

Impact of Dark Specialty Malts on Extract Composition and Wort Fermentation

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ABSTRACT

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Dark specialty malts are important ingredients for the production of several beer styles. These malts not only impart colour, flavour and antioxidative activity to wort and beer, they also affect the course of wort fermentations and the production of flavour-active yeast metabolites. The application of considerable levels of dark malt was found to lower the attenuation, mainly as a result of lower levels of fermentable sugars and amino acids in dark wort samples. In fact, from the darkest caramel malts and from roasted malts, practically no fermentable material can be hydrolysed by pilsner malt enzymes during mashing. Compared to wort brewed with 50% pilsner malt and 50% dark caramel malt or roasted malt, wort brewed with 100% pilsner malt contained nearly twice as much fermentable sugars and amino acids. Reduced levels of yeast nutrients also lowered the fermentation rate, ranging from 1.7°P/day for the reference pilsner wort of 9 EBC to 1.1°P/day for the darkest wort (890 EBC units), brewed with 50% roasted malt. This additionally indicates that lower attenuation values for dark wort are partially due to the inhibitory effects of Maillard compounds on yeast metabolism. The application of dark caramel or roasted malts further led to elevated levels of the vicinal diketones diacetyl and 2,3-pentanedione. Only large levels of roasted malt gave rise to two significant diacetyl peaks during fermentation. The level of ethyl acetate in beer was inversely related to colour, whereas the level of isoamyl acetate appeared to be affected by the use of roasted malt. With large levels of this malt type, negligible isoamyl acetate was generated during fermentation.

Key words: Maillard reaction, wort sugars and amino acids, fermentation, attenuation, vicinal diketones, esters.

INTRODUCTION

Dark specialty malts can be defined as all malts cured at higher temperatures than pilsner malt or roasted in a roasting drum. Depending on the production process, these malts are usually classified into three groups: colour malts, caramel malts and roasted malts¹⁰. The most important properties of dark malts (colour, flavour and antioxidative activity) result from non-enzymatic browning, also known as the Maillard reaction^{6,8}. Colour malts, but also caramel malts and roasted malts, which are rarely used in levels above 5%^{2,36}, can have an appreciably high impact

on the final beer characteristics as they contribute to the colour and flavour of several beer styles⁵. An unlimited number of colours and flavours can be attained by the use of different dark specialty malts, but also by the variation of the dark malt level or by the combination of different malt types^{14,17,18}. Dark malts may also improve the foam stability and the mouthfeel of beer, presumably by the presence of melanoidins^{21,23}. Furthermore, due to a higher level of antioxidants, beers brewed with dark malts normally have a better flavour stability and a longer shelf life than pale beers⁴.

The influence of non-enzymatic browning on both physico-chemical as well as sensory characteristics of malt was assessed in previous work^{7,9,10}. Conversely, this study was mainly concerned with the effect of dark malts and, as a consequence, of non-enzymatic browning on the course of the fermentation and on the development of flavour-active yeast metabolites. During fermentation, brewing yeast strains utilise a wide variety of nutrients to support growth and to generate energy. Of these, carbohydrates and amino acids are of most significance for fermentation performance and beer quality³. As, during thermal treatment of malt, Maillard reactions are initiated by the reaction between reducing sugars and amino compounds, it can be deduced that wort brewed with considerable levels of dark malt contains lower levels of carbohydrates and amino acids. This undoubtedly affects several aspects of the fermentation process, including attenuation and fermentation rate. Since the amino acid metabolism of brewers' yeast is closely associated with the formation of fusel alcohols, esters and vicinal diketones, lower amino acid levels in wort brewed with dark malt might substantially affect the generation of these important flavour-active compounds.

To gain more knowledge on the levels of available yeast nutrients, fermentable carbohydrates and amino acids were quantified in Congress wort. Furthermore, pilot scale wort samples were brewed with different levels and different types of dark specialty malts and assessed for fermentation rate and attenuation, while flavour compounds were evaluated in the resulting beers. In order to better detect the impact of Maillard compounds, brews were prepared with excessive levels of dark malt and without the addition of hops.

MATERIALS AND METHODS

Materials

All dark specialty malts were commercial samples provided by Dingemans (Stabroek, Belgium) and Weyermann (Bamberg, Germany). Standard pilsner malt was supplied by Cargill Malt (Herent, Belgium).

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A top-fermenting *Saccharomyces cerevisiae* brewery strain (CMBS 301) was obtained from the CMBS (Centre for Malting and Brewing Science) yeast collection, Leuven, Belgium.

The reagents sodium azide, ninhydrin, dabsyl chloride, 3,3'-thiodipropionic acid (TDPA) and dimethylformamide, the internal standards norvaline and rhamnose, and also the 20 calibration amino acids (aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, glycine, arginine, alanine, proline, valine, methionine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, lysine, histidine and tyrosine) and the 5 calibration saccharides (glucose, fructose, maltose, maltotriose and maltotetraose) were purchased from Sigma-Aldrich Chemie GmbH, (Munich, Germany). Triethylamine was obtained from Fisher Scientific (Hanover Park, IL, USA).

Production of pilot scale wort and beer

Standard 12°P wort was prepared as described by Delvaux *et al.*¹². In brewing experiments with 100% pilsner malt or 100% dark specialty malt, the total amount of malt required for 1 litre of 12°P wort was calculated using equation (1). For brews made with different levels of pilsner and specialty malt, the amount of malt was deduced from equations (2) and (3).

$$\text{Total amount of malt (g)} = \frac{132}{\left(\frac{E \cdot (100 - M)}{10000}\right)} \quad (1)$$

Amount of pilsner malt (g)

$$= \frac{132}{\left(\frac{E_{\text{pils}} \cdot (100 - M_{\text{pils}})}{10000}\right) + \left(\frac{\%_{\text{sp}}}{\%_{\text{pils}}} \cdot \frac{E_{\text{sp}} \cdot (100 - M_{\text{sp}})}{10000}\right)} \quad (2)$$

Amount of dark specialty malt (g)

$$= \text{Amount of pilsner malt} \cdot \frac{\%_{\text{sp}}}{\%_{\text{pils}}} \quad (3)$$

where:

- E = extract content of dry malt, % (w/w)
- E_{pils} = extract content of dry pilsner malt, % (w/w)
- E_{sp} = extract content of dry specialty malt, % (w/w)
- M = moisture content of malt, % (w/w)
- M_{pils} = moisture content of pilsner malt, % (w/w)
- M_{sp} = moisture content of specialty malt, % (w/w)
- %_{pils} = level of pilsner malt, % (w/w)
- %_{sp} = level of specialty malt, % (w/w)

For the production of beer, wort samples were boiled for 1 h in a glycerol bath (106°C), cooled and subsequently aerated to obtain an oxygen concentration of 8 ppm. Wort was pitched with 10 × 10⁶ yeast cells per millilitre and fermented for 10 days at 20°C. On the clarified

beer, real attenuation, ethanol content and real extract were measured using a digital density meter (Paar DSA 48, Anton Paar, Graz, Austria).

