Technische Universität München
Fakultät für Chemie
Fachgebiet für Lebensmittelchemie

# Odor-Active Compounds in German Flavor Hop Varieties

**Silva Denise Neiens**

Vollständiger Abdruck der von der Fakultät für Chemie der Technischen Universität München zur Erlangung des akademischen Grades des

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

genehmigten Dissertation.

| | | |
|---|---|---|
| Vorsitzender: | | Prof. Dr. Michael Rychlik |
| Prüfer der Dissertation: | 1. | Priv.-Doz. Dr. Martin Steinhaus |
| | 2. | Prof. Dr. Peter Schieberle |

Die Dissertation wurde am 20.02.2019 bei der Technischen Universität München eingereicht und durch die Fakultät für Chemie am 06.05.2019 angenommen.

# Danksagung

Meinem Doktorvater, Herrn Priv.-Doz. Dr. Martin Steinhaus danke ich ganz herzlich für die Überlassung dieses Promotionsthemas, für die hervorragende Betreuung, für die Unterstützung, für die Ratschläge, für die Hilfe beim Erstellen der Publikationen, für das gute Arbeitsklima sowie für das mir stets entgegengebrachte Vertrauen. Vielen Dank auch für die Korrektur zahlreicher Abstracts, Poster, Vorträge und Berichte sowie ganz besonders auch für die Ermöglichung der Teilnahmen an internationalen und nationalen Symposien.

Bei Herrn Prof. Dr. Peter Schieberle möchte ich mich für die zusätzliche Betreuung während der ersten zwei Jahre der Promotion bedanken sowie für die Übernahme des Zweitprüfers.

Herrn Prof. Michael Rychlik danke ich für die Übernahme des Prüfungsvorsitzes bei der mündlichen Prüfung.

Teile der vorliegenden Arbeit wurden als Projekt des Forschungskreises der Ernährungsindustrie e.V. (FEI) über die AiF im Rahmen des Programms zur Förderung der Industriellen Gemeinschaftsforschung (IGF) vom Bundesministerium für Wirtschaft und Energie aufgrund eines Beschlusses des Deutschen Bundestages finanziell unterstützt und gefördert. Hierfür mein herzlicher Dank.

Bei allen Mitarbeitern der Deutschen Forschungsanstalt für Lebensmittelchemie bzw. des Leibniz-Instituts für Lebensmittel-Systembiologie an der Technischen Universität München möchte ich mich für ihre Hilfsbereitschaft, die gute Zusammenarbeit und die Teilnahme an den Sensoriken bedanken.

Meinen Laborkollegen aus Labor E.44 Nadine Fischer, Dr. Mario Flaig, Johanna Grimm, Dr. Michaela Jonas, Dr. Johanna Kreißl, Dr. Luca Nicolotti, Philipp Schlumpberger, Dr. Christina Schmid, Julia Schmidt, Tatsu Sotaro und Johanna Sprenger sowie auch Daniela Füllemann, Inge Kirchmann und Christine Stübner danke ich für die Unterstützung, für die tolle Zusammenarbeit, für das gute Laborklima, für die Hilfsbereitschaft, für die zahlreichen Diskussionen im Labor, die unterhaltsamen Mittagspausen sowie auch die zusammen verbrachte Zeit außerhalb der Arbeit.

Bei Dr. Johanna Kreißl und Dr. Veronika Mall bedanke ich mich für jegliche Hilfe bei den Gaschromatographen und Massenspektrometern. Dr. Johanna Kreißl sei herzlich gedankt für die NMR-Messungen und die Hilfe beim Auswerten der NMR Spektren.

Dr. Stephanie Frank, Dr. Johanna Kreißl, Dr. Veronika Mall, Dr. Klaas Reglitz und Jörg Stein danke ich, dass sie mir stets mit Rat und Tat weiterhalfen, für ihre Tipps sowie Unterstützung, sei es bei Fragen zu Synthesen, Standards oder auch rund ums Bier. Ein Dank geht auch an Dr. Petra Steinhaus für das Korrekturlesen der Publikationen sowie sämtlicher Abstracts.

Für die Hilfe im Labor und die Aufarbeitungen unzähliger Hopfen- sowie Bierproben möchte ich mich ganz herzlich bei Julia Bock, Anita Herzner, Inge Kirchmann, Anja Matern und Vanessa Wehner bedanken, sowie bei Sami Kaviani-Nejad und Ines Otte für die Aufnahme zahlreicher Chromatogramme und Spektren.

Dr. Michaela Jonas, Dr. Johanna Kreißl, Dr. Veronika Mall und Dr. Christina Schmid danke ich für die vielen gemeinsamen Aktivitäten und vor allem für die schönen Tage in den letzten Jahren am See oder auch auf der Isar.

Meinem Bruder Dr. Patrick Neiens sowie Dr. Vanessa Welk danke ich für ihre Unterstützung, für ihr stetes Interesse an meiner Arbeit, für die vielen Aufmunterungen und auch für die manchmal nötige Ablenkung durch die ein oder anderen Barabende.

Mein größter Dank gilt meinen Eltern Dr. Gina Bühler-Neiens und Rüdiger Neiens, die mir meine Ausbildung ermöglichten und ohne deren Hilfe das alles nicht erreichbar gewesen wäre. Danke für eure immerwährende Unterstützung in allen Lebenslagen, euer Vertrauen in meine Entscheidungen, eure Liebe, eure Geduld, euren Zuspruch sowie Aufmunterungen. Danke, dass ihr einfach immer für mich da seid!

The experimental part of the present work was performed between July 2014 and October 2017 at the Germany Research Centre for Food Chemistry (since September 15, 2017: 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.

# Contents

# 1 Summary

In recent years, the international hop market faced an increasing demand for novel hop cultivars with special aroma characteristics suitable for the growing number of craft breweries. In Germany, several new special flavor hop varieties were developed. Among them, Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Polaris have been introduced to the market.

The flavor hop variety Hallertau Blanc shows a white wine-like aroma note, Huell Melon a strong fruity, cantaloupe-like note, Mandarina Bavaria a fruity, tangerine-like note, and Polaris exhibits pronounced minty and fruity notes. To clarify the molecular background of these specific aroma characteristics, volatiles isolated from hop pellets by solvent extraction and solvent-assisted flavor evaporation (SAFE) were subjected to a comparative aroma extract dilution analysis (cAEDA). Results revealed myrcene, (3*R*)-linalool, 2-methylbutanoic acid, and 3-methylbutanoic acid as well as geraniol as most potent odorants. In Huell Melon hops, high flavor dilution (FD) factors were additionally determined for the fruity smelling esters ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanaote, which corresponded to the intense fruitiness of this variety. The minty, fruity aroma note of Polaris was reflected by odor-active amounts of 3-methylbutyl acetate and 1,8-cineole. Clove-like, herbaceous smelling (1*R*,4*S*)-calamenene was identified for the first time as hop odorant. The compound was present in all varieties investigated. Quantitation experiments using stable isotope dilution assays (SIDA) confirmed the results of the cAEDA. In the Huell Melon sample, extraordinary high concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate were found. Concentrations were up to 100 times higher than the respective concentrations in the other flavor hop varieties. Data thus confirmed the prominent role of these three esters for the pronounced fruity note in the sensory profile of Huell Melon. By contrast, the banana-like smelling 3-methylbutyl acetate and the eucalyptus-like smelling 1,8-cineole as well as fruity smelling methyl 2-methylbutanoate were clearly higher in Polaris. These compounds reflect the characteristic aroma note of Polaris hops.

To get a deeper insight into the influence of variety-specific hop odorants on the aroma of beer, bottom-fermented beers and top-fermented beers, both either late or dry hopped with Huell Melon hops, were subjected to a screening for odor-active compounds by application of a cAEDA. Beer without hop addition was included in the study as reference. Results revealed 11 odor-active compounds that were present in the late hopped beer and in the dry hopped beer but absent in the reference beer. Among these were geraniol, linalool, myrcene, and propyl 2-methylbutanoate. Ethyl 2-methylpropanoate, methyl 2-methylbutanoate, and ethyl 2-methylbutanoate exhibited clearly higher FD factors in the late hopped beer and in the dry hopped beer than in the reference beer. To substantiate the screening results, the hop-derived compounds were quantitated by SIDA. Results showed minimal transfer of myrcene from hops into beer and a moderate transfer for propyl 2-methylbutanoate, geraniol, and linalool. Rates clearly beyond a direct transfer were observed for ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and methyl 2-methylbutanoate and thus suggested a potential formation from the corresponding hop-derived carboxylic acids by the yeast. In a series of spiking experiments, the reference beer was spiked with one hop odorant at a time and the sensory beers were compared to the unspiked sample in 3-AFC tests. Results finally revealed that particularly linalool and propyl 2-methylbutanoate contributed to the characteristic aroma of beers flavored with Huell Melon hops.

# 2 Zusammenfassung

Aufgrund der wachsenden Craft-Bier-Szene stieg in den letzten Jahren das Interesse an neuartigen Hopfensorten mit besonderen Aromaeigenschaften. In Deutschland wurden neue Special-Flavor-Hopfensorten entwickelt und auf dem Hopfenmarkt eingeführt, darunter die Sorten Hallertau Blanc, Hüll Melon, Mandarina Bavaria und Polaris.

Die Hopfensorte Hallertau Blanc zeigt eine weißweinartige Aromanote, Hüll Melon eine honigmelonenartige Aromanote, Mandarina Bavaria eine fruchtige, mandarinenartige Aromanote und die Sorte Polaris weist fruchtige und minzartige Noten auf. Um den molekularen Hintergrund dieser spezifischen Aromanoten zu klären, wurden die flüchtigen Bestandteile durch Lösungsmittelextraktion und Solvent-Assisted Flavor Evaporation (SAFE) aus Hopfenpellets isoliert und einer vergleichenden Aromaextraktverdünnungsanalyse (AEVA) unterzogen. Die Ergebnisse zeigten Myrcen, (3*R*)-Linalool, 2-Methylbuttersäure und 3-Methylbuttersäure sowie Geraniol als die potentesten geruchsaktiven Substanzen. In Hüll Melon wurden hohe Flavor-Dilution (FD) Faktoren zusätzlich für die fruchtig riechenden Ester Ethyl-2-methylpropanoat, Ethyl-2-methylbutanoat und Propyl-2-methylbutanaot bestimmt. Dies korrespondierte mit der intensiven fruchtigen Note dieser Sorte. Die minzartige, fruchtige Aromanote von Polaris spiegelte sich in geruchsaktiven Mengen von 3-Methylbutylacetat und 1,8-Cineol wider. Das nelkenartig, krautig riechende (1*R*,4*S*)-Calamenen wurde erstmals als geruchsaktive Substanz in Hopfen identifiziert. Die Verbindung fand sich in allen untersuchten Sorten. Quantifizierungsexperimente mittels Stabilisotopenverdünnungsassays (SIVA) bestätigten die Ergebnisse der vergleichenden AEVA. In der Sorte Hüll Melon wurden außergewöhnlich hohe Konzentrationen an Ethyl-2-methylpropanoat, Ethyl-2-methylbutanoat und Propyl-2-methylbutanoat bestimmt. Die Konzentrationen waren bis zu 100-fach höher als die jeweiligen Konzentrationen in den anderen Flavor-Hopfen. Die Daten bestätigten somit die Rolle dieser drei Ester für die ausgeprägte fruchtige Note von Hüll Melon. Im Gegensatz dazu waren die Konzentrationen des bananenartig riechenden 3-Methylbutylacetats und des eukalyptusartig riechenden 1,8-Cineols sowie des fruchtig riechenden Methyl-2-methylbutanoats in Polaris deutlich höher. Diese Verbindungen spiegeln das charakteristische Aroma der Sorte Polaris wider.

Um einen tieferen Einblick in den Einfluss von Hopfenaromastoffen auf das Aroma von Bier zu erhalten, wurden untergärige und obergärige Biere jeweils mit Späthopfung oder Kalthopfung mit Hopfen der Sorte Hüll Melon gebraut und zum Screening auf geruchsaktive Verbindungen mittels vergleichender AEVA eingesetzt. Bier ohne Zugabe von Hopfen diente als Referenz. Die Ergebnisse zeigten 11 geruchsaktive Verbindungen, die im spätgehopften und im kaltgehopften Bier vorhanden waren, jedoch nicht im Referenzbier. Darunter waren Geraniol, Linalool, Myrcen und Propyl-2-methylbutanoat. Ethyl-2-methylpropanoat, Methyl-2-methylbutanoat und Ethyl-2-methylbutanoat wiesen im spätgehopften und im kaltgehopften Bier deutlich höhere FD-Faktoren auf als im Referenzbier. Um die Ergebnisse des Screenings zu überprüfen, wurden die aus Hopfen stammenden Verbindungen mittels SIVA quantifiziert. Die Ergebnisse zeigten einen minimalen Transfer von Myrcen aus Hopfen ins Bier und einen mäßigen Transfer für Propyl-2-methylbutanoat, Geraniol und Linalool. Raten, die deutlich über einen direkten Transfer hinaus gingen, wurden für Ethyl-2-methylpropanoat, Ethyl-2-methylbutanoat und Methyl-2-methylbutanoat ermittelt und deuteten auf eine zusätzliche Bildung, z.B. aus den entsprechenden aus dem Hopfen stammenden Carbonsäuren, durch die Hefe hin. In sensorischen Tests wurde das Referenzbier jeweils mit einem einzelnen Hopfenaromastoff versetzt und die gespikten Biere wurden in einem 3-AFC-Test mit der nicht gespikten Probe verglichen. Die Ergebnisse zeigten, dass insbesondere Linalool und Propyl-2-methylbutanoat zum charakteristischen Aroma von mit Hüll Melon Hopfen gebrauten Bieren beitragen.

# 3 Abbreviations and Nomenclature

*Abbreviations:*

| | |
|---|---|
| AEDA | aroma extract dilution analysis |
| cAEDA | comparative aroma extract dilution analysis |
| 3-AFC | 3-alternative forced choice |
| CI | chemical ionization |
| EI | electron ionization |
| FD | flavor dilution |
| FFAP | free fatty acid phase |
| FID | flame ionization detector |
| GC | gas chromatography |
| GC-FID | gas chromatography–flame ionization detector |
| GC-FPD | gas chromatography–flame photometric detector |
| GC-MS | gas chromatography–mass spectrometry |
| GC-O | gas chromatography–olfactometry |
| GC-GC-MS | two-dimensional heart-cut gas chromatography–mass spectrometry |
| GC×GC-TOFMS | comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry |
| HC 2013 | Hallertau Blanc, harvest 2013 |
| HC 2014 | Hallertau Blanc, harvest 2014 |
| HN 2013 | Huell Melon, harvest 2013 |
| HN 2014 | Huell Melon, harvest 2014 |
| HT 2014 | Hallertau Tradition, harvest 2014 |
| MB 2012 | Mandarina Bavaria, harvest 2012 |
| MB 2013 | Mandarina Bavaria, harvest 2013 |
| MCSS | moving column stream switching |
| NIST | National Institute of Standards and Technology |
| NMR | nuclear magnetic resonance |
| OAV | odor activity value |
| PA 2012 | Polaris, harvest 2012 |
| PA 2013 | Polaris, harvest 2013 |
| RI | retention index |
| SAFE | solvent-assisted flavor evaporation |
| SIDA | stable isotope dilution assay |

*Nomenclature:*

| | |
|---|---|
| abhexone | 3-hydroxy-4-methyl-5-ethylfuran-2(5*H*)-one |
| 2-acetyl-1-pyrroline | 1-(3,4-dihydro-2H-pyrrol-5-yl)ethanone |
| *α*-amorphene | (1*R*,4a*S*,8a*R*)-4,7-dimethyl-1-(propan-2-yl)-1,2,4a,5,6,8a-hexahydronaphthalene |
| bergamotene | 6-methyl-2-methylidene-6-(4-methylpent-3-en-1-yl)-bicyclo[3.1.1]heptane |
| calamenene | 1,6-dimethyl-4-(propan-2-yl)-1,2,3,4-tetrahydro-naphthalene |
| *α*-caryophyllene | (1*E*,4*E*,8*E*)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene |
| *β*-caryophyllene | (1*R*,4*E*,9*S*)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene |
| 1,8-cineole | 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane |
| citronellol | 3,7-dimethyloct-6-en-1-ol |
| citronellyl acetate | 3,7-dimethyloct-6-en-1-yl acetate |
| (*E*)-*β*-damascenone | (*E*)-1-(2,6,6-trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one |
| DDQ | 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile |
| *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 |
| *β*-farnesene | (6*E*)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene |
| geraniol | (2*E*)-3,7-dimethylocta-2,6-dien-1-ol |
| geranyl acetate | (2*E*)-3,7-dimethylocta-2,6-dien-1-yl acetate |
| HDMF | 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (Furaneol®) |
| *β*-ionone | 4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one |
| LDA | lithium diisopropylamide |
| limonene | 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene |
| linalool | 3,7-dimethylocta-1,6-dien-3-ol |
| linalool oxide | 2-(5-ethenyl-5-methyloxolan-2-yl)propan-2-ol |
| menthone | 5-methyl-2-(propan-2-yl)cyclohexanone |
| myrcene | 7-methyl-3-methylideneocta-1,6-diene |
| nerol | (2*Z*)-3,7-dimethylocta-2,6-dien-1-ol |
| (*Z*)-*β*-ocimene | (3*Z*)-3,7-dimethylocta-1,3,6-triene |
| perillene | 3-(4-methyl-3-pentenyl)furan |
| *α*-pinene | 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene |
| *β*-pinene | 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane |
| *α*-selinene | (2*R*,4a*R*,8a*R*)-2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene |
| *β*-selinene | (4a*R*,7*R*,8a*S*)-7-isopropenyl-4a-methyl-1-methylene-decahydronaphthalene |
| sotolon | 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one |
| *α*-terpineol | 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol |
| vanillin | 4-hydroxy-3-methoxybenzaldehyde |

# 4 Introduction

## 4.1 Molecular Sensory Science

### 4.1.1 Odor-Active Compounds and Aroma Perception

Aroma is one of the most important characteristics of a food product. Aroma is caused by odor-active compounds, which can be perceived by the human nose. Over the lifetime of a food product, the aroma impacts food selection, correct recognition of food and differentiation from other food products, and detection of spoilage. The odor-active compounds present in a food product are thus important contributors to food quality.

Odor-active compounds are volatiles which are able to bind to one of ~400 types of olfactory G-protein-coupled receptors present on the surface of the olfactory epithelium (Figure 1). Aroma is typically evoked by a mixture of various odor-active compounds. An individual odor-active compound can activate one or more types of receptors. In turn, one type of receptor can typically be activated by several compounds. This results in activation patterns characterizing each aroma impression.[1-3]

The odor-active compounds reach the olfactory epithelium either through the nostrils during inhalation (orthonasally) or through the mouth and throat during the chewing process (retronasally). The binding of an odorant causes a conformational change of the receptor, which starts an intracellular reaction cascade that finally leads to the depolarization of the cell membrane. The depolarization proceeds as a neural impuls via the axon of the olfactory neuron to the olfactory bulb (*bulbus olfactorius*). In the olfactory bulb, axons of receptor cells of the same receptor type bundle in a glomerulus. Activation of a defined set of glomeruli results in a characteristic activation pattern. These activation patterns are transmitted via mitral-cells to higher regions of the brain where the patterns are recognized as a specific aroma.[1-7]

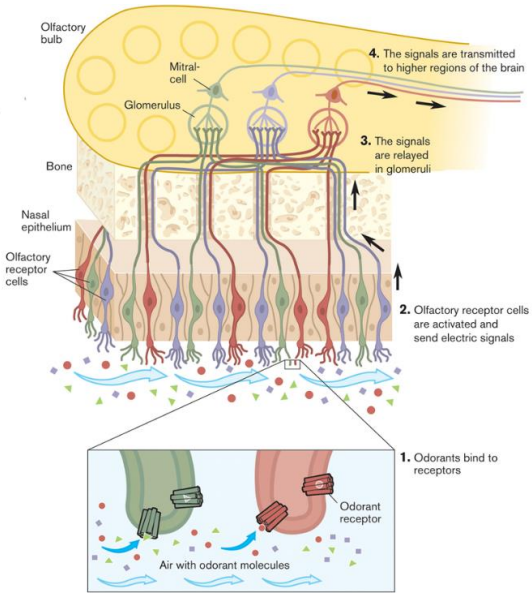



Figure 1: Organization of the olfactory system[2]

An essential requirement for the odor activity of a compound is its volatility. Only a sufficiently volatile compound can be released from food into the ambient air. The volatility of a compound depends on the molecular weight of the compound and its polarity. However, most volatile compounds emanated from food are odorless, because they are not able to interact with any odorant receptor of the olfactory epithelium or because they are present in a quantity below the compound-specific threshold concentration, which is necessary to activate the intracellular reaction cascade in the olfactory epithelium. The concentration of a compound in the nose depends on its concentration in the food and the specific release characteristics influenced by the food matrix and the chemical properties of the compound. The odor thresholds of various odor-active compounds occurring in food are extremely different, e.g. ethanol has an odor threshold in water of 990000 µg/kg[8], whereas the odor threshold value of 4-methyl-4-sulfanylpentan-2-one, an odor-active compound characterized by a black currant-like aroma note, amounts to 0.00055 µg/kg in the same matrix.[9] Only a few odor-active substances in a food product are relevant for the overall aroma. For the identification of these odor-active key compounds, the sensomics concept is utilized.

### 4.1.2 The Sensomics Concept

For the characterization of odor-active key compounds, the following concept is applied (Figure 2).

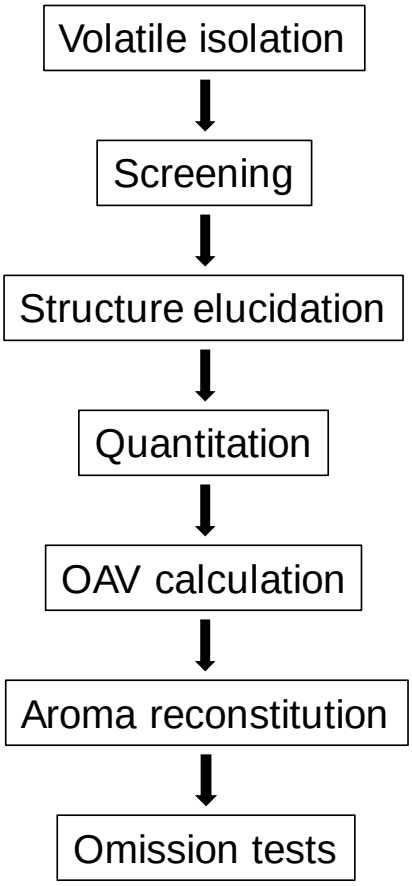


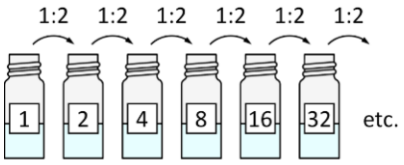

Figure 2: Identification of odor-active key compounds according to Schieberle, 1995[10] and Grosch, 2001[11]

Identification of odor-active key compounds starts with the isolation of the volatile compounds. After extraction with a low boiling organic solvent, e.g. dichloromethane or diethyl ether, the separation of volatiles from nonvolatile compounds is accomplished by using the solvent-assisted flavor evaporation (SAFE).[12] The SAFE method offers profound advantages over other methods described in literature, e.g. the simultaneous distillation/extraction.[13] During SAFE distillation, the temperature is kept below 40 °C. With low temperatures, the risk of compound degradation and formation of artifacts is minimized.

To distinguish between odor-active compounds and the bulk of odorless volatiles, the concentrated SAFE distillate is applied to gas chromatography-olfactometry (GC-O). After chromatographic separation on the column, the gas flow is splitted into two parts. One part is directed to a flame ionization detector (FID) while the second part is transferred to a heated exit, named sniffing port. The FID signal is printed by a recorder and the odor quality perceived at the sniffing port is simultaneously marked in the chromatogram (Figure 3).


Figure 3: GC-O, basic principle (left) and application (right) (illustration: Martin Steinhaus)

An aroma extract dilution analysis (AEDA)[14] is performed by stepwise dilution of the initial extract with solvent to obtain dilutions of 1:2, 1:4, 1:8 etc. Each diluted sample is subjected to GC-O analysis until no odor-active compound remains detectable. In AEDA, a flavor dilution (FD) factor is assigned to each odor-active compound, representing the dilution factor of the highest diluted sample in which the odorant was detected by GC-O (Figure 4).




GC-O →

| 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 4: Aroma extract dilution analysis: stepwise extract dilution, GC-O, and FD factor calculation (illustration: Martin Steinhaus)

The structure elucidation of odor-active compounds is performed by comparing the odor quality perceived at the sniffing port, retention indices (RIs) on two capillary columns with different polarities, and mass spectra acquired in electron ionization (EI) mode as well as in chemical ionization (CI) mode with respective data of authentic reference compounds analyzed under the same conditions. Reference compounds, which are commercially not available, have to be synthesized and their structure has to be verified by nuclear magnetic resonance (NMR) spectrometry.

AEDA is a valuable screening methode for distinguishing odor-active compounds from the bulk of odorless volatiles. However, it does not allow to clearly determine the contribution of individual odorants to the overall aroma of the analyzed food. For that reason, it is important to carry out quantitative determinations of the odor-active compounds which can be done by using stable isotope dilution assays (SIDA).[15] In SIDA, internal standards are added at the beginning to the workup which are deuterium- or $^{13}C$-substituted analogues[16] of the target analytes (Figure 5). The mixture is homogenized until an equilibrium has been reached between the target analyte and the added standard. Since an isotopically substituted standard and its corresponding target analyte are nearly identical in their chemical and physical properties, the ratio of concentrations of the two compounds remains constant during the workup. In subsequent GC-MS analyses, the isotopically substituted standard and the target analyte can be differenciated by their different molecular weights and the peak area ratio of the two isotopologues can be determined. The concentration of the target analyte in the sample is calculated from the area ratio of the target analyte and the isotopically substituted standard, the sample amount, and the amount of standard added, by using a calibration line equation previously obtained from the analysis of target analyte/standard mixtures in different concentration ratios.

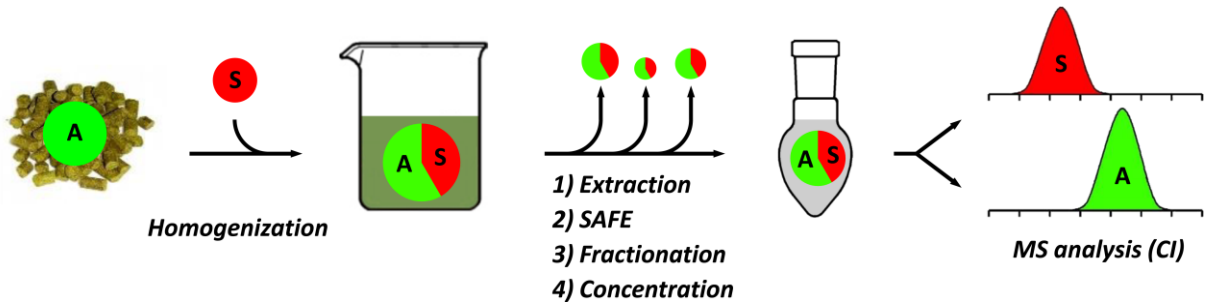



Figure 5: Application of SIDA for odorant quantitation: compensation of workup losses (illustration: Martin Steinhaus)

The odor activity value (OAV) is defined as ratio of the concentration of the odor-active compound to its odor threshold.[17,18] Determination of the odor threshold should be performed in a matrix that is as similar as possible to the matrix of the foodstuff. The OAV indicates the factor by which the concentration of an odor-active compound in a foodstuff is above its odor threshold value. Odor-active compounds exhibiting an OAV < 1 normally do not contribute to the overall aroma, compounds with an OAV ≥ 1 may contribute to the overall aroma. Odor thresholds are determined according to the American Society for Testing and Materials (ASTM) procedure for the determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[19]

Based on the quantitative results, an aroma reconstitution model is prepared from a model matrix mimicking the situation in the original food and the odor-active compounds for which OAVs ≥ 1 have been calculated. The model matrix should at least represent the water content, the lipid content, and the pH of the original food. In reconstitution tests, the model is then compared to the original food product in a quantitative olfactory profile analysis. A high similarity in the profiles of reconstitution model and food indicates that the qualitative and quantitative results are correct.

After successful aroma reconstitution, the final step of the sensomics concept is omission tests. In an omission test, a single odor-active compound is omitted from the aroma reconstitution model. The incomplete model is then tested against the complete aroma reconstitution model in a 3-alternative forced choice (3-AFC) test. If the 3-AFC test result in a significant difference, the omitted odor-active compound has shown its relevance for the overall aroma of the complete reconstitution model and the *p*-value may be used as numeric approximation of its importance.[20,21]

## 4.2 Hops and Beer

Since the ninth century, hops have been used for brewing. Cultivation in central Europe started in the thirteenth century. Hops are primarily used to enhance the shelf-life and bitterness of beer. Additionally, hops increase foam stability, assist in the precipitation of proteins and, depending on the time of hop addition, can influence the aroma of beer.[22]

### 4.2.1 The Hop Plant

Hop plants are classified as *Humulus lupulus* L. They belong to the taxonomic family of hemp (*Cannabaceae*) and to the order of nettles (*Urticaceae*). Wild hops occur in Europe, North America, and Asia. Hop plants are twiners. With the aid of hooked hair located on the stem, the plants entwine clockwise around other plants. The leaves of hop plants are located in pairs at node. Hop plants are dioecious, that means on a single hop plant either male or female inflorescences are produced. Male flowers are produced in loose panicles and have a perianth of five sepals and five anthers on short filaments. Female flowers occur in inflorescences that consist of a condensed central axis. On each node of the female flowers, a pair of bracts is located. Each bract subtends a pair of bracteoles. The bracteole has a small flower enclosed in a fold at the base. As the inflorescences mature, the central axis extends. The bracts and bracteoles enlarge to produce the cones, which are the parts of the hop plants used in brewing (Figure 6). On the inside of the bracts, glands are located that produce a secretion. The yellow and sticky glands are called lupulin. Lupulin is the valuable component of hops and contain the essential oil and the bitter compounds. Male hop flowers develop only a small number of glands and are therefore not used in brewing.[22-24]

Figure 6: Structure of a hop cone; whole cone and cross-section

Hop plants are perennials. Each winter the aerial parts of the hop plants die to ground and in spring new buds sprout. The rootstock can survive for many years and in a mature plant, the rootstock can extend downward for more than 1.5 m and laterally for more than 2 m.[24]

### 4.2.2 Hop Cultivation

For the commercial exploitation of hops, only female plants are cultivated. Male hops are only planted to be used for crossbreeding in order to create new hop varieties. The male flowers have to be separated from the hop cultivation area to prevent unwanted fertilization of the female flowers and the development of seeds. The high fatty acid content in seeds can negatively impact the foam and flavor stability of beer.[22,23,25-29]

Every spring, new shoots of female hop plants are produced from buds located on the branched stem tissue. More buds are generated than are needed to grow hops. Therefore, most of the buds have to be removed by the hop growers. Two or three of the strongest buds are trained onto wires or twines used as climbing aids and then grow up to 30 cm in length per day. The growing speed of hops depends on soil and climatic conditions as well as lighting conditions and lengths of day. Hops are harvested before their physiological maturity, typically at the time the hop plants have grown up their climbing aids completely (5.5–8 m). Yield, *α*-acids, oil content, aroma, appearance, and deficiencies of hop cones are the parameters used to determine the optimal harvest period for each variety (Figure 7). The time frame for harvesting hops of an individual variety is between seven and 14 days. In Germany, the complete harvest time for hops extends from end of August to end of September. In commercial hop production, plants are capable of producing hop cones every season for up to 25 years.[22,24,29,30]

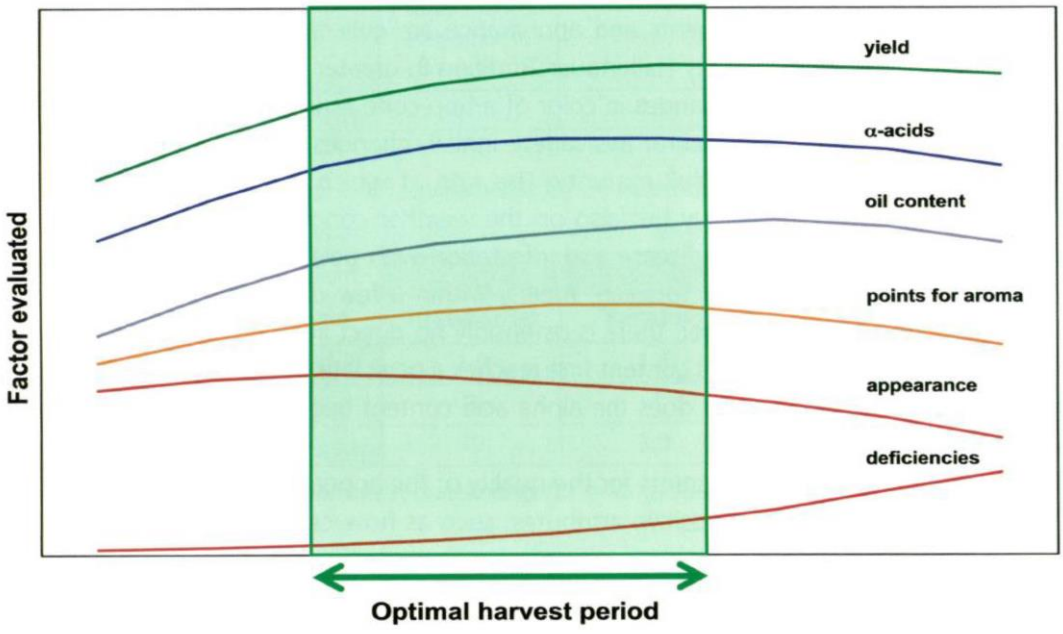



Figure 7: Schematic diagram of the optimal harvest period[22]

The world hop cultivation extends in two belts encircling the globe between latitudes 35° and 55° in the southern and northern hemisphere. With more than 45000 t of hop production in 2017, the United States of America are the leading hop producing country, followed by Germany with nearly the same amount (Table 1). The biggest hop growing regions in the USA are in the Yakima Valley (WA), in Oregon, and in Idaho. In Europe, hops are additionally grown in the Czech Republic, Slovenia, Poland, and the United Kingdom.[22,29,31]

Table 1: World Harvest of Hops, Estimate for 2017[31]

| country | production (t) | country | production (t) |
|---|---|---|---|
| United States of America | 45110 | Slovenia | 2736 |
| Germany | 41556 | Poland | 2500 |
| Czech Republic | 6750 | United Kingdom | 1781 |
| China | 5513 | Others | 7956 |
| | | Total | 113902 |

The largest region of hop cultivation in Germany is in the Hallertau to the north of Munich, followed by Elbe-Saale and Tettnang. In 2017, around 19500 ha of hops were cultivated in Germany, of which 83% were grown in the Hallertau region (Table 2).[31]

Table 2: Hops Growing Area in Germany, 2017[31]

| growing area | acreage (ha) | growing area | acreage (ha) |
|---|---|---|---|
| Hallertau | 16310 | Tettnang | 1353 |
| Elbe-Saale | 1466 | Spalt, Baden, Bitburg, and Rheinland-Pfalz | 391 |
| | | Total | 19543 |

Hop varieties are traditionally divided into aroma hop varieties and bitter hop varieties, whereby today no clear differentiation between aroma hops and bitter hops is possible anymore. Basically, bitter hops have a high content of *α*-acids, whereas aroma hops are characterized by their pleasant aroma, a higher content of polyphenols, and a lower *α*-acid content. With aroma hops, a traditional hoppy aroma note can be achieved, whereas bitter hops are primarily used to give the beer its bitterness. However, the respective varieties are not limited to the usage as bitter hops or aroma hops. Aroma hops can also contribute to the bitterness of the beer as well as bitter hops can influence its aroma. In addition to aroma hop varieties and bitter hop varieties, in recent years, a rising demand for new hop varieties, which exhibit unique and special aroma characteristics evolved. These new varieties are referred to as flavor hops. The aroma notes of the flavor hops in some cases significantly differ from the aroma notes of traditional hop varieties. Furthermore, some of their sensory characteristics are even considered atypical for hops.[22,32]

In 2017, the acreage of aroma hops and bitter hops in Germany was nearly on the same level. Perle, Hallertau Tradition, Hersbrucker Spät, Hallertau Mittelfrüh, Tettnanger, Spalter Select, and Saphir are common aroma hop varieties currently grown in Germany, whereas the varieties Herkules, Hallertau Magnum, and Hallertau Taurus belong to the bitter hop varieties. With about 30%, the bitter hop variety Herkules is currently the most widely cultivated variety in Germany, followed by the aroma hop varieties Perle and Hallertau Tradition (Table 3).[23,33]

Table 3: Varieties Cultivated in Germany in 2017[33]

| variety | acreage (%) | variety | acreage (%) |
|---|---|---|---|
| Herkules | 29.7 | Hallertau Mittelfrüh | 3.7 |
| Perle | 15.2 | Spalter Select | 2.7 |
| Hallertau Tradition | 13.8 | Saphir | 2.4 |
| Hallertau Magnum | 10.3 | Mandarina Bavaria | 1.8 |
| Hersbrucker Spät | 4.7 | Northern Brewer | 1.5 |
| Tettnanger | 3.8 | Others | 10.4 |

The term flavor hops accrued approximately 20 years ago and was first introduced by the Brewers Association in the United States. The international hop market faced an increasing demand for novel hop varieties with special aroma characteristics suitable for the growing number of craft breweries. In breeding of new hop varieties, various methods such as selection, crossing, and gene transfer are possible to increase disease resistance, yield, and bitterness, and create new flavors. More emphasis was placed on fruity, exotic, and other extraordinary aroma notes than on typical hoppy notes. American hop growers quickly responded to the increasing demand for flavor hop varieties. Between 2010 and 2017, the acreage of flavor hops in the USA increased from 12% to 70%, while the acreage of bitter hop varieties decreased from over 70% to 22%. Flavor hop varieties make it possible to transfer very fruity and extraordinary aroma impressions into beer. This in turn inspired more and more brewers worldwide to develop special flavor beers.[22,33-35]

The German flavor hop variety Mandarina Bavaria accounted for 1.8% of the total acreage of hop varieties in 2017 (Table 3). Cultivation of flavor hops in Germany is still a small sector but it is strongly growing in recent years. In 2013, only 0.7% of the total acreage of hops were flavor hops. In the following four years, the acreage of flavor hops increased to 6.3% and flavor hops were cultivated on a total acreage of 1231 ha (Figure 8).[33,36]

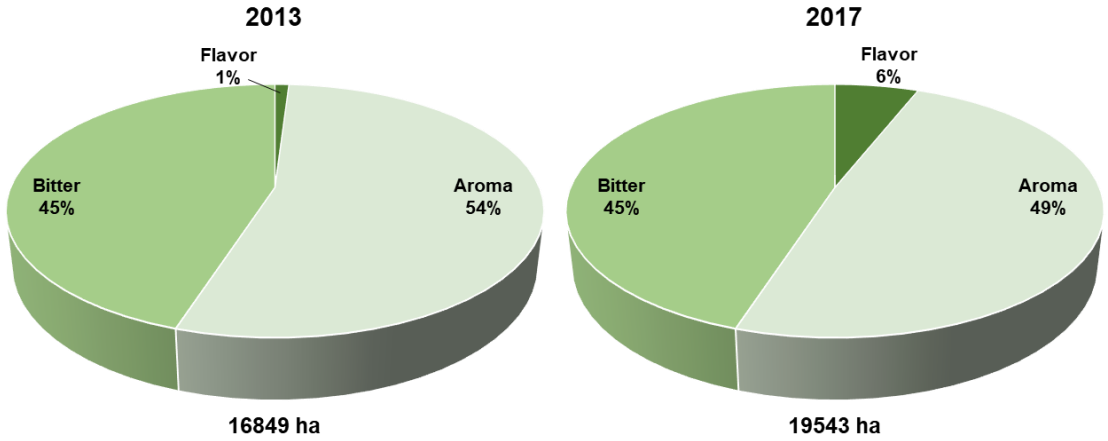



Figure 8: Development of the acreage of hop varieties in Germany between 2013 and 2017[33,36]

In 2006, the breeding team at the Hop Research Center (HRC) Huell in the Hallertau generated the first hybrids of German flavor hop varieties through fertilization of hop blossoms of the US variety Cascade with the pollen of Huell breeding lines. In this way, the fruity, citrusy aroma notes of hop varieties with North American background were combined with the traditional, classic aroma notes of hops with European background from Huell (Figure 9). After the harvest of October 2011, the first eight “Hueller Special Flavor Hops” were presented to the German and the international hop and brewing community. In first brewing experiments, especially dry hopped beers showed very fruity notes. The aroma of the beers was described as grapefruit-like, mango-like, mandarine-like, lime-like, and melon-like, as well as minty. In 2012, the four breeding lines Hallertau Blanc (2007/019/008), Huell Melon (2009/002/706), Mandarina Bavaria (2007/018/013), and Polaris (2000/109/728) were released for cultivation. Hallertau Blanc is described as having a white wine-like aroma note, Huell Melon a fruity, cantaloupe-like aroma note, Mandarina Bavaria a citrusy, tangerine-like aroma note, whereas the specific aroma note of Polaris is characterized as fruity and minty. The hop varieties Hallertau Blanc, Huell Melon, and Mandarina Bavaria are crossbreedings of the North American variety Cascade and a male Huell breeding line. The hop variety Polaris is a high *α*-acid hop variety, which was bred from diverse Huell germplasm.[34,37-41]

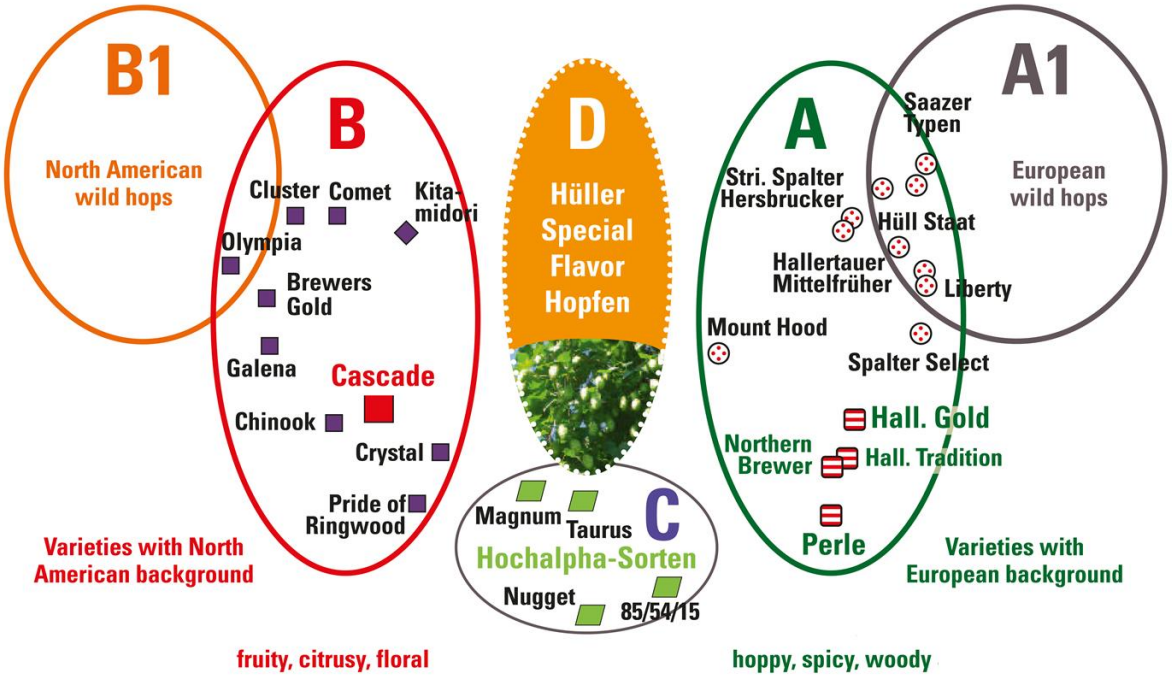



Figure 9: Genetic background of the Huell Special Flavor Hops (figure modified from Seigner et al. 2012[34])

For the flavor hop varieties Hallertau Blanc, Huell Melon, and Mandarina Bavaria a total oil content between 1.4 mL and 2.1 mL per 100 g of fresh hop cones and an *α*-acid content of 7.0–11.0% was determined.[38-40] With 4.4–4.8 mL per 100 g of fresh hop cones, Polaris has a significantly higher content of essential oil and also a high *α*-acid content of 18.0–24.0%, which results in a better utilization as bitter hop variety.[41]

### 4.2.3 Composition of Hop Cones

Freshly harvested hop cones consist of approximately 80% water. They are dried directly after the harvest to a water content of approximately 10%. Dried hop cones are basically composed of 60% cellulose, lignins, proteins, amino acids, minerals, lipids, carbohydrates, and pectins, as well as of 24% bitter compounds and 5% polyphenols (Table 4).[22,42]

Table 4: Composition of Dried Hop Cones[22]

| ingredient | % |
|---|---|
| cellulose, lignins, proteins, amino acids, minerals, lipids, carbohydrates, pectins | 60 |
| bitter compounds | 24 |
| water | 10 |
| polyphenols | 5 |
| essential oil | 1 |

The bitter compounds in hops are divided into hard resins and soft resins. The hard resins account for 0.2–1.2% of the bitter compounds in dried hop cones with 10% moisture. The soft resins include the *α*-acids (humulone, cohumulone, adhumulone) with 2–20% and the *β*-acids (lupulone, colupulone, and adlupulone) with 3–10%.[22,43]

The content of essential oil is only about 1% of the total dried hop cones. The composition of the essential oil strongly depends on the hop variety, the harvest year, as well as the growing area.

