

M. Féchir, T. Kraus-Weyermann and J. Voigt

Identification of marker volatiles in malt to predict malt-derived aroma properties of bottom-fermented beers

It is commonly known the malt composition for brewing significantly affects beer sensory properties. Besides the common kilned malt, specialty malts like caramel or roasted malts are often added to produce beers with a range of unique aroma and color properties. However, it has not yet been systematically studied, in what way different malt types quantitatively impact specific beer aroma properties. One pale malt, two caramel malts and two roasted malts were selected to produce five different bottom fermented beers. HS-trap-GC-MS was used to identify volatiles which were correlated with specific beer aroma properties, as judged by a sensory panel. The analysis identified 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde, hexanal, 2-methylbut-2-enal, pentan-1-ol and 2-pentylfuran as suitable marker volatiles in malt to predict malt-derived beer aroma properties that can be described with attributes like toffee, honey, chocolate, vanilla or biscuit. Statistical analysis revealed strong correlations between ten specific beer aroma attributes and these six marker volatiles in malts. Thus, we suggest this approach as an efficient and relatively simple method to improve quality management and product design in the brewing and malting industry and to partially replace resource-intensive trial-and-error testing in pilot plant scale.

Descriptors: specialty barley malt, HS-trap-GC-MS, malt marker volatiles, beer aroma prediction, beer sensory

1 Introduction

Malt is one of the main raw materials for beer production and refers to germinated, heated, dried and thereby preserved cereals. By applying additional technological processes during malting, specialty malts with a unique color and aroma can be manufactured. The characteristics of these specialty malts can be influenced by the variation in drying to induce caramelization [1, 2] or roasting [3–5] to obtain a wide range of malt products featuring various aroma properties and designed for specific demands [6]. In the brewing industry, especially barley malt is of high importance [7]. Besides the common kilned base malt, which is mostly used to provide fermentable carbohydrates, specialty malts are often applied to produce beers with a high variety of unique aroma and color properties compared to traditional, light colored beer types [7, 8].

It is commonly known, that the malt composition significantly affects beer sensory properties [9–11], in case of caramel or roasted malts especially the beer aroma [12, 13]. However, it has not yet been systematically studied, in what way different malt types quantitatively influence the perception of specific olfactory attributes in

beers produced with these malts and how this knowledge can be implemented into new methods for applications in quality management and product design in the malting and brewing industry.

On the one hand, the overall beer aroma and the influence of kilned base malt on beer sensory properties have already been sufficiently studied [14, 15]. On the other hand, to our knowledge, there is no data published on the formation of aroma compounds in specialty malts depending on the applied malting technology, the specific transfer of these compounds to beer and the contribution of specialty malts to the overall beer aroma until now. For other flavor-providing raw materials for beer production, sensory and analytical studies are already available, focusing on aroma compounds in hops [16, 17], the transfer of these compounds to beer [18, 19] and aroma compounds formed by yeast during fermentation [20–22].

The impact of specialty malts on the beer aroma has only been investigated to a very limited extend by applying sensory evaluation but without establishing a connection to quantifiable properties already present in the malts, that would allow for a prediction of beer aroma based on malt composition [6]. Voigt et al. developed and presented a new Malt Aroma Wheel® to practically apply sensory findings for the development of new beer types [23]. Other previous studies predominantly focused on the flavor stability of a specific final product [24], on nitrogen containing heterocycles only [25], on a single malt type [26, 27] or did not distinguish between aroma compounds and volatiles [28, 29]. Herb et al. recently reported that barley genotype and growing conditions as well as the degree of modification affect the contribution of barley malt to

<https://doi.org/10.23763/BrSc21-01fechir>

Authors

Michael Féchir, Jens Voigt, Trier University of Applied Sciences, department of Food Technology, Trier, Germany; Thomas Kraus-Weyermann, Weyermann Specialty Malts, Bamberg, Germany; corresponding author: michael.fechir@oregonstate.edu

the beer aroma [30, 31]. However, these studies mostly focused on sensory evaluation and technological quality parameters of individual barley cultivars for the production of kilned base malts, not specialty malts. Thus, the influence of different malting technologies and the analytical identification of aroma compounds were not considered. Furthermore, only beer, but no raw materials or intermediates were evaluated.

The aim of this study was to identify marker volatiles in malt to predict major malt-derived aroma properties of beer. The aroma of five bottom-fermented lager-type beers produced with different malt types (industrially produced kilned base malt, caramel malts and roasted malts all made from barley) was evaluated with a sensory panel and volatiles in the malts were identified and quantified, which allow for a correlation-based prediction of specific beer aroma properties depending on the malt composition. In this context, it was our goal to introduce a relatively simple and fast analytical method that is able to predict major malt-derived aroma properties of beer by analyzing just the malts selected for brewing without the need for extensive trial-and-error testing in pilot plant scale followed by sensory evaluation of beers with a trained panel to improve quality management and product design in the brewing and malting industry.

2 Materials and methods

2.1 Malts

Kilned base malt (KM), caramel malts (CM1, CM2) and roasted malts (RM1, RM2), each produced from a single batch of barley grains (variety Barke®) of harvest 2016 were provided by Mich. Weyermann (Bamberg, Germany). These malts were industrially produced, caramelization and roasting were performed in a roasting drum. As determined by routine analyses [32], malts complied with product specifications, malting process parameters and technical data are shown below (Table 1).

2.2 Beers

Five beers were brewed using a Braumeister Plus 50 L brewing device (Speidel, Ofterdingen, Germany) by applying different barley malt compositions as follows: I. kilned base malt beer (KMB): 100 % kilned base malt (KM), II. caramel malt beer one (CMB1): 70 % kilned base malt (KM) + 30 % caramel malt one (CM1), III. caramel malt beer two (CMB2): 70 % kilned base malt (KM) + 30 % caramel malt two (CM2), IV. roasted malt beer one

(RMB1): 98 % kilned base malt (KM) + 2 % roasted malt one (RM1), and V. roasted malt beer two (RMB2): 98 % kilned base malt (KM) + 2 % roasted malt two (RM2). A total of 11 kg barley malt were used for each brew and 50 L of water were added to the ground malt, followed by mashing for 20 min at 50 °C, 55 min at 63 °C, 30 min at 73 °C and 10 min at 78 °C. Spent grains were separated by filtration and washed with 10 L of water, which was subsequently unified with the filtrate and boiled for 60 min. After 10 min of boiling, 37.5 g pelletized hops (*Humulus lupulus*), type Hallertauer Perle (Hopsteiner, Mainburg, Germany) were added. After 50 min of boiling, additional 12.5 g were added, resulting in a total dosage of 50 g hop pellets and 20 IBU with an original extract of ≥ 12 °P. The hot trub was separated from the wort and after cooling to 20 °C, 20 g of rehydrated dry yeast *Saccharomyces cerevisiae*, strain W34/70 (Fermentis Lesaffre, Marcq-en-Barœul, France) were added to initiate fermentation in cooled cylindrical tanks (Speidel) at 14 °C. After reaching 80–82 % apparent attenuation as determined with an extract and alcohol measuring device (ALEX 500, Anton Paar, Graz, Austria), yeast was separated by decantation and fermentation was stopped. Beers were then stored for one week at 8 °C and for additional three weeks at 2 °C in 50 L kegs for maturation. Dissolved CO₂ was adjusted to 4.5 g/L before beers were bottled in 0.5 L amber glass bottles sealed with crown caps. With an ALEX 500 extract and alcohol measuring device (Anton Paar), the final ethanol concentrations by volume were determined as 5.08 % (KMB), 4.95 % (CMB1), 4.84 % (CMB2), 4.65 % (RMB1) and 4.27 % (RMB2).

