Research article

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

Klaas Reglitz,[1] ⓘ Michael Féchir,[1,2] Veronika Mall,[1] Jens Voigt[2] and Martin Steinhaus[1]* ⓘ

Top-fermented wheat beers are known for their unique aroma. However, the impact of speciality wheat malts on the aroma of these beers and the transfer of odour active compounds from malt to the beer has not been investigated in detail. Three beers were brewed with different malt composition. The grist for each beer contained 50% kilned barley malt and 50% different wheat malts - beer (1) kilned wheat malt, beer (2) kilned wheat malt and caramel wheat malt, and beer (3) kilned wheat malt and roasted wheat malt. The odour active compounds in the beers were identified by aroma extract dilution analysis and their individual impact on aroma was evaluated by quantitation and calculation of odour activity values (OAVs). The results were verified sensorially by comparing aroma reconstitution models with the original beers. Characteristic odour active compounds in the beer brewed with caramel wheat malt were earthy compounds 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, caramel-like compounds 4-hydroxy-2,5-dimethylfuran-3(2H)-one and maltol, and sotolon with a soup seasoning-like aroma. The aroma of the roasted wheat malt beer was characterised by smoky and phenolic compounds 2-methoxyphenol and 4-methylphenol. Important beer odorants were quantified in the malts to assess their transfer from malt to beer. The results suggest that direct transfer of the odour active compounds in beers was not significant and that they were formed and/or released during the brewing process, confirming earlier results with different barley malts and bottom-fermented beers. © 2022 The Authors. *Journal of the Institute of Brewing* published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling.

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

Introduction

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

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

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* Correspondence to: Martin Steinhaus, Leibniz Institute for Food Systems Biology at the Technical University of Munich (Leibniz-LSB@TUM), Lise-Meitner-Straße 34, 85354 Freising, Germany. Email: martin.steinhaus@tum.de

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

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

[2] Trier University of Applied Sciences, Schneidershof, 54293, Trier, Germany

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

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

Materials and methods

Barley and wheat malts

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

Preparation of beers

A Braumeister Plus 50 L (Speidel, Ofterdingen, Germany) was used to brew three top-fermented wheat beers. Each beer was made with 50% barley malt and 50% wheat malt. The kilned wheat malt beer (KWB) was brewed with 50% KBM and 50% KWM, the caramel wheat malt beer (CWB) was brewed with a mixture of 50% KBM, 30% CWM, and 20% KWM, and the roasted wheat malt beer (RWB) was brewed with a mixture of 50% KBM, 48% KWM, and 2% RWM. Each malt mixture (11 kg) was ground and added to 50 L of water. Mashing was performed at 50°C for 20 min, 63°C for 55 min, 73°C for 30 min, and 78°C for 10 min. After lautering, spent grains were washed with water (10 L). The wash water was combined with the first wort and the mixture was boiled (60 min). Hop pellets (37.5 g), variety Hallertau Perle (Hopsteiner, Mainburg, Germany) were added 10 min after starting the boil. A second portion of hops (12.5 g) was added 40 min later. The total hop dosage corresponded to an expected bitterness of 20 IBU (international bitterness units). The original extract was ≥12 °P. After

removal of the hot trub, the wort was cooled to 20°C. Dried yeast - Saccharomyces cerevisiae WB06 (20 g) (Fermentis Lesaffre, Marcq-en-Barœul, France) - was rehydrated and added to the wort. Fermentation was in cylindroconical tanks (Speidel) at 19°C and was monitored using an ALEX 500 Alcohol and Extract Meter (Anton Paar, Graz, Austria). Data can be found in Supporting Information, Table S2. At an apparent relative degree of 79–82%, fermentation was stopped, and the yeast was removed by decantation. The wheat beers were stored in 50 L kegs at 8°C for 1 week and then matured at 2°C for 2 weeks. CO_2 was adjusted to 4.5 g/L before bottling in 0.5 L amber glass bottles. The bottles were sealed with crown caps. Final ethanol concentrations were 5.05% ABV (KWB), 4.39% ABV. (CWB), and 4.59% ABV (RWB) and pH values were 4.45 (KWB), 4.46 (CWB), and 4.43 (RWB). All wheat beers were stored for 3 weeks before analysis.

Reference odorants (numbering refers to Table 2).

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

Stable isotopically substituted odorants

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

Miscellaneous chemicals and reagents

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

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

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

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

Compound	Standard	quantifier ion (m/z)		calibration line equation[a]	R²
		analyte	standard		
3	(^{2}H$_3$)-**3**	102	105	y = 1.6533x + 0.5000	0.999
5	(^{2}H$_3$)-**5**	57	63	y = 0.8807x − 0.0692	1.000
6	(^{2}H$_{11}$)-**6**	131	142	y = 1.7430x − 0.4544	0.999
7a	(^{2}H$_{11}$)-**7b**	71	82	y = 1.1539x − 0.4007	0.994
7b	(^{2}H$_{11}$)-**7b**	71	82	y = 1.6042x − 0.5521	0.993
8	(^{2}H$_{11}$)-**8**	145	156	y = 0.8998x + 0.0390	1.000
9	(^{13}C$_5$)-**9**	111	116	y = 1.4451x − 0.1637	0.997
11a	(^{2}H$_3$)-**11a**	135–136	138–139	y = 0.8047x + 0.6997	0.998
11b	(^{2}H$_3$)-**11b**	135–136	138–139	y = 0.4568x + 0.0817	0.994
12	(^{2}H$_3$)-**12**	75	78	y = 0.6656x + 0.1366	0.995
13	(^{2}H$_3$)-**13**	104	107	y = 0.7746x − 0.1736	0.998
14	(^{2}H$_3$)-**14**	135	138	y = 0.8082x + 0.0292	1.000
17	(^{2}H$_7$)-**17**	103	110	y = 1.0390x − 0.0492	0.999
18	(^{2}H$_2$)-**18**	103	105	y = 0.7257x + 0.0649	1.000
19	(^{13}C$_2$)-**19**	120	122	y = 1.0042x + 0.1568	0.998
20	(^{2}H$_2$)-**20b**	117	119	y = 0.9328x + 0.0974	0.999
22	(^{2}H$_3$)-**22**	106	109	y = 0.8860x + 0.0376	1.000
23	(^{2}H$_3$)-**23**	117	120	y = 0.8565x + 0.0783	1.000
24	(^{2}H$_7$)-**24**	121	123–129	y = 1.8565x + 0.3444	0.998
26	(^{2}H$_3$)-**26**	124	127	y = 1.0985x − 0.0876	1.000
27	(^{2}H$_5$)-**27**	91	96	y = 0.9150x − 0.1010	0.998
28	(^{13}C$_2$)-**28**	126	128	y = 1.1378x + 0.1735	0.998
30	(^{13}C$_2$)-**30**	128	130	y = 1.3031x − 0.2360	0.997
32	(^{2}H$_7$)-**32**	108	115	y = 0.3767x − 0.0454	1.000
33	(^{13}C$_6$)-**33**	150	156	y = 0.4151x + 0.0340	0.999
34	(^{13}C$_2$)-**34**	128	130	y = 1.0632x − 0.0127	1.000
35	(^{2}H$_3$)-**35**	135	138	y = 0.7675x − 0.0178	1.000
37	(^{13}C$_2$)-**37**	136	138	y = 1.1324x − 0.0082	1.000
38	(^{2}H$_3$)-**38**	151+152	154+155	y = 0.8910x + 0.0488	0.999
39	(^{2}H$_2$)-**39**	150	152	y = 0.5267x − 0.0576	0.995

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

Gas chromatography-mass spectrometry (GC-MS)

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

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

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

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

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

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

Aroma extract dilution analysis (AEDA)

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

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

Quantitation

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

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

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

Odour threshold value

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

Aroma reconstitution

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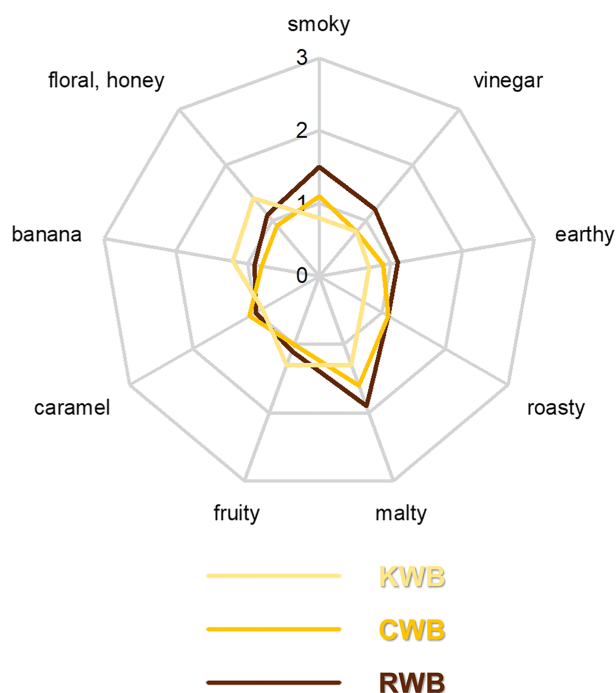
Quantitative olfactory profile

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

Results and discussion

Quantitative olfactory profiles of the wheat beers

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

Figure 1. Quantitative olfactory profiles of kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB). [Colour figure can be viewed at wileyonlinelibrary.com]
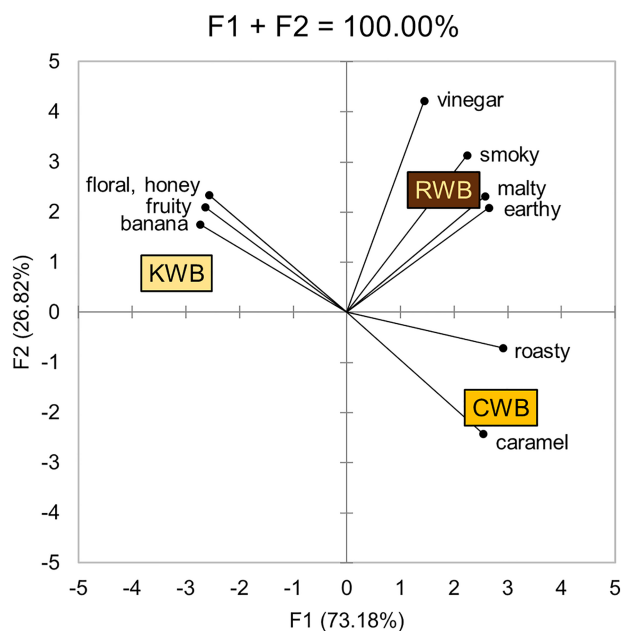
Figure 2. Principal component analysis applied to the sensorial data of kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB). [Colour figure can be viewed at wileyonlinelibrary.com]

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

Statistical evaluation of the sensory data by principal component analysis is reported in Figure 2. Principal component F1 accounted for 73.18% of the variation in the dataset and predominantly allowed for differentiation between the speciality malt beers CWB and RWB located in the positive range of axis F1 and

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

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

Screening for odour active compounds in the wheat beers

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

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

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

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

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

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

[a] Compounds were identified by comparing the retention indices (RIs) on two GC columns of different polarities (DB-FFAP, DB-5), mass spectrum obtained by GC-MS, together with odour from the sniffing port during GC-O to data obtained from authentic reference compounds analysed under equal conditions.
[b] Retention index; calculated from the retention time of the compound and the retention times of adjacent n-alkanes by linear interpolation.
[c] Flavour dilution factor.
[d] Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine.

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

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

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

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

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

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

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

and 39) and phenolic 4-methylphenol (**32**; 15 vs. <1), which was reflected by the most intense smoky note in the olfactory profile (cf. Figure 1).

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

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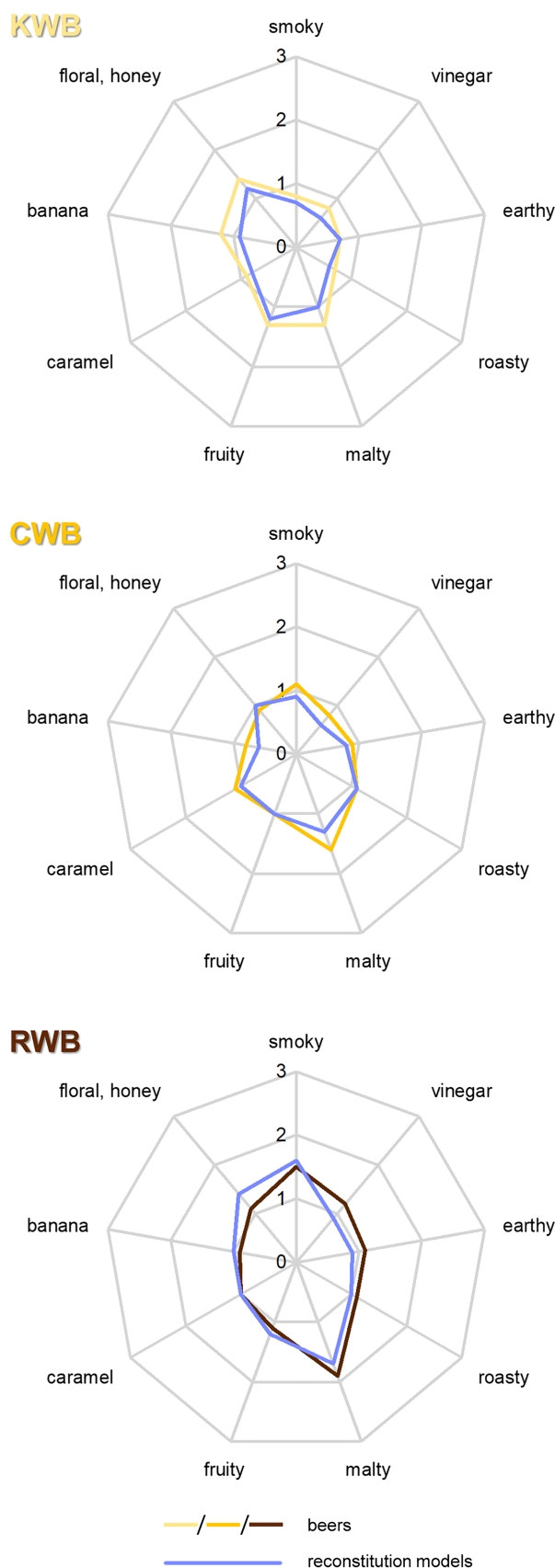
Wheat beer aroma reconstitution

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

Quantitation of the wheat beer odorants in malt

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

Transfer of odorants from malt to wheat beers

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

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

Table 4. Concentration of selected odour active compounds in malts

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

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

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

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

[a] Kilned wheat malt beer; data was calculated as the concentration in KBM/KWM 50/50 (Table S9) × grist load (kg malt per kg beer).
[b] Caramel wheat malt beer; data was calculated as the concentration in KBM/CWM/KWM 50/30/20 (Table S9) × grist load.
[c] Roasted wheat malt beer; data was calculated as the concentration in KBM/KWM/RWM 50/48/2 (Table S9) × grist load.
[d] 4-Hydroxy-2,5-dimethylfuran-3(2H)-one.
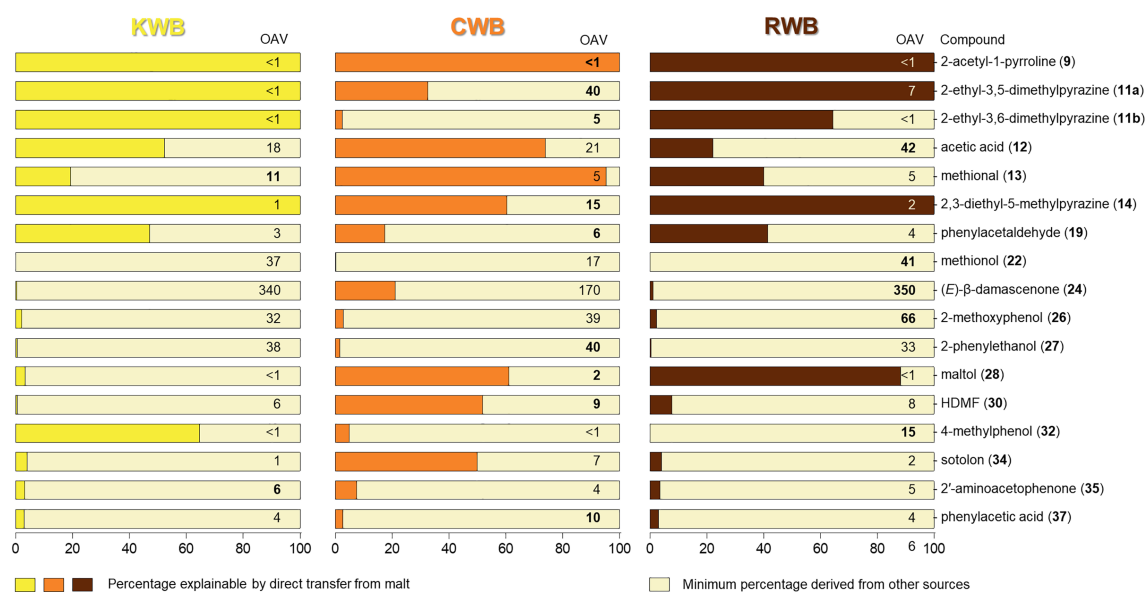
Figure 4. Percentage concentration of compounds in kilned wheat malt beer (KWB), caramel wheat malt beer (CWB), and roasted wheat malt beer (RWB) explained by direct transfer from malt. [Colour figure can be viewed at wileyonlinelibrary.com]

Compound	KWB OAV	CWB OAV	RWB OAV
2-acetyl-1-pyrroline (9)	<1	<1	<1
2-ethyl-3,5-dimethylpyrazine (11a)	<1	40	7
2-ethyl-3,6-dimethylpyrazine (11b)	<1	5	<1
acetic acid (12)	18	21	42
methional (13)	11	5	5
2,3-diethyl-5-methylpyrazine (14)	1	15	2
phenylacetaldehyde (19)	3	6	4
methionol (22)	37	17	41
(E)-β-damascenone (24)	340	170	350
2-methoxyphenol (26)	32	39	66
2-phenylethanol (27)	38	40	33
maltol (28)	<1	2	<1
HDMF (30)	6	9	8
4-methylphenol (32)	<1	<1	15
sotolon (34)	1	7	2
2'-aminoacetophenone (35)	6	4	5
phenylacetic acid (37)	4	10	4

Percentage explainable by direct transfer from malt — Minimum percentage derived from other sources

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

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

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

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

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

Nomenclature

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

Author contributions

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

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Conflict of interest

The authors declare there are no conflicts of interest.

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Supporting information

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