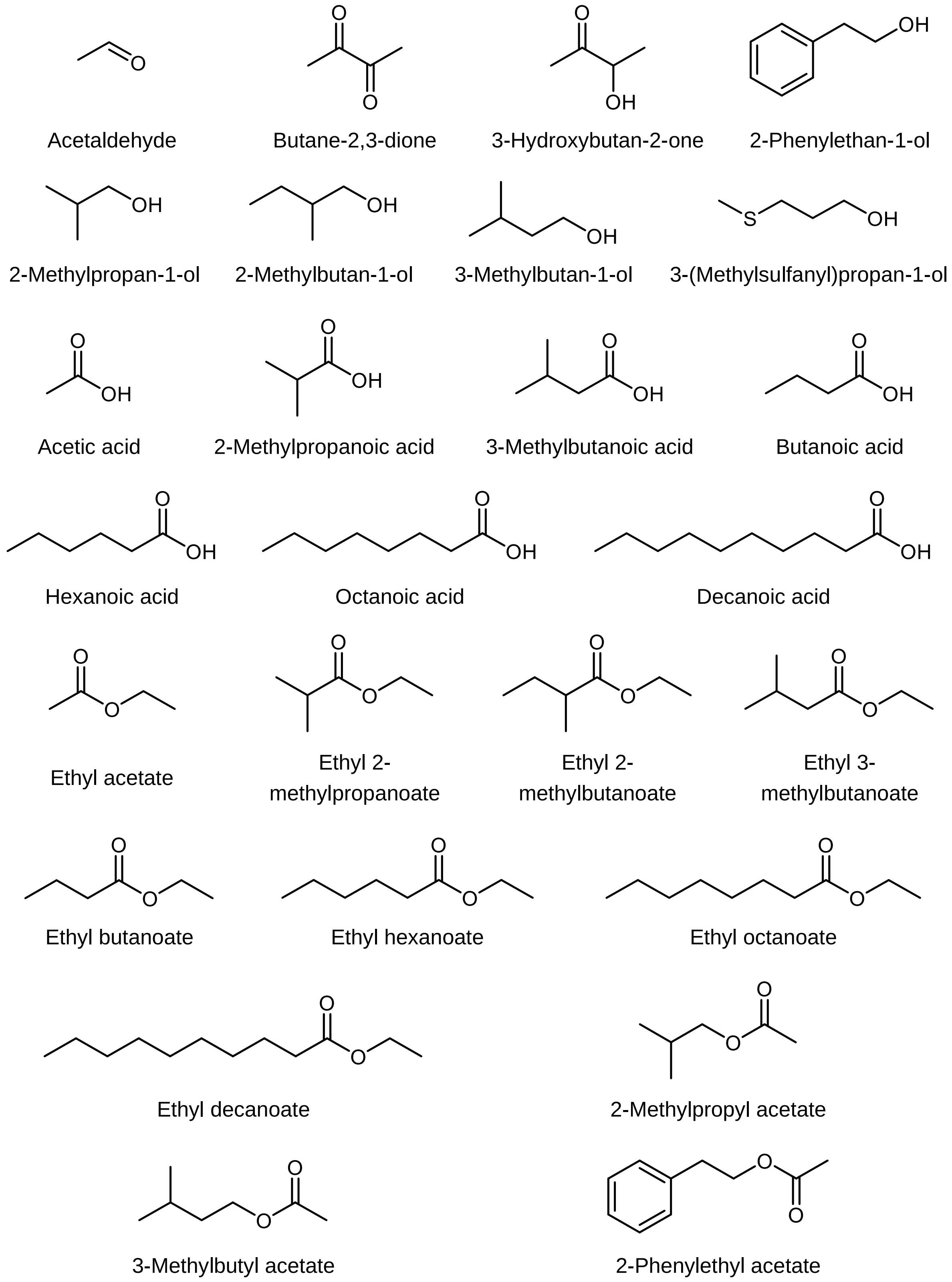
Grape juice and wine largely differ in aroma, primarily due to the involvement of microorganisms during fermentation. The fermentation typically comprises alcoholic fermentation (AF) and malolactic fermentation (MLF). AF is essential to convert grapes into wine and predominated by *Saccharomyce*s strains. The most widely used *Saccharomyce*s species in the wine industry is *S. cerevisiae*.[49,110,111] During AF, yeast does not only convert sugar into ethanol and carbon dioxide but also produces numerous fermentation by-products, including a wide variety of volatiles.[49] MLF is optional and conducted by lactic acid bacteria, among which the most widely applied species is *Oenococcus oeni*.[49,111-113] The aims of MLF are deacidification via converting dicarboxylic L-malic acid to monocarboxylic L-lactic acid and carbon dioxide,[49,110,111,114,115] and further modification of the wine flavor beyond sour taste reduction.[49,110,113-115] MLF can lead to the formation of odor-active volatiles, but also to the decrease of odorants previously formed during AF.[49,114] In particular, MLF can reduce the green, vegetative aroma and enhance fruity and buttery aroma characters.[111,114] Both AF and MLF involve the formation of odorants by hydrolysis of precursors.[49,113-115] In general, the volatiles formed during fermentation constitute the largest percentage of the total volatile content in wine and also a majority of wine odorants originate in fermentation,[48,116] among which are particularly carbonyl compounds, alcohols, acids, and esters (Table 2, Figure 11).

Important odor-active carbonyl compounds originating in fermentation include acetaldehyde, butane-2,3-dione, and 3-hydroxybutan-2-one (Table 2, Figure 11). Acetaldehyde is mainly produced during AF as an intermediate of ethanol formation. Minor amounts can also be formed by lactic acid bacteria during MLF. Acetaldehyde can be further oxidized to acetic acid.[49] Butane-2,3-dione is produced in both AF and MLF. However, the major portion originates in MLF as a metabolite of citric acid.[49,113,114] It plays an important role in the buttery note of wine aroma.[49,110,112-114] It can also act as an intermediate compound in the formation of 3-hydroxybutan-2-one and subsequently butane-2,3-diol.[49,113,114] 3-Hydroxybutan-2-one is another buttery smelling odorant; however, its OTC (155000 µg/L) is much higher than that of butane-2,3-dione (100 µg/L).

Table 2: Odor and OTC of important wine odorants originating from microbial metabolism during fermentation

| odorant | CAS no. | odor[a] | OTC[b] (µg/L) | reference[c] |
|---|---|---|---|---|
| acetaldehyde | 75-07-0 | fresh, green | 500 | 65 |
| butane-2,3-dione | 431-03-8 | buttery | 100 | 65 |
| 3-hydroxybutan-2-one | 513-86-0 | butter-like, carrot-like | 155000 | 72 |
| 2-methylpropan-1-ol | 78-83-1 | malty | 40000 | 65 |
| 2-methylbutan-1-ol | 137-32-6 | malty | not found | |
| 3-methylbutan-1-ol | 123-51-3 | malty | 30000 | 65 |
| 2-phenylethan-1-ol | 60-12-8 | floral, honey-like | 14000 | 68 |
| 3-(methylsulfanyl)propan-1-ol | 505-10-2 | cooked potato-like | 1000 | 68 |
| acetic acid | 64-19-7 | vinegar-like | 300000 | 69 |
| butanoic acid | 107-92-6 | sweaty | 173 | 68 |
| 2-methylpropanoic acid | 79-31-2 | sweaty, cheesy | 2300 | 68 |
| 3-methylbutanoic acid | 503-74-2 | sweaty | 33.4 | 68 |
| hexanoic acid | 142-62-1 | sweaty | 420 | 68 |
| octanoic acid | 124-07-2 | carrot-like, musty | 500 | 68 |
| decanoic acid | 334-48-5 | soapy, musty | 1000 | 68 |
| ethyl acetate | 141-78-6 | solvent-like | 12300 | 69 |
| ethyl butanoate | 105-54-4 | fruity | 125 | 117 |
| ethyl 2-methylpropanoate | 97-62-1 | fruity | 15 | 68 |
| ethyl 2-methylbutanoate | 7452-79-1 | fruity | 18 | 68 |
| ethyl 3-methylbutanoate | 108-64-5 | fruity, blueberry-like | 3 | 68 |
| ethyl hexanoate | 123-66-0 | fruity, pineapple-like | 14 | 68 |
| ethyl octanoate | 106-32-1 | fruity, green | 5 | 68 |
| ethyl decanoate | 110-38-3 | soapy, pear-like | 200 | 68 |
| 2-methylpropyl acetate | 110-19-0 | fruity | 1605 | 118 |
| 3-methylbutyl acetate | 123-92-2 | banana-like, fruity | 30 | 65 |
| 2-phenylethyl acetate | 103-45-7 | honey-like, floral | 250 | 65 |

[a]The odor of each odorant is taken from the Leibniz-LSB@TUM Odorant Database.[20] [b]Orthonasal odor threshold concentration. An OTC of 2-methylbutan-1-ol determined in wine or model wine was not found in the literature. [c]Reference from which the OTC value has been taken. The OTCs from the references were determined in different matrices, including water/ethanol (90 + 10, w/w),[65] 14% (v/v) ethanol solution adjusted to pH 3.5 with tartaric acid,[72] synthetic wine (11% ethanol, v/v, 7 g/L glycerol, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH),[68] synthetic wine (10% ethanol, v/v, 5 g/L tartaric acid, pH 3.2),[69] 10% water/ethanol mixture containing 5 g/L of tartaric acid at pH 3.2,[117] and 10% hydroalcoholic solution at pH 3.2.[118]



Acetaldehyde
Butane-2,3-dione
3-Hydroxybutan-2-one
2-Phenylethan-1-ol
2-Methylpropan-1-ol
2-Methylbutan-1-ol
3-Methylbutan-1-ol
3-(Methylsulfanyl)propan-1-ol
Acetic acid
2-Methylpropanoic acid
3-Methylbutanoic acid
Butanoic acid
Hexanoic acid
Octanoic acid
Decanoic acid
Ethyl acetate
Ethyl 2-methylpropanoate
Ethyl 2-methylbutanoate
Ethyl 3-methylbutanoate
Ethyl butanoate
Ethyl hexanoate
Ethyl octanoate
Ethyl decanoate
2-Methylpropyl acetate
3-Methylbutyl acetate
2-Phenylethyl acetate

Figure 11: Chemical structures of important wine odorants originating from microbial metabolism during fermentation

Important odor-active alcohols originating in fermentation include 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, 2-phenylethan-1-ol, and 3-(methylsulfanyl)propan-1-ol (Table 2 and Figure 11). These compounds are formed through the Ehrlich pathway from the respective amino acids valine, isoleucine, leucine, phenylalanine, and methionine during

AF.[119] 2-Phenylethan-1-ol is also formed from its glycoside precursor, but only in minor quantities.[57,120-122] All of these alcohols are present in wine in comparably high concentrations, i.e., the mg/L level; especially 3-methylbutan-1-ol is often the most abundant odorant. Despite their role as being odorants themselves, these alcohols are also important precursors of the corresponding highly odor-active ethyl esters. Formally, also ethanol is to be mentioned here as it is the Ehrlich product of alanine; however, it is often considered a matrix component rather than a wine odorant.

Important odor-active carboxylic acids originating in fermentation include acetic acid, butanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic acid, and decanoic acid (Table 2 and Figure 11). Among them, the vinegar-like smelling acetic acid is the most abundant volatile acid in wine.[49,50,104,123] The branched-chain carboxylic acids 2-methylpropanoic acid and 3-methylbutanoic acid are the oxidation products of the respective Ehrlich aldehydes.[114,119] Butanoic acid, hexanoic acid, octanoic acid, and decanoic acid are primarily produced in the fatty acid metabolism of yeast during AF.[124] They directly contribute to wine aroma, but also serve as precursors in the formation of ethyl esters.[111]

Esters are one of the most important odorant groups in wine, vitally contributing to the fruity and floral notes of wine aroma.[49,51,114,116,123] The majority of wine esters are produced by yeast during AF.[49,51,110] They are often divided into two subgroups, namely (1) ethyl esters of carboxylic acids and (2) acetates of higher alcohols. As detailed in Table 2 and Figure 11, important odor-active ethyl esters are ethyl butanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, and typical acetate esters in wine include 2-methylpropyl acetate, 3-methylbutyl acetate, and 2-phenylethyl acetate. Ethyl acetate is often also grouped into the acetate esters.[59] It usually shows the highest concentration among the esters, thus corresponding to the high concentrations of its precursors ethanol and acetic acid.[123] Banana-like, fruity smelling 3-methylbutyl acetate is another ester with a particularly high concentration in wine.[116] In general, the concentrations of acetate esters are typically higher than those of ethyl esters,[51] while the OTCs of the ethyl esters are substantially lower than those of the corresponding acetate esters (Table 2).

### 3.2.3 Odorants Originating from Storage and Aging

When the fermentation is completed, the wine is still not suitable for consumption. It needs storage and aging to improve the overall quality, including physical, chemical, and sensory qualities.[125,126] During this period, the wine composition continuously changes due to ongoing chemical and biochemical reactions within this complex system.[125,126] Depending on the aspired wine style, the selection of storage and aging conditions differ. Usually, there are two steps, namely an initial aging in a stainless steel tank or a wooden barrel (storage or maturation) and a final aging in the bottle.[125,127] For producing high-quality wine, aging in oak barrels is still the most popular option,[125] however, alternatives exist and include non-oak wood barrels like cherry and chestnut barrels,[128-130] the use of stainless steel tanks with oak chips,[131-133] and others.[126,134]

During aging in oak barrels, the quality of wine is modified by two main mechanisms: (1) the permeability of the wood material allows oxygen to enter into the wine, resulting in a slow oxidation of wine components;[125,126,135] (2) components of the oak barrel migrate into the wine.[125] Migration depends on oak species and origin (American or French oak),[136-139] barrel production method including cooperage, seasoning,[135,137] and toasting degree (light, medium or heavy),[135,140,141] volume of the barrel,[136] age of the barrel,[135,136] wine aging time,[135,138] etc. Important odorants originating in oak barrel aging include 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol, 2-methoxyphenol, 2-methoxy-4-(prop-2-en-1-yl)phenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and whisky lactone (Table 3, Figure 12). These compounds are mainly formed during the production of the barrels. Whisky lactone, which shows a coconut-like smell, is one of the most important odorants extracted from oak barrels, especially the *cis*-isomer.[48,104,125,135,136,142-144] It is derived from oak lipids,[139] and mainly formed from the galloylglucoside, glucoside, or rutinoside of its open-ring form 3-methyl-4-hydroxyoctanoic acid[142,144,145] via acid hydrolysis or pyrolysis. Whisky lactone has four stereoisomers. However, only two of them occur naturally in oak, namely the (4*S*,5*S*)-isomer (a *cis*-isomer) and the (4*S*,5*R*)-isomer (a *trans*-isomer).[143,146] The phenolic odorants 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol, 2-methoxyphenol, 2-methoxy-4-(prop-2-en-1-yl)phenol, and 4-hydroxy-3-methoxybenzaldehyde are derived from lignin and polyphenols during toasting.[135,139,147,148] Furanone compounds, including the caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, are formed through the thermal degradation of carbohydrates.[135,139,147]

If the wine has a pronounced horse stable-like, medicinal, or phenolic off-flavor, it may be caused by elevated concentrations of 4-ethylphenol (OTC 620 µg/L) and 4-ethylguaiacol (OTC 140 µg/L) formed in the course of a microbiological contamination with *Dekkera* (formerly *Brettanomyces*) yeast. This species is able to produce substantial amounts of 4-ethylphenol and 4-ethylguaiacol from hydroxycinnamic acids (ferulic, *p*-coumaric, caffeic, and sinapic acids).[149,150]

The second stage of wine aging happens in the bottle. Bottle aging is also influenced by many factors, such as temperature,[127,151] bottle orientation (vertical or horizontal),[127,151,152] closure,[127,152-154] humidity,[127] and aging time.[151] In contrast to the oak barrels, glass bottles are chemically inert, thus migration of vessel components into the wine is not an issue. However, minor amounts of oxygen can still reach the wine through the closure,[127] leaving the microenvironment in wine, however, basically reductive. The oxygen transfer through the closure varies considerably depending on the material the stoppers are made of.[127,155]

Important odor-active compounds originating in bottle aging of wine include strecker aldehydes such as 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and 3-(methylsulfanyl)propanal (Table 3, Figure 12). They are formed via the degradation of the respective amino acids valine, isoleucine, leucine, phenylalanine, and methionine, or via oxidation of the respective alcohols, which are generally present in a high concentrations in wine (cf. Table 2).[155,156] The concentration of the aldehydes may increase with aging time.[157] Compared to the alcohols, the aldehydes have much lower OTCs, thus even a minor change in their concentrations can have a substantial effect on the aroma of wine. Among them, phenylacetaldehyde and 3-(methylsulfanyl)propanal are the most potent odorants owing to their low OTCs[138,157-160] (Table 3). Sotolon is another frequently detected odorant related to oxidation; it is a product of the degradation of ascorbic acid and *α*-ketobutyric acid.[161]

Table 3: Odor and OTC of important wine odorants originating from oak barrel and bottle aging

| odorant | CAS no. | odor[a] | OTC[b] (µg/L) | reference[c] |
|---|---|---|---|---|
| 4-ethylphenol | 123-07-9 | phenolic | 620 | 149 |
| 4-vinylphenol | 2628-17-3 | phenolic, earthy | 770 | 162 |
| 4-ethylguaiacol | 2785-89-9 | smoky, gammon-like | 140 | 149 |
| 4-vinylguaiacol | 7786-61-0 | smoky, clove-like | 440 | 162 |
| 2-methoxyphenol | 90-05-1 | smoky, gammon-like | 9.5 | 68 |
| 2-methoxy-4-(prop-2-en-1-yl)phenol | 97-53-0 | clove-like | 6 | 68 |
| 4-hydroxy-3-methoxybenzaldehyde | 121-33-5 | vanilla-like, sweet | 60 | 163 |
| 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one | 3658-77-3 | caramel-like | 5 | 118 |
| *cis*-whisky lactone | 147254-32-8 | coconut-like | 35 | 164 |
| *trans*-whisky lactone | 39638-67-0 | coconut-like | 122 | 164 |
| 2-methylpropanal | 78-84-2 | malty | 6.0 | 157 |
| 2-methylbutanal | 96-17-3 | malty | 16 | 157 |
| 3-methylbutanal | 590-86-3 | malty | 4.6 | 157 |
| phenylacetaldehyde | 122-78-1 | floral, honey-like | 1 | 157 |
| 3-(methylsulfanyl)propanal | 3268-49-3 | cooked potato-like | 0.5 | 165 |
| sotolon | 28664-35-9 | fenugreek-like, lovage-like | 15 | 166 |
| hydrogen sulfide | 7783-06-4 | rotten egg-like | 1.1–1.6 | 167 |
| dimethyl sulfide | 75-18-3 | cooked asparagus-like | 25 | 168 |
| (furan-2-yl)methanethiol | 98-02-2 | sulfuric, cabbage-like | 0.0004 | 169 |
| phenylmethanethiol | 100-53-8 | sulfuric, burned | 0.0003 | 170 |

[a]The odor of each odorant is taken from the Leibniz-LSB@TUM Odorant Database.[20] [b]Orthonasal odor threshold concentration. [c]Reference from which the OTC value has been taken. The OTCs from the references were determined in different matrices, including red wine,[149] white wine,[162] synthetic wine (11% ethanol, v/v, 7 g/L glycerol, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH),[68] 10% water/ethanol mixture containing 5 g/L of tartaric acid at pH 3.2,[163] 10% hydroalcoholic solution at pH 3.2,[118] 14% (v/v) ethanol/water,[164] 10% water/ethanol solution containing 5 g/L tartaric acid and pH adjusted to 3.2,[157] synthetic wine (11% ethanol, v/v, 5 g/L tartaric acid, 7 g/L glycerin, pH 3.4),[165] white wine,[166] red wine and white wine,[167] white wine,[168] synthetic wine (12% ethanol, v/v, 8 g/L glycerol, and different salts),[169] and model hydroalcoholic solution with 12% (v/v) ethanol, 5 g/L tartaric acid, pH 3.5.[170]

Wine after bottle aging may have an unpleasant “reductive” aroma for which rotten egg-like smelling hydrogen sulfide ($H_2S$) is held responsible.[127,171] The production of $H_2S$ may relate to the $SO_2$ addition during winemaking and storage or to specific precursors in wine.[155] $H_2S$ can also act as an intermediate in the generation of other odorants. For example, the roasted coffee-like smelling 2-furanmethanethiol can be formed through the reaction between $H_2S$ and furan-2-carbaldehyde.[172] Furan-2-carbaldehyde originates in oak wood,[147] however, its concentration is usually below its OTC.[68] Similarly, smoky smelling phenylmethanethiol is supposed to be formed from the reaction between $H_2S$ and benzaldehyde.[170] Another crucial sulfury odorant in aged wine is dimethyl sulfide. It may be generated from the precursor dimethyl sulfoxide by reduction.[173]

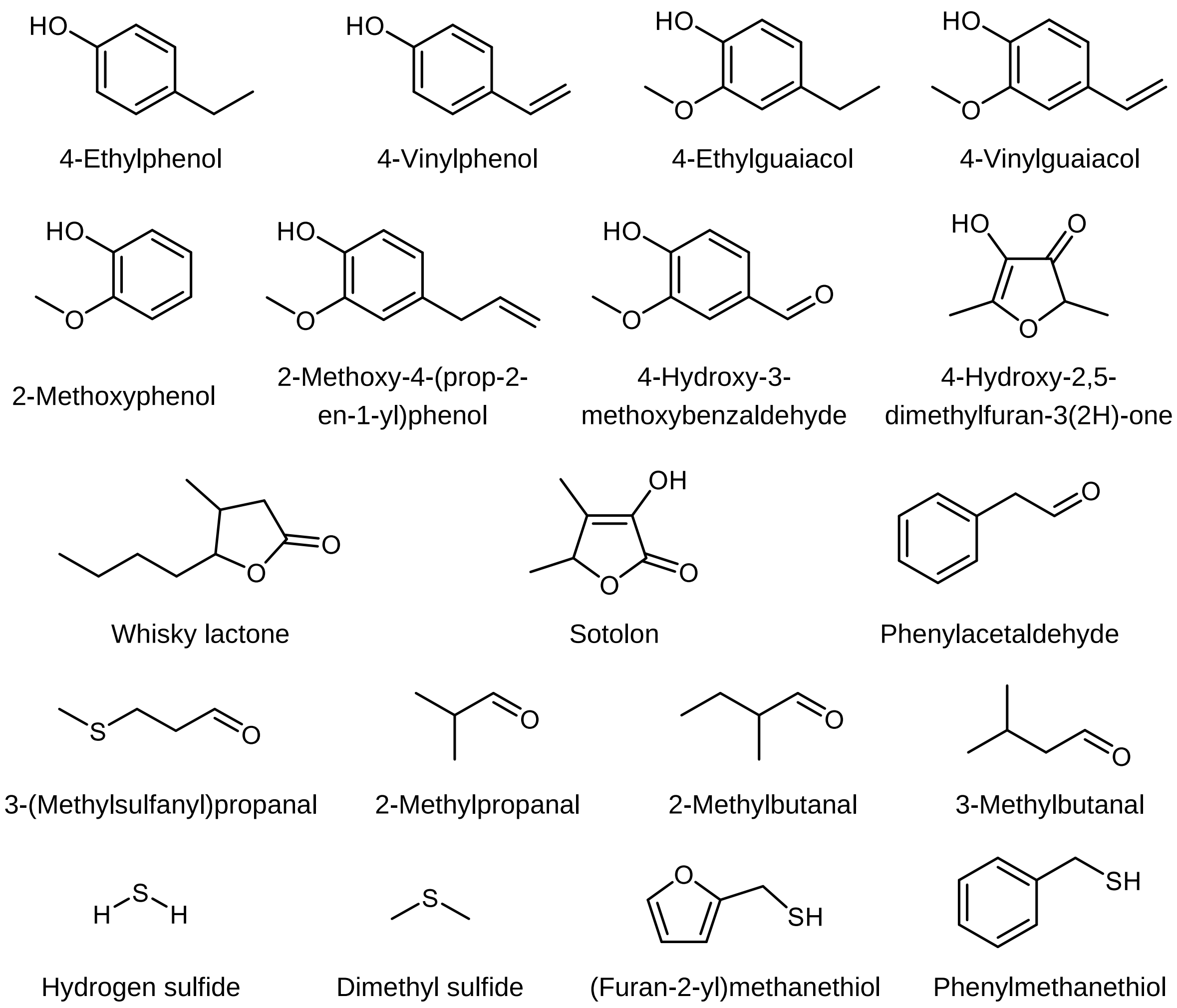


Figure 12: Chemical structures of important wine odorants originating from oak barrel and bottle aging

Although the inert bottle material does not provide a source of odorants in wine, the stopper material may do so. Wines may show the cork taint off-flavor when using contaminated stoppers. 1,3,5-Trichloro-2-methoxybenzene has been identified as the odorant responsible for this unpleasant aroma note.[174] Its formation can occur at any time during stopper processing, from the forest to shipping. Finally, the compound can migrate from natural cork stoppers into the wine during bottle aging.[175]

### 3.2.4 Molecular Background of Lychee Aroma in Wine

The descriptors used for drawing a picture of wine aroma can be roughly divided into two groups: rather general ones, like fruity, floral, and woody; and more specific terms, like rosy, apple-like, and coffee-like. The latter require that the specific aroma can be clearly recognized.

Lychee (or litchi) is a specific term used in the description of wine aroma. Until now, a lychee note has only been reported in wines made from a few grape varieties, particularly those made from Gewürztraminer grapes.[66,67,176,177] A previous study investigated the similarity of Gewürztraminer wine, fresh lychees, and canned lychees by comparative GC–O analysis.[66] Twelve odorants (*cis*-rose oxide, ethyl hexanoate/isohexanoate, *β*-damascenone, linalool, ethyl 2-methylpropanoate, geraniol, ethyl 2-methylbutanoate, 2-phenylethan-1-ol, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 4-hydroxy-3-methoxybenzaldehyde, citronellol, and

2-phenylethyl acetate) were common to all the three samples. The authors concluded that these twelve odorants contributed to the lychee note of the Gewürztraminer wine. Quantitation and OAV calculation revealed that the concentrations of *cis*-rose oxide, *β*-damascenone, ethyl 2-methylpropanoate, linalool, and geraniol were beyond their OTCs in the three samples. Thus, among the twelve odorants, these five compounds were considered to be particularly important for the lychee note.[66] In any case, it seemed obvious that the lychee note in Gewürztraminer wines is not due to a single compound but generated by the combination of odorants. Four of the five odorants typically originate from grapes, as detailed before. Among them, *cis*-rose oxide showed the highest OAV. Another study investigated the impact of individual odorants in Gewürztraminer wine by omission experiments. Data revealed that the absence of *cis*-rose oxide most significantly decreased the similarity between the odor reconstitution model and the original Gewürztraminer wine.[65] Thus, its importance to Gewürztraminer wine was evident. In fact, *cis*-rose oxide is commonly detected in various lychee cultivars;[178] even though the concentration is not high, its OAV is consistently among the highest, not only in fresh lychee juice,[179-182] but also in heat-sterilized lychee juice[182] and modified lychee juice (with adjustment of sugar, acid, pH, amino acid or $SO_2$ levels) for fermentation,[183-188] indicating its importance to lychee flavor.

Actually, *cis*-rose oxide appeared to be the only odorant commonly associated with a lychee aroma note in wine.[59,60,92,93,189] At harvest, its concentration in Gewürztraminer grape berries is already beyond the OTC,[190] however, it also depends on the harvest time. A study found that its concentration in the final wine significantly increased from normal to late harvest and furthermore from late to ice harvest.[189]

The metabolic pathway leading to *cis*-rose oxide in grape berries was investigated by in vivo-feeding experiments. Results showed that deuterated geraniol spiked into the mesocarp of ripening grape berries of *Vitis vinifera* L. Scheurebe generated deuterated *cis*- and *trans*-rose oxides via a series of transformations. The stereoselective reduction of geraniol produced (*S*)-citronellol, the key intermediate product, and further cyclization of (*S*)-citronellol finally generated the rose oxides.[55,191]

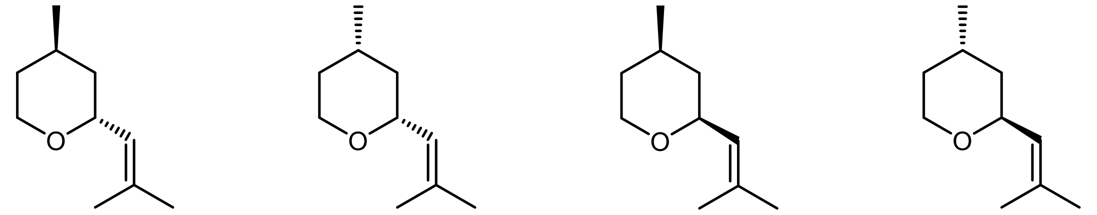
The rose oxide structure includes two stereocenters resulting in four stereoisomers, among which the (2*R*,4*S*)- and (2*S*,4*R*)-isomers are the *cis*-rose oxides and the (2*S*,4*S*)- and (2*R*,4*R*)-isomers are the *trans*-rose oxides (Table 4, Figure 13). *Cis*-rose oxides have been more frequently mentioned as grape and wine constituents than the *trans*-isomers, especially in Muscat and aromatic grape varieties and wines made thereof.[60,70,192-194] Currently, OTCs of *cis*-rose oxide of unknown enantiomeric composition are available and amount to 0.1 µg/L in water[66] and 0.2 µg/L in wine matrix.[65] However, individual OTCs of the four stereoisomers in grape or wine matrix have not been published. Nevertheless, information in the literature indicates that the odor potencies of the stereoisomers substantially differ. The odor potency of the (2*S*,4*R*)-isomer is reported to be the highest, followed by that of the (2*R*,4*S*)-isomer, whereas the two *trans*-rose oxides are less potent.[195] In line with this, a recent publication reported OTCs in hydroalcoholic solution of 0.5 µg/L for the most potent (2*S*,4*R*)-isomer, 50 µg/L for the second most potent, but already 100 times less potent (2*R*,4*S*)-isomer, and OTCs of 80 µg/L and 160 µg/L for the least potent (2*S*,4*S*)- and (2*R*,4*R*)-isomers, respectively.[196] The odor descriptions of the four stereoisomers, however, are very similar. All of them were assigned green, geranium-type odors by Wüst and Mosandl.[195] In another study, the odor of the (2*S*,4*R*)-isomer was described as floral and green, the odor of the (2*R*,4*S*)-isomer as herbal, green, and floral, the odor of the (2*S*,4*S*)-isomer as herbal, green, and floral, and the odor of the (2*R*,4*R*)-isomer as floral and green.[196]

Table 4: Odors and OTCs of rose oxide stereoisomers

| stereoisomer | CAS no. | odor | OTC[a] (µg/L) | reference[b] |
|---|---|---|---|---|
| 2*R*,4*R* | 876-18-6 | floral, green | 160 | 196 |
| 2*R*,4*S* | 4610-11-1 | herbal, green, floral | 50 | 196 |
| 2*S*,4*R* | 876-17-5 | floral, green | 0.5 | 196 |
| 2*S*,4*S* | 5258-10-6 | herbal, green, floral | 80 | 196 |

[a]Orthonasal odor threshold concentration. [b]The OTCs from the reference were determined in a hydroalcoholic solution (10 µL of ethanol and 20 mL water).[196]

Given the differences in the OTCs, the enantiomeric distribution of the *cis*-rose oxides is vitally important to assess their odor impact, e.g., in wine. Helmut Guth reported an enantiomeric distribution of 70% (2*S*,4*R*)-isomer and 30% (2*R*,4*S*)-isomer in Gewürztraminer wine.[197] Further studies found different ratios among wines with percentages of the (2*S*,4*R*)-isomer of 58–76% in Gewürztraminer wine, 43–70% in Muscat wines, and 38–41% in Silvaner wines.[70] It is, however, yet unknown, how the different enantiomeric ratios of *cis*-rose oxide impact the typical lychee note in wine.



(2*R*,4*R*)-Rose oxide (2*R*,4*S*)-Rose oxide (2*S*,4*R*)-Rose oxide (2*S*,4*S*)-Rose oxide

Figure 13: Chemical structures of rose oxide stereoisomers

A booklet published by the State Institute of Viticulture Freiburg, Germany, assigns a lychee aroma also to wine made from *Vitis vinifera* L. ‘Muscaris’ grapes. Muscaris is a relatively new fungus-resistant grape variety with an intensive aroma and good resistance to downy mildew, powdery mildew, and botrytis. It was bred at the State Institute of Viticulture Freiburg in 1987 from the mother variety Solaris and the father variety Gelber Muskateller, also known as Muscat à petits grains blancs.[198,199] The breeding aim was to combine the advantages of both grape varieties, i.e., the disease tolerance and environmental adaptability of Solaris and the intense aroma of Gelber Muskateller. Muscaris is early-ripening with green berries even at high must weights. It is particularly suitable for producing dessert wine due to its high sugar level, but it can also be used for dry wine as long as the grape berries are harvested at a certain controlled sugar level.[199] As a promising variety, the cultivation area of the Muscaris grape increased in recent years, reaching 117 ha in Germany in 2022.[200]

To date, there are only a few studies on the aroma of this new grape variety. In wine from Muscaris grapes numerous volatiles have been reported, including four of the five important lychee-related odorants, i.e., *cis*-rose oxide, *β*-damascenone, ethyl 2-methylpropanoate, and linalool. The exception is geraniol. Its concentration might have been too low to be detected in the studied Muscaris wine.[201,202] Nevertheless, there is no information available about the crucial aroma components of Muscaris grape berries. Only some phenolic volatiles, including 4-vinylphenol, 4-vinylguaiacol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, and 4-hydroxy-3-ethoxybenzaldehyde, have been identified in Muscaris grape; their aroma impact, however, is unclear.[203]

### 3.2.5 Comparison of Odorants between Wine and Beer

In addition to wine, beer is another popular alcoholic drink worldwide. Between 2000 and 2022, its average annual production volume was 1.78 billion hectoliters, with 1.89 billion hectoliters in 2022 (Figure 14).[204] Thus, the production volume of beer is ~7 times higher than that of wine. Beer and wine are both fermentative products. However, the basic raw materials used are different, i.e., malt, hop, and water for beer, and grape for wine. Moreover, the whole production chains in beer and wine making are substantially different. In addition, beer usually requires less maturation time. Consequently, it is not surprising that the aroma of beer is different from the aroma of wine.