### 4.2.4 Hop Processing

Unprocessed whole hops are rarely used for brewing today. Instead, industrially manufactured hop products are applied. The advantages of hop products are their homogeneity as well as their reduced volume in comparison to whole hops, whereby the storage capacity is increased and transport costs as well as packaging costs are reduced.[44] The distribution of hop products is illustrated in Figure 10.

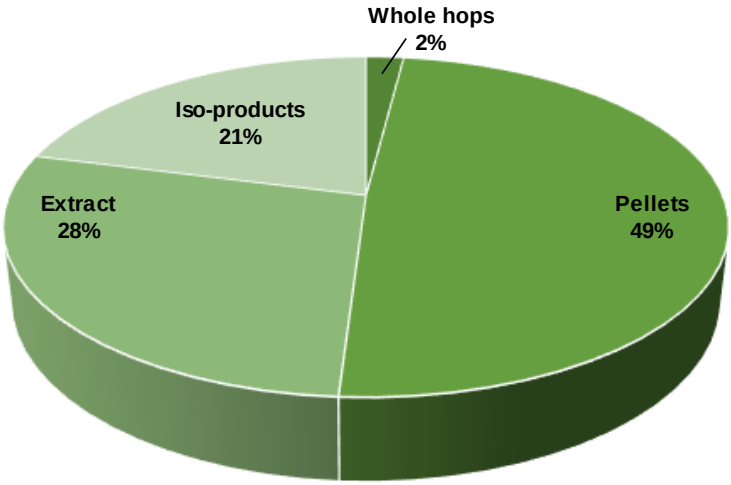



Figure 10: Share of hop products on the world market[22]

Whole hops are only used in a small number of small-sized and medium-sized breweries, especially in traditional and craft breweries. The main part of the whole hops harvested is processed to hop pellets. For the production of hop pellets, leaves, stems, pieces of metal, and stones are removed after the harvest. Hop cones are dried to a water content of 8–10% and subsequently ground to a hop powder. After pelletization, the packaging is proceeded in an oxygen-free atmosphere. In type 90 pellets, 100 kg of whole hop cones produce approximately 95 kg of pellets. For the production of enriched hop pellets called type 45 pellets, 100 kg of whole hops yield approximately 45–50 kg of pellets. After grinding and sieving of hop powder at −30 to −34 °C, there is a separation of bracts and strings. This results in an enrichment of lupulin in type 45 pellets. For stabilized pellets, the production includes the addition of magnesium oxide, whereby *α*-acids are preserved in the form of magnesium salts.

Isomerized pellets or iso-pellets are standardized pellets which underwent heating at a temperature of not more than 50 °C for 8–14 days. This leads to a conversion of *α*-acids to iso-*α*-acids.[22,26]

Hop extract is produced by extraction of hop pellets with ethanol (ethanol extract) or by extraction with carbon dioxide ($CO_2$ extract). As ethanol and carbon dioxide occur naturally in beer, they are well-accepted for the production of hop extracts. The ethanol extraction is a continuous process with ethanol serving as solvent. The final product is obtained after evaporation of the solvent. The resulting extract contains all bitter substances present in the whole hops (*α*-acids, *β*-acids, soft and hard resins) in a variety-specific composition. $CO_2$ extracts are made by extraction of hop pellets with carbon dioxide in liquid or supercritical state at a pressure between 20 MPa and 25 MPa. The $CO_2$ extract contains *α*-acids, *β*-acids, and essential oil, whereby the spectrum of bitter compounds shows little changes compared to the whole hops.[22,26]

Additionally, hop oil products are manufactured from hops. Hop oils are obtained by specific steam distillation approaches or by vacuum distillation applied to $CO_2$ extracts.[22,26]

### 4.2.5 Hop Volatiles

Numerous studies have been conducted on hop volatiles, which led to the identification of more than 500 compounds in hops and hop products (Table 5).[45,46] Among them, hydrocarbons, alcohols, sulfur compounds, esters, and ketones represent the largest compound groups.

Table 5: Classes of Volatile Compounds in Hops (VCF 2018)[46]

| substance class | compounds | substance class | compounds |
|---|---|---|---|
| hydrocarbons | 103 | furans | 8 |
| alcohols | 83 | amines | 6 |
| sulfur compounds | 82 | further nitrogen compounds | 4 |
| esters | 77 | ethers | 3 |
| ketones | 59 | phenols | 3 |
| acids | 39 | acetals | 1 |
| aldehydes | 28 | halogenated compounds | 1 |
| epoxides, pyrans, coumarins | 15 | | |

By using modern instrumentation, for instance GC×GC-TOFMS, the separation of more than 1500 hop volatiles in essential hop oil is possible today.[47]

About 80% of the compounds found in essential hop oil of freshly harvested hop cones are allocated to the substance class of hydrocarbons, such as monoterpenes and sesquiterpenes, while 20% belong to the substance class of oxygenated compounds (Table 6).[22,48]

Table 6: Substance Classes Found in the Essential Oil from Freshly Harvested Hop Cones[22]

| | | concentration (%) |
|---|---|---|
| hydrocarbons | monoterpenes | 40 |
| | sesquiterpenes | 40 |
| | aliphatic hydrocarbons | < 1 |
| oxygenated derivates | carboxylic acid esters | 15 |
| | carboxylic acids | 1 |
| | monoterpene oxides | 1 |
| | sesquiterpene oxides | 1 |
| | aldehydes and ketones | 1 |
| | thiols | < 1 |

The first targeted investigations on the identification of volatile compounds in hop oil were accomplished by Chapman between the years 1893 and 1929. Analyses of distillated hop oil led to the identification of *α*-humulene, myrcene, linalool, farnesene, *β*-caryophyllene, undecane-2-one, geraniol, nerol, and *γ*-caryophyllene.[49-64]

Analysis of hop oil by gas chromatography was first accomplished by Howard in 1956 and resulted in the separation of 18 compounds.[65] The essential oil was pre-fractionated by chromatography on silica gel. A mixture of hydrocarbons and oxygenated substances was detected. The hydrocarbons included myrcene, *β*-caryophyllene or *γ*-caryophyllene, and humulene, whereas the oxygenated fraction consisted of esters of at least 12 fatty acids.[66]

By the application of gas chromatography coupled with a mass spectrometer as a detector, the identification of further volatile compounds in hop oil was possible.[67-87] Analyzing the volatile fraction of hops from the variety Spalter Select by using GC-MS led to the identification and quantitation of more than 140 volatile compounds.[78]

Variety-specific differences of hop oils were investigated by several researchers. Compounds like myrcene, *β*-caryophyllene, *α*-humulene, and farnesene were used as marker substances for the differentiation of hop varieties.[87-90]

Narziß and Forster demonstrated losses of volatile compounds of up to 50% during drying of hop cones. For most of the investigated volatiles, for example monoterpenes, sesquiterpenes, linalool, and esters, concentrations decreased during the process. In contrast, an increase in oxidation products of sesquiterpenes and in compounds assumed to be degradation products of hop bitter substances was observed.[91]

In most of these studies, the focus has been on the identification of volatile compounds in the hops or in the hop oils, whereas the individual contribution of the volatiles to the overall aroma

of hops was not assessed. However, it is well established that the majority of volatiles present in food, beverages, and their raw materials have no or only little impact on the overall aroma (cf. chapter 4.1).

### 4.2.6 Odor-Active Compounds in Hops

A first study on the identification of odor-active compounds in hops was published by Guadagni et al. in 1966. Based on the calculation of odor activity values, the authors concluded that myrcene represented the main part of the total hop aroma followed by *S*-methyl hexanethioate, methyl dec-4-enoate, caryophyllene, and humulene. For linalool, only a minor contribution to the aroma was estimated.[86]

Evaluation of the volatile fraction of Hallertau Tradition hops and two US hop varieties resulted in the identification of linalool and oxidation products of caryophyllene and humulene. These compounds were suggested to contribute significantly to the overall odor of all three hop varieties.[92] A crucial role of linalool for the aroma of hops had already been suggested as early as 1929.[57]

Application of AEDA on the volatiles obtained from dried hop cones of different hop varieties revealed high FD factors for *tran*s-4,5-epoxy-(2*E*)-dec-2-enal, 4-ethenyl-2-methoxyphenol, ethyl 2-methylpropanoate, geraniol, linalool, methyl 2-methylbutanoate, myrcene, nonanal, (5*Z*)-octa-1,5-dien-3-one, oct-1-en-3-one, propyl 2-methylbutanoate, (3*E*,5*Z*)-undeca-1,3,5,9-tetraene, and (3*E*,5*Z*)-undeca-1,3,5-triene, suggesting an impact of these compounds on the aroma of hops.[93,94] Analysis of the odor-active compounds in five different hop varieties resulted in the identification of further aroma-relevant compounds, among them 3-methylbutyl 2-methylpropanoate, undecan-2-one, *β*-citronellol, methyl geranate, bergamotene, *β*-farnesene, *α*-amorphene, and *α*- and *β*-selinene as well as 5 thioesters and 41 thiols.[95]

By using headspace solid phase microextraction and GC-MS analysis on the floral hop oil essence isolated from Spalter Select hops, dodecan-2-one, ethyl nonanoate, methyl 4-methyloctanoate, methyl non-3-enoate, methyl octanoate, *cis*-*β*-ocimene, perillene, and undecan-2-one were suggested as important contributors to the floral, fruity, and citrusy hop aroma. The compounds commonly associated with the floral flavor of hops, namely linalool and geraniol, were not detected in this hop oil essence.[96]

Depending on the variety, numerous thiols were identified as odor-active compounds in hops. The black currant-like smelling 4-methyl-4-sulfanylpentan-2-one (4MSP) was first detected in Cascade hops. A significant contribution of 4MSP to the overall aroma of hops was especially demonstrated for US varieties, for modern German breeds, and for a Japanese hop variety. In the hop variety Nelson Sauvin, 3-sulfanylpentan-1-ol, 3-sulfanylhexan-1-ol, 3-sulfanyl-4-methylpentan-1-ol, and 3-sulfanyl-4-methylpentyl acetate were identified as odor-active thiols. The grapefruit-like smelling 3-sulfanyl-4-methylpentan-1-ol was identified as variety-specific compound in the hop variety Hallertau Blanc.[94,97-100]

An effect of maturity on the chemical composition of Cascade and Willamette hops harvested at three points of time (early, typical, and late) was demonstrated by Sharp et al.[101] The content of hop acids did not change during plant maturation for the period examined, whereas hop oil content and the concentrations of limonene, linalool, methyl heptanoate, myrcene, *α*- and

*β*-pinene increased. In addition, the influence of the harvest date on the volatile organosulfur compounds dimethyl disulfide, *S*-methyl 3-methylbutanethioate, and *S*-methyl 4-methyl-pentanethioate in the flavor hop varieties Cascade, Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Polaris was analyzed. Results showed that the concentrations of these compounds were clearly dependent on the variety and increased by a factor of up to ten by late harvest dates. High concentrations were found in the hop variety Polaris. In sensory tests, late-harvested hops were associated with onion-like and garlic-like aroma notes.[102]

### 4.2.7 Beer and the Usage of Hops in Brewing

The major part of hops is utilized in breweries for the production of beer. A minor amount is used in the pharmaceutical industry.

Beer is an alcoholic beverage, produced from water, malt, hops, and yeast by fermentation. The main components of beer are water, residual extract, and ethanol (Table 7). The residual extract is very complex and consists of 80–85% carbohydrates, 4.5–5.2% proteins, 3–5% glycerin, 3–4% minerals, 2–3% bitter compounds, and 0.7–1% organic acids. It also contains tannins, colorants as well as small amounts of vitamins.[23]

Table 7: Composition of Beer[23]

| component | % |
|---|---|
| water | 90 – 92 |
| residual extract | 4.0 – 4.5 |
| ethanol | 3.8 – 4.2 |

According to the German beer law (“Biergesetz”) from 1993, which is based on Bavarian regulations dating back to 1516, also known as “Reinheitsgebot“, only the use of water, malt, hops, and yeast is allowed for the brewing process in Germany.[103] In other countries, corn and rice are permitted for the production of beer, too.[104]

In the last 20 years, the world beer production has increased by 35%. The highest production of beer in 2017 was estimated for the PR of China (489 million hL), followed by the United States of America (197 million hL), Brasil (131 million hL), Mexico (116 million hL), and Germany (96 million hL).[31,105] In 2017, the highest per capita consumption of beer was reported for the Czech Republic with 137 liters, followed by Poland with 98.1 liters and Germany, with a consumption of 96.0 liters. The United States of America was on the twelfth place in the world with a consumption of 74.9 liters beer per capita.[106]

The beer brewing process (Figure 11) starts with milling of the malt. In the mashing step, water is added to the milled malt initiating the conversion of starch into fermentable sugars by enzymes naturally present in the malt. In the lautering step, the sugar-rich liquid called wort is separated from the malt draff. The wort is boiled and hops are added. During the boiling process, the wort is sterilized and hop components like hop volatiles including odor-active compounds, bitter compounds, and polyphenols are transferred into the wort. The *α*-acids isomerize into iso-*α*-acids, which enhances the bitterness of beer. The final amount of iso-*α*-

acids present in the beer depends on the beer style. Weakly hopped beers contain only 10 mg/L iso-*α*-acids, whereas extremely hopped beers may contain more than 100 mg/L iso-*α*-acids. The separation of hop solids from the wort occurs after wort boiling in the whirlpool. The wort is then cooled to a temperature between 15 °C and 20 °C. Bottom-fermenting yeast or top-fermenting yeast is added and sugars from the malt are converted into alcohol and carbon dioxide. The pH level decreases to about 4.5. Additionally, fermentation byproducts are formed, among them higher alcohols, esters, organic acids, and diacetyl. Diacetyl is an undesirable product with a buttery aroma, which decreases again in the ongoing fermentation process. During conditioning, beers are stored for up to one month at about 0 °C. Yeast, hop draff, and proteins are removed by filtration, e.g. with diatomaceous earth. Filtered beers are ready to be filled into bottles, cans, and barrels.[22,23,104,107,108]

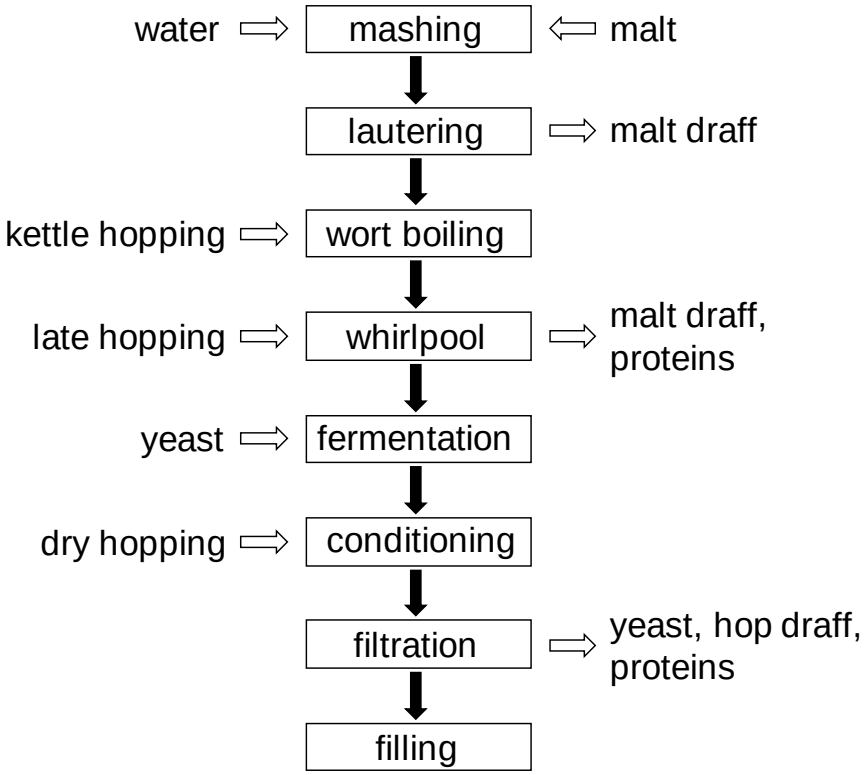



Figure 11: Schematic diagram of beer production according to Biendl et al., 2014[22]

During the brewing process, there are three different options for the addition of hops (cf. Figure 11). Hop addition at the beginning of wort boiling or during wort boiling is called kettle hopping. In kettle hopping, hop volatiles are almost completely lost with the exhaust vapors. Hop addition at the end of wort boiling or to the whirlpool is known as late hopping, which leads to an increased transfer of hop oil components than to kettle hopping, but without significant increase of the bitterness. The third possibility is dry hopping. This option includes hop addition during conditioning. Dry hopping is particularly used by craft brewers in combination with flavor hop addition in high dosages to achieve a strong hop aroma in the beer.[22,35]

### 4.2.8 Transfer of Odor-Active Compounds from Hops into Beer

The aroma is an important aspect for the consumers` beer brand selection. Similar to hops, research on beer aroma was first focused on the identification of volatiles. In various studies on beer volatiles, more than 600 compounds have been identified (Table 8).[46] Important substance classes were found to be acids, esters, alcohols, and aldehydes.

Table 8: Classes of Volatile Compounds in Beer (VCF 2018)[46]

| substance class | compounds | substance class | compounds |
|---|---|---|---|
| acids | 126 | hydrocarbons | 13 |
| esters | 100 | pyridines | 13 |
| alcohols | 72 | further nitrogen compounds | 11 |
| aldehydes | 71 | lactones | 11 |
| sulfur compounds | 50 | pyrroles | 11 |
| ketones | 39 | nitriles and amides | 8 |
| phenols | 28 | pyrrolines | 6 |
| epoxides, pyrans, coumarins | 25 | ethers | 2 |
| furans | 24 | acetals | 1 |
| pyrazines | 24 | oxazolines | 1 |
| amines | 23 | | |

The first analysis of beer volatiles by West et al. was focused on the identification of esters and higher alcohols.[109] Between 1964 and 1995, the volatile fraction of beer was analyzed in more detail. By GC analyses, alcohols, esters, thiols, furanes, furanones, lactones, oxygen heterocycles, phenols, and *γ*-pyrones were identified as volatile compounds in beer.[110-121] Later, also terpene hydrocarbons, like caryophyllene, farnesene, and myrcene were identified as hop-derived constituents in beer.[122]

The role of hops and hop oil in beer was first analyzed by Buttery in 1967. The study showed that volatile substances from hops play only a minor part in the overall aroma of beer. The major loss of volatile compounds was suspected to occur in the kettle boiling and hopped wort filtering stages of the brewing process. Further studies confirmed later that hop volatiles are almost completely lost during wort boiling with the exhaust vapors and residual amounts reaching the final beer would stay clearly below their odor threshold values. This was later verified by Deinzer and Yang, who found out that aroma hop varieties had high concentrations of *α*-humulene and *β*-caryophyllene, but their odor thresholds are too high in order to result in a significant influence on beer flavor.[123-126]

As the majority of volatiles present in beer is basically odorless and has no or only little impact on the overall aroma, the identification of the odor-active compounds among the beer volatiles is crucial.

The addition of hops directly before the end of the boil or to the whirlpool (late hopping), in particular in combination with the use of traditional European varieties, results in a floral note in the overall beer aroma. Peacock and further researchers showed that this floral note is predominantly caused by linalool.[80-82,127-130] In addition, by application of gas chromatography coupled with mass spectrometry Peacock showed that geraniol, which is included in the hop varieties Cascade and Cluster, can be transferred into the beer in substantial amounts when late hop addition is applied.[82]

Further studies focused on beers brewed with hops of special aroma properties. Beers hopped with Cascade showed floral and citrusy notes whereas beers brewed with Hallertauer Mittelfrüh were characterized by herbaceous and spicy odor impressions. Cascade beers showed significantly higher concentrations of geraniol. On the other hand, in beers with Hallertauer Mittelfrüh higher amounts of linalool were determined. Additionally, it was demonstrated that myrcene can not be transferred in odor-active amounts from hops into the final beer due to major losses during of the brewing process. Therefore, this compound is not contributing to the aroma of late-hopped beer.[131-135]

In contrast, by using different hop varieties, it was demonstrated that hop-derived compounds like ethyl 3-methylbutanoate, ethyl 4-methylpentanoate, geraniol, hexanal, 3-sulfanylhexan-1-ol, 3-methylbut-2-enal, 4MSP, (2*E*,6*Z*)-nona-2,6-dienal, and *trans*-4,5-epoxy-(2*E*)-dec-2-enal can exceed their odor threshold values in the final beer and therefore, can have an impact on the overall aroma of beer.[9,99,136,137]

Hop-derived compounds in beers brewed with eighteen different hop varieties from the United States, Germany, and New Zealand were analyzed in another study. The concentrations of *β*-citronellol, ethyl heptanoate, geraniol, geranyl acetate, 3-methylbutyl 2-methylpropanoate, 2-methylpropyl 2-methylpropanoate, linalool, *cis*-linalool oxide, and 2-methylbutyl 2-methylpropanoate varied widely amongst the beers. However, the concentrations of nerol and *β*-ionone showed almost no difference.[138]

In addition to a simple transfer of volatiles from hops into beer, biotransformation of hop-derived precursors can significantly influence the overall aroma of beer. Different studies showed that particularly terpene alcohol glycosides, cysteine adducts, and glutathione adducts originating in hops can undergo biotransformation reactions to odor-active compounds during fermentation. The type of yeast (bottom-fermenting or top-fermenting) further influences the release of these compounds.[139-151]

To achieve an intense hoppy aroma in beer, dry hopping is more effective than late hop addition. In many craft beers, usage of extremely high amounts of hops results in a very strong hop flavor in the beer.[35,132,152] In brewing trials with Cascade and hop additions between 200 g/hL and 1600 g/hL the most pleasant hop aroma note was perceived at a hopping rate between 386 g/hL and 800 g/hL. The highest transfer rates of linalool, geraniol, and myrcene, however, were calculated in beers with a hopping rate of 386 g/hL.[135]

# 5 Objectives

Although hops were initially used in brewing to enhance the shelf-life of beer and impart bitterness, depending on hopping time and brewing technology, hops may also influence the aroma of beer. Earlier research has shown that the floral aroma of traditional German beers is mainly caused by the hop-derived compound linalool. However, driven by the growing number of microbreweries in the United States, in recent years, a rising demand for special flavor hops with more diverse aromatizing potential evolved. The demand for more aromatic hops also increased in Germany and new flavor hop varieties were bred at the German Hop Research Center in Huell, Germany. In 2012, four German flavor hop varieties, namely Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Polaris were released for cultivation. Hallertau Blanc is said to possess a white wine-like aroma note, Huell Melon is characterized by a strong fruity, cantaloupe-like note, Mandarina Bavaria shows a fruity, tangerine-like aroma note, and Polaris exhibits pronounced minty and fruity notes. Identification of odor-active compounds in hops has been the subject of various studies. However, little was known about the odorants responsible for the characteristic aroma notes in the above mentioned flavor hop varieties, their changes during the brewing process, and their impact on the aroma of the final beer.

The first aim of the current research project was the identification of the variety-characterizing odor-active compounds in the four German flavor hop varieties Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Polaris. Further research was focused on the identification of hop-derived odorants in beers brewed with the flavor hop variety Huell Melon using different hopping approaches. By quantitation of selected hop-derived odor-active compounds in beer, transfer rates/formation rates were calculated and the influence of the timing of the hop dosage was assessed. Finally, on the basis of the quantitative data, the impact of individual hop-derived odorants on the overall aroma of beer was shown in sensory experiments.

# 6 Results and Discussion

This thesis is a publication-based dissertation. Data was summarized in three articles published in international scientific peer reviewed journals. For each publication a copy of the original, a summary including the individual contributions of the authors, as well as the reprint permission of the publisher can be found in the appendix.

## 6.1 Screening for Odor-Active Compounds in Hops

The screening was applied to samples of the varieties Hallertau Blanc, Huell Melon, Mandarina Bavaria, and Polaris. An industry panel selected samples that were considered to exhibit the characteristic olfactory profile associated with each variety. The selected samples were all grown in the Hallertau region, Germany. For varieties Mandarina Bavaria und Polaris, samples of the harvest year 2012 were selected, whereas samples of Hallertau Blanc and Huell Melon were from the harvest year 2014. The traditional aroma hop variety Hallertau Tradition (harvest year 2014) was additionally included in the research project. All samples were used as pellets, type 90, and were stored in vacuum-sealed bags at −20 °C.

To isolate the volatile compounds, pellets of the five hop varieties were homogenized and hop powders were extracted with organic solvent. Nonvolatiles were removed by SAFE. The SAFE distillates were concentrated and subjected to cAEDA. Results revealed 46 odor-active regions with FD ≥ 16 in at least one of the five hop varieties (Table 9).[153] Structure assignment of the odor-active compounds was achieved by comparing of the retention indices on two columns of different polarity, the odor quality as perceived at the sniffing port of the GC-O system, and the mass spectra in EI mode and in CI mode with data from authentic reference compounds analyzed under the same conditions. To avoid coelution during GC-MS analysis, SAFE distillates were first fractionated by acid-base extraction into a fraction containing the neutral and basic volatiles and a fraction containing the acidic volatiles. The neutral and basic volatiles were further fractionated into five sub-fractions of different polarity by using silica gel chromatography. Additionally, a fraction containing only the volatile thiols was isolated by means of mercurated agarose gel.[154] Finally, 38 out of 46 odor-active regions could be unequivally assigned to the causative compounds.

The highest FD factors in all five samples were determined for the geranium leaf-like smelling myrcene (**8**; FD factors 1024–2048), the citrusy, bergamot-like smelling linalool (**24**; FD 128–1024), and the cheesy smelling compounds 2-methylbutanoic acid and 3-methylbutanoic acid (**28** and **29**; FD 64–1024). This confirmed the importance of these three compounds in hops reported in the literature.[93,94] It has also been reported that the more odor-active (3*R*)-isomer of the chiral compound linalool is predominating in hops.[93,94,128,155] Thus the enantiomeric distributions of linalool in the five hop varieties were determined by GC-enantioGC-MS analysis. Results confirmed the dominance of (3*R*)-linalool in hops. The percentage of (3*R*)-linalool in the five hop varieties was between 86% (Huell Melon) and 95% (Mandarina Bavaria).

Further 23 odor-active compounds showed FD factors of ≥ 16 in at least one of the five different hop varieties. These included the fruity smelling esters ethyl 2-methylpropanoate (**1**), methyl 2-methylbutanoate (**3**), ethyl 2-methylbutanoate (**4**), and propyl 2-methylbutanoate (**7**), the buttery smelling butane-2,3-dione (**2**), the citrusy, soapy smelling octanal (**12**), the mushroom-like smelling oct-1-en-3-one (**13**), the roasty, popcorn-like smelling 2-acetyl-1-pyrroline (**14**), and the geranium leaf-like smelling (5*Z*)-octa-1,5-dien-3-one (**16**). 4-Methyl-4-sulfanylpentan-2-one (**17**), which is mainly responsible for the typical black currant aroma in Cascade hops,[98,99] was also identified as an odor-active compound in all five hop varieties. This was to

be expected for the three varieties Hallertau Blanc, Huell Melon, and Mandarina Bavaria due to their genetic derivation from the variety Cascade, but the highest FD factor was determined in the variety Polaris.[38-41]

Additional odor-active compounds present in the four flavor hop varieties as well as in Hallertau Tradition were identified as (3*E*,5*Z*)-undeca-1,3,5-triene and nonanal (fresh, fruity, floral; **18** and **19**), acetic acid (vinegar; **20**), 3-(methylsulfanyl)propanal (cooked potato; **22**), propanoic acid, 2-methylpropanoic acid, butanoic acid (all cheesy; **23**, **25**, and **27**), geraniol (rose, floral; **37**), *trans*-4,5-epoxy-(2*E*)-dec-2-enal (metallic; **42**), 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (caramel; **43**), sotolon (seasoning; **44**), phenylacetic acid (honey; **45**), and vanillin (vanilla; **46**). Eight odor-active compounds could not be structurally identified (**9**, **11**, **32**–**34**, **38**, **40**, and **41**). Structure assignment of the clove-like, herbaceous smelling compound **36** is detailed in the following chapter.

In summary, high FD factors of the fruity smelling esters ethyl 2-methylpropanoate (512), ethyl 2-methylbutanoate (1024), and propyl 2-methylbutanoate (256) suggested these compounds to contribute to the fruity, cantaloupe note of Huell Melon hops. These compounds were detected in the cAEDA with significantly higher FD factors in the variety Huell Melon than in the other hop varieties (FD factors ≤ 64). In the variety Polaris, fuity, banana-like smelling 3-methylbutyl acetate and eucalyptus-like smelling 1,8-cineole were identified as variety-specific odor-active compounds. For both, an FD factor of 64 was determined in Polaris, whereas in Hallertau Blanc, Huell Melon, Mandarina Bavaria, and in Hallertau Tradition, they were not detected by GC-O (FD < 1). Additionally, the high FD factor of fruity smelling methyl 2-methylbutanoate (128) suggested a contribution to the fruity aroma note of Polaris.

No variety-characteristic odorants became visible in this study for the flavor hop varieties Hallertau Blanc and Mandarina Bavaria.

Table 9: Odor-Active Compounds in the SAFE Distillates Obtained from the Flavor Hop Varieties Hallertau Blanc (HB), Huell Melon (HN), Mandarina Bavaria (MB), Polaris (PA), and the Classic Aroma Hop Variety Hallertau Tradition (HT)[153]

| no. | odorant[a] | odor[b] | RI[c] | | FD factor[d] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | FFAP | ZB-5 | HB 2014[e] | HN 2014[e] | MB 2012[e] | PA 2012[e] | HT 2014[e] |
| **1** | ethyl 2-methylpropanoate | fruity | 973 | 863 | 16 | 512 | 32 | 16 | 4 |
| **2** | butane-2,3-dione | buttery | 993 | 605 | 64 | 64 | 64 | 32 | 64 |
| **3** | methyl 2-methylbutanoate | fruity | 1024 | 780 | 32 | 16 | 32 | 128 | 32 |
| **4** | ethyl 2-methylbutanoate | fruity | 1056 | 757 | 32 | 1024 | 32 | 16 | 64 |
| **5** | hexanal | green, grassy | 1088 | 802 | 4 | 4 | < 1 | 2 | 16 |
| **6** | 3-methylbutyl acetate | fruity, banana | 1119 | 883 | < 1 | < 1 | < 1 | 64 | < 1 |
| **7** | propyl 2-methylbutanoate | fruity | 1140 | 946 | 16 | 256 | 4 | 8 | 8 |
| **8** | myrcene | geranium leaf | 1167 | 994 | 1024 | 1024 | 1024 | 2048 | 1024 |
| **9** | unknown | malty, fruity | 1191 | - | 16 | 32 | < 1 | < 1 | 32 |
| **10** | 1,8-cineole | minty, eucalyptus | 1206 | 1027 | < 1 | < 1 | < 1 | 64 | < 1 |
| **11** | unknown | mushroom | 1258 | - | < 1 | 4 | 16 | 64 | < 1 |
| **12** | octanal | citrusy, soapy | 1287 | 1010 | 16 | 32 | 16 | 4 | 4 |
| **13** | oct-1-en-3-one | mushroom | 1302 | 976 | 8 | 16 | 16 | 64 | 32 |
| **14** | 2-acetyl-1-pyrroline | roasty, popcorn | 1343 | 925 | 32 | 64 | 8 | 16 | 32 |
| **15** | dimethyl trisulfide | cabbage | 1367 | 971 | < 1 | < 1 | < 1 | < 1 | 256 |
| **16** | (5*Z*)-octa-1,5-dien-3-one | geranium leaf | 1372 | 983 | 128 | 64 | 64 | 32 | 256 |
| **17** | 4-methyl-4-sulfanylpentan-2-one | black currant | 1381 | 940 | 32 | 16 | 32 | 64 | 16 |
| **18** | (3*E*,5*Z*)-undeca-1,3,5-triene | fresh, pineapple | 1389 | 1185 | 128 | 256 | 128 | 256 | 32 |
| **19** | nonanal | citrusy, soapy | | 1117 | | | | | |
| **20** | acetic acid | vinegar, pungent | 1446 | 626 | 16 | 16 | 16 | 16 | 32 |
| **21** | 1,3,5,8-undecatetraene[f] | fresh, citrusy | 1457 | 1185 | < 1 | 32 | < 1 | 8 | < 1 |
| **22** | 3-(methylsulfanyl)propanal | cooked potato | 1460 | 907 | 32 | 64 | 64 | 64 | 64 |
| **23** | propanoic acid | cheesy, pungent | 1486 | 839 | 2 | 32 | 4 | 64 | 4 |
| **24** | linalool | citrusy, bergamot | 1546 | 1100 | 256 | 128 | 256 | 512 | 1024 |
| **25** | 2-methylpropanoic acid | cheesy | 1560 | 802 | 64 | 32 | 32 | 16 | 256 |
| **26** | (2*E*,6*Z*)-nona-2,6-dienal | cucumber | 1597 | 1145 | 64 | 64 | 32 | 32 | 32 |

Table 9 (continued): Odor-Active Compounds in the SAFE Distillates Obtained from the Flavor Hop Varieties Hallertau Blanc (HB), Huell Melon (HN), Mandarina Bavaria (MB), Polaris (PA), and the Classic Aroma Hop Variety Hallertau Tradition (HT)[153]

| no. | odorant[a] | odor[b] | RI[c] | | FD factor[d] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | FFAP | ZB-5 | HB 2014[e] | HN 2014[e] | MB 2012[e] | PA 2012[e] | HT 2014[e] |
| **27** | butanoic acid | cheesy | 1621 | 810 | 16 | 32 | 32 | 128 | 16 |
| **28** | 2-methylbutanoic acid | cheesy | 1678 | 847 | 256 | 256 | 64 | 256 | 1024 |
| **29** | 3-methylbutanoic acid | | | | | | | | |
| **30** | (2*E*,4*E*)-nona-2,4-dienal | fatty, cucumber | 1700 | 1214 | < 1 | 16 | < 1 | < 1 | 32 |
| **31** | pentanoic acid | cheesy | 1731 | 914 | 1 | 1 | < 1 | 1 | 128 |
| **32** | unknown | citrusy | 1736 | - | < 1 | 32 | < 1 | < 1 | < 1 |
| **33** | unknown | onion | 1758 | - | 2 | 16 | 1 | 8 | < 1 |
| **34** | unknown | sulfury, sweaty | 1762 | - | < 1 | 64 | < 1 | < 1 | < 1 |
| **35** | geranyl acetate | floral, rose | 1770 | 1353 | < 1 | 2 | < 1 | 16 | < 1 |
| **36** | unknown | clove, herbaceous | 1831 | 1538 | 64 | 128 | 16 | 32 | 128 |
| **37** | geraniol | rose, floral | 1842 | 1258 | 16 | 16 | 64 | 32 | 8 |
| **38** | unknown | pungent, onion | 1922 | - | 2 | 64 | 2 | 8 | < 1 |
| **39** | heptanoic acid | cheesy | 1946 | 1080 | < 1 | < 1 | < 1 | 2 | 64 |
| **40** | unknown | cabbage | 1991 | - | < 1 | 2 | < 1 | < 1 | 16 |
| **41** | unknown | sweaty, onion | 2011 | - | 2 | 32 | 2 | 8 | < 1 |
| **42** | *trans*-4,5-epoxy-(2*E*)-dec-2-enal[g] | metallic | 2020 | 1382 | 16 | 32 | 8 | 4 | 32 |
| **43** | HDMF[h] | caramel | 2040 | 1068 | 8 | 8 | 8 | 64 | 8 |
| **44** | sotolon | seasoning | 2221 | 1107 | 8 | 8 | 32 | 16 | 32 |
| **45** | phenylacetic acid | honey | 2562 | 1266 | 8 | 16 | 8 | 8 | 16 |
| **46** | vanillin | vanilla | 2585 | 1404 | 16 | 8 | 8 | 8 | 32 |

[a]Odorants exhibiting an FD factor of ≥ 16 in at least one of the five hop samples; order reflects elution order on the FFAP column; structure assignments were based on the comparison of the retention indices on two GC capillaries (FFAP, ZB-5), the mass spectra obtained by GC-MS, the odor as perceived at the sniffing port during GC-O with data obtained from authentic reference compounds. [b]Odor as perceived at the sniffing port during GC-O. [c]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]Flavor dilution factor; dilution factor of the highest dilution of the hop volatile isolate in which the odorant was detected during GC-O. [e]Number indicates harvest year. [f]No reference compound was available; mass spectral data suggested a mixture of isomeric 1,3,5,8-undecatetraenes. [g]GC-MS analysis did not result in a clear mass spectrum, but comparison of RIs and odor quality with respective data of an authentic reference compound allowed for unequivocal structure assignment. [h]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

## 6.2 Identification of (1*R*,4*S*)-Calamenene as an Odor-Active Compound

During the work targeted at the structure assignment of the clove-like and herbaceous smelling odorant **36** (Table 9) in hops, it became apparent that the compound was also odor-active in the fruit pulp of *Spondias mombin* L.[156]

*S. mombin* belongs to the sumac family (*Anacardiaceae*). The fruit of *S. mombin* is known by more than 50 names, among them cajá, yellow mombin, hog plum, and taperebá. *S. mombin* is native to southern Mexico, Brazil, and the Caribbean islands where it is very popular as a thirst quencher. The yellow fruit with a diameter of approximately 4 cm combines a sweet and sour taste with a fruity, sweet, and slightly turpentine-like aroma. Application of GC-O in combination with an AEDA to the volatiles isolated from the fruit pulp of cajá by solvent extraction and SAFE revealed 39 odor-active compounds with FD factors ranging from 4 to 1024. Based on the comparison of the retention indices, the odor qualities, and the mass spectra with data from authentic reference compounds, 33 out of the 39 compounds could be structurally identified. The fruity smelling ethyl butanoate showed the highest FD factor of 1024, followed by fruity, banana-like smelling 3-methylbutyl acetate (512) and caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (512). Further odorants detected in the SAFE distillate of *S. mombin* fruit pulp included the fruity smelling esters ethyl 3-methylbutanoate, ethyl hexanoate, methyl 3-hydroxybutanoate, and ethyl 2-methylbutanoate, *α*-pinene, myrcene, (3*Z*)-hex-3-en-1-ol, linalool, 2-phenylethan-1-ol, vanillin, and the clove-like, herbaceous smelling compound also detected in hops (all 256). Results suggested that the fruity and sweet note of cajá fruit pulp is mainly caused by ethyl butanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, and methyl 3-hydroxybutanoate in combination with the caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, whereas the turpentine-like aroma note is due to the presence of *α*-pinene, myrcene, and (*Z*)-*β*-ocimene.