2.3 Chemicals

Acetic acid, 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde and 2-phenylethan-1-ol were purchased from Alfa Aesar (Karlsruhe, Germany), 3-hydroxybutan-2-one, maltol, 2-methoxyphenol, methyl 2-methylbutanoate, 2-methylbut-2-enal and pentane-2,3-dione from Fluka (Munich, Germany), butane-2,3-dione, hexanal, 3-methylbutanoic acid, 4-nonanol, pentanal, pentan-1-ol, 2-pentylfuran and pyrazine from Merck (Darmstadt, Germany). Methanol (SupraSolv) and ethanol (SupraSolv) were purchased from VWR (Darmstadt, Germany).

2.4 HS-trap-GC-MS

A Clarus 680 gas chromatograph (Perkin Elmer, Waltham, MA) was equipped with a Zebron ZB-Wax fused silica column, 30 m × 0.25 mm i.d., 0.25 µm film (Phenomenex, Torrance, CA) and connected to a Clarus SQ 8 mass spectrometer (Perkin Elmer) via a heated (250 °C) transfer line. Helium was used as carrier gas at 1.00 mL/

Table 1 Process Parameters and Technical Data of Malt Types used in this study

malt	trade name	RH _{germ} (%)	T _{kiln} (°C)	T _{cara/roast} (°C)	color (EBC)	extract ^a (%)	RH _{malt} (%)
KM	Barke® Pilsner	43 – 45	80 – 90	–	2.5 – 4.5	80.5	<5.0
CM1	CARAHELL®	42 – 44	^b	110 – 120	110 – 130	73.0	<4.5
CM2	CARAMÜNCH® 2	42 – 44	^b	120 – 130	280 – 320	73.0	<4.5
RM1	CARAFA® 1	x	80 – 90 ^c	190 – 200	800 – 1000	65.0	<3.8
RM2	CARAFA® 3	x	80 – 90 ^c	210 – 220	1300 – 1500	65.0	<3.8

^a Soluble extract, dry basis. ^b Green malt was directly transferred to roasting drum without previous kilning step. ^c Malts were kilned like a regular kilned base malt and subsequently transferred to roasting drum.

min constant flow. Column temperature was set to 35 °C, held for 5 min, ramped at 2 °C/min to 150 °C, then ramped at 3 °C/min to 240 °C and held for 20 min. Mass spectra were obtained in EI mode at 70 eV and 240 °C with a scan range of *m/z* 35–350 and evaluated using TurboMass software (Perkin Elmer). The GC-MS system was connected to a HS-40 autosampler equipped with an air monitoring trap (Perkin Elmer), which was operated using the TurboMatrix software (Perkin Elmer).

2.5 Beer Olfactory Profiles

Beer samples were stored in bottles at 5 °C and incubated at ambient temperature for 1 h prior to opening and sensory evaluation in amber beer sensory glasses by a trained panel (5 females, 7 males; age 22–60). The odor of beers was evaluated on a scale from 0 to 5 with increments of 0.1 using 16 predefined attributes (almond, biscuit, bread, caramel, chocolate, clove, cocoa, coffee, dried fruit, hazelnut, honey, malty-sweet, roasted almond, toffee, vanilla, wood smoke) based on Voigt et al. [23], to describe the malt-derived olfactory properties of these beers. For the training of panelists with respect to the sample matrix, food extracts representing each attribute were added to water/ethanol solution (95.2/4.8; v/v) at different concentrations and compared to beer samples. Beers were evaluated three times by each panelist on three separate days. The final rating for each sample and attribute represents the average of the panelist means.

2.6 Screening of Malt Volatiles

Malt samples were stored vacuum-packed at –15 °C. Prior to analysis, samples were ground frozen using a centrifugal laboratory mill ZM200 (Retsch, Haan, Germany) at 6000 rpm with a 0.5 mm ring sieve. 0.5 g of malt grist were weighed into 20 ml headspace vials and 100 µg 4-nonanol (as 100 µL of a 1 g/L methanolic solution) were added as internal standard (IS). Vials were then sealed with crimp top PTFE/butyl caps and were tempered at 80 °C for 15 min using the HS-40 autosampler prior to analysis. Volatiles were then isolated by applying three successive trap load cycles of 3 min, each with 1.2 min decay time and extraction pressure of 21 psi, 10 min trap dry purge, low trap temperature of 30 °C, high trap temperature of 280 °C, 0.2 min desorb time and trap hold for 20 min. All detected compounds were tentatively identified by matching obtained mass spectra (MS) with the MS database of the American National Institute of Standards and Technology (NIST v. 2.0g, Gaithersburg, MD). Results were verified by comparing mass spectra and retention indices with those of high purity compounds.

2.7 Quantitation of Malt Volatiles

Semi-quantitative determination of identified volatiles was performed by calculating the relative peak area of analytes based on

Table 2 Selected Volatiles Identified in Malts and Calibrations applied for Quantitation

volatile ^a	RI ^b	calibration range (µg/g) ^c	approximation function	R ²
pentanal	978	0.25 – 2048	$y = 0.0813 x^{0.8457}$	0.999
butane-2,3-dione	1001	0.25 – 512	$y = 0.0052 x^{0.9499}$	0.996
methyl 2-methylbutanoate	1025	0.25 – 512	$y = 0.0387 x^{0.9311}$	0.996
pentane-2,3-dione	1075	0.25 – 1024	$y = 0.0086 x^{1.1004}$	0.998
hexanal	1091	0.25 – 512	$y = 0.0332 x^{0.8774}$	0.996
2-methylbut-2-enal	1116	1 – 512	$y = 0.0055 x^{0.9834}$	0.997
pyrazine	1229	0.5 – 512	$y = 0.0459 x^{0.9474}$	0.995
2-pentylfuran	1255	0.5 – 256	$y = 0.0053 x^{1.2914}$	0.997
pentan-1-ol	1295	0.5 – 256	$y = 0.0055 x^{1.0811}$	0.994
3-hydroxybutan-2-one	1311	1 – 512	$y = 0.0037 x^{0.8991}$	0.995
2-ethyl-3,5-dimethylpyrazine	1471	0.5 – 256	$y = 0.0009 x^{1.1723}$	0.997
acetic acid	1474	1 – 8192	$y = 0.0232 x^{0.4112}$	0.997
furan-2-carbaldehyde	1487	0.25 – 1024	$y = 0.0121 x^{0.8772}$	0.995
3-methylbutanoic acid	1885	0.25 – 256	$y = 0.0006 x^{1.1728}$	0.992
2-methoxyphenol	2094	0.5 – 512	$y = 0.0075 x^{1.0681}$	0.996
2-phenylethan-1-ol	2103	0.5 – 512	$y = 0.0035 x^{0.6107}$	0.998
maltol	2228	2 – 1024	$y = 0.0018 x^{0.6305}$	0.997