Comparable to wine, the raw material has a major impact on the aroma of the final product. Next to the raw material, i.e., malt[205] and hops,[206] also brewing parameters such as top fermentation or bottom fermentation,[207] and hopping time[208] lead to beers with different aroma styles. Moreover, odorant concentrations also differ between fresh and aged beer.[209] In summary, beer odorants mainly originate from the malt, the hops, the microbial metabolism during fermentation, and the aging processes.

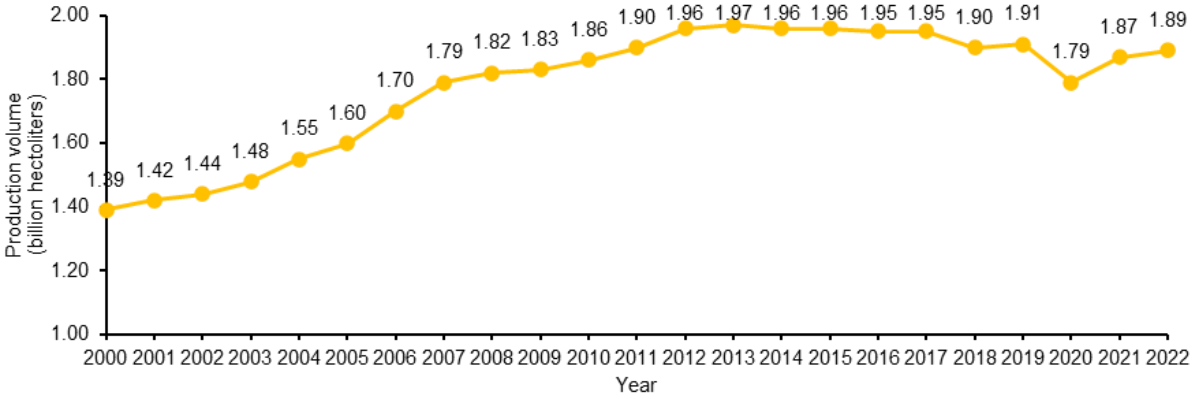


Figure 14: The world production volume of beer from 2000 to 2022[204](citation)

Even though there is a large number of publications on beer aroma, the malt-derived odorants in beer are barely clarified.[36] However, it has been shown that different malts lead to different concentrations of some odorants in beer. For example, the concentration of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one in dark larger beer produced with roasted malt was much higher than in pale lager beer; the result suggested that part of this odorant was directly transferred from the malt used.[205] Phenolic compounds 4-vinylphenol[210] and 4-vinylguaiacol,[205,210] were identified as odorants responsible for the characteristic clove-like aroma[211] of beer made with Bavarian wheat malt (≥50%). Moreover, 4-vinylguaiacol was also proposed to contribute to the clove-like aroma of Tsingtao wheat beers.[212] 4-Ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 4-vinylguaiacol, and 4-hydroxy-3-methoxybenzaldehyde were found to play an important role in the aroma of fresh Belgian specialty beers produced with different roasted malts.[213] Phenolic odorants can also influence wine aroma if wood materials are involved in aging, while their concentrations originating from grapes are small.

Beer odorants derived from hops include terpenoids, thiols, and esters. The most representative odorants are the monoterpene alcohols linalool[206,208,214-217] and geraniol,[206,208,215-217] and the thiol 4-MSP.[216,218] Other terpenes and terpenoids like myrcene,[208,217] nerol, geranyl acetate, and *α*-terpineol,[215,216,219] and esters of branched-chain carboxylic acids like methyl 2-methylbutanoate, propyl 2-methylbutanoate,[208] ethyl 3-methylbutanoate,[206] ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate[206,208] were also identified as odorants in some beers. In wine, odorant terpenoids and thiols also originate from the raw material, the grape berries, while the ethyl esters are produced mainly during fermentation; nevertheless, methyl and propyl esters known in beer are scarcely reported as wine odorants.

Beer odorants originating from yeast metabolism during fermentation are similar to those produced during wine fermentation. They also contribute most to the total number of odorants in beer. Crucial beer odorants formed in the fermentation step include acetic acid, phenylacetic acid, 3-methylbutan-1-ol, 2-phenylethan-1-ol, 3-(methylsulfanyl)propan-1-ol, ethyl hexanoate, ethyl 2-methylbutanoate,[36,220] ethyl octanoate, ethyl butanoate, and acetaldehyde.[221]

Similar to wine, in beer strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and 3-(methylsulfanyl)propanal) constitute an important group of odorants related to aging. They can contribute to the stale character of beer.[222-224] During storage or on the shelf, beer may generate a skunky aroma, predominantly caused by the light-induced formation of 3-methylbut-2-ene-1-thiol.[222,225] It is noteworthy that this compound is not a common odorant in wine.[226]

Given that in both beer and wine the majority of important odorants are formed during fermentation from microbial metabolism, the question arises, which compounds actually account for the aroma difference between beer and wine. The fact that substantial aroma differences are also present within beers of different styles and within wines of different styles, makes this question even more difficult to answer. To simplify the task, an option could be to only consider odorants that are commonly found in either beer or wine. Odorants typically related to highly specific raw materials or very special processing methods could be excluded. For example, the petrol-like smelling TDN is a grape-derived odorant in wine[71,80] made with Riesling grapes, but generally absent in beer. A petrol-like note would thus push the aroma recognition to wine-like, despite the fact that this note is also absent from all non-Riesling wines. Likewise, the coconut-like smelling *cis*-whisky lactone is a characteristic odorant in wine aged in the oak barrel,[163,227] but also absent from beer and all wines not aged in oak barrels.

In 2010, Vicente Ferreira characterized 27 wine volatiles present in odor-active amounts in more or less all different kinds of wine as “the base of wine aroma”. All of them are products of the fermentation and were subdivided into fusel alcohols (2-methylpropan-1-ol, 3-methylbutan-1-ol, hexan-1-ol, 2-phenylethan-1-ol, 3-(methylsulfanyl)propan-1-ol), straight-chain acids (acetic acid, butanoic acid, hexanoic acid, octanoic acid, decanoic acid), isoacids (2-methylpropanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid), straight-chain acid ethyl esters (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate), fusel alcohol acetates (2-methylpropyl acetate, 3-methylbutyl acetate, 2-phenylethyl acetate), isoacid ethyl esters (ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate), and miscellaneous compounds (ethanol, butane-2,3-dione, acetaldehyde).[228] Later, “aroma base” was coined for these group of compounds.[229-233] By spiking the aroma base with additional odorants, any type of specialty wine aroma can be created. For example, to create a red wine model with a woody aroma, whisky lactone, 2-methoxyphenol, 2-methoxy-4-(prop-2-en-1-yl)phenol, 4-hydroxy-3-methoxybenzaldehyde, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one need to be added.[229,231,232]

The sensory impression of the wine aroma base created with 27 crucial odorants was described as “vinous,” “slightly sweet, pungent, alcoholic and a little bit fruity.”[228] Unfortunately, the concentrations of the individual compounds were not provided, making it impossible to double-check the aroma profile of the wine aroma base. Moreover, as far as we know, there is currently no report on a comparable set of odorants that would define the aroma base of beer.

# 4 Objectives

A pleasant aroma is a major quality attribute of wines. A lychee aroma note in wine is particularly appreciated, but limited to a few grape varieties. Among these is the relatively new and promising fungus-resistant Muscaris variety bred from Solaris as the mother and Gelber Muskateller as the father. Given the lack of information in the literature, the first part of the study aimed to 1) investigate the major odorants in Muscaris grapes, 2) compare them with the odorants in Muskateller grapes, and 3) decipher the molecular background of the lychee note in the aroma of the Muscaris grapes.

The second part of the study was focused on the aroma difference between beer and wine. In wine, a certain set of odorants has been suggested to form what was called the aroma base. However, no concentration data on individual compounds were available to allow sensory verification and the concept had not been applied to beer at all. Thus, the aims of this part were to 1) perform a literature-based meta-analysis on the occurrence and concentrations of odorants in both beer and wine, 2) establish beer and wine aroma base models based on the literature data that reflect their basic olfactory difference, and 3) identify the odorant group(s) responsible for the aroma differences between the beer and wine aroma base models through sensory analyses using concentration leveling tests.

# 5 Results and Discussion

This thesis is a publication-based dissertation. Data were summarized in two articles published in an international scientific peer-reviewed journal. For each article, a copy of the original publication, a summary including the individual contributions of the authors, and the reprint permission of the publisher are included in the appendix.

## 5.1 Characterization of Odorants Responsible for the Lychee Aroma of *Vitis vinifera* L. ‘Muscaris’

### 5.1.1 Odorant Screening

Odorant screening was in parallel applied to Muscaris grapes and grapes of its father variety, Muskateller. Fresh, ripe, and uninjured grape berries were crushed by hand using a spoon and a kitchen sieve without breaking the seeds. The volatiles were isolated by extraction with dichloromethane followed by aSAFE[26] at 40 °C and concentrated to a final volume of 1 mL using a Vigreux column and a Bemelmans microdistillation device.[30] The concentrates were subjected to GC–O analysis in combination with cAEDA.

The screening results showed 39 odor-active compounds in Muscaris grapes, 35 of which were also present in Muskateller grapes. The FD factors of the 39 Muscaris grape odorants ranged from 1 to 32768 and the FD factors of the 35 Muskateller grape odorants ranged from 1 to 8192 (Table 5). Preliminary structure assignments were achieved by comparing the RIs of the grape odorants on two GC columns with different polarity (DB-FFAP and DB-5) together with the associated odor descriptions to data compiled in the Leibniz-LSB@TUM Odorant Database.[20] The structure proposals were confirmed by GC–O and GC–MS analysis of authentic reference compounds. To minimize coelution problems, a comprehensive two-dimensional GC×GC–MS instrument was employed. For enantiomeric assignments of chiral odorants, two chiral GC columns (BGB-174E and BGB-176) with differently substituted *β*-cyclodextrins were used. To clarify the elution order, an enantiomeric mixture and at least one individual enantiomer were analyzed under equal conditions.

Ultimately, the structures of 38 odorants were successfully assigned using this approach, only compound **33** remained unknown. Among the odorants identified in Muscaris grapes, only vanilla-like smelling 4-hydroxy-3-methoxybenzaldehyde (vanillin; **39**) had previously been reported;[203] all other compounds were reported for the first time in this grape variety.

Table 5: Odorants in the volatile isolates obtained from Muscaris and Muskateller grapes

| no. | odorant[a] | odor[b] | RI[c] | | FD factor[d] | |
|---|---|---|---|---|---|---|
| | | | FFAP | DB-5 | Muscaris | Muskateller |
| **1** | butane-2,3-dione | butter | 969 | 591 | 8 | 16 |
| **2** | hexanal | green, grassy | 1079 | 800 | 16 | 64 |
| **3** | (3*E*)-hex-3-enal | green, grassy | 1134 | 800 | 4 | 4 |
| **4** | (3*Z*)-hex-3-enal | grassy, green | 1139 | 802 | 128 | 256 |
| **5** | heptanal | fatty, green | 1180 | 900 | 32 | 16 |
| **6** | 2-/3-methylbutan-1-ol[g] | malty | 1200 | 735/738 | 1 | 2 |
| **7** | (2*E*)-hex-2-enal | green apple | 1214 | 848 | 4 | 16 |
| **8** | oct-1-en-3-one | mushroom | 1295 | 981 | 16 | 32 |
| **9** | 2-acetyl-1-pyrroline[e] | popcorn, roasted | 1331 | 918 | 32 | 16 |
| **10** | (2*S*,4*R*)-rose oxide[f] | floral, lychee | 1343 | 1111 | 64 | 64 |
| **11** | 2,4,5-trimethyl-1,3-thiazole[e] | roasted, earthy | 1371 | 1000 | 64 | 64 |
| **12** | acetic acid | vinegar | 1447 | 613 | 8 | 2 |
| **13** | 3-(methylsulfanyl)propanal[e] | cooked potato | 1453 | 904 | 512 | 2048 |
| **14** | (3*R*)-citronellal[f] | citrusy, soapy | 1469 | 1151 | 16 | 16 |
| **15** | decanal | citrusy, soapy | 1492 | 1210 | 8 | 16 |
| **16** | 2-methoxy-3-(2-methylpropyl)pyrazine[e] | bell pepper | 1517 | 1180 | 8 | <1 |
| **17** | (2*E*)-non-2-enal | fatty | 1528 | 1159 | 32 | 8 |
| **18** | (3*R*)-/(3*S*)-linalool[f,g] | citrusy, floral | 1542 | 1100 | 64 | 512 |
| **19** | (2*E*,6*Z*)-nona-2,6-dienal | cucumber | 1579 | 1152 | 32 | 32 |
| **20** | undecanal | citrusy, soapy | 1598 | 1308 | 4 | 2 |
| **21** | phenylacetaldehyde | floral, honey | 1640 | 1038 | 128 | 256 |
| **22** | 2-/3-methylbutanoic acid[g] | sweaty | 1662 | 855/866 | 8 | 4 |
| **23** | 3-methylnonane-2,4-dione[e] | hay, anise, fishy | 1700 | 1243 | 32 | 16 |
| **24** | (3*S*)-*β*-citronellol[f] | soapy, rose | 1760 | 1233 | 64 | 8 |
| **25** | (*E*)-*β*-damascenone[e] | cooked apple | 1813 | 1390 | 16 | 32 |
| **26** | geraniol | floral, rose | 1844 | 1262 | 32768 | 8192 |
| **27** | 2-methoxyphenol[e] | smoky | 1860 | 1088 | 8 | 8 |
| **28** | 2-phenylethan-1-ol | honey, floral | 1908 | 1117 | 4 | 2 |
| **29** | *β*-ionone | floral, violet | 1932 | 1488 | 8 | 16 |
| **30** | *trans*-4,5-epoxy-(2*E*)-dec-2-enal[e] | metallic | 2006 | 1380 | 32 | 16 |
| **31** | 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one[e] | caramel | 2035 | 1072 | 2 | 1 |
| **32** | octanoic acid | sour, musty | 2058 | 1185 | 4 | 4 |
| **33** | unknown | fresh, herb, licorice | 2139 | 1368 | 4 | 1 |
| **34** | 2-methoxy-4-(prop-2-en-1-yl)phenol | clove | 2162 | 1358 | 128 | <1 |
| **35** | 4-ethylphenol | phenolic | 2175 | 1169 | 4 | <1 |
| **36** | sotolon[e] | fenugreek | 2198 | 1110 | 32 | 16 |
| **37** | undecanoic acid | soapy, oily | 2371 | 1471 | 16 | 4 |
| **38** | phenylacetic acid[e] | floral, honey | 2567 | 1261 | 1 | <1 |
| **39** | 4-hydroxy-3-methoxybenzaldehyde | vanilla | 2576 | 1400 | 4 | 4 |

[a] Odorants showing an FD factor of ≥1 in either of the two samples; odorants were identified by comparing the retention indices on two columns of different polarity (DB-FFAP, DB-5), the mass spectra obtained by GC–MS as well as the odor quality as perceived at the sniffing port during GC–O to data obtained from authentic reference compounds analyzed under equal conditions. [b] Odor as perceived at the sniffing port during GC–O. [c] Retention index; calculated from the retention time of the odorant and the retention times of adjacent *n*-alkanes by linear interpolation. [d] Flavor dilution factor; dilution factor of the highest diluted grape volatile isolate in which the odorant was detected during GC–O by any of three assessors. [e] An unequivocal mass spectrum of the compound could not be obtained; identification was based on the remaining criteria detailed in footnote a and by spiking experiments using GC–O/FID. [f] Odor-active enantiomers as identified by analysis of the volatile isolates using GC–O/FID in combination with a chiral column. [g] The compounds were not separated on the column used for AEDA; the FD factor refers to the mixture.

Among the odorants identified in Muscaris grapes, floral, rose-like smelling geraniol (**26**) showed the highest FD factor of 32768. With 8192, geraniol also showed the highest FD factor among the Muskateller grape odorants. Nineteen odorants were detected with FD factors ≥32 in at least one of the two samples. Twelve of them showed rather similar FD factors in both grape varieties, including green, grassy smelling (3*Z*)-hex-3-enal (**4**; FD factors 128 and 256), floral, honey-like smelling phenylacetaldehyde (**21**; FD factors 128 and 256), floral, lychee-like smelling (2*S*,4*R*)-rose oxide (**10**; FD factors 64 and 64), roasted, earthy smelling 2,4,5-trimethyl-1,3-thiazole (**11**; FD factors 64 and 64), cucumber-like smelling (2*E*,6*Z*)-nona-2,6-dienal (**19**; FD factors 32 and 32), fatty, green smelling heptanal (**5**; FD factors 32 and 16), popcorn-like, roasted smelling 2-acetyl-1-pyrroline (**9**; FD factors 32 and 16), hay-, anise-like, and fishy smelling 3-methylnonane-2,4-dione (**23**; FD factors 32 and 16), metallic smelling *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**30**; FD factors 32 and 16), fenugreek-like smelling sotolon (**36**; FD factors 32 and 16), mushroom-like smelling oct-1-en-3-one (**8**; FD factors 16 and 32), and cooked apple-like smelling (*E*)-*β*-damascenone (**25**; FD factors 16 and 32). Four of the odorants with FD factors ≥32 in at least one of the two samples had higher FD factors in Muscaris grapes, namely geraniol (**26**; floral, rose-like; FD factors 32768 and 8192), 2-methoxy-4-(prop-2-en-1-yl)phenol (**34**; clove-like; FD factors 128 and <1), (3*S*)-*β*-citronellol (**24**; soapy, rose-like; FD factors 64 and 8), and (2*E*)-non-2-enal (**17**; fatty; FD factors 32 and 8). The remaining three odorants of the nineteen odor-active compounds with FD factors ≥32 in at least one of the two samples showed higher FD factors in Muskateller grapes. These compounds were cooked potato-like smelling 3-(methylsulfanyl)propanal (**13**; FD factors 512 and 2048), citrusy, floral smelling (3*R*)-linalool and (3*S*)-linalool (**18**; FD factors 64 and 512) and green, grassy smelling hexanal (**2**; FD factors 16 and 64). In summary, the odorant compositions and their assigned FD factors were quite similar in both grape varieties, reflecting the close genetic relation of Muscaris and Muskateller grapes. Among these odorants, the monoterpenoids *cis*-rose oxide, linalool, and geraniol are considered highly characteristic for Muskateller grapes. Their biosynthetic formation pathways in grapes have previously been published.[55]

### 5.1.2 Quantitation and OAV Calculation

The odorants selected for quantitation included those with FD factors ≥32 in at least one of the two grape samples (c.f. Table 5). Odorants with green and grassy notes were additionally included even if their FD factors were lower. In total, twenty odorants were quantitated in both grape varieties by GC–MS using stable isotopically substituted analogues of the target odorants as internal standards. Isotopologues were not available for (3*E*)-hex-3-enal (**3**) and 2-methoxy-4-(prop-2-en-1-yl)phenol (**34**), thus, the deuterated isotopologues of (3*Z*)-hex-3-enal (**4**) and 2-methoxy-4-[(1*E*)-prop-1-en-1-yl]phenol were used as internal standards. Since the screening and structure assignment steps required a considerable amount of time, fresh grapes were not available anymore when carrying out the quantitations. Instead, grape berries of the same batch which had been shock-frozen with liquid nitrogen and stored in vacuum-sealed bags at −20 °C were used for most of the odorant quantitations. However, two crucial odorants originating from the lipoxygenase pathway,[234] namely (3*Z*)-hex-3-enal (**4**) and (3*E*)-hex-3-enal (**3**), were quantitated beforehand in the fresh grape berries, because it had been reported that their concentrations can substantially differ between fresh and frozen-thawed plant material.[235,236]

The workup for quantitation was roughly the same as detailed for odorant screening. However, the stable isotopically substituted internal standards were added to the dichloromethane used for extraction. Depending on the expected concentrations of the target odorants, the amount of grape berries, the amount of the stable isotopically substituted odorants added, and the final concentrate volume differed between workups. Finally, the concentrates were analyzed using either a one-dimensional GC–MS system, a two-dimensional heart-cut GC–GC–HRMS system, or a comprehensive two-dimensional GC×GC–MS system.

The individual enantiomer concentrations of the crucial chiral odorants were obtained from the sum of enantiomers as determined by achiral GC–MS via stable isotopically substituted internal standards in the quantitation assays and the enantiomeric distribution as determined by chiral GC–MS. The separation of the enantiomers of *cis*-rose oxide was achieved on the BGB-176 column, i.e., 30% 2,3-dimethyl-6-tert-butyldimethylsilyl-*β*-cyclodextrin dissolved in 15% phenyl-, 85% methylpolysiloxane, while the separation of the enantiomers of linalool and *β*-citronellol was realized on the BGB-174E column, i.e., 30% 2,3-diacetyl-6-tert-butyldimethylsilyl-*β*-cyclodextrin dissolved in 6% cyanopropylphenyl-, 94% methylpolysiloxane. The relevant chromatograms are depicted in the Supporting Information file of Publication 1.[37] The enantiomeric distributions of *cis*-rose oxide, linalool, and *β*-citronellol are shown in Table 6. With 71% and 75% in Muscaris and Muskateller grapes, respectively, the odor-active (2*S*,4*R*)-isomer was the major enantiomer of *cis*-rose oxide. This was in line with previous studies which reported (2*S*,4*R*)-rose oxide as the predominant enantiomer in six Muscat grape varieties and percentages of 88–97%.[192] In linalool and *β*-citronellol, the (*S*)-isomers dominated with more than 90%. (3*S*)-Linalool was previously reported as the major enantiomer in two other Muscat grape samples with 94% and 99.8%, respectively, the percentages reported were similar to our findings.[237] As far as we know, there is currently no report on the enantiomeric distribution of *β*-citronellol in Muscat grapes. In total, the results showed comparable enantiomeric distributions of *cis*-rose oxide, linalool, and *β*-citronellol in Muscaris and Muskateller grapes, once more reflecting their close genetic relation.

Table 6: Enantiomeric distribution of important chiral odorants in Muscaris and Muskateller grapes

| odorant | enantiomer | odor | enantiomeric distribution[a] (%) in | |
|---|---|---|---|---|
| | | | Muscaris | Muskateller |
| *cis*-rose oxide | 2*R*,4*S* | floral, lychee (weak) | 29 | 25 |
| | 2*S*,4*R* | floral, lychee (strong) | 71 | 75 |
| linalool | 3*R* | citrusy, floral | 9 | 2 |
| | 3*S* | citrusy, floral | 91 | 98 |
| *β*-citronellol | 3*R* | soapy, rose | 2 | 3 |
| | 3*S* | soapy, rose | 98 | 97 |

[a]Mean of triplicates.

The quantitation results of the 20 odorants (Table 7) showed a wide range of concentrations covering over six orders of magnitude. The lowest concentrations were below 0.01 μg/kg and were obtained for 2-acetyl-1-pyrroline (**9**) in both grapes, while the highest concentrations were 1160 μg/kg for geraniol (**26**) in Muscaris grapes and 700 μg/kg for (2*E*)-hex-2-enal (**7**) in Muskateller grapes, respectively. Further odorants with high concentrations (>100 μg/kg) included (2*E*)-hex-2-enal (**7**; 325 μg/kg), hexanal (**2**; 294 μg/kg), and (3*S*)-linalool (**18b**; 113 μg/kg) in Muscaris grapes, and hexanal (**2**; 548 μg/kg), (3*S*)-linalool (**18b**; 518 μg/kg), and geraniol (**26**; 442 µg/kg) in Muskateller grapes. In general, the concentrations of most odorants differed only slightly between both grape varieties.

Table 7: Concentrations and OAVs of important odorants in Muscaris and Muskateller grapes

| no.[a] | odorant[b] | concentration[c] (µg/kg) | | OTC[d] (µg/kg) | OAV[e] | |
|---|---|---|---|---|---|---|
| | | Muscaris | Muskateller | | Muscaris | Muskateller |
| **26** | geraniol | 1160 | 442 | 1.1[f] | 1100 | 400 |
| **19** | (2*E*,6*Z*)-nona-2,6-dienal | 1.35 | 0.464 | 0.0045[f] | 300 | 100 |
| **4** | (3*Z*)-hex-3-enal | 21.8 | 26.6 | 0.12[f] | 180 | 220 |
| **18a** | (3*R*)-linalool[g] | 11.2 | 10.3 | 0.087[f] | 130 | 120 |
| **2** | hexanal | 294 | 548 | 2.4[f] | 120 | 230 |
| **3** | (3*E*)-hex-3-enal | 13.7 | 22.0 | 0.23[h] | 59 | 95 |
| **18b** | (3*S*)-linalool[g] | 113 | 518 | 2.7[f] | 42 | 190 |
| **23** | 3-methylnonane-2,4-dione | 1.23 | 0.724 | 0.046[f] | 27 | 16 |
| **8** | oct-1-en-3-one | 0.413 | 0.293 | 0.016[f] | 26 | 18 |
| **10** | (2*S*,4*R*)-rose oxide[g] | 0.873 | 1.19 | 0.045[h] | 19 | 26 |
| **24** | (3*S*)-*β*-citronellol[g] | 56.0 | 28.7 | 4.9[i] | 11 | 5.9 |
| **17** | (2*E*)-non-2-enal | 2.00 | 1.75 | 0.19[f] | 11 | 9.2 |
| **21** | phenylacetaldehyde | 53.2 | 38.0 | 5.2[f] | 10 | 7.3 |
| **13** | 3-(methylsulfanyl)propanal | 3.66 | 4.55 | 0.43[f] | 8.5 | 11 |
| **7** | (2*E*)-hex-2-enal | 325 | 700 | 110[f] | 3.0 | 6.4 |
| **25** | (*E*)-*β*-damascenone | 0.0106 | 0.0157 | 0.0060[f] | 1.8 | 2.6 |
| **34** | 2-methoxy-4-(prop-2-en-1-yl)phenol | 0.886 | 0.116 | 1.8[f] | <1 | <1 |
| **5** | heptanal | 1.86 | 1.51 | 6.1[h] | <1 | <1 |
| **36** | sotolon | 0.0563 | 0.0728 | 1.7[f] | <1 | <1 |
| **9** | 2-acetyl-1-pyrroline | <0.01 | <0.01 | 0.053[f] | <1 | <1 |

[a]Numbering according to Table 5. [b]Odorants in order of decreasing OAVs in Muscaris grapes. [c]Mean of duplicates or triplicates; individual values and standard deviations are available in the Supporting Information file of Publication 1.[37] [d]Orthonasal odor threshold concentration in water. [e]Odor activity value; calculated as ratio of concentration to odor threshold concentration. [f]Data taken from the Leibniz-LSB@TUM Odorant Database.[20] [g]Concentrations of individual enantiomers were calculated from the concentration of the sum of enantiomers as obtained in the quantitation assays and the enantiomeric distribution depicted in Table 6. [h]Data obtained in the current study.[42] [i]Data from literature.[238]

To assess the odor contribution potential of each odorant, its OAV was calculated as the ratio of the concentration determined in the grapes and the OTC determined in water. The results (Table 7) showed OAVs ≥1 in both grape varieties for 16 of the 20 quantitated odorants. The highest OAV was determined for geraniol (**26**) in both Muscaris (OAV 1100) and Muskateller (OAV 400) grapes. Geraniol has not been reported in Muscaris grapes; however, a huge dataset of geraniol concentrations in Muskateller grapes was available, most of them being in the range of ~70 µg/kg[239] to ~300 µg/kg,[240] corresponding to OAVs of ~60 to ~300.

Four odorants showed OAVs ≥100 in both grapes, namely (2*E*,6*Z*)-nona-2,6-dienal (**19**; OAVs 300 and 100), (3*Z*)-hex-3-enal (**4**; OAVs 180 and 220), (3*R*)-linalool (**18a**; OAVs 130 and 120), and hexanal (**2**; OAVs 120 and 230). These odorants have also not been reported in Muscaris grapes; however, linalool was frequently reported in Muskateller grapes, with concentrations mainly in the range of ~300 µg/kg[190] to ~600 µg/kg.[241] Nevertheless, the lack of enantiospecific concentration data in combination with the huge difference in the OTCs of (*R*)- and (*S*)-linalool (cf. Table 7) made it impossible to translate the concentrations into OAVs.

Five further odorants showed OAVs in the range of <100, but >10 in both grape varieties, namely (3*E*)-hex-3-enal (**3**; OAVs 59 and 95), (3*S*)-linalool (**18b**; OAVs 42 and 190), 3-methylnonane-2,4-dione (**23**; OAVs 27 and 16), oct-1-en-3-one (**8**; OAVs 26 and 18), and

(2*S*,4*R*)-rose oxide (**10**; OAV 19 and 26). There were no data available for comparison in Muscaris grapes; however, data were particularly available for *cis*-rose oxide in Muskateller grapes. The concentrations of this compound in Muskateller grapes primarily ranged between ~0.6 µg/kg[190] and ~4 µg/kg.[242] With the enantiomeric distribution of 25% (2*R*,4*S*)- and 75% (2*S*,4*R*)-isomer determined by us (cf. Table 6), this would correspond to OAVs of the odor-active (2*S*,4*R*)-isomer in the range of ~10 to ~70.

To better compare the two grape varieties, the OAV ratio of each odorant with an OAV ≥1 was determined by dividing the higher OAV by the lower one. The majority of the sixteen odorants showed factors ≤2. However, the two odorants with the highest OAVs in Muscaris grapes, geraniol (**26**) and (2*E*,6*Z*)-nona-2,6-dienal (**19**), showed factors of ~3. An even higher factor was calculated for (3*S*)-linalool (**18b**). Interestingly, although the OAV of (3*S*)-linalool (**18b**) in Muskateller grapes was about five times that in Muscaris grapes, the OAV sum of both citrusy, floral smelling linalool enantiomers (**18a**, **18b**) in Muskateller grapes was only about twice that in Muscaris grapes (cf. Table 7). In summary, the results of quantitation and OAV calculation of the major odorants showed only minor differences between the two grape varieties. The analytical data thus suggested that paternal Muskateller genes strongly influenced the aroma of the Muscaris grapes.

### 5.1.3 Sensory Analysis

To verify the analytical data and identify the odorants responsible for the distinct lychee note, odor reconstitution and omission experiments were performed. In a preliminary experiment, the concentrations of tartaric acid and the pH were determined in the juices of both Muscaris and Muskateller grapes. Results were 3.57 g/L tartaric acid in Muscaris grapes, 4.23 g/L tartaric acid in Muskateller grapes, and a pH of 3.8 in both. An individual ethanolic stock solution was prepared for each odorant with an OAV ≥1. The odor reconstitution models were then prepared by adding distinct volumes of the stock solutions corresponding to the target concentrations (cf. Table 7) to the respective aqueous tartaric acid solutions. Final pH adjustment was achieved with aqueous potassium hydroxide.

The results obtained with a trained sensory panel revealed olfactory profiles of the odor reconstitution models of Muscaris and Muskateller grapes that were pretty close. Green-grassy, floral, lychee and green apple-like notes were balanced in the profiles of both models (Figure 15). The lychee note, which was distinct in the aroma of fresh Muscaris grape berries, was well reproduced in the odor reconstitution model. Unfortunately, a direct comparison of the odor reconstitution models with the corresponding fresh materials was not possible due to the short shelf-life of the grapes. Given a generally huge influence of the vintage,[50] fresh grapes harvested a year later were not considered an alternative. A direct comparison, however, was possible between the frozen-thawed grapes and an adjusted odor reconstitution model.

Adjustment affected the concentrations of (3*E*)-hex-3-enal (**3**) and (3*Z*)-hex-3-enal (**4**), for which quantitative data had been obtained in both, the fresh grapes as well as the frozen-thawed grapes. The concentrations in the frozen-thawed Muscaris grapes were 6.02 µg/kg (**3**) and 145 µg/kg (**4**) and in the frozen-thawed Muskateller grapes were 14.8 µg/kg (**3**) and 78.0 µg/kg (**4**).[37]

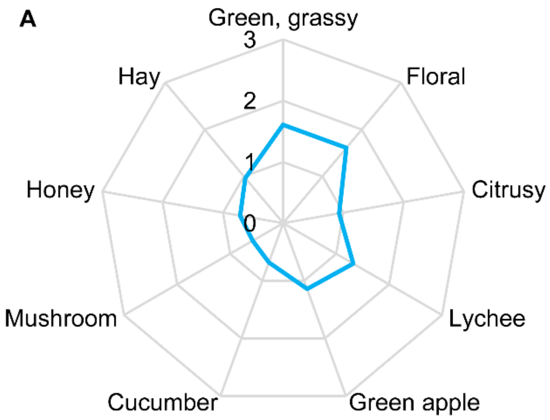


Figure 15: Quantitative olfactory profiles of the odor reconstitution models of Muscaris (A) and Muskateller (B) grapes. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

Quantitative olfactory profile analyses revealed good agreement between model and frozen-thawed grapes for both varieties as detailed in Figure 16.[37] This result proved that the major odorants contributing to the aroma of both Muscaris and Muskateller grapes were correctly identified and quantitated.

