PROJECT REPORT No. 286

PREDICTION OF THE SWEET/CARAMEL AROMA OF BEER FROM MALT ANALYSIS

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by

J C SLAUGHTER

ICBD, Department of Biological Sciences
Heriot-Watt University, Edinburgh EH14 4AS

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CONTENTS

ABSTRACT

Three important food flavour compounds, MHF [5-methyl -4-hydroxy-3(2H)-furanone], DMHF [2,5-dimethyl-4-hydroxy-3(2H)-furanone] and EMHF [5-(or 2)-ethyl-2(or 5)-methyl-4-hydroxy-3(2H)-furanone] have been identified earlier in a wide range of beers. Both DMHF and EMHF have distinctive sweet/caramel like flavours and aroma thresholds of 0.16 mg/litre and 0.02 mg/litre in water and in beer 0.3 mg/litre and 0.7 mg/litre respectively. MHF has a meaty/brothy flavour and a much higher threshold of 8.3 mg/litre. The most significant furanone in beer seems to be DMHF, which has been shown to contribute to the sweet/caramel flavour of dark lager, experimental light lagers and a range of UK ales. MHF is always found but at levels below the flavour threshold. EMHF seems to occur in measurable amounts only rarely. These compounds are known to arise during heating of sugars and amino acids and are common in a variety of thermally processed foods. DMHF is also found in fruits. Both DMHF and EMHF can be formed during yeast fermentation. However, it is not known how the whole series of steps in beer production influence the furanone content of the final beer.

The aim of this study was to identify the key steps during brewery operations which influence furanone concentration in beer and therefore may impact significantly on the flavour of the product. Laboratory beers were produced on a small scale and samples analysed for the flavour-active hydroxyfuranones throughout the process. The length and temperature of mashing, the length of boiling, the rate of cooling the worts and the effects of grist composition were investigated . Fermentation temperature and the use of stabilising agents, PVPP and Lucilite PC5 were also investigated. The results demonstrated that several aspects of beer production procedures affect the furanone content of beer but in practice because of the existing constraints on brewery procedures from other requirements, the most important factors are grist composition and fermentation temperature. Darker malts tend to produce beers with higher furanone contents but fermentation has a major affect on final concentration as yeast produces both DMHF and EMHF from, as yet, unidentified precursors. The results suggest that precursor compounds may prove to be more important than the malt furanones in determining the final furanone content of beer. A clearer understanding of the nature of the precursors should allow manipulation of their production and raises the possibility of separating beer colour and typical malt flavours.

Analysis of samples from full scale commercial ale production gave essentially the same results as the laboratory experiments but indicated that the final conditioning steps lead to reduction in furanone concentrations, particularly of EMHF.

SUMMARY

Introduction and Aims

Three important food flavour compounds, MHF [5-methyl -4-hydroxy-3(2H)-furanone], DMHF [2,5-dimethyl-4-hydroxy-3(2H)-furanone] and EMHF [5-(or 2)-ethyl-2(or 5)-methyl-4-hydroxy-3(2H)-furanone] have been identified earlier in a wide range of beers. Both DMHF and EMHF have distinctive sweet/caramel like flavours and aroma thresholds of 0.16 mg/litre and 0.02 mg/litre in water and in beer 0.3 mg/litre and 0.7 mg/litre respectively. MHF has a meaty/brothy flavour and a much higher threshold of 8.3 mg/litre. The most significant furanone in beer seems to be DMHF, which has been shown to contribute to the sweet/caramel flavour of dark lager, experimental light lagers and a range of UK ales. MHF is always found but at levels below the flavour threshold. EMHF seems to occur in measurable amounts only rarely.

These compounds are know to be products of the Maillard reaction between reducing sugars and amino acids and are common in thermally treated foods such as coffee, bread, popcorn and barley malt. DMHF also occurs naturally in some fruits, for example strawberries and pineapples, as well as in the fermented soy products soy sauce and miso which, in addition, contain high levels of EMHF. DMHF and MHF have been found in laboratory and commercial malts in approximate proportion to the heating regime during kilning but EMHF has never been detected.

During fermentation yeast is thought to transform Maillard intermediates to DMHF and EMHF rather make direct use of sugars and amino acids. It is likely, therefore, that during the production of beer, yeast fermentation will increase the final furanone content of the beer to different degrees depending on the extent of high temperature processing of the original malt and wort. During malting and brewhouse operations there are various steps which involve heating. Kilning the malt following germination obviously provides the elevated temperatures required for the Maillard reaction and the strong influence of kilning conditions on the final concentration of furanones in

wort has been shown. In separate studies worts obtained from pale lager malts were found to contain fewer furanones than the worts from dark malts.

However, it is not known how the whole series of steps in beer production influence the furanone content of the final beer. The preparation of the wort by mashing and its subsequent boiling are processes likely to favour the Maillard reaction and therefore may affect production of intermediates and furanones. Other aspects of beer production, for example the use of adjuncts, wort strength, fermentation temperature and the use of stabilizing agents, all appear to have the potential to affect the final furanone content.

The aim of this study was to identify the key steps during brewery operations which influence furanone concentration in beer and to determine to what extent beer furanone composition can be predicted from analysis of the malt.