^a Selected volatiles identified in malts listed in order of increasing retention index. ^b Retention index on ZB-WAX capillary column determined with a homologous series of alkanes (C6 – C26). ^c Given as the range between lower and upper limit of quantitation; calibration was performed with at least nine different concentrations in triplicate; calibration range was defined as concentration range with CV < 20 % between individual signal intensities of triplicates.

the IS (peak area analyte/peak area IS). For this approach, a linear context between peak area or concentration of analytes and peak area or known concentration of the IS had to be assumed as an approximation to identify significant quantitative differences between samples based on a one-way Analysis of Variance (ANOVA). For subsequent exact quantitation of selected volatiles, serial dilutions of pure compounds in methanol were created and 100 µl of each dilution including IS were added to 0.5 g starch (from wheat, Merck). Calibrations were then performed by measuring at least nine dilutions for each compound in triplicate and applying exponential regression. Calibration range between lower and upper limit of quantitation was determined as concentration range with CV < 20 %. Calibration details are shown in table 2.

2.8 Statistical Analysis

Principal Component Analysis (PCA), Partial Least Squares Regression (PLS-R), linear and exponential regression as well as one-way Analysis of Variance (ANOVA) were performed using XLSTAT Premium 2020.4.1 (Addinsoft, Boston, MA), confidence intervals were set to 95 %.

3 Results and discussion

3.1 Beer Olfactory Profiles

Beers KMB, CMB1, CMB2, RMB1 and RMB2 were evaluated by a trained sensory panel and olfactory profiles were determined, revealing significant differences between all five beers. Thereby,

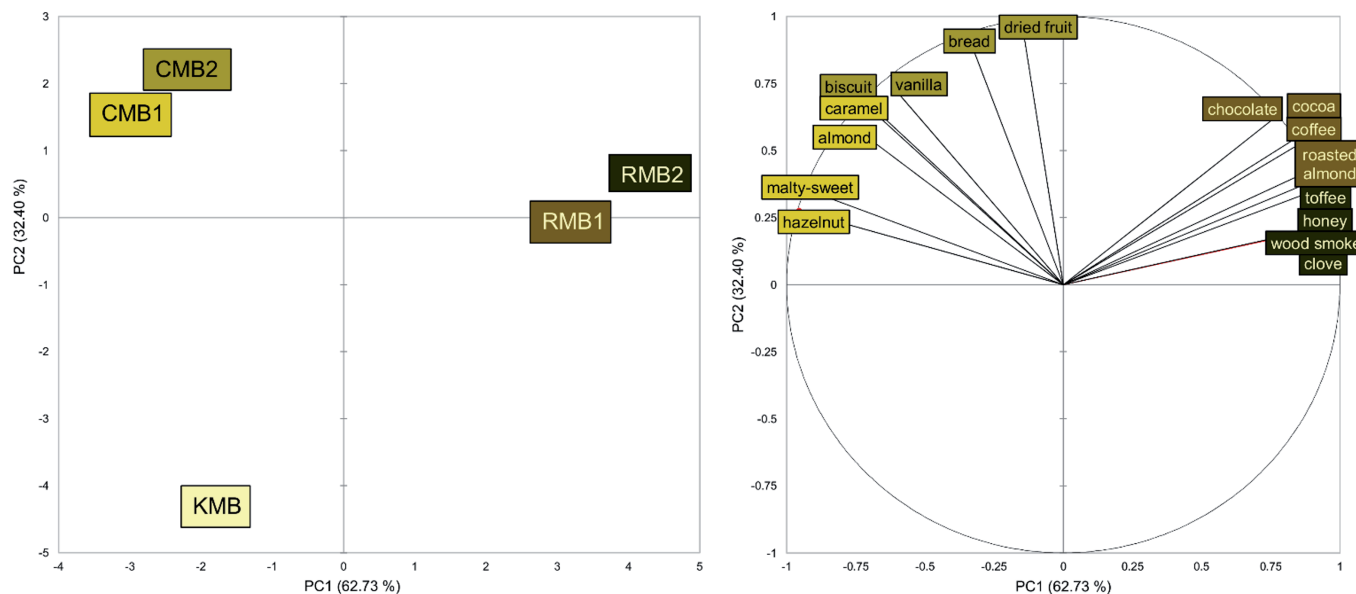


Fig. 1 Overall differences between beers KMB, CMB1, CMB2, RMB1, and RMB2 (left) and contribution of sensory attributes (right) based on PCA of beer olfactory profiles

KMB exhibited the overall weakest olfactory profile. The partial substitution of kilned base malt with caramel or roasted malts to produce beers CMB1, CMB2, RMB1 and RMB2 resulted in a significant increase in intensity for almost all attributes, which is well in line with recent literature [6].

These observations were verified by PCA, displaying strongest differences in olfactory profiles between beers produced with 100% kilned base malt, 30% caramel or 2% roasted malts as shown by a clear separation in figure 1 (left). Principal component 1 (PC1) clearly separates KMB from the specialty malt beers, whereas principal component 2 (PC2) separates caramel and roasted malt beers from each other. Differences between CMB1 and CMB2 as well as RMB1 and RMB2 were less pronounced for both components but still significant. PC1 (62.73%) and PC2 (32.40%) in sum covered 95.13% of the total variation in the data set. Based on correlations on axes PC1 or PC2 (Fig. 1, right), olfactory attributes can be grouped into 1) hazelnut and malty-sweet, 2) almond, caramel, biscuit and vanilla, 3) bread and dried fruit, 4) chocolate, cocoa and coffee, 5) roasted almond, toffee and honey and 6) wood smoke and clove. The closer to parallel an attribute line is to axes PC1 or PC2, the higher the contribution of that attribute to the respective principal component. Thus, attributes hazelnut, malty-sweet, wood smoke and clove mostly contribute to PC1, whereas PC2 was mostly defined by attributes bread and dried fruit. Attributes like biscuit, vanilla, chocolate and cocoa equally contributed to both principal components. The attributes in group 1) were predominantly present in the kilned base malt and were negatively correlated with the attributes in group 5), which were most dominant in the roasted malts. The odor of CMB1 was mostly defined by the attributes in groups 1) and 2), whereas CMB2 was mainly characterized by the attributes in groups 2) and 3). Furthermore, the attributes in groups 4) and 5) were most dominant in RMB1. In contrast, the odor of RMB2 could best be described by applying attributes in groups 5) and 6). These findings were well in line with recent studies on the sensory properties of beers produced with different types of specialty malts [6, 24–26].