Based on the comparison of its mass spectrum with mass spectra of the NIST database,[157] the initially unknown clove-like and herbaceous smelling compound detected in the SAFE distillates obtained from hops and cajá fruit pulp was tentatively identified as the aromatized sesquiterpenoid calamenene. Literature research revealed that *cis*-calamenene as well as *trans*-calamenene had already been reported as constituents of various essential oils,[46] however, none of them has ever been reported as an odor-active compound. Published retention indices of *cis*- and *trans*-calamenenes suggested that the compound in hops and cajá fruit pulp was the *trans*-isomer.[46,158,159] To confirm this assumption, racemic *trans*-calamenene was synthesized from racemic menthone in a multistep reaction (Figure 12).[156,160] In the first step, menthone was deprotonated by lithium diisopropylamide followed by a nucleophilic attack of the formed carbanion on allyl bromide which afforded allylmenthone. This was reacted with 2-methylallylmagnesium chloride, followed by a ring closing metathesis. Elimination of water enforced by phosphoryl chloride resulted in a mixture of dihydrocalamenenes which, in the final step, underwent oxidative aromatization to *trans*-calamenene by means of 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile (DDQ).[156,161] After purification of the raw product by argentation chromatography with silver-nitrate coated silica gel, the structure of *trans*-calamenene was confirmed by $^{1}H$, $^{13}C$, and various 2D NMR experiments.[156]

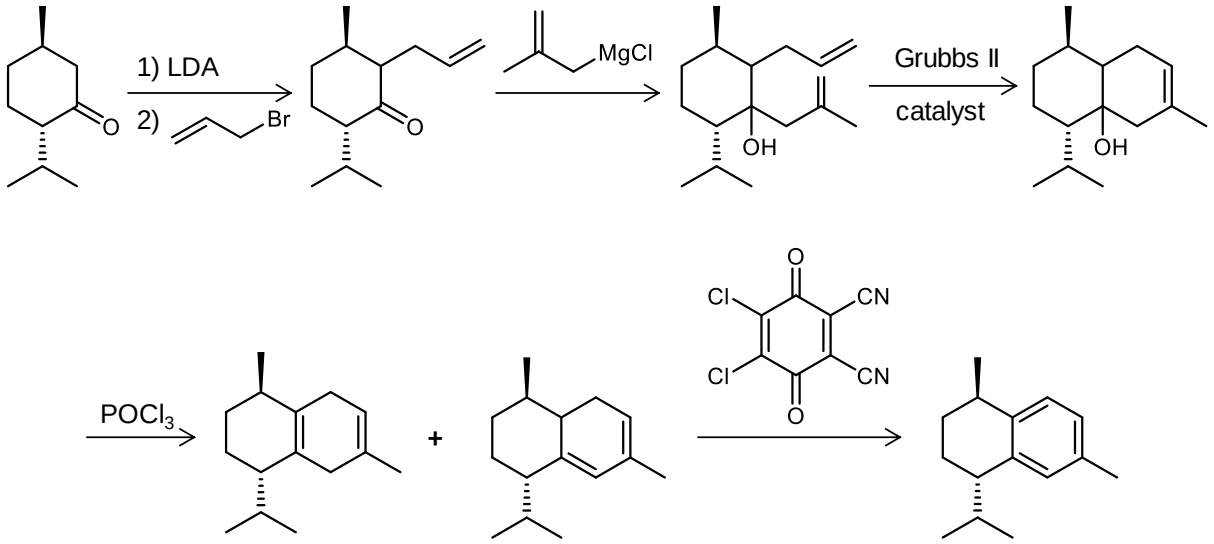



Figure 12: Synthetic approach to *trans*-calamenenes from menthones (exemplified by the synthesis of (1*R*,4*S*)-calamenene from (2*S*,5*R*)-menthone)

As retention indices, odor quality, and mass spectra of the unknown, clove-like, herbaceous smelling compound agreed with respective data of the synthesized product, the compound in hops and cajá fruit pulp was identified as *trans*-calamenene. Thus, for the first time *trans*-calamenene was identified as an odor-active compound.

To clarify the enantiomeric ratio of *trans*-calamenene in hops and in cajá fruit pulp, enantiopure (1*R*,4*S*)-calamenene and enantiopure (1*S*,4*R*)-calamenene were synthesized using the approach detailed above (cf. Figure 12), but starting from enantiopure (2*S*,5*R*)-menthone and enantiopure (2*R*,5*S*)-menthone, respectively.[153] For both enantiomeric *trans*-calamenenes, the odor quality perceived at the sniffing port of the GC-O was described as clove-like and herbaceous. The odor thresholds of (1*R*,4*S*)-calamenene and (1*S*,4*R*)-calamenene in air were determined by aroma extract dilution analysis using (2*E*)-dec-2-enal as internal standard.[162,163] (1*R*,4*S*)-Calamenene was identified as the more potent *trans*-isomer. Its odor threshold amounted to 2.5 ng/L and thus was slightly lower than the odor threshold of (1*S*,4*R*)-calamenene (9.5 ng/L).[153]

Analytical separation of the two enantiomeric *trans*-calamenenes was achieved by GC-enantioGC-MS analysis using a *β*-cyclodextrin based GC column. Results indicated that the *trans*-calamenene in both, hops and cajá was pure (1*R*,4*S*)-calamenene, whereas (1*S*,4*R*)-calamenene was absent (Figure 13).[153,164] The same was found for the *trans*-calamenene identified in the leaves of the curry tree (*Bergera koenigii syn. Murraya koenigii*).[164,165] Consistent with our results, pure (1*R*,4*S*)-calamenene was also identified in the essential oil of *Cedrela odorata* and *Bauania tricrenata*, whereas (1*S*,4*R*)-calamenene and *cis*-calamenenes were absent.[166] The additional peak at 21.75 min observed in the chromatograms of the isolates obtained from hops, cajá fruit pulp, and curry leaves showed basically the same mass spectrum as (1*R*,4*S*)-calamenene and (1*S*,4*R*)-calamenene. This peak may correspond to one of the *cis*-calamenenes. However, no effort was made to clarify the identity of this compound as GC-O showed that it was odorless.

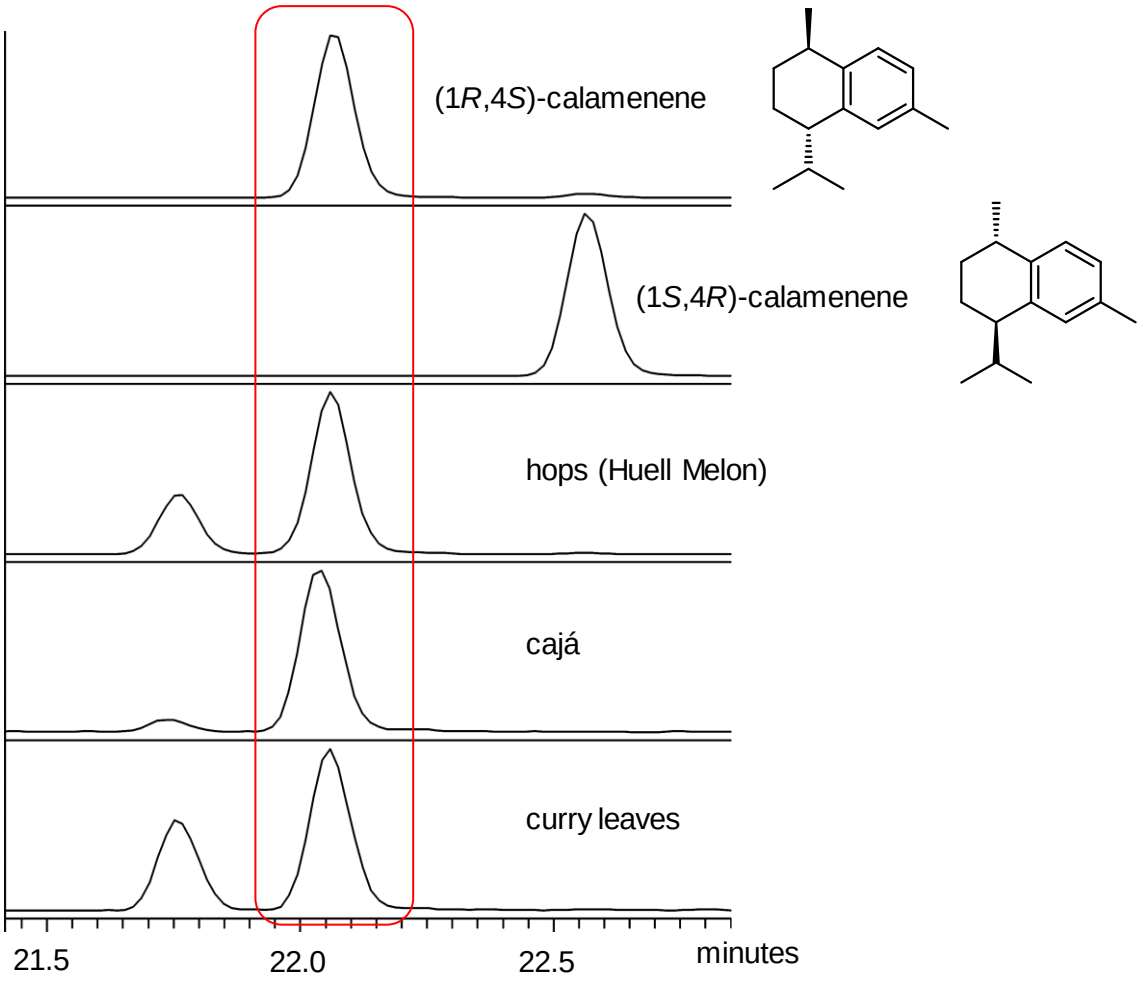



Figure 13: GC-enantioGC-MS separation of calamenene isomers in SAFE distillates obtained from hops (Huell Melon), from cajá fruit pulp, and from the leaves of the curry tree in comparison to the reference compounds (1*R*,4*S*)-calamenene and (1*S*,4*R*)-calamenene

## 6.3 Quantitation of Odor-Active Compounds in Hops

To substantiate the results of the screening experiments and to get deeper insights into the role of the individual odor-active compounds in hops, the concentrations of 18 major odorants were determined in the pellets of Hallertau Blanc, Huell Melon, Mandarina Bavaria, Polaris, and Hallertau Tradition. Among the 18 compounds were in particular those odorants for which the results of the cAEDA suggested a contribution to the variety-specific aroma notes. To examine the influence of the harvest year, hop pellets of the four flavor hop varieties from a second harvest year were additionally included in the quantitation study.[153]

The majority of quantitations was accomplished by using SIDAs in combination with GC-MS analysis. Hop pellets were first homogenized and the powders were extracted with organic solvent. During the extraction procedure, isotopically substituted analogues of the target analytes were added as internal standards. Nonvolatiles were removed by SAFE, volatile isolates were concentrated, and concentrates were subjected to heart-cut GC-GC-MS(CI) or GC×GC-TOFMS(EI) analysis. Myrcene was quantitated by GC-FID analysis after simultaneous extraction and fractionation using tetradecane as internal standard.[167]

Results of the quantitations revealed concentrations between 0.223 µg/kg and 31 g/kg (Table 10).[153] Data clearly confirmed the results of the screening experiments. For the fruity smelling esters ethyl 2-methylpropanoate (**1**), ethyl 2-methylbutanoate (**4**), and propyl 2-methyl-butanoate (**7**), the highest concentrations were found in the sample of Huell Melon of harvest year 2014. The samples of all other hop varieties showed lower concentrations. The variety-specific role of ethyl 2-methylpropanoate (**1**), ethyl 2-methylbutanoate (**4**), and propyl 2-methylbutanoate (**7**) for the aroma of Huell Melon was thus confirmed. However, concentrations in the Huell Melon 2013 sample were not quite as high as in the Huell Melon 2014 sample. Likewise, the quantitations confirmed the variety-specific role of banana-like smelling 3-methylbutyl acetate (**6**) and eucalyptus-like smelling 1,8-cineole (**10**) in Polaris hops. Only little differences were observed between the Polaris 2012 sample and the Polaris 2013 sample.

Of all quantitated odorants in the different hop varieties, the highest concentrations were determined for the geranium leaf-like smelling myrcene (**8**). With more than 30 g/kg, extraordinary high concentrations were determined for the Polaris 2012 sample. A possible explanation for that high amount could be inhomogeneous pelletizing. The concentrations of citrusy smelling (3*R*)-linalool ((3*R*)-**24**) were between 9 mg/kg and 80 mg/kg, a typical range in hops.[9] Concentrations of rose-like smelling geraniol (**37**) were between 4 mg/kg and 90 mg/kg, well in accordance with published data.[9] Both, linalool and geraniol concentrations, are apparently much less dependent on the harvest year than on the hop variety. The cheesy smelling compounds 2-methylbutanoic acid (**28**) and 3-methylbutanoic acid (**29**), by contrast, showed clear differences between harvest years. In all four flavor hop varieties, concentrations of 2-methylbutanioc acid and 3-methylbutanoic acid were clearly higher in the sample of harvest year 2013 than in the sample of harvest year 2012 and 2014. The same was observed for acetic acid (**20**) and butanoic acid (**27**) at least for varieties Hallertau Blanc, Huell Melon, and Mandarina Bavaria, whereas concentrations were nearly equal for the variety Polaris. For the geranium leaf-like smelling (5*Z*)-octa-1,5-dien-3-one (**16**), concentrations were determined to be between 0.552 µg/kg and 5.52 µg/kg. For varieties Huell Melon, Mandarina Bavaria, and Polaris concentrations were nearly equal in the samples of both harvest years. In the variety Hallertau Blanc, concentration of (5*Z*)-octa-1,5-dien-3-one (**16**) was ten times higher in the

sample of harvest 2014 than in the sample of harvest 2013. The concentrations of the black currant-like smelling 4-methyl-4-sulfanylpentan-2-one (**17**) were between 0.223 µg/kg and 2.83 µg/kg, thus rather low. In some hop varieties from the United States like Citra and Eureka, concentrations of up to 114 µg/kg were reported.[98,99] For octanal (**12**), (3*E*,5*Z*)-undeca-1,3,5-triene (**18**), and nonanal (**19**), concentrations did not differ substantially between the hop varieties and the harvest years.

Table 10: Concentrations of Selected Hop Odorants in the Flavor Hop Varieties Hallertau Blanc (HB), Huell Melon (HN), Mandarina Bavaria (MB), Polaris (PA), and the Classic Aroma Hop Variety Hallertau Tradition (HT)[153]

| **odorant**[a] | **concentration (µg/kg)**[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **HB 2013**[c] | **HB 2014**[c] | **HN 2013**[c] | **HN 2014**[c] | **MB 2012**[c] | **MB 2013**[c] | **PA 2012**[c] | **PA 2013**[c] | **HT 2014**[c] |
| ethyl 2-methylpropanoate **(1)** | 16.8 | 48.8 | 262 | 2140 | 19.7 | 50.0 | 62.0 | 130 | 18.9 |
| methyl 2-methylbutanaote **(3)** | 327 | 233 | 1590 | 162 | 241 | 662 | 3020 | 2930 | 985 |
| ethyl 2-methylbutanoate **(4)** | 4.37 | 9.07 | 149 | 650 | 5.49 | 9.33 | 6.75 | 12.0 | 8.93 |
| 3-methylbutyl acetate **(6)** | 605 | 474 | 101 | 91.1 | 85.5 | 180 | 4490 | 2300 | 16.5 |
| propyl 2-methylbutanoate **(7)** | 19.2 | 157 | 348 | 1330 | 16.8 | 21.8 | 48.3 | 58.8 | 31.1 |
| myrcene **(8)** | 1550000 | 4270000 | 491000 | 1710000 | 1670000 | 1460000 | 31600000 | 6070000 | 1830000 |
| 1,8-cineole **(10)** | 1.46 | 1.82 | 1.22 | 1.33 | 2.75 | 1.71 | 63.2 | 62.4 | 1.58 |
| octanal **(12)** | 498 | 370 | 217 | 954 | 198 | 787 | 392 | 2610 | 263 |
| (5*Z*)-octa-1,5-dien-3-one **(16)** | 0.594 | 5.52 | 0.708 | 0.552 | 0.994 | 1.11 | 0.792 | 1.02 | 2.40 |
| 4-methyl-4-sulfanylpentan-2-one **(17)** | 1.88 | 0.899 | 0.273 | 0.223 | 1.26 | 1.49 | 2.83 | 2.53 | 0.525 |
| (3*E*,5*Z*)-undeca-1,3,5-triene **(18)** | 2.86 | 5.31 | 3.14 | 8.42 | 7.21 | 6.20 | 19.8 | 42.7 | 1.21 |
| nonanal **(19)** | 844 | 3000 | 586 | 1740 | 397 | 592 | 616 | 301 | 640 |
| acetic acid **(20)** | 610000 | 359000 | 600000 | 358000 | 669000 | 141000 | 367000 | 385000 | 329000 |
| (3*R*)-linalool **(**(3*R*)-**24)** | 25000 | 30700 | 9030 | 10200 | 23600 | 19400 | 52500 | 48900 | 79700 |
| butanoic acid **(27)** | 1010 | 441 | 2770 | 770 | 775 | 1360 | 1720 | 1670 | 145 |
| 2-methylbutanoic acid **(28)** | 21600 | 4050 | 33100 | 11000 | 2510 | 15300 | 4620 | 25900 | 63900 |
| 3-methylbutanoic acid **(29)** | 38500 | 18500 | 151000 | 41200 | 6460 | 69900 | 15500 | 73700 | 233000 |
| geraniol **(37)** | 15800 | 17800 | 11900 | 23100 | 47300 | 26300 | 88300 | 90300 | 3590 |

[a]Numbers in parentheses refer to Table 9. [b]Mean of triplicates, standard deviations were consistently < 20%. [c]Number indicates harvest year.

## 6.4 Impact of Huell Melon Hops on the Odor-Active Compounds in Beer

To get an insight into the influence of the variety-specific odorants of Huell Melon hops on the aroma of beer, beers were brewed with Huell Melon pellets of the harvest year 2014. The pellets were from the same batch as used for odorant screening and odorant quantitation in hops (cf. chapters 6.1 and 6.3). One beer was produced with late hop addition and a second beer was brewed with dry hopping. For both, hop dosage was 2.5 g/L. A beer without addition of hop pellets served as reference. Fermentations were carried out by using a bottom-fermenting yeast (type W34/70) or a top-fermenting yeast (type OK3). Thus, in total six beers were available for analysis, three bottom-fermented beers (late hopped, dry hopped, reference) and three top-fermented beers (late hopped, dry hopped, reference).[168]

At first, the three bottom-fermented beers were analyzed for odor-active compounds by application of a cAEDA. Results revealed a total of 35 odorants with FD factors of 16 to 1024 in at least one of the three beers. The odorants with the highest FD factors in the reference beer, in the late hopped beer, and in the dry hopped beer were identified as honey-like, floral smelling 2-phenylethan-1-ol (FD factor 1024), malty smelling 2- and 3-methylbutan-1-ol (512–1024), and malty smelling 2-methylpropan-1-ol (512–1024). These compounds are all well-known byproducts of the fermentation.[121,129,169] (*E*)-*β*-Damascenone (cooked apple-like; FD factor 256–512) was also detected with high FD factors in all three beer samples. It originates from malt.[169,170] Fourteen compounds were identified as hop-derived odorants. Among them, the fruity smelling esters ethyl 2-methylbutanaote, ethyl 2-methylpropanoate, and methyl 2-methylbutanaote showed the highest FD factors in the late hopped beer and in the dry hopped beer. Geraniol (floral, rose-like), linalool (citrusy, floral), 4-methyl-4-sulfanylpentan-2-one (black currant-like), myrcene (geranium leaf-like), (1*R*,4*S*)-calamenene (clove-like, herbaceous), (5*Z*)-octa-1,5-dien-3-one (geranium leaf-like), propyl 2-methylbutanoate (fruity), and (3*E*,5*Z*)-undeca-1,3,5-triene (fresh, pineapple-like) were only detected in the late hopped beer and in the dry hopped beer, but not in the reference beer. Analysis of the three top-fermented beers by a comparative odorant screening revealed the same set of hop-derived compounds in the beers (data not shown).

To substantiate the results of the screening experiments, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, geraniol, linalool, methyl 2-methylbutanoate, myrcene, and propyl 2-methylbutanaote were quantitated in the three bottom-fermented beers and in the three top-fermented beers by application of SIDAs. Concentrations of the hop-derived odor-active compounds in the beers were between 2 ng/L and 57 µg/L. For all these odorants, the lowest concentrations were determined in the reference beers, whereas concentrations in the late hopped beers and in the dry hopped beers were significantly higher. For ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and geraniol, furthermore the concentrations in the dry hopped beers were much higher than the concentrations in the late hopped beers. Almost no difference was determined between the concentrations in the late hopped beers and the dry hopped beers for linalool, myrcene, methyl-2-methylbutanoate, and propyl 2-methylbutanoate. The concentrations of ethyl 2-methylpropanoate and linalool were higher in the late hopped and dry hopped beers, brewed by top-fermentation than the concentrations in the late hopped and dry hopped bottom-fermented beers. For the other five odorants, no considerable difference between the beers brewed by bottom-fermentation and the beers brewed by top-fermentation was observed.

Transfer rates from hops into beer were calculated for each odorant from the hop-derived concentration in beer divided by the concentration in hops multiplied by the hop dosage. The

hop-derived concentration in beer was calculated by subtracting the concentration in the reference beer from the concentration in the late or dry hopped beer, respectively. The results revealed percentages between 0.15% and 2000%. A minimal transfer well below 1% from hops into beer was shown for geranium-leaf like smelling myrcene, which was in agreement with previous research.[131-135] Moderate transfer was observed for the fruity smelling propyl 2-methylbutanoate, the rose-like, floral smelling geraniol, and the citrusy smelling linalool. With transfer rates between 10% and 19% into the bottom-fermented and into the top-fermented hopped beers, recovery of propyl 2-methylbutanoate was comparably low. Geraniol showed substantial differences in the transfer rates between late and dry hopped beers. With 7.8% and 18% into the late hopped bottom-fermented and the late hopped top-fermented beers, respectively, transfer was clearly lower than the transfer into the dry hopped beers, where transfer rates between 47% and 51% were calculated. It is well known from literature that geraniol can be converted by yeast to compounds such as geranyl acetate, citronellol, citronellyl acetate, linalool, and α-terpineol and that there is a more effective transformation of geraniol during late hopping in contrast to dry hopping.[138,140,141,146,147] Calculated transfer rates for linalool were nearly equal for the late and dry hopped beers, but showed significant differences between the bottom-fermented and the top-fermented beers. For the bottom-fermented beers, transfer rates of 100% suggested an effective transfer from hops into beer. This was well in agreement with data from literature.[9,132] The calculated rates for the transfer of linalool into the top-fermented beers with 190% clearly exceeded a simple transfer and thus suggested an additional formation of linalool in the brewing process from precursors supplied with the hops. Linalool can be formed enzymatically from hop-derived linalyl glycosides by yeast during fermentation.[139,142-144] Another potential source of additional linalool is the biotransformation of geraniol and nerol into linalool and citronellol, which has been reported to be more effective during fermentation with a top-fermenting yeast than during fermentation with a bottom-fermenting yeast.[140,141,146,147,150]

Process-induced changes beyond a direct transfer from hops into beer were also observed for the fruity smelling esters ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and methyl 2-methylbutanoate. Transfer rates of 35% and 33% were calculated for ethyl 2-methylbutanoate in the late hopped beers, but rates of 250% and 320% observed for the dry hopped beers were clearly beyond a simple transfer. Extraordinary high rates were also calculated for ethyl 2-methylpropanoate (170%–1000%) and methyl 2-methylbutanoate (1200%–2000%). The results suggest a formation from hop-derived precursors, probably the corresponding carboxylic acids. This hypothesis has to be confirmed in future experiments, e.g. by spiking the hops used in a brewing trial with isotopically substituted analogues of 2-methylpropanoic acid, 2-methylbutanoic acid, and 3-methylbutanoic acid and subsequent analysis the isotopologue ratios of the esters in the final beers.

For an estimation of the influence of the individual odorants on the overall beer aroma, sensory tests were finally carried out in the form of spiking experiments. The bottom-fermented and the top-fermented reference beers were spiked with individual odorants to reach the concentrations in the bottom-fermented and the top-fermented late hopped beers as well as the concentrations in the bottom-fermented and the top-fermented dry hopped beers. These spiked beers were orthonasally compared to the respective reference beers in 3-AFC tests. Spiking experiments confirmed the generally outstanding role of linalool for the hoppy aroma of beer. The effect of geraniol spiking was less pronounced. Despite its low concentrations in beer and low transfer rates, spiking of myrcene was significantly detected in three out of four beers. Individual spiking of the four esters revealed a high impact of propyl 2-methylbutanoate

on the aroma of all four hopped beers and an aroma contribution of methyl 2-methylbutanoate and ethyl 2-methylbutanoate, particularly to the dry hopped beers. By contrast, addition of ethyl 2-methylpropanoate was not significantly detected in three out of four beers.

In summary, the results of this study confirmed the contribution of linalool, geraniol, and myrcene for the aroma of hop-flavored beers and suggested a formation of some potent esters after late and particularly after dry hopping.

# 7 References


1. Buck, L.; Axel, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **1991**, *65*, 175–187.
2. The Nobel Foundation. The Nobel Prize in Physiology or Medicine 2004. https://www.nobelprize.org/prizes/medicine/2004/press-release/ (December 29, 2018).
3. Olender, T.; Waszak, S. M.; Viavant, M.; Khen, M.; Ben-Asher, E.; Reyes, A.; Nativ, N.; Wysocki, C. J.; Ge, D. L.; Lancet, D. Personal receptor repertoires: olfaction as a model. *Bmc Genomics* **2012**, 13.
4. Malnic, B.; Hirono, J.; Sato, T.; Buck, L. B. Combinatorial receptor codes for odors. *Cell* **1999**, *96*, 713–723.
5. Krautwurst, D. Human olfactory receptor families and their odorants. *Chem. Biodiversity* **2008**, *5*, 842–852.
6. Prinz zu Waldeck, C.; Frings, S. Wie wir riechen, was wir riechen: Die molekularen Grundlagen der Geruchswahrnehmung. *Biol. Unserer Zeit* **2005**, *35*, 302–310.
7. Mori, K.; Sakano, H. How is the olfactory map formed and interpreted in the mammalian brain? *Annu Rev Neurosci* **2011**, *34*, 467–499.
8. Franitza, L.; Granvogl, M.; Schieberle, P. Influence of the production process on the key aroma compounds of rum: from molasses to the spirit. *J. Agric. Food Chem.* **2016**, *64*, 9041–9053.
9. Steinhaus, M.; Schieberle, P. Transfer of the potent hop odorants linalool, geraniol and 4-methyl-4-sulfanyl-2-pentanone from hops into beer. In *European Brewery Convention, Proceedings of the 31st EBC Congress, Venice* 2007; Fachverlag Hans Carl, Nuremberg, Germany, 2007; Chapter 112, pp 1–8.
10. Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. In *Characterization of Food*; Goankar, A., Ed.; Elsevier: Amsterdam, The Netherlands, 1995; pp 403–431.
11. Grosch, W. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem. Senses* **2001**, *26*, 533–545.
12. 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.
13. Likens, S. T.; Nickerson, G. B. Detection of certain hop oil constituents in brewing products. *Am. Soc. Brew. Chem. Proc.* **1964**, 5–13.
14. 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.
15. Schieberle, P.; Grosch, W. Quantitative analyses of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252–257.
16. IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC), Nomenclature of Organic Chemistry. Section H: Isotopically Modified Compounds. Eur. J. Biochem. **1987**, *86*, 9–25.

17. Rothe, M.; Thomas, B. Aromastoffe des Brotes, Versuch einer Auswertung chemischer Geschmacksanalysen mit Hilfe des Schwellenwertes. *Z. Lebensm.-Unters. Forsch.* **1963**, *119*, 302–310.

18. Grosch, W. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragrance J.* **1994**, *9*, 147–158.

19. American Society of Testing and Materials. Standard E679-04. Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. In: ASTM Book of Standards, Vol. 15.08. American Society of Testing and Materials, West Conshohocken, PA, USA, 2005, pp. 38-44.

20. 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.

21. 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**, *63*, 639–647.

22. Biendl, M.; Engelhard, B.; Forster, A.; Gahr, A.; Lutz, A.; Mitter, W.; Schmidt, R.; Schönberger, C. Hops - Their Cultivation, Composition and Usage. Fachverlag Hans Carl: Nuremberg, Germany, 2014.

23. Neve, R. A. Hops: Botany. Chapman and Hall, London, 1991.

24. Narziß, L.; Back, W.; Gastl, M.; Zarnkow, M. Abriss der Bierbrauerei. 8th Ed. Wiley-VCH Verlag: Weinheim, Germany, 2017.

25. Narziß, L.; Back, W. Die Bierbrauerei - Band 2: Die Technologie der Würzebereitung. 8th Ed. Wiley-VCH Verlag: Weinheim, Germany, 2009.

26. European Brewery Convention, Technology and Engineering Forum. Hops and Hop Products. Manual of Good Practice. Getränke-Fachverlag Hans Carl: Nuremberg, Germany, 1997.

27. Lieberei, R.; Reisdorff, C.; Franke, W. Nutzpflanzen. 8th Ed. Georg Thieme Verlag: Stuttgart, Germany, 2012.

28. Barth H. J.; Klinke, C.; Schmidt, C. Der grosse Hopfenatlas: Geschichte und Geographie einer Kulturpflanze. Getränke-Fachverlag Hans Carl: Nuremberg, Germany, 1994.

29. Almaguer, C.; Schönberger, C.; Gastl, M.; Arendt, E. K.; Becker, T. *Humulus lupulus* - a story that begs to be told. A review. *J. Inst. Brew.* **2014**, *120*, 289–314.

30. Verzele M, De Keukeleire D. Chemistry and analysis of hop and beer bitter acids. Elsevier Science: Amsterdam, The Netherlands, 1991.

31. Hopsteiner; Steiner, S. H.; Germany, 2017, Entscheidungsdaten für den Hopfeneinkauf. https://www.hopsteiner.com/wp-content/uploads/2018/01/Entscheidungsdaten-2017.pdf (December 29, 2018).

32. Forster, A.; Beck, B.; Schmidt, R. Hop polyphenols – do more than just cause turbidity in beer. *Hopfenrundschau Int.* **1999**, 68–74.

33. Bayerische Landesanstalt für Landwirtschaft – Institut für Pflanzenbau und Pflanzenzüchtung – und Gesellschaft für Hopfenforschung e.V.; Jahresbericht 2017, Sonderkultur Hopfen; Bayerische Landesanstalt für Landwirtschaft (LfL), ed.; Freising-Weihenstephan, Germany, 2018.

34. Seigner, E.; Lutz, A.; Kammhuber, K. Neuer Trend in der Hüller Hopfenzüchtung - Hüller Special Flavor Hopfen. New German special flavor hops from Hüll. *Hopfen-Rundschau International*, Edition of the German Hop Growers' Magazine. **2012**, 40–49.

35. Lutz, A.; Kammhuber, K.; Seigner, E. New trend in hop breeding at the hop research center Huell. *Brew. Sci.* **2012**, *65*, 24–32.

36. Bayerische Landesanstalt für Landwirtschaft – Institut für Pflanzenbau und Pflanzenzüchtung – und Gesellschaft für Hopfenforschung e.V.; Jahresbericht 2013, Sonderkultur Hopfen; Bayerische Landesanstalt für Landwirtschaft (LfL), ed.; Freising-Weihenstephan, Germany, 2014.

37. Bayerische Landesanstalt für Landwirtschaft, LfL; Institut für Pflanzenbau und Pflanzenzüchtung; Die Hüller Special Flavor - Hopfensorten – aktueller Kenntnisstand; Lutz, A.; Kneidl, J.; Seigner, E.; Team Züchtungsforschung Hüll. https://-www.lfl.bayern.de/mam/cms07/ipz/dateien/hopfen_hüller_special-flavor.pdf (December 29, 2018).

38. Lutz, A.; Kneidl, J.; Seefelder, S.; Kammhuber, K.; Seigner, E. New German flavor hop variety Hallertau Blanc (2007/019/008). LfL, Institut für Pflanzenbau und Pflanzenzüchtung, Hopfenforschungszentrum Hüll (Bavarian state research center for agriculture institute for crop science and plant breeding hop research center Hüll). https://-www.lfl.bayern.de/mam/cms07/ipz/dateien/hopfen_blanc_2015_engl.pdf (December 29, 2018).

39. Lutz, A.; Kneidl, J.; Seefelder, S.; Kammhuber, K.; Seigner, E. New German flavor hop variety Huell Melon (2009/002/706). LfL, Institut für Pflanzenbau und Pflanzenzüchtung, Hopfenforschungszentrum Hüll (Bavarian state research center for agriculture institute for crop science and plant breeding hop research center Hüll). https://www.-lfl.bayern.de/mam/cms07/ipz/dateien/hopfen_melon_2015_engl.pdf (December 29, 2018).

40. Lutz, A.; Kneidl, J.; Seefelder, S.; Kammhuber, K.; Seigner, E. New German flavor hop variety Mandarina Bavaria (2007/018/013). LfL, Institut für Pflanzenbau und Pflanzenzüchtung, Hopfenforschungszentrum Hüll (Bavarian state research center for agriculture institute for crop science and plant breeding hop research center Hüll). https://www.lfl.bayern.de/mam/cms07/ipz/dateien/hopfen_manadarina_engl_2015.pdf (December 29, 2018).

41. Lutz, A.; Kneidl, J.; Seigner, E.; Kammhuber, K. New German flavor hop variety Polaris (2000/109/728) LfL, Institut für Pflanzenbau und Pflanzenzüchtung, Hopfenforschungszentrum Hüll (Bavarian state research center for agriculture institute for crop science and plant breeding hop research center Hüll). https://www.lfl.bayern.de/mam/cms07/ipz/dateien/hopfen_polaris_poster.pdf (December 29, 2018).

42. Stevens, R. The chemistry of hop constituents. *Chem. Rev.* **1967**, *67*, 19–71.

43. Hough, J. S.; Briggs, D. E.; Stevens, R.; Young, T. W. Malting and Brewing Science, Volume 2: Hopped Wort and Beer, 2nd Ed, Chapman Hall, New York, 1982.
44. Kaltner, D. Moderne Hopfenprodukte - Aus vielen guten Zutaten kann der Brauer ein spitzen Bier-Image brauen. In *Getränkeherstellung*, 2005.
45. Roberts, M. T.; Lewis, A. C. Rapid characterization of hop essential oils using gas chromatography-time of flight mass spectrometry. *J. Am. Soc. Brew. Chem.* **2002**, *60*, 116–121.
46. In VCF Volatile Compounds in Food: Database, Version 16.3; Nijssen, L. M., Ingen-Visscher, C. A. v., Donders, J. J. H., Eds.; TNO Triskelion: Zeist, The Netherlands, 2018.
47. Roberts, M. T.; Dufour, J.-P.; Lewis, A. C. Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC×GC-TOFMS) for high resolution analysis of hop essential oil. *J. Sep. Sci.* **2004**, *27*, 473–478.
48. Lermusieau, G.; Collin, S. Hop Aroma Extraction and Analysis. In: *Analysis of Taste and Aroma, Molecular Methods of Plant Analysis*; Jackson J. F., Linskens H. F., eds.; Springer Verlag: Berlin, Heidelberg, Germany, *21*, 2002.
49. Chapman, A. C. The essential oil of hops. Preliminary notice. *Proc. Chem. Soc*. **1893**, *9*, 177.
50. Chapman, A. C. Essential oil of hops. *Proc. Chem. Soc.* **1894**, *10*, 227–229.
51. Chapman, A. C. Essential oil of hops. *J. Chem. Soc. Trans.* **1895**, *67*, 54–63.
52. Chapman, A. C. Some derivatives of humulene. *J. Chem. Soc. Trans.* **1895**, *67*, 780–784.
53. Chapman, A. C. The essential oil of hops. *J. Inst. Brew.* **1898**, *4*, 224–235.
54. Chapman, A. C. Essential oil of hops. *J. Chem. Soc. Trans.* **1903**, *83*, 505–513.
55. Chapman, A. C. On the chemical individuality of humulene. *J. Chem. Soc.* **1928**, 785–789.
56. Chapman, A. C. Higher-boiling constituents of the essential oil of hops. *J. Chem. Soc.* **1928**, 1303–1306.
57. Chapman, A. C. The essential oil of hops. *J. Inst. Brew.* **1929**, *35*, 247–255.
58. Chapman, A. C. Individuality of humulene. *J. Chem. Soc.* **1929**, 359–360.
59. Hartsuck, J. A.; Paul, I. C. Molecular structure of humulene: x-ray analysis of the silver nitrate adducts. *Chem. Ind.* **1964**, 977-978.
60. Šorm, F.; Mleziva, J.; Arnold, Z. On terpenes XII. On the composition of oil of hops. *Collect. Czech. Chem. Commun.* **1949**, *14*, 693–698.
61. Šorm, F.; Mleziva, J.; Arnold, Z.; Pliva, J. On terpenes XIII. On the sesquiterpenes from the essential oil of hops. *Collect. Czech. Chem. Commun.* **1949**, *14*, 699–714.
62. Herout, V.; Streibl, M.; Mleziva, J.; Šorm, F. On terpenes. XIV. On the identity of humulene and *α*-caryophyllene. *Collect. Czech. Chem. Commun.* **1949**, *14*, 716–722.
63. Šorm, F.; Arient, J. On terpenes XIX. On the constitution of nat. farnesene. Synthesis of 2,6,10-trimethyldodecane and 2,7,10-trimethyldodecane. *Collect. Czech. Chem. Commun.* **1950**, *15*, 175–185.

64. Rigby, F. L.; Bethune, J. L. Separation of the aromatic constituents of hops. *Am. Soc. Brew. Chem. Proc.* **1955**, 174–183.

65. Howard, G. A. New approach to the analysis of hop oil. *J. Inst. Brew.* **1956**, *62*, 158–159.

66. Howard, G. A. Evaluation of hops. V. The essential oil of hops. *J. Inst. Brew.* **1957**, *63*, 126–138.

67. Buttery, R. G.; McFadden, W. H.; Teranishi, R.; Kealy, M. P.; Mon, T. R. Constituents of hop oil. *Nature* **1963**, *200*, 435–436.

68. Buttery, R. G.; Lundin, R. E.; McFadden, W. H.; Jahnsen, V. J.; Kealy, M. P. Volatile hop constituents: Identification of methyl dec-4-enoate and methyl deca-4,8-dienoate. *Chem. Ind.* **1963**, 1981–1982.

69. Buttery, R. G.; McFadden, W. H.; Lundin, R. E.; Kealy, M. P. Volatile hop constituents: conventional and capillary gaschromatographic separation with characterization by physical methods. *J. Inst. Brew.* **1964**, *70*, 396–401.

70. Stevens, R. Chemistry of hop constituents. Part XIX. The essential oil of the variety OW-153. *J. Chem. Soc.* **1964**, 956–958.

71. Buttery, R. G.; Black, D. R.; Guadagni, D. G.; Kealy, M. P. A study of the volatile oxygenated constituents in different hop varieties. *Proc. Am. Soc. Brew. Chem.* **1965**, 103–111.

72. Buttery, R. G.; Black, D. R.; Kealy, M. P. Volatile oxigenated consituents of hops. Identification by combined gas chromatography and mass spectrometry. *J. Chromatog.* **1965**, *18*, 399–402.

73. Buttery, R. G.; Lundin, R. E.; Ling, L. Sesquiterpene hydrocarbons of hops. *Chem. Ind.* **1966**, 1225–1226.

74. Buttery, R. G.; Black, D. R.; Ling, L. Methyl geranate in hop oil. *J. Inst. Brew.* **1966**, *72*, 202–204.

75. Buttery, R. G.; Ling, L. Identification of hop varieties by gas chromatographie analysis of their essential oils. Capillary gas chromatography patterns and analyses of hop oils from American grown varieties. *J. Agric. Food Chem.* **1967**, *15*, 531–535.

76. Buttery, R. G.; Lundin, R. E.; Ling, L. Characterization of some $C_{15}$ constituents of hop oil. *J. Agric. Food Chem.* **1967**, *15*, 58–65.

77. Buttery, R. G.; Teranishi, R.; Mon, T. R.; Ling, L. Volatile hop constituents: Characterization of *β*-copaene and pentadec-6-ene-2-one. *J. Sci. Fd Agric.* **1969**, *20*, 721–722.

78. Tressl, R.; Friese, L. Gaschromatographisch-massenspektrometrische Untersuchung der Aromastoffe von Spalter Hopfen. *Z. Lebensm.-Unters. Forsch*. **1978**, 350–354.

