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Odor-Active Compounds in Malt Extracts for the Baking Industry – Identification, Sources, and Impact on Bread Aroma

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Contents

1 Summary

In the recent years, malts and malt extracts have gained importance as quality improving ingredients in the baking industry. Liquid malt extracts (LMEs) are used as all-natural ingredients to enhance enzyme activity, color, and aroma of bakery products. The current study focused on a light and a dark LME and their use in bread making.

In addition to a strong malty odor note, the aroma of the light and the dark LME showed pronounced honey-like, caramel-like, soup seasoning-like, roasty, and smoky notes, the intensities of which strongly dependent on the malt extract type. To clarify the molecular background of the aroma differences, the volatiles from both LMEs were isolated by solvent extraction and solvent-assisted flavor evaporation (SAFE) and subjected to aroma extract dilution analysis (AEDA). To substantiate the screening results, major odorants were quantitated using gas chromatography-mass spectrometry (GC-MS) and isotopically substituted odorants as internal standards. Odor activity values (OAVs) were calculated to evaluate the contribution of the individual compounds to the overall aroma. Important odorants in the light LME were 3-(methylsulfanyl)propanal, (*E*)-β-damascenone, and 4-ethenyl-2-methoxyphenol. In the dark LME, high OAVs were calculated for sotolon, 3-(methylsulfanyl)propanal, (*E*)-β-damascenone, acetic acid, and maltol. The pronounced honey-like odor note in the profile of the light LME was reflected by a high OAV of phenylacetaldehyde. Sotolon, maltol, and 2-methoxyphenol, known to be formed by thermal reactions, showed higher OAVs in the dark LME, corresponding to the more intense soup seasoning-like, caramel-like, and smoky odor notes. To investigate the aroma changes during processing from malt to extract, the odorants were additionally quantitated in the Pilsner malt that served as starting material for both LMEs. Comparing the concentrations in the malt and the LMEs revealed seven odorants mainly formed by malt extract processing. In the light LME, (*E*)-β-damascenone and 4-ethenyl-2-methoxyphenol and in the dark LME, maltol, sotolon, (*E*)-β-damascenone, and 2-methoxyphenol were identified as important process-induced odorants.

To investigate the impact of LME addition on the aroma of bread, first the volatile isolates of crust and crumb of breads made without and with the addition of the dark LME were subjected to AEDA. Results revealed only minor differences between the bread crust samples and slightly larger differences between the bread crumb samples. In the volatile isolate of the crumb of the bread made with the dark LME, higher FD factors were determined for maltol and sotolon. Quantitations and OAV calculations carried out for the reference bread and the breads made with the light and the dark LME resulted in high OAVs for roasty, popcorn-like smelling 2-acteyl-1-pyrroline in all bread crust samples. For most of the bread crust odorants, only slightly higher OAVs were found after adding LME to the bread recipe. Clearly higher OAVs, however, were obtained for 3-(methylsulfanyl)propanal, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF), and phenylacetaldehyde in the crust of the bread made with the light LME, and for sotolon in the crust of the bread made with the dark LME. Larger differences in the OAVs were observed between the bread crumbs. Slightly higher OAVs in the crumb of the bread made with the light LME were found for phenylacetaldehyde, phenylacetic acid, and 3-(methylsulfanyl)propanal. Clearly higher OAVs in the crumb of the bread made with the dark LME were obtained for the Maillard reaction products sotolon, maltol, and HDMF.

To get a deeper understanding of the impact of the LME addition on the bread aroma, the concentrations in the breads were compared to the amounts added with the LMEs. The differences between the reference bread and the bread made with the light LME could mainly

be explained by the formation of odorants from precursors during bread making, the impact on the bread aroma, however, was low. In contrast, adding dark LME to the dough resulted in a significant impact on the bread aroma, with the impact on the crumb aroma being higher than the impact on the crust aroma. The higher concentration of sotolon in bread crust and crumb and of maltol in bread crumb was primarily explainable by a direct transfer from the dark LME to the bread. In contrast, the additional amount of HDMF in the crumb of the bread made with the dark LME must have been newly formed from LME-derived precursors during bread making. In summary, the results showed that the addition of LME to the dough had a particular impact on the molecular basis of the bread aroma, with the impact of the dark LME addition being greater than that of the light LME addition. Moreover, the data showed that the compounds responsible for this effect are sotolon, maltol, and HDMF. To further intensify the aroma of bread crust and crumb, the production of the dark LME should be optimized for a targeted formation of sotolon, maltol, and HDMF.

2 Zusammenfassung

Malze und Malzextrakte haben in den letzten Jahren als qualitätsverbessernde Zutaten in der Backindustrie immer mehr an Bedeutung gewonnen. Flüssige Malzextrakte (Liquid Malt Extracts, LMEs) werden als natürliche Zutat zur Verbesserung der Enzymaktivität, der Farbe und des Aromas von Backwaren verwendet. Die vorliegende Arbeit befasste sich mit einem hellem und einem dunklem LME und deren Verwendung bei der Brotherstellung.

Das Aroma von hellem und dunklem LME zeigte neben einer starken malzigen Aromanote auch ausgeprägte honigartige, karamellartige, suppenwürzeartige, röstige und rauchige Noten, deren Intensität stark von der Malzextraktsorte abhing. Um den molekularen Hintergrund der Aromaunterschiede zu klären, wurden die flüchtigen Bestandteile aus beiden LMEs durch Lösungsmittelextraktion und Solvent-Assisted Flavor Evaporation (SAFE) isoliert und einer Aromaextraktverdünnungsanalyse (AEVA) unterzogen. Um die Ergebnisse des Screenings zu objektivieren, wurden wichtige Geruchstoffe mit Hilfe der Gaschromatographie-Massenspektrometrie (GC-MS) und mit isotopensubstituierten Geruchsstoffen als interne Standards quantifiziert. Odor Activity Values (OAVs) wurden berechnet, um den Beitrag der einzelnen Verbindungen zum Gesamtaroma zu bewerten. Wichtige Geruchstoffe im hellem LME waren 3-(Methylsulfanyl)propanal, (*E*)-β-Damascenon und 4-Ethenyl-2-methoxyphenol. Im dunklem LME wurden für Sotolon, 3-(Methylsulfanyl)propanal, (*E*)-β-Damascenon, Essigsäure und Maltol hohe OAVs berechnet. Die ausgeprägte honigartige Note im Profil des hellen LME spiegelte sich im hohen OAV von Phenylacetaldehyd wider. Sotolon, Maltol und 2-Methoxyphenol, die bekanntermaßen durch thermische Reaktionen gebildet werden, wiesen im dunklen LME höhere OAVs auf, was mit den intensiven suppenwürzeartigen, karamellartigen und rauchigen Noten korrespondierte. Um die Aromaveränderungen während der Verarbeitung von Malz zu Extrakt zu untersuchen, wurden die geruchsaktiven Verbindungen zusätzlich im Pilsner Malz quantifiziert, das als Ausgangsmaterial für beide LMEs diente. Ein Vergleich der Konzentrationen im Malz und in den LMEs ergab sieben Verbindungen, die hauptsächlich bei der Malzextraktherstellung entstehen. Im hellen LME wurden (*E*)-β-Damascenon und 4-Ethenyl-2-methoxyphenol und im dunklen LME Maltol, Sotolon, (*E*)-β-Damascenon und 2-Methoxyphenol als wichtige prozessinduzierte Geruchsstoffe identifiziert.

Um die Auswirkungen des LME-Zusatzes auf das Brotaroma zu untersuchen, wurden die flüchtigen Verbindungen von Brotkruste und Brotkrume, die ohne und mit Zusatz von dunklem LME hergestellt worden waren, einer AEDA unterzogen. Die Ergebnisse zeigten nur geringe Unterschiede zwischen den beiden Krusten, aber etwas größere Unterschiede zwischen den beiden Krumen. In der mit Zusatz von dunklem LME hergestellten Krume wurden höhere FD-Faktoren für Maltol und Sotolon ermittelt. Quantifizierungen und OAV-Berechnungen, die für das Referenzbrot und die mit hellem und dunklem LME hergestellten Brote durchgeführt wurden, ergaben in allen Krusten den höchsten OAV für das röstig, popcornartig riechende 2-Acteyl-1-pyrrolin. Für die meisten Verbindungen der Brotkruste wurden nur geringfügig höhere Konzentrationen nach Zugabe von LME zur Brotrezeptur festgestellt. Deutlich höhere OAVs wurden jedoch für 3-(Methylsulfanyl)propanal, 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-on (HDMF) und Phenylacetaldehyd in der Kruste des mit hellem LME hergestellten Brotes und für Sotolon in der Kruste des mit dunklem LME hergestellten Brotes ermittelt. Größere Unterschiede in den OAVs zeigten sich zwischen den Krumen der Brote. Für Phenylacetaldehyd, Phenylessigsäure und 3-(Methylsulfanyl)propanal wurden leicht höhere OAVs in der Krume des mit hellem LME hergestellten Brotes festgestellt. Deutlich höhere OAVs wurden

jedoch in der Krume des Brotes mit Zusatz von dunklem LME für die Maillard-Reaktionsprodukte Sotolon, Maltol und HDMF gefunden.

Um die Auswirkungen des LME-Zusatzes auf das Brotaroma besser zu verstehen, wurden die Konzentrationen in den Broten mit den mit den LMEs zugesetzten Mengen verglichen. Die Unterschiede zwischen dem Referenzbrot und dem mit hellem LME hergestellten Brot ließen sich hauptsächlich durch die Bildung von geruchsaktiven Verbindungen aus Vorläufern während der Brotherstellung erklären. Die Auswirkungen auf das Brotaroma waren jedoch gering. Im Gegensatz dazu führte die Zugabe von dunklem LME zum Teig zu einer signifikanten Beeinflussung des Brotaromas, wobei die Auswirkung auf das Aroma der Krume größer war als die auf das Aroma der Kruste. Der Konzentrationsanstieg von Sotolon in Brotkruste und -krume und von Maltol in der Brotkrume war in erster Linie durch einen direkten Transfer der Verbindungen aus dem dunklen LME in das Brot erklärbar. Im Gegensatz dazu musste die zusätzliche Menge an HDMF in der Krume des Brotes, das mit dunklem LME hergestellt wurde, während der Brotherstellung aus Vorläufern im LME neu gebildet worden sein. Zusammenfassend zeigten die Ergebnisse, dass der Zusatz von LME zum Teig einen wesentlichen Einfluss auf die molekulare Grundlage des Brotaromas hatte, wobei der Einfluss von dunklem LME größer war als der von hellem LME. Darüber hinaus wurde gezeigt, dass die für diesen Effekt verantwortlichen Verbindungen Sotolon, Maltol und HDMF sind. Um das Aroma der Kruste und Krume des mit dunklen LME hergestellten Brotes zu intensivieren, sollte die Herstellung des dunklen LME auf eine gezielte Bildung von Sotolon, Maltol und HDMF optimiert werden.

3 Abbreviations and Nomenclature

Abbreviations

AEDA	Aroma extract dilution analysis
ASTM	American Society for Testing and Materials
ATP	Adenosine triphosphate
3-AFC	3-Alternative forced-choice
b.p.	Boiling point
cAMP	Cyclic adenosine monophosphate
CI	Chemical ionization
Diff.	Difference
DME	Dry malt extract
EI	Electron ionization
FD	Flavor dilution
FFAP	Free fatty acid phase
FID	Flame ionization detector
GC	Gas chromatography
GC×GC	Comprehensive two-dimensional gas chromatography
GC-O	Gas chromatography-olfactometry
LME	Liquid malt extract
MS	Mass spectrometry
NAD	Nicotinamide adenine dinucleotide
NMR	Nuclear magnetic resonance
OAV	Odor activity value
OTV	Odor threshold value
RI	Retention index
SAFE	Solvent-assisted flavor evaporation
SDE	Simultaneous distillation/extraction
SIDA	Stable isotope dilution assay
SPME	Solid phase microextraction
TOF	Time of flight

Nomenclature

2-Acetyl-1-pyrroline	1-(3,4-Dihydro-2*H*-pyrrol-5-yl)ethan-1-one
2-Acetyl-2-thiazoline	1-(4,5-Dihydro-1,3-thiazol-2-yl)ethan-1-one
2'-Aminoacetophenone	1-(2-Aminophenyl)ethanone
(*E*)-β-Damascenone	(2*E*)-1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one
Dihydromaltol	5-Hydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one
Maltol	3-Hydroxy-2-methyl-4*H*-pyran-4-one
HDMF	4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one (Furaneol®)
Sotolon	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one
Vanillin	4-Hydroxy-3-methoxybenzaldehyde

4 Introduction

4.1 Molecular Sensory Science

4.1.1 Odor-Active Compounds and Aroma Perception

The customers' decision to purchase foods is influenced by various aspects. Next to healthiness, factors such as regionality, seasonality, freshness, sustainability, and food safety have gained importance in the recent years. Different diets, such as vegetarian, vegan, gluten-free, and lactose-free diets, are also playing an increasingly important role. Nevertheless, sensory impressions, such as aroma and taste, are the most important decision drivers.[1] The aroma of a food is evoked by the combination of various odor-active compounds, which are thus all-important for food quality and consumer acceptance.

To be an odor-active compound, a food constituent must be volatile. Sufficient volatility of the compound is necessary for its release from the food matrix into the air and thus its ability to enter the nose. The volatility depends on the molecular weight and the polarity of the compound. Odorants are rather low molecular weight compounds.[2]

In addition, odorants must be able to bind to and activate one of the ~400 different olfactory receptor proteins. The odorants released from the food matrix before consumption reach the olfactory epithelium in the nasal cavity with the inhaled air through the nostrils (orthonasally). Odorants released during chewing of the food enter the nasal cavity mainly directly after swallowing with the exhaled air from behind (retronasally).[2]

The odorants bind to G protein-coupled receptor proteins located in the membrane of the olfactory receptor cells' cilia. This leads to the activation of the G protein mediated intracellular reaction cascade. First, the adenylyl cyclase is activated which produces cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The secondary messenger cAMP then binds to cation channels, thereby opening them. This allows cations (e.g., Ca^{2+}) to pass through the ion channels, which in turn activates the chloride ion channels and finally leads to a depolarization of the cell membrane. The action potential generated by the depolarization is then transmitted via the axon to the olfactory bulb of the brain. There, the axons of olfactory receptor cells with the same type of receptor protein are grouped together in glomeruli. The qualitative and quantitative combination of different odorants activates a defined set of glomeruli and thus results in a specific activation pattern. Via the mitral cells, this pattern is transmitted to higher regions of the brain, where it is processed and finally results in the perception of a specific aroma (Figure 1).[3-9]

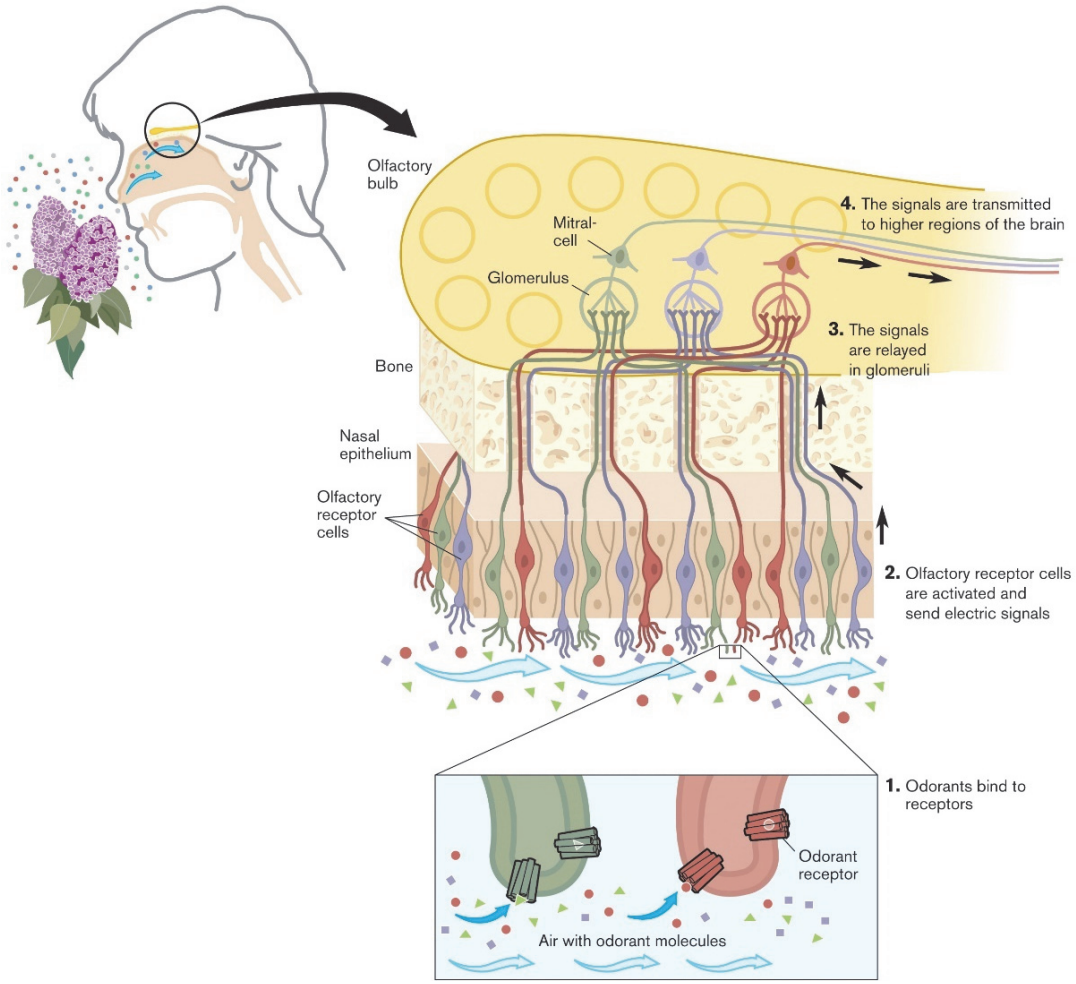

Figure 1: Odorant receptors and organization of the olfactory system[9]

To be perceived as an odor, the concentration of the odorant has to exceed its individual odor threshold value (OTV) in air. The OTV in a food is dependent on the chemical properties of the odorant and the specific release characteristics from the food matrix. The OTVs of the odorants cover a wide range of concentrations, in detail, more than ten decimal powers. For example, alcoholic smelling ethanol has an OTV of 990000 µg/kg[10] in water whereas for sulfury, burnt smelling (1*S*)-1-phenylethane-1-thiol an OTV as low as 0.00021 µg/kg[11] has been determined in the same matrix. In addition, OTVs can be extremely divergent in different matrices. For example, malty smelling 2-methylpropanal shows an OTV of 0.49 µg/kg[10] in water and an OTV of 56 µg/kg[12] in starch, which is over 100 times higher.

In summary, if a food compound is volatile, is able to bind to an olfactory receptor protein, and is present in a concentration above its specific OTV, the compound is odor-active and thus has the potential to contribute to the overall aroma of the food. The specific aroma of a food is evoked only by a few of these odor-active compounds, the so-called key odorants. The key odorants which have been found in many types of food are named generalists, whereas the so-called specialists and individualists are key odorants only in a small number of foods or a single food, respectively.[13] In order to identify the key odorants in a specific food, a well-established approach is available.

4.1.2 The Sensomics Concept for Key Odorant Identification

The concept for the identification of the key odorants includes seven major steps (Figure 2). The first step is the isolation of the volatile compounds. Organic solvents with low boiling points (b.p.), like diethyl ether (b.p. 35 °C) or dichloromethane (b.p. 40 °C) are used for solvent extraction. The volatiles are separated from the nonvolatile components in the solvent extract by solvent-assisted flavor evaporation (SAFE)[14] (Figure 3). The application of high vacuum allows to maintain the temperature ≤ 40 °C, thus avoiding temperature-induced compound degradation and artifact formation. This is a major advantage of SAFE over other methods such as the simultaneous distillation/extraction (SDE).[2, 15, 16] The SAFE distillate is concentrated to volumes of approximately 100 µL to 1 mL, e.g., using a Vigreux column and a Bemelmans microdistillation device.[17]

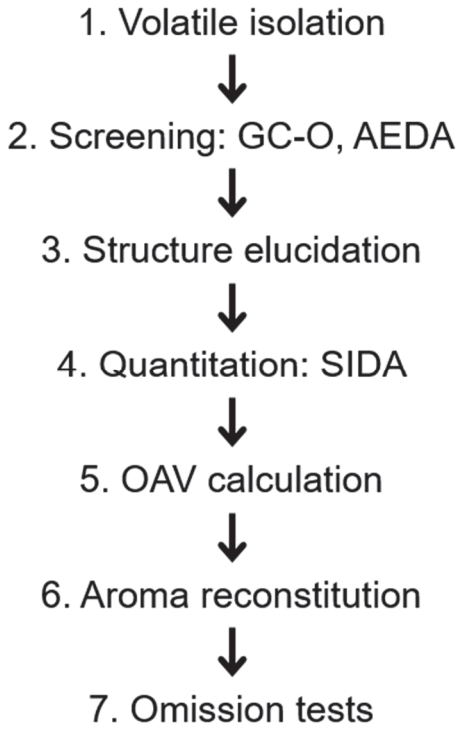

Figure 2: The Sensomics concept for key odorant identification (modified according to Schieberle, 1995[18] and Grosch, 2001[19])

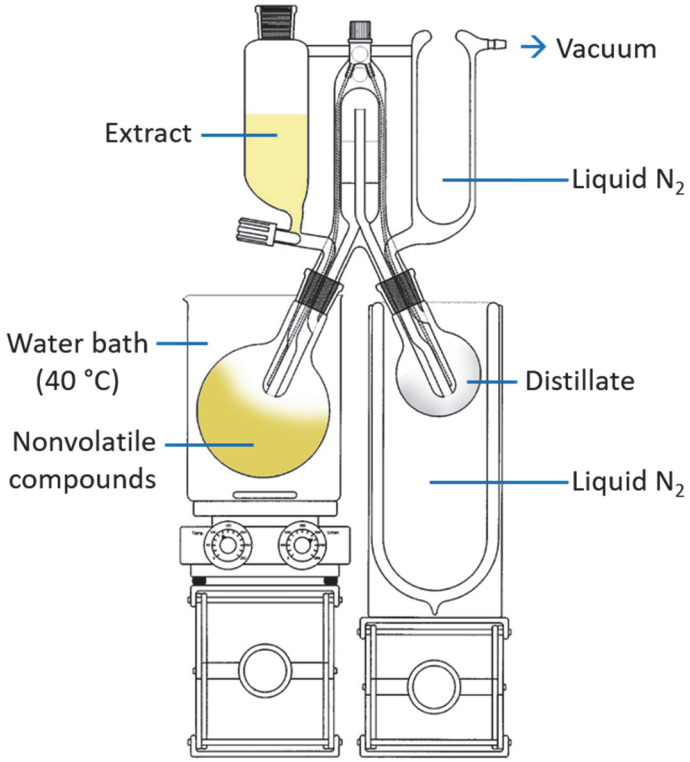

Figure 3: SAFE equipment (illustration: Martin Steinhaus, modified from Engel et al., 1999[14])

The concentrated SAFE distillate containing the volatiles is subjected to gas chromatography-olfactometry (GC-O). Coupling the human nose with a gas chromatograph allows for the detection of the odor-active compounds among the volatiles, the major part of which is odorless. Approximately 1 µL of the volatile isolate is applied onto the column using an in-oven cold on-column injection technique. After chromatographic separation of the volatiles on the column, the effluent is splitted by a Y-shaped splitting device. Two deactivated capillaries direct the effluent halves simultaneously to a flame ionization detector (FID) and a heated exit serving as sniffing port. The FID signal is recorded by a computer software or an analogue recorder. Trained sniffers place their noses above the sniffing port to mark the odorous regions with their odor qualities in the FID chromatogram (Figure 4).

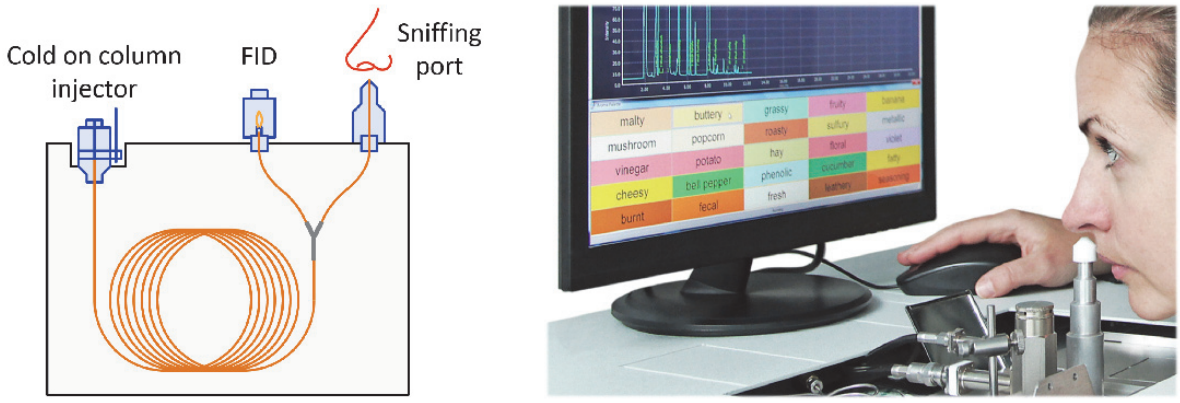

Figure 4: GC-O system (illustration and photo: Martin Steinhaus)

The ranking of the odorants detected during GC-O screening is best performed by applying a dilution to threshold approach. In an aroma extract dilution analysis (AEDA),[20] the initial volatile isolate is diluted stepwise with organic solvent, usually by a factor of two. Subsequently, the dilution series (1:2, 1:4, 1:8, 1:16, etc.) is subjected to GC-O analysis until not a single odorous region can be detected at the sniffing port during the entire GC run. A flavor dilution factor (FD factor) is assigned to each odorant representing the dilution factor of the sample with the highest dilution in which the odorant was detected during GC-O analysis (Figure 5).

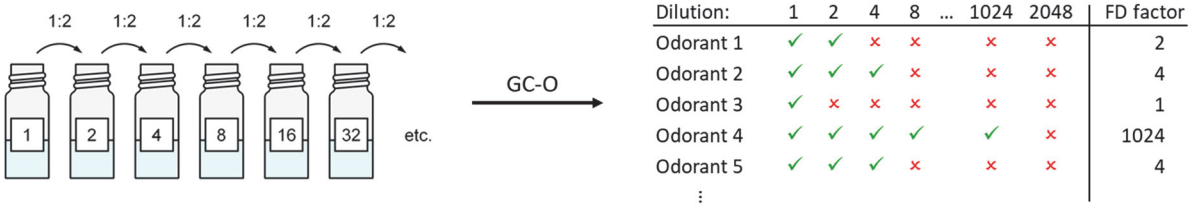

Figure 5: AEDA: stepwise dilution of the volatile isolate and calculation of FD factors after GC-O analysis of the diluted samples (illustration: Martin Steinhaus)

The next step is to elucidate the structures of the odorants detected during odorant screening by GC-O and AEDA. Structural assignments of the odorants are achieved by comparing the retention indices (RIs) on two columns of different polarities (e.g. DB-FFAP, DB-5), the odor quality perceived at the sniffing port, the odor intensities at adequate concentration levels, as well as the mass spectra generated by gas chromatography-mass spectrometry (GC-MS) in electron ionization (EI) and chemical ionization (CI) mode to data obtained from authentic reference compounds analyzed under the same conditions. To reduce co-elution problems in GC-MS, different separation techniques are applied. For example, the volatile isolates can be fractionated by acid-base extraction,[21, 22] silica gel chromatography,[21-23] or mercurated agarose gel for selective thiol isolation.[22-25] The odorants in the individual fractions are localized by GC-O before the fractions are subjected to GC-MS analysis. On the other hand, improved separation can also be achieved by using two-dimensional GC, such as GC×GC-TOFMS[25, 26] or GC-GC-MS with heart-cutting.[22, 27] If a reference substance is not commercially available, it needs to be synthesized to allow for the unequivocal identification of the unknown compound. The structure of the synthezised compound is confirmed by nuclear magnetic resonance (NMR) spectrometry. Then the compound is analyzed under the same conditions as the volatile isolate or a fraction by GC-O and GC-MS.

The screening for odorants with GC-O in combination with AEDA is a useful tool to distingush between odor-active and odorless volatiles and to get a first idea on the importance of individual compounds. However, GC-O results cannot provide a clear assessment of the contribution of individual odorants to the overall aroma of the analyzed food sample. To substantiate the screening results, exact concentrations of the identified compounds are required. Odorant quantiation is preferentially performed by stable isotope dilution assays (SIDA) (Figure 6).[28] In SIDA, stable isotopically substituted analogues of the target compounds, commonly deuterated or ^{13}C-substitiuted compounds, are employed as internal standards. The standards are added to the sample at the beginning of the workup. The mixture is homogenzied until an equilibrium is reached between the target compounds and the added internal standards. Since the target analyte and the corresponding isotopically substituted standard possess almost identical chemical and physical properties, losses of the analyte

during sample workup, such as during SAFE, fractionation, and concentration, are compensated by the same loss of the internal standard. Accordingly, the ratio of the analyte to its isotopically substituted standard remains constant. Subsequently, the peak area ratio of analyte and standard is monitored by GC-MS. Bearing the different molecular weights of the analyte and its isotopologue in mind, molecule ions or fragments including the isotopical substitution are used to distinguish between analyte and standard. The odorant concentration is finally calculated from the area ratio associated with the target analyte and the isotopically substituted standard, the amount of sample used, and the amount of standard added, by employing a calibration line equation obtained from the analysis of analyte/standard mixtures in different concentration ratios.