3.2 Screening of Malt Volatiles

As previously reported, HS-GC-MS is a versatile and promising tool to analyze the volatile composition of specialty malts [33]. Thus, malts KM, CM1, CM2, RM1 and RM2, which were used to produce the respective beers, were analyzed by applying HS-trap-GC-MS to perform a screening of volatiles. A total of 56 volatile compounds was identified in at least one of the malts by first matching obtained mass spectra (MS) with the NIST MS database and subsequently comparing MS and retention indices (RI) with those of high purity compounds. In accordance with the overall weakest olfactory profile of beer KMB, only 33 of 56 volatile compounds were detected and identified in malt KM. In contrast, 44 different compounds were detected and identified in CM1, 41 in CM2, 51 in RM1 and 46 in RM2. Most of the identified compounds were already reported as volatiles in kilned base malts [33], caramel malts [24, 34] or roasted malts [3, 5, 23].

3.3 Quantitation of Malt Volatiles

To approximate major quantitative differences in volatile composition between the malts, all 56 previously identified volatiles were semi-quantitatively determined based on an internal standard. Results were then statistically analyzed by ANOVA, which revealed most significant differences between malts for a specific set of 17 volatiles. To verify these findings and to determine these compounds in malts in a more precise way, calibrations with at least nine different concentrations per compound were performed in starch to determine the calibration range with respect to the sample matrix.

Quantitation was then performed by applying the respective approximation functions with coefficients of determination (R^2) of ≥ 0.992 as obtained by calibration. As a result of calibrations being performed with serial dilutions to cover a wide range of concentrations, exponential regression was selected to best approximate calibration measurements. Obtained concentrations in malts varied between $< 0.25 \mu\text{g/g}$ for methyl 2-methylbutanoate in CM1 and

RM2 and 6300 µg/g for acetic acid in CM2, CV was consistently <20% (Table 3). Overall, concentrations of most quantified volatiles were highest in CM2 or RM1, whereas KM exhibited significantly lower concentrations for almost all volatiles except for maltol. A high number of volatiles showed comparable concentration ranges in RM1 and RM2 with the exception of primarily hexanal, maltol and methyl 2-methylbutanoate. In contrast, CM1 showed greater similarities to KM than to CM2. Nevertheless, the overall volatile composition was significantly different between all five malt samples, which was well in line with the previously discussed olfactory profiles of beers produced with these malts. Volatiles maltol and 2-ethyl-3,5-dimethylpyrazine were previously reported as important aroma compounds in caramel and roasted malts [3, 33]. Furthermore, acetic acid and 2-phenylethan-1-ol were identified as odorants in caramel malt [25, 34], whereas volatiles pentane-2,3-dione, pentan-1-ol, 2-methylbut-2-enal, hexanal, 2-pentylfuran and furan-2-carbaldehyde were already identified in roasted malt in 1994 [35]. The phenolic compound 2-methoxyphenol, which has a smoky smell, is a well-known malt-derived odorant in especially darker beers [23].

Based on the results displayed in table 3, concentrations of these volatiles in malt mixtures applied for producing beers KMB, CMB1, CMB2, RMB1 and RMB2 were calculated according to the percentages of each malt used for brewing (100 % KM for beer KMB, 70 % KM + 30 % CM1 for beer CMB1, 70 % KM + 30 % CM2 for beer CMB2, 98 % KM + 2 % RM1 for beer RMB1, 98 % KM + 2 % RM2 for beer RMB2, Table 4).

Concentrations of volatiles introduced into the brewing process by malt mixtures as shown in table 4 were used as a basis for correlations with previously discussed beer olfactory profiles determined with a trained sensory panel to identify volatile compounds in malts suitable for predicting specific sensory attributes in beers as described in the following chapter.

3.4 Correlation of Beer Olfactory Profiles and Malt Volatiles

Correlation models created by combining sensory and analytical data on volatiles have already been proven to be a valuable tool in other malt-related food categories like single-malt whisky [36, 37]. With regards to beer, correlation studies have only been conducted to trace back

beer flavor stability to technological malt quality parameters [38] or to investigate hop-related beer flavor properties [39]. In contrast, quantitatively predicting beer aroma properties solely based on

Table 3 Concentration of Selected Volatiles in KM, CM1, CM2, RM1 and RM2

volatile	RI	concentration in malt (µg/g) ^a				
		KM	CM1	CM2	RM1	RM2
pentanal	978	41	47	55	2100	1400
butane-2,3-dione	1001	1.6	19	160	93	64
methyl 2-methylbutanoate	1025	1.2	< 0.25	0.77	1.73	< 0.25
pentane-2,3-dione	1075	1.5	1.1	12	11	11
hexanal	1091	1.6	2.8	3.1	150	26
2-methylbut-2-enal	1116	0.49	29	80	4.3	3.4
pyrazine	1229	n.d. ^b	n.d.	n.d.	23	17
2-pentylfuran	1255	1.5	1.6	6.8	15	6.8
pentan-1-ol	1295	n.d.	3.2	2.8	15	23
3-hydroxybutan-2-one	1311	6.3	1.5	740	430	720
2-ethyl-3,5-dimethylpyrazine	1471	1.6	1.9	3.2	39	86
acetic acid	1474	130	2200	6300	3200	1400
furan-2-carbaldehyde	1487	1.2	6.1	19	420	490
3-methylbutanoic acid	1885	0.29	0.32	57	14	35
2-methoxyphenol	2094	n.d.	n.d.	n.d.	100	180
2-phenylethan-1-ol	2103	18	69	1300	1400	1200
maltol	2228	110	23	4.1	6.4	73

^a Displayed with 2 significant digits as mean of triplicates with CV < 20 %. ^b Compound not detected in the respective sample.