79. Peacock, V. E.; Deinzer, M. L. Non-bitter hop contributions to beer flavor. *ACS Symp. Ser.* **1980**, *170*, 119–127.

80. Peacock, V. E.; Deinzer, M. L.; McGill, L. A.; Wrolstad, R. E. Hop aroma in American beer. *J. Agric. Food Chem.* **1980**, *28*, 774–777.

81. Peacock, V. E.; Deinzer, M. L. Chemistry of hop aroma in beer. *J. Am. Soc. Brew. Chem.* **1981**, *39*, 136–141.

82. 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.

83. Seaton, J. C.; Suggett, A.; Moir, M. The flavor contribution of sulfur compounds in hops. *Tech. Q. Master Brew. Assoc. Am.* **1981**, *18*, 26–30.

84. Seaton, J. C.; Suggett, A.; Moir, M. The role of sulfur compounds in beer flavour. In *European Brewery Convention Monograph VII, Flavor Symposium*. Copenhagen. 1981, pp. 143–155.

85. Tressl, R.; Engel, K. H.; Kossa, M.; Koeppler, H. Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus* var. Hersbrucker Spaet). *J. Agric. Food Chem.* **1983**, *31*, 892–897.

86. Guadagni, D. G.; Buttery, R. G.; Harris, J. Odour intensities of hop oil components. *J. Sci. Food Agric.* **1966**, *17*, 142–144.

87. Likens, S. T.; Nickerson, G. B. Identification of hop varieties by gas chromatograph analysis of their essential oil. Constency of oil composition under various enviromental influences. *J. Agric. Food Chem.* **1967**, *15*, 525–530.

88. Freundorfer, J.; Maier, J.; Reiner, L. Rechnergestützte Verfahren zur Sortenerkennung bei Hopfen und Hopfenprodukten auf der Basis der ätherischen Öle. *Brew. Sci.–Monatsschr. Brauwiss.* **1991**, *6*, 221–238.

89. De Cooman, L.; Everaert, E.; De Keukeleire, D. Quantitative analysis of hop acids, essential oils and flavonoids as a clue to the identification of hop varieties. *Phytochem. Anal.* **1998**, *9*, 145–150.

90. De Keukeleire, D.; David, F.; Haghebaert, K.; Sandra, P. Automated reporting on the quality of hops and hop products. *J. Inst. Brew.* **1998**, *104*, 75–82.

91. Narziß, L.; Forster, A., Gaschromatographic studies on aroma compounds in hops and their products. II. Influence of drying on the composition of hop aroma constituents (in German). *Brauwissenschaft* **1971**, *24*, 145–151.

92. Sanchez, N. B.; Lederer, C. L.; Nickerson, G. B.; Libbey, M. L.; McDaniel, M. R. Sensory and analytical evaluation of hop oil oxygenated fractions. In *Food Science and Human Nutrition*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; pp 371–402.

93. Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter Select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776–1783.

94. Steinhaus, M.; Wilhelm, W.; Schieberle, P. Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis. *Eur. Food Res. Technol.* **2007**, *226*, 45–55.

95. Gros, J.; Nizet, S.; Collin, S. Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. *J. Agric. Food Chem.* **2011**, *59*, 8853–8865.

96. Van Opstaele, F.; De Causmaecker, B.; Aerts, G.; De Cooman, L. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography–mass spectrometry/olfactometry. *J. Agric. Food Chem.* **2012**, *60*, 12270–12281.

97. Takoi, K.; Degueil, M.; Shinkaruk, S.; Thibon, C.; Maeda, K.; Ito, K.; Bennetau, B.; Dubourdieu, D.; Tominaga, T. Identification and characteristics of new volatile thiols derived from the hop (*Humulus luplus* L.) cultivar Nelson Sauvin. *J. Agric. Food Chem.* **2009**, *57*, 2493–2502.

98. Kishimoto, T.; Kobayashi, M.; Yako, N.; Iida, A.; Wanikawa, A. Comparison of 4-mercapto-4-methylpentan-2-one contents in hop cultivars from different growing regions. *J. Agric. Food Chem.* **2008**, *56*, 1051–1057.

99. Reglitz, K.; Steinhaus, M. Quantitation of 4-methyl-4-sulfanylpentan-2-one (4MSP) in hops by a stable isotope dilution assay in combination with GCxGC-TOFMS: method development and application to study the influence of variety, provenance, harvest year, and processing on 4MSP concentrations. *J. Agric. Food Chem.* **2017**, *65*, 2364–2372.

100. Kankolongo Cibaka, M.-L.; Gros, J.; Nizet, S.; Collin, S. Quantitation of selected terpenoids and mercaptans in the dual-purpose hop varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace. *J. Agric. Food Chem.* **2015**, *63*, 3022–3030.

101. Sharp, D. C.; Townsend, M. S.; Qian, Y.; Shellhammer, T. H. Effect of harvest maturity on the chemical composition of Cascade and Willamette hops. *J. Am Soc. Brew. Chem.* **2014**, *72*, 231–238.

102. Kammhuber, K.; Mundhammer, M.; Weihrauch, S. Influence of the date of harvest on the sulphur compounds of the “Special Flavour Hop” varieties Cascade, Mandarina Bavaria, Hallertau Blanc, Huell Melon and Polaris. *Brew. Sci.* **2017**, *70*, 124–130.

103. Biergesetz Bundesgesetzblatt, Jahrgang 1993, Teil I, Vorläufiges Biergesetz, § 9, Bundesgestzblatt online, Bürgerzugang. https://www.bgbl.de/xaver/bgbl/start.xav?start-=%2F%2F*%5B%40attr_id%3D%27bgbl193s1399.pdf%27%5D#__bgbl__%2F%2F*%5B%40attr_id%3D%27bgbl193s1399.pdf%27%5D__1544875986458 (December 29, 2018).

104. Belitz, H.-D.; Grosch, W.; Schieberle, P. Lehrbuch der Lebensmittelchemie. 6th Ed. Springer Verlag: Berlin Heidelberg, Germany, 2008.

105. Hopsteiner; Steiner, S. H.; Germany, 2008, Entscheidungsdaten für den Hopfeneinkauf. https://www.hopsteiner.com/wp-content/uploads/2017/06/Entscheidungsdaten_fuer_-den_Hopfeneinkauf_2008.pdf (December 29, 2018).

106. Statista Alcoholic Drinks Report 2018 – Beer. The countries drinking the most beer, beer sales per capita in 2017 (in litres). https://www.statista.com/chart/12510/the-countries-drinking-the-most-beer/ (December 29, 2018).

107. Intelmann, D.; Haseleu, G.; Dunkel, A.; Lagemann, A.; Stephan, A.; Hofmann, T. Comprehensive sensomics analysis of hop-derived bitter compounds during storage of beer. *J. Agric. Food Chem.* **2011**, *59*, 1939–1953.

108. Malowicki, M. G.; Shellhammer, T. H. Isomerization and degradation kinetics of hop (*Humulus lupulus*) acids in a model wort-boiling system. *J. Agric. Food Chem.* **2005**, *53*, 4434–4439.

109. West, D. B.; Evans, R. F; Becker, K. Studies of the ester and higher-alcohol contents of beer. *Proc. Am. Soc. Brew.* **1951**, 51–59.

110. Harold, F. V.; Hildebrand, R. P.; Murieson, A. S.; Murray, P. J. Trace volatioles of beer. Part I. *J. Inst. Brew.* **1961**, *67*, 161–172.

111. Strating, J.; Venema, A. Gas-chromatographic study of an aroma concentrate from beer. *J. Inst. Brew.* **1961**, *67*, 525–528.

112. Schreier, P.; Drawert, F.; Junker, A. Flüchtige Schwefelverbindungen des Bieraromas. *Brauwissenschaft* **1974**, *27*, 205–207.

113. Schreier, P.; Drawert, F.; Junker, A. Gaschromatographische Bestimmung der Inhaltsstoffe von Gärungsgetränken. *Brauwissenschaft* **1975**, *28*, 73–79.

114. Tressl, R.; Kossa, T.; Renner, R. Gaschromatographisch-massenspektrometrische Untersuchungen flüchtiger Inhaltsstoffe von Hopfen, Würze und Bier und deren Genese. II. Phenole und Sauerstoffhaltige Heterocyclen in Würze und Bier. In: *European Brewery Convention: Proceedings of the 15th Congress. Nice*, 1975, pp. 737–756.

115. Meilgaard, M. C. Flavor chemistry of beer: part II: flavour and threshold of 239 aroma volatiles. *Tech. Q. Master Brew. Assoc. Am.* **1975**, *12*, 151–168.

116. Engan, S. Beer composition: volatile substances. In *Brewing Science*; Pollock, J. R. A., Ed.; Academic Press: London, UK, 1981; pp. 93–165.

117. Nykänen, L.; Suomalainen, H. Aroma of beer, wine and distilled alcoholic beverages. Akademie Verlag, Berlin, 1983.

118. Meilgaard, M. C.; Peppard, T. L. The flavor of beer. In *Food Flavors Part B: The Flavor of Beverages*; Morton, I. D., Macleod, A. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1986; pp. 99–170.

119. Angelino, S. A. G. F. Beer. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Marcel Dekker, Inc.: New York, USA, 1991; pp. 581–616.

120. Hardwick, W. A. The properties of beer. In*, Handbook of Brewing*; Hardwick, W. A., Ed., Marcel Dekker Inc.: New York, USA, 1995; pp. 551–586.

121. Schieberle, P. Primary odorants of pale lager beer. *Z. Lebensm.-Unters. Forsch*. **1991**, 558–565.

122. Harold, F. V.; Hildebrand, R. P.; Morieson, A. S.; Murray, P. J. Influence of hop oil constituents on the flavour and aroma of beer. *J. Inst. Brew.* **1960**, *66*, 395–398.

123. Buttery, R. G.; Black, D. R.; Lewis, M. J.; Ling, L. A. study of the fate of volatile hop constituents in beer. *J. Food Sci.* **1967**, 414–419.

124. Kaltner, D.; Thum, B.; Forster, C.; Back, W. Untersuchungen zum Hopfenaroma in Pilsner Bieren bei Variation technischer Parameter. *Monatsschrift f. Brauwiss.* **2001**, 199–205.

125. Kishimoto, T.; Wanikawa, A.; Kagami, N.; Kawatsura, K. Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agric. Food Chem.* **2005**, *53*, 4701–4707.

126. Deinzer, M.; Yang, X. Hop aroma: character impact compounds found in beer, methods of formation of individual compounds. In *EBC monograph 22nd symposium on hops, Zoeterwoude, The Netherlands 1994*; Getränke-Fachverlag Hans Carl: Nuremberg, Germany, 1994; pp 181–197.

127. 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.

128. 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.

129. 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.

130. Hanke, S.; Herrmann, M.; Rückerl, J.; Back, W. The transfer of hop aroma compounds during the brewing process. *Brauwelt International* **2009**, *27*, 191–194.

131. Lam, K. C.; Foster, R. T.; Deinzer, M. L. Aging of hops and their contribution to beer flavor. *J. Agric. Food Chem.* **1986**, *34*, 763–770.

132. Forster, A.; Gahr, A. On the fate of certain hop substances during dry hopping. *Brew. Sci.* **2013**, *65*, 93–103.

133. Haefliger, O. P.; Jeckelmann, N. Stripping of aroma compounds during beer fermentation monitored in real-time using an automatic cryotrapping sampling system and fast gas chromatography/mass spectrometry. *Anal. Methods* **2013**, *5*, 4409–4418.

134. Haslbeck, K.; Bub, S.; Schönberger, C.; Zarnkow, M.; Jacob, F.; Coelhan, M. On the fate of *β*-myrcene during fermentation - The role of stripping and uptake of hop oil components by brewer`s yeast in dry-hopped wort and beer. *Brew. Sci.* **2017**, *70*, 159–169.

135. Lafontaine, S. R.; Shellhammer, T. H. Impact of static dry-hopping rate on the sensory and analytical profiles of beer. *J. Inst. Brew.* **2018**, 124, 434–442.

136. 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.

137. 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.

138. Takoi, K.; Tokita, K.; Sanekata, Y.; Usami, Y.; Itoga, Y.; Koie, K.; Matsumoto, I.; Nakayama, Y. Varietal differences of hop-derived flavour compounds in late-hopped/dry-hopped beers. *Brew. Sci.* **2016**, *69*, 1–7.

139. Goldstein, H.; Ting, P.; Navarro, A.; Ryder, D. Water-soluble hop flavor precursors and their role in beer flavor. In *European Brewery Convention, Proceedings of the 27st EBC Congress, Cannes* 1999; Fachverlag Hans Carl: Nuremberg, Germany, 1999; pp 53–62.

140. King, A. J.; Dickinson, J. R. Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast* **2000**, *16*, 499–506.

141. King, A. J.; Dickinson, J. R. Biotransformation of hop aroma terpenoids by ale and lager yeasts. *FEMS Yeast Res.* **2003**, *3*, 53–62.

142. Kollmannsberger, H.; Biendl, M.; Nitz, S. Occurence of glycosidically bound flavour compounds in hops, hop products and beer. *Monatsschr. Brauwiss.* **2006**, 83–89.

143. Daenen, L.; Saison, D.; De Cooman, L.; Derdelinckx, G.; Verachtert, H.; Delvaux, F. R. Flavour enhancement in beer: hydrolysis of hop glycosides by yeast *β*-glucosidase. *Cerevisia* **2007**, *32*, 24–36.

144. Hanke, S.; Hermann, M.; Rückerl, J.; Schönberger, C.; Back, W. Hop volatile compounds (Part II): transfer rates of hop compounds from hop peletts to wort and beer. *Brew. Sci.* **2008**, *61*, 140–147.

145. Takoi, K.; Itoga, Y.; Koie, K.; Kosugi, T. The Contribution of geraniol metabolism to the citrus flavour of beer: synergy of geraniol and citronellol under coexistence with excess linalool. *J. Inst. Brew.* **2010**, *116*, 251–260.

146. Takoi, K.; Koie, K.; Itoga, Y.; Katayama, Y.; Shimase, M.; Nakayama, Y.; Watari, J. Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer. *J. Agric. Food Chem.* **2010**, *58*, 5050–5058.

147. Praet, T.; Van Opstaele, F.; Jaskula-Goiris, B.; Aerts, G.; De Cooman, L. Biotransformations of hop-derived aroma compounds by *Saccharomyces cerevisiae* upon fermentation. *Cerevisia* **2012**, *36*, 125–132.

148. Gros, J.; Peeters, F.; Collin, S. Occurrence of odorant polyfunctional thiols in beers hopped with different cultivars. First evidence of an *S*-cysteine conjugate in hop (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2012**, *60*, 7805–7816.

149. Dresel, M.; Praet, T.; Van Opstaele, F.; Van Holle, A.; Naudts, D.; De Keukeleire, D.; De Cooman, L.; Aerts, G. Comparison of the analytical profiles of volatiles in single-hopped worts and beers as a function of the hop variety. *Brew. Sci.* **2015**, *68*, 8–28.

150. Sharp, D. C.; Steensels, J.; Shellhammer, T. H. The effect of hopping regime, cultivar and *β*-glucosidase activity on monoterpene alcohol concentrations in wort and beer. *J. Inst. Brew.* **2017**, *123*, 185–191.

151. Kankolongo Cibaka, M.-L.; Decourrière, L.; Lorenzo-Alonso, C.-J.; Bodart, E.; Robiette, R.; Collin, S. 3-Sulfanyl-4-methylpentan-1-ol in dry-hopped beers: first evidence of glutathione *S*-conjugates in hop (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2016**, *64*, 8572–8582.

152. Schönberger, C.; Kostelecky, T. 125th Anniversary review: the role of hops in brewing. *The Institute of Brewing and Disilling* **2011**, *117*, 259–267.

153. Neiens, S. D.; Steinhaus, M. Odor-active compounds in the special flavor hops Huell Melon and Polaris. *J. Agric. Food Chem.* **2018**, *66*, 1452–1460.

154. Full, G.; Schreier, P. Covalent chromatography. A valuable method for the aroma analysis of thiols at trace levels. *Lebensmittelchemie* **1994**, *48*, 1–4.

155. Kaltner, D.; Steinhaus, M.; Mitter, W.; Biendl, M.; Schieberle, P. (*R*)-Linalool as key compound for hop aroma in beer and its behavior during beer staling (in German). *Monatsschrift f. Brauwiss.* **2003**, *56*, 192–196.

156. Neiens, S. D.; Geißlitz, S. M.; Steinhaus, M. Aroma-active compounds in *Spondias mombin* L. fruit pulp. *Eur. Food Res. Technol.* **2017**, *243*, 1073–1081.

157. U.S. Department of Commerce. Mass spectral library NIST17. National Institute of Standards and Technology, Gaithersburg.

158. Courtois, E. A.; Paine, C. E.; Blandinieres, P. A.; Stien, D.; Bessiere, J. M.; Houel, E.; Baraloto, C.; Chave, J. Diversity of the volatile organic compounds emitted by 55 species of tropical trees: a survey in French Guiana. *J. Chem. Ecol.* **2009**, *35*, 1349–1362.

159. Mebazaa, R.; Mahmoudi, A.; Fouchet, M.; Dos Santos, M.; Kamissoko, F.; Nafti, A.; Ben Cheikh, R.; Rega, B.; Camel, V. Characterisation of volatile compounds in Tunisian fenugreek seeds. *Food Chem.* **2009**, *115*, 1326–1336.

160. Nakashima, K.; Imoto, M.; Sono, M.; Tori, M.; Nagashima, F.; Asakawa, Y. Total synthesis of (-)-(7*S*,10*R*)-calamenene and (-)-(7*S*,10*R*)-2-hydroxycalamenene by use of a ring-closing metathesis reaction. A comparison of the *cis*- and *trans*-isomers. *Molecules* **2002**, *7*, 517–527.

161. Halton, B.; Randall, C. J.; Grainsford, G. J.; Stang, P. J. Cycloproparenes: Synthesis, structure and spectral properties of alkylidenecycloproparenes. *J. Am. Chem. Soc.* **1986**, *108*, 5949–5956.

162. Teranishi, R.; Buttery, R. G.; Guadagni, D. G. Odor quality and chemical structure in fruit and vegetable flavors. *Ann. N. Y. Acad. Sci.* **1974**, 209–216.

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

164. Neiens, S.; Geißlitz., S.; Steinhaus, M. Zum Vorkommen geruchsaktiver Mengen an (1*R*,4*S*)-Calamenen in verschiedenen pflanzlichen Lebensmitteln. *Lebensmittelchemie* **2017**, *71*, 132.

165. Steinhaus, M. Characterization of the major odor-active compounds in the leaves of the curry tree *Bergera koenigii* L. by aroma extract dilution analysis. *J. Agric. Food Chem.* **2015**, *63*, 4060–4067.

166. König, W. A.; Rieck, A.; Hardt, I.; Gehrcke, B.; Kubeczka, K.-H.; Muhle, H. Enantiomeric composition of the chiral constituents of essential oils. Part 2: sesquiterpene hydrocarbons. *J. High Resolut. Chromatogr.* **1994**, *17*, 315–320.

167. Steinhaus, M. Confirmation of 1-phenylethane-1-thiol as the character impact aroma compound in curry leaves and its behavior during tissue disruption, drying, and frying. *J. Agric. Food Chem.* **2017**, *65*, 2141–2146.

168. 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.

169. Buiatti, S. Beer composition: an overview. In *Beer in Health and Disease Prevention*; Preedy, V. R., Ed.; Academic Press: Burlington, MA, USA., 2009; pp 213–226.

170. 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.

# 8 Appendix

## 8.1 Publication 1: Odor-Active Compounds in the Special Flavor Hops Huell Melon and Polaris

### 8.1.1 Bibliographic Data


| | |
|---|---|
| Title: | Odor-active compounds in the special flavor hops Huell Melon and Polaris |
| Authors: | Silva D. Neiens and Martin Steinhaus |
| Journal: | Journal of Agricultural and Food Chemistry |
| Publication year: | 2018 |
| Volume: | 66 |
| Issue: | 6 |
| Pages: | 1452–1460 |
| DOI: | 10.1021/acs.jafc.7b05859 |
| Hyperlink: | https://pubs.acs.org/doi/pdf/10.1021/acs.jafc.7b05859 |


### 8.1.2 Publication Reprint

A reprint of publication 1, odor-active compounds in the special flavor hops Huell Melon and Polaris, follows starting with the next page.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 1452−1460

pubs.acs.org/JAFC

# Odor-Active Compounds in the Special Flavor Hops Huell Melon and Polaris

Silva D. Neiens and Martin Steinhaus*

Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Straße 34, 85354 Freising, Germany

Supporting Information

**ABSTRACT:** The volatiles isolated from samples of the special flavor hop varieties, Huell Melon and Polaris, and from the aroma hop variety, Hallertau Tradition, by solvent extraction and solvent-assisted flavor evaporation (SAFE) were subjected to a comparative aroma extract dilution analysis (cAEDA), which resulted in 46 odor-active compounds in the flavor dilution (FD) factor range of 16 to 2048. On the basis of high FD factors, myrcene, (3*R*)-linalool, and 2- and 3-methylbutanoic acid were confirmed as important variety-independent hop odorants. (1*R*,4*S*)-Calamenene was identified for the first time as an odor-active compound in hops. Clear differences in the FD factors and their subsequent objectification by stable isotope dilution quantitation suggested that high concentrations of the esters ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and propyl 2-methylbutanoate cause the characteristic fruity, cantaloupe-like odor note in Huell Melon hops, whereas the fruity and minty odor notes in Polaris are associated with high amounts of 3-methylbutyl acetate and 1,8-cineole.

**KEYWORDS:** *hops, Humulus lupulus, aroma extract dilution analysis, AEDA, stable isotope dilution assay, SIDA, myrcene, linalool, ethyl 2-methylbutanoate, 1,8-cineole, 3-methylbutyl acetate, calamenene*

## INTRODUCTION

For a long time, hops were primarily used to enhance shelf-life and bitterness of beer. However, hops may also have a vital impact on beer aroma, in particular, when added late in the brewing process.[1] Multiple, or at least dual, hop additions have, therefore, become common practice.[2] To best exploit the bittering potential of hops, a first portion is added at the beginning of wort boiling.[3] Further portions are added toward the end of the boil, to the whirlpool, or even later.[4] Hop addition after fermentation, a procedure known as dry-hopping, is extensively used by craft brewers and in combination with a high hop dosage leads to an intense hoppy aroma in the finished beer.[2,5] The craft beer market is currently booming and is driving the global demand of aromatic hops. This pushed the development of new hop varieties with exceptional sensory characteristics, particularly in the United States and in Germany, which are the leading hop producing countries, with acreages of 22900 and 19500 ha, respectively.[6] Among the most promising varieties bred at the hop research center in Hüll, Germany, are Huell Melon and Polaris, both released in 2012.[5] Their unique aroma properties are much appreciated by craft brewers. Polaris additionally exhibits a high bittering potential with $\alpha$-acid contents of ∼20%. The acreage of Huell Melon and Polaris is still low (∼157 ha and ∼174 ha, respectively, in 2017), but the demand is high, thus acreage is steadily increasing.[7] Huell Melon exhibits an intense fruity, cantaloupe-like aroma note, whereas the specific aroma note characterizing Polaris combines fruity and minty nuances. The compounds responsible for these sensory characteristics, however, have not been previously reported.

Numerous studies have been conducted on hop volatiles of different varieties, which has led to the identification of >500 compounds.[8,9] With modern instrumentation, such as GC×GC-TOFMS, simultaneous separation of >1000 hop volatiles is possible.[10] However, it is well established that the majority of volatiles present in food, beverages, and their raw materials have no or little impact on the overall aroma.[11] Identification of the crucial odor-active compounds, however, is possible by using activity-guided techniques, such as gas chromatography−olfactometry (GC-O). First applications of GC-O to hops revealed the presence of distinct odor-active compounds, but structure assignments could not be achieved.[12,13] Later, linalool, neral, and humulene epoxide were suggested as potent hop odorants.[14] A crucial role of linalool for the aroma of hops had already been suggested as early as 1929.[15]

In the first comprehensive GC-O study on hops,[16] the volatiles isolated from Spalter Select hops were screened for odor-active compounds by application of an aroma extract dilution analysis (AEDA).[17] On the basis of high flavor dilution (FD) factors, *trans*-4,5-epoxy-(2*E*)-dec-2-enal, linalool, myrcene, ethyl 2-methylpropanoate, methyl 2-methylbutanoate, (5*Z*)-octa-1,5-dien-3-one, nonanal, (3*E*,5*Z*)-undeca-1,3,5-triene, and (3*E*,5*Z*,9*E*)-undeca-1,3,5,9-tetraene were identified as potent odorants. Application of a comparative AEDA (cAEDA) to hops of 5 different varieties revealed further odor-active hop compounds, such as geraniol and 4-methyl-4-sulfanyl-pentan-2-one (4MSP), in Cascade hops.[18] Black currant-like smelling 4MSP significantly contributes to the aroma of various hops, in particular those of some US varieties and modern German breeds, whereas it is absent from traditional German varieties and English hops.[19,20] GC-O in combination with GC-FPD applied to the thiol fraction obtained from Nelson Sauvin hops

Received: December 13, 2017
Revised: January 23, 2018
Accepted: January 24, 2018
Published: January 27, 2018





DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

led to the identification of further odor-active thiols, namely 3-sulfanylpentan-1-ol, 3-sulfanylhexan-1-ol, 3-sulfanyl-4-methylpentan-1-ol, and 3-sulfanyl-4-methylpentyl acetate.[21] Gros et al.[22] reported data on 60 odor-active compounds in Tomahawk, Nelson Sauvin, Nugget, Cascade, and Saaz hops, among which were 3-methylbutyl 2-methylpropanoate and undecan-2-one, 5 terpenoids (myrcene, linalool, β-citronellol, geraniol, and methyl geranate), 7 sesquiterpenoids (β-caryophyllene, α-humulene, bergamotene, β-farnesene, α-amorphene, and α- and β-selinene), as well as 5 thioesters and 41 thiols. Van Opstaele et al.[23] applied headspace solid phase microextraction in combination with GC-O to a floral essence isolated from Spalter Select hops by supercritical fluid extraction and solid phase extraction/fractionation. In addition to well-known hop odorants, myrcene, nonanal, methyl nonanoate, and 3-methylbutyl 2-methylpropanoate, perillene, *cis*-β-ocimene, undecan-2-one, dodecan-2-one, methyl octanoate, methyl non-3-enoate, methyl 4-methyloctanoate, and ethyl nonanoate were identified as odor-active compounds.

The aim of the present study was to identify the major odor-active compounds in hop pellets of the varieties Huell Melon and Polaris, with a special focus on the compounds accounting for the specific nuances characterizing either variety, i.e. the fruity, cantaloupe-like note in Huell Melon and the fruity and minty notes in Polaris. For that purpose, the volatiles isolated from the hop pellets by solvent extraction and solvent-assisted flavor evaporation (SAFE)[24] were subjected to a cAEDA. Hallertau Tradition was included in the cAEDA as a third variety and reference. Hallertau Tradition exhibits a typical hop aroma profile lacking any extraordinary notes. To objectify the results, compounds showing high FD factors and/or clear differences in the FD factors between the three hop varieties were subsequently quantitated, preferentially by stable isotope dilution assays (SIDAs).[25]

## MATERIALS AND METHODS

**Hop Samples.** Commercial blends with representative aroma characteristics were provided by Hopsteiner (Mainburg, Germany). Type 90 pellets of the following hop cultivars and harvest years were obtained: Huell Melon 2013, Huell Melon 2014, Polaris 2012, Polaris 2013, and Hallertau Tradition 2014. Samples were stored in vacuum-sealed bags at −20 °C.

**Reference Odorants.** Compounds **1**−**6**, **8**, **10**, **12**, **15**, **19**, **20**, **22**−**24**, (*R*)-**24**, **25**−**31**, **35**, **37**, **39**, and **43**−**45** were purchased from Sigma-Aldrich (Taufkirchen, Germany), **13** and **17** were obtained from Alfa Aesar (Karlsruhe, Germany), and **46** was obtained from Merck (Darmstadt, Germany). Compounds **7**,[26] **14**,[27] **16**,[28] **18**,[16] **36**,[29] and **42**[30] were synthesized as detailed in the literature. Compounds (1*R*,4*S*)-**36** and (1*S*,4*R*)-**36** were synthesized from the enantiopure compounds (2*R*,5*S*)-menthone and (2*S*,5*R*)-menthone (Sigma-Aldrich), respectively, using the approach recently described for the racemate.[29]

**Isotopically Substituted Odorants.** ($^2H_2$)-1,8-Cineole (1,3,3-trimethyl(5,6-$^2H_2$)-2-oxabicyclo[2.2.2]octane (($^2H_2$)-**10**),[31] 4-($^{13}$C)-methyl-4-sulfanyl(1,3,5-$^{13}C_3$)pentan-2-one (($^{13}C_4$)-**17**),[32] (3*E*,5*Z*)-(10,10,11,11-$^2H_4$)undeca-1,3,5-triene (($^2H_4$)-**18**),[33] ($^2H_2$)linalool (3,7-dimethyl(1,2-$^2H_2$)octa-1,6-dien-3-ol (($^2H_2$)-**24**),[34] and ($^2H_2$)-geraniol ((2*E*)-3,7-dimethyl(1,1-$^2H_2$)octa-2,6-dien-1-ol (($^2H_2$)-**37**),[35] were synthesized as detailed in the literature. ($^2H_5$)Ethyl 2-methylpropanoate (($^2H_5$)-**1**), ($^2H_3$)methyl 2-methylbutanoate (($^2H_3$)-**3**), (2,2,2-$^2H_3$)ethyl 2-methylbutanoate (($^2H_3$)-**4**), and (3,3,3-$^2H_3$)propyl 2-methylbutanoate (($^2H_3$)-**7**) were synthesized from the respective alcohols and carboxylic acids (all Sigma-Aldrich) using the approach detailed above for the synthesis of isotopically unmodified **7**. In an analogous manner, 3-($^2H_3$)methyl($^2H_8$)butyl acetate (($^2H_{11}$)-**6**) was synthesized from 3-($^2H_3$)methyl($^2H_8$)butan-1-ol (CDN Isotopes, Pointe-Claire, QC, Canada) and acetic acid. (5*Z*)-(5,6-$^2H_2$)-Octa-1,5-dien-3-one (($^2H_2$)-**16**) was synthesized from hex-3-yn-1-ol as suggested earlier,[36] but using Dess-Martin periodinane[37] (Sigma-Aldrich) instead of PCC for the conversion of the alcohol intermediates to the carbonyl compounds. (3,3,4,4-$^2H_4$)Nonanal (($^2H_4$)-**19**) was synthesized from non-3-yn-1-ol using the approach detailed for the synthesis of (5,5,6,6-$^2H_4$)hexanal from 5-hexyn-1-ol.[38] (3,4-$^2H_2$)Butanoic acid (($^2H_2$)-**27**) and 3-methyl(3,4-$^2H_2$)butanoic acid (($^2H_2$)-**29**) were synthesized from the corresponding unsaturated alcohols but-3-en-1-ol and 3-methylbut-3-en-1-ol (both Sigma-Aldrich) by homogeneous deuteration using the approach detailed for the synthesis of (5,5,6,6-$^2H_4$)hexan-1-ol,[38] followed by oxidation of the saturated alcohol with potassium permanganate.[39]

**Miscellaneous Chemicals.** Tetradecane and (2*E*)-dec-2-enal were purchased from Sigma-Aldrich. Dichloromethane, diethyl ether, and pentane were freshly distilled before use. Silica gel 60 (0.040−0.063 mm) was purchased from VWR (Darmstadt, Germany) and purified as detailed recently.[40] Mercurated agarose gel was prepared from Affi-Gel 10 (Bio-Rad, Munich, Germany).[41]

**GC-O/FID.** A Trace GC Ultra gas chromatograph (Thermo Scientific, Dreieich, Germany) was equipped with a cold-on-column injector, a flame ionization detector (FID), a tailor-made sniffing port,[42] and one of the following fused silica columns: (1) ZB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film (Phenomenex, Aschaffenburg, Germany), (2) ZB-5, 30 m × 0.32 mm i.d., 0.25 μm film, (Phenomenex), and (3) BGB-176, 30 m × 0.25 mm i.d., 0.25 μm film (BGB Analytik, Rheinfelden, Germany). The carrier gas was helium at 90 kPa (ZB-FFAP), 60 kPa (ZB-5), and 75 kPa (BGB-176). The oven temperature was 40 °C for 2 min, then ramped at 6 °C/min (ZB-FFAP and ZB-5) or 2 °C/min (BGB-176) to 230 °C (ZB-FFAP), 240 °C (ZB-5), and 200 °C (BGB-176). The end of the analytical column was connected to a deactivated Y-shaped glass splitter which divided the column effluent into two equal parts that were directed via deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) to the FID (250 °C) and the sniffing port (230 °C), respectively. During a GC-O analysis, a trained person placed the nose closely above the top of the sniffing port and evaluated the effluent. Odorous regions were marked in the FID chromatogram printed by a recorder and the associated odor qualities were noted. Retention indices (RI) of the odorous regions were calculated from their retention times and the retention times of adjacent *n*-alkanes by linear interpolation.

**GC-MS.** A HP 5890 Series II gas chromatograph (Hewlett-Packard, Heilbronn, Germany) was equipped with a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film or DB-5, 30 m × 0.25 mm i.d., 0.25 μm film (both Agilent Technologies, Waldbronn, Germany), and connected to an MAT 95 sector field mass spectrometer (Finnigan, Bremen, Germany). The carrier gas was helium at 1.9 mL/min constant flow. All other GC conditions were equivalent to those used in the GC-O/FID analyses. MS(EI) spectra were generated at 70 eV using a scan range of *m*/*z* 35−300. MS(CI) spectra were obtained at 150 eV using isobutane as reagent gas and a scan range of *m*/*z* 85−350. For the evaluation of the mass spectra the Xcalibur software (Thermo) was used.

**GC-GC-MS.** A Trace GC Ultra (Thermo) was equipped with a cold-on-column injector and a fused silica column, DB-FFAP, 30 m × 0.32 mm i.d., 0.25 μm film (Agilent). The column end was connected to a moving column stream switching (MCSS) device (Thermo), conveying the eluate via uncoated fused silica capillaries time-programmed either simultaneously to an FID (250 °C) and a sniffing port (230 °C) or via a heated (250 °C) hose to a cold trap located in the oven of a CP 3800 GC (Varian, Darmstadt, Germany). The cold trap consisted of a piece of steel tubing housing the capillary and could be cooled by liquid nitrogen. The end of the capillary was connected to a fused silica column, DB-1701, 30 m × 0.25 mm i.d., 0.25 μm film (Agilent); DB-5, 30 m × 0.25 mm i.d., 1.00 μm film (Agilent); or BGB-176, 30 m × 0.25 mm i.d., 0.25 μm film (BGB). The end of this column was connected to a Saturn 2200 mass spectrometer (Varian) operated in EI mode or in CI mode with methanol as the reagent gas. Helium served as the carrier gas (100 kPa) and make-up gas for the MCSS device (50 kPa). The oven temperature in the first dimension


DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

Table 1. Isotopically Substituted Compounds and Quantitation Parameters Used in the Stable Isotope Dilution Assays

| target analyte(s) | isotopically substituted internal standard | quantifier ion ($m/z$) analyte | quantifier ion ($m/z$) standard | calibration line[a] |
|---|---|---|---|---|
| butanoic acid | (3,4-$^2H_2$)butanoic acid | 89 | 91 | $y = 0.780x + 0.065$ |
| 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo[2,2,2]octane) | 1,3,3-trimethyl(5,6-$^2H_2$)-2-oxabicyclo[2,2,2]octane | 137 | 139 | $y = 1.150x - 0.089$ |
| ethyl 2-methylbutanoate | (2,2,2-$^2H_3$)ethyl 2-methylbutanoate | 131 | 134 | $y = 0.987x - 0.065$ |
| ethyl 2-methylpropanoate | ($^2H_5$)ethyl 2-methylpropanoate | 117 | 122 | $y = 0.920x + 0.019$ |
| geraniol ((2*E*)-3,7-dimethylocta-2,6-dien-1-ol) | (2*E*)-3,7-dimethyl(1,1-$^2H_2$)octa-2,6-dien-1-ol | 137 | 139 | $y = 1.005x - 0.007$ |
| linalool (3,7-dimethylocta-1,6-dien-3-ol) | 3,7-dimethyl(1,2-$^2H_2$)octa-1,6-dien-3-ol | 137 | 139 | $y = 0.967x + 0.073$ |
| 4-methyl-4-sulfanylpentan-2-one | 4-($^{13}$C)methyl-4-sulfanyl(1,3,5-$^{13}C_3$)pentan-2-one | 99 | 103 | $y = 0.893x - 0.063$ |
| 2- and 3-methylbutanoic acid (sum of isomers) | 3-methyl(3,4-$^2H_2$)butanoic acid | 117 | 119 | $y = 0.949x - 0.027$ |
| 3-methylbutyl acetate | 3-($^2H_3$)methyl($^2H_8$)butyl acetate | 131 | 142 | $y = 1.155x - 0.078$ |
| methyl 2-methylbutanoate | ($^2H_3$)methyl 2-methylbutanoate | 117 | 120 | $y = 0.993x - 0.009$ |
| nonanal | (3,3,4,4-$^2H_4$)-nonanal | 143 | 147 | $y = 1.166x - 0.036$ |
| (5*Z*)-octa-1,5-dien-3-one | (5*Z*)-(5,6-$^2H_2$)-octa-1,5-dien-3-one | 125 | 127 | $y = 0.995x - 0.015$ |
| propyl 2-methylbutanoate | (3,3,3-$^2H_3$)propyl 2-methylbutanoate | 145 | 148 | $y = 1.076x - 0.051$ |
| (3*E*,5*Z*)-undeca-1,3,5-triene | (3*E*,5*Z*)-(10,10,11,11-$^2H_4$)undeca-1,3,5-triene | 151 | 155 | $y = 1.142x - 0.089$ |

[a] $y$ = peak area standard/peak area analyte; $x$ = concentration standard ($\mu$g/mL)/concentration analyte ($\mu$g/mL).

was 40 °C for 2 min, then it ramped up at 6 °C/min to 230 °C. The oven temperature in the second dimension was 40 °C for 2 min, then it ramped up at 4 or 6 °C/min to 240 °C (DB-5, DB-1701) or 200 °C (BGB-176).

**GC×GC-TOFMS.** The system consisted of a 6890 Plus gas chromatograph (Agilent) and a Pegasus III TOFMS (Leco, Mönchengladbach, Germany). The GC was equipped with a KAS4 injector (Gerstel, Mühlheim/Ruhr, Germany). The injector was connected to a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 $\mu$m film (Agilent). The end of this column was connected to a second fused silica column, DB-5, 2 m × 0.15 mm i.d., 0.30 $\mu$m film (Agilent). The front part of this column was passed through a liquid nitrogen-cooled dualstage quad-jet thermal modulator (Leco), the major part was installed in a secondary oven mounted inside the primary GC oven, and the column end was connected via a heated (250 °C) transfer line to the MS inlet. Helium at 2 mL/min constant flow served as the carrier gas. The temperature of the first oven was 40 °C for 2 min, it ramped up at 6°/min to 230 °C, and held for 5 min at 230 °C. The modulation time was 4 s. The temperature of the secondary oven was 70 °C for 2 min, it ramped up at 6°/min to 250 °C, and held for 5 min at 250 °C. The mass spectrometer was operated in the EI mode at 70 eV, with a scan range of $m/z$ 35−350, and a scan rate of 100 spectra/s. Data evaluation was performed by means of GC Image (GC Image, Lincoln, NE, USA).

**Isolation of Hop Volatiles.** Hop pellets were immersed in liquid nitrogen and processed into a powder with mortar and pestle. The hop powder (5 g) was added to dichloromethane (100 mL). Under stirring, anhydrous sodium sulfate (5 g) was added and stirring was continued for 3 h at ambient temperature. After filtration, nonvolatile compounds were removed from the extract by SAFE during 30 min at 30 °C. The distillate was concentrated (1 mL) by using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device.[43] Hop volatile isolates were stored at −20 °C. Odor evaluation of small amounts of the hop volatile isolates using fragrance test strips demonstrated their sensory equivalence to the starting materials, particularly the presence of the specific notes characterizing the Huell Melon and Polaris samples.

**AEDA.** The hop volatile isolates were analyzed by GC-O using an FFAP column. Analyses were carried out by three trained and experienced GC-O sniffers (two females, one male; age, 24−46). Training included weekly sensory evaluation sessions with aqueous solutions of reference odorants and GC-O analyses of reference odorant mixtures. Each sniffer repeated the GC-O analysis until results were reproducible (~10−20 runs). Then, hop volatile isolates were stepwise diluted 1:2 with dichloromethane to obtain dilutions of 1:2, 1:4, 1:8, 1:16, etc., and each diluted sample was also subjected to GC-O. Each odor-active compound was assigned a flavor dilution (FD) factor, representing the dilution factor of the highest diluted sample in which the odorant was detected by any of the three sniffers.