Determination of volatile compounds by headspace GC

Beer volatiles were quantified by headspace gas chromatography using a Perkin Elmer AutoSystem XL (Perkin Elmer, Norwalk CT), equipped with a flame ionisation detector (FID) and an electron capture detector (ECD). Samples of 5 mL were heated for 16 min at 60°C in the HS-40 auto sampler. Separation was achieved on a 50 m WCOT fused silica capillary column coated with CP-Wax 52CB (length 50 m, internal diameter 0.32 mm and layer thickness 1.2 µm; Varian, Middelburg, The Netherlands). 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; FID detector temperature 250°C, ECD detector temperature 220°C. Helium was used as carrier gas. The obtained chromatograms were analysed using Turbochrom Navigator software (Perkin-Elmer, Norwalk CT).

Determination of saccharides by HPLC

Prior to HPLC analysis, ionic extract components were removed from wort and beer by means of ion exchange resins. To this end, a 200 µL sample was loaded on a 'mixed bed' ion-exchanger (Dowex-50H⁺/Dowex-1-acetate, Dow Chemical Company, Midland, MI, USA) and rinsed 6 times with 200 µL Milli-Q water (Millipore, New Bedford, MA, USA). Neutralised samples were centrifuged (1500g, 5 min) to remove resin particles and further diluted in sodium azide water (0.02%, w/v).

Saccharides were analysed by HPLC according to Van den Ende *et al.*³², using a Dionex DX-500 high-performance anion exchange chromatography device (Dionex, Sunnyvale, CA, USA). After separation on a Dionex CarboPac PA100 column (4 × 250 mm), saccharides were quantified with a pulsed amperometric detector (Dionex ED40) equipped with a gold working electrode. The eluent was pumped at a flow rate of 1.0 mL/min using a Dionex GP40 gradient pump and consisted of a mixture of 100 mM NaOH and increasing concentrations of Na acetate (30–350 mM gradient in 45 min). The column was regenerated with 1 M NaOH for 10 min and equilibrated for 20 min after every run. Quantification was performed using rhamnose as an internal standard. Conversion factors (relative to rhamnose) of glucose, fructose, maltose, maltotriose and maltotetraose were obtained using the external-standard method. PeakNet chromatography software (Dionex, Sunnyvale, CA, USA) was used for system control and data analysis.

Determination of amino acids by HPLC

Amino acids were analysed as described by Krause *et al.*²² with some modifications. After membrane filtration over 0.45 µm filters (Alltech, Deerfield, IL, USA), a 300 µL sample was diluted with 300 µL internal standard (Norvaline 100 mg/L in 0.1 M HCl–0.2% TDPA). The diluted sample was deproteinised by ultrafiltration through a low-binding, regenerated cellulose 5 kDa membrane (Milli-

Table I. Elution programme for amino acid analysis by high performance liquid chromatography.

| Time (min) | 10 mM Sodium acetate (% (v/v)) | 80% (v/v) Acetonitrile (% (v/v)) |
|------------|--------------------------------|----------------------------------|
| 0 | 92 | 8 |
| 8.75 | 80 | 20 |
| 35 | 65 | 35 |
| 50 | 20 | 80 |
| 59 | 20 | 80 |
| 61 | 92 | 8 |
| 68 | 92 | 8 |

pore, Bedford, MA, USA) and then derivatised by dabsyl chloride reagent (12.4 mM in acetone) using an automated pre-column dabsylation device (AS3500, ThermoFinnigan, San Jose, CA, USA). To this end, 10 µL sample, 100 µL dabsyl chloride reagent and 90 µL reaction buffer (150 mM NaHCO₃, pH 8.6) were subsequently loaded to an empty glass vial with screw cap (1.5 mL). The vial was incubated at 70°C for 15 min, during which it was vortexed for the first and last 5 min. After cooling at 12°C for 5 min, 200 µL of dilution buffer (mixture of 50 mL acetonitrile, 25 mL ethanol and 25 mL acetate buffer (10 mM, pH 6.55, containing 4% dimethylformamide and 0.16% triethylamine)) was added and the sample was kept at 12°C for 20 min before injection.

Following a specific elution programme (Table I), derivatised samples (10 µL) were separated on an Alltima C18 column (length, 150 mm; ID, 4.6 mm; particle size, 5 µm, Alltech, Deerfield, IL, USA) and detected at 365 nm with a UV detector (UV3000HR, ThermoFinnigan, San Jose, CA, USA). The column was thermostated at 50°C. Results were analysed using PC1000 System software (ThermoFinnigan, San Jose, CA, USA). Before analyses, the HPLC column was calibrated with a series of amino acid standard solutions at concentrations ranging from 10 to 100 ppm.

Free amino nitrogen (FAN)

Free amino nitrogen was measured by colorimetry using the ninhydrin method¹³. Wort or beer samples were diluted 100 times with distilled water. Exactly 1.0 mL of

colour reagent (100 g/L NaHPO₄, 60 g/L KH₂PO₄, 5 g/L ninhydrin and 3 g/L fructose) was added to 2.0 mL of the diluted sample in a glass tube with a screw cap. The sample was placed in a water bath (100°C) for exactly 16 min. After cooling in a water bath (20°C) for 20 min, 5.0 mL of a dilution reagent (2 g KIO₃ in 1 L H₂O/EtOH (600:400, v/v)) was added to the sample and thoroughly mixed. The absorbance of the sample was measured at 570 nm. A blank (distilled water) and a glycine standard solution (10.72 mg/L) were also analysed following the same procedure. A colour correction was included by taking into account the absorbance caused by coloured compounds.

