Technische Universität München
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Molecular Insights into the Contribution of Specialty Malts to the Beer Aroma

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1 Summary

Malt is a major raw material in the brewing industry and is of high importance for the beer's sensory properties. By applying intense thermal treatment during malting, specialty malts are produced, which are commonly added to kilned base malt to create beers with unique color and aroma properties. However, the contribution of specialty malts such as caramel and roasted malt to the beer aroma and the transfer of odorants from these malts to beer have not yet been studied on the molecular level. Therefore, six beers, three bottom-fermented Lager beers and three top-fermented wheat beers were produced comprising a reference beer, a caramel malt beer, and a roasted malt beer each. The reference beers were exclusively produced with kilned base malt while the specialty malt beers were brewed by substituting a portion of the kilned barley or wheat base malt with caramel and roasted malt of the respective type. The bottom-fermented beers were solely produced with kilned, caramel, and roasted barley malt. In contrast, the top-fermented beers were brewed with equal parts of barley and wheat malt, while the wheat malt portion included the respective specialty malts. The aroma of the beers showed mostly banana-like, floral, honey-like, and fruity notes in the reference beers, caramel-like and malty notes in the caramel malt beers, and earthy and roasty notes in the roasted malt beers. Overall, the bottom-fermented specialty malt beers had a stronger caramel-like, earthy, and roasty aroma than the top-fermented specialty malt beers, whereas the malty note was comparable between the beer types with the reference beers representing the overall lowest aroma intensity in both cases. Major odorants responsible for the aroma differences were identified in the beers using aroma extract dilution analysis followed by quantitation and calculation of odor activity values (OAVs). In total, 30 odorants exhibited OAVs ≥1 in at least one of the beers. A number of known secondary fermentation products showed similar OAVs in the three beers of each type. 19 of the 30 compounds exhibited substantial differences among the bottom-fermented beers and 15 showed clear differences among the top-fermented beers. The two beer types differed in OAVs for acetic acid, 2-acetyl-1-pyrroline, and (*E*)-β-damascenone. 2-Acetyl-1-pyrroline was one of the major odorants in the bottom-fermented beers, especially in the caramel malt beer, but not in the top-fermented beers. Overall, the caramel malt beers were characterized by high OAVs of fruity, caramel-like, roasty, and earthy smelling aldehydes, furanones, pyranones, and pyrazines, whereas the roasted malt beers exhibited high OAVs for phenolic, smoky, and sweet smelling phenols and some earthy smelling pyrazines. To assess their transfer from malt to beer, the odorants were quantitated in the malts used for brewing and hypothetical concentrations in the beers were calculated assuming 100% transfer and the absence of other sources. A comparison to the actual concentrations in the beers revealed that a direct transfer played only a minor role in the amount of odorants in the beers, suggesting a substantial formation from precursors and/or a release of encapsulated odorants during brewing.

2 Zusammenfassung

Malz ist ein wichtiger Rohstoff in der Brauindustrie und von großer Bedeutung für die sensorischen Biereigenschaften. Mithilfe von intensiven thermischen Prozessen beim Mälzen werden Spezialmalze hergestellt, die üblicherweise dem gedarrten Basismalz zugesetzt werden, um Biere mit einzigartigen Farb- und Aromaeigenschaften herzustellen. Der Beitrag von Spezialmalzen wie Karamell- und Röstmalz zum Bieraroma und der Transfer von Geruchsstoffen vom Malz ins Bier wurden bisher jedoch nicht auf molekularer Ebene untersucht. Daher wurden sechs Biere, darunter drei untergärige Lagerbiere und drei obergärige Weizenbiere hergestellt, jeweils ein Referenzbier, ein Karamellmalzbier und ein Röstmalzbier. Die Referenzbiere wurden ausschließlich mit gedarrtem Basismalz hergestellt, bei den Spezialmalzbieren wurde hingegen ein Teil des gedarrten Gersten- oder Weizenbasismalzes durch Karamell- oder Röstmalz der jeweiligen Getreideart ersetzt. Die untergärigen Biere wurden ausschließlich mit Gerstenmalz hergestellt, wohingegen die obergärigen Biere zu gleichen Teilen aus Gersten- und Weizenmalz gebraut wurden, wobei der Weizenmalzanteil die jeweiligen Spezialmalze beinhaltete. Das Aroma zeigte vor allem bananenartige, blumige, honigartige und fruchtige Noten in den Referenzbieren, karamellartige und malzige Noten in den Karamellmalzbieren sowie erdige und röstige Noten in den Röstmalzbieren. Insgesamt wiesen die untergärigen Spezialmalzbiere ein stärker karamellartiges, erdiges und röstiges Aroma auf, während die malzige Note zwischen den Biersorten vergleichbar war. Beide Referenzbiere wiesen eine insgesamt geringere Aromaintensität auf. Die Geruchsstoffe wurden in den Bieren mittels Aromaextraktverdünnungsanalyse identifiziert, quantifiziert und anschließend wurden ihre Odor Activity Values (OAVs) berechnet. Insgesamt wiesen 30 Geruchsstoffe OAVs ≥1 in mindestens einem der Biere auf. Eine Reihe bekannter Gärungsnebenprodukte zeigte ähnliche OAVs in den Bieren der jeweiligen Sorte. 19 der 30 Verbindungen wiesen jedoch erhebliche Unterschiede in den untergärigen Bieren auf sowie 15 innerhalb der obergärigen Biere. Die Biersorten unterschieden sich in den OAVs für Essigsäure, 2-Acetyl-1-pyrrolin und (*E*)-β-Damascenon. 2-Acetyl-1-pyrrolin war ein wichtiger Geruchsstoff in den untergärigen Bieren, insbesondere im Karamellmalzbier, nicht jedoch in den obergärigen Bieren. Insgesamt wiesen die Karamellmalzbiere hohe OAVs für fruchtige, karamellartige, röstige und erdig riechende Aldehyde, Furanone, Pyranone und Pyrazine auf, während die Röstmalzbiere hohe OAVs für phenolische, rauchige und süßlich riechende Phenole und einige erdig riechende Pyrazine aufwiesen. Um den Transfer der Geruchsstoffe vom Malz ins Bier zu bewerten, wurden diese in den Malzen quantifiziert und die Konzentrationen in den Bieren berechnet, die einem 100%igen Transfer entsprechen würden. Ein Vergleich mit den tatsächlichen Konzentrationen in den Bieren ergab, dass ein direkter Transfer nur eine geringe Rolle für die Menge der Geruchsstoffe in den Bieren spielte, was auf eine wesentliche Bildung aus Vorläufern und/oder eine Freisetzung von eingeschlossenen Geruchsstoffen während des Brauens hindeutete.

3 Abbreviations and Nomenclature

Abbreviations:

AEDA	Aroma extract dilution analysis
ASTM	American Society for Testing and Materials
AV	Acidic volatiles
CI	Chemical ionization
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane
EI	Electron ionization
FD	Flavor dilution
FFAP	Free fatty acid phase
FID	Flame ionization detector
GC-O	Gas chromatography-olfactometry
GC-MS	Gas chromatography-mass spectrometry
GC × GC-MS	Comprehensive two-dimensional gas chromatography-mass spectrometry
HDMF	4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one
HS-SPME-GC-MS	Headspace-solid phase microextraction-gas chromatography-mass spectrometry
IBU	International bitter units
i.d.	Inner diameter
NBV	Neutral/basic volatiles
OAV	Odor activity value
OTV	Odor threshold value
PDMS/DVB	Polydimethylsiloxane/Divinylbenzene
POF	Phenolic off-flavor
RI	Retention index
SAFE	Solvent-assisted flavor evaporation
SIDA	Stable isotope dilution assay
TOF	Time-of-flight

Nomenclature:

Abhexone	5-Ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one;
2-Acetyl-1-pyrroline	1-(3,4-Dihydro-2*H*-pyrrol-5-yl)ethan-1-one
2′-Aminoacetophenone	1-(2-Aminophenyl)ethan-1-one
Cyclotene	3-Methylcyclopentane-1,2-dione
(*E*)-β-Damascenone	(2*E*)-1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one
γ-Decalactone	5-Hexyldihydrofuran-2(3*H*)-one
Eugenol	2-Methoxy-4-(prop-2-en-1-yl)phenol
2,9-Humuladien-6-one	(4*E*,8*E*)-2,2,6,10-Tetramethylcycloundeca-4,8-dien-1-one
Isomaltol	1-(3-Hydroxyfuran-2-yl)ethan-1-one
Linalool	3,7-Dimethyl-1,6-octadien-3-ol
Maltol	3-Hydroxy-2-methyl-4*H*-pyran-4-one
Methional	3-(Methylsulfanyl)propanal
Methionol	3-(Methylsulfanyl)propan-1-ol
γ-Nonalactone	5-Pentyldihydrofuran-2(3*H*)-one
2-Propanoyl-1-pyrroline	1-(3,4-Dihydro-2*H*-pyrrol-5-yl)propan-1-one
2-Acetylpyrazine	1-(Pyrazin-2-yl)ethan-1-one
Sotolon	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one
2-Acetylthiazole	1-(1,3-Thiazol-2-yl)ethan-1-one
Vanillin	4-Hydroxy-3-methoxybenzaldehyde

4 Introduction

4.1 Molecular Sensory Science

4.1.1 The Olfactory System and Odorants

The quality of food is determined by several factors like freshness, nutritional value, absence of contaminants, environmental aspects, and sensory properties. Multiple studies in recent years have shown that among these, the sensory properties, which are perceived by the primary human senses, are the main determinants for the selection or the rejection of food, ultimately resulting in a purchase decision by the consumer.[1-3] These sensory properties also account for the pleasure experienced during the consumption of food and can be categorized into appearance, aroma, irritation, taste, and texture. However, the primary human senses do not contribute equally to this experience and it has been shown that olfaction is clearly the main contributor to the hedonic value of food.[4-6]

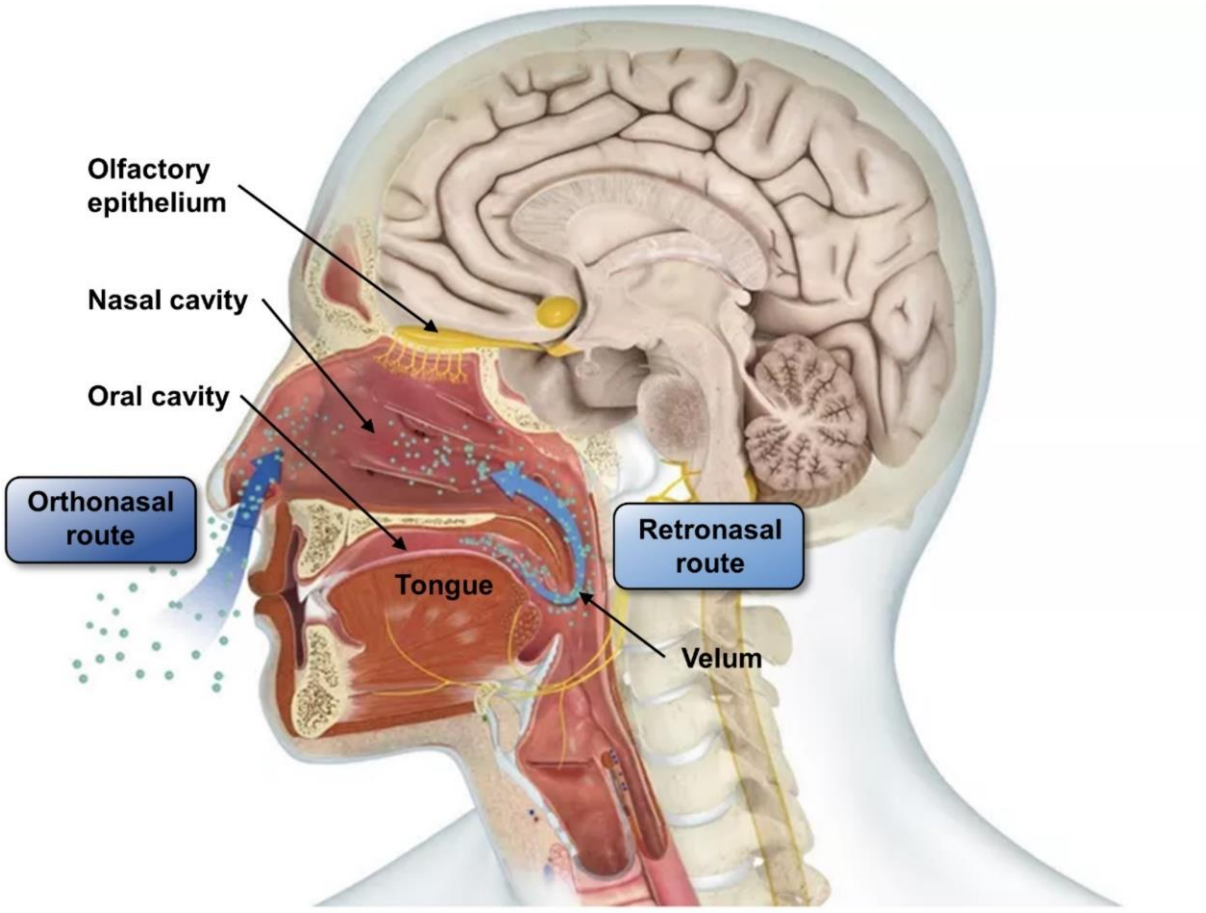

Figure 1 The olfactory system and the two routes of olfactory perception[7]

Olfaction enables the perception of an aroma via the olfactory epithelium, which is located in the nasal cavity and comprises approximately 10 million receptor neurons. The perception of an aroma is caused by the evaporation of odorants from the food that have to reach the olfactory epithelium in a sufficient number of molecules. This can occur through the nostrils (orthonasally) or the throat (retronasally).

Retronasal aroma perception mainly takes place after swallowing due to a reflexive exhalation which leads to a transfer of odorants previously deposited in the throat into the nasal cavity from the rear (Figure 1).[8]

After entering the olfactory system, the odorants are dissolved in the olfactory mucosa and bind to specific proteins, which have a high affinity towards the odorants, potentially resulting in a transport of the odorants to the receptor. After binding to one of the approximately 400 types of G-protein coupled receptors in the membrane of the olfactory receptor cell's cilia (Figure 2, 1.), the odorants trigger an intracellular reaction cascade due to conformational changes of the receptor molecule finally leading to the depolarization of the cell membrane.

This depolarization creates an action potential (Figure 2, 2.) which is then propagated to the olfactory bulb via glomeruli that bundle odorant receptor cells expressing the same type of receptor protein (Figure 2, 3.). These glomeruli then activate specific mitral cells, which relay the frequency-encoded action potentials to the limbic system and the cerebral cortex in the brain via the axon (Figure 2, 4.).[8-12]

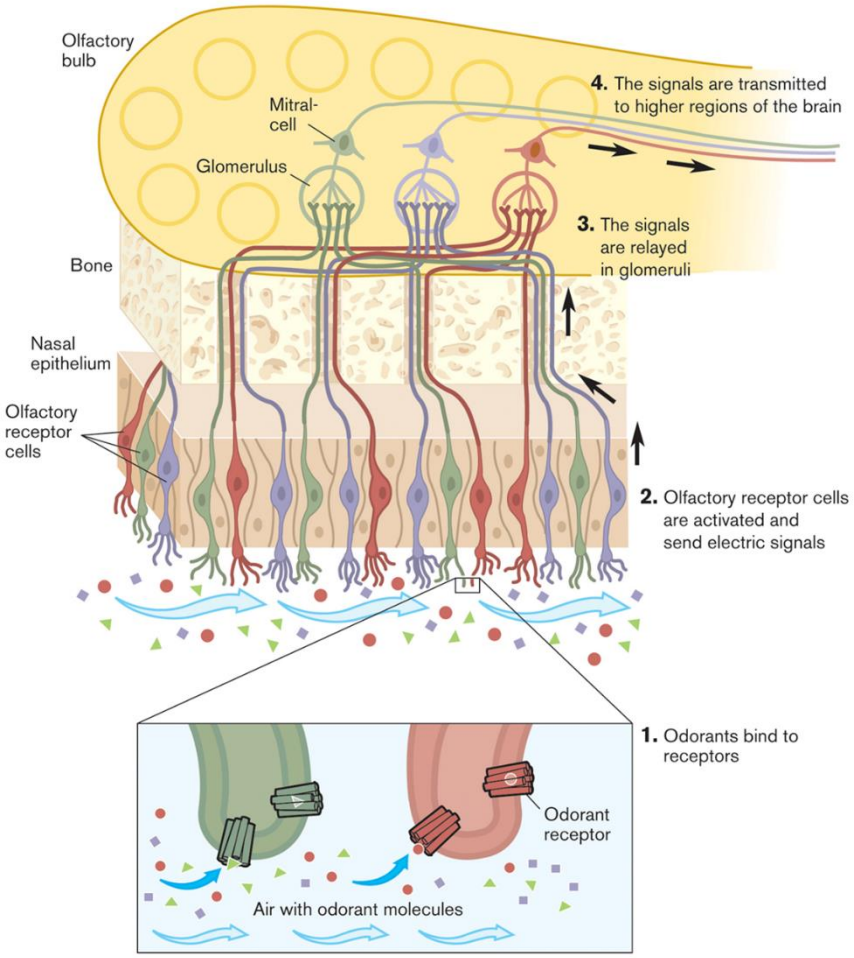

Figure 2 The process of olfactory perception divided into its four main steps[13]

Typically, a specific odorant can bind to multiple different receptors, just as each type of receptor can be activated by several different odorants. In most cases, the aroma of food is caused by a mixture of odorants,[4, 14-16] which results in a specific activation pattern of different receptors leading to the overall aroma impression or olfactory profile.[11, 17-22]

A compound has to fulfill several criteria to be perceived as an odorant. Besides being volatile, it must be able to bind to the respective receptor proteins to activate them. That is why odorants typically possess a functional group as well as hydrophobic regions.[8, 20]

Furthermore, an odorant has to be present at a certain minimum concentration in the air to trigger an odor event. This is referred to as the odor threshold value (OTV), which can vary greatly between different odorants. The OTV in a food is influenced by the specific food matrix, from which the odorant is released and by its physico-chemical properties.[23-27] As an example, the OTV of the cooked apple-like smelling compound (*E*)-β-damascenone in water is 0.006 µg/kg, while the OTV of the cheesy smelling compound 2-methylpropanoic acid in the same matrix is 60000 µg/kg.[28]

In general, all odorants that are present above their specific OTV may play a role in the olfactory profile of the food. However, it is typically just a small number of odorants that significantly contribute to the overall aroma, often referred to as the key odorants.[4]

Throughout the literature, several compounds, so-called generalists, have been identified to play an important role in many different food products while others, so-called individualists, are unique to a certain type of food.[29-30] A Sensomics-based concept has been developed to identify these key odorants in different food systems.

4.1.2 Identification of Key Odorants

The following concept was developed by Schieberle[31] and Grosch[32] to identify and characterize the key odorants (Figure 3).

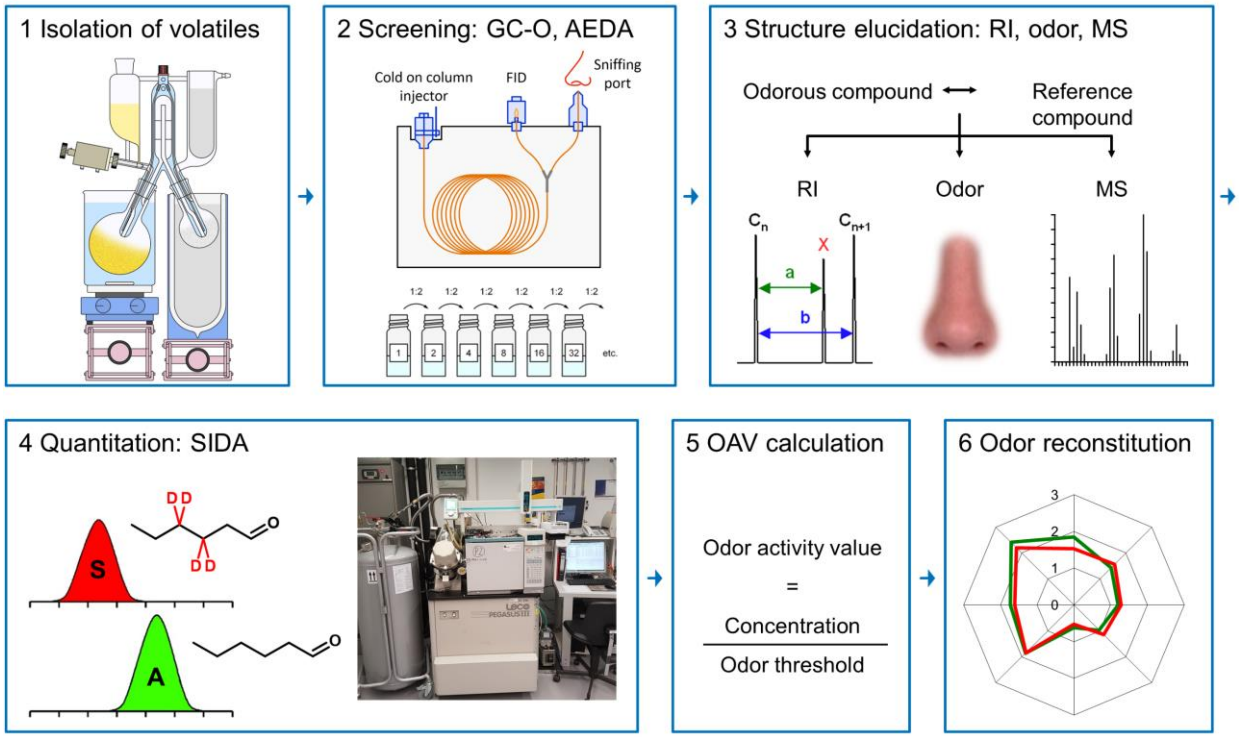

Figure 3 Identification of key odorants (illustrations: Martin Steinhaus)

In a first step, the volatiles are isolated from a sample by using an organic solvent with a low boiling point and polarity such as diethyl ether and by applying a solvent-assisted flavor evaporation (SAFE).[33] Utilizing a high vacuum and liquid nitrogen as a coolant for recondensation, this method, in contrast to other separation methods described in the literature,[34] allows for a gentle evaporation of the volatiles at relatively low temperatures (≤40 °C) thus minimizing the risk of compound degradation and artifact formation.[4]

By applying an acid-base extraction, the volatiles can be further separated into a fraction containing the acidic volatiles (AV) and a fraction containing the neutral and basic volatiles (NBV). The organic phase containing the isolated volatiles or the two phases after optional fractionation into AV and NBV are then concentrated to a volume of approximately 500 µl by using a Vigreux column and a Bemelmans microdistillation device.[35]

To screen the isolated volatiles for odorants and to differentiate the odorants from the multitude of odorless volatiles, the concentrate is subjected to gas chromatography-olfactometry (GC-O).[4]

After separating the volatiles using a capillary column and a specific temperature program, a Y-shaped glass splitter equally divides the effluent between a stream leading to a flame ionization detector (FID) and a stream leading to a heated exit, which is used as a sniffing port by the GC-O assessor.

By placing the nose directly above the sniffing port, the assessor is able to perceive odorants during the GC-O analysis while the FID signal is simultaneously plotted by a recorder or computer (Figure 4). Any detected odorants are recorded by the assessor based on the perceived odor quality and the respective retention time.

The result of a GC-O analysis is a combination of the FID chromatogram and the olfactory data obtained by the assessor.[4]

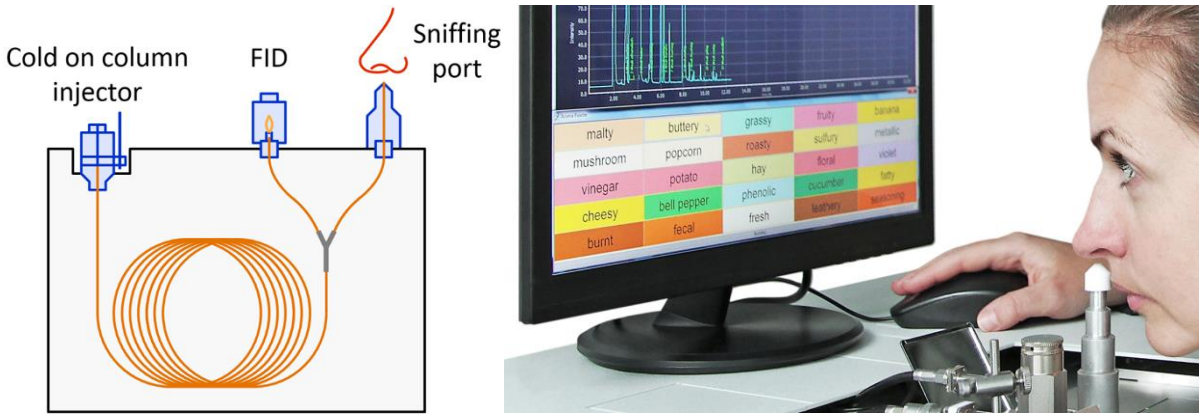

Figure 4 Basic principle (left) and application (right) of a GC-O analysis (illustrations: Martin Steinhaus)

Following the initial GC-O analysis, an aroma extract dilution analysis (AEDA) is applied by stepwise diluting the concentrated volatile isolate with diethyl ether to obtain dilutions of 1:2, 1:4, 1:8, and so on, and subjecting the diluted samples to GC-O. Each odorant is then assigned a flavor dilution (FD) factor, representing the dilution factor of the most diluted sample, in which the odorant was detected by the assessor, resulting in a ranking of odorants according to their odor potency (Figure 5).[4]

Furthermore, AEDA is particularly suitable for comparing two or more samples with respect to their odorants.[31] Due to variations in sensing thresholds between individuals and the potential occurrence of anosmia, it is necessary that this type of analysis is performed by multiple assessors for each sample and dilution.

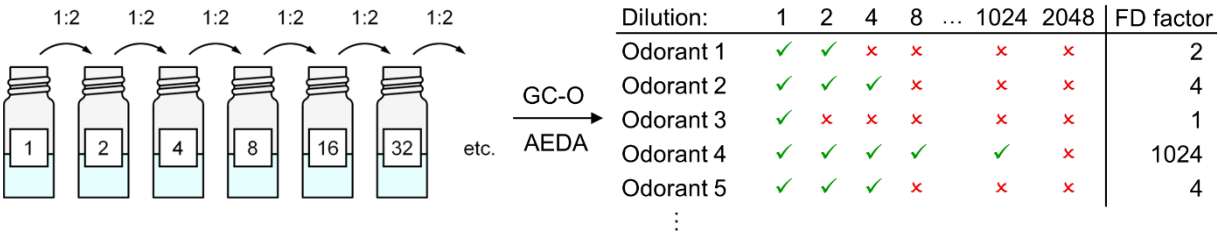

Figure 5 AEDA including stepwise dilution of the concentrated volatile isolate and the calculation of FD factors (illustration: Martin Steinhaus)

The structure elucidation of the odorants detected by AEDA is based on comparing several parameters with those of authentic reference compounds analyzed at the same conditions and in appropriate dilution. This includes the odor quality perceived at the sniffing port, the retention index (RI), which is calculated by comparing the retention time of the odorant to the retention times of a series of *n*-alkanes, and the mass spectra obtained by gas chromatography-mass spectrometry (GC-MS) in electron ionization (EI) and chemical ionization (CI) modes.[4]

Although AEDA provides valuable information on the odorants present in a sample and approximates the potency of specific odorants based on FD factors, this type of analysis cannot finally clarify the contribution of the identified odorants to the overall olfactory profile. This is a result of matrix effects in the sample that are not considered during AEDA as well as workup losses of volatiles that are not compensated. Furthermore, during GC-O, all volatiles in the sample are fully evaporated regardless of their volatility. To address these issues, the results of the AEDA are further substantiated by a quantitation of the identified odorants using stable isotopically substituted odorants as internal standards, referred to as a stable isotope dilution assay (SIDA).[4, 31, 36]

These internal standards are ^{2}H- (deuterium-) or ^{13}C-substituted analogues[37] of the target odorants and are added to the sample prior to the workup process (Figure 6). A significant benefit of applying this technique is that the almost identical physical and chemical properties of the analyte and the isotopically substituted analogue account for any losses that occur during the workup process thus leaving the ratio of analyte to analogue unchanged. To ensure this result, however, the mixture of sample and internal standard has to be sufficiently homogenized and thus equilibrated, the time required strongly depending on the sample matrix.[38]

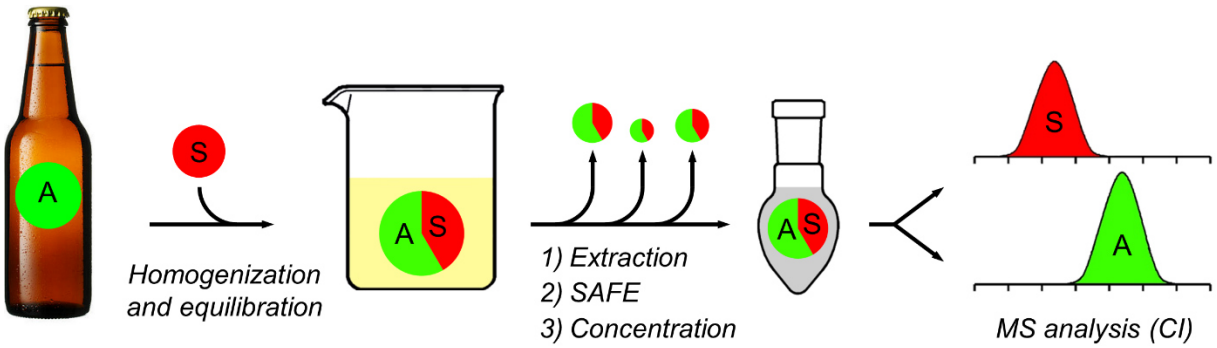

Figure 6 The process of quantitating analytes (A) by applying stable isotopically substituted odorants (S) as internal standards (illustration: Martin Steinhaus and Klaas Reglitz)

The analyte is quantitated via GC-MS by monitoring characteristic quantifier ions of the analyte and the internal standard, preferably in CI mode to obtain an intense signal for the molecular ion.[4] The analyte concentration is then calculated from the peak areas of the analyte and the standard, the amount of sample used, and the amount of standard added to the sample. This is achieved by applying a calibration line equation obtained from the analysis of analyte/standard mixtures in at least five different concentration ratios followed by linear regression.