Table 4 Concentration of Selected Volatiles in Malt Mixtures used for Brewing Beers KMB, CMB1, CMB2, RMB1 and RMB2

volatile	RI	concentration in malt mixtures for brewing (µg/g)				
		KMB ^a	CMB1 ^b	CMB2 ^c	RMB1 ^d	RMB2 ^e
pentanal	978	41	43	45	82	68
butane-2,3-dione	1001	1.6	6.8	49	3.4	2.8
methyl 2-methylbutanoate	1025	1.2	0.92	1.1	1.2	1.2
pentane-2,3-dione	1075	1.5	1.4	4.7	1.7	1.7
hexanal	1091	1.6	2.0	2.1	4.6	2.1
2-methylbut-2-enal	1116	0.49	9.0	24	0.57	0.55
pyrazine	1229	n.d.	n.d.	n.d.	0.46	0.34
2-pentylfuran	1255	1.5	1.5	3.1	1.8	1.6
pentan-1-ol	1295	n.d.	0.96	0.84	0.30	0.46
3-hydroxybutan-2-one	1311	6.3	4.9	230	15	21
2-ethyl-3,5-dimethylpyrazine	1471	1.6	1.7	2.1	2.3	3.3
acetic acid	1474	130	750	1900	770	410
furan-2-carbaldehyde	1487	1.2	2.7	6.5	9.6	11
3-methylbutanoic acid	1885	0.29	0.30	17	0.56	0.98
2-methoxyphenol	2094	n.d.	n.d.	n.d.	2.0	0.020
2-phenylethan-1-ol	2103	18	33	400	46	42
maltol	2228	110	84	78	110	110

^a Calculated as $\text{conc}_{\text{KMB}} = \text{conc}_{\text{KM}} (\text{Table 3})$. ^b Calculated as $\text{conc}_{\text{CMB1}} = 0.7 \times \text{conc}_{\text{KM}} (\text{Table 3}) + 0.3 \times \text{conc}_{\text{CM1}} (\text{Table 3})$. ^c Calculated as $\text{conc}_{\text{CMB2}} = 0.7 \times \text{conc}_{\text{KM}} (\text{Table 3}) + 0.3 \times \text{conc}_{\text{CM2}} (\text{Table 3})$. ^d Calculated as $\text{conc}_{\text{RMB1}} = 0.98 \times \text{conc}_{\text{KM}} (\text{Table 3}) + 0.02 \times \text{conc}_{\text{RM1}} (\text{Table 3})$. ^e Calculated as $\text{conc}_{\text{RMB2}} = 0.98 \times \text{conc}_{\text{KM}} (\text{Table 3}) + 0.02 \times \text{conc}_{\text{RM2}} (\text{Table 3})$.

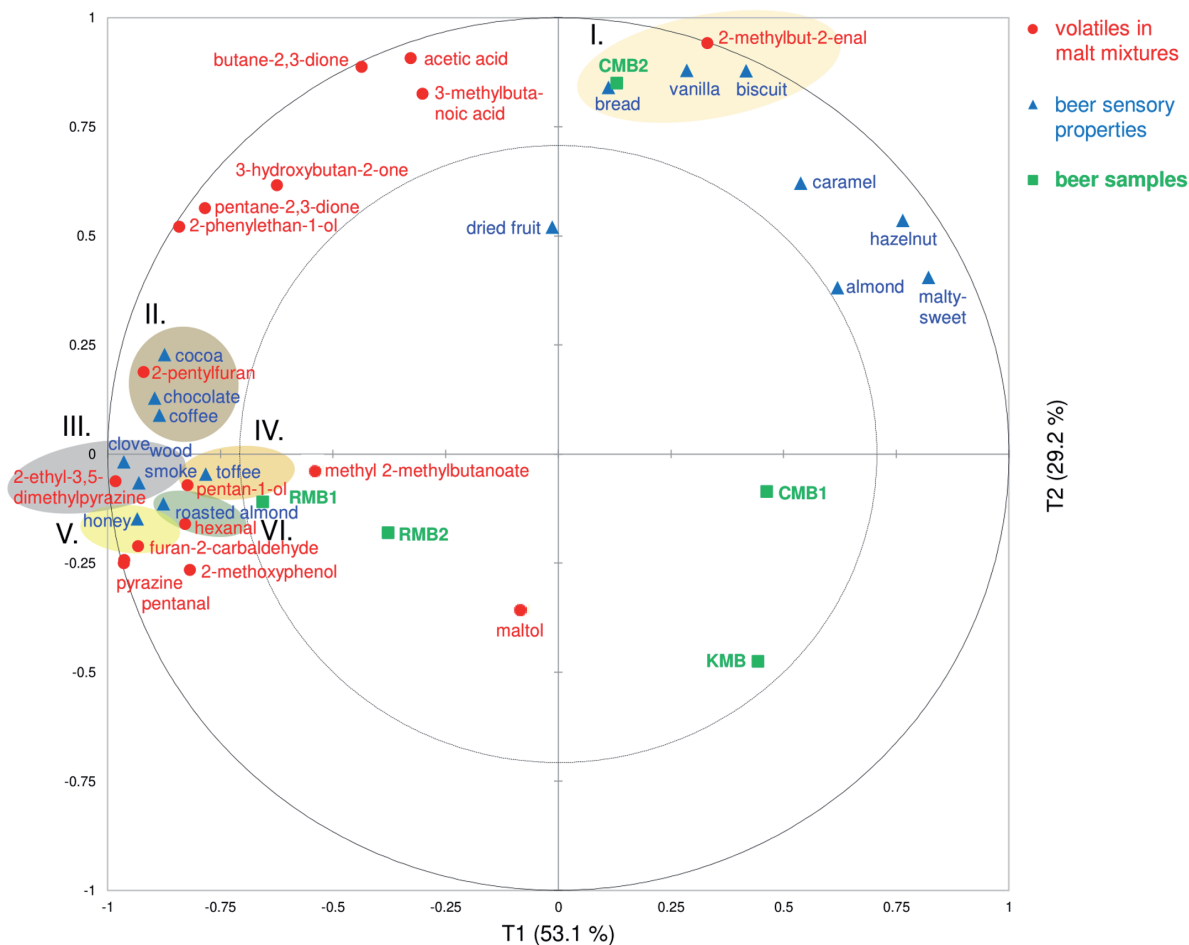


Fig. 2 Correlation of volatiles quantified in malts and olfactory profiles of respective beers by means of PLS-R; colored areas show strongest correlations between volatiles in malt mixtures and olfactory attributes in beers

the concentration of specific volatiles in malt is a new approach, that has not been systematically studied until now.

To identify marker volatiles among the 17 volatiles quantified in malts that allow for such a prediction of specific beer olfactory properties, obtained volatile concentrations in malt mixtures applied for brewing (Table 4) and beer olfactory profiles were further statistically evaluated and correlated by means of PLS-R (Fig. 2). As shown by this two-dimensional model, which covers 82.2 % of the variation in the data set by combining factors T1 (53.1 %) and T2 (29.2 %), a close physical proximity reflects a strong correlation between displayed properties and samples. The further away a volatile or an attribute is located from the central intersection, the higher their contribution to the factors T1 or T2. This is further illustrated by the two concentric circles in the diagram with the smaller circle showing the 50 % contribution threshold and the larger circle indicating the 100 % contribution threshold. Thus, attributes and volatiles located within the small circle (maltol, methyl 2-methylbutanoate, and dried fruit) only contribute with less than 50 % to each of the factors, indicating that they are less significant for differentiating the malts and beers from each other. In contrast, the majority of attributes and volatiles contribute with 50–100 % to each factor and are therefore located between the two concentric circles. Depending on the proximity to either the T1 or T2 axes, volatiles and sensory attributes contribute more towards the respective factor.