RESULTS AND DISCUSSION

Dark malts lower the attenuation of wort

In a preliminary experiment, as many as 40 pilot scale beers were brewed with different levels of 9 dark specialty malts. As darker malts have a lower extract yield¹⁰, higher quantities of these malts were needed to obtain an original extract of about 12°P. Table II represents some significant properties of beer brewed with various levels of one malt type (caramel malt of 300 EBC units), whereas Table III summarises the same properties of beer brewed with a specific level (30%) of different dark malts. It was found that the colour of beer increased with increasing levels of a specific malt or with the application of darker malts. Remarkably, although all worts were more or less of the same original extract (~12°P), attenuation and ethanol levels were lower while density and real extract were higher for the darker beers.

The data in Table II and Table III suggest that dark wort samples contain less fermentable sugars or that Maillard compounds inhibit the metabolism of yeast cells. Less fermentable extract might be partially due to a lowered malt enzymatic activity. In comparison with pilsner malt, dark malts have little or no enzymatic activity. As a result, the application of dark malt leads to less hydrolytic reactions during mashing and less fermentable extract. A lower fermentable extract content might also be ascribed to Maillard reactions during the production of dark malt.

Table II. Properties of beer brewed with various levels of caramel malt (300 EBC units).

| Malt type | Level of malt (%) | Beer colour (EBC units) | Original extract (°P) | Real extract (°P) | Real attenuation (%) | Ethanol (v/v) |
|-----------|-------------------|-------------------------|-----------------------|-------------------|----------------------|---------------|
| Caramel | 5 | 21 | 11.8 | 3.7 | 69 | 5.3 |
| Caramel | 15 | 49 | 11.7 | 3.6 | 69 | 5.3 |
| Caramel | 30 | 96 | 11.8 | 4.1 | 64 | 5.0 |
| Caramel | 50 | 162 | 12.0 | 5.3 | 56 | 4.5 |

Table III. Properties of beer brewed with 30% of dark malt and 70% of pilsner malt.

| Malt type | Malt colour (EBC units) | Beer colour (EBC units) | Original extract (°P) | Real extract (°P) | Real attenuation (%) | Ethanol (v/v) |
|-----------|-------------------------|-------------------------|-----------------------|-------------------|----------------------|---------------|
| Colour | 7 | 9.5 | 12.2 | 3.8 | 69 | 5.5 |
| Caramel | 20 | 15 | 11.8 | 3.7 | 69 | 5.3 |
| Colour | 50 | 33 | 11.9 | 4.0 | 67 | 5.2 |
| Caramel | 150 | 75 | 12.3 | 4.3 | 65 | 5.2 |
| Caramel | 300 | 96 | 11.8 | 4.1 | 64 | 5.0 |
| Roasted | 900 | 390 | 11.5 | 5.2 | 55 | 4.1 |

Table IV. Fermentable sugars in Congress wort brewed with 100% pilsner malt (P), 50% pilsner and 50% kiln-dried colour malt (K), 50% pilsner and 50% caramel malt (C) and 50% roasted malt (R).

| Malt name | Malt type | Wort colour (EBC units) | Level of saccharides in Congress wort (g/L) | | | | |
|----------------|-----------|-------------------------|---|----------|---------|---------|-------------|
| | | | Glucose | Fructose | Sucrose | Maltose | Maltotriose |
| Scarlett | P | 5 | 6.4 | 1.7 | 4.8 | 38.2 | 9.9 |
| Carahell | C | 19 | 5.6 | 1.4 | 5.6 | 34.0 | 10.6 |
| Carared | C | 25 | 6.3 | 1.4 | 5.7 | 34.1 | 11.1 |
| Melanoidin | K | 37 | 6.4 | 1.7 | 5.5 | 33.0 | 10.9 |
| Caramunich II | C | 79 | 5.3 | 1.3 | 4.9 | 31.1 | 10.3 |
| Caramunich III | C | 110 | 5.7 | 1.4 | 4.1 | 26.1 | 9.2 |
| Cara-roma | C | 240 | 5.8 | 1.3 | 4.4 | 27.7 | 8.3 |
| Carafa | R | 610 | 4.4 | 1.1 | 3.2 | 21.3 | 5.9 |

It is plausible that the Maillard compounds produced are unavailable for enzymatic hydrolysis. Consequently, it is unlikely that brewer's yeast can metabolise these Maillard reaction products.

The results presented in Table II and Table III were obtained after 10 days of fermentation. As the recorded values are only momentary, low attenuation values with dark wort could also result from a slow fermentation process. Indeed, some Maillard compounds are known for their toxic effects towards *Saccharomyces cerevisiae*^{1,31}. When considerable levels of dark malts are used, Maillard reaction products might reach harmful levels in wort, leading to an inhibition of yeast metabolism and a stuck or sluggish fermentation.

Composition of wort extract

The analysis of fermentable wort extract might help to explain the origin of the lower attenuation of dark wort. The levels of different saccharides and amino acids were determined in Congress worts, brewed with 50% pilsner malt (Scarlett) and 50% dark malt. Reference wort was prepared with 100% pilsner malt (Scarlett).

Table IV illustrates the profile of the most important fermentable saccharides in Congress wort brewed with diverse malt types. Fructose and sucrose presumably originate from malted barley where they mainly occur in the free form. Malt also contains limited amounts of free glucose, maltose and maltotriose. In wort, however, these three saccharides chiefly derive from the amylolysis of starch during the mashing process.

The level of maltose, the most abundant saccharide in wort, was noticeably decreased with increasing wort colour and its concentration in the darkest wort sample was nearly half the concentration in the reference wort. For the other saccharides, the relation between the concentration in wort and wort colour was less clear. Compared to the reference wort, samples brewed with malts of intermediate colour (<80 EBC units) contained even more sucrose and maltotriose. Only in wort prepared with 50% roasted malt were levels of glucose, fructose, sucrose and maltotriose significantly lower than the levels in pilsner wort.

During the production of colour malts or caramel malts, the application of a withering scheme or a saccharification phase results in a considerable hydrolysis of starch polymers. However, this was not translated into elevated levels of starch degradation products in wort. This is likely the result of a partial consumption of reducing sugars by non-enzymatic browning reactions during the sub-

sequent drying stages of the malt production process. Yet, the obtained data do not make it possible to estimate the effect of non-enzymatic browning as too many factors are involved. Indeed, the level of a specific saccharide in wort depends on the level of this saccharide in malt, the participation in non-enzymatic browning reactions and the release by malt enzymes during mashing. To gain more knowledge on the influence of the Maillard reaction, it could be interesting to determine saccharides during malt production.