The next step in the process of identifying key odorants is the calculation of odor activity values (OAVs) by dividing the concentration determined for each odorant by its odor threshold value (OTV),[39] which is obtained with a trained sensory panel by adding authentic reference compounds to a suitable model system to represent the respective food matrix according to the American Society for Testing and Materials (ASTM) standard practice for the determination of odor and taste thresholds by a forced choice ascending concentration series method of limits.[40] Thus, all odorants whose concentrations exceed the OTV in the respective matrix exhibit an OAV ≥1 and are therefore potential contributors to the overall aroma of the food. In contrast, the compounds whose concentrations do not exceed the OTV exhibit an OAV <1 and do normally not contribute to the overall aroma of the food.[4]

During the consumption of food, in contrast to GC-O analysis, the odorants are perceived as a mixture. Thus, the perception of a food aroma is sometimes subject to additive effects but more often to suppressive effects.[4] To take these effects into account and to verify that all important odorants have been identified and quantitated correctly, an aroma reconstitution is performed. In this context, all odorants exhibiting OAVs ≥1 are combined in a model matrix best representing the original food sample at the concentrations previously determined.

This model matrix should match the investigated food sample at least in its water content, pH, concentration of sugars and lipids, and, in the case of alcoholic beverages, also its ethanol concentration.

The aroma reconstitution model is then sensorially compared to the original sample by a trained panel to determine quantitative olfactory profiles.[31] If the quantitative olfactory profiles of the aroma reconstitution model and the original food sample do not substantially differ, the aroma reconstitution is considered successful and all important odorants have been correctly identified and quantitated.

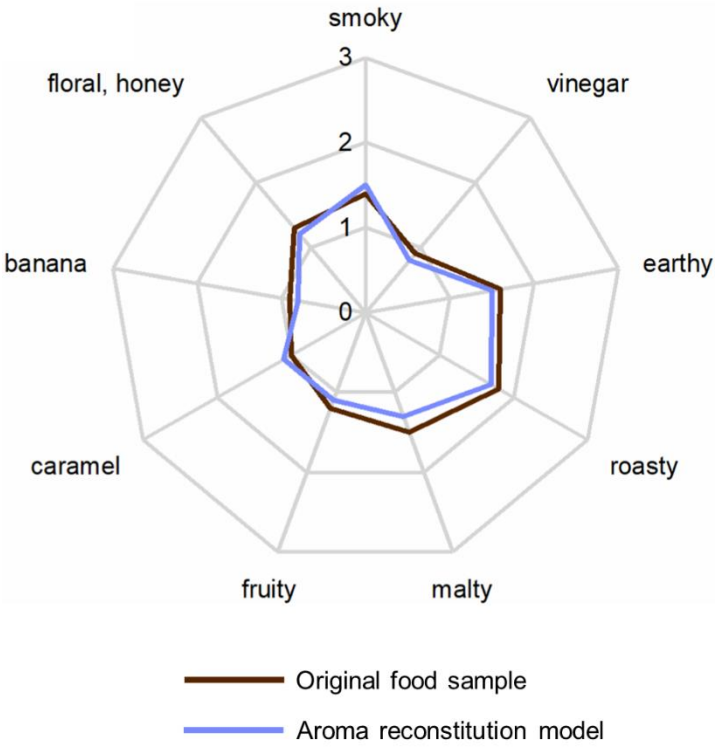

Figure 7 Exemplary results of a successful aroma reconstitution experiment comparing the quantitative olfactory profile of an aroma reconstitution model with that of the original food sample (illustration: Michael Féchir and Klaas Reglitz)

4.1 Malt

4.1.1 Barley

Barley (*Horderum vulgare*) is a member of the grass family and a major cereal grain ranking among the top five grain types produced by volume worldwide in 2021.[41] It is globally grown in temperate climates and was one of the first cultivated grains with records dating back to approximately 10,000 years ago.[42] Besides its use as animal fodder[43] and cereal for bread making, the main application of barley is the production of malt as a fermentable substrate for alcoholic beverages, primarily beer.[44] While six-row barley is mostly used as animal fodder due to its higher protein content, two-row barley varieties are mainly used for malting due to their higher amount of carbohydrates, their lower amount of protein, and more uniform germination during malting.[45] Traditionally, spring barley has been the preferred choice for malting due to its beneficial malting behavior. However, in recent years, higher yields as a result of longer growth periods in most countries have led to the increased popularity of winter barley varieties for malting.[46]

The barley grain consists of several distinct components (Figure 8). The husk forms the outermost layer and protects the grain from physical damage. It contains mostly silica, cellulose, lignin, and pentonans and accounts for approximately 10% of the dry grain weight.

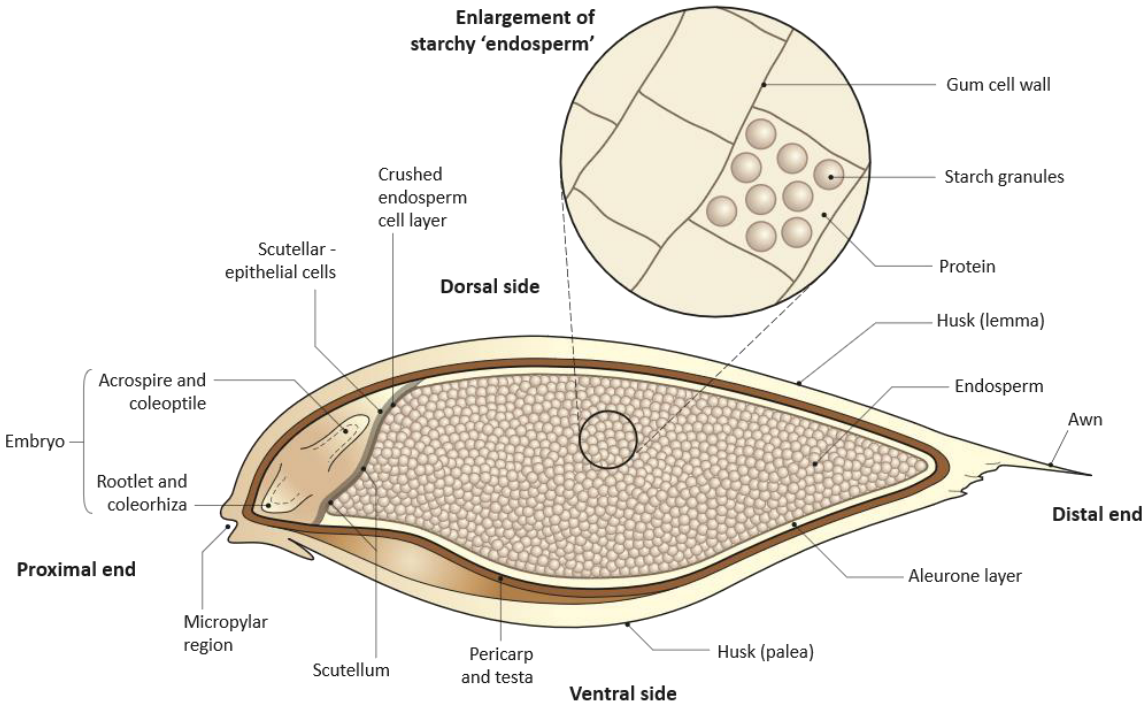

Figure 8 Structure of a two-row barley grain and its major components[47]

The embryo contains the majority of the lipids in the grain and is responsible for the production of growth regulators like gibberellic acid, which triggers the formation and release of enzymes in the aleurone layer during germination. The thin aleurone layer is the main production site for enzymes such as α-amylase, limit dextrinase, and endoprotease.

The largest portion of the barley grain is formed by the endosperm, which contains about 75% of the grain's dry matter and mostly consists of non-living tissue in the form of starch granules embedded in a protein matrix.[44, 47-49] The average composition of barley grains determined at air-dried conditions and in the grain dry matter is shown in Table 1 below.[50]

Table 1 Average Composition of Barley Grains[50]

Component	Air-dried grain (%)	Grain dry matter (%)
Water	11.7	-
Available carbohydrates	63.3	71.7
Protein	10.6	12.0
Fiber	9.8	11.1
Lipids	2.1	2.4
Minerals	2.3	2.6

4.1.2 Wheat

Wheat (*Triticum aestivum*) is the most widely cultivated member of the grass family and is primarily grown in temperate regions of the Northern Hemisphere accounting for the largest total acreage among food crops worldwide with a global production of 760 million tons in 2020.[51-52] Although wheat is primarily used for the production of bread, pasta, biscuits, and pastries it also plays a major role in malting, being the second most commonly malted cereal after barley.[53]

Due to its characteristic impact on the beer aroma and its beneficial effect on the foam stability as a result of a higher protein content, wheat malt is often applied to partially substitute barley malt in the brewing industry, especially in Europe.[54-56] Barley and wheat grains are very similar in shape and structure. However, in contrast to barley grains, wheat grains do not possess a husk, which is of significant importance as a natural filter aid in the traditional brewing process.[57] As a result, barley malt is the main raw material for the production of most beers while substantial quantities of barley malt are substituted by wheat malt in some cases to obtain certain quality traits unique to specific beer types.[53-54] The average composition of wheat grains determined at air-dried conditions and in the grain dry matter is shown in Table 2.[50]

Table 2 Average Composition of Wheat Grains[50]

Component	Air-dried grain (%)	Grain dry matter (%)
Water	13.2	-
Available carbohydrates	59.6	71.7
Protein	11.7	12.0
Fiber	13.3	11.1
Lipids	2.2	2.4
Minerals	1.5	2.6
Water	11.7	-

4.1.3 The Malting Process

Malting describes the limited germination of cereal grains under controlled conditions and is perhaps the oldest biotechnological process having been practiced for at least 6,000 years.[54] The objective of malting is the biological conversion of stored biopolymers in the grain, mostly starch and proteins, to fermentable sugars (primarily maltose and glucose), amino acids, and low molecular weight peptides to be used in the production of alcoholic beverages such as beer or whiskey. To this day, the major steps of the malting process are steeping, germination, and kilning of mostly barley and wheat[58] but also rye, oats, triticale, and other cereals.[53, 59-60]

The initial phase of steeping refers to the process of increasing the moisture content of stored grains from approximately 12% up to 48% over typically 2 to 3 days alternating between periods of water treatment (immersion) and air treatment at 10 to 13 °C. After reaching a moisture content of 30% or higher, the grains swell substantially and increase their metabolic activity. At a moisture content above 43%, mostly amylolytic and proteolytic enzymes are formed, which are responsible for the degradation of the endosperm during the following steps. At this point, the grains have a high demand for oxygen due to an increase in aerobic metabolic activity (respiration), which is why continuous ventilation with fresh air has to be applied.[54, 61]

After reaching the target moisture content of up to 48%, the germination process is initiated, for which several conditions aside from the moisture content have to be met such as a sufficient oxygen supply and a temperature between 14 and 18 °C. Germination refers to the physiological process of forming organs, rootlets, and plumules in the seedling by partially consuming the energy reserves stored in the endosperm, mostly in the form of starch, which is enzymatically converted to sugars. During germination, the grains, which are stored in piles, have to be rearranged at regular intervals to ensure a homogeneous oxygen supply and a sufficient dissipation of heat over typically 7 days.

The germination not only results in a degradation of starch and proteins while forming cavities in the endosperm but also in a partial deconstruction of cell walls and other structural grain elements by cytolytic enzymes leading to an increase in friability. In parallel, the components of the seedling increase in size and the rootlets become visible, representing a reliable method of tracking the progress of the germination (Figure 9).[62-64]

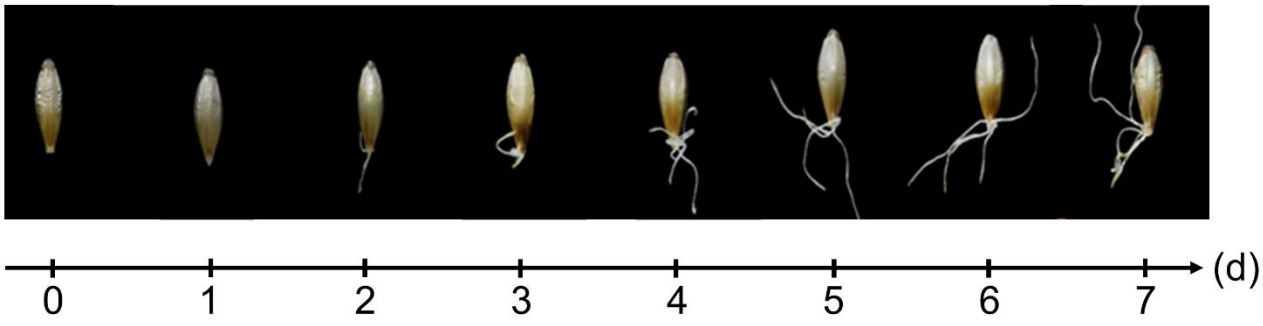

Figure 9 Physiological changes of a barley grain over 7 days of germination (illustration: Weyermann Specialty Malts and Michael Féchir)

After the grains have reached a certain friability and most of the starch has been enzymatically converted to sugars but only a small portion of these sugars have been consumed by the growing seedling, the grains are referred to as green malt. At this point, the germination is slowed down and finally stopped by substantially reducing the moisture content with the primary goal of preserving the sugars and enzymes in the green malt to make them long-term stable. The kilning process is performed by applying a high airflow whose temperature is gently increased to a final level of 80 °C or higher. However, it is important to dry the grains to a low moisture content at comparatively low temperatures and to slowly increase the heat load minimizing denaturation of the enzymes, thus ensuring that the enzymatic activity in the grains can continue to a certain extent until the target moisture content of 4% or lower is reached. Another goal of the kilning process is to preserve the increased volume of the grains as a result of the previous swelling during steeping and to keep the cavities formed in the endosperm intact ensuring a high friability of the kilned malt.[64-66]

During kilning, several additional changes occur in the grains mostly due to the application of heat. This highly depends on the applied temperatures and includes a formation of colorants and odorants as a result of thermal processes such as the Maillard reaction and the Strecker degradation.[67-70] Furthermore, kilning removes most of the unwanted green-grain aroma from the malt.[54]

The final step in the malting process is the deculming during which the dried rootlets or culms are physically removed from the malt. The separated rootlets are rich in protein and are typically processed or sold as animal feed. The finished malt can be stored in dry silos for up to one year before being used for brewing or other applications.[53-54] A typical volume and mass balance from the raw material to the final product of the malting process is shown in Table 3.[53]

Table 3 Typical Volume and Mass Balance During the Malting Process[53]

Processing level	From 100 hL of barley	From 100 kg of barley	Moisture content (%)
Barley	100 hL	100 kg	16
Steeped barley	145 hL	155 kg	45
Green malt	220 hL	147 kg	48
Kilned malt	118 hL	78 kg	3.5
Stored finished malt	120 hL	79 kg	4.5

4.1.4 Specialty Malts

By applying additional processing steps during malting, different types of specialty malts can be obtained. These specialty malts possess several unique characteristics that differentiate them from the common kilned malt, primarily a more intense color and aroma. These characteristics can be influenced by variations in the applied drying technology to obtain a wide range of malt products featuring different color and aroma properties and designed for specific demands (Figure 10).[67, 71-76] The two main categories of specialty malts are caramel malts and roasted malts, which exhibit distinct differences in their sensory properties.[72, 77]

The amber to light brown colored caramel malts, which typically possess a caramel, vanilla-like, nutty, or bread crust-like aroma,[72, 78] are produced by drying the green malt at final temperatures of 110 to 140 °C instead of 80 to 90 °C, which is common during the production of standard kilned malt. This drying process can be either performed in a kiln, similar to the standard malting procedure, or in a roasting drum. In contrast, the dark brown colored roasted malts typically possess a roasty, chocolate-like, coffee-like, or earthy aroma[72, 79-80] and are obtained by first producing a standard kilned malt and then subjecting this kilned malt to an intense roasting process in a roasting drum at temperatures of up to 220 °C.[81] The main differences between the manufacturing of caramel malts and roasted malts are the amount of the applied heat load and the moisture content at which the increased temperatures are applied. The kilned malt that is further processed into roasted malt is already very dry (<4% moisture) at the beginning of roasting.

In contrast, the heating process applied for manufacturing caramel malts is used instead of the standard kilning process, not in addition, as is the case with roasting. Thus, the green malt is heated to temperatures above 110 °C right after the germination has been completed while still having a high moisture content with the result of liquefying large parts of the endosperm and forming semicrystalline structures on cooling.

This has a substantial effect on the color- and aroma-forming thermal reactions taking place in the grains such as the Maillard reaction, Strecker degradation, caramelization, and pyrolysis which are more intense during the manufacturing of caramel and roasted malts compared to standard kilned malts.[67-69, 73-74, 82-83] As a result, specialty malts typically contain a lower amount of sugars, amino acids, peptides, and preserved enzymes compared to standard kilned malts due to the more intense thermal treatment.[84]

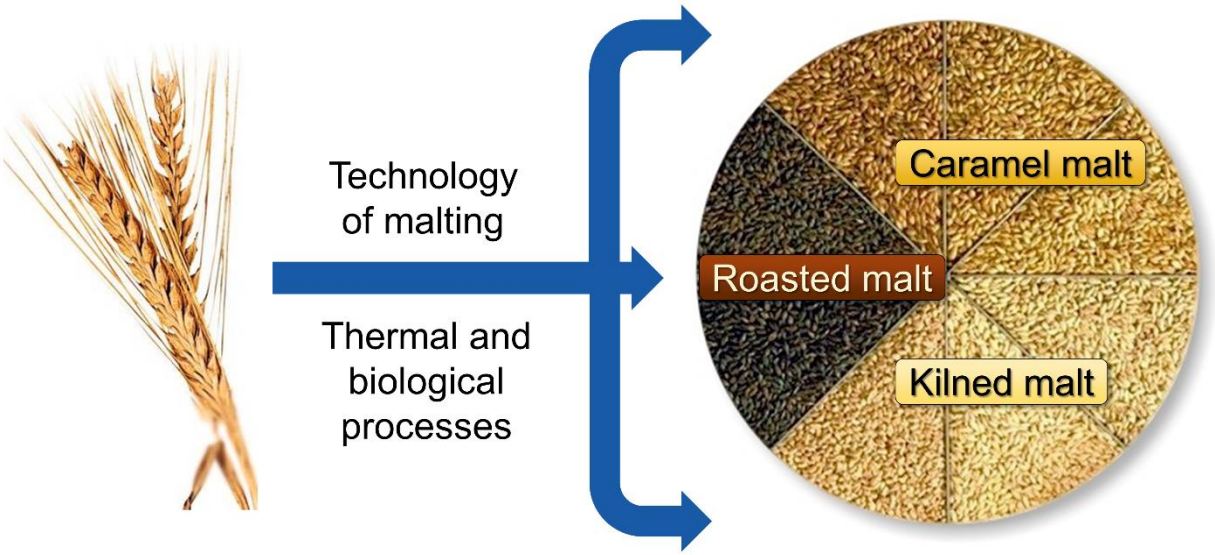

Figure 10 Range of kilned, caramel, and roasted malts produced from cereal grains[85]

4.2 Beer

4.2.1 The Brewing Process

Brewing describes the process of using water, malt, hops, and yeast to produce a fermented, alcoholic beverage called beer. Among the brewing raw materials, malt has the most important impact on the properties of the final product by providing carbohydrates and proteins as well as the enzymes that are responsible for breaking these biopolymers down into fermentable sugars, peptides, and amino acids. In this context, malt also has a crucial influence on the beer sensory properties. Beer is primarily produced by applying standard kilned malt. However, a part of the kilned malt can be substituted by specialty malts such as caramel and roasted malt to enhance the color and aroma of the beer.[53, 86-89]

The first step in the brewing process is grinding the malt mixture to a coarse powder while keeping the husks from the barley malt mostly intact. The grist is then added to water (Figure 11). During the mashing step, the mixture of ground malt and water, the so-called mash, is heated and a temperature program is applied comprising typically four consecutive resting periods at 50, 62, 72, and 78 °C, respectively, to provide the optimal conditions for the activity of the different amylolytic and proteolytic enzymes from the malt. During this mashing step, which usually takes up to 2 h, the remaining starch and proteins in the mash are degraded to peptides, amino acids, and sugars (mostly glucose and maltose). The amount of malt added to a defined volume of water for mashing is selected based on the target soluble extract of the wort, which is measured in degrees Plato (°P) while 1 °P represents the density of a solution of 1% sucrose in water.[53, 88]

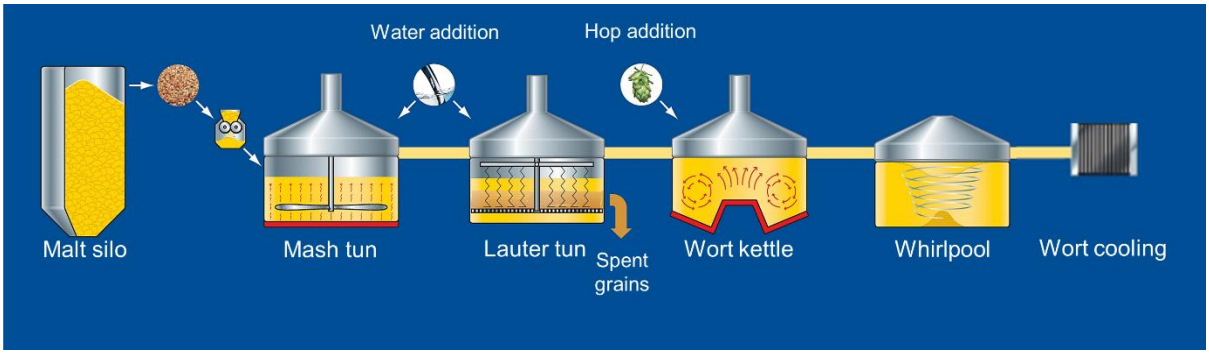

Figure 11 Steps on the “hot side” of the brewing process consisting of malt grinding, mashing, lautering, wort boiling, and whirlpool[90]

After the mashing is complete, the liquid fraction of the mash is separated from the spent grains by lautering, resulting in a clear sugary wort, referred to as the first wort. This is achieved by mechanical filtration using the barley husks as a natural filter aid. To increase the yield of this extraction process, the spent grains are typically washed with water, which is then unified with the first wort. The spent grains are rich in fiber and are typically processed or sold as animal feed.[53, 88, 91]

Following the lautering step, the clear wort is boiled for approximately one hour with the goal of adjusting the amount of soluble extract in the wort by evaporating water, precipitating the hot trub, which mostly consists of denatured protein, and providing the typical bitter taste of beer by adding hops and thermally isomerizing the hop bitter acids contained therein. Thereby, the typical bitter taste is provided by adding hops that are rich in α- and β-acids at the start of boiling while the hoppy aroma in the beer is achieved by adding aroma hops towards the end of boiling, finally resulting in a product that is referred to as the cast wort.[53, 86-87]

While cooling, the boiled wort is transferred to a so-called whirlpool and is set in a circular motion by tangentially entering the respective vessel to concentrate the trub that has been precipitated during boiling in a cone-shaped trub pile at the bottom of the whirlpool. After separating the hot trub in the whirlpool, the wort is cooled to 12 – 20 °C depending on the temperature required for fermentation.[53, 86-87]

The cooled wort is then inoculated with a yeast strain that has been selected for fermentation (Figure 12). During fermentation, the sugars, amino acids, and peptides in the wort are metabolized by the yeast resulting in a continuous decrease in soluble extract while primarily forming ethanol and CO_2 but also secondary fermentation products. These include compounds that play a role as odorants in beer such as higher alcohols and esters, which are typically associated with a positive impact on the aroma but also odorants such as diacetyl (butane-2,3-dione), which exhibits a buttery aroma that is undesired in most beers. The fermentation is completed as soon as a target concentration of remaining soluble extract is reached, there are no more fermentable sugars left or the concentration of ethanol exceeds the tolerance threshold of the respective yeast strain.[53, 86, 92-93]

Furthermore, there is a clear distinction between two categories of yeast strains used for brewing, the top-fermenting strains and the bottom-fermenting strains. These differ substantially in their metabolism, their optimal fermentation temperature, and the secondary products formed during fermentation.[53, 86, 92-93]

Bottom-fermenting yeast strains are traditionally applied for the production of beer types such as “Lager”, “Pilsner”, and “Helles”. The term “bottom-fermenting” originates from the fact that the yeast sinks to the bottom of the respective vessel at the end of the fermentation. The optimal fermentation temperature of bottom-fermenting yeast strains typically lies between 12 and 14 °C while the fermentation is usually completed within a period of 8 to 12 days depending on the starting cell count. Bottom-fermenting yeast is commonly known for its “clean” fermentation only forming a relatively limited amount of secondary fermentation products.[53, 86, 92-93]

In contrast, top-fermenting yeast strains are primarily applied for the production of wheat beers and ales including beer types such as “Kölsch” and “Alt”. The term “top-fermenting” originates from the fact that the yeast rises to the top of the respective vessel at the end of the fermentation due to multiple cells clustering together, thereby enclosing part of the formed CO_2, which causes buoyancy. The optimal fermentation temperature of top-fermenting yeast strains typically lies between 15 and 20 °C and the fermentation is usually completed after a period of 4 to 7 days depending on the starting cell count.

