Sensory and Instrumental Flavour Analysis of Wort Brewed with Dark Specialty Malts

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ABSTRACT

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In order to explore the flavour produced by dark specialty malts, wort samples were brewed with different malts and evaluated by sensory and instrumental analysis. With increasing wort colour, a trained tasting panel detected more intense bitter and burnt flavours, whereas sweet and husky flavour notes decreased. Conversely, caramel and bread-like flavour attributes had a maximal intensity for the intermediate wort colours. Tasting of 20 EBC worts indicated that the flavour profile was significantly affected not only by malt level and malt colour but also by malt origin. Furthermore, the darkest caramel malt (480 EBC units) was found to contain most Maillard aldehydes as determined by the reaction with thiobarbituric acid. Similarly, other intermediate products of the Maillard reaction such as acetic acid, diacetyl and 2,3-pentanedione were found to arise in a higher concentration in dark caramel malts (220–480 EBC units) than in roasted malt (1200 EBC units). Dynamic headspace GC/MS further revealed that brewing with dark specialty malts considerably increased the level of 3-methylbutanal, its aldol condensation product (2-isopropyl-5-methyl-2-hexenal) and heterocyclic Maillard compounds. In contrast, dark malts drastically reduced the amount of hexanal in wort. By means of HPLC, it was established that only extreme roasting temperatures lead to the thermal degradation of ferulic acid to 4-vinylguaiacol in malt.

Key words: Maillard reaction, purge and trap GC/MS, sensory assessment, thiobarbituric acid number, wort tasting.

INTRODUCTION

Food flavour can be investigated not only by instrumental methods[27] but also by sensory evaluation[28]. Although sensory assessment is rather subjective in nature, it is widely accepted because current detectors do not exhibit the same sensitivity and selectivity as the human olfactory system[25]. Major sources of flavour in beer are raw materials, processing conditions and post-fermentation treatments[30].

Barley malt can contribute both desirable and undesirable flavours to wort and beer. A broad spectrum of malt flavours can be acquired by varying barley variety and malting parameters, particularly degree of modification and kilning or roasting temperature profiles[12,38]. Dark specialty malts can be categorized into three major groups: colour malts, caramel malts and roasted malts[11]. Typical flavours associated with these malts have been described by several authors[2,17,22,37]. Malt flavour volatiles can be classified according to chemical structure and mode of formation into phenolic compounds, volatile sulphur components, Maillard reaction products and compounds formed by the oxidation of lipids[40]. Not all of the nearly 250 volatile components identified in dark malt, contribute considerably to the overall flavour. The impact of a particular compound is a combination of the absolute level and its threshold value. In malt, a limited number of key flavour compounds were identified by a comparison of the relative aroma values of several flavourants[46] and by the application of flavour dilution analysis[1,14]. Nevertheless, it is generally accepted that Strecker aldehydes and heterocyclic Maillard compounds have a substantial impact on the flavour of thermally processed foods. Using gas chromatography-mass spectrometry, dark specialty malts were found to contain several heterocyclic Maillard reaction products[10,34,42,43]. In colour malts and in lightly coloured caramel malts, oxygen heterocyclic components such as pyrones, furans and furanones predominate while nitrogen containing heterocycles such as pyrazines, pyridines and pyrroles contribute most to the flavour of dark caramel malts and roasted malts[15,30,37,40].

The main specifications for dark specialty malts are colour, moisture content and extract. These specifications are inadequate to accurately predict the brewing performance and flavour potential of dark malts and the flavour stability of the resulting beers. Therefore, the specification set for dark malts may well be extended with other physico-chemical properties, characterised in a previous study[11]. As the flavour of beer of a particular colour depends on the malt types used[2,16], and as malts with similar colour can produce different flavours in the final product depending on the malting conditions[6], it might also be valuable to add flavour descriptions to the set of specifications.

The main objective of this work was to explore the flavour attributes of wort samples brewed with dark malts. As these malts significantly differ in degree of browning, mainly Maillard reaction products were considered. Unboiled wort was selected as the study material in order to exclude the effects of flavour-active compounds originat-

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ing from hops, from boiling or from the fermentation process. In the first part of the present paper, the flavour profile of wort was evaluated by a trained tasting panel. The influence of dark specialty malts on the level of several important volatile compounds was examined in the second part.

MATERIALS AND METHODS

Malts

Pilsner malt was provided by Cargill Malt Division (Herent, Belgium). Dark specialty malts were obtained from various European malting plants: Belgomalt (Gembloux, Belgium), Dingemans (Stabroek, Belgium), Malteries Franco-Belges (Pithiviers, France), Pauls malt (Knapton, United Kingdom), Thywissen (Hürth, Germany) and Weyermann (Bamberg, Germany).

Reagents

2-Acetylpyridine, 2-acetylpyrrole, 2-acetylthiazole, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, dimethyl sulfide (DMS), furfural, furfuryl alcohol, 3-hydroxy-2-methyl-4-pyrone (maltol), 2-methoxy-4-vinylphenol (4-vinylguaiacol), 3-methylbutanal, 2-methylpyrazine, pyrazine and 2-thiobarbituric acid were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany).

Wort production

Wort samples were prepared according to Analytica EBC[13]. Reference pilsner wort was brewed with 100% pilsner malt. Unless otherwise stated, dark worts were made with 50% pilsner malt and 50% dark malt. For some experiments, wort of 12° Plato was evaluated. This wort was made, using higher rates of malt (a total of 70 g malt instead of 50 g).

Malt and wort colour

Malt colour was evaluated according to the EBC standards of analysis[13]. Some properties were plotted against wort colour (± half of the malt colour for dark malts). To this end, the colour of wort was not further converted to malt colour.

