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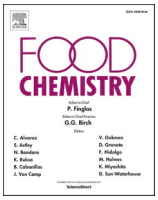

Identification of potential odorant markers to monitor the aroma formation in kilned specialty malts

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ABSTRACT

Specialty malts are strategic ingredients regarding their contribution to colour and flavour of beer. Malts with the same colour may present distinct flavour characteristics and intensities. Contradictorily, colour is the benchmark in practical quality control. To investigate the correlation between colour and flavour of kilned barley specialty malts, odorants of commercial products of pale ale (5–9 EBC), Vienna (6–10 EBC), Munich (11–35 EBC) and melanoidin malts (80–90 EBC) were screened *via* solvent-assisted flavour evaporation (SAFE) and compared *via* comparative aroma extract dilution analysis (cAEDA). Subsequently, selected odorants were quantified using solid-phase microextraction (SPME). A total of 34 odorants were detected, of which 12 exhibited a concentration increase as the coloration increased, whereas 4 suggested the influence of temperature and modification degree on aroma formation. Such odorants are thus elected as potential markers for monitoring the influence of process variations on the formation of aroma in commercial kilned specialty malts.

1. Introduction

As the principal raw material in beer production, malt influences a variety of characteristics of the final beer. Flavour and colour are the primary ones (Liscomb, Bies, & Hansen, 2015), where flavour characteristics arise from the combination between aromas and tastes, and colour as the result of pigmented substances such as melanoidins (Coghe, Derdelinckx, & Delvaux, 2004). Malting barley (*Hordeum vulgare*) is the principal cereal used to produce malt, given its biochemical properties, as well as cultural and economic values (Narziß, Back, Gastl, & Zarnkow, 2017). In the case of basic malts, the process of malting comprises in germinating the grains in a controlled manner, aiming to achieve a suitable amount of enzymes and modification level of its endosperm content. In practical manners, the malting process translates into the three main steps named steeping, germination and kilning (Back, Gastl, Krottenthaler, Narziß, & Zarnkow, 2019). Specialty malts are generally produced with the intention of granting distinct flavour and colour features to beer. Depending on the technology of production, specialty malts can be categorised into colour (kilned), roasted, smoked, sour or chit malt (Prado, Gastl, & Becker, 2021). For the kilned barley specialty malts, the malting parameters are varied aiming to extend the germination of the grains and increase the thermal load during the kilning phase. This guarantees plenty of substrate for the formation of pigmented substances and flavour active compounds *via* non-enzymatic browning reactions (e.g., lard reactions, caramelisation and pyrolysis). Here, it is important to deliberate the differences between the kilning and the roasting process and their impact on the final malts. The kilning process consists of slowly drying the green malt usually at temperatures between 80 °C and 130 °C. This process takes several hours, aiming to maintain the integrity of the malt enzymes. On the other hand, the roasting process aims to produce distinct colour and flavours. Therefore, the grains are gradually heated to higher temperatures (usually above 200 °C). This process is shorter, taking only a few hours (Kunze & Mieth, 2014; Narziß, Back, Gastl, & Zarnkow, 2017; Prado, 2019). lard reactions happen already at temperatures of 50 °C, while caramelisation occurs at > 120 °C and pyrolysis at > 200 °C. In this manner, roasted specialties have more influence from caramelisation and pyrolysis reactions than kilned specialties, which are subjected almost exclusively to the effects of lard-reactions (Coghe, Derdelinckx, & Delvaux, 2004; Prado, Gastl, & Becker, 2021).

Given its feasibility, the coloration of the malt is monitored in order to control the production quality of kilned specialty malts and determine the end-point of the kilning phase. In practical terms, the degree of darkness of the husks indicates the intensity of the thermal process applied in its production. As a coloration index, EBC (European Brewery Convention) colour units are generally adopted, determined *via* the

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measurement of the absorbance of Congress wort at a wavelength of 430 nm or the direct comparison to coloured glasses (ranging between 2 and 27 EBC) (Coghe, Derdelinckx, & Delvaux, 2004; MEBAK, 2016). On the other hand, several researches have addressed the inconsistency of the correlation between colour and aroma formation (Mackie & Slaughter, 2000; Vandecan, Daems, Schouppe, Saison, & Delvaux, 2011; Yahya, Linforth, & Cook, 2014). Here, factors intrinsic to the formation of aroma compounds such as precursor availability, evaporation, degradation and cross-reactions are responsible for the non-linearity of such correlation. In practice, relevant variables of the malting process (e.g., thermal load, malt modification and moisture degree) influence the formation of flavours differently (O'Shaughnessy, Chandra, Fryer, Robbins, & Wedzicha, 2003; Parr, Bolat, & Cook, 2021; S. Vandecan, Daems, Schouppe, Saison, & Delvaux, 2011; Yahya, Linforth, & Cook, 2014). This leads to a scenario where specialty products with similar colour indices can present different flavour profiles, leading to a dilemma where aroma and flavour specifications are undermined.

When studying the aroma composition of food matrixes, several methodologies for aroma extraction, identification and quantification are available (Coghe, Martens, D'Hollander, Dirinck, & Delvaux, 2004; Deki & Yoshimura, 1974a, 1974b, 1974c; Dong, Piao, Zhang, Zhao, Hou, & Shi, 2013; Fickert & Schieberle, 1998; Fors & Eriksson, 1986; Jackson & Hudson, 1978; Kim, Lee, & Kim, 1998; Mackie & Slaughter, 2000; Reichel, Carvalho, Santos, Bednar, Rodrigues, & Guido, 2021; Salmerón, Loeza-Serrano, Pérez-Vega, & Pandiella, 2015; Vandecan, Daems, Schouppe, Saison, & Delvaux, 2011; Vandecan, Saison, Schouppe, Delvaux, & Delvaux, 2010; Yahya, Linforth, & Cook, 2014). Solvent-assisted flavour evaporation (SAFE) is currently considered the most effective method for extracting aromas of food matrixes. Nevertheless, drawbacks such as complexity, non-automatization, time and solvent consumption make such a technique unfeasible for the analysis of a large number of samples. On the other hand, solid-phase microextraction (SPME) is a technique that is sensitive enough to extract aroma compounds that are volatile under mild conditions (40 °C to 60 °C and atmospheric pressure), being a more realistic technique for the analysis of odour-relevant compounds when compared to the human olfactory system. Furthermore, SPME can be fully automated, resulting in a high throughput capacity.