Regardless of the involvement of saccharides in the Maillard reaction, the determination of sugars in wort might shed more light on the observed lower attenuation of wort brewed with dark malts. For this purpose, the percentage of fermentable sugars in the extract (ratio of the sum of the saccharides glucose, fructose, sucrose, maltose and maltotriose to the total extract) was plotted against wort colour (Fig. 1)). From this graph, it appears unambiguous that the fermentable fraction of the extract decreases with an increasing wort colour. Most of the extract of the reference pilsner wort (8.8°P) consisted of fermentable sugars (6.1°P). Congress wort brewed with Caramunich III (8.5°P) and Cara-roma (8.4°P) included somewhat more than 50% fermentable sugars, whereas wort brewed with 50% roasted malt (8.5°P) only contained 3.6°P as fermentable carbohydrates, chiefly deriving from pilsner malt. As the extract of the darkest wort already comprises nearly 5°P of compounds other than fermentable sugars, this inevitably leads to lower attenuation values.

Table V and Table VI represent the levels of the most common amino acids in descending order of concentration in pilsner wort. For all Congress worts, proline, leucine, arginine, phenylalanine and valine were the five most

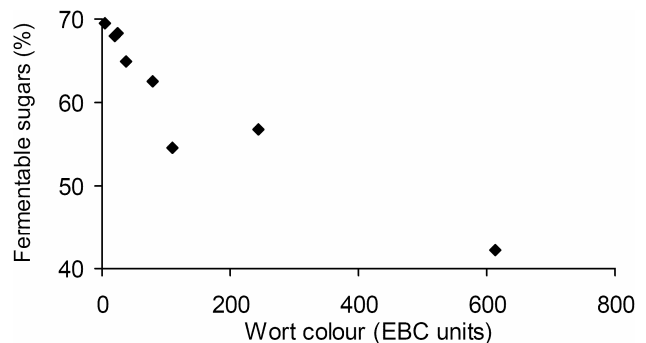


Fig. 1. Percentage of extract consisting of fermentable sugars as a function of the colour of Congress wort.

abundant amino acids which all have, with the exception of arginine, apolar side chains. Wort samples were poor in glycine and methionine and contained negligible levels of cysteine.

In general, the levels of the separate amino acids in wort decreased with increasing wort colour. The application of darker malts seems to generate less amino acids in wort. This was not so for Melanoidin malt, a kiln-dried colour malt. Although this malt was produced at higher temperatures than pilsner malt, wort brewed with 50% Melanoidin malt recurrently contained as much or more amino acids than the reference wort, made with 100% pilsner malt. It even contained significantly more amino acids than paler worts brewed with caramel malt (Carahell and Carared), probably as a result of the saccharification and caramelisation stages used for the production of caramel malt. It was further established that, without exception, the level of a specific amino acid in wort brewed with 50% pilsner malt and 50% dark caramel malt or roasted malt was about half the level in wort prepared with 100% pilsner malt. This suggests that in the darkest wort samples, practically all amino acids were derived from the pilsner malt. The same can be deduced from Fig. 2, representing the total level of amino acids (Fig. 2A) and the FAN content (Fig. 2B) in different Congress worts. When the separate amino acids from Table V and Table VI are plotted against wort colour, similar graphs as Fig. 2A are obtained (data not shown).

Dark caramel malts and roasted malt appear to be extremely poor in free and hydrolysable amino acids. In contrast to what was found for model media, all amino acids seem to be equally reactive under intensive roasting conditions. The low amino acid levels in dark wort samples also indicate that malt, when heated to very high temperatures, no longer contains proteins accessible to pilsner

malt proteases during mashing. This might be due to both protein denaturation and incorporation of proteins in the melanoidin structure¹⁹.

Uptake of extract during fermentation

In this work, it was demonstrated that the application of considerable levels of dark specialty malts results in a decreased attenuation due to less fermentable wort extract. Nevertheless, dark malts may also cause stuck or sluggish fermentations owing to a toxic effect of Maillard compounds towards the *S. cerevisiae* metabolism. To unravel whether dark malts also delay wort fermentations, the evolution of the extract was followed in the course of fermentation. For this purpose, wort was brewed with 4 different malt types, covering the largest possible colour range: pilsner malt (Scarlett), colour malt (Melanoidin), caramel malt (Cara-aroma) and roasted malt (Carafa). Instead of Congress wort, pilot scale wort of 12°P was used for the following experiments. Some general properties of wort brewed with the 4 different malt types are indicated in Table VII. Boiled wort was pitched with 10×10^6 yeast cells/mL and samples were collected at regular time intervals during fermentation.

Despite the similar original extract content (Table VII), the course of the fermentation curves markedly depended on the malt type (Fig. 3). During the first 90 h, the fermentation rate was the highest for the reference wort, at 1.74°P per day. Wort prepared with 50% Melanoidin malt or with 50% Cara-aroma was fermented at a rate of 1.38 and 1.34°P, respectively. In contrast, the extract utilisation by *S. cerevisiae* was hampered for wort brewed with 50% roasted malt and a rate of only 1.11°P per day was observed.

Although Melanoidin wort contained as much fermentable material as the reference wort, the apparent extract

Table V. Level of the amino acids proline (pro), leucine (leu), arginine (arg), phenylalanine (phe), valine (val), glutamine (gln), alanine (ala), tyrosine (tyr), lysine (lys) and isoleucine (ile) in wort made with 100% pilsner malt (P), 50% kiln-dried colour malt (K), 50% caramel malt (C) and 50% roasted malt (R).

| Malt name | Malt type | Wort colour (EBC units) | Level of amino acids in Congress wort (mg/L) | | | | | | | | | |
|----------------|-----------|-------------------------|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | Pro | Leu | Arg | Phe | Val | Gln | Ala | Tyr | Lys | Ile |
| Scarlett | P | 5 | 340 | 117 | 105 | 103 | 102 | 89 | 86 | 74 | 65 | 62 |
| Carahell | C | 19 | 270 | 99 | 106 | 83 | 84 | 70 | 77 | 63 | 51 | 55 |
| Carared | C | 25 | 305 | 101 | 61 | 83 | 86 | 64 | 83 | 63 | 46 | 55 |
| Melanoidin | K | 37 | 315 | 125 | 109 | 102 | 107 | 73 | 91 | 82 | 64 | 69 |
| Caramunich II | C | 79 | 220 | 85 | 82 | 73 | 78 | 59 | 59 | 70 | 48 | 47 |
| Caramunich III | C | 110 | 210 | 87 | 83 | 67 | 66 | 50 | 56 | 81 | 51 | 46 |
| Cara-aroma | C | 240 | 190 | 72 | 70 | 56 | 52 | 47 | 43 | 82 | 42 | 35 |
| Carafa | R | 610 | 190 | 70 | 55 | 55 | 49 | 45 | 44 | 44 | 36 | 33 |