Due to the more rapid fermentation at higher temperatures and the differences in metabolism, top-fermenting yeast strains are known to produce a higher amount of secondary fermentation products compared to bottom-fermenting strains. These secondary fermentation products substantially contribute to the unique aroma of most top-fermented beers but can also have negative effects as off-flavors.[53, 86, 92-93]

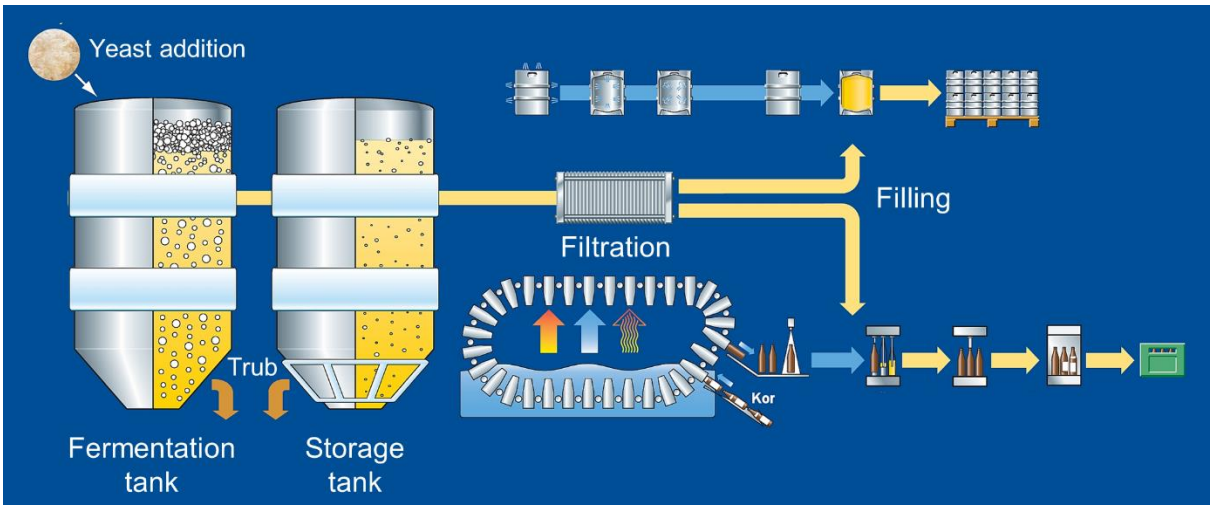

Figure 12 Steps on the "cold side" of the brewing process consisting of fermentation, maturation, filtration, and filling[90]

Within the group of the top-fermenting yeasts, there is a subcategory, which is known to produce a relatively high amount of phenolic secondary fermentation products such as 2-methoxyphenol and 4-ethenyl-2-methoxyphenol by enzymatic decarboxylation of phenolic acids like ferulic, *p*-coumaric, cinnamic, vanillic, caffeic, and sinapic acid resulting from a specific gene that is not present in other yeast strains. These yeasts are referred to as "positive for phenolic off-flavor" (POF+) and are typically applied for the production of German wheat beers, in which the smoky or clove-like aroma caused by these odorants is desired.[94-100]

In many cases, most of the yeast is subsequently separated by filtration and/or centrifugation leading to a product referred to as green beer. This green beer then has to undergo a resting period of approximately 7 days at 8 °C during which most of the diacetyl (butane-2,3-dione), an undesired odorant in beer (buttery smell), is enzymatically converted by the few remaining yeast cells to 3-hydroxybutan-2-one and finally to butane-2,3-diol,[53] which has a substantially higher sensory threshold.[101-102] This is then followed by maturation for approximately 2 weeks at 2 °C during which the remaining yeast becomes resuspended and metabolizes most of the remaining fermentable extract and large parts of undesired odorants by secondary fermentation at a reduced rate controlled by the low temperature and a low cell count.[53, 93, 103]

After maturation, the beer may be subjected to filtration and the amount of dissolved CO_2 is adjusted. Subsequently, the beer is filled into bottles, cans, or kegs. The shelf life of finished beer is typically 6 to 12 months when stored below 8 °C.[53]

4.2.2 Odorants in Beer

The beer aroma is defined by an interplay of various odorants, whose occurrences and concentrations highly depend on the raw materials used for brewing and the technological parameters applied during the brewing process. Thus, the odorants in the beer directly or indirectly originate from the malt, the hops, or the fermentation.

The basic odorants in bottom-fermented Pilsner[104] and Lager beer[26] as well as top-fermented German wheat beer[55-56, 99] have been extensively studied resulting in the conclusion that many potent odorants found in these beers, especially esters and alcohols such as ethyl butanoate, ethyl hexanoate, ethyl methylpropanoate, 3-methylbutan-1-ol, and 3-methylbutyl acetate are secondary products of the yeast metabolism. The contribution of different hop varieties to the beer aroma has been investigated in detail revealing geranyl acetate, geraniol, 2,9-humuladien-6-one, linalool, and 4-methyl-4-sulfanylpentan-2-one as important hop-derived beer odorants.[105-109]

On the contrary, knowledge on the contribution of malt to the beer aroma is scarce. Although it is commonly known that malt, particularly specialty malt has a substantial impact on the aroma of top- and bottom-fermented beers,[71, 84, 110-111] the data on the specific odorants responsible for this contribution is very limited. 4-Hydroxy-2,5-dimethylfuran-2(3*H*)-one (HDMF) has been identified as a major odorant in beer produced with roasted malt by Schieberle.[26] Furthermore, the phenolic odorants 4-methylphenol, 2-methoxyphenol, 4-ethylphenol, 4-ethenylphenol, 4-ethyl-2-methoxyphenol, 4-ethenyl-2-methoxyphenol, vanillin, and 4-(1-hydroxyethyl)-2-methoxyphenol were characterized as major contributors to the aroma of Belgian specialty beers produced with roasted malt.[112] However, it remained unclear if these compounds directly originated from the roasted malt or if the malt merely provided phenolcarboxylic acids, which were then converted to phenolic odorants by POF+ yeasts.

The odorants in malt itself have been investigated by multiple studies. In this context, Farley and Nursten first applied gas chromatography-olfactometry (GC-O) to malt extract.[113] However, the detected odorants were not identified. In contrast, Beal and Mottram characterized 2-methylbutanal and 3-methylbutanal, which exhibit a malty smell, as the most important odorants in kilned barley malt. The process of malt roasting was identified as a method to substantially increase the concentration of these compounds.[69] Furthermore, alkylpyrazines and maltol were suggested as important odorants, particularly in roasted malts. Fickert and Schieberle analyzed the odorants of a caramel malt by aroma extract dilution analysis (AEDA) and quantitated the compounds with high FD factors to evaluate their contribution to the aroma by calculating odor activity values (OAVs).[114] In accordance with the results of Beal and Mottram, they verified 2-methylbutanal and 3-methylbutanal, which both exhibit a malty smell, as major malt odorants with OAVs of 130 and 235, respectively. Additionally identified major odorants in caramel malt included (2*E*,4*E*)-deca-2,4-dienal (fatty), dimethyl sulfide (cabbage-like), dimethyl trisulfide (sulfurous), HDMF (caramel-like), methional (potato-like), 2-methylpropanal (malty), and 1-octen-3-one (mushroom-like).

Using a different approach, Vandecan et al. used the concentrations of 12 selected compounds to distinguish between different malt types.[73, 82] Thus, the concentrations of 2-acetylpyrrole, (*E*)-β-damascenone, and HDMF were highest in kilned malt. In contrast, the concentrations of 4-hydroxy-5-methylfuran-2(3*H*)-one and maltol were highest in caramel malt, whereas the concentrations of cyclotene and different pyrazines were highest in roasted malt. Using different target compounds, Yahya et al. applied a comparable approach, revealing significantly higher concentrations of acetic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, isomaltol, and pentane-2,3-dione in caramel malt but higher concentrations of HDMF, maltol, methylpyrazine, and phenylacetaldehyde in roasted malt.[74]

4.2.3 Transfer of Odor-Active Compounds from Malt to Beer

As discussed in the previous chapter, the major odorants responsible for the typical aroma of different top- and bottom-fermented beer types have been identified and studied in detail. Furthermore, there are several studies investigating the odorants responsible for the aroma of kilned, caramel, and roasted wheat and barley malt.

It has become clear that the aroma properties of a beer are determined by the raw materials used for brewing such as the malt mixture but also by a variety of technological parameters applied during beer production. In this context, it is known that the different stages of the brewing process, namely mashing, lautering, wort boiling, whirlpool, fermentation, and maturation all have an impact on the beer sensory properties including the aroma. This is mostly due to the addition of water, different thermal treatments, and the yeast metabolism, which cause major changes in the product composition. However, the chemical processes taking place during these steps that convert the ingredients provided by the raw materials to the compounds that are responsible for the properties of the final beer, are not yet fully understood. While the analysis of the brewing raw materials can provide some insights into the final product properties,[78] the causal link between malt and beer aroma properties, in particular, is still missing. Thus, the previous assumption in this field of research has been that the impact of malt, particularly that of specialty malt on the beer aroma mostly results from a direct transfer of odorants from the malt to the beer without a large number of chemical changes during the brewing process. However, this hypothesis needs to be tested.

5 Objectives

On one hand, the odorants responsible for the typical aroma of several beer types have been studied in detail by now. On the other hand, several important odorants in kilned, caramel, and roasted wheat and barley malt have been identified. However, the processes involved in the transfer and potential change of odorants from the malt to the beer that take place during the brewing process are not yet fully understood.

Thus, the objectives of this work were (1) to brew two bottom-fermented Lager beers with the addition of specialty barley malts and two top-fermented wheat beers with the addition of specialty wheat malts, namely, a caramel malt and a roasted malt each, (2) to investigate how the aroma properties of the caramel and roasted malt beers differ from each other for each beer type and from the aroma properties of standard beers exclusively brewed with kilned base malts, (3) to screen the volatiles isolated from the beers for odorants by application of GC-O and AEDA, (4) to substantiate these results by quantitating important odorants, calculating their OAVs, and verifying the identified odorants by aroma reconstitution experiments, and finally, (5) to assess the efficiency of the odorant transfer from malt to beer.

6 Results and Discussion

This thesis is based on two research articles that have been published in international peer-reviewed scientific journals. Copies of the two articles, the summaries highlighting the individual author contributions as well as reprint permissions of the publishers are included in the appendix.

6.1 Quantitative Olfactory Profiles of Beers

A standardized brewing protocol was applied to produce six different beers in pilot scale, namely three bottom-fermented Lager beers including a kilned barley malt beer (KBB), a caramel barley malt beer (CBB), and a roasted barley malt beer (RBB)[28] as well as three top-fermented wheat beers including a kilned wheat malt beer (KWM), a caramel wheat malt beer (CWB), and a roasted wheat malt beer (RWB).[115] The malt mixtures applied for the production of the six beers are shown in Table 4 below and were inspired by a common practice in the brewing industry.

Table 4 Composition of Malt Mixtures Applied for Brewing Six Different Beers

Beer	Beer type	Percentage of malts used in brewing recipe (%)					
		KBM[a]	CBM[b]	RBM[c]	KWM[d]	CWM[e]	RWM[f]
KBB	Bottom-fermented	100	0	0	0	0	0
CBB	Bottom-fermented	70	30	0	0	0	0
RBB	Bottom-fermented	98	0	2	0	0	0
KWB	Top-fermented	50	0	0	50	0	0
CWB	Top-fermented	50	0	0	20	30	0
RWB	Top-fermented	50	0	0	48	0	2

[a]Kilned barley malt. [b]Caramel barley malt. [c]Roasted barley malt. [d]Kilned wheat malt. [e]Caramel wheat malt. [f]Roasted wheat malt.

Using a trained sensory panel consisting of 15 individuals (11 female and 4 male, aged 23−50), the quantitative olfactory profiles of the six beers were determined orthonasally by rating the intensities of nine descriptors (“banana”, “caramel”, “earthy”, “roasty”, “floral, honey”, “fruity”, “malty”, “smoky”, and “vinegar”) on a scale from 0 (not detectable) to 3 (strong) with 0.5 increments. The descriptors were predefined based on the odor of reference compounds dissolved in water at concentrations of approximately 100-fold their respective odor threshold values.[28, 115]

The quantitative olfactory profiles of the bottom-fermented beers KBB, CBB, and RBB exhibited significant differences (Figure 13). Beer KBB was characterized by a banana-like, floral, honey-like, and fruity aroma. In contrast, beer CBB demonstrated a strong caramel-like and malty aroma, whereas the aroma of beer RBB was perceived as particularly earthy and roasty.[28]

On the one hand, the aroma notes detected in KBB were also present in the beers CBB and RBB, although slightly weaker. On the other hand, however, the characteristic aroma notes perceived in CBB and RBB were very weak in beer KBB.[28]

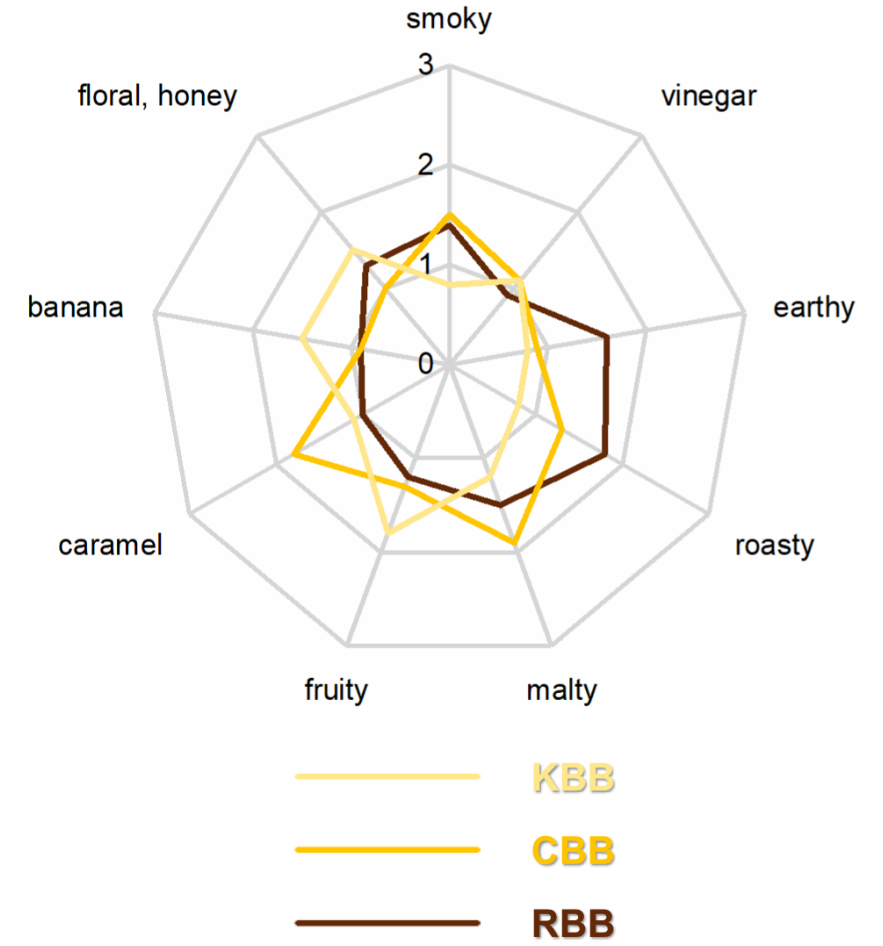

Figure 13 Quantitative olfactory profiles of the three bottom-fermented beers KBB, CBB, and RBB[28]

Similar to the bottom-fermented beers, the olfactory profiles of the top-fermented wheat beers also showed clear differences, which were, however, a bit smaller in scale. Beer KWB was primarily characterized as banana-like, floral, honey-like, and fruity. In contrast, beer CWB exhibited significantly stronger caramel-like, earthy, malty, roasty, and smoky notes. Beer RWB showed an even stronger earthy, malty, roasty, and smoky aroma, whereas the caramel-like note was slightly weaker than in CWB but stronger than in KWB.[115]

Overall, statistical evaluation of the quantitative olfactory profiles using principal component analysis revealed that for both beer types, the aroma differences between the reference beer KBB and KWB, respectively, and the beers produced with specialty malts were approximately twice as large as the differences between the beers produced with caramel and roasted malts.[28, 115]

There were minor variations between the nine olfactory descriptors in their contribution to the differences between the beers but overall, every descriptor substantially contributed to the differentiation.[28, 115]

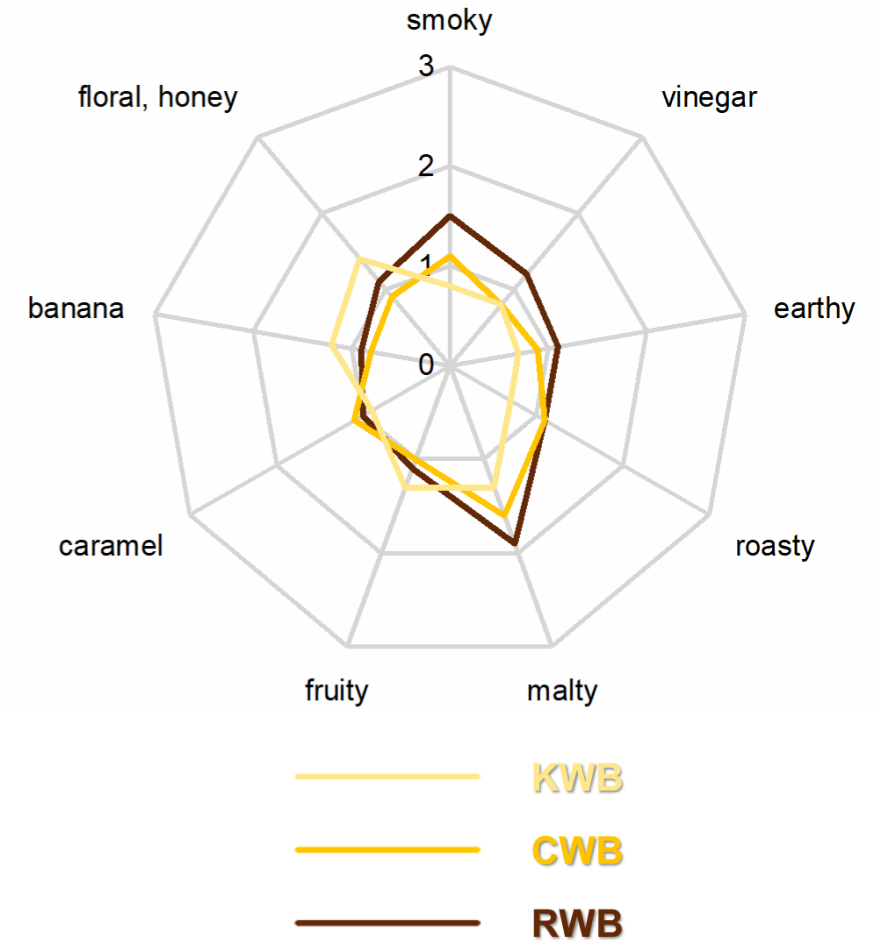

Figure 14 Quantitative olfactory profiles of the three top-fermented beers KWB, CWB, and RWB[115]

Furthermore, there were clear differences in olfactory profiles between the three bottom-fermented and the three top-fermented beers as a result of the variation in grain type and the different yeast strains applied for fermentation. While the bottom-fermented specialty malt beers CBB and RBB had a stronger caramel-like, earthy, and roasty aroma than the top-fermented specialty malt beers CWB and RWB, the intensity of the malty note was comparable between the beer types with the respective reference beer clearly representing the lowest intensity.

6.2 Screening for Odorants in Specialty Malt Beers

As a first step in the identification of the compounds responsible for the differences in olfactory profiles between the beers, the volatiles isolated by solvent extraction and SAFE from the specialty malt beers CBB, RBB, CWB, and RWB were subjected to AEDA comparing the caramel and the roasted malt beer of each beer type. The reference beers KBB and KWB were exclusively produced from kilned base malts, which also made up major portions (≥70%) of the malt mixtures applied to brew the specialty malt beers. Thus, the odorants in the reference beers KBB and KWB were already covered by the analysis of CBB, RBB, CWB, and RWB.

Overall, the comparative AEDA revealed 44 odor-active compounds exhibiting FD factors between 1 and 1024 in at least one of the beers. Each odor-active compound was identified by comparing its retention indices on two GC capillaries of different polarity (DB-FFAP and DB-5), its mass spectrum obtained by GC-MS, as well as its odor quality as perceived at the sniffing port during GC-O to data obtained from authentic reference compounds analyzed under equal conditions. A selection of the odorants with the highest FD factors in the volatiles isolated from the four specialty malt beers is shown in Table 5 below.[28, 115]

Table 5 Odorants with FD Factors of ≥32 in at Least One of the Four Specialty Malt Beers Sorted by Ascending Retention Index[28, 115]

Odorant	Odor	RI[a]	FD Factor			
		FFAP	CBB	RBB	CWB	RWB
Ethanol	Ethanolic	925	1024	1024	1024	1024
Ethyl 2-methylbutanoate	Fruity	1045	64	8	126	256
Methylpropan-1-ol	Malty	1090	2	1	64	64
2-/3-Methylbutan-1-ol	Malty	1206	128	256	512	1024
Ethyl hexanoate	Fruity, pineapple	1226	16	8	32	64
2-Acetyl-1-pyrroline	Roasty, popcorn	1329	256	16	4	2
2-Ethyl-3,5(6)-dimethylpyrazine[b]	Earthy	1432	64	32	126	4
Acetic acid	Vinegar, pungent	1449	256	64	256	512
Methional	Cooked potato	1456	256	256	126	64
2,3-Diethyl-5-methylpyrazine	Earthy	1485	64	16	64	16
2-Methylpropanoic acid	Cheesy	1558	64	16	4	4
Butanoic acid	Cheesy	1624	64	64	2	8
Phenylacetaldehyde	Honey	1642	8	4	64	32
2-/3-Methylbutanoic acid	Cheesy	1661	256	256	1024	1024
Methionol	Cooked potato	1717	32	16	256	512
(*E*)-β-Damascenone	Cooked apple	1811	256	16	64	256
2-Methoxyphenol	Smoky, sweet	1859	4	16	64	256
2-Phenylethanol	Floral, honey	1918	1024	1024	1024	512
Maltol	Caramel	1972	64	16	512	4
γ-Nonalactone	Coconut	2023	<1	<1	32	16
HDMF	Caramel	2048	256	128	1024	256
4-Methylphenol	Phenolic	2086	1	16	4	64
Sotolon	Soup seasoning	2200	256	64	1024	126
2′-Aminoacetophenone	Foxy	2207	64	64	64	256
2,6-Dimethoxyphenol	Smoky, clove	2271	<1	<1	32	<1
Phenylacetic acid	Honey, beeswax	2562	64	16	16	64
Vanillin	Vanilla	2578	256	256	1024	126
3-Phenylpropanoic acid	Floral	2623	128	128	32	16

[a]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [b]Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine.

Similarly high FD factors in all four specialty malt beers were determined for ethanol (FD 1024) and 2-phenylethanol (FD 512−1024). Overall, the bottom-fermented beers CBB and RBB exclusively brewed with barley malts showed substantially higher FD factors for 2-acetyl-1-pyrroline, methional, 2-methylpropanoic acid, butanoic acid, and 3-methylpropanoic acid, whereas the top-fermented wheat malt beers CWB and RWB exhibited higher FD factors for ethyl hexanoate, HDMF methionol, 2-methoxyphenol, ethyl 2-methylbutanoate, 2-/3-methylbutanoic acid, 2-/3-methylbutan-1-ol, methylpropan-1-ol, and phenylacetaldehyde as a result of the variation in grain type and the different yeast strains applied for fermentation.[28, 115]

Comparing CBB and RBB, clearly higher FD factors in CBB were found for 2-acetyl-1-pyrroline (FD 256 vs 16), acetic acid (FD 256 vs 64), (*E*)-β-damascenone (FD 256 vs 16), 2,3-diethyl-5-methylpyrazine (FD 64 vs 16), ethyl 2-methylbutanoate (FD 64 vs 8), maltol (FD 64 vs 16), 2-methylpropanoic acid (FD 64 vs 16), phenylacetic acid (FD 64 vs 16), and sotolon (FD 256 vs 64) suggesting higher concentrations in CBB originating from CBM. In contrast, higher FD factors in RBB were obtained for 4-ethenyl-2-methoxyphenol (FD 16 vs 4, not displayed) and 2-methoxyphenol (FD 16 vs 4) suggesting higher concentrations in RBB originating from RBM.[28]

Comparing CWB and RWB, clearly higher FD factors were determined for 2′-aminoacetophenone (FD 256 vs 64), HDMF (FD 1024 vs 256), maltol (FD 512 vs 4), sotolon (FD 1024 vs 126), and vanillin (FD 1024 vs 126) suggesting higher concentrations in CWB, which originated from CWM. In contrast, higher FD factors in beer RWB were obtained for (*E*)-β-damascenone (FD 256 vs 64), 2-methoxyphenol (FD 256 vs 64), 4-methylphenol (FD 64 vs 5), and phenylacetic acid (FD 64 vs 16) suggesting higher concentrations in RWB originating from RWM.[115]

6.3 Quantitation of Odorants and Calculation of Odor Activity Values

Based on the previous odorant screening and in consideration of literature data, 30 major odor-active compounds in the bottom-fermented beers and 23 major odor-active compounds in the top-fermented beers were selected for quantitation to substantiate the results of the comparative AEDA and to gain deeper insights into the contribution of individual odorants to the overall olfactory profiles. To compensate for workup losses and to ensure the highest possible accuracy of the obtained concentrations, stable isotopically substituted odorants containing ^{2}H or ^{13}C were applied as internal standards.

The concentrations of the odorants determined in the three bottom-fermented beers ranged from 4.1 ng/kg for 2,3-diethyl-5-methylpyrazine in KBB to 790 mg/kg for acetic acid in CBB (Table 6), whereas the concentrations of odorants determined in the three top-fermented beers ranged from 51 ng/kg for 4-methylphenol in KWB to 240 mg/kg for acetic acid in RWB (Table 7). Subsequentl, the OAVs of the 30 quantitated compounds were calculated as ratio of the concentration to the orthonasal odor threshold value (OTV), which was previously determined in water by applying a trained sensory panel and the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[40] The calculation of OAVs allowed for an approximation of the odor impact the individual compounds had on the overall olfactory profiles. The results for the bottom-fermented beers revealed OAVs of up to 250 for (*E*)-β-Damascenone in CBB (Table 6), whereas the OAVs in the top-fermented beers ranged up to 350 for (*E*)-β-Damascenone in RWB (Table 7).[28, 115]

Among the quantitated compounds, butanoic acid, 4-ethenyl-2-methoxyphenol, 2-methylbutan-1-ol, 3-methylbutyl acetate, 2-methylpropanoic acid, methylpropan-1-ol, 3-phenylpropanoic acid, and vanillin exhibited OAVs of <1 in all three bottom-fermented barley malt beers and were therefore considered irrelevant for the aroma (Table 6).[28] For the top-fermented wheat malt beers, this was only the case for 2-acetyl-1-pyrroline (Table 7),[115] which was, however, among the most important odorants in the bottom-fermented beers with OAVs of up to 73 in CBB.

The low OAVs of these compounds were in contrast to relatively high FD factors obtained by the comparative AEDA, particularly for 3-phenylpropanoic acid and vanillin, which might have been overestimated during AEDA. Their comparatively high boiling points have little influence on the FD factors but reduce their release into the headspace of the beers, thus leading to low OAVs. This clearly demonstrates, why the exact quantitation and the calculation of OAVs is essential for obtaining an accurate assessment of the contribution of individual compounds to the overall olfactory profile.[4]

Table 6 Concentrations and OAVs of Selected Odor-Active Compounds in Bottom-Fermented Barley Malt Beers[28]

Odorant	OTV (µg/kg)	Concentration[a] (µg/kg)			OAV		
		KBB	CBB	RBB	KBB	CBB	RBB
Ethyl 2-methylbutanoate	0.13	4.1	14	3.3	32	110	26
Methylpropan-1-ol	1900	780	620	660	<1	<1	<1
3-Methylbutyl acetate	7.2	1.8	2.2	1.8	<1	<1	<1
2-Methylbutan-1-ol	1200	370	340	280	<1	<1	<1
3-Methylbutan-1-ol	220	1200	1200	1000	6	6	5
Ethyl hexanoate	1.2	8.4	12	9.1	7	10	8
2-Acetyl-1-pyrroline	0.053	0.10	3.9	0.10	2	73	2
2-Ethyl-3,5-dimethylpyrazine	0.28	2.3	11	7.0	8	38	25
2-Ethyl-3,6-dimethylpyrazine	0.28	0.010	4.3	9.4	<1	15	34
Acetic acid	5600	630000	790000	120000	110	140	21
Methional	0.43	2.7	9.1	4.6	6	21	11
2,3-Diethyl-5-methylpyrazine	0.031	0.0041	0.26	0.051	<1	8	1
2-Methylpropanoic acid	60000	850	1300	920	<1	<1	<1
Butanoic acid	2400	1400	2000	1300	<1	<1	<1
Phenylacetaldehyde	5.2	10	27	19	2	5	4
2-/3-Methylbutanoic acid[b]	490	1100	1500	1100	2[c]	3[c]	2[c]
Methionol	36	610	2000	720	17	54	20
(*E*)-β-Damascenone	0.006	1.1	1.5	0.80	190	250	130
2-Methoxyphenol	0.84	15	35	57	18	42	67
2-Phenylethanol	140	14000	14000	14000	100	100	100
Maltol	5000	110	14000	1800	<1	3	<1
HDMF	87	330	1100	400	4	12	5
4-Methylphenol	3.9	0.17	0.61	26	<1	<1	7
Eugenol	1.8	0.83	0.55	5.5	<1	<1	3
4-Ethenyl-2-methoxyphenol	4.4	0.043	0.16	1.05	<1	<1	<1
Sotolon	1.7	2.5	16	3.3	1	10	2
2′-Aminoacetophenone	0.27	2.2	1.8	1.3	8	7	5
Phenylacetic acid	68	640	950	580	9	14	9
Vanillin	53	7.0	11	8.3	<1	<1	<1
3-Phenylpropanoic acid	120	14	43	13	<1	<1	<1

[a]Mean of duplicates or triplicates. [b]Concentrations are given as the sum of the isomers 2-methylbutanoic acid and 3-methylbutanoic acid. [c]Calculated with the odor threshold value of 3-methylbutanoic acid (490 µg/kg).