Although the focus has been on the aroma formation and composition of roasted malts, kilned specialties also exert relevant impact on the aroma and flavour characteristics of the final beer. To this regard, the present work focused exclusively on the aroma composition of kilned barley specialty malts of a colour spectrum from 5 to 90 EBC. The objective comprised in developing a hybrid strategy that combines the effectiveness of SAFE alongside the high throughput capacity of SPME to identify and quantify odour-relevant compounds of kilned specialty malts. It was hypothesised that such analysis might point out the impact of the malting technology on the aroma formation and the incongruences within the simple correlation between aroma and colour formation.

2. Materials and methods

2.1. Chemicals and malt samples

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO) as analytical standards and/or at least 90% purity degree: (*E*)-2-nonenal, (*E*)-β-damascenone, (*E,E*)-2,4-nonadienal, (*E,Z*)-2,6-nonadienal, 1-octen-3-ol, 2,3,5-trimethylpyrazine, butane-2,3-dione, 2-ethyl-3,6(5)-dimethylpyrazine, 2-methoxy-4-vinylphenol, 2-methoxyphenol, 2-methylbutanal, 2-methylpropanal, 2-phenylethan-1-ol, 3-(methylthio)propanal, 3-methylbutanal, 5-ethyl-2,3-dimethylpyrazine, anhydrous sodium carbonate (Na_2CO_3), anhydrous sodium sulfate (Na_2SO_4), benzaldehyde, benzaldehyde-d_6, diethyl ether, dimethyl sulfide, ethanol, hexanal, hexanal-d_{12}, menthol, phenylacetaldehyde, pyrazine-d_4, and γ-nonalactone.

Commercial kilned specialty malt samples were kindly donated by IREKS (Kulmbach, Germany) and base malt purchased from Weyermann® Malzfabrik (Bamberg, Germany). Barley starch was kindly donated by Altia Industrial (Helsinki, Finland).

2.2. Solvent assisted flavour evaporation (SAFE) extraction

Malt samples were frozen using liquid nitrogen and ground into a fine powder using a universal laboratory disc mill (DLFU; Bühler-Miag GmbH, Braunschweig, Germany). From each sample, 20 g were diluted in 200 mL of 95% (*v/v*) diethyl ether in distilled water and incubated overnight at room temperature, under 150 rpm. After incubation, the liquid phase was filtered by a filter paper (Ø 320 mm), dehydrated over Na_2SO_4 and filtered again. The filtrate was poured directly in the dropping funnel of the SAFE distillation unit (BÆNG; Glasbläserei Bahr, Manching, Germany) (Engel, Bahr, & Schieberle, 1999). The system was thermostatted at 40 °C and kept under high vacuum (10^{-4} to 10^{-5} mbar). Organic acids were removed with the addition of a saturated solution of Na_2CO_3 and residues of water with the addition of Na_2SO_4. The extract was concentrated to a final volume of 2 mL using a Vigreux Column (60 × 1 cm) at 40 °C (Bemelmans, 1979) and immediately stored at –20 °C. Each malt sample had its aroma extracted twice in a parallel manner. Extracts were analysed in a period no longer than 7 days.

2.3. Comparative aroma extract dilution analysis (cAEDA)

Each SAFE extract was submitted to a serial dilution on a 1:2 (*v/v*) basis (Elmore, 2015a). From each dilution, 3 μL were injected splitless at 220 °C by an autosampler (TriPlus RSH; Thermo Scientific Inc., Waltham, MA), into a gas chromatograph (GC) (GC Trace 1300; Thermo Scientific Inc., Waltham, MA) equipped with a TG-5MS column (length: 60 m, inner diameter: 0.25 mm, film thickness: 0.25 μm), and coupled to a single quadrupole mass spectrometer (MS) (ISQ 7000; Thermo Scientific Inc., Waltham, MA) and an olfactory detection port (ODP 3; Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). As carrier gas, helium was used with a flow rate of 1.9 mL/min. The initial temperature was 60 °C and held for 4.5 min, followed by heating at 5 °C/min until a final temperature of 220 °C and held for 8 min. To minimise potential sniffing fatigue, a maximum of 3 dilution-samples were analysed per day, per panellist. Furthermore, panellists were instructed to alternate in between sample analyses. Peak detection was performed using Xcalibur 4.1.31.9 (Thermo Scientific Inc., Waltham, USA) and identification was performed *via* the NIST database. The odour intensity was rated on a scale ranging from 1 to 3 (1 = weak, 2 = medium, 3 = strong). Each dilution series was analysed in duplicate, by two trained panellists, until no odour was perceived anymore.

2.4. Solid phase Microextraction–Gas Chromatography–Mass spectrometry (SPME–GC–MS)

Malt samples were crushed using a ceramic mortar and pestle, from which four grams were placed in a 20-mL headspace vial together with 10 μL of the internal standard (IS) and 50 μL of ethanol. Samples were stored in a cooled autosampler tray at 17 °C. Prior to extraction, an SPME fibre 50/30 μm DVB/CAR/PDMS (Supelco, Bellefonte, PA) was conditioned for 1 min at 250 °C. Samples were then incubated at 40 °C for 30 min under cycles of vigorous agitation for 600 s followed by a rest period of 5 s. Injection was carried out via split mode of 1:5 at 250 °C by an autosampler (TriPlus RSH; Thermo Scientific Inc., Waltham, MA), into a GC (GC Trace 1310; Thermo Scientific Inc., Waltham, MA) equipped with a TG-5MS column (length: 60 m, inner diameter: 0.25 mm, film thickness: 0.25 μm), and coupled to a single quadrupole MS (ISQ 7000; Thermo Scientific Inc., Waltham, MA). As carrier gas, helium was used with a flow rate of 1.425 mL/min. The initial temperature was 60 °C and held for 4 min, followed by heating at 5 °C/min until a final temperature of 210 °C, then followed by heating at 10 °C/min until a

final temperature of 250 °C. Peak detection was performed using Xcalibur 4.1.31.9 (Thermo Scientific Inc., Waltham, MA) and identification was performed *via* the NIST database. All quantification assays were carried out in a 4-fold analysis.