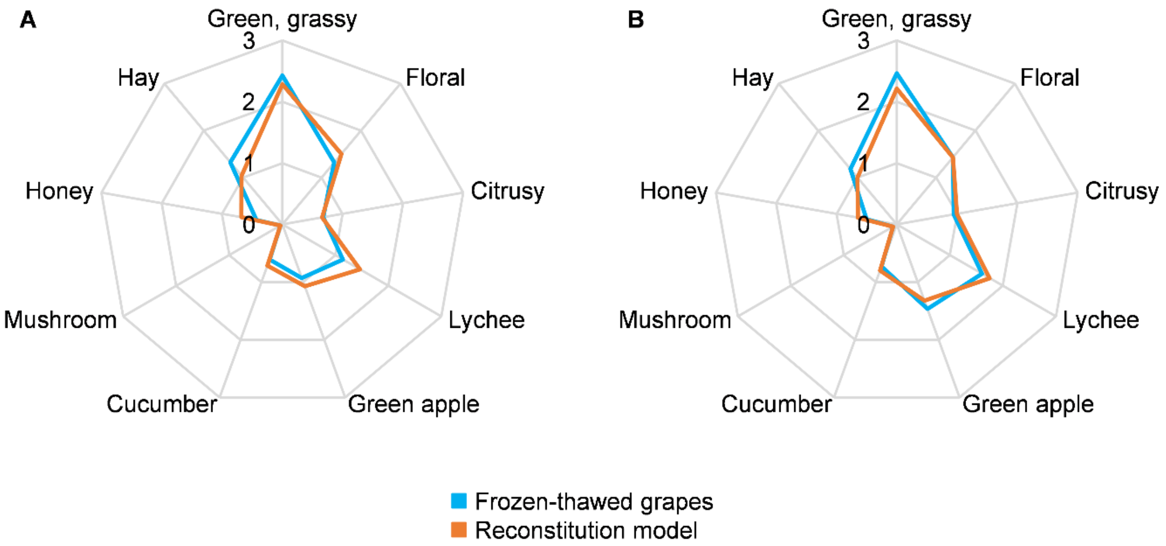


Figure 16: Quantitative olfactory profiles of the frozen-thawed Muscaris (A) and Muskateller (B) grapes in comparison to the quantitative olfactory profiles of the respective odor reconstitution models. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

To decipher the molecular background of the lychee note, omission experiments were performed based on the odor reconstitution models depicted in Figure 15. Incomplete odor reconstitution models from which odorants had been omitted were compared with the corresponding complete models in quantitative olfactory profile analyses performed by trained assessors.

Surprisingly, the omission of (2*S*,4*R*)-rose oxide (**10**), the only odorant with a specific lychee-like odor in both Muscaris and Muskateller grapes, did not result in a significant change of the intensity rating of the lychee note in the models (Figure 17). The *p*-values calculated by paired *t*-tests were 0.8 and 0.1 for the Muscaris model and the Muskateller model, respectively. The results indicated that (2*S*,4*R*)-rose oxide (**10**) alone did not play a significant role in the lychee note. Floral, rose-like smelling geraniol (**26**) was chosen for further omission experiments due to its high OAVs in the Muscaris and Muskateller grapes (cf. Table 7). However, the omission of geraniol in both models resulted only in a minor difference in the intensity rating of the lychee note. Thus, the effect was similar to that observed after omission of (2*S*,4*R*)-rose oxide. *p*-Values calculated from paired *t*-tests were 1 in the Muscaris models and 0.2 in the Muskateller models, suggesting that geraniol (**26**) alone was also not responsible for the lychee note. However, after omitting both (2*S*,4*R*)-rose oxide (**10**) and geraniol (**26**), the intensity of the lychee note substantially decreased, namely by 0.6 and 0.7, for the models of Muscaris and Muskateller grapes, respectively. Paired *t*-tests suggested a significant difference between the complete and incomplete odor reconstitution models with respect to the lychee note, as the *p*-values were 0.03 and 0.01 for the Muscaris models and the Muskateller models, respectively. Thus, it is obviously the combination of (2*S*,4*R*)-rose oxide and geraniol that is responsible for the distinct lychee note in Muscaris grapes as well as in Muskateller grapes. As far as we know, the combination of the two odorants has not been reported as the molecular basis for the lychee note.

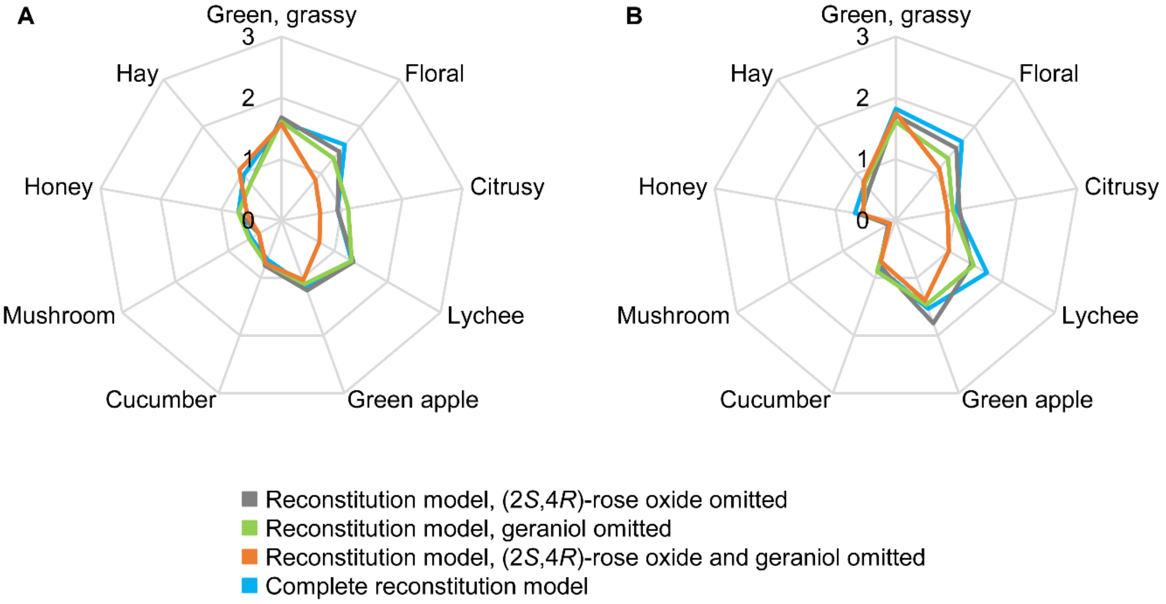


Figure 17: Quantitative olfactory profiles of the odor reconstitution models of Muscaris (A) and Muskateller (B) grapes from which (2*S*,4*R*)-rose oxide, geraniol, or both were omitted. The complete models depicted in Figure 15 are additionally included for comparison. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

## 5.2 Investigation of the Aroma Difference between Beer and Wine

### 5.2.1 Literature Survey and Meta-Analysis

To define the aroma bases of beer and wine, a comprehensive literature survey was performed. The search was based on the collection of Web of Science and Google Scholar and focused on research articles written in English and published between 2000 and 2021. The keywords used for searching were “aroma”, “volatile”, “odor-active compound”, and “odorant” in combination with “beer” and “wine”. This search procedure resulted in ~950 and ~6650 articles for beer and wine, respectively. The article collection was filtered with the aim to concentrate on general beer and wine samples and sort out specialty beers and wines. The remaining literature was further concentrated by removing articles which lacked reliable methodology in the identification and quantitation of odorants.

At the compound level, we concentrated on odorants that are commonly present in beer or wine. Thus, we did not consider odorants limited to specific raw materials such as certain grape or hop varieties, or specific processing such as dry-hopping of beer or barrel-aging of wine. For example, TDN and 4-MSP in wine,[80,94] hop-derived linalool and geraniol in beer[208] as well as oak barrel-derived *cis*-whisky lactone in wine[227] were excluded. However, hop-derived 3-methylbut-2-ene-1-thiol was included as beer odorant, as it is formed from the nonvolatile hop bitter acids generally present in beer.[222] Among the volatiles finally classified as basic odorants in beer and wine were mainly compounds generated during alcoholic fermentation.[49,114,223,243-246] In total, the numbers of compounds regarded as basic odorants were 42 for beer and 42 for wine, respectively. These compounds are listed in Table 8 for beer and Table 9 for wine, respectively. Forty of the compounds were common to beer and wine.

As a next step, the concentration values of the basic beer and wine odorants were extracted from 32 articles covering 160 beer samples and 252 articles covering 904 wine samples. The individual concentration values are provided in the Supporting Information file of Publication 2.[247] The arithmetic mean concentrations of each odorant in beer and wine are included in Tables 8 and 9. In total, the odorant concentrations covered a wide range. In beer, concentrations ranged from 0.00645 µg/L for 3-methylbut-2-ene-1-thiol to 311000 µg/L for acetic acid. In wine, the low end was 14.1 µg/L for dimethyl sulfide and the highest concentration was 219000 µg/L for acetic acid. High concentrations of ≥10000 µg/L were also obtained for 3-methylbutan-1-ol (30000 µg/L), 2-phenylethan-1-ol (25700 µg/L), ethyl acetate (23700 µg/L), and 2-methylbutan-1-ol (10300 µg/L) in beer, and 3-methylbutan-1-ol (172000 µg/L), 2-methylbutan-1-ol (70100 µg/L), ethyl acetate (69100 µg/L), acetaldehyde (49100 µg/L), 2-methylpropan-1-ol (33000 µg/L), 2-phenylethan-1-ol (28700 µg/L), and 3-hydroxybutan-2-one (16600 µg/L) in wine. Moreover, seven and thirteen compounds showed concentrations of ≥1000 µg/L, but <10000 µg/L in beer and wine, respectively. Among the 40 odorants shared, 32 showed higher concentrations in wine than in beer.

Table 8: Mean concentrations of beer odorants based on published data and the corresponding OAVs

| compound[a] | concentration[b] (µg/L) | OTC[c] (µg/kg) | OAV[d] | dataset size[e] |
|---|---|---|---|---|
| ethyl acetate | 23700 | 5[f] | 4700 | 81 |
| 3-methylbutyl acetate | 2070 | 7.2[g] | 290 | 96 |
| ethyl hexanoate | 239 | 1.2[g] | 200 | 74 |
| 2-phenylethan-1-ol | 25700 | 140[g] | 180 | 92 |
| 3-methylbutan-1-ol | 30000 | 220[g] | 140 | 73 |
| acetaldehyde | 1800 | 16[g] | 110 | 10 |
| ethyl 3-methylbutanoate | 2.41 | 0.023[g] | 100 | 22 |
| dimethyl sulfide | 31.0 | 0.30[g] | 100 | 3 |
| ethyl butanoate | 70.6 | 0.76[g] | 93 | 69 |
| 3-methylbutanal | 35.0 | 0.50[g] | 70 | 52 |
| ethyl octanoate | 581 | 8.7[g] | 67 | 68 |
| 2-methylpropanal | 28.4 | 0.49[g] | 58 | 42 |
| acetic acid | 311000 | 5600[g] | 55 | 3 |
| ethyl 2-methylpropanoate | 3.37 | 0.089[g] | 38 | 7 |
| octanoic acid | 5930 | 190[g] | 31 | 56 |
| butane-2,3-dione | 16.6 | 1.0[g] | 17 | 53 |
| phenylacetic acid | 821 | 68[g] | 12 | 4 |
| 3-(methylsulfanyl)propan-1-ol | 421 | 36[g] | 12 | 6 |
| ethyl 2-methylbutanoate | 1.30 | 0.13[g] | 10 | 19 |
| 3-(methylsulfanyl)propanal | 3.94 | 0.43[g] | 9.2 | 24 |
| 2-methylbutan-1-ol | 10300 | 1200[g] | 8.6 | 64 |
| ethyl propanoate | 85.8 | 10[f] | 8.6 | 19 |
| 3-methylbut-2-ene-1-thiol | 0.00645 | 0.00076[g] | 8.5 | 2 |
| 2-methylbutanal | 8.22 | 1.5[g] | 5.5 | 48 |
| decanoic acid | 2360 | 500[f] | 4.7 | 41 |
| 2-methylpropyl acetate | 205 | 66[f] | 3.1 | 40 |
| phenylacetaldehyde | 13.8 | 5.2[g] | 2.7 | 30 |
| 2-phenylethyl acetate | 788 | 360[g] | 2.2 | 59 |
| 1,1-diethoxyethane | 50 | 25[g] | 2.0 | 1 |
| ethyl decanoate | 84.8 | 122[f] | <1 | 57 |
| butanoic acid | 1380 | 2400[g] | <1 | 16 |
| 2-methylpropan-1-ol | 9600 | 19000[g] | <1 | 78 |
| 3-methylbutanoic acid | 245 | 490[g] | <1 | 15 |
| hexanoic acid | 1780 | 4800[g] | <1 | 55 |
| octan-1-ol | 35.2 | 110[f] | <1 | 5 |
| 2-methylbutanoic acid | 561 | 3100[g] | <1 | 3 |
| hexan-1-ol | 35.1 | 590[g] | <1 | 14 |
| benzaldehyde | 8.37 | 150[g] | <1 | 50 |
| ethyl dodecanoate | 35.0 | 3500[f] | <1 | 4 |
| 2-methylpropanoic acid | 448 | 60000[g] | <1 | 4 |
| ethyl 2-phenylacetate | 0.66 | 155.55[f] | <1 | 1 |
| butan-1-ol | 1.54 | 1900[g] | <1 | 9 |

[a]Compounds in order of decreasing OAVs. [b]Arithmetic mean of individual values resulting from the literature survey; individual values are available in the Supporting Information file of Publication 2.[247] [c]Orthonasal odor threshold concentration in water. [d]Odor activity value; approximated as ratio of the mean concentration in beer to the odor threshold concentration. [e]Number of concentration values used to calculate the mean. [f]Data from literature.[248-253] [g]Data taken from the Leibniz-LSB@TUM Odorant Database.[20]

Table 9: Mean concentrations of wine odorants based on published data and the corresponding OAVs

| compound[a] | concentration[b] (µg/L) | OTC[c] (µg/kg) | OAV[d] | dataset size[e] |
|---|---|---|---|---|
| ethyl acetate | 69100 | 5[f] | 14000 | 464 |
| acetaldehyde | 49100 | 16[g] | 3100 | 144 |
| butane-2,3-dione | 1400 | 1.0[g] | 1400 | 85 |
| ethyl hexanoate | 1570 | 1.2[g] | 1300 | 658 |
| ethyl 3-methylbutanoate | 27.5 | 0.023[g] | 1200 | 320 |
| ethyl 2-methylpropanoate | 93.5 | 0.089[g] | 1100 | 264 |
| 3-methylbutan-1-ol | 172000 | 220[g] | 780 | 555 |
| 3-methylbutyl acetate | 3650 | 7.2[g] | 510 | 719 |
| ethyl butanoate | 374 | 0.76[g] | 490 | 596 |
| ethyl 2-methylbutanoate | 42.7 | 0.13[g] | 330 | 270 |
| ethyl octanoate | 2460 | 8.7[g] | 280 | 687 |
| 3-methylbutanal | 119 | 0.50[g] | 240 | 104 |
| 2-phenylethan-1-ol | 28700 | 140[g] | 200 | 684 |
| 2-methylpropanal | 36.5 | 0.49[g] | 74 | 112 |
| 2-methylbutan-1-ol | 70100 | 1200[g] | 58 | 128 |
| dimethyl sulfide | 14.1 | 0.30[g] | 47 | 82 |
| acetic acid | 219000 | 5600[g] | 39 | 229 |
| 3-(methylsulfanyl)propan-1-ol | 1360 | 36[g] | 38 | 311 |
| 3-(methylsulfanyl)propanal | 14.6 | 0.43[g] | 34 | 122 |
| ethyl propanoate | 295 | 10[f] | 30 | 150 |
| octanoic acid | 5580 | 190[g] | 29 | 582 |
| 3-hydroxybutan-2-one | 16600 | 590[g] | 28 | 164 |
| 2-methylbutanal | 40.2 | 1.5[g] | 27 | 31 |
| phenylacetic acid | 452 | 68[g] | 6.6 | 48 |
| ethyl decanoate | 741 | 122[f] | 6.1 | 595 |
| decanoic acid | 2460 | 500[f] | 4.9 | 489 |
| hexan-1-ol | 2710 | 590[g] | 4.6 | 657 |
| 2-phenylethyl acetate | 682 | 360[g] | 1.9 | 607 |
| 2-methylpropan-1-ol | 33000 | 19000[g] | 1.7 | 486 |
| 3-methylbutanoic acid | 814 | 490[g] | 1.7 | 264 |
| 2-methylpropyl acetate | 101 | 66[f] | 1.5 | 241 |
| hexanoic acid | 4060 | 4800[g] | <1 | 533 |
| benzaldehyde | 108 | 150[g] | <1 | 323 |
| butan-1-ol | 1120 | 1900[g] | <1 | 259 |
| butanoic acid | 1180 | 2400[g] | <1 | 241 |
| octan-1-ol | 44.7 | 110[f] | <1 | 169 |
| ethyl 2-phenylacetate | 53.8 | 155.55[f] | <1 | 208 |
| 2-methylbutanoic acid | 545 | 3100[g] | <1 | 64 |
| ethyl dodecanoate | 269 | 3500[f] | <1 | 247 |
| propanoic acid | 1490 | 20000[g] | <1 | 64 |
| 2-methylpropanoic acid | 2180 | 60000[g] | <1 | 235 |

[a]Compounds in order of decreasing OAVs. [b]Arithmetic mean of individual values resulting from the literature survey; individual values are available in the Supporting Information file of Publication 2.[247] [c]Orthonasal odor threshold concentration in water. [d]Odor activity value; approximated as ratio of the mean concentration in wine to the odor threshold concentration. [e]Number of concentration values used to calculate the mean. [f]Data from literature.[248-253] [g]Data taken from the Leibniz-LSB@TUM Odorant Database.[20]

To gain information on the individual odor activity of the odorants, the OAV of each odorant was calculated as the ratio of the mean concentration and the respective OTC in water. The results revealed 29 and 32 compounds with OAVs ≥1 in beer (Table 8) and wine (Table 9), respectively. These compounds were thus considered important for the aroma bases of beer and wine. Among them, 27 odorants were common in the aroma bases of beer and wine. The OAVs of nine of these odorants were quite similar in beer and wine; they did not differ by more than a factor of two. Two odorants, namely dimethyl sulfide and 2-methylpropyl acetate, showed higher OAVs in beer, whereas the remaining 16 odorants showed higher OAVs in wine. Large OAV differences (>10-fold) were obtained for butan-2,3-dione, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, acetaldehyde, and ethyl 3-methylbutanoate. The highest OAVs were calculated for ethyl acetate in both beer (OAV 4700) and wine (OAV 14000). Except for ethyl acetate, no odorant showed an OAV ≥1000 in beer, while additional five odorants showed OAVs ≥1000 in wine, namely acetaldehyde (OAV 3100), butane-2,3-dione (1400), ethyl hexanoate (OAV 1300), ethyl 3-methylbutanoate (OAV 1200), and ethyl 2-methylpropanoate (OAV 1000). The OAV range of ≥100 but <1000 included seven odorants in both beer and wine. These odorants were 3-methylbutyl acetate (OAV 290), ethyl hexanoate (OAV 200), 2-phenylethan-1-ol (OAV 180), 3-methylbutan-1-ol (OAV 140), acetaldehyde (OAV 110), ethyl 3-methylbutanoate (OAV 100), and dimethyl sulfide (OAV 100) in beer, and 3-methylbutan-1-ol (OAV 780), 3-methylbutyl acetate (OAV 510), ethyl butanoate (OAV 490), ethyl 2-methylbutanoate (OAV 330), ethyl octanoate (OAV 280), 3-methylbutanal (OAV 240), and 2-phenylethan-1-ol (OAV 200) in wine. Moreover, eleven and ten odorants showed OAVs ≥10, but <100 and ten and nine odorants showed OAVs ≥1 but <10 in beer and wine, respectively.

Vicente Ferreira identified 27 compounds that form the base of wine aroma.[228] Except for ethanol, which we regarded as a matrix component, the other 26 odorants were all included in the initial selection of basic wine odorants. Twenty-two of them showed mean concentrations beyond their OTCs in water and, thus, were included in the current wine aroma base. However, the remaining four, all of which were carboxylic acids (2-methylpropanoic, 2-methylbutanoic, butanoic, and hexanoic acids) were excluded as their OAVs were <1. Besides, our wine aroma base included ten additional odorants which were not included in Vicente Ferreira's selection (Table 9). These compounds were 3-methylbutanal (OAV 240), 2-methylpropanal (OAV 74), 2-methylbutan-1-ol (OAV 58), dimethyl sulfide (OAV 47), 3-(methylsulfanyl)propanal (OAV 34), ethyl propanoate (OAV 30), 3-hydroxybutan-2-one (OAV 28), 2-methylbutanal (OAV 27), 2-phenylacetic acid (OAV 6.6), and phenylacetaldehyde (OAV 4.1). Five of them were Strecker aldehydes formed by the degradation of the respective amino acids.[223,254]

### 5.2.2 Aroma Base Models of Beer and Wine

The 29 and 32 odorants with OAVs ≥1 and their corresponding mean concentrations, all resulting from our comprehensive literature survey and the subsequent meta-analysis, were the data basis for the aroma base models of beer and wine, respectively. The concentrations of ethanol, major acids, and glycerol as well as pH and carbonation were additionally considered when preparing the aroma base models (Supporting Information file of Publication 2[247]). A trained sensory panel was able to reliably assign the two models in a blind test to beer and wine, respectively. The aroma of the two base models was considered to well reflect the basic olfactory difference between beer and wine.

### 5.2.3 Concentration Leveling Tests

To finally elucidate the odorants responsible for the aroma difference between the aroma base models of beer and wine, concentration leveling tests were performed. Thus, the concentration levels of selected odorants in the one model were adjusted to the concentration levels in the other model, and the effect on the overall odor was evaluated in a sensory test. For this purpose, the odorants in the aroma base models of beer and wine were grouped into “buttery”, “fruity”, “malty”, “honey”, “sweaty”, “sulfury”, or “miscellaneous” according to their predominant odor qualities[20] as shown in Table 10.

Table 10: OAV sums of odorant groups in the aroma base models of beer and wine

| group[a] | odorant | OAV sum[b] | | OAV sum ratio wine/beer[c] |
|---|---|---|---|---|
| | | beer | wine | |
| “buttery” | butane-2,3-dione<br>3-hydroxybutan-2-one | 17 | 1400 | 86 |
| “fruity” | 1,1-diethoxyethane<br>ethyl butanoate<br>ethyl decanoate<br>ethyl hexanoate<br>ethyl 2-methylbutanoate<br>ethyl 3-methylbutanoate<br>ethyl 2-methylpropanoate<br>ethyl octanoate<br>ethyl propanoate<br>3-methylbutyl acetate<br>2-methylpropyl acetate | 810 | 5200 | 6.4 |
| “malty” | 2-methylbutanal<br>3-methylbutanal<br>2-methylbutan-1-ol<br>3-methylbutan-1-ol<br>2-methylpropanal<br>2-methylpropan-1-ol | 280 | 1200 | 4.2 |
| “miscellaneous” | acetaldehyde<br>acetic acid<br>ethyl acetate<br>hexan-1-ol | 4900 | 17000 | 3.5 |
| “honey” | phenylacetaldehyde<br>phenylacetic acid<br>2-phenylethan-1-ol<br>2-phenylethyl acetate | 200 | 220 | 1.1 |
| “sweaty” | decanoic acid<br>3-methylbutanoic acid<br>octanoic acid | 36 | 36 | 1.0 |
| “sulfury” | dimethyl sulfide<br>3-methylbut-2-ene-1-thiol<br>3-(methylsulfanyl)propanal<br>3-(methylsulfanyl)propan-1-ol | 130 | 120 | 0.89 |

[a] Odorants were grouped into “buttery”, “fruity”, “malty”, “honey”, “sweaty”, “sulfury” or “miscellaneous” according to their predominant odor qualities.[20] [b] Sum of the OAVs of the individual odorants within the group. [c] For each group, a ratio was calculated by dividing the OAV sum in wine by the OAV sum in beer.

The OAV sum of individual odorants within each odorant group was calculated (Table 10). Results showed that the miscellaneous odorants group had the highest OAV sum in both aroma base models, followed by the fruity odorants group. To better visualize the differences in the OAV sums, for each group of odorants a ratio was calculated by dividing the OAV sum in wine by the OAV sum in beer. The highest ratio of 86 was calculated for the buttery odorant group with an OAV sum of 1400 in the wine aroma base model and an OAV sum of only 17 in the beer aroma base model. The ratios calculated for the fruity, malty, and miscellaneous odorant groups amounted to 6.4, 4.2, and 3.5, respectively, corresponding to OAV sums in the wine aroma base model of 5200, 1200, and 17000, and OAV sums in the beer aroma base model of 810, 280, and 4900. For the groups of honey, sweaty, and sulfury odorants, the differences between the beer and wine models were small resulting in OAV sum ratios close to 1.

In the sensory tests, the assessors were provided with the test samples that had odorant concentrations adjusted, as well as with the two aroma base models as references. The assessors were asked to evaluate the odor similarity of each test sample to the references by positioning a mark on a 20 cm ruler, of which the leftmost position was defined as 100% beer-like and 0% wine-like, represented by the beer aroma base model, and the rightmost position was defined as 100% wine-like and 0% beer-like, represented by the wine aroma base model. The mark on the ruler was converted to the percentages of both beer-like and wine-like. For each test sample, the arithmetic mean percentages of both beer-like and wine-like were calculated from the individual results of the assessors.

The results of the concentration leveling tests are depicted in Figure 18. As detailed in Figure 18A, when the concentration levels of the malty, miscellaneous, sweaty, and sulfury odorant groups in the beer aroma base model were adjusted to the concentration levels in the wine aroma base model, respectively, the test samples were still evaluated as more beer-like corresponding to percentages on the unstructured scale of 61–76% beer-like. When the buttery odorant group was adjusted in the same way, the test sample was not clearly attributable with 53% beer-like and 47% wine-like, although this odorant group showed the largest difference in the OAV sum between the beer and wine aroma base models. In the honey odorant group, even though the OAV sums in the aroma base models of beer and wine were quite close, the adjustment of the concentrations in the beer model to the levels in the wine model made it difficult to clearly assign the test sample to beer-like or wine-like. Interestingly, when the concentrations of the fruity odorants in the beer aroma base model were adjusted to the concentration levels of the wine aroma base model, i.e., the OAV sum of the fruity odorant group increased by ~5.4 times, the test sample was evaluated as more wine-like (70%). This result suggested that higher concentrations of esters in the wine model were crucial for the aroma difference to the beer model.

As shown in Figure 18B, the odorant concentration adjustment had a generally smaller effect on the evaluation of the wine aroma base model than was the case with the beer aroma base model. Six of the seven test samples were still rated as more wine-like with percentages of 62–78%. Although adjustment of the concentrations of the fruity odorants was able to switch the odor impression of the beer aroma base model from beer-like to wine-like, no such effect was observed when the concentrations of the fruity odorants in the wine aroma base model were adjusted to the levels in the beer aroma base model. Interestingly, when the concentrations of the honey odorants in the wine aroma base model were adjusted to the concentrations in the beer aroma base model, the test sample was evaluated as slightly more beer-like.

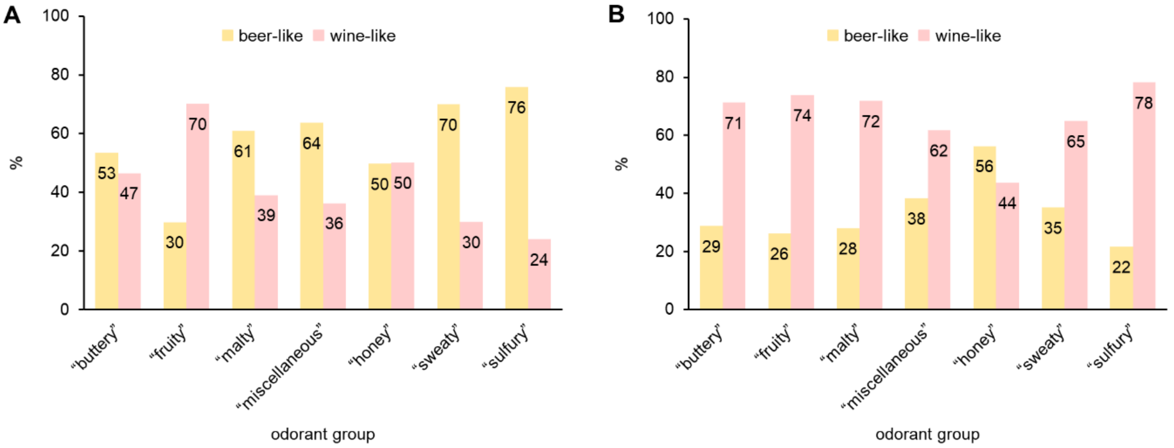


Figure 18: Results of the concentration leveling tests based on the beer (A) and the wine (B) aroma base models. Assessors rated the odor similarity of test samples to the beer and wine aroma base models.

Additionally, the influence of the matrix composition on the beer- and wine-like ratings was investigated by concentration leveling test (data not shown). The test sample with the odorant concentrations of the beer base model in combination with the wine matrix composition was evaluated as more beer-like (65%) and the test sample with the odorant concentrations of the wine base model in combination with the beer matrix composition was rated more wine-like (66%). The percentages obtained for these two models were lower than those obtained for the full beer aroma base model (88%) and the full wine aroma base model (83%), respectively. The results thus suggested an influence of the matrix composition on the overall odor impression of the models, however, the influence of the odorants dominated. As far as we know, the influence of the matrix composition has so far only been investigated in studies focused on specific odor notes of beer[255] and wine[104,256,257] but not on the overall beer-like and wine-like odor impression.

# 6 References

1. GfK Panel Services Deutschland und Bundesvereinigung der Deutschen Ernährungsindustrie e.V., BVE. Consumers' Choice '11. https://www.bve-online.de/presse/infothek/publikationen-jahresbericht/consumers-choice2011 (accessed December 10, 2024)

2. Bundesministerium für Ernährung und Landwirtschaft. Deutschland, wie es isst. Berlin, 2021; https://www.bmel.de/SharedDocs/Downloads/DE/Broschueren/ernaehrungsreport-2021.pdf?__blob=publicationFile&v=6 (accessed December 10, 2024)

3. International Food Information Council. 2022 Food & Health Survey; https://foodinsight.org/wp-content/uploads/2022/05/IFIC-2022-Food-and-Health-Survey-Report.pdf (accessed December 10, 2024)

4. Steinhaus, M. Gas chromatography–olfactometry: principles, practical aspects and applications in food analysis. In *Advanced Gas Chromatography in Food Analysis*; Tranchida, P., Ed.; The Royal Society of Chemistry: Cambridge, UK, 2019; pp 337–399.

5. Nelson, D. L.; Cox, M. M. Lehninger principles of biochemistry. 5th edition; W. H. Freeman and Company: New York, USA, 2008; pp 465–467.

6. Krautwurst, D. Human olfactory receptor families and their odorants. *Chem. Biodiversity* **2008**, *5*, 842–852.

7. Olender, T.; Lancet, D.; Nebert, D. W. Update on the olfactory receptor (OR) gene superfamily. *Hum. Genomics*. **2008**, *3*, 1–11.

8. Niimura, Y.; Nei, M. Evolution of olfactory receptor genes in the human genome. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 12235–12240.

9. Adipietro, K. A.; Mainland, J. D.; Matsunami, H. Functional evolution of mammalian odorant receptors. *PLoS Genet.* **2012**, *8*, e1002821.

10. Buck, L.; Axel, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **1991**, *65*, 175–187.

11. Firestein, S. How the olfactory system makes sense of scents. *Nature* **2001**, *413*, 211–218.

12. The Nobel Foundation. The Nobel Prize in Physiology or Medicine 2004; https://www.nobelprize.org/prizes/medicine/2004/press-release/ (accessed December 10, 2024)

13. Malnic, B.; Hirono, J.; Sato, T.; Buck, L. B. Combinatorial receptor codes for odors. *Cell* **1999**, *96*, 713–723.

14. Frings, S. Chemoelectrical signal transduction in olfactory sensory neurons of air-breathing vertebrates. *Cell. Mol. Life Sci.* **2001**, *58*, 510–519.

15. Haag, F.; Krautwurst, D. Olfaction and the complex interaction between odourant ligands and their receptors. In *Comprehensive Analytical Chemistry*; Elsevier: Amsterdam, Netherlands, 2022; pp 1–40.

16. Geithe, C.; Noe, F.; Kreissl, J.; Krautwurst, D. The broadly tuned odorant receptor OR1A1 is highly selective for 3-methyl-2,4-nonanedione, a key food odorant in aged wines, tea, and other foods. *Chem. Senses* **2017**, *42*, 181–193.

17. Haag, F.; Di Pizio, A.; Krautwurst, D. The key food odorant receptive range of broadly tuned receptor OR2W1. *Food Chem.* **2022**, *375*, 131680.

18. Gerkin, R. C.; Castro, J. B. The number of olfactory stimuli that humans can discriminate is still unknown. *eLife* **2015**, *4*, e08127.

19. McGann, J. P. Poor human olfaction is a 19th-century myth. *Science* **2017**, *356*, eaam7263.

20. Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. Leibniz-LSB@TUM Odorant Database, Version 1.2; Leibniz Institute for Food Systems Biology at the Technical University of Munich: Freising, Germany, 2022. https://www.leibniz-lsb.de/en/databases/leibniz-lsbtum-odorant-database (accessed December 10, 2024).

21. Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology. *Angew. Chem., Int. Ed.* **2014**, *53*, 7124–7143.

22. Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the key aroma compounds in pink guava (*Psidium guajava* L.) by means of aroma re-engineering experiments and omission tests. *J. Agric. Food Chem.* **2009**, *57*, 2882–2888.

23. Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food: Emerging Methods*; Gaonkar, A. G., Ed.; Elsevier Science B.V.: Amsterdam, Netherlands, 1995; pp 403–431.

24. Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545.

25. Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237–241.

26. Schlumpberger, P.; Stübner, C. A.; Steinhaus, M. Development and evaluation of an automated solvent-assisted flavour evaporation (aSAFE). *Eur. Food Res. Technol.* **2022**, *248*, 2591–2602.

27. Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the aroma-active compounds in pink guava (*Psidium guajava*, L.) by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* **2008**, *56*, 4120–4127.

28. Li, J. X.; Schieberle, P.; Steinhaus, M. Characterization of the major odor-active compounds in Thai durian (*Durio zibethinus* L. 'Monthong') by aroma extract dilution analysis and headspace gas chromatography–olfactometry. *J. Agric. Food Chem.* **2012**, *60*, 11253–11262.

29. Tatsu, S.; Matsuo, Y.; Nakahara, K.; Hofmann, T.; Steinhaus, M. Key Odorants in Japanese roasted barley tea (Mugi-Cha)–Differences between roasted barley tea

prepared from naked barley and roasted barley tea prepared from hulled barley. *J. Agric. Food Chem.* **2020**, *68*, 2728–2737.

30. Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in Flavour Research*; Land, G. G., Nursten, H.E., Eds.; Applied Science Publishers: London, UK, 1979; pp 79–88.
31. Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm. -Unters. Forsch.* **1987**, *185*, 111–113.
32. Keller, A.; Zhuang, H. Y.; Chi, Q. Y.; Vosshall, L. B.; Matsunami, H. Genetic variation in a human odorant receptor alters odour perception. *Nature* **2007**, *449*, 468–472.
33. Trimmer, C.; Keller, A.; Murphy, N. R.; Snyder, L. L.; Willer, J. R.; Nagai, M. H.; Katsanis, N.; Vosshall, L. B.; Matsunami, H.; Mainland, J. D. Genetic variation across the human olfactory receptor repertoire alters odor perception. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 9475–9480.
34. Munafo, J. P., Jr.; Didzbalis, J.; Schnell, R. J.; Schieberle, P.; Steinhaus, M. Characterization of the major aroma-active compounds in mango (*Mangifera indica* L.) cultivars Haden, White Alfonso, Praya Sowoy, Royal Special, and Malindi by application of a comparative aroma extract dilution analysis. *J. Agric. Food Chem.* **2014**, *62*, 4544–4551.
35. Schaller, T.; Schieberle, P. Comparison of the key aroma compounds in fresh, raw ginger (*Zingiber officinale* Roscoe) from China and roasted ginger by application of aroma extract dilution analysis. *J. Agric. Food Chem.* **2020**, *68*, 15292–15300.
36. Fechir, M.; Reglitz, K.; Mall, V.; Voigt, J.; Steinhaus, M. Molecular insights into the contribution of specialty barley malts to the aroma of bottom-fermented lager beers. *J. Agric. Food Chem.* **2021**, *69*, 8190–8199.
37. Wang, X. J.; Frank, S.; Steinhaus, M. Molecular background of the lychee aroma of *Vitis vinifera* L. 'Muscaris'. *J. Agric. Food Chem.* **2024**, *72*, 1674–1682.
38. Kiefl, J.; Pollner, G.; Schieberle, P. Sensomics analysis of key hazelnut odorants (*Corylus avellana* L. 'Tonda Gentile') using comprehensive two-dimensional gas chromatography in combination with time-of-flight mass spectrometry (GC×GC-TOF-MS). *J. Agric. Food Chem.* **2013**, *61*, 5226–5235.
39. Füllemann, D.; Steinhaus, M. Characterization of odorants causing smoky off-flavors in cocoa. *J. Agric. Food Chem.* **2020**, *68*, 10833–10841.
40. Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252–257.
41. Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68–73.
42. ASTM International. *E679−19 Standard Practice for Determination of Odor and Taste Thresholds by a Forced-choice Ascending Concentration Series Method of Limits*; ASTM: West Conshohocken, PA, 2019.

43. Munafo, J. P.; Didzbalis, J.; Schnell, R. J.; Steinhaus, M. Insights into the key aroma compounds in mango (*Mangifera indica* L. 'Haden') fruits by stable isotope dilution quantitation and aroma simulation experiments. *J. Agric. Food Chem.* **2016**, *64*, 4312–4318.

44. Li, J. X.; Schieberle, P.; Steinhaus, M. Insights into the key compounds of durian (*Durio zibethinus* L. 'Monthong') pulp odor by odorant quantitation and aroma simulation experiments. *J. Agric. Food Chem.* **2017**, *65*, 639–647.

45. World wine production outlook - OIV first estimates. International Organisation of Vine and Wine, Dijon, France, 2023. https://www.oiv.int/sites/default/files/documents/OIV_World_Wine_Production_Outlook_2023.pdf (accessed December 10, 2024)

46. Gómez-Míguez, M. J.; Cacho, J. F.; Ferreira, V.; Vicario, I. M.; Heredia, F. J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473.

47. Lorrain, B.; Tempere, S.; Iturmendi, N.; Moine, V.; de Revel, G.; Teissedre, P. L. Influence of phenolic compounds on the sensorial perception and volatility of red wine esters in model solution: an insight at the molecular level. *Food Chem*. **2013**, *140*, 76–82.

48. Polášková, P.; Herszage, J.; Ebeler, S. E. Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* **2008**, *37*, 2478–2489.

49. Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173.

50. Robinson, A. L.; Boss, P. K.; Solomon, P. S.; Trengove, R. D.; Heymann, H.; Ebeler, S. E. Origins of grape and wine aroma. Part 1. chemical components and viticultural impacts. *Am. J. Enol. Vitic.* **2014**, *65*, 1–24.

51. Gambetta, J. M.; Bastian, S. E. P.; Cozzolino, D.; Jeffery, D. W. Factors Influencing the aroma composition of Chardonnay wines. *J. Agric. Food Chem.* **2014**, *62*, 6512–6534.

52. Longo, R.; Carew, A.; Sawyer, S.; Kemp, B.; Kerslake, F. A review on the aroma composition of *Vitis vinifera* L. Pinot noir wines: origins and influencing factors. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 1589–1604.

53. He, Y.; Wang, X. Y.; Li, P. H.; Lv, Y. C.; Nan, H. L.; Wen, L. K.; Wang, Z. T. Research progress of wine aroma components: a critical review. *Food Chem.* **2023**, *402*, 134491.

54. Gonzalez-Barreiro, C.; Rial-Otero, R.; Cancho-Grande, B.; Simal-Gandara, J. Wine aroma compounds in grapes: a critical review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 202–218.

55. Schwab, W.; Wüst, M. Understanding the constitutive and induced biosynthesis of mono- and sesquiterpenes in grapes (*Vitis vinifera*): a key to unlocking the biochemical secrets of unique grape aroma profiles. *J. Agric. Food Chem.* **2015**, *63*, 10591–10603.

56. Rienth, M.; Vigneron, N.; Darriet, P.; Sweetman, C.; Burbidge, C.; Bonghi, C.; Walker, R. P.; Famiani, F.; Castellarin, S. D. Grape berry secondary metabolites and their

modulation by abiotic factors in a climate change scenario–a review. *Front. Plant Sci.* **2021**, *12*, 643258.

57. Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr. A* **1985**, *331*, 83–90.
58. D'Onofrio, C.; Matarese, F.; Cuzzola, A. Study of the terpene profile at harvest and during berry development of *Vitis vinifera* L. aromatic varieties Aleatico, Brachetto, Malvasia di Candia aromatica and Moscato bianco. *J. Sci. Food Agric.* **2017**, 9*7*, 2898–2907.
59. Francis, I. L.; Newton, J. L. Determining wine aroma from compositional data. *Aust. J. Grape Wine Res.* **2005**, *11*, 114–126.
60. Black, C. A.; Parker, M.; Siebert, T. E.; Capone, D. L.; Francis, I. L. Terpenoids and their role in wine flavour: recent advances. *Aust. J. Grape Wine Res.* **2015**, *21*, 582–600.
61. Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vossing, T.; Widder, S.; Krammer, G.; Sefton, M. A.; Herderich, M. J. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, 3738–3744.
62. Siebert, T. E.; Wood, C.; Elsey, G. M.; Pollnitz, A. P. Determination of rotundone, the pepper aroma impact compound, in grapes and wine. *J. Agric. Food Chem.* **2008**, *56*, 3745–3748.
63. Vázquez, L. C.; Pérez-Coello, M. S.; Cabezudo, M. D. Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes. *Anal. Chim. Acta* **2002**, *458*, 39–44.
64. Palomo, E. S.; Díaz-Maroto, M. C.; Viñas, M. A. G.; Soriano-Pérez, A.; Pérez-Coello, M. S. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. *Food Control* **2007**, *18*, 398–403.
65. Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
66. Ong, P. K. C.; Acree, T. E. Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. *J. Agric. Food Chem.* **1999**, *47*, 665–670.
67. Katarína, F.; Katarína, M.; Katarína, Ď.; Ivan, Š.; Fedor, M. Influence of yeast strain on aromatic profile of Gewürztraminer wine. *LWT–Food Sci. Technol.* **2014**, *59*, 256–262.
68. Ferreira, V.; López, R.; Cacho, J. F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
69. Escudero, A.; Gogorza, B.; Melús, M. A.; Ortin, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524.
70. Koslitz, S.; Renaud, L.; Kohler, M.; Wüst, M. Stereoselective formation of the varietal aroma compound rose oxide during alcoholic fermentation. *J. Agric. Food Chem.* **2008**, *56*, 1371–1375.

71. Dein, M.; Kerley, T.; Munafo, J. P. Characterization of odorants in a 10-Year-Old Riesling wine. *J. Agric. Food Chem.* **2021**, *69*, 11372–11381.

72. Moyano, L.; Zea, L.; Moreno, J.; Medina, M. Analytical study of aromatic series in sherry wines subjected to biological aging. *J. Agric. Food Chem.* **2002**, *50*, 7356–7361.

73. Li, Z. Z.; Howell, K.; Fang, Z. X.; Zhang, P. Z. Sesquiterpenes in grapes and wines: occurrence, biosynthesis, functionality, and influence of winemaking processes. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 247–281.

74. Mendes-Pinto, M. M. Carotenoid breakdown products the–norisoprenoids–in wine aroma. *Arch. Biochem. Biophys.* **2009**, *483*, 236–245.

75. Winterhalter, P.; Rouseff, R. Carotenoid-derived aroma compounds: an introduction. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R., Eds.; ACS Publications: ACS Symposium Series 802; American Chemical Society: Washington, DC, USA, 2002; pp 1–19.

76. Parker, M.; Capone, D. L.; Francis, I. L.; Herderich, M. J. Aroma precursors in grapes and wine: flavor release during wine production and consumption. *J. Agric. Food Chem.* **2018**, *66*, 2281–2286.

77. Sefton, M. A.; Skouroumounis, G. K.; Elsey, G. M.; Taylor, D. K. Occurrence, sensory impact, formation, and fate of damascenone in grapes, wines, and other foods and beverages. *J. Agric. Food Chem.* **2011**, *59*, 9717–9746.

78. Plotto, A.; Barnes, K. W.; Goodner, K. L. Specific anosmia observed for *β*-Ionone, but not for *α*-Ionone: significance for flavor research. *J. Food Sci.* **2006**, *71*, S401–S406.

79. Jaeger, S. R.; McRae, J. F.; Bava, C. M.; Beresford, M. K.; Hunter, D.; Jia, Y. L.; Chheang, S. L.; Jin, D.; Peng, M.; Gamble, J. C.; Atkinson, K. R.; Axten, L. G.; Paisley, A. G.; Tooman, L.; Pineau, B.; Rouse, S. A.; Newcomb, R. D. A Mendelian trait for olfactory sensitivity affects odor experience and food selection. *Curr. Biol.* **2013**, *23*, 1601–1605.

80. Sacks, G. L.; Gates, M. J.; Ferry, F. X.; Lavin, E. H.; Kurtz, A. J.; Acree, T. E. Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and concentrations in young Riesling and non-Riesling wines. *J. Agric. Food Chem.* **2012**, *60*, 2998–3004.

81. Winterhalter, P. 1,1,6-Trimethyl-1, 2-dihydronaphthalene (TDN) formation in wine. 1. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol rationalizing the origin of TDN and related $C_{13}$ norisoprenoids in Riesling wine. *J. Agric. Food Chem.* **1991**, *39*, 1825–1829.

82. Gök, R.; Bechtloff, P.; Ziegler, M.; Schmarr, H. G.; Fischer, U.; Winterhalter, P., Synthesis of deuterium-labeled 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and quantitative determination of TDN and isomeric vitispiranes in Riesling wines by a stable-isotope-dilution assay. *J. Agric. Food Chem.* **2019**, *67*, 6414–6422.

83. Janusz, A.; Capone, D. L.; Puglisi, C. J.; Perkins, M. V.; Elsey, G. M.; Sefton, M. A. (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene: a potent grape-derived odorant in wine. *J. Agric. Food Chem.* **2003**, *51*, 7759–7763.

84. Cox, A.; Capone, D. L.; Elsey, G. M.; Perkins, M. V.; Sefton, M. A. Quantitative analysis, occurrence, and stability of (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene in wine. *J. Agric. Food Chem.* **2005**, *53*, 3584–3591.

85. Ziegler, M.; Gök, R.; Bechtloff, P.; Winterhalter, P.; Schmarr, H. G.; Fischer, U. Impact of matrix variables and expertise of panelists on sensory thresholds of 1,1,6-trimethyl-1,2-dihydronaphthalene known as petrol off-flavor compound in Riesling wines. *Food Qual. and Prefer.* **2019**, *78*, 103725.

86. Tominaga, T.; Murat, M. L.; Dubourdieu, D. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **1998**, *46*, 1044–1048.

87. Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109–112.

88. Pickering, G. J.; Karthik, A.; Inglis, D.; Sears, M.; Ker, K. Determination of ortho- and retronasal detection thresholds for 2-isopropyl-3-methoxypyrazine in wine. *J. Food Sci.* **2007**, *72*, S468–S472.

89. Swiegers, J. H.; Capone, D. L.; Pardon, K. H.; Elsey, G. M.; Sefton, M. A.; Francis, I. L.; Pretorius, I. S. Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. *Yeast* **2007**, *24*, 561–574.

90. Swiegers, J. H.; Pretorius, I. S. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 954–960.

91. Roland, A.; Schneider, R.; Razungles, A.; Cavelier, F. Varietal thiols in wine: discovery, analysis and applications. *Chem. Rev.* **2011**, *111*, 7355–7376.

92. Ferreira, V.; Lopez, R. The actual and potential aroma of winemaking grapes. *Biomolecules* **2019**, *9*, 818.

93. Ruiz, J.; Kiene, F.; Belda, I.; Fracassetti, D.; Marquina, D.; Navascués, E.; Calderón, F.; Benito, A.; Rauhut, D.; Santos, A.; Benito, S. Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 7425–7450.

94. Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.

95. Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification of 3-mercaptohexyl acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor. *Vitis* **1996**, *35*, 207–210.

96. Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.

97. Capone, D. L.; Sefton, M. A.; Jeffery, D. W. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *J. Agric. Food Chem.* **2011**, *59*, 4649–4658.

98. Capone, D. L.; Pardon, K. H.; Cordente, A. G.; Jeffery, D. W. Identification and quantitation of 3-*S*-cysteinyiglycinehexan-1-ol (Cysgly-3-MH) in Sauvignon blanc grape juice by HPLC-MS/MS. *J. Agric. Food Chem.* **2011**, *59*, 11204–11210.

99. Bonnaffoux, H.; Roland, A.; Rémond, E.; Delpech, S.; Schneider, R.; Cavelier, F. First identification and quantification of *S*-3-(hexan-1-ol)-γ-glutamyl-cysteine in grape must as a potential thiol precursor, using UPLC-MS/MS analysis and stable isotope dilution assay. *Food Chem.* **2017**, *237*, 877–886.

100. Lei, Y. J.; Xie, S.; Guan, X. Q.; Song, C. Z.; Zhang, Z. W.; Meng, J. F. Methoxypyrazines biosynthesis and metabolism in grape: a review. *Food Chem.* **2018**, *245*, 1141–1147.

101. Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Factors affecting the presence of 3-alkyl-2-methoxypyrazines in grapes and wines. A review. *J. Agric. Food Chem.* **2004**, *55*, 153–159.

102. Sidhu, D.; Lund, J.; Kotseridis, Y.; Saucier, C. Methoxypyrazine analysis and influence of viticultural and enological procedures on their levels in grapes, musts, and wines. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 485–502.

103. de Boubée, D. R.; Van Leeuwen, C.; Dubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834.

104. Villamor, R. R.; Ross, C. F. Wine matrix compounds affect perception of wine aromas. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 1–20.

105. Zhao, X. F.; Ju, Y. L.; Wei, X. F.; Dong, S.; Sun, X. Y.; Fang, Y. L. Significance and transformation of 3-alkyl-2-methoxypyrazines through grapes to wine: olfactory properties, metabolism, biochemical regulation, and the HP-MP cycle. *Molecules* **2019**, *24*, 4598.

106. Kotseridis, Y.; Baumes, R. L.; Bertrand, A.; Skouroumounis, G. K. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chromatogr. A* **1999**, *841*, 229–237.

107. Pickering, G. J.; Spink, M.; Kotseridis, Y.; Inglis, D.; Brindle, I. D.; Sears, M.; Beh, A. L. Yeast strain affects 3-isopropyl-2-methoxypyrazine concentration and sensory profile in Cabernet Sauvignon wine. *Aust. J. Grape Wine Res.* **2008**, *14*, 230–237.

108. Allen, M.; Lacey, M. Methoxypyrazines of grapes and wines. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Publications: ACS Symposium Series 714; American Chemical Society: Washington, DC, USA, 1998; pp 31–38.

109. Hjelmeland, A. K.; Wylie, P. L.; Ebeler, S. E. A comparison of sorptive extraction techniques coupled to a new quantitative, sensitive, high throughput GC–MS/MS method for methoxypyrazine analysis in wine. *Talanta* **2016**, *148*, 336–345.

110. Belda, I.; Ruiz, J.; Esteban-Fernández, A.; Navascués, E.; Marquina, D.; Santos, A.; Moreno-Arribas, M. V. Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules* **2017**, *22*, 189.

111. Carpena, M.; Fraga-Corral, M.; Otero, P.; Nogueira, R. A.; Garcia-Oliveira, P.; Prieto, M. A.; Simal-Gandara, J. Secondary aroma: influence of wine microorganisms in their aroma profile. *Foods* **2021**, *10*, 51.

112. Bartowsky, E. J.; Borneman, A. R. Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wine. *Appl. Microbiol. Biotechnol.* **2011**, *92*, 441–447.

113. Cappello, M. S.; Zapparoli, G.; Logrieco, A.; Bartowsky, E. J. Linking wine lactic acid bacteria diversity with wine aroma and flavour. *Int. J. Food Microbiol.* **2017**, *243*, 16–27.

114. Styger, G.; Prior, B.; Bauer, F. F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1145–1159.

115. Virdis, C.; Sumby, K.; Bartowsky, E.; Jiranek, V. Lactic acid bacteria in wine: technological advances and evaluation of their functional role. *Front. Microbiol.* **2021**, *11*, 612118.

116. Ebeler, S. E.; Thorngate, J. H. Wine chemistry and flavor: looking into the crystal glass. *J. Agric. Food Chem.* **2009**, *57*, 8098–8108.

117. Juan, F. S.; Cacho, J.; Ferreira, V.; Escudero, A. Aroma chemical composition of red wines from different price categories and its relationship to quality. *J. Agric. Food Chem.* **2012**, *60*, 5045–5056.

118. Ferreira, V.; Ortin, N.; Escudero, A.; López, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048–4054.

119. Hazelwood, L. A.; Daran, J. M.; van Maris, A. J. A.; Pronk, J. T.; Dickinson, J. R. The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. *Appl. Environ. Microbiol.* **2008**, *74*, 2259–2266.

120. Fernández-González, M.; Di Stefano, R.; Briones, A. Hydrolysis and transformation of terpene glycosides from muscat must by different yeast species. *Food Microbiol.* **2003**, *20*, 35–41.

121. Fernández-González, M.; Di Stefano, R. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces cerevisiae*. *LWT-Food Sci. Technol.* **2004**, *37*, 467–473.

122. Ghaste, M.; Narduzzi, L.; Carlin, S.; Vrhovsek, U.; Shulaev, V.; Mattivi, F. Chemical composition of volatile aroma metabolites and their glycosylated precursors that can uniquely differentiate individual grape cultivars. *Food Chem.* **2015**, *188*, 309–319.

123. Bell, S. J.; Henschke, P. A. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* **2005**, *11*, 242–295.

124. Waterhouse, A. L.; Sacks, G. L.; Jeffery, D. W. Understanding wine chemistry; 1st ed.;. John Wiley & Sons, Inc.: Chichester, UK, 2016; pp 205–213

125. Carpena, M.; Pereira, A. G.; Prieto, M. A.; Simal-Gandara, J. Wine aging technology: fundamental role of wood barrels. *Foods* **2020**, *9*, 1160.

126. Tao, Y.; García, J. F.; Sun, D. W. Advances in wine aging technologies for enhancing wine quality and accelerating wine aging process. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 817–835.

127. Echave, J.; Barral, M.; Fraga-Corral, M.; Prieto, M. A.; Simal-Gandara, J. Bottle aging and storage of wines: a review. *Molecules* **2021**, *26*, 713.

128. De Rosso, M.; Panighel, A.; Vedova, A. D.; Stella, L.; Flamini, R. Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels. *J. Agric. Food Chem.* **2009**, *57*, 1915–1920.

129. de Simón, B. F.; Martínez, J.; Sanz, M.; Cadahía, E.; Esteruelas, E.; Muñoz, A. M. Volatile compounds and sensorial characterisation of red wine aged in cherry, chestnut, false acacia, ash and oak wood barrels. *Food Chem.* **2014**, *147*, 346–356.

130. de Simón, B. F.; Sanz, M.; Cadahía, E.; Martínez, J.; Esteruelas, E.; Muñoz, A. M., Polyphenolic compounds as chemical markers of wine ageing in contact with cherry, chestnut, false acacia, ash and oak wood. *Food Chem.* **2014**, *143*, 66–76.

131. Wilker, K. L.; Gallander, J. F. Comparison of Seyval blanc wine aged in barrels and stainless steel tanks with oak chips. *Am. J. Enol. Vitic.* **1988**, *39*, 38–43.

132. Cano-López, M.; Bautista-Ortín, A. B.; Pardo-Mínguez, F.; López-Roca, J. M.; Gómez-Plaza, E. Sensory descriptive analysis of a red wine aged with oak chips in stainless steel tanks or used barrels: effect of the contact time and size of the oak chips. *J. Food Qual.* **2008**, *31*, 645–660.

133. Bautista-Ortín, A. B.; Lencina, A. G.; Cano-López, M.; Pardo-Mínguez, F.; López-Roca, J. M.; Gómez-Plaza, E. The use of oak chips during the ageing of a red wine in stainless steel tanks or used barrels: effect of the contact time and size of the oak chips on aroma compounds. *Aust. J. Grape Wine Res.* **2008**, *14*, 63–70.

134. Santos, M. C.; Nunes, C.; Ferreira, A. S.; Jourdes, M.; Teissedre, P. L.; Rodrigues, A.; Amado, O.; Saraiva, J. A.; Coimbra, M. A. Comparison of high pressure treatment with conventional red wine aging processes: impact on phenolic composition. *Food Res. Int.* **2019**, *116*, 223–231.

135. Garde-Cerdán, T.; Ancín-Azpilicueta, C. Review of quality factors on wine ageing in oak barrels. *Trends Food Sci. Technol.* **2006**, *17*, 438–447.

136. Pérez-Prieto, L. J.; López-Roca, J. M.; Martínez-Cutillas, A.; Mínguez, F. P.; Gómez-Plaza, E. Maturing wines in oak barrels. Effects of origin, volume, and age of the barrel on the wine volatile composition. *J. Agric. Food Chem.* **2002**, *50*, 3272–3276.

137. Spillman, P. J.; Sefton, M. A.; Gawel, R. The effect of oak wood source, location of seasoning and coopering on the composition of volatile compounds in oak-matured wines. *Aust. J. Grape Wine Res.* **2004**, *10*, 216–226.

138. Jarauta, I.; Cacho, J.; Ferreira, V. Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: an analytical study. *J. Agric. Food Chem.* **2005**, *53*, 4166–4177.

139. Martínez-Gil, A. M.; del Alamo-Sanza, M.; del Barrio-Galán, R.; Nevares, I. Alternative woods in oenology: volatile compounds characterisation of woods with respect to traditional oak and effect on aroma in wine, a review. *Appl. Sci.* **2022**, *12*, 2101.

140. de Simón, B. F.; Cadahía, E.; del Álamo, M.; Nevares, I. Effect of size, seasoning and toasting in the volatile compounds in toasted oak wood and in a red wine treated with them. *Anal. Chim. Acta* **2010**, *660*, 211–220.

141. Martínez-Gil, A. M.; del Alamo-Sanza, M.; Nevares, I.; Sánchez-Gómez, R.; Gallego, L. Effect of size, seasoning and toasting level of *Quercus pyrenaica* Willd. wood on wine phenolic composition during maturation process with micro-oxygenation. *Food Res. Int.* **2020**, *128*, 108703.

142. Wilkinson, K. L.; Elsey, G. M.; Prager, R. H.; Tanaka, T.; Sefton, M. A. Precursors to oak lactone. Part 2: synthesis, separation and cleavage of several $\beta$-D-glucopyranosides of 3-methyl-4-hydroxyoctanoic acid. *Tetrahedron* **2004**, *60*, 6091–6100.

143. Brown, R. C.; Sefton, M. A.; Taylor, D. K.; Elsey, G. M. An odour detection threshold determination of all four possible stereoisomers of oak lactone in a white and a red wine. *Aust. J. Grape Wine Res.* **2006**, *12*, 115–118.

144. Wilkinson, K. L.; Prida, A.; Hayasaka, Y. Role of Glycoconjugates of 3-methyl-4-hydroxyoctanoic acid in the evolution of oak lactone in wine during oak maturation. *J. Agric. Food Chem.* **2013**, *61*, 4411–4416.

145. Hayasaka, Y.; Wilkinson, K. L.; Elsey, G. A.; Raunkjær, M.; Sefton, M. A. Identification of natural oak lactone precursors in extracts of American and French oak woods by liquid chromatography–tandem mass Spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 9195–9201.

146. Maga, J. A. Oak lactones in alcoholic beverage. *Food Rev. Int.* **1996**, *12*, 105–130.

147. Arapitsas, P.; Antonopoulos, A.; Stefanou, E.; Dourtoglou, V. G. Artificial aging of wines using oak chips. *Food Chem.* **2004**, *86*, 563–570.

148. Spillman, P. J.; Pollnitz, A. P.; Liacopoulos, D.; Skouroumounis, G. K.; Sefton, M. A. Accumulation of vanillin during barrel-aging of white, red, and model wines. *J. Agric. Food Chem.* **1997**, *45*, 2584–2589.

149. Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Pons, M. The origin of ethylphenols in wines. *J. Sci. Food Agric.* **1992**, *60*, 165–178.

150. Suárez, R.; Suárez-Lepe, J. A.; Morata, A.; Calderón, F. The production of ethylphenols in wine by yeasts of the genera *Brettanomyces* and *Dekkera*: a review. *Food Chem.* **200**7, *102*, 10–21.

151. Hernanz, D.; Gallo, V.; Recamales, A. F.; Meléndez-Martínez, A. J.; González-Miret, M. L.; Heredia, F. J. Effect of storage on the phenolic content, volatile composition and

colour of white wines from the varieties Zalema and Colombard. *Food Chem.* **2009**, *113*, 530–537.

152. Mas, A.; Puig, J.; Lladó, N.; Zamora, F. Sealing and storage position effects on wine evolution. *J. Food Sci.* **2002**, *67*, 1374–1378.
153. Silva, M. A.; Julien, M.; Jourdes, M.; Teissedre, P. L. Impact of closures on wine post-bottling development: a review. *Eur. Food Res. Technol.* **2011**, *233*, 905–914.
154. Han, G. M.; Ugliano, M.; Currie, B.; Vidal, S.; Diéval, J. B.; Waterhouse, A. L. Influence of closure, phenolic levels and microoxygenation on Cabernet Sauvignon wine composition after 5 years' bottle storage. *J. Sci. Food Agric.* **2015**, *95*, 36–43.
155. Ugliano, M. Oxygen contribution to wine aroma evolution during bottle aging. *J. Agric. Food Chem.* **2013**, *61*, 6125–6136.
156. Oliveira, C. M.; Ferreira, A. C. S.; De Freitas, V.; Silva, A. M. S. Oxidation mechanisms occurring in wines. *Food Res. Int.* **2011**, *44*, 1115–1126.
157. Culleré, L.; Cacho, J.; Ferreira, V. An assessment of the role played by some oxidation-related aldehydes in wine aroma. *J. Agric. Food Chem.* **2007**, *55*, 876–881.
158. Bueno, M.; Carrascón, V.; Ferreira, V. Release and formation of oxidation-related aldehydes during wine oxidation. *J. Agric. Food Chem.* **2016**, *64*, 608–617.
159. Ferreira, V.; Bueno, M.; Franco-Luesma, E.; Culleré, L.; Fernández-Zurbano, P. Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *J. Agric. Food Chem.* **2014**, *62*, 10015–10027.
160. Bueno, M.; Marrufo-Curtido, A.; Carrascón, V.; Fernández-Zurbano, P.; Escudero, A.; Ferreira, V. Formation and accumulation of acetaldehyde and strecker aldehydes during red wine oxidation. *Front. Chem.* **2018**, *6*, 20.
161. Pons, A.; Lavigne, V.; Landais, Y.; Darriet, P.; Dubourdieu, D. Identification of a sotolon pathway in dry white wines. *J. Agric. Food Chem.* **2010**, *58*, 7273–7279.
162. Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Lavigne, V. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J. Sci. Food Agric.* **1993**, *62*, 191–202.
163. Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653–1660.
164. Chaves, M.; Zea, L.; Moyano, L.; Medina, M. Changes in color and odorant compounds during oxidative aging of Pedro Ximenez sweet wines. *J. Agric. Food Chem.* **2007**, *55*, 3592–3598.
165. Escudero, A.; Hernández-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.
166. Martin, B.; Etiévant, P. X.; Le Quéré, J. L.; Schlich, P. More clues about sensory impact of sotolon in some flor sherry wines. *J. Agric. Food Chem.* **1992**, *40*, 475–478.

167. Siebert, T.; Bramley, B.; Solomon, M. Hydrogen sulfide: aroma detection threshold study in red and white wine. *AWRI Tech. Rev*. **2009**, *183*, 14–16.

168. Goniak, O. J.; Noble, A. C. Sensory study of selected volatile sulfur compounds in white wine. *Am. J. Enol. Vitic.* **1987**, *38*, 223–227.

169. Tominaga, T.; Blanchard, L.; Darriet, P.; Dubourdieu, D. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2000**, *48*, 1799–1802.

170. Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution of benzenemethanethiol to smoky aroma of certain *Vitis vinifera* L. wines. *J. Agric. Food Chem.* **2003**, *51*, 1373–1376.

171. Ugliano, M.; Kwiatkowski, M.; Vidal, S.; Capone, D.; Siebert, T.; Dieval, J. B.; Aagaard, O.; Waters, E. J. Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc Wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *J. Agric. Food Chem.* **2011**, *59*, 2564–2572.

172. Blanchard, L.; Tominaga, T.; Dubourdieu, D. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. *J. Agric. Food Chem.* **2001**, *49*, 4833–4835.

173. Demora, S. J.; Lee, P.; Shooter, D.; Eschenbruch, R. The analysis and importance of dimethylsulfoxide in wine. *Am. J. Enol. Vitic.* **1993**, *44*, 327–332.

174. Simpson, R. F.; Sefton, M. A. Origin and fate of 2,4,6-trichloroanisole in cork bark and wine corks. *Aust. J. Grape Wine Res.* **2007**, *13*, 106–116.

175. Mazzoleni, V.; Maggi, L. Effect of wine style on the perception of 2,4,6-trichloroanisole, a compound related to cork taint in wine. *Food Res. Int.* **2007**, *40*, 694–699.

176. Reynolds, A. G.; Wardle, D. A.; Dever, M. Vine performance, fruit composition, and wine sensory attributes of Gewürztraminer in response to vineyard location and canopy manipulation. *Am. J. Enol. Vitic.* **1996**, *47*, 77–92.

177. Rusjan, D.; Strlič, M.; Kosmerl, T.; Prosen, H. The response of monoterpenes to different enzyme preparations in Gewürztraminer (*Vitis vinifer*a L.) wines. *S. Afr. J. Enol. Vitic.* **2009**, *30*, 56–64.

178. Liu, Z. Y.; Zhao, M. L.; Li, J. G. Aroma volatiles in litchi fruit: a mini-review. *Horticulturae* **2022**, *8*, 1166.

179. Ong, P. K. C.; Acree, T. E. Gas chromatography/olfactory analysis of lychee (*Litchi chinesis* Sonn.). *J. Agric. Food Chem.* **1998**, *46*, 2282–2286.

180. Wu, Y. W.; Pan, Q. H.; Qu, W. J.; Duan, C. Q. Comparison of volatile profiles of nine litchi (*Litchi chinensis* Sonn.) cultivars from Southern China. *J. Agric. Food Chem.* **2009**, *57*, 9676–9681.

181. Wu, Y. W.; Zhu, B. Q.; Tu, C.; Duan, C. Q.; Pan, Q. H. Generation of volatile compounds in litchi wine during winemaking and short-term bottle storage. *J. Agric. Food Chem.* **2011**, *59*, 4923–4931.

182. An, K. J.; Liu, H. C.; Fu, M. Q.; Qian, M. C.; Yu, Y. S.; Wu, J. J.; Xiao, G. S.; Xu, Y. J. Identification of the cooked off-flavor in heat-sterilized lychee (*Litchi chinensis* Sonn.) juice by means of molecular sensory science. *Food Chem.* **2019**, *301*, 125282.

183. Chen, D.; Liu, S. Q. Chemical and volatile composition of lychee wines fermented with four commercial *Saccharomyces cerevisiae* yeast strains. *Int. J. Food Sci. Technol.* **2014**, *49*, 521–530.

184. Chen, D.; Chia, J. Y.; Liu, S. Q. Impact of addition of aromatic amino acids on non-volatile and volatile compounds in lychee wine fermented with *Saccharomyces cerevisiae* MERIT.ferm. *Int. J. Food Microbiol.* **2014**, *170*, 12–20.

185. Chen, D.; Vong, W. C.; Liu, S. Q. Effects of branched-chain amino acid addition on chemical constituents in lychee wine fermented with *Saccharomyces cerevisiae*. *Int. J. Food Sci. Technol.* **2015**, *50*, 2519–2528.

186. Chen, D.; Liu, S. Q. Impact of simultaneous and sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on non-volatiles and volatiles of lychee wines. *LWT-Food Sci. Technol.* **2016**, *65*, 53–61.

187. Tang, Z. S.; Zeng, X. A.; Brennan, M. A.; Han, Z.; Niu, D. B.; Huo, Y. J. Characterization of aroma profile and characteristic aromas during lychee wine fermentation. *J. Food Process. Preserv.* **2019**, *43*, e14003.

188. Zhao, L. L.; Ruan, S. L.; Yang, X. K.; Chen, Q. L.; Shi, K.; Lu, K.; He, L.; Liu, S. W.; Song, Y. B. Characterization of volatile aroma compounds in litchi (Heiye) wine and distilled spirit. *Food Sci. Nutr.* **2021**, *9*, 5914–5927.