Overall, 22 compounds showed OAVs of >1 in at least one of the three beers within each group. 2-/3-Methylbutanoic acid (OAV 2−3), 3-methylbutan-1-ol (OAV 5−6), and 2-phenylethanol (OAV 100) exhibited identical or similar OAVs in the beers KBB, CBB, and RBB, whereas butanoic acid (OAV 1−2), ethyl hexanoate (OAV 4−5), ethyl 2-methyl-butanoate (OAV 75−92), 2-/3-methylbutanoic acid (2−3), 2-methylbutan-1-ol (OAV 11), 3-methylbutan-1-ol (OAV 3), and 2-phenylethanol (OAV 33−40) showed comparable OAVs in the beers KWB, CWB, and RBB.[28, 115] These compounds are well-known secondary fermentation products and their biosynthesis by the two different yeast strains was obviously not influenced by any malt components.[116-119] These results also confirmed a “cleaner” fermentation by bottom-fermenting yeasts with fewer fermentation by-products compared to top-fermenting yeast strains as it has been reported throughout the literature.[53, 86, 92-93]

A total of 19 compounds showed substantial differences in OAVs between the bottom-fermented beers, whereas 15 compounds showed clear differences between the top-fermented beers.[28, 115] Despite exhibiting the highest OAV in all 6 beers (OAV 130–340), the cooked apple-like odor of (*E*)-β-damascenone is known to be easily suppressed in mixtures of odorants, which is why this compounds most likely only plays a minor role in the overall beer aroma.[120-121]

Overall, the two reference beers KBB and KWB, which were exclusively brewed with kilned malts exhibited the lowest OAVs for most of the compounds when compared to the caramel and roasted malt beers of the same type (Tables 6 and 7).[28, 115]

Among the bottom-fermented barley malt beers, CBB showed the highest OAVs for 14 of the compounds such as fruity smelling ethyl 2-methylbutanoate, earthy smelling 2-ethyl-3,5-dimethylpyrazine, vinegar-like smelling acetic acid, potato-like smelling methional, honey-like smelling phenylacetaldehyde, caramel-like smelling maltol, and soup seasoning-like smelling sotolon. Especially the higher OAVs of the caramel-like smelling maltol and HDMF compared to the other beers corresponded well with the pronounced caramel-like aroma determined in the quantitative olfactory profile of CBB. This observation was well in line with previous results reporting HDMF as an important odorant in caramel malt beer.[26]

The OAVs of some important odor-active compounds such as methional, phenylacetaldehyde, methionol, HDMF, and sotolon in beer RBB were between those in KBB and CBB, which corresponded well with the quantitative olfactory profile of RBB. However, several phenolic compounds such as 2-methoxyphenol, 4-methyl-phenol, and eugenol exhibited substantially higher OAVs in RBB compared to the other barley malt beers. Although this was very likely the result of the intense thermal treatment during the roasting of RBM, which was applied to produce beer RBB, the higher OAVs of these compounds in RBB did not correspond well to the quantitative olfactory profile of RBB, whose rating for the descriptor smoky was higher than in KBB but weaker than in CBB.[28]

Among the top-fermented wheat malt beers, 10 compounds such as earthy smelling 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, and 2,3-diethyl-5-methyl-pyrazine as well as soup seasoning-like smelling sotolon exhibited the highest OAVs in beer CWB. Although CWB showed the highest OAVs for the caramel-like smelling compounds HDMF and maltol, the caramel note of CWB as determined in the quantitative olfactory profile was only slightly stronger than in the beers CWB and RWB. Similar to the roasted barley malt beer RBB, the roasted wheat malt beer RWB showed the highest OAVs of phenolic compounds such as 2-methoxyphenol and 4-methylphenol compared to the other two wheat malt beers KWB and CWB.[115]

Table 7 Concentrations and OAVs of Selected Odor-Active Compounds in Top-Fermented Wheat Malt Beers[115]

Odorant	OTV (µg/kg)	Concentration[a] (µg/kg)			OAV		
		KWB	CWB	RWB	KWB	CWB	RWB
Ethyl 2-methylbutanoate	0.013	0.98	1.0	1.2	75	77	92
2-Methylbutan-1-ol	1200	13000	13000	13000	11	11	11
3-Methylbutan-1-ol	220	670	740	760	3	3	3
Ethyl hexanoate	1.2	5.2	5.6	5.8	4	5	5
2-Acetyl-1-pyrroline	0.053	0.012	0.037	0.014	<1	<1	<1
2-Ethyl-3,5-dimethylpyrazine	0.28	0.10	11	2.1	<1	40	7
2-Ethyl-3,6-dimethylpyrazine	25	0.10	120	2.7	<1	5	<1
Acetic acid	5600	100000	120000	240000	18	21	42
Methional	0.43	4.6	2.3	2.2	11	5	5
2,3-Diethyl-5-methylpyrazine	0.031	0.029	0.46	0.070	1	15	2
Butanoic acid	2400	2100	2100	5700	1	1	2
Phenylacetaldehyde	5.2	17	29	20	3	6	4
2-/3-Methylbutanoic acid[b]	490	980[g]	1400[g]	1200[g]	2[c]	3[c]	3[c]
Methionol	36	1300	630	1500	37	17	41
(*E*)-β-Damascenone	0.006	2.1	1.0	2.1	340	170	350
2-Methoxyphenol	0.84	27	33	55	32	39	66
2-Phenylethanol	140	5300	5600	4700	38	40	33
Maltol	5000	110	7900	1600	<1	2	<1
HDMF	87	550	780	650	6	9	8
4-Methylphenol	3.9	0.051	1.6	58	<1	<1	15
Sotolon	1.7	2.3	12	3.4	1	7	2
2′-Aminoacetophenone	0.27	1.5	1.2	1.4	6	4	5
Phenylacetic acid	68	270	700	290	4	10	4

[a]Mean of duplicates or triplicates. [b]Concentrations are given as the sum of the isomers 2-methylbutanoic acid and 3-methylbutanoic acid. [c]Calculated with the odor threshold value of 3-methylbutanoic acid (490 µg/kg).

Although the overall trends were comparable, there were substantial differences between the three barley malt beers and the three wheat malt beers. The roasty popcorn-like smelling compound 2-acetyl-1-pyrroline was more odor-active in the three bottom-fermented beers, especially in CBB, represented by an OAV of 73. In contrast, the OAV of 2-acetyl-1-pyrroline was <1 in all the top-fermented wheat malt beers, suggesting no contribution to the aroma of these beers. Furthermore, the compound (*E*)-β-damascenone, although known to be overestimated by OAV calculations exhibited the highest OAV in CBB compared to KBB and RBB (OAV 250 vs 190 and 130) but the lowest OAV in CWB compared to the kilned and roasted wheat malt beers (OAV 170 vs 340 and 350). Finally, among the wheat malt beers, the OAV of acetic acid was highest in RWB, which corresponded well with the relatively strong vinegar-like note in the respective olfactory profile. However, the OAV of acetic acid in the bottom fermented beers was clearly lower in RBB than in both other beers (OAV 21 vs 110 and 140).[28, 115]

6.4 Beer Aroma Reconstitution

Aroma reconstitution models were prepared for each of the six beers using all odorants exhibiting OAVs ≥1 in the respective beers (17 in KBB, 20 in CBB, 21 in RBB, 18 in KWB, 21 in CWB, and 20 in RWB). These odorants were added to a hydroalcoholic solution at the concentrations previously determined in the beer samples. The final mixtures were adjusted to pH values (4.68 in KBB, 4.69 in CBB, 4.74 in RBB, 4.45 in KWB, 4.46 in CWB, and 4.43 in RWB) and ethanol concentrations (5.08% vol. for KBB, 4.47% vol. for CBB, 4.95% vol. for RBB, 5.06% vol. for KWB, 4.39% vol. for CWB, and 4.59% vol. for RWB) representing those of the original beers.[28, 115]

Quantitative olfactory profiles were determined for the aroma reconstitution models and compared to those of the beer samples. Overall, the reconstitution models very well represented the aroma of the beers, thus verifying that all key odorants had been correctly identified and quantitated in the beers (Figures 15). Minor differences between the beers and the reconstitution models were observed for the floral, honey-like note in KBB and RWB, the earthy and vinegar-like notes in CBB and RWB, the malty note in RBB and CWB, and the banana-like note in KWB. However, these deviations were small.[28, 115]

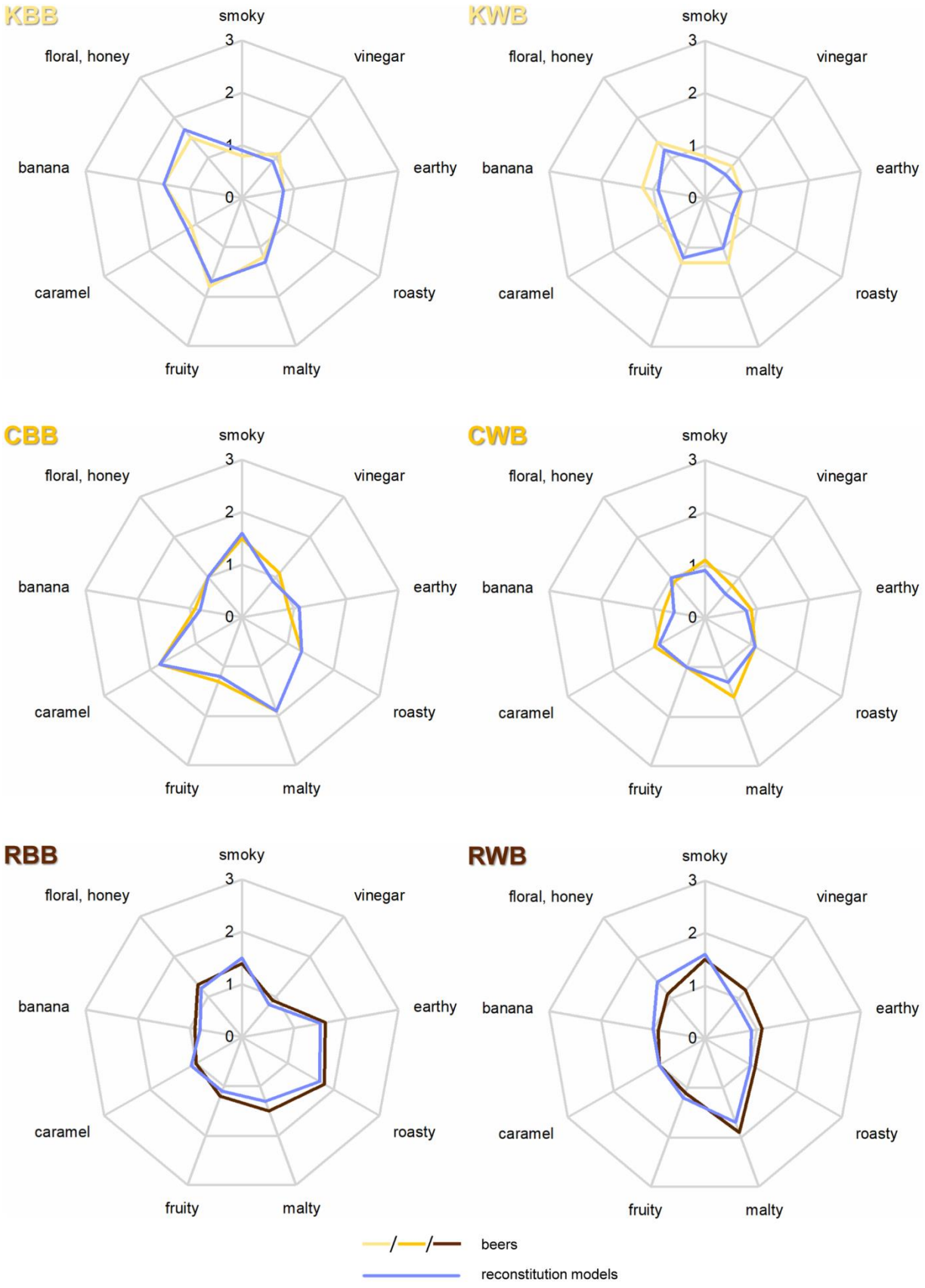

Figure 15 Quantitative olfactory profiles of the aroma reconstitution models in comparison to those of the beers KBB, KWB, CBB, CWB, RBB, and RWB[28, 115]

6.5 Quantitation of Beer Odorants in Malts

In the previous experiments, the odorants responsible for the characteristic aroma of the bottom-fermented barley malt beers KBB, CBB, and RBB, the top-fermented wheat malt beers KWB, CWB, and RWB, and the aroma differences between these beers were identified, quantitated, and verified. Furthermore, the results suggested that especially the odorants responsible for the aroma differences between the beers originated from the malts applied for brewing. Thus, the next step was to assess the efficiency of the odorant transfer from the malts to the beers. For this, all compounds exhibiting OAVs ≥1 in at least one of the beers as well as substantial differences in OAVs between the three beer samples of each type were quantitated in the 6 malts applied for brewing.[28, 115]

For an accurate assessment of the odorant transfer from the malts to the beers, it is crucial to consider the free form as well as the bound form of the odorants in the malt, which is released by contact with water. According to the literature, the amount of the bound form of some Strecker aldehydes and other compounds not related to Strecker degradation in malt can exceed the amount of the free form by a factor of up to 58.[122-124] To account for this, the free and the bound forms of the odorants were quantitated in sum by subjecting the malt to extraction with a mixture of 95% diethyl ether and 5% of water by volume before applying SAFE. The volatile isolate was then separated into the acidic volatile fraction (AV) and the neutral and basic volatile fraction (NBV) before GC–MS analysis.[28]

After the odor-active compounds had been quantitated in KBM, CBM, RBM, KWM, CWM, and RWM, the concentrations determined in these malts were used to calculate the concentrations in the malt mixtures applied for producing the beers KBB, CBB, RBB, KWB, CWB, and RWB (Table 8) according to the respective recipes (cf. Table 4).

Table 8 Concentrations of Selected Odor-Active Compounds in the Malt Mixtures Applied for Producing the Beers KBB, CBB, RBB, KWB, CWB, and RWB

Odorant	Concentration in the malt mixtures applied for producing beers[a] (µg/kg)					
	KBB	CBB	RBB	KWB	CWB	RWB
Ethyl 2-methylbutanoate	0.14	0.52	0.14	n.d.[b]	n.d.	n.d.
Ethyl hexanoate	2.6	2.5	2.7	n.d.	n.d.	n.d.
2-Acetyl-1-pyrroline	1.5	2.1	1.6	1.6	52	1.8
2-Ethyl-3,5-dimethylpyrazine	3.6	5.5	8.3	7.3	16	16
2-Ethyl-3,6-dimethylpyrazine	0.16	3.4	1.4	1.4	14	8.0
Acetic acid	96,000	230,000	120,000	240,000	400,000	240,000
Methional	4.8	18	4.7	4.1	10	4.0
2,3-Diethyl-5-methylpyrazine	0.0059	0.33	0.49	0.14	1.3	0.58
Phenylacetaldehyde	24	25	25	37	23	38
Methionol	5.5	3.9	6.3	6.3	4.3	6.3
(*E*)-β-Damascenone	0.051	0.46	0.20	0.034	0.96	0.11
2-Methoxyphenol	1.4	4.3	6.8	2.7	4.4	6.0
2-Phenylethanol	n.d.	n.d.	n.d.	25	95	25
Maltol	19	5400	1500	17	22000	6400
HDMF	17	300	83	12.3	1800	230
4-Methylphenol	0.17	0.40	0.91	0.15	0.35	0.31
Eugenol	0.088	0.069	0.23	n.d.	n.d.	n.d.
Sotolon	0.19	2.4	0.43	0.42	2	0.62
2′-Aminoacetophenone	0.38	0.43	0.38	0.22	0.41	0.22
Phenylacetic acid	57	370	70	37	86	40

[a]Mean of duplicates or triplicates. The malt mixtures applied for producing the beers were KBB: 100% KBM; CBB: 70% KBM and 30% CBM; RBB: 98% KBM and 2% RBM; KWB: 50% KBM and 50% KWM; CWB: 50% KBM, 30% CWM, and 20% KWM; RWB: 50% KBM, 48% KWM, and 2% RWM. [b]Not determined due to the compound not exhibiting an OAV ≥1 in at least one of the beers within each beer type or due to only minor differences in the OAVs between these beers indicating that the compound is not malt-derived.

The obtained concentrations ranged from 0.0059 µg/kg for 2,3-diethyl-5-methylpyrazine in malt KBM used to brew beer KBB to 400 mg/kg for acetic acid in the malt mixture applied to produce beer CWB. Overall, the concentration of most odorants in the malts substantially increased with the intensity of the thermal treatment during malting. Thus, the majority of odor-active compounds exhibited the lowest concentrations in the kilned malts KBM and KWM and the highest concentrations in the roasted malts RBM and RWM. The difference in concentration was most extreme for the odorants 2,3-diethyl-5-methylpyrazine and maltol. However, due to the beers CBB and CWB being produced with 30% caramel malts but the beers RBB and RWB only being produced with 2% roasted malts, the concentrations of most odorants were highest in the malt mixtures applied for brewing the caramel malt beers (Table 8) except for 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-methoxyphenol, and 4-methylphenol, whose concentrations were still highest in one of the two roasted malt mixtures despite the lower amounts in the brewing recipe compared to the caramel malts.[28, 115]

6.6 Transfer of Odorants from Malt to Beer

The odorant concentrations in the malt mixtures were used to calculate the hypothetical concentrations to be expected in the beers assuming 100% transfer from malt to beer and the absence of other sources. Subsequently, these hypothetical concentrations were compared to the odorant concentrations previously determined in the beers to assess the efficiency of their transfer.

The results for the three bottom-fermented beers are displayed in Figure 16. The full bars represent the odorant concentrations determined in the beers and the yellow, orange, and brown bars indicate the calculated hypothetical concentrations in the beers KBB, CBB, and RBB, consequently representing the concentrations explainable by a direct transfer from the malt mixtures to the beers. Thus, the differences between the full bars and the colored bars display the minimum percentages of the odorant concentrations that do not originate from the malt directly. To illustrate the impact of each odorant on the aroma of the beers, the OAVs were copied from Table 6 and the highest OAV of each odorant was highlighted in bold.[28]

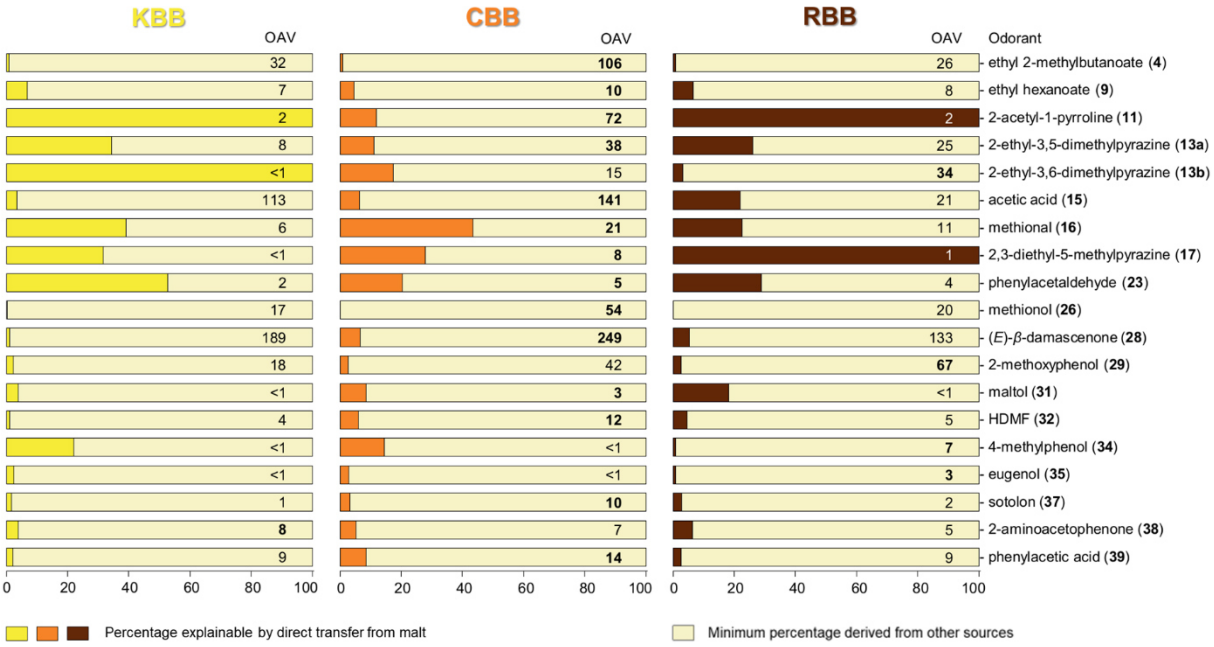

Figure 16 Percentage of odorant concentrations in bottom-fermented beers explain- able by a direct transfer from malt[28]

Overall, for most odorants, a direct transfer only accounted for less than 50% of the actual concentrations in the beers, in many cases even less than 20%. However, considering the substantially different OAVs between the beers and the fact that the malt mixtures were the only differences between these beers, the impact of the malt composition on the final odorant concentrations in the beer was evident.[28]

This suggested an additional formation of the odorants from malt-derived precursors during the brewing process. It has been reported that compounds such as 2-acetyl-1-pyrroline, (*E*)-β-damascenone, HDMF, maltol, phenols, pyrazines, and sotolon are formed during thermal treatment while it has been observed that (*E*)-β-damascenone and sotolon are also formed during beer aging.[125-126] The formation of these compounds might occur by thermal processing during brewing or during malt kilning and roasting. Since the majority of the odorants were found in substantially lower amounts in the malt mixtures than in the beers, odorants might also have been encapsulated in malt biopolymers such as starch resulting in adducts that are stable during water contact at room temperature but release the odorants at higher temperatures and/or by biopolymer degrading enzymes in the brewing process. However, this hypothesis has to be tested in future studies.[28]

In contrast, the concentrations of the odorants 2-acetyl-1-pyrroline and 2-ethyl-3,6-dimethylpyrazine in KBB and 2-ethyl-3,6-dimethylpyrazine and 2,3-diethyl-5-methyl-pyrazine in RBB were equal to or lower than the hypothetical concentrations, suggesting only a direct transfer or even a partial loss during the brewing process. However, the OAVs of these odorants in the respective beers were relatively low (≤2) suggesting that they only played a minor role in the overall aroma.[28]

The results for the three top-fermented wheat malt beers are depicted in Figure 17. Similar to the bottom-fermented beers, only minor percentagess of most odorants in KWB, CWB, and RWB could be explained by a direct transfer from the malt mixtures.[115] This was the case for the known secondary fermentation products methionol, phenylacetic acid, and 2-phenylethanol as well as for (*E*)-β-damascenone, HDMF, 2-methoxyphenol, and 4-methylphenol, which are most likely formed during thermal processing as previously suggested based on the results obtained from the bottom-fermented beers.[115]

An exception was the roasty, popcorn-like smelling compound 2-acetyl-1-pyrroline, which was additionally formed during the production of the bottom-fermented beers resulting in an OAV of 2 in KBB and CBB and in an OAV as high as 72 in CWB, in which 2-acetyl-1-pyrroline was the 4th most potent odorant. However, in the top-fermented beers, this compound was only present at ≤4% of the hypothetical concentrations not even reaching the odor threshold concentrations in any of the three beers despite 2-acetyl-1-pyrroline being present in the malt mixtures used to brew the top-fermented beers at equal or higher concentrations (1.5–2.1 µg/kg) compared to those applied for brewing the bottom-fermented beers (1.6–52.0 µg/kg).[28, 115]

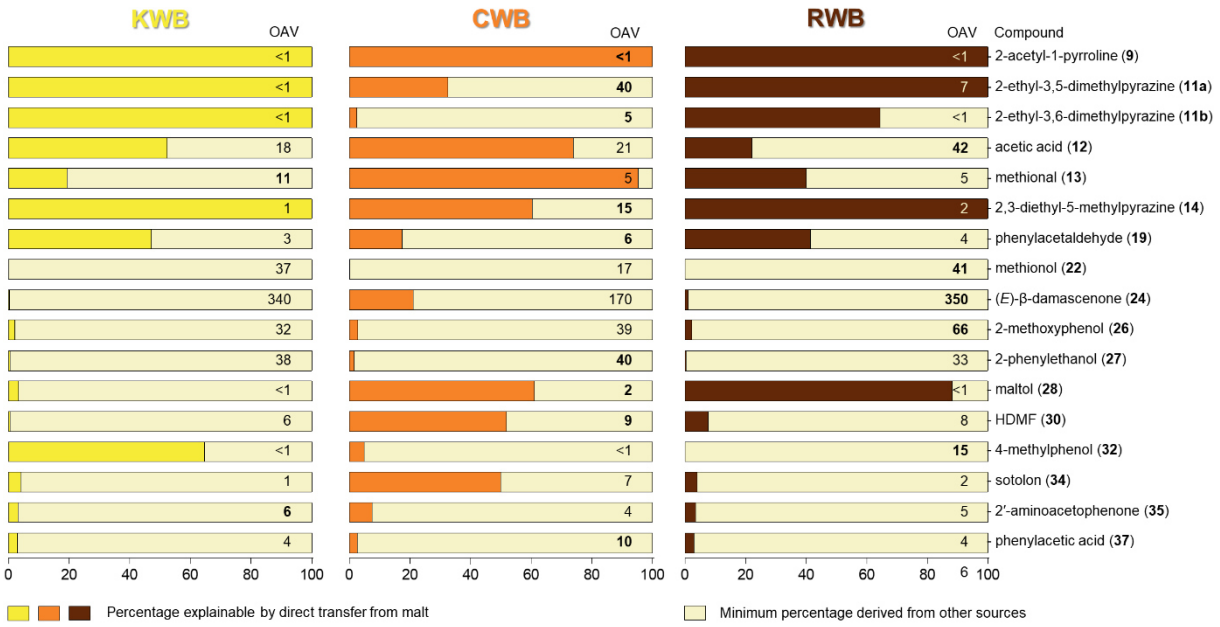

Figure 17 Percentage of odorant concentrations in top-fermented wheat beers explain- able by a direct transfer from malt[115]

Although the OAVs of the individual compounds differed between the two beer types, the overall trends were comparable between the bottom- and top-fermented beers (except for 2-acetyl-1-pyrroline), leaving two potential conclusions. Either, currently unknown precursors of the beer odorants are formed during thermal treatment in the malting process, which are themselves not odor-active but are converted to odorants during mashing, wort boiling, or fermentation in the brewing process, or the odorants are already formed during malt production but are then encapsulated in biopolymers such as starch to which they might be non-covalently bound. In the latter case, large parts of most odorants could be released by the simultaneous application of water and increased temperatures as is the case during brewing, associated with a gelatinization and enzymatic degradation of starch. This could also explain why a full release of the odorants from the malt was not achieved during our workup when by applying extraction of the volatiles with diethyl ether and water at ambient temperature. Depending on the odorant, either one or both of the explanations apply. However, this hypothesis has to be tested in future studies.

In conclusion, this work identified and quantitated the compounds responsible for the aroma of bottom- and top-fermented beers produced from kilned, caramel, and roasted barley and wheat malts. The results revealed that a large number of the major odorants in the beers were either directly or indirectly derived from the malts applied for brewing. The conducted experiments showed that especially the caramel and roasted malts provided a distinct aroma to the beers produced with these specialty malts, which was characterized based on quantitative olfactory profiles determined by a trained sensory panel as well as the identification and quantitation of the responsible odorants with

analytical methods. While the aroma of the caramel malt beers was primarily characterized by caramel-like, earthy, malty, and roasty smelling pyrazines, furanones, and pyranones, the aroma of the roasted malt beers was mostly determined by phenolic, smoky, and sweet smelling phenols and some earthy smelling pyrazines. The reference beers solely produced with kilned malts had an overall weaker aroma that was mostly defined by floral, honey-like, and fruity notes. The quantitation of the odor-active compounds in the malts to assess their transfer from malt to beer surprisingly revealed substantially lower amounts of most major odorants in the malt mixtures applied for brewing compared to the beers, indicating that they were either formed from malt-derived precursors during brewing and/or are formed during the malting process, subsequently bound by biopolymers, and liberated from these complexes during brewing. Thus, the significance of sensory and analytical results obtained by evaluating dry malt or malt pretreated with water at room temperature to predict the beer aroma is limited. However, it has to be investigated if methods like congress mash[127] and hot steep[128] are better suited to achieve this goal by simultaneously applying water and increased temperatures, thus representing a simplified version of the brewing process.