Table VI. Level of the amino acids asparagine (asn), aspartic acid (asp), serine (ser), glutamic acid (glu), threonine (thr), tryptophan (trp), histidine (his), glycine (gly) and methionine (met) in wort made with 100% pilsner malt (P), 50% kiln-dried colour malt (K), 50% caramel malt (C) and 50% roasted malt (R).

| Malt name | Malt type | Wort colour (EBC units) | Level of amino acids in Congress wort (mg/L) | | | | | | | | |
|----------------|-----------|-------------------------|--|-----|-----|-----|-----|-----|-----|-----|-----|
| | | | Asn | Asp | Ser | Glu | Thr | Trp | His | Gly | Met |
| Scarlett | P | 5 | 53 | 49 | 44 | 43 | 43 | 41 | 41 | 27 | 21 |
| Carahell | C | 19 | 54 | 43 | 37 | 39 | 46 | 30 | 33 | 23 | 15 |
| Carared | C | 25 | 42 | 39 | 35 | 35 | 42 | 27 | 29 | 21 | 13 |
| Melanoidin | K | 37 | 51 | 44 | 41 | 46 | 46 | 32 | 44 | 26 | 22 |
| Caramunich II | C | 79 | 44 | 32 | 31 | 32 | 31 | 25 | 28 | 19 | 13 |
| Caramunich III | C | 110 | 39 | 28 | 29 | 31 | 29 | 19 | 23 | 19 | 12 |
| Cara-aroma | C | 240 | 33 | 23 | 23 | 28 | 24 | 18 | 20 | 17 | 10 |
| Carafa | R | 610 | 29 | 22 | 20 | 28 | 19 | 19 | 17 | 14 | 8 |

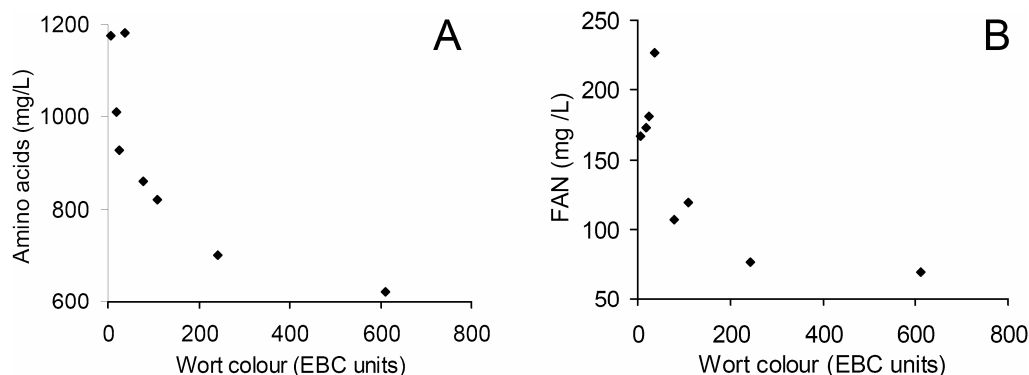


Fig. 2. (A) Relation between total level of amino acids in Congress wort and wort colour. (B) Relation between the free amino nitrogen (FAN) content of Congress wort and wort colour.

Table VII. Grist composition, original extract, colour, pH, radical scavenging (RS) and reducing activity (RA) of 12°P wort samples brewed with different malt types (Scarlett = pilsner malt, Melanoidin malt = colour malt, Cara-aroma = caramel malt and Carafa = roasted malt).

| Wort sample | Grist constitution | Original extract (°P) | Wort colour (EBC units) | pH | RS (% DPPH discolouration) | RA (Fe-Dip absorption) |
|-------------|-------------------------------------|-----------------------|-------------------------|------|----------------------------|------------------------|
| 1 | 100% Scarlett | 12.5 | 9 | 5.79 | 16 | 8 |
| 2 | 50% Scarlett 50% Melanoidin malt | 12.5 | 62 | 5.32 | 56 | 14 |
| 3 | 50% Scarlett 50% Cara-aroma | 11.9 | 390 | 4.93 | 80 | 31 |
| 4 | 50% Scarlett 50% Carafa | 12.0 | 890 | 5.10 | 89 | 62 |

consumed by yeast after 190 h of fermentation was significantly lower (8.26 versus 10.22°P). This supports the suggestion that not only the level of fermentable sugars, but also the presence of Maillard compounds can affect the rate of extract conversion. Further evidence for the equal amount of fermentable extract in Scarlett and Melanoidin wort samples can be deduced from the nearly identical values of the apparent extract, recorded after 430 h.

Despite the fact that the curves representing the uptake of extract from Melanoidin and Cara-aroma wort were found to overlap during the first days of the fermentation,

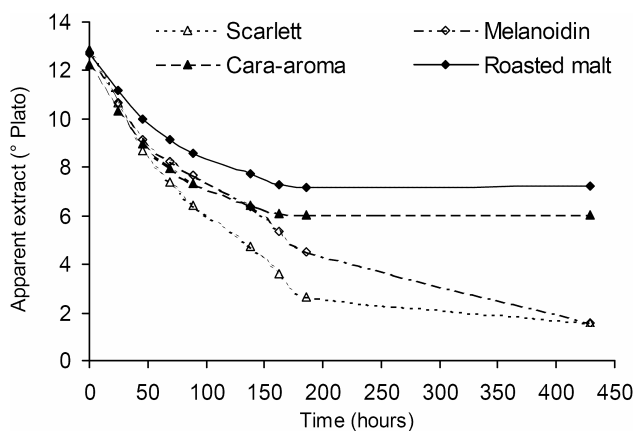


Fig. 3. Evolution of the apparent extract content during the fermentation of wort brewed with 100% Scarlett or with 50% Scarlett and 50% of dark malt (Melanoidin, Cara-aroma and roasted malt). Properties of these wort samples are presented in Table VII.

they diverged after 140 h. From then on, the curve for Melanoidin wort approached the reference curve (Scarlett wort), whereas for Cara-aroma wort, no further decrease in extract was found after reaching 6.0°P. The curve for wort brewed with roasted malt ran nearly parallel with the curve for Cara-aroma wort. After fermentation, the former wort still contained at least 7.1°P unfermentable material. The results for wort from Cara-aroma and roasted malt were not due to a stuck fermentation as the high values for the limit extract agree with the data on fermentable carbohydrates presented earlier.