Methods

Malts

Malts were prepared using a Seeger Micro-maltings unit. Barley, Prisma 1.8%N (Triumph x Cambinus) x Piccolo) was steeped at 16ºC for 8 h then 24 h following a 16 h air rest. Germination was then carried out at 16ºC with daily turning of the grain to ensure even development. Samples were taken on day 3 and kilned as follows: Lager malt: 50ºC (16 h) and ale malt: 65ºC (16 h) & 80ºC (2 h). A stewed malt was also prepared[9] by heating the green malt at 70ºC for 4 hours in an enclosed environment, which allowed no recirculation of air, followed by kilning at 110ºC for 2 hours. Once kilned, all samples were derooted by hand and stored in airtight containers until required for extraction.

Mashing

Standard preparation of malt hot water extract.

An all malt wort was prepared by grinding 75g of malt in a Buhler Universal Laboratory disc mill (type DLFU) set with a 0.2mm disc gap. A CM4 mashing bath (Cannongate Technology Ltd., UK) was used for mashing. The grist was stirred into 350 ml distilled water at 4º, 20º, 45º, 65º, 75º or 85ºC, and this temperature was maintained for 30, 60 or 90 minutes with continuous stirring. The worts were then filtered using Elderol filter paper and stored at 4ºC until required.

Mashing with adjuncts.

Four cereal adjuncts were used to supplement the barley malts. Mashing was carried out as above but the weight of either ale or lager malt used was reduced by 20% and the total weight maintained by the addition of unmalted cereal. The final volume of water in the mash was 350 ml. The adjuncts used & their preparation prior to mashing were as follows: 20g of barley (Prisma, Bairds) or wheat (Riband, United distillers) was finely ground in a Buhler Universal Laboratory disc mill (type DLFU) set with a 0.2mm disc gap and added to 100 ml water at 65ºC & incubated with stirring at 92ºC for 10 minutes. 20g of maize flakes (crushed, Micronized Food Products) was added to 100 ml water at 65ºC and incubated at 92ºC for 40 minutes. The hot adjuncts were cooled to 65ºC, water (250 ml) and the fine ground malt was added and mashed for one hour at 65ºC. Stewed malt was also used as an adjunct. Here the two finely ground malts were mixed together dry and then mashed using the standard procedure. Samples of the worts were taken and stored at 4ºC for analysis within 48 h.

Mash Boiling

Once filtered, the worts were boiled for 50 minutes in a round bottom flask using a condenser to prevent loss of water though evaporation. Once cooled on the bench or on ice the extracts were cleared of precipitate by centrifugation at 2500 rpm for 5 minutes.

Boiling with hops

An all ale malt and an all lager malt wort were boiled, as described above, for 50 minutes but with the addition of hops. A sample of pelleted hops (Slovenian Golding Crop 198 (SCB Edinburgh (epo 9156))) was added to the wort prior to boiling to give a level of bitterness units calculated as 11BU for the lager wort and 20 BU for the ale wort.

Boiling with maltose syrup adjunct

When boiling with a syrup adjunct, the worts used were prepared as described above except that the quantity of malt used was reduced to 60g (80%) or 45g (60%). Once filtered and cooled, maltose syrup (ABR Foods, U.K.) was added to give gravities as near as possible to that seen with a normal all malt mash. The wort was then boiled for 50 minutes as described previously.

Fermentation.

Once cooled, boiled worts were inoculated with *Saccharomyces cerevisae* NCYC1108 to give a final concentration of 10^7 cells/ml. Fermentations were carried out using 30 ml aliquots in loosely capped 50 ml centrifuge tubes held at 26ºC for 72 hours. After this time the cells were removed by centrifugation and the samples degassed overnight at 4ºC. The following day gravity, colour and furanones were measured. Samples of boiled ale worts were also fermented at 25º, 20º and 13ºC in an orbital incubator set at 150 rpm.

Treatment Of Green Beer With Stabilizing Agents.

A standard ale malt wort was prepared and fermented using the conditions described above. After 72 hours fermentation the yeast was removed by centrifugation and the sample degassed overnight at 4ºC. A sample of the cold green beer was treated with either the silica hydrogel, Lucilite PC5 (Crosfield, UK) or PVPP (polyvinylpolypyrrolidone, Sigma UK), by stirring continuously for 10 minutes at 4 ºC. Lucilite PC5 was added at a rate of 1g/litre of green beer. PVPP was first slurried

at 10% wt/vol in water for 30 minutes before adding 200mg/litre of green beer. The agents were removed by centrifugation at 13000rpm and the furanone content was analysed. A third sample of the green beer was taken and treated as above but omitting the addition of a stabilising agent, to give the level of furanones detectable in the beer.

GC-MS Analysis.

20 ml of sample was saturated with sodium chloride and shaken with 24 ml methyl acetate for 10 minutes using a bench top shaker (Stuart Scientific, U.K.) set at 800osc/min. After centrifugation at 2000 xg for 10 minutes, the organic layer was rotary evaporated to 1 ml and decanol was added to a concentration of 10 mg/litre. The GC-MS analysis was performed using a GC-MS HP6890 fitted with an HP-5MS 0.25mm x 30m column. The temperature ran from an initial 40ºC, with a 2 minute hold, to 200ºC at a rate of 5ºC/min. The final temperature of 200ºC was held for 40 minutes and helium was used as the carrier gas. Used in the SIM mode, the following monitor ions were measured: m/z 128 for DMHF, m/z 142 for EMHF, m/z 114 for MHF and m/z 83 for decanol, the internal standard. A calibration curve was constructed for DMHF (Aldrich, U.K.) and EMHF (Tokyo Kasei Kogyo Co., Ltd., Japan) using standard solutions in methyl acetate. MHF is not commercially available therefore the curve for DMHF was used to determine values for MHF.