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8 Appendix

8.1 Publication 1: Molecular Insights into the Contribution of Specialty Barley Malts to the Aroma of Bottom-Fermented Lager Beers

8.1.1 Bibliographic Data

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8.1.2 Publication Reprint

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

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Molecular Insights into the Contribution of Specialty Barley Malts to the Aroma of Bottom-Fermented Lager Beers

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ABSTRACT: Specialty barley malts provide unique aroma characteristics to beer; however, the transfer of specialty malt odorants to beer has not yet been systematically studied. Therefore, three beers were brewed: (1) exclusively with kilned base barley malt, (2) with the addition of a caramel barley malt, and (3) with the addition of a roasted barley malt. Major odorants in the beers were identified by aroma extract dilution analysis followed by quantitation and calculation of odor activity values (OAVs). The caramel malt beer was characterized by outstandingly high OAVs for odorants such as (*E*)-*β*-damascenone, 2-acetyl-1-pyrroline, methionol, 2-ethyl-3,5-dimethylpyrazine, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, whereas the highest OAV for 2-methoxyphenol was obtained in the roasted malt beer. Quantifying odorants in the malts revealed that the direct transfer from malt to beer played only a minor role in the amount of malt odorants in the beers, suggesting a substantial formation from precursors and/or a release of encapsulated odorants during brewing.

KEYWORDS: *specialty barley malt beer, Hordeum vulgare, aroma extract dilution analysis (AEDA), stable isotopically substituted odorants, odorant transfer*

INTRODUCTION

Malting involves a three-step process of steeping, germinating, and kilning cereal grains. Kilned malt, particularly from barley (*Hordeum vulgare*), is a major raw material in beer brewing. It provides carbohydrates and proteins and the enzymes that break down these biopolymers into fermentable sugars, peptides, and amino acids during mashing. Additionally, malt has a vital influence on the sensory properties of beer.[1,2] To enhance color and aroma of beer, a part of the kilned malt in the recipe can be substituted by so-called specialty malts such as caramel malt and roasted malt.[3] Specialty malts are obtained when higher temperatures are applied during malting, which enhances the formation of colorants and odorants produced via thermal processes such as the Maillard reaction and Strecker degradation.[4−9] Higher steeping degrees and/or germination temperatures can be applied to further increase this effect.

The basic odor-active compounds in beer have been studied in detail.[10,11] The majority of these compounds are secondary products of yeast metabolism such as 2-phenylethanol, 3-methylbutan-1-ol, ethyl butanoate, and ethyl hexanoate.[10,12,13] Hops also provide a number of odor-active compounds to beer aroma, among which linalool, geraniol, and 4-methyl-4-sulfanylpentan-2-one are of particular importance.[11,14−17] By contrast, the knowledge of contributors from malt, particularly the role of specialty malts in beer aroma, is scarce. Schieberle characterized caramel-like 4-hydroxy-2,5-dimethylfuran-2(3*H*)-one (HDMF) as a major odorant in beer produced with roasted malt.[10] Scholtes et al. identified phenols such as 4-methylphenol, 2-methoxyphenol, 4-ethylphenol, 4-ethenylphenol, 4-ethyl-2-methoxyphenol, 4-ethenyl-2-methoxyphenol, vanillin, and 4-(1-hydroxyethyl)-2-methoxyphenol as important aroma contributors in some Belgian specialty beers brewed with roasted malts.[18] However, it remained unclear, if these roasted malts provided the phenols themselves or the corresponding phenolcarboxylic acids, which were converted to phenols by POF+ yeasts. A large number of studies have been performed on odor-active compounds present in barley malt itself. Farley and Nursten were the first to apply gas chromatography-olfactometry (GC-O) to a malt extract but did not identify the odor-active compounds.[19] Also, using GC-O, Beal and Mottram identified 3-methylbutanal and 2-methylbutanal as the most potent odorants in a kilned barley malt.[6] Furthermore, roasting significantly increased their concentrations. Alkylpyrazines and maltol were suggested as additional important malt odorants. Fickert and Schieberle applied an aroma extract dilution analysis (AEDA) to a caramel malt, quantitated compounds with high FD factors, and assessed their aroma impact by calculating odor activity values (OAVs).[20] With high OAVs of 235 and 130, 3-methylbutanal and 2-methylbutanal were confirmed as key odorants in malt. Further important odorants included methional (potato-like), dimethyl sulfide (cabbage-like), dimethyl trisulfide (sulfurous), 2-methylpropanal (malty), HDMF (caramel-like), (2*E*,4*E*)-deca-2,4-dienal (fatty), and 1-octen-3-one (mushroom-like). Vandecan et al. compared differently processed specialty malts based on the concen-

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trations of 12 selected compounds.[7,8] HDMF, 2-acetylpyrrole, and β-damascenone concentrations were the highest in kilned malt, whereas 4-hydroxy-5-methylfuran-2(3*H*)-one and maltol were highest in caramel malt, and cyclotene and pyrazines were highest in roasted malt. A similar approach was applied by Yahya et al., but target compounds differed.[9] For example, pentane-2,3-dione, acetic acid, isomaltol, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one showed significantly higher concentrations in caramel malt, whereas methylpyrazine, phenylacetaldehyde, maltol, and HDMF were higher in roasted malt.

Although there is some knowledge of odor-active compounds in malt including specialty malts, very little information is available on their transfer into beer and their role in the final beer aroma. Thus, the objectives of our study were (1) to brew beers with the addition of specialty barley malts, namely, a caramel malt and a roasted malt, (2) to characterize how the specific aroma profiles differ from each other and from the aroma profile of a standard lager beer exclusively brewed with a kilned base malt, (3) to screen the volatiles isolated from the beers for odor-active compounds by application of GC-O and AEDA, (4) to substantiate the screening results by quantitating potent odorants and calculating their OAVs, and finally, (5) to assess the efficiency of the odorant transfer from malt to beer.

MATERIALS AND METHODS

Malts. A kilned barley malt (KBM), a caramel barley malt (CBM), and a roasted barley malt (RBM) were provided by Mich. Weyermann (Bamberg, Germany). All three malts were made from a single batch of barley, variety Barke, harvest 2016; however, thermal treatments differed. KBM was kilned at 80−90 °C. For CBM, green malt was directly transferred to a roasting drum without a previous kilning step and treated at 120−130 °C. RBM was kilned at 80−90 °C and subsequently roasted in the roasting drum at 210−220 °C. Further malting parameters and product specifications determined with standard approaches[21] are provided in the Supporting Information, Table S1.

Beers. A Braumeister Plus 50 L device (Speidel, Ofterdingen, Germany) was used to brew a kilned barley malt beer (KBB), a caramel barley malt beer (CBB), and a roasted barley malt beer (RBB). KBB was brewed with 100% KBM, CBB was brewed with a mixture of 70% KBM and 30% CBM, and RBB was brewed with a mixture of 98% KBM and 2% RBM. Malt (11 kg) was ground and added to 50 L of water. Mashing was performed at 50 °C for 20 min followed by 63 °C for 55 min, 73 °C for 30 min, and 78 °C for 10 min. After lautering, spent grains were washed with 10 L of water. The first wort and the washing water were combined, and the mixture was boiled for 60 min. Pelletized hops (37.5 g), variety Hallertau Perle (Hopsteiner, Mainburg, Germany), were added after 10 min of boiling. A second portion of hops (12.5 g) was added after 50 min of boiling. The total hop dosage corresponded to an expected bitterness of 20 IBU. The original extract was ≥12 °P. The hot trub was separated, and the wort was cooled to 20 °C. The rehydrated dry yeast (20 g), *Saccharomyces cerevisiae*, strain W34/70 (Fermentis Lesaffre, Marcq-en-Barœul, France) was added. This strain is a well-established lager beer yeast, yielding a product with a neutral aroma and a low amount of fermentation byproducts.[22,23] Fermentation was performed at 14 °C in cylindro−conical tanks (Speidel). Fermentation was monitored daily (Supporting Information, Table S2) with an ALEX 500 alcohol and extract meter (Anton Paar, Graz, Austria) and finally stopped at an apparent relative degree of fermentation of 74−77%. The yeast was separated by decantation, and beers were stored in 50 L kegs at 8 °C for 1 week. Maturation was performed at 2 °C for 2 weeks. Levels of dissolved CO_2 were adjusted to 4.5 g/L, and the beers were bottled in 0.5 L amber glass bottles sealed with crown caps. Final ethanol concentrations were 5.08% vol. (KBB), 4.47% vol. (CBB), and 4.95% vol. (RBB). The beers were stored for 3 weeks before analysis.

Reference Odorants. Compounds **1**, **3**−**10**, **12**−**41**, and 3-methylbutyl acetate were purchased from Merck (Darmstadt, Germany), and compound **2** was from Alfa Aesar (Karlsruhe, Germany). Compound **11** was synthesized as detailed in the literature.[24]

Stable Isotopically Substituted Odorants. The following isotopically substituted compounds were used as internal standards in the quantitation assays: ($^2H_3$)-**4**, ($^2H_3$)-**6**, ($^2H_{11}$)-**7**, ($^2H_{11}$)-**8b**, ($^2H_{11}$)-**9**, ($^{13}C_5$)-**11**, ($^2H_3$)-**13a**, ($^2H_3$)-**13b**, ($^2H_3$)-**15**, ($^2H_3$)-**16**, ($^2H_3$)-**17**, ($^2H_7$)-**20**, ($^2H_2$)-**22**, ($^{13}C_2$)-**23**, ($^2H_2$)-**24b**, ($^2H_3$)-**26**, ($^2H_3$)-**27**, ($^2H_7$)-**28**, ($^2H_3$)-**29**, ($^2H_5$)-**30**, ($^{13}C_2$)-**32**, ($^2H_7$)-**34**, ($^{13}C_6$)-**36**, ($^{13}C_2$)-**37**, ($^2H_3$)-**38**, ($^{13}C_2$)-**39**, ($^2H_3$)-**40**, ($^2H_2$)-**41**, and ($^2H_2$)-2-methoxy-4-propylphenol. The sources are detailed in the Supporting Information, Table S3.

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

GC-O/FID. The GC-O/FID system detailed previously was employed.[17]

GC−MS. A 7890B gas chromatograph equipped with a GC Sampler 80 and a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film, was connected to an Ion Trap 240 mass spectrometer via a heated (250 °C) transfer line (Agilent, Waldbronn, Germany). The carrier gas was helium at 1.00 mL/min constant flow. The oven temperature was initially 40 °C, held for 5 min, and then ramped at 6 °C/min to 230 °C and held for 5 min. Mass chromatograms were obtained in the CI mode using methanol as reagent gas and a scan range of m/z 40−250. MS workstation software (Agilent) was used for data evaluation.

HS-SPME-GC-MS. The system described above was equipped with a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film, or a fused silica column, DB-5, 30 m × 0.25 mm i.d., 1.00 μm film (both Agilent). The GC sampler was operated with a 65 μm PDMS/DVB SPME fiber or with a 50 μm DVB/CAR/PDMS SPME fiber (both Merck). Volatiles were extracted at 30 °C for 5 min and desorbed at 250 °C for 1.5 min. After analysis, fibers were baked out at 270 °C for 10 min. For the analysis of compounds **6** and **7**, the oven temperature was 35 °C for 5 min, ramped at 20 °C/min to 240 °C, and held for 10 min. For the analysis of compounds **8** and **9**, the oven temperature was 40 °C for 2 min, ramped at 6 °C/min to 230 °C, and held for 5 min. MS parameters were as described above.

GC × GC-TOFMS. A 6890 Plus gas chromatograph (Agilent) was equipped with a PAL autosampler (CTC Analytics, Zwingen, Switzerland), a CIS 4 injector (Gerstel, Mülheim a. d. Ruhr, Germany), a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film, in the first dimension, and a fused silica column, DB-5, 2 m × 0.15 mm i.d., 0.30 μm film, in the second dimension (both Agilent). The GC was connected to a Pegasus III TOFMS system (Leco, Mönchengladbach, Germany) and was operated as previously described.[25] The temperature of the first oven was 40 °C for 2 min, ramped at 6°/min to 230 °C, and held for 5 min. The modulation time was 4 s. The temperature of the second oven was 70 °C for 2 min, ramped at 6°/min to 250 °C, and held for 5 min. GC Image software (Lincoln, NE) was used for data evaluation.

Aroma Extract Dilution Analysis. Beer (250 mL) was decarbonated by filtration and then stirred with diethyl ether (300 mL) at ambient temperature for 1 h. The phases were separated, and the aqueous phase was stirred with a second portion of diethyl ether (300 mL) for 1 h. The combined organic phases were washed with brine (200 mL) and dried with anhydrous sodium sulfate. After filtration, volatile compounds were separated from non-volatiles by solvent-assisted flavor evaporation (SAFE).[26] The distillate was concentrated to a final volume of 500 μL by using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device.[27]

Beer volatile isolates were analyzed by GC-O/FID. The analyses were carried out by three experienced GC-O sniffers (aged 27−36). The volatile isolates were stepwise diluted with diethyl ether to obtain

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dilutions of 1:2, 1:4, 1:8, and so on, and the diluted samples were additionally subjected to GC-O/FID analysis. Each odor-active compound was assigned a flavor dilution (FD) factor, representing the dilution factor of the most diluted sample, in which the odorant was detected.[28]

Odorant Quantitation. The filtered beer (250 mL) was stirred with diethyl ether (300 mL) at ambient temperature for 24 h. Malt grains were frozen with liquid nitrogen and ground into a fine powder using a laboratory mill Grindomix GM 200 (Retsch, Haan, Germany) at 4000 rpm (10 s) and 10,000 rpm (10 s). Diethyl ether (0.5−5 mL) and water (9.5−95 mL) were added to the powder (1−10 g), and the mixture was stirred at ambient temperature for 24 h. In both cases, the diethyl ether portion had been spiked with stable isotopically substituted odorants as internal standards (cf. Supporting Information, Table S3). The filtration, washing, drying, and volatile-isolation steps were performed as detailed above. The isolates were separated into the acidic volatile fraction (AV) and the neutral/basic volatile fraction (NBV) as detailed previously.[29] The compounds **15**, **20**, **22**, **24**, and **27** were quantitated by GC−MS analysis of AV; **26**, **37**, **39**, **40**, and **41** were quantitated by GC × GC-TOFMS analysis of AV; and **4**, **11**, **13**, **16**, **17**, **23**, **28**, **29**, **30**, **31**, **32**, **34**, **35**, and **38** were quantitated by GC × GC-TOFMS analysis of NBV.

The compounds **6**−**9** were quantitated by HS-SPME-GC-MS using the PDMS/DVB fiber (**6**, **7**) or the DVB/CAR/PDMS fiber (**8**, **9**). For this purpose, beer samples were degassed and diluted 1:100 with water. The diluted samples (1 mL) were placed in 20 mL headspace vials and spiked with stable isotopically substituted odorants. The vials were sealed, and the samples were subjected to HS-SPME-GC-MS analysis. Powdered malt samples (2 g) were mixed with water (1 mL) and spiked with stable isotopically substituted odorants. The vials were sealed, and the samples were equilibrated at ambient temperature for 30 min and finally subjected to HS-SPME-GC-MS analysis.

Characteristic quantifier ions of the analyte and internal standard were monitored by MS. The concentration of each target compound in the malt and beer samples was calculated from the peak areas of the analyte and standard, the amount of malt or beer used, and the amount of standard added by employing a calibration line equation. The calibration line equation was obtained from the analysis of analyte/standard mixtures in at least five different concentration ratios (∼1:20−50:1) followed by linear regression. Individual quantifier ions and calibration line equations are provided in the Supporting Information, Table S3.

Odor Threshold Values. The American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits was applied.[30] The matrix was water and the trained panel consisted of 15−20 employees (aged 24−56) of the Leibniz-LSB@TUM.

Aroma Reconstitution. For each of the three beer aroma models, defined volumes (0.05−2 mL) of ethanolic stock solutions of the odorants were combined and made up to 10 mL with water. A volume of 0.1 mL of such a mixture was added to a hydroalcoholic solution with an ethanol concentration corresponding to the respective beer sample (5.08% vol. for KBB, 4.47% vol. for CBB, and 4.95% vol. for RBB). The pH was adjusted to the value in the original beer (4.68 in KBB, 4.69 in CBB, and 4.74 in RBB). The concentrations of the stock solutions and the volumes used were adjusted to obtain final concentrations of each odorant in the beer aroma model solutions that represented the concentrations previously determined in the beer samples.

Quantitative Olfactory Profiles. The beers and the reconstitution models (10 mL) were evaluated in cylindrical ground neck glasses (height 7 cm and i.d. 3.5 cm) with lids (Merck) at ∼15 °C. 15 trained panelists (11 female and 4 male, aged 23−50) evaluated the olfactory profiles of the beer samples orthonasally by rating the intensities of nine predefined descriptors on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Individual descriptors were defined using the odor of a reference compound dissolved in water at a concentration of ∼100-fold its respective odor threshold value. The nine descriptors and the corresponding reference compounds were "banana" (3-methylbutyl acetate), "caramel" (**32**), "earthy" (**13a**), "roasty" (**11**), "floral, honey" (**30**), "fruity" (**9**), "malty" (**8b**), "smoky" (**29**), and "vinegar" (**15**). Ratings of all panelists were averaged by calculating the arithmetic mean. For data analysis, XLSTAT-Biomed 2019.3.1 software (Addinsoft, Boston, MA) was used.

RESULTS AND DISCUSSION

Quantitative Olfactory Profiles of Beers. Two specialty malt lager beers were brewed using a standardized protocol. For beer CBB, a mixture of 70% KBM and 30% CBM was used, whereas beer RBB was brewed with 98% KBM and 2% RBM. These percentages of caramel and roasted malt were inspired by a common practice in the brewing industry. A third beer was prepared from 100% KBM and served as a reference. The olfactory profiles of the three beers showed significant differences (Figures 1 and 2). Beer KBB showed strong floral,

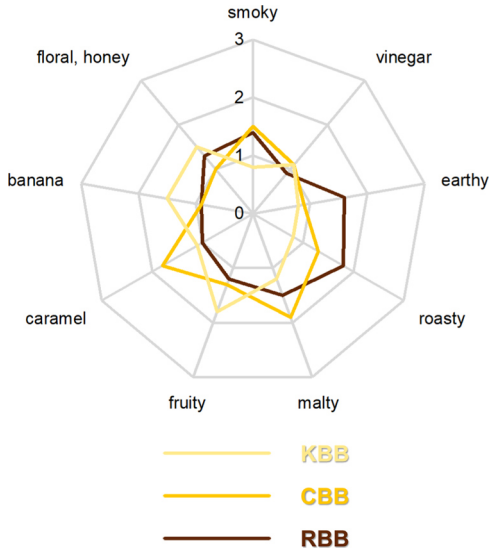

Figure 1. Quantitative olfactory profiles of beers KBB, CBB, and RBB.

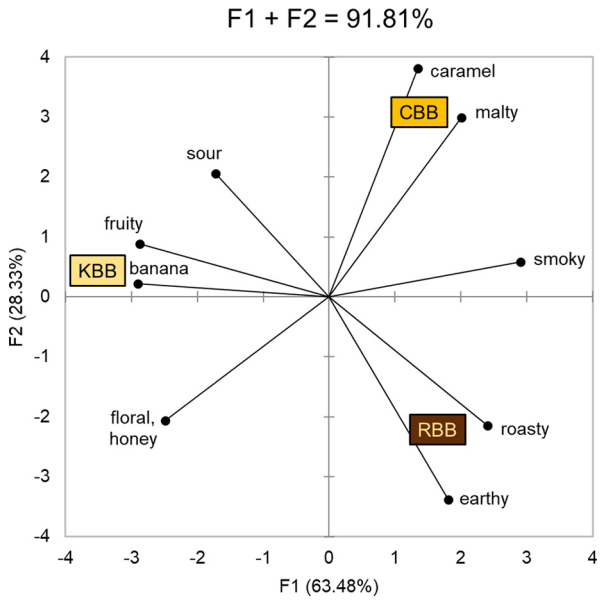

Figure 2. Principal component analysis applied to the quantitative olfactory profiles of beers KBB, CBB, and RBB.

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Table 1. Odorants in the SAFE Distillates Obtained from Barley Malt Beers

				FD factor[c]	
no.	odorant[a]	odor	RI[b] FFAP	CBB	RBB
1	2-methylpropanal	malty	833	8	4
2	ethanol	ethanolic	925	1024	1024
3	butane-2,3-dione	butter	991	4	1
4	ethyl 2-methylbutanoate	fruity	1045	64	8
5	ethyl 3-methylbutanoate	fruity	1059	8	4
6	methylpropan-1-ol	malty	1090	2	1
7	3-methylbutyl acetate	fruity, banana	1117	4	4
8	2-/3-methylbutan-1-ol	malty	1206	128	256
9	ethyl hexanoate	fruity, pineapple	1226	16	8
10	oct-1-en-3-one	mushroom	1295	1	4
11	2-acetyl-1-pyrroline	roasty, popcorn	1329	256	16
12	3-isopropyl-2-methoxypyrazine	earthy	1427	2	<1
13	2-ethyl-3,5(6)-dimethylpyrazine[d]	earthy	1432	64	32
14	ethyl octanoate	fruity, green	1441	<1	8
15	acetic acid	vinegar, pungent	1449	256	64
16	methional	cooked potato	1456	256	256
17	2,3-diethyl-5-methylpyrazine	earthy	1485	64	16
18	propanoic acid	cheesy, pungent	1538	1	<1
19	linalool	citrus, bergamot	1542	4	8
20	2-methylpropanoic acid	cheesy	1558	64	16
21	MDMF[e]	caramel	1592	1	<1
22	butanoic acid	cheesy	1624	64	64
23	phenylacetaldehyde	honey	1642	8	4
24	2-/3-methylbutanoic acid	cheesy	1661	256	256
25	(2*E*,4*E*)-nona-2,4-dienal	fatty	1695	1	8
26	methionol	cooked potato	1717	32	16
27	pentanoic acid	cheesy	1726	<1	1
28	(*E*)-*β*-damascenone	cooked apple	1811	256	16
29	2-methoxyphenol	smoky, sweet	1859	4	16
30	2-phenylethanol	floral, honey	1918	1024	1024
31	maltol	caramel	1972	64	16
32	HDMF[f]	caramel	2048	256	128
33	octanoic acid	sour, musty	2062	<1	4
34	4-methylphenol	phenolic	2086	1	16
35	eugenol	clove	2169	<1	4
36	4-ethenyl-2-methoxyphenol	phenolic	2178	4	16
37	sotolon	seasoning	2200	256	64
38	2′-aminoacetophenone	foxy	2207	64	64
39	phenylacetic acid	honey, bees-wax	2562	64	16
40	vanillin	vanilla	2578	256	256
41	3-phenylpropanoic acid	floral	2623	128	128

[a]Each odorant was identified by comparing its retention indices on two GC columns of different polarity (DB-FFAP and DB-5), its mass spectrum obtained by GC−MS, and its odor quality as perceived at the sniffing port during GC-O to the data obtained from authentic reference compounds analyzed under the same conditions. [b]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [c]Flavor dilution factor. [d]Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine. [e]4-Methoxy-2,5-dimethylfuran-3(2*H*)-one. [f]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

honey-like, banana-like, and fruity odor notes. In the beers CBB and RBB produced with specialty malts, these notes were also present but weaker. Beer CBB exhibited particularly strong caramel and malty odor notes, whereas the profile of beer RBB was dominated by earthy and roasty notes. These traits were very weak in the reference beer KBB (Figure 1).

To further substantiate the aroma differences between the beers, a principal component analysis was applied to the quantitative olfactory profiles. The factor F1 covered 2/3 of the variation in the data set and separated the two specialty malt beers CBB and RBB on the left side of the plot from the reference beer KBB on the right side of the plot (Figure 2). The factor F2 covered the remaining 1/3 of the variation and primarily separated the two specialty malt beers from each other with CBB being located in the upper half and RBB being located in the lower half of the plot. Thus, the overall aroma differences between CBB and RBB on the one hand and the reference beer KBB on the other hand were roughly twice as large as the differences between the two specialty malt beers. Furthermore, the principal component analysis revealed that the attributes fruity, banana-like, and smoky primarily contributed to the factor F1 separating CBB and RBB from KBB. In contrast, attributes caramel and earthy mostly contributed to the factor F2 distinguishing CBB from RBB.

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Table 2. Concentrations and OAVs of Selected Odor-Active Compounds in Barley Malt Beers

			concentration[b] (μg/kg)			OAV[c]		
no.	odorant	OTV[a] (μg/kg)	KBB	CBB	RBB	KBB	CBB	RBB
4	ethyl 2-methylbutanoate	0.13	4.1	14	3.3	32	110	26
6	methylpropan-1-ol	1900	780	620	660	<1	<1	<1
7	3-methylbutyl acetate	7.2	1.8	2.2	1.8	<1	<1	<1
8a	2-methylbutan-1-ol	1200	370	340	280	<1	<1	<1
8b	3-methylbutan-1-ol	220	1200	1200	1000	6	6	5
9	ethyl hexanoate	1.2	8.4	12	9.1	7	10	8
11	2-acetyl-1-pyrroline	0.053	0.10	3.9	0.10	2	73	2
13a	2-ethyl-3,5-dimethylpyrazine	0.28	2.3	11	7.0	8	38	25
13b	2-ethyl-3,6-dimethylpyrazine	0.28	0.010	4.3	9.4	<1	15	34
15	acetic acid	5600	630,000	79,0000	120,000	110	140	21
16	methional	0.43	2.7	9.1	4.6	6	21	11
17	2,3-diethyl-5-methylpyrazine	0.031	0.0041	0.26	0.051	<1	8	1
20	2-methylpropanoic acid	60,000	850	1300	920	<1	<1	<1
22	butanoic acid	2400	1400	2000	1300	<1	<1	<1
23	phenylacetaldehyde	5.2	10	27	19	2	5	4
24	2-/3-methylbutanoic acid[d]	490	1100	1500	1100	2[e]	3[e]	2[e]
26	methionol	36	610	2000	720	17	54	20
28	(*E*)-β-damascenone	0.006	1.1	1.5	0.80	190	250	130
29	2-methoxyphenol	0.84	15	35	57	18	42	67
30	2-phenylethanol	140	14,000	14,000	14,000	100	100	100
31	maltol	5000	110	14,000	1800	<1	3	<1
32	HDMF[f]	87	330	1100	400	4	12	5
34	4-methylphenol	3.9	0.17	0.61	26	<1	<1	7
35	eugenol	1.8	0.83	0.55	5.5	<1	<1	3
36	4-ethenyl-2-methoxyphenol	4.4	0.043	0.16	1.05	<1	<1	<1
37	sotolon	1.7	2.5	16	3.3	1	10	2
38	2′-aminoacetophenone	0.27	2.2	1.8	1.3	8	7	5
39	phenylacetic acid	68	640	950	580	9	14	9
40	vanillin	53	7.0	11	8.3	<1	<1	<1
41	3-phenylpropanoic acid	120	14	43	13	<1	<1	<1

[a]Odor threshold value; orthonasally determined in water. [b]Mean of duplicates or triplicates; individual data and standard deviations are included in the Supporting Information, Tables S4−S6. [c]Odor activity value. [d]Concentrations are given as sum of the isomers 2-methylbutanoic acid (**24a**) and 3-methylbutanoic acid (**24b**); OAVs were calculated with the OTV of 3-methylbutanoic acid (490 μg/kg). [e]Calculated with the odor threshold value of 3-methylbutanoic acid. [f]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

The remaining attributes malty, vinegar-like, floral, honey-like, and roasty contributed equally to both factors. As indicated by a longer distance from the origin of the plot, attributes caramel and earthy contributed slightly more to the overall separation, especially compared to the attributes smoky, banana-like, and sour whose locations were closer to the origin. Overall, however, all nine variables substantially contributed to the differentiation between the beers.