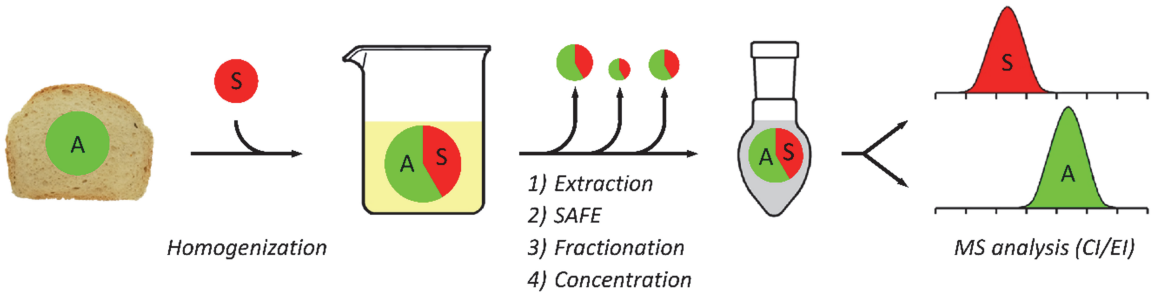

Figure 6: Workup procedure for odorant quantitation by application of SIDA (illustration: Martin Steinhaus, photo: Nadine Rögner)

In the next step, the odorant concentration is related to the OTV to approximate the relevance of the individual compound to the overall aroma of the food. The OTV should be determined in a matrix as similar as possible to the foodstuff. For example, in case of bread, OTVs in starch were used.[12, 29, 30] OTVs are determined orthonasally according to the American Society for Testing and Materials (ASTM) procedure for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[31] The odor activity value (OAV) is defined as the ratio of the odorant concentration to the OTV (Formula 1).[32, 33] The OAV indicates the factor by which concentration of an odorant exceeds its OTV. Odorants showing an OAV ≥ 1 may contribute to the overall aroma, while compounds with an OAV < 1 normally do not play a role in the perception of the overall aroma of the investigated food.

Formula 1: Calculation of the OAV

$$\mathrm{OAV} = \frac{\text{Odorant concentration } \frac{\mu g}{kg}}{\text{Odor threshold value } \frac{\mu g}{kg}}$$

During food consumption, odorants are not perceived as single substances but as a mixture of odorants imbedded in a matrix. For the verification of the identification and quantitation experiments, an aroma reconstitution model is prepared from a model matrix mimicking the investigated food and the odorants with OAVs ≥ 1 at their respective concentrations. The model matrix should at least represent the main composition of the original food, including the water content, the lipid content, and the pH value. The aroma reconstitution model is then orthonasally compared to the original food by a trained sensory panel in a quantitative olfactory profile analysis. If the profiles show a good agreement, it can be assumed that all important

odorants have been correctly identified and quantitated. The aroma reconstitution was thus successful.[2]

The final step in the concept are omission tests. An individual odorant or a group of odorants with similar odor properties is/are omitted from the aroma reconstitution model. In a 3-alternative forced-choice (3-AFC) test, the incomplete model is compared to the complete reconstitution model. If a significant difference is found between the incomplete and the complete model in the 3-AFC test, the omitted odorant is obviously important for the overall odor profile of the complete aroma reconstitution model and can thus be classified as a key odorant of the food.[34, 35] Finally, the application of the Sensomics concept for key odorant identification has reduced the huge number of volatiles to typically 10–20 key odorants which evoke the specific aroma of the analyzed food.[2] As of 2014, a total of 226 key odorants in 227 food samples were identified.[13]

4.2 Malt, Malt Extract, and Bread

Cereal products are among the most important stable foods. For at least 30000 years, mankind has been feeding on cereal porridge. It has been baked for ~22000 years as flatbread.[36] Today, in particular bread as a baked cereal product is of utmost importance. Rich in carbohydrates it is a significant source of energy for humans in many countries. In 2020, German households purchased a total of ~1.7 million tons of bread which corresponded to ~37.9 kg of bread per household.[37] The importance of bread is also shown by the large number of over 3200 different bread types in the German market.[38] Adding different ingredients to the basic recipes leads to variations in the baking properties, the color, and particularly in the aroma of the breads. Among the commonly used all-natural ingredients for bread making are malt flours and malt extracts obtained from barley.[39-42]

4.2.1 Barley

Barley (*Hordeum vulgare* L.) is mainly used as animal feed and as a raw material for malts to be used for making alcoholic beverages, such as beer. The versatility and adaptability to unfavorable climatic and soil conditions of barley are important factors in cultivation and application.[43]

Among the worldwide production of cereals, barley ranks fourth behind corn, wheat, and rice. Approximately 60% of the world`s barley production occurs in Europe. In 2020, the leading barley-producing country was the Russian Federation (Table 1). This was followed by Spain, Germany, Canada, France, and Australia.[44]

Table 1: Barley production in the leading producer countries in 2020[44]

Country	Production (million tons)	Country	Production (million tons)
Russian Federation	20.94	Australia	10.13
Spain	11.47	Turkey	8.30
Germany	10.77	United Kingdom	8.12
Canada	10.74	Ukraine	7.64
France	10.27	Others	58.66
		Total	157.03

Barley is a member of the grass family Poaceae. The barley kernel consists of three main components: the embryo (germ), the endosperm, and the grain coverings (Figure 7). The embryo, which represents ~2.5% of the barley grain weight, contains mainly storage proteins, lipids, sugars, minerals, and enzymes. The main part, with ~75% of the barley grain weight, is the endosperm, a starchy mass embedded in a protein matrix and the main source of nutrients for the embryo. The endosperm is surrounded by the aleurone layer, rich in proteins and lipids. This layer also contains enzymes important for the germination during the malting process.

The grain coverings consist mainly of cellulose and are divided from the inside to the outside into testa, pericarp, and two protecting husks.[43, 45]

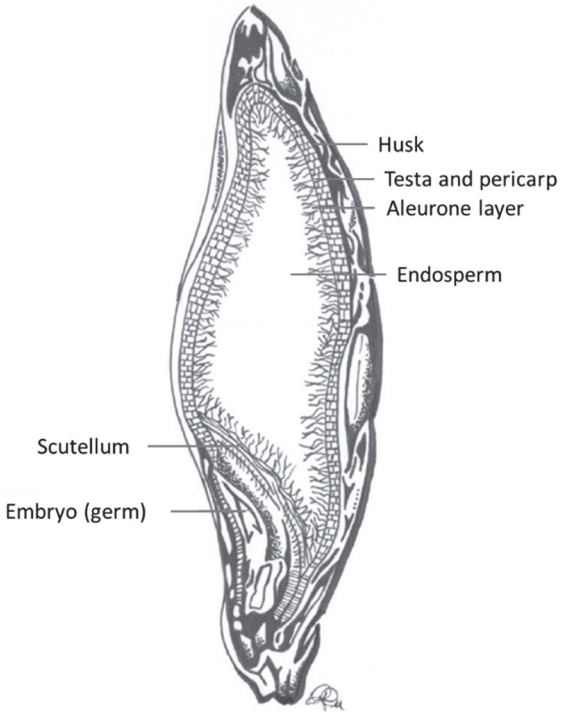

Figure 7: Longitudinal cross section of a barley kernel (modified from Arendt and Zannini, 2013[43])

Barley without husks consist basically of 63% carbohydrates, 12% water, 10% proteins, 9.8% dietary fiber, 2.3% minerals, and 2.1% fat (Table 2).[46] The starch is the most abundant carbohydrate (~60–68% of the dry matter), followed by non-starch polysaccharides (cellulose and hemicellulose), and sugars (glucose, fructose, sucrose, and maltose).[43]

Table 2: Composition of barley without husks[46]

Component	Amount (g/100 g)
Available carbohydrates	63
Water	12
Proteins	10
Total dietary fiber	9.8
Minerals	2.3
Fat	2.1
Total nitrogen	1.8

The content of active enzymes in unprocessed barley is rather low. Enzymes are mostly present in a bound form. The enzymes are important for producing soluble nutrients from

insoluble substances in the endosperm for the growing embryo during germination. A major aim of the malting of barley and other cereal grains is the activation of these enzymes in order to modify the grain composition with respect to the further use.[43, 45, 47]

4.2.2 Malt and Malt Extract Production

Malt is in general germinated and re-dried cereal grains. The major part is produced from barley. The production of malt is divided into three basic steps: steeping, germination, and kilning (Figure 8). In the first step, water is added to the cleaned cereal grains at 12–15 °C. The moisture content increases from ~12% to ~43–48%. This initiates the germination step, during which the kernels begin to grow. Under controlled conditions, such as sufficient humidity, oxygen, and temperature (14–18 °C), enzymes start to degrade high molecular weight compounds. The enzymes of particular interest are the starch degrading amylases. Further enzymes include cytolytic, proteolytic, fat degrading, and phosphate ester splitting enzymes. α-Amylase, formed during germination, and β-amylase, activated during germination, degrade starch to reducing sugars, in particular maltose. The sugar content increases from ~2% in barley to ~8% in barley malt. The cell walls, consisting mainly of β-glucans and pentosans, are degraded and solubilized by cytolytic enzymes. This softens the kernel and enables the enzymes to reach the endosperm. Endo- and exopeptidases degrade ~38–42% of the protein into peptides and amino acids. After a total of 4–6 days of germination, the so-called green malt is obtained. In the final stage of the malting process, the green malt is kilned to stop the germination, to reduce the moisture content, and to form characteristic color and aroma. Initial drying to a moisture content of ~10% is performed by slowly increasing the temperature to 40–55 °C, depending on the desired malt type. The final kilning temperature for pale malt is 80–85 °C leading to a residual moisture content of 3.5–4%. Dark malt is kilned at 102–105 °C resulting in a residual moisture content of 1.5–2%.[43, 45, 47, 48]

Specialty malts such as caramel malts and roasted malts as well as malt extracts are created by modifying the kilning process or by further processing the kilned malt (Figure 8). These malt products differ in color, aroma, taste, and enzyme activity.

Caramel malt is produced from green malt with a water content of up to 48%. Slightly increasing the temperature at the end of germination to 45–50 °C results in further enzymatic degradation and consequently in an extended formation of sugars and amino acids. In a roasting drum, the malt is saccharified at 60–70 °C for 1–1.5 h or at 60–75 °C for 3 h. Subsequently, the malt is heated, depending on the caramel malt type, up to 170 °C. During drying, thermal reactions such as the Maillard reaction and the Strecker degradation provide colorants and odorants from the previously formed degradation products. Caramel malts are characterized by their predominant caramel-like odor note.[45, 47]

For the production of roasted malt, moistened pale malt is heated in a roasting drum at 180–220 °C under uniform rotation. During the roasting process, a strong increase in color and a deactivation of enzymes is observed. The aroma of roasted malt is influenced by the formation of caramel-like, malty, roasty, and smoky odor notes.[45, 47, 49]

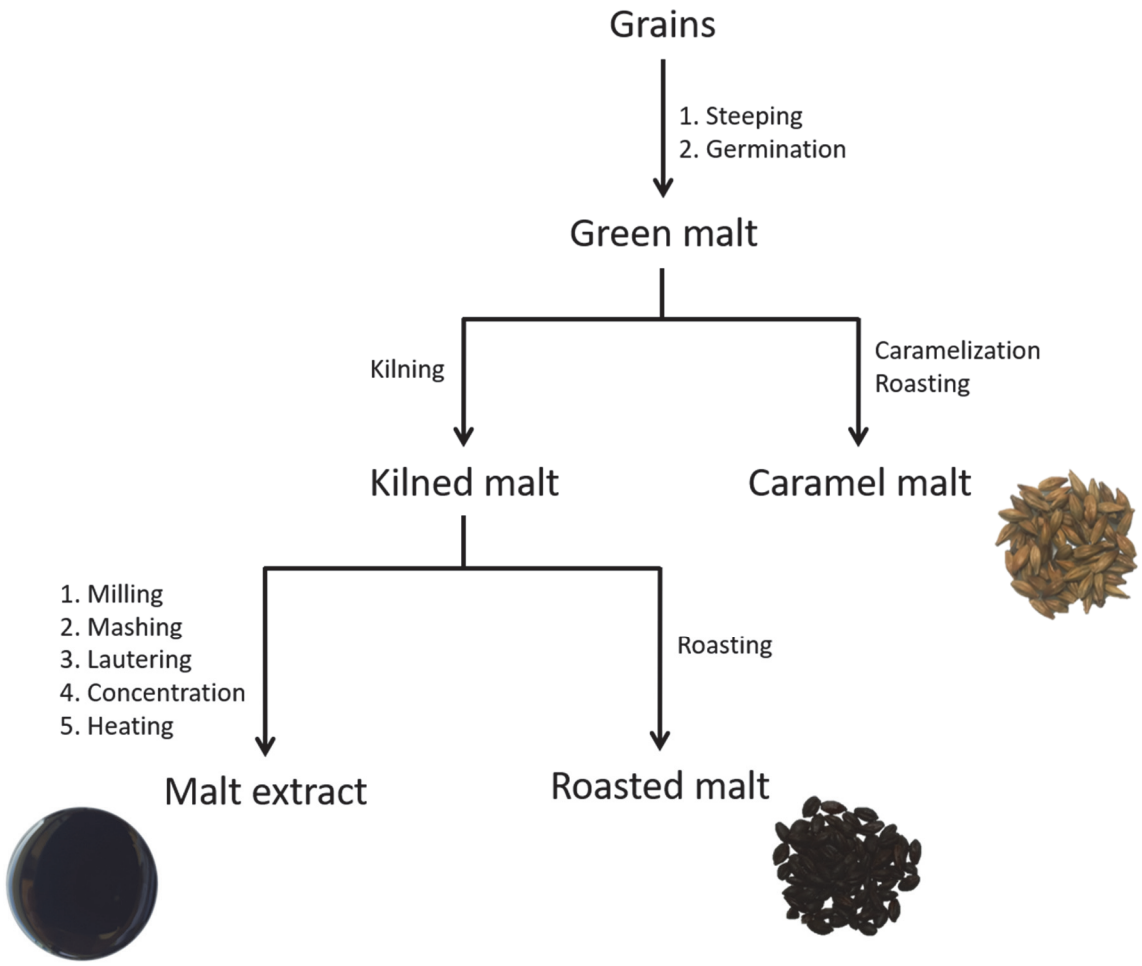

Figure 8: Schematic diagram of malt and malt extract production (photos: Martin Steinhaus and Nadine Rögner)

Malts can be further processed to malt extracts (Figure 8). The kilned malt is milled, mashed with warm water, and the mixture is heated to temperatures between 50 and 70 °C for several hours. This process results in a substantial enzymatic degradation of biopolymers such as starch and proteins. Starch is almost completely degraded to maltose and other reducing sugars. The contents of maltose and glucose are finally ~50% and ~10% of the total carbohydrates, respectively. In the lautering step, the insoluble grain particles, the malt draff, is removed resulting in a sugar-rich aqueous liquid. This phase is gently concentrated under vacuum to a water content of no more than ~28%. This approach finally yields a liquid malt extract (LME) with a syrup-like consistency. Properties such as aroma and color of the individual LMEs can be influenced by specific temperature programs. Basically, LMEs are divided into light and dark LMEs. An LME can be further processed into a dried malt extract (DME). The drying to a crystalline powder with a low water content of ~4% is carried out, e.g., by means of spray drying.[39, 47, 48, 50]

4.2.3 Malt and Malt Extract Volatiles

Around 250 volatiles have been identified in malts (Table 3). The substance classes with the largest number of identified volatiles are aldehydes, pyrazines, alcohols, and furans.[51]

Table 3: Classes of volatile compounds in malt[51]

Substance class	Number of compounds
Aldehydes	39
Pyrazines	36
Alcohols	31
Furans	28
Sulfur compounds	19
Further nitrogen compounds	14
Ketones	13
Acids	13
Phenols	12
Pyrroles	10
Pyridines	8
Amines	6
Oxazol(in)es	6
Esters	3
Lactones	2
Hydrocarbons	2
(Ep)oxides, pyrans, coumarins	2

In an early investigation on volatile compounds in malt, Brand[52] isolated a compound which he called maltol from an “empyreumatic and pungent-smelling condensate” obtained from malt. Some years later, the structure of maltol was correctly determined as 3-hydroxy-2-methyl-4*H*-pyran-4-one.[53, 54]

Extensive experiments on the identification of volatile compounds in peated malt were carried out by Deki et al.[55-57] After steam distillation, they identified several compounds by comparison of RIs and mass spectra with data obtained from authentic reference compounds analyzed under the same conditions. In the group of phenolic compounds, 17 volatiles were detected, including for example phenol, 2-, 3-, and 4-methylphenol, as well as 2-methoxyphenol.[55] Furthermore, a large number of alcohols, such as 2-phenylethan-1-ol, aldehydes, such as acetaldehyde and 3-methylbutanal, pyrazines, such as 2,5-dimethylpyrazine and 2-methyl-5-aceteylpyrazine, and acids, such as acetic acid and propanoic acid were identified.[55-57]

Other studies focused on the influence of various parameters, such as the roasting time and the roasting temperature on the volatiles. Furthermore, the differences between various malt types were evaluated.[49, 58-64]

By application of SPME-GC-MS, Dong et al.[60] analyzed the volatile compounds in barley, green malts at different malting stages, and of the final product malt. A total of 47 volatile compounds were identified. The main groups of volatiles were aldehydes, ketones, alcohols, carboxylic acids, and furans. The amount of Strecker aldehydes, such as 2- and 3-methylbutanal and 2-methylpropanal, and the amount of lipid oxidation products, such as hexanal, increased continuously during the germination process. The Strecker aldehydes reached their maximum during roasting, whereas the aldehydes derived from lipid oxidation decreased at this stage.

Woffenden et al.[58] produced malts at five different roasting temperatures. The contents of pyrazines, such as 2-methylpyrazine and aldehydes, such as furan-2-carbaldehyde, increased with increasing temperatures during roasting. Up to a roasting temperature of 130 °C, the contents of the highly volatile compounds 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal increased. However, a decrease of these compounds was observed at higher roasting temperatures.

Parr et al.[61] investigated the course of formation of 20 compounds during roasting of barley, green malt, and pale malt at different roasting times and temperatures. They assumed that the initial moisture content of the samples, along with the roasting time and the roasting temperature, played an important role in volatile compound formation. Compounds such as maltol and phenylacetaldehyde showed the highest concentrations during roasting of green malt which had a higher initial moisture content than barley and pale malt. During roasting of barley and pale malt, predominantly pyrazines, pyridines, 2-methylfuran, 2-pentylfuran, and 2-acteyl-5-methylfuran were formed.

The influence of different roasting temperatures (100–180 °C) on 14 compounds was evaluated by Vandecan et al.[62] The compound selection was based on literature data of odorants in malt, chocolate, other roasted foods, and wort. Each sample was first held at 60 °C for 60 min before the temperature was raised to the final value. The caramel-like smelling compounds 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF), 4-hydroxy-5-methylfuran-3(2*H*)-one, and maltol were found in the highest concentrations in the malt roasted at 100 °C. In contrast, the pyrazines 2,3,5-trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine showed the highest concentrations after roasting at 180 °C.

The differences between malt types, such as kilned malts, caramel malts, roasted malts, and other specialty malts, were also investigated.[49, 63, 64] The concentrations of volatiles were typically higher when the applied temperature was higher. Extraordinary concentration differences were found for caramel-like smelling maltol and earthy smelling 2,3-diethyl-5-methylpyrazine. The concentration of cooked potato-like smelling 3-(methylsulfanyl)propanal was highest in the caramel malt and lowest in the roasted malt.[64] Vandecan et al.[63] and Yahya et al.[49] also showed that various pyrazines were highest in the roasted malts. The authors thus assumed that pyrazines were important for the aroma of the roasted malts.

All the studies mentioned above focused mainly on the identification of major volatile compounds. However, most volatile compounds have no or only little impact on the overall aroma. To characterize the odor-active compounds in malts and malt extracts and to assess

the contribution of the individual compounds to the overall aroma, an odorant screening by GC-O and AEDA followed by odorant quantitation and OAV calculation is the method of choice (cf. Figure 2).

In 1980, Farley and Nursten[65] obtained a volatile isolate from a malt extract by SDE. Application of GC-O and GC-MS resulted in ~47 odor-active compounds and 38 structure proposals. Among others, 3-methylbutan-1-ol, 2-phenylethan-1-ol, phenylacetaldehyde, damascenone, 4-ethenyl-2-methoxyphenol, and 3-(methylsulfanyl)propanal were identified. The two malty smelling odorants 2- and 3-methylbutanal were highlighted as important contributors to the organoleptic properties of the LME.

By using liquid-liquid extraction, Przybylski and Kamiński,[66] obtained an isolate from a rye malt extract. After gas chromatographic separation of the volatiles, the odor of the fractions was orthonasally evaluated. GC-MS analysis revealed 32 structure proposals including, for example, acetic acid, propanoic acid, 2,5-dimethylpyrazine, 2-methylphenol, and maltol. The role of the identified compounds for the odor of the fractions and the malt extract was not further evaluated.

In comparison to the odorants in malt extracts, the odorants in different malt types have been studied more extensively. Beal et al.[67] applied GC-O in combination with dilution techniques to evaluate the odor-active regions in the chromatogram of a malted barley extract previously obtained by steam distillation. The two malty smelling aldehydes 2- and 3-methylbutanal were assigned the highest dilution factors. Other odor-active compounds included 2-acetylfuran, 2-ethyl-6-methylpyrazine, maltol, and 2-phenylethan-1-ol.

Application of GC-O and a comparative AEDA to volatile isolates obtained from barley, green malt, and dark malt elucidated the formation of odorants during the malting process. Important malt odorants were already present in the barley, but with low FD factors (≤ 16). In the green malt, high FD factors were found for lipid oxidation products, such as (2*E*,6*Z*)-nona-2,6-dienal (FD factor 512), (2*E*)-non-2-enal (FD factor 256), and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (FD factor 256). During kilning, the aldehydes decreased significantly. On the other hand, new compounds were formed, mainly in the course of thermal reactions like the Maillard reaction. High FD factors in the dark malt with significant differences to the other samples were revealed for HDMF (FD factor 2048), 3-methylbutanal (FD factor 256), 4-ethenyl-2-methoxyphenol (FD factor 128), sotolon, maltol, and (*E*)-β-damascenone (each FD factor 64).[68]

Application of GC-O in combination with AEDA to a volatile isolate obtained from a caramel malt by solvent extraction and SAFE revealed high FD factors for malty smelling 3-methylbutanal as well as for mushroom-like smelling oct-1-en-3-one, cooked potato-like smelling 3-(methylsulfanyl)propanal, fatty smelling (2*E*,4*E*)-deca-2,4-dienal, cheesy smelling 2- and 3-methylbutanoic acid, caramel-like smelling HDMF, and vanilla-like smelling vanillin. Precise odorant quantitation using SIDA, calculation of OAVs, aroma reconstitution, and omission tests finally showed that the malty smelling aldehydes 3-methylbutanal (OAV 235) and 2-methylpropanal (OAV 70) were the key odorants that contributed most the aroma of the caramel malt.[68, 69]

4.2.4 Use of Malts and Malt Extracts in Bakery Products

Along with water, hops, and yeast, malt is one of the main raw materials used in the production of alcoholic and non-alcoholic beverages by fermentation, such as malt drinks, whisky, and beer. A major part of the malt produced worldwide is used for brewing beer. Using malt extracts instead of malts for brewing allows craft brewers to skip the mashing and lautering steps of the traditional brewing process. In addition to the beverage sector, malt flours and malt extracts are used as an ingredient in bakery products, breakfast cereals, and confectionery. In the baking industry, malt products are used to enhance enzyme activity, color, aroma, sweetness, and nutritional value.[39-41, 43, 47, 70]

Malt flours and malt extracts for bakery products are distinguished according to their different enzyme activities. While diastatic malt products contain active enzymes, the enzymes are inactivated by high temperatures in non-diastatic malt products. Diastatic malt flours are added during dough making. The enzymes convert starch into sugars and reduce the fermentation time. Due to a very low amount of < 1% of the flour weight, most diastatic malt flours have little to no effect on the bread aroma and color. In contrast, diastatic specialty malt flours, in which only a small proportion of the enzymes are still active, are added in higher amounts (~3%) and therefore also can enhance the bread aroma and color. Non-diastatic specialty malt flours, e.g., caramel and roasted malt flours do not show enzyme activity. Depending on the applied amount, which is usually between 1 and 5% in the dough, an impact on the color and the aroma of the bakery product is possible.[39]

Diastatic malt extracts are produced under milder conditions than non-diastatic malt extracts. Rather thermostable enzymes such as α-amylase thus remain active.[39] α-Amylase is only slowly degraded at temperatures above 80 °C.[71] Malt extracts replace up to 5% of the flour in bread recipes. Malt extracts provide sugars such as maltose and glucose, thus increasing the germination power of the yeast. The fermentation time is shorter, the bread volume increases, and the crumb porosity improves.[39-41] Diastatic malt extracts act as dough conditioners. Their active malt enzymes produce even more digestible sugars during dough preparation. This is beneficial, especially when flours with low baking values are used. Malt extracts also play a significant role for the color of crust and crumb. Malt extract derived reducing sugars promote the browning of the crust during baking via the Maillard reaction.[72] Intrinsic malt extract colorants tint the crumb from golden brown to dark brown. This darker color can be more appealing to consumers. Moreover, malt extracts contain high amounts of sugars and thus increase the sweet taste of crust and crumb. Last but not least, malt extracts and specialty malt flours can significantly enhance the aroma of bakery products.[39]

4.2.5 Bread Making

Only a few ingredients are needed to make leavened bread. The minimum recipe consists of only four ingredients: flour, water, yeast, and salt. Non-essential ingredients can be added during bread making to improve the physical and sensory properties of the bread, e.g., towards softer texture, longer shelf-life, more intensive color, and improved aroma. The bread making process can be divided into three main steps: dough formulation, dough processing, and baking. First, all ingredients are mixed together. After a first fermentation period, the dough is kneaded and formed. The dough piece is further fermented until the volume has increased to the desired value. Baking finally turns the dough into bread.[73]

Depending on the bread type, the baking temperature, and the baking time, the crumb to crust ratio is between 70/30 and 85/15.[74] A whole bread basically consists of carbohydrates (48%) and water (39%), followed by proteins and dietary fiber (Table 4).[46]

Table 4: Composition of a standard bread[46]

Component	Amount (g/100 g)
Available carbohydrates	48
Water	39
Proteins	6.2
Total dietary fiber	4.6
Minerals	1.5
Fat	1.1
Total nitrogen	1.1

To assess the quality of breads, nutritional properties as well as physical attributes such as loaf volume, crumb softness, crust crunchiness, crust and crumb color, porosity, and elasticity, are evaluated. Additionally, the bread aroma is of particular importance for bread quality and consumer acceptance.