Statistical Analysis And Recovery Rates.

The overall coefficient of variation for the method of extraction and analysis of furanones was calculated by extracting a malt at 65ºC for 50 minutes as described above followed by solvent extraction and GC-MS analysis 5 separate times. The coefficient of variation for DMHF was 5.4% and 13% for MHF. The recovery rate of DMHF from a malt wort was also established. The furanone was added to a wort to increase the concentration by 1 mg/litre. This sample was then extracted and analysed by GC-MS and the recovery rate of DMHF was calculated as 54%. In the absence of standard compound a recovery rate for MHF could not be established. The

concentrations for both furanones in the text are the experimentally derived values unadjusted for recovery rates and are the averages of duplicate experiments.

Key Results

Furanones are not present in lager or ale malts but MHF and DMHF are formed during mashing of ale malt

Extraction temperatures over 45^{o}C are needed for furanones to form during extraction of ale malts. Higher temperatures have little effect on DMHF but HMF concentration was doubled by extraction at 85^{o}C.

Both MHF and DMHF are present in stewed malt and no further production occurs during mashing

Two successive cold water extractions yielded the same amount of furanones from a stewed malt as did extraction at 65^{o}C.

Length of mashing has little effect on wort furanone content

Extraction of ale and stewed malt for between 30 and 90 minutes yielded worts with very similar furanone concentrations but extraction for 120 minutes showed a slight decline, particularly with HMF from stewed malt.

Boiling an ale malt for more than 30 minutes with or without hops had no effect on DMHF content but HMF increased with the length of the boil

Use of cereal adjuncts and wort syrups at 20% with ale malts yields sweet worts with various levels of DMHF but the differences largely disappear on boiling for 90 minutes

Barley and stewed malt adjuncts gave higher DMHF levels in sweet worts than with ale malt alone whereas maize and wheat gave worts with no detectable furanones. After boiling all worts had

very similar concentrations except the stewed malt adjunct wort where the concentration increased by 50%.

Rate of wort cooling affects the DMHF content of the beer

Fermentation of wort cooled at room temperature yielded lower amounts of DMHF than worts cooled more rapidly on ice.

Adjuncts affect the concentration of furanones in beer

Fermentation of boiled adjunct worts of similar DMHF contents yielded beers with quite different amounts but the results also depended on the type of malt used. For both ale and lager malts barley adjunct had no effect, wort syrup reduced the furanone concentration whilst stewed malt caused a substantial rise.

All the adjunct beers showed similar levels of EMHF except for the wort syrup beers where EMHF was not detectable.

Fermentation temperature affects beer furanone content

Fermentation at 25^{o}C produced nearly twice the amount of DMHF as fermentation at 13^{o}C. The amount of EMHF was little affected by temperature.

The IoB fermentability test can be used to give an estimate of beer DMHF content

Comparison of the amount of DMHF produced in a conventional laboratory fermentation and an IoB fermentability test with both ale and lager malt showed that the fermentability test could be used to predict the outcome of the higher gravity fermentation.

Furanones follow the same pattern in commercial brewing as in the laboratory experiments

Analysis of samples from a large brewery at the end of lautering, boiling and fermentation showed exactly the same pattern of changes in the concentration of MHF, DMHF and EMHF as found in the laboratory experiments. By the end of cold conditioning in the brewery DMHF had declined, MHF had disappeared whilst EMHF remained constant. In the bright beer tank neither MHF nor EMHF could be detected but the concentration of DMHF remained significant.

Conclusions

1. The three furanones did not change as a group during beer production but each compound followed its own pattern which was the same in full-scale brewing as in laboratory experiments.
2. Most of the brewhouse operations which affect furanones are constrained by other considerations and so, whilst furanones are undoubtedly formed in the brewhouse, there is little scope to influence their production at this stage except at the traditional level of grist composition.
3. Fermentation is a critical stage in the formation of DMHF and the only point at which EMHF is formed. Whilst fermentation temperature and yeast strain (from other work) can be used to control furanone production there is a major impact of the grist. It may be that the role of the grist in providing precursor compounds which the yeast can convert into DMHF and EMHF is more important than the amount of furanone present in the malt itself.
4. A prediction of beer furanone from instrumental malt analysis is currently impossible due largely due to the unknown nature of the yeast precursor compounds present in the malt and cereals. However, the IoB fermentability test seems able to give an empirical measure of fermentation performance and could be used now by any brewer wishing to control this aspect of the beer flavour profile.

Implications for Levy Payers

Whilst the main determinant of beer furanone content is clearly the grist, this work has shown that fermentation is critical. All the EMHF and much of the DMHF is produced at this stage and so there is a need for greater understanding of the compounds which yeast use to produce the furanones and then, the malting factors which control formation of the compounds at that stage.

From the laboratory results with ale malt and adjuncts, fermentation increases the concentration of DMHF two to five times. In other words, most of the compound which contributes towards a typical aspect of malt flavour in beer is actually produced by the yeast.

TECHNICAL DETAILS

Draft paper submitted for publication in the Journal of the Institute of Brewing

Formation of 4-hydroxyfuranones and their precursors during production of worts and beers.