2.5. Calibration and method validation

As a feasible matrix for the calibration of the malt odorants in dry ground malt, pale malt was ground into fine powder using a universal laboratory disc mill (DLFU; Bühler-Miag GmbH, Braunschweig, Germany) and incubated with diethyl ether 1:3 (*v/v*) overnight, at room temperature, at 120 rpm. After discarding the liquid fraction, this procedure was repeated once again, and the washed malt was left to dry.

Standard solutions were prepared by diluting reference compounds in ethanol, in a broad concentration range aiming for expected concentrations in malt based on previous research (Dong, Piao, Zhang, Zhao, Hou, & Shi, 2013; Prado, Gastl, & Becker, 2021; Vandecan, Saison, Schouppe, Delvaux, & Delvaux, 2010) and preliminary studies. In order to calibrate all odorant compounds, 4 g of the washed malt were spiked with 50 μL of the calibration solutions. Matrix effect was controlled by the addition of 10 μL of the IS (benzaldehyde-d_6, hexanal-d_{12}, menthol and pyrazine-d_4). To quantify the compounds, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*,*Z*)-2,6-nonadienal, butnae-2,3-dione, 2-methylbutanal, 2-methylpropanal, 3-(methylthio)propanal, 3-methylbutanal, dimethyl sulfide, and hexanal, hexanal-d_{12} was used. For the compounds 2-methoxy-4-vinylphenol, 2-methoxyphenol, 2-phenylethan-1-ol, benzaldehyde and phenylacetaldehyde, benzaldehyde-d_6 was used. For the pyrazines 2,3,5-trimethylpyrazine, 2-ethyl-3,6(5)-dimethylpyrazine and 5-ethyl-2,3-dimethylpyrazine, pyrazine-d_4 was used. Finally, for the compounds (*E*)-β-damascenone, 1-octen-3-ol and γ-nonalactone, menthol was used. The analytes were calibrated in a 3-fold manner, with a minimum of 5 calibration points. Limit of detection (LD) and limit of quantification (LQ) were statistically determined as 3 *s/m* and 10 *s/m*, respectively, where *s* corresponds to the signal deviation intercepted at the vertical axis, and *m* the slope of the regression line. Recovery was determined by spiking samples with a known concentration and calculation of the spike recoveries.

2.6. Determination of odour thresholds (OT) and odour activity values (OAVs)

In order to determine the individual odour impact of the selected odorants in each malt sample, individual OT were determined. Due to its purity and odour absence, barley starch was used to mimic the malt matrix. The determination of individual OT was performed by spiking reference compounds diluted in ethanol, at various concentrations, in 5 g of barley starch. Samples were incubated in an orbital shaker (Reax 2 overhead shaker; Heidolph Instruments, Schwabach, Germany) for 15 min at room temperature. After incubation, samples were presented to a trained panel consisting of 10–13 panellists, in triangle tests with ascending concentrations. Individual OT were calculated according to Czerny et al. (2008). OAVs were calculated by dividing the odour concentrations by their OT in barley starch (Dach & Schieberle, 2021; Rychlik & Grosch, 1996; Schoenauer & Schieberle, 2019).

2.7. Malt colour analysis

To assess the colouration of the kilned specialty malt products approached in this study, a spectrophotometric colour measurement was carried out according to MEBAK procedures (R-205.07.110 [2016–03]) (MEBAK, 2016).

2.8. Statistical analysis

Statistical analysis was carried out using the software JMP®, Version 16 Pro. (SAS Institute GmbH, Heidelberg, Germany). Statistical significance was analysed by one-way ANOVA followed by Tukey's test, where a p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Screening of odorants in kilned specialty malts

To identify the odour-relevant compounds in the malts in study, their aroma composition was extracted *via* SAFE and compared *via* cAEDA. Aroma extracts of the four specialty malts were subjected to cAEDA in a 4-fold analysis (aroma extractions were carried out in duplicates, and each analysed twice), resulting in the detection of 34 odorants, from which 31 were identified (Table 1). Adopting SAFE as the method for aroma extraction, Rögner, Mall, and Steinhaus (2021) identified 13 of the same odorants in liquid malt extracts (80% dry matter + 48% sugar): 2- and 3-methylbutanal, acetic acid, phenylacetaldehyde, (*E*)-β-damascenone, 2-methoxyphenol, 2-phenylethan-1-ol, *trans*-4,5-epoxy-(*E*)-2-decenal, γ-nonalactone, furaneol, sotolon, phenylacetic acid and vanillin.

As a result of the cAEDA, the highest flavour dilution (FD) factor was assigned to the isomers 2-ethyl-3,6(5)-dimethylpyrazine (FD 8192), followed by 3-(methylthio)propanal (FD 4096), 3-methylbutanal (FD 2048), phenylacetaldehyde (FD 1024) and 4-hydroxy-2,5-dimethyl-2(5*H*)-furanone (FD 1024). FD values in brackets relate to the melanoidin malt sample. As for the Munich malt, the highest FD factors were also assigned to 3-(methylthio)propanal (FD 2048), 2-ethyl-3,6(5)-dimethylpyrazine (FD 2048), 3-methylbutanal (FD 512), phenylacetaldehyde (FD 512) and 4-hydroxy-2,5-dimethyl-2(5*H*)-furanone (FD 512). In Vienna malt, the highest FD factors were assigned to 3-(methylthio)propanal (FD 512) and 4-hydroxy-2,5-dimethyl-2(5*H*)-furanone (FD 128). Finally, the highest FD factors in pale ale malt were assigned to 3-(methylthio)propanal (FD 128) and *trans*-4,5-epoxy-(*E*)-2-decenal (FD 128).

By analysing each odorant individually, it is noticeable that pale ale malt generally presented the lowest FD factors, whereas melanoidin malt presented the highest. When comparing all of the four malt samples, 12 of the identified compounds, namely 2-methylpropanal, butane-2,3-dione, 3-methylbutanal, 2-methylbutanal, 3-(methylthio)propanal, 1-octen-3-ol, phenylacetaldehyde, 2-methoxyphenol, 2-phenylethan-1-ol, (*E*,*E*)-2,4-nonadienal, 2-methoxy-4-vinylphenol and (*E*)-β-damascenone, revealed FD factors to increase as the coloration of the malts increase. Such findings indicate a correlation between the formation of these odorants and of colour, suggesting a higher concentration of odorants in darker kilned specialty malts. Nevertheless, the qualitative character of such data alongside the propensity of under- or over-estimations precludes a conclusion to be drawn about the concentration of the odorants from the FD factors alone. To this purpose, a quantification method based on SPME–GC–MS was developed.