Among beers, RMB1 and RMB2 showed the overall greatest similarities to each other in olfactory profiles and volatiles in applied malt mixtures. A comparable level of similarities could be observed between beers CMB1 and KMB, whereas CMB1 and CMB2 as well as every other sample pairing exhibited a significantly lower level of similarities.

As previously discussed, KMB and CMB1 were characterized by the olfactory attributes almond, malty-sweet, hazelnut and caramel (CMB1 slightly more intense than KMB) with a low to medium concentration range of most volatiles in the respective malt mixtures compared to the other samples. Beer CMB2 could best be described by the attributes bread, vanilla, biscuit and dried fruit. The respective malt mixture consisting of 70 % KM and 30 % CM2 exhibited high concentrations of acetic acid, butane-2,3-dione, 3-methylbutanoic acid and 2-methylbut-2-enal. The odor of beers RMB1 and RMB2 was mostly characterized by attributes like toffee, roasted almond, coffee, chocolate and wood smoke, whereby coffee and roasted almond were most dominant in RMB1. In malt mixtures for producing beers RMB1 and RMB2, volatiles 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde, pentanal and pyrazine were present in high concentrations compared to the kilned base malt and the caramel malts, whereas the volatile compound 2-methoxyphenol was not detected in any of the other malts but only in the roasted malts RM1 and RM2.

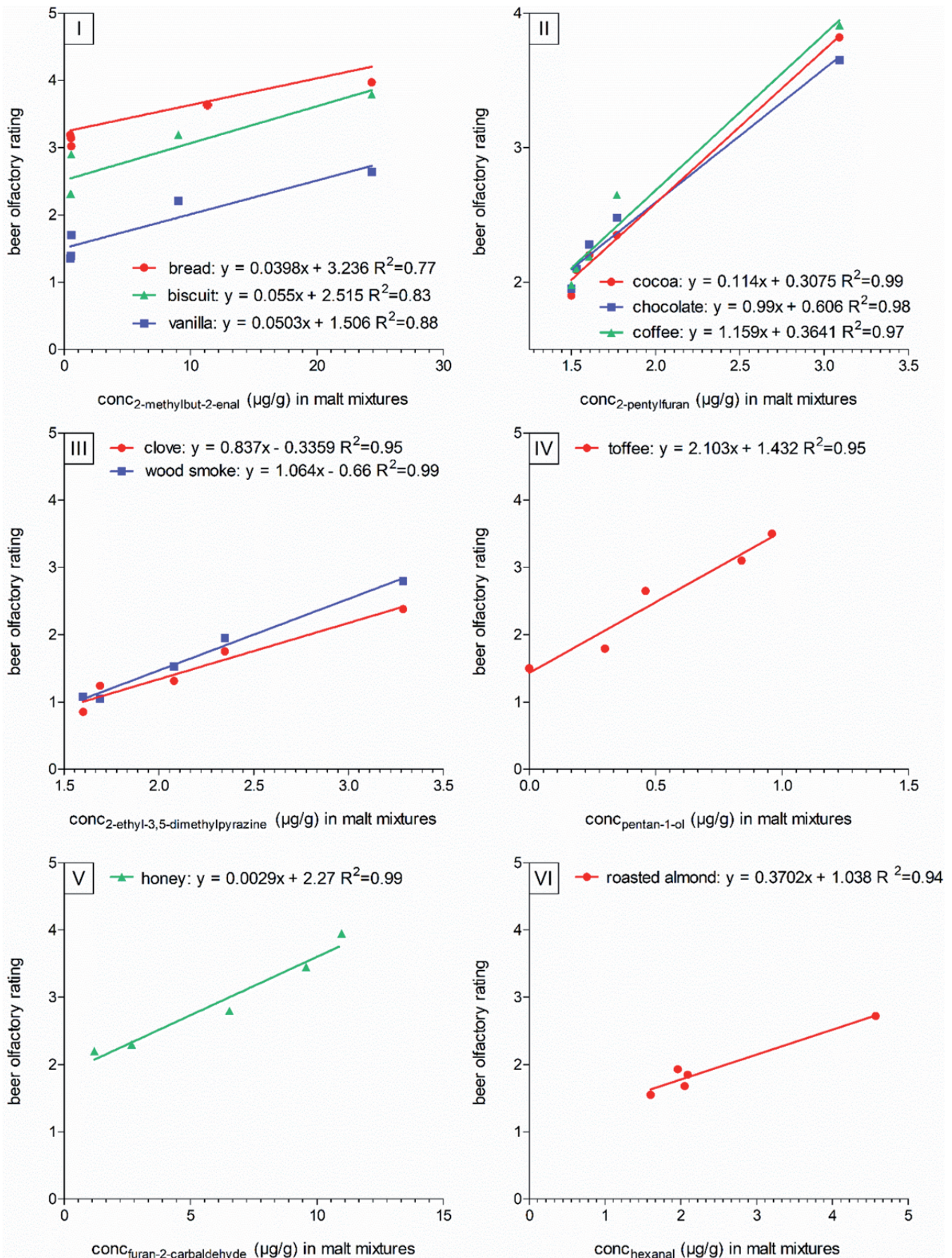


Fig. 3 Correlation of selected volatiles in malt mixtures applied for brewing and olfactory profiles of respective beers by means of linear regression

Based on the PLS-R model, six of the quantified malt volatiles exhibited strong correlations with specific olfactory attributes in beers as follows: I) 2-methylbut-2-enal with bread, vanilla and biscuit, II) 2-pentylfuran with cocoa, chocolate and coffee, III) 2-ethyl-3,5-dimethylpyrazine with clove and wood smoke, IV) pentan-1-ol with toffee, V) furan-2-carbaldehyde with honey and VI) hexanal with dried fruit as indicated by colored markings in figure 2.

To verify the correlations between beer sensory attributes and malt volatiles as shown by PLS-R and to determine the accuracy of potential predictions, linear regression was performed for previously identified correlation groups I) - VI) (Fig. 3). Approximation functions were determined and coefficients of variation ranged between 0.77 and 0.99, confirming these six compounds as suitable marker volatiles in malt for predicting specific beer olfactory properties. Linear functions for correlations between 2-ethyl-3,5-dimethylpyrazine and attributes clove and wood smoke exhibited negative y -intercepts, suggesting that these two correlations might not be applicable for malt mixtures with concentrations below 1.6 $\mu\text{g/g}$. However, considering that this concentration of the known Maillard reaction product 2-ethyl-3,5-dimethylpyrazine [3, 33] was observed in the kilned base malt, it is unlikely to detect lower concentrations in other malt mixtures, for which this model might be used in routine applications.