Worts of 20 EBC units

A Congress wort colour of 20 EBC units was obtained by the application of different levels of dark malts, originating from the three malting plants (Table I).

Table I. Level of malt required for the production of 20 EBC wort.

Malt type	Malt colour (EBC units)	Level of malt	Malting plant
Caramel malt	155	10%	A
Caramel malt	105	15%	A
Caramel malt	150	10%	B
Colour malt	20	100%	C

Flavour descriptions

In order to generate a terminology for the evaluation of malt flavours and to obtain accurate and reproducible data, a tasting panel was trained to identify and describe typical malt and wort components. Initially, the panel was trained to recognize the characteristic flavour impressions associated with the compounds dimethyl sulfide, 3-methylbutanal, 2-methylpyrazine, 2-acetylthiazole, 2-acetylpyrrole, 2-acetylpyridine, maltol, 4-vinylguaiacol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. These compounds were first assessed in water and later in pilsner wort. In a next step, quantitative descriptive analysis (QDA) was performed on wort samples prepared with different dark specialty malts. The tasting panel consisting of 12 members was encouraged to develop a vocabulary for detectable flavour characteristics. Together with the flavour descriptors of the chemical compounds, flavours typically originating from pilsner malt (sweet, astringency, husky and musty) and dark malt (toffee, chocolate, biscuit, tobacco, coffee) were included in the tasting form.

Congress wort tasting

Congress wort tasting was conducted in a sensory evaluation laboratory. Wort samples brewed with dark malts were presented in black tasting glasses in order to mask colour differences between samples. In every session, wort prepared with 100% pilsner malt was included as a reference. Water and crackers were used to cleanse the palate between samples. Sensory profiling was performed by the 12 trained panellists. Wort flavours were assigned a number on a 0–5 intensity scale, 0 being absent and 5 being extremely strong. The intensities recorded by the different assessors were averaged for each flavour attribute.

Thiobarbituric acid number

The thiobarbituric acid number (TBN) was determined according to Thalacker and Birkenstock[41]. The assay was started by the addition of 5 mL thiobarbituric acid solution (0.02 M, 90% acetic acid) to 10 mL of Congress wort. This reaction mixture was incubated at 70°C for 70 min. The absorption of the acquired yellowish solution was determined at a wavelength of 448 nm and the absorption value was corrected for wort colour. Thiobarbituric acid numbers were obtained by multiplying the adjusted absorption values with a factor 10 and with the dilution factor.

Purge and trap GC/MS

Wort samples were evaluated by purge and trap GC/MS according to Vanderhaegen *et al.*[44], using a slightly modified procedure. Prior to analysis, 200 µL internal standard (200 mg/L pentanol) and 200 µL of a 10% antifoam solution (Sigma-Aldrich Chemie GmbH, Munich, Germany) were added to 50 mL of wort. Then, a 5 mL aliquot was transferred to the Tekmar Dohrman 3000 (Emerson, Mason, OH, USA) purge and trap concentrator unit with a Vocarb 3000 trap (Supelco, Bellefonte, PA, USA). Helium was used as carrier gas. The following conditions were applied: 15 min purge and 15 min dry purge at 35°C for Congress wort or 10 min purge and 8 min dry purge at 100°C for 12° Plato wort. This was followed by 6 min desorption at 250°C and 10 min bake at 260°C. The indicated temperatures are those of the adsorbing trap. The wort sample temperature was kept at 20°C during purging. Before entering the GC, volatiles were focused using a cold trap with MFA 815 control unit (ThermoFinnigan, San Jose, CA, USA). The initial temperature was set to –70°C, the final temperature to 220°C. Gas chromatography was performed using a Fisons GC 8000 gas chromatograph (Fisons, Mainz, Germany) equipped with a Chrompack CP-WAX-52-CB column (length 50 m, internal diameter 0.32 mm, film thickness 1.2 µm; Varian, Palo Alto, CA, USA). The oven temperature was maintained at 60°C for 5 min after injection and then programmed at 5°C min^{-1} to 250°C. Total ion mass chromatograms were obtained from the Fisons MD 800 quadripole mass spectrometer (ionization energy: 70 eV, source temperature: 250°C; Fisons, Mainz, Germany) and analysed using the Masslab software program (ThermoQuest, Manchester, UK) for identification and quantification of volatiles.

Determination of 4-vinylguaiacol

Wort samples were filtered through 0.45 µm regenerated cellulose syringe filters (Alltech, Deerfield, IL, USA). Levels of 4-vinylguaiacol were determined by HPLC according to a method described by Coghe *et al.*[9] For this purpose, a Dionex (Sunnyvale, CA, USA) DX 500 chromatography system equipped with a Rheodyne (Rohnert Park, CA, USA) model 9125 automatic sample injector (40 µL sample loop), a Dionex GP40 gradient pump and a Dionex ED40 electrochemical detector were used. The detector was operated with a glassy carbon working electrode at a potential of +0.9 V versus Ag/AgCl and an output range of 20 nA. The analysis was performed on a 25 cm × 4 mm Nucleosil C18 10 µm column (Machery-Nagel, Düren, Germany) eluted with $H_2O/CH_3OH/H_3PO_4$ (640:350:10, v/v).

Acetic acid determination

Acetic acid was determined by an enzymatic assay kit (R-Biopharm GmbH, Darmstadt, Germany). The spectrophotometric measurement evaluated the production of NADH at 340 nm.