Evolution of flavour-active yeast metabolites during fermentation

The lowered levels of amino acids in wort brewed with dark malts most certainly have an effect on the formation of flavour, as several important flavour compounds result from the amino acid metabolism of yeast. The evolution of the flavour profile during fermentation of the wort samples presented in Table VII, was examined by headspace GC. While a FID allowed the determination of acetaldehyde, DMS, ethyl acetate, isoamyl acetate, ethyl caproate, n-propanol, isobutanol and isoamyl alcohol, the ECD could be used for the quantification of the vicinal diketones 2,3-butanedione (diacetyl) and 2,3-pentanedione.

Vicinal diketones

In a preliminary experiment, beer samples were obtained with 30% of different dark malts (Table III) and analysed for diacetyl and 2,3-pentanedione (Fig. 4). Al-

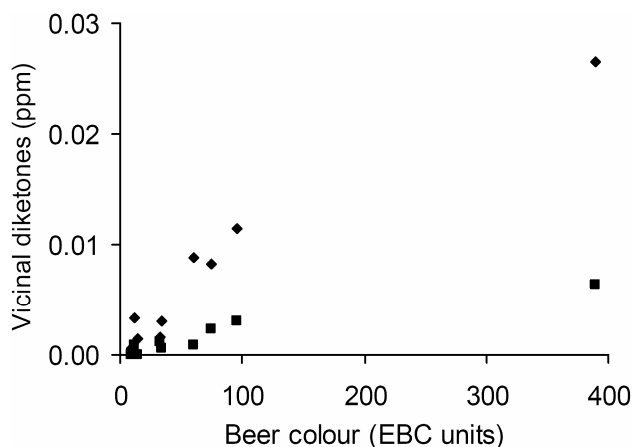


Fig. 4. Relation between the vicinal diketone level and beer colour (♦ = diacetyl, ■ = 2,3-pentanedione). Beer samples were prepared with 30% of diverse dark malts (see Table III).

though vicinal diketone levels increased with beer colour, beer contained less diacetyl and 2,3-pentanedione than wort⁹.

In previous work, it was described that thermal treatment of malt and wort leads to the formation of diacetyl and 2,3-pentanedione as well as to further reactions⁹. In contrast, diacetyl and 2,3-pentanedione generated during fermentation indirectly result from yeast metabolism as overflow products of valine and isoleucine biosynthesis^{3,11}. During this biosynthesis, some valine and isoleucine precursors (α -acetoxy acids) leak out of the yeast cells into the fermenting wort and chemically decarboxylate to diacetyl and 2,3-pentanedione. Yeast cells are able to enzymatically reduce diacetyl to acetoin and further to 2,3-butanediol and 2,3-pentanedione to 2,3-pentanediol.

Fig. 5 represents the diacetyl profiles during the fermentation of the wort samples described in Table VII. The curves obtained for Scarlett, Melanoidin and Cara-aroma wort are clearly distinguishable from the curve for wort brewed with roasted malt. For three malt samples, only one extended diacetyl peak was observed early in fermentation. Conversely, the profile obtained with roasted malt revealed two peaks. The first was small and coincided with the appearance of the single peak seen for the other wort samples, whereas the second peak was sharper and larger. The findings for wort brewed with 50% roasted malt are in line with Nakatani *et al.*²⁸, indicating that two diacetyl peaks result from low FAN contents. The first increase in diacetyl is attributed to valine biosynthesis in the beginning of the fermentation when specific amino acids categorised in group A²⁰ are consumed. When wort is depleted in group A amino acids (glutamic acid, aspartic acid, serine, threonine, lysine, arginine), yeast cells start to take up valine, which leads to an inhibition of its biosynthesis and a decrease in the level of vicinal diketones by yeast reductases. Only low FAN levels can give rise to valine depletion, resulting in a renewed biosynthesis and the formation of the second diacetyl peak³⁰. It may be concluded that large levels of roasted malts generate similar results as adjuncts¹⁶. Both raw materials cause a dilution of the FAN content of wort, the former by the deple-

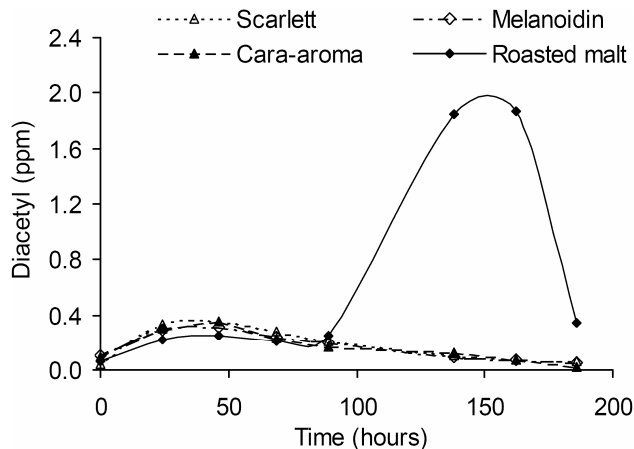


Fig. 5. Evolution of diacetyl levels during fermentation of wort prepared with 100% Scarlett or with 50% Scarlett and 50% of dark malt (Melanoidin, Cara-aroma and roasted malt). Properties of these wort samples are presented in Table VII.

tion of amino compounds through the Maillard reaction, the latter mainly by the resistance of adjunct proteins to enzymatic degradation by barley malt enzymes.