3.2. Quantification of selected odorants

In order to quantify the odorants detected via cAEDA, a method was developed based on SPME for aroma extraction and GC–MS for detection and quantification. To this study, dimethyl sulfide, 2,3,5-trimethylpyrazine and 5-ethyl-2,3-dimethylpyrazine were included in the quantification strategy given their pertinence and relevance to the brewing literature. On the other hand, acetic acid, 3-methylbutanoic acid, 2-acetyl-1-pyrroline, norfuraneol, furaneol, sotolon, 2-acetyl-1,4,5,6-tetrahydopyridine, β-ionone, phenylacetic acid, 2-aminoacetophenone, *trans*-4,5-epoxy-(*E*)-decenal, 1-methyl-(1*H*)-indole and vanillin were not added to the quantification strategy. This was due to either lack of authentic standards, synthesis inconvenience or incompatibility with the analytical system (e.g., column polarity and detection sensitivity). The resulting calibration strategy comprised a total of 22 odorants. Coefficients of correlation were above 0.98 and recovery rates ranged from 97 to 103% (Table 2).

Table 1
Odour-active compounds identified in four commercial samples of kilned specialty malts ($n = 4$).

N°	Odorant	Odour Quality[a]	RI[b]	FD[c]			
				Pale ale	Vienna	Munich	Melanoidin
1	acetic acid	vinegar	355	<1	64	<1	<1
2	2-methylpropanal	malty	421	<1	4	8	64
3	butane-2,3-dione	butter	440	2	8	8	16
4	3-methylbutanal	grainy	500	64	64	512	2048
5	2-methylbutanal	grainy	513	2	2	4	16
6	hexanal	green	779	2	4	<1	1
7	3-methylbutanoic acid	cheesy, malty	875	2	4	<1	8
8	3-(methylthio)propanal	potatoes	1099	128	512	2048	4096
9	2-acetyl-1-pyrroline	popcorn	1156	64	32	256	64
10	1-octen-3-ol	mushrooms	1344	2	4	4	64
11	unknown	popcorn	1495	8	8	128	32
12	4-hydroxy-5-methyl-3-(2*H*)-furanone[d]	caramel	1532	8	2	8	4
13	phenylacetaldehyde	flowers	1566	32	32	512	1024
14	4-hydroxy-2,5-dimethyl-2(5*H*)-furanone[e]	sugar cotton	1590	32	128	512	1024
15	2-ethyl-3,6(5)-dimethylpyrazine	roasted	1691	64	64	2048	8192
16	2-methoxyphenol	smoky	1721	1	2	1	32
17	sotolon	spicy	1765	2	2	16	64
18	2-phenylethan-1-ol	roses	1790	4	4	4	16
19	2-acetyl-1,4,5,6-tetrahydropyridine	popcorn	1886	16	8	128	64
20	(*E,Z*)-2,6-nonadienal	cucumber	1911	4	1	32	32
21	(*E*)-2-nonenal	cardboard	1923	4	8	4	32
22	β-ionone	violets	1947	2	1	<1	<1
23	unknown	spicy	1990	<1	<1	2	16
24	(*E,E*)-2,4-nonadienal	spicy	2092	2	4	4	16
25	(*E,E*)-2,6-decadienal	cellar	2165	<1	<1	2	<1
26	unknown	smoky	2180	<1	<1	2	8
27	phenylacetic acid	flowers	2189	4	4	4	4
28	2-aminoacetophenone	cellar, spicy	2360	2	4	16	32
29	2-methoxy-4-vinylphenol	smoky	2385	16	16	32	64
30	γ-nonalactone	fruity	2520	4	2	2	8
31	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	2548	128	64	256	256
32	(*E*)-β-damascenone	peaches	2585	<1	<1	4	16
33	1-methyl-(1*H*)-indole	faecal	2600	<1	<1	4	2
34	vanillin	vanilla	2618	16	32	256	128

[a] Odour characteristics perceived *via* GC–O.
[b] Retention index, calculated from the retention time of the compounds on TG-5MS.
[c] Flavour dilution factor.
[d] Norfuraneol.
[e] Furaneol.

Table 2
Method validation for the quantification of selected odour-active compounds of kilned specialty malts via SPME–GC–MS ($n = 3$).

RI[a]	**Odorant**	***m/z***[b]	r^2	**LD** ($\mu g \cdot kg^{-1}_{malt}$)[c]	**LQ** ($\mu g \cdot kg^{-1}_{malt}$)[d]	**Recovery (%)**
403	dimethyl sulfide	62	0.9996	5.67	18.91	99.19
422	2-methylpropanal	72	0.9945	11.65	38.83	98.80
440	butane-2,3-butanedione	86	0.9996	5.99	19.95	99.04
511	3-methylbutanal	44	0.9993	18.07	60.23	98.05
523	2-methylbutanal	41	0.9938	5.30	17.66	99.38
796	hexanal	56	0.9984	15.04	50.14	97.69
1120	3-(methylthio)propanal	104	0.9947	11.94	39.80	97.55
1317	benzaldehyde	106	0.9996	7.85	26.17	99.32
1361	1-octen-3-ol	57	0.9965	3.17	10.57	100.36
1443	2,3,5-trimethylpyrazine	122	0.9906	0.29	0.97	100.07
1588	phenylacetaldehyde	91	0.9969	109.97	366.57	100.03
1699	2-ethyl-3,6-dimethylpyrazine	135	0.9902	0.07	0.23	99.98
1716	2-ethyl-3,5-dimethylpyrazine	135	0.9988	0.20	0.65	99.97
1722	5-ethyl-2,3-dimethylpyrazine	135	0.9939	0.25	0.82	101.09
1739	2-methoxyphenol	124	0.9959	2.65	8.85	98.35
1820	2-phenylethan-1-ol	122	0.9997	3.37	11.24	100.63
1930	(*E,Z*)-2,6-nonadienal	70	0.9932	0.68	2.26	99.44
1949	(*E*)-2-nonenal	43	0.9832	4.49	14.95	99.26
2111	(*E,E*)-2,4-nonadienal	81	0.9851	8.49	28.29	103.19
2408	2-methoxy-4-vinylphenol	135	0.9947	0.07	0.24	101.51
2557	γ-nonalactone	85	0.9914	0.85	2.84	98.05
2616	(*E*)-β-damascenone	121	0.9906	0.10	0.35	99.66

[a] Retention index, calculated from the retention time of the compounds on TG-5MS.
[b] Mass fragment given as mass-to-charge ratio.
[c] Lower limit of detection.
[d] Lower limit of quantification.