**Fractionation of Hop Volatiles.** A SAFE distillate was prepared as described above. Acidic volatiles were extracted with aqueous sodium carbonate solution (0.5 mol/L) in three portions (200 mL total). The organic phase containing the neutral and basic volatiles was dried over anhydrous sodium sulfate and concentrated to 0.5 mL (fraction NBV). The aqueous phase was washed with dichloromethane (50 mL), acidified (pH 2) with hydrochloric acid (32%), and the acidic volatiles were re-extracted with dichloromethane in three portions (300 mL total). The combined organic phases were dried over anhydrous sodium sulfate and concentrated to 0.5 mL (fraction AV). Fraction NBV was applied onto a slurry of purified silica gel (9 g) in pentane in a water-cooled (12 °C) glass column (1 cm i.d.). Elution was performed with pentane/diethyl ether mixtures of 100 + 0, 90 + 10, 70 + 30, 50 + 50, and 0 + 100 (v+v; 50 mL each). The eluate was collected in five portions of 50 mL each and eluate portions were concentrated to 0.5 mL (NBV1−NBV5). A separate SAFE distillate was prepared to isolate the volatile hop thiols (fraction VT) by covalent trapping on mercurated agarose gel using the basic approach published earlier[44] with the modifications detailed recently.[40]

**Determination of the Enantiomeric Distribution of Linalool and *trans*-Calamenene.** Separation of enantiomeric linalools and *trans*-calamenenes was achieved by GC-enantioGC-MS(CI) analysis of the hop volatile isolates (linalools) and NBV1 fractions (calamenenes) using the GC-GC-MS system described above with the DB-FFAP column in the first dimension and the chiral BGB-176 column in the second dimension.

**Quantitation of Myrcene.** Myrcene was quantitated by the simultaneous extraction and fractionation approach detailed recently[31] using tetradecane as internal standard.

**Stable Isotope Dilution Assays.** Hop powder (1−15 g) and the same amount of anhydrous sodium sulfate were added to solvent (80−300 mL). The solvent was either diethyl ether (quantitation of **1**, **3**, **4**, **6**, and **7**) or dichloromethane (quantitation of **10**, **16**−**19**, **24**, **27**−**29**, and **37**). The mixture was spiked with the stable isotopically substituted hop odorants (0.05−50 $\mu$g) and stirred for 3 h at ambient temperature. After filtration, nonvolatile compounds were removed by SAFE. For the quantitation of **24**, the SAFE distillate was concentrated (10 mL) and subjected to GC-GC-MS(CI) analysis. For the quantitation of all other compounds, the SAFE distillate was fractionated using the approaches detailed above and concentrated (0.1−0.5 mL). Quantitations of **1**, **3**, **4**, **6**, **7**, **16**, **18**, **19**, **27**, **28** + **29** (sum of isomers), and **37** were accomplished by GC-GC-MS(CI) of fractions NBV2 (**1**, **3**, **4**, **6**, **7**, **16**, **18**, and **19**), NBV3 (**37**), and AV (**27**, and **28** + **29**) using either the DB-5 column (**18**) or the DB-1701 column (all other compounds) in the second dimension. Quantita-


DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

**Table 2. Odor-Active Compounds in the SAFE Distillates Obtained from the Flavor Hop Cultivars, Huell Melon (HN) and Polaris (PA), and the Aroma Hop Cultivar, Hallertau Tradition (HT)**

| | | | RI[c] | | FD factor[d] | | |
|---|---|---|---|---|---|---|---|
| no. | odorant[a] | odor[b] | FFAP | DB-5 | HN 2014[e] | PA 2012[e] | HT 2014[e] |
| 1 | ethyl 2-methylpropanoate | fruity | 973 | 863 | 512 | 16 | 4 |
| 2 | butane-2,3-dione | buttery | 993 | 605 | 64 | 32 | 64 |
| 3 | methyl 2-methylbutanoate | fruity | 1024 | 780 | 16 | 128 | 32 |
| 4 | ethyl 2-methylbutanoate | fruity | 1056 | 757 | 1024 | 16 | 64 |
| 5 | hexanal | green, grassy | 1088 | 802 | 4 | 2 | 16 |
| 6 | 3-methylbutyl acetate | fruity, banana | 1119 | 883 | <1 | 64 | <1 |
| 7 | propyl 2-methylbutanoate | fruity | 1140 | 946 | 256 | 8 | 8 |
| 8 | myrcene | geranium leaf | 1167 | 994 | 1024 | 2048 | 1024 |
| 9 | unknown | malty, fruity | 1191 | | 32 | <1 | 32 |
| 10 | 1,8-cineole | minty, eucalyptus | 1206 | 1027 | <1 | 64 | <1 |
| 11 | unknown | mushroom | 1258 | | 4 | 64 | <1 |
| 12 | octanal | citrusy, soapy | 1287 | 1010 | 32 | 4 | 4 |
| 13 | oct-1-ene-3-one | mushroom | 1302 | 976 | 16 | 64 | 32 |
| 14 | 2-acetyl-1-pyrroline | roasty, popcorn | 1343 | 925 | 64 | 16 | 32 |
| 15 | dimethyl trisulfide | cabbage | 1367 | 971 | <1 | <1 | 256 |
| 16 | (5*Z*)-octa-1,5-dien-3-one | geranium leaf | 1372 | 983 | 64 | 32 | 256 |
| 17 | 4-methyl-4-sulfanylpentan-2-one | black currant | 1381 | 940 | 16 | 64 | 16 |
| 18 | (3*E*,5*Z*)-undeca-1,3,5-triene | fresh, pineapple | 1389 | 1185 | 256 | 256 | 32 |
| 19 | nonanal | citrusy, soapy | 1389 | 1117 | | | |
| 20 | acetic acid | vinegar, pungent | 1446 | 626 | 16 | 16 | 32 |
| 21 | undeca-1,3,5,8-tetraene[f] | fresh, citrusy | 1457 | 1185 | 32 | 8 | <1 |
| 22 | methional | cooked potato | 1460 | 907 | 64 | 64 | 64 |
| 23 | propanoic acid | cheesy, pungent | 1486 | 839 | 32 | 64 | 4 |
| 24 | linalool | citrusy, bergamot | 1546 | 1100 | 128 | 512 | 1024 |
| 25 | 2-methylpropanoic acid | cheesy | 1560 | 802 | 32 | 16 | 256 |
| 26 | (2*E*,6*Z*)-nona-2,6-dienal | cucumber | 1597 | 1145 | 64 | 32 | 32 |
| 27 | butanoic acid | cheesy | 1621 | 810 | 32 | 128 | 16 |
| 28 | 2-methylbutanoic acid | cheesy | 1678 | 847 | 256 | 256 | 1024 |
| 29 | 3-methylbutanoic acid | cheesy | 1678 | 847 | | | |
| 30 | (2*E*,4*E*)-nona-2,4-dienal | fatty, cucumber | 1700 | 1214 | 16 | <1 | 32 |
| 31 | pentanoic acid | cheesy | 1731 | 914 | 1 | 1 | 128 |
| 32 | unknown | citrusy | 1736 | | 32 | <1 | <1 |
| 33 | unknown | onion | 1758 | | 16 | 8 | <1 |
| 34 | unknown | sulfury, sweaty | 1762 | | 64 | <1 | <1 |
| 35 | geranyl acetate | flowery, rose | 1770 | 1353 | 2 | 16 | <1 |
| 36 | *trans*-calamenene | clove, herbaceous | 1831 | 1538 | 128 | 32 | 128 |
| 37 | geraniol | flowery, rose | 1842 | 1258 | 16 | 32 | 8 |
| 38 | unknown | pungent, onion | 1922 | | 64 | 8 | <1 |
| 39 | heptanoic acid | cheesy | 1946 | 1080 | <1 | 2 | 64 |
| 40 | unknown | cabbage | 1991 | | 2 | <1 | 16 |
| 41 | unknown | sweaty, onion | 2011 | | 32 | 8 | <1 |
| 42 | *trans*-4,5-epoxy-(2*E*)-dec-2-enal[g] | metallic | 2020 | 1382 | 32 | 4 | 32 |
| 43 | HDMF[h] | caramel | 2040 | 1068 | 8 | 64 | 8 |
| 44 | sotolon | soup-seasoning | 2221 | 1107 | 8 | 16 | 32 |
| 45 | phenylacetic acid | honey | 2562 | 1266 | 16 | 8 | 16 |
| 46 | vanillin | vanilla | 2585 | 1404 | 8 | 8 | 32 |

[a]Odorants exhibiting an FD factor of ≥16 in at least one of the three hop samples; order reflects elution order on the FFAP column; structure assignments were based on the comparison of the retention indices on two GC capillaries of different polarity (FFAP, DB-5), the mass spectra were obtained by GC-MS as well as the odor as perceived at the sniffing port during GC-O with data obtained from authentic reference compounds analyzed under the same conditions. [b]Odor as perceived at the sniffing port during GC-O. [c]Retention index: calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]Flavor dilution factor: dilution factor of the highest dilution of the hop volatile isolate in which the odorant was detected during GC-O by any of three panelists. [e]Number indicates harvest year. [f]No reference compound was available; mass spectral data suggested a mixture of isomeric 1,3,5,8-undecatetraenes. [g]GC-MS analysis did not result in a clear mass spectrum, but comparison of retention indices and odor quality with respective data of an authentic reference compound allowed for unequivocal structure assignment. [h]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

tions of **10** and **17** were done by GC × GC-TOFMS of fractions NBV2 (**10**) and fraction VT (**17**). Peak areas corresponding to analyte and internal standard were obtained from the extracted ions chromatograms using the quantifier ions detailed in Table 1. The concentration of each target compound in the hop pellet samples was then calculated from the area counts of the analyte peak, the area



DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

counts of the standard peak, the amount of hop powder used, and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in known concentrations. The individual concentrations of **28** and **29** were calculated from the sum of the concentrations as determined by GC-GC-MS(CI) and the ratio **28**/**29** as determined by GC-GC-MS(EI).[42]

**Determination of Odor Thresholds.** Odor thresholds in air were determined by aroma extract dilution analysis using (2*E*)-dec-2-enal as internal standard.[45,46]

## RESULTS AND DISCUSSION

**Odorant Screening.** Application of a cAEDA to the concentrates obtained from pellets of Huell Melon, Polaris, and Hallertau Tradition hops by solvent extraction and SAFE resulted in 44 odorous regions in the chromatogram with FD factors ≥16 in at least one of the three hop samples. As a first step toward structure elucidation, the retention indices and odor descriptors of the odorous chromatogram regions recorded during AEDA were compared to previously published data of hop odorants[16,18] and to data on roughly 1600 food odorants compiled in an in-house database. In case of matching data, authentic reference compounds were analyzed by GC-O to confirm the structure proposals. Further confirmation was achieved by comparative GC-O analysis of the hop volatile isolates and the reference compounds using a second GC column of different polarity (DB-5). Final structure confirmation was realized by mass spectrometry. To avoid coelutions during GC-MS analysis, the hop volatile isolates were fractionated by acid−base extraction into a fraction of acidic volatiles and a fraction of neutral and basic volatiles. The latter was further fractionated into five fractions by silica gel liquid chromatography. In a separate experiment, the thiols among the hop volatiles were selectively isolated by covalent trapping using mercurated agarose gel. Each fraction was then subjected to GC-O analysis to localize the previously detected odorants and then analyzed by GC-MS (EI and CI) in parallel to reference compounds using the FFAP as well as the DB-5 column.

Based on the concordance of odor, retention index on FFAP, retention index on DB-5, and the mass spectra obtained for the hop odorants with the respective data obtained from the analysis of authentic reference compounds, the structures of 39 hop odorants could be allocated (Table 2). A fresh, citrusy smelling region at RI 1389 was shown to be comprised of two odorants that were coeluted on the FFAP column, namely fresh, pineapple-like smelling (3*E*,5*Z*)-undeca-1,3,5-triene (**18**) and citrusy, soapy smelling nonanal (**19**). However, the compounds could be separated by GC-O using the DB-5 column, which showed that both substances were present in odor-active amounts in all three hop volatile isolates. Another case of unseparated odorants was found with compounds **28** and **29**. Cheesy smelling 2- and 3-methylbutanoic acids could not be separated either using the FFAP column or using the DB-5 column. The mass spectra recorded in EI mode, however, showed that in all three hop pellet samples, mixtures of the isomers were present in which 3-methylbutanoic acid dominated.

The FD factors of the 46 hop odorants depicted in Table 2 covered a range of 16−2048. In all three samples, high FD factors were obtained for geranium leaf-like smelling myrcene (**8**; FD 1024−2048); the cheesy smelling methylbutanoic acid isomers **28** and **29** (FD 256−1024); and for citrusy, bergamot-like smelling linalool (**24**; FD 128−1024). The importance of these compounds for the aroma of hops has already been demonstrated in previous studies.[16,18] It was also reported previously that chiral linalool in hops is mainly comprised of the more odor-active (3*R*)-isomer, whereas the *S*-antipode is of minor importance.[16,18,34,47] In the Huell Melon, Polaris, and Hallertau Tradition hop pellets, the enantiomeric distribution of linalool was determined by GC-enantioGC-MS. Results (Table 3) confirmed the dominance of (3*R*)-linalool.

**Table 3. Enantiomeric Distribution of Linalool in the Three Hop Samples**

| | R/S[a] |
|---|---|
| Huell Melon 2014 | 89/11 |
| Polaris 2012 | 94/6 |
| Hallertau Tradition 2014 | 97/3 |

[a]Ratio of (3*R*)- to (3*S*)-linalool as determined by GC-enantioGC-MS; mean of duplicates.

Further compounds with high FD factors in the variety Hallertau Tradition were dimethyl trisulfide (**15**; FD 256), (5*Z*)-octa-1,5-dien-3-one (**16**; FD 256), 2-methylpropanoic acid (**25**; FD 256), pentanoic acid (**31**; FD 128), and *trans*-calamenene (**36**; FD 128). In the variety Huell Melon, high FD factors were additionally obtained for the fruity smelling esters, ethyl 2-methylbutanoate (**4**; FD 1024), ethyl 2-methylpropanoate (**1**; FD 512), and propyl 2-methylbutanoate (**7**; FD 256); for (3*E*,5*Z*)-undeca-1,3,5-triene and nonanal (**18**/**19**; FD 256); as well as for *trans*-calamenene (**36**; FD 128). In the variety Polaris further compounds with high FD factors included (3*E*,5*Z*)-undeca-1,3,5-triene and nonanal (**18**/**19**; FD 256), methyl 2-methylbutanoate (**3**; FD 128), butanoic acid (**27**; FD 128), 3-methylbutyl acetate (**6**; FD 64), and 1,8-cineole (**10**; FD 64).

The clove-like and herbaceous smelling sesquiterpenoid *trans*-calamenene (**36**) is a widespread essential oil component that was also reported in hop essential oils.[48] However, it has never been identified as an odor-active compound in hops so far. Recently, it was also found among the odor-active compounds in *Spondias mombin* fruits.[29] To clarify its stereochemistry in hops, the approach published for the synthesis of racemic *trans*-calamenene from racemic *trans*-menthone[29] was applied to the synthesis of enantiopure (1*R*,4*S*)-calamenene and enantiopure (1*S*,4*R*)-calamenene from (2*S*,5*R*)-menthone and (2*R*,5*S*)-menthone, respectively (Figure 1). Both compounds showed the same clove-like, herbaceous odor, however, with an odor-threshold in air of 2.5 ng/L, (1*R*,4*S*)-calamenene was slightly more potent than (1*S*,4*R*)-calamenene, for which a threshold of 9.5 ng/L was determined. Using the synthesized calamenenes as reference compounds, the enantiomeric distribution of *trans*-calamenene in Huell Melon, Polaris, and Hallertau Tradition hop pellets was determined by GC-enantioGC-MS. Results (Figure 2) clearly showed that the *trans*-calamenene was pure (1*R*,4*S*)-calamenene and (1*S*,4*R*)-calamenene was absent. The same result was obtained for the *trans*-calamenene in *Spondias mombin* fruit pulp and the calamenene earlier reported in curry leaves[40] (data not shown). The calamenene previously detected in *Cedrela odorata* and *Bauania tricrenata* was also pure (1*R*,4*S*)-calamenene, whereas (1*S*,4*R*)-calamenene and *cis*-calamenenes were absent.[49] In the chromatograms of the hop samples, an additional peak appeared (cf. Figure 2 C−E, peak at 21.75 min) that showed basically the same mass spectrum as


DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

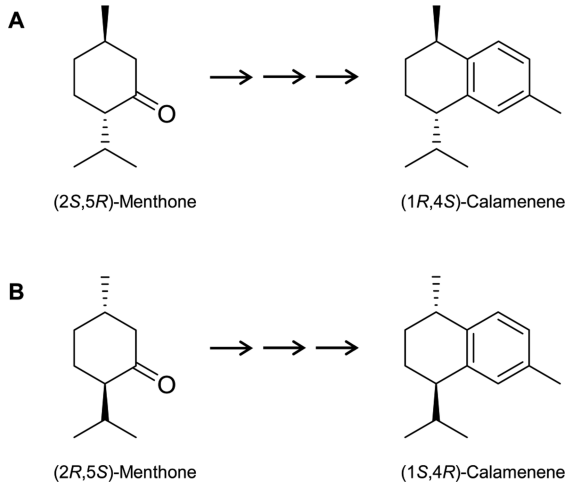



**Figure 1.** Enantiopure *trans*-calamenenes were synthesized from enantiopure menthones using the approach detailed by Neiens et al.[29] (2*S*,5*R*)-Menthone yielded (1*R*,4*S*)-calamenene (A), and (2*R*,5*S*)-menthone yielded (1*S*,4*R*)-calamenene (B).

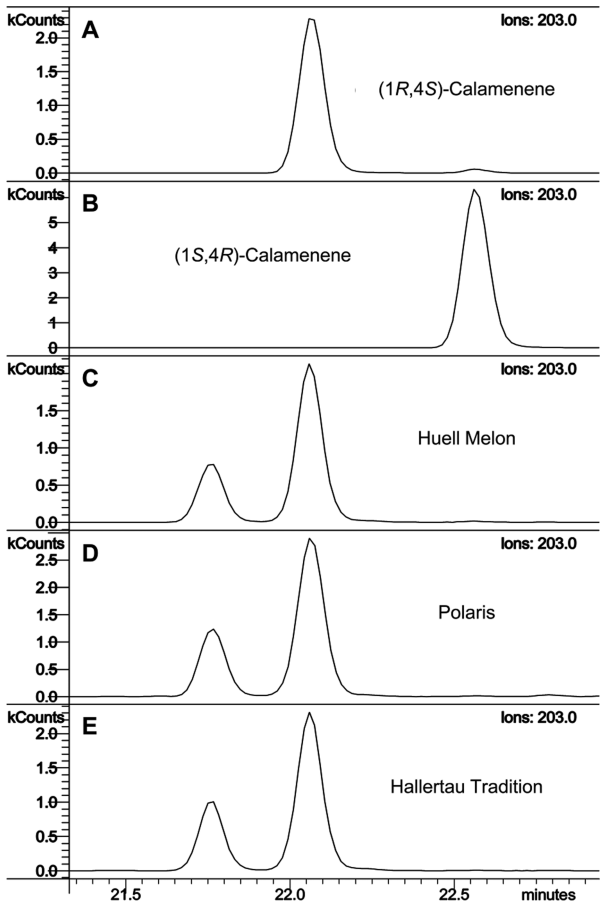



**Figure 2.** GC-enantioGC-MS separation of calamenene isomers in hops, varieties Huell Melon (C), Polaris (D), and Hallertau Tradition (E), in comparison to the reference compounds (1*R*,4*S*)-calamenene (A) and (1*S*,4*R*)-calamenene (B).

the *trans*-calamenenes and may therefore correspond to a *cis*-calamenene. However, as this compound was odorless, no further efforts were made to unequivocally clarify its structure.

In summary of the screening experiments, odor qualities in combination with the FD factors suggested that ethyl 2-methylbutanoate (**4**), ethyl 2-methylpropanoate (**1**), and propyl 2-methylbutanoate (**7**) contributed to the intense fruity, cantaloupe-like aroma note of Huell Melon hops. Their FD factors in the Huell Melon sample (1024, 512, and 256) were clearly higher than the respective FD factors in the Hallertau Tradition sample (64, 4, and 8) and the Polaris sample (16, 16, and 8). For Polaris hops, data suggested that the specific minty aroma note is due to 1,8-cineole (**10**), whereas 3-methylbutyl acetate (**6**) contributes to the fruity note. Both compounds were found in odor-active amounts in the Polaris extract (FD 64), but were not detected in the Hallertau Tradition sample and the Huell Melon sample (FD <1). Methyl 2-methylbutanoate (**3**), which showed a clearly higher FD factor in Polaris (128) as compared to that of Hallertau Tradition (32) and Huell Melon (16), might further contribute to the fruitiness of Polaris.

**Odorant Concentrations.** To get a deeper insight into the role of the individual hop odorants discussed above and to objectify the aroma differences between the three hop varieties, 16 compounds were quantitated in the samples previously used for the screening experiments. Quantitation of myrcene was achieved by GC-FID after simultaneous extraction and fractionation, all other compounds were quantitated by stable isotope dilution assays. Results revealed concentrations in the range of <1 μg/kg to >1 g/kg (Table 4). Quantitative data confirmed the variety-specific role of ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and propyl 2-methylbutanoate in the aroma of Huell Melon hops. Concentrations of these three esters in the Huell Melon sample were 30 to 100 times higher than their concentrations in any of the two other varieties. Quantitations also supported a variety-specific role of 1,8-cineole, 3-methylbutyl acetate, and methyl 2-methylbutanoate in the aroma of Polaris hops. The 1,8-cineole and 3-methylbutyl acetate concentrations in Polaris exceeded the respective concentrations in Huell Melon and Hallertau Tradition by a factor of ≥40. Differences were less pronounced for methyl 2-methylbutanoate, but nevertheless the Polaris sample clearly showed the highest concentration among the three varieties.

Concentration differences among the three samples were also found for other odor-active compounds. An extraordinary high concentration of myrcene was determined in Polaris. This sample also showed the highest value for 4-methyl-4-sulfanylpentan-2-one. However, compared to the concentrations in some US flavor hops, such as Simcoe, Eureka, and Citra (up to 114 μg/kg),[19,20] the 4-methyl-4-sulfanylpentan-2-one concentration in the Polaris sample was still low. Concentrations of geraniol and (3*E*,5*Z*)-undeca-1,3,5-triene were clearly higher in the two flavor hops, whereas concentrations of (3*R*)-linalool, (5*Z*)-octa-1,5-dien-3-one, and methylbutanoic acids were higher in the reference sample of Hallertau Tradition.

**Influence of the Harvest Year.** To get the first idea whether the concentration differences discussed above were either associated with the variety or influenced by other parameters, the odor-active compounds were additionally quantitated in samples of Huell Melon and Polaris of another harvest year, namely 2013. Results (Table 5) confirmed higher amounts of ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and propyl 2-methylbutanoate in Huell Melon than in Polaris, however, concentrations in the 2013 Huell Melon sample were not as high as in the 2014 sample analyzed before. 1,8-Cineole and 3-methylbutyl acetate were confirmed as variety-specific odor-active compounds in Polaris. 1,8-Cineole


DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

**Table 4. Concentrations of Selected Odor-Active Compounds in the Hop Cultivars, Huell Melon (HN), Polaris (PA), and Hallertau Tradition (HT)**

| odorant[a] | concentration (μg/kg)[b] | | |
|---|---|---|---|
| | HN 2014 | PA 2012 | HT 2014 |
| ethyl 2-methylpropanoate (**1**) | 2140 | 62.0 | 18.9 |
| methyl 2-methylbutanoate (**3**) | 162 | 3020 | 985 |
| ethyl 2-methylbutanoate (**4**) | 650 | 6.75 | 8.93 |
| 3-methylbutyl acetate (**6**) | 91.1 | 4490 | 16.5 |
| propyl 2-methylbutanoate (**7**) | 1330 | 48.3 | 31.1 |
| myrcene (**8**) | 1710000 | 31600000 | 1830000 |
| 1,8-cineole (**10**) | 1.33 | 63.2 | 1.58 |
| (5*Z*)-octa-1,5-dien-3-one (**16**) | 0.552 | 0.792 | 2.40 |
| 4-methyl-4-sulfanylpentan-2-one (**17**) | 0.223 | 2.83 | 0.525 |
| (3*E*,5*Z*)-undeca-1,3,5-triene (**18**) | 8.42 | 19.8 | 1.21 |
| nonanal (**19**) | 1740 | 616 | 640 |
| (3*R*)-linalool ((3*R*)-**24**)[c] | 10200 | 52500 | 79700 |
| butanoic acid (**27**) | 770 | 1720 | 145 |
| 2-methylbutanoic acid (**28**) | 11000 | 4620 | 63900 |
| 3-methylbutanoic acid (**29**) | 41200 | 15500 | 233000 |
| geraniol (**37**) | 23100 | 88300 | 3590 |

[a]Numbers in parentheses refer to Table 2. [b]Mean of triplicates; standard deviations were <20%; individual values and standard deviations are available in the Supporting Information. [c]Concentrations were calculated from the total amount of linalool as determined by SIDA (HN 2014:11500 μg/kg, PA 2012:55900 μg/kg, HT 2014:82200 μg/kg) and the enantiomeric ratios depicted in Table 3.

**Table 5. Concentrations of Selected Odor-Active Compounds in the Hop Cultivars, Huell Melon (HN) and Polaris (PA), of Harvest Year 2013**

| odorant[a] | concentration (μg/kg)[b] | |
|---|---|---|
| | HN 2013 | PA 2013 |
| ethyl 2-methylpropanoate (**1**) | 262 | 130 |
| methyl 2-methylbutanoate (**3**) | 1590 | 2930 |
| ethyl 2-methylbutanoate (**4**) | 149 | 12.0 |
| 3-methylbutyl acetate (**6**) | 101 | 2300 |
| propyl 2-methylbutanoate (**7**) | 348 | 58.8 |
| myrcene (**8**) | 491000 | 6070000 |
| 1,8-cineole (**10**) | 1.22 | 62.4 |
| (5*Z*)-octa-1,5-dien-3-one (**16**) | 0.708 | 1.02 |
| 4-methyl-4-sulfanylpentan-2-one (**17**) | 0.273 | 2.53 |
| (3*E*,5*Z*)-undeca-1,3,5-triene (**18**) | 3.14 | 42.7 |
| nonanal (**19**) | 586 | 301 |
| (3*R*)-linalool ((3*R*)-**24**)[c] | 9030 | 48900 |
| butanoic acid (**27**) | 2770 | 1670 |
| 2-methylbutanoic acid (**28**) | 33100 | 25900 |
| 3-methylbutanoic acid (**29**) | 151000 | 73700 |
| geraniol (**37**) | 11900 | 90300 |

[a]Numbers in parentheses refer to Table 2. [b]Mean of triplicates; standard deviations were <20%; individual values and standard deviations are available in the Supporting Information. [c]Concentrations were calculated from the total amount of linalool as determined by SIDA (HN 2013:10500 μg/kg, PA 2013:53100 μg/kg) and the enantiomeric ratios as determined by heart-cut-GC-enantioGC-MS (HN 2013: R/S = 86/14, PA 2013: R/S = 92/8).

concentrations in the two Polaris samples were virtually identical and much higher than the concentrations in the Huell Melon samples. The 3-methylbutyl acetate concentration in the 2013 sample of Polaris, however, was lower than the concentration in the 2012 sample, but both were clearly higher than the 3-methylbutyl acetate concentrations in the Huell Melon samples. The concentration of methyl 2-methylbutanoate in Polaris 2013 was similar to its concentration in Polaris 2012, but in Huell Melon the concentration in the 2013 sample was clearly higher than the concentration in the 2014 sample and rather in the range of the Polaris samples, thus suggesting that high concentrations of this compound are not limited to Polaris.

Myrcene concentrations in the 2013 samples were lower for both varieties, particularly the amount in Polaris was much lower than the extraordinary high value found in the 2012 sample. (3*R*)-Linalool concentrations in the 2013 samples were virtually the same as the concentrations in the samples used for the screening experiments, suggesting that (3*R*)-linalool concentrations are, to a major extent, genetically controlled and hardly influenced by other factors. The same was found for the enantiomeric distribution of linalool. For both harvests, the ratios (3*R*)-linalool/(3*S*)-linalool were somewhat higher in Polaris (2012:94/6; 2013:92/8) than in Huell Melon (2013:86/14; 2014:89/11).

Little influence of the harvest year was also found in the concentrations of 4-methyl-4-sulfanylpentan-2-one and (5*Z*)-octa-1,5-dien-3-one, whereas variability was higher in geraniol, nonanal, (3*E*,5*Z*)-undeca-1,3,5-triene, and, in particular, in the carboxylic acids butanoic acid, 2-methylbutanoic acid, and 3-methylbutanoic acid.

In summary, the results of this study suggested that the major odor-active compounds in Huell Melon and Polaris hops include common hop odorants, such as myrcene, (3*R*)-linalool, and 2- and 3-methylbutanoic acids, whereas the specific fruity, cantaloupe-like note characterizing the Huell Melon variety is caused by ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and propyl 2-methylbutanoate, and the fruity and minty notes in Polaris are due to 3-methylbutyl acetate and 1,8-cineole, respectively. Our findings provide the basis for further studies targeting the fate of the variety-specific odorants in the brewing process and their role in the aroma of different types of beer brewed with Huell Melon and Polaris hops. These studies are currently underway.



DOI: 10.1021/acs.jafc.7b05859
J. Agric. Food Chem. 2018, 66, 1452−1460

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b05859.

Detailed concentration data of the hop odorants including standard deviations (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*Phone: +49 8161 71 2991; Fax: +49 8161 71 2970; E-mail: martin.steinhaus@tum.de.

### ORCID

Martin Steinhaus: 0000-0002-9879-1474



### Funding

This IGF Project of the FEI was supported via AiF within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economics and Energy (BMWi), based on a resolution of the German Parliament. Project No. 18069 N.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS


The authors thank Julia Bock, Anja Matern, and Vanessa Wehner for technical assistance and Dr. Martin Biendl, Hopsteiner, Mainburg, Germany, for providing the hop pellets.


## ■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; CI, chemical ionization; EI, electron ionization; FD factor, flavor dilution factor; FFAP, free fatty acid phase; FID, flame ionization detector; GC-FID, gas chromatography-flame ionization detector; GC-GC-MS, two-dimensional heart-cut gas chromatography−mass spectrometry; GC×GC-TOFMS, comprehensive two-dimensional gas chromatography−time-of-flight mass spectrometry; GC-MS, gas chromatography−mass spectrometry; GC-O, gas chromatography−olfactometry; MCSS, moving column stream switching system; RI, retention index; SAFE, solvent-assisted flavor evaporation; SIDA, stable isotope dilution assay

### Nomenclature

2-Acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone; calamenene, 1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene; 1,8-cineole, 1,3,3-trimethyl-2-oxabicyclo[2,2,2]-octane; *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; geranyl acetate, (2*E*)-3,7-dimethylocta-2,6-dien-1-yl acetate; HDMF, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, also known as Furaneol; linalool, 3,7-dimethylocta-1,6-dien-3-ol; menthone, 2-isopropyl-5-methylcyclohexan-1-one; methional, 3-(methylsulfanyl)propanal; myrcene, 7-methyl-3-methylideneocta-1,6-diene; sotolon, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; vanillin, 4-hydroxy-3-methoxybenzaldehyde

## ■ REFERENCES


(1) Kaltner, D.; Forster, C.; Flieher, M.; Nielsen, T. P. The Influence of dry hopping on three different beer styles. *Brauwelt International* **2013**, *6*, 355−359.

(2) Forster, A.; Gahr, A. On the fate of certain hop substances during dry hopping. *Brewing Science* **2013**, *66*, 93−103.

(3) Steenackers, B.; De Cooman, L.; De Vos, D. Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. *Food Chem.* **2015**, *172*, 742−756.

(4) Kishimoto, T.; Wanikawa, A.; Kagami, N.; Kawatsura, K. Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agric. Food Chem.* **2005**, *53*, 4701−4707.

(5) Lutz, A.; Kammhuber, K.; Seigner, E. New trend in hop breeding at the hop research center Huell. *Brewing Science* **2012**, *65*, 24−32.

(6) International Hop Growers' Convention. Economic Commission - Summary Reports. http://www.hmelj-giz.si/ihgc/doc/2017%20NOV%20IHGC%20Country%20Reports.pdf (accessed January 17, 2018).

(7) Brunner, W. Changes in acreage in the German hop growing regions 2017. In *Hopfen-Rundschau, International ed. of the German Hop Growers Magazine 2017/2018*; Verband Deutscher Hopfenpflanzer, e. V., Ed.; Hopfen-Rundschau: Wolnzach, Germany, 2017; pp 16−18.

(8) Roberts, M. T.; Lewis, A. C. Rapid characterization of hop essential oils using gas chromatography-time of flight mass spectrometry. *J. Am. Soc. Brew. Chem.* **2002**, *60*, 116−121.

(9) In VCF *Volatile Compounds in Food: Database*, Version 16.3; Nijssen, L. M., Ingen-Visscher, C. A. v., Donders, J. J. H., Eds.; TNO Triskelion: Zeist, The Netherlands, 2017.

(10) Roberts, M. T.; Dufour, J.-P.; Lewis, A. C. Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC−TOFMS) for high resolution analysis of hop essential oil. *J. Sep. Sci.* **2004**, *27*, 473−478.

(11) 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.

(12) Seaton, J. C.; Suggett, A.; Moir, M. The flavor contribution of sulfur compounds in hops. *MBAA TQ* **1981**, *18*, 26−30.

(13) Seaton, J. C.; Suggett, A.; Moir, M. The role of sulfur compounds in beer flavour. *Monogr. Eur. Brew. Conv.* **1981**, *7*, 143−155.

(14) McDaniel, M. R.; Miranda-Lopez, R.; Watson, B. T.; Micheals, N. J.; Libbey, L. M. Pinot noir aroma: a sensory/gas chromatographic approach. In *Flavors and Off-Flavors*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1992; pp 23−36.

(15) Chapman, A. C. The essential oil of hops. *J. Inst. Brew.* **1929**, *35*, 247−255.

(16) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter Select) based on GC−olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776−1783.

(17) 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.

(18) Steinhaus, M.; Wilhelm, W.; Schieberle, P. Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis. *Eur. Food Res. Technol.* **2007**, *226*, 45−55.

(19) Kishimoto, T.; Kobayashi, M.; Yako, N.; Iida, A.; Wanikawa, A. Comparison of 4-mercapto-4-methylpentan-2-one contents in hop cultivars from different growing regions. *J. Agric. Food Chem.* **2008**, *56*, 1051−1057.

(20) Reglitz, K.; Steinhaus, M. Quantitation of 4-methyl-4-sulfanylpentan-2-one (4MSP) in hops by a stable isotope dilution assay in combination with GC × GC-TOFMS: Method development and application to study the influence of variety, provenance, harvest year, and processing on 4MSP concentrations. *J. Agric. Food Chem.* **2017**, *65*, 2364−2372.

(21) Takoi, K.; Degueil, M.; Shinkaruk, S.; Thibon, C.; Maeda, K.; Ito, K.; Bennetau, B.; Dubourdieu, D.; Tominaga, T. Identification and characteristics of new volatile thiols derived from the hop (*Humulus luplus* L.) cultivar Nelson Sauvin. *J. Agric. Food Chem.* **2009**, *57*, 2493−2502.




DOI: 10.1021/acs.jafc.7b05859
*J. Agric. Food Chem.* 2018, 66, 1452−1460

(22) Gros, J.; Nizet, S.; Collin, S. Occurrence of odorant polyfunctional thiols in the super alpha Tomahawk hop cultivar. Comparison with the thiol-rich Nelson Sauvin bitter variety. *J. Agric. Food Chem.* **2011**, *59*, 8853−8865.
(23) Van Opstaele, F.; De Causmaecker, B.; Aerts, G.; De Cooman, L. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography−mass spectrometry/olfactometry. *J. Agric. Food Chem.* **2012**, *60*, 12270−12281.
(24) 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.
(25) Schieberle, P.; Grosch, W. Quantitative analyses of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252−257.
(26) 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.
(27) 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.
(28) Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1313−1317.
(29) Neiens, S. D.; Geißlitz, S. M.; Steinhaus, M. Aroma-active compounds in *Spondias mombin* L. fruit pulp. *Eur. Food Res. Technol.* **2017**, *243*, 1073−1081.
(30) Schieberle, P.; Grosch, W. Potent odorants of the wheat bread crumb differences to the crust and effect of a longer dough fermentation. *Z. Lebensm.-Unters. Forsch.* **1991**, *192*, 130−135.
(31) Steinhaus, M. Confirmation of 1-phenylethane-1-thiol as the character impact aroma compound in curry leaves and its behavior during tissue disruption, drying, and frying. *J. Agric. Food Chem.* **2017**, *65*, 2141−2146.
(32) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.
(33) 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.
(34) 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.
(35) Fischer, A.; Schieberle, P. Characterisation of the key aroma compounds in the peel oil of Pontianak oranges (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) by aroma reconstitution experiments. *Eur. Food Res. Technol.* **2009**, *229*, 319−328.
(36) Guth, H.; Grosch, W. Comparison of stored soy-bean oil and rapeseed oils by aroma extract dilution analysis. *Lebensm. Wiss. Technol.* **1990**, *23*, 59−65.
(37) Dess, D. B.; Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones. *J. Org. Chem.* **1983**, *48*, 4155−4156.
(38) 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.
(39) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.
(40) Steinhaus, M. Characterization of the major odor-active compounds in the leaves of the curry tree *Bergera koenigii* L. by aroma extract dilution analysis. *J. Agric. Food Chem.* **2015**, *63*, 4060−4067.
(41) Bio-Rad Laboratories, Munich, Germany, http://www.bio-rad.com/webroot/web/html/lsr/tech_support_faqs/FAQ268439460.html (accessed December 8, 2017).
(42) 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.
(43) Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in Flavour Research*; Land, G. G., Nursten, H. E., Eds.; Applied Science: London, UK, 1979; pp 79−88.
(44) Full, G.; Schreier, P. Covalent chromatography. A valuable method for the aroma analysis of thiols at trace levels. *Lebensmittelchemie* **1994**, *48*, 1−4.
(45) Teranishi, R.; Buttery, R. G.; Guadagni, D. G. Odor quality and chemical structure in fruit and vegetable flavors. *Ann. N. Y. Acad. Sci.* **1974**, *237*, 209−216.
(46) Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm.-Unters. Forsch.* **1987**, *184*, 277−282.
(47) Kaltner, D.; Steinhaus, M.; Mitter, W.; Biendl, M.; Schieberle, P. (*R*)-linalool as key compound for hop aroma in beer and its behavior during beer staling (in German). *Monatsschr. Brauwiss.* **2003**, *56*, 192−196.
(48) Nance, M. R.; Setzer, W. N. Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing. *J. Brew. Distill.* **2011**, *2*, 16−22.
(49) König, W. A.; Rieck, A.; Hardt, I.; Gehrcke, B.; Kubeczka, K.-H.; Muhle, H. Enantiomeric composition of the chiral constituents of essential oils. Part 2: Sesquiterpene hydrocarbons. *J. High Resolut. Chromatogr.* **1994**, *17*, 315−320.


DOI: 10.1021/acs.jafc.7b05859
*J. Agric. Food Chem.* 2018, 66, 1452−1460

### 8.1.3 Summary and Individual Contributions

Driven by US Craft Breweries, the demand for special flavor hops steadily increases. In Germany new flavor hop varieties were bred, among them Huell Melon and Polaris. The aim of the study was the identification of the odor-active compounds in Huell Melon and Polaris, whose specific aroma notes are characterized as fruity, cantaloupe-like and fruity, minty, respectively. Hallertau Tradition was included as a third variety and reference.

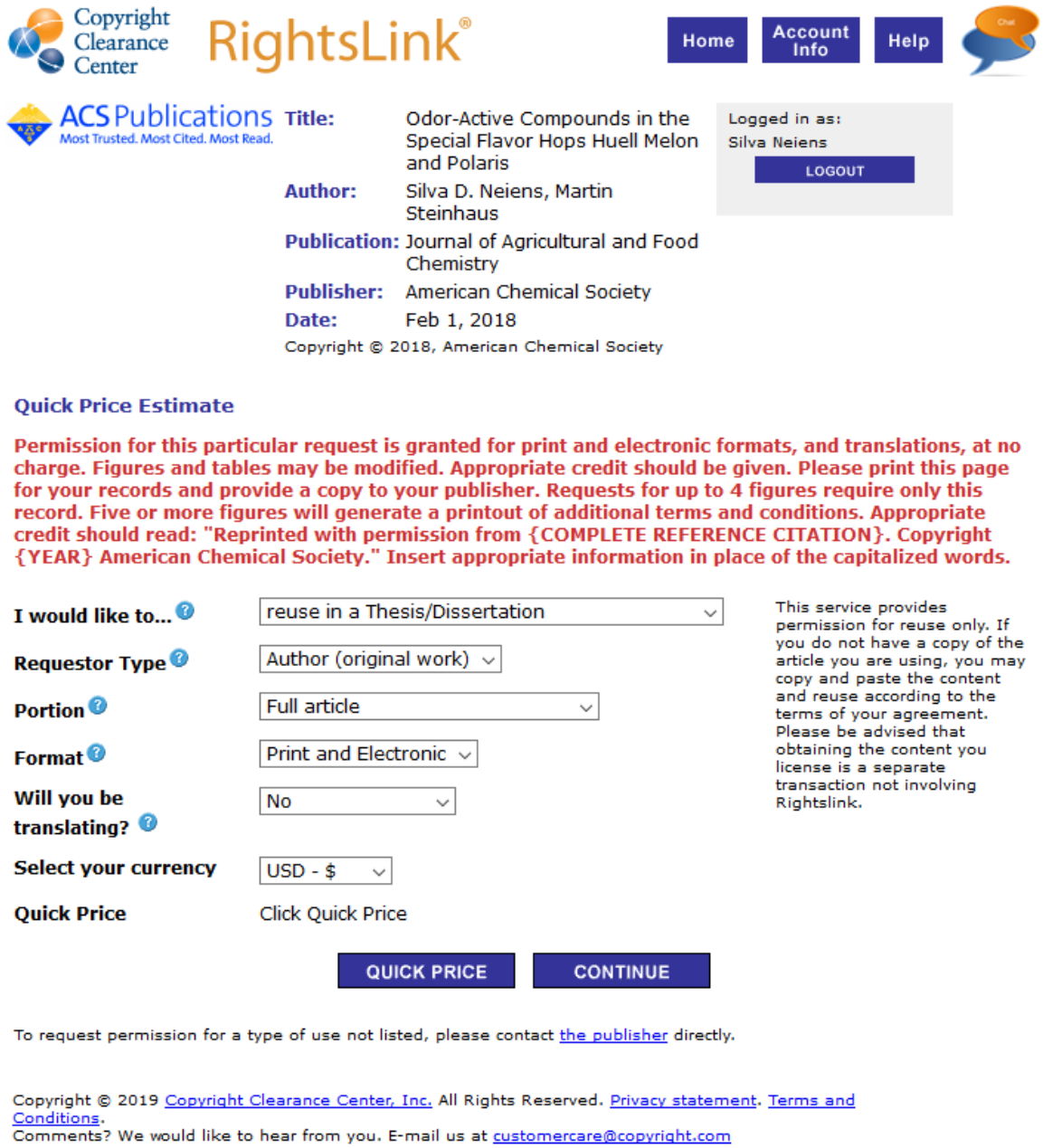
Application of a cAEDA on the volatile isolates obtained from Huell Melon, Polaris, and Hallertau Tradition hops resulted in a total of 46 odorants with FD factors between 16 and 2048. High FD factors in all three varieties were found for myrcene, 2- and 3-methylbutanoic acid as well as (3*R*)-linalool, all of which are well established odor-active hop constituents. Clove-like, herbaceous smelling (1*R*,4*S*)-calamenene was identified for the first time as an odor-active compound in hops after synthesis of enantiopure *trans*-calamenenes and GC-enantioGC-MS analysis. In Huell Melon hops, conspicuously high FD factors were found for ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate, suggesting their contribution to the fruity, cantaloupe-like aroma. The fruity, minty aroma of Polaris corresponded to the presence of odor-active amounts of 3-methylbutyl acetate and 1,8-cineole, both of which were not detected in the extracts of Huell Melon and Hallertau Tradition.