4.2.6 Wheat Bread Volatiles

More than 300 volatiles have been reported in wheat bread, including aldehydes, ketones, esters, furans, and pyrazines (Table 5).[51]

Table 5: Classes of volatile compounds in wheat bread[51]

Substance class	Number of compounds
Aldehydes	50
Ketones	36
Esters	30
Furans	28
Pyrazines	25
Acids	22
Alcohols	23
Amines	22
Sulfur compounds	20
Hydrocarbons	11
Pyridines	11
Pyrroles	9
Further nitrogen compounds	7
Lactones	6
Acetals	4
Phenols	4
(Ep)oxides, pyrans, coumarins	2
Ethers	1

The formation of volatiles is significantly influenced by different chemical and enzymatic reactions that take place during the individual steps of wheat bread making. In the bread crumb, the volatiles are mainly formed by lipid oxidation and yeast metabolism during fermentation. In contrast, the crust is exposed to higher temperatures during the baking process. Hence, the crust volatiles are mainly formed by the Maillard reaction.[75]

Oxidation of the unsaturated fatty acids of the wheat flour occurs mainly during kneading of the dough in the presence of oxygen. Both, enzymatic reactions catalyzed by lipoxygenases and autoxidation reactions lead to hydroperoxides as first intermediates. These primary products are odorless and unstable. In the further process of bread making, the hydroperoxides are degraded enzymatically by hydroperoxide lyases or by non-enzymatic reactions to numerous volatile compounds, in particular aldehydes and ketones.[75, 76] The most abundant polyunsaturated fatty acid in wheat is linoleic acid, which accounts for over 50% of the total

fatty acid content.[73] It has been shown in model reactions that aldehydes, such as hexanal, (2*Z*)-oct-2-enal, (2*E*)-non-2-enal, and (2*E*,4*E*)-deca-2,4-dienal are formed during the autoxidation of linoleic acid.[77]

During fermentation, the yeast metabolizes mono- and disaccharides to ethanol and carbon dioxide. The carbon dioxide produced during alcoholic fermentation loosens the dough and raises it to the desired volume. In parallel, numerous volatiles are formed as side products of yeast metabolism including alcohols, aldehydes, acids, ketones, esters, and lactones. The degradation of amino acids to the corresponding aldehydes, alcohols, and acids via the Ehrlich pathway is one of the most important sources of volatiles. In Figure 9, the degradation of leucine via the Ehrlich pathway is illustrated. Transamination and decarboxylation of the amino acid leucine leads to the corresponding aldehyde 3-methylbutanal, which is subsequently reduced to the alcohol 3-methylbutan-1-ol.[75, 76] Spiking of ($^{13}C_6$)leucine to yeast doughs showed an efficient conversion of 84% to the corresponding alcohol, ($^{13}C_5$)-3-methylbutan-1-ol.[78]

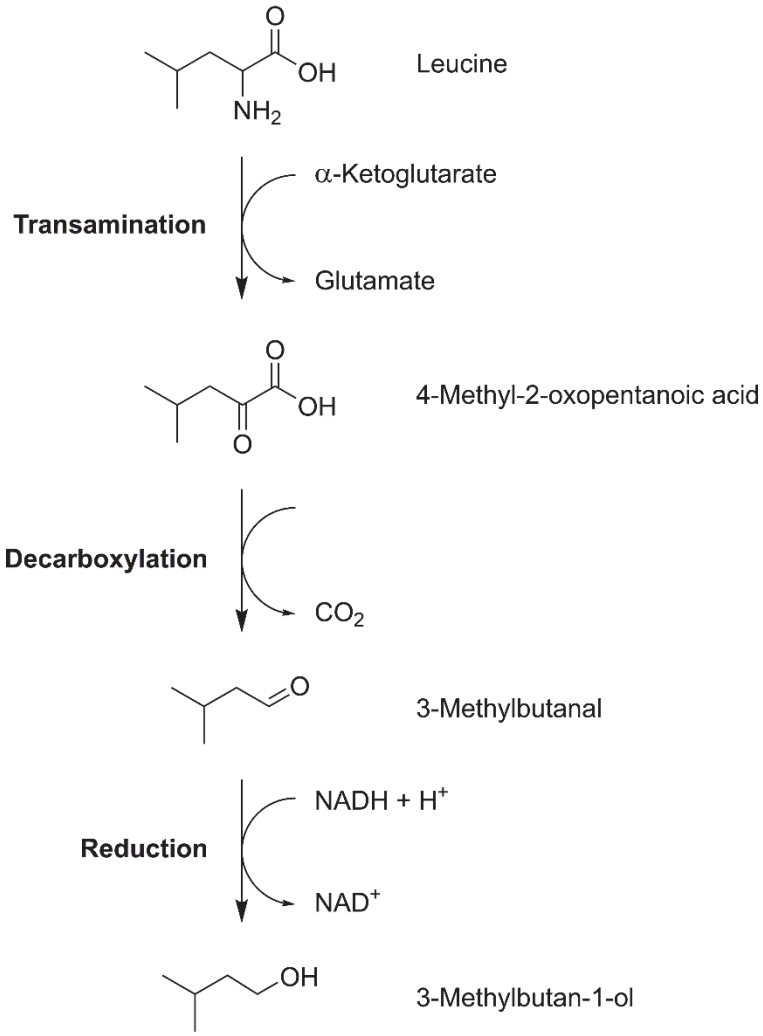

Figure 9: Formation of 3-methylbutanal and 3-methylbutan-1-ol from leucine via the Ehrlich pathway[76]

In the last step of bread making, the baking process, thermal reactions predominate. They mainly take place on the surface, the part that will become the bread crust. The Maillard reaction between reducing sugars and amino acids results in pyrroles, pyrrolines, furans, and pyranones and generates intermediates for further thermal reactions. The Strecker degradation describes the reaction of Maillard reaction derived α-dicarbonyl compounds with amino acids. Oxidative decarboxylation of the amino acids results in the Strecker aldehydes, which are important for the bread aroma. Furthermore, the second products of Strecker degradation are aminoketones and aminoaldehydes. These are precursors of pyrazines.[72, 74, 75, 79] Figure 10 shows the formation of 2-acteyl-1-pyrroline, an important odorant in the bread crust. The Strecker degradation of ornithine, an abundant amino acid in yeast, and subsequent cyclization of the resulting aldehyde leads to 1-pyrroline. This heterocyclic compound is an intermediate in the formation of 2-acetyl-1-pyrroline.[80, 81]

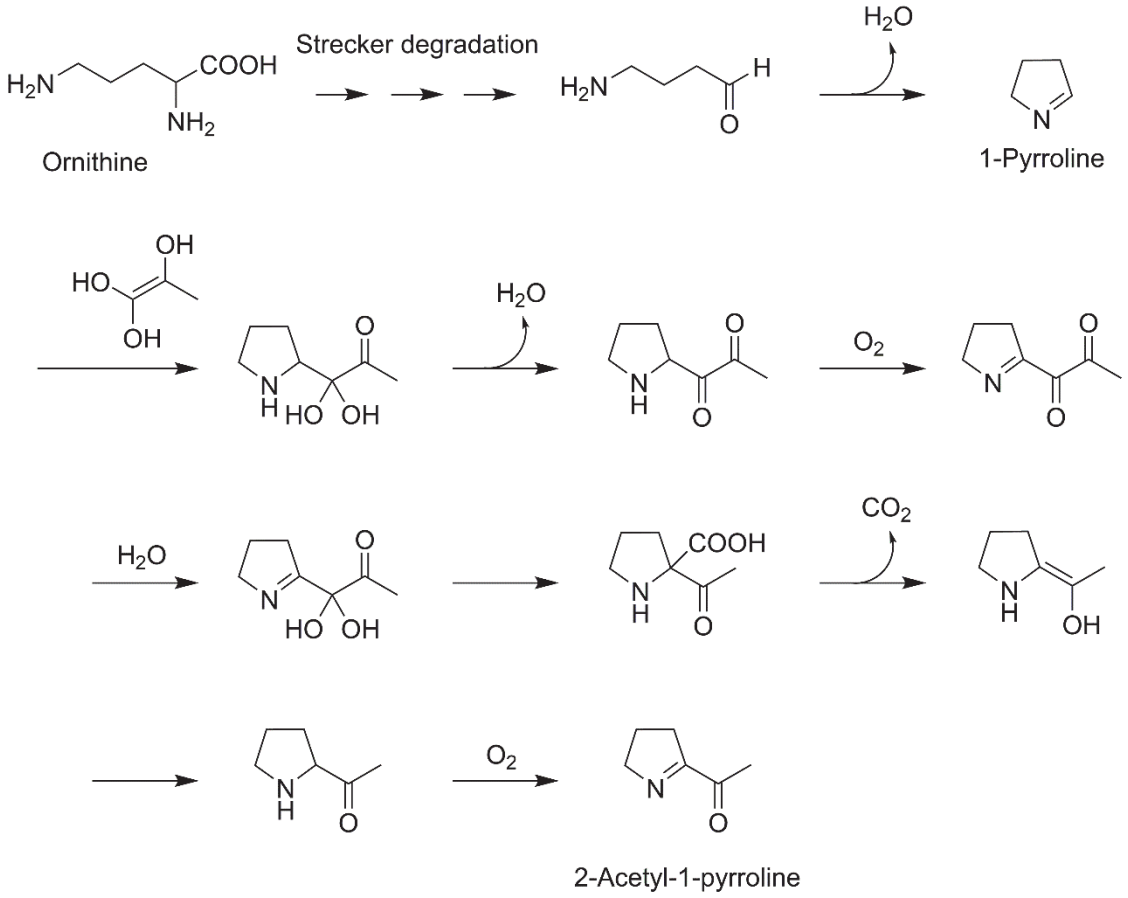

Figure 10: Formation of 2-acetyl-1-pyrroline from ornithine via 1-pyrroline[81]

Most of the volatiles formed by various reactions during bread making are odorless and have thus no impact on the overall wheat bread aroma. Therefore, the characterization of the odor-active compounds is crucial (cf. section 4.1.2).

Application of GC-O in combination with AEDA on the volatile isolates obtained from wheat bread crumb revealed high FD factors for 2-phenylethan-1-ol, (2*E*)-non-2-enal, (2*E*,4*E*)-deca-2,4-dienal, 3-methylbutan-1-ol, *trans*-4,5-epoxy-(2*E*)-dec-2-enal, 2- and 3-methylbutanoic acid, 3-(methylsulfanyl)propanal, ethyl octanoate, vanillin, phenylacetic acid, sotolon, and 4-ethenyl-2-methoxyphenol.[76, 82-86] Quantitation experiments showed that mainly odorants

derived from lipid oxidation and yeast metabolism, like 2- and 3-methylbutanoic acid, 3-methylbutan-1-ol, phenylacetic acid, 2-phenylethan-1-ol, 1-octen-3-one, acetic acid, hexanal, (2*E*)-non-2-enal, (2*E*,4*E*)-deca-2,4-dienal, and 4-ethenyl-2-methoxyphenol were present in concentrations above their individual OTVs.[29] Studies on the odorants in a steamed yeast dough made from wheat flour with a similar aroma profile to that of wheat bread crumb confirmed these findings but revealed further odorants with OAVs ≥ 1. The latter group included *trans*-4,5-epoxy-(2*E*)-dec-2-enal, γ-nonalactone, 2-acetyl-1-pyrroline, butane-2,3-dione, vanillin, (2*E*,6*Z*)-deca-2,6-dienal, 3-methylbutanal, butanoic acid, phenylacetaldehyde, and HDMF.[85]

GC-O and AEDA of volatile isolates obtained from wheat bread crust resulted in high FD factors for 2-acetyl-1-pyrroline, 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine, maltol, vanillin, sotolon, 3-(methylsulfanyl)propanal, HDMF, 4-ethenyl-2-methoxyphenol, (2*E*,4*E*)-deca-2,4-dienal, and phenylacetic acid.[20, 30, 75, 82, 87-89] Quantitation and OAV calculation revealed the roasty and popcorn-like smelling 2-acetyl-1-pyrroline as a key odorant in the wheat bread crust. With an exceptionally low OTV of 0.0073 µg/kg in starch,[12] it reached by far the highest OAV in the wheat bread crust.[30, 87, 90] In addition, high OAVs were calculated for 3-methylbutanal, 3-(methylsulfanyl)propanal, 2-ethyl-3,5-dimethylpyrazine, HDMF, and (2*E*)-non-2-enal.[30, 86, 90] A similar profile to that of the wheat bread crust is obtained when wheat bread slices are toasted. Studies on toasted wheat bread odorants showed high OAVs for 2-acetyl-1-pyrroline, HDMF, (2*E*)-non-2-enal, 3-(methylsulfanyl)propanal, butane-2,3-dione, and 3-methylbutanoic acid.[12]

Further studies focused on the influence of processing parameters and recipe on wheat bread crust and crumb aroma.

Birch et al.[91] investigated the influence of different commercial baker's yeasts of the species *Saccharomyces cerevisiae* on wheat bread crumb volatiles. Significantly different concentrations were obtained for 3-methylbutanal, phenylacetaldehyde, butane-2,3-dione, propan-1-ol, 2-methylpropan-1-ol, ethyl acetate, and 2-phenylethan-1-ol. The results suggested that the commercial products differed in the yeast strain.

Further studies focused on the influence of the amount of yeast in the recipe. Frasse et al.[92] compared doughs prepared without and with the addition of yeast. Application of GC-O and AEDA proved that typical bread crumb odorants, such as 3-methylbutanal, butane-2,3-dione, 3-methylbutan-1-ol, and 2-phenylethan-1-ol were present in significantly higher amounts in the dough prepared with yeast. In the dough sample without yeast addition, higher FD factors were found for lipid oxidation products, such as (2*E*,4*E*)-deca-2,4-dienal. Birch et al.[93] baked three breads with different yeast levels. The dough prepared with the highest amount of yeast resulted in a bread crumb with higher concentrations of compounds known to be formed by yeast metabolism, such as phenylacetaldehyde and butane-2,3-dione. Gassenmeier et al.[83] prepared doughs with liquid and soft pre-ferments and yeast concentrations in the final dough of 1.5% and 4.6%, respectively. The pre-ferments were prepared in advance and contained different amounts of water, flour, and yeast. In the bread crumbs with 1.5% and 4.6% yeast, 12 and 19 odorants were identified with FD factors ≥ 8, respectively. The alcohols 3-methylbutan-1-ol and 2-phenylethan-1-ol showed significantly higher concentrations in the bread crumb prepared with the liquid pre-ferment and the lower yeast concentration in the dough. The two alcohols were formed in higher concentrations in the liquid pre-ferment than in the soft

pre-ferment. Preparing the dough with a liquid pre-ferment was thus more efficient for the formation of important bread crumb odorants.

Dough fermentation is the processing step in bread making with the greatest influence on the formation of important crumb odorants. Due to economic reasons, however, the reduction of fermentation temperature and fermentation time is of great advantage for the baking industry.[75]

Increasing the temperature from 25 °C to 35 °C in a liquid pre-ferment enhanced the formation of 3-methylbutan-1-ol and 2-phenylethan-1-ol by factors of 3.3 and 1.7, respectively. A higher temperature of 40 °C led to lower concentrations.[83] Birch et al.[93] investigated the influence of fermentation temperature on bread volatiles. The dough was fermented at 5 °C, 15 °C, and 35 °C, respectively. Fermentation was stopped after different periods when a specific dough height was reached. Higher fermentation temperatures were associated with higher concentrations of some lipid oxidation products, such as hexanal and heptanal, in the bread crumb.

The influence of different fermentation times on the bread crumb odorants was investigated by Schieberle and Grosch.[84] The odorants in bread crumb prepared with a standard protocol were compared with the odorants in a bread crumb made from a longer fermented dough. In the bread crumb of the longer fermented dough, six odorants showed slightly higher FD factors and two odorants, namely 3-methylbutan-1-ol and 2-phenylethan-1-ol, showed significantly higher FD factors. Gassenmeier et al.[83] also showed that the formation of the two alcohols depends on the fermentation procedure. In the first 8 h of fermentation, the concentrations of 3-methylbutan-1-ol and 2-phenylethan-1-ol increased continuously. A further extension of the fermentation time up to 16 h did not lead to a significant further increase.

Zehentbauer et al.[94] focused on the influence of yeast amount and fermentation parameters on the bread crust aroma. A higher amount of yeast resulted in higher concentrations of the odorants 2-acetyl-1-pyrroline and 3-(methylsulfanyl)propanal in the crust. A significantly longer fermentation time at a lower temperature led to higher concentrations of Strecker aldehydes, such as 2- and 3-methylbutanal, 2-methylpropanal, and 3-(methylsulfanyl)propanal.

One of the main ingredients of any bread recipe is flour. Its contribution to the final bread aroma was the objective of a study by Moskowitz et al.[89] They compared odorants in a crust of a bread made with whole wheat flour with those in a crust of a bread made with refined wheat flour. In the latter, higher concentrations of 2-acetyl-1-pyrroline, HDMF, 2-phenylethan-1-ol, 2-acetyl-2-thiazoline, and 2,4-dihyroxy-2,5-dimethyl-3(2*H*)-furanone but lower concentrations of 2-ethyl-3,5-dimethylpyrazine, (2*E*,4*E*)-deca-2,4-dienal, and (2*E*)-non-2-enal were found. The concentrations of important roasty and caramel-like smelling bread crust odorants were thus lower in the bread baked with whole wheat flour. It was assumed that the release of ferulic acid from the whole meal flour during the baking process led to this lower amount of crust odorants. The authors suggested that ferulic acid reacts with 2-oxopropanal and hence inhibits the Maillard reaction related formation of 2-acetyl-1-pyrroline.[89, 95]

Rychlik et al.[12] investigated the odorants formed by toasting of wheat bread slices in relation to the intensity of browning, ranging from unroasted to burnt. The concentration of 2-acetyl-1-pyrroline increased continuously with the browning grade of the wheat bread slices. The concentration of HDMF was high especially in toasts with a medium brown color. The concentration of the pyrazine 2-ethyl-3,5-dimethylpyrazine increased continuously until the weak toasted stage and showed a strong increase from the medium toasted to the burnt stage.

3-(Methylsulfanyl)propanal showed a maximum concentration in toasted slices with a medium brown color and lower concentrations with a higher roasting degree of the bread slices.

The storage of bread also leads to changes in the aroma. The concentrations of important bread crust odorants, such as roasty, popcorn-like smelling 2-acetyl-1-pyrroline and malty smelling 3-methylbutanal decreased rapidly during storage of wheat bread. After 96 h of storage, only 90% and 66% of the initial odorant concentrations remained, respectively.[96] The losses of roasty and malty smelling bread crust odorants and the higher stability of fatty smelling lipid oxidation products, e.g., (2*E*)-non-2-enal, were considered to be responsible for the stale odor after bread storage.[30, 96, 97]

By application of GC-O and AEDA, Roth et al.[86] analyzed the crust and the crumb of breads made without and with the addition of distiller`s grain. A major key odorant in wheat bread crust, namely 2-acetyl-1-pyrroline, was not found during GC-O screening of the bread sample in which 20% of the wheat flour was substituted by distiller`s grain. Ferulic acid was assumed to be the reason for the low amount of 2-acetyl-1-pyrroline and the resulting stale odor of the crust, as it was the case when using whole meal flour.[89] In the crumb of the bread with distiller`s grain, higher FD factors of sotolon, HDMF, 2-ethyl-3,5-dimethylpyrazine, and phenylacetic acid were determined than in the reference crumb.[86] Sotolon was found with the highest FD factor of all odorants in the distiller`s grain,[98] therefore a transfer from distiller`s grain to the bread crumb was assumed.

5 Objectives

Previous studies on malt aroma were mainly focused on the identification of volatile compounds and the impact of roasting on the volatile composition. So far, only the key odorants in a caramel malt have been comprehensively studied. Data on odorants in malt extracts are scarcely available and only little is known about the odorant changes during processing of malt to LME. While malts and malt extracts have a long tradition in beer making, they have just recently gained importance as quality improving ingredients in the baking industry. Both are added to bakery products in order to enhance enzyme activity and to improve color and aroma. Lately, there has been an increasing demand for specialty malts and malt extracts in the baking industry with the aim of providing the consumers a wider range of products with improved aroma. However, the impact of malt or malt extract addition on the odorants in wheat bread crust and crumb had not been systematically investigated on the molecular level. In particular, it was unclear whether the malt products are a direct source of bread odorants or rather provide precursors from which bread odorants are formed during the bread making.

The fist aim of the current study was the identification of major odorants in a light and a dark LME for the baking industry, both produced from the same Pilsner malt. In order to get an insight into the sources of the LME odorants, selected odorants were quantitated in both, the raw material and the LMEs. This answered the question, whether the LME odorants mainly originated from a transfer of malt odorants or from the de novo formation during LME production. The second part of the study was dedicated to the impact of LME addition on the aroma of wheat bread crust and crumb. On the basis of quantitative data, the role of a direct odorant transfer from the LMEs to the bread and the role of odorant formation from precursors provided by the LMEs during bread making were finally evaluated.

6 Results and Discussion

This thesis is a publication-based dissertation. The data was summarized in two peer-reviewed papers published in international scientific journals. The appendix contains reprints of both publications, summaries including the individual authors` contributions, and the reprint permissions obtained from the publishers.

6.1 Odor-Active Compounds in Malt Extracts

For the odorant screening, a light and a dark LME to be used in the baking industry were selected. Both had been produced from the same batch of Pilsner malt. The light LME was obtained after milling and mashing of the Pilsner malt followed by vacuum concentration of the aqueous extract. To produce the dark LME, the light LME was additionally heated followed by dilution with water.

To get a first insight into the aroma differences between the light and the dark LME, the extracts were orthonasally compared in a quantitative olfactory profile analysis (Figure 11).[99] The profile of the dark LME showed higher intensities in smoky, earthy, roasty, soup seasoning-like, malty, caramel-like, and clove-like odor notes, while the light LME was characterized as more honey-like. The differences between the olfactory profiles could be attributed to the additional heating step in the dark LME production, which presumably lead to an increased formation of Maillard reaction products.[72, 100]

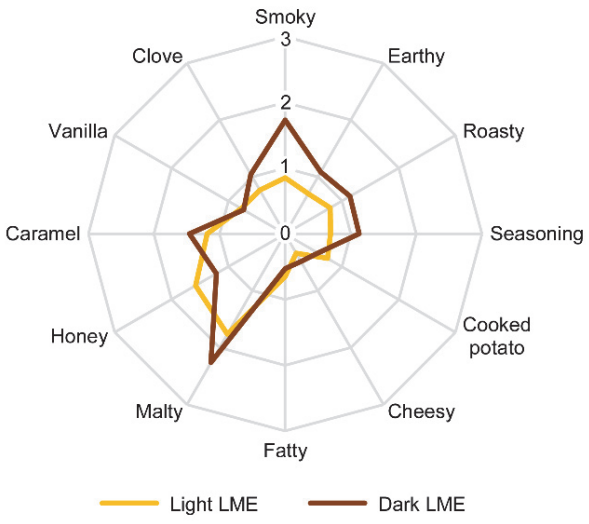

Figure 11: Quantitative olfactory profiles of the light and the dark LME.[99] Panelists rated the intensity of each descriptor on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong

To elucidate the odorants responsible for the olfactory differences, as a first step, volatile isolates obtained from the light and the dark LME were prepared by solvent extraction, SAFE, and concentration. The isolates were subsequently subjected to GC-O and a rough AEDA

using 1:10 dilutions. The screening revealed 28 odorants with an FD factor ≥ 1 in at least one sample (cf. 8.1.2, Paper 1, Table 1).[99] Comparing gas chromatographic and odor characteristics of the odorants with data in the Leibniz-LSB@TUM odorant database[101] and published data on malt odorants[68, 69] led to preliminary structure proposals. The structure assignments were confirmed by comparing the RIs on two columns of different polarity, the odor quality perceived at the sniffing port, and the mass spectra to data obtained by the analysis of authentic reference substances under the same conditions. Following this approach, 24 out of the 28 LME odorants could be unequivocally identified. All of them had already been reported as odorants in malts or malt extracts.[65, 66, 68, 69]

In both LMEs, the odorants with the highest FD factor of 1000 were honey-like smelling phenylacetic acid and vanilla-like smelling vanillin. Higher FD factors in the light LME than in the dark LME were determined for caramel-like smelling HDMF (FD factors 1000 vs. 10), metallic smelling *trans*-4,5-epoxy-(2*E*)-dec-2-enal (FD factors 100 vs. 1), clove-like smelling 4-ethenyl-2-methoxyphenol (FD factors 100 vs. < 1), and honey-like smelling phenylacetaldehyde (FD factors 100 vs. 10). The set of odorants with higher FD factors in the dark LME than in the light LME included the two caramel-like smelling compounds maltol (FD factors 1000 vs. 100) and dihydromaltol (FD factors 100 vs. 1), soup seasoning-like smelling sotolon (FD factors 1000 vs. 10), smoky smelling 2-methoxyphenol (FD factors 100 vs. 10), and the carboxylic acids acetic acid (FD 100 vs. 10) and 2- and 3-methylbutanoic acid (FD factors 100 vs. 10).[99]

To substantiate the results of the screening experiments, 15 important LME odorants were quantitated in the light and the dark LME by means of SIDA. Additionally, eight important bread odorants were included in the quantitation assays (cf. section 6.3). The concentration data were later needed to assess the impact of LME addition on the bread aroma (cf. section 6.4). Precise odorant concentrations were determined using stable isotopically substituted odorants as internal standards to compensate for losses during the sample workup. After solvent extraction, SAFE distillation, fractionation, and concentration, the volatile isolates were analyzed by GC-MS (CI), GC-GC-MS (CI), or GC×GC-TOFMS (EI).

The quantitation assays revealed concentrations between 0.0266 µ/kg and 869000 µg/kg (Table 6).[99, 102] In order to estimate the odor potencies of the LME odorants, OAVs were calculated by dividing the concentrations by the orthonasal OTVs in water. As a result, 15 and 14 of the 23 quantitated compounds in the light and the dark LME, respectively, exhibited OAVs ≥ 1. In the light LME, high OAVs were calculated for cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**; OAV 1500), cooked apple-like smelling (*E*)-β-damascenone (**3**; OAV 430), clove-like smelling 4-ethenyl-2-methoxyphenol (**6**; OAV 91), and honey-like smelling phenylacetaldehyde (**7**; OAV 70). Important odorants in the dark LME were soup seasoning-like smelling sotolon (**2**; OAV 780), cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**; OAV 550), cooked apple-like smelling (*E*)-β-damascenone (**3**; OAV 410), vinegar-like smelling acetic acid (**4**; OAV 160), and caramel-like smelling maltol (**5**; OAV 120).

Major differences between the quantitative olfactory profiles of the light and the dark LME could be substantiated by the OAV calculations. For example, in the light LME, the stronger honey-like odor note corresponded to the higher OAV of phenylacetaldehyde (**7**; OAVs 70 vs. 20). In the dark LME, higher OAVs were calculated for soup seasoning-like smelling sotolon (**2**; OAVs 780 vs. 7.4), caramel-like smelling maltol (**5**; OAVs 120 vs. 1.8), roasty, popcorn-like smelling 2-acetyl-1-pyrroline (**9**; OAVs 60 vs. 26), and smoky smelling 2-methoxyphenol (**12**; OAVs 26

vs. 2.6). The differences between the OAVs of the Maillard reaction products[72, 100] sotolon, maltol, and 2-acetyl-1-pyrroline, as well as in the OAVs of 2-methoxyphenol corresponded to the higher intensities of the soup seasoning-like, the caramel-like, the roasty, and the smoky odor note in the olfactory profile of the dark LME. However, the OAV data could not provide an explanation for the higher intensities of the malty and the earthy odor notes in the dark LME.

Table 6: Concentrations and OAVs of important odorants in the light and the dark LME[99, 102]

No.	Odorant	Concentration (µg/kg)[a]		OTV in water (µg/kg)[b]	OAV[c]	
		Light LME	Dark LME		Light LME	Dark LME
1	3-(Methylsulfanyl)propanal	631	235	0.43[10]	1500	550
2	Sotolon	12.6	1330	1.70[34]	7.4	780
3	(*E*)-β-Damascenone	2.59	2.45	0.0060[103]	430	410
4	Acetic acid	186000	869000	5600[13]	33	160
5	Maltol	9170	613000	5000[104]	1.8	120
6	4-Ethenyl-2-methoxyphenol	1910	14.0	21[105]	91	< 1
7	Phenylacetaldehyde	362	107	5.2[106]	70	20
8	Phenylacetic acid	3970	4220	68[107]	58	62
9	2-Acetyl-1-pyrroline[d]	1.40	3.19	0.053[10]	26	60
10	Vanillin	1190	1710	53[10]	22	32
11	(2*E*,4*E*)-Deca-2,4-dienal	0.862	0.519	0.027[10]	32	19
12	2-Methoxyphenol	2.18	21.9	0.84[10]	2.6	26
13	2'-Aminoacetophenone[d]	2.42	0.540	0.27[10]	9.0	2.0
14	(2*E*)-Non-2-enal[d]	1.38	1.10	0.19[10]	7.3	5.8
15	3-Methylbutanoic acid	335	1580	490[10]	< 1	3.2
16	HDMF	221	35.9	87[108]	2.5	< 1
17	2-Methylbutanoic acid	89.4	309	3100[107]	< 1	< 1
18	2-Phenylethan-1-ol	113	115	140[10]	< 1	< 1
19	2-Methylpropanoic acid[d]	151	599	60000[64]	< 1	< 1
20	Butanoic acid[d]	750	229	2400[10]	< 1	< 1
21	2-Acetyl-2-thiazoline[d]	0.0296	0.0266	0.079[109]	< 1	< 1
22	3-Methylbutan-1-ol[d]	29.4	3.18	220[10]	< 1	< 1
23	2-Methylbutan-1-ol[d]	11.3	0.615	1200[10]	< 1	< 1

[a]Mean of duplicates or triplicates; standard deviations were < 20%. [b]Orthonasal odor threshold values in water. [c]Odor activity value; calculated as ratio of concentration to OTV.
[d]Bread odorants additionally quantitated in the LME samples (cf. section 6.3).

6.2 Sources of Malt Extract Odorants

Next to the general characterization of important odorants in the light and in the dark LME, the source of these odorants was of great interest. Comparing the concentrations of important odorants in both LMEs (cf. Table 6) already led to the assumption that the processing steps from malt to extract had a major impact on the odorant concentrations, at least for the dark LME that was exposed to a higher thermal impact. However, it was still unclear, to which extent malt odorants were directly transferred from malt to LME and to which extent odorants were newly formed during malt extract production. To elucidate this, the 15 LME odorants were also quantitated in the Pilsner malt that served as starting material for the production of both LMEs.