189. Lukić, I.; Radeka, S.; Grozaj, N.; Staver, M.; Peršurić, D. Changes in physico-chemical and volatile aroma compound composition of Gewürztraminer wine as a result of late and ice harvest. *Food Chem.* **2016**, *196*, 1048–1057.

190. Li, X. Y.; Wen, Y. Q.; Meng, N.; Qian, X.; Pan, Q. H. Monoterpenyl glycosyltransferases differentially contribute to production of monoterpenyl glycosides in two aromatic *Vitis vinifera* varieties. *Front. Plant Sci.* **2017**, *8*, 1226.

191. Luan, F.; Mosandl, A.; Múnch, A.; Wüst, M. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, *E*/*Z*-isomerization, oxidation and glycosylation. *Phytochemistry* **2005**, *66*, 295–303.

192. Luan, F.; Mosandl, A.; Gubesch, M.; Wüst, M. Enantioselective analysis of monoterpenes in different grape varieties during berry ripening using stir bar sorptive extraction- and solid phase extraction-enantioselective-multidimensional gas chromatography–mass spectrometry. *J. Chromatogr. A.* **2006**, *1112*, 369–374.

193. Fenoll, J.; Manso, A.; Hellín, P.; Ruiz, L.; Flores, P. Changes in the aromatic composition of the *Vitis vinifera* grape Muscat Hamburg during ripening. *Food Chem.* **2009**, *114*, 420–428.

194. Ruiz-García, L.; Hellín, P.; Flores, P.; Fenoll, J. Prediction of Muscat aroma in table grape by analysis of rose oxide. *Food Chem.* **2014**, *154*, 151–157.

195. Wüst, M.; Mosandl, A. Important chiral monoterpenoid ethers in flavours and essential oils -enantioselective analysis and biogenesis. *Eur. Food Res. Technol.* **1999**, *209*, 3–11.

196. Yamamoto, T.; Matsuda, H.; Utsumi, Y.; Hagiwara, T.; Kanisawa, T. Synthesis and odor of optically active rose oxide. *Tetrahedron Lett.* **2002**, *43*, 9077–9080.

197. Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.

198. Maul, E.; Töpfer, R.; Röckel, F.; Brühl, U.; Hundemer, M.; Mahler-Ries, A.; Walk, M.; Kecke, S.; Wolck, A.; Ganesch, A. Vitis International Variety Catalogue. Julius Kühn-Institute (JKI) - Federal Research Center for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof: Siebeldingen, Germany, 2023. https://www.vivc.de/ (accessed December 10, 2024).

199. Fungus-resistant grape varieties. State Institute of Viticulture Freiburg, Freiburg, Germany, 2023. https://wbi.landwirtschaft-bw.de/pb/site/pbs-bw-mlr/get/documents_E-1412531467/MLR.LEL/PB5Documents/wbi/051%20Aufgaben%20und%20Fachbereiche/0513%20Fachbereiche/05136%20Referat%20Resistenz%20und%20Klonenz%C3%BCchtung/051325%20Listen%20zu%20PIWI%20Keltertrauben/WBI_Fungus_resistant_grape_varieties_EN.pdf (accessed December 10, 2024).

200. Land und Forstwirtschaft, Fischerei: Landwirtschaftliche Bodennutzung-Rebflächen-Fachserie 3 Reihe 3.1.5-2022. Federal Statistical Office (Destatis), Wiesbaden, Germany, 2023. https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Wein/Publikationen/Downloads-Wein/rebflaechen-2030315227004.pdf?__blob=publicationFile (accessed December 10, 2024).

201. van Wyk, N.; Pretorius, I. S.; von Wallbrunn, C. Assessing the oenological potential of *Nakazawaea ishiwadae*, *Candida railenensis* and *Debaryomyces hansenii* strains in mixed-culture grape must fermentation with *Saccharomyces cerevisiae*. *Fermentation*. **2020**, *6*, 49.

202. van Wyk, N.; Scansani, S.; Beisert, B.; Brezina, S.; Fritsch, S.; Semmler, H.; Pretorius, I. S.; Rauhut, D.; von Wallbrunn, C. The use of *Hanseniaspora occidentalis* in a sequential must inoculation to reduce the malic acid content of wine. *Appl. Sci.* **2022**, *12*, 6919.

203. Barnaba, C.; Dellacassa, E.; Nicolini, G.; Giacomelli, M.; Villegas, T. R.; Nardin, T.; Larcher, R. Targeted and untargeted high resolution mass approach for a putative profiling of glycosylated simple phenols in hybrid grapes. *Food Res. Int.* **2017**, *98*, 20–33.

204. Barth-Haas Group. Beer production worldwide from 1998 to 2022 (in billion hectoliters). Statista, Hamburg, Germany, 2023. https://www.statista.com/statistics/270275/worldwide-beer-production/ (accessed December 10, 2024)

205. Schieberle, P. Primary odorants of pale lager beer. Differences to other beers and changes during storage. *Z. Lebensm.-Unters. Forsch.* **1991**, *193*, 558–565.

206. Kishimoto, T.; Wanikawa, A.; Kono, K.; Shibata, K. Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *J. Agric. Food Chem.* **2006**, *54*, 8855–8861.

207. Van Opstaele, F.; De Rouck, G.; Janssens, P.; Montandon, G. An exploratory study on the impact of the yeast strain on hop flavour expressions in heavily hopped beers: New England IPA. *Brew. Sci.* **2020**, *73*, 26–40.

208. Neiens, S. D.; Steinhaus, M. Investigations on the impact of the special flavor hop variety Huell Melon on the odor-active compounds in late hopped and dry hopped beers. *J. Agric. Food Chem.* **2019**, *67*, 364–371.

209. Saison, D.; De Schutter, D. P.; Vanbeneden, N.; Daenen, L.; Delvaux, F.; Delvaux, F. R. Decrease of aged beer aroma by the reducing activity of brewing yeast. *J. Agric. Food Chem.* **2010**, *58*, 3107–3115.

210. Langos, D.; Granvogl, M.; Schieberle, P. Characterization of the key aroma compounds in two Bavarian wheat beers by means of the sensomics approach. *J. Agric. Food Chem.* **2013**, *61*, 11303–11311.

211. Langos, D.; Granvogl, M. Studies on the simultaneous formation of aroma-active and toxicologically relevant vinyl aromatics from free phenolic acids during wheat beer brewing. *J. Agric. Food Chem.* **2016**, *64*, 2325–2332.

212. Yin, H.; Dong, J. J.; Yu, J. H.; Chang, Z. M.; Qian, Z. H.; Liu, M. L.; Huang, S. L.; Hu, X. C.; Liu, X. L.; Deng, Y.; Wang, D. F. A preliminary study about the influence of high hydrostatic pressure processing on the physicochemical and sensorial properties of a cloudy wheat beer. *J. Inst. Brew.* **2016**, *122*, 462–467.

213. Scholtes, C.; Nizet, S.; Collin, S. Guaiacol and 4-methylphenol as specific markers of torrefied malts. fate of volatile phenols in special beers through aging. *J. Agric. Food Chem.* **2014**, *62*, 9522–9528.

214. Steinhaus, M.; Fritsch, H. T.; Schieberle, P. Quantitation of (*R*)- and (*S*)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100–7105.

215. Peacock, V. E.; Deinzer, M. L.; Likens, S. T.; Nickerson, G. B.; McGill, L. A. Floral hop aroma in beer. *J. Agric. Food Chem.* **1981**, *29*, 1265–1269.

216. Rettberg, N.; Biendl, M.; Garbe, L. A. Hop aroma and hoppy beer flavor: chemical backgrounds and analytical tools–a review. *J. Am. Soc. Brew. Chem.* **2018**, *76*, 1–20.

217. Forster, A.; Gahr, A. On the fate of certain hop substances during dry hopping. *Brew. Sci*. **2013**, *66*, 93–103.

218. Reglitz, K.; Lemke, N.; Hanke, S.; Steinhaus, M. On the behavior of the important hop odorant 4-mercapto-4-methylpentan-2-one (4MMP) during dry hopping and during storage of dry hopped beer. *Brew. Sci.* **2018**, *71*, 96–99.

219. Lermusieau, G.; Bulens, M.; Collin, S. Use of GC–Olfactometry to identify the hop aromatic compounds in beer. *J. Agric. Food Chem.* **2001**, *49*, 3867–3874.

220. Reglitz, K.; Féchir, M.; Mall, V.; Voigt, J.; Steinhaus, M. The impact of caramel and roasted wheat malts on aroma compounds in top-fermented wheat beer. *J. Inst. Brew.* **2022**, *128*, 138–149.

221. Fritsch, H. T.; Schieberle, P. Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. *J. Agric. Food Chem.* **2005**, *53*, 7544–7551.

222. Schönberger, C.; Kostelecky, T. 125th Anniversary review: the role of hops in brewing. *J. Inst. Brew.* **2011**, *117*, 259–267.

223. Baert, J. J.; De Clippeleer, J.; Hughes, P. S.; De Cooman, L.; Aerts, G. On the origin of free and bound staling aldehydes in beer. *J. Agric. Food Chem.* **2012**, *60*, 11449–11472.

224. Wietstock, P. C.; Kunz, T.; Methner, F. J. Relevance of oxygen for the formation of strecker aldehydes during beer production and storage. *J. Agric. Food Chem.* **2016**, *64*, 8035–8044.

225. Kobayashi, M.; Shimizu, H.; Shioya, S. Beer volatile compounds and their application to low-malt beer fermentation. *J. Biosci. Bioeng.* **2008**, *106*, 317–323.

226. San-Juan, F.; Cacho, J.; Ferreira, V.; Escudero, A. 3-Methyl-2-butene-1-thiol: identification, analysis, occurrence and sensory role of an uncommon thiol in wine. *Talanta* **2012**, *99*, 225–231.

227. Frank, S.; Schieberle, P. Changes in the major odorants of grape juice during manufacturing of Dornfelder red wine. *J. Agric. Food Chem.* **2022**, *70*, 13979–13986.

228. Ferreira, V. Volatile aroma compounds and wine sensory attributes. In *Managing Wine Quality: Viticulture and Wine Quality*; Reynolds, A. G., Ed.; Woodhead Publishing: Cambridge, UK, 2010; pp 3–28.

229. de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Ferreira, V. On the effects of higher alcohols on red wine aroma. *Food Chem.* **2016**, *210*, 107–114.

230. Ferreira, V.; Sáenz-Navajas, M. P.; Campo, E.; Herrero, P.; de la Fuente, A.; Fernández-Zurbano, P. Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. *Food Chem.* **2016**, *199*, 447–456.

231. de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Ferreira, V. Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions. *Aust. J. Grape Wine Res.* **2017**, *23*, 162–169.

232. de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Valentin, D.; Ferreira, V. Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. *Food Chem.* **2020**, *307*, 125553.

233. Arias-Pérez, I.; Sáenz-Navajas, M. P.; de-la-Fuente-Blanco, A.; Ferreira, V.; Escudero, A. Insights on the role of acetaldehyde and other aldehydes in the odour and tactile nasal perception of red wine. *Food Chem.* **2021**, *361*, 130081.

234. Kalua, C. M.; Boss, P. K. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2009**, *57*, 3818–3830.

235. Kreissl, J.; Schieberle, P. Characterization of aroma-active compounds in Italian tomatoes with emphasis on new odorants. *J. Agric. Food Chem.* **2017**, *65*, 5198–5208.

236. Sellami, I.; Mall, V.; Schieberle, P. Changes in the key odorants and aroma profiles of hamlin and valencia orange juices not from concentrate (NFC) during chilled storage. *J. Agric. Food Chem.* **2018**, *66*, 7428–7440.

237. Luan, F.; Hampel, D.; Mosandl, A.; Wüst, M. Enantioselective analysis of free and glycosidically bound monoterpene polyols in *Vitis vinifera* L. cvs. Morio Muscat and Muscat Ottonel: evidence for an oxidative monoterpene metabolism in grapes. *J. Agric. Food Chem.* **2004**, *52*, 2036–2041.

238. Schaller, T.; Schieberle, P. Quantitation of key aroma compounds in fresh, raw ginger (*Zingiber officinale* Roscoe) from China and roasted ginger by stable isotope dilution assays and aroma profiling by recombination experiments. *J. Agric. Food Chem.* **2020**, *68*, 15284–15291.

239. Río Segade, S.; Vilanova, M.; Pollon, M.; Giacosa, S.; Torchio, F.; Rolle, L. Grape VOCs response to postharvest short-term ozone treatments. *Front. Plant Sci.*, **2018**, *9*, 1826.

240. Ribéreau-Gayon, P.; Boidron, J. N.; Terrier, A. Aroma of Muscat grape varieties. *J. Agric. Food Chem.*, **1975**, *23*, 1042–1047.

241. Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components cv Muscat during first development and maturation. *J. Sci. Food Agric.*, **1985**, *36*, 857–862.

242. Matarese, F.; Cuzzola, A.; Scalabrelli, G.; D'Onofrio, C. Expression of terpene synthase genes associated with the formation of volatiles in different organs of *Vitis vinifera*. *Phytochemistry*, **2014**, *105*, 12–24.

243. Procopio, S.; Qian, F.; Becker, T. Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. *Eur. Food Res. Technol.* **2011**, *233*, 721–729.

244. Olaniran, A. O.; Hiralal, L.; Mokoena, M. P.; Pillay, B. Flavour-active volatile compounds in beer: production, regulation and control. *J. Inst. Brew.* **2017**, *123*, 13–23.

245. Holt, S.; Miks, M. H.; de Carvalho, B. T.; Foulquié-Moreno, M. R.; Thevelein, J. M. The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiol. Rev.* **2019**, *43*, 193–222.

246. Castejón-Musulén, O.; Aragón-Capone, A. M.; Ontañón, I.; Peña, C.; Ferreira, V.; Bueno, M. Accurate quantitative determination of the total amounts of strecker aldehydes contained in wine. Assessment of their presence in table wines. *Food Res. Int.* **2022**, *162*, 112125.

247. Wang, X. J.; Frank, S.; Steinhaus, M. Molecular insights into the aroma difference between beer and wine: a meta-analysis-based sensory study using concentration leveling tests. *J. Agric. Food Chem.* **2024**, *72*, 22250–22257.

248. Pang, X.; Guo, X.; Qin, Z.; Yao, Y.; Hu, X.; Wu, J. Identification of aroma-active compounds in Jiashi muskmelon juice by GC–O–MS and OAV calculation. *J. Agric. Food Chem.* **2012**, *60*, 4179–4185.

249. Mall, V.; Schieberle, P. Evaluation of key aroma compounds in processed prawns (whiteleg shrimp) by quantitation and aroma recombination experiments. *J. Agric. Food Chem.* **2017**, *65*, 2776–2783.

250. Yilmaztekin, M. Characterization of potent aroma compounds of cape gooseberry (*Physalis peruviana* L.) fruits grown in Antalya through the determination of odor activity values. *Int. J. Food Prop.* **2014**, *17*, 469–480.

251. Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006–1009.

252. Niu, Y.; Wang, P.; Xiao, Z.; Zhu, J.; Sun, X.; Wang, R. Evaluation of the perceptual interaction among ester aroma compounds in cherry wines by GC–MS, GC–O, odor threshold and sensory analysis: an insight at the molecular level. *Food Chem.* **2019**, *275*, 143–153.

253. Giri, A.; Osako, K.; Ohshima, T. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chem.* **2010**, *120*, 621–631.

254. Marrufo-Curtido, A.; Ferreira, V.; Escudero, A. Factors that affect the accumulation of strecker aldehydes in standardized wines: the importance of pH in oxidation. *Molecules* **2022**, *27*, 3056.

255. Ickes, C. M.; Cadwallader, K. R. Effects of ethanol on flavor perception in alcoholic beverages. *Chem. Percept.* **2017**, *10*, 119–134.

256. Sáenz-Navajas, M. P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010**, *58*, 5574–5585.

257. Frank, S.; Wollmann, N.; Schieberle, P.; Hofmann, T. Reconstitution of the flavor signature of Dornfelder red wine on the basis of the natural concentrations of its key aroma and taste compounds. *J. Agric. Food Chem.* **2011**, *59*, 8866–8874.

# 7 Appendix

## 7.1 Publication 1: Molecular Background of the Lychee Aroma of *Vitis vinifera* L. ‘Muscaris’

### 7.1.1 Bibliographic Data

Title: Molecular Background of the Lychee Aroma of *Vitis vinifera* L. ‘Muscaris’

Authors: Xingjie Wang, Stephanie Frank, Martin Steinhaus

Journal: Journal of Agricultural and Food Chemistry

Publisher: American Chemical Society

Publication date: January 12, 2024 (online)

Issue date: January 24, 2024

Volume: 72

Issue: 3

Pages: 1674−1682

DOI: https://doi.org/10.1021/acs.jafc.3c08298

Hyperlink: https://pubs.acs.org/doi/full/10.1021/acs.jafc.3c08298

### 7.1.2 Publication Reprint

For a reprint of publication 1, please turn to the next page.

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

pubs.acs.org/JAFC

Article

# Molecular Background of the Lychee Aroma of *Vitis vinifera* L. 'Muscaris'

Xingjie Wang, Stephanie Frank,* and Martin Steinhaus*

**Cite This:** *J. Agric. Food Chem.* 2024, 72, 1674−1682

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Muscaris is a modern white grape variety with good fungal resistance and a pleasant aroma, the molecular background of which was unknown. A comparative aroma extract dilution analysis applied to Muscaris grapes and grapes of the father variety Muskateller revealed little differences and resulted in 39 and 35 odorants, respectively. Sixteen odorants exceeded their odor threshold concentrations. Odor reconstitution and omission experiments showed that the distinct lychee note in the aroma of the Muscaris grapes was generated by the combination of (2*S*,4*R*)-rose oxide and geraniol. This finding will guide further molecular research on the transfer of the lychee note into wine and may also be helpful for the targeted breeding of new grape varieties.

**KEYWORDS:** *Muscaris, Muskateller, Vitis vinifera L., lychee aroma, (2S,4R)-rose oxide, geraniol, aroma extract dilution analysis (AEDA), stable isotopically substituted odorant, odor activity value (OAV), odor reconstitution*

## ■ INTRODUCTION

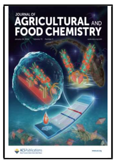
Muscaris is a relatively new *Vitis vinifera* variety with an intense aroma and good resistance to downy mildew, powdery mildew, and botrytis. Muscaris is early ripening. The berries remain green, even at high must weights. It is particularly suitable for producing dessert wine and dry wine.[1] The cultivation area of Muscaris increased in recent years, reaching 117 ha in Germany in 2022.[2]

Muscaris was bred at the State Institute of Viticulture Freiburg, Germany, in 1987 from the mother variety Solaris and the father variety Gelber Muskateller, also known as Muscat à petits grains blancs or Muscat Blanc,[3] with the idea to combine the disease tolerance and environmental adaptability of Solaris with the intense aroma of Gelber Muskateller. Solaris is a white grape variety bred in 1975, whose fungal resistance originates from the wild Asian species *Vitis amurensis*.[3,4] Actually, it was shown that Muscaris has a similar resistance to downy mildew, powdery mildew, and botrytis as Solaris.[1,5] Gelber Muskateller is one of the most widely planted white Muscat varieties. It is of Greek origin and has a long cultivation history in Germany. Its fungal resistance is low;[6] however, it is highly appreciated for its floral and fruity notes, which are intense in the grapes[6,7] as well as in the wine.[8,9] Muskateller grapes are often used as a blending partner for other white grape varieties to boost the aroma of wine.[6]

Several studies have been conducted on Muscaris and addressed fungal resistance,[5,10,11] viticultural characteristics,[12,13] mechanical grape properties,[13] and grape composition including phenolic compounds,[14] minerals, and ascorbic acid.[15] Further studies focused on the composition of Muscaris must[10,12] and Muscaris wine.[14,16−20] However, there is currently no information in the literature on odor-active compounds in Muscaris grapes. Some phenolic compounds including 4-ethenylphenol, 4-ethenyl-2-methoxyphenol, 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, and 4-hydroxy-3-ethoxybenzaldehyde were identified as Muscaris grape volatiles, but their odor contribution was not assessed.[14] We found that the aroma of Muscaris grapes is characterized by a distinct lychee note, the molecular background of which piqued our interest. Lychee notes had previously been reported in Muscaris wine and also in Gewürztraminer wine.[1,21−24] A comparative gas chromatography−olfactometry (GC−O) analysis applied to Gewürztraminer wine, fresh lychees, and canned lychees detected 12 odorants common to all three samples.[22] Among them, *cis*-rose oxide, *β*-damascenone, ethyl 2-methylpropanoate, linalool, and geraniol consistently showed concentrations beyond their odor threshold concentrations (OTCs). *cis*-Rose oxide, linalool, and geraniol had also been reported in odor-active amounts, that is in concentrations exceeding their OTCs, in Muskateller grapes and wines.[8,9,25,26]

Given the gaps in the literature detailed above, our aims were to characterize the major odor-active compounds in Muscaris grapes, compare them with the odor-active compounds in the grapes of its father variety, Muskateller, and elucidate the molecular background of the lychee note in the aroma of the Muscaris grapes. This knowledge is a prerequisite for further research, e.g., on odorant transfer from Muscaris grapes into wine and for targeted breeding. Thus, our study included (i) the screening for odor-active compounds in Muscaris and Muskateller grapes by application of a comparative aroma extract dilution analysis (cAEDA), (ii) the quantitation of potent odorants in grapes of both varieties

**Received:** November 8, 2023
**Revised:** December 23, 2023
**Accepted:** December 26, 2023
**Published:** January 12, 2024



followed by the calculation of odor activity values (OAVs), (iii) the verification of the analytical data by sensory evaluation of odor reconstitution models, and finally (iv) the identification of the odorants responsible for the characteristic lychee note via omission experiments.

## ■ MATERIALS AND METHODS

**Grapes.** Muscaris and Muskateller grapes were harvested at the State Institute of Viticulture Freiburg, Germany, in early September 2022 and sent refrigerated to the Leibniz-LSB@TUM within 1 day. Unripe and injured berries were removed. The fresh grapes were directly used for odorant screening, as well as for the quantitation of selected compounds. The residual material was shock-frozen with liquid nitrogen and stored in vacuum-sealed bags at −20 °C.

**Reference Odorants.** The following reference compounds were purchased at the highest purity available from the following commercial sources: **1**, **2**, **6**−**8**, **11**−**18**, **18a**, **19**, **20**, **22**, **24**, **26**−**29**, **31**, **32**, **34**−**39**, rose oxide (mixture of isomers), citronellal (racemic), (3*S*)-citronellal, and *β*-citronellol (racemic) (Merck; Darmstadt, Germany), **5** (TCI; Eschborn, Germany), **21** (Thermo Fisher Scientific; Dreieich, Germany), **23** (Chemos; Altdorf, Germany), and **30** (Cayman Chemicals Company; Ann Arbor, MI, USA). Compound **25** was a gift from Symrise (Holzminden, Germany). Compounds **3**, **4**, and **9** were synthesized according to published procedures.[27−29] Compound **21** was freshly distilled before use. Enantiopure **18b** was obtained from racemic **18** by preparative HPLC.[30]

**Stable Isotopically Substituted Odorants.** Compound ($^2H_{13-14}$)-**5** was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Compounds ($^2H_2$)-**2**, ($^2H_2$)-**4**, ($^2H_2$)-**7**, ($^2H_{2-4}$)-**8**, ($^{13}C_5$)-**9**, ($^2H_{2-4}$)-**10**, ($^2H_3$)-**13**, ($^2H_2$)-**17**, ($^2H_2$)-**18**, ($^2H_2$)-**19**, ($^{13}C_2$)-**21**, ($^2H_3$)-**23**, ($^2H_2$)-**24**, ($^2H_{3-6}$)-**25**, ($^2H_2$)-**26**, ($^{13}C_2$)-**36**, and 2-[($^2H_3$)methoxy]-4-[(1*E*)-prop-1-en-1-yl]phenol were synthesized as detailed in the references provided in the Table S1.

**Miscellaneous Chemicals.** Potassium hydroxide and (2*R*,3*R*)-tartaric acid were purchased from Merck. Dichloromethane (CLN; Freising, Germany) was freshly distilled through a column (120 × 5 cm) packed with Raschig rings prior to use.

**Gas Chromatography.** GC−O analyses were performed by using a GC−O/FID instrument. For GC−MS analyses, three different instruments were used: a GC−MS instrument with an ion trap mass spectrometer, a two-dimensional heart-cut GC−GC−HRMS instrument with an orbitrap mass spectrometer, and a comprehensive two-dimensional GC × GC−MS instrument with a time-of-flight (TOF) mass spectrometer. Details of the GC instruments are available in the Supporting Information

**Enantioselective Odorant Analysis.** Enantioselective analysis of *cis*-rose oxide, citronellal, linalool, and *β*-citronellol was accomplished by combining the GC−O/FID instrument and the GC−MS instrument with a chiral GC column. Citronellal and *β*-citronellol enantiomers were separated with a BGB-174E column, whereas linalool and *cis*-rose oxide enantiomers were separated with a BGB-176 column (both BGB Analytik; Lörrach, Germany). In the case of citronellal, *β*-citronellol, and linalool enantiomers, the elution order was determined by a comparative analysis of the enantiomeric mixture and at least one of the individual enantiomers. The elution order of *cis*-rose oxide enantiomers was determined from chromatographic and sensory information available in the literature.[31−33]

**Aroma Extract Dilution Analysis.** Fresh grapes (200 g) were crushed by hand with the help of a spoon and a kitchen sieve (stainless steel, sieve mesh size of 1 mm), taking care not to break the seeds. After a fixed period (10 min), dichloromethane (400 mL) was added and the mixture was stirred at ambient temperature for 2 h. Skin and seeds were removed with a sieve, and the organic phase was isolated by centrifugation (4500 rpm, 5 °C, 10 min) using a Heraeus Multifuge X3FR (Thermo Fisher Scientific) and dried with anhydrous sodium sulfate. Nonvolatiles were removed by automated solvent-assisted flavor evaporation (aSAFE)[34] at 40 °C using an open/closed time combination for the pneumatic valve of 0.2 s/10 s. The distillate was concentrated to a final volume of 1.0 mL using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device.[35]

The grape volatile isolate was stepwise diluted 1:2 with dichloromethane to obtain dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048 1:4096, 1:8192, 1:16,384, 1:32,768, and 1:65,536. The undiluted sample, as well as each diluted sample, was subjected to GC−O analysis[36] using the GC−O/FID instrument detailed in the Supporting Information with the FFAP column. Each odorant was assigned a flavor dilution (FD) factor, representing the dilution factor of the highest diluted sample in which the odorant was detected at the sniffing port during GC−O.

**Odorant Quantitation.** The workup of the grapes (20−200 g) basically followed the procedure detailed in the AEDA section. In general, the starting material was frozen-thawed grapes. (3*E*)-Hex-3-enal (**3**) and (3*Z*)-hex-3-enal (**4**) were additionally quantitated in fresh grapes. The stable isotopically substituted odorants used as internal standards (Table S2) were added to the dichloromethane portion used for extraction. Depending on the expected target compound concentrations, amounts of the added internal standards varied between 0.001 and 15 *μ*g. The aSAFE distillates were concentrated to final volumes of 0.1−1.0 mL, and the concentrates were analyzed either with the GC−MS system (**2**−**5**, **7**, **8**, **10**, **13**, **17**−**19**, **21**, **23**, **24**, **26**), the two-dimensional heart-cut GC−GC−HRMS system (**9**, **36**), or the comprehensive two-dimensional GC × GC−MS system (**25**, **34**). All quantitations were carried out in duplicate or triplicate analyses.

Peak areas of the analytes and the respective internal standards were collected from the extracted ion chromatograms using the quantifier ions detailed in Table S2. The concentration of each odorant in the grapes was calculated from the area counts of the analyte peak, the area counts of the internal standard peak, the amount of grapes used in the workup, and the amount of internal standard added by employing a calibration line equation. To obtain the calibration line equation, solutions of the analyte and the respective internal standard were mixed in different concentration ratios and analyzed under the same conditions, followed by linear regression. The calibration line equations are available in Table S2. Individual concentrations and standard deviations can be found in Tables S3−S5.

**Odor Threshold Concentrations.** Orthonasal OTCs of (3*E*)-hex-3-enal (**3**), heptanal (**5**), and (2*S*,4*R*)-rose oxide (**10**) were determined in pure water according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[37] Assessors (6 males and 12−14 females, aged 21−60 years) were recruited from the trained panel of the Leibniz-LSB@TUM. The tests were carried out in separate booths of a room exclusively dedicated to sensory evaluations. The room temperature was 22 ± 2 °C. Further details including the GC−O approach for the purity testing of the odorants prior to their use in the OTC determinations are available in the literature.[38]

**Odor Reconstitution Models.** The basis of the odor reconstitution models was aqueous solutions of tartaric acid (3.57 g/L for the Muscaris grape models and 4.23 g/L for the Muskateller grape models). The tartaric acid concentrations in the grapes were previously determined using an enzymatic test kit (R-Biopharm; Darmstadt, Germany). An individual ethanolic stock solution was prepared for each odorant for which an ≥1 OAV had been determined. The absence of odor-active impurities in the reference odorants was checked by GC−O.[38] Aliquots of the stock solutions were combined and diluted with the appropriate tartaric acid solution to obtain final odorant concentrations in the models that represented the concentrations previously determined in the grapes. Final ethanol concentrations were kept below 200 *μ*L/kg. Using aqueous potassium hydroxide (2 mol/L), the pH of the models was adjusted to 3.8, the value previously determined in both the Muscaris and the Muskateller grapes. The complete odor reconstitution models contained 16 odorants; incomplete models were additionally prepared from which either (2*S*,4*R*)-rose oxide (**10**), geraniol (**26**), or both were omitted.

**Table 1. Odorants in the Volatile Isolates Obtained from Muscaris and Muskateller Grapes**

| no. | odorant[a] | odor[b] | RI[c] | | FD factor[d] | |
|---|---|---|---|---|---|---|
| | | | FFAP | DB-5 | Muscaris | Muskateller |
| **1** | butane-2,3-dione | butter | 969 | 591 | 8 | 16 |
| **2** | hexanal | green, grassy | 1079 | 800 | 16 | 64 |
| **3** | (3*E*)-hex-3-enal | green, grassy | 1134 | 800 | 4 | 4 |
| **4** | (3*Z*)-hex-3-enal | grassy, green | 1139 | 802 | 128 | 256 |
| **5** | heptanal | fatty, green | 1180 | 900 | 32 | 16 |
| **6** | 2-/3-methylbutan-1-ol[g] | malty | 1200 | 735/738 | 1 | 2 |
| **7** | (2*E*)-hex-2-enal | green apple | 1214 | 848 | 4 | 16 |
| **8** | oct-1-en-3-one | mushroom | 1295 | 981 | 16 | 32 |
| **9** | 2-acetyl-1-pyrroline[e] | popcorn, roasted | 1331 | 918 | 32 | 16 |
| **10** | (2*S*,4*R*)-rose oxide[f] | floral, lychee | 1343 | 1111 | 64 | 64 |
| **11** | 2,4,5-trimethyl-1,3-thiazole[e] | roasted, earthy | 1371 | 1000 | 64 | 64 |
| **12** | acetic acid | vinegar | 1447 | 613 | 8 | 2 |
| **13** | 3-(methylsulfanyl)propanal[e] | cooked potato | 1453 | 904 | 512 | 2048 |
| **14** | (3*R*)-citronellal[f] | citrusy, soapy | 1469 | 1151 | 16 | 16 |
| **15** | decanal | citrusy, soapy | 1492 | 1210 | 8 | 16 |
| **16** | 2-methoxy-3-(2-methylpropyl)pyrazine[e] | bell pepper | 1517 | 1180 | 8 | <1 |
| **17** | (2*E*)-non-2-enal | fatty | 1528 | 1159 | 32 | 8 |
| **18** | (3*R*)-/(3*S*)-linalool[f,g] | citrusy, floral | 1542 | 1100 | 64 | 512 |
| **19** | (2*E*,6*Z*)-nona-2,6-dienal | cucumber | 1579 | 1152 | 32 | 32 |
| **20** | undecanal | citrusy, soapy | 1598 | 1308 | 4 | 2 |
| **21** | phenylacetaldehyde | floral, honey | 1640 | 1038 | 128 | 256 |
| **22** | 2-/3-methylbutanoic acid[g] | sweaty | 1662 | 855/866 | 8 | 4 |
| **23** | 3-methylnonane-2,4-dione[e] | hay, anise, fishy | 1700 | 1243 | 32 | 16 |
| **24** | (3*S*)-*β*-citronellol[f] | soapy, rose | 1760 | 1233 | 64 | 8 |
| **25** | (*E*)-*β*-damascenone[e] | cooked apple | 1813 | 1390 | 16 | 32 |
| **26** | geraniol | floral, rose | 1844 | 1262 | 32,768 | 8192 |
| **27** | 2-methoxyphenol[e] | smoky | 1860 | 1088 | 8 | 8 |
| **28** | 2-phenylethan-1-ol | honey, floral | 1908 | 1117 | 4 | 2 |
| **29** | *β*-ionone | floral, violet | 1932 | 1488 | 8 | 16 |
| **30** | *trans*-4,5-epoxy-(2*E*)-dec-2-enal[e] | metallic | 2006 | 1380 | 32 | 16 |
| **31** | 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one[e] | caramel | 2035 | 1072 | 2 | 1 |
| **32** | octanoic acid | sour, musty | 2058 | 1185 | 4 | 4 |
| **33** | unknown | fresh, herb, licorice | 2139 | 1368 | 4 | 1 |
| **34** | 2-methoxy-4-(prop-2-en-1-yl)phenol | clove | 2162 | 1358 | 128 | <1 |
| **35** | 4-ethylphenol | phenolic | 2175 | 1169 | 4 | <1 |
| **36** | sotolon[e] | fenugreek | 2198 | 1110 | 32 | 16 |
| **37** | undecanoic acid | soapy, oily | 2371 | 1471 | 16 | 4 |
| **38** | phenylacetic acid[e] | floral, honey | 2567 | 1261 | 1 | <1 |
| **39** | 4-hydroxy-3-methoxybenzaldehyde | vanilla | 2576 | 1400 | 4 | 4 |

[a] Odorants showing an FD factor of ≥1 in either of the two samples; odorants were identified by comparing the retention indices on two columns of different polarity (DB-FFAP, DB-5), the mass spectra obtained by GC−MS as well as the odor quality as perceived at the sniffing port during GC−O to data obtained from authentic reference compounds analyzed under equal conditions. [b] Odor as perceived at the sniffing port during GC−O. [c] Retention index; calculated from the retention time of the odorant and the retention times of adjacent *n*-alkanes by linear interpolation. [d] Flavor dilution factor; dilution factor of the highest diluted grape volatile isolate in which the odorant was detected during GC−O by any of three assessors. [e] An unequivocal mass spectrum of the compound could not be obtained; identification was based on the remaining criteria detailed in footnote a and by spiking experiments using GC−O/FID. [f] Odor-active enantiomers as identified by analysis of the volatile isolates using GC−O/FID in combination with a chiral column. [g] The compounds were not separated on the column used for AEDA; the FD factor refers to the mixture.