Although Nakatani *et al.*²⁸ indicated that low wort FAN contents may give rise to a second diacetyl peak due a renewed biosynthesis of valine, this does not explain the difference in diacetyl formation between on the one hand wort prepared with roasted malt and on the other hand wort brewed with Cara-aroma malt. As shown in Table V, Table VI and Fig. 2B, the levels of most individual amino acids were low and almost the same for both wort samples, leading to comparable FAN contents. The increased formation of diacetyl at the end of the fermentation of wort made with roasted malt is probably be due to more specific effects. The following possibilities might be put forward. (1) A renewed synthesis of valine becomes inhibited at the level of the acetoxy acid isomero-reductase. As a consequence more acetolactate is excreted and oxidised to diacetyl. The possibility of enzyme inhibitions by roasted malt compounds is also reflected by the reduced synthesis of isoamyl acetate and ethyl acetate (see further). (2) Maillard compounds from roasted malt affect the yeast cell membrane, resulting in a more rapid excretion of acetolactate or a slower uptake of diacetyl. (3) Compared to Cara-aroma wort, conditions of wort made with roasted malt lead to a faster oxidation of acetolactate to diacetyl, at a higher rate than the rate of diacetyl uptake and reduction.

Fusel alcohols and esters

Apart from ethanol, fermenting yeast cells also produce higher alcohols (fusel alcohols) imparting alcoholic, solvent-like or flowery flavours. Although the individual alcohols occur in levels below their threshold values, fusel alcohols may contribute to the overall aroma of fermented wort due to synergistic effects²⁶. Furthermore, fusel alcohols are of importance because they provide the alcohol moieties for certain esters.

In general, wort composition, in particular the free amino nitrogen content, determines the formation of certain fusel alcohols. Of importance is the relationship be-

tween fusel alcohol formation and the sequential uptake of amino acids. The aliphatic alcohols isobutanol, isoamyl alcohol and amyl alcohol are by-products of group B amino acids (valine, leucine and isoleucine), whereas the aromatic alcohols 2-phenylethanol, tyrosol and tryptophol derive from group C amino acids (phenylalanine, tyrosine and tryptophan)²⁰. Fig. 6 illustrates the development of isoamyl alcohol and isobutanol during the fermentation of the 12°P worts presented in Table VII. The formation of these alcohols was hardly influenced by the malt type used. Similarly, a decrease of FAN by the use of adjuncts has little effect on alcohols derived from group B amino acids²⁹. The formation of aromatic alcohols, however, was significantly enhanced by FAN dilution, especially for top-fermented beers. From Fig. 6B, it can further be seen that only wort brewed with Cara-aroma contained slightly higher levels of isobutanol. This might have partially resulted from the reduction of isobutanol, the Strecker aldehyde from valine.

Esters are possibly the most important set of flavour-active beer components, arising from yeast metabolism. The main volatile esters are ethyl acetate (solvent-like flavour), isoamyl acetate (fruity, banana flavour) and phenyl

ethyl acetate (flowery, roses, honey). These acetate esters frequently occur in levels above the threshold value. As for fusel alcohols, the presence of different esters can have a synergistic effect.

Fig. 7 represents the development of isoamyl acetate and ethyl acetate during fermentation of the wort samples characterised in Table VII. Unlike fusel alcohol formation, acetate ester formation is considerably affected by dark malts. In contrast to other malts, high levels of roasted malt completely prevented the synthesis of isoamyl acetate. On the other hand, the rate of ethyl acetate formation consistently decreased with increasing wort colour.

Two factors are of importance for the rate of isoamyl acetate and ethyl acetate synthesis: (1) the concentration of the two substrates, alcohol (isoamyl alcohol or ethanol) and acetyl-coenzyme A and (2) the total enzymatic activity³⁴. Since the concentration of isoamyl alcohol was practically unaffected by the malt type (Fig. 6) and since it has been demonstrated that the availability of acetyl-CoA is not a limiting factor for ester synthesis²⁴, the results in Fig. 7 are probably due to the activity of enzymes involved in ester formation. In ale yeasts, two alcohol acetyl transferase enzymes, encoded by the genes *ATF1* and *ATF2*,

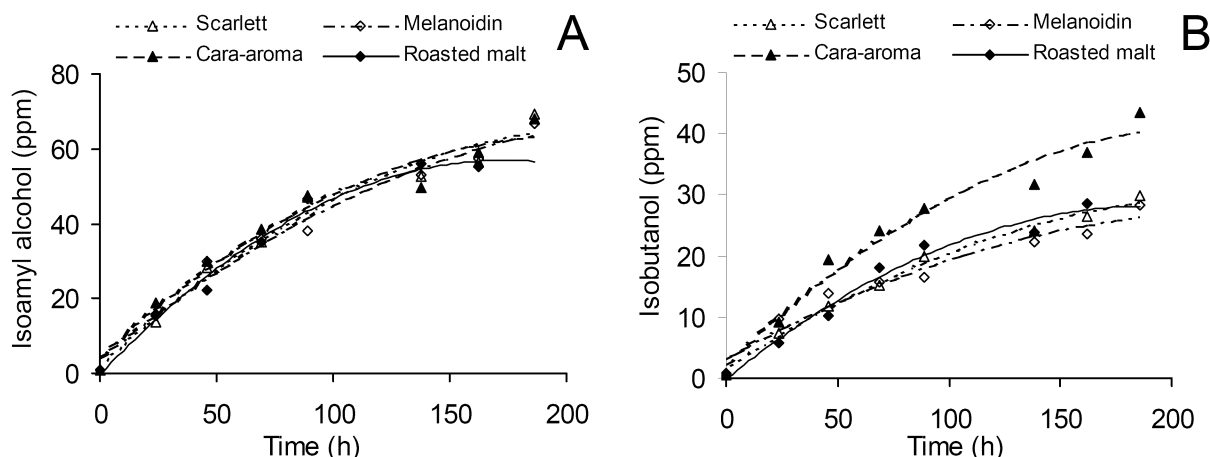


Fig. 6. Evolution of isoamyl alcohol (A) and isobutanol (B) levels during fermentation of wort brewed with 100% Scarlett or 50% Scarlett and 50% of dark malt (Melanoidin, Cara-aroma and roasted malt). Properties of these wort samples are presented in Table VII.