A.E. Mackie* and J.C. Slaughter

The International Centre for Brewing and Distilling,
Dept. of Biological Sciences,
Heriot-Watt University,
Edinburgh EH14 4AS,
Scotland, U.K.
Telephone (+44) 131 449 5111
Fax (+44) 131 449 5153

* corresponding author.

ABSTRACT

Laboratory beers and samples taken at each stage of production, were analysed for the flavour active 4-hydroxyfuranones; 5-methyl -4-hydroxy-3(2H)-furanone (MHF), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) and 5-(or 2)-ethyl-2(or 5)-methyl-4-hydroxy-3(2H)-furanone (EMHF). The length and temperature of mashing, the length of boiling, the rate of cooling the worts and the effects of grist composition were investigated to identify the 4-hydroxyfuranone content of worts and subsequent beers. Fermentation temperature and the use of the stabilising agents, PVPP and Lucilite PC5, on the 4-hydroxyfuranone content of the beer was also investigated. The results demonstrated that several aspects of beer production procedures affect the furanone content of the beer, but in practice the important factors are grist composition, the rate at which the boiled wort is cooled and fermentation temperature. Fermentation has a major effect on final furanone content as yeast produces both DMHF and EMHF. The results suggest that malt levels of precursor compounds, which can be converted to 4-hydroxyfuranones by the Maillard reaction or by yeast, may prove to be more important than the quantities of the furanones found in malt in determining the final furanone content of beer. A clearer understanding of the nature of the precursors should allow manipulation of their production and beer furanone content.

INTRODUCTION

Three important flavour compounds, 5-methyl -4-hydroxy-3(2H)-furanone (MHF), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) and 5-(or 2)-ethyl-2(or 5)-methyl-4-hydroxy-3(2H)-furanone (EMHF) have been identified in various concentrations in a wide range of beers. Both DMHF and EMHF have distinctive sweet/caramel like flavours and aroma thresholds of 0.16 mg/litre and 0.02 mg/litre in water and in beer of 0.3 mg/litre and 0.7 mg/litre[13] respectively. MHF has a meaty/brothy flavour and a much higher aroma threshold of 8.3 mg/litre in water. The most significant furanone in beer seems to be DMHF, which has been shown to contribute to the sweet/caramel flavour of dark lager [16] , experimental light lagers [13] and also a range of U.K. ales[8] .

MHF is always found but at levels below the flavour threshold. EMHF seems to occur in measurable amounts only rarely[8].

These compounds are known to be products of the Maillard reaction between reducing sugars and amino acids and are common to a variety of thermally processed foods such as coffee[18], bread[15], popcorn[1] and barley malt[3,9]. DMHF is also known to occur naturally in fruits, for example strawberries[17] and pineapples[11] and also in the fermented products soy sauce[10] and miso[4] which also contain high levels of EMHF [4,10].

During fermentation yeast is thought to transform Maillard intermediates to DMHF and EMHF rather make direct use of sugars and amino acids[12,5]. It is likely, therefore, that during the production of beer, yeast fermentation will increase the final furanone content of the beer to different degrees depending on the extent of high temperature processing of the original malt and wort. During malting & brewhouse operations there are various steps which involve heating. Kilning the malt following germination obviously provides the elevated temperatures required for the Maillard reaction and the strong influence of kilning conditions on the final concentration of furanones in wort has been shown[9]. In separate studies worts obtained from pale lager malts were found to contain fewer furanones than the worts from dark malts[8,3].

However, it is not known how the whole series of steps in beer production influence the furanone content of the final beer. The preparation of the wort by mashing and its subsequent boiling are processes likely to favour the Maillard reaction and therefore may affect production of intermediates and furanones. Other aspects of beer production, for example the use of adjuncts, wort strength, fermentation temperature and the use of stabilizing agents, all appear to have the potential to affect the final furanone content.

The aim of this study was to identify the key steps during brewery operations which influence furanone concentration in beer and therefore may impact significantly on the flavour of the product.

MATERIALS AND METHODS

Malts

Malts were prepared using a Seeger Micro-maltings unit. Barley, Prisma 1.8%N (Triumph x Cambinus) x Piccolo) was steeped at 16ºC for 8 h then 24 h following a 16 h air rest. Germination was then carried out at 16ºC with daily turning of the grain to ensure even development. Samples were taken on day 3 and kilned as follows: lager malt: 50ºC (16 h) and ale malt: 65ºC (16 h) & 80ºC (2 h). A stewed malt was also prepared[9] by heating the green malt at 70ºC for 4 hours in an enclosed environment, which allowed no recirculation of air, followed by kilning at 110ºC for 2 hours.

Once kilned, all samples were derooted by hand and stored in airtight containers until required for extraction.

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Mashing with adjuncts.

Four cereal adjuncts were used to supplement the barley malts. Mashing was carried out as above but the weight of either ale or lager malt used was reduced by 20% and the total weight maintained by the addition of unmalted cereal. The final volume of water in the mash was 350 ml. The adjuncts used & their preparation prior to mashing were as follows: 20g of barley (Prisma, Bairds) or wheat (Riband, United distillers) was finely ground in a Buhler Universal Laboratory disc mill

(type DLFU) set with a 0.2mm disc gap and added to 100 ml water at 65ºC & incubated with stirring at 92ºC for 10 minutes. 20g of maize flakes (crushed, Micronized Food Products) was added to 100 ml water at 65ºC and incubated at 92ºC for 40 minutes. The hot adjuncts were cooled to 65ºC, water (250 ml) and the fine ground malt was added and mashed for one hour at 65ºC. Stewed malt was also used as an adjunct. Here the two finely ground malts were mixed together dry and then mashed using the standard procedure. Samples of the worts were taken and stored at 4ºC for analysis within 48 h.