Determination of vicinal diketones

Diacetyl and 2,3-pentanedione were quantified in wort by headspace gas chromatography. To this end, a Perkin Elmer AutoSystem XL (Perkin Elmer, Norwalk, CT, USA) equipped with an electron capture detector (ECD) was used. Samples of 5 mL were heated for 16 min at 60°C in the HS-40 autosampler. Separation was achieved on a 50 m WCOT fused silica capillary column coated with CP-WAX-52-CB (length, 50 m; ID, 0.32 mm; and layer thickness, 1.2 µm; Varian, Palo Alto, CA, USA). The following conditions were applied: injection temperature 180°C; oven temperature 75°C for 6 min, increase at 25°C/min to 110°C, hold for 3.5 min; ECD detector temperature 220°C. Helium was used as carrier gas. The obtained chromatograms were analysed using Turbochrom Navigator software (Perkin-Elmer, Norwalk, CT, USA).

RESULTS AND DISCUSSION

The sensory evaluation of raw materials gives an immediate indication of the quality, the balance of flavours present and/or the presence of any flavour taints[5,32]. In dark specialty malts, the majority of the flavour-active compounds originate from the Maillard reaction and are produced during malt kilning or roasting. Higher kilning or roasting temperatures normally result in darker malts and more intense flavour contributions[37]. Dark malt flavour has been previously assessed by the sensory analysis of milled, wetted malt[32]. This method of tasting allowed the determination of the sensorial contributions from both the husk and the malted endosperm. In contrast to this procedure, only flavour components solubilised during Congress mashing were evaluated in our study. In a first series of tests, the influence of the malt level on the flavour profile of wort was assessed. Subsequently, the impact of the application of different malt types on the flavour of wort brewed with 50% of dark malt was investigated. For a final series of experiments, different levels of diverse dark malts were used to evaluate the flavour of wort samples with a fixed colour of 20 EBC units.

Influence of malt level on sensory profile

Initially, multiple sessions were organised to study the effect of the specialty malt level on several flavour attributes. Four colour malts (7, 14, 23 and 44 EBC units) and three caramel malts (50, 90 and 130 EBC units) were investigated. Per session, one dark malt was assessed at rates of 5, 15, 30 and 50% of total grist. For each flavour note, average values of triplicate tasting sessions were represented in spider web diagrams. An example for a colour malt of 44 EBC units is shown in Fig. 1. In general, bitter, burnt, bread and caramel flavour impressions increased while sweet and husky flavours decreased with increasing specialty malt level. For all analysed malts, it was observed that burnt and bitter flavours drastically increased with the amount of malt used. Caramel and bread flavours changed gradually for lightly coloured malts (< 40 EBC units). Conversely, bread and caramel flavours mainly increased in worts brewed with a small proportion (5, 15 and 30%) of darker malts (> 40 EBC units). Grists composed of 50% of these darker malts did not further contribute to bread and caramel flavour attributes.

Influence of malt type on sensory profile

Another series of tasting sessions was conducted to determine the flavour contribution of different malt types. To this end, wort samples were prepared with 50% of dark

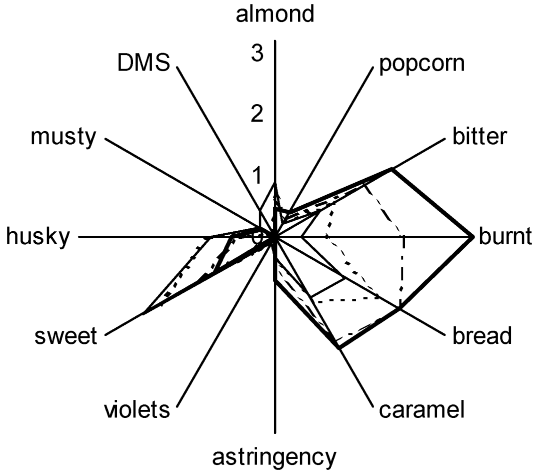

Fig. 1. Spider web diagram resulting from tasting of wort brewed with 5% (—), 15% (· · ·), 30% (– · –) and 50% (▬) colour malt of 44 EBC units.

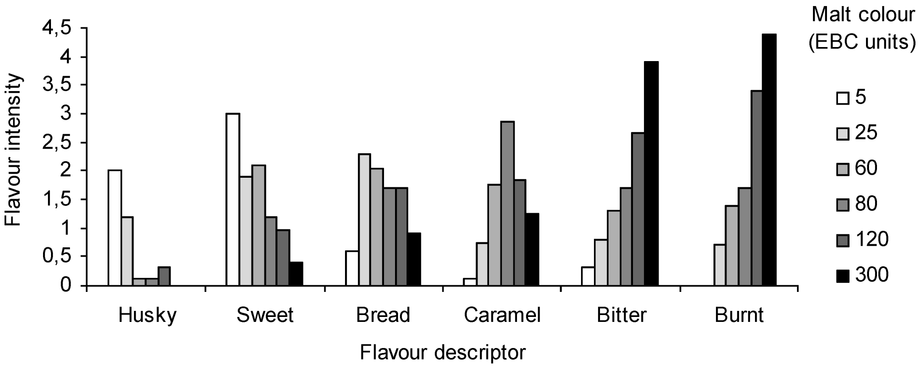

Fig. 2. Flavour intensity of several flavour attributes detected in wort by a trained tasting panel. Wort samples were brewed with 100% pilsner malt (5 EBC units), 50% colour malt (25 EBC units) or 50% caramel malt (60, 80, 120 and 300 EBC units).

malt. It was found that, even for a specific malt colour, flavour was significantly affected by the malting plant of origin. Indeed, the application of slightly different production parameters can have a considerable impact on malt flavour[39]. In order to better observe the effect of the malt type used, consequent tasting sessions were performed using malt from one malting plant. In agreement with previous findings[5], the predominant flavour attributes for pilsner malt were green, sweet and sulphury whereas those for colour malts were malty, nutty, bread and biscuit-like. As also reported by other authors[37,45], flavours associated with caramel malts were frequently referred to as malty, toffee, and caramel, while flavours resulting from the roasting of pilsner malt were mainly described by the terms burnt, bitter, astringent, chocolate and coffee.