Screening for Odorants in Beers by AEDA. As a first step toward the identification of the substances responsible for the differences in the olfactory profiles, a comparative AEDA was applied to the volatiles isolated from beers CBB and RBB by solvent extraction and SAFE. Beer KBB was not separately analyzed because it was brewed with 100% KBM. Thus, odorants originating from KBM were already covered by analysis of CBB and RBB, for which KBM was used at percentages of 70 and 98%, respectively. Moreover, the assessment of the differences between the three beers was finally performed on the basis of OAV calculations, and AEDA was primarily used to aid in the selection of the target odorants for the quantitation assays.

Results of the comparative AEDA revealed 41 odor-active compounds with FD factors between 1 and 1024 (Table 1). High FD factors in both beers were found for ethanol (**2**; FD 1024), 2-phenylethanol (**30**; FD 1024), methional (**16**; FD 256), 2-/3-methylbutanoic acid (**24**; FD 256), vanillin (**40**; FD 256), 2-/3-methylbutan-1-ol (**8**; FD 128 and 256), HDMF (**32**; FD 256 and 128), and 3-phenylpropanoic acid (**41**; FD 128). Clearly, higher FD factors in CBB were obtained for ethyl 2-methylbutanoate (**4**; FD 64 vs 8), 2-acetyl-1-pyrroline (**11**; FD 256 vs 16), acetic acid (**15**; FD 256 vs 64), 2,3-diethyl-5-methylpyrazine (**17**; FD 64 vs 16), 2-methylpropanoic acid (**20**; FD 64 vs 16), (*E*)-β-damascenone (**28**; FD 256 vs 16), maltol (**31**; FD 64 vs 16), sotolon (**37**; FD 256 vs 64), and phenylacetic acid (**39**; FD 64 vs 16), suggesting higher amounts in CBB which originated from CBM. Likewise, higher FD factors in RBB obtained for 2-methoxyphenol (**29**; FD 16 vs 4) and 4-ethenyl-2-methoxyphenol (**36**; FD 16 vs 4) suggested higher amounts in RBB which originated from RBM.

Quantitation and OAV Calculation of Beer Odorants. On the basis of the results obtained by AEDA and in consideration of the literature data, 29 major odor-active compounds were selected for quantitation. Stable isotopically substituted beer odorants were used as internal standards to compensate for workup losses and to ensure the highest possible accuracy of the concentration data generated. The results (Table 2) revealed concentrations ranging from 4.1 ng/kg for 2,3-diethyl-5-methylpyrazine (**17**) in KBB to 790 mg/kg

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for acetic acid (**15**) in CBB. To approximate the odor impact of the individual compounds, OAVs were calculated as a ratio of the concentration to the orthonasal odor threshold value in water. The results revealed OAVs up to 250. 8 out of 30 compounds, namely, **6**, **7**, **8a**, **20**, **22**, **36**, **40**, and **41** showed OAVs <1 in all three beers and were thus considered irrelevant for the aroma. Some of these low OAVs were in contrast to comparatively high FD factors obtained in the AEDA, particularly for compounds **40** and **41**. These compounds might have been overestimated during AEDA. Their relatively high boiling points have little influence on the FD factors but clearly reduce their release into the headspace of the beers, thus leading to low OAVs. This illustrates, why the exact quantitation and the calculation of OAVs is essential to obtain a more accurate assessment of the odor contribution of individual compounds.[28] 22 out of 30 compounds showed an OAV of ≥1 in at least one of the three beers. Among these, three compounds showed comparable OAVs in all three beers, namely, 3-methylbutan-1-ol (**8b**; OAV 5−6), 2-/3-methylbutanoic acid (**24**; OAV 2−3), and 2-phenylethanol (**30**; OAV 100). These compounds are well-known fermentation by-products,[12,13,31,32] and their biosynthesis by the yeast was obviously not influenced by malt components. The other 19 odorants showed more or less pronounced differences among the three beers. In all beers, (*E*)-*β*-damascenone (**28**) exhibited the highest OAV (130−250). However, in mixtures, its odor is known to be easily suppressed by other odor-active compounds, resulting in a minor importance for the overall aroma.[33,34] In most cases, the KBB brewed exclusively with KBM showed the lowest OAVs among the three beers. CBB, which was brewed with 30% CBM in addition to 70% KBM, showed the highest OAVs among the three beers for fruity smelling compounds **4**, **9**, and **28**; roasty smelling compound **11**; earthy smelling compounds **13a** and **17**; vinegar-like smelling **15**; potato-like smelling compounds **16** and **26**; honey-like smelling compounds **23** and **39**; caramel-like smelling compounds **31** and **32**; and seasoning-like smelling compound **37**. The high OAVs for **31** and **32** corresponded well to the high intensity of the caramel-like note in beer CBB (cf. Figure 1). An important role of HDMF (**32**) in beers brewed with caramel malt has previously been reported.[10]

RBB, which was brewed with 2% RBM in addition to 98% KBM, showed OAVs that were in between KBB and CBB for some important odorants such as **16**, **23**, **26**, **32**, and **37**. This corresponded well to the olfactory profile of RBB (cf. Figure 1). Clearly, higher OAVs in RBB than in the other two beers were calculated for phenolic compounds 2-methoxyphenol (**29**; OAV 67 vs 18 & 42), 4-methylphenol (**34**; OAV 7 vs <1 & <1), and eugenol (**35**; OAV 3 vs <1 & <1), reflecting the higher thermal impact on the malt during roasting of RBM. However, this was not visible in the olfactory profile of RBB, where the rating of the attribute smoky was higher than that in the profile of KBB but not higher than that in the profile of CBB.

Beer Aroma Reconstitution. Reconstitution models were prepared on the basis of all odorants with OAVs ≥1 in the three beers (17 in KBB, 20 in CBB, and 21 in RBB) and hydroalcoholic solutions representing the ethanol concentrations and pH values of the original beers. Overall, the reconstitution models very well represented the characteristic aroma of the beers, thus indicating that all key odorants were correctly identified and quantitated (Figure 3). Differences between the beers and the respective reconstitution models were particularly observed for the floral, honey-like, and vinegar-like notes in KBB, the earthy and vinegar-like notes in CBB, and the malty note in RBB. However, these differences were small.

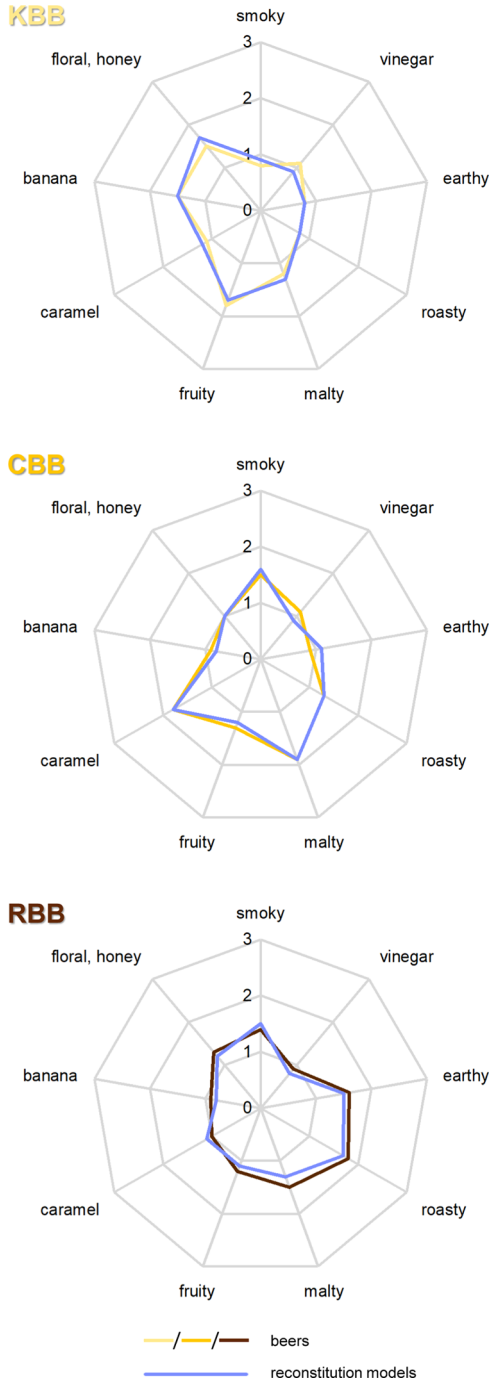

Figure 3. Quantitative olfactory profiles of the aroma reconstitution models in comparison to the profiles of the beers KBB, CBB, and RBB.

Quantitation of Beer Odorants in Malts. The experiments discussed above had revealed the odor-active compounds responsible for the characteristic aroma of CBB and RBB and the difference in the aroma of KBB. Our next aim was to assess the efficiency of their transfer from the malts to beers. As a first step, the 19 compounds with OAVs > 1 in at least

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Table 3. Concentrations of Selected Odor-Active Compounds in Barley Malts and Malt Mixtures

		concentration in malts[a] (μg/kg)			concentration in malt mixtures (μg/kg)	
no.	odorant	KBM	CBM	RBM	KBM/CBM 70/30[b]	KBM/RBM 98/2[c]
4	ethyl 2-methylbutanoate	0.14	1.4	0.17	0.52	0.14
9	ethyl hexanoate	2.6	2.1	7.4	2.5	2.7
11	2-acetyl-1-pyrroline	1.5	3.6	5.7	2.1	1.6
13a	2-ethyl-3,5-dimethylpyrazine	3.6	9.9	240	5.5	8.3
13b	2-ethyl-3,6-dimethylpyrazine	0.16	11	60	3.4	1.4
15	acetic acid	96,000	550,000	1,200,000	230,000	120,000
16	methional	4.8	50	0.081	18	4.7
17	2,3-diethyl-5-methylpyrazine	0.0059	1.1	24	0.33	0.49
23	phenylacetaldehyde	24	27	59	25	25
26	methionol	5.5	0.091	44	3.9	6.3
28	(*E*)-β-damascenone	0.051	1.4	7.3	0.46	0.20
29	2-methoxyphenol	1.4	11	270	4.3	6.8
31	maltol	19	18,000	73,000	5400	1500
32	HDMF[d]	17	970	3300	300	83
34	4-methylphenol	0.17	0.93	37	0.40	0.91
35	eugenol	0.088	0.026	7.3	0.069	0.23
37	sotolon	0.19	7.5	12	2.4	0.43
38	2′-aminoacetophenone	0.38	0.53	0.39	0.43	0.38
39	phenylacetic acid	57	1100	700	370	70

[a]Mean of duplicates or triplicates; individual data and standard deviations are included in the Supporting Information, Tables S7−S9. [b]Calculated as 0.7 × concentration in KBM + 0.3 × concentration in CBM. [c]Calculated as 0.98 × concentration in KBM + 0.02 × concentration in RBM. [d]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

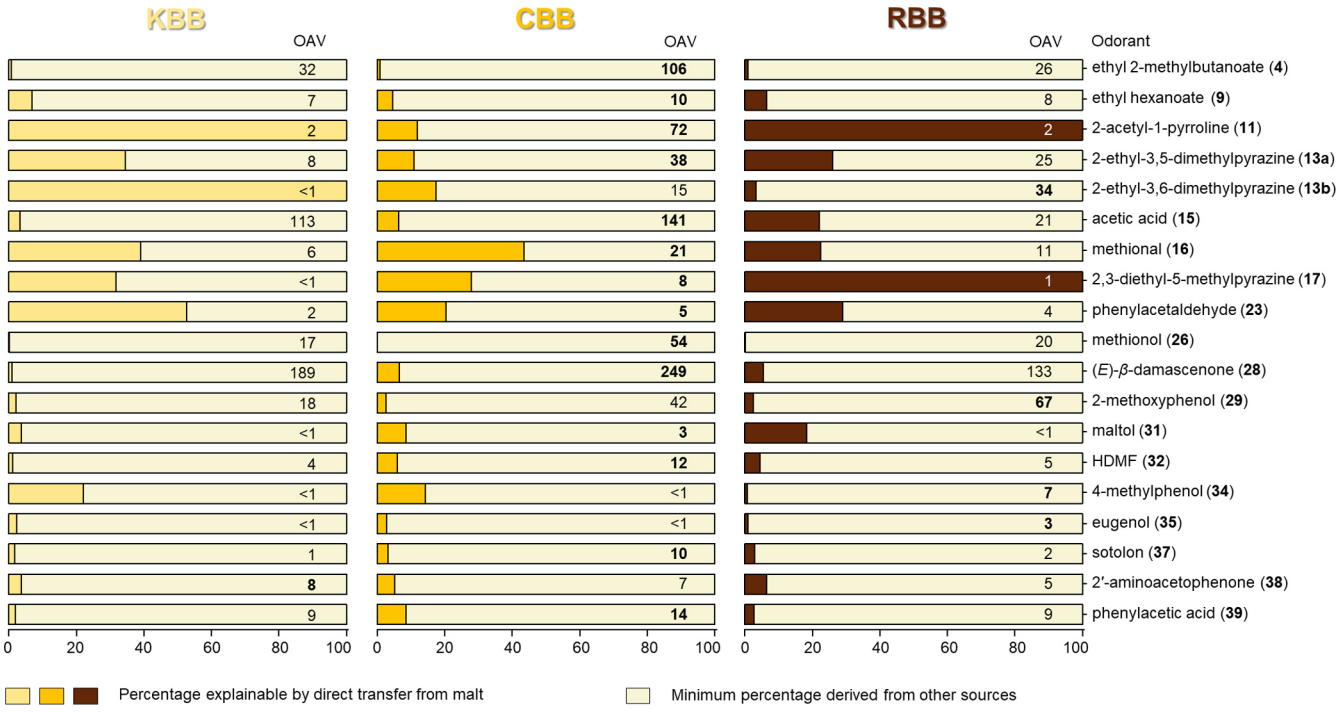

Figure 4. Percentages of odorant concentrations in beer explainable by a direct transfer from malt.

one of the beers **and** with a clear difference in the OAVs among the three beers were quantitated in the three malts.

For these quantitation experiments, one must not only consider the free form of the odorants in malt but also their bound counterpart which is released by water contact. It has been shown that for some Strecker aldehydes, the amount of the bound form exceeds the amount of the free form by a factor of up to 58.[35] Intermediate formation of 3-oxazolines during Strecker degradation was suggested to account for this.[36] However, a recent study reported a high amount of bound odorants in malt not only for Strecker aldehydes but also for compounds not related to Strecker degradation.[37] We therefore quantitated the odorants in KBM, CBM, and RBM as the sum of free and bound volatiles. The majority of compounds showed an increasing concentration in the order of increasing thermal impact during malting, that is, the lowest concentration was determined in KBM and the highest concentration was determined in RBM (Table 3). Extreme

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differences were found for 2,3-diethyl-5-methylpyrazine (**17**) and maltol (**31**). Different from the majority of compounds, methional showed the highest concentration in CBM, whereas for methionol, the concentration in CBM was the lowest.

Transfer of Odorants from Malt to Beer. From the concentrations of the odorants in the individual malts and their percentages in the brewing recipes, the concentrations in the malt mixtures used for CBB and RBB were calculated (Table 3, columns 6 and 7). No such calculation was necessary for KBB as it was brewed with 100% KBM. These odorant concentrations were then used to calculate the hypothetical concentrations to be expected in the beers, assuming 100% transfer from malt and the absence of other sources (Supporting Information, Table S10). These hypothetical concentrations were then compared to the actual concentrations of the compounds previously determined in the beers (cf. Table 2). Results are depicted in Figure 4. Full bars represent the concentrations of the odorants determined in the beers. The percentages explainable by a direct transfer from malt are indicated by the yellow, light brown, and dark brown bars. OAVs were copied from Table 2 to illustrate the aroma impact of the individual compounds in the three beers.

For the majority of compounds, less than 50% of the total concentration in beer could be attributed to direct transfer from the malt, indicating that the final odorant concentrations in the beers mainly originated from other sources. Considering the clear differences in the OAVs among the three beers, an influence of the different malt mixtures, however, was evident. We therefore hypothesize a substantial odorant formation to occur from malt-derived precursors during the brewing process, albeit this conclusion is somewhat speculative. The transformation of precursors to odorants might occur during mashing, boiling, and due to the metabolic activity of the yeast. For example, the yeast may convert free amino acids provided by the malts to a variety of compounds by means of the Ehrlich pathway. Methionine might be converted to methionol (**26**) and methional (**16**). The yeast might also form esters such as ethyl 2-methylbutanoate (**4**) and ethyl hexanoate (**9**) from malt-derived free carboxylic acids. Given the fact that these compounds showed the highest OAVs in CBB, the concentrations of the precursor acids should have been the highest in CBM. Unfortunately, no data on the concentrations of amino acids and other carboxylic acids were available for the malts used in this study. Compounds such as 2-acetyl-1-pyrroline (**11**), the pyrazines (**13a**, **13b**, and **17**), (*E*)-*β*-damascenone (**28**), the phenols (**29**, **34**, and **35**), maltol (**31**), HDMF (**32**), 4-methylphenol (**34**), and sotolon (**37**) are typically formed by thermal reactions. However, formation of (*E*)-*β*-damascenone (**28**) and sotolon (**37**) has also been reported during beer aging.[38,39] The other option is that they are thermally formed during kilning or roasting of malt but are then encapsulated in malt biopolymers such as starch. The adducts would be stable during the treatment with water at room temperature but release the odorants at higher temperature and/or by the activity of enzymes in the brewing process.

In summary, our results showed that the direct transfer from malt to beer plays only a minor role in the total concentration of important malt-derived beer odorants. The characterization of specialty malts by using sensory evaluation methods or by quantitating key odorants to conclude on the sensory properties of specialty malt beers should therefore be of little significance. This applies to the direct analysis of dry malt as well as to malt pretreated with water at room temperature. Whether the hot steep method[40] or the congress mash method[41] is suitable to convert the precursors from malt to odorants similar to the brewing process is yet to be clarified.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c01846.

> Malting parameters and malt product specifications; fermentation data obtained by daily measurements; details on syntheses and suppliers of isotopically substituted compounds; quantifier ions and calibration line data used in the quantitations; and individual concentration data used for calculation of means and standard deviations/coefficients of variation (PDF)

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■ 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-O, gas chromatography-olfactometry; GC-MS, gas chromatography-mass spectrometry; GC × GC-MS, comprehensive two-dimensional gas chromatography-mass spectrometry; HS-SPME-GC-MS, headspace-solid phase microextraction-gas chromatography-mass spectrometry; IBU, international bitter units; i.d., inner diameter; OAV, odor activity value; RI, retention index; SAFE, solvent-assisted flavor evaporation; TOF, time-of-flight

■ NOMENCLATURE

abhexone, 5-ethyl-3-hydroxy-4-methylfuran-2(5*H*)-one; 2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethanone; 2-amino-1-phenylethanone, 2′-aminoacetophenone; cyclotene, 3-methylcyclopentane-1,2-dione; (*E*)-*β*-damascenone, (2*E*)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one; *γ*-decalactone, 5-hexyldihydrofuran-2(3*H*)-one; eugenol, 2-methoxy-4-(prop-2-en-1-yl)phenol; linalool, 3,7-dimethyl-1,6-octadien-3-ol; maltol, 3-hydroxy-2-methyl-4*H*-pyran-4-one; methional, 3-(methylsulfanyl)propanal; methionol, 3-(methylsulfanyl)propan-1-ol; 2-methoxy-3-(propan-2-yl)-pyrazine, 3-isopropyl-2-methoxypyrazine; *γ*-nonalactone, 5-pentyldihydrofuran-2(3*H*)-one; 2-propanoyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)propan-1-one; 1-(pyrazin-2-yl)-ethanone, 2-acetylpyrazine; sotolon, 3-hydroxy-4,5-dimethyl-furan-2(5*H*)-one; 1-(1,3-thiazol-2-yl)ethanone, 2-acetylthiazole; vanillin, 4-hydroxy-3-methoxybenzaldehyde

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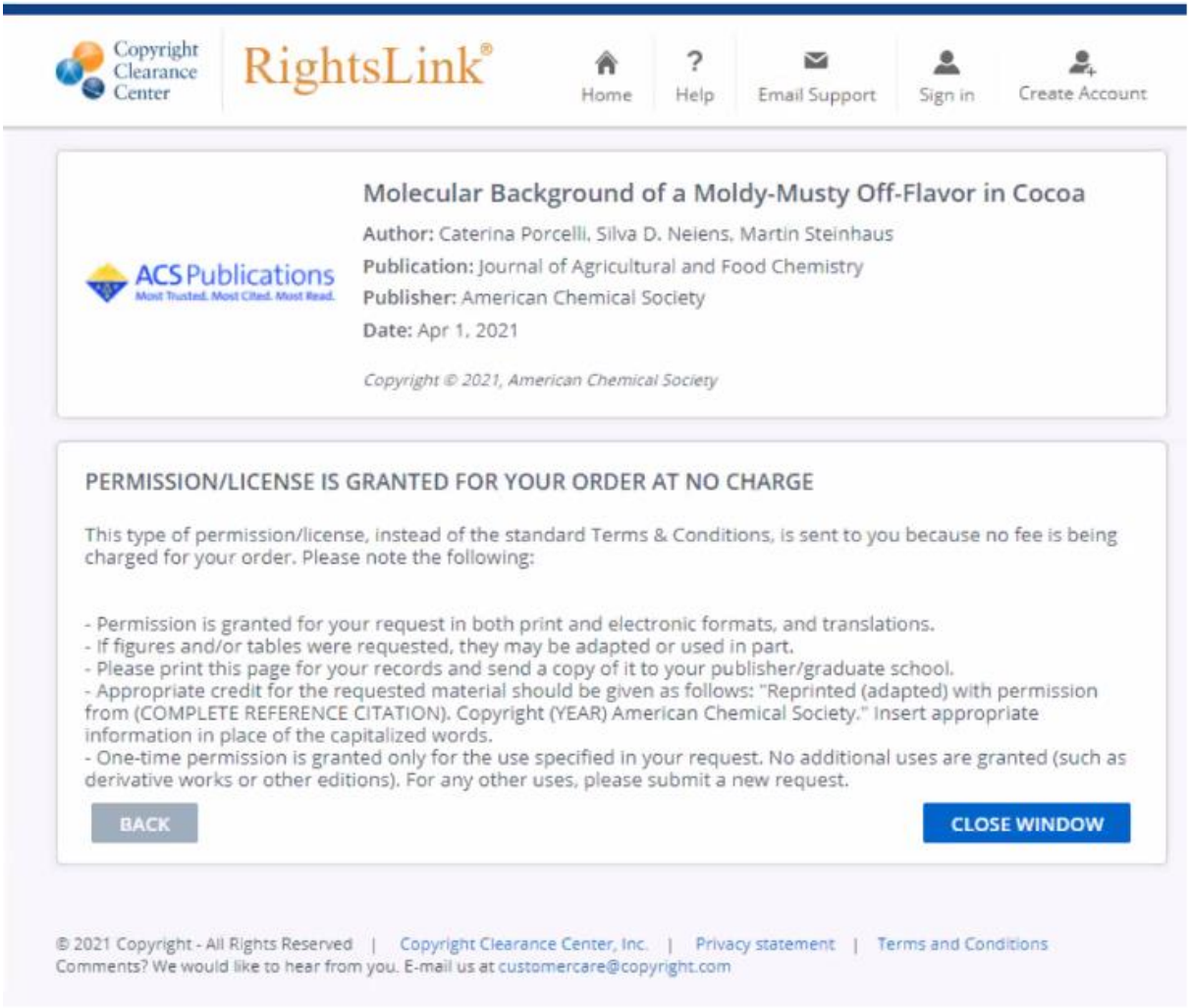
8.1.3 Summary and Individual Contributions

Malt produced from cereal grains is one of the main raw materials in brewing and has a major sensory impact on the final product beer. By applying intense thermal processes during malting, specialty malts are produced, which are often used alongside common kilned malt to brew bottom-fermented beers with unique color and aroma properties. The contribution of these specialty malts, particularly caramel and roasted malt to the aroma of bottom-fermented beer and the odorant transfer from the malt to the beer have, however, not yet been studied on a molecular level.

Therefore, three bottom-fermented Lager beers were produced, namely a reference beer (KBB), a caramel malt beer (CBB), and a roasted malt beer (RBB). The reference beer was exclusively produced with kilned barley malt while the specialty malt beers were brewed by substituting 30% and 2% of the kilned malt with caramel and roasted barley malt, respectively. Quantitative olfactory profiles were determined with a trained sensory panel revealing banana-like, floral, honey-like, and fruity notes in KBB, caramel-like and malty notes in CBB, and earthy and roasty notes in RBB. The reference beer KBB exhibited the overall lowest aroma intensity. Major odorants in the beers were identified by applying aroma extract dilution analysis complemented by quantitation and calculation of odor activity values (OAVs) for all relevant odorants. In total, 22 odorants showed OAVs ≥1 in at least one of the beers while a number of known fermentation by-products exhibited comparable OAVs in the beers, suggesting that they were not influenced by the malt type applied for brewing. In contrast, 19 out of the 22 compounds exhibited substantial differences in OAVs between the beers. The aroma of beer CBB was mostly characterized by fruity, caramel-like, roasty, and earthy smelling aldehydes, furanones, pyranones, and pyrazines, whereas the aroma of RBB was primarily determined by phenolic, smoky, and sweet smelling phenols and some earthy smelling pyrazines with high OAVs. The odorants were then quantitated in the malts used for brewing to assess their transfer from malt to beer. Based on the concentrations in the malts, hypothetical concentrations in the beers were calculated assuming 100% direct transfer and the absence of other sources. Comparing these hypothetical concentrations to the actual concentrations in the beers revealed that a direct transfer only accounted for a small portion of the odorant concentrations in the beers, indicating that a substantial amount of the odorants was formed from precursors and/or released from a bound form during brewing.

Michael Féchir designed and conducted the brewing experiments performed to produce the beers KBB, CBB, and RBB. Klaas Reglitz, Veronika Mall, and Michael Féchir designed and conducted the volatile isolations, the GC-O screenings, the structure assignments, the quantitations of the odorants, and the calculation of the OAVs in the beers as well as the volatile isolations and the quantitations of the odorants in the malts. Klaas Reglitz, Veronika Mall, and Michael Féchir designed and conducted the determination of OTVs in water and starch, the determination of quantitative olfactory profiles as well as the aroma reconstitution experiments. Michael Féchir assessed the odorant transfer and performed the statistical evaluation of the quantitative olfactory profiles. Michael and Klaas evaluated the data and prepared the manuscript, which was revised by Veronika Mall, Jens Voigt, and Martin Steinhaus. Jens Voigt, Veronika Mall, and Michael Féchir conceived this study, which was directed and supervised by Jens Voigt and Martin Steinhaus.

8.1.4 Reprint Permission



8.2 Publication 2: The Impact of a Caramel and a Roasted Wheat Malt on Important Aroma-Relevant Compounds in a Top-Fermented Wheat Beer

8.2.1 Bibliographic Data

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8.2.2 Publication Reprint

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The impact of caramel and roasted wheat malts on aroma compounds in top-fermented wheat beer

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Top-fermented wheat beers are known for their unique aroma. However, the impact of speciality wheat malts on the aroma of these beers and the transfer of odour active compounds from malt to the beer has not been investigated in detail. Three beers were brewed with different malt composition. The grist for each beer contained 50% kilned barley malt and 50% different wheat malts - beer (1) kilned wheat malt, beer (2) kilned wheat malt and caramel wheat malt, and beer (3) kilned wheat malt and roasted wheat malt. The odour active compounds in the beers were identified by aroma extract dilution analysis and their individual impact on aroma was evaluated by quantitation and calculation of odour activity values (OAVs). The results were verified sensorially by comparing aroma reconstitution models with the original beers. Characteristic odour active compounds in the beer brewed with caramel wheat malt were earthy compounds 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, caramel-like compounds 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and maltol, and sotolon with a soup seasoning-like aroma. The aroma of the roasted wheat malt beer was characterised by smoky and phenolic compounds 2-methoxyphenol and 4-methylphenol. Important beer odorants were quantified in the malts to assess their transfer from malt to beer. The results suggest that direct transfer of the odour active compounds in beers was not significant and that they were formed and/or released during the brewing process, confirming earlier results with different barley malts and bottom-fermented beers.