To confirm the trends observed by cAEDA, the calibrated odorants were quantified in the same malt samples in a 3-fold analysis as shown in Table 3. The resulting values were below the LD for butane-2,3-dione and (*E*)-β-damascenone in pale ale malt; 2-methoxyphenol in pale ale, Vienna and Munich malts; and (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal and γ-nonalactone in all malts. Values between the LD and LQ are still shown for the sake of comparison of the commercial samples. In a similar approach, S. M. Vandecan, Saison, Schouppe, Delvaux, and Delvaux (2010) quantified much higher concentrations of (*E*)-β-damascenone (1805 $\mu g \cdot kg_{malt}^{-1}$), 2,3,5-trimethylpyrazine (472 $\mu g \cdot kg_{malt}^{-1}$) and 2-ethyl-3,5-dimethylpyrazine (458 $\mu g \cdot kg_{malt}^{-1}$) directly from the malt matrix. Such difference is most likely due to numerous variables concerning both genetic and epigenetic backgrounds, as well as process variations applied during the production of the samples. Malt characteristics, such as barley variety, year of harvesting, endosperm content and malting technology applied, are very likely to differ from the samples approached in this study, influencing thus the results obtained in both studies.

A similar trend was observed regarding the correlation between the formation of specific odorants and of colour found *via* cAEDA. Concentrations were found to increase as the colour index of the malt samples also increased for the following odorants: 2-methylpropanal, butane-2,3-dione, 3-methylbutanal, 2-methylbutanal, 3-(methylthio) propanal, benzaldehyde, 1-octen-3-ol, phenylacetaldehyde, 2-methoxyphenol, 2-phenylethan-1-ol, 2-methoxy-4-vinylphenol and (*E*)-β-damascenone. Nonetheless, such a relationship is not always uniform. Fig. 1 illustrates typical results found for the correlations between colour index and the concentration of measured odorants. Strecker degradation aldehydes such as 3-methylbutanal demonstrated a linear correlation (Fig. 1A), while other compounds such as benzaldehyde and (E)-β-damascenone suggested a logarithmic correlation (Fig. 1B and D).

In contrast with the results from the cAEDA, where the isomers 2-ethyl-3,6(5)-dimethylpyrazine had a consistent increase of its FD factor as coloration increased, the quantified concentrations for all pyrazines increased only from pale ale to Munich malts in a logarithmic manner, consistently decreasing in melanoidin (Fig. 1C). In a similar manner, previous studies have shown meaningful differences in the concentrations of pyrazines when comparing different aroma extraction techniques (Liu, Su, & Song, 2017; Wieczorek, Majcher, & Jelen, 2020). Here, it is important to consider the different natures of each technique. While SAFE is based on a high-vacuum transfer of volatile material leading to higher yields of high-boiling and polar compounds in a liquid solution, the aroma extraction in SPME depends on the equilibrium between the fibre, headspace and, in this case, the solid sample (Elmore, 2015b). In this manner, the susceptibility of the pyrazines to be extracted may differ considerably from one technique to the other. Furthermore, FD factors might have been overestimated, corroborating to the necessity of the quantification method in order to provide more assertive conclusions (Tranchida, 2019).

Table 3
Quantification of selected odour-active compounds of kilned specialty malts in commercial samples *via* SPME–GC–MS.

Odorant	Concentration ($\mu g \cdot kg_{malt}^{-1}$)[1]			
	Pale ale	Vienna	Munich	Melanoidin
dimethyl sulfide	56.3 ± 7.5 ^{a}	37.3 ± 2.7 ^{b}	15.2 ± 2.2* ^{c}	42.1 ± 1.3 ^{b}
2-methylpropanal	23.9 ± 3.2* ^{c}	53.1 ± 3.7 ^{bc}	78.7 ± 6.03^{b}	404 ± 27.7 ^{a}
butane-2,3-dione	<LD[2]	5.7 ± 0.3* ^{b}	6.6 ± 0.5* ^{b}	22.7 ± 1.3 ^{a}
3-methylbutanal	83.7 ± 24.8^{c}	149 ± 7.6 ^{bc}	176 ± 5.9 ^{b}	911 ± 61.7 ^{a}
2-methylbutanal	37.3 ± 10.1^{c}	76.9 ± 3.9 ^{bc}	130 ± 8.7 ^{b}	580 ± 42.2 ^{a}
hexanal	137 ± 25.1 ^{a}	143 ± 9.7 ^{a}	95.1 ± 6.4 ^{b}	110 ± 9.2 ^{ab}
3-(methylthio)propanal	17.0 ± 3.2* ^{c}	25.4 ± 1.06* ^{bc}	30.0 ± 0.4 ^{b}	113 ± 5.5 ^{a}
benzaldehyde	11.2 ± 0.3* ^{c}	14.2 ± 0.5* ^{b}	18.0 ± 0.3* ^{a}	19.4 ± 1.6* ^{a}
1-octen-3-ol	5.0 ± 0.4* ^{b}	7.7 ± 0.6* ^{b}	9.5 ± 1.2* ^{b}	16.4 ± 3.3 ^{a}
2,3,5-trimethylpyrazine	3.5 ± 0.2 ^{d}	6.8 ± 0.6^{c}	15.1 ± 0.8 ^{a}	9.5 ± 1.2 ^{b}
phenylacetaldehyde	179 ± 18.3* ^{c}	269 ± 18.2* ^{c}	361 ± 13.6 ^{b}	976 ± 102 ^{a}
2-ethyl-3,6-dimethylpyrazine	6.2 ± 0.1^{c}	11.1 ± 0.6 ^{b}	16.6 ± 1.07 ^{a}	7.7 ± 0.7^{c}
2-ethyl-3,5-dimethylpyrazine	1.16 ± 0.05^{c}	1.10 ± 0.05^{c}	2.5 ± 0.1 ^{a}	1.6 ± 0.1 ^{b}
5-ethyl-2,3-dimethylpyrazine	0.307 ± 0.01* ^{d}	0.76 ± 0.09* ^{c}	1.9 ± 0.1 ^{a}	1.3 ± 0.1 ^{b}
2-methoxyphenol	<LD	<LD	<LD	2.4 ± 0.2*
2-phenylethan-1-ol	4.9 ± 0.4* ^{c}	8.92 ± 1.06* ^{b}	10.1 ± 1.09^{b}	12.3 ± 0.4 ^{a}
(*E*,*Z*)-2,6-nonadienal	<LD	<LD	<LD	<LD
(*E*)-2-nonenal	<LD	<LD	<LD	<LD
(*E*,*E*)-2,4-nonadienal	<LD	<LD	<LD	<LD
2-methoxy-4-vinylphenol	0.21 ± 0.02^{c}	0.60 ± 0.1 ^{b}	0.90 ± 0.2 ^{a}	1.1 ± 0.1 ^{a}
γ-nonalactone	<LD	<LD	<LD	<LD
(*E*)-β-damascenone	<LD	0.14 ± 0.01* ^{b}	0.39 ± 0.07 ^{a}	0.41 ± 0.03 ^{a}

[1] Values are shown as the average mean ± standard deviation ($n = 3$).
[2] Value below limit of detection.
[a,b,c,d] Superscripted letters represent statistical groups based on Tukey's test.
* Value between limit of detection and limit of quantification.

3.3. OAV calculation of malt odorants

After the quantification of the relevant odorants, their OAVs were calculated aiming the estimation of their individual odour impact in each malt specialty. The results revealed values up to 416 (Table 4). Most of the odorants presented OAVs above 1, indicating their relevance to the odour quality of the malt samples. The highest values were attributed to 3-(methylthio)propanal and 1-octen-3-ol. In contrast, 6 out of the 18 compounds presented OAVs below 1 in all malt samples (2,3,5-trimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 5-ethyl-2,3-dimethylpyrazine, 2-methoxyphenol, 2-phenylethan-1-ol and 2-methoxy-4-vinylphenol), being thus considered poorly relevant for the odour quality of the malts. Apart from 2,3,5-trimethylpyrazine and 5-ethyl-2,3-dimethylpyrazine, these compounds were in contrast with the results obtained from the cAEDA. A possible explanation relies on their relatively high boiling point, which although might have low influence on the FD factors, it decreases their concentration in the headspace samples, leading to a low OAV (Fechir, Reglitz, Mall, Voigt, & Steinhaus, 2021). Furthermore, the irrelevance attributed by the OAVs of 2-ethyl-3,6-dimethylpyrazine suggest that its identification by cAEDA could possibly refer to the isomer 2-ethyl-3,5-dimethylpyrazine, which was, at first, difficult to distinguish due to their close retention time but similar odour quality. Nevertheless, it is important to point out the individuality offered by the analysis of OAVs and its ignorance to interaction effects. Within complex mixtures, the interaction between aromas may offer additive, antagonistic and synergistic effects (Parker, 2015). In this manner, even in concentrations below threshold, 2-ethyl-3,6-dimethylpyrazine, as well as the other odorants, may still contribute to the complexity of odour qualities.

3.4. Variation of aroma composition within the malt colour spectrum 5 to 65 EBC

After considering both cAEDA and quantification results, a comparison between both groups of data was performed aiming to establish a correlation between the variation of aroma and the colouration of the malt samples.

In a comparative manner, the cAEDA results and the quantification of the selected odorants revealed a general trend where 12 odorants had