Mash boiling.

Once filtered, the worts were boiled for 50 minutes in a round bottom flask using a condenser to prevent loss of water though evaporation. Once cooled on the bench or on ice the extracts were cleared of precipitate by centrifugation at 2500 rpm for 5 minutes.

Boiling with hops.

An all ale malt and an all lager malt wort were boiled, as described above, for 50 minutes but with the addition of hops. A sample of pelleted hops (Slovenian Golding Crop 198 (SCB Edinburgh (epo 9156))) was added to the wort prior to boiling to give a level of bitterness units calculated as 11BU for the lager wort and 20 BU for the ale wort.

Boiling with maltose syrup adjunct.

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Fermentation.

Once cooled, boiled worts were inoculated with *Saccharomyces cerevisae* NCYC1108 to give a final concentration of 10^7 cells/ml. Fermentations were carried out using 30 ml aliquots in loosely capped 50 ml centrifuge tubes held at 26ºC for 72 hours. After this time the cells were removed by centrifugation and the samples degassed overnight at 4ºC. The following day gravity, colour and furanones were measured. Samples of boiled ale worts were also fermented at 25º, 20º and 13ºC in an orbital incubator set at 150 rpm.

Treatment of green beer with stabilizing agents.

A standard ale malt wort was prepared and fermented using the conditions described above. After 72 hours fermentation the yeast was removed by centrifugation and the sample degassed overnight at 4ºC. A sample of the cold green beer was treated with either the silica hydrogel, Lucilite PC5 (Crosfield, UK) or PVPP (polyvinylpolypyrrolidone, Sigma UK), by stirring continuously for 10 minutes at 4 ºC. Lucilite PC5 was added at a rate of 1g/litre of green beer. PVPP was first slurried at 10% wt/vol in water for 30 minutes before adding 200mg/litre of green beer. The agents were removed by centrifugation at 13000rpm and the furanone content was analysed. A third sample of the green beer was taken and treated as above but omitting the addition of a stabilising agent, to give the level of furanones detectable in the beer.

GC-MS Analysis.

20 ml of sample was saturated with sodium chloride and shaken with 24 ml methyl acetate for 10 minutes using a bench top shaker (Stuart Scientific, U.K.) set at 800osc/min. After centrifugation at 2000 xg for 10 minutes, the organic layer was rotary evaporated to 1 ml and decanol was added to a concentration of 10 mg/litre. The GC-MS analysis was performed using a GC-MS HP6890 fitted with an HP-5MS 0.25mm x 30m column. The temperature ran from an initial 40ºC, with a 2 minute hold, to 200ºC at a rate of 5ºC/min. The final temperature of 200ºC was held for 40 minutes and helium was used as the carrier gas. Used in the SIM mode, the following monitor ions were measured: m/z 128 for DMHF, m/z 142 for EMHF, m/z 114 for MHF and m/z 83 for decanol, the internal standard. A calibration curve was constructed for DMHF (Aldrich, U.K.) and EMHF (Tokyo Kasei Kogyo Co., Ltd., Japan) using standard solutions in methyl acetate. MHF is not commercially available therefore the curve for DMHF was used to determine values for MHF.

Statistical analysis and recovery rates.

The overall coefficient of variation for the method of extraction and analysis of furanones was calculated by extracting a malt at 65ºC for 50 minutes as described above followed by solvent extraction and GC-MS analysis 5 separate times. The coefficient of variation for DMHF was 5.4% and 13% for MHF. The recovery rate of DMHF from a malt wort was also established. The furanone was added to a wort to increase the concentration by 1 mg/litre. This sample was then extracted and analysed by GC-MS and the recovery rate of DMHF was calculated as 54%. In the absence of standard compound a recovery rate for MHF could not be established. The concentrations for both furanones in the text are the experimentally derived values unadjusted for recovery rates and are the averages of duplicate experiments.

RESULTS AND DISCUSSION

Influence of the length and temperature of mashing on furanones in wort.

The furanone content, gravity and colour of ale and stewed malt worts prepared by mashing for increasing lengths of time are shown in Table I. Lager malt worts were also prepared in the same way but did not contain detectable levels of furanones. Only two of the furanones, DMHF and MHF, were detected in the malt worts.

The results indicate that the length of mashing had little effect on the DMHF concentration with either malt type as the results were unaltered from 30 minutes though to 120 minutes of mashing. In contrast, MHF concentration fell progressively with time when stewed malt was used. With ale malt, although steady from 30-90 minutes, by 120 minutes the concentration had fallen.