It has been reported that products with low moisture content such as chocolate, cornflakes, crackers, oat flour, oat products, rye bread crust, and also malt, not only contain odor-active compounds in a free form, but also in a bound form from which odorants are released by water contact.[68, 110-115] During the mashing step included in the LME production, the malt also comes into contact with water and a release of odorants from their bound form was assumed. In order to confirm this water-related odorant release, preliminary quantitation experiments in malt were carried out with three selected odorants, namely vanillin, phenylacetaldehyde, and phenylacetic acid. To quantitate only the free odorants, the volatiles were extracted with pure diethyl ether. To cover the sum of free and bound odorants, diethyl ether and a minor amount of water were added simultaneously before extraction.[68, 69] An increase in concentration was observed for all three compounds when water was added together with the extraction solvent (Figure 12). Water addition resulted in a 33-fold increase in the concentration of the Strecker aldehyde phenylacetaldehyde and 14-fold and 9-fold increases in the concentrations of phenylacetic acid and vanillin, respectively.[99]

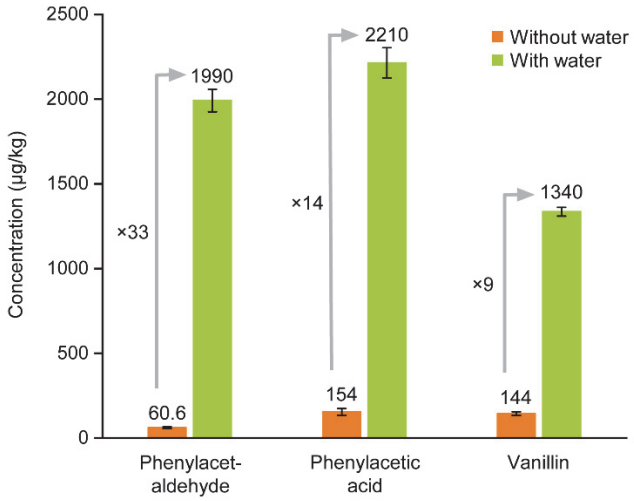

Figure 12: Concentrations of phenylacetaldehyde, phenylacetic acid, and vanillin in malt determined without and with water addition before solvent extraction[99]

While the general water-induced release of odorant concentrations in foods with low moisture content has been interpreted as cleavage of starch complexes in the literature,[111-113] 3-oxazolines were additionally suggested as hydrolabile precursors of Strecker aldehydes.[114] Hence, the excessive higher concentration of phenylacetaldehyde after water contact could be explained by an ensemble of both mechanisms. In order to clarify if the extraction protocol

including the addition of a minor amount of water led to an exhaustive extraction of bound odorants, further experiments were carried out. For this purpose, the water treatment and the solvent extraction step were temporally separated and the water contact time was varied. Results revealed a very quick release of the bound odorants. Already after one minute the plateau of the maximum odorant concentrations were reached and these concentrations were equivalent to the odorant concentrations found by simultaneous addition of a minor amount of water and diethyl ether (cf. 8.1.2, Paper 1, Figure 3).[99] Thus, the latter approach was considered to be suitable to quantify the sum of free and bound odorants in malt.

The approach was then applied for odorant quantitation experiments in the Pilsner malt. Results revealed concentrations between 0.135 µg/kg for (*E*)-β-damascenone and 404000 µg/kg for acetic acid (cf. 8.1.2, Paper 1, Table 3).[99] Finally, the percentages of change were calculated from the odorant concentrations in the malt, the odorant concentrations in the LMEs, and the process yields associated with the production of light and dark LME from the Pilsner malt. Percentages between 1.8% and 90000% were obtained (cf. 8.1.2, Paper 1, Table 3).[99] Most of the odorants featured recoveries below 100%, indicating losses during LME production. Potential causes include thermal reactions and evaporation during vacuum concentration.

Seven compounds exhibited percentages above 100% in at least one of the LMEs, indicating a formation during LME production (cf. 8.1.2, Paper 1, Table 3).[99] An increase was observed for vinegar-like smelling acetic acid, smoky smelling 2-methoxyphenol, clove-like smelling 4-ethenyl-2-methoxy-phenol, vanilla-like smelling vanillin, cooked apple-like smelling (*E*)-β-damascenone, soup seasoning-like smelling sotolon, and caramel-like smelling maltol. While acetic acid showed a loss during the production of the light LME (43%), its high percentage after the production of the dark LME (260%) indicated a formation, possibly via sugar degradation and Strecker degradation of alanine.[116-119] The phenolic compound vanillin showed similar percentages in the light LME (150%) and in the dark LME (270%). 4-Ethenyl-2-methoxyphenol showed an increase from malt to the light LME (+300%), but a decrease from malt to the dark LME (–96%). 2-Methoxyphenol showed a moderate (140%) and a pronounced (1800%) higher amount in the light LME and in the dark LME, respectively. The higher amounts of these phenolic compounds could be explained by the thermal decomposition of ferulic acid.[120-123] The different percentages of 4-ethenyl-2-methoxyphenol and 2-methoxyphenol could be associated with the additional heating step during the production of the dark LME. 4-Ethenyl-2-methoxyphenol is formed first. Further thermal impact then induces the conversion of 4-ethenyl-2-methoxyphenol to 2-methoxyphenol,[120, 121] explaining the lower amount of 4-ethenyl-2-methoxyphenol and the higher amount of 2-methoxyphenol in the dark LME. (*E*)-β-Damascenone showed a vast higher amount in the light and the dark LME (1800% and 2200%) than in the malt. Probably, this odorant was formed thermally or released from glycoside precursors during mashing.[124-131] While the Maillard reaction products maltol and sotolon already showed clearly higher amounts in the light LME (1100% and 290%) than in the malt, the amounts in the dark LME were even higher, with percentages of 90000% and 39000%, respectively. The additional heating step in the production protocol of the dark LME obviously boosted the formation of Maillard reaction products. Sotolon could be formed from butane-2,3-dione and hydroxyacetaldehyde,[132] and maltol from the disaccharide maltose.[68, 133] In summary, for the aroma of the light and the dark LME, the formation of process-induced odorants was shown to be more important than a transfer of malt odorants.

6.3 Impact of Malt Extract Addition on Bread Aroma

To get a first insight into the impact of malt extract addition on the aroma of wheat bread, crusts and crumbs of breads made with the previously analyzed light and dark LMEs (cf. section 6.1) were orthonasally characterized in a quantitative olfactory profile analysis. A bread without LME addition served as reference (Figure 13). All breads were made using a standardized recipe and a standardized production protocol.[102] The olfactory profile of the reference bread crust showed strong roasty, malty, and caramel-like odor notes (Figure 13, left). The olfactory profile of the crust of the bread made with the light LME was evaluated very similar to the reference crust, only the smoky odor note was rated slightly more intensive. The differences between the crust of the bread made with the dark LME and the reference bread crust were slightly larger, but still small. The malty, honey-like, caramel-like, smoky, and clove-like notes were rated slightly more intensive in the crust of the bread made with the dark LME. The reference bread crumb exhibited a strong malty note followed by roasty, fatty, cheesy, and cooked potato-like notes (Figure 13, right). Again, the differences in the aroma between the two LME breads and the reference bread were small. Adding the dark LME to the bread recipe resulted in slightly more changes in the olfactory profile of the bread crumb than adding the light LME. In the crumb of the bread made with the light LME, only smoky and honey-like notes were rated higher, whereas in the crumb of the bread made with the dark LME, smoky, earthy, roasty, soup seasoning-like, malty, and caramel-like notes were rated more intensive.

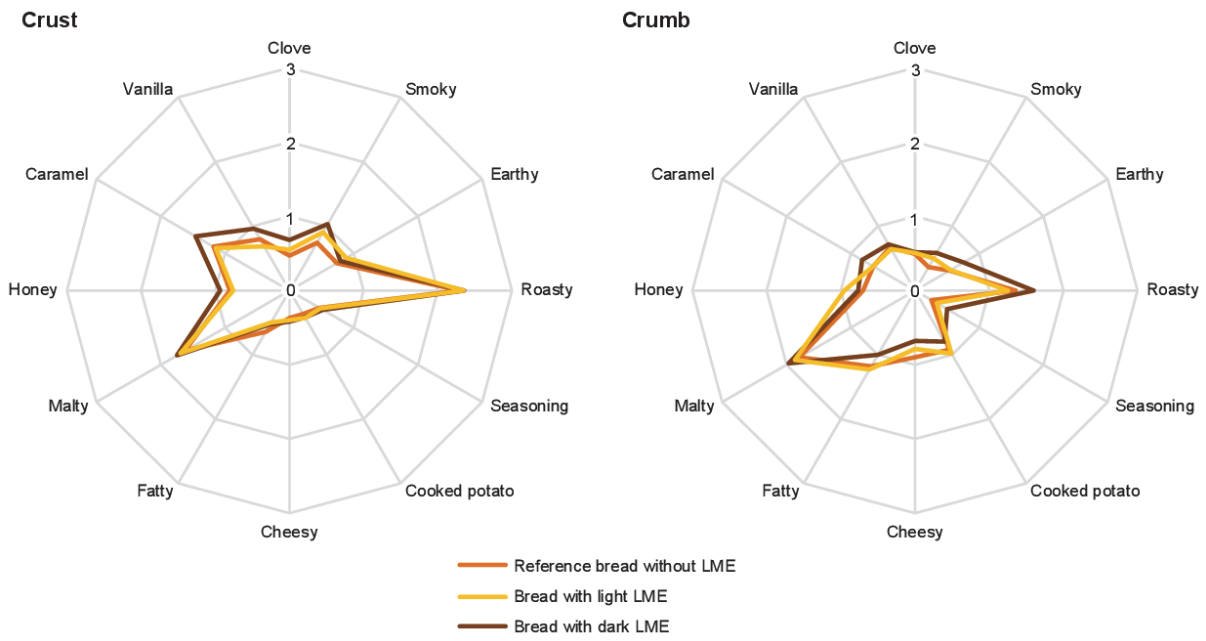

Figure 13: Quantitative olfactory profiles of crust and crumb of breads made without LME, with the light LME, or with the dark LME. Panelists rated the intensity of each descriptor on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong

The rather minor differences in the olfactory profiles between the bread made with the light LME and the reference bread were confirmed in 3-AFC tests. The crust and the crumb of the bread made with the light LME showed no significant difference to the reference bread crust and crumb (p > 10%). On the other hand, the crust and the crumb of the bread made with the dark LME were significantly distinguishable from the reference bread crust and crumb,

indicating a clear impact of dark LME addition on the bread aroma (crust: *p* 0.19%, crumb: *p* 0.0061%).

To elucidate the odorants responsible for the aroma impact of LME addition, the volatiles were isolated from bread crusts and crumbs by solvent extraction and SAFE. Since a water-induced release of bound odorants by saliva during the consumption of bread had to be considered, the extraction step included the simultaneous addition of water and solvent, as performed in the analysis of the Pilsner malt (cf. section 6.2). The influence of water addition on bread crust and crumb odorants had been investigated in preliminary experiments (cf. 8.2.2, Paper 2, Figure 2).[102] In the bread crust, the concentrations of phenylacetaldehyde and phenylacetic acid increased ~8-fold and ~5-fold, respectively, when water was added before solvent extraction. This was in accordance with the findings obtained from foods with low moisture contents[68, 110-115] including malt (cf. Figure 12). No significant differences were observed between the concentrations of bread crumb odorants.[102] This observation corresponded to the higher moisture content in the bread crumb.

A screening for odorants was applied to the volatile isolates obtained from the crust and the crumb of the bread made with the dark LME in comparison to the volatile isolates obtained from the reference bread crust and crumb. No screening was applied to the bread made with the light LME. Given that the light LME showed the same set of odorants,[99] its impact on bread aroma was low (cf. Figure 13), and the result was primarily used to select the odorants to be quantitated in the following step, odorant screening in the light LME bread was considered unnecessary. Application of GC-O, AEDA, and identification experiments finally resulted in 48 odorants with FD factors ≥ 10 in at least one of the two breads without and the with dark LME (cf. 8.2.2, Paper 2, Table 1).[102] The identified odorants were consistent with previously characterized wheat bread crust and crumb odorants.[12, 20, 30, 75, 76, 82-84, 87]

In both bread crust samples, without and with the dark LME, high FD factors were obtained for roasty, popcorn-like smelling 2-acetyl-1-pyrroline, cooked potato-like smelling 3-(methylsulfanyl)propanal, cheesy smelling 2- and 3-methylbutanoic acid, caramel-like smelling compounds maltol and HDMF, soup seasoning-like smelling sotolon, honey-like smelling phenylacetic acid, and vanilla-like smelling vanillin. In both bread crumb samples, high FD factors were obtained for cooked potato-like smelling 3-(methylsulfanyl)propanal, cheesy smelling 2- and 3-methylbutanoic acid, honey-like smelling 2-phenylethan-1-ol, and vanilla-like smelling vanillin. No significant differences were found between the two crust samples based on the FD factors. However, significant differences in the FD factors were found between the bread crumb samples. Important odorants in the dark LME (cf. section 6.1), such as caramel-like smelling maltol (FD factors 1000 vs. 10) and soup seasoning-like smelling sotolon (FD factors 1000 vs. 10) showed clearly higher FD factors in the crumb of the bread made with the dark LME.[102]

To get a deeper insight in the role of malt extract addition on the bread aroma, odorant quantitations were carried out in the crust and the crumb of breads made without and with the addition of the light and the dark LME, respectivley.[102] Based on the outcomes of the odorant screening in the reference bread and the bread made with the dark LME, 23 important odorants were selected for the quantitation experiments. The concentrations in the bread crusts ranged from 0.224 µg/kg to 269000 µg/kg (Table 7). For 18 odorants in the reference bread crust, 18 odorants in the crust of the bread made with the light LME, and 20 odorants in the crust of the bread made with the dark LME, respectively, concentrations above the OTVs in starch were found, i.e., OAVs were ≥ 1. The OAVs verified the importance of roasty, popcorn-like smelling

2-acetyl-1-pyrroline (**9**) as a major wheat bread crust odorant.[12, 30, 75] Ranging from 1800 to 3600, the compound exhibited by far the highest OAVs of all bread crust odorants. High OAVs in the bread crust samples were additionally determined for cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**; OAVs 160–1200), caramel-like smelling HDMF (**16**; OAVs 160–360), clove-like smelling 4-ethenyl-2-methoxyphenol (**6**; OAVs 130–190), and honey-like smelling phenylacetaldehyde (**7**; OAVs 37–180). For most of the bread crust odorants, only minor differences in the OAVs were detected between the reference bread and the breads made with the light LME and the dark LME. Odorants with clearly higher OAVs in the crust of the bread made with the light LME than in the crust of the bread made with the dark LME and the reference bread crust were found for cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**; OAVs 1200 vs. 230 vs. 160), caramel-like smelling HDMF (**16**; OAVs 360 vs. 180 vs. 160), and honey-like smelling phenylacetaldehyde (**7**; OAVs 180 vs. 59 vs. 37). However, these differences were not reflected in the sensory data (cf. Figure 13). 2-Acetyl-1-pyrroline with its extraordinarily high OAV may have masked the aroma notes evoked by the latter three odorants. In the crust of the bread made with the dark LME, a higher OAV was calculated for soup seasoning-like smelling sotolon (**2**; OAV 15) than in the other bread crusts (OAVs 3.4–6.4). In summary, only slight differences were detected between the crust samples of the breads made without and with LME addition. This corresponded very well with the AEDA data (cf. 8.2.2, Paper 2, Table 1)[102] and may be assigned to the fact that bread crust odorants are mainly generated by thermal reactions during bread baking in the course of the Maillard reaction.[72, 75, 79, 100]

Table 7: Concentrations, orthonasal OTVs in starch, and OAVs of odorants in the crust of breads made without (reference) and with LMEs[102]

No.[a]	Odorant	Concentration in bread crust (µg/kg)[b]			OTV in starch (µg/kg)[c]	OAV in bread crust[d]		
		Reference	Light LME	Dark LME		Reference	Light LME	Dark LME
9	2-Acetyl-1-pyrroline	20.9	26.0	12.8	0.0073[12]	2900	3600	1800
1	3-(Methylsulfanyl)propanal	42.2	331	61.4	0.27[12]	160	1200	230
16	HDMF	2110	4680	2300	13[12]	160	360	180
6	4-Ethenyl-2-methoxyphenol	1200	825	884	6.4[102]	190	130	140
7	Phenylacetaldehyde	216	1020	341	5.8[134]	37	180	59
15	3-Methylbutanoic acid	1030	620	831	13[29]	79	48	64
5	Maltol	47500	66600	57700	1400[134]	34	48	41
22	3-Methylbutan-1-ol	1820	1650	2180	98[102]	19	17	22
8	Phenylacetic acid	287	491	398	23[29]	12	21	17
11	(2*E*,4*E*)-Deca-2,4-dienal	45.9	43.7	47.6	2.7[12]	17	16	18
19	2-Methylpropanoic acid	2640	1860	3030	190[134]	14	9.8	16
2	Sotolon	7.08	13.4	32.4	2.1[126]	3.4	6.4	15
20	Butanoic acid	1220	1280	1280	100[12]	12	13	13
4	Acetic acid	150000	269000	138000	30000[134]	5.0	9.0	4.6
14	(2*E*)-Non-2-enal	98.0	93.4	89.2	12[29]	8.2	7.8	7.4
17	2-Methylbutanoic acid	542	263	362	77[29]	7.0	3.4	4.7
18	2-Phenylethan-1-ol	1490	1380	1380	470[134]	3.2	2.9	2.9
3	(*E*)-β-Damascenone	0.224	0.444	0.271	0.15[126]	1.5	3.0	1.8
12	2-Methoxyphenol	2.25	2.59	5.29	4.2[12]	< 1	< 1	1.3
23	2-Methylbutan-1-ol	651	459	761	760[29]	< 1	< 1	1.0
21	2-Acetyl-2-thiazoline	1.82	1.51	1.60	2.4[134]	< 1	< 1	< 1
13	2'-Aminoacetophenone	0.524	0.726	0.612	2.9[29]	< 1	< 1	< 1
10	Vanillin	263	237	221	440[29]	< 1	< 1	< 1

[a]Numbers refer to Table 6. [b]Mean of duplicates or triplicates; standard deviations were < 20%. [c]Orthonasal odor threshold values in starch. [d]Odor activity value; calculated as ratio of concentration to OTV.

The concentrations in the bread crumbs ranged from 0.169 µg/kg to 240000 µg/kg (Table 8). OAV calculation revealed 15, 16 and 19 odorants with an OAV ≥ 1 in the crumb of the breads made without LME, with the light LME, and with the dark LME, respectively. High OAVs in the bread crumbs were assigned to cheesy smelling 3-methylbutanoic acid (**15**; OAVs 67–110), cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**; OAVs 50–110), roasty, popcorn-like smelling 2-acetyl-1-pyrroline (**9**; OAVs 62–90), and malty smelling 3-methylbutan-1-ol (**22**; OAV 35–42). Most of the odorants showed OAVs that were in the same range in all crumb samples, suggesting little influence of malt extract addition. Formation of these compounds could be predominantly assigned to yeast metabolism during fermentation (**4**, **6**, **15**, **17**, **18**, **19**, **20**, **22**, **23**) and to lipid oxidation (**3**, **11**, **14**).[75, 76, 87] Nevertheless, for three odorants slight differences were found between the reference crumb and the crumb of the bread made with the light LME. These odorants included the two honey-like smelling compounds phenylacetaldehyde (**7**) and phenylacetic acid (**8**), and cooked potato-like smelling 3-(methylsulfanyl)propanal (**1**), which increased 2.8-fold, 2.4-fold, and 2.2-fold, respectively, when the light LME was added to the dough. More pronounced differences were present between the crumb of the bread made with the dark LME and the reference bread crumb. Soup seasoning-like smelling sotolon (**2**) and the two caramel-like smelling compounds maltol (**5**) and HDMF (**16**) exhibited 57-fold, 114-fold, and 6-fold higher OAVs, respectively, in the crumb of the bread made with the dark LME. While these odorants did not exceed their OTVs in the reference crumb, they reached OAVs clearly above 1 in the crumb of the bread made with the dark LME, namely 11, 7.1, and 4.8, respectively, implying a contribution to the overall aroma in the latter.

Table 8: Concentrations, orthonasal OTVs in starch, and OAVs of odorants in the crumb of breads made without (reference) and with LMEs[102]

No.[a]	Odorant	Concentration in bread crumb (µg/kg)[b]			OTV in starch (µg/kg)[c]	OAV in bread crumb[d]		
		Reference	Light LME	Dark LME		Reference	Light LME	Dark LME
15	3-Methylbutanoic acid	1410	865	1210	13[29]	110	67	93
1	3-(Methylsulfanyl)propanal	13.8	30.6	13.6	0.27[12]	51	110	50
9	2-Acetyl-1-pyrroline	0.544	0.657	0.453	0.0073[12]	74	90	62
22	3-Methylbutan-1-ol	3390	3580	4150	98[102]	35	37	42
19	2-Methylpropanoic acid	3960	3360	4610	190[134]	21	18	24
8	Phenylacetic acid	210	507	301	23[29]	9.1	22	13
6	4-Ethenyl-2-methoxyphenol	136	132	127	6.4[102]	21	21	20
20	Butanoic acid	1830	1600	1880	100[12]	18	16	19
7	Phenylacetaldehyde	34.6	96.3	72.4	5.8[134]	6.0	17	12
11	(2*E*,4*E*)-Deca-2,4-dienal	29.9	43.8	43.0	2.7[12]	11	16	16
17	2-Methylbutanoic acid	836	436	577	77[29]	11	5.7	7.5
2	Sotolon	0.417	1.13	23.9	2.1[126]	< 1	< 1	11
4	Acetic acid	118000	240000	160000	30000[134]	3.9	8.0	5.3
5	Maltol	87.1	521	9890	1400[134]	<1	< 1	7.1
18	2-Phenylethan-1-ol	2120	2430	1880	470[134]	4.5	5.2	4.0
16	HDMF	10.3	25.7	62.1	13[12]	< 1	2.0	4.8
14	(2*E*)-Non-2-enal	50.8	34.1	38.9	12[29]	4.2	2.8	3.2
23	2-Methylbutan-1-ol	1130	1260	1300	760[29]	1.5	1.7	1.7
3	(*E*)-β-Damascenone	0.0684	0.142	0.177	0.15[126]	< 1	< 1	1.2
10	Vanillin	156	166	182	440[29]	< 1	< 1	< 1
12	2-Methoxyphenol	0.965	1.45	1.40	4.2[12]	< 1	< 1	< 1
21	2-Acetyl-2-thiazoline	0.229	0.203	0.169	2.4[134]	< 1	< 1	< 1
13	2'-Aminoacetophenone	0.572	0.683	0.583	2.9[29]	< 1	< 1	< 1

[a]Numbers refer to Table 6. [b]Mean of duplicates or triplicates; standard deviations were < 20%. [c]Orthonasal odor threshold values in starch. [d]Odor activity value; calculated as ratio of concentration to OTV.

6.4 The Role of Odorant Precursors in Bread Crust and Crumb

Malt extract addition can influence the final bread crust and crumb aroma either by a simple transfer of important LME odorants into the bread or by a formation of odorants from LME-derived precursors during the bread making. To assess the origin of the bread odorants, the odorant concentrations in the breads were compared to the amounts of the odorants added with the LME. In order to compare the data from the LMEs (Table 6) and the bread crusts (Table 7) and crumbs (Table 8), the concentrations of all important bread odorants (OAV ≥ 1) were converted to µg per kg dough (Table 9 and Table 10).[102]

In a portion of the crust of the bread made with the dark LME originating from 1 kg dough, sotolon (**2**) was present at a level of 4.92 µg/kg when no dark LME was added and of 22.5 µg/kg when dark LME was added, corresponding to an increase of 360% (Table 9). Since 23.9 µg/kg sotolon were introduced to the dough by adding dark LME, 100 % of the higher concentration in the crust could be explained by a direct transfer from the dark LME to the bread crust, with a transfer efficiency of 74%. The higher amounts of maltol (**5**), (*E*)-β-damascenone (**3**), and phenylacetic acid (**8**) were also explainable by a direct transfer (98–100%), but with lower relative increases of 21–39%. For other compounds, the higher concentrations in the crust of the bread made with the dark LME could not be fully explained by a direct transfer. Hence, a formation of these compounds during the bread making from precursors provided by the LME played a major role. For 3-(methylsulfanyl)propanal (**1**), 2-methoxyphenol (1**2**), and phenylacetaldehyde (**7**), the concentrations in the dark LME could explain only 32%, 19%, and 2.2% of the higher amounts in the bread made with the dark LME, respectively. In the case of the comparison of the reference bread crust and the crust of the bread made with the light LME, no higher odorant concentration could be fully explained by a direct transfer from light LME to the bread crust. This indicated that the light LME provided higher amounts of odorant precursors than the dark LME. Regarding the high relative concentration increases of 3-(methylsulfanyl)propanal (**1**; +690%), phenylacetaldehyde (**7**; +370%), HDMF (**16**; +120%), (*E*)-β-damascenone (**3**; +98%), and sotolon (**2**; +89%), only small percentages between <1% (**16**; HDMF) and 30% (**3**; (*E*)-β-damascenone) could be explained by a direct transfer. The two Strecker aldehydes phenylacetaldehyde (**7**) and 3-(methylsulfanyl)propanal (**1**), which showed the highest relative increases of 370% and 690%, respectively, after adding the light LME to the bread recipe, might have been formed by Strecker degradation from light LME-derived precursors during baking. At high temperatures, such as on the surface of the bread, the Strecker aldehydes are formed by oxidative decarboxylation of amino acids.[75, 87, 116] In both crusts of breads made with LME addition, rather small relative differences were found between the other compounds, indicating that the crust aroma was mainly affected by the key odorant 2-acetyl-1-pyrroline, typically formed in the course of the Maillard reaction.[72, 79, 100]

Table 9: Amounts of odorants in the bread crusts in relation to the amounts added with the light and the dark LME[102]

No.[a]	Odorant	Concentration in crust (µg/kg dough)[b]			Diff. light LME bread vs. reference bread	Diff. dark LME bread vs. reference bread	Amount added with light LME		Amount added with dark LME	
		Reference bread	Light LME bread	Dark LME bread	µg/kg dough	µg/kg dough	µg/kg dough[c]	Explains % diff. between breads	µg/kg dough[c]	Explains % diff. between breads
1	3-(Methylsulfanyl)propanal	29.3	230	42.6	+201 (+690%)	+13.3 (+45%)	11.3	5.6	4.22	32
7	Phenylacetaldehyde	150	709	237	+559 (+370%)	+86.8 (+58%)	6.50	1.2	1.92	2.2
2	Sotolon	4.92	9.30	22.5	+4.38 (+89%)	+17.6 (+360%)	0.227	5.2	23.9	100
12	2-Methoxyphenol	1.56	1.80	3.68	+0.236 (+15%)	+2.11 (+140%)	0.0391	17	0.393	19
16	HDMF	1470	3250	1600	+1790 (+120%)	+132 (+9.0%)	3.97	< 1	0.645	< 1
3	(*E*)-β-Damascenone	0.156	0.309	0.189	+0.153 (+98%)	+0.0328 (+21%)	0.0465	30	0.0440	100
4	Acetic acid	104000	187000	95900	+82700 (+79%)	−8340 (−8.0%)	3340	4.0	15600	–
8	Phenylacetic acid	200	341	277	+141 (+71%)	+76.9 (+39%)	71.3	51	75.8	98
5	Maltol	33000	46300	40100	+13300 (+40%)	+7090 (+21%)	165	1.2	11000	100
9	2-Acetyl-1-pyrroline	14.5	18.1	8.91	+3.56 (+24%)	−5.63 (−39%)	0.0251	< 1	0.0573	–
22	3-Methylbutan-1-ol	1260	1150	1510	−118 (−9.3%)	+250 (+20%)	0.529	–	0.0571	< 1
23	2-Methylbutan-1-ol	452	319	529	−134 (−30%)	+76.7 (+17%)	0.203	–	0.0111	< 1
19	2-Methylpropanoic acid	1830	1290	2110	−542 (−30%)	+271 (+15%)	2.72	–	10.8	4.0
20	Butanoic acid	848	890	889	+42.0 (+4.9%)	+41.7 (+4.9%)	13.5	32	4.11	9.9
11	(2*E*,4*E*)-Deca-2,4-dienal	31.9	30.4	33.1	−1.53 (−4.8%)	+1.14 (+3.6%)	0.0155	–	0.00932	< 1
14	(2*E*)-Non-2-enal	68.1	64.9	62.0	−3.17 (−4.7%)	−6.09 (−8.9%)	0.0249	–	0.0198	–
18	2-Phenylethan-1-ol	1040	956	959	−79.2 (−7.6%)	−76.6 (−7.4%)	2.04	–	2.07	–
15	3-Methylbutanoic acid	716	431	578	−285 (−40%)	−138 (−19%)	6.01	–	28.4	–
6	4-Ethenyl-2-methoxyphenol	834	573	614	−261 (−31%)	−220 (−26%)	34.3	–	0.252	–
17	2-Methylbutanoic acid	377	183	252	−194 (−52%)	−125 (−33%)	1.61	–	5.55	–

[a]Numbers refer to Table 6. [b]µg of the odorant in a portion of the bread crust originating from 1 kg of dough; calculated by multiplying the odorant concentrations in crust (Table 7) by conversion factors of 0.695; the conversion factor for the crust was approximated as (dry weight crust × amount crust + dry weight crumb × amount crumb) / (amount dough × dry weight crust).[102] [c]Odorant concentration in the dough derived from the addition of the LME; calculated by multiplying the odorant concentration in the LME (Table 6) with the LME content in the dough (15 g/835 g).

In the bread crumb (Table 10), the concentrations of thermally formed odorants, e.g., maltol, sotolon, and HDMF, were lower than in the crust, corresponding to the lower temperatures reached in the crumb during baking. Due to these lower initial concentrations, LME addition to the bread recipe showed a greater impact on the final amount of Maillard reaction-related compounds. The addition of the dark LME resulted in 114-fold and 57-fold higher concentrations of maltol (**5**; from 86.8 to 9860 µg/kg dough) and of sotolon (**2**; from 0.416 to 23.8 µg/kg dough) in the bread crumb, respectively. The differences between the reference bread and the bread made with the dark LME were fully explainable by a direct transfer of these odorants from the dark LME with efficacies of 89% and 98%, respectively. Significantly lower relative increases of ~40% were calculated for 2-methoxyphenol (**12**) and phenylacetic acid (**8**). A direct transfer of the latter compounds from the dark LME to the bread crumb could explain 91% and 84% of the higher concentrations in the crumb of the bread made with the dark LME. However, the concentration of smoky smelling 2-methoxyphenol remained below its OTV in both crumbs, without and with dark LME addition, indicating that this odorant does not contribute to the overall bread crumb aroma. Regarding HDMF (**16**), only 1% of its concentration increase of 500% could be explained by a direct transfer from the dark LME to the bread crumb. Thus, the major part of HDMF must have been formed from LME-derived precursors during bread making. Presumably, these precursors were reducing sugars or reactive C_3-intermediates of the Maillard reaction formed during dark LME production.[135, 136] As in the case of the crust of the bread made with the light LME (cf. Table 9), none of the concentration increases of bread crumb odorants could be fully explained by a direct transfer from the light LME to the bread crumb. Up to 68% of the more than doubled concentration of (*E*)-β-damascenone (**3**; +110%) and 3-(methylsulfanyl)propanal (**1**; +120%) in the crumb of the bread made with the light LME could be ascribed to a direct transfer from the light LME to the bread crumb. Adding light LME to the bread recipe had the greatest impact on the concentration of maltol (**5**), resulting in a relative increase of 500%. However, only 38% could be explained by a direct transfer from the light LME to the bread crumb, thus a major part of the maltol was formed from light LME-derived precursors during bread making.

In summary, differences between the reference bread and both LME breads were demonstrated on the molecular level. The addition of the dark LME had a greater impact on the final odorant concentrations than the addition of the light LME. Adding light LME to the dough caused only small increases in the odorant concentrations. The differences could mainly be explained by the formation of odorants from precursors during bread making, but the final amounts were not sufficient to have a significant influence on the bread aroma. However, adding dark LME to the dough resulted in a significant impact on the bread aroma, with the impact on the crumb aroma being higher than on the crust aroma. The higher concentrations were primarily explainable by a direct transfer of odorants from the dark LME to the bread, but also partly by the formation of odorants from LME-derived precursors during bread making. Due to the differences in LME production, malt-derived precursors were possibly already reacted in case of the dark LME, resulting in higher odorant concentrations that could be directly transferred to the bread. On a molecular level, sotolon, maltol, and HDMF were responsible for the aroma differences between the reference bread and the bread made with the dark LME. To further improve the aroma of bread crust and crumb, the production of the dark LME should be optimized for a targeted formation of these three compounds.

Table 10: Amounts of odorants in the bread crumbs in relation to the amounts added with the light and the dark LME[102]

No.[a]	Odorant	Concentration in crumb (µg/kg dough)[b]			Diff. light LME bread vs. reference bread	Diff. dark LME bread vs. reference bread	Amount added with light LME		Amount added with dark LME	
		Reference bread	Light LME bread	Dark LME bread	µg/kg dough	µg/kg dough	µg/kg dough[c]	Explains % diff. between breads	µg/kg dough[c]	Explains % diff. between breads
5	Maltol	86.8	520	9860	+433 (+500%)	+9770 (+11000%)	165	38	11000	100
2	Sotolon	0.416	1.12	23.8	+0.708 (+170%)	+23.4 (+5600%)	0.227	32	23.9	100
16	HDMF	10.3	25.7	61.9	+15.4 (+150%)	+51.6 (+500%)	3.97	26	0.645	1.2
7	Phenylacetaldehyde	34.5	96.0	72.2	+61.5 (+180%)	+37.7 (+110%)	6.50	11	1.92	5.1
3	(*E*)-β-Damascenone	0.0680	0.142	0.177	+0.0736 (+110%)	+0.109 (+160%)	0.465	63	0.0440	41
8	Phenylacetic acid	210	505	300	+296 (+140%)	+90.1 (+43%)	71.3	24	75.8	84
1	3-(Methylsulfanyl)propanal	13.7	30.5	13.5	+16.8 (+120%)	−0.200 (−1.5%)	11.3	68	4.22	–
4	Acetic acid	118000	239000	160000	+122000 (+100%)	+41900 (+36%)	3340	2.7	15600	37
12	2-Methoxyphenol	0.960	1.44	1.40	+0.479 (+50%)	+0.433 (+45%)	0.0391	8.2	0.393	91
11	(2*E*,4*E*)-Deca-2,4-dienal	29.8	43.6	42.9	+13.8 (+46%)	+13.1 (+44%)	0.0155	< 1	0.00932	< 1
22	3-Methylbutan-1-ol	3380	3570	4140	+189 (+5.6%)	+757 (+22%)	0.529	< 1	0.0571	< 1
9	2-Acetyl-1-pyrroline	0.542	0.655	0.452	0.113 (+21%)	−0.0903 (−17%)	0.0251	22	0.0573	–
19	2-Methylpropanoic acid	3950	3350	4600	−589 (−15%)	+648 (+16%)	2.72	–	10.8	1.7
23	2-Methybutan-1-ol	1130	1260	1300	+129 (+11%)	+173 (+15%)	0.203	< 1	0.0111	< 1
18	2-Phenylethan-1-ol	2110	2420	1870	+309 (+15%)	−239 (−11%)	2.04	< 1	2.07	–
20	Butanoic acid	1820	1600	1870	−229 (−13%)	+49.8 (+2.7%)	13.5	–	4.11	8.3
6	4-Ethenyl-2-methoxyphenol	136	132	126	−3.84 (−2.8%)	−9.37 (−6.9%)	34.3	–	0.252	–
15	3-Methylbutanoic acid	1410	862	1210	−544 (−39%)	−199 (−14%)	6.01	–	28.4	–
14	(2*E*)-Non-2-enal	50.6	34.0	38.8	−16.6 (−33%)	−11.9 (−23%)	0.0249	–	0.0198	–
15	2-Methylbutanoic acid	834	435	576	−399 (−48%)	−258 (−31%)	1.61	–	5.55	–

[a]Numbers refer to Table 6. [b]µg of the odorant in a portion of the bread crumb originating from 1 kg of dough; calculated by multiplying the odorant concentrations in crumb (Table 8) by conversion factors of 0.997; the conversion factor for the crumb was approximated as (dry weight crust × amount crust + dry weight crumb × amount crumb) / (amount dough × dry weight crumb).[102] [c]Odorant concentration in the dough derived from the addition of the LME; calculated by multiplying the odorant concentration in the LME (Table 6) with the LME content in the dough (15 g/835 g).

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8 Appendix

8.1 Publication 1: Odour-Active Compounds in Liquid Malt Extracts for the Baking Industry

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ORIGINAL PAPER

Odour-active compounds in liquid malt extracts for the baking industry

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Abstract
An odorant screening by gas chromatography–olfactometry (GC–O) and a crude aroma extract dilution analysis (AEDA) applied to the volatiles isolated from a light and a dark liquid malt extract (LME) by solvent extraction and solvent-assisted flavour evaporation (SAFE) identified 28 odorants. Fifteen major odorants were subsequently quantitated and odour activity values (OAVs) were calculated as ratio of the concentration to the respective odour threshold value (OTV). Important odorants in the light LME included 3-(methylsulfanyl)propanal (OAV 1500), (*E*)-β-damascenone (OAV 430), and 4-ethenyl-2-methoxyphenol (OAV 91). In the dark LME, sotolon (OAV 780), 3-(methylsulfanyl)propanal (OAV 550), (*E*)-β-damascenone (OAV 410), acetic acid (OAV 160), and maltol (OAV 120) were of particular importance. To get an insight into the changes during malt extract production, the quantitations were extended to the malt used as the starting material for both LMEs. Addition of a minor amount of water to malt before volatile extraction was shown to be effective to cover the free as well as the bound malt odorants. Results showed that some LME odorants originated from the starting material whereas others were formed during processing. Important process-induced LME odorants included (*E*)-β-damascenone and 4-ethenyl-2-methoxyphenol in the light LME as well as maltol, sotolon, (*E*)-β-damascenone, and 2-methoxyphenol in the dark LME. In summary, the odorant formation during LME production was shown to be more important than the transfer of odorants from the malt.

Keywords Liquid malt extract · Pilsner malt · Aroma extract dilution analysis (AEDA) · Odour activity value (OAV) · Stable isotopically substituted odorant · Free and bound odorants

Abbreviations

AEDA	Aroma extract dilution analysis
AV	Acidic volatiles
CI	Chemical ionisation
DME	Dry malt extract
EI	Electron ionisation
FD factor	Flavour dilution factor
FFAP	Free fatty acid phase
FID	Flame ionisation detector
GC	Gas chromatography
GC × GC	Comprehensive two-dimensional gas chromatography
GC–O	Gas chromatography–olfactometry
HDMF	4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one
i.d.	Inner diameter
LME	Liquid malt extract
MCSS	Moving column stream switching
MS	Mass spectrometry
NBV	Neutral and basic volatiles
TOF	Time of flight
OAV	Odour activity value
OTV	Odour threshold value
RI	Retention index
SAFE	Solvent-assisted flavour evaporation
SDE	Simultaneous distillation/extraction

Nomenclature

Cyclotene	2-Hydroxy-3-methylcyclopent-2-en-1-one
(*E*)-β-Damascenone	(2*E*)-1-(2,6,6-Trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one
Dihydromaltol	5-Hydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one

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Maltol	3-Hydroxy-2-methyl-4*H*-pyran-4-one
γ-Nonalactone	5-Pentyloxolan-2-one
Sotolon	3-Hydroxy-4,5-dimethylfuran-2(5*H*)-one
Vanillin	4-Hydroxy-3-methoxybenzaldehyde

Introduction

Malts are germinated and re-dried grains, primarily of barley, but also of wheat, rye, and other cereals. The grains are first treated with water until the moisture content increases from ~ 12% to 43–48%. This initiates germination, during which enzymes start to break down starch to reducing sugars. After 4–6 days, the so-called green malt is dried in a kiln to obtain pale malt with a residual moisture content of 3.5–4% or dark malt with a residual moisture content of 1.5–2% [1, 2]. Malts can be further processed to malt extracts. For this purpose, the malt grains are milled and mashed with warm water. Application of a specific temperature program results in a substantial enzymatic degradation of biopolymers such as starch and proteins. After separation from the solid grain particles (malt draff), the aqueous phase is concentrated to yield a liquid malt extract (LME) with a syrup-like consistency. The LME can be further dried, e.g. by spray drying, to obtain a crystalline material marketed as dried malt extract (DME) [2, 3].

Malt extracts are mainly used in the baking industry. There is also a significant use of malt extracts for the production of confectionery, breakfast cereals, and other food products as well as for home brewing. In the baking industry, malt extracts are added to flours with low diastatic activity to provide fermentable sugars [4, 5], but also to enhance colour and aroma of bakery products. Particularly the contribution of malt extracts to the aroma of bakery products is yet poorly understood. The olfactory profile of malt extracts is characterized by malty, caramel-like, and honey-like odour notes. However, little is known on their molecular background.

In 1980, Farley and Nursten [6] applied gas chromatography–olfactometry (GC–O) and gas chromatography–mass spectrometry (GC–MS) to a volatile isolate obtained from an LME by simultaneous distillation/extraction (SDE) [7]. Forty-seven odour-active compounds were detected. GC–MS resulted in 38 structure proposals. However, structure assignments were not confirmed by GC–O of reference substances. Furthermore, the fact that SDE leads to extensive artefact formation [8] was not addressed. The compounds 2- and 3-methylbutanal were highlighted as odorants with malty characteristics and a high importance for the organoleptic properties of the LME. Przybylski and Kamiński [9] used GC to separate the volatiles isolated from a rye malt extract by liquid–liquid extraction into 39 fractions and evaluated their odour. GC–MS analysis led to 32 structure proposals, but no attempt was made to assess the role of the identified compounds for the odour of the fractions.

Unlike the scarce scientific literature on malt extract volatiles, numerous papers have been published on the volatiles in different malts, including green malt [10, 11], peated malt [12–14], dark malt [10, 15], crystal malt [11, 15, 16], caramel malt [10, 17–19], and roasted malt [15, 18, 20]. In summary, ~ 250 volatiles have been identified [21]. The odour contribution of individual malt volatiles was assessed in a study on caramel malt [10, 17]. Application of GC–O in combination with an aroma extract dilution analysis (AEDA) [22] to a volatile isolate obtained by solvent extraction and solvent-assisted flavour evaporation (SAFE) [23] revealed high flavour dilution (FD) factors for 3-methylbutanal, oct-1-en-3-one, 3-(methylsulfanyl)propanal, (2*E*,4*E*)-deca-2,4-dienal, 2- and 3-methylbutanoic acid, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF), and vanillin. Quantitation experiments, the calculation of odour activity values (OAVs), aroma reconstitution, and omission tests finally showed that the malty smelling aldehydes 3-methylbutanal (OAV 235) and 2-methylpropanal (OAV 70) were key compounds in the aroma of the caramel malt.

In summary of the literature overview, a comprehensive study on the key odorants in malt extracts for the baking industry was still lacking. In particular, it was unknown, whether malt extract odorants mainly originate from a transfer of malt odorants or whether a de novo formation during the processing of the malt to the malt extract also makes a contribution. This knowledge, however, is crucial for a targeted optimization of the aroma of malt extracts. The objective of the present study was to elucidate the major odour-active compounds in a light LME and in a dark LME for the baking industry, both produced from the same malt, and relate their concentrations to the concentrations that were initially present in the malt.

Materials and methods

Malt and malt extract samples

Light LME (77.5–81.0% dry matter, 48.2% sugar), dark LME (59.0–65.0% dry matter, 22.1% sugar), and the Pilsner malt from which both malt extracts had been produced, were obtained from Ireks (Kulmbach, Germany). The light LME was obtained from the Pilsner malt after milling, mashing with water, and application of vacuum concentration to the aqueous extract. The dark LME was obtained from the light LME by an additional heating step followed by dilution with water. 1 kg Pilsner malt yielded 0.94 kg light LME and 1.20 kg dark LME.

Reference odorants

The following compounds were purchased from commercial sources: **1**, **22** (Alfa Aesar, Karlsruhe, Germany), **2**–**5**, **8**–**10**, **12**, **14**, **16**, **17**, **19**, **20**, **23**, **24**, and **26**–**28** (Merck, Darmstadt, Germany). Compound **11** was a gift from Symrise (Holzminden, Germany). The following compounds were synthesised as detailed in the literature: **6** [24] and **18** [25].

Stable isotopically substituted odorants

The following compounds were purchased from commercial sources: ($^2H_3$)-**4** and ($^{13}C_2$)-**26** (Merck), ($^2H_5$)-**16** (CDN Isotopes, Quebec, Canada), and ($^{13}C_2$)-**20** (aromaLAB, Planegg, Germany). The following compounds were synthesised as detailed in the literature: ($^2H_3$)-**5** [26], ($^{13}C_2$)-**8** [27], ($^2H_2$)-**9** [28], ($^2H_6$)-**11** [29], ($^2H_3$)-**14** [30], ($^{13}C_6$)-**22** [31], ($^{13}C_2$)-**23** [32], and ($^2H_3$)-**27** [33]. ($^{13}C_2$)-**10** was prepared using the approach detailed in [31], but starting from (2*E*)-oct-2-enal instead of (2*E*)-(1,2-$^{13}C_2$)oct-2-enal. ($^{13}C_2$)-**17** was synthesised as detailed below.

Miscellaneous chemicals

The following chemicals were purchased from commercial sources: (^{13}C)ethanoic anhydride (Euriso-top, Saint-Aubin, France), furan, 2-methylpropan-2-ol, and sodium borohydride (Merck), nitromethane, aqueous sodium hypochlorite solution with 5% available chlorine, and tin(II) trifluoromethanesulfonate (VWR, Darmstadt, Germany). Diethyl ether, dichloromethane (CLN, Freising, Germany), and pentane (VWR) were freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings.

Synthesis of ($^{13}C_2$)maltol [($^{13}C_2$)-17]

1-(Furan-2-yl)($^{13}C_2$)ethan-1-one

Following the approach published for the isotopically unmodified compound [34], Friedel–Crafts acylation of furan (75 mg, 1.1 mmol) with (^{13}C)ethanoic anhydride (250 mg, 2.4 mmol) in dichloromethane (1 mL) using tin(II) trifluoromethanesulfonate (23 mg, 0.055 mmol) as Lewis acid resulted in 1-(furan-2-yl)($^{13}C_2$)ethan-1-one.

1-(Furan-2-yl)($^{13}C_2$)ethan-1-ol

The 1-(furan-2-yl)($^{13}C_2$)ethan-1-one obtained above was dissolved in methanol (5 mL) and sodium borohydride (60 mg, 1.6 mmol) was slowly added, while maintaining the temperature below 40 °C. The solvent was removed in vacuo, water (20 mL) was added and the product was extracted with diethyl ether (3 × 50 mL). After drying over anhydrous sodium sulphate, the solvent was removed in vacuo to yield 1-(furan-2-yl)($^{13}C_2$)ethan-1-ol [10].

Synthesis of ($^{13}C_2$)maltol [($^{13}C_2$)-17]

Following the approach for the synthesis of the isotopically unmodified compound [35], the 1-(furan-2-yl)($^{13}C_2$)ethan-1-ol obtained above was added to acetic acid (5 mL) and reacted with *tert*-butyl hypochlorite (1.3 g, 12 mmol) previously prepared as detailed in [36]. The resulting product was purified by silica gel chromatography with a pentane/diethyl ether gradient to afford 3.3 mg of 3-hydroxy-2-(^{13}C)methyl(2-^{13}C)-4*H*-pyran-4-one, that is ($^{13}C_2$)maltol, in 99.95% purity (GC–FID) equivalent to 2.4% overall yield. MS (EI): *m/z* (%) 71 (100), 128 (91), 45 (78), 43 (50), 57 (39), 55 (38), 99 (28), 54 (27), 44 (23), 42 (23), 56 (17), 53 (17), 41 (17), 69 (16), 58 (11), 46 (9), 39 (8), 72 (8), 40 (7), 126 (7), 52 (6), 70 (5). The mass spectrum in comparison with the mass spectrum of the isotopically unmodified compound (Supplementary file 1, Fig. S1 and Fig. S2) confirmed the incorporation of two ^{13}C atoms and its suitability as internal standard in quantitation assays.

GC–O

A Trace Ultra series GC (Thermo Fisher Scientific, Dreieich, Germany) was equipped with a cold on-column injector, an FID (250 °C base temperature), and a custom-made sniffing port (230 °C base temperature) [13]. Two different fused silica columns were used, either a J&W DB-FFAP, 30 m × 0.32 mm i.d., 0.25 µm film thickness, or a J&W DB-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness (both Agilent, Waldbronn, Germany). The end of the column was connected to a Y-shaped quick-seal glass connector (CHM, Fridolfing, Germany) that directed the effluent via two deactivated fused silica capillaries, 30 cm × 0.2 mm i.d. (Agilent) to the FID and the sniffing port, respectively. The carrier gas was helium at a constant pressure of 70 kPa for the DB-FFAP column and 95 kPa for the DB-5 column. The injection volume was 1 µL. The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min. The final temperatures were 230 °C for the DB-FFAP column and 240 °C for the DB-5 column. During GC–O analysis, a panellist placed his nose above the sniffing port and evaluated the effluent. The panellist marked odorous regions in the FID chromatogram plotted by a recorder and noted the odour quality [8]. A linear retention index (RI) was calculated for each odour-active compound from its retention time and the retention times of adjacent *n*-alkanes by linear interpolation [37].

GC–MS

A 7890B GC was equipped with a GC 80 autosampler, a multimode injector, and a J&W DB-FFAP column, 30 m × 0.25 mm i.d., 0.25 µm film thickness and was connected to a Saturn 220 ion trap mass spectrometer (Agilent). The carrier gas was helium at a constant flow of 1.0 mL/min. The injection volume was 1 µL. The injection temperature was 40 °C. The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min to a final temperature of 230 °C. Mass spectra were generated in the chemical ionisation (CI) mode with methanol as reagent gas. For data analysis the MS Workstation 7.0.2 software (Agilent) was used.

Heart-cut GC–GC–MS

A Trace GC Ultra (Thermo Fisher Scientific) was equipped with a PAL autosampler (CTC Analytics, Zwingen, Switzerland), a cold on-column injector, and a J&W DB-FFAP column, 30 m × 0.32 mm i.d., 0.25 µm film thickness (Agilent). The carrier gas was helium at a constant pressure of 110 kPa. The injection volume was 1–2 µL. The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min to a final temperature of 230 °C. The end of the column was connected to a moving column stream switching (MCSS) device (Thermo Fisher Scientific), which allowed for a time-programmed transfer of the eluate via deactivated fused silica capillaries (0.32 mm i.d.) either simultaneously to an FID (250 °C base temperature) and a sniffing port (230 °C base temperature) or via another deactivated fused silica capillary passed through a heated (250 °C) hose to a liquid nitrogen-cooled trap located in the oven of a CP 3800 GC (Agilent). Helium served as make-up gas for the MCSS device (50 kPa). The capillary in the second oven was a J&W DB-1701, 30 m × 0.25 mm i.d., 0.25 µm film thickness (Agilent). The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min to a final temperature of 240 °C. The end of this column was connected to a Saturn 2200 ion trap mass spectrometer (Agilent). Mass spectra were generated in the CI mode with methanol as reagent gas. For data analysis the MS Workstation 6.9.3 software (Agilent) was used.

Comprehensive two-dimensional gas chromatography–time of flight MS (GC × GC–TOFMS)

A 6890 GC (Agilent) was equipped with Combi PAL autosampler (CTC Analytics), a CIS 4 injector (Gerstel, Mülheim an der Ruhr, Germany), and a J&W DB-FFAP column, 30 m × 0.25 mm i.d., 0.25 µm film thickness (Agilent) used as first column. The carrier gas was helium at a constant flow of 2.0 mL/min. The injection volume was 1 µL. The initial oven temperature of 40 °C was held for 2 min, followed by a gradient of 6 °C/min to a final temperature of 230 °C. The end of the column was connected to a second column, J&W DB-1701, 2.7 m × 0.18 mm i.d., 0.18 µm film thickness (Agilent). A liquid nitrogen-cooled dual stage quad-jet thermal modulator was installed at the beginning of the second column and operated with a modulation period of 4 s. The major part of the second column was installed in a secondary oven mounted inside the primary GC oven. The initial oven temperature of the secondary oven was 45 °C and was held for 2 min, followed by a gradient of 6 °C/min to a final temperature of 240 °C. The end of the column was connected to a Pegasus II TOF mass spectrometer (Leco, Mönchengladbach, Germany) via a heated (250 °C) transfer line. Mass spectra were generated in the electron ionisation (EI) mode at 70 eV with a scan range of *m*/z 35 – 350 and a scan rate of 100 spectra/s. For data analysis, the GC Image (Lincoln, NE, USA) software was used.

Isolation of volatiles

LME samples (10 g) were diluted with water (20 mL) and stirred with diethyl ether (80 mL) at room temperature for 2 h. The aqueous phase was separated and stirred with a second portion of diethyl ether (80 mL). The combined organic phases were dried over anhydrous sodium sulphate and non-volatile matrix components were removed by SAFE at 40 °C [23]. The distillate was concentrated to a final volume of 1 mL using a Vigreux column (50 × 1 cm) and a Bemelmans microdistillation device [38].

AEDA

Volatile isolates prepared from light and dark LME as described above were repeatedly subjected to GC–O analysis (FFAP column) by three trained and experienced sniffers. After the results had become reproducible, the volatile isolates were stepwise diluted 1:10 with diethyl ether to obtain dilutions of 1:10, 1:100, 1:1000, and 1:10,000 of the initial solution. The diluted samples were also analysed by GC–O (three sniffers) and each odorant was assigned an FD factor defined as the dilution factor of the highest diluted sample in which the odorant was detected during GC–O analysis by any of the three panellists [8].

Odorant quantitation

LME samples (0.5–20 g) were diluted with water (4–40 mL). Diethyl ether (16–160 mL) and stable isotopically substituted odorants (cf. Supplementary file, Table S1; 0.03–400 µg) were added, and the mixture was stirred at room temperature overnight. The aqueous phase was separated, shaken with a

second portion of diethyl ether (16–160 mL), and the organic phases were combined.

Commercial malt powder (2 g) was either added to diethyl ether (20 mL), or malt powder (0.5 g) was added to a mixture of diethyl ether (19 mL) and water (1 mL), or malt powder (0.5 g) was first mixed with water (2 mL), the mixture was allowed to stand for 30 s, 1 min, 2 min, 5 min, 15 min, or 180 min, and finally anhydrous sodium sulphate (10 g) suspended in diethyl ether (50 mL) was added. Stable isotopically substituted odorants (0.1–2 µg) were added and the mixture was stirred at room temperature overnight. The supernatant was decanted, the residue was stirred with a second portion of diethyl ether (20 mL), and the organic phases were combined.

Pilsner malt was 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 (19–190 mL) and water (1–10 mL) were added to the powder (0.5 – 20 g) followed by stable isotopically substituted odorants (0.01–20 µg), and the mixture was stirred at room temperature overnight. The supernatant was decanted, the residue was stirred with a second portion of diethyl ether (20–200 mL), and the organic phases were combined.

The ethereal extracts obtained from the different materials were dried over anhydrous sodium sulphate and non-volatiles were removed by SAFE at 40 °C. The SAFE distillates were separated into neutral and basic volatiles (NBV) and acidic volatiles (AV) as detailed in [39]. Fractions NBV and AV were concentrated to final volumes between 0.2 mL and 5 mL. Concentrates were analysed by using the GC–MS system (**4**), the heart-cut GC–GC–MS system (**9**), or the GC × GC–TOFMS system (**5**, **8**, **10**, **11**, **14**, **16**, **17**, **20**, **22**, **23**, **26**, and **27**). Odorant concentrations were finally calculated from the area counts of the analyte peak and the internal standard peak as obtained from the extracted ion chromatograms of characteristic quantifier ions, the amount of LME or malt used, and the amount of standard added by employing a calibration line equation previously obtained from the analysis of analyte/standard mixtures in five different concentration ratios (5:1, 2:1, 1:1, 1:2, and 1:5). Quantifier ions and calibration line equations are available in the Supplementary file, Table S1. The individual concentrations of the two isomers of **9** were determined from the concentrations obtained for the sum of isomers and the ratios of isomers, which were determined by GC × GC–TOFMS using the approach detailed in [40].

Quantitative olfactory profiles

LME samples (5 g) were placed in cylindrical ground neck glasses (7 cm height, 3.5 cm i.d.) with lids (VWR, Darmstadt, Germany). A panel of 16–18 trained assessors (males and females, ages 21–49) orthonasally evaluated the intensities of pre-defined descriptors on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong. Descriptors had previously been collected by free-choice profiling. Each descriptor was defined by the odour of a reference compound dissolved in water in a concentration ~ 100 times above its orthonasal odour threshold value (OTV). Reference compounds were selected on the basis of their odour and their occurrence in malt and other thermally treated foods [41, 42]. The twelve descriptors and the corresponding reference compounds were "smoky" (2-methoxyphenol; OTV 0.84 µg/kg [43]), "earthy" (2,3,5-trimethylpyrazine; OTV 11 µg/kg [44]), "roasty" (2-acetyl-2-thiazoline; OTV 0.079 µg/kg [44]), "seasoning-like" (sotolon; OTV 1.7 µg/kg [45]), "cooked potato-like" (3-(methylsulfanyl)propanal; OTV 0.43 µg/kg [43]), "cheesy" (3-methylbutanoic acid; OTV 490 µg/kg [43]), "fatty" ((2*E*,4*E*)-deca-2,4-dienal; OTV 0.027 µg/kg [43]), "malty" (3-methylbutanal; OTV 0.5 µg/kg [43]), "honey-like" (phenylacetic acid; OTV 68 µg/kg [46]), "caramel-like" (HDMF; OTV 87 µg/kg [47]), "vanilla-like" (vanillin; OTV 53 µg/kg [43]), and "clove-like" (4-allyl-2-methoxyphenol; OTV 1.8 µg/kg [48]). The results of the individual panellists were averaged by calculating the arithmetic mean.

Results and discussion

Sensory characterization of light and dark LME

A light LME and a dark LME, both obtained from the same batch of malt, were orthonasally compared by a trained sensory panel in a quantitative olfactory profile analysis using 12 pre-defined descriptors. Results (Fig. 1) showed clear differences between the samples. The aroma of the dark LME was characterized by stronger smoky, earthy, roasty, seasoning-like, and malty notes and slightly more intense caramel-like and clove-like notes compared to the light LME. The profile of the light LME showed a higher intensity only in the honey-like odour note. The differences in the olfactory profiles corresponded to the different production protocols. In particular, the processing of the dark LME included an additional heating step, suggesting an increased formation of Maillard reaction products [49, 50].

Screening for odour-active compounds in light and dark LME

To get a first insight into the odorants responsible for the different olfactory profiles of the light LME and the dark LME, the volatiles were isolated by solvent extraction and SAFE, and screened for odour-active compounds by GC–O and a crude AEDA using 1:10 dilutions. This resulted in a

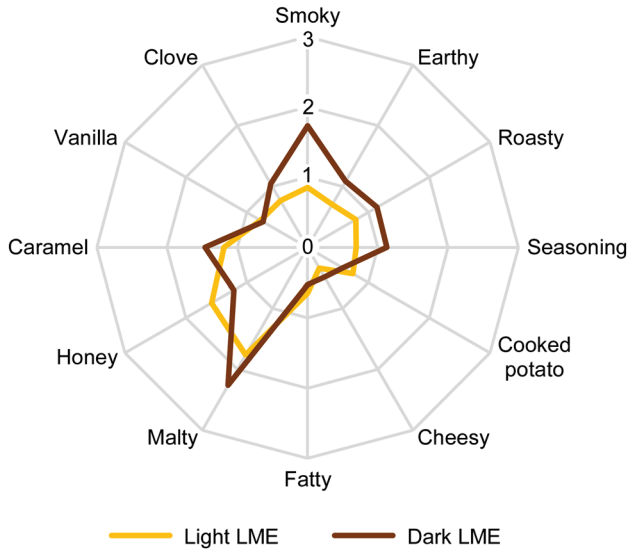

Fig. 1 Quantitative olfactory profiles of light and dark LME. Panellists rated the intensity of each descriptor on a scale from 0 to 3 in 0.5 increments with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong

total of 28 odorants (Table 1). Preliminary structural assignments were achieved by comparing odour quality and RIs with data from literature [17] and from the Leibniz-LSB@TUM odorant database [42]. Assignments were confirmed by comparing the odour quality, the RIs on two columns of different polarity (DB-FFAP, DB-5), and the odour intensity at adequate concentration levels, as well as the mass spectra recorded by GC × GC–TOFMS to data of authentic reference substances analysed under the same conditions. Using this approach, 21 out of the 28 LME odorants were unequivocally identified. Due to low concentrations, no mass spectra were obtained for oct-1-en-3-one (**3**) and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**18**). Nevertheless, their identification was considered unambiguous due to their highly characteristic odour quality. As no reference compound was available, caramel-like smelling odorant **13** was only tentatively identified as dihydromaltol on the basis of a comparison of the odour quality, the RI, and the mass spectrum with literature data [17]. In summary, structures could be assigned to 24 out of the 28 malt extract odorants, all of which had already been reported as malt or malt extract components [6, 9, 17].

In both malt extract samples, a high FD factor of 1000 was determined for honey-like smelling phenylacetic acid (**26**) and vanilla-like smelling vanillin (**27**). Higher FD factors in the light LME than in the dark LME were in particular found for caramel-like smelling HDMF (**20**; FD 1000 vs. 10), metallic smelling *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**18**; FD 100 vs. 1), clove-like smelling 4-ethenyl-2-methoxyphenol (**22**; FD 100 vs. < 1), and honey-like smelling phenylacetaldehyde (**8**; FD 100 vs. 10). Higher FD factors in the dark LME were obtained for the two caramel-like smelling compounds maltol (**17**; FD 1000 vs. 100) and dihydromaltol (**13**; FD 100 vs. 1), seasoning-like smelling sotolon (**23**; FD 1000 vs. 10), smoky smelling 2-methoxyphenol (**14**; FD 100 vs. 10), and the carboxylic acids acetic acid (**4**; FD 100 vs. 10) and 2-/3-methylbutanoic acid (**9**; FD 100 vs. 10). In both LMEs, the FD factors of highly volatile compounds such as 2- and 3-methylbutanal (**1**), and oct-1-en-3-one (**3**) were considerably lower than in malt [17], indicating losses in the concentration step during the malt extract production. Similar losses of such highly volatile malt compounds have been reported during wort boiling [51].

Odorant quantitation in light and dark LME and OAV calculation

To substantiate the differences in the odorants between the light LME and the dark LME and lift the investigations to a higher level of accuracy, 15 selected odorants were quantitated by GC–MS. Selection was based on the FD factors obtained in the screening experiments and according to literature on the relevance of the compounds for bread aroma [42, 52, 53]. Stable isotopically substituted odorants were employed as internal standards to compensate for losses during the sample workup. Results (Table 2) revealed concentrations in a range between 0.519 µg/kg for (2*E*,4*E*)-deca-2,4-dienal (**11**) and 869,000 µg/kg for acetic acid (**4**). To assess the odour potency of the odorants, OAVs were calculated by dividing the individual concentrations by the OTVs of the compounds in water.

In the light LME, 12 of 15 compounds showed OAVs ≥ 1. High OAVs were calculated for cooked potato-like smelling 3-(methylsulfanyl)propanal (**5**; OAV 1500), cooked apple-like smelling (*E*)-β-damascenone (**11**; OAV 430), clove-like smelling 4-ethenyl-2-methoxyphenol (**22**; OAV 91), and the two honey-like smelling odorants phenylacetaldehyde (**8**; OAV 70) and phenylacetic acid (**26**; OAV 58). In the dark LME, 11 of 15 compounds showed OAVs ≥ 1. High OAVs were calculated for seasoning-like smelling sotolon (**23**; OAV 780), cooked potato-like smelling 3-(methylsulfanyl)propanal (**5**; OAV 550), cooked apple-like smelling (*E*)-β-damascenone (**11**; OAV 410), vinegar-like smelling acetic acid (**4**; OAV 160), caramel-like smelling maltol (**17**; OAV 120), and honey-like smelling phenylacetic acid (**26**; OAV 62).

The differences found in the OAVs between the light LME and the dark LME very well corresponded to the differences in the quantitative olfactory profiles (cf. Fig. 1). For example, the stronger honey-like odour note in the light LME reflected the higher OAV of phenylacetaldehyde (**8**; OAV 70 vs. 20). With OAVs of 58 and 62, phenylacetic acid (**26**) did obviously not contribute to this sensory difference. In the dark LME, clearly higher OAVs

Table 1 Odorants in the SAFE distillates obtained from the light LME and the dark LME

No	Odorant[a]	Odour[b]	RI[c]		FD factor[d]	
			DB-FFAP	DB-5	Light LME	Dark LME
1	2- and 3-Methylbutanal	Malty	938	663	10	10
2	Pentane-2,3-dione	Buttery	1068	706	< 1	10
3	Oct-1-en-3-one[e]	Mushroom	1300	980	1	< 1
4	Acetic acid	Vinegar, sour	1451	638	10	100
5	3-(Methylsulfanyl)propanal	Cooked potato	1458	904	100	100
6	(2*Z*)-Non-2-enal	Fatty	1505	1149	< 1	1
7	Unknown	Sweet	1531		< 1	1
8	Phenylacetaldehyde	Honey	1645	1043	100	10
9	2- and 3-Methylbutanoic acid	Cheesy	1668	867	10	100
10	(2*E*,4*E*)-Deca-2,4-dienal	Fatty	1818	1316	1	1
11	(*E*)-β-Damascenone	Cooked apple	1819	1386	10	10
12	Cyclotene	Seasoning	1839	1031	< 1	10
13	Dihydromaltol[f]	Caramel	1868		1	100
14	2-Methoxyphenol	Smoky	1868	1086	10	100
15	Unknown	Fruity	1892		10	< 1
16	2-Phenylethan-1-ol	Honey	1919	1117	10	10
17	Maltol	Caramel	1981	1117	100	1000
18	*trans*-4,5-Epoxy-(2*E*)-dec-2-enal[e]	Metallic	2009	1374	100	1
19	γ-Nonalactone	Coconut	2045	1363	< 1	10
20	HDMF[g]	Caramel	2034	1076	1000	10
21	Unknown	Seasoning	2079		< 1	10
22	4-Ethenyl-2-methoxyphenol	Clove	2187	1314	100	< 1
23	Sotolon	Seasoning	2218	1123	10	1000
24	2,6-Dimethoxyphenol	Smoky	2288	1307	1	10
25	Unknown	Sweet	2493		< 1	10
26	Phenylacetic acid	Honey	2587	1278	1000	1000
27	Vanillin	Vanilla	2593	1398	1000	1000
28	3-Phenylpropanoic acid	Cinnamon	2625	1353	< 1	10

[a]Each odorant was identified by comparing the RIs on two GC columns of different polarity (DB-FFAP, DB-5), the mass spectrum obtained by GC×GC–TOFMS, as well as the odour quality perceived at the sniffing port during GC–O to data obtained from authentic reference compounds analysed under equal conditions

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

[c]Retention index; calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation[37]

[d]Flavour dilution factor; dilution factor of the highest dilution of the volatile isolate in which the odorant was detected during GC–O analyses

[e]GC–MS analysis did not result in a clear mass spectrum, but comparison of RIs and odour quality with respective data of an authentic reference compound allowed for unequivocal structure assignment

[f]The compound was tentatively identified by comparing the odour quality, the RI, and the mass spectrum with data obtained from[17, 42]

[g]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one

were obtained for the well-known Maillard reaction products sotolon (**23**; OAV 780 vs. 7.4) and maltol (**17**; OAV 120 vs. 1.8) [49, 50] as well as for 2-methoxyphenol (**14**; OAV 26 vs. 2.6). This corresponded well to the higher intensities of the seasoning-like, the caramel-like, and the smoky odour notes in the quantitative olfactory profile. However, OAV data did not provide an explanation for the higher ratings obtained for the earthy, roasty, and malty notes in the dark LME.

Sources of LME odorants

The differences in the concentrations of important odorants between the light and the dark LME already indicated

Table 2 Concentrations, orthonasal OTVs in water, and OAVs of important odour-active compounds in the light and the dark LME

No.[a]	Odorant	OTV (µg/kg)[b]	Light LME		Dark LME	
			Conc. (µg/kg)[c]	OAV[d]	Conc. (µg/kg)[c]	OAV[d]
5	3-(Methylsulfanyl)propanal	0.43 [43]	631	1500	235	550
23	Sotolon	1.7 [45]	12.6	7.4	1330	780
11	(*E*)-β-Damascenone	0.0060 [39]	2.59	430	2.45	410
4	Acetic acid	5600 [41]	186,000	33	869,000	160
17	Maltol	5000 [76]	9170	1.8	613,000	120
22	4-Ethenyl-2-methoxyphenol	21 [77]	1910	91	14.0	< 1
8	Phenylacetaldehyde	5.2 [78]	362	70	107	20
26	Phenylacetic acid	68 [46]	3970	58	4220	62
27	Vanillin	53 [43]	1190	22	1710	32
10	(2*E*,4*E*)-Deca-2,4-dienal	0.027 [43]	0.862	32	0.519	19
14	2-Methoxyphenol	0.84 [43]	2.18	2.6	21.9	26
9a	3-Methylbutanoic acid	490 [43]	335	< 1	1580	3.2
20	HDMF[e]	87 [47]	221	2.5	35.9	< 1
9b	2-Methylbutanoic acid	3100 [46]	89.4	< 1	309	< 1
16	2-Phenylethan-1-ol	140 [43]	113	< 1	115	< 1

[a]Numbers refer to Table 1

[b]Orthonasal odour threshold values in water; OTVs were taken from the references specified and had been determined according to ASTM [79]

[c]Mean of duplicates or triplicates; standard deviations were < 20%; individual concentration values and standard deviations are available in Supplementary file 1, Tables S2 and S3

[d]Odour activity value; calculated as ratio of concentration to OTV

[e]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one

a major impact of the processing steps from malt to extract, at least for the dark LME which faced a higher thermal impact. However, it was still unclear, whether a transfer of malt odorants or the odorant formation during malt extract processing is generally more important for the amount of odorants finally present in the LMEs. To clarify this, we aimed at quantifying all odorants previously quantitated in the light LME and the dark LME (cf. Table 2) also in the malt that served as starting material for both extracts.

It has been reported that in products with low moisture content such as chocolate, cornflakes, crackers, and malt, odour-active compounds are not only present in the free form, but to a major extent in a bound form from which the odorants are released by water contact [19, 54]. As during LME production the malt also gets into contact with water, we attempted to quantitate the free and bound odorants in the malt as sum. It was shown that in malt, the Strecker aldehydes 2- and 3-methylbutanal, phenylacetaldehyde, and 3-(methylsulfanyl)propanal increase 10–140-fold after water treatment [19]. 3-Oxazolines formed during Strecker degradation were suggested as the crucial hydrolabile precursors of these compounds [54]. Own preliminary experiments, however, showed that not only Strecker aldehydes, but also other odour-active compounds increase in malt upon water contact. Figure 2 exemplifies this for phenylacetaldehyde (**8**), phenylacetic acid (**26**), and vanillin (**27**). The substances

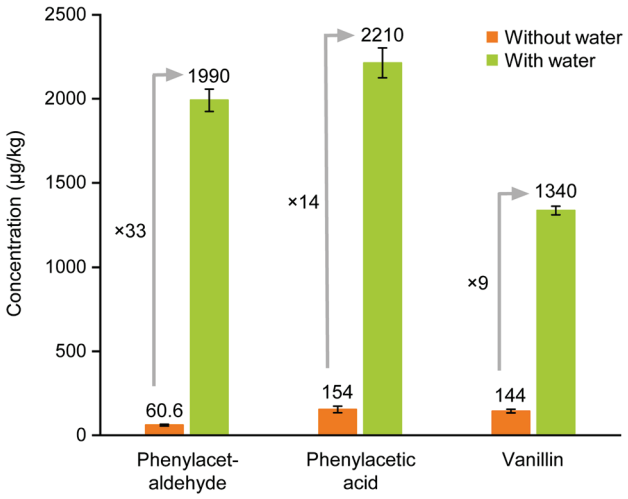

Fig. 2 Concentrations of phenylacetaldehyde, phenylacetic acid, and vanillin in malt powder determined without and with water addition before solvent extraction

were quantitated in a commercial malt powder by using stable isotopically substituted odorants as internal standards and two different workup procedures. To quantitate only the free compounds, pure diethyl ether was used as extraction solvent. By contrast, to cover additionally the bound compounds, a minor amount of water was added together with

the diethyl ether. A similar approach had been suggested earlier for this purpose [10, 17].

Results showed clearly higher concentrations of all three odorants when water was added. Water addition increased the concentration of phenylacetaldehyde 33-fold from 60.6 µg/kg to 1990 µg/kg. The concentrations of phenylacetic acid and vanillin also significantly increased. With factors of 14 and 9, however, the increase was less pronounced. These observations suggested a combination of a general release mechanism applicable to volatiles in general and an additional formation of Strecker aldehydes as suggested before. Similar results were reported for oat products [55–57]. These authors suggested the cleavage of starch complexes as a general odorant release mechanism.

Although the experiments with water addition indicated a simultaneous quantitation of free and bound odorants in malt, it yet remained unclear whether the release of the bound odorants was exhaustive. To clarify this, we conducted a series of quantitation experiments during which water treatment and solvent extraction were temporally separated and the duration of the water treatment was varied. Experiments were again carried out with phenylacetaldehyde (**8**), phenylacetic acid (**26**), and vanillin (**27**). Results (Fig. 3) showed a very quick release of the bound odorants. The maximum value was already reached after 1 min of water contact and equalled the concentrations previously obtained after simultaneous addition of water and diethyl ether. Thus, the simultaneous addition of water and diethyl ether led to an exhaustive release of bound odorants and is an appropriate approach to quantitate the sum of free and bound volatiles in malt.

Application of this approach to the quantitation of important LME odorants in the malt that served as starting material for both, the light and the dark LMEs, resulted in concentrations ranging from 0.135 µg/kg for (*E*)-β-damascenone (**11**) to 404,000 µg/kg for acetic acid (**4**) (Table 3). High concentrations were additionally determined for phenylacetaldehyde (**8**; 7000 µg/kg), phenylacetic acid (**26**; 6250 µg/kg), 3-methylbutanoic acid (**9a**; 3810 µg/kg), 2-phenylethan-1-ol (**16**; 1360 µg/kg), and 3-(methylsulfanyl)propanal (**5**; 1190 µg/kg). To assess odorant loss and odorant formation on the way from the malt to the LMEs, the percentage of change was calculated from the odorant concentrations in the malt, the odorant concentrations in the LMEs, and the process yields. Results revealed huge differences: percentages ranged from 1.8% to 90,000% (Table 3).

Approximately half of the analysed compounds exhibited recoveries below 100% in both extracts. These included three compounds with comparatively high OAVs, namely the cooked potato-like smelling 3-(methylsulfanyl)propanal (**5**) and the honey-like smelling compounds phenylacetaldehyde (**8**) and phenylacetic acid (**26**). The recoveries for 3-(methylsulfanyl)propanal were 50% and 24% in the

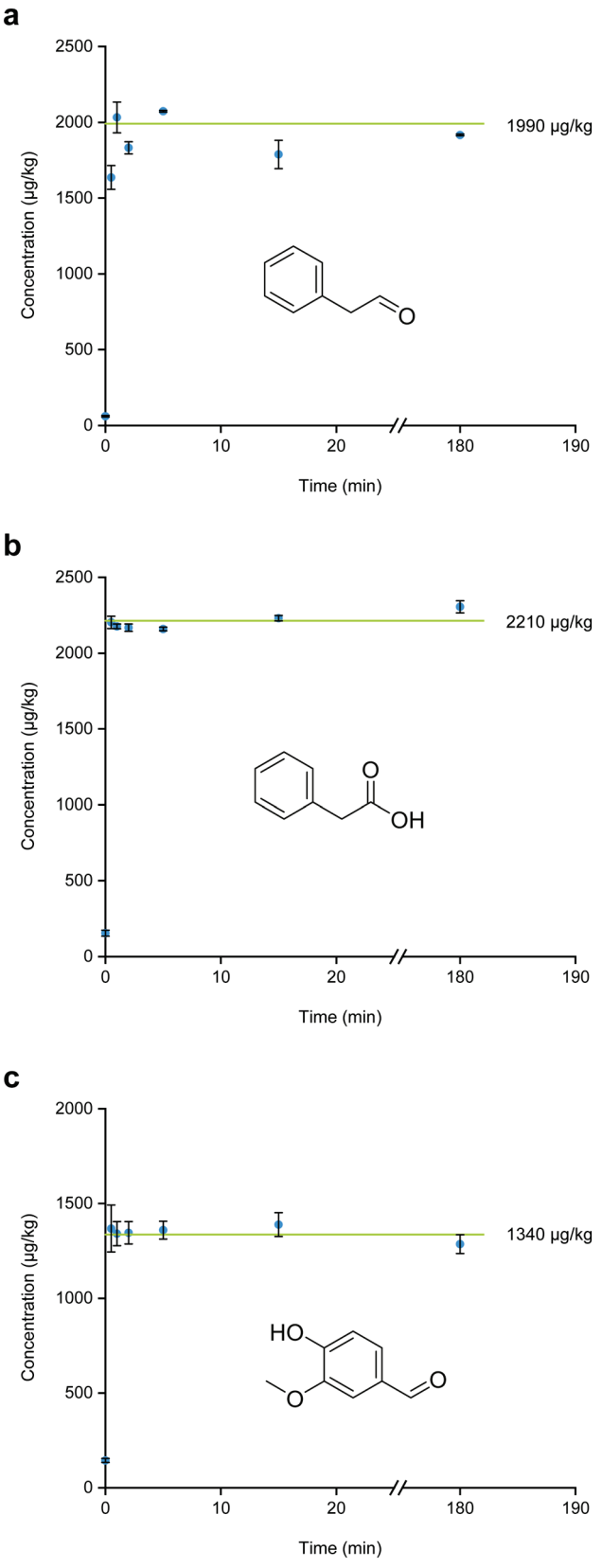

Fig. 3 Concentrations of phenylacetaldehyde (**a**), phenylacetic acid (**b**), and vanillin (**c**) in malt powder after stirring with water for different periods (dots) in comparison to the concentrations determined after simultaneous addition of water and diethyl ether (straight line)

Table 3 Concentrations of important LME odorants in the Pilsner malt and changes during the production of the light LME and the dark LME from the malt

No.[a]	Odorant	Conc. in malt (µg/kg)[b]	Change (%)[c]	
			Malt to light LME	Malt to dark LME
17	Maltol	815	1100	90,000
23	Sotolon	4.07	290	39,000
11	(*E*)-β-Damascenone	0.135	1800	2200
14	2-Methoxyphenol	1.47	140	1800
22	4-Ethenyl-2-methoxyphenol	447	400	3.8
27	Vanillin	759	150	270
4	Acetic acid	404,000	43	260
26	Phenylacetic acid	6250	60	81
20	HDMF[d]	321	65	13
5	3-(Methylsulfanyl)propanal	1190	50	24
9a	3-Methylbutanoic acid	3810	8.3	50
9b	2-Methylbutanoic acid	792	11	47
16	2-Phenylethan-1-ol	1360	7.8	10
10	(2*E*,4*E*)-Deca-2,4-dienal	13.1	6.2	4.7
8	Phenylacetaldehyde	7000	4.9	1.8

[a]Numbers refer to Table 1

[b]Mean of duplicates or triplicates; standard deviations were < 20%; individual concentration values and standard deviations are available in Supplementary file 1, Table S4

[c]Calculated as (concentration in malt extract/concentration in malt) × process yield; concentrations in malt extracts were taken from Table 2, process yields were 0.94 for light LME and 1.2 for dark LME

[d]4-Hydroxy-2,5-dimethylfuran-3(2*H*)-one

light LME and the dark LME, respectively. With 4.9% and 1.8%, phenylacetaldehyde showed the lowest recoveries of all compounds analysed. Higher recoveries were calculated for phenylacetic acid, namely 60% and 81%. Losses might be associated with thermal reactions and with evaporation during the vacuum concentration process.

Seven compounds showed an increase in at least one of the two LMEs, indicating a formation during malt extract production. Among them were vinegar-like smelling acetic acid (**4**), the phenolic compounds 2-methoxyphenol (**14**; smoky), 4-ethenyl-2-methoxyphenol (**22**; clove-like), and vanillin (**27**; vanilla-like), cooked apple-like smelling (*E*)-β-damascenone (**11**) as well as seasoning-like smelling sotolon (**23**) and caramel-like smelling maltol (**17**). Acetic acid showed a decrease from the malt to the light LME (43%) but an increase to the dark LME (260%). During the production of the dark LME, acetic acid might have been formed via sugar degradation and Strecker degradation of alanine [58–61]. Within the group of phenolic compounds, vanillin showed similar percentages in the light LME (150%) and the dark LME (270%), whereas 4-ethenyl-2-methoxyphenol showed an increase in the light LME (400%), but a decrease in the dark LME (3.8%), and 2-methoxyphenol showed a moderate increase in the light LME (140%), but a clearly higher increase in the dark LME (1800%). These observations are in line with the higher thermal impact associated with the additional heating step during the production of the dark LME. It has been demonstrated that the thermal decomposition of ferulic acid first results in 4-ethenyl-2-methoxyphenol [62–65] which presumably was accumulated in the light LME. A higher thermal impact converts 4-ethenyl-2-methoxyphenol to 2-methoxyphenol [62, 63], explaining the lower amount of 4-ethenyl-2-methoxyphenol and the higher amount of 2-methoxyphenol in the dark LME. With percentages of 1800% and 2200%, (*E*)-β-damascenone revealed a significant gain on the way from the malt to the LMEs, but only a small difference between the light and the dark LME. Thermal formation of (*E*)-β-damascenone is well known, but the compound also might have been formed from glycosides during mashing [66–73]. The highest increase of all compounds investigated was found for the Maillard reaction products maltol and sotolon in the dark LME, namely 90,000% and 39,000%. For both compounds, the percentages in the light LME were clearly lower (1100% and 290%) indicating that the additional heating step was crucial for their elevated formation in the dark LME. It has been demonstrated that sotolon can be formed by an aldol-reaction from butane-2,3-dione and hydroxyacetaldehyde, both being previously generated by retro-aldol cleavage of sugars [74]. The precursor of maltol is the disaccharide maltose [10, 75] resulting from the enzymatic breakdown of starch during germination and mashing.

Conclusions

The odour-active compounds in LMEs clearly differ from the odour-active compounds in the malt used for their production. Whereas some malt odorants decrease during extract production, others show an enormous increase. Thus, the formation of odorants during LME production is much more important than the mere transfer of odorants from malt to LMEs.

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Declarations

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

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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8.1.3 Summary and Individual Contributions

Malts are germinated and re-dried cereal grains and can be further processed into malt extracts. The aims of the study were to identify the odor-active compounds in a light and a dark LME for the baking industry and to clarify their origin.

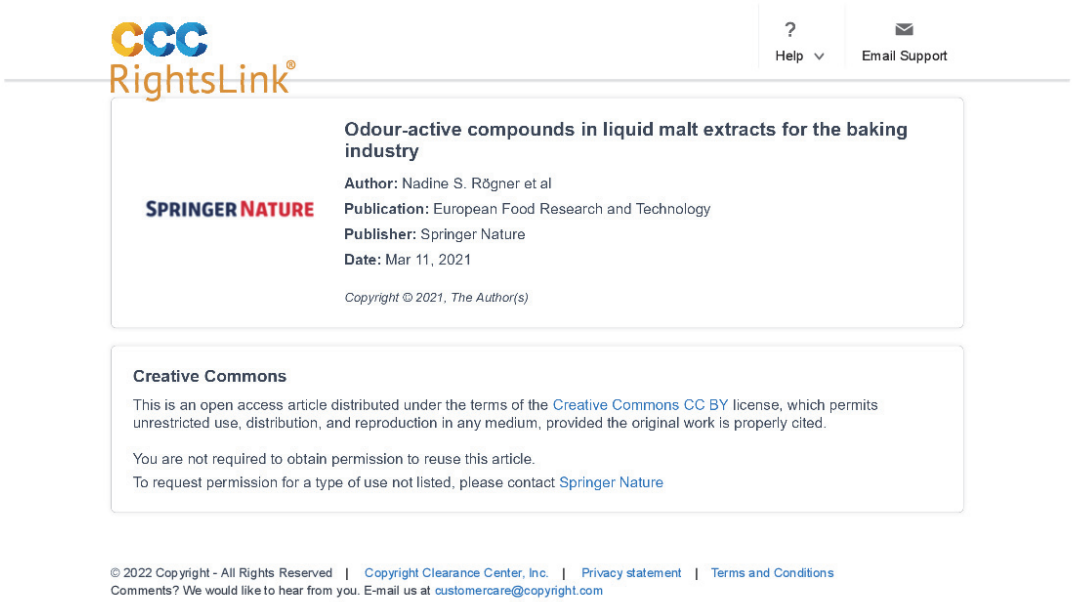
The volatiles from a light and a dark LME were isolated by solvent extraction and SAFE. Application of AEDA on the concentrated distillates resulted in 28 odorants with FD factors between 1 and 1000 in at least one LME. The structures of 24 odorants could be assigned. In both LMEs, high FD factors were found for vanillin and phenylacetic acid. Higher FD factors in the light LME than in the dark LME were determined for HDMF, *trans*-4,5-epoxy-(2*E*)-dec-2-enal, 4-ethenyl-2-methoxyphenol, and phenylacetaldehyde. In the dark LME, higher FD factors were obtained for maltol, dihydromaltol, sotolon, 2-methoxyphenol, acetic acid, as well as 2- and 3-methylbutanoic acid.

To substantiate the results of the AEDA, 15 odorants were quantitated in the light and the dark LME using GC-MS and stable isotopically substituted odorants as internal standards. OAVs were calculated by dividing the odorant concentrations by the OTVs in water. In the light and the dark LME, 12 and 11 odorants, respectively, showed OAVs ≥ 1. In the light LME, high OAVs were calculated for 3-(methylsulfanyl)propanal, (*E*)-β-damascenone, 4-ethenyl-2-methoxyphenol, phenylacetaldehyde, and phenylacetic acid. In the dark LME, sotolon, 3-(methylsulfanyl)propanal, (*E*)-β-damascenone, acetic acid, maltol, and phenylacetic acid exhibited high OAVs. The higher OAV of honey-like smelling phenylacetaldehyde in the light LME was in agreement with the stronger honey-like odor note in the quantitative olfactory profile. Clearly higher OAVs in the dark LME were found for odorants formed by thermal reactions, such as sotolon, maltol, and 2-methoxyphenol. This corresponded to the higher thermal impact during dark LME production.

To get an insight into the odorant changes during LME production, the 15 selected odorants were additionally quantitated in the malt that served as starting material for the two LMEs. To cover both, the free and the bound malt odorants, an approach with the simultaneous addition of the extraction solvent and a minor amount of water was applied. For most odorants the amount in the extracts was clearly lower than in the malt, whereas seven odorants showed significantly higher amounts in at least one LME. In the light LME, (*E*)-β-damascenone and 4-ethenyl-2-methoxyphenol and in the dark LME, maltol, sotolon, (*E*)-β-damascenone, and 2-methoxyphenol were identified as important process-induced odorants. The odorant formation during malt extract production was more important than the mere odorant transfer from the malt to the LMEs.

Nadine S. Rögner designed and performed the experiments including volatile isolation, GC-O analyses, structure assignments, sensory tests, quantitation experiments, and GC-MS analyses. Nadine evaluated the resulting data, designed the graphics, and prepared the manuscript. Veronika Mall conceived the study, performed the synthesis of ($^{13}C_2$)maltol, and revised the manuscript. Martin Steinhaus directed the study, supervised Nadine's work, and revised the manuscript. Veronika and Martin participated in the GC-O analyses and the sensory evaluation.

8.1.4 Reprint Permission



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Odour-active compounds in liquid malt extracts for the baking industry

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8.2 Publication 2: Impact of Malt Extract Addition on Odorants in Wheat Bread Crust and Crumb

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8.2.2 Publication Reprint

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Impact of Malt Extract Addition on Odorants in Wheat Bread Crust and Crumb

Nadine S. Rögner, Veronika Mall, and Martin Steinhaus*

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ABSTRACT: Application of gas chromatography−olfactometry and aroma extract dilution analysis to the volatiles isolated from (1) crust and (2) crumb of a wheat bread made with the addition of a dark liquid malt extract (LME) to the dough and (3) crust and (4) crumb of a reference bread made without addition resulted in the identification of 23 major odorants. Their quantitation followed by the calculation of odor activity values (OAV = ratio of concentration to odor threshold value) suggested that LME addition influenced the aroma of the bread predominantly by increasing seasoning-like smelling sotolon in crust and crumb, and caramel-like smelling compounds maltol and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF) in the crumb. The increase in sotolon and maltol was explainable by direct transfer from the LME to the bread, whereas HDMF must have been formed from LME-derived precursors. This difference needs to be considered in the targeted optimization of LMEs for bread making.

KEYWORDS: *liquid malt extract, wheat bread, aroma extract dilution analysis (AEVA), odor activity value (OAV)*

INTRODUCTION

Liquid malt extracts (LMEs) are concentrated syrup-like malt products, which are frequently used as a bread dough ingredient in the baking industry. LMEs provide fermentable sugars to flours with low diastatic activity, enhance the color of bread crumb and crust, and can also influence bread aroma.

The compounds contributing to the aroma of wheat bread have been studied in detail.[1−10] The aroma of the bread crust is mainly associated with odorants formed by thermal reactions during baking. A key compound in wheat bread crust aroma is roasty, popcorn-like smelling 2-acetyl-1-pyrroline, which shows an exceptionally low odor threshold value (OTV) of 0.0073 μg/kg in starch[5] and thus a high odor activity value (OAV; ratio of concentration to OTV) in the crust. Further important bread crust odorants are caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (HDMF), malty smelling 3-methylbutanal, and cooked potato-like smelling 3-(methylsulfanyl)-propanal.[3,5−7] In contrast, the odorants contributing to wheat bread crumb aroma are predominantly formed during fermentation and include aldehydes such as (2*E*)-non-2-enal and (2*E*,4*E*)-deca-2,4-dienal, alcohols such as 2- and 3-methylbutan-1-ol and 2-phenylethan-1-ol, ketones such as butane-2,3-dione, and acids such as 2- and 3-methylbutanoic acid.[2−4,6,11] Detailed studies elucidated the impact of yeast level, time, and temperature during fermentation,[4,12] the addition of enzymes,[3] and the addition of the distiller's grain[13] on wheat bread odorants. However, to the best of our knowledge, the impact of malt extract addition during dough preparation on the odorants in the finished bread has not been systematically studied yet. Poiana *et al.*[14] and Man *et al.*[15] showed that malt extract addition improved the quality of breads in terms of porosity, bread diameter, and height, but bread aroma was not assessed.

We recently characterized the odor-active compounds of a light and a dark LME.[16] Both LMEs showed intense malty, caramel-like, and honey-like odor notes. Particularly in the dark LME, also roasty, smoky, and seasoning-like odor impressions were perceptible. The volatiles isolated by solvent extraction and solvent-assisted flavor evaporation (SAFE) were screened for odorants by gas chromatography−olfactometry (GC−O) in combination with aroma extract dilution analysis (AEDA). Results were substantiated by quantitation and OAV calculation. In the light LME, potent odorants included cooked potato-like smelling 3-(methylsulfanyl)propanal (OAV 1500), cooked apple-like smelling (*E*)-β-damascenone (OAV 430), clove-like smelling 4-ethenyl-2-methoxyphenol (OAV 91), and honey-like smelling phenylacetaldehyde (OAV 70). In the dark LME, the highest OAVs were determined for seasoning-like smelling sotolon (OAV 780), 3-(methylsulfanyl)propanal (OAV 550), (*E*)-β-damascenone (OAV 410), acetic acid (OAV 160), and caramel-like smelling maltol (OAV 120).

The aim of the present investigation was to study the impact of LME addition on the aroma of wheat bread on a molecular level. The dark extract was chosen because dark LMEs are more widely used to influence the aroma of bread than light LMEs. We screened the volatiles isolated from crusts and crumbs of breads with and without LME addition for odor-active compounds by AEDA and substantiated the results by odorant quantitation and OAV calculation. Finally, the amounts of the odorants added with the LME were compared

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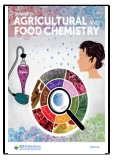

to the amounts of the odorants in the bread to assess the potential role of direct transfer and the role of formation from precursors during bread making.

MATERIALS AND METHODS

Malt Extract. A dark LME was obtained from Ireks (Kulmbach, Germany). The malt extract was from the same batch as used in our previous study on malt extract odorants.[16]

Breads. In a kneading machine (Universal, Robert Bosch Hausgeräte, Munich, Germany), 500 g of wheat flour, type 550 according to the German flour classification system (Rosenmühle, Ergolding, Germany), 20 g of fresh baker's yeast (F. X. Wieninger, Passau, Germany), and 10 g of sodium chloride (Merck, Darmstadt, Germany) were mixed at speed 1 for 30 s. For the reference bread, 290 g of water was added. For the bread with LME addition, 290 g of water and 15 g of LME were premixed and the mixture was added. Doughs were kneaded for 1 min at speed 1 followed by 7 min at speed 3. Dough portions of 400 g were fermented in a proofing cabinet (Heraeus, Hanau, Germany) at 30 °C and 90% relative humidity for 15 min. The doughs were placed in non-stick coated loaf pans (20 cm × 11.5 cm × 7 cm) and further fermented for 35 min. Baking was done in an oven (Piccolo, Winkler Wachtel, Hilden, Germany) at 230 °C for 35 min. The breads were allowed to cool at room temperature for 2 h. The crust (5 mm) was separated from the crumb using a bread slicing machine (Graef, Arnsberg, Germany).

Chemicals. The following reference odorants were purchased from commercial sources: **1**, **3**, **41** (Alfa Aesar, Karlsruhe, Germany), **2**, **4**−**6**, **11**, **12**, **14**, **17**, **20**−**23**, **25**, **26**, **28**, **29**, **31**, **32**, **35**, **36**, **39**, **42**, **43**, and **45**−**48** (Merck, Darmstadt, Germany). Odorant **27** was a gift from Symrise (Holzminden, Germany). Odorants **7**,[17] **8**,[18] **13**,[19] **30**,[20] and **34**[6] were synthesized as detailed in the literature.

The following isotopically substituted odorants were purchased from commercial sources: ($^{13}C_2$)-**36** (aromaLAB, Martinsried, Germany), ($^{2}H_{11}$)-**5a**, ($^{2}H_7$)-**17**, ($^{2}H_5$)-**31** (CDN Isotopes, Quebec, Canada), ($^{2}H_3$)-**11**, and ($^{13}C_2$)-**47** (Merck, Darmstadt, Germany). Compounds ($^{13}C_5$)-**7**,[21] ($^{2}H_3$)-**12**,[22] ($^{2}H_2$)-**14**,[23] ($^{2}H_2$)-**20**,[24] ($^{13}C_2$)-**21**,[25] ($^{2}H_2$)-**22a**,[26] ($^{2}H_4$)-**25**,[27] ($^{2}H_6$)-**27**,[28] ($^{2}H_3$)-**29**,[27] ($^{13}C_2$)-**33**,[16] ($^{13}C_6$)-**41**,[21] ($^{13}C_2$)-**42**,[29] ($^{2}H_3$)-**43**,[30] and ($^{2}H_3$)-**48**[31] were synthesized as detailed in the literature. ($^{13}C_2$)-**26** was prepared according to the approach published by Kiefl *et al.* for the synthesis of ($^{13}C_4$)-**26** but using ethyl (diethoxyphosphoryl)($^{13}C_2$) acetate in combination with (2*E*)-oct-2-enal instead of (2*E*)-(1,2-$^{13}C_2$)oct-2-enal.[21]

Diethyl ether (CLN, Freising, Germany) was freshly distilled through a column (120 cm × 5 cm) packed with Raschig rings.

Aroma Extract Dilution Analysis (AEDA). Bread crust samples and bread crumb samples were frozen with liquid nitrogen and ground into fine powders using a laboratory mill Grindomix GM 200 (Retsch, Haan, Germany) at 4000 rpm (10 s) and 10,000 rpm (10 s). Diethyl ether (285 mL) and water (15 mL) were added to the powder (60 g) and the mixture was stirred at room temperature for 2 h. The supernatant was decanted and the residue was stirred with a second portion of diethyl ether (300 mL). The combined organic phases were dried over anhydrous sodium sulfate. Non-volatiles were removed by SAFE at 40 °C.[32] The volatile fraction was concentrated to a final volume of 1 mL using a Vigreux column (50 cm × 1 cm) and a microdistillation device at a water bath temperature of 40 °C.[33]

The volatile isolates were subjected to GC−O analysis using an FFAP column, 30 m × 0.32 mm i.d., 0.25 μm film thickness (Agilent, Waldbronn, Germany) and the GC−O/flame ionization detector (FID) system detailed in Rögner *et al.*[16] The duration of each GC−O run was 36 min. GC−O analyses were repeatedly performed by three trained and experienced assessors until reproducible results were obtained. Then, the volatile isolates were stepwise diluted to 1:10 with diethyl ether to obtain dilutions of 1:10, 1:100, 1:1000, and 1:10000 of the initial solution. The diluted samples were also analyzed by GC−O. Finally, a flavor dilution (FD) factor was assigned to each odorant, representing the dilution factor of the highest diluted sample in which the odorant was detected during GC−O analysis by any of the three assessors.[34]

Odorant Quantitation. Bread crust and crumb powders were prepared as detailed before. In preliminary experiments on the effect of water addition, either pure diethyl ether (100 mL) or diethyl ether (95 mL) plus water (5 mL) was added to the powder (10 g). For the quantitation of major odorants in breads made without and with LME addition, diethyl ether (19−380 mL) and water (1−20 mL) were added to the powder (0.5−50 g). In all cases, stable isotopically substituted odorants (0.01−100 μg; cf. Supporting Information, Table S1) in diethyl ether (20−200 μL) were added as internal standards. The mixture was stirred at room temperature overnight. The supernatant was decanted and the residue was stirred with a second portion of diethyl ether (20−400 mL). The combined organic phases were dried over anhydrous sodium sulfate, and non-volatiles were removed by SAFE at 40 °C.[32] To reduce background during GC−mass spectrometry (MS) analysis, the SAFE distillate was fractionated into neutral and basic volatiles (NBV) and acidic volatiles (AV) using aqueous sodium carbonate and the procedure detailed by Tatsu *et al.*[35] Fractions NBV and AV were concentrated to final volumes of 0.2−5 mL, and the concentrates were subjected to GC−MS. For the quantitation of odorants **7**, **14**, **17**, **20**, **25**, and **43** in the LME, the workup was done as described by Rögner *et al.*[16] Except for compound **5**, all GC−MS analyses were accomplished using the one-dimensional GC−MS system (**11**, **17**, and **20**), the two-dimensional heart-cut GC−GC−MS system (**14** and **22**), or the comprehensive two-dimensional GC×GC−TOFMS system (**7**, **12**, **21**, **25**−**27**, **29**, **31**, **33**, **36**, **41**−**43**, **47**, and **48**), previously detailed by Rögner *et al.*[16] For the quantitation of compound **5**, the GC−GC−MS system was equipped with a J&W DB-FFAP column, 30 m × 0.32 mm i.d. and 0.25 μm film thickness, in the first oven and a J&W DB-5 column, 30 m × 0.25 mm i.d. and 1 μm film thickness (both Agilent), in the second oven. The temperature of the second oven was kept at 35 °C during analysis.

Odorant concentrations were calculated from the peak area counts of the analyte peak and the internal standard peak in the extracted ion chromatograms of characteristic quantifier ions, the amount of bread or LME used, and the amount of standard added, by employing a calibration line equation obtained by linear regression after the analysis of analyte/standard mixtures in five different concentration ratios, namely, 5:1, 2:1, 1:1, 1:2, and 1:5. The quantifier ions and the calibration line equations are summarized in the Supporting Information, Table S1. Compound **22** was initially quantitated as the sum of isomers; individual concentrations were then determined as recently reported.[16,18]

Determination of the Moisture Content. Bread crust and crumb powders obtained as detailed before were heated to 99 °C until constant weight using an infrared Moisture Analyzer MA35 (Sartorius, Göttingen, Germany).

Sensory Tests. A specially designed, odor-free sensory room with separated booths was kept at 22 ± 2 °C. Tests were performed orthonasally by a trained panel of 16−19 assessors (males and females, aged 21−49). Samples were presented in cylindrical PTFE beakers (5.7 cm height, 4.1 cm i.d.) with lids (VWR, Darmstadt, Germany). Quantitative olfactory profile analyses and three-alternative forced-choice (3-AFC) tests were done with crust and crumb samples (4 g) obtained from freshly baked breads. In the quantitative olfactory profile analyses, the twelve pre-defined descriptors detailed by Rögner *et al.*[16] were used. 3-AFC tests were performed with eyes closed to exclude visual influences. OTVs were determined in odorless starch (Roth, Karlsruhe, Germany) according to the American Society for Testing and Materials procedure[36] and the approach detailed by Schoenauer and Schieberle.[37]

RESULTS AND DISCUSSION

Sensory Characterization of Breads. To get a first idea about the influence of dark LME addition on the aroma of wheat bread, quantitative olfactory profile analyses were applied to a wheat bread with malt extract addition and the reference bread without malt extract addition. Both breads were made on the basis of a standard recipe and a standardized

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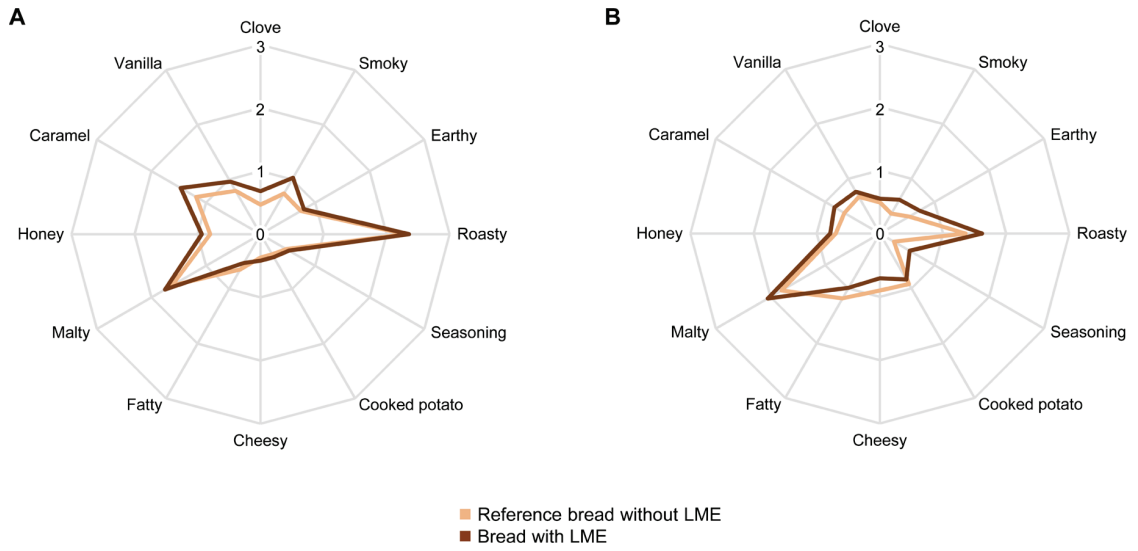

Figure 1. Quantitative olfactory profiles of crust (A) and crumb (B) of breads made without (reference bread) and with addition of LME. Assessors rated the intensity of each descriptor on a scale from 0 to 3 with 0.5 increments and 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong.

procedure. Bread crusts (Figure 1A) and bread crumbs (Figure 1B) were separately evaluated by a trained sensory panel. The olfactory profile of the reference bread crust was dominated by the roasty aroma note, malty and caramel-like notes were also rated comparably high. In the olfactory profile of the bread with LME addition, the malty, honey-like, caramel-like, smoky, and clove-like notes were rated slightly more intense; however, the differences to the reference bread were small. The olfactory profile of the reference bread crumb was characterized by the high intensity of the malty note followed by roasty, fatty, cheesy, and cooked potato-like notes. Again, the aroma of the LME bread showed only slight differences. Smoky, earthy, roasty, seasoning-like, malty, and caramel-like notes were rated higher, whereas fatty, cheesy, and cooked potato-like notes were rated lower.

In view of the minor differences between the olfactory profiles of the reference breads and the breads with LME addition, we applied 3-AFC tests to verify that the samples were distinguishable. Results indeed confirmed a difference for both, the bread crusts and the bread crumbs (cf. Supporting Information, Table S2). The crust of the bread with LME addition showed a highly significant difference to the crust of the reference bread (p value of 0.19%). The test with the crumbs even resulted in a very high significance (p value of 0.0061%). Despite the high similarity in the olfactory profiles, the LME addition thus had a clear impact on the aroma of the bread crust and the aroma of the bread crumb.

Screening for Odor-Active Compounds in Breads. To elucidate the compounds responsible for the aroma impact of LME addition, the volatiles were isolated from the crust and crumb of the breads without and with LME addition by solvent extraction and SAFE. Water was added to the powdered crust and crumb samples before solvent extraction to consider the release of bound odorants by saliva during bread consumption. Particularly in foods with low moisture content such as malt, oat products, and also bread crust, a considerable increase of the odorant concentrations after water addition has previously been observed.[16,38−42] We recently showed that the concentrations of phenylacetaldehyde, phenylacetic acid, and vanillin in malt increased 9−33-fold after water addition. Furthermore, we demonstrated that the addition of a minor amount of water together with the extraction solvent is sufficient to achieve an exhaustive release of the bound odorants.[16] In the current bread study, a preliminary experiment on the influence of water addition before solvent extraction was performed using phenylacetaldehyde and phenylacetic acid as exemplary compounds. Results clearly indicated an effect of water addition to the crust (Figure 2). The concentrations of phenylacetaldehyde and phenylacetic acid increased ∼8-fold and ∼5-fold after water addition. In the bread crumb, however, the differences were negligible. These observations corresponded well to the different water contents of the bread crust and the bread crumb, which were ∼20 and ∼44%, respectively (cf. Supporting Information, Table S3).

The volatile isolates obtained from the crust and crumb of the breads without and with LME addition were subjected to AEDA.[10] A coarse approach with 1:10 dilutions was considered sufficient at this point. On the one hand, there was already extensive knowledge on wheat bread odorants in general available from the literature[1−10] as well as detailed information on the key odorants in the malt extract employed from our previous paper.[16] Thus, discovery of novel important odorants was unlikely. On the other hand, the differences between the breads were later assessed by quantitation in a much more detailed way than possible with AEDA.

The AEDA resulted in 48 odorants with FD factors ≥10 in at least one of the four samples (Table 1). Structure assignments were achieved in three steps. First, retention indices (RIs) and odor qualities were compared to literature data compiled in the Leibniz-LSB@TUM odorant database.[43] The structure proposals were then confirmed by parallel GC−O analyses of the bread volatile isolates and authentic reference compounds using two columns of different polarity (DB-FFAP and DB-5) and finally by parallel GC−MS analyses using a GC×GC−TOFMS system. To reduce coelution problems, the bread volatile isolates were fractionated by acid−base extraction before GC×GC−TOFMS analysis.[35]

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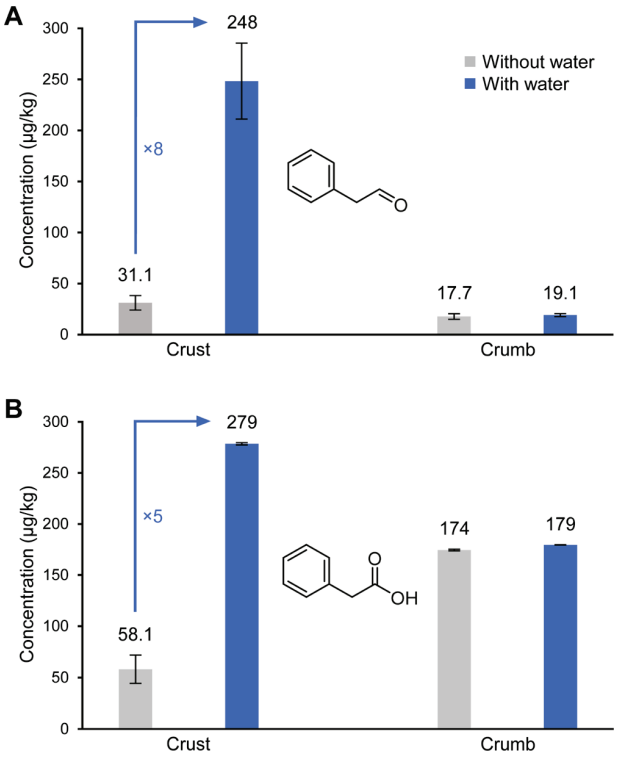

Figure 2. Concentrations of phenylacetaldehyde (A) and phenylacetic acid (B) in bread crust and crumb without and with water addition before extraction. Individual concentration values and standard deviations are available in the Supporting Information, Table S4.

Using the approach detailed above, the structures of 33 out of 48 compounds could be assigned. Due to their low concentrations, the pure mass spectra could not be obtained for oct-1-en-3-one (**6**), (5*Z*)-octa-1,5-dien-3-one (**8**), (2*E*,4*E*,6*Z*)-nona-2,4,6-trienal (**30**), and *trans*-4,5-epoxy-(2*E*)-dec-2-enal (**34**). However, their characteristic odor properties in combination with RI values matching those of authentic reference compounds analyzed under the same conditions allowed for an unequivocal structure assignment. In summary, 37 out of 48 odorants were identified. These 37 odorants included all compounds with FD factors of 100 and 1000 in any of the four samples. Compounds with FD factors of 10 and below were considered of minor importance for the overall aroma.

The outcome of the AEDA agreed well with previous data on wheat bread odorants.[2−7,10] In the bread crust samples, high FD factors were obtained for the popcorn-like smelling 2-acetyl-1-pyrroline (**7**), the cooked potato-like smelling 3-(methylsulfanyl)propanal (**12**), the cheesy smelling 2- and 3-methylbutanoic acids (**22**), the caramel-like smelling compounds maltol (**33**) and HDMF (**36**), the seasoning-like smelling sotolon (**42**), the honey-like smelling phenylacetic acid (**47**), and the vanilla-like smelling vanillin (**48**). On the level of the FD factors, no significant differences were observed between the crust of the reference bread without LME addition and the crust of the bread with LME addition.

In the bread crumb samples, high FD factors were obtained for the cooked potato-like smelling 3-(methylsulfanyl)propanal (**12**), the cheesy smelling 2- and 3-methylbutanoic acids (**22**), the honey-like smelling 2-phenylethan-1-ol (**31**), and the vanilla-like smelling vanillin (**48**). Different from the bread crust samples, the FD factors revealed a clear difference between the crumb of the reference bread without LME addition and the crumb of the bread with LME addition, namely, in the caramel-like smelling maltol (**33**; FD factors 1000 vs 10) and in the seasoning-like smelling sotolon (**42**; FD factors 1000 vs 10). Both compounds are key odorants in the LME used.[16]

In summary, the odorant screening experiments suggested that LME addition led to substantial increases in maltol and sotolon in the bread crumb, which could be a crucial factor for the aroma difference.

Odorant Quantitation and OAV Calculations. To get a more substantial insight into the influence of malt extract addition on the aroma of the bread crust and the bread crumb, 23 odorants were selected for quantitation and OAV calculations. The selection was based on the results of the AEDA, reports on important bread crust and crumb odorants in the literature, as well as on the results of our previous study on the key odorants in the LME used.[16] Quantitations were accomplished by GC−MS using stable isotopically substituted odorants as internal standards to compensate for any losses during the sample work-up.

The results (Table 2) showed odorant concentrations ranging from 0.0684 µg/kg for (*E*)-β-damascenone (**27**) in the reference bread crumb to 160,000 µg/kg for acetic acid (**11**) in the LME bread crumb. To approximate the odor relevance of the compounds in the bread samples, OAVs were calculated as the ratio of the individual concentrations to the OTVs in starch. Three odorants (**25**, **43**, **48**) showed OAVs <1 in all four samples. They were therefore considered irrelevant for the aroma and thus excluded from further experiments.

In contrast, 18 and 20 odorants revealed OAVs >1, i.e., they showed concentrations above their OTVs in the crusts of the reference bread and the bread with LME addition, respectively. In both bread crusts, the highest OAV by far (2900 and 1800) was calculated for roasty, popcorn-like smelling 2-acetyl-1-pyrroline (**7**). This agreed well with previous reports on the importance of this compound in wheat bread crust.[3,5,7] Further compounds with high OAVs in both bread crusts included clove-like smelling 4-ethenyl-2-methoxyphenol (**41**; OAVs 190 and 140), cooked potato-like smelling 3-(methylsulfanyl)-propanal (**12**; OAVs 160 and 230), and caramel-like smelling HDMF (**36**; OAVs 160 and 180). Most odor-active compounds showed only minor differences in the OAVs between the reference bread crust and the crust of the bread with LME addition. An exception was seasoning-like smelling sotolon (**42**), whose OAV increased more than 4-fold from 3.4 to 15 after LME addition.

In the crumbs of the reference bread and the bread with LME addition, 15 and 19 odorants, respectively, showed OAVs >1. High OAVs in both bread crumbs were calculated for cheesy smelling 3-methylbutanoic acid (**22a**; OAVs 110 and 93), popcorn-like smelling 2-acetyl-1-pyrroline (**7**; OAVs 74 and 62), cooked potato-like smelling 3-(methylsulfanyl)-propanal (**12**; OAVs 51 and 50), and malty smelling 3-methylbutan-1-ol (**5a**; OAVs 35 and 42). Like in the bread crusts, also in the bread crumbs, the differences between the reference bread and the bread with LME addition were small for most of the odor-active compounds. This included typical fermentation byproducts (**22a**, **12**, **5a**, **17**, **41**, **20**, **47**, **21**, **22b**, **11**, **31**, and **5b**) and compounds formed by lipid oxidation (**26**, **14**, and **27**).[3,4] Clear differences, however, were obtained for seasoning-like smelling sotolon (**42**) and the caramel-like

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Table 1. Odorants in the Volatile Isolates Obtained from Crust and Crumb of Wheat Breads Made without (Reference Bread) and with Addition of LME (LME Bread)

					FD factor[d]			
			RI[c]		crust		crumb	
no.	odorant[a]	odor[b]	DB-FFAP	DB-5	reference bread	LME bread	reference bread	LME bread
1	2-/3-methylbutanal	malty	938	663	10	10	<1	<1
2	butane-2,3-dione	buttery	992	613	10	10	<1	<1
3	pentane-2,3-dione	buttery	1068	706	10	10	<1	<1
4	hexanal	green	1084	802	<1	<1	10	1
5	2-/3-methylbutan-1-ol	malty	1227	<600	10	10	100	100
6	oct-1-en-3-one[e]	mushroom	1300	980	100	100	10	1
7	2-acetyl-1-pyrroline	popcorn	1333	924	1000	1000	10	1
8	(5*Z*)-octa-1,5-dien-3-one[e]	geranium leaf	1371	982	100	100	<1	<1
9	unknown	earthy	1407		10	10	<1	<1
10	unknown	roasty, earthy	1423		10	10	<1	<1
11	acetic acid	vinegar	1451	638	100	100	100	100
12	3-(methylsulfanyl)propanal	cooked potato	1458	904	1000	1000	1000	100
13	(2*Z*)-non-2-enal	fatty	1505	1149	100	10	10	10
14	(2*E*)-non-2-enal	fatty	1535	1162	100	100	100	100
15	unknown	earthy	1544		10	1	<1	<1
16	unknown	earthy	1556		10	10	<1	<1
17	2-methylpropanoic acid	cheesy	1559	<600	10	10	10	10
18	unknown	roasty	1574		10	<1	<1	<1
19	unknown	fatty	1591		10	1	10	10
20	butanoic acid	cheesy	1630	807	100	100	100	10
21	phenylacetaldehyde	honey	1645	1042	10	10	10	10
22	2-/3-methylbutanoic acid	cheesy	1668	858	1000	1000	1000	1000
23	(2*E*,4*E*)-nona-2,4-dienal	fatty	1708	1216	10	100	10	1
24	unknown	sweaty, fatty	1738		<1	<1	10	<1
25	2-acetyl-2-thiazoline	popcorn	1770	1100	10	10	<1	<1
26	(2*E*,4*E*)-deca-2,4-dienal	fatty	1818	1316	100	100	100	10
27	(*E*)-*β*-damascenone	cooked apple	1819	1386	10	10	<1	<1
28	geraniol	floral	1842	1245	<1	<1	10	1
29	2-methoxyphenol	smoky	1868	1086	10	10	10	10
30	(2*E*,4*E*,6*Z*)-nona-2,4,6-trienal[e]	oat, sweet	1882	1271	10	10	10	10
31	2-phenylethan-1-ol	honey	1919	1117	100	100	1000	1000
32	*γ*-octalactone	coconut	1928	1273	10	10	10	10
33	maltol	caramel	1981	1117	1000	1000	10	1000
34	*trans*-4,5-epoxy-(2*E*)-dec-2-enal[e]	metallic	2009	1374	100	100	100	100
35	*γ*-nonalactone	coconut	2045	1363	1	10	<1	<1
36	HDMF	caramel	2034	1076	1000	1000	100	1000
37	unknown	coconut	2086		<1	<1	10	10
38	unknown	phenolic	2109		10	1	<1	<1
39	*γ*-decalactone	peach	2153	1475	10	1	1	10
40	unknown	phenolic	2200		10	10	10	1
41	4-ethenyl-2-methoxyphenol	clove	2187	1314	100	100	100	10
42	sotolon	seasoning	2218	1123	100	1000	10	1000
43	2′-aminoacetophenone	foxy	2228	1306	10	10	10	10
44	unknown	pungent	2252		10	1	10	10
45	1*H*-indole	fecal	2470	1293	10	10	<1	<1
46	3-methyl-1*H*-indole	fecal	2517	1384	10	10	<1	<1
47	phenylacetic acid	honey	2587	1278	1000	1000	100	100
48	vanillin	vanilla	2593	1398	1000	1000	1000	1000

[a]Each odorant was identified by comparing the RIs on two GC columns of different polarity (DB-FFAP and DB-5), the mass spectrum obtained by GC×GC−TOFMS, as well as the odor quality perceived at the sniffing port during GC−O to data obtained from authentic reference compounds analyzed under equal conditions. [b]Odor quality as perceived at the sniffing port during GC−O. [c]Retention index, calculated from the retention time of the compound and the retention times of adjacent *n*-alkanes by linear interpolation. [d]Flavor dilution factor, the dilution factor of the highest diluted sample obtained from the volatile isolate in which the odorant was detected during GC−O analysis by any of three assessors. [e]GC−MS analysis did not result in a clear mass spectrum, but a comparison of RIs and odor quality with respective data of an authentic reference compound allowed for unequivocal structure assignment.