**Quantitative Olfactory Profiles.** Samples (10 g), either freshly crushed frozen-thawed grapes or odor reconstitution models, were placed in cylindrical polytetrafluoroethylene vessels (5.7 cm height, 3.5 cm i.d., 50 mL nominal volume) with lids (Bohlender; Grünsfeld, Germany) and presented to 14 assessors (5 males and 9 females, aged 24−60 years) recruited from the trained panel of the Leibniz-LSB@TUM. The tests were carried out in the room described before. Assessors were asked to orthonasally rate the intensities of nine predefined descriptors on a seven-point scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Each descriptor was defined by the odor of a reference compound dissolved in water at a concentration 100 times above its respective orthonasal OTC. The nine odor descriptors and the corresponding reference compounds were "green, grassy" (hexanal), "floral" (geraniol), "citrusy" (linalool, racemic), "lychee" ((2*S*,4*R*)-rose oxide), "green apple" ((2*E*)-hex-2-enal), "cucumber" ((2*E*,6*Z*)-nona-2,6-dienal), "mushroom" (oct-1-en-3-one), "honey" (phenylacetaldehyde), and "hay" (3-methylnonane-2,4-dione). Ratings of all assessors were averaged by calculating the arithmetic mean. Statistical analysis was performed with Excel (Microsoft; Redmond, WA, USA).

## ■ RESULTS AND DISCUSSION

**Odorant Screening.** GC−O in combination with cAEDA applied to the volatile isolates obtained from fresh Muscaris and Muskateller grapes, both of which showed a distinct lychee

**Table 2. Enantiomeric Distribution of Important Chiral Odorants in Muscaris and Muskateller Grapes**

| odorant | enantiomer | odor | enantiomeric distribution[a] (%) in | |
|---|---|---|---|---|
| | | | Muscaris | Muskateller |
| *cis*-rose oxide | 2*R*,4*S* | floral, lychee (weak) | 29 | 25 |
| | 2*S*,4*R* | floral, lychee (strong) | 71 | 75 |
| linalool | 3*R* | citrusy, floral | 9 | 2 |
| | 3*S* | citrusy, floral | 91 | 98 |
| *β*-citronellol | 3*R* | soapy, rose | 2 | 3 |
| | 3*S* | soapy, rose | 98 | 97 |

[a] Mean of triplicates.

note, resulted in 39 odor-active compounds, 35 of which were present in both samples. FD factors ranged from 1 to 32,768 for Muscaris grapes and from 1 to 8192 for Muskateller grapes (Table 1). Structure assignments were achieved with the following approach: the RIs obtained for Muscaris and Muskateller grape odorants with two GC columns of different polarity (DB-FFAP and DB-5) in combination with the odor descriptions were compared to published data, foremost those compiled in the Leibniz-LSB@TUM Odorant Database.[39] The resulting structure proposals were confirmed by GC−O and GC−MS analyses of the grape volatile isolates in parallel with authentic reference compounds. In the case of coelution problems, the comprehensive two-dimensional GC × GC−MS instrument was employed for the mass spectral analyses. For enantiospecific structure assignments, the approach was repeated using two chiral GC columns with differently substituted *β*-cyclodextrin phases.

As a result, the structures of 38 odorants were successfully determined. Only compound **33**, despite all efforts, remained unknown. Among the odorants identified in Muscaris grapes, vanilla-like smelling 4-hydroxy-3-methoxybenzaldehyde (vanillin; **39**) was the only compound which had previously been reported in grapes of this variety.[14]

The compound with by far the highest FD factor among the Muscaris grape odorants was floral, rose-like smelling geraniol (**26**; FD factor 32,768). With 8192, geraniol also showed the highest FD factor among the Muskateller grape odorants. Nineteen odorants were assigned FD factors ≥32 in at least one of the two samples. Twelve of them showed comparable FD factors in both grape varieties. This compound group included green, grassy smelling (3*Z*)-hex-3-enal (**4**; FD factors 128 and 256), floral, honey-like smelling phenylacetaldehyde (**21**; FD factors 128 and 256), floral, lychee-like smelling (2*S*,4*R*)-rose oxide (**10**; FD factors 64 and 64), roasted, earthy smelling 2,4,5-trimethyl-1,3-thiazole (**11**; FD factors 64 and 64), cucumber-like smelling (2*E*,6*Z*)-nona-2,6-dienal (**19**; FD factors 32 and 32), fatty, green smelling heptanal (**5**; FD factors 32 and 16), popcorn-like, roasted smelling 2-acetyl-1-pyrroline (**9**; FD factors 32 and 16), hay-, anise-like, and fishy smelling 3-methylnonane-2,4-dione (**23**; FD factors 32 and 16), metallic smelling *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**30**; FD factors 32 and 16), fenugreek-like smelling sotolon (**36**; FD factors 32 and 16), mushroom-like smelling oct-1-en-3-one (**8**; FD factors 16 and 32), and cooked apple-like smelling (*E*)-*β*-damascenone (**25**; FD factors 16 and 32). Apart from geraniol, clearly higher FD factors in the Muscaris grapes than in the Muskateller grapes were also obtained for 2-methoxy-4-(prop-2-en-1-yl)phenol (**34**; clove-like; FD factors 128 and <1), (3*S*)-*β*-citronellol (**24**; soapy, rose-like; FD factors 64 and 8), and (2*E*)-non-2-enal (**17**; fatty; FD factors 32 and 8). In contrast, higher FD factors in the Muskateller grapes were found for cooked potato-like smelling 3-(methylsulfanyl)-propanal (**13**; FD factors 512 and 2048), citrusy, floral smelling isomers (3*R*)- and (3*S*)-linalool (**18**; FD factors 64 and 512), and green, grassy smelling hexanal (**2**; FD factors 16 and 64). In summary, however, the odorant spectrum in the Muscaris grapes showed a huge similarity to the odorant spectrum in the Muskateller grapes, thus reflecting the close genetic relationship between the two varieties. This was particularly the case for some monoterpenes considered iconic for Muskateller such as *cis*-rose oxide, linalool, and geraniol. A detailed review on their biosynthesis in grapes has been published by Schwab and Wüst.[40]

**Odorant Quantitation and OAV Calculation.** Twenty odorants for which high FD factors in at least one of the two grape samples had been determined during the odorant screening (cf. Table 1) were selected. Quantitation was accomplished by GC−MS using stable isotopically substituted odorants as internal standards. The grape samples subjected to quantitation were from the same batch as those used for odorant screening. Given the time required to collect the screening data and assign the structures, it was impossible to perform the quantitations with fresh material. Accordingly, a majority of odorants were quantitated in frozen-thawed grapes. Exceptions were the lipoxygenase products[41] (3*Z*)-hex-3-enal (**4**) and (3*E*)-hex-3-enal (**3**), which were quantitated in the fresh grapes in parallel to the screening experiments. This was done because it had been reported that the concentration of (3*Z*)-hex-3-enal can substantially differ between fresh and stored as well as frozen-thawed plant materials.[28,42]

Enantiospecific concentrations of important chiral odorants were calculated from the sum of enantiomers as determined via stable isotopically substituted internal standards in the quantitation assays and the enantiomeric distribution as determined by GC with chiral columns. Chromatograms showing the separation of the enantiomers are available in Figure S1. The enantiomeric distributions of *cis*-rose oxide, linalool, and *β*-citronellol are listed in Table 2. In both linalool and *β*-citronellol, the (*S*)-isomer clearly predominated with percentages of 91−98%. With 94 and 99.8%, similar data were reported for linalool in two other Muscat grape varieties.[43] To the best of our knowledge, the enantiomeric distribution of *β*-citronellol in Muscat grapes has not been examined so far. In *cis*-rose oxide, the major enantiomer was the odor-active (2*S*,4*R*)-isomer with 71% in the Muscaris grapes and 75% in the Muskateller grapes. In other six Muscat grape varieties previously investigated, the (2*S*,4*R*)-isomer showed even higher percentages of 88−97%.[32] In total, the data obtained for Muscaris and Muskateller grapes were highly similar, again illustrating their close genetic relation.

The quantitation of the 20 odorants resulted in concentrations between the nanogram per kilogram range and 1160

**Table 3. Concentrations and OAVs of Important Odorants in Muscaris and Muskateller Grapes**

| no.[a] | odorant[b] | concentration[c] (μg/kg) | | OTC[d] (μg/kg) | OAV[e] | |
|---|---|---|---|---|---|---|
| | | Muscaris | Muskateller | | Muscaris | Muskateller |
| **26** | geraniol | 1160 | 442 | 1.1[f] | 1100 | 400 |
| **19** | (2*E*,6*Z*)-nona-2,6-dienal | 1.35 | 0.464 | 0.0045[f] | 300 | 100 |
| **4** | (3*Z*)-hex-3-enal | 21.8 | 26.6 | 0.12[f] | 180 | 220 |
| **18a** | (3*R*)-linalool[g] | 11.2 | 10.3 | 0.087[f] | 130 | 120 |
| **2** | hexanal | 294 | 548 | 2.4[f] | 120 | 230 |
| **3** | (3*E*)-hex-3-enal | 13.7 | 22.0 | 0.23[h] | 59 | 95 |
| **18b** | (3*S*)-linalool[g] | 113 | 518 | 2.7[f] | 42 | 190 |
| **23** | 3-methylnonane-2,4-dione | 1.23 | 0.724 | 0.046[f] | 27 | 16 |
| **8** | oct-1-en-3-one | 0.413 | 0.293 | 0.016[f] | 26 | 18 |
| **10** | (2*S*,4*R*)-rose oxide[g] | 0.873 | 1.19 | 0.045[h] | 19 | 26 |
| **24** | (3*S*)-*β*-citronellol[g] | 56.0 | 28.7 | 4.9[i] | 11 | 5.9 |
| **17** | (2*E*)-non-2-enal | 2.00 | 1.75 | 0.19[f] | 11 | 9.2 |
| **21** | phenylacetaldehyde | 53.2 | 38.0 | 5.2[f] | 10 | 7.3 |
| **13** | 3-(methylsulfanyl)propanal | 3.66 | 4.55 | 0.43[f] | 8.5 | 11 |
| **7** | (2*E*)-hex-2-enal | 325 | 700 | 110[f] | 3.0 | 6.4 |
| **25** | (*E*)-*β*-damascenone | 0.0106 | 0.0157 | 0.0060[f] | 1.8 | 2.6 |
| **34** | 2-methoxy-4-(prop-2-en-1-yl)phenol | 0.886 | 0.116 | 1.8[f] | <1 | <1 |
| **5** | heptanal | 1.86 | 1.51 | 6.1[h] | <1 | <1 |
| **36** | sotolon | 0.0563 | 0.0728 | 1.7[f] | <1 | <1 |
| **9** | 2-acetyl-1-pyrroline | <0.01 | <0.01 | 0.053[f] | <1 | <1 |

[a] Numbering according to Table 1. [b] Odorants in order of decreasing OAVs in Muscaris grapes. [c] Mean of duplicates or triplicates; individual values and standard deviations are available in Tables S3 and S4. [d] Orthonasal odor threshold concentration in water. [e] Odor activity value; calculated as the ratio of concentration to odor threshold concentration. [f] Data taken from the Leibniz-LSB@TUM Odorant Database.[39] [g] Concentrations of individual enantiomers were calculated from the concentration of the sum of enantiomers as obtained in the quantitation assays and the enantiomeric distribution depicted in Table 2. [h] Data obtained in the current study.[37] [i] Data from literature.[44]

μg/kg for geraniol (**26**) in Muscaris grapes and 700 μg/kg for (2*E*)-hex-2-enal (**7**) in Muskateller grapes (Table 3). High concentrations of >100 μg/kg were additionally determined for (2*E*)-hex-2-enal (**7**; 325 μg/kg), hexanal (**2**; 294 μg/kg), and (3*S*)-linalool (**18b**; 113 μg/kg) in Muscaris grapes, and hexanal (**2**; 548 μg/kg), (3*S*)-linalool (**18b**; 518 μg/kg), and geraniol (**26**; 442 μg/kg) in Muskateller grapes. Again, the data showed only minor differences between the two varieties.

To gain more information on the odor activity of the individual odorants, OAVs were calculated as the ratios of the odorant concentrations in the grapes and the OTCs in water. The results (Table 3) revealed an OAV of ≥1 in both grape varieties for 16 of the 20 quantitated odorants. The highest OAV was calculated for geraniol (**26**) in both Muscaris (OAV 1100) and Muskateller (OAV 400) grapes. Whereas in Muscaris grapes no previous data was available in the literature, a huge data set was found for geraniol in Muskateller grapes. The majority of the geraniol concentrations in Muskateller grapes ranged between ~70[45] and ~300 μg/kg,[46] corresponding to OAVs of ~60 and ~300.

Four additional odorants showed an OAV of ≥100 in both grapes in our study, namely, (2*E*,6*Z*)-nona-2,6-dienal (**19**; OAVs 300 and 100), (3*Z*)-hex-3-enal (**4**; OAVs 180 and 220), (3*R*)-linalool (**18a**; OAVs 130 and 120), and hexanal (**2**; OAVs 120 and 230). None of the four compounds had previously been quantitated in Muscaris grapes, but data were particularly available for linalool in Muskateller grapes. Concentrations were typically in the range of ~300[26] to ~600 μg/kg.[47] However, in no case, the enantiomeric distribution was considered. Given the huge difference in the OTCs of (*R*)- and (*S*)-linalool (cf. Table 3), estimation of the corresponding OAVs was thus not possible.

The OAV range of <100, but >10 in both grapes included five odorants in Table 3. These odorants were (3*E*)-hex-3-enal (**3**; OAVs 59 and 95), (3*S*)-linalool (**18b**; OAVs 42 and 190), 3-methylnonane-2,4-dione (**23**; OAVs 27 and 16), oct-1-en-3-one (**8**; OAVs 26 and 18), and (2*S*,4*R*)-rose oxide (**10**; OAVs 19 and 26). Literature data were available for *cis*-rose oxide in Muskateller grapes. Concentrations varied between ~0.6[26] and ~4 μg/kg[48] for the enantiomeric mixture. Assuming an enantiomeric distribution of 25/75 as determined by us (cf. Table 2), this would correspond to OAVs of ~10 to ~70 for the odor-active (2*S*,4*R*)-rose oxide.

Comparing the OAVs of individual odorants provided in Table 3 between Muscaris and Muskateller grapes resulted in little differences for a majority of compounds; that is, the OAVs did not differ by more than a factor of 2. Important exceptions were geraniol (**26**) and (2*E*,6*Z*)-nona-2,6-dienal (**19**), which not only featured the highest two OAVs in the Muscaris grapes but additionally showed OAVs ~3 times lower in the Muskateller grapes. Another exception was (3*S*)-linalool (**18b**) with an OAV in the Muskateller grapes almost 5 times higher than that in the Muscaris grapes. However, when the OAVs of both citrusy, floral smelling linalool enantiomers (cf. Table 2) were summed, the combined values differed only by a factor of ~2 between the Muskateller and the Muscaris grapes.

**Odor Reconstitution and Omission Experiments.** To verify the analytical data and identify the odorants responsible for the characteristic lychee note, odor reconstitution and omission experiments were performed. First, an aqueous odor reconstitution model was prepared for each of the two grape varieties containing all 16 odorants with OAVs ≥1 in the previously determined concentrations (cf. Table 3). Orthonasal evaluation of the two models by a trained sensory panel resulted in quite similar olfactory profiles for both grape

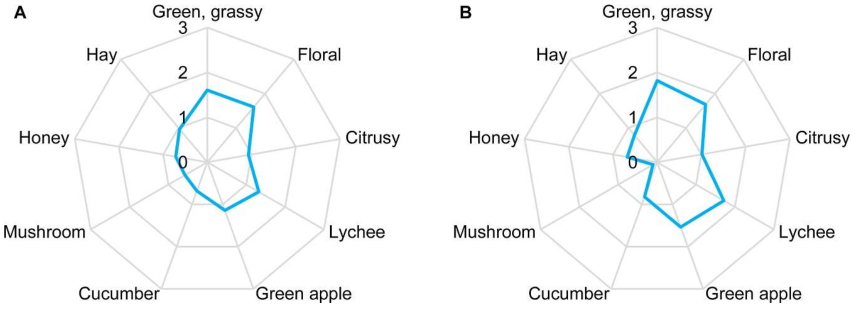


**Figure 1.** Quantitative olfactory profiles of the odor reconstitution models of Muscaris (A) and Muskateller (B) grapes. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

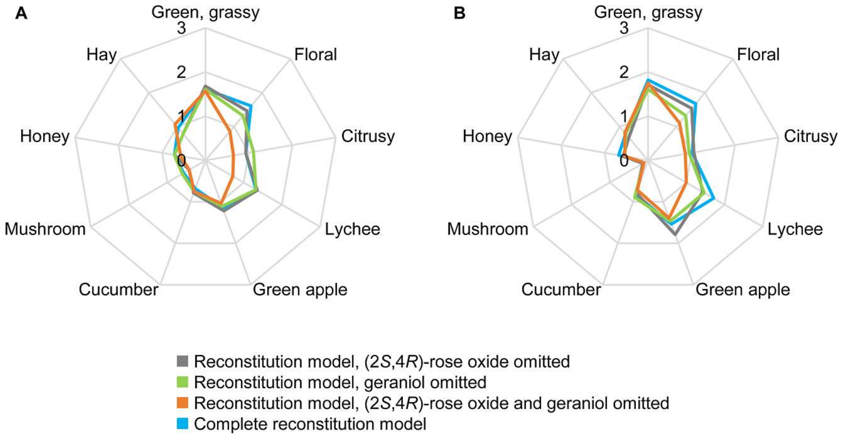


**Figure 2.** Quantitative olfactory profiles of the odor reconstitution models of Muscaris (A) and Muskateller (B) grapes from which (2*S*,4*R*)-rose oxide, geraniol, or both were omitted. The complete models depicted in Figure 1 are additionally included for comparison. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

varieties. The Muscaris model as well as the Muskateller model showed a balanced combination of green-grassy, floral, lychee, and green apple-like notes (Figure 1). Moreover, the distinct lychee note perceived in the fresh Muscaris grapes was well reflected in the aroma of the Muscaris reconstitution model. A direct comparison of the two reconstitution models with the fresh materials, however, was not possible due to the short shelf life of the grapes. Using fresh grapes of the following harvest as a reference was not considered appropriate as it is known that the vintage can have a huge influence on the grape aroma.[49]

As an alternative to evidence of the correctness of the analytical data, the frozen-thawed Muscaris and Muskateller grapes were used as references for reconstitution models fully based on odorant concentrations in the frozen-thawed materials. This included (3*E*)-hex-3-enal (**3**) and (3*Z*)-hex-3-enal (**4**), which therefore were additionally quantitated in the frozen-thawed grapes (Table S5). The resulting quantitative olfactory profiles (Figure S2) revealed good agreements between the models and the grapes, thus providing evidence that all major aroma-contributing compounds in Muscaris and Muskateller grapes were correctly identified and quantitated.

The primary aim of the omission experiments was to elucidate the molecular basis of the lychee note. The tests were based on the reconstitution models depicted in Figure 1. In the first experiment, (2*S*,4*R*)-rose oxide (**10**) was omitted from the models as it was the only compound among the odorants identified in Muscaris and Muskateller grapes with a specific lychee-like odor (cf. Table 1). The incomplete odor reconstitution models were then compared to the complete odor reconstitution models in quantitative olfactory profile analyses. Surprisingly, the omission of (2*S*,4*R*)-rose oxide did not result in a substantially reduced intensity rating for the lychee note, in either the Muscaris or the Muskateller model (Figure 2). Using paired *t*-tests, *p*-values of 0.8 (Muscaris) and 0.1 (Muskateller) were calculated, suggesting that there was no significant difference in the lychee note. In contrast, when geraniol (**26**) was additionally omitted, the intensity rating for the lychee note dropped considerably: in the Muscaris model by 0.6 units and in the Muskateller model by 0.7 units. The *t*-tests resulted in *p*-values of 0.03 (Muscaris) and 0.01 (Muskateller), suggesting a significant difference between the complete and incomplete reconstitution models. Interestingly, when only geraniol was omitted, the effect on the lychee note was small, similar to the effect observed when only (2*S*,4*R*)-rose oxide was omitted. *p*-Values were 1 in the Muscaris models and 0.2 in the Muskateller models. Thus, it became obvious that the lychee note in the Muscaris grapes, but also in the Muskateller grapes, was generated by the combination of (2*S*,4*R*)-rose oxide and geraniol. Whereas (2*S*,4*R*)-rose oxide showed a specific lychee odor but rather moderate OAVs of 19 and 26 in Muscaris and Muskateller grapes, respectively, geraniol showed high OAVs of 1100 and 400 in combination with a floral and rosy odor not distinctly lychee-like. To the best of our knowledge, the combinatorial effect of (2*S*,4*R*)-rose oxide and geraniol on the lychee note in the aroma of Muscaris and Muskateller grapes has not yet been described in the literature. Further studies are necessary to better understand the interaction of (2*S*,4*R*)-rose oxide and geraniol during olfactory perception.

In summary, this work bridged some of the gaps in the literature. It revealed, for the first time, the major odorants in Muscaris grapes and demonstrated that the combination of (2*S*,4*R*)-rose oxide and geraniol was responsible for the characteristic lychee note in the aroma. These results form the basis for further studies, including research on the transfer of Muscaris grape odorants into wine and their role in the hedonic value of wine. Furthermore, the knowledge of the molecular background of the lychee note may be useful in the targeted breeding of new grape varieties with distinct aroma properties.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c08298.

> Additional information on GC instruments; references on synthetic procedures to isotopically substituted odorants; stable isotopically substituted internal standards, quantifier ions, and calibration lines used in the quantitation assays; individual odorant concentrations used for mean calculations and standard deviations; chromatograms showing the enantiomeric separation of *cis*-rose oxide, linalool, and β-citronellol by GC−MS with chiral columns; and quantitative olfactory profiles of the frozen-thawed Muscaris and Muskateller grapes in comparison to the quantitative olfactory profiles of the respective odor reconstitution models (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Authors

**Stephanie Frank** − *Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany*; orcid.org/0000-0001-5853-4839; Phone: +49 8161 71 2990; Email: s.frank.leibniz-lsb@tum.de

**Martin Steinhaus** − *Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany; Technical University of Munich, TUM School of Natural Sciences, Department of Chemistry, 85748 Garching, Germany*; orcid.org/0000-0002-9879-1474; Phone: +49 8161 71 2991; Email: martin.steinhaus@tum.de

### Author

**Xingjie Wang** − *Technical University of Munich, TUM School of Natural Sciences, Department of Chemistry, 85748 Garching, Germany; Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.3c08298

### Funding

X.W. gratefully acknowledges funding from the China Scholarship Council (CSC), grant no. 201906300007.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors thank Monika Riedmaier and Jörg Stein for skillful technical assistance. Special thanks go to Dr. Rainer Amann from the State Institute of Viticulture Freiburg, Germany, for kindly providing grape samples.

## ■ ABBREVIATIONS

aSAFE, automated solvent-assisted flavor evaporation; ASTM, American Society for Testing and Materials; cAEDA, comparative aroma extract dilution analysis; FD, flavor dilution; FFAP, free fatty acid phase; FID, flame ionization detector; GC, gas chromatography; HR, high resolution; HPLC, high-performance liquid chromatography; i.d., inner diameter; MS, mass spectrometry; O, olfactometry; OAV, odor activity value; OTC, odor threshold concentration; RI, retention index; TOF, time-of-flight

## ■ NOMENCLATURE

| | |
|---|---|
| 2-acetyl-1-pyrroline | 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethan-1-one |
| (3*R*)-/(3*S*)-citronellal | (3*R*)-/(3*S*)-3,7-dimethyloct-6-enal |
| (3*R*)-/(3*S*)-β-citronellol | (3*R*)-/(3*S*)-3,7-dimethyloct-6-en-1-ol |
| (*E*)-β-damascenone | (2*E*)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one |
| *trans*-4,5-epoxy-(2*E*)-dec-2-enal | (2*E*)-3-[(2*R*,3*R*)/(2*S*,3*S*)-3-pentyloxiran-2-yl]prop-2-enal |
| geraniol | (2*E*)-3,7-dimethylocta-2,6-dien-1-ol |
| β-ionone | (3*E*)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one |
| (3*R*)-/(3*S*)-linalool | (3*R*)-/(3*S*)-3,7-dimethylocta-1,6-dien-3-ol |
| *cis*-rose oxide | (2*R*,4*S*)-/(2*S*,4*R*)-rose oxide, (2*R*,4*S*)-/(2*S*,4*R*)-4-methyl-2-(2-methylprop-1-en-1-yl)-oxane |
| sotolon | 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one |

## ■ REFERENCES

(1) Fungus-resistant grape varieties; State Institute of Viticulture Freiburg, Freiburg, Germany, 2023. https://wbi.landwirtschaft-bw.de/pb/site/pbs-bw-mlr/get/documents_E-1412531467/MLR.LEL/PB5Documents/wbi/051%20Aufgaben%20und%20Fachbereiche/0513%20Fachbereiche/05136%20Referat%20Resistenz%20und%20Klonenz%C3%BCchtung/051325%20Listen%20zu%20PIWI%20Keltertrauben/WBI_Fungus_resistant_grape_varieties_EN.pdf (accessed Nov 7, 2023).

(2) Land und Forstwirtschaft, Fischerei: Landwirtschaftliche Bodennutzung-Rebflächen-Fachserie 3 Reihe 3.1.5−2022. Federal Statistical Office (Destatis), Wiesbaden, Germany, 2023. https://www.destatis.de/DE/Themen/Branchen-Unternehmen/Landwirtschaft-Forstwirtschaft-Fischerei/Wein/Publikationen/Downloads-Wein/rebflaechen-2030315227004.pdf?__blob=publicationFile (accessed Nov 7, 2023).

(3) Maul, E.; Töpfer, R.; Röckel, F.; Brühl, U.; Hundemer, M.; Mahler-Ries, A.; Walk, M.; Kecke, S.; Wolck, A.; Ganesch, A. Vitis International Variety Catalogue. Julius Kühn-Institute (JKI) - Federal Research Center for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof: Siebeldingen, Germany, 2023. https://www.vivc.de/(accessed Nov 7, 2023).

(4) Schwander, F.; Eibach, R.; Fechter, I.; Hausmann, L.; Zyprian, E.; Töpfer, R. Rpv10: a new locus from the Asian Vitis gene pool for pyramiding downy mildew resistance loci in grapevine. *Theor. Appl. Genet.* **2012**, *124*, 163−176.

(5) Vezzulli, S.; Vecchione, A.; Stefanini, M.; Zulini, L. Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy). *Eur. J. Plant Pathol.* **2018**, *150*, 485−495.

(6) Gelber Muskateller. The Palatinate Magazine, Rohrbach, Germany, 2023. https://www.das-pfalz-magazin.de/weinlexikon/weisse-rebsorten/gelber-muskateller/?L=0 (accessed Nov 7, 2023).

(7) Bureau, S. M.; Razungles, A. J.; Baumes, R. L. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *J. Sci. Food Agric.* **2000**, *80*, 2012−2020.

(8) Palomo, E. S.; Díaz-Maroto, M.; Viñas, M. G.; Soriano-Pérez, A.; Pérez-Coello, M. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. *Food Control* **2007**, *18*, 398−403.

(9) Marcon, Â. R.; Delamare, A. P. L.; Schwarz, L. V.; Pasini, L.; Versari, A.; Parpinello, G. P.; Echeverrigaray, S. Volatile and sensory composition of Brazilian Muscat sparkling wine and Asti. *J. Food Process. Preserv.* **2021**, *45*, No. e15240.

(10) Casanova-Gascón, J.; Ferrer-Martín, C.; Bernad-Eustaquio, A.; Elbaile-Mur, A.; Ayuso-Rodríguez, J. M.; Torres-Sánchez, S.; Jarne-Casasús, A.; Martín-Ramos, P. Behavior of vine varieties resistant to fungal diseases in the Somontano region. *Agronomy* **2019**, *9*, 738.

(11) Zini, E.; Dolzani, C.; Stefanini, M.; Gratl, V.; Bettinelli, P.; Nicolini, D.; Betta, G.; Dorigatti, C.; Velasco, R.; Letschka, T.; Vezzulli, S. R-loci arrangement versus downy and powdery mildew resistance level: a Vitis hybrid survey. *Int. J. Mol. Sci.* **2019**, *20*, 3526.

(12) Pedò, S.; Bottura, M.; Porro, D. Development, yield potential and nutritional aspects of resistant grapevine varieties in Trentino Alto Adige. *BIO Web of Conferences*; Poni, S., Ed.; EDP Sciences S.A.: Les Ulis, France, 2019; Vol. *13*, p 02004.

(13) Porro, D.; Wolf, M.; Pedò, S. Evaluation of mechanical properties of berries on resistant or tolerant varieties of grapevine. *BIO Web of Conferences*; Poni, S., Ed.; EDP Sciences S.A.: Les Ulis, France, 2019; Vol. *13*, p 01005.

(14) Barnaba, C.; Dellacassa, E.; Nicolini, G.; Giacomelli, M.; Roman Villegas, T.; Nardin, T.; Larcher, R. Targeted and untargeted high resolution mass approach for a putative profiling of glycosylated simple phenols in hybrid grapes. *Food Res. Int.* **2017**, *98*, 20−33.

(15) Czaplicka, M.; Parypa, K.; Szewczuk, A.; Gudarowska, E.; Rowinska, M.; Zubaidi, M. A.; Nawirska-Olszanska, A. Assessment of selected parameters for determining the internal quality of white grape cultivars grown in cold climates. *Appl. Sci.* **2022**, *12*, 5534.

(16) Leis, D.; Renner, W.; Leitner, E. Characterisation of wines produced from fungus resistant grape varieties. In *Flavour Science, Proceedings of the XV Weurman Flavour Research Symposium*; Siegmund, B., Leitner, E., Eds.; Verlag der Technischen Universität Graz: Graz, Austria, 2018; pp 511−514.

(17) Román, T.; Nicolini, G.; Barp, L.; Malacarne, M.; Tait, F.; Larcher, R. Shikimic acid concentration in white wines produced with different processing protocols from fungus-resistant grapes growing in the Alps. *Vitis* **2018**, *57*, 41−46.

(18) Ruocco, S.; Perenzoni, D.; Angeli, A.; Stefanini, M.; Rühl, E.; Patz, C. D.; Mattivi, F.; Rauhut, D.; Vrhovsek, U. Metabolite profiling of wines made from disease-tolerant varieties. *Eur. Food Res. Technol.* **2019**, *245*, 2039−2052.

(19) van Wyk, N.; Pretorius, I. S.; von Wallbrunn, C. Assessing the oenological potential of *Nakazawaea ishiwadae*, *Candida railenensis* and *Debaryomyces hansenii* strains in mixed-culture grape must fermentation with *Saccharomyces cerevisiae*. *Fermentation* **2020**, *6*, 49.

(20) van Wyk, N.; Scansani, S.; Beisert, B.; Brezina, S.; Fritsch, S.; Semmler, H.; Pretorius, I. S.; Rauhut, D.; von Wallbrunn, C. The use of *Hanseniaspora occidentalis* in a sequential must inoculation to reduce the malic acid content of wine. *Appl. Sci.* **2022**, *12*, 6919.

(21) Reynolds, A. G.; Wardle, D. A.; Dever, M. Vine performance, fruit composition, and wine sensory attributes of Gewürztraminer in response to vineyard location and canopy manipulation. *Am. J. Enol. Vitic.* **1996**, *47*, 77−92.

(22) Ong, P. K. C.; Acree, T. E. Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. *J. Agric. Food Chem.* **1999**, *47*, 665−670.

(23) Rusjan, D.; Strlič, M. S.; Košmerl, T.; Prosen, H. The response of monoterpenes to different enzyme preparations in Gewürztraminer (*Vitis vinifera* L.) wines. *S. Afr. J. Enol. Vitic.* **2009**, *30*, 56−64.

(24) Katarína, F.; Katarína, M.; Katarína, Ď.; Ivan, Š.; Fedor, M. Influence of yeast strain on aromatic profile of Gewürztraminer wine. *LWT—Food Sci. Technol.* **2014**, *59*, 256−262.

(25) Čuš, F.; Zabukovec, P.; Schroers, H. Indigenous yeasts perform alcoholic fermentation and produce aroma compounds in wine. *Czech J. Food Sci.* **2017**, *35*, 329−345.

(26) Li, X. Y.; Wen, Y. Q.; Meng, N.; Qian, X.; Pan, Q. H. Monoterpenyl glycosyltransferases differentially contribute to production of monoterpenyl glycosides in two aromatic *Vitis vinifera* varieties. *Front. Plant Sci.* **2017**, *8*, 1226.

(27) Steinhaus, M.; Sinuco, D.; Polster, J.; Osorio, C.; Schieberle, P. Characterization of the aroma-active compounds in pink guava (*Psidium guajava*, L.) by application of the aroma extract dilution analysis. *J. Agric. Food Chem.* **2008**, *56*, 4120−4127.

(28) Kreissl, J.; Schieberle, P. Characterization of aroma-active compounds in Italian tomatoes with emphasis on new odorants. *J. Agric. Food Chem.* **2017**, *65*, 5198−5208.

(29) Buttery, R. G.; Ling, L. C.; Juliano, B. O.; Turnbaugh, J. G. Cooked rice aroma and 2-acetyl-1-pyrroline. *J. Agric. Food Chem.* **1983**, *31*, 823−826.

(30) Reglitz, K.; Stein, J.; Ackermann, J.; Heigl, V.; Brass, L.; Ampenberger, F.; Zarnkow, M.; Steinhaus, M. Enantiospecific determination of the odour threshold concentrations of (*R*)- and (*S*)-linalool in water and beer. *Brew. Sci.* **2023**, *76*, 92−96.

(31) Wüst, M.; Beck, T.; Mosandl, A. Conversion of citronellyl diphosphate and citronellyl *β*-D-glucoside into rose oxide by *Pelargonium graveolens*. *J. Agric. Food Chem.* **1999**, *47*, 1668−1672.

(32) Luan, F.; Mosandl, A.; Gubesch, M.; Wüst, M. Enantioselective analysis of monoterpenes in different grape varieties during berry ripening using stir bar sorptive extraction- and solid phase extraction-enantioselective-multidimensional gas chromatography-mass spectrometry. *J. Chromatogr. A* **2006**, *1112*, 369−374.

(33) Wüst, M.; Mosandl, A. Important chiral monoterpenoid ethers in flavours and essential oils -enantioselective analysis and biogenesis. *Eur. Food Res. Technol.* **1999**, *209*, 3−11.

(34) Schlumpberger, P.; Stübner, C. A.; Steinhaus, M. Development and evaluation of an automated solvent-assisted flavour evaporation (aSAFE). *Eur. Food Res. Technol.* **2022**, *248*, 2591−2602.

(35) Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in Flavour Research*; Land, G. G., Nursten, H. E., Eds.; Applied Science Publishers: London, UK, 1979, pp 79−88.

(36) Steinhaus, M. Gas chromatography-olfactometry: principles, practical aspects and applications in food analysis. In *Advanced Gas Chromatography in Food Analysis*; Tranchida, P., Ed.; The Royal Society of Chemistry: Cambridge, UK, 2019; pp 337−399.

(37) ASTM International. *E679−19 Standard Practice for Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits*; ASTM: West Conshohocken, PA, 2019.

(38) Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.

(39) Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. Leibniz-LSB@TUM Odorant Database, Version 1.2; Leibniz Institute for Food Systems Biology at the Technical University of Munich: Freising,

Germany, 2022. https://www.leibniz-lsb.de/en/databases/leibniz-lsbtum-odorant-database (accessed Nov 7, 2023).

(40) Schwab, W.; Wüst, M. Understanding the constitutive and induced biosynthesis of mono-and sesquiterpenes in grapes (*Vitis vinifera*): a key to unlocking the biochemical secrets of unique grape aroma profiles. *J. Agric. Food Chem.* **2015**, *63*, 10591−10603.

(41) Kalua, C. M.; Boss, P. K. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2009**, *57*, 3818−3830.

(42) Sellami, I.; Mall, V.; Schieberle, P. Changes in the key odorants and aroma profiles of hamlin and valencia orange juices not from concentrate (NFC) during chilled storage. *J. Agric. Food Chem.* **2018**, *66*, 7428−7440.

(43) Luan, F.; Hampel, D.; Mosandl, A.; Wüst, M. Enantioselective analysis of free and glycosidically bound monoterpene polyols in *Vitis vinifera* L. cvs. Morio Muscat and Muscat Ottonel: evidence for an oxidative monoterpene metabolism in grapes. *J. Agric. Food Chem.* **2004**, *52*, 2036−2041.

(44) Schaller, T.; Schieberle, P. Quantitation of key aroma compounds in fresh, raw ginger (*Zingiber officinale* Roscoe) from China and roasted ginger by stable isotope dilution assays and aroma profiling by recombination experiments. *J. Agric. Food Chem.* **2020**, *68*, 15284−15291.

(45) Río Segade, S.; Vilanova, M.; Pollon, M.; Giacosa, S.; Torchio, F.; Rolle, L. Grape VOCs response to postharvest short-term ozone treatments. *Front. Plant Sci.* **2018**, *9*, 1826.

(46) Ribéreau-Gayon, P.; Boidron, J. N.; Terrier, A. Aroma of Muscat grape varieties. *J. Agric. Food Chem.* **1975**, *23*, 1042−1047.

(47) Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components cv Muscat during first development and maturation. *J. Sci. Food Agric.* **1985**, *36*, 857−862.

(48) Matarese, F.; Cuzzola, A.; Scalabrelli, G.; D'Onofrio, C. Expression of terpene synthase genes associated with the formation of volatiles in different organs of *Vitis vinifera*. *Phytochemistry* **2014**, *105*, 12−24.

(49) Robinson, A. L.; Boss, P. K.; Solomon, P. S.; Trengove, R. D.; Heymann, H.; Ebeler, S. E. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *Am. J. Enol. Vitic.* **2014**, *65*, 1−24.

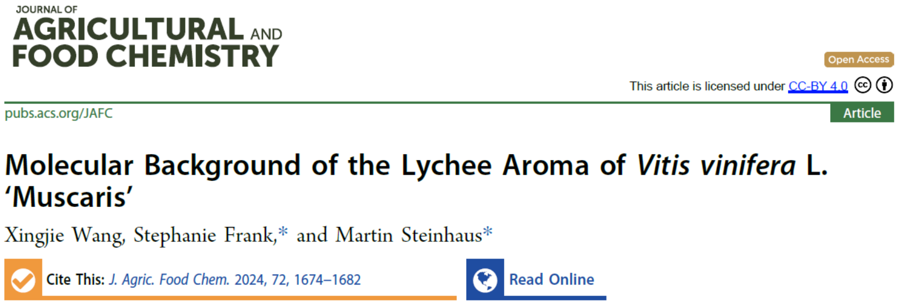
### 7.1.3 Summary and Individual Contributions

Lychee aroma is a highly appreciated aroma note in wine, however, it is limited to only a few grape varieties among which are Gewürztraminer and Muscaris. The Muscaris grape variety was bred by the State Institute of Viticulture Freiburg, Germany with the aim to combine the disease tolerance and environmental adaptability of the mother variety, Solaris, with the intense aroma of the father variety, Gelber Muskateller. Given that Muscaris was a relatively new white grape variety, little was known about the compounds responsible for its aroma including the characteristic lychee note and the relation to the compounds aroma-active in Muskateller.

A comparative aroma extract dilution analysis (cAEDA) applied to volatile isolates of both Muscaris and Muskateller grapes resulted in 39 and 35 odorants, respectively. Among them, only 4-hydroxy-3-methoxybenzaldehyde had earlier been reported in Muscaris grapes. The flavor dilution (FD) factors of the odorants ranged from 1 to 32768 in Muscaris grapes and from 1 to 8192 in Muskateller grapes. The highest FD factor was assigned to geraniol in both grape varieties. Enantioselective analyses revealed that the (2*S*,4*R*)-*cis*-rose oxide and the (*S*)-*β*-citronellol were the odor-active isomers in the grape samples, while in linalool, both the (*R*)- and (*S*)-isomers were odor-active, however, the (*S*)-isomer predominated. Quantitative data revealed relatively high concentrations for geraniol (1160 µg/kg and 442 µg/kg), (2*E*)-hex-2-enal (325 µg/kg and 700 µg/kg), hexanal (294 µg/kg and 548 µg/kg) and (3*S*)-linalool (113 µg/kg and 518 µg/kg) in Muscaris and Muskateller grapes, respectively. The concentrations of 16 odorants exceeded their odor threshold concentrations (OTCs). Geraniol had the highest odor activity value (OAV) in both grape varieties (1100 and 400). (2*E*,6*Z*)-Nona-2,6-dienal (OAVs 300 and 100), (3*Z*)-hex-3-enal (OAVs 180 and 220), (3*R*)-linalool (OAVs 130 and 120), and hexanal (OAVs 120 and 230) also showed relatively high OAVs. The results demonstrated a huge similarity in the odorants of both grape varieties, thus reflecting their close genetic relation. Odor reconstitution models based on odorants with OAVs ≥1 were orthonasally in good agreement with the grape samples, thus illustrating that the major odorants had been correctly identified and quantitated. Omission experiments showed that the combination of (2*S*,4*R*)-rose oxide and geraniol was responsible for the distinct lychee note in the aroma of the Muscaris grapes.

Xingjie Wang, Stephanie Frank, and Martin Steinhaus jointly designed the research. Xingjie Wang performed the practical work, including volatile isolation, GC–O screening, structural identification and quantitation of odorants, calculation of OAVs, and preparation of the sensory tests. Xingjie Wang processed the data and drafted the manuscript. Stephanie Frank and Martin Steinhaus conceived and directed the study, supervised Xingjie Wang’s work, participated in the sensory tests, and revised the manuscript.

### 7.1.4 Reprint Permission



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC

Open Access

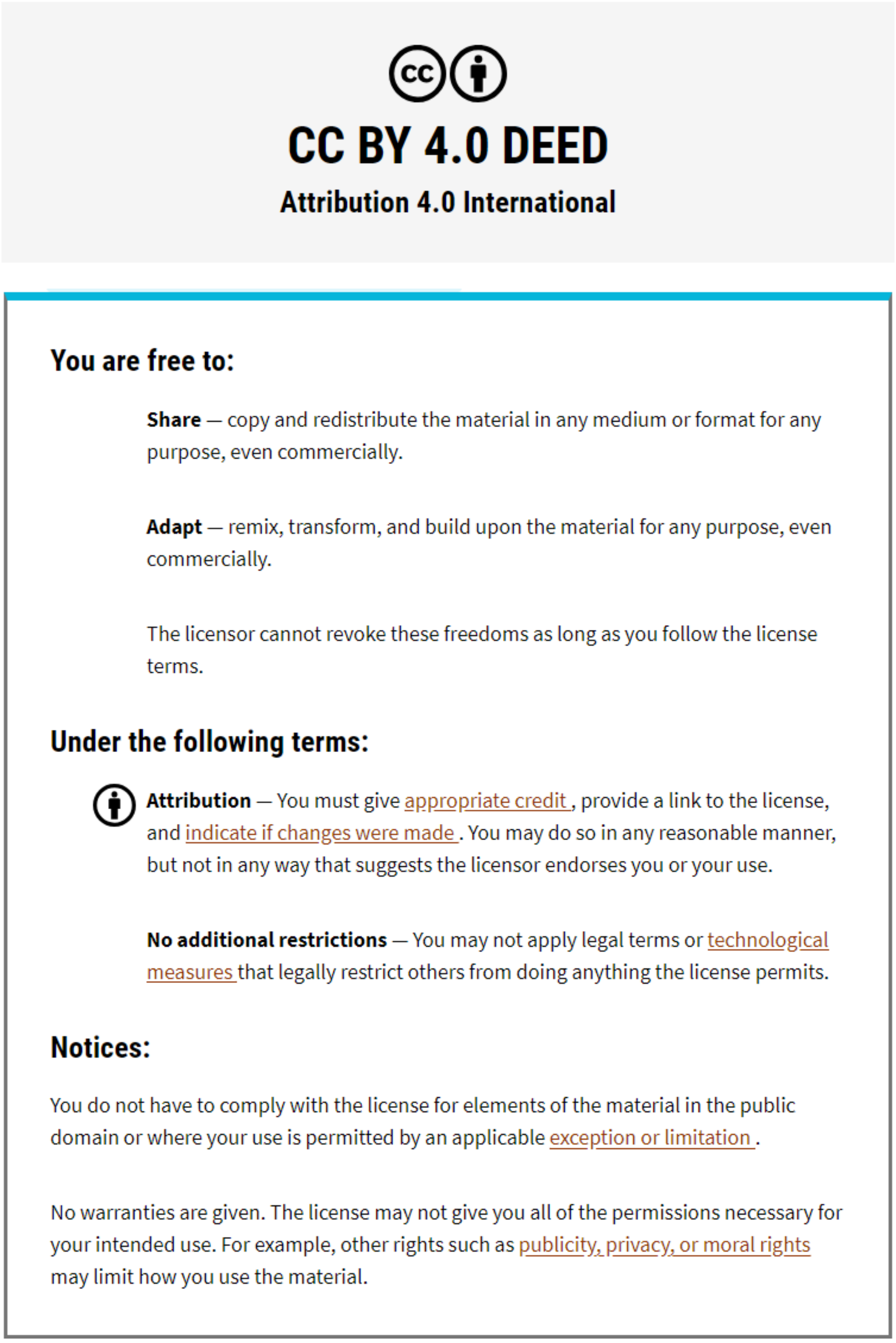
Article

**Molecular Background of the Lychee Aroma of *Vitis vinifera* L. 'Muscaris'**

Xingjie Wang, Stephanie Frank,* and Martin Steinhaus*

Cite This: *J. Agric. Food Chem.* 2024, 72, 1674−1682

Read Online



**CC BY 4.0 DEED**

**Attribution 4.0 International**

**You are free to:**

**Share** — copy and redistribute the material in any medium or format for any purpose, even commercially.

**Adapt** — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

**Under the following terms:**

**Attribution** — You must give appropriate credit , provide a link to the license, and indicate if changes were made . You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

**No additional restrictions** — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.

**Notices:**

You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable exception or limitation .

No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as publicity, privacy, or moral rights may limit how you use the material.

## 7.2 Publication 2: Molecular Insights into the Aroma Difference between Beer and Wine: A Meta-Analysis-Based Sensory Study Using Concentration Leveling Tests

### 7.2.1 Bibliographic Data

Title: Molecular Insights into the Aroma Difference between Beer and Wine: A Meta-Analysis-Based Sensory Study Using Concentration Leveling Tests

Authors: Xingjie Wang, Stephanie Frank, Martin Steinhaus

Journal: Journal of Agricultural and Food Chemistry

Publisher: American Chemical Society

Publication date: September 30, 2024 (online)

Issue date: October 9, 2024

Volume: 72

Issue: 40

Pages: 22250−22257

DOI: https://doi.org/10.1021/acs.jafc.4c06838

Hyperlink: https://pubs.acs.org/doi/10.1021/acs.jafc.4c06838

### 7.2.2 Publication Reprint

For a reprint of publication 2, please turn to the next page.

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

# Molecular Insights into the Aroma Difference between Beer and Wine: A Meta-Analysis-Based Sensory Study Using Concentration Leveling Tests

Xingjie Wang, Stephanie Frank,* and Martin Steinhaus*

**Cite This:** *J. Agric. Food Chem.* 2024, 72, 22250−22257

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

**ABSTRACT:** Beer and wine are popular beverages with clearly different aroma characters, the molecular background of which has not yet been systematically investigated. A comprehensive literature survey returned 14 845 concentration values obtained from 160 beer and 904 wine samples, covering 42 basic beer and 42 basic wine odorants, among which 40 were common to both beverages. Based on mean concentrations and a comparison with threshold data, 29 beer and 32 wine odorants were finally selected to build aroma base models that reflected the basic olfactory difference between beer and wine. Orthonasal concentration leveling tests applied to groups of odorants with similar odor characteristics finally revealed the crucial role of fruity smelling compounds. When 11 fruity compounds, predominantly esters, in the beer aroma base model were adjusted to the respective concentration levels in the wine aroma base model, the sensory panel no longer described the sample as beer-like but as wine-like.

**KEYWORDS:** *beer, wine, aroma base, fruity odorants, literature survey, meta-analysis, concentration leveling test*

## ■ INTRODUCTION

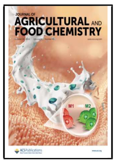
With production volumes of 189 and 26.2 billion liters in 2022, beer and wine are undoubtedly the most important alcoholic beverages in the world.[1,2] Whereas winemaking has a history of approximately 8000 years, humankind started even earlier making beer, presumably around 13 000 bp.[3,4] An important factor that adds to the popularity of beer and wine is their pleasant aroma, which is basically caused by volatile compounds. In both beer and wine, several hundred volatiles have been structurally characterized so far.[5] However, only the volatiles present in concentrations above their respective substance-specific odor threshold concentrations (OTCs) have the potential to contribute to the aroma, which considerably reduces the number of compounds.[6] Among these odor-active volatiles are compounds originating in the starting materials as well as compounds formed during processing and storage.[7−21] For example, malt[7,8] and hops[9−11] are important sources of beer odorants, and so are grapes for wine odorants.[12−17] A major proportion of beer and wine odorants, however, are formed during fermentation.[12,14,18−20] This contributes to the fact that beer and wine have many key odorants in common, which in turn raises the question of which compounds actually account for the aroma difference between beer and wine. In his groundbreaking work on wine aroma, Vicente Ferreira defined an "aroma base" consisting of ethanol and 26 other, mainly also fermentation-derived compounds that produced a basic wine aroma.[22] Further odorants, either individually or in combination, can, if present in sufficient concentration, break the "aroma buffer" formed by the aroma base odorants and generate specific aroma notes, including the woody character of barrel-aged wines[23−25] and specific varietal notes such as the 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) note specifically for Riesling wines.[16,17] No such research was ever undertaken with beer aroma compounds.

The aims of our study were to (i) perform a literature-based meta-analysis on the occurrence and concentrations of beer and wine odorants, (ii) based on the literature data establish aroma base models for beer and wine that reflect the basic olfactory difference between the two, and (iii) identify the odorant group(s) responsible for the aroma difference between the beer and wine base models by sensory analyses using concentration leveling tests.

## ■ MATERIALS AND METHODS

**Literature Survey and Data Extraction.** Web of Science and Google Scholar database searches were conducted using the strings "aroma", "volatile", "odor-active compound", and "odorant" in combination with "beer" and "wine", respectively. Papers written in English and published in 2000 or later were screened for odorant concentration data. Data extraction was applied to papers that fulfilled the following sample and quality criteria: (1) Beer data were obtained from bottom-fermented beers predominantly brewed with barley malt; wine data were obtained from dry red, rosé, or white wines exclusively made from *Vitis vinifera* berries. (2) Fermentation was accomplished with a single commercial *Saccharomyces* yeast strain. (3) The ethanol concentration was within typical limits (3.5−7.5% ALC/VOL in beer, 8.5−17.9% ALC/VOL in wine). And finally, (4) structure assignments complied with the JAFC guidelines for reporting flavor constituents, and, in addition, quantitation methods

**Received:** July 29, 2024
**Revised:** September 6, 2024
**Accepted:** September 18, 2024
**Published:** September 30, 2024



https://doi.org/10.1021/acs.jafc.4c06838

Table 1. Mean Concentrations of Beer Odorants Based on Published Data and the Corresponding OAVs

| compound[a] | concentration[b] (μg/L) | OTC[c] (μg/kg) | OAV[d] | data set size[e] |
|---|---|---|---|---|
| ethyl acetate | 23700 | 5[f] | 4700 | 81 |
| 3-methylbutyl acetate | 2070 | 7.2[g] | 290 | 96 |
| ethyl hexanoate | 239 | 1.2[g] | 200 | 74 |
| 2-phenylethan-1-ol | 25700 | 140[g] | 180 | 92 |
| 3-methylbutan-1-ol | 30000 | 220[g] | 140 | 73 |
| acetaldehyde | 1800 | 16[g] | 110 | 10 |
| ethyl 3-methylbutanoate | 2.41 | 0.023[g] | 100 | 22 |
| dimethyl sulfide | 31.0 | 0.30[g] | 100 | 3 |
| ethyl butanoate | 70.6 | 0.76[g] | 93 | 69 |
| 3-methylbutanal | 35.0 | 0.50[g] | 70 | 52 |
| ethyl octanoate | 581 | 8.7[g] | 67 | 68 |
| 2-methylpropanal | 28.4 | 0.49[g] | 58 | 42 |
| acetic acid | 311000 | 5600[g] | 55 | 3 |
| ethyl 2-methylpropanoate | 3.37 | 0.089[g] | 38 | 7 |
| octanoic acid | 5930 | 190[g] | 31 | 56 |
| butane-2,3-dione | 16.6 | 1.0[g] | 17 | 53 |
| phenylacetic acid | 821 | 68[g] | 12 | 4 |
| 3-(methylsulfanyl)propan-1-ol | 421 | 36[g] | 12 | 6 |
| ethyl 2-methylbutanoate | 1.30 | 0.13[g] | 10 | 19 |
| 3-(methylsulfanyl)propanal | 3.94 | 0.43[g] | 9.2 | 24 |
| 2-methylbutan-1-ol | 10300 | 1200[g] | 8.6 | 64 |
| ethyl propanoate | 85.8 | 10[f] | 8.6 | 19 |
| 3-methylbut-2-ene-1-thiol | 0.00645 | 0.00076[g] | 8.5 | 2 |
| 2-methylbutanal | 8.22 | 1.5[g] | 5.5 | 48 |
| decanoic acid | 2360 | 500[f] | 4.7 | 41 |
| 2-methylpropyl acetate | 205 | 66[f] | 3.1 | 40 |
| phenylacetaldehyde | 13.8 | 5.2[g] | 2.7 | 30 |
| 2-phenylethyl acetate | 788 | 360[g] | 2.2 | 59 |
| 1,1-diethoxyethane | 50 | 25[g] | 2.0 | 1 |
| ethyl decanoate | 84.8 | 122[f] | <1 | 57 |
| butanoic acid | 1380 | 2400[g] | <1 | 16 |
| 2-methylpropan-1-ol | 9600 | 19000[g] | <1 | 78 |
| 3-methylbutanoic acid | 245 | 490[g] | <1 | 15 |
| hexanoic acid | 1780 | 4800[g] | <1 | 55 |
| octan-1-ol | 35.2 | 110[f] | <1 | 5 |
| 2-methylbutanoic acid | 561 | 3100[g] | <1 | 3 |
| hexan-1-ol | 35.1 | 590[g] | <1 | 14 |
| benzaldehyde | 8.37 | 150[g] | <1 | 50 |
| ethyl dodecanoate | 35.0 | 3500[f] | <1 | 4 |
| 2-methylpropanoic acid | 448 | 60000[g] | <1 | 4 |
| ethyl 2-phenylacetate | 0.66 | 155.55[f] | <1 | 1 |
| butan-1-ol | 1.54 | 1900[g] | <1 | 9 |

[a] Compounds in order of decreasing OAVs. [b] Arithmetic mean of individual values resulting from the literature survey; individual values are available in the Supporting Information, Table S3. [c] Orthonasal odor threshold concentration in water. [d] Odor activity value; approximated as ratio of the mean concentration in beer to the orthonasal odor threshold concentration in water. [e] Number of concentration values used to calculate the mean. [f] Data from literature.[44−49] [g] Data taken from the Leibniz-LSB@TUM Odorant Database.[39]

used GC−FID, GC−MS, or LC−MS, included the use of internal standards, and compensated for detector response differences by appropriate calibration approaches. Data not further considered included, for example, data obtained from light and extra high alcohol beers, dealcoholized beers and wines, data obtained from beers and wines made with mixed fermentation, and data obtained after storage at elevated temperature.

**Beer and Wine Samples.** German Bitburger Premium Pils beer (4.8% ALC/VOL; vintage 2022), Spanish Airén dry white wine (10.5% ALC/VOL; vintage 2021), German Silvaner dry white wine (12.0% ALC/VOL; vintage 2019), and German Weissburgunder dry white wine (12.0% ALC/VOL; vintage 2021) were purchased from a local shop in Germany.

**Reference Odorants.** Commercially available compounds were purchased at the highest purity available. Acetaldehyde (≥99%), acetic acid (≥99%), butane-2,3-dione (97%), decanoic acid (≥98%), 1,1-diethoxyethane (99%), dimethyl sulfide (≥99%), ethyl acetate (≥99%), ethyl butanoate (≥99%), ethyl decanoate (≥99%), ethyl hexanoate (≥99%), ethyl 2-methylbutanoate (99%), ethyl 3-methylbutanoate (98%), ethyl 2-methylpropanoate (≥98%), ethyl octanoate (≥98%), 3-hydroxybutan-2-one (97%), 2-methylbutanal (95%), 3-methylbutanal (≥97%), 3-methylbutanoic acid (99%), 2-methylbutan-1-ol (≥99%), 3-methylbutan-1-ol (≥98%), 3-methylbutyl acetate (≥99%), 2-methylpropanal (≥99%), 2-methylpropyl acetate (99%), 3-(methylsulfanyl)propanal (96%), 3-(methylsulfanyl)propan-1-ol (≥98%), octanoic acid (≥98%), phenylacetic acid (99%), 2-phenylethan-1-ol (≥99%) were from Merck (Darmstadt, Germany); ethyl propanoate (≥99%), hexan-1-ol (99%), phenylacetaldehyde (95%), 2-phenylethyl acetate (98%) were from Thermo Fisher Scientific (Dreieich, Germany); 2-methylpropan-1-ol

**Table 2. Mean Concentrations of Wine Odorants Based on Published Data and the Corresponding OAVs**

| compound[a] | concentration[b] (μg/L) | OTC[c] (μg/kg) | OAV[d] | data set size[e] |
|---|---|---|---|---|
| ethyl acetate | 69100 | 5[f] | 14000 | 464 |
| acetaldehyde | 49100 | 16[g] | 3100 | 144 |
| butane-2,3-dione | 1400 | 1.0[g] | 1400 | 85 |
| ethyl hexanoate | 1570 | 1.2[g] | 1300 | 658 |
| ethyl 3-methylbutanoate | 27.5 | 0.023[g] | 1200 | 320 |
| ethyl 2-methylpropanoate | 93.5 | 0.089[g] | 1100 | 264 |
| 3-methylbutan-1-ol | 172000 | 220[g] | 780 | 555 |
| 3-methylbutyl acetate | 3650 | 7.2[g] | 510 | 719 |
| ethyl butanoate | 374 | 0.76[g] | 490 | 596 |
| ethyl 2-methylbutanoate | 42.7 | 0.13[g] | 330 | 270 |
| ethyl octanoate | 2460 | 8.7[g] | 280 | 687 |
| 3-methylbutanal | 119 | 0.50[g] | 240 | 104 |
| 2-phenylethan-1-ol | 28700 | 140[g] | 200 | 684 |
| 2-methylpropanal | 36.5 | 0.49[g] | 74 | 112 |
| 2-methylbutan-1-ol | 70100 | 1200[g] | 58 | 128 |
| dimethyl sulfide | 14.1 | 0.30[g] | 47 | 82 |
| acetic acid | 219000 | 5600[g] | 39 | 229 |
| 3-(methylsulfanyl)propan-1-ol | 1360 | 36[g] | 38 | 311 |
| 3-(methylsulfanyl)propanal | 14.6 | 0.43[g] | 34 | 122 |
| ethyl propanoate | 295 | 10[f] | 30 | 150 |
| octanoic acid | 5580 | 190[g] | 29 | 582 |
| 3-hydroxybutan-2-one | 16600 | 590[g] | 28 | 164 |
| 2-methylbutanal | 40.2 | 1.5[g] | 27 | 31 |
| phenylacetic acid | 452 | 68[g] | 6.6 | 48 |
| ethyl decanoate | 741 | 122[f] | 6.1 | 595 |
| decanoic acid | 2460 | 500[f] | 4.9 | 489 |
| hexan-1-ol | 2710 | 590[g] | 4.6 | 657 |
| phenylacetaldehyde | 21.5 | 5.2[g] | 4.1 | 185 |
| 2-phenylethyl acetate | 682 | 360[g] | 1.9 | 607 |
| 2-methylpropan-1-ol | 33000 | 19000[g] | 1.7 | 486 |
| 3-methylbutanoic acid | 814 | 490[g] | 1.7 | 264 |
| 2-methylpropyl acetate | 101 | 66[f] | 1.5 | 241 |
| hexanoic acid | 4060 | 4800[g] | <1 | 533 |
| benzaldehyde | 108 | 150[g] | <1 | 323 |
| butan-1-ol | 1120 | 1900[g] | <1 | 259 |
| butanoic acid | 1180 | 2400[g] | <1 | 241 |
| octan-1-ol | 44.7 | 110[f] | <1 | 169 |
| ethyl 2-phenylacetate | 53.8 | 155.55[f] | <1 | 208 |
| 2-methylbutanoic acid | 545 | 3100[g] | <1 | 64 |
| ethyl dodecanoate | 269 | 3500[f] | <1 | 247 |
| propanoic acid | 1490 | 20000[g] | <1 | 64 |
| 2-methylpropanoic acid | 2180 | 60000[g] | <1 | 235 |

[a] Compounds in order of decreasing OAVs. [b] Arithmetic mean of individual values resulting from the literature survey; individual values are available in the Supporting Information, Table S6. [c] Orthonasal odor threshold concentration in water. [d] Odor activity value; approximated as ratio of the mean concentration in wine to the orthonasal odor threshold concentration in water. [e] Number of concentration values used to calculate the mean. [f] Data from literature.[44−49] [g] Data taken from the Leibniz-LSB@TUM Odorant Database.[39]

(≥99%) was from TCI (Eschborn, Germany). 3-Methylbut-2-ene-1-thiol was synthesized according to a previously published procedure.[26] The absence of odor-active impurities in the reference odorants was confirmed by GC−O.[27]

**Miscellaneous Chemicals.** Citric acid monohydrate, glycerol, potassium hydroxide, and (2*R*,3*R*)-tartaric acid were purchased from Merck. Ethanol (99.9%) was obtained from Honeywell (Seelze, Germany).

**Aroma Models.** For the beer aroma base model, glycerol (1.3 g) and citric acid (150 mg) were added to a 1 L volumetric flask and dissolved in a minimum amount of deionized water (~10 mL).[28] Distinct volumes (40 μL−1.3 mL) of individual ethanolic stock solutions prepared from the reference odorants or aqueous dilutions thereof were added to achieve final concentrations in agreement with the data compiled in Table 1. Further ethanol was added to achieve a final concentration of 5% ALC/VOL. The solution was first made up to ~990 mL with deionized and carbonated water, and the pH was adjusted to 4.5 using aqueous potassium hydroxide (2 mol/L) before the solution was finally made up to 1 L. For the wine aroma base model, glycerol (7.5 g) and tartaric acid (1.3 g) were added to a 1 L volumetric flask and dissolved in a minimum amount of deionized water (~10 mL).[28] Distinct volumes (30 μL−3 mL) of individual ethanolic stock solutions prepared from the reference odorants were added to achieve final concentrations in agreement with the data compiled in Table 2. Further ethanol was added to achieve a final concentration of 12.9% ALC/VOL. The solution was first made up to ~990 mL with deionized water, and the pH was adjusted to 3.4 using aqueous potassium hydroxide (2 mol/L) before the solution was finally made up to 1 L.

Aroma models used in the concentration leveling tests were prepared on the basis of either the beer aroma base model or the wine aroma base model; however, the concentrations of defined groups of odorants were adjusted to the levels present in the aroma base model of the other beverage.

**Sensory Evaluations.** All sensory tests were carried out in separate booths of a room exclusively dedicated to sensory evaluations. The room temperature was 22 ± 2 °C. Samples (10 mL) were provided in cylindrical polytetrafluoroethylene vessels (5.7 cm height, 3.5 cm inner diameter, 50 mL nominal volume) with lids (Bohlender; Grünsfeld, Germany). The sample temperature was 10 °C. Samples were evaluated orthonasally.

Three initial sensory sessions were dedicated to training and panel member selection. In session 1, 15 assessors recruited from the sensory panel of the Leibniz-LSB@TUM were asked to familiarize themselves with the aroma characteristics of beer and wine using nine test samples. Samples 1–4 consisted of a commercial beer sample and three commercial wine samples. The beer was made with a single hop addition at the beginning of the boil and thus did not show a hoppy aroma character. The wines did not show distinct varietal characters and were not barrel-aged. The beer was additionally presented after defoaming by filtration through a paper filter (sample 5). Samples 6–9 were volatile isolates of the four commercial beverages obtained by automated solvent-assisted flavor evaporation (aSAFE)[29] at 40 °C using valve open/closed time combinations of 0.2 s/30 s (beer) and 0.2 s/60 s (wine). In training session 2, the ability of the assessors to correctly assign the samples used in session 1 to beer or wine was tested. For this purpose, the samples were presented in a random order and tested blindly. Likewise, in training session 3, the ability of the assessors to correctly assign the aroma base models of beer and wine was tested. Nine assessors with 100% correct answers in training sessions 2 and 3 were finally selected to perform the concentration leveling tests. This expert panel consisted of four males and five females aged 29–54. From time to time, they were retested according to training session 3 to ensure their expert status.

In the concentration leveling tests, the assessors were provided with the test sample, as well as with the aroma base models as references. They were asked to evaluate the test sample for its similarity with beer and wine and accordingly mark a position on a 20 cm ruler, of which the left margin was defined as 100% beer-like and 0% wine-like, represented by the beer aroma base model, whereas the right margin was defined as 100% wine-like and 0% beer-like and was represented by the wine aroma base model. The information and evaluation sheets given to the assessors are available in the Supporting Information. The marks on the ruler were finally converted to percentages of beer-like and wine-like depending on the exact position of the mark between the extremes. From the results of the individual assessors, the panel result was calculated as an arithmetic mean.

## RESULTS AND DISCUSSION

**Meta-Analysis.** The literature survey initially resulted in a total of ~950 and ~6650 articles for beer and wine, respectively. After applying the sample and quality criteria filters detailed in the Materials and Methods section, the numbers substantially decreased to 32 papers containing beer odorant concentrations and 252 papers containing wine odorant concentrations (cf. Supporting Information, Tables S1 and S4). The papers covered a total of 160 beer samples and 904 wine samples (cf. Supporting Information, Tables S2 and S5). Concentration data were subsequently extracted for all odorants that were considered to contribute to the aroma of beer or wine in general, i.e., independently of special raw materials and processing variants.

Compounds *not* considered included wine odorants associated with specific grape varieties and wine odorants originating from barrel aging. For example, TDN characterizes wines made from Riesling grapes, 4-methyl-4-sulfanylpentan-2-one characterizes Sauvignon Blanc wines, and odor-active amounts of *cis*-whisky lactone are associated with barrel aging.[16,21,30] Likewise, beer odorants simply transferred from hops were excluded from the meta-analysis because due to evaporation they do not appear in beers brewed with a single hop dosage at the beginning of wort boiling. For this reason, e.g., linalool and geraniol were not considered. Hop-derived 3-methylbut-2-ene-1-thiol, however, was included in the meta-analysis, as it is formed in beer from nonvolatile hop bitter constituents. Among the compounds finally classified as basic beer and wine odorants were, as expected, particularly compounds formed during fermentation.[31−37] For example, compounds 2-phenylethan-1-ol, 3-methylbutan-1-ol, and 2-methylbutan-1-ol are typical fermentation byproducts.