As shown in figure 3, higher concentrations of 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde, hexanal, 2-methylbut-2-enal, pentan-1-ol and 2-pentylfuran in malt mixtures applied for brewing were connected to an increased perception of the respective olfactory attributes in beers produced with these malts, confirming the results of recent studies, which identified these volatiles as important aroma compounds in caramel and roasted malts by GC-O and GC-MS [4, 35, 40]. Among other volatiles identified in the analyzed malts, furan-2-carbaldehyde and 2-pentylfuran are known as Maillard-reaction products formed during intense thermal processing of cereals like caramelization or roasting [41]. 2-methylbut-2-enal exhibited highest concentrations in the caramel malts, proposing that this compound is mostly formed at medium temperatures of 120–180 °C during caramel malt production and is partially evaporated or decomposed at temperatures above 200 °C during malt roasting. In contrast, 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde, hexanal and pentan-1-ol were mostly present in the roasted malts, suggesting that the formation of these compounds is highly increased at temperatures above 200 °C during malt roasting compared to lower temperatures during the production of kilned base malts and caramel malts.

Although these marker volatiles were reported as aroma compounds in the past, the results presented in this study do not allow for a conclusion, if suggested marker volatiles 2-ethyl-3,5-dimethylpyrazine, furan-2-carbaldehyde, hexanal, 2-methylbut-2-enal, pentan-1-ol and 2-pentylfuran are themselves odor-active and thereby the cause for the increased perception of specific olfactory attributes in beers. However, it is at least very likely, that these marker volatiles are formed during malting and transferred to beer at a similar rate compared to possibly unknown odorants, which are in comparison either present in much lower concentrations or significantly harder to detect. Since analyzing these odorants as part of the quality management in small- to medium-sized

breweries or malting companies is not feasible without substantial investment in specialized analytical equipment [14, 17, 19] and personnel, we suggest the described method based on analyzing marker volatiles in malts as an efficient and relatively simple way to predict major malt-derived aroma properties of beer without the need for time- and resource-consuming brewing trials followed by sensory evaluation.

4 Conclusion/Summary

This study represents the first approach to introduce a relatively simple and fast analytical method that is able to predict major malt-derived aroma properties of bottom-fermented beers by solely analyzing the malts selected for brewing without the need for extensive trial-and-error testing in pilot plant scale followed by sensory evaluation of beers with a trained panel. This approach will not be able to fully replace beer sensory evaluation as part of the quality control, however, it is a useful tool especially for product design and the development of new brewing recipes and beer styles.

To further optimize this method for industrial routine application, it should be possible to reduce the number of calibration points for each marker volatile to five without a significant decline in performance, depending on expected concentration ranges or malt types. Since the batch size for brewing in this study was only 50 L and malt compositions were relatively simple with either 30 % caramel malt or 2 % roasted malt in addition to kilned base malt, however, further research has to be conducted to investigate, if the prediction model exhibits the same accuracy when applied to industrial scale brewing of specialty malt beers with more complex malt compositions. Furthermore, future studies have to show, how fermentation with different yeast strains will affect the predictability of malt-derived beer aroma properties and if this method is also applicable to beers produced with other malted cereals besides barley like wheat, which is most commonly used to produce top-fermented beers.

Acknowledgements

This IGF Project of the FEI (18669N) was supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

Conflict of Interest

The authors declare no competing financial interest.

5 References

1. Narziss, L.; Miedaner, H. and Koch, M.: Studies of volatile substances during malt and beer production with special consideration of those arising as a result of heating. 1. Analysis, identification and effects on aroma, *Monatsschrift für Brauwissenschaft*, **41** (1988), no. 9, pp. 344-352.
2. Liscomb, C. and Hansen, R.: Kilned Versus Roasted: A Differentiation of Caramelized Specialty Malts, *Tech. Q. Master Brew. Assoc. Am.*, **54**