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Table 2. Concentrations, Orthonasal OTVs in Starch, and OAVs of Important Odorants in Crust and Crumb of Wheat Breads Made without (Reference Bread) and with Addition of LME (LME Bread)

		concentration (μg/kg)[b]			OAV[d]	
no.[a]	odorant	reference bread	LME bread	OTV in starch (μg/kg)[c]	reference bread	LME bread
Crust						
7	2-acetyl-1-pyrroline	20.9	12.8	0.0073[e]	2900	1800
12	3-(methylsulfanyl)propanal	42.2	61.4	0.27[e]	160	230
41	4-ethenyl-2-methoxyphenol	1200	884	6.4	190	140
36	HDMF	2110	2300	13[e]	160	180
22a	3-methylbutanoic acid	1030	831	13[f]	79	64
21	phenylacetaldehyde	216	341	5.8[g]	37	59
33	maltol	47,500	57,700	1400[g]	34	41
5a	3-methylbutan-1-ol	1820	2180	98	19	22
26	(2*E*,4*E*)-deca-2,4-dienal	45.9	47.6	2.7[e]	17	18
47	phenylacetic acid	287	398	23[f]	12	17
17	2-methylpropanoic acid	2640	3030	190[g]	14	16
42	sotolon	7.08	32.4	2.1[h]	3.4	15
20	butanoic acid	1220	1280	100[e]	12	13
14	(2*E*)-non-2-enal	98.0	89.2	12[f]	8.2	7.4
22b	2-methylbutanoic acid	542	362	77[f]	7.0	4.7
11	acetic acid	150,000	138,000	30000[g]	5.0	4.6
31	2-phenylethan-1-ol	1490	1380	470[g]	3.2	2.9
27	(*E*)-β-damascenone	0.224	0.271	0.15[h]	1.5	1.8
29	2-methoxyphenol	2.25	5.29	4.2[e]	<1	1.3
5b	2-methylbutan-1-ol	651	761	760[f]	<1	1.0
25	2-acetyl-2-thiazoline	1.82	1.60	2.4[g]	<1	<1
43	2′-aminoacetophenone	0.524	0.612	2.9[f]	<1	<1
48	vanillin	263	221	440[f]	<1	<1
Crumb						
22a	3-methylbutanoic acid	1410	1210	13[f]	110	93
7	2-acetyl-1-pyrroline	0.544	0.453	0.0073[e]	74	62
12	3-(methylsulfanyl)propanal	13.8	13.6	0.27[e]	51	50
5a	3-methylbutan-1-ol	3390	4150	98	35	42
17	2-methylpropanoic acid	3960	4610	190[g]	21	24
41	4-ethenyl-2-methoxyphenol	136	127	6.4	21	20
20	butanoic acid	1830	1880	100[e]	18	19
26	(2*E*,4*E*)-deca-2,4-dienal	29.9	43.0	2.7[e]	11	16
47	phenylacetic acid	210	301	23[f]	9.1	13
21	phenylacetaldehyde	34.6	72.4	5.8[g]	6.0	12
22b	2-methylbutanoic acid	836	577	77[f]	11	7.5
42	sotolon	0.417	23.9	2.1[h]	<1	11
33	maltol	87.1	9890	1400[g]	<1	7.1
11	acetic acid	118,000	160,000	30000[g]	3.9	5.3
36	HDMF	10.3	62.1	13[e]	<1	4.8
31	2-phenylethan-1-ol	2120	1880	470[g]	4.5	4.0
14	(2*E*)-non-2-enal	50.8	38.9	12[f]	4.2	3.2
5b	2-methylbutan-1-ol	1130	1300	760[f]	1.5	1.7
27	(*E*)-β-damascenone	0.0684	0.177	0.15[h]	<1	1.2
25	2-acetyl-2-thiazoline	0.229	0.169	2.4[g]	<1	<1
29	2-methoxyphenol	0.965	1.40	4.2[e]	<1	<1
43	2′-aminoacetophenone	0.572	0.583	2.9[f]	<1	<1
48	vanillin	156	182	440[f]	<1	<1

[a]Numbers refer to Table 1. [b]Mean of duplicates or triplicates; standard deviations were <20%; individual concentration values and standard deviations are available in the Supporting Information, Tables S5−S8. [c]Orthonasal odor threshold value in starch. [d]Odor activity value, calculated as the ratio of concentration to OTV. [e]Data taken from Rychlik and Grosch.[5] [f]Data taken from Rohleder *et al.*[1] [g]Data taken from Schoenauer and Schieberle.[37] [h]Data approximated using the orthonasal OTV in cellulose taken from Czerny and Grosch.[46]

smelling compounds maltol (**33**) and HDMF (**36**). These compounds increased 57-fold, 114-fold, and 6-fold. All three were not odor-active in the crumb of the reference bread (OAVs <1) but showed concentrations clearly exceeding their odor threshold values in the crumb of the bread with LME addition, corresponding to OAVs of 11, 7.1, and 4.8.

In summary, the OAV calculations confirmed the conclusions drawn from the odorant screening experiments but also provided a more detailed picture. OAV data indicated that

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LME addition influenced the aroma of the bread predominantly by increasing the concentration of seasoning-like smelling sotolon in the crust and by increasing the concentrations of sotolon and the caramel-like smelling compounds maltol and HDMF in the crumb.

The Role of Odorant Precursors. Although sotolon, maltol, and HDMF had been clearly identified as the compounds being causative for the bread aroma changes associated with LME addition and sotolon and maltol were also major odor-active compounds in the LME employed,[16] it remained unclear whether these compounds were simply transferred from the LME to the bread or whether their formation from precursors provided by the LME during bread making additionally played a role. This information, however, is essential if a targeted optimization of LMEs is to be achieved.

To get an insight into the role of odorant precursors, the odorant concentrations in the breads were compared to the amounts of the odorants added with the LME. The odorant concentrations in the LME are depicted in Table 3. They were determined by GC−MS using stable isotopically substituted odorants as internal standards or were taken from our previous paper.[16] To allow for a direct comparison, all data were converted to concentration values in μg per kg dough. To obtain a more comprehensive picture, these calculations were not only applied to sotolon, maltol, and HDMF but included all compounds found to be odor-active in the breads (cf. Table 2).

Table 3. Odorant Concentrations in the LME

no.[a]	odorant	concentration (μg/kg)[b]
11	acetic acid	869000[c]
33	maltol	613000[c]
47	phenylacetic acid	4220[c]
22a	3-methylbutanoic acid	1580[c]
42	sotolon	1330[c]
17	2-methylpropanoic acid	599[d]
22b	2-methylbutanoic acid	309[c]
12	3-(methylsulfanyl)propanal	235[c]
20	butanoic acid	229[d]
31	2-phenylethan-1-ol	115[c]
21	phenylacetaldehyde	107[c]
36	HDMF	35.9[c]
29	2-methoxyphenol	21.9[c]
41	4-ethenyl-2-methoxyphenol	14.0[c]
7	2-acetyl-1-pyrroline	3.19[d]
5a	3-methylbutan-1-ol	3.18[d]
27	(*E*)-β-damascenone	2.45[c]
14	(2*E*)-non-2-enal	1.10[d]
5b	2-methylbutan-1-ol	0.615[d]
26	(2*E*,4*E*)-deca-2,4-dienal	0.519[c]

[a]Numbers refer to Table 1. [b]Mean of duplicates or triplicates; standard deviations were <20%. [c]Data taken from our previous paper on malt extract odorants.[16] [d]Individual concentration values used for mean calculations and standard deviations are available in the Supporting Information, Table S9.

The results are depicted in Table 4. The portion of the bread crust originating from 1 kg of dough contained 4.92 μg of sotolon when no LME was added and 22.5 μg of sotolon when LME was added. This difference of 17.6 μg equaled to an increase of 360% induced by the LME addition, which could be fully explained by direct transfer of sotolon from the LME to the bread. The efficacy of this transfer, calculated by dividing the difference between the two breads (17.6 μg/kg dough) by the amount added with the LME (23.9 μg/kg dough), would then amount to 74%. Further, compounds whose amounts in the bread crust were fully explainable by a direct transfer from the LME included maltol (64% transfer efficacy) and (*E*)-β-damascenone (75% transfer efficacy). However, with a relative increase associated with LME addition of only 21%, their contribution to the sensory difference was only minor. A higher relative increase of 39% was shown by phenylacetic acid whose concentration in the LME was able to explain 98% of the higher amount in the LME bread.

For other compounds showing clearly higher concentrations in the bread with LME addition than in the reference bread, this difference was only to a minor extent explainable by direct transfer, thus indicating that the major part was formed during bread making from precursors present in the LME. This was in particular the case for 2-methoxyphenol (19% of the increase is explainable by direct transfer), 3-(methylsulfanyl)propanal (32% of the increase is explainable by direct transfer), and phenylacetaldehyde (2.2% of the increase is explainable by direct transfer). The large remainder of compounds (**5a** and below in Table 4, crust) were characterized by rather small differences between the reference bread and the bread with LME addition in combination with negligible amounts added with the LME.

In the crumb, the situation was somewhat different from the crust. The concentration levels of important thermally formed compounds in the reference bread crumb were clearly lower than in the reference bread crust, which corresponded to the lower temperatures during baking. This was, for example, the case for maltol (86.8 vs 33,000 μg/kg dough), sotolon (0.416 vs 4.92 μg/kg dough), and HDMF (10.3 vs 1470 μg/kg dough). Consequently, the additional amounts that could be attributed to the LME addition showed a greater effect. For example, a portion of bread crumb originating from 1 kg of dough contained 86.8 μg of maltol when no LME was added but 9860 μg of maltol when LME was added. This corresponded to a 114-fold increase resulting from the LME addition, which was fully explainable by a direct transfer of maltol from the LME to the bread with an efficacy of 89%. An even higher efficacy was calculated for sotolon. Addition of the LME resulted in a 57-fold increase in sotolon in the crumb, namely, from 0.416 to 23.8 μg/kg, which was explainable by a direct transfer from the LME with an efficacy of 98%. Among all other compounds, a major contribution of a direct transfer from the LME was only possible for 2-methoxyphenol (up to 91%) and phenylacetic acid (up to 84%). 2-Methoxyphenol, however, remained below its odor threshold value (cf. Table 2) and the relative increase in phenylacetic acid associated with LME addition amounted only to 1.4-fold, which was low when compared to the data obtained for maltol (114-fold) and sotolon (56-fold).

For HDMF, a substantial contribution of a direct transfer could be excluded. In a portion of the bread crumb originating from 1 kg of dough, HDMF was present at a level of 10.3 μg when no LME was added and 61.9 μg when LME was added. This corresponded to an increase of 500% of which only 1% could be explained by a direct transfer of HDMF from the LME. Thus, 99% of the additional HDMF in the crumb of the bread with LME addition must have been formed from LME-

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Table 4. Amounts of Odorants in the Bread Crusts and Crumbs in Relation to the Amounts Added with the LME

		concentration(μg/kg dough)[b]		difference LME bread vs ref. bread		amount added with LME	
no.[a]	odorant	reference bread	LME bread	μg/kg dough	%	μg/kg dough[c]	explains % difference between breads
Crust							
42	sotolon	4.92	22.5	+17.6	+360	23.9	100
29	2-methoxyphenol	1.56	3.68	+2.11	+140	0.393	19
21	phenylacetaldehyde	150	237	+86.8	+58	1.92	2.2
12	3-(methylsulfanyl)propanal	29.3	42.6	+13.3	+45	4.22	32
47	phenylacetic acid	200	277	+76.9	+39	75.8	98
33	maltol	33,000	40,100	+7090	+21	11,000	100
27	(*E*)-β-damascenone	0.156	0.189	+0.0328	+21	0.0440	100
5a	3-methylbutan-1-ol	1260	1510	+250	+20	0.0571	<1
5b	2-methylbutan-1-ol	452	529	+76.7	+17	0.0111	<1
17	2-methylpropanoic acid	1830	2110	+271	+15	10.8	4.0
36	HDMF	1470	1600	+132	+9.0	0.645	<1
20	butanoic acid	848	889	+41.7	+4.9	4.11	9.9
26	(2*E*,4*E*)-deca-2,4-dienal	31.9	33.1	+1.14	+3.6	0.00932	<1
31	2-phenylethan-1-ol	1040	959	−76.6	−7.4	2.07	
11	acetic acid	104,000	95,900	−8340	−8.0	15,600	
14	(2*E*)-non-2-enal	68.1	62.0	−6.09	−8.9	0.0198	
22a	3-methylbutanoic acid	716	578	−138	−19	28.4	
41	4-ethenyl-2-methoxyphenol	834	614	−220	−26	0.252	
22b	2-methylbutanoic acid	377	252	−125	−33	5.55	
7	2-acetyl-1-pyrroline	14.5	8.91	−5.63	−39	0.0573	
Crumb							
33	maltol	86.8	9860	+9770	+11,000	11,000	100
42	sotolon	0.416	23.8	+23.4	+5600	23.9	100
36	HDMF	10.3	61.9	+51.6	+500	0.645	1.2
27	(*E*)-β-damascenone	0.0680	0.177	+0.109	+160	0.0440	41
21	phenylacetaldehyde	34.5	72.2	+37.7	+110	1.92	5.1
29	2-methoxyphenol	0.960	1.40	+0.433	+45	0.393	91
26	(2*E*,4*E*)-deca-2,4-dienal	29.8	42.9	+13.1	+44	0.00932	<1
47	phenylacetic acid	210	300	+90.1	+43	75.8	84
11	acetic acid	118,000	160,000	+41,900	+36	15,600	37
5a	3-methylbutan-1-ol	3380	4140	+757	+22	0.0571	<1
5b	2-methylbutan-1-ol	1130	1300	+173	+15	0.0111	<1
17	2-methylpropanoic acid	3950	4600	+648	+16	10.8	1.7
20	butanoic acid	1820	1870	+49.8	+2.7	4.11	8.3
12	3-(methylsulfanyl)propanal	13.7	13.5	−0.200	−1.5	4.22	
41	4-ethenyl-2-methoxyphenol	136	126	−9.37	−6.9	0.252	
31	2-phenylethan-1-ol	2110	1870	−239	−11	2.07	
22a	3-methylbutanoic acid	1410	1210	−199	−14	28.4	
7	2-acetyl-1-pyrroline	0.542	0.452	−0.0903	−17	0.0573	
14	(2*E*)-non-2-enal	50.6	38.8	−11.9	−23	0.0198	
22b	2-methylbutanoic acid	834	576	−258	−31	5.55	

[a]Numbers refer to Table 1. [b]Microgram of the odorant in a portion of the bread crust/crumb originating from 1 kg of dough, calculated by multiplying the odorant concentrations in crust and crumb (Table 2) by conversion factors of 0.695 and 0.997, respectively; the conversion factor for the crust was approximated from the data provided in the Supporting Information, Tables S3 and S10, as (dry weight crust × amount crust + dry weight crumb × amount crumb)/(amount dough × dry weight crust); the conversion factor for the crumb was likewise approximated as (dry weight crust × amount crust + dry weight crumb × amount crumb)/(amount dough × dry weight crumb). [c]Odorant concentration in the dough derived from the addition of the LME, calculated by multiplying the odorant concentration in the LME (Table 3) with the LME content in the dough (15 g/835 g).

derived precursors, presumably reducing sugars or reactive C_3 intermediates of the Maillard reaction formed during LME production.[44,45] Similar to the crust, about half of the odorants (**5a** and below in Table 4, crumb) were characterized by rather small differences between the reference bread and the bread with LME addition in combination with negligible amounts added with the LME.

In conclusion, the addition of a dark LME to wheat bread dough was able to significantly influence the aroma of the bread, with the aroma impact on the crumb being greater than that on the crust. Seasoning-like smelling sotolon was a key odorant for the aroma change in the crust, whereas the aroma change in the crumb was due to a substantial increase in the concentrations of sotolon and the caramel-like smelling compounds maltol and HDMF. When aiming at a targeted optimization of LMEs for bread making, it needs to be considered that sotolon and maltol can be directly transferred from the LME to the bread, whereas a direct transfer plays only

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a negligible role for the additional HDMF in the crumb, of which 99% were formed from precursors and a maximum of 1% could be explained by a direct transfer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c05638.

Quantifier ions and calibration line data used in the quantitations, detailed results of the 3-AFC tests, moisture contents and dry weight values of bread crust and crumb, individual concentration data used for mean calculations and standard deviations, and relation of the amount of dough to the amounts of bread crust and crumb obtained after baking (PDF)

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■ ABBREVIATIONS

AEDA, aroma extract dilution analysis; 3-AFC, three-alternative forced-choice; AV, acidic volatiles; FD, flavor dilution; FFAP, free fatty acid phase; FID, flame ionization detector; GC, gas chromatography; GC×GC, comprehensive two-dimensional gas chromatography; GC−O, gas chromatography−olfactometry; HDMF, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one; i.d., inner diameter; LME, liquid malt extract; MCSS, moving column stream switching; MS, mass spectrometry; NBV, neutral and basic volatiles; TOF, time of flight; OAV, odor activity value; OTV, odor threshold value; RI, retention index; SAFE, solvent-assisted flavor evaporation

■ NOMENCLATURE

2-acetyl-1-pyrroline, 1-(3,4-dihydro-2*H*-pyrrol-5-yl)ethan-1-one; 2-acetyl-2-thiazoline, 1-(4,5-dihydro-1,3-thiazol-2-yl)-ethan-1-one; 2′-aminoacetophenone, 1-(2-aminophenyl)-ethanone; (*E*)-*β*-damascenone, (2*E*)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one; *γ*-decalactone, 5-hexyloxolan-2-one; geraniol, (2*E*)-3,7-dimethylocta-2,6-dien-1-ol; maltol, 3-hydroxy-2-methyl-4*H*-pyran-4-one; *γ*-nonalactone, 5-pentyloxolan-2-one; *γ*-octalactone, 5-butyloxolan-2-one; sotolon, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one; vanillin, 4-hydroxy-3-methoxybenzaldehyde

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J. Agric. Food Chem. 2021, 69, 13586−13595

8.2.3 Summary and Individual Contributions

Malt extracts are used in the baking industry as an all-natural ingredient to provide fermentable sugars and to enhance color and aroma of bakery products. The aim of the current study was to investigate the impact of dark LME addition on major odor-active compounds in wheat bread crust and crumb. A bread made without LME addition served as reference.

Bread crusts and crumbs were stirred with solvent and a minor amount of water and the extracted volatiles were separated from nonvolatile compounds by SAFE. The concentrated distillates obtained from the crust and the crumb of breads made without and with the addition of the dark LME were screened for odor-active compounds by GC-O and AEDA. Results showed 48 odorants with FD factors ≥ 10 in at least one of the four samples. The structures of 37 odorants could be assigned. High FD factors in both crust samples were obtained for 2-acetyl-1-pyrroline, 3-(methylsulfanyl)propanal, 2- and 3-methylbutanoic acid, maltol, HDMF, sotolon, phenylacetic acid, and vanillin. In both bread crumbs, high FD factors were found for 3-(methylsulfanyl)propanal, 2- and 3-methylbutanoic acid, 2-phenylethan-1-ol, and vanillin. In the crumb of the bread made with LME addition, high FD factors were additionally determined for maltol and sotolon, indicating that these compounds could be responsible for the aroma differences.

To substantiate the results of the AEDA, 23 odorants were quantitated in the crust and the crumb of breads made without and with LME addition using GC-MS and stable isotopically substituted odorants as internal standards. Results revealed concentrations between 0.0684 µg/kg and 160000 µg/kg. OAVs were calculated by dividing the odorant concentrations by the OTVs in starch. The highest OAV in both bread crusts was determined for 2-acteyl-1-pyrroline, followed by 4-ethenyl-2-methoxyphenol, 3-(methylsulfanyl)propanal, and HDMF. As in AEDA, only minor differences were found between the two crusts. However, for sotolon a higher OAV was calculated in the crust of the LME bread. In the reference bread crumb and the crumb of the bread made with LME addition, high OAVs were found for 3-methylbutanoic acid, 2-acetyl-1-pyrroline, 3-(methylsulfanyl)propanal, and 3-methylbutan-1-ol. Clearly higher OAVs in the crumb of the bread made with LME addition were found for the Maillard reaction products sotolon, maltol, and HDMF.

To get a deeper understanding of the impact of the LME addition on the bread aroma, the odorant concentrations in the breads were compared to the amounts added with the LME. The bread crumb aroma was clearly more affected than the bread crust aroma. The higher concentration of sotolon in the crust and the crumb and of maltol in the crumb of the LME bread was explainable by a direct transfer from the LME to the bread. The additional amount of HDMF in the crumb of the bread made with LME addition must have been newly formed from LME-derived precursors during bread making.

Nadine S. Rögner designed and performed the experiments including bread making, volatile isolation, GC-O analyses, structure assignments, sensory tests, quantitation experiments, and GC-MS analyses. Nadine evaluated the resulting data, designed the graphics, and prepared the manuscript. Veronika Mall conceived the study and revised the manuscript. Martin Steinhaus directed the study, supervised Nadine’s work, and revised the manuscript. Veronika and Martin participated in the GC-O analyses and the sensory tests, such as the quantitative olfactory profiles and the 3-AFC tests.

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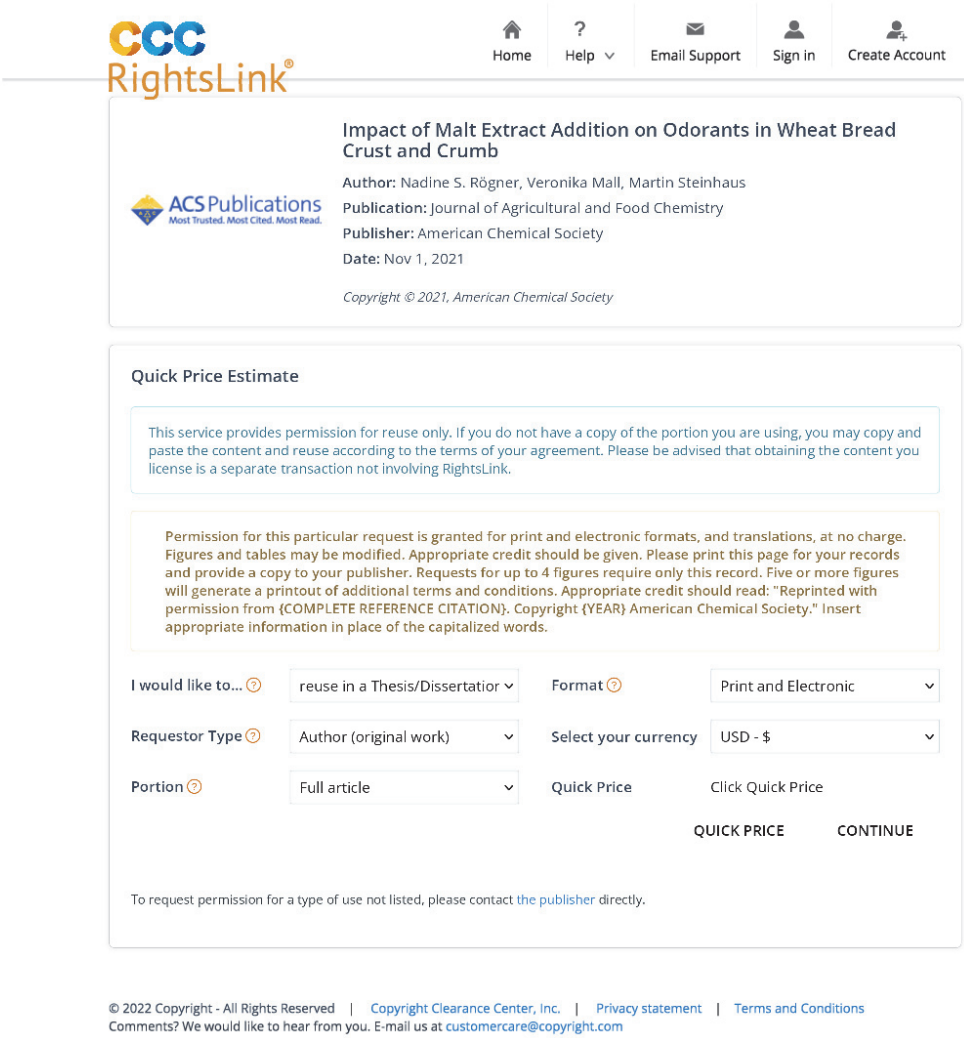

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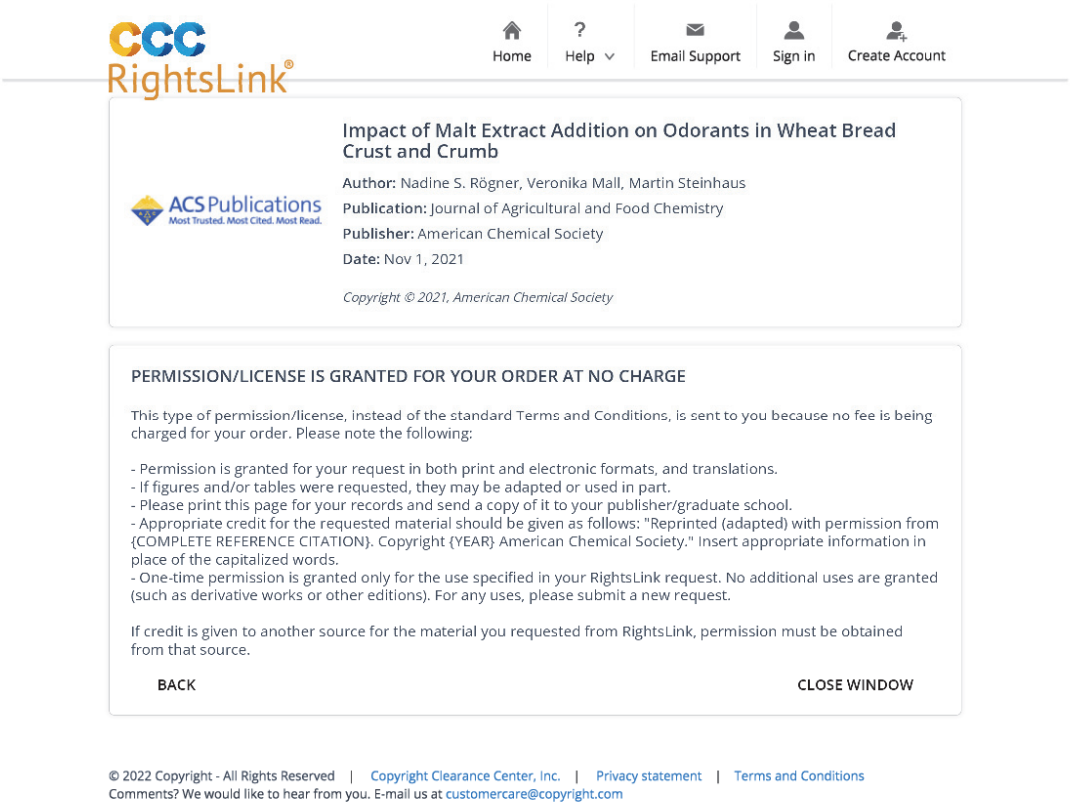

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8.3 List of Publications, Talks, and Poster Presentations

Publications

Publications in peer reviewed journals

Fischer, N. S.; Steinhaus, M. Identification of an important odorant precursor in durian: first evidence of ethionine in plants. *J. Agric. Food Chem.* **2020**, *68*, 38, 10397–10402. DOI: 10.1021/acs.jafc.9b07065

Rögner, N. S.; Mall, V.; Steinhaus, M. Odour-active compounds in liquid malt extracts for the baking industry. *Eur. Food Res. Technol.* **2021**, *247*, 5, 1263–1275. DOI: 10.1007/s00217-021-03707-z

Rögner, N. S.; Mall, V.; Steinhaus, M. Impact of malt extract addition on odorants in wheat bread crust and crumb. *J. Agric. Food Chem.* **2021**, *69*, 13586–13595. DOI: 10.1021/acs.jafc.1c05638

Publications in non-peer reviewed journals

Fischer, N.; Mall, V.; Reglitz, K.; Steinhaus, M. Malz als aromatisierende Zutat beim Brotbacken: Untersuchungen zu Veränderungen der Schlüsselgeruchsstoffe. *Lebensmittel-chemie*. **2020**, *73*, S1, S114. DOI: 10.1002/lemi.201951114

Talks

Characterization of odor-active compounds in malt and their transfer into wheat bread. Forschungsseminar der Lebensmittelchemie, Freising, Germany, November 11, 2019.

Poster presentations

Malz als aromatisierende Zutat beim Brotbacken: Untersuchungen zu Veränderungen der Schlüsselgeruchstoffe. Lebensmittelchemische Gesellschaft (LChG), Fachgruppe in der Gesellschaft Deutscher Chemiker (GDCh), 48. Deutscher Lebensmittelchemikertag (Society of Food Chemistry, a division of the German Chemical Society, 48th National Meeting). Dresden, Germany, September 16–18, 2019.

Bioactive compounds in durian (*Durio zibethinus* L.): first report of ethionine in the plant kingdom. 3rd Global Bioeconomy Alliance Symposium. São Paulo/Ubatuba, Brazil, September 30–October 04, 2019.