The results for several flavour notes typically depended on malt colour (Fig. 2). It was observed that the pronounced sulphury flavour attribute (DMS) associated with pilsner malt was masked by the application of dark specialty malts. Other white malt flavours such as sweet and husky also decreased with increasing malt colour. Although the panellists could not observe the colour of the wort samples, burnt and bitter flavour notes were linearly related to the colour of the applied malts. Bread, popcorn and caramel flavour impressions showed a maximum intensity for the intermediate malt colours. For the darkest malts, it appears as if these flavours are masked by other, more potent flavourants, which are responsible for bitter and burnt flavour attributes.

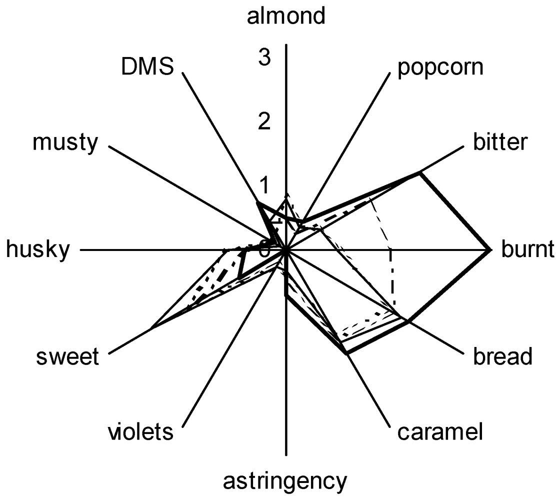

Fig. 3. Spider web diagram resulting from wort tasting. Congress worts of 20 EBC units were prepared with different levels of dark malts: 10% caramel malt of 155 EBC units, malting plant A (—), 15% caramel malt of 105 EBC units, malting plant A (· · ·), 10% caramel malt of 150 EBC units, malting plant B (– · –) and 100% colour malt of 20 EBC units, malting plant C (▬).

Sensory profile of 20 EBC wort

In a final series of tasting experiments, the impact of dark malts on the flavour of 20 EBC wort was investigated. In order to obtain this preset colour, tested malts were used in different ratios (Table I). A colour of 20 EBC units was selected because of the difficulty to generate flavour profiles for less coloured wort samples and because of the flavour masking by burnt and bitter flavours in highly coloured worts. Despite the identical wort colour, different malts result in clearly different flavour profiles (Fig. 3). These results confirm that wort colour and flavour cannot be equated. Bitter and burnt flavours are usually ascribed to roasted malts. Nevertheless, the application of a high level of lightly coloured malt resulted in more pronounced burnt and bitter notes and a less sweet character. Furthermore, it was observed that wort samples prepared with differently coloured caramel malts (105 versus 155 EBC units) but originating from a particular malting plant (plant A), had similar flavour profiles. In contrast, malt made by different plants but of practically the same colour (155 versus 150 EBC units) resulted in totally different profiles. As indicated earlier, flavour can be significantly affected by the malting plant of origin. In line with these results, it has previously been reported that the level of some volatile N-heterocyclic compounds was not only affected by malt colour but also by the origin of the malt[18]. In some cases, it appears as if the 'house flavour' of a specific malting plant is more important for the overall flavour profile than the malt colour.

Malt volatiles

Malt processing leads to the development of a whole range of highly volatile compounds. Flavourants contributing most to the flavour of dark specialty malts are intermediate Maillard reaction products of low molecular weight[45]. The end products of the Maillard reaction, the brown polymeric melanoidins of high molecular weight, are not flavour-active[24]. It has been postulated that the stereo-chemical arrangement of some structural elements significantly correlates with the sensory perception of heterocyclic compounds[19]. A planar arrangement of carbonyl groups, enolic hydroxyl groups and methyl groups on oxygen heterocyclic compounds appears to elicit caramel-like flavours. On the other hand, bread-like flavours might result from planar, unsaturated heterocyclic compounds with one or two nitrogen atoms in the ring structure and with an acetyl group in the 2 position.

The rate and extent of the Maillard reaction can be evaluated by malt colour but also by volatile intermediates of the non-enzymatic browning reaction. An important objective of this study was to better comprehend the development of flavour compounds during kilning or roasting of malt. Therefore, levels of several Maillard volatiles were determined in wort samples brewed with 50% of dark malt and related to wort colour. The overall thermal charge, to which malt was subjected during its production, was evaluated by the thiobarbituric acid number. Furthermore, dynamic headspace gas chromatography mass spectrometry was used to determine the difference in the volatile composition of worts brewed with different dark malts. In addition, a selection of several volatiles typically formed by thermal reactions was evaluated.

Thermal charge received during curing or roasting

The thiobarbituric acid number (TBN) provides information on the levels of substances present in malt and wort, which give a yellow colouration with thiobarbituric acid in acetic acid solution. Thiobarbituric acid reacts with carbonyl compounds such as 5-hydroxymethylfurfural (HMF) and structural similar compounds. The determination of TBN is still a useful and simple technique for estimating the extent of the Maillard reaction and the heat treatment given to malt or wort[41]. Previously, Narziss and Stippler[36] revealed that the level of HMF in malt increased with both, temperature and duration of drying. It was also found that, in comparison with kiln dried colour malts, HMF values were much higher in caramel malts (4–125 EBC units) and roasted malt (1450 EBC units). Furthermore, HMF levels were found to consistently increase with malt colour[36]. From the data presented in Fig. 4 it can be seen that the TBN indeed increased with colour for most malts. However, the thiobarbituric acid number of 240 EBC wort prepared with 50% of the darkest caramel malt (480 EBC units) was higher than the TBN of 650 EBC wort brewed with 50% of roasted malt (1300 EBC units).

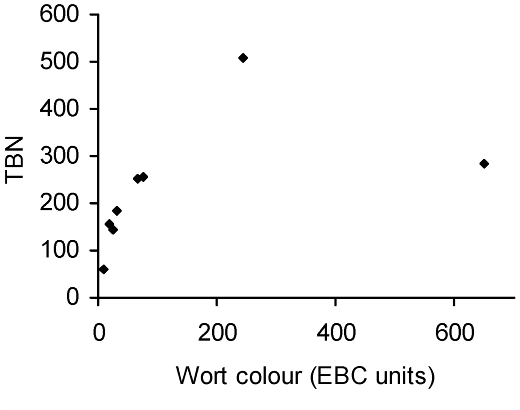

Fig. 4. Thiobarbituric acid number (TBN) versus wort colour.

HMF and other heterocyclic aldehydes develop during the heating of malt. These intermediary compounds of the Maillard reaction are highly reactive and can take part in further reactions such as the formation of high molecular weight melanoidins. Therefore, levels of these intermediary aldehydes in malt depend on formation reactions as well as on subsequent reactions. With the exception of roasted malt, a net formation of carbonyl compounds was observed. The lower thiobarbituric acid number of wort brewed with roasted malt might be explained by the higher roasting temperatures (> 200°C) used for the production of this malt type. Due to different reaction conditions, carbonyl precursors might additionally form other intermediary Maillard compounds, leading to less formation of carbonyl compounds. It is also possible that thiobarbituric acid reactive groups are lost by the transformation of aldehydes or by the formation of bonds with other compounds. In roasted malt, aldehydes might be involved in polymerisation reactions resulting in the formation of melanoidins which are of significantly higher molecular weight than melanoidins of other dark specialty malts[7]. Taken together the thiobarbituric acid number appears to be a good estimator for the thermal treatment received during the production of pilsner, colour and caramel malt. For roasted malt, it is a less useful indicator.

Purge and trap GC/MS analysis of Congress wort

Apart from very concentrated compounds, such as furfural and 5-methylfurfural, malt contains a number of aromatic compounds in trace levels[34]. The prevalence of heterocyclic dark malt constituents has been intensively studied earlier[33,35,42,43]. It has been reported that curing or roasting temperatures have the greatest influence on the level of volatile malt compounds[33]. In this work, dynamic headspace GC/MS was applied to evaluate the volatile composition of wort. Furthermore, it was investigated which volatiles were noticeably influenced by malt colour.

Purge and trap GC/MS allowed the detection and identification of several compounds. The largest group of detectable wort volatiles consisted of aldehydes, including linear aldehydes (C2–C10), unsaturated aldehydes (e.g. 2-octenal, 2,4-decadienal), branched aldehydes (e.g. 2-methylpropanal, 2-methylbutanal, 3-methylbutanal), and branched unsaturated aldehydes (e.g. 2-isopropyl-5-methyl-2-hexenal). Other identifiable groups were ketones, organic acids (C2–C4), sulphur compounds (SO_2, DMS, dimethyltrisulfide) and different types of Maillard reaction products. The latter group contained oxygen heterocyclic compounds such as furans and furanones and, at trace levels, nitrogen heterocyclic compounds such as pyrroles and pyrazines.

Using purge and trap GC/MS, five main wort compounds were found to significantly alter with malt colour (Fig. 5). Worts brewed with different dark specialty malts

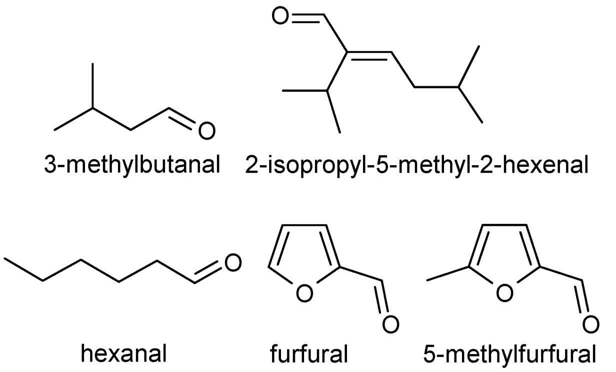

Fig. 5. Chemical structure of five Congress wort volatiles which are, according to purge and trap GC/MS analysis, clearly influenced by malt colour.

notably differed in 3-methylbutanal (isovaleraldehyde), 2-isopropyl-5-methyl-2-hexenal (isodihydro lavandulal), hexanal, 2-furancarboxaldehyde (furfural), and 5-methyl-2-furancarboxaldehyde (5-methylfurfural). Relative concentrations of these five compounds are represented in Fig. 6. For each compound, the highest concentration was set to 100%.

It was observed that 3-methylbutanal reached a maximal level in wort brewed with malt of 150 EBC units. Previously, it has been demonstrated that 3-methylbutanal is one of the most important contributors to the overall intensive malty flavour of dark malt[1,14,46]. This aldehyde, which is formed by the Strecker degradation of leucine, can be reduced to 3-methylbutanol during fermentation. However, 3-methylbutanal is also reported to be responsible for an agreeable sweet malty flavour in dark beers[15].

From Fig. 6, it was also established that the compound 2-isopropyl-5-methyl-2-hexenal gradually increased with malt colour. This component was probably formed by an aldol condensation between two 3-methylbutanal molecules. The product of this reaction is an alpha-beta-hydroxyaldehyde or aldehyde-alcohol (aldol). During curing or roasting, this aldol product might undergo dehydration to give a conjugated system, namely the alpha-beta-unsaturated aldehyde 2-isopropyl-5-methyl-2-hexenal.

In contrast to 3-methylbutanal and its condensation product, two Maillard compounds (furfural and 5-methylfurfural) appeared to increase exponentially with malt colour. These results suggest that the formation of furfural and 5-methylfurfural requires more thermal energy than the formation of the other two compounds.

From the data presented in Fig. 6, it can also be seen that hexanal was the only compound noticeably decreasing with increasing malt colour. As this compound is the most important degradation product of linoleic acid[26], it is a useful indicator molecule for oxidation reactions. Hexanal is found in considerable levels in green malt and pale malt. It is formed during germination, subsequent thermal processing, and storage of malt[46]. Dark malt usually contains less of this aldehyde. This can be due to inactivation of lipoxygenase enzymes during curing or roasting[11], intensive evaporation[46] or interaction between lipid degradation products and Maillard reaction products during heating[31]. In this study, the reference wort was prepared with 100% of pilsner malt, whilst the other samples were brewed with 50% of pilsner malt and 50% of dark malt. It was indeed observed that worts brewed with dark malt contained less hexanal. Remarkably, the level of hexanal in the darkest wort sample was less than half of the level in pilsner wort. Therefore, it is plausible that part of the hexanal in pilsner wort is also formed by oxidation reactions during mashing. Darker worts seem to be better protected against oxidation during mashing and filtration. The lower amounts in wort samples of higher colour value might also be due to the binding of hexanal to certain Maillard reaction products during wort production.

Purge and trap analysis of 12° Plato wort

The volatile composition of Congress wort was clearly affected by the malt type used. Nevertheless, few heterocyclic Maillard compounds were detected in these Congress worts. To better observe the influence of the malt type used on the level of heterocyclic Maillard compounds, more concentrated wort samples (12° Plato) were brewed according to the Congress method and evaluated by means of purge and trap GC/MS. In these worts, more heterocyclic compounds could be detected including oxygen heterocycles: 2-furfural, 5-methyl-2-furfural, acetyl-

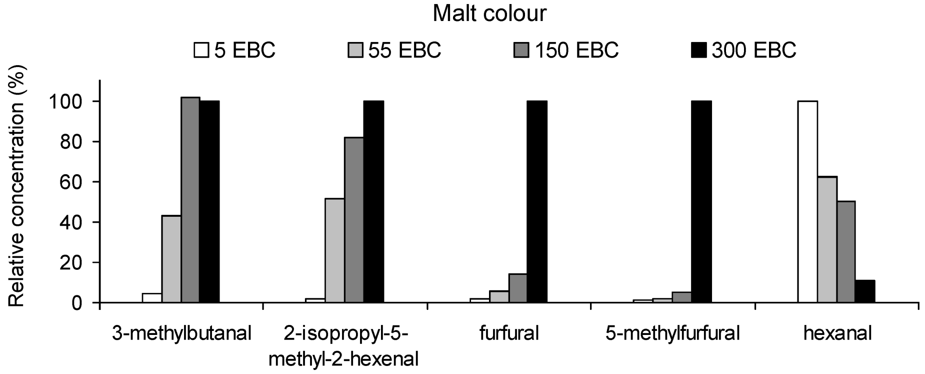

Fig. 6. Semi quantitative purge and trap GC/MS analysis of five volatile compounds in wort. Wort samples were brewed with 100% of pilsner malt (5 EBC units) or with 50% of pilsner malt and 50% of dark malt (55, 150 and 300 EBC units). For each chemical compound, the highest concentration was set to 100%.

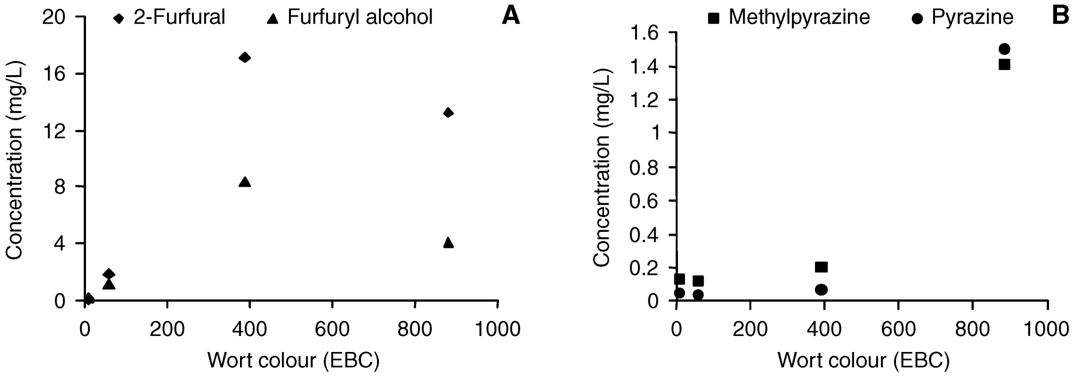

Fig. 7. **(A)** Levels of the oxygen heterocyclic compounds 2-furfural and furfuryl alcohol in 12°P wort. **(B)** Levels of the nitrogen heterocyclic compounds pyrazine and methylpyrazine in 12°P wort. Wort samples were brewed with 100% Scarlett malt of 5 EBC units or with 50% Scarlett and 50% dark malt (Melanoidin malt of 67 EBC units, Cara-aroma of 480 EBC units and Roasted malt of 1200 EBC units). Corresponding wort colours were 8, 60, 390 and 880 EBC units, respectively.

furan and furfuryl alcohol but also nitrogen containing heterocycles: pyrazine, methylpyrazine, 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine and 2-ethyl-3-methylpyrazine. The relationship between the amounts of these heterocyclic compounds and the wort colour was found to depend on the type of heterocyclic atom in the carbon ring (Fig. 7). The levels of oxygen containing heterocyclic compounds furfural, furfuryl alcohol, 5-methyl-2-furfural and acetylfuran were the highest in 390 EBC wort brewed with Cara-aroma, a caramel malt with a colour of 480 EBC units. The lower levels of oxygen heterocycles in roasted malt indicate that these compounds can be involved in reactions occurring at higher temperatures. Only when roasted malt was used as part of the grist, pyrazines and pyrazine derivatives (methylpyrazine, 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine and 2-ethyl-3-methylpyrazine) were observed in considerable levels. These nitrogen-containing compounds can be formed by the condensation of aminoketones, which originate from the Strecker degradation or by the condensation of retroaldol fragments. Fig. 7 suggests that pyrazine formation requires more thermal energy than the formation of oxygen heterocyclic compounds. Furthermore, the former heterocycles seem to be less reactive than the latter.