- (2017), no. 1, pp. 47-51.
3. Vandecan, S. M. G.; Daems, N.; Schoupe, N.; Saison, D. and Delvaux, F. R.: Formation of flavor, color, and reducing power during the production process of dark specialty malts, *J. Am. Soc. Brew. Chem.*, **69** (2011), no. 3, pp. 150-157.
 4. Yahya, H.; Linforth, R. S. T. and Cook, D. J.: Flavour generation during commercial barley and malt roasting operations: A time course study, *Food Chem.*, **145** (2014), pp. 378-387.
 5. Coghe, S.; Gheeraert, B.; Michiels, A. and Delvaux, F. R.: Development of Maillard reaction related characteristics during malt roasting, *J. Inst. Brew.*, **112** (2006), no. 2, pp. 148-156.
 6. Liscomb, C.; Bies, D. and Hansen, R.: Specialty Malt Contributions to Wort and Beer, *Tech. Q. Master Brew. Assoc. Am.*, **52** (2015), no. 4, pp. 181-190.
 7. Gruber, M. A.: The flavor contributions of kilned and roasted products to finished beer styles, *Tech. Q. Master Brew. Assoc. Am.*, **38** (2001), no. 4, pp. 227-233.
 8. Blenkinsop, P.: The manufacture, characteristics and uses of speciality malts, *Tech. Q. Master Brew. Assoc. Am.*, **28** (1991), no. 4, pp. 145-149.
 9. Yu, J.; Huang, S.; Dong, J.; Fan, W.; Huang, S.; Liu, J.; Chang, Z.; Tian, Y.; Hao, J. and Hu, S.: The influence of LOX-less barley malt on the flavour stability of wort and beer, *J. Inst. Brew.*, **120** (2014), no. 2, pp. 93-98.
 10. Bettenhausen, H. H.; Barr L.; Broeckling, C. D.; Chaparro, J. M.; Holbrook, C.; Sedin, D. and Heuberger A. L.: Influence of malt source on beer chemistry, flavor, and flavor stability, *Food Res. Int.*, **113** (2018), pp. 487-504.
 11. Liguori, L.; De Francesco, G.; Orlio, P.; Peretti, D. A. and Albanese, D.: Influence of malt composition on the quality of a top fermented beer, *J. Food Sci. Technol.*, (2020), pp. 1-9.
 12. Chandra, S.; Booer, C.; Proudlove, M. and Jupp, D.: Speciality malt-Targetting specific flavours and colour for brewing, European Brewery Convention Congress, 1999, Cannes.
 13. Mackie, A. E. and Slaughter, J. C.: Key steps during barley malting that influence the concentration of flavor compounds, *J. Am. Soc. Brew. Chem.*, **58** (2000), no. 2, pp. 69-72.
 14. Fritsch, H. T. and Schieberle, P.: Identification Based on Quantitative Measurements and Aroma Recombination of the Character Impact Odorants in a Bavarian Pilsner-type Beer, *J. Agric. Food Chem.*, **53** (2005), no. 19, pp. 7544-7551.
 15. Fritsch, H. T.; Stephan, A.; Stettner, G.; Methner, F.-J. and Schieberle, P.: Total mapping and sensory simulation of beer aroma, European Brewery Convention Congress, 2005, Prague.
 16. Neiens, S. D.; Steinhaus, M.: Odor-active compounds in the special flavor hops Huell Melon and Polaris, *J. Agric. Food Chem.*, **66** (2018), no. 6, pp. 1452-1460.
 17. Brendel, S.; Hofmann, T. and Granvogel, M.: Characterization of Key Aroma Compounds in Pellets of Different Hop Varieties (*Humulus Lupulus L.*) by Means of the Sensomics Approach, *J. Agric. Food Chem.*, **67** (2019), no. 43, pp. 12044-12053.
 18. Lermusieau, G.; Bulens, M. and Collin, S.: Use of GC-olfactometry to identify the hop aromatic compounds in beer, *J. Agric. Food Chem.*, **49** (2001), no. 8, pp. 3867-3874.
 19. Neiens, S. D. and Steinhaus, M.: Investigations on the Impact of the Special Flavor Hop Variety Huell Melon on the Odor-Active Compounds in Late Hopped and Dry Hopped Beers, *J. Agric. Food Chem.*, **67** (2018), no. 1, pp. 364-371.
 20. Peppard, T. L. and Halsey, S. A.: Malt flavour—Transformation of carbonyl compounds by yeast during fermentation, *J. Inst. Brew.*, **87** (1981), no. 6, pp. 386-390.
 21. Dack, R. E.; Black, G. W. and Koutsidis, G.: The effect of Maillard reaction products and yeast strain on the synthesis of key higher alcohols and esters in beer fermentations, *Food Chem.*, **232** (2017), pp. 595-601.
 22. Hayashida, Y. and Slaughter, J. C.: Biosynthesis of flavour-active furanones by *Saccharomyces cerevisiae* during fermentation depends on the malt type used in medium preparation, *Biotechnol. Lett.*, **19** (1997), no. 5, pp. 429-431.
 23. Voigt, J.; Kraus-Weyermann, T. and Richter, A.: A new beer design tool – Use of malt-aroma-wheels for the prediction of beer characteristics, Master Brewers Conference, 2013, Texas.
 24. Scholtes, C.; Nizet, S. and Collin, S.: Guaiacol and 4-methylphenol as specific markers of torrefied malts. Fate of volatile phenols in special beers through aging, *J. Agric. Food Chem.*, **62** (2014), no. 39, pp. 9522-9528.
 25. Herent, M. F.; Vanthourhout, C.; Gijs, L. and Collin, S.: Influence of the composition of nitrogenous heterocycles of special malts on the aromatic profile of the beer, European Brewery Convention Congress, 1997, Maastricht.
 26. Fickert, B. and Schieberle, P.: Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses, *Food/Nahrung*, **42** (1998), no. 06, pp. 371-375.
 27. Coghe, S.; Martens, E.; D'Hollander, H.; Dirinck, P. J. and Delvaux, F. R.: Sensory and instrumental flavour analysis of wort brewed with dark specialty malts, *J. Inst. Brew.*, **110** (2004), no. 2, pp. 94-103.
 28. Narziss, L.; Miedaner, H.; Koch, M.: Examination of volatile substances during malting and beer processing with particular consideration of those originating from thermal loading of products. Part 2: Influence of the malting and mashing parameters, *Monatsschrift für Brauwissenschaft*, **42** (1989), no. 6, pp. 232-242.
 29. Foster, C.; Narziss, L. and Back, W.: Investigations of flavor and flavor stability of dark beers brewed with different kinds of special malts, *Tech. Q. Master Brew. Assoc. Am.*, **35** (1998), no. 2, pp. 73-77.
 30. Herb, D.; Filichkin, T.; Fisk, S.; Helgerson, L.; Hayes, P.; Meints, B.; Jennings, R.; Monsour, R.; Tynan, S. and Vinkemeier, K.: Effects of barley (*Hordeum vulgare L.*) variety and growing environment on beer flavor, *J. Am. Soc. Brew. Chem.*, **75** (2017), no. 4, pp. 345-353.
 31. Herb, D.; Filichkin, T.; Fisk, S.; Helgerson, L.; Hayes, P.; Benson, A.; Vega, V.; Carey, D.; Thiel, R. and Cistue, L.: Malt modification and its effects on the contributions of barley genotype to beer flavor, *J. Am. Soc. Brew. Chem.*, **75** (2017), no. 4, pp. 354-362.
 32. Pfenninger, H.: *Brautechnische Analysenmethoden*, Auflage: MEBAK, 1993.
 33. Vandecan, S. M. G.; Saison, D.; Schoupe, N.; Delvaux, F. and Delvaux, F. R.: Optimisation of specialty malt volatile analysis by headspace solid-phase microextraction in combination with gas chromatography and mass spectrometry, *Anal. Chim. Acta*, **671** (2010), no. 1, pp. 55-60.
 34. Fickert, B. and Schieberle, P.: Comparative study on the flavour of caramalt and caramalt beer, European Brewery Convention Congress, 1999, Cannes.
 35. Beal, A. D. and Mottram, D. S.: Compounds contributing to the characteristic aroma of malted barley, *J. Agric. Food Chem.*, **42** (1994), no. 12, pp. 2880-2884.
 36. Swan, J. S. and Howie, D.: Correlation of sensory and analytical data in flavour studies into Scotch malt whisky, ALKO symposium on flavour research of alcoholic beverages, 1984, Helsinki.
 37. Swan, J. S. and Howie, D.: Correlation of Sensory data in Flavour Studies into Scotch Malt Whiskies', ALKO symposium on flavour research

- of alcoholic beverages, 1985, Helsinki.
38. Guido, L. F.; Curto, A. F.; Boivin, P.; Benismail, N.; Gonçalves, C. R. and Barros, A. A.: Correlation of malt quality parameters and beer flavor stability: multivariate analysis, *J. Agric. Food Chem.*, **55** (2007), no. 3, pp. 728-733.
 39. Peppard, T. L.; Ramus, S. A.; Witt, C. A. and Siebert, K. J.: Correlation of sensory and instrumental data in elucidating the effect of varietal differences on hop flavor in beer, *J. Am. Soc. Brew. Chem.*, **47** (1989), no. 1, pp. 18-26.
 40. Parr, H.; Bolat, I.; Miller, P.; Clegg, S. and Cook, D.: The Flavour Properties of Roasted Malts: A Gas Chromatography-Olfactometry Study, *Trends in Brewing*, 2018, Ghent.
 41. Schieberle, P. and Hofmann, T.: New results on the formation of important maillard aroma compounds, *Adv. Flavours Fragr.*, **277** (2002), pp. 163-177.

Received 21 December 2020, accepted 8 February 2021