The effect of different mash temperatures on the furanones in ale and lager malt worts was also investigated. Mashing at temperatures of 4, 20, 45, 65, 75 and 85ºC was carried out as described in the methods. The results for the ale malt are shown in Table II. Furanones were not detected in the lager malt worts regardless of the mashing temperature. Furanones were not detected in the ale malt worts produced at 4 and 20ºC but both MHF and DMHF were detected in worts mashed at 45ºC. At temperatures above this the level of DMHF rose only slightly with each temperature increase but the concentration of MHF was twice as high at 85ºC as 45ºC. The wort produced at 4ºC had a low colour which was doubled when mashing was carried out at 20ºC. However, at temperatures above this changes in colour were not significant. The gravity of the wort was similar at 4ºC and 20ºC but increased with temperature up to 65ºC whereafter it declined with increasing temperature.

The results can be interpreted in two ways; it could be that at the low temperatures of 4 and 20ºC the mash was too cold to extract the furanones directly from the malt or, alternatively, the malt itself did not contain furanones and the temperature was too low to convert any precursors to the two furanones. To investigate this further, samples of the worts prepared at 4 and 20 ºC were

heated to 65ºC for one hour following filtration. No furanones were detected in the heated wort originating from the 4ºC mash but levels found in the heated 20ºC mash (DMHF 0.08 mg/litre and MHF 0.13 mg/litre) were comparable with those found in wort prepared at 65ºC (DMHF 0.10 mg/litre and MHF 0.18 mg/litre). This suggests that in an ale malt, furanone precursors or Maillard intermediates are formed during kilning but furanones themselves are not present. The precursors are extracted at temperatures greater than 20ºC and converted to DMHF and MHF during mashing at temperatures greater than 45 ºC.

However, a different pattern emerged with the stewed malt wort. An extract of the stewed malt at 4ºC gave detectable levels of both MHF and DMHF (0.32 mg/litre DMHF and 1.62 mg/litre MHF). Recovery of the spent grains and their repeated extraction yielded a further 0.13 mg/litre DMHF and 0.32 mg/litre MHF. Combining the results for the two extractions gave a wort containing 0.45 mg/litre DMHF and 1.94 mg/litre MHF. These levels are similar to those found in the standard 65ºC mash (0.45 mg/litre DMHF and MHF 1.49 mg/litre) suggesting that the furanones in a stewed malt wort are formed principally during the stewing and kilning process and are simply extracted from the malt during mashing.

The results suggest that at a practical level, although furanones are formed during mashing of ale malts, the process will not be a critical one. Mashing in a brewery is performed at temperatures greater than 45ºC and within the time limits investigated here so it is unlikely that any changes in the process will significantly alter the furanone content of the wort.

Influence of boiling and cooling of wort on the furanone content.

The effect of the length of a wort boil on furanone content was investigated by boiling an ale malt wort for periods of time between 30 to 70 minutes. The results (Table III) showed that increased boiling caused a slight increase in DMHF concentration. MHF concentration was clearly affected by boiling and a steady increase with boil time was evident. Boiling caused only a slight increase in the wort colour and had no effect of the gravity of the wort as the process was conducted under

reflux. The addition of hops to either ale or lager malt wort during boiling as described in methods had no effect on DMHF content (Table IV).

To determine the effect of the rate of cooling the wort after boiling, samples of freshly boiled wort were left on the lab bench for 0, 10, 20, 30 or 40 minutes and then transferred to ice to bring the temperature to 20ºC as rapidly as possible. However, altering the time allowed for the wort to cool in this way did not affect the concentration of DMHF detected in the wort (Table IV).

Mashing with adjuncts.

The effect of a number of adjuncts on wort furanone concentration was investigated. Tables VI & VII detail the levels of furanones detected in ale and lager malt worts mashed with and without adjuncts as described in the methods section.

The DMHF content of the ale malt worts (Table VI) varied depending upon the adjunct used. The use of barley or stewed malt gave elevated levels of the furanone. Conversely the use of maize or wheat gave worts with no detectable DMHF. However boiling the worts eliminated any differences as all the boiled worts contained similar levels of DMHF regardless of grist composition with the exception of stewed malt. This adjunct is known to contain high levels of DMHF and therefore an elevated content in the mixed wort was expected. DMHF was not detected in any lager malt adjunct worts except in the case of stewed malt.

Table VII details the MHF detected in the worts which, although not detected in three of the lager malt based sweet worts, increased considerably on boiling regardless of malt or adjunct used.

Fermentation

Influence of wort cooling time

The boiled worts which had been allowed to cool for increasing lengths of time as described above were fermented as described in the methods. As noted above, prior to fermentation no differences in the DMHF content of the worts were evident (Table V). However, following fermentation, the beer derived from the wort which had taken the longest to cool (40 minutes at room temperature

followed by 4 minutes on ice) contained a significantly lower level of DMHF than the other fermented worts. It has been suggested that once the Maillard reaction has started in an aqueous solution it continues even if the temperature of the solution decreases[6] . It may be that during the extended wort cooling there is a depletion of the Maillard intermediates required for furanone formation by yeast which results in a beer containing lower levels of DMHF than one made from a wort cooled rapidly. This, however, is unlikely to cause a major problem in most breweries as here hot wort is normally cooled as rapidly as possible to fermenter temperature (10-16ºC), usually in enclosed heat exchangers[7] .