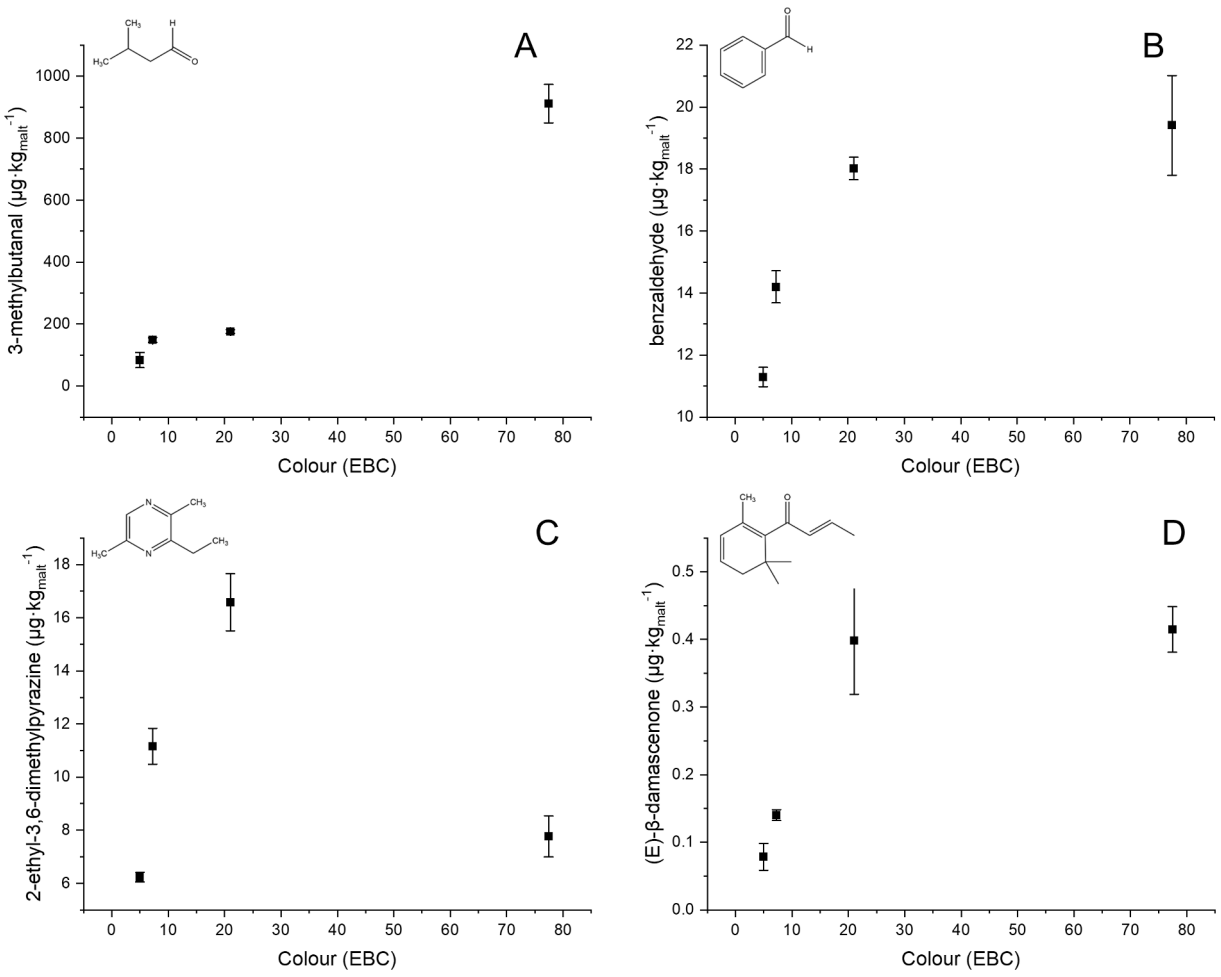

Fig. 1. Relation between malt colour and the concentration of (A) 3-methylbutanal, (B) benzaldehyde, (C) 2-ethyl-3,6-dimethylpyrazine and (D) (E)-β-damascenone.

Table 4
Odour-activity values (OAVs) of selected odour-active compounds of kilned specialty malts in commercial samples.

Odorant	Odour Threshold (µg·kg$_{starch}^{-1}$)	OAV[a]			
		Pale ale	Vienna	Munich	Melanoidin
2-methylpropanal	20[b]	1	3	4	20
butane-2,3-dione	6.5[c]	–	<1	1	3
3-methylbutanal	32[c]	3	5	5	28
2-methylbutanal	53[c]	<1	1	2	11
hexanal	30[c]	5	5	3	4
3-(methylthio)propanal	0.27[e]	63	94	111	418
benzaldehyde	7[d]	2	2	3	3
1-octen-3-ol	0.08[d]	63	97	120	205
2,3,5-trimethylpyrazine	28[c]	<1	<1	<1	<1
phenylacetaldehyde	28[c]	6	10	13	35
2-ethyl-3,6-dimethylpyrazine	90[c]	<1	<1	<1	<1
2-ethyl-3,5-dimethylpyrazine	0.17[b]	7	7	15	10
5-ethyl-2,3-dimethylpyrazine	50[d]	<1	<1	<1	<1
2-methoxyphenol	4.2[b]	–	–	–	<1
2-phenylethan-1-ol	470[e]	<1	<1	<1	<1
(E,Z)-2,6-nonadienal	0.34[e]	–	9	8	11
2-methoxy-4-vinylphenol	18[b]	<1	<1	<1	<1
(*E*)-β-damascenone	0.2[c]	–	<1	2	2

[a] Odour-activity value.
[b] Odour threshold according to Rychlik and Grosch (1996).
[c] Odour threshold according to Dach and Schieberle (2021).
[d] Own determination.
[e] Odour threshold according to Schoenauer and Schieberle (2019).