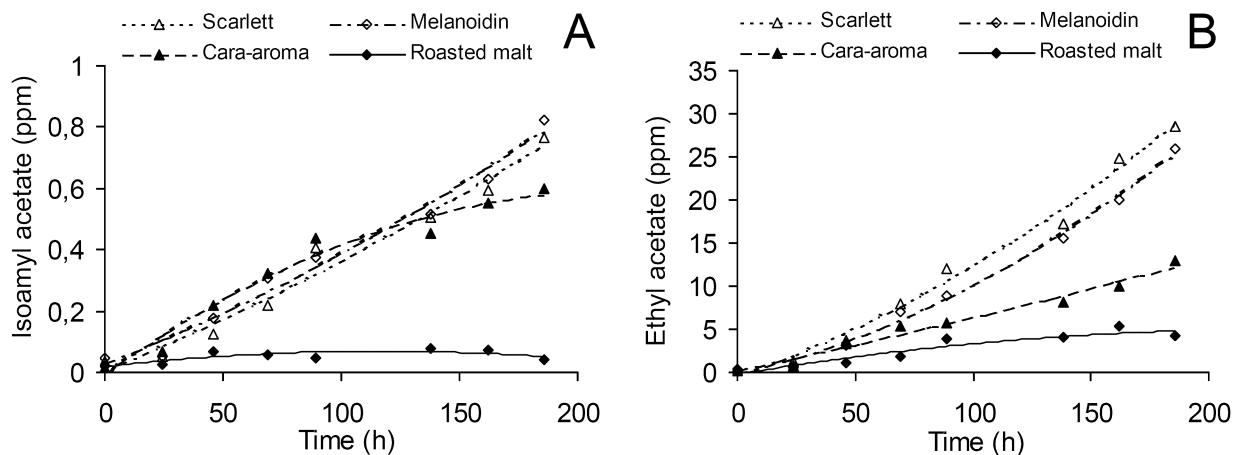


Fig. 7. Evolution of isoamyl acetate (A) and ethyl acetate (B) levels during fermentation of wort brewed with 100% Scarlett or 50% Scarlett and 50% of dark malt (Melanoidin, Cara-aroma and roasted malt). Properties of these wort samples are presented in Table VII.

are the key enzymes involved in the biosynthesis of acetate esters^{15,25,27}. Deletion and overexpression studies indicated that the *ATF1* gene is responsible for the formation of both isoamyl acetate and ethyl acetate, whereas *ATF2* mainly leads to isoamyl acetate³⁵. As the transcription of the *ATF* genes appears to be an important limiting factor for ester formation, any fermentation factor that changes the transcription rate of *ATF1* or *ATF2* will have an effect on the production of acetate esters. It has been observed that *ATF* is repressed at the end of fermentation, probably due to low wort nutrient levels and/or several stress factors that act upon yeast cells³³. Unfermented wort brewed with 50% roasted malt already contains highly reduced nutrient levels and elevated concentrations of Maillard compounds that may exert toxic effect towards yeast metabolism. Therefore, it is plausible that in this wort, *ATF* genes are repressed during fermentation. Nevertheless, it is also possible that Maillard compounds inhibit or inactivate alcohol acetyl transferase enzymes.

CONCLUSIONS

Whereas previous work on dark malt merely focused on the physico-chemical and sensory characteristics of malt or unfermented wort^{7,9,10}, this paper treated the influence of dark specialty malts on wort fermentations. More specifically, the availability of yeast nutrients, the course of the fermentation and the formation of flavour-active compounds were investigated.

In a preliminary experiment, different dark beers were brewed with diverse malt types. Although the resulting worts nearly had an identical original extract content, it was found that dark worts were considerably lower attenuated than lightly coloured worts. Therefore, it was further examined whether this was due to a lower level of fermentable extract in darker wort samples or to sluggish fermentations in the presence of Maillard components. The level of fermentable carbohydrates and amino acids significantly decreased with increasing wort colour, probably as a result of non-enzymatic browning reactions during curing or roasting of malt. The results even suggest that the darkest malts (Cara-aroma and roasted malt) do not contribute at all to the fermentable extract in wort. During the production of the darkest malts, browning reactions might be so intensive that the resulting Maillard compounds are unavailable for enzymatic hydrolysis during mashing. Indeed, compared to wort brewed with 100% pilsner malt, wort samples brewed with 50% pilsner malt and 50% Cara-aroma or 50% roasted malt contained about half the level of fermentable saccharides and amino acids. The unfermentable Maillard compounds in these wort samples are presumably responsible for the higher viscosity and improved foam stability of beer brewed with dark malts.

Lower levels of yeast nutrients in some worts had an impact on the course of the fermentation and on the formation of flavour compounds. It was found that the lower fermentable extract of dark wort is the main source of the reduced attenuations. Nevertheless, Maillard compounds also affect the course of the fermentation. Even at the start, the rate of extract uptake was inversely related to wort colour. During the first 4 days, fermentation rate was

1.7°P/day for the reference wort, 1.4–1.3°P/day for worts of intermediate colour and 1.1°P/day for wort brewed with 50% roasted malt. This indicates that yeast metabolism might be hampered by lower nutrient levels and/or by the presence of Maillard compounds. Further evidence for this may be obtained by the refermentation of dark beer with variable levels of glucose or maltose as we have indications that a combination of high levels of ethanol and Maillard compounds block fermentation processes. Fermentations with different fractions of malt compounds can possibly reveal which group of Maillard reaction products has the largest impact on yeast growth.

Dark malts can also affect the production of flavour-active yeast metabolites. This was particularly the case for vicinal diketones and esters. In contrast to other dark malts, high levels of roasted malt resulted in the appearance of a large second diacetyl peak. This malt type also completely prevented the biosynthesis of isoamyl acetate. The production of a second important ester, ethyl acetate, decreased more consistently with wort colour. The evaluation of the transcription of *ATF* genes could help to understand whether either transcription is hampered or enzymatic activity is inhibited in the presence of large levels of Maillard compounds.

Brewers should not worry about the results presented in this work as some were obtained for extreme levels of dark caramel malt and roasted malt (50% of total grist). In contrast to adjuncts, these malts are not used at rates above 10%. Thus, in normal brewing practice, no drastically reduced fermentation rates and ester profiles should be expected. Nevertheless, the experiments described in this paper are of scientific relevance as they allowed us to estimate the content of fermentable extract in dark specialty malts. Furthermore, trends with regard to flavour formation can be enlightened. In previous studies, it was pointed out that dark malts are excellent reference materials for the evaluation of Maillard reactions in food products. With this work, we demonstrate that such malts are also useful to study the response of *S. cerevisiae* towards severe stress conditions. However, additional research is required to enlighten the origin of these effects. For this purpose, it might be useful to determine the accumulation of trehalose, a disaccharide used by yeast to withstand stressful situations.

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