The data acquired by purge and trap GC/MS analysis of 12° Plato wort, are in good agreement with the results for wort tasting. More specifically, in the experiment where the influence of the malt type on the sensory profile of wort was investigated, it was established that bread and caramel-like flavours reached a maximal intensity for the intermediate malt colours, whereas burnt and bitter flavours persistently increased with malt colour. The same trends were observed for oxygen and nitrogen heterocyclic compounds, respectively. This is presumably no coincidence as bread and caramel-like flavours are evoked by several oxygen heterocycles while pyrazines are known to contribute bitter and burnt flavours to many roasted food products.

4-Vinylguaiacol in Congress wort

With a flavour threshold value of 0.3 ppm[29], 4-vinylguaiacol is the most flavour-active phenolic compound in wort and beer. The compound, which is formed by the decarboxylation of ferulic acid, has a flavour often referred to as smoky, burnt, spicy or clove-like[37].

When high levels of 4-vinylguaiacol occur in beer, this phenolic compound was most likely formed during fermentation with yeast strains of the Pof$^+$ phenotype[9]. However, thermal decomposition of ferulic acid can also lead to elevated levels of this compound. Thermal decarboxylation reactions have been reported to occur during malt curing[23] and wort boiling[42]. In this work, it was investigated which type of thermal treatment generates the most 4-vinylguaiacol in unfermented wort.

Congress worts brewed with 50% of pilsner malt and 50% of specialty malt were analysed for 4-vinylguaiacol. Although a highly sensitive HPLC method was used (detection limit < 0.01 ppm), the phenolic compound was only observed in wort brewed with 50% of roasted malt (0.44 ppm of 4-vinylguaiacol). It seems that only the highest roasting temperatures, used for the production of roasted barley and roasted malt (> 200°C), allow thermal degradation of ferulic acid. In agreement with previous studies, harsher roasting conditions can result in medicinal and smoky flavours[30]. However, since roasted barley or roasted malt make up only a small proportion of the grist (< 5%), phenolic off-flavours would not be expected to appear under normal circumstances[30]. In order to evaluate the effect of boiling, wort brewed with 100% pilsner malt was boiled for different periods of time (Table II). The phenolic compound gradually developed during boiling and could already be detected after half an hour. Despite the lower temperatures, boiling of wort appears to generate more 4-vinylguiacol than curing or roasting of green malt. These results suggest that thermal degradation of ferulic acid is favoured under aqueous reaction conditions.

Table II. Levels of 4-vinylguaiacol formed during boiling of Congress wort brewed with 100% pilsner malt.

Boiling time (h)	4-Vinylguaiacol (ppm)
0.5	0.06
1	0.15
2	0.22
3	0.29

Acetic acid in Congress wort

Organic acids arising in beer originate mainly from wort fermentation and are derived directly from pyruvate or from the branched tricarboxylic acid cycle[3]. Their excretion into beer results in a lowering of the pH and can be explained by the need to maintain a neutral intracellular pH and the fact that yeast cells do not require these acids for anabolic reactions[3]. Acetic acid is rather volatile and can produce an acid taste and a flavour reminiscent of vinegar. Nevertheless, levels in beer are usually below the threshold of 175 ppm[29]. As it has been reported that the Maillard reaction can also generate acetic acid[21], the influence of dark malt on the content of acetic acid in Congress wort was investigated. From Table III, it can be seen that the malt type used has a clear influence on the acetic acid concentration. The level of this volatile organic acid had a maximal value for wort brewed with the second darkest caramel malt. The lower values in wort samples brewed with darker malts might result from higher roasting temperatures, which in turn can lead to more evaporation but also to further reactions involving acetic acid. The results obtained in this study confirm that acetic acid is formed by the Maillard reaction. Yet, it is not clear whether this occurs mainly during curing and roasting of malt or during mashing. Although the levels in wort can approach the threshold value, this will not lead to problems in beer as acetic acid largely evaporates during wort boiling.

Diacetyl and 2,3-pentanedione in Congress wort

Diacetyl (2,3-butanedione) and 2,3-pentanedione can impart distinct flavours to beer, typically referred to as butterscotch and honey, respectively. Of the two, diacetyl is the most significant vicinal diketone as it usually occurs in larger amounts and as it has a lower flavour threshold than 2,3-pentanedione. The threshold concentration of diacetyl is reported as 0.15 ppm whereas 2,3-pentanedione has a flavour threshold of about 0.9 ppm[29]. Diacetyl and 2,3-pentanedione are typically associated with the fermentation process where they are generated as side-products of the pathways by which yeast produces valine and isoleucine[3]. Nevertheless, these vicinal diketones have also been detected in different Maillard model systems. In the cascade of Maillard reactions, diacetyl and 2,3-pentanedione can be formed by retro-aldolisation or cleavage[47]. These reactive intermediates possess a much higher browning activity than monosaccharides[20]. Furthermore, α-dicarbonyl compounds such as diacetyl and 2,3-pentanedione play an important role in the formation of flavour compounds as they can react with α-amino acids according to the Strecker degradation. The formed aminoketones may subsequently be converted to the corresponding pyrazines[43]. The Maillard reaction can also lead to the development of vicinal diketones during beer aging[44]. The importance of vicinal diketones during aging has partially been elucidated. The addition of a vicinal diketone trapping reagent to beer was found to inhibit the formation of HMF and furfural during the storage at 28°C and delayed the development of bread-like, caramel and burnt flavour notes[4].

Table III. Levels of acetic acid in Congress worts brewed with 50% of pilsner malt and 50% of different dark malts.

Malt type	Wort colour (EBC units)	Acetic acid (ppm)
Pilsner	5	25
Caramel	19	56
Caramel	25	63
Colour	37	69
Caramel	79	66
Caramel	110	165
Caramel	240	75
Roasted	610	36

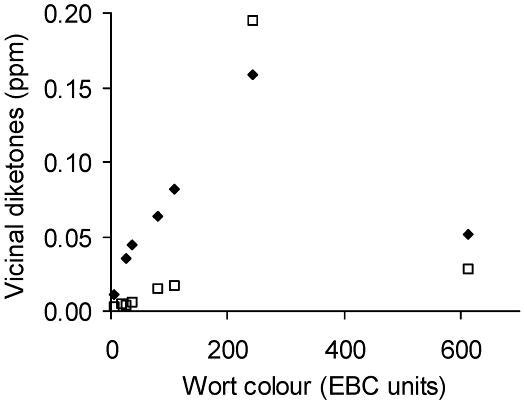

Fig. 8. Relation between wort colour and vicinal diketone level in Congress wort (◆ = diacetyl, □ = 2,3-pentanedione).

In this work, it was examined whether vicinal diketones can be found in unfermented wort brewed with 50% of dark malt. From Fig. 8 it can be seen that considerable levels indeed occurred in dark worts. A significant part of these vicinal diketones was reduced during fermentation, which resulted in lower levels of diacetyl and 2,3-pentanedione in beer brewed with dark specialty malts[8]. The amounts of diacetyl and 2,3-pentanedione in Congress wort were highest in the 240 EBC sample. In this sample, the diacetyl level slightly exceeded the threshold value. Furthermore, this was the only sample where the level of 2,3-pentanedione was higher than the level of diacetyl. In line with the results obtained by the TBN assay, reaction conditions used for the production of the darkest caramel malt seem to leave a maximal amount of reactive Maillard intermediates in wort.

CONCLUSIONS

Brewers mainly select dark specialty malts based on the colour specification. However, this characteristic does not allow predicting the flavour contributions to wort or beer. The present work was undertaken to better understand the origin and the distribution of several flavour compounds in different dark specialty malts. Malt flavour was assessed by both, sensory profiling and instrumental assessment of wort. Regardless of the technique used, it was concluded that malt flavour and colour cannot be equated.

Initially, the flavour evoked by dark malts was evaluated by Congress wort tasting. In order to increase the accuracy of the obtained data, a tasting panel was trained to recognize typical malt flavours. Subsequently, a comprehensive terminology for the assessment of malt flavour was developed. Wort tasting proved to be a useful tool to generate a fingerprint of dark malts. Some Maillard reaction related flavour attributes such as 'burnt' and 'bitter' tended to increase with malt colour or malt level. Conversely other flavour attributes such as 'bread' and 'caramel' appeared to have a maximal intensity for the intermediate wort colours. Sensory assessment further revealed that wort flavour profiles not only depended on malt level and malt colour, but also on malt type and even on where the malt was produced.

Several components of low volatility can significantly impart the flavour of wort brewed with dark malt. Important representatives from various chemical classes were determined by different instrumental analyses. Purge and Trap GC-MS allowed the differentiation of Congress wort by five main volatile compounds. As expected, Maillard compounds increased with increasing wort colour. However, not all Maillard reaction products evolved to the same extent. The Strecker aldehyde 3-methylbutanal had a maximal level in wort produced with caramel malt of 150 EBC units. The aldol condensation product of two 3-methylbutanal molecules (2-isopropyl-5-methyl-2-hexenal) increased steadily with the colour of wort. In contrast, furfural and 5-methylfurfural were mainly observed in the darkest wort samples. Hexanal was the only compound clearly decreasing with wort colour. A protective effect of malt antioxidants during the brewing process and the binding of hexanal to Maillard reaction products might contribute to this effect.

The thermal decarboxylation of ferulic acid to 4-vinylguaiacol was compared for two thermal processes: malt drying and wort boiling. The effect of curing or roasting was examined by the determination of 4-vinylguaiacol in Congress wort. The phenolic flavour compound could only be detected in wort brewed with roasted malt. On the other hand, even short boiling of pilsner wort already resulted in the formation of detectable levels of 4-vinylguaiacol, suggesting that thermal decarboxylation of ferulic acid is favoured in an aqueous medium.

This study furthermore indicates that there is a net formation of flavour compounds in most dark specialty malts. The thiobarbituric acid number, the acetic acid level and the vicinal diketone content of Congress wort as well as the amount of oxygen heterocyclic compounds in 12° Plato wort revealed that dark caramel malts contain most intermediary Maillard reaction products. Although roasted malts are much darker, they appear to have less of these intermediates. It is still unclear whether the lower levels of volatile products in roasted malt are primarily due to pyrolysis, advanced polymerisations, abundant evaporation and/or lack of substrates for the Maillard reaction. The analysis of the evolution of intermediate compounds during the production of roasted malts could help to elucidate these findings. Only pyrazines and pyrazine derivates increased throughout the colour range. Whereas these compounds only occurred in trace levels in most malt types, they seem to be formed in large amounts during the production of roasted malt. Moreover, it was established that the concentration of oxygen and nitrogen heterocycles correlated well with the intensity of caramel-like and burnt flavours, respectively.

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