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords: specialty wheat malt beer; *Triticum aestivum*; aroma extract dilution analysis (AEDA); stable isotopically substituted odorant; odorant transfer

Introduction

Wheat beer is brewed by substituting up to 80% of barley malt with malted or unmalted wheat (*Triticum aestivum*) (Briggs, 1998) and by using top-fermenting *Saccharomyces cerevisiae* yeast instead of bottom-fermenting *S. pastorianus*. This results in a unique aroma profile. Fermentation by-products formed during the brewing process contribute fruity and clove-like notes to the aroma of wheat beer (Yin et al, 2016; Lermusieau et al, 2001). The fruity character is associated with a high concentration of esters and relatively low concentrations of higher alcohols (Meier-Dörnberg et al, 2017). Important compounds contributing to the fruitiness of wheat beers are (*E*)-β-damascenone, 3-methylbutyl acetate, ethyl methylpropanoate, ethyl butanoate, and 3-methylbutyl acetate (Langos et al, 2013). The clove-like aroma note is from volatile phenols, particularly 2-methoxy-4-vinylphenol (Goodey and Tubb, 1982; Schieberle, 1991). Volatile phenols originate from the enzymatic decarboxylation of phenolic acids including ferulic, *p*-coumaric, cinnamic, vanillic, caffeic, and sinapic acid by top-fermenting yeast characterised as POF+ (phenolic off-flavour). Most of the phenolic acids have comparable concentrations in barley and wheat malt, but the amount of ferulic acid is higher in wheat malt (Kalb et al, 2020; Langos et al, 2015; Langos and Granvogl, 2016).

In recent years, it has become increasingly popular to replace part of the kilned malt by specialty malts to develop new beer styles for expanding speciality beer markets (Meier-Dörnberg et al, 2017). Speciality malts such as caramel and roasted malt provide a characteristic colour but also impact taste and beer aroma (Prado et al, 2021). Higher temperatures during the production of these malts lead to the formation of colourants and odorants through thermal reactions including the Maillard reaction and Strecker degradation (Gasior et al, 2020). Whereas the impact of specialty hops on the aroma of beer and the transfer of odour active compounds to beer have already been studied at a molecular level (Peacock et al, 1981; Lermusieau and Collin, 2003; Neiens and Steinhaus, 2018a; Reglitz et al, 2018; Silva Ferreira and Collin, 2021), corresponding studies with speciality malts are scarce. We recently

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investigated the role of caramel barley malt and roasted barley malt for the aroma of bottom-fermented beers (Féchir et al, 2021). The results revealed (*E*)-β-damascenone, 2-acetyl-1-pyrroline, methionol, 2-ethyl-3,5-dimethylpyrazine, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one as important odour active compounds characterising the caramel malt beer and 2-methoxyphenol as an important aroma contributor in the roasted malt beer. Moreover, the direct transfer from malt to beer is of minor importance for typical malt odorants in beer, whereas the major part is formed or released from malt derived precursors during the brewing process.

The aim of the present study was to extend the above research to top-fermented wheat beers. The objectives were (1) to brew two top-fermented wheat beers at a small scale (50 L) using caramel wheat malt and roasted wheat malt, respectively, (2) to sensorially characterise the wheat beers in comparison to a reference wheat beer brewed with kilned base malts, (3) to identify odour active compounds in the wheat beers using gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA) applied to volatile isolates obtained by solvent extraction and solvent-assisted flavour evaporation (SAFE), (4) to assess the impact of these compounds on the aroma of the wheat beers by quantitation and calculation of odour activity values (OAVs), and (5) to evaluate their transfer from the malts to the wheat beers.

Materials and methods

Barley and wheat malts

Kilned barley malt (KBM), kilned wheat malt (KWM), caramel wheat malt (CWM), and roasted wheat malt (RWM) were obtained from Mich. Weyermann (Bamberg, Germany). The barley malt was made from variety Barke, harvest 2016. The three wheat malts were made from a single batch of wheat, variety Elixer, harvest 2016. KBM and KWM were kilned at 80-90°C. For CWM, green malt was transferred to a roasting drum without a kilning step and treated at 120-130°C. RWM was kilned at 80-90°C and then roasted in the roasting drum at 210-220°C. Further malting parameters and product specification are provided in Supporting Information, Table S1. The product specifications were determined with standard methods (Pfenninger, 1993).

Preparation of beers

A Braumeister Plus 50 L (Speidel, Ofterdingen, Germany) was used to brew three top-fermented wheat beers. Each beer was made with 50% barley malt and 50% wheat malt. The kilned wheat malt beer (KWB) was brewed with 50% KBM and 50% KWM, the caramel wheat malt beer (CWB) was brewed with a mixture of 50% KBM, 30% CWM, and 20% KWM, and the roasted wheat malt beer (RWB) was brewed with a mixture of 50% KBM, 48% KWM, and 2% RWM. Each malt mixture (11 kg) was ground and added to 50 L of water. Mashing was performed at 50°C for 20 min, 63°C for 55 min, 73°C for 30 min, and 78°C for 10 min. After lautering, spent grains were washed with water (10 L). The wash water was combined with the first wort and the mixture was boiled (60 min). Hop pellets (37.5 g), variety Hallertau Perle (Hopsteiner, Mainburg, Germany) were added 10 min after starting the boil. A second portion of hops (12.5 g) was added 40 min later. The total hop dosage corresponded to an expected bitterness of 20 IBU (international bitterness units). The original extract was ≥12 °P. After removal of the hot trub, the wort was cooled to 20°C. Dried yeast - *Saccharomyces cerevisiae* WB06 (20 g) (Fermentis Lesaffre, Marcq-en-Barœul, France) - was rehydrated and added to the wort. Fermentation was in cylindroconical tanks (Speidel) at 19°C and was monitored using an ALEX 500 Alcohol and Extract Meter (Anton Paar, Graz, Austria). Data can be found in Supporting Information, Table S2. At an apparent relative degree of 79–82%, fermentation was stopped, and the yeast was removed by decantation. The wheat beers were stored in 50 L kegs at 8°C for 1 week and then matured at 2°C for 2 weeks. CO_2 was adjusted to 4.5 g/L before bottling in 0.5 L amber glass bottles. The bottles were sealed with crown caps. Final ethanol concentrations were 5.05% ABV (KWB), 4.39% ABV. (CWB), and 4.59% ABV (RWB) and pH values were 4.45 (KWB), 4.46 (CWB), and 4.43 (RWB). All wheat beers were stored for 3 weeks before analysis.

Reference odorants (numbering refers to Table 2).

The compounds **1**, **3**-**8**, and **10**-**39** were purchased from Merck (Darmstadt, Germany), compound **2** was purchased from Alfa Aesar (Karlsruhe, Germany), and compound **9** was synthesised (Schieberle and Grosch, 1987).

Stable isotopically substituted odorants

The following compounds were synthesised as detailed in the literature: ($^2H_3$)-**3** (Li et al, 2017), ($^2H_{11}$)-**6** (Neiens and Steinhaus, 2018b), ($^{13}C_5$)-**9** (Kiefl et al, 2013), ($^2H_3$)-**11a** (Cerny and Grosch, 1993), ($^2H_3$)-**11b** (Cerny and Grosch, 1993), ($^2H_3$)-**13** (Grimm and Steinhaus, 2019), ($^2H_3$)-**14** (Cerny and Grosch, 1993), ($^2H_2$)-**18** (Neiens and Steinhaus, 2018b), ($^{13}C_2$)-**19** (Münch and Schieberle, 1998), ($^2H_2$)-**20b** (Neiens and Steinhaus, 2018b), ($^2H_3$)-**22** (Grimm and Steinhaus, 2019), ($^2H_3$)-**23** (Jagella and Grosch, 1999), ($^2H_7$)-**24** (Sen et al, 1991), ($^2H_3$)-**26** (Kiefl et al, 2013), ($^2H_5$)-**27** (Münch and Schieberle, 1998), ($^{13}C_2$)-**28** (Rögner et al, 2021), ($^{13}C_6$)-**33** (Kiefl et al, 2013), ($^{13}C_2$)-**34** (Blank et al, 1993), ($^2H_3$)-**35** (Dollmann et al, 1996), ($^2H_3$)-**38** (Cerny and Grosch, 1993), and ($^2H_2$)-**39** (Ruisinger and Schieberle, 2012). ($^2H_3$)-**9**, ($^2H_3$)-**12**, ($^2H_7$)-**32**, and ($^{13}C_2$)-**37** were purchased from Merck (Darmstadt, Germany); ($^2H_3$)-**5**, ($^2H_{11}$)-**7b**, ($^2H_{11}$)-**8**, and ($^2H_7$)-**17** were purchased from CDN Isotopes (Quebec, Canada) via EQ Laboratories (Augsburg, Germany); ($^{13}C_2$)-**30** was purchased from aromaLAB (Planegg, Germany).

Miscellaneous chemicals and reagents

Diethyl ether and dichloromethane were purchased from VWR (Darmstadt, Germany). Before use, both solvents were freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings.

Gas chromatography-olfactometry/flame ionisation detector (GC-O/FID)

A gas chromatograph was equipped with a cold on-column injector, a free fatty acid phase (DB-FFAP) or a DB-5 capillary column, an effluent splitter, a flame ionisation detector (FID), and a heated exit serving as sniffing port. Details of the system are reported in Neiens and Steinhaus (2018a).

Table 1. Internal standards, quantifier ions, and calibration lines used for quantitation

Compound	Standard	quantifier ion (*m/z*)		calibration line equation[a]	R^2
		analyte	standard		
3	($^2H_3$)-**3**	102	105	y = 1.6533x + 0.5000	0.999
5	($^2H_3$)-**5**	57	63	y = 0.8807x − 0.0692	1.000
6	($^2H_{11}$)-**6**	131	142	y = 1.7430x − 0.4544	0.999
7a	($^2H_{11}$)-**7b**	71	82	y = 1.1539x − 0.4007	0.994
7b	($^2H_{11}$)-**7b**	71	82	y = 1.6042x − 0.5521	0.993
8	($^2H_{11}$)-**8**	145	156	y = 0.8998x + 0.0390	1.000
9	($^{13}C_5$)-**9**	111	116	y = 1.4451x − 0.1637	0.997
11a	($^2H_3$)-**11a**	135–136	138–139	y = 0.8047x + 0.6997	0.998
11b	($^2H_3$)-**11b**	135–136	138–139	y = 0.4568x + 0.0817	0.994
12	($^2H_3$)-**12**	75	78	y = 0.6656x + 0.1366	0.995
13	($^2H_3$)-**13**	104	107	y = 0.7746x − 0.1736	0.998
14	($^2H_3$)-**14**	135	138	y = 0.8082x + 0.0292	1.000
17	($^2H_7$)-**17**	103	110	y = 1.0390x − 0.0492	0.999
18	($^2H_2$)-**18**	103	105	y = 0.7257x + 0.0649	1.000
19	($^{13}C_2$)-**19**	120	122	y = 1.0042x + 0.1568	0.998
20	($^2H_2$)-**20b**	117	119	y = 0.9328x + 0.0974	0.999
22	($^2H_3$)-**22**	106	109	y = 0.8860x + 0.0376	1.000
23	($^2H_3$)-**23**	117	120	y = 0.8565x + 0.0783	1.000
24	($^2H_7$)-**24**	121	123–129	y = 1.8565x + 0.3444	0.998
26	($^2H_3$)-**26**	124	127	y = 1.0985x − 0.0876	1.000
27	($^2H_5$)-**27**	91	96	y = 0.9150x − 0.1010	0.998
28	($^{13}C_2$)-**28**	126	128	y = 1.1378x + 0.1735	0.998
30	($^{13}C_2$)-**30**	128	130	y = 1.3031x − 0.2360	0.997
32	($^2H_7$)-**32**	108	115	y = 0.3767x − 0.0454	1.000
33	($^{13}C_6$)-**33**	150	156	y = 0.4151x + 0.0340	0.999
34	($^{13}C_2$)-**34**	128	130	y = 1.0632x − 0.0127	1.000
35	($^2H_3$)-**35**	135	138	y = 0.7675x − 0.0178	1.000
37	($^{13}C_2$)-**37**	136	138	y = 1.1324x − 0.0082	1.000
38	($^2H_3$)-**38**	151+152	154+155	y = 0.8910x + 0.0488	0.999
39	($^2H_2$)-**39**	150	152	y = 0.5267x − 0.0576	0.995

[a] y = peak area standard/peak area analyte; x = concentration standard (μg/mL)/concentration analyte (μg/mL).

Gas chromatography-mass spectrometry (GC-MS)

A 7890B gas chromatograph equipped with a GC Sampler 80 and a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d, 0.25 μm film, was connected to an Ion Trap 240 mass spectrometer via a heated (250°C) transfer line (Agilent, Waldbronn, Germany). The carrier gas was helium at 1 mL/min constant flow. The oven temperature was 40°C (5 min), then ramped at 6°C/min to 230°C (5 min). Mass chromatograms were obtained in chemical ionisation (CI) mode using methanol as reagent gas and a scan range of *m/z* 40–250. The MS workstation software (Agilent) was used for data evaluation.

Headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS)

The previously described GC-MS system was equipped with a DB-FFAP column, 30 m × 0.25 mm i.d, 0.25 μm film, or a DB-5 column, 30 m × 0.25 mm i.d, 1 μm film (both Agilent). The GC sampler was operated with a 65 μm PDMS/DVB (Polydimethylsiloxane/Divinylbenzene) SPME fibre or with a 50 μm DVB/CAR/PDMS SPME (Divinylbenzene/Carboxen/Polydimethylsiloxane) fibre (both Merck). Volatiles were extracted at 30°C for 5 min and desorbed at 250°C for 1.5 min. After analysis, fibres were baked out at 270°C for 10 min. For the analysis of compounds **5** and **6**, the oven temperature was 35°C (5 min), ramped at 20°C/min to 240°C (10 min). For the analysis of compounds **7** and **8**, the oven temperature was 40°C (2 min), ramped at 6°C/min to 230°C (5 min).

Comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOFMS)

A 6890 Plus gas chromatograph (Agilent) was equipped with a PAL autosampler (CTC Analytics, Zwingen, Switzerland), a CIS 4 injector (Gerstel, Mülheim a. d. Ruhr, Germany), a fused silica column, DB-FFAP, 30 m × 0.25 mm i.d, 0.25 μm film, in the first dimension, and a fused silica column, DB-5, 2 m × 0.15 mm i.d, 0.30 μm film, in the second dimension (both Agilent). The GC was connected to a Pegasus III time of flight (TOF) MS (Leco, Mönchengladbach, Germany). The temperature of the first oven was 40°C (2 min), ramped at 6°C/min to 230°C (5 min). Modulation time was 4 s.

The temperature of the second oven was 70°C (2 min), ramped at 6°C/min to 250°C (5 min). The GC Image software (Lincoln, NE, USA) was used for data evaluation.

Aroma extract dilution analysis (AEDA)

Wheat beer (250 mL) was degassed by filtration. Diethyl ether (300 mL) was added, and the mixture was stirred at room temperature for 1 h. After phase separation, the aqueous phase was stirred with a second portion (300 mL) of diethyl ether for 1 h. The combined organic phases were washed with saturated aqueous sodium chloride (200 mL) and dried with anhydrous sodium sulphate. After filtration, the volatiles were isolated by solvent-assisted flavour evaporation (SAFE) (Engel et al, 1999). The distillate was concentrated (500 μL) by using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device (Bemelmans, 1979).

Beer volatiles were analysed by GC-O/FID. Analysis was performed by three experienced GC-O sniffers (aged 27–36). The volatile isolates were stepwise diluted with diethyl ether to obtain dilutions of 1:2, 1:4, 1:8, etc. Each diluted sample was subjected to GC-O/FID analysis. The odour active compounds were assigned flavour dilution (FD) factors representing the dilution factor of the most diluted sample, in which the odour of the compound was detected at the sniffing port (Steinhaus, 2019).

Quantitation

Filtered wheat beer (250 mL) was stirred with diethyl ether (300 mL) at room temperature for 24 h. Malt grains were frozen in liquid nitrogen and ground into a fine powder using a laboratory mill Grindomix GM 200 (Retsch, Haan, Germany) at 4000 rpm (10 s) and 10,000 rpm (10 s). Diethyl ether (0.5–5 mL) and water (9.5–95 mL) were added to the powder (1–10 g) and the mixture was stirred at room temperature for 24 h. In both cases, the extraction solvent contained known amounts of stable isotopically substituted odorants as internal standards (Table 1). Filtration, washing, drying, and SAFE were performed as above. The isolates were separated into acidic volatiles (AV) and neutral/basic volatiles (NBV) as described by Neiens and Steinhaus (2018b). The compounds **12**, **17**, **18**, **20**, and **23** were quantitated by GC-MS analysis of fraction AV; **22**, **34**, **37**, **38,** and **39** were quantitated by GC×GC-TOFMS analysis of fraction AV; and **3**, **9**, **11**, **13**, **14**, **19**, **24**, **26**, **27**, **28**, **30**, **32**, and **35** were quantitated by GC×GC-TOFMS analysis of fraction NBV.

The compounds **5**–**8** were quantitated after headspace sampling with the PDMS/DVB fibre (**5**, **6**) or the DVB/CAR/PDMS fibre (**7**, **8**). Before analysis, beer samples were degassed and diluted with water (1:100). The diluted samples (1 mL) were placed in 20 mL headspace vials and spiked with stable isotopically substituted compounds. The vials were sealed, and the samples were subjected to HS-SPME-GC-MS analysis. Powdered malt samples (2 g) were mixed with water (1 mL) and spiked with the stable isotopically substituted compounds and the vials were sealed. After equilibration at room temperature (30 min), the samples were subjected to HS-SPME-GC-MS analysis.

During GC-MS analyses, characteristic quantifier ions of analyte and internal standard were monitored. The concentration was calculated from the peak areas of analyte and standard, the amount of malt or beer used, and the amount of standard added, by employing a calibration line equation. This was obtained from the analysis of analyte/standard mixtures with at least five different concentration ratios (~1:20–50:1) followed by linear regression. Individual quantifier ions and calibration line equations are reported in Table 1.

Odour threshold value

OTVs were determined according to the American Society for Testing and Materials (ASTM) standard practice for determination of odour and taste thresholds by a forced-choice ascending concentration series method of limits (ASTM International, 2019). The thresholds were determined in pure water. The trained panel consisted of 15–20 people, male and female aged 24-56, all of whom are employees of the Leibniz-LSB@TUM.

Aroma reconstitution

Defined volumes (0.05-2 mL) of ethanolic stock solutions with the individual odour active compounds were combined and made up to 10 mL with water. A volume (0.1 mL) was added to a hydroalcoholic solution with an ethanol concentration corresponding to the respective beer sample. The pH was adjusted to that of the original wheat beer. The concentration of the stock solutions and the volumes used were adjusted to obtain a final concentration of each compound in the beer aroma reconstitution solutions that represented the concentrations previously determined in the wheat beer samples.

Quantitative olfactory profile

The degassed wheat beers and the reconstitution models (10 mL) were evaluated in cylindrical ground neck glasses (height 7 cm, i.d. 3.5 cm) with lids (Merck) at ~15°C. In three separate sessions, 15 trained panellists (11 female, 4 male, aged 23–50) orthonasally evaluated the aroma of one of the wheat beers and the corresponding reconstitution model by rating the intensities of 9 predefined descriptors on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Individual descriptors were defined by the odour of a reference compound dissolved in water at a concentration exceeding its respective odour threshold value by a factor of ~100. The following nine descriptors and reference compounds were used: 'banana' (**6**), 'caramel' (**30**), 'earthy' (**11a**), 'roasty' (**9**), 'floral, honey' (**27**), 'fruity' (**8**), 'malty' (**7b**), 'smoky' (**26**), and 'vinegar' (**12**). Ratings of all panellists were combined by calculating the arithmetic mean. Data analysis was accomplished with the XLSTAT-Biomed 2019.3.1 software (Addinsoft, Boston, MA, USA).

Results and discussion

Quantitative olfactory profiles of the wheat beers

Orthonasal evaluation revealed clear differences in aroma between the caramel wheat malt beer (CWB), the roasted wheat malt beer (RWB), and the reference kilned wheat malt beer (KWB) (Figure 1). Beer KWB made with a 1:1 mixture of kilned barley malt and kilned wheat malt showed dominant floral, honey-like, banana-like, and fruity aroma notes. These attributes were rated lower in both speciality wheat malt beers. Beer CWB made with 30% caramel wheat malt showed higher intensities of smoky, earthy, roasty, malty, and caramel-like notes than beer KWB. Beer RWB brewed with 2% roasted wheat malt showed higher scores

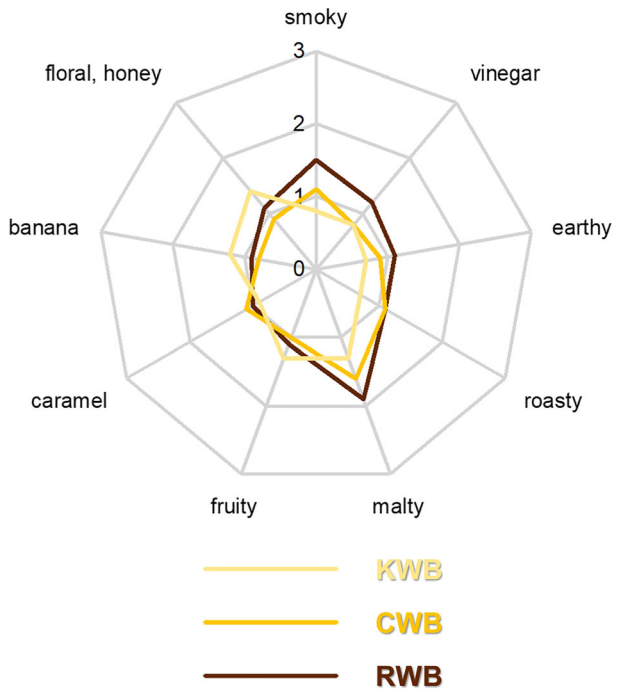

Figure 1. Quantitative olfactory profiles of kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB). [Colour figure can be viewed at wileyonlinelibrary.com]

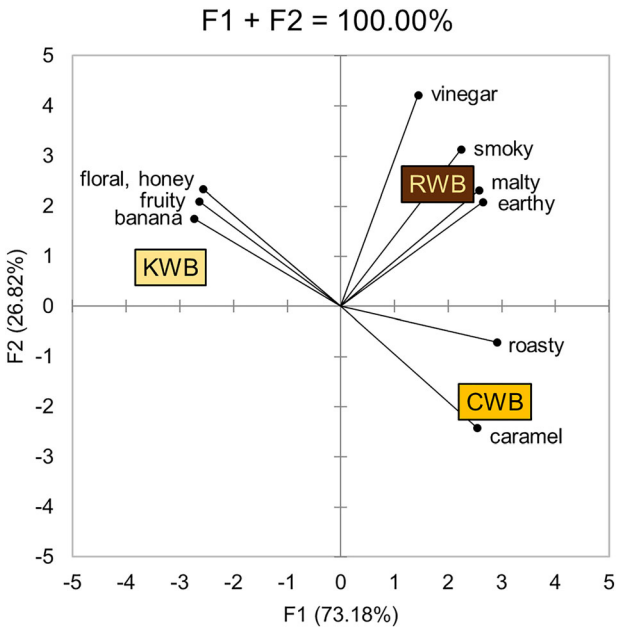

Figure 2. Principal component analysis applied to the sensorial data of kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB). [Colour figure can be viewed at wileyonlinelibrary.com]

for smoky, earthy, roasty, and malty notes, but not for the caramel-like note which was highest in beer CWB.

Statistical evaluation of the sensory data by principal component analysis is reported in Figure 2. Principal component F1 accounted for 73.18% of the variation in the dataset and predominantly allowed for differentiation between the speciality malt beers CWB and RWB located in the positive range of axis F1 and reference wheat beer KWB located in the negative range of axis F1. Principal component F2 accounted for the remaining 26.82% of the variation and allowed for a separation of the two different speciality malt beers with CWB being in the negative range of axis F2 and RWB being in the positive range of axis F2. In the PCA plot, the distance between the speciality wheat malt beers and the reference wheat beer KWB (~7 on axis F1) was almost twice as large as the distance between speciality wheat malt beer CWB and speciality wheat malt beer RWB (~4 on axis F2). This confirmed the substantial effect of speciality wheat malts on the aroma of top-fermented wheat beers.

Principal component F1 was mostly defined by the attributes roasty, caramel, earthy, and banana-like, and separated wheat beers CWB and RWB from KWB. Principal component F2 was primarily characterised by vinegar-like and smoky attributes and distinguished the two wheat beers, CWB and RWB. The attributes malty, floral, honey-like, caramel, and fruity contributed equally to both components. As indicated by a longer distance from the intersection of the two axes, attributes caramel-like, vinegar-like, floral, honey-like, and smoky contributed slightly more to the overall separation, whereas roasty and earthy attributes were located closer to the intersection, contributing less to the overall separation.

Screening for odour active compounds in the wheat beers

Application of a comparative aroma extract dilution analysis (AEDA) to the volatile isolates obtained from the caramel wheat malt beer (CWB) and the roasted wheat malt beer (RWB) by solvent extraction, SAFE, and concentration, resulted in 39 odorants with FD factors between 1 and 1024 (Table 2). The primary aim of this was to facilitate the selection of compounds for quantitation and OAV calculation and not to identify differences between the beers. For this reason, the kilned wheat malt beer (KWB) was not included in the screening. Given that wheat beer KWB was brewed with only kilned barley malt and kilned wheat malt, both of which were also in the malt mixtures of wheat beers CWB and RWB, unique odorants were not to be expected to be present in beer KWB.

The AEDA revealed high FD factors for ethanol (**2**; FD 1024), 2-/3-methylbutanoic acid (**20**; FD 1024), 2- and 3-methylbutan-1-ol (**7**; FD 512–1024), 2-phenylethanol (**27**; FD 512–1024), acetic acid (**12**; FD 256–512), methionol (**22**; FD 256–512), and ethyl 2-methylbutanoate (**3**; FD 126–256). In the caramel wheat malt beer (CWB), high FD factors were additionally obtained for HDMF (**30**; FD 1024), sotolon (**34**; FD 1024), vanillin (**38**; FD 1024), maltol (**28**; FD 512), and 2′-aminoacetophenone (**35**; FD 256), suggesting that these compounds originated from the caramel wheat malt. In contrast, higher FD factors in the roasted wheat malt beer (RWB) were found for (*E*)-β-damascenone (**24**; FD 256), 2-methoxyphenol (**26**; FD 256), 4-methylphenol (**32**; FD 64), and phenylacetic acid (**37**; FD 64), implying their origin from the roasted wheat malt.

Quantitation of odour active compounds in the wheat beers and OAV calculation

Considering the results of the AEDA screening and the literature on beer odorants in speciality barley malt beers (Féchir et al, 2021), 23 compounds were selected for quantitation by GC-MS. Stable isotopically substituted odorants were used as internal standards. The concentrations ranged from 51 ng/kg for 4-methylphenol

Table 2. Odour active compounds in the volatile isolates obtained from the caramel wheat malt beer (CWB) and the roasted wheat malt beer (RWB)

no.	Compound	Odour	RI[b] (FFAP)	FD factor[c] CWB	FD factor[c] RWB
1	2-methylpropanal	malty	833	4	4
2	ethanol	ethanolic	925	1024	1024
3	ethyl 2-methylbutanoate	fruity	1045	126	256
4	ethyl 3-methylbutanoate	fruity	1059	8	<1
5	methylpropan-1-ol	malty	1090	64	64
6	3-methylbutyl acetate	fruity, banana	1117	16	16
7	2-/3-methylbutan-1-ol	malty	1206	512	1024
8	ethyl hexanoate	fruity, pineapple	1226	32	64
9	2-acetyl-1-pyrroline	roasty, popcorn	1329	4	2
10	2-methoxy-3-(propan-2-yl) pyrazine	earthy	1427	<1	1
11	2-ethyl-3,5(6)-dimethylpyrazine[d]	earthy	1432	126	4
12	acetic acid	vinegar, pungent	1449	256	512
13	methional	cooked potato	1456	126	64
14	2,3-diethyl-5-methylpyrazine	earthy	1485	64	16
15	propanoic acid	cheesy, pungent	1538	4	<1
16	linalool	citrusy, bergamot	1542	1	2
17	2-methylpropanoic acid	cheesy	1558	4	4
18	butanoic acid	cheesy	1624	2	8
19	phenylacetaldehyde	honey	1642	64	32
20	2-/3-methylbutanoic acid	cheesy	1661	1024	1024
21	(2*E*,4*E*)-nona-2,4-dienal	fatty	1695	4	<1
22	methionol	cooked potato	1717	256	512
23	pentanoic acid	cheesy	1726	16	16
24	(*E*)-β-damascenone	cooked apple	1811	64	256
25	2-phenylethyl acetate	floral, honey	1814	16	4
26	2-methoxyphenol	smoky, sweet	1859	64	256
27	2-phenylethanol	floral, honey	1918	1024	512
28	maltol	caramel	1972	512	4
29	ɣ-nonalactone	coconut	2023	32	16
30	HDMF	caramel	2048	1024	256
31	octanoic acid	sour, musty	2062	16	16
32	4-methylphenol	phenolic	2086	4	64
33	4-ethenyl-2-methoxyphenol	phenolic	2178	16	4
34	sotolon	soup seasoning	2200	1024	126
35	2′-aminoacetophenone	foxy	2207	64	256
36	2,6-dimethoxyphenol	smoky, clove	2271	32	<1
37	phenylacetic acid	honey, beeswax	2562	16	64
38	vanillin	vanilla	2578	1024	126
39	3-phenylpropanoic acid	floral	2623	32	16

[a] Compounds were identified by comparing the retention indices (RIs) on two GC columns of different polarities (DB-FFAP, DB-5), mass spectrum obtained by GC-MS, together with odour from the sniffing port during GC-O to data obtained from authentic reference compounds analysed under equal conditions.