To substantiate the results of cAEDA, 16 selected odorants were quantitated in samples of all three hop varieties by using SIDAs. Results revealed concentrations between 0.223 µg/kg and 31 g/kg. Quantitation of the esters confirmed the results of the screening. Extraordinary high concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate were quantitated in Huell Melon hops. By contrast, concentrations of 3-methylbutyl acetate, 1,8-cineole as well as methyl 2-methylbutanoate were considerably higher in Polaris than in Huell Melon and Hallertau Tradition, which suggested these compounds to be responsible for the characteristic aroma note of Polaris.

To examine the influence of the harvest year, Huell Melon and Polaris hops from a second harvest year were analyzed. Concentrations of ethyl 2-methylpropanoate, ethyl 2-methyl-butanoate, and propyl 2-methylbutanaote in Huell Melon were higher compared to Polaris, but not as high as in the samples of Huell Melon from the other harvest year. The variety-specific odorants 1,8-cineole and 3-methylbutyl acetate showed nearly identical concentrations in the Polaris samples of both harvest years. Little influence of the harvest year was also found for the concentrations of (3*R*)-linalool, 4-methyl-2-sulfanylpentan-2-one, and (5*Z*)-octa-1,5-dien-3-one.

Silva D. Neiens designed and performed the experiments including volatile isolations, GC-O screenings, structure assignments, syntheses, particularly the syntheses of enantiopure calamenenes, quantitations, and sensory experiments. Silva evaluated the resulting data and prepared the manuscript. Martin Steinhaus conceived and directed the study, supervised Silva`s work, and revised the manuscript. Martin additionally participated in the sensory tests, including the GC-O analyses.

### 8.1.4 Reprint Permission



Copyright Clearance Center RightsLink®

Home | Account Info | Help

ACS Publications
Most Trusted. Most Cited. Most Read.

Title: Odor-Active Compounds in the Special Flavor Hops Huell Melon and Polaris
Author: Silva D. Neiens, Martin Steinhaus
Publication: Journal of Agricultural and Food Chemistry
Publisher: American Chemical Society
Date: Feb 1, 2018
Copyright © 2018, American Chemical Society

Logged in as:
Silva Neiens
LOGOUT

Quick Price Estimate

Permission for this particular request is granted for print and electronic formats, and translations, at no charge. Figures and tables may be modified. Appropriate credit should be given. Please print this page for your records and provide a copy to your publisher. Requests for up to 4 figures require only this record. Five or more figures will generate a printout of additional terms and conditions. Appropriate credit should read: "Reprinted with permission from {COMPLETE REFERENCE CITATION}. Copyright {YEAR} American Chemical Society." Insert appropriate information in place of the capitalized words.

I would like to... reuse in a Thesis/Dissertation
Requestor Type Author (original work)
Portion Full article
Format Print and Electronic
Will you be translating? No
Select your currency USD - $
Quick Price Click Quick Price

QUICK PRICE CONTINUE

This service provides permission for reuse only. If you do not have a copy of the article you are using, you may copy and paste the content and reuse according to the terms of your agreement. Please be advised that obtaining the content you license is a separate transaction not involving Rightslink.

To request permission for a type of use not listed, please contact the publisher directly.

Copyright © 2019 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

**Title:** Odor-Active Compounds in the Special Flavor Hops Huell Melon and Polaris

**Author:** Silva D. Neiens, Martin Steinhaus

**Publication:** Journal of Agricultural and Food Chemistry

**Publisher:** American Chemical Society

**Date:** Feb 1, 2018



**PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE**

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

## 8.2 Publication 2: Aroma-Active Compounds in *Spondias mombin* L. Fruit Pulp

### 8.2.1 Bibliographic Data


| | |
|---|---|
| Title: | Aroma-active compounds in *Spondias mombin* L. fruit pulp |
| Authors: | Silva D. Neiens, Sabrina M. Geißlitz, and Martin Steinhaus |
| Journal: | European Food and Research Technology |
| Publication year: | 2017 |
| Volume: | 243 |
| Issue: | 6 |
| Pages: | 1073–1081 |
| DOI: | 10.1007/s00217-016-2825-7 |
| Hyperlink: | https://link.springer.com/content/pdf/10.1007%2Fs00217-016-2825-7.pdf |


### 8.2.2 Publication Reprint

A reprint of publication 2, aroma-active compounds in *Spondias mombin* L. fruit pulp, follows starting with the next page.

Eur Food Res Technol (2017) 243:1073–1081
DOI 10.1007/s00217-016-2825-7

ORIGINAL PAPER

# Aroma-active compounds in *Spondias mombin* L. fruit pulp

**Silva D. Neiens[1] · Sabrina M. Geißlitz[1] · Martin Steinhaus[1]**

Received: 26 September 2016 / Revised: 28 October 2016 / Accepted: 12 November 2016 / Published online: 23 November 2016


**Abstract** Application of an aroma extract dilution analysis to the volatiles isolated from sweet, fruity, and slightly turpentine-like smelling *Spondias mombin* L. fruit pulp by solvent extraction and solvent-assisted flavour evaporation afforded 39 aroma-active compounds with flavour dilution (FD) factors ranging from 4 to 1024, 33 of which were identified and eight that had not been reported in *S. mombin* fruit before. The highest FD factors were obtained for ethyl butanoate (fruity; FD 1024), 3-methylbutyl acetate (fruity, banana-like; FD 512), and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (sweet, caramel-like; FD 512). High FD factors were also found for $\alpha$-pinene (resinous), ethyl 3-methylbutanoate (fruity), myrcene (geranium leaf-like), ethyl hexanoate (fruity), (3*Z*)-hex-3-en-1-ol (green, grassy), methyl 3-hydroxybutanoate (fruity), linalool (citrusy), *trans*-calamenene (clove-like, herbaceous), 2-phenylethanol (flowery), and vanillin (vanilla-like) (all FD 256). Data suggest that the sweet and fruity aroma of *S. mombin* fruit pulp is mainly caused by a group of potent aroma-active esters including ethyl butanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, and methyl 3-hydroxybutanoate, in combination with sweet, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, whereas the turpentine-like note is primarily due to $\alpha$-pinene and myrcene.

✉ Martin Steinhaus
martin.steinhaus@lrz.tum.de

[1] Deutsche Forschungsanstalt für Lebensmittelchemie (German Research Centre for Food Chemistry), Lise-Meitner-Straße 34, 85354 Freising, Germany

**Keywords** Yellow mombin · Hog plum · Cajá · *Spondias mombin* · Aroma extract dilution analysis · *trans*-Calamenene

**Abbreviations**

| | |
|---|---|
| AEDA | Aroma extract dilution analysis |
| FFAP | Free fatty acid phase |
| FD | Flavour dilution |
| GC–O | Gas chromatography–olfactometry |
| GC–MS | Gas chromatography–mass spectrometry |
| RI | Retention index |
| SAFE | Solvent-assisted flavour evaporation |

**Nomenclature**

| | |
|---|---|
| 2-Acetyl-1-pyrroline | 1-(3,4-Dihydro-2*H*-pyrrol-5-yl) ethanone |
| Cadalene | 1,6-Dimethyl-4-(propan-2-yl) naphthalene |
| *trans*-Calamenene | (1*R*,4*S*)-/(1*S*,4*R*)-1,6-Dimethyl-4-(propan-2-yl)-1,2,3,4-tetrahydronaphthalene |
| DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) | 4,5-Dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile |
| *trans*-5,8-Dihydrocalamenene | (1*R*,4*S*)-/(1*S*,4*R*)-1,6-Dimethyl-4-(propan-2-yl)-1,2,3,4,5,8-hexahydronaphthalene |
| *trans*-4a,5-Dihydrocalamenene | (1*R*,4*S*)-/(1*S*,4*R*)-1,6-Dimethyl-4-(propan-2-yl)-1,2,3,4,4a,5-hexahydronaphthalene |

| | |
|---|---|
| DMPU (*N*,*N*′-dimethylpropylene urea) | 1,3-Dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one |
| Grubbs II catalyst (2nd generation Grubbs' catalyst) | Benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium |
| LDA (lithium diisopropylamide) | Di(propan-2-yl)azanide |
| Linalool | 3,7-Dimethylocta-1,6-dien-3-ol |
| Menthone | (2*R*,5*S*)-/(2*S*,5*R*)-5-Methyl-2-(propan-2-yl)cyclohexanone |
| Myrcene | 7-Methyl-3-methylideneocta-1,6-diene |
| (*Z*)-$\beta$-Ocimene | (3*Z*)-3,7-Dimethylocta-1,3,6-triene |
| $\alpha$-Pinene | 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene |
| Sotolon | 3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one |
| Vanillin | 4-Hydroxy-3-methoxybenzaldehyde |

## Introduction

*Spondias mombin* L. is a small deciduous tree in the sumac family (Anacardiaceae) [1]. It is native to the American tropics between southern Mexico and Brazil including some Caribbean islands, but nowadays also widely grown in Africa and Southeast Asia [1]. The fruit of *S. mombin* is known by more than 50 different names. Common English names are yellow mombin and hog plum [1–4]. In Spanish, the fruit is called ciruelo amarillo or jobo, among others [1, 4, 5], whereas in Brazil, it is known as cajá or taperebá [1, 4, 6–9]. The fruits are arranged in hanging panicles of 12 or more [1]. Individual fruits are drupes of ellipsoid shape and about 4 cm in length [1, 4]. They consist of a yellow skin, a translucently yellow, juicy pulp, and a huge white pit [1]. They are rich in potassium, phenolic compounds, antioxidant activity, and vitamin A active carotenoids [4]. Fresh fruit is a popular thirst quencher [1]. However, fruit is also processed into preserves, frozen pulp, juice, jam, jelly, ice cream, pickles, and wine [1, 4].

A major factor contributing to the popularity of *S. mombin* fruit is its pleasant flavour, combining a sour–sweet taste with an exotic aroma [4]. The aroma includes sweet and fruity notes, with a hint of turpentine [1]. Studies targeted at the molecular background of *S. mombin* fruit aroma are rare. In the pioneering work, hept-2-ene, ocimene, methyl benzoate, ethyl benzoate, ethyl octanoate, and ethyl cinnamate were identified in the steam distillate obtained from Brazilian *S. mombin* fruit pulp by gas chromatography–mass spectrometry (GC–MS) [8]. A first comprehensive study revealed 46 volatiles in Nigerian *S. mombin* fruit among which cinnamic acid, ethanol, ethyl 3-hydroxybutanoate, propyl 3-hydroxybutanoate, and benzaldehyde predominated [2]. Twenty-eight volatiles were shown to be present as glycosides [2]. Further reports on *S. mombin* fruit volatiles utilised plant material grown in Brazil [6, 7, 9], Mexico [5], and Tahiti [3] and led to a total number of >200 identified compounds. Despite the large number of structurally characterised volatiles, little is known about the aroma activity of the individual compounds in *S. mombin* fruit yet. Only a single study applied gas chromatography–olfactometry (GC–O) to *S. mombin* fruit volatiles [2]. Twenty-five odorant zones were detected in the chromatogram, most of which showed fruity, sweet, floral, and wine-like odours, but individual descriptions also included lemony, putrid, eucalyptus-like, cod liver oil-like, and baked bread-like. However, no attempt was made to unequivocally assign all the 25 odorant zones to the causative odour-active volatiles. Only one zone, showing an intense odour closely resembling the overall odour of the fruit pulp, was assigned to a compound, namely 2-methylpropyl 3-hydroxybutanoate. This compound was suggested to be the character impact compound of *S. mombin* fruit.

To gain a deeper insight into the compounds contributing to *S. mombin* fruit aroma, the aim of the present study was to apply an aroma extract dilution analysis (AEDA) [10] to the volatiles isolated from the fruit pulp by solvent extraction and solvent-assisted flavour evaporation (SAFE) [11] and elucidate the structures of potent aroma-active compounds.

## Materials and methods

### Fruit material

Pure frozen *S. mombin* fruit pulp (polpa de cajá), manufactured by a commercial fruit processing company in Jundiai, SP, Brazil, was purchased from a local vendor in Germany. The material was kept frozen at −18 °C. Immediately before workup, the pulp was allowed to thaw at room temperature.

### Chemicals

Reference odorants **1**–**8**, **10**–**14**, **18**, **20**–**22**, **24**, **26**, **29**, **34**–**36,** and **39** were purchased from Sigma-Aldrich (Taufkirchen, Germany). Compounds **9**, **28,** and **38** were from Merck (Darmstadt, Germany). The following reference odorants were synthesised according to the literature

procedures: **16** [12], **17** [13], **19** [14], and **33** [15]. Odorants **23** and **32** were synthesised as detailed below.

Allyl bromide, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), *N*,*N*′-dimethyl-propylene urea (DMPU), Grubbs II catalyst, lithium diisopropylamide (LDA) in THF/hexane (1 M), menthone, 2-methylallylmagnesium bromide in THF (0.5 M), and phosphoryl chloride were purchased from Sigma-Aldrich. Sodium methanolate was from Alfa Aesar (Karlsruhe, Germany). Silica gel 60 (0.040–0.063 mm) was purchased from VWR (Darmstadt, Germany) and purified as follows: after extraction with hydrochloric acid (32%; 3 h), the gel was washed with water until the eluate was acid-free, dried at 120 °C until constant weight, and deactivated by water addition (7% final water content). Silver nitrate-coated silica gel was prepared from purified, yet undeactivated silica gel and silver nitrate (3 + 1, w/w). The mixture was wetted with water, homogenised by stirring, and then dried and deactivated as detailed above. Dichloromethane, diethyl ether, and pentane were freshly distilled before use.

## Syntheses

### *Methyl 3-hydroxybutanoate (**23**)*

The compound was obtained from the corresponding ethyl ester by transesterification. Under argon, sodium methanolate (0.5 g) in methanol (20 mL) was dropwise added to ethyl 3-hydroxybutanoate (0.66 g, 5 mmol) in methanol (10 mL). The mixture was refluxed for 90 min. After cooling, water (100 mL) was added and the mixture was extracted with diethyl ether (100 mL). The organic phase was washed with water (3 × 100 mL) and dried over anhydrous sodium sulphate. The solvent was removed in vacuo. The raw synthesis showed a purity of 7% (GC–FID). GC–MS suggested elimination products as major compounds. Therefore, the raw synthesis was subjected to column chromatography (1.5 cm i.d.) with purified silica gel (25 g). After elution of by-products with pentane/diethyl ether (70 + 30, v + v; 150 mL), the target compound was eluted with pentane/diethyl ether (50 + 50, v + v; 150 mL). Removal of the solvent in vacuo afforded **23** as colourless liquid, purity 83% (GC–FID), yield 1.19 mg (10.1 µmol) = 0.2% (GC–FID, internal standard ethyl 3-hydroxybutanoate), RI FFAP 1479, RI DB-5 920. MS (EI): *m*/*z* (%) 43 (100), 74 (69), 45 (51), 71 (33), 103 (24), 87 (21), 42 (16), 61 (12), 59 (11), 44 (7), 85 (6), 41 (5), 69 (5). MS (CI): *m*/*z* (%) 119 (100), 101 (9), 120 (6), 87 (5).

### *trans-Calamenene (**32**)*

Starting from menthone, 1,6-dimethyl-4-(propan-2-yl)-1,3,4,5,8,8a-hexahydronaphthalen-4a(2*H*)-ol (**II**) was synthesised as detailed in [16]. One minor modification was the substitution of the carcinogenic solvent HMPT for the less toxic DMPU [17] in the preparation of allylmenthone.

Bicyclic alcohol **II** (170 mg, 760 µmol) dissolved in pyridine (60 mL) and phosphoryl chloride (10 mL) were stirred overnight under argon. Water (50 mL) was added under ice cooling, and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with hydrochloric acid (1 M; 6 × 100 mL) and brine (3 × 50 mL) and dried over anhydrous sodium sulphate. The solvent was removed in vacuo, and the raw synthesis was subjected to column chromatography (1.5 cm i.d.) with purified silica gel (25 g). Elution with pentane (150 mL) afforded a mixture of four products, *m*/*z* 204 (GC–MS), purity 70% (GC–FID), yield 7.2 mg (35 µmol) = 4.6% (GC-FID, internal standard pentadecane).

The above mixture was dissolved in 1,4-dioxane (30 mL), DDQ (4 g, 17.6 mmol) in 1,4-dioxane (50 mL) was added under argon at 15 °C, and the mixture was stirred for ten minutes. After filtration, the solvent was evaporated in vacuo and the residue was subjected to column chromatography (1.5 cm i.d.) with purified silica gel (25 g). Elution with pentane (150 mL) afforded **32**, purity 50% (GC–FID), yield 1.2 mg (5.9 µmol) = 17% (GC-FID, internal standard pentadecane), RI FFAP 1829, RI DB-5 1533. MS (EI): *m*/*z* (%) 159 (100), 202 (36; $M^+$), 131 (20), 129 (14), 160 (12), 117 (8), 128 (8), 144 (8), 41 (7), 105 (7), 117 (7), 158 (7), 143 (6), 39 (5). MS (CI): m/z (%) 203 (100), 202 (22), 119 (22), 201 (17), 204 (15), 133 (9), 147 (7), 63 (5), 65 (5), 67 (5), 159 (5).

The raw product was further purified by argentation chromatography (1.5 cm i.d.) with silver nitrate-coated silica gel (25 g) and pentane (150 mL) as eluent. The eluate was collected in fractions of 10 mL, and fractions were monitored by GC–O. Fractions containing the clove-like, herbaceous smelling target compound were combined, resulting in a purity of 94% (GC–FID). The solvent was evaporated in vacuo. The residue was taken up in $CDCl_3$ and subjected to NMR measurements. NMR data (carbon nos. refer to Fig. 1): $^1$H NMR (500 MHz, $CDCl_3$, 300 K): $\delta$ 0.71 (d, 3H, $J$ = 7 Hz, H–C10 or H–C12), 1.00 (d, 3H, $J$ = 7, H–C10 or H–C12), 1.26 (d, 3H, $J$ = 7 Hz, H–C9), 1.28–1.38 (m, 1H, H–C2), 1.54–1.64 (m, 1H, H–C3), 1.79–1.87 (m, 1H, H–C3), 1.91–2.00 (m, 1H, H–C2), 2.19–2.28 (m, 1H, H–C11), 2.30 (s, 3H, H–C13), 2.65–2.81 (m, 2H, H–C1 and H–C4), 6.94 (d, 1H, $J$ = 8 Hz, H–C7), 7.02 (s, 1H, H–C5), 7.12 (d, 1H, $J$ = 8 Hz, H–C8); $^{13}$C NMR (125 MHz, $CDCl_3$, 300 K): $\delta$ 17.3 (C10 or C12), 21.1 (C13), 21.3 (C3), 21.5 (C10 or C12), 22.3 (C9), 30.8 (C2), 31.9 (C11), 32.5 (C1), 43.8 (C4), 126.1 (C7), 126.8 (C8), 128.7 (C5), 134.5 (C6), 139.9 (C4a or C8a), 140.1 (C4a or C8a).

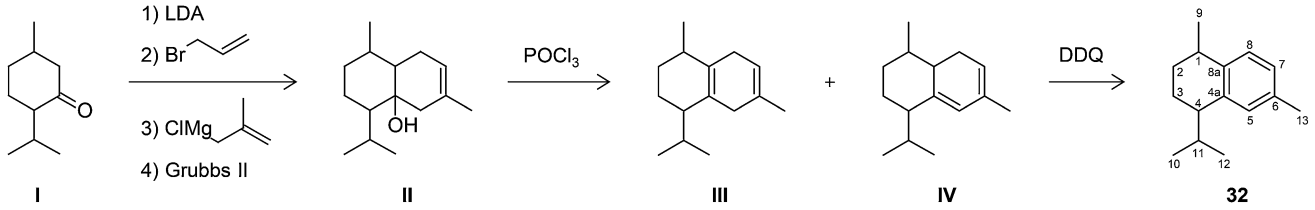



**Fig. 1** Synthetic approach leading from menthone (**I**) to calamenene (**32**)

### Isolation of *S. mombin* fruit pulp volatiles

Fruit pulp (10 g) and dichloromethane (30 mL) were homogenised with a commercial stainless steel blender. Under ice cooling and continuous blending, sodium sulphate (40 g) was added in small portions during 10 min. The mixture was filtered through defatted cotton wool and sea sand, the residue was rinsed with dichloromethane (2 × 10 mL), and nonvolatiles were removed from the combined organic extracts by SAFE during 45 min at 40 °C. The distillate was concentrated to a final volume of 1 mL, first using a Vigreux column (50 × 1 cm) and subsequently a Bemelmans microdistillation device [18].

### Fractionation of *S. mombin* fruit pulp volatiles

Using the approach detailed above, but employing 150 g sodium sulphate and 300 mL + 2 × 50 mL dichloromethane, the volatiles from 300 g *S. mombin* fruit pulp were isolated. The SAFE distillate was extracted with aqueous sodium hydrogen carbonate (0.5 mol/L; 1 × 100 mL, 2 × 50 mL). The combined aqueous extracts were washed with dichloromethane (50 mL), acidified (pH 2) with hydrochloric acid (16%), and re-extracted with dichloromethane (1 × 100 mL, 2 × 50 mL). The organic re-extracts were combined, dried over anhydrous sodium sulphate, and concentrated (1 mL) to obtain the acidic volatiles fraction (AF). The acid-free SAFE distillate representing the neutral and basic volatiles fraction (NBF) was dried over anhydrous sodium sulphate, concentrated (1 mL), and further fractionated by column chromatography (1 cm i.d.) using purified silica gel (8 g) and the following pentane/diethyl ether mixtures (v + v; 50 mL each) as eluents: 100 + 0, 90 + 10, 70 + 30, 50 + 50, and 0 + 100. The eluate was collected in five portions of 50 mL, and each portion was concentrated (1 mL) to obtain fractions NBF1 to NBF5.

### GC–O

A gas chromatograph 5160 (Carlo Erba, Hofheim, Germany) was equipped with a cold on-column injector, an FID, and a tailor-made sniffing port [13]. Two different fused silica columns were used, either a DB-FFAP, 30 m × 0.32 mm i.d., 0.25-µm film or a DB-5, 25 m × 0.32 mm i.d., 0.25-µm film (Agilent, Waldbronn, Germany). The carrier gas was helium at 70 kPa (FFAP) and 50 kPa (DB-5). Injection volume was 1 µL. The start temperature was 40 °C, held for 2 min, and followed by a gradient of 6 °C/min. The end temperatures were 230 °C for DB-FFAP and 240 °C for DB-5. The end of the column was connected to a Y-shaped glass splitter that directed the effluent via two deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) to the FID and the sniffing port. During GC–O analyses, a panellist placed his nose above the sniffing port. Whenever an odour was detected, the panellist marked the position in the FID chromatogram plotted by a recorder and noted the odour quality. A linear retention index (RI) was calculated for each odour-active compound from its retention time and the retention times of adjacent *n*-alkanes by linear interpolation.

### AEDA

The concentrated cajá volatiles isolate (1 mL) was stepwise diluted 1:2 with dichloromethane to obtain dilutions of 1:2, 1:4, 1:8, 1:16, etc. Each diluted sample was then analysed by GC–O using the FFAP column. Dilution was continued until no odorant was detected in the entire GC–O run. Each odour-active compound was assigned a flavour dilution (FD) factor, representing the dilution factor of the highest diluted sample in which the respective odorant was detected by GC–O [10].

### GC–MS

Mass spectra in the EI mode were recorded from m/z 35–300 at 70 eV using either a sector field system or a GC×GC–TOFMS system. The sector field system consisted of a HP 5890 gas chromatograph (Hewlett-Packard, Heilbronn, Germany) connected to a MAT 95 S mass spectrometer (Finnigan, Bremen, Germany). Columns and GC conditions were as detailed in the GC–O part. The GC×GC–TOFMS system was a Pegasus III (Leco, Mönchengladbach, Germany) [19] with a DB-FFAP column, 25 m × 0.25 mm i.d., 0.25-µm film (Agilent) in the

first and a DB-5 column, 2 m × 0.15 mm i.d., 0.30-μm film (Agilent) in the second dimension. Temperature programs were 40 °C (2 min), 6°/min to 230 °C (5 min), for the first oven and 70 °C (2 min), 6°/min to 250 °C (6 min), for the second oven. Modulation time was 4 s. Mass spectra in the CI mode were recorded from m/z 85–300 using the sector field system at 150 eV and isobutane as reagent gas.

## NMR spectroscopy

$^{1}$H and $^{13}$C NMR spectra were recorded at 300 K using an Avance III 500 MHz spectrometer with Topspin software, version 2.1 (Bruker, Rheinstetten, Germany), and tetramethylsilane as the internal standard ($\delta = 0.00$ ppm). Correct signal assignment was confirmed by 2D NMR spectroscopy using COSY, HSQC, and HMBC experiments (data not shown).

## Results and discussion

### Screening *S. mombin* fruit pulp volatiles for aroma-active compounds by AEDA

The volatile fraction was isolated from *S. mombin* fruit pulp by solvent extraction and SAFE. A scent strip dipped into the SAFE distillate, after evaporation of the solvent, fully reflected the characteristic aroma of the fruit material including its sweet, fruity, and turpentine-like notes. Application of an AEDA revealed 39 aroma-active compounds with FD factors ranging from 4 to 1024 (Table 1). The highest FD factors were determined for fruity smelling compound **4** (FD 1024), fruity, banana-like smelling compound **8** (FD 512) and sweet, caramel-like smelling compound **35** (FD 512) followed by compounds **3** (resinous), **6**, **12**, and **23** (fruity), **10** (geranium leaf-like), **18** (green, grassy), **25** (sweet), **26** (citrusy), **32** (clove-like, herbaceous), **34** (flowery), and **38** (vanilla-like) (all FD 256).

### Structure elucidation of aroma-active *S. mombin* fruit pulp volatiles

Structure assignment was approached by comparing RI and odour of the aroma-active *S. mombin* fruit pulp volatiles as obtained by GC–O to compiled data of reference compounds. Resulting preliminary assignments were then confirmed by analysing the respective reference compounds by GC–O and GC–MS in parallel to the *S. mombin* volatiles on two separation systems of different polarity (DB-5 and FFAP). To avoid coelution of aroma-active *S. mombin* volatiles and interfering compounds, the fruit pulp volatile isolates were fractionated before GC–MS analysis by acid–base extraction and silica gel chromatography. The acidic volatiles and five fractions of neutral and basic volatiles of different polarity were then separately analysed by GC–O to localise the previously detected aroma-active compounds and finally subjected to GC–MS analysis.

Using this approach, initially 27 aroma-active compounds (**1**–**14**, **18**–**21**, **23**, **24**, **26**, **28**, **29**, **34**, **35**, **38**, and **39**) were positively identified. Mass spectral confirmation failed for trace compounds **16**, **17**, **22**, **33**, and **36**. However, due to their highly specific odour qualities, GC–O analyses in comparison with authentic reference compounds performed on both separation systems, nevertheless, allowed for their unequivocal identification as 2-acetyl-1-pyrroline (**16**), (5*Z*)-octa-1,5-dien-3-one (**17**), 3-(methylsulfanyl)propanal (**22**), (2*E*,4*E*,6*Z*)-2,4,6-nonatrienal (**33**), and sotolon (**36**).

Based on a comparison of its mass spectrum with database spectra [20], clove-like, herbaceous smelling compound **32** was tentatively identified as *trans*-calamenene, an aromatised sesquiterpene hydrocarbon. This structure was in agreement with the occurrence of **32** in the hydrocarbon fraction NBF1. However, no reference compound was available to confirm the structure assignment. Therefore, we attempted to synthesise *trans*-calamenene from menthone as reported by Nakashima et al. [16]. Menthone (**I**) was reacted with LDA and allyl bromide to obtain allylmenthone. Reaction with 2-methylallylmagnesium bromide yielded the diene alcohol 3-methyl-1-(2-methylprop-2-en-1-yl)-6-(propan-2-yl)-2-(prop-2-en-1-yl)cyclohexanol that was further reacted with Grubbs' II catalyst to yield the bicyclic alcohol 1,6-dimethyl-4-(propan-2-yl)-1,3,4,5,8,8a-hexahydronaphthalen-4a(2*H*)-ol (**II**) (Fig. 1). This compound was treated with phosphoryl chloride. According to Nakashima et al., this reaction resulted in a mixture containing 13% *trans*-calamenene and 17% *trans*-5,8-dihydrocalamenene, indicating a partial oxidative aromatisation of the cyclohexadiene ring. However, we found only traces of *trans*-calamenene in the reaction mixture. Instead, we observed four products that showed mass spectra with a molecular ion of *m/z* 204 in agreement with the presence of *trans*-dihydrocalamenene isomers *trans*-5,8-dihydrocalamenene (**III**) and *trans*-4a,5-dihydrocalamenene (**IV**) (Fig. 1).

When Nakashima et al. treated *trans*-5,8-dihydrocalamenene with DDQ at room temperature overnight, they observed aromatisation of both rings, resulting in the naphthalene derivative cadalene. However, Halton et al. [21] reported the conversion of a 1,2,3,4,5,8-hexahydronaphthaline derivative to the corresponding 1,2,3,4-tetrahydronaphthalene derivative, when DDQ was applied for a short period of time at sub-ambient temperature. Indeed, with Halton's approach we were able to convert the *trans*-dihydrocalamenene mixture to *trans*-calamenene (Fig. 1).

**Table 1** Aroma-active compounds in the SAFE distillate obtained from *S. mombin* fruit pulp

| No. | Odorant[a] | Odour[b] | RI | | FD factor | Reported earlier[c] |
|---|---|---|---|---|---|---|
| | | | FFAP | DB-5 | | |
| 1 | Ethyl 2-methylpropanoate | Fruity | <1000 | 758 | 16 | |
| 2 | Butane-2,3-dione | Buttery | <1000 | <700 | 4 | [7] |
| 3 | $\alpha$-Pinene | Resinous | 1015 | 934 | 256 | [3, 6, 7, 9] |
| 4 | Ethyl butanoate | Fruity | 1033 | 800 | 1024 | [2, 3, 5–7, 9] |
| 5 | Ethyl 2-methylbutanoate | Fruity | 1049 | 849 | 128 | |
| 6 | Ethyl 3-methylbutanoate | Fruity | 1068 | 877 | 256 | [3] |
| 7 | Butyl acetate | Fruity, apple | 1076 | 931 | 32 | [3, 5–7, 9] |
| 8 | 3-Methylbutyl acetate | Fruity, banana | 1122 | 934 | 512 | [3, 5–7, 9] |
| 9 | Butan-1-ol | Malty | 1141 | <700 | 64 | [3, 6, 7] |
| 10 | Myrcene | Geranium leaf | 1160 | 990 | 256 | [3, 6, 9] |
| 11 | 2- and 3-Methylbutan-1-ol | Malty | 1206 | 728 | 16 | [3, 5, 7] |
| 12 | Ethyl hexanoate | Fruity | 1231 | 996 | 256 | [2, 3, 5–7, 9] |
| 13 | (*Z*)-$\beta$-Ocimene | Terpeny | 1249 | 1168 | 64 | [3, 5, 7–9] |
| 14 | 3-Hydroxybutan-2-one | Buttery | 1284 | 711 | 64 | [3, 7] |
| 15 | Unknown | Terpeny | 1304 | – | 16 | |
| 16 | 2-Acetyl-1-pyrroline[d] | Roasty, popcorn | 1337 | 921 | 128 | |
| 17 | (5*Z*)-Octa-1,5-dien-3-one[d] | Geranium leaf | 1373 | 983 | 64 | |
| 18 | (3*Z*)-Hex-3-en-1-ol | Green, grassy | 1384 | 854 | 256 | [2, 3, 5–7, 9] |
| 19 | Hexyl butanoate | Fruity | 1413 | 1196 | 64 | [2, 3, 5, 6] |
| 20 | Ethyl octanoate | Fruity | 1434 | 1197 | 64 | [2, 3, 5–9] |
| 21 | Acetic acid | Vinegar | 1451 | <700 | 32 | [3, 7] |
| 22 | 3-(Methylsulfanyl)propanal[d] | Cooked potato | 1455 | 902 | 128 | |
| 23 | Methyl 3-hydroxybutanoate | Fruity | 1479 | 920 | 256 | [3, 7] |
| 24 | Ethyl 3-hydroxybutanoate | Fruity | 1518 | 932 | 32 | [2, 3, 7] |
| 25 | Unknown | Sweet | 1527 | – | 256 | |
| 26 | Linalool | Citrusy | 1543 | 1102 | 256 | [2, 3, 5, 6, 9] |
| 27 | Unknown | Fruity | 1560 | – | 64 | |
| 28 | Butanoic acid | Cheesy | 1626 | n.d.[e] | 128 | [2, 3, 7] |
| 29 | 2- and 3-Methylbutanoic acid | Cheesy | 1671 | n.d.[e] | 8 | [3] |
| 30 | Unknown | Citrusy | 1715 | – | 32 | |
| 31 | Unknown | Phenolic | 1785 | – | 64 | |
| 32 | *trans*-Calamenene | Clove, herbaceous | 1828 | 1533 | 256 | [3] |
| 33 | (2*E*,4*E*,6*Z*)-2,4,6-Nonatrienal[d] | Oat flake | 1879 | 1272 | 64 | |
| 34 | 2-Phenylethanol | Flowery | 1919 | 1112 | 256 | [3, 7] |
| 35 | 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one | Sweet, caramel | 2043 | 1066 | 512 | |
| 36 | Sotolon[d] | Soup seasoning | 2215 | 1113 | 64 | |
| 37 | Unknown | Phenolic | 2350 | – | 8 | |
| 38 | Vanillin | Vanilla | 2605 | 1409 | 256 | [3] |
| 39 | 3-Phenylpropanoic acid | Flowery | 2647 | 1338 | 128 | |

[a] Each odorant was identified by comparing its RIs on two GC capillaries of different polarity (FFAP, DB-5), its mass spectrum obtained by GC–MS, as well as its odour quality as perceived at the sniffing port during GC–O with data obtained from authentic reference compounds analysed in parallel

[b] Odour quality as perceived at the sniffing port during GC–O

[c] References reporting the compound in *S. mombin* fruit before

[d] A clear mass spectrum could not be obtained in the *S. mombin* fruit volatile isolates; identification was based on the remaining criteria detailed in footnote a

[e] Not determined; due to bad peak shapes on column DB-5, no RI was calculated

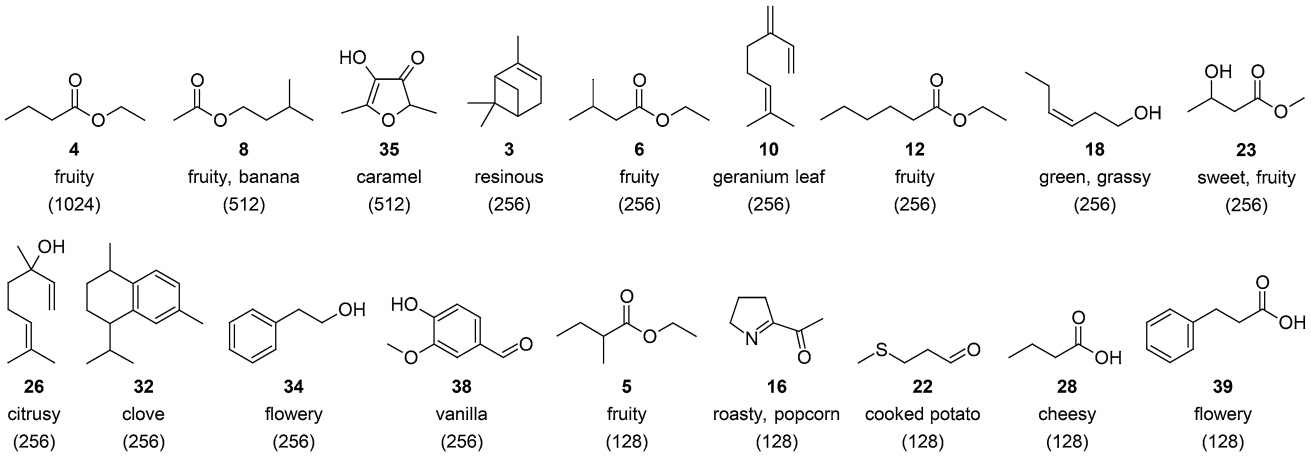



**Fig. 2** Most aroma-active compounds (FD factor ≥128) in the SAFE distillate obtained from *S. mombin* fruit pulp; FD factors are given in *parentheses*

The raw synthesis, which contained 50% (GC–FID) of the target compound, was purified by argentation chromatography to yield *trans*-calamenene in 94% purity. The structure was corroborated by NMR. Finally, GC–O and GC–MS analyses of the synthesised compound confirmed that compound **32** in the *S. mombin* fruit pulp aroma isolate was *trans*-calamenene. However, it remained unclear whether **32** was (+)-*trans*-calamenene or (−)-*trans*-calamenene or a mixture of both. To clarify this, stereospecific synthesis of (+)-*trans*-calamenene or (−)-*trans*-calamenene from stereopure (+)- and (−)-menthone and their separate evaluation by GC–O, preferentially by using a chiral stationary phase, is necessary. This will be subject to further research.

In summary, the structures of 33 aroma-active fruit pulp volatiles were elucidated, among which eight (**1**, **5**, **16**, **17**, **22**, **33**, **35**, and **39**) had not been reported in *S. mombin* fruit before. Six compounds (**15**, **25**, **27**, **30**, and **31**) remained unidentified.

## Discussion

The aroma-active compound, for which the highest FD factor (1024) was determined, was fruity smelling ethyl butanoate (**4**) (Fig. 2). This compound has been found in *S. mombin* fruit before [2, 3, 5–7, 9], and in some studies, it was reported to be one of the major volatiles [6, 9]. Further fruity smelling esters detected with high FD factors in the *S. mombin* fruit extract included 3-methylbutyl acetate (**8**, FD 512), ethyl 3-methylbutanoate (**6**, FD 256), ethyl hexanoate (**12**, FD 256), methyl 3-hydroxybutanoate (**23**, FD 256), and ethyl 2-methylbutanoate (**5**, FD 128). Among these, ethyl 2-methylbutanoate has not been reported in *S. mombin* fruit before. Ethyl butanoate, ethyl 2- and 3-methylbutanoate, and ethyl hexanoate exhibit comparably low odour thresholds (0.01–1 µg/L in water, [22]) and are important generalists [23] among the aroma-active compounds in fruits. Individually or in combination, they play a key role in the aroma of many other fruits such as orange [24], durian [25], kiwifruit [19], and mangoes [22]. By contrast, 3-methylbutyl acetate and methyl 3-hydroxybutanoate are less commonly found aroma-active in fruits. The banana-like aroma note of 3-methylbutyl acetate in combination with its high FD factor (512) might therefore add some individuality to the aroma of *S. mombin* fruit. 2-Methylpropyl 3-hydroxybutanoate, previously suggested as character impact compound of *S. mombin* fruit pulp by Adedeji et al. [2], was not present in aroma-active amounts.

The highest FD factor of a non-ester compound was 512 and was determined for sweet, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (**35**). Despite its aroma potency, it has not been reported in *S. mombin* fruit yet. 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one is another important generalist compound, not only in fruits, but in foods in general [23]. It has long been known as key compound in strawberry and pineapple aroma [23] and more recently was also found among the most aroma-active compounds in mango [26] and kiwifruit [19]. In *S. mombin* fruit aroma, it may contribute to the overall sweet–fruity note.

The turpentine note, on the other hand, is most likely linked to resinous smelling $\alpha$-pinene (**3**, FD 256) and geranium leaf-like smelling myrcene (**10**, FD 256), probably with some further contribution by terpeny smelling (*Z*)-$\beta$-ocimene (**13**, FD 64). The same compounds are also responsible for the turpentine note in some mango varieties [27].