The total numbers of compounds classified as basic beer and wine odorants were 42 for beer and 42 for wine. The vast majority, namely, 40 odorants, were common to both beverages. The concentration data extracted from the literature for the compounds consisted of 14 845 individual concentration values. These are compiled in the Supporting Information, Tables S3 and S6. For each odorant, a mean concentration value was then calculated in beer and wine, respectively (Tables 1 and 2). The overall range of the mean odorant concentrations in beer was 6.45 ng/L (3-methylbut-2-ene-1-thiol) to 311 mg/L (acetic acid), whereas in wine, the values ranged from 14.1 μg/L (dimethyl sulfide) to 219 mg/L (acetic acid).

To assess the aroma relevance of the individual compounds, odor activity values (OAVs) were calculated from the concentration values (cf. Tables 1 and 2, fourth column). This approach resulted in 13 beer odorants and 10 wine odorants whose mean concentrations were below the OTCs, i.e., showed OAVs <1. These compounds were thus not further considered, leaving 29 beer odorants and 32 wine odorants that were considered essential for the aroma base models. Among them, 27 odorants were common to beer and wine. The compound with the highest mean OAV was ethyl acetate in both beer (OAV 4700) and wine (OAV 14 000). The mean OAVs of nine of these 27 odorants were quite similar in beer and wine, i.e., they did not differ by more than a factor of 2. Only two compounds, dimethyl sulfide and 2-methylpropyl acetate, showed slightly higher OAVs in beer, whereas 16 odorants showed higher OAVs in wine. Particularly high differences (factor wine/beer >10) were obtained for butane-2,3-dione, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, acetaldehyde, and ethyl 3-methylbutanoate.

Of the 32 wine odorants with mean OAVs >1 in our study, 22 were also included in the aroma base of wine suggested by Ferreira.[22] Among the five additional compounds included in Ferreira's set were ethanol, which we treated as a matrix component rather than an odorant (cf. section below) and the four carboxylic acids hexanoic, butanoic, 2-methylbutanoic, and 2-methylpropanoic acid, which were part of our initial compound selection but were finally discarded because their mean OAVs were <1. On the other hand, our wine base compound selection included 10 additional odorants not considered by Ferreira, namely 3-methylbutanal (OAV 240), 2-methylpropanal (OAV 74), 2-methylbutan-1-ol (OAV 58), dimethyl sulfide (OAV 47), 3-(methylsulfanyl)propanal (OAV 34), ethyl propanoate (OAV 30), 3-hydroxybutan-2-one (OAV 28), 2-methylbutanal (OAV 27), phenylacetic acid (OAV 6.6), and phenylacetaldehyde (OAV 4.1). Five of these additional

Table 3. OAV Sums of Odorant Groups in the Aroma Base Models of Beer and Wine

| group[a] | odorant | OAV sum[b] |  | OAV sum ratio wine/beer[c] |
|---|---|---|---|---|
|  |  | beer | wine |  |
| "buttery" | butane-2,3-dione<br>3-hydroxybutan-2-one | 17 | 1400 | 86 |
| "fruity" | 1,1-diethoxyethane<br>ethyl butanoate<br>ethyl decanoate<br>ethyl hexanoate<br>ethyl 2-methylbutanoate<br>ethyl 3-methylbutanoate<br>ethyl 2-methylpropanoate<br>ethyl octanoate<br>ethyl propanoate<br>3-methylbutyl acetate<br>2-methylpropyl acetate | 810 | 5200 | 6.4 |
| "malty" | 2-methylbutanal<br>3-methylbutanal<br>2-methylbutan-1-ol<br>3-methylbutan-1-ol<br>2-methylpropanal<br>2-methylpropan-1-ol | 280 | 1200 | 4.2 |
| "miscellaneous" | acetaldehyde<br>acetic acid<br>ethyl acetate<br>hexan-1-ol | 4900 | 17000 | 3.5 |
| "honey" | phenylacetaldehyde<br>phenylacetic acid<br>2-phenylethan-1-ol<br>2-phenylethyl acetate | 200 | 220 | 1.1 |
| "sweaty" | decanoic acid<br>3-methylbutanoic acid<br>octanoic acid | 36 | 36 | 1.0 |
| "sulfury" | dimethyl sulfide<br>3-methylbut-2-ene-1-thiol<br>3-(methylsulfanyl)propanal<br>3-(methylsulfanyl)propan-1-ol | 130 | 120 | 0.89 |

[a]Odorants were grouped into "buttery", "fruity", "malty", "honey", "sweaty", "sulfury", or "miscellaneous" according to their predominant odor qualities.[39] [b]Sum of the OAVs of the individual odorants within the group. [c]For each group, a ratio was calculated by dividing the OAV sum in wine by the OAV sum in beer.

compounds were aldehydes formed by amino acid degradation.[36,38]

**Beer and Wine Aroma Base Models.** The outcome of the literature survey and the subsequent meta-analysis, i.e., the 29 beer and 32 wine odorants showing OAVs >1 and their corresponding mean concentrations (cf. Tables 1 and 2), formed the data basis for our aroma base models. The models were hydroalcoholic solutions of the odorants and additionally included major acids, glycerol, pH adjustment, and, in the case of the beer model, carbonation (Supporting Information, Table S7). An expert panel of nine trained assessors was repeatedly able to correctly assign the aroma base models in blind tests to beer and wine, respectively. Thus, the aroma base models for beer and wine reflected the basic olfactory difference between the two. This was the prerequisite for the subsequent concentration leveling tests.

**Concentration Leveling Tests.** To get an idea of which compounds contribute to the aroma difference between the beer and wine aroma base models, the concentration levels of selected odorants in one model were adjusted to the concentration levels in the other model, and the effect on the overall odor was evaluated in a sensory test. For this purpose, the odorants were classified into seven groups according to their predominant odor character,[39] namely "buttery", "fruity", "malty", "honey", "sweaty", "sulfury", or "miscellaneous" (Table 3). Based on the OAV sums, the biggest difference between the beer and wine aroma models was in the buttery group, followed by the fruity, malty, and miscellaneous groups. No substantial difference between beer

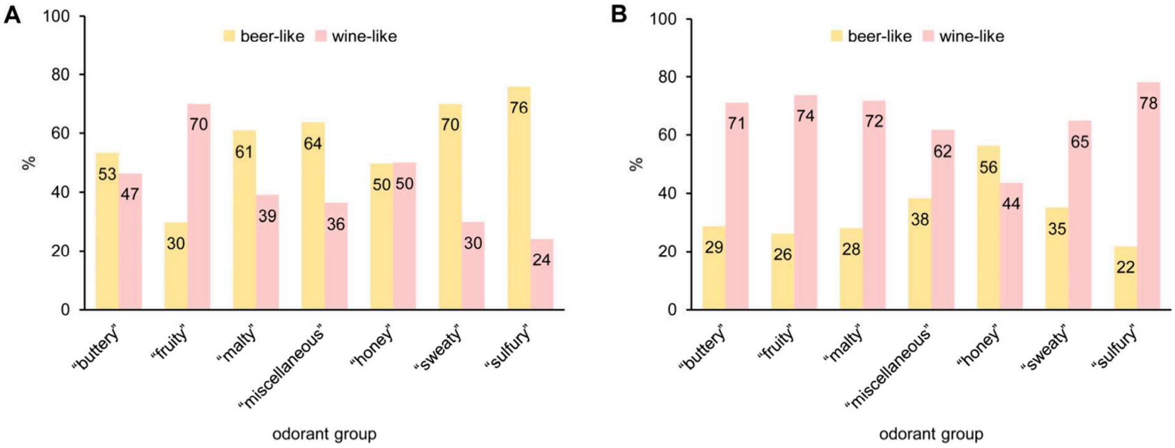


**Figure 1.** Results of the concentration leveling tests based on the beer (A) and wine (B) aroma base models. Assessors rated the odor similarity of test samples to the beer and wine aroma base models.

and wine was found in the OAV sums of the honey, sweaty, and sulfury groups.

The results of the concentration leveling tests are summarized in Figure 1. Figure 1A depicts the results of the sensory tests based on the beer aroma base model. This means the test samples equaled the beer aroma base model in all matrix compound concentrations (including the ethanol concentration) and all odorant concentrations except those assigned to the odorant group given on the *x*-axis. As detailed in Figure 1A, when the concentrations of the sulfury, sweaty, miscellaneous, or malty odorants were adjusted to the levels in the wine aroma base model, the panel still evaluated the overall odor as more beer-like, with percentages of 61−76%. This was considered a clear result, given that even an experiment with the full beer aroma base model as a test sample, i.e., the test sample was identical to the reference sample defining 100% beer-like, did not give a result of 100% but only of 88% (data not shown). No clear assignment to beer or wine was possible after the buttery or honey-like odorants were adjusted to the wine aroma base levels. However, the biggest effect was observed when the concentrations of the fruity odorants, which were clearly more odor-active in the wine model (cf. Table 3), were adjusted. In this case, the test sample was rated more wine-like (70%) than beer-like (30%), suggesting higher ester levels in wine as a crucial parameter for the aroma difference to beer.

The overall olfactory impression of the wine aroma base model (Figure 1B) was even more stable than that of the beer aroma base model. Six of the seven leveling tests still resulted in a clear assignment to wine, even including the test in which the fruity compounds were adjusted to the beer aroma base levels. The corresponding percentages obtained for wine-like ranged from 62 to 78%; the full wine aroma base model as a test sample resulted in 83% wine-like (data not shown). The only leveling test using the wine aroma base model that resulted in an inversion of the sensory evaluation was the one in which the honey-like odorants were adjusted to the levels in the beer aroma base model. In this case, the test sample was rated slightly more beer-like than wine-like. This result, on the one hand, was surprising because the concentration differences between the beer and wine aroma base models in the four honey-like smelling odorants were only minor, but, on the other hand, was in line with the result obtained with the beer aroma base model depicted in Figure 1A.

To answer the question of to what extent the matrix composition contributed to the assignment of a test sample to beer or wine, two further sensory experiments were conducted. Test sample 1 combined the matrix composition of the wine aroma base model with all the odorant concentrations in the beer aroma base model, and test sample 2 combined the matrix composition of the beer aroma base model with all the odorant concentrations in the wine aroma base model. The test sample with the beer aroma base odorants in the wine matrix was evaluated as more beer-like (65%), and the test sample with the wine aroma base odorants in the beer matrix was evaluated as more wine-like (66%). Thus, percentages were somewhat lower than those obtained for the models with the beer aroma base odorants in the beer matrix (88%) and the wine aroma base odorants in the wine matrix (83%). This indicated an influence of the matrix. However, in both cases, the odorant composition and not the matrix composition determined whether the model was evaluated as more beer-like or more wine-like. In other words, the odorant composition dominated over the matrix composition. The influence of the matrix composition has been studied for beer[40] and wine[41−43] before, however, only separately and never in combination with a simultaneous assessment of the beer- and wine-like aroma character.

In summary, this study provided an extensive literature survey of basic odor-active compounds in beer and wine and their concentration ranges. The majority of these basic odorants originate in the fermentation step and are common to both beer and wine; however, substantial differences exist between beer and wine in the concentrations of some odorants. The sensory experiments revealed that beer and wine can be olfactorily differentiated on the basis of the basic odorants and that particularly higher ester concentrations in wine are a crucial discriminating parameter, whereas the impact of the matrix composition, including the different alcohol content, was only minor. In real life, however, further factors not investigated in this study may additionally contribute to the different perceptions of beer and wine aroma. For example, the drinking temperature of beer is normally lower than the drinking temperature of wine, whereas in our study an intermediate temperature of 10 °C was used for all sensory tests. Furthermore, the effect of beer foam on the release of odorants was also not considered, as no foaming agent was present in our beer model. Nevertheless, the finding

https://doi.org/10.1021/acs.jafc.4c06838
J. Agric. Food Chem. 2024, 72, 22250−22257

that the odorant composition dominated over the matrix composition might be used in the beverage industry to develop novel and innovative low-alcohol beverages and to meet changing consumer preferences.

The concentration leveling tests used in our study proved to be a promising tool to clarify the molecular background of aroma differences between two samples in general and are therefore predestined for more applications in the future. These may include the comparison of fresh and stored samples, differently processed samples, samples of different varieties, and samples with and without off-flavor.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c06838.

> References used for data extraction, sample characteristics, and extracted odorant concentrations, ethanol concentrations, and pH data; matrix compositions of the aroma base models; additional information on sensory analyses (PDF)

## AUTHOR INFORMATION

### Corresponding Authors

**Stephanie Frank** — *Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany;* orcid.org/0000-0001-5853-4839; Phone: +49 8161 71 2990; Email: s.frank.leibniz-lsb@tum.de

**Martin Steinhaus** — *Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany; Technical University of Munich, TUM School of Natural Sciences, Department of Chemistry, 85748 Garching, Germany;* orcid.org/0000-0002-9879-1474; Phone: +49 8161 71 2991; Email: m.steinhaus.leibniz-lsb@tum.de

### Author

**Xingjie Wang** — *Technical University of Munich, TUM School of Natural Sciences, Department of Chemistry, 85748 Garching, Germany; Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), 85354 Freising, Germany;* orcid.org/0000-0001-8185-2739

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.4c06838

### Funding

Xingjie Wang gratefully acknowledges funding from the China Scholarship Council (CSC), grant no. 201906300007.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank Jörg Stein for the skillful technical assistance.

## ABBREVIATIONS

aSAFE, automated solvent-assisted flavor evaporation; FID, flame ionization detector; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; O, olfactometry; OAV, odor activity value; OTC, odor threshold concentration; TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene

### Nomenclature

citric acid monohydrate,2-hydroxypropane-1,2,3-tricarboxylic acid hydrate; dimethyl sulfide,(methylsulfanyl)methane; glycerol,propane-1,2,3-triol; (2*R*,3*R*),tartaric acid,; (2*R*,3*R*)-2,3-dihydroxybutanedioic; *cis*-whisky lactone,(4*R*,5*R*)/(4*S*,5*S*)-5-butyl-4-methyloxolan-2-one

## REFERENCES

(1) Barth-Haas Group. *Beer Production Worldwide from 1998 to 2022 (in Billion Hectoliters)*; Statista: Hamburg, Germany, 2023; https://www.statista.com/statistics/270275/worldwide-beer-production/ (accessed 2024-07-24).

(2) *World Wine Production Outlook—OIV First Estimates*; International Organisation of Vine and Wine: Dijon, France, 2023; https://www.oiv.int/sites/default/files/documents/OIV_World_Wine_Production_Outlook_2023.pdf (accessed 2024-07-24).

(3) McGovern, P.; Jalabadze, M.; Batiuk, S.; Callahan, M. P.; Smith, K. E.; Hall, G. R.; Kvavadze, E.; Maghradze, D.; Rusishvili, N.; Bouby, L.; Failla, O.; Cola, G.; Mariani, L.; Boaretto, E.; Bacilieri, R.; This, P.; Wales, N.; Lordkipanidze, D. Early Neolithic wine of Georgia in the South Caucasus. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E10309−E10318.

(4) Liu, L.; Wang, J.; Rosenberg, D.; Zhao, H.; Lengyel, G.; Nadel, D. Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: investigating Natufian ritual feasting. *J. Archaeol. Sci. Rep.* **2018**, *21*, 783−793.

(5) Van Dongen, W. D.; Donders, J. J. H. Database, version 16.10, 1963−2024. *Introduction to Volatile Compounds in Food*; BeWiDo BV: Reeuwijk, The Netherlands, 2024; https://www.vcf-online.nl/VcfHome.cfm (accessed 2024-07-24).

(6) Steinhaus, M. Gas chromatography-olfactometry: principles, practical aspects and applications in food analysis. In *Advanced Gas Chromatography in Food Analysis*; Tranchida, P., Ed.; The Royal Society of Chemistry: Cambridge, UK, 2019; pp 337−399.

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

(8) Langos, D.; Granvogl, M.; Schieberle, P. Characterization of the key aroma compounds in two Bavarian wheat beers by means of the sensomics approach. *J. Agric. Food Chem.* **2013**, *61*, 11303−11311.

(9) Steinhaus, M.; Fritsch, H. T.; Schieberle, P. Quantitation of (*R*)- and (*S*)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100−7105.

(10) Reglitz, K.; Lemke, N.; Hanke, S.; Steinhaus, M. On the behavior of the important hop odorant 4-mercapto-4-methylpentan-2-one (4MMP) during dry hopping and during storage of dry hopped beer. *Brew. Sci.* **2018**, *71*, 96−99.

(11) Neiens, S. D.; Steinhaus, M. Investigations on the impact of the special flavor hop variety Huell Melon on the odor-active compounds in late hopped and dry hopped beers. *J. Agric. Food Chem.* **2019**, *67*, 364−371.

(12) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.

(13) Ong, P. K. C.; Acree, T. E. Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. *J. Agric. Food Chem.* **1999**, *47*, 665−670.

(14) Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J. Agric. Food Chem.* **2004**, *52*, 1653−1660.

(15) Benkwitz, F.; Nicolau, L.; Lund, C.; Beresford, M.; Wohlers, M.; Kilmartin, P. A. Evaluation of key odorants in Sauvignon Blanc wines using three different methodologies. *J. Agric. Food Chem.* **2012**, *60*, 6293−6302.

(16) Sacks, G. L.; Gates, M. J.; Ferry, F. X.; Lavin, E. H.; Kurtz, A. J.; Acree, T. E. Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaph-

thalene (TDN) and concentrations in young Riesling and non-Riesling wines. *J. Agric. Food Chem.* **2012**, *60*, 2998−3004.

(17) Dein, M.; Kerley, T.; Munafo, J. P. Characterization of odorants in a 10-Year-Old Riesling wine. *J. Agric. Food Chem.* **2021**, *69*, 11372−11381.

(18) Féchir, M.; Reglitz, K.; Mall, V.; Voigt, J.; Steinhaus, M. Molecular insights into the contribution of specialty barley malts to the aroma of bottom-fermented lager beers. *J. Agric. Food Chem.* **2021**, *69*, 8190−8199.

(19) Reglitz, K.; Féchir, M.; Mall, V.; Voigt, J.; Steinhaus, M. The impact of caramel and roasted wheat malts on aroma compounds in top fermented wheat beer. *J. Inst. Brew.* **2022**, *128*, 138−149.

(20) Ferreira, V.; Ortín, N.; Escudero, A.; López, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048−4054.

(21) Frank, S.; Schieberle, P. Changes in the major odorants of grape juice during manufacturing of Dornfelder red wine. *J. Agric. Food Chem.* **2022**, *70*, 13979−13986.

(22) Ferreira, V. Volatile aroma compounds and wine sensory attributes. In *Managing Wine Quality: Viticulture and Wine Quality*; Reynolds, A. G., Ed.; Woodhead Publishing: Cambridge, UK, 2010; pp 3−28.

(23) de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Ferreira, V. On the effects of higher alcohols on red wine aroma. *Food Chem.* **2016**, *210*, 107−114.

(24) de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Ferreira, V. Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions. *Aust. J. Grape Wine Res.* **2017**, *23*, 162−169.

(25) de-la-Fuente-Blanco, A.; Sáenz-Navajas, M. P.; Valentin, D.; Ferreira, V. Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. *Food Chem.* **2020**, *307*, 125553.

(26) Holscher, W.; Vitzthum, O. G.; Steinhart, H. Prenyl alcohol source for odorants in roasted coffee. *J. Agric. Food Chem.* **1992**, *40*, 655−658.

(27) Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.

(28) Souci, S. W.; Fachmann, W.; Kraut, H. *Food Composition and Nutrition Tables*, 8th ed.; Medpharm Scientific Publishers: Stuttgart, Germany, 2016; pp 1151−1169.

(29) Schlumpberger, P.; Stübner, C. A.; Steinhaus, M. Development and evaluation of an automated solvent-assisted flavour evaporation (aSAFE). *Eur. Food Res. Technol.* **2022**, *248*, 2591−2602.

(30) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385−392.

(31) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139−173.

(32) Styger, G.; Prior, B.; Bauer, F. F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1145−1159.

(33) Procopio, S.; Qian, F.; Becker, T. Function and regulation of yeast genes involved in higher alcohol and ester metabolism during beverage fermentation. *Eur. Food Res. Technol.* **2011**, *233*, 721−729.

(34) Olaniran, A. O.; Hiralal, L.; Mokoena, M. P.; Pillay, B. Flavour-active volatile compounds in beer: production, regulation and control. *J. Inst. Brew.* **2017**, *123*, 13−23.

(35) Holt, S.; Miks, M. H.; de Carvalho, B. T.; Foulquié-Moreno, M. R.; Thevelein, J. M. The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. *FEMS Microbiol. Rev.* **2019**, *43*, 193−222.

(36) Baert, J. J.; De Clippeleer, J.; Hughes, P. S.; De Cooman, L.; Aerts, G. On the origin of free and bound staling aldehydes in beer. *J. Agric. Food Chem.* **2012**, *60*, 11449−11472.

(37) Castejón-Musulén, O.; Aragón-Capone, A. M.; Ontañón, I.; Peña, C.; Ferreira, V.; Bueno, M. Accurate quantitative determination of the total amounts of strecker aldehydes contained in wine. Assessment of their presence in table wines. *Food Res. Int.* **2022**, *162*, 112125.

(38) Marrufo-Curtido, A.; Ferreira, V.; Escudero, A. Factors that affect the accumulation of strecker aldehydes in standardized wines: the importance of pH in oxidation. *Molecules* **2022**, *27*, 3056.

(39) Kreissl, J., Mall, V., Steinhaus, P., Steinhaus, M. *Leibniz-LSB@TUM Odorant Database*, version 1.2; Leibniz Institute for Food Systems Biology at the Technical University of Munich: Freising, Germany, 2022; https://www.leibniz-lsb.de/en/databases/leibniz-lsbtum-odorant-database (accessed July 24, 2024).

(40) Ickes, C. M.; Cadwallader, K. R. Effects of ethanol on flavor perception in alcoholic beverages. *Chem. Percept.* **2017**, *10*, 119−134.

(41) Sáenz-Navajas, M. P.; Campo, E.; Culleré, L.; Fernández-Zurbano, P.; Valentin, D.; Ferreira, V. Effects of the nonvolatile matrix on the aroma perception of wine. *J. Agric. Food Chem.* **2010**, *58*, 5574−5585.

(42) Frank, S.; Wollmann, N.; Schieberle, P.; Hofmann, T. Reconstitution of the flavor signature of Dornfelder red wine on the basis of the natural concentrations of its key aroma and taste compounds. *J. Agric. Food Chem.* **2011**, *59*, 8866−8874.

(43) Villamor, R. R.; Ross, C. F. Wine matrix compounds affect perception of wine aromas. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 1−20.

(44) Pang, X.; Guo, X.; Qin, Z.; Yao, Y.; Hu, X.; Wu, J. Identification of aroma-active compounds in Jiashi muskmelon juice by GC−O−MS and OAV calculation. *J. Agric. Food Chem.* **2012**, *60*, 4179−4185.

(45) Mall, V.; Schieberle, P. Evaluation of key aroma compounds in processed prawns (whiteleg shrimp) by quantitation and aroma recombination experiments. *J. Agric. Food Chem.* **2017**, *65*, 2776−2783.

(46) Yilmaztekin, M. Characterization of potent aroma compounds of cape gooseberry (*Physalis peruviana* L.) fruits grown in Antalya through the determination of odor activity values. *Int. J. Food Prop.* **2014**, *17*, 469−480.

(47) Buttery, R. G.; Turnbaugh, J. G.; Ling, L. C. Contribution of volatiles to rice aroma. *J. Agric. Food Chem.* **1988**, *36*, 1006−1009.

(48) Niu, Y.; Wang, P.; Xiao, Z.; Zhu, J.; Sun, X.; Wang, R. Evaluation of the perceptual interaction among ester aroma compounds in cherry wines by GC−MS, GC−O, odor threshold and sensory analysis: an insight at the molecular level. *Food Chem.* **2019**, *275*, 143−153.

(49) Giri, A.; Osako, K.; Ohshima, T. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chem.* **2010**, *120*, 621−631.

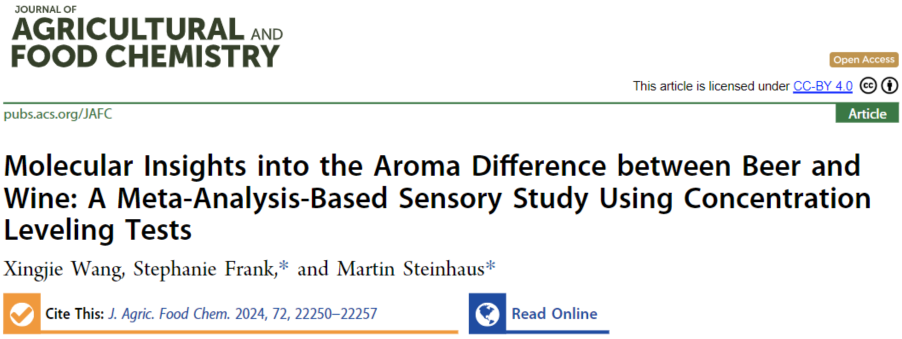
### 7.2.3 Summary and Individual Contributions

Beer and wine clearly differ in their aroma. However, which substances account for the aroma difference between the two has not been systematically studied yet. It is undoubtful that special raw materials or processing can facilitate recognition, however, we hypothesized that there is a basic olfactory difference between beer and wine that is reflected in the composition of more general odorants. Odorants constituting a basic wine aroma have already been suggested in the literature and included primarily compounds formed during fermentation. However, no quantitative data on these odorants were available and the concept of an aroma base has not been applied to beer yet.

As a first step towards elucidating the aroma difference between beer and wine on the molecular level, a comprehensive literature survey of beer and wine was performed. By focusing on general beer and wine odorants and on data based on reliable identification and quantitation methodology, the number of articles was considerably reduced. Finally, mean concentrations of 42 basic odorants generally present in beer and wine were calculated from data of 160 beer samples extracted from 32 articles and data of 904 wine samples extracted from 252 articles. The mean concentrations of 32 of the 40 shared odorants were higher in wine than in beer. The highest concentrations were calculated for acetic acid in both beer (311 mg/L) and wine (219 mg/L). The calculation of odor activity values (OAVs) resulted in 29 and 32 compounds with OAVs ≥1 in beer and wine, respectively. Ethyl acetate showed the highest OAVs in both beer (OAV 4700) and wine (OAV 14000). Big differences in the OAVs (>10-fold) were obtained for butan-2,3-dione, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, acetaldehyde, and ethyl 3-methylbutanoate. Aroma base models based on the mean concentration data reflected the basic olfactory difference between beer and wine. Finally, concentration leveling tests were performed to elucidate the odorants responsible for the aroma difference between the two models by sensory evaluation. For this purpose, the odorants in the two models were classified according to their predominant odor qualities into seven groups, namely “buttery”, “fruity”, “malty”, “honey”, “sweaty”, “sulfury”, or “miscellaneous”. The test samples for the leveling tests were prepared by adjusting the concentrations of the individual odorants in each odorant group in the beer model to the concentration in the wine model or vice versa. The results showed that the fruity smelling odorants played a crucial role in the aroma difference between the beer and wine aroma base models.

Xingjie Wang, Stephanie Frank, and Martin Steinhaus jointly designed the research. Xingjie Wang performed the literature research, the data extraction and analysis, he conducted the sensory tests, analyzed the experimental data, and drafted the manuscript. Stephanie Frank and Martin Steinhaus conceived and directed the study, supervised Xingjie Wang’s work, participated in the sensory tests, and revised the manuscript.

## 7.2.4 Reprint Permission



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Open Access

This article is licensed under CC-BY 4.0

pubs.acs.org/JAFC

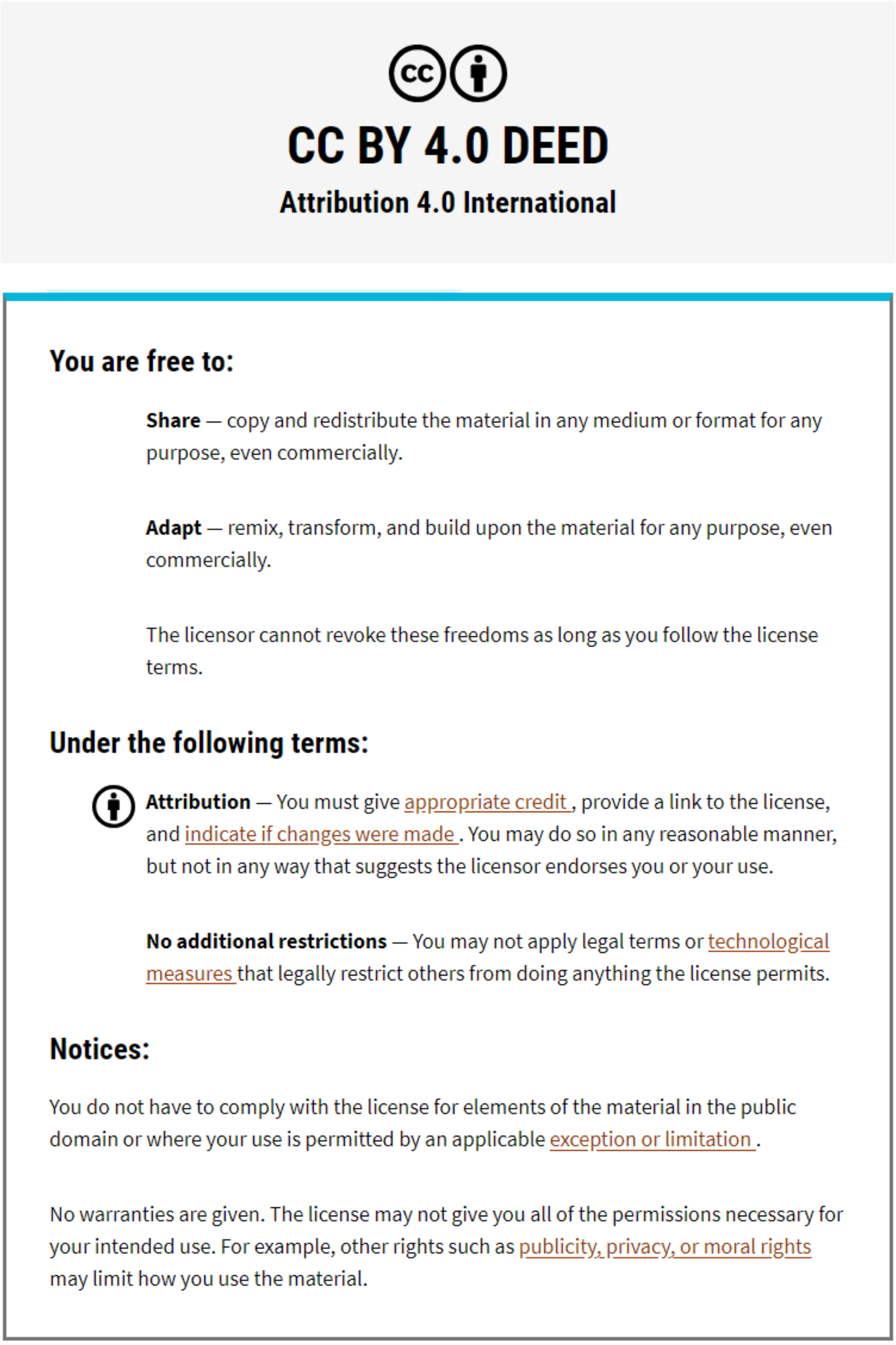
Article

**Molecular Insights into the Aroma Difference between Beer and Wine: A Meta-Analysis-Based Sensory Study Using Concentration Leveling Tests**

Xingjie Wang, Stephanie Frank,* and Martin Steinhaus*

Cite This: *J. Agric. Food Chem.* 2024, 72, 22250−22257

Read Online



**CC BY 4.0 DEED**

**Attribution 4.0 International**

**You are free to:**

**Share** — copy and redistribute the material in any medium or format for any purpose, even commercially.

**Adapt** — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

**Under the following terms:**

**Attribution** — You must give appropriate credit , provide a link to the license, and indicate if changes were made . You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

**No additional restrictions** — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.

**Notices:**

You do not have to comply with the license for elements of the material in the public domain or where your use is permitted by an applicable exception or limitation .

No warranties are given. The license may not give you all of the permissions necessary for your intended use. For example, other rights such as publicity, privacy, or moral rights may limit how you use the material.

## 7.3 List of Publications, Talks, and Poster Presentations

### Publications

Publications in peer-reviewed journals:

Wang, X.; Frank, S.; Steinhaus, M. Molecular background of the lychee aroma of *Vitis vinifera* L. ‘Muscaris’. *J. Agric. Food Chem*. **2024**, *72*(3), 1674–1682. DOI: https://doi.org/10.1021/acs.jafc.3c08298

Wang, X.; Frank, S.; Steinhaus, M. Molecular insights into the aroma difference between beer and wine: a meta-analysis-based sensory study using concentration leveling tests. *J. Agric. Food Chem*. **2024**, *72*(40), 22250–22257. DOI: https://doi.org/10.1021/acs.jafc.4c06838

Miscellaneous journal contributions:

Wang, X.; Frank, S.; Steinhaus, M. Deciphering the molecules behind the lychee aroma note of *Vitis vinifera* L. 'Muscaris' grapes. *Lebensmittelchemie* **2024**, *78*(S3), S3-113. DOI: https://doi.org/10.1002/lemi.202459095

### Talks

Wang, X.; Frank, S.; Steinhaus, M. Key odorants in Muscaris grapes. Food Chemistry and Food Quality Seminar, Freising, Germany, November 6, 2023

Wang, X.; Frank, S.; Steinhaus, M. Molecular background of the lychee odor of *Vitis Vinifera* L. cv. Muscaris. LSB’s Young Researcher Seminar, Freising, Germany, July 20, 2023

Wang, X.; Frank, S.; Steinhaus, M. What makes the aroma difference between beer and wine–a sensory study using concentration leveling tests. LSB’s Young Researcher Seminar, Freising, Germany, June 6, 2024

### Poster Presentations

Wang, X.; Frank, S.; Steinhaus, M. Deciphering the molecules behind the lychee aroma note of *Vitis vinifera* L. ‘Muscaris’ grapes. Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 52. Deutsche Lebensmittelchemietage (German Society of Food Chemistry, a division of the German Chemical Society, 52nd National Meeting), Freising, Germany, September 16–18, 2024