[b] Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation.

[c] Flavour dilution factor.

[d] Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine.

(**32**) in KWB to 240 mg/kg for acetic acid (**12**) in RWB (Table 3). By dividing the concentrations by the odour threshold values in water, OAVs were calculated to approximate the impact of the odorants on the aroma of the wheat beers.

A total of 22 compounds exhibited OAVs ≥1 in at least one of the three beers. Most of the fermentation by-products (Steensels et al, 2014, Rossouw et al, 2008) such as higher alcohols (**7a**, **7b**, **22**, **27**), carboxylic acids (**18**, **20**), and esters (**3**, **8**) showed only minor differences between the three beers, indicating that their synthesis was barely influenced by malt composition. These compounds are formed in the anabolism or catabolism of amino acids via the Ehrlich pathway and, in the case

Table 3. Concentration and OAVs of selected odour active compounds in the wheat beers

no.	Compound	OTV[a] (μg/kg)	Concentration[b] (μg/kg)			OAV[c]		
			KWB[d]	CWB[e]	RWB[f]	KWB[d]	CWB[e]	RWB[f]
3	ethyl 2-methylbutanoate	0.013	0.98	1.0	1.2	75	77	92
7a	2-methylbutan-1-ol	1200	13000	13000	13000	11	11	11
7b	3-methylbutan-1-ol	220	670	740	760	3	3	3
8	ethyl hexanoate	1.2	5.2	5.6	5.8	4	5	5
9	2-acetyl-1-pyrroline	0.053	0.012	0.037	0.014	<1	<1	<1
11a	2-ethyl-3,5-dimethylpyrazine	0.28	0.10	11	2.1	<1	40	7
11b	2-ethyl-3,6-dimethylpyrazine	25	0.10	120	2.7	<1	5	<1
12	acetic acid	5600	100000	120000	240000	18	21	42
13	methional	0.43	4.6	2.3	2.2	11	5	5
14	2,3-diethyl-5-methylpyrazine	0.031	0.029	0.46	0.070	1	15	2
18	butanoic acid	2400	2100	2100	5700	1	1	2
19	phenylacetaldehyde	5.2	17	29	20	3	6	4
20	2-/3-methylbutanoic acid	490	980[g]	1400[g]	1200[g]	2[h]	3[h]	3[h]
22	methionol	36	1300	630	1500	37	17	41
24	(*E*)-β-damascenone	0.006	2.1	1.0	2.1	340	170	350
26	2-methoxyphenol	0.84	27	33	55	32	39	66
27	2-phenylethanol	140	5300	5600	4700	38	40	33
28	maltol	5000	110	7900	1600	<1	2	<1
30	HDMF	87	550	780	650	6	9	8
32	4-methylphenol	3.9	0.051	1.6	58	<1	<1	15
34	sotolon	1.7	2.3	12	3.4	1	7	2
35	2′-aminoacetophenone	0.27	1.5	1.2	1.4	6	4	5
37	phenylacetic acid	68	270	700	290	4	10	4

[a] Odour threshold value orthonasally determined in water.
[b] Mean of duplicates or triplicates; individual data and standard deviations are included in the Supporting Information, Tables S3-S5.
[c] Odour activity value.
[d] Kilned wheat malt beer.
[e] Caramel wheat malt beer.
[f] Roasted wheat malt beer.
[g] Concentrations are given as the sum of the isomers 2-methylbutanoic acid (**20a**) and 3-methylbutanoic acid (**20b**).
[h] OAVs were calculated with the OTV of 3-methylbutanoic acid (490 μg/kg)

of esters, by enzymatic condensation of organic acids and alcohols (Pires et al, 2014, Holt et al, 2019). The minor differences in the OAVs were most likely a result of small variations between brewing batches.

In the caramel wheat malt beer (CWB), comparatively high OAVs were obtained for earthy smelling pyrazines, 2-ethyl-3,5-dimethylpyrazine (**11a**; 40 vs. <1 and 7), 2-ethyl-3,6-dimethylpyrazine (**11b**; 5 vs. <1), and 2,3-diethyl-5-methylpyrazine (**14**; 15 vs. 1 and 2) as well the lactone sotolon with a soup seasoning-like aroma (**14**; 7 vs. 1 and 2). Although wheat beer CWB, in accordance with a somewhat stronger caramel note in the olfactory profile (Figure 1), also showed the highest OAVs for caramel-like smelling compounds, the differences to the other two beers were smaller than the OAVs of the pyrazines. In detail, CWB showed OAVs of 2 vs. <1 for maltol (**28**) and 9 vs. 6 and 8 for HDMF (**30**). The roasted wheat malt beer (RWB) was characterised by comparatively high OAVs for the two phenolic odorants, namely smoky 2-methoxyphenol (**26**; 66 vs. 32 and 39) and phenolic 4-methylphenol (**32**; 15 vs. <1), which was reflected by the most intense smoky note in the olfactory profile (cf. Figure 1).

Similar results we obtained for beers brewed with corresponding barley malt mixtures (Féchir et al, 2021). However, a clear difference was observed in the roasty popcorn aroma of 2-acetyl-1-pyrroline (**9**). Among the barley malt beers, 2-acetyl-1-pyrroline was highly odour active in the caramel malt beer with an OAV of 73 vs. 2 in the kilned barley malt beer and the roasted barley malt beer (Féchir et al, 2021). Whereas 2-acetyl-1-pyrroline showed OAVs of <1 in all three wheat malt beers (Table 3). Other differences between barley malt beers and wheat malt beers were found for cooked apple-like (*E*)-β-damascenone and vinegar-like acetic acid. (*E*)-β-damascenone showed the highest OAV in all three wheat beers, but it is well known that its aroma contribution is typically overestimated using OAV calculations, as it tends to be easily suppressed in mixtures. Nevertheless, among the barley malt beers, the caramel malt beer showed the highest OAV for

(*E*)-β-damascenone with 250 vs. 190 and 130 (Féchir et al, 2021), whereas among the wheat malt beers, the caramel malt beer showed the lowest OAV with 170 vs. 340 and 350 (Table 3). Acetic acid, with an OAV of 42 was highest in the roasted wheat malt beer (RWB), which was in accordance with the stronger vinegar note in the olfactory profile (Figure 1). In the corresponding roasted barley malt beer, the OAV of acetic acid was relatively low (21 vs. 110 and 140; Féchir et al, 2021).

Wheat beer aroma reconstitution

All odour active compounds with OAVs of ≥1 in the three wheat beers (18 in KWB, 21 in CWB and RWB) were used to prepare hydroalcoholic aroma reconstitution models with ethanol concentration and pH according to the original products. The olfactory profiles of the reconstitution models were then compared to those of the beers (Figure 3). Subtle differences were observed between the models and beers. For example, the floral, honey-like, malty, and banana-like notes were slightly underrepresented in the KWB model, as well as the banana-like, vinegar-like, and malty notes in the CWB model, and the floral, honey-like, vinegar-like, and malty notes in the RWB model. Nevertheless, the overall similarities between the models and the beers were high and the models also reflected the characteristic differences between the three beers. Therefore, the key compounds in the beers were considered to have been identified with no relevant odorant having been overlooked.

Quantitation of the wheat beer odorants in malt

To assess the transfer of odorants from malt to the beer, 16 compounds were quantitated in the malts used to brew the beers. To cover the free odorants and also the portion bound as hydrolabile precursors, a small amount of water was added during the volatile extraction process (Rögner et al, 2021). The results are reported in Table 4. As was expected from the different thermal treatments during wheat malt production, clear differences were obtained in important odorants. For example, pyrazines (**11a**, **11b**, **14**) and phenols (**26**, **32**), but also the caramel-like compounds maltol (**28**) and HDMF (**30**) showed the highest concentrations in the roasted wheat malt (RWM). These findings were in good agreement with the data reported for the corresponding barley malts (Féchir et al, 2021). In the caramel wheat malt (CWM), extraordinarily high concentrations were obtained for 2-acetyl-1-pyrroline (**9**), methional (**22**), and sotolon (**34**). The concentration of 2-acetyl-1-pyrroline and sotolon in CWM were not only clearly higher than in KWM and RWM, but also far higher than in the corresponding barley malts (Féchir et al, 2021).

Transfer of odorants from malt to wheat beers

The odorant concentration in the malt mixtures used for brewing the kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB) were calculated from the concentration in the individual malts (Table 4) and their percentage in the mixtures. From these data (Supporting Information, Table S9) and the grist loads, the hypothetical concentration of the odour active compounds in the beers were calculated assuming 100% transfer (Table 5). These hypothetical values were compared to the actual concentrations in Table 3 and the results are shown in Figure 4. The full bars represent the actual concentrations of the odour active compounds in the beers, the parts highlighted in

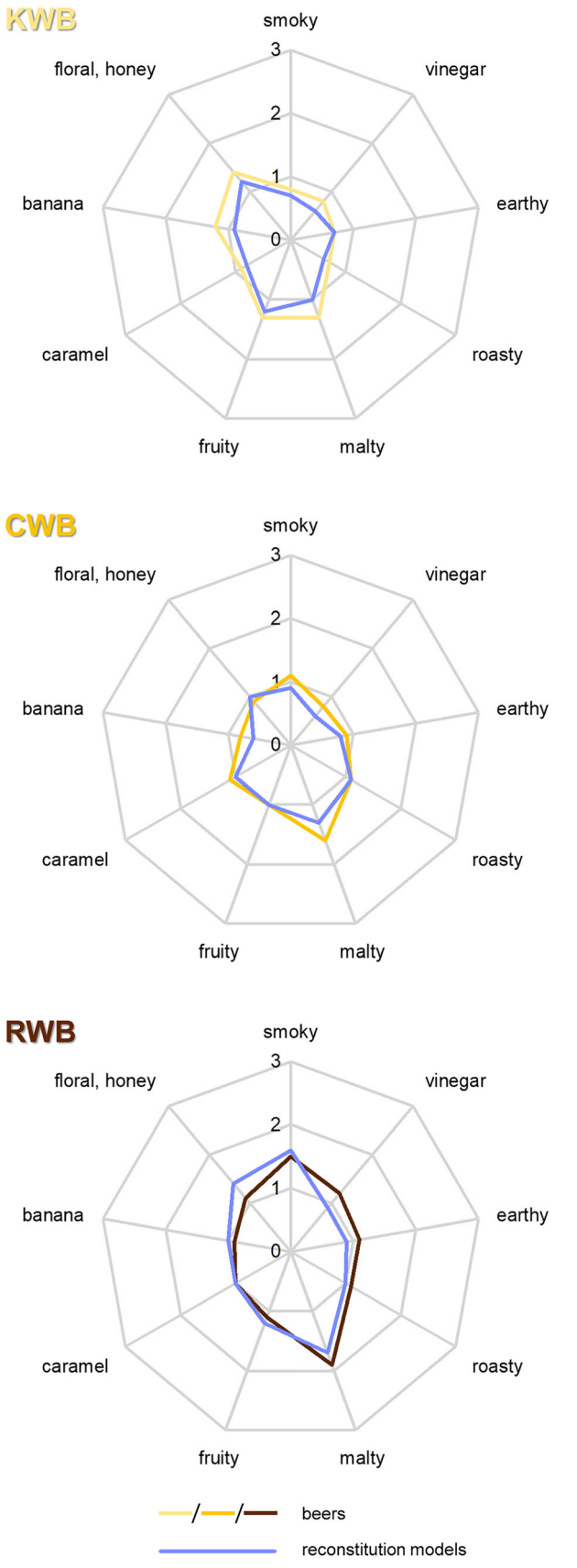

Figure 3. Quantitative olfactory profiles of the aroma reconstitution models in comparison to the profiles of kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4. Concentration of selected odour active compounds in malts

no.	Compound	Concentration[a] (μg/kg) KBM[b]	KWM[c]	CWM[d]	RWM[e]
9	2-acetyl-1-pyrroline	1.5	1.7	170	11
11a	2-ethyl-3,5-dimethylpyrazine	3.6	11	41	440
11b	2-ethyl-3,6-dimethylpyrazine	0.16	2.6	45	330
12	acetic acid	96000	380000	930000	540000
13	methional	4.8	3.3	23	1.2
14	2,3-diethyl-5-methylpyrazine	0.0059	0.28	4.0	22
19	phenylacetaldehyde	24	49	4.0	110
22	methionol	5.5	7.0	0.45	7.1
24	(*E*)-β-damascenone	0.051	0.016	3.1	4.0
26	2-methoxyphenol	1.4	4.0	9.5	170
27	2-phenylethanol	180	51	1100	63
28	maltol	19	15	73000	320000
30	HDMF	17	7.5	6100	11000
32	4-methylphenol	0.17	0.13	0.81	8.1
34	sotolon	0.19	0.64	90	11
35	2′-aminoacetophenone	0.38	0.050	0.71	0.40
37	phenylacetic acid	57	16	180	210

[a] Mean of duplicates or triplicates; individual data and standard deviations are included in the Supporting Information, Tables S6-S8.
[b] Kilned barley malt; concentrations were taken from Féchir et al, (2021).
[c] Kilned wheat malt.
[d] Caramel wheat malt.
[e] Roasted wheat malt.

Table 5. Hypothetical concentration of selected odour active compounds in wheat beers assuming 100% transfer from malt mixtures to beer

no.	Compound	Hypothetical concentration in beer (μg/kg) KWB[a]	CWB[b]	RWB[c]
9	2-acetyl-1-pyrroline	0.35	12	0.39
11a	2-ethyl-3,5-dimethylpyrazine	1.6	3.6	3.5
11b	2-ethyl-3,6-dimethylpyrazine	0.30	3.1	1.8
12	acetic acid	52000	89000	53000
13	methional	0.89	2.2	0.88
14	2,3-diethyl-5-methylpyrazine	0.031	0.28	0.13
19	phenylacetaldehyde	8.0	5.1	8.3
22	methionol	1.4	0.9	1.4
24	(*E*)-β-damascenone	0.0074	0.21	0.025
26	2-methoxyphenol	0.59	0.96	1.3
27	2-phenylethanol	25	95	25
28	maltol	3.7	4800	1400
30	HDMF[d]	2.7	410	51
32	4-methylphenol	0.033	0.078	0.068
34	sotolon	0.091	6.0	0.14
35	2′-aminoacetophenone	0.047	0.091	0.049
37	phenylacetic acid	8.0	19	8.9

[a] Kilned wheat malt beer; data was calculated as the concentration in KBM/KWM 50/50 (Table S9) × grist load (kg malt per kg beer).
[b] Caramel wheat malt beer; data was calculated as the concentration in KBM/CWM/KWM 50/30/20 (Table S9) × grist load.
[c] Roasted wheat malt beer; data was calculated as the concentration in KBM/KWM/RWM 50/48/2 (Table S9) × grist load.
[d] 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one.

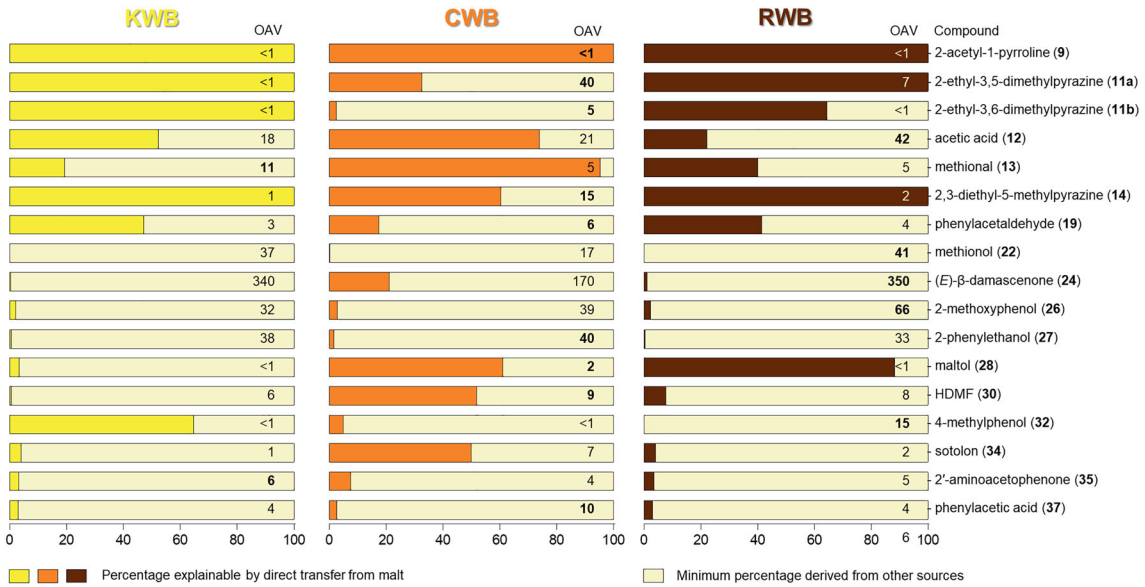

Figure 4. Percentage concentration of compounds in kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB) explained by direct transfer from malt. [Colour figure can be viewed at wileyonlinelibrary.com]

yellow, orange, and brown represent the percentage of each compound in the beers that can be explained by a direct transfer from the respective malt mixture. To indicate the impact of each compound on the aroma of the three beers, OAVs taken from Table 3 were included and the highest OAV of each odorant highlighted in bold.

In most cases, only a minor percentage of the odorant concentration in the wheat beers could be explained by direct transfer from the malts. Similar results have been reported for the corresponding barley malt beers (Féchir et al, 2021). This was to be expected for compounds known to originate from other sources than malt. For example, methionol (**22**), 2-phenylethanol (**27**), and phenylacetic acid (**37**) are fermentation by-products. It was, however, surprising to obtain similar results for compounds (*E*)-β-damascenone (**24**), 2-methoxyphenol (**26**), HDMF (**30**), and 4-methylphenol (**32**), presumably formed by elevated temperatures during malt production. Potential explanations for this include the following. (1) The malts contain thermally formed precursor compounds rather than the odorants and the conversion of the precursors to the odorants occurs during brewing (mashing, boiling, or fermentation). (2) The odorants are formed by the thermal treatment during malt production but are entrapped in unknown aggregates to which they might be non-covalently bound. Indeed, it is suggested that starch might play a role in the encapsulation of odorants during malting. This could also explain why full liberation is not achieved in our approach, but in brewing where the starch is gelatinised and enzymatically degraded.

Different behaviour was observed for 2-acetyl-1-pyrroline. The amounts recovered in the beers were low, 3% in KWB, 0.3% in CWB, and 4% in RWB, with concentrations below the OTV (Table 3), suggesting this compound was degraded in the brewing process. By contrast, in the corresponding beer brewed with caramel barley malt, the concentration of 2-acetyl-1-pyrroline was higher than expected. As only 12% could be explained by a direct transfer from malt, a substantial amount was formed during the brewing process (Féchir et al, 2021).

In conclusion, this study has identified the compounds contributing to the specific aroma of a caramel wheat and roasted wheat malt beer. Pyrazines, furanones, and the pyranone maltol characterised the aroma of the caramel wheat malt beer, whereas phenols contributed the typical aroma of the beer brewed with the roasted wheat malt.

Analyses of the malts showed lower amounts of important odorants than were present in beers, suggesting their formation from malt derived precursors during brewing and/or liberation from complexes. This limits the significance of sensory and analytical data from malts for the prediction of beer aroma properties. Nevertheless, the study confirmed the essential contribution of speciality wheat malt to the aroma of beer at a molecular level. The chemistry behind the increase of malt derived key compounds during brewing, however, is still to be investigated.

Nomenclature

2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethan-1-one; 2'-aminoacetophenone, 1-(2-aminophenyl)ethan-1-one; (*E*)-β-damascenone, (2*E*)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one; HDMF, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one; linalool, 3,7-dimethylocta-1,6-dien-3-ol; maltol, 3-hydroxy-2-methyl-4*H*-pyran-4-one; methional, 3-(methylsulfanyl) propanal; methionol, 3-(methylsulfanyl)propan-1-ol; ɣ-nonalactone, 5-pentyldihydrofuran-2(3*H*)-one; sotolon, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; vanillin, 4-hydroxy-3-methoxybenzaldehyde

Author contributions

Klaas Reglitz: investigation, methodology, visualisation, writing (review and editing), project administration.

Michael Féchir: investigation, resources, formal analysis, writing (original draft), visualisation, project administration.

Veronika Mall: conceptualisation, investigation, writing (review and editing), project administration, funding acquisition.

Jens Voigt: conceptualisation, supervision, project administration, funding acquisition.

Martin Steinhaus: supervision, methodology, writing (review and editing), project administration.

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Conflict of interest

The authors declare there are no conflicts of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

8.2.3 Summary and Individual Contributions

Top-fermented beers are often produced by complementing the kilned barley malt commonly applied for brewing with kilned wheat malt resulting in beers with unique characteristics. To obtain wheat malt beers with a wide range of aroma properties, a part of the kilned barley and wheat malt can be substituted by specialty wheat malts such as caramel wheat malt and roasted wheat malt, which are produced by applying additional thermal processing during malting. However, the impact of these malts on the aroma of top-fermented beers and the transfer of the responsible odorants from the malt to the beer have not yet been studied on a molecular level.

Therefore, three top fermented wheat malt beers were brewed, namely a reference beer solely produced with kilned wheat and barley malts (KWB), a caramel wheat malt beer (CWB), and a roasted wheat malt beer (RWB). The three beers were brewed with equal parts of kilned barley malt and wheat malt, while the wheat malt portion included the specialty malts applied for producing CWB and RWB. The aroma of the beers was characterized by a trained sensory panel revealing substantial differences between the two specialty malt beers CWB and RWB but also between the specialty malt beers and the reference beer KWB. The responsible odorants in the beers were identified by aroma extract dilution analysis and quantitated followed by a calculation of odor activity values (OAVs) to assess the impact of the individual compounds on the aroma of each beer. Major odorants in CWB were earthy smelling 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine, caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and maltol, and soup seasoning-like smelling sotolon. In contrast, the aroma of beer RWB was primarily determined by phenolic and smoky smelling 2-methoxyphenol and 4-methylphenol. The results were verified by comparing aroma reconstitution models to the original beers.

The transfer from malt to beer was then assessed by quantitating the odorants in the malts applied for brewing and comparing hypothetical concentrations in the beers calculated assuming 100% transfer and the absence of other sources to the actual concentrations. The results revealed that substantial amounts of the odorants in the beers did not originate from a direct transfer but were formed and/or released during the brewing process, thus confirming earlier results obtained with different barley malts and bottom-fermented beers.

Michael Féchir designed and conducted the brewing experiments performed to produce the beers KWB, CWB, and RWB. Klaas Reglitz, Veronika Mall, and Michael Féchir designed and conducted the volatile isolations, the GC-O screenings, the structure assignments, the quantitations of the odorants, and the calculation of the OAVs in the beers as well as the volatile isolations and the quantitations of the odorants in the malts. Klaas Reglitz, Veronika Mall, and Michael Féchir designed and conducted the determination of OTVs in water and starch, the determination of quantitative olfactory profiles as well as the aroma reconstitution experiments. Michael Féchir assessed the odorant transfer and performed the statistical evaluation of the quantitative olfactory profiles. Michael and Klaas evaluated the data and prepared the manuscript, which was revised by Veronika Mall, Jens Voigt, and Martin Steinhaus. Jens Voigt, Veronika Mall, and Michael Féchir conceived this study, which was directed and supervised by Jens Voigt and Martin Steinhaus.

8.2.4 Reprint Permission

Research article | Open Access

The impact of caramel and roasted wheat malts on aroma compounds in top-fermented wheat beer

Klaas Reglitz, Michael Féchir, Veronika Mall, Jens Voigt, Martin Steinhaus

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Klaas Reglitz and Michael Féchir contributed equally to this work and share first authorship

8.3 List of Publications, Talks, and Poster Presentations

Peer-reviewed publications:

Morissy, C. P.; Halstead, M. A.; Féchir, M.; Carrijo, D.; Fisk, S. P.; Johnson, V.; Shellhammer, T. H., Hayes, P. M. Investigation of barley variety contribution to beer flavor using elite germplasm in commercial-type malts and beers. *J. Am. Soc. Brew. Chem.* **2022**.

Féchir, M.; Weaver, G.; Roy, C.; Shellhammer, T. Exploring the regional identity of Cascade and Mosaic hops grown at different locations in Oregon and Washington. *J. Am. Soc. Brew. Chem.* **2022**.

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Féchir, M.; Kraus-Weyermann, T.; Voigt, J. Identification of marker volatiles in malt to predict malt-derived aroma properties of bottom-fermented beers. *Brewing Science*. **2021**, *74*, 17−26.

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Van Simaeys, K. R.; Féchir, M.; Gallagher, A.; Stokholm, A.; Weaver, G.; Shellhammer, T. H. Examining chemical and sensory differences of new American aroma hops grown in the Willamette valley, Oregon, Oregon. *J. Am. Soc. Brew. Chem.* **2021**, 1−9.

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Reports and other publications:

Féchir, M.; Kraus-Weyermann, T.; Voigt, J. Predicting the impact of malt composition on the beer aroma. *Brauwelt International.* **2022**.

Féchir, M.; Kraus-Weyermann, T.; Voigt, J. Vorhersage des Einflusses der Malzzusammensetzung auf das Bieraroma. *Brauwelt International.* **2022**.

Féchir, M.; Reglitz, K.; Voigt, J.; Steinhaus, M. *Einfluss der Prozesstechnologie auf die Bildung von Schlüsselaromastoffen und Markerverbindungen in Gersten- und Weizenmalz sowie Malzextrakt und Bestimmung der Transferraten in Bier und Brot*. Teaching & Research Report 2017 & 2018, Trier University of Applied Sciences, Germany, 2019.

Féchir, M.; Mall, V.; Voigt, J.; Steinhaus, M. *Einfluss der Prozesstechnologie auf die Bildung von Schlüsselaromastoffen und Markerverbindungen in Gersten- und Weizenmalz sowie Malzextrakt und Bestimmung der Transferraten in Bier und Brot*. Project Report 2017, Research Association of the German Food Industry (FEI), Bonn, Germany, 2018.

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Oral presentations:

Shellhammer, T.; Féchir, M.; Gallagher, A.; Weaver, G.; Van Simaeys, K. R. Impact of regionality on hop flavor and quality. *Brewing Summit 2022*, Providence, RI, 2022.

Féchir, M.; Van Simaeys, K.; Weaver, G.; Roy, C.; Gallagher, A.; Shellhammer, T. Exploring the regional identity of hops in the Pacific Northwest: A case study involving Cascade and Mosaic hops grown in Oregon and Washington. *38th EBC Congress*, Madrid, Spain, 2022.

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Féchir, M.; Mall, V.; Reglitz, K.; Voigt, J.; Steinhaus, M. Technologische Einflussfaktoren auf die Bildung von Markersubstanzen in Malz und deren Transfer in Bier und Zwischenprodukte. *48. Internationales Braugerstenseminar*, Versuchs- und Lehranstalt für Brauerei in Berlin (VLB), Berlin, Germany, 2019.

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Voigt, J.; Féchir, M. Characterisation of key aroma compounds in two specialty malts by means of aroma extract dilution analysis. *36th Congress EBC European Brewery Convention*, Ljubljana, Slovenia, 2017.

Poster presentations:

Féchir, M.; Mall, V.; Reglitz, K.; Voigt, J.; Steinhaus, M. Characterization of malts by sensory analysis and analysis of volatiles. *37th EBC Congress*, Antwerp, Belgium, 2019.

Féchir, M.; Voigt, J.; Mall, V.; Cryer, D.; Richter, A. A new analytical approach to malt aroma Characterization. *IBD Asia Pacific Convention*, Wellington, New Zealand, 2018.

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