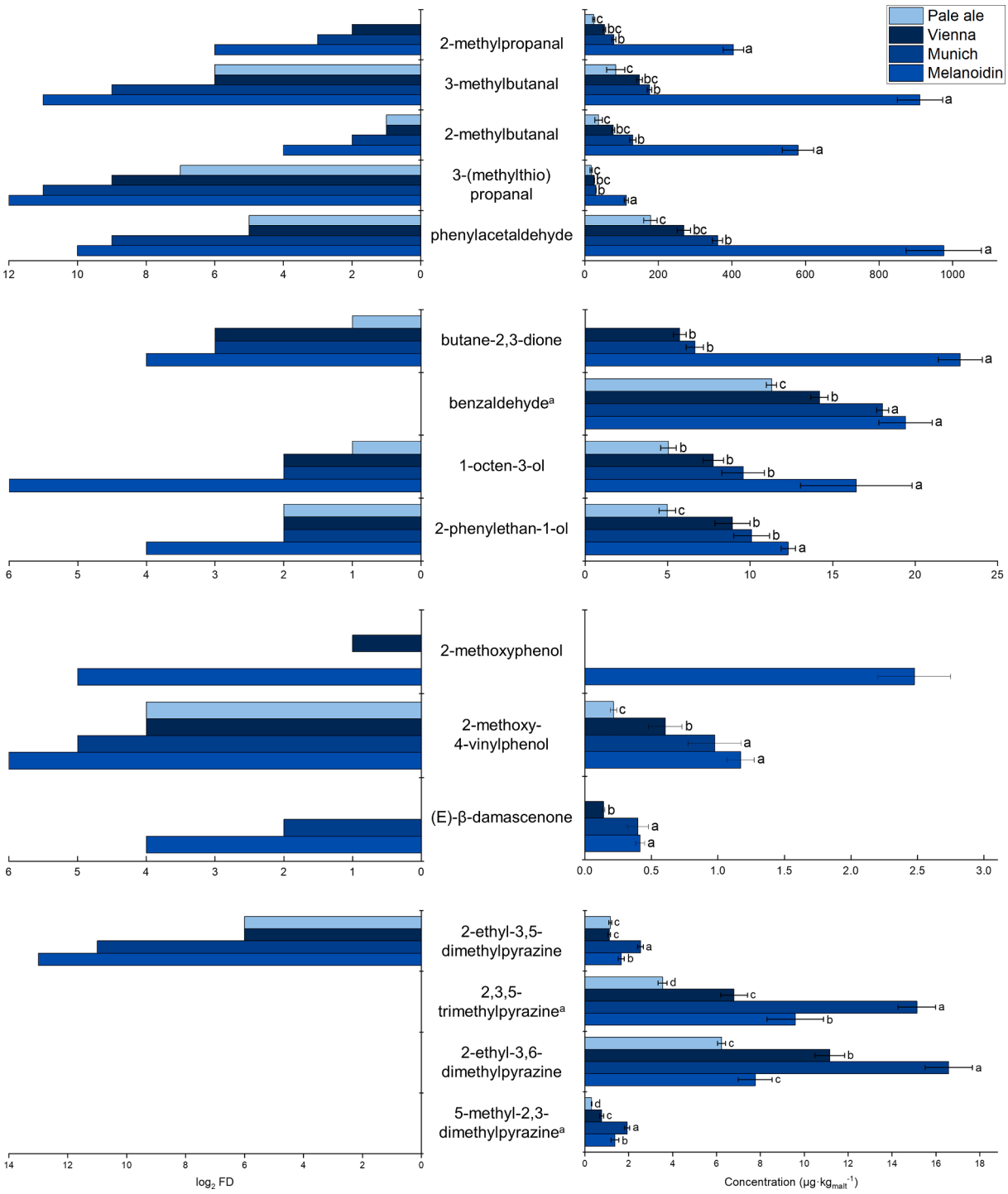

Fig. 2. Comparison of flavour dilution factors (log_2 FD) and concentration of selected odour-active compounds of commercial samples of kilned specialty malts ($n = 3$). [a] Benzaldehyde, 2,3,5-trimethylpyrazine and 5-methyl-2,3-dimethylpyrazine were not detected *via* cAEDA.

their concentration increased as the colour of the malt also increased (Fig. 2). Although not all of these compounds were determined to be relevant to the malt aroma, they still present a pattern of concentration increase alongside the coloration index. As previously discussed, this correlation is complex and has singular patterns to the many groups of odorants that compose the total aroma quality of the malts. In fact, variables related to the malting technology applied during the production of each sample are more likely to explain such patterns rather than simply the colour index.

Diverging from the traditional process conditions to produce base malt, pale ale malts are produced after increased curing temperatures (around 90 °C). Vienna malt is produced after a higher steeping degree (up to 46%) and curing temperatures between 90 °C and 100 °C. Munich malt is also produced after a higher steeping degree (up to 47%) and curing temperatures above 100 °C (Prado, 2019; Prado, Gastl, & Becker, 2021). As for melanoidin malts, a prolonged and intense germination is applied, where the green malt is left to self-heat under a closed environment at approximately 36 h before its ending. Here, the intense metabolism of the grains promotes the increase of ambient temperatures up to 40–50 °C. At this stage, the metabolism is then slowed down due to

the accumulation of CO_2, bringing the growth to a standstill. The nutrients generated from the malt modification are no longer consumed for the growth, thus increasing the concentration of reducing sugars and amino acids, and consequentially leading to an abundance of lard products. In this manner, relatively low curing temperatures (between 70 °C and 85 °C) are already enough to produce the malt (Narziß, Back, Gastl, & Zarnkow, 2017).

When interpreting the results illustrated by Figs. 1 and 2 in the light of such malting conditions, it is deducible that the concentration increase of most of the odorants reflect the higher malt modification degree (proportioned by the higher steeping degrees) and curing temperatures. More specifically, the elevated concentrations observed in the melanoidin malt suggest the influence of the high endosperm modification alongside the curing temperatures in terms of the odorant markers formation. During the production of melanoidin malt, the extreme modification of the endosperm allows a rich availability of low-molecular sugars, amino acids, small peptides and organic acids, which then lead to an abundance of lard products during the kilning phase (Narziß, Back, Gastl, & Zarnkow, 2017).

However, the pattern of all four pyrazines analysed in this study presented a different pattern, where their concentration increased only from pale ale to Munich malt, decreasing considerably in melanoidin malt. Since an extreme modification of the endosperm is promoted during its malting process, the lower concentration of pyrazines suggests their formation to be less dependent to the malt modification degree, and negatively affected by the higher steeping degree. Several researches have described the non-linear dependency of pyrazine formation to temperature and its susceptibility to humidity (Channell, Yahya, & Cook, 2010; Fors & Eriksson, 1986; Vandecan, Daems, Schouppe, Saison, & Delvaux, 2011; Yahya, Linforth, & Cook, 2014). Although such works approached roasted malts, they demonstrate the complexity of the formation of such odorants and the insufficiency of the aroma–-colour correlation in order to control flavour quality specifications. Along these lines, the odorant and their concentrations pointed out in Fig. 2 reflect crucial variables of the malting process. In this manner, such compounds are eligible as potential candidate markers for analysing commercial kilned malt samples and the influence of their production methods on the formation of aroma. Furthermore, an in-depth study becomes necessary, aiming to better understand the dependency of such odorant markers to the variables herein discussed and thus provide further insights towards a better controllability of the aroma formation in kilned specialty malts.

4. Conclusion

The present study provided the first step for a comprehensive analysis of the correlation between the formation of aroma and colour in kilned specialty malts. SAFE was applied in specialty malts for the first time, aiming the screening of odorant-active compounds in commercial products of different colour indices. In sequence, a method based on SPME–GC–MS was developed in order to quantify such compounds in a high throughput manner.

In malt specialties pale ale (5–9 EBC), Vienna (6–10 EBC), Munich (11–35 EBC) and melanoidin (80–90 EBC), the concentrations of 2-methylpropanal, butane-2,3-dione, 3-methylbutanal, 2-methylbutanal, 3-(methylthio)propanal, benzaldehyde, 1-octen-3-ol, phenylacetaldehyde, 2-methoxyphenol, 2-phenylethan-1-ol, 2-methoxy-4-vinylphenol and (*E*)-β-damascenone were found to increase alongside the colouration index, not always in a linear manner. These findings suggest the effect of malt modification degree and curing temperatures on the formation aroma. The concentrations of pyrazines increased only from pale ale to Munich malts, presenting a significant decrease in melanoidin malt, and thus suggesting their higher dependency to kilning temperatures than to malt modification degree. These findings illustrate the suitability of such hybrid strategy in providing information regarding the malting process, thus naming potential candidate markers for monitoring process variations and its influence on the formation of aroma in kilned specialty malts.

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CRediT authorship contribution statement

Raphael Prado: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Writing – original draft. **Anna Celina Marie Hartung:** Investigation, Validation, Data curation. **Martina Gastl:** Supervision, Funding acquisition, Writing – review & editing. **Thomas Becker:** Supervision, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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