Further compounds with high FD factors in *S. mombin* fruit included citrusy smelling linalool (**26**), green, grassy smelling (3*Z*)-hex-3-en-1-ol (**18**), clove-like, herbaceous smelling *trans*-calamenene (**32**), flowery smelling 2-phenylethanol (**34**), and vanilla-like smelling vanillin (**38**) (all FD

256), followed by roasty, popcorn-like smelling 2-acetyl-1-pyrroline (**16**), cooked potato-like smelling 3-(methylsulfanyl)propanal (**22**), cheesy smelling butanoic acid (**28**), and flowery smelling 3-phenylpropanoic acid (**39**) (all FD 128). The aroma contribution of all these compounds is not as obvious as that of the esters and the terpene hydrocarbons; however, they might at least possess a modifying influence on the overall aroma of *S. mombin* fruit pulp.

*trans*-Calamenene is a common compound in various essential oils. It has also been found in *S. mombin* fruit pulp before [3]. However, it is noteworthy to mention that it has never been mentioned as aroma-active compound in food yet. *cis*-Calamenene, however, was tentatively identified with a "fresh" odour quality in dried leaves of the Chinese fragrant plant *Lysimachia foenum-graecum* [28], and calamenene of undefined stereochemistry was reported with a "herb spice" odour from adhesive [29]. In both cases, identification was not confirmed by analysis of the reference compound. Therefore, the present study is the first to unequivocally report a calamenene as aroma-active compound in a food. Another interesting aspect about *trans*-calamenene is its odour quality, because clove-like odours are typically associated with alkylated 2-methoxyphenols such as 2-methoxy-4-vinylphenol or eugenol, but not with hydrocarbons.

## Conclusions

In summary, our results suggest that the sweet and fruity aroma of *S. mombin* fruit pulp is mainly caused by ethyl butanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, methyl 3-hydroxybutanoate, and ethyl 2-methylbutanoate in combination with sweet, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, whereas $\alpha$-pinene, myrcene, and (*Z*)-$\beta$-ocimene are responsible for the turpentine-like aroma note.

**Acknowledgements** The authors thank Julia Bock for excellent technical assistance.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights statement** This article does not contain any studies with human or animal subjects.

## References

1. Morton JF (2013) Fruits of warm climates. Echo Point Books & Media, Brattleboro
2. Adedeji J, Hartman TG, Rosen RT, Ho CT (1991) Free and glycosidically bound aroma compounds in hog plum (*Spondias mombins* L.). J Agric Food Chem 39:1494–1497
3. Teai T, Claude-Lafontaine A, Schippa C, Cozzolino F (2005) Volatile compounds in fresh yellow mombin fruits (*Spondias mombin* L.) from French Polynesia. J Essent Oil Res 17:368–372. doi:10.1080/10412905.2005.9698933
4. Tiburski JH, Rosenthal A, Deliza R, de Oliveira Godoy RL, Pacheco S (2011) Nutritional properties of yellow mombin (*Spondias mombin* L.) pulp. Food Res Int 44:2326–2331
5. Sagrero-Nieves L, De Pooter HL (1992) Volatiles of the jobo fruit (*Spondias mombin* L.). J Essent Oil Res 4:535–537. doi:10.1080/10412905.1992.9698127
6. Augusto F, Valente ALP, Dos Santos Tada E, Rivellino SR (2000) Screening of Brazilian fruit aromas using solid-phase microextraction−gas chromatography−mass spectrometry. J Chromatogr A 873:117–127
7. Allegrone G, Barbeni M (1992) Identification of volatile components of caja fruit (*Spondias lutea* L.) and chiral analysis of 3-hydroxy aliphatic esters. Flavour Fragr J 7:337–342. doi:10.1002/ffj.2730070609
8. Alves S, Jennings WG (1979) Volatile composition of certain Amazonian fruits. Food Chem 4:149–159
9. Ceva-Antunes PMN, Bizzo HR, Alves SM, Antunes OAC (2003) Analysis of volatile compounds of taperebá (*Spondias mombin* L.) and cajá (*Spondias mombin* L.) by simultaneous distillation and extraction (SDE) and solid phase microextraction (SPME). J Agric Food Chem 51:1387–1392
10. Schieberle P, Grosch W (1987) Evaluation of the flavor of wheat and rye bread crusts by aroma extract dilution analysis. Z Lebensm Unters Forsch 185:111–113. doi:10.1007/Bf01850088
11. Engel W, Bahr W, Schieberle P (1999) 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 209:237–241. doi:10.1007/s002170050486
12. Buttery RG, Ling LC, Juliano BO, Turnbaugh JG (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agric Food Chem 31:823–826
13. Steinhaus M, Sinuco D, Polster J, Osorio C, Schieberle P (2008) Characterization of the aroma-active compounds in pink guava (*Psidium guajava*, L.) by application of the aroma extract dilution analysis. J Agric Food Chem 56:4120–4127. doi:10.1021/jf8005245
14. Fischer E, Speier A (1895) Darstellung der Ester. Chem Ber 28:3252–3258
15. Schuh C, Schieberle P (2005) Characterization of (E,E,Z)-2,4,6-nonatrienal as a character impact aroma compound of oat flakes. J Agric Food Chem 53:8699–8705. doi:10.1021/jf051601i
16. Nakashima K, Imoto M, Masakazu S, Tori M, Nagashima F, Asakawa Y (2002) Total synthesis of (–)-(7S,10R)-calamenene and (–)-(7S,10R)-2-hydroxycalamenene by use of a ring-closing metathesis reaction. A comparison of the *cis*- and *trans*-isomers. Molecules 7:517–527
17. Mukhopadhyay T, Seebach D (1982) Substitution of HMPT by the cyclic urea DMPU as a cosolvent for highly reactive nucleophiles and bases. Helv Chim Acta 65:385–391
18. Bemelmans JMH (1979) Review of isolation and concentration techniques. In: Land GG, Nursten HE (eds) Progress in flavour research. Applied Science Publishers, London, pp 79–98
19. Lindhorst AC, Steinhaus M (2016) Aroma-active compounds in the fruit of the hardy kiwi (*Actinidia arguta*) cultivars Ananasnaya, Bojnice, and Dumbarton Oaks: differences to common kiwifruit (*Actinidia deliciosa* 'Hayward'). Eur Food Res Technol 242:967–975. doi:10.1007/s00217-015-2603-y
20. U.S. Department of Commerce. Mass spectral library NIST11. National Institute of Standards and Technology, Gaithersburg

21. Halton B, Randall CJ, Gainsford GJ, Stang PJ (1986) Cycloproparenes: synthesis, structure, and spectral properties of alkylidenecyloproparenes. J Am Chem Soc 108:5949–5956
22. Munafo JP, Didzbalis J, Schnell RJ, Steinhaus M (2016) 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 64:4312–4318. doi:10.1021/acs.jafc.6b00822
23. Dunkel A, Steinhaus M, Kotthoff M, Nowak B, Krautwurst D, Schieberle P, Hofmann T (2014) Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology. Angew Chem Int Ed 53:7124–7143. doi:10.1002/anie.201309508
24. Buettner A, Schieberle P (2001) Evaluation of aroma differences between hand-squeezed juices from valencia late and navel oranges by quantitation of key odorants and flavor reconstitution experiments. J Agric Food Chem 49:2387–2394. doi:10.1021/jf001363l
25. Li J-X, Schieberle P, Steinhaus M (2012) 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 60:11253–11262. doi:10.1021/jf303881k
26. Munafo JP, Didzbalis J, Schnell RJ, Schieberle P, Steinhaus M (2015) 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone, a cultivar-independent key compound in mango aroma. In: Taylor AJ, Mottram DS (eds) Flavour science: proceedings of the XIV Weurman flavour research symposium, Context Products, Packington, pp 107–110
27. Munafo JP Jr, Didzbalis J, Schnell RJ, Schieberle P, Steinhaus M (2014) 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 62:4544–4551. doi:10.1021/jf5008743
28. Shu N, Shen H (2009) Aroma-impact compounds in *Lysimachia foenum-graecum* extracts. Flavour Fragr J 24:1–6. doi:10.1002/ffj.1908
29. Vera P, Uliaque B, Canellas E, Escudero A, Nerin C (2012) Identification and quantification of odorous compounds from adhesives used in food packaging materials by headspace solid phase extraction and headspace solid phase microextraction coupled to gas chromatography–olfactometry–mass spectrometry. Anal Chim Acta 745:53–63. doi:10.1016/j.aca.2012.07.045

### 8.2.3 Summary and Individual Contributions

*Spondias mombin* L. is a small tree in the sumac family (*Anacardiaceae*) and is native to the American tropics between southern Mexico and Brazil including some Caribbean islands. Today, *S*. *mombin* is also grown in Africa and Southeast Asia. The Brazilian name of the fruit of *S. mombin* is cajá and the fruit is popular due to its sweet, sour taste and an exotic aroma. The aim of the present study was to get an insight into the compounds responsible for the characteristic aroma of *S. mombin* fruit pulp.

Application of an AEDA to the volatiles isolated from the fruit pulp by solvent extraction and SAFE resulted in 39 odor-active compounds with FD factors between 4 and 1024. Their structure elucidation was accomplished by comparison of the retention indices obtained by gas chromatography with two columns of different polarity, the odor quality as perceived at the sniffing port of the GC-O system, and the mass spectra (EI and CI) with data from authentic reference compounds. A total of 33 odorants were identified, among them eight odorants that had not been reported in *S. mombin* fruit before. The highest FD factors were obtained for fruity smelling ethyl butanoate (FD 1024), banana-like smelling 3-methylbutyl acetate (512), and sweet, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (512). Further odorants detected with high FD factors were *α*-pinene (resinous), ethyl 3-methylbutanoate (fruity), myrcene (geranium leaf), ethyl hexanoate (fruity), (3*Z*)-hex-3-en-1-ol (green, grassy), methyl 3-hydroxybutanoate (fruity), linalool (citrusy), *trans*-calamenene (clove, herbaceous), 2-phenylethan-1-ol (flowery), and vanillin (vanilla) (all FD 256), as well as ethyl 2-methylbutanoate (fruity), 2-acetyl-1-pyrroline (roasty, popcorn), methional (cooked potato), butanoic acid (cheesy), and 3-phenylpropanoic acid (flowery) (all FD 128). The clove-like, herbaceous smelling *trans*-calamenene was identified for the first time as an odor-active compound in cajá fruit pulp. The reference compound was synthesized from racemic menthone in a multistep reaction. In a first step, menthone was deprotonated by lithium diisopropylamide followed by a nucleophilic attack of the formed carbanion to allyl bromide. The allylmenthone formed was reacted with 2-methylallylmagnesium chloride, followed by a ring closing metathesis. Elimination of water enforced by phosphoryl chloride resulted in a mixture of dihydrocalamenenes which, in the final step, underwent oxidative aromatization to *trans*-calamenene by means of DDQ. After purification of the synthesized product by argentation chromatography, the structure was verified by NMR experiments.

The results of this study suggested that the sweet and fruity aroma of *S. mombin* fruit pulp is mainly caused by a combination of potent odor-active esters including ethyl butanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, ethyl hexanoate, and methyl 3-hydroxy-butanoate, with the sweet, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one. An additional turpentine-like note is most likely due to *α*-pinene, myrcene, and (*Z*)-*β*-ocimene.

Silva D. Neiens performed the synthesis of *trans*-calamenene together with Sabrina M. Geißlitz. Silva verified the results of the synthesis by GC-MS and NMR experiments. Silva evaluated all data and prepared the manuscript. Additionally, Silva participated in the sensory tests, including the GC-O analyses. Sabrina performed the synthesis of *trans*-calamenene together with Silva. Sabrina performed the screening experiments, structure assignments, and ester syntheses. Martin Steinhaus conceived and directed the study, supervised Silva`s and Sabrina`s work, and revised the manuscript. Martin additionally participated in the sensory tests, including the GC-O analyses.

### 8.2.4 Reprint Permission

**SPRINGER NATURE LICENSE**
**TERMS AND CONDITIONS**

Jan 07, 2019

This Agreement between Ms. Silva Neiens ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

| | |
|---|---|
| License Number | 4503630663380 |
| License date | Jan 07, 2019 |
| Licensed Content Publisher | Springer Nature |
| Licensed Content Publication | European Food Research and Technology |
| Licensed Content Title | Aroma-active compounds in Spondias mombin L. fruit pulp |
| Licensed Content Author | Silva D. Neiens, Sabrina M. Geißlitz, Martin Steinhaus |
| Licensed Content Date | Jan 1, 2016 |
| Licensed Content Volume | 243 |
| Licensed Content Issue | 6 |
| Type of Use | Thesis/Dissertation |
| Requestor type | non-commercial (non-profit) |
| Format | print and electronic |
| Portion | full article/chapter |
| Will you be translating? | no |
| Circulation/distribution | <501 |
| Author of this Springer Nature content | yes |
| Title | Odor-Active Compounds in German Flavor Hop Varieties |
| Institution name | Leibniz-Institute for Food Systems Biology at the Technical University of Munich |
| Expected presentation date | May 2019 |
| Requestor Location | Ms. Silva Neiens<br>Lise-Meitner-Strasse 34<br><br>Freising, 85354<br>Germany<br>Attn: Ms. Silva Neiens |
| Billing Type | Invoice |
| Billing Address | Ms. Silva Neiens<br>Lise-Meitner-Strasse 34<br><br>Freising, Germany 85354<br>Attn: Ms. Silva Neiens |
| Total | 0.00 USD |

Terms and Conditions

**Springer Nature Terms and Conditions for RightsLink Permissions**

**Springer Nature Customer Service Centre GmbH (the Licensor)** hereby grants you a non-exclusive, world-wide licence to reproduce the material and for the purpose and requirements specified in the attached copy of your order form, and for no other use, subject to the conditions below:

1. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of this material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the copyright of another entity (as credited in the published version).

   If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Where **print only** permission has been granted for a fee, separate permission must be obtained for any additional electronic re-use.

3. Permission granted **free of charge** for material in print is also usually granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

4. A licence for 'post on a website' is valid for 12 months from the licence date. This licence does not cover use of full text articles on websites.

5. Where **'reuse in a dissertation/thesis'** has been selected the following terms apply: Print rights of the final author's accepted manuscript (for clarity, NOT the published version) for up to 100 copies, electronic rights for use only on a personal website or institutional repository as defined by the Sherpa guideline (www.sherpa.ac.uk/romeo/).

6. Permission granted for books and journals is granted for the lifetime of the first edition and does not apply to second and subsequent editions (except where the first edition permission was granted free of charge or for signatories to the STM Permissions Guidelines http://www.stm-assoc.org/copyright-legal-affairs/permissions/permissions-guidelines/), and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence.

7. Rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

8. The Licensor's permission must be acknowledged next to the licensed material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

9. Use of the material for incidental promotional use, minor editing privileges (this does not include cropping, adapting, omitting material or any other changes that affect the meaning, intention or moral rights of the author) and copies for the disabled are permitted under this licence.

10. Minor adaptations of single figures (changes of format, colour and style) do not require the Licensor's approval. However, the adaptation should be credited as shown in Appendix below.

**Appendix — Acknowledgements:**

**For Journal Content:**
Reprinted by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

For **Advance Online Publication papers:**
Reprinted by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM].)

**For Adaptations/Translations:**
Adapted/Translated by permission from **[the Licensor]**: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

**Note: For any republication from the British Journal of Cancer, the following credit line style applies:**

Reprinted/adapted/translated by permission from **[the Licensor]**: on behalf of Cancer Research UK: : **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication)

For **Advance Online Publication** papers:
Reprinted by permission from The **[the Licensor]**: on behalf of Cancer Research UK: **[Journal Publisher** (e.g. Nature/Springer/Palgrave)] **[JOURNAL NAME] [REFERENCE CITATION** (Article name, Author(s) Name), **[COPYRIGHT]** (year of publication), advance online publication, day month year (doi: 10.1038/sj.[JOURNAL ACRONYM])

**For Book content:**
Reprinted/adapted by permission from **[the Licensor]**: **[Book Publisher** (e.g. Palgrave Macmillan, Springer etc) **[Book Title]** by **[Book author**(s)] **[COPYRIGHT]** (year of publication)

**Other Conditions**:

Version 1.1

**Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.**

## 8.3 Publication 3: Investigations on the Impact of the Special Flavor Hop Variety Huell Melon on the Odor-Active Compounds in Late Hopped and Dry Hopped Beers

### 8.3.1 Bibliographic Data


| | |
|---|---|
| Title: | Investigations on the impact of the special flavor hop variety Huell Melon on the odor-active compounds in late hopped and dry hopped beers |
| Authors: | Silva D. Neiens and Martin Steinhaus |
| Journal: | Journal of Agricultural and Food Chemistry |
| Publication year: | 2019 |
| Volume: | 67 |
| Issue: | 1 |
| Pages: | 364–371 |
| DOI: | 10.1021/acs.jafc.8b05663 |
| Hyperlink: | https://pubs.acs.org/doi/10.1021/acs.jafc.8b05663 |


### 8.3.2 Publication Reprint

A reprint of publication 3, investigations on the impact of the special flavor hop variety Huell Melon on the odor-active compounds in late hopped and dry hopped beers, follows starting with the next page.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2019, 67, 364−371

pubs.acs.org/JAFC

# Investigations on the Impact of the Special Flavor Hop Variety Huell Melon on the Odor-Active Compounds in Late Hopped and Dry Hopped Beers

Silva D. Neiens and Martin Steinhaus*

Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Straße 34, 85354 Freising, Germany

Supporting Information

**ABSTRACT:** Bottom-fermented and top-fermented beers, both either late or dry hopped with Huell Melon hops, and respective reference beers without late or dry hopping were subjected to a comparative odorant screening by aroma extract dilution analyses. On the basis of differences in the FD factors, 14 odorants were identified as hop-derived. Among them were ethyl 2-methylpropanoate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, myrcene, linalool, and geraniol. Differences between late hopped, dry hopped, and reference beers were substantiated by quantitation. Results showed minimal transfer of myrcene from hops into beer. Moderate transfer was observed for propyl 2-methylbutanoate, geraniol, and linalool. Process-induced changes of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and methyl 2-methylbutanoate were beyond a direct transfer from hops into beer, suggesting a formation from the corresponding hop-derived carboxylic acids by yeast. Spiking experiments revealed that linalool and propyl 2-methylbutanoate contributed particularly to the characteristic aroma of beers flavored with Huell Melon hops.

**KEYWORDS:** *hops, Humulus lupulus, Huell Melon, late hopping, dry hopping, aroma extract dilution analysis, AEDA, stable isotope dilution assay, SIDA, transfer rate, spiking experiment*



## INTRODUCTION

The impact of hops on the olfactory properties of beer has been a mystery for a long time. Whereas the preservative and bittering properties of $\alpha$-acids and their isomerization products in beer are well-known,[1] the contribution of hop volatiles to beer aroma is still not fully understood today. The basis of beer aroma is constituted by fermentation byproducts such as 2-phenylethan-1-ol and 3-methylbutan-1-ol originating in yeast metabolism. Malts contribute compounds such as cooked apple-like smelling (*E*)-$\beta$-damascenone and caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (furaneol).[2] Hops do not necessarily contribute to the aroma of beer. If only a single hop addition at the beginning of the boil is applied, hop volatiles are almost completely lost with the exhaust vapors, and residual amounts reaching the final beer would stay clearly below their odor threshold values.[3,4] If a perceivable hop aroma in the beer is desired, a second portion of hops has to be added later in the process, such as shortly before the end of the boil or to the whirlpool.[5] Particularly when using traditional European varieties for this process, known as late hopping, the result is a floral aroma note in the beer. This note is predominantly caused by a single compound, namely linalool.[6−14] Depending on the variety, further hop-derived compounds may exceed their odor threshold values in the final beer. Among them are geraniol, 4-methyl-4-sulfanylpentan-2-one, ethyl 3-methylbutanoate, hexanal, ethyl 4-methylpentanoate, 3-methylbut-2-enal, (2*E*,6*Z*)-nona-2,6-dienal, and *trans*-4,5-epoxy-(2*E*)-dec-2-enal.[15−17] The influence of hops on the aroma of beer is not limited to a simple transfer of odorants from hops into beer but may also include hop-derived precursors, such as terpene alcohol glycosides, cysteine adducts, and glutathione adducts, that undergo biotransformation to odor-active compounds during fermentation.[18−28]

An approach even more effective than late hopping to achieve a hoppy aroma in the beer is dry hopping.[29] During dry hopping, hops are added even later in the process, typically to the green beer after the main fermentation. Dry hopping is widely used by craft brewers. The increasing number of microbreweries in combination with their preference for rather higher hop dosages has not only tremendously pushed the production of hops in recent years but also promoted the development of new varieties with novel aroma characteristics. Among the varieties recently launched by the German Hop Research Center in Hüll, Germany, is Huell Melon.[30] The aroma of Huell Melon is characterized by a strong fruity note reminiscent of cantaloupe. We recently screened the volatiles isolated by solvent extraction and solvent-assisted flavor evaporation (SAFE) from different hop varieties including Huell Melon for odor-active compounds using aroma extract dilution analysis (AEDA).[31] Among the most potent odor-active compounds in the Huell Melon hops were geranium leaf-like smelling myrcene, citrusy and floral smelling (3*R*)-linalool, and cheesy smelling 2- and 3-methylbutanoic acids, all of which were also major odorants in other hop varieties. By contrast, fruity smelling compounds ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and propyl 2-methylbutanoate were revealed as variety specific odorants in Huell Melon hops.

Received: October 15, 2018
Revised: December 10, 2018
Accepted: December 12, 2018
Published: December 12, 2018




DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

Stable isotope dilution quantitation of these esters confirmed clearly higher concentrations in Huell Melon than in other varieties, suggesting that they were causative for the fruity and cantaloupe-like aroma note.

The aim of the present study was to brew beers late hopped and dry hopped with Huell Melon hops and to study the process-induced changes of important odor-active compounds from the hops to the final beer.

## MATERIALS AND METHODS

**Hops.** Type 90 pellets of Huell Melon hops, harvest 2014, 5.8% α-acid, were obtained from Hopsteiner (Mainburg, Germany). The pellets were from the same batch as recently used for odorant screening and odorant quantitation in hops.[31]

**Beers.** A total of six beers were brewed, among which were a reference beer, a late hopped beer, and a dry hopped beer produced with a bottom-fermenting yeast as well as a reference beer, a late hopped beer, and a dry hopped beer produced with a top-fermenting yeast. Each beer was brewed starting from industrial wort (10 L; 100% Pilsen malt). Wort gravity was 11.5°P (density equivalent to 11.5 g sucrose per 100 g of wort). The wort boiling time was 60 min. Worts were bittered with aroma-free α-extract (20% α-acid; 4.5 g α/hL for reference and dry hopped beers, 1.3 g α/hL for late hopped beers; Hopsteiner) added at the beginning of the boil. For the production of the late hopped beers, hops (2.5 g/L) were added in one portion to the whirlpool. Fermentations were conducted either with a bottom-fermenting yeast (W34/70; $10^6$ cells/mL) at 14 °C or with a top-fermenting yeast (OK3; $10^5$ cells/mL) at 20 °C. During fermentations, residual extract and diacetyl concentration were continuously monitored. For the production of the dry hopped beers, hops (2.5 g/L) were added in one portion after the residual extract had decreased to <3%. No agitation was applied during dry hopping (static dry hopping). After the diacetyl concentration had decreased to <0.12 mg/L, beers were cooled to −2 °C. After 7 days of cold storage, hops were removed from the dry hopped beers. After 14 days of cold storage, yeast was removed by filtration through diatomaceous earth. Filtered beers were bottled (330 mL, brown glass) under $CO_2$ and stored at 6 °C. Final alcohol content of the finished beers ranged between 4.9 and 5.4 vol %.

**Reference Odorants.** Compounds **1**−**6**, **9**, **10**, **14**−**18**, **20**−**22**, **24**, **26**−**28**, **30**, **32**−**34**, and **36** were purchased from Sigma-Aldrich (Taufkirchen, Germany). Compound **23** was obtained from Symrise (Holzminden, Germany). Compounds **31** and **38** were from Alfa Aesar (Karlsruhe, Germany), and compound **35** was from Merck (Darmstadt, Germany). Compounds **7**,[32] **11**,[33] **12**,[34] **37**,[35] and **40**[31] were synthesized as detailed in the literature.

**Isotopically Substituted Odorants.** The following compounds were prepared as described previously: ($^2H_2$)-**17** [3,7-dimethyl-(1,2-$^2H_2$)octa-1,6-dien-3-ol],[10] ($^2H_2$)-**24** [(2*E*)-3,7-dimethyl-(1,1-$^2H_2$)octa-2,6-dien-1-ol],[36] ($^2H_5$)-**2** [($^2H_5$)ethyl 2-methylpropanoate],[31] ($^2H_3$)-**3** [($^2H_3$)methyl 2-methylbutanoate],[31] ($^2H_3$)-**4** [(2,2,2-$^2H_3$)ethyl 2-methylbutanoate],[31] and ($^2H_3$)-**7** [(3,3,3-$^2H_3$)-propyl 2-methylbutanoate].[31] ($^{13}C_3$)-**36** [7-($^{13}$C)methyl-3-methylidene-(7,8-$^{13}C_2$)octa-1,6-diene] was purchased from AromaLab (Planegg, Germany).

**Miscellaneous Chemicals.** Diethyl ether and ethanol (LiChrosolv) were purchased from VWR (Darmstadt, Germany). Diethyl ether was freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings.

**GC-O/FID.** A Trace GC Ultra gas chromatograph (Thermo Scientific, Dreieich, Germany) was equipped with a cold on-column injector, a flame ionization detector (FID), and a tailor-made sniffing port.[37] The following fused silica columns were used, a (1) ZB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film and a (2) ZB-5, 30 m × 0.32 mm i.d., 0.25 μm film (both Phenomenex, Aschaffenburg, Germany). The carrier gas was helium at 90 kPa (ZB-FFAP) and 60 kPa (ZB-5) constant pressure, respectively. The injection volume was 1 μL. The initial oven temperature was 40 °C (2 min), and the gradients were typically 6 °C/min to 230 °C for ZB-FFAP and to 240 °C for ZB-5. The column effluents were divided 1:1 using a deactivated Y-shaped glass splitter and two deactivated fused silica capillaries (50 cm × 0.25 mm i.d.) connecting the splitter to the FID and the sniffing port. The sniffing port was mounted on a heated (250 °C) detector base of the GC.

**GC-MS.** A HP 5890 Series II gas chromatograph (Hewlett-Packard, Heilbronn, Germany) was equipped with a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film or DB-5, 30 m × 0.25 mm i.d., 0.25 μm film (both Agilent Technologies, Waldbronn, Germany), and connected to an MAT 95 sector field mass spectrometer (Finnigan, Bremen, Germany). The carrier gas was helium at a 1.9 mL/min constant flow. The injection volume was 0.5 μL. All other GC conditions were equivalent to those used in the GC-O/FID analyses. MS(EI) spectra were generated at 70 eV using a scan range of $m/z$ 35−300. MS(CI) spectra were obtained at 150 eV using isobutane as the reagent gas and a scan range of $m/z$ 85−350. For the evaluation of the mass spectra, the Xcalibur software (Thermo) was used.

**GC-GC-MS(CI).** A Trace GC Ultra (Thermo) was equipped with a cold on-column injector and a fused silica column, DB-FFAP, 30 m × 0.32 mm i.d., 0.25 μm film (Agilent) or DB-WAX, 30 m × 0.32 mm i.d., 1.00 μm film (Agilent). The column end was connected to a moving column stream switching (MCSS) device (Thermo), conveying the eluate via uncoated fused silica capillaries time-programmed either simultaneously to an FID (250 °C) and a sniffing port (230 °C) or via a heated (250 °C) hose to a cold trap located in the oven of a CP 3800 GC (Varian, Darmstadt, Germany). The cold trap consisted of a piece of steel tubing housing the capillary and could be cooled by liquid nitrogen. The end of the capillary was connected to a fused silica column, DB-1701, 30 m × 0.25 mm i.d., 0.25 μm film (Agilent). The end of this column was connected to a Saturn 2200 mass spectrometer (Varian) operated in CI mode with methanol as the reagent gas. Helium served as a carrier gas (100 kPa for DB-FFAP; 120 kPa for DB-WAX) and was also used as a makeup gas for the MCSS device (50 kPa). The injection volume was 2 μL. The oven temperature in the first dimension was 40 °C for 2 min and then was ramped at 6 °C/min to 230 °C. The oven temperature in the second dimension was 40 °C for 2 min and then was ramped at 6 °C/min to 240 °C.

**Aroma Extract Dilution Analysis (AEDA).**[38] The beer (250 mL) was shaken with diethyl ether (2 × 300 mL). The organic phases were combined and subjected to SAFE[39] at 30 °C. The distillate was dried over anhydrous sodium sulfate and concentrated (1 mL), first using a Vigreux column (50 × 1 cm) and subsequently using a Bemelmans microdistillation device.[40] Beer volatile isolates were analyzed by GC-O/FID.

During a GC-O/FID run, the sniffer placed her or his nose closely above the top of the sniffing port and evaluated the effluent. Whenever an odor was perceived, the retention time and the odor quality were noted in the FID chromatogram printed by a recorder. GC-O/FID analyses of all beer volatile isolates were carried out by three experienced sniffers (two females, one male; age 26−48) using the FFAP column as well as the ZB-5 column. Each sniffer repeated the GC-O/FID analyses of each individual beer volatile isolate until the outcome was reproducible. For each odorant a linear retention index (RI) was calculated on both columns (FFAP, ZB-5) from its retention time and the retention times of adjacent *n*-alkanes by linear interpolation.

The beer volatile isolates were stepwise diluted 1:2 with diethyl ether to obtain dilutions of 1:2, 1:4, 1:8, 1:16, etc. Diluted samples were analyzed by GC-O/FID using the FFAP column. A flavor dilution (FD) factor was assigned to each odor-active compound, representing the dilution factor of the highest diluted sample in which the odorant was detected during GC-O/FID analysis by any of the three sniffers.

The structures of the odorants were assigned by comparing their retention indices on FFAP and ZB-5, their mass spectrum obtained by GC-MS, and their odor quality as perceived at the sniffing port during GC-O/FID with data obtained from authentic reference compounds analyzed in parallel (same instrument, consecutive runs).



DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

**Table 1. Odorants in the SAFE Distillates Obtained from the Bottom-Fermented Beers: Reference Beer (RB), Late Hopped Beer (LHB), and Dry Hopped Beer (DHB)**

| | | | RI[d] | | FD factor[e] | | |
|---|---|---|---|---|---|---|---|
| no. | odorant[a,b] | odor[c] | FFAP | ZB-5 | RB | LHB | DHB |
| **1** | methyl 2-methylpropanoate | fruity | 939 | <700 | 32 | 32 | 64 |
| **2** | ethyl 2-methylpropanoate | fruity | 956 | 760 | 32 | 128 | 512 |
| **3** | methyl 2-methylbutanoate | fruity | 1003 | 785 | 16 | 64 | 128 |
| **4** | ethyl 2-methylbutanoate | fruity | 1023 | 852 | 16 | 64 | 512 |
| **5** | 2-methylpropan-1-ol | malty | 1087 | <700 | 1024 | 512 | 512 |
| **6** | 3-methylbutyl acetate | fruity, banana | 1110 | 893 | 32 | 32 | 64 |
| **7** | propyl 2-methylbutanoate | fruity | 1157 | 961 | <1 | 16 | 32 |
| **8** | unknown | fruity | 1185 | | 8 | 8 | 16 |
| **9** | 2-/3-methylbutan-1-ol | malty | 1202 | 744 | 512 | 1024 | 1024 |
| **10** | ethyl hexanoate | fruity, pineapple | 1225 | 1008 | 32 | 64 | 64 |
| **11** | 2-acetyl-1-pyrroline | roasty, popcorn | 1334 | 934 | 64 | 64 | 128 |
| **12** | (3*E*,5*Z*)-undeca-1,3,5-triene | fresh, pineapple | 1386 | 1186 | <1 | 16 | 32 |
| **13** | unknown | fruity | 1416 | | <1 | 4 | 16 |
| **14** | acetic acid | vinegar, pungent | 1424 | <700 | 64 | 128 | 128 |
| **15** | 3-(methylsulfanyl)propanal | cooked potato | 1446 | 914 | 64 | 32 | 64 |
| **16** | propanoic acid | cheesy, pungent | 1528 | 843 | 8 | 8 | 16 |
| **17** | linalool | citrusy, floral | 1538 | 1106 | <1 | 32 | 64 |
| **18** | 2-methylpropanoic acid | cheesy | 1558 | 792 | 32 | 32 | 64 |
| **19** | unknown | roasty | 1579 | | 32 | 16 | 32 |
| **20** | butanoic acid | cheesy | 1623 | 829 | 64 | 64 | 128 |
| **21** | 2-/3-methylbutanoic acid | cheesy | 1688 | 879 | 128 | 256 | 256 |
| **22** | 3-(methylsulfanyl)propan-1-ol | cooked potato | 1718 | 993 | 32 | 16 | 32 |
| **23** | (*E*)-*β*-damascenone | cooked apple | 1808 | 1384 | 512 | 256 | 256 |
| **24** | geraniol | floral, rose | 1858 | 1250 | <1 | 2 | 16 |
| **25** | unknown | clove, vanilla | 1875 | | <1 | <1 | 16 |
| **26** | 2-phenylethan-1-ol | honey, floral | 1913 | 1125 | 1024 | 1024 | 1024 |
| **27** | *β*-ionone | floral, violet | 1945 | 1490 | 8 | 8 | 16 |
| **28** | EHMF[f] | caramel | 2077 | 1139 | 128 | 128 | 64 |
| **29** | unknown | caramel | 2131 | | 16 | 32 | 32 |
| **30** | sotolon | seasoning | 2212 | 1113 | 64 | 128 | 64 |
| **31** | 2-methoxy-4-vinylphenol | smoky, clove | | 1310 | | | |
| **32** | 2-aminoacetophenone | foxy | 2215 | 1295 | 16 | 8 | 16 |
| **33** | abhexone | seasoning | 2271 | 1200 | 32 | 32 | 64 |
| **34** | phenylacetic acid | honey | 2548 | 1257 | 16 | 16 | 16 |
| **35** | vanillin | vanilla | 2578 | 1411 | 32 | 16 | 32 |

[a]Odorants exhibiting an FD factor of ≥16 in at least one of the three beer samples; odorants are listed in the order of increasing RI on FFAP. [b]Structure assignment of each odorant was based on the comparison of the compound's retention indices on FFAP and ZB-5, its mass spectrum obtained by GC-MS, as well as its odor quality as perceived at the sniffing port during GC-O with data obtained from authentic reference compounds analyzed in parallel. [c]Odor quality as perceived at the sniffing port during GC-O. [d]Retention index, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [e]Flavor dilution factor; the dilution factor of the highest dilution of the concentrated SAFE distillate in which the odorant was detected during GC-O by any of three panelists. [f]2-Ethyl-4-hydroxy-5-methylfuran-3(2*H*)-one.

**Stable Isotope Dilution Assays (SIDAs).** To beer (1−150 mL), isotopically substituted analogues of the target compounds (0.03−1 μg) were added in diethyl ether (10−200 mL) as internal standards, and the mixture was vigorously stirred for 1 h at ambient temperature. The organic phase was separated, and the aqueous phase was shaken with a second portion of diethyl ether (10−200 mL). The combined organic phases were subjected to SAFE. The distillate was dried over anhydrous sodium sulfate and concentrated (200 μL).

Aliquots of the concentrates were analyzed by two-dimensional heart-cut gas chromatography−mass spectrometry using the GC-GC-MS(CI) system and a DB-FFAP column (compounds **17** and **24**) or a DB-WAX column (compounds **2**−**4**, **7**, and **36**) in the first dimension. The retention times of the target compounds and their isotopically substituted analogues in the first and second dimensions had previously been determined by analysis of reference mixtures and by using the FID and the sniffing port as monitor detectors. At the elution time of the target compounds, a heart-cut (∼1 min) of the eluate of the first column containing the respective target compound and its isotopically substituted analogue was transferred via the MCSS and the transfer line to the second oven. Transferred substances were refocused at the cold trap inside the second oven. Cooling was turned off, and the second oven and the mass spectrometer were started.

Peak areas corresponding to the analyte and internal standard were obtained from the extracted ion chromatograms using characteristic quantifier ions. The concentration of each target compound in the beer samples was then calculated from the area counts of the analyte peak, the area counts of the standard peak, the amount of beer used, and the amount of standard added, by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in five different concentration ratios (5:1, 2:1, 1:1, 1:2, and 1:5). Individual quantifier ions and calibration line equations are available in the Supporting Information.

**Spiking Experiments.** Reference beers (50 mL) were spiked with odorants dissolved in water/ethanol (95/5, v/v; 50−200 μL) to reach



DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

Table 2. Hop-Derived Odorants in the SAFE Distillates Obtained from the Bottom-Fermented Late Hopped Beer (LHB) and the Bottom-Fermented Dry Hopped Beer (DHB)

| | | | RI[d] | | FD factor[e] | | |
|---|---|---|---|---|---|---|---|
| no. | odorant[a,b] | odor[c] | FFAP | ZB-5 | RB | LHB | DHB |
| **2** | ethyl 2-methylpropanoate | fruity | 956 | 760 | 32 | 128 | 512 |
| **3** | methyl 2-methylbutanoate | fruity | 1003 | 785 | 16 | 64 | 128 |
| **4** | ethyl 2-methylbutanoate | fruity | 1023 | 852 | 16 | 64 | 512 |
| **7** | propyl 2-methylbutanoate | fruity | 1157 | 961 | <1 | 16 | 32 |
| **36** | myrcene | geranium leaf | 1168 | 998 | <1 | 2 | 2 |
| **37** | (5*Z*)-octa-1,5-dien-3-one | geranium leaf | 1366 | 995 | <1 | 1 | 4 |
| **38** | 4-methyl-4-sulfanylpentan-2-one | black currant | 1376 | 948 | <1 | 2 | 4 |
| **12** | (3*E*,5*Z*)-undeca-1,3,5-triene | fresh, pineapple | 1386 | 1186 | <1 | 16 | 32 |
| **13** | unknown | fruity | 1416 | | <1 | 4 | 16 |
| **17** | linalool | citrusy, floral | 1538 | 1106 | <1 | 32 | 64 |
| **39** | unknown | sweaty | 1688 | | <1 | 8 | 4 |
| **40** | (1*R*,4*S*)-calamenene | clove, herbaceous | 1831 | 1538 | <1 | 1 | 1 |
| **24** | geraniol | floral, rose | 1858 | 1250 | <1 | 2 | 16 |
| **25** | unknown | clove, vanilla | 1875 | | <1 | <1 | 16 |

[a]Odorants exhibiting an FD factor ≥ 1 in the late hopped beer and/or the dry hopped beer samples and a clearly lower FD factor in the reference beer; odorants are listed in the order of increasing RI on FFAP. [b,c,d,e]cf. Table 1.

the concentrations in the late hopped and dry hopped beers. Spiked beers were orthonasally compared to the respective reference beer to which the same amount of water/ethanol (95/5, v/v) but no odorant had been added. Comparison was performed in 3-AFC tests using a sensory panel (18−23 assessors, males and females, age 21−49). The panel was specifically trained to recognize the odor characteristics of the added compounds by using individual solutions of the odorants dissolved in water/ethanol (95/5; v/v) in concentrations of 100× their odor threshold values in water.

## RESULTS AND DISCUSSION

**Comparative Screening for Odor-Active Compounds in the Reference Beer, the Late Hopped Beer, and the Dry Hopped Beer.** Application of AEDA to the bottom-fermented beers revealed a total of 35 odorants exhibiting an FD factor of ≥16 in at least one of the three samples (Table 1). By comparing their retention indices on FFAP and ZB-5, their mass spectra as obtained by GC-MS, and their odor properties as perceived at the sniffing port with data obtained from authentic reference compounds, 30 out of the 35 beer odorants could be assigned.

In all three beers, the highest FD factors (512−1024) were determined for honey, floral smelling 2-phenylethan-1-ol (**26**), malty smelling 2-methylpropan-1-ol (**5**), and malty smelling 2- and 3-methylbutan-1-ol (**9**). These compounds are well-known as major odor-active compounds in beer.[2,11] They originate from the fermentation process and are byproducts of the yeast metabolism.[41] Consequently, their FD factors did not significantly differ between the three beers. The same was true for cooked apple-like smelling (*E*)-β-damascenone (**23**), for which FD factors of 256−512 were found. This important beer aroma compound originates from malt.[2,11,42,43] In the same FD factor range, the fruity smelling esters ethyl 2-methylpropanoate (**2**) and ethyl 2-methylbutanoate (**4**), previously identified as variety specific odorants in Huell Melon hops,[31] appeared in the dry hopped beer. Both compounds showed comparably low FD factors in the reference beer (32 and 16) and higher FD factors in the late hopped beer (128 and 64), but both compounds were among the most potent odorants in the dry hopped beer (FD factor 512). Although these compounds could also have originated from yeast metabolism during fermentation,[44] the clear differences in their FD factors between the reference beer, the late hopped beer, and the dry hopped beer in combination with their prominent role in the aroma of Huell Melon hops in the first instance suggested a simple transfer from hops into beer.

Further compounds that showed FD factors in the hopped beers that were clearly higher than those in the reference beer are depicted in Table 2. Among them was also propyl 2-methylbutanoate (**7**), the third compound previously suggested as a contributor to the characteristic fruity, cantaloupe-like odor note in Huell Melon hops. With 16 and 32, its FD factors in the hopped beers were not as high as the FD factors of ethyl 2-methylpropanoate and ethyl 2-methylbutanoate; however, its absence in the reference beer (FD factor < 1) also identified it as hop-derived compound. Different from ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and propyl 2-methylbutanoate, methyl 2-methylbutanoate (**3**) was not among the esters detected with very high FD factors in the Huell Melon hops. Actually, the concentration of methyl 2-methylbutanoate in the hop sample used for the current study (Huell Melon 2014) was even lower (162 μg/kg) than that in the Polaris and Hallertau Tradition hop samples analyzed in parallel (3020 and 985 μg/kg, respectively).[31] Nevertheless, the FD factors obtained for methyl 2-methylbutanoate in the late hopped beer (64) and in the dry hopped beer (128) were clearly higher than its FD factor in the reference beer (16).

Ten more compounds were suggested to be derived from hops, as they were found odor-active in the hopped beers (FD factors 1−64) but not in the reference beer (FD factor < 1). Among them were linalool (**17**), geraniol (**24**), and 4-methyl-4-sulfanylpentan-2-one (**38**), the impact of which to the aroma of beer has already been demonstrated,[15,16] (3*E*,5*Z*)-undeca-1,3,5-triene (**12**), (5*Z*)-octa-1,5-dien-3-one (**37**), myrcene (**36**), previously characterized (1*R*,4*S*)-calamenene (**40**),[31] and three unknown compounds (**13**, **25**, **39**).

The comparative screening approach detailed above for the identification of hop-derived compounds in bottom-fermented beers was likewise applied to beers produced with a standard top-fermenting yeast. GC-O/FID results (data not shown) were virtually identical to the data obtained from the bottom-


DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

**Table 3. Concentrations of Selected Hop-Derived Odorants in the Bottom-Fermented and in the Top-Fermented Beers: Reference Beer (RB), Late Hopped Beer (LHB), and Dry Hopped Beer (DHB)**

| | | concentration ($\mu$g/L)[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | bottom-fermented beers | | | top-fermented beers | | |
| odorant[a] | odor threshold[b] ($\mu$g/kg) | RB | LHB | DHB | RB | LHB | DHB |
| ethyl 2-methylpropanoate (**2**) | 0.089 | 2.10 | 11.3 | 33.6 | 3.78 | 13.8 | 57.2 |
| methyl 2-methylbutanoate (**3**) | 0.048 | 1.91 | 9.23 | 9.90 | 3.17 | 8.17 | 9.55 |
| ethyl 2-methylbutanoate (**4**) | 0.008 | 0.326 | 0.890 | 4.35 | 0.223 | 0.761 | 5.40 |
| propyl 2-methylbutanoate (**7**) | 0.020 | 0.002 | 0.512 | 0.499 | 0.001 | 0.338 | 0.633 |
| myrcene (**36**) | 1.2 | 0.179 | 7.77 | 6.65 | 0.244 | 15.0 | 8.20 |
| linalool (**17**) | 0.58 | 0.609 | 28.3 | 31.3 | 0.840 | 54.0 | 56.3 |
| geraniol (**24**) | 1.1 | 2.47 | 6.96 | 29.7 | 2.31 | 12.9 | 31.6 |

[a]Numbers in parentheses refer to Tables 1 and 2. [b]Orthonasal odor detection threshold values in aqueous solution. [c]Mean of triplicates; standard deviations were <10%.

fermented beers; in particular, data revealed the same set of hop-derived compounds in the beers.

**Concentrations of Hop-Derived Odorants in Beers.** To substantiate the results of the screening experiments, selected odor-active compounds were quantitated in the reference beers, in the late hopped beers, and in the dry hopped beers. Quantitations were applied to the bottom-fermented beers as well as to the top-fermented beers and included ethyl 2-methylpropanoate (**2**), methyl 2-methylbutanoate (**3**), ethyl 2-methylbutanoate (**4**), propyl 2-methylbutanoate (**7**), myrcene (**36**), linalool (**17**), and geraniol (**24**). All quantitations were accomplished by SIDA using stable isotopically substituted analogues of the target compounds as internal standards to compensate for potential losses during the workup.

Results (Table 3) confirmed the impact of hopping on the amounts of all analyzed compounds in the final beers, as concentrations were clearly higher in the late hopped beers and in the dry hopped beers than in the reference beers. Concentrations in the hopped beers were well above the odor detection threshold values of the individual compounds. Linalool (**17**) and geraniol (**24**) contents were in a typical range previously reported in beer.[3,10,11,16,45] Among the seven compounds, four showed virtually the same concentrations in the dry hopped beers as in the late hopped beers, namely, methyl 2-methylbutanoate (**3**), propyl 2-methylbutanoate (**7**), myrcene (**36**), and linalool (**17**), whereas ethyl 2-methylpropanoate (**2**), ethyl 2-methylbutanoate (**4**), and geraniol (**24**) exhibited clearly higher concentrations in the dry hopped beers than in the late hopped beers. Thus, linalool (**17**) and geraniol (**24**), although both being monoterpene alcohols, in that respect substantially differed in their behavior. Similarly, methyl 2-methylbutanoate (**3**) and propyl 2-methylbutanoate (**7**) behaved differently from the ethyl esters ethyl 2-methylpropanoate (**2**) and ethyl 2-methylbutanoate (**4**). These observations suggested that effects beyond a direct transfer of compounds from hops into beer had occurred. The assumption was substantiated by calculating for each odorant the ratio of the absolute amount quantitated in the late or dry hopped product corrected for the amount present in the reference beer and the amount initially added with the hop pellets. Results (Table 4) revealed percentages between 0.15% and 2000%, indicating that some compounds might have underwent a simple transfer from hops into beer, whereas others, in major parts, were newly formed from hop-derived precursors in the process. Independent of the type of hopping and the type of fermentation, very low transfer rates well below 1% were calculated for myrcene (**36**). This was in agreement with data from the literature. Due to its nonpolar nature, myrcene is effectively adsorbed to yeast cells and further losses occur with carbon dioxide by stripping. Thus, myrcene is almost completely removed during fermentation and filtering, even after dry hopping.[20,29,46,47]

**Table 4. Transfer/Formation Rates of Selected Hop-Derived Odorants in Bottom-Fermented Beers and Top-Fermented Beers: Late Hopped Beer (LHB) and Dry Hopped Beer (DHB)**

| | transfer/formation rates (%)[b] | | | |
|---|---|---|---|---|
| | bottom-fermented beers | | top-fermented beers | |
| odorant[a] | LHB | DHB | LHB | DHB |
| ethyl 2-methylpropanoate (**2**) | 170 | 590 | 190 | 1000 |
| methyl 2-methylbutanoate (**3**) | 1800 | 2000 | 1200 | 1600 |
| ethyl 2-methylbutanoate (**4**) | 35 | 250 | 33 | 320 |
| propyl 2-methylbutanoate (**7**) | 15 | 15 | 10 | 19 |
| myrcene (**36**) | 0.18 | 0.15 | 0.35 | 0.19 |
| linalool (**17**) | 96 | 110 | 190 | 190 |
| geraniol (**24**) | 7.8 | 47 | 18 | 51 |

[a]Numbers in parentheses refer to Tables 1 and 2. [b]Transfer/formation rates were calculated as (concentration in late/dry hopped beer − concentration in reference beer)/(concentration in hops × hop dosage); concentrations in hops were taken from reference 31 and are also available in the Supporting Information, and the hop dosage was 2.5 g/L.

Linalool (**17**) showed little differences in the transfer/formation rates between late and dry hopped beers, but significant differences between the bottom-fermented and the top-fermented beers. Percentages in the bottom-fermented beers were close to 100%, thus suggesting an effective transfer of the compound from hops to beer. This was in agreement with data reported earlier.[16,29] In beers brewed with different hop varieties and hop dosages of 60% and 40% at the beginning of the boil and to the whirlpool, respectively, results for linalool were 32−44%, which was interpreted as a more or less complete transfer of the linalool added with the second portion.[16] Another study also revealed complete recovery of linalool after dry hopping. Four beers dry hopped with novel



DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

German special flavor hops, among them Huell Melon, showed results for linalool of 100−111%.[29]

With 190%, rates for linalool (**17**) in the top-fermented beers were clearly beyond 100%, thus pointing to an additional formation of the compound in the brewing process from precursors supplied with the hops. Linalool formation has been observed during fermentation.[22,23] It may enzymatically be formed from hop-derived linalyl glycosides by yeast.[18,21,22] Another potential source of additional linalool is the metabolic transformation of other terpenoids by yeast. It has been shown that geraniol and nerol can be converted into linalool and citronellol.[19,20,24,25,28] In line with the differences between the bottom-fermented and the top-fermented beers in the current study, this conversion was reported to be more effective during top-fermentation than during bottom-fermentation.[20]

Different from linalool, geraniol (**24**) showed clear differences between late and dry hopped beers in both the bottom-fermented beers and the top-fermented beers. With 7.8% and 18% in the late hopped bottom-fermented beer and the late hopped top-fermented beer, respectively, results were clearly lower than in the dry hopped beers, where percentages were 47%−51%. A higher recovery of geraniol by dry hopping than by late hopping was reported in another comparative investigation earlier; however, no transfer rates were calculated in this study.[45] Transfer rates for geraniol after late hopping were reported in another publication and amounted to 14%−37%. However, in this study only 40% of the hops had been added to the whirlpool, whereas 60% had been added at the beginning of the boil.[16] Transfer rates for geraniol after dry hopping with four different hop varieties were recently calculated, and results ranged between 49% (Huell Melon) and 178% (Hallertau Blanc).[29] Thus, the rate reported for Huell Melon was virtually the same as the rate calculated in the current study (47%). In general, recoveries of geraniol were clearly lower than those obtained for linalool. One reason might be that cleavage of glycosides does not contribute as much to geraniol formation as it does to linalool formation.[18,48] Furthermore, it has been shown that geraniol can be transferred by yeast to compounds such as geranyl acetate, citronellol, citronellyl acetate, linalool, and $\alpha$-terpineol.[19,20,24,25] Differences between the late hopped beers and the dry hopped beers could thus be associated with a more effective transformation of geraniol during late hopping.

The most interesting results were obtained when rates were calculated for the fruity smelling esters ethyl 2-methylpropanoate (**2**), methyl 2-methylbutanoate (**3**), ethyl 2-methylbutanoate (**4**), and propyl 2-methylbutanoate (**7**). With 15% in the bottom-fermented beers and 10%−19% in the top-fermented beers, recovery of propyl 2-methylbutanoate (**7**) was comparably low and in the range suggesting a simple transfer from the hops into the beer. With rates of 35% and 33%, the same could be assumed for ethyl 2-methylbutanoate (**4**) in the late hopped beers, but with 250% and 320%, results in the dry hopped beers were clearly beyond a simple transfer and suggested a formation of the compound during the secondary fermentation. Even higher rates were calculated for ethyl 2-methylpropanoate (**2**) and methyl 2-methylbutanoate (**3**), not only in the dry hopped beers but also in the late hopped beers. For these compounds, formation from hop-derived precursors seems to be more relevant than the direct transfer from hops into beer. The most evident potential precursors would be the corresponding carboxylic acids. 2-Methylpropanoic acid and 2-methylbutanoic acid are abundant in hops, and their conversion into ethyl esters during alcoholic fermentation would be highly comprehensible. The low rates for propyl 2-methylbutanoate (**7**) would also fit, as propan-1-ol is not available in the beer. The high formation of methyl 2-methylbutanoate (**3**), on the other hand, is then somewhat surprising. It is also noteworthy that neither methyl nor ethyl 3-methylbutanoate were detected among the odor-active compounds in the hopped beers (cf. Table 1), although their odor threshold values are in the same low range as those of the 2-methylbutanoic acid esters,[32,49] and 3-methylbutanoic acid is even more abundant in hops than 2-methylbutanoic acid.[31] The absence of ethyl 3-methylbutanoate in hopped beers was recently also reported in another study.[50] To confirm the role of hop-derived carboxylic acids for the formation of highly odor-active esters in late and dry hopped beers, spiking the hops with isotopically substituted analogues of 2-methylpropanoic acid, 2-methylbutanoic acid, and 3-methylbutanoic acid and analysis of the isotopologue ratios of the esters in the final beers would be the method of choice; however, this was beyond the scope of the current study. Another open question is why the esters are more effectively formed when the hops are added after primary fermentation.

**Spiking Experiments.** To assess the contribution of the hopping-associated odorants to the overall aroma of the hopped beers, reference beers were spiked with the individual compounds to reach their concentrations in the late hopped and dry hopped beers. In orthonasal 3-AFC tests, spiked reference beers were compared to the respective reference beer without addition. Results (Figure 1) confirmed the generally

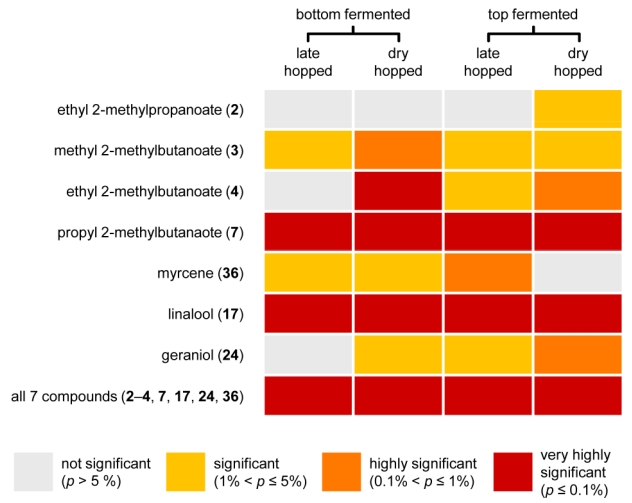



**Figure 1.** Olfactory effect of spiking the reference beers with individual hop odorants at the concentration levels corresponding to the late and dry hopped beers (detailed data on the 3-AFC tests are included in the Supporting Information).

outstanding role of linalool for the hoppy aroma of beer. Independent of the type of fermentation of the base beer, linalool (**17**) addition resulted in a clear sensory difference between the spiked beer and the unspiked beer (all $p$ values ≤ 0.1%). The effect of geraniol spiking was less pronounced. Nevertheless, geraniol (**24**) was significantly detected when added to the respective reference beer in the amounts previously quantitated in the dry hopped bottom-fermented beer ($p$ value ≤ 5%), the late hopped top-fermented beer ($p$ value ≤ 5%), and the dry hopped top-fermented beer ($p$ value


DOI: 10.1021/acs.jafc.8b05663
J. Agric. Food Chem. 2019, 67, 364−371

≤ 1%). Despite its low concentrations, even myrcene (**36**) was significantly detected in three out of four beers. Individual spiking of the four esters revealed in particular a high impact of propyl 2-methylbutanoate (**7**) on the aroma of the hopped beers. Although propyl 2-methylbutanoate showed a rather low transfer from hops into beer (cf. Table 4), its addition was clearly detectable in the 3-AFC tests and $p$ values were low (≤0.1%). Spiking experiments also suggested an aroma contribution of methyl 2-methylbutanoate (**3**) and ethyl 2-methylbutanoate (**4**), particularly to the dry hopped beers. By contrast, addition of ethyl 2-methylpropanoate (**2**) was not significantly detected in three out of four spiking experiments. Only addition of the highest amount associated with the dry hopped top-fermented beer revealed significance, but only at the lowest level ($p$ value ≤ 5%). In a final test, the reference beers were spiked with all seven compounds simultaneously. All four spiked beers could clearly be differentiated from the respective unspiked beers. However, this was to be expected, as already the individual spiking of linalool (**17**) and propyl 2-methylbutanoate (**7**) had returned the highest significance.

In summary, the results of this study confirmed the contribution of linalool, geraniol, and myrcene for the aroma of hop-flavored beers and shed new light on the role of some esters, which are obviously not only transferred from hops into beer but in major parts are newly formed after late and particularly after dry hopping. Their precursors are presumably the corresponding carboxylic acids which are alkylated by the yeast. This hypothesis, however, needs to be confirmed in further studies.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b05663.

Quantitation parameters (quantifier ions and calibration line equations) used in the stable isotope dilution assays; previously published data on odorant concentrations in the Huell Melon hops; and detailed data on the 3-AFC tests employed in the spiking experiments (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: martin.steinhaus@tum.de. Tel.: +49 8161 71 2991. Fax: +49 8161 71 2970.

### ORCID

Martin Steinhaus: 0000-0002-9879-1474



### Funding

This IGF Project of the FEI was supported via AiF within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament (project no. 18069 N).


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS


The authors thank Anita Herzner and Inge Kirchmann for excellent technical assistance, Dr. Martin Biendl, Hopsteiner, Mainburg, Germany, for providing the hops, and Dr. Stefan Hanke, Bitburger Braugruppe GmbH, Bitburg, Germany, for performing the brewing trials.


## ■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; AFC, alternative forced choice; CI, chemical ionization; EI, electron ionization; FD factor, flavor dilution factor; FFAP, free fatty acid phase; FID, flame ionization detector; GC, gas chromatography; GC-O, gas chromatography−olfactometry; MCSS, moving column stream switching; MS, mass spectrometry; RI, retention index; SAFE, solvent-assisted flavor evaporation; SIDA, stable isotope dilution assay

## ■ NOMENCLATURE

abhexone, 3-hydroxy-4-methyl-5-ethylfuran-2(5*H*)-one; 2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone; (1*R*,4*S*)-calamenene, (1*R*,4*S*)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene; citronellol, 3,7-dimethyloct-6-en-1-ol; citronellyl acetate, 3,7-dimethyloct-6-en-1-yl acetate; (*E*)-β-damascenone, (2*E*)-1-(2,6,6-trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one; geraniol, (2*E*)-3,7-dimethylocta-2,6-dien-1-ol; geranyl acetate, (2*E*)-3,7-dimethylocta-2,6-dien-1-yl acetate; β-ionone, 4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one; linalool, 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; sotolon, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; α-terpineol, 2-(4-methylcyclohex-3-en-1-yl)-propan-2-ol; vanillin, 4-hydroxy-3-methoxybenzaldehyde

## ■ REFERENCES


(1) Steenackers, B.; De Cooman, L.; De Vos, D. Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. *Food Chem.* **2015**, *172*, 742−756.

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

(3) Kaltner, D.; Thum, B.; Forster, C.; Back, W. Investigations into hoppy aroma in Pilsner beers under variation in technological parameters (in German). *Monatsschr. Brauwiss.* **2001**, *54*, 199−205.

(4) Kishimoto, T.; Wanikawa, A.; Kagami, N.; Kawatsura, K. Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. *J. Agric. Food Chem.* **2005**, *53*, 4701−4707.

(5) Mitter, W.; Cocuzza, S. Revival of a process. Dry hopping - basics and techniques. *Brew. Beverage Ind. Int.* **2013**, *3*, 28−30.

(6) Peacock, V. E.; Deinzer, M. L.; McGill, L. A.; Wrolstad, R. E. Hop aroma in American beer. *J. Agric. Food Chem.* **1980**, *28*, 774−777.

(7) Peacock, V. E.; Deinzer, M. L. Chemistry of hop aroma in beer. *J. Am. Soc. Brew. Chem.* **1981**, *39*, 136−141.

(8) 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.

(9) 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.

(10) 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.

(11) 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.

(12) Fritsch, H. T.; Kaltner, D.; Schieberle, P.; Back, W. Unlocking the secret behind hop aroma in beer. *Brauwelt International* **2005**, *23*, 22−23.




DOI: 10.1021/acs.jafc.8b05663
*J. Agric. Food Chem.* 2019, 67, 364−371

(13) Hanke, S.; Herrmann, M.; Rückerl, J.; Back, W. The transfer of hop aroma compounds during the brewing process. *Brauwelt International* **2009**, *27*, 191−194.
(14) Nielsen, T. P. Character impact of hop aroma compounds in ale, hop flavor, and aroma. In *Hop flavour and aroma, Proceedings of the 1st International Brewers Symposium*; Shellhammer, T. H., Ed.; Master Brewers Association of the Americas: St. Paul, MN, 2009; pp 59−77.
(15) 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.
(16) Steinhaus, M.; Schieberle, P. Transfer of the potent hop odorants linalool, geraniol, and 4-methyl-4-sulfanyl-2-pentanone from hops into beer. In *European Brewery Convention, Proceedings of the 31st EBC Congress, Venice 2007*; Fachverlag Hans Carl: Nuremberg, Germany, 2007; Chapter 112, pp 1−8.
(17) Reglitz, K.; Steinhaus, M. Quantitation of 4-methyl-4-sulfanylpentan-2-one (4MSP) in hops by a stable isotope dilution assay in combination with GCxGC-TOFMS: Method development and application to study the influence of variety, provenance, harvest year, and processing on 4MSP concentrations. *J. Agric. Food Chem.* **2017**, *65*, 2364−2372.
(18) Goldstein, H.; Ting, P.; Navarro, A.; Ryder, D. Water-soluble hop flavor precursors and their role in beer flavor. In *European Brewery Convention, Proceedings of the 27st EBC Congress, Cannes 1999*; Fachverlag Hans Carl: Nuremberg, Germany, 1999; pp 53−62.
(19) King, A.; Richard Dickinson, J. Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae, Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast* **2000**, *16*, 499−506.
(20) King, A. J.; Dickinson, J. R. Biotransformation of hop aroma terpenoids by ale and lager yeasts. *FEMS Yeast Res.* **2003**, *3*, 53−62.
(21) Kollmannsberger, H.; Biendl, M.; Nitz, S. Occurence of glycosidically bound flavour compounds in hops, hop products and beer. *Monatsschr. Brauwiss.* **2006**, 83−89.
(22) Daenen, L.; Saison, D.; De Cooman, L.; Derdelinckx, G.; Verachtert, H.; Delvaux, F. R. Flavour enhancement in beer: hydrolysis of hop glycosides by yeast. *Cerevisia* **2007**, *32*, 24−36.
(23) Hanke, S.; Herrmann, M.; Rueckerl, J.; Schoenberger, C.; Back, W. Hop volatile compounds (Part II): transfer rates of hop compounds from hop pellets to wort and beer. *Brew. Sci.* **2008**, *61*, 140−147.
(24) Takoi, K.; Koie, K.; Itoga, Y.; Katayama, Y.; Shimase, M.; Nakayama, Y.; Watari, J. Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer. *J. Agric. Food Chem.* **2010**, *58*, 5050−5058.
(25) Praet, T.; Van Opstaele, F.; Jaskula-Goiris, B.; Aerts, G.; De Cooman, L. Biotransformations of hop-derived aroma compounds by *Saccharomyces cerevisiae* upon fermentation. *Cerevisia* **2012**, *36*, 125−132.
(26) Gros, J.; Peeters, F.; Collin, S. Occurrence of odorant polyfunctional thiols in beers hopped with different cultivars. First evidence of an S-cysteine conjugate in hop (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2012**, *60*, 7805−7816.
(27) Dresel, M.; Praet, T.; Van Opstaele, F.; Van Holle, A.; Naudts, D.; De Keukeleire, D.; De Cooman, L.; Aerts, G. Comparison of the analytical profiles of volatiles in single-hopped worts and beers as a function of the hop variety. *Brew. Sci.* **2015**, *68*, 8−28.
(28) Sharp, D. C.; Steensels, J.; Shellhammer, T. H. The effect of hopping regime, cultivar and beta-glucosidase activity on monoterpene alcohol concentrations in wort and beer. *J. Inst. Brew.* **2017**, *123*, 185−191.
(29) Forster, A.; Gahr, A. On the fate of certain hop substances during dry hopping. *Brew. Sci.* **2013**, *66*, 93−103.
(30) Lutz, A.; Kammhuber, K.; Seigner, E. New trend in hop breeding at the Hop Research Center Huell. *Brew. Sci.* **2012**, *65*, 24−32.
(31) Neiens, S. D.; Steinhaus, M. Odor-active compounds in the special flavor hops Huell Melon and Polaris. *J. Agric. Food Chem.* **2018**, *66*, 1452−1460.
(32) 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.
(33) 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.
(34) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter Select) based on GC−olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776−1783.
(35) Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of methyl linolenate at room-temperature. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1313−1317.
(36) Fischer, A.; Schieberle, P. Characterisation of the key aroma compounds in the peel oil of Pontianak oranges (*Citrus nobilis* Lour. var. *microcarpa* Hassk.) by aroma reconstitution experiments. *Eur. Food Res. Technol.* **2009**, *229*, 319−328.
(37) 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.
(38) Schieberle, P.; Grosch, W. Evaluation of the flavor of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 111−113.
(39) 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.
(40) Bemelmans, J. M. H. Review of isolation and concentration techniques. In *Progress in Flavour Research*; Land, G. G., Nursten, H. E., Eds.; Applied Science: London, U.K., 1979; pp 79−88.
(41) Buiatti, S. Beer composition: an overview. In *Beer in Health and Disease Prevention*; Preedy, V. R., Ed.; Academic Press: Burlington, MA, U.S.A., 2009; pp 213−226.
(42) 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.
(43) Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses. *Nahrung* **1998**, *42*, 371−375.
(44) Saerens, S. M. G.; Verstrepen, K. J.; Thevelein, J. M.; Delvaux, F. R. Ethyl ester production during brewery fermentation: a review. *Cerevisia* **2008**, *33*, 82−90.
(45) Takoi, K.; Tokita, K.; Sanekata, Y.; Usami, Y.; Itoga, Y.; Koie, K.; Matsumoto, I.; Nakayama, Y. Varietal differences of hop-derived flavour compounds in late-hopped/dry-hopped beers. *Brew. Sci.* **2016**, *69*, 1−7.
(46) Haefliger, O. P.; Jeckelmann, N. Stripping of aroma compounds during beer fermentation monitored in real-time using an automatic cryotrapping sampling system and fast gas chromatography/mass spectrometry. *Anal. Methods* **2013**, *5*, 4409−4418.
(47) Haslbeck, K.; Bub, S.; Schönberger, C.; Zarnkow, M.; Jacob, F.; Coelhan, M. On the fate of $\beta$-myrcene during fermentation − The role of stripping and uptake of hop oil components by brewers yeast in dry-hopped wort and beer. *Brew. Sci.* **2017**, *70*, 159−169.
(48) Ohta, T.; Morimitsu, Y.; Sameshima, Y.; Samuta, T.; Ohba, T. Transformation from geraniol, nerol and their glucosides into linalool and $\alpha$-terpineol during shochu distillation. *J. Ferment. Bioeng.* **1991**, *72*, 347−351.
(49) 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.
(50) Matsche, B.; Insa, A. M.; Wiesen, E.; Schönberger, C.; Krottenthaler, M. The influence of yeast strains and hop varieties on the aroma of beer. *Brew. Sci.* **2018**, *71*, 31−38.


DOI: 10.1021/acs.jafc.8b05663
*J. Agric. Food Chem.* 2019, 67, 364−371

### 8.3.3 Summary and Individual Contributions

Previous investigations on the German flavor hop variety Huell Melon revealed outstanding high concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanaote, and propyl 2-methylbutanaote and suggested these compounds to be mainly responsible for the pronounced fruitiness of this hop variety. To get a deeper insight into the influence of these odorants on the aroma of beer, bottom-fermented and top-fermented beers were brewed, both either late hopped or dry hopped with Huell Melon hops. Beers without late hopping and dry hopping were included in the study as reference.

The beers were extracted with solvent and nonvolatiles were removed by SAFE. The SAFE distillates were concentrated and screened for odor-active compounds by cAEDA. Results showed a total of 35 odorants in the FD factor range of 16 to 1024. The odorants with the highest FD factors in all beers were 2-phenylethan-1-ol, 2-/3-methylbutan-1-ol, 2-methyl-propan-1-ol, and (*E*)-*β*-damascenone. Fourteen odorants were identified as hop-derived compounds, among them ethyl 2-methylpropanoate, ethyl 2-methylbutanaote, methyl 2-methylbutanaote, linalool, geraniol, 4-methyl-4-sulfanylpentan-2-one, and myrcene, as well as (3*E*,5*Z*)-undeca-1,3,5-triene, propyl 2-methylbutanoate, (5*Z*)-octa-1,5-dien-3-one, and (1*R*,4*S*)-calamenene.

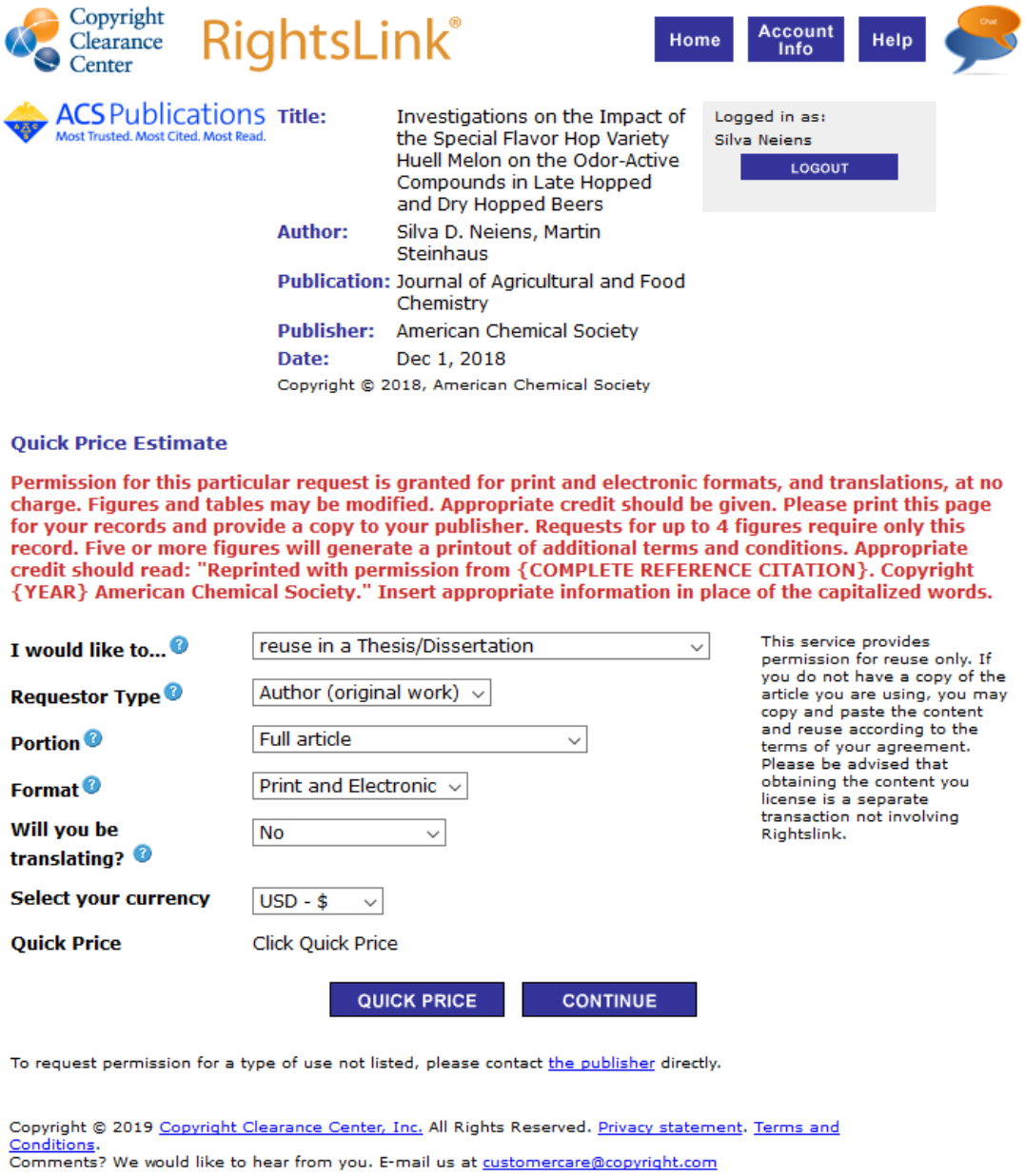
To substantiate the differences in the FD factors, the odorants ethyl 2-methylpropanoate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanaote, myrcene, linalool, and geraniol were quantitated in the beers by using SIDAs. The concentrations were in a range between 2 ng/L and 57 µg/L. For all compounds, concentrations in the dry hopped beers were clearly higher than the concentrations in the late hopped beers.

Calculation of the transfer rates showed a minimal transfer of myrcene from hops into beer and a moderate transfer for propyl 2-methylbutanoate, geraniol, and linalool. Process-induced changes in the concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and methyl 2-methylbutanoate were beyond a direct transfer from hops into beer, suggesting a formation from the corresponding hop-derived carboxylic acids by yeast.

In order to estimate the influence of the individual odorants on the overall beer aroma, sensory tests were carried out in form of spiking experiments. Reference beers were spiked with individual odorants to reach the concentrations of the late and dry hopped beers. The spiked beers were orthonasally compared to the respective reference beer in 3-AFC tests. Spiking experiments revealed that particularly linalool and propyl 2-methylbutanoate contributed to the characteristic aroma of beers flavored with Huell Melon hops.

Silva D. Neiens designed and performed the experiments including volatile isolations, GC-O screenings, structure assignments, syntheses, quantitations, and sensory experiments. Silva evaluated the resulting data and prepared the manuscript. Martin Steinhaus conceived and directed the study, supervised Silva`s work, and revised the manuscript. Martin additionally participated in the sensory tests, including the GC-O analyses.

### 8.3.4 Reprint Permission



Copyright Clearance Center RightsLink®

Home | Account Info | Help

ACS Publications
Most Trusted. Most Cited. Most Read.

Logged in as:
Silva Neiens
LOGOUT

**Title:** Investigations on the Impact of the Special Flavor Hop Variety Huell Melon on the Odor-Active Compounds in Late Hopped and Dry Hopped Beers

**Author:** Silva D. Neiens, Martin Steinhaus

**Publication:** Journal of Agricultural and Food Chemistry

**Publisher:** American Chemical Society

**Date:** Dec 1, 2018

Copyright © 2018, American Chemical Society

**Quick Price Estimate**

**Permission for this particular request is granted for print and electronic formats, and translations, at no charge. Figures and tables may be modified. Appropriate credit should be given. Please print this page for your records and provide a copy to your publisher. Requests for up to 4 figures require only this record. Five or more figures will generate a printout of additional terms and conditions. Appropriate credit should read: "Reprinted with permission from {COMPLETE REFERENCE CITATION}. Copyright {YEAR} American Chemical Society." Insert appropriate information in place of the capitalized words.**

| | |
|---|---|
| **I would like to...** | reuse in a Thesis/Dissertation |
| **Requestor Type** | Author (original work) |
| **Portion** | Full article |
| **Format** | Print and Electronic |
| **Will you be translating?** | No |
| **Select your currency** | USD - $ |
| **Quick Price** | Click Quick Price |

This service provides permission for reuse only. If you do not have a copy of the article you are using, you may copy and paste the content and reuse according to the terms of your agreement. Please be advised that obtaining the content you license is a separate transaction not involving Rightslink.

QUICK PRICE | CONTINUE

To request permission for a type of use not listed, please contact the publisher directly.

Copyright © 2019 Copyright Clearance Center, Inc. All Rights Reserved. Privacy statement. Terms and Conditions.
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

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

***Publications***

Publications in peer reviewed journals:

Neiens, S.D.; Geißlitz, S.M.; Steinhaus, M. Aroma-active compounds in *Spondias mombin* L. fruit pulp. *Eur. Food Res. Technol.* **2017**, *243*, 6, 1073–1081. DOI: 10.1007/s00217-016-2825-7

Neiens, S.D.; Steinhaus, M. Odor-active compounds in the special flavor hops Huell Melon and Polaris. *J. Agric. Food Chem.* **2018**, *66*, 6, 1452–1460. DOI: 10.1021/acs.jafc.7b05859

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*, 1, 364–371. DOI: 10.1021/acs.jafc.8b05663

Annual reports and conference proceedings:

Neiens, S.; Steinhaus, M. Aroma-active compounds in the flavour hops Hallertauer Blanc, Hüll Melon, Mandarina Bavaria and Polaris. In: Deutsche Forschungsanstalt für Lebensmittelchemie, Bericht 2015 (German Research Center for Food Chemistry, Annual Report 2015). Deutsche Forschungsanstalt für Lebensmittelchemie, Freising, 2015, pp. 32-35, 151. ISBN 978-3-946117-01-8

Neiens, S.; Steinhaus, M. Screening for hop-derived aroma compounds in beers dry-hopped with novel German flavor hops. In: Deutsche Forschungsanstalt für Lebensmittelchemie, Bericht 2016 (German Research Center for Food Chemistry, Annual Report 2016). Deutsche Forschungsanstalt für Lebensmittelchemie, Freising, 2016, pp. 46–49, 158. ISBN 978-3-00-056386-7

Neiens, S.; Steinhaus, M. Characterization of odor-active compounds in Huell Melon and Polaris Hops. In: International Hop Growers' Convention, I.H.G.C., Proceedings of the Scientific-Technical Commission, St. Stefan am Walde, Austria, 25-29 June 2017. Weihrauch, F. (Ed.). Scientific-Technical Commission of the International Hop Growers' Convention, I.H.G.C. c/o Hop Research Center Hüll, Wolnzach, Germany, pp. 67–70. ISSN 2512-3785

Neiens, S.D.; Geißlitz, S.M.; Steinhaus, M. Aroma-active compounds in *Spondias mombin* fruit pulp. In: Leibniz-Institut für Lebensmittel-Systembiologie an der Technischen Universität München, Bericht 2017 (Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Annual Report 2017). Leibniz-Institut für Lebensmittel-Systembiologie an der Technischen Universität München, Freising, 2017, pp. 18–20, 76. ISBN 978-3-00-058295-0

Neiens, S.D.; Steinhaus, M. Objectifying the aroma characteristics of important German flavor hops by quantitation of key compounds. In: Leibniz-Institut für Lebensmittel-Systembiologie an der Technischen Universität München, Bericht 2017 (Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Annual Report 2017). Leibniz-Institut für Lebensmittel-Systembiologie an der Technischen Universität München, Freising, 2017, pp. 23–25, 77. ISBN 978-3-00-058295-0

Miscellaneous journal contributions:

Geißlitz, S.; Neiens, S.; Steinhaus, M. Identifizierung aromaaktiver Verbindungen in Cajá (Identification of aroma-active compounds in cajá). *Lebensmittelchemie* **2016**, *70*, 113. DOI: 10.1002/lemi.201690041

Neiens, S.; Steinhaus, M. Identifizierung aromaaktiver Verbindungen in den Flavor-Hopfen Hüll Melon und Polaris mit Hilfe einer vergleichenden Aromaextraktverdünnungsanalyse (Identification of aroma-active compounds in the flavor hops Hüll Melon and Polaris by application of a comparative aroma extract dilution analysis). *Lebensmittelchemie* **2016**, *70*, 115–116. DOI: 10.1002/lemi.201690041

Neiens, S.; Steinhaus, M. Charakteristische Sortenunterschiede in den Schlüsselaromastoffen deutscher Flavor-Hopfen (Characteristic variety-dependent differences in the key aroma compounds of German flavor hops). *Lebensmittelchemie* **2016**, *70*, 146. DOI: 10.1002/lemi.201690051

Neiens, S.; Geißlitz, S; Steinhaus, M. Zum Vorkommen geruchsaktiver Mengen an (1*R*,4*S*)-Calamenen in verschiedenen pflanzlichen Lebensmitteln (On the occurrence of odor-active amounts of (1*R*,4*S*)-calamenene in different plant-based food). *Lebensmittelchemie* **2017**, *71*, 132. DOI: 10.1002/lemi.201770504

## ***Talks***

Oral presentations at international scientific meetings:

Aroma-active compounds in novel German flavor hops. European Brewery Convention, 36th Congress. Ljubljana, Slovenia, May 14–18, 2017.

Characterization of odor-active compounds in Huell Melon and Polaris Hops. Meeting of the Scientific-Technical Commission (STC) of the International Hop Growers' Convention, I.H.G.C. St. Stefan am Walde, Austria, June 25–29, 2017.

Aroma-active compounds in the hop variety Huell Melon and their influence on beer aroma. Young Scientist Symposium, 6th International Young Scientists Symposium on Malting, Brewing and Distilling, Bitburg/Trier, Germany, September 12–14, 2018.

Identification of the major odor-active compounds in cajá, the fruit of *Spondias mombin*. American Chemical Society, 257th National Meeting, Agricultural and Food Chemistry Division, Symposium: The flavor of subtropical and tropical fruits, Orlando, FL, USA, March 31–April 04, 2019. (Invited talk)

Oral presentations at miscellaneous national meetings:

Charakteristische Sortenunterschiede in den Schlüsselaromastoffen deutscher Flavor-Hopfen (Characteristic variety-dependent differences in the key aroma compounds of German flavor hops). Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 67. Arbeitstagung des Regionalverbands Bayern (German Society of Food Chemistry, a division of the German Chemical Society, 67th Bavarian Regional Meeting). Erlangen, Germany, March 10, 2016.

Zum Vorkommen geruchsaktiver Mengen an (1*R*,4*S*)-Calamenen in verschiedenen pflanzlichen Lebensmitteln (On the occurrence of odor-active amounts of (1*R*,4*S*)-calamenene in different plant-based food). Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 68. Arbeitstagung des Regionalverbands Bayern (German Society of Food Chemistry, a division of the German Chemical Society, 68th Bavarian Regional Meeting). Bayreuth, Germany, March 22, 2017.

***Poster presentations***

Identifizierung aromaaktiver Verbindungen in den Flavor-Hopfen Hüll Melon und Polaris mit Hilfe einer vergleichenden Aromaextraktverdünnungsanalyse (Identification of aroma-active compounds in the flavor hops Hüll Melon and Polaris by application of a comparative aroma extract dilution analysis). Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 44. Deutscher Lebensmittelchemikertag (Society of Food Chemistry, a division of the German Chemical Society, 44$^{th}$ National Meeting). Karlsruhe, Germany, September 14–16, 2015.