Influence of grist composition

The DMHF content of beer prepared from the range of adjunct worts described earlier are shown in Table VI. Regardless of malt type or adjunct used the DMHF content was higher after fermentation than before, confirming that this compound is a product of yeast metabolism as well as the Maillard reaction[3,5,9,13]. The influence adjuncts varied depending upon the adjunct type. No differences are seen between a fermented all ale malt wort and one supplemented with barley, or wheat so these adjuncts presumably provide the same levels of DMHF substrate for the yeast as the ale malt. However, maize adjunct does not appear to contribute DMHF substrates to the fermentation as the DMHF content was around 80% of that detected in an all ale malt fermentation. The use of maltose syrup adjunct seemed to disproportionately reduce levels of DMHF detected in the green beers. Similar results were evident with the lager malt worts, although the DMHF content of the beer prepared with the maize adjunct was similar to that of the ale malt and the wheat adjunct gave a distinctly raised DMHF concentration. It may be that in this case the maize and wheat interact with enzymes present in the lager malt, which are absent from the ale malt, to produce furanone precursors.

With both malt types, the largest increases of DMHF after fermentation were seen when stewed malt was used as adjunct. An increase of over 5-fold occurred with the ale malt based wort and

over 4-fold with the lager malt based wort. The stewed malt was prepared by liquefying the endosperm and then kilning at 110ºC, thus allowing the Maillard reaction to proceed. Therefore the elevated levels of DMHF are likely to reflect increased quantities of Maillard intermediates for the yeast to convert to DMHF[5,12].

Table VII details the MHF detected in the fermented worts. With only the one exception of the maize wort, MHF content decreased after fermentation. This result was to be expected as this furanone is a product of the Maillard reaction but not of yeast metabolism[4]. At its highest, MHF was found at only one fifth of its aroma threshold in water and is therefore unlikely to play an important role in the flavour of beer.

EMHF (Table VIII) was not detected in any wort pre or post boil, but was, however, detected in the fermented worts so confirming it as a product of yeast metabolism[3,14]. The level of EMHF found in the fermented worts did not appear to be influenced by the nature of the malt used or by the use of barley, maize, wheat or stewed malt as adjunct with all these fermented worts containing similar levels of around 0.10mg/litre. No EMHF was detectable in the fermented wort prepared with maltose syrups and it is possible that the syrups contained an inhibitor for EMHF production. However, it is more likely that the syrup does not provide substrates for EMHF production and the concentration in the beer is therefore reduced to values below the detection limit of 0.05mg/litre. A more sensitive analytical protocol could confirm this.

Influence of fermentation temperature

Ale malt worts were fermented at 25, 20 and 13ºC with continuous agitation. The DMHF content of the green beer, shown in Table IX, was highest in the wort fermented at 25ºC and declined with each decrease in fermentation temperature. The temperature had no influence on the quantity of EMHF produced by the yeast.

Treatment of green beer with stabilising agents

Two commonly used stabilising agents were checked for their ability to remove furanones from a beer when applied to bind to polyphenols and haze-forming proteins. Treatment with either PVPP or Lucilite PC5, as described in the methods, had no effect on the furanone content (Table X).

Prediction of beer furanone content

Worts were produced from an ale and lager malt as described in the method section and also using the IOB fermentability method (2.16). The furanone concentrations were measured in the resulting beers. The concentrations of DMHF detected, shown in Table XI, are expressed not only as mg/litre but also as the quantity of DMHF produced per degree of gravity of the original wort. The worts are of different strengths and the beer furanone concentrations are also different but both types of fermentation produce the same concentration of DMHF per degree of wort gravity given the same malt. EMHF was not detected in the IOB fermentation. This is likely to be a result of the dilute nature of the wort used, which reduced the EMHF content below the detection limits.

Measurement of the furanone produced per degree of gravity during an IOB fermentability test appears to allow prediction of the beer DMHF which will result from fermentation of wort at industrial strength.

4-Hydroxyfuranone content of beer produced on industrial scale.

To verify our results with the situation in full scale commercial breweries, samples were obtained from a large U.K. brewery during the production of an ale and analysed for hydroxyfuranone content. The results (Table XII) clearly mirror those achieved in the laboratory. DMHF and MHF were evident on mashing and their concentrations increased during the boiling of the wort. DMHF increased further on fermentation and EMHF became detectable for the first time . MHF content decreased after fermentation as in the laboratory experiments. Post fermentation samples were provided from the cold conditioning tank and the bright beer tank. A decrease in both MHF and DMHF was evident in the cold conditioning tank. This area was not investigated in our laboratory

work and may be due to the holding time allowing spontaneous reactions to occur or the addition of primary and auxiliary finnings. A second decrease in the DMHF content in the bright beer tank appears to be related to the dilution of the beer to sales gravity. EMHF is not detectable in the bright beer tank and it is possible that the dilution of the beer decreased the EMHF content to below its detection limits.

CONCLUSIONS

The results reported in this paper indicate that, in addition to being formed during kilning of green malt, the 4-hydroxyfuranones are also produced during the brewery operations of mashing, boiling and fermentation. The degree of heating during kilning appears to influence not only the level of DMHF and MHF in the malt but also the levels of precursors suitable for conversion to furanones by heating during brewhouse operations and by yeast fermentation (Figure 1). A lager malt, kilned at low temperatures has no furanones, no heat precursors and relatively low amounts of yeast precursors. An ale malt kilned at higher temperatures, still has no furanones but contains greater amounts of both groups of precursors. At the other end of the scale the stewed malt kilned at elevated temperatures has the highest levels of both heat and yeast precursors as well as furanones themselves.

The order of appearance of the two types of precursor with increasing extent of heating of the malt suggests that yeast utilises the Amadori compounds which are the first Maillard intermediates to be formed. The heating steps in the brewhouse appear to convert the later intermediates, possibly the 1-deoxydiketones.

With regards to practical brewing the concentration of the heat and yeast precursors seems likely to be just as important in determining the final furanone content of the beer as the furanone content of the malt itself. However, variation in the limits of brewhouse operations within the limit set by other criteria, are unlikely to result in variation in the flavour and precursor content of the worts.

Thus the types and proportions of malts used are the most important factors available to give the brewer control over wort composition.

Although not investigated in the current work different yeast strains produce different amounts of furanones from the same worts[2,13]. Therefore it would seem feasible that suitable choice of yeast strain could be used to increase or decrease beer furanones from the same worts. The details of yeast metabolism involved and the nature of the Maillard intermediates utilised by yeast need to be investigated to allow full advantage to be taken at the practical level. The possibility arises that better understanding of these areas could allow production of beers with a wider range of properties than at the moment. Pale beers could be given aspects of a malty aroma whilst darker beers could have this property reduced or modified.

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Table I. The effect of the length of mashing on the properties of sweet malt worts

	Ale malt				Stewed malt			
Time	DMHF	MHF	Colour	Gravity	DMHF	MHF	Colour	Gravity
30	0.11	0.24	6.3	61.6	0.44	1.85	29.8	57.0
60	0.09	0.24	6.1	64.0	0.45	1.49	30.3	58.2
90	0.10	0.26	6.45	64.0	0.40	1.30	30.6	59.4
120	0.08	0.14	6.9	65.0	0.41	1.28	30.4	57.8

DMHF (mg/litre), MHF (mg/litre), colour (EBC) and excess degrees of gravity of worts prepared from ale and stewed malts mashed at 65ºC for 30, 60, 90 and 120 minutes.

Table II. The effect of the mashing temperature on the properties of sweet malt worts

Temperature	DMHF	MHF	Colour	Gravity
4ºC	nd	nd	3.1	15.0
20ºC	nd	nd	6.3	17.2
45ºC	0.11	0.21	6.8	25.9
65ºC	0.10	0.18	6.1	61.0
75ºC	0.13	0.22	7.4	57.7
85ºC	0.14	0.40	6.1	52.9

Furanone (mg/litre) content, colour (EBC) and excess gravity of ale malt worts mashed at a range of temperatures. nd: none detected.

Table III. Properties of ale malt worts boiled for increasing lengths of time

Time	Colour	Gravity	DMHF	MHF
0	4.9	56.3	0.08	0.25
30	6.2	55.0	0.10	0.62
50	6.7	55.5	0.10	0.69
70	7.2	55.8	0.12	0.86
90	8.8	55.7	0.14	1.00
110	8.1	55.8	0.12	1.13

Furanone content (mg/litre), colour (EBC) and excess gravity of ale malt worts boiled for increasing time periods (minutes) using a isomantle and condenser.

Table IV. Comparisons of malt worts boiled with or without the addition of hops

malt	Colour	Gravity	DMHF	MHF
Ale malt	9.3	59.7	0.10	0.35
Ale malt & hops	13	60.2	0.10	0.61
Lager malt	7.0	56.4	nd	0.11
Lager malt & hops	13.8	62.4	nd	0.14

Furanone content (mg/litre), colour (EBC) and excess gravity of ale and lager malt worts boiled for 50 minutes with or without the addition of pelleted hops. nd: none detected.

Table V. The effect of the rate of wort cooling on 4-hydroxyfuranone content of worts and beers

Time before transfer to ice (minutes)	Total time cooling (minutes)	DMHF content (mg/litre) pre-fermentation	post-fermentation
0	10	0.12	0.40
10	17	0.09	0.39
20	26	0.10	0.38
30	35	0.10	0.39
40	44	0.10	0.30

Table VI. DMHF in ale and lager malt adjunct worts and beers

	DMHF concentration (mg/litre)					
	Ale malt			Lager malt		
adjunct	wort	Boiled wort	Beer	wort	Boiled wort	Beer
none	0.08	0.11	0.34	nd	nd	0.21
barley	0.14	0.12	0.36	nd	nd	0.21
maize	nd	0.10	0.26	nd	nd	0.24
wheat	nd	0.11	0.40	nd	nd	0.31
stewed malt	0.14	0.20	1.11	0.10	0.12	0.54
syrup (40%)	*	0.09	0.24	*	nd	0.15
syrup (20%)	*	0.10	0.20	*	nd	0.12

* No DMHF analysis on sweet wort as syrup was added during the boil as described in the methods. nd: none detected

Table VII. MHF in ale and lager malt adjunct worts & beers

	MHF concentration (mg/litre)					
adjunct	Ale malt			Lager malt		
	wort	Boiled wort	Beer	wort	Boiled wort	Beer
none	0.12	0.35	0.15	0.08	0.23	0.09
barley	0.41	0.92	0.75	nd	0.12	0.09
maize	0.15	0.38	0.29	nd	0.10	0.11
wheat	0.15	0.43	0.10	nd	0.19	0.08
stewed malt	0.69	1.80	1.68	0.19	0.76	0.69
syrup (40%)	*	0.27	0.12	*	0.13	0.11
syrup (20%)	*	0.18	0.10	*	0.14	0.09

* as Table VI. nd: none detected.

Table VIII. EMHF in beers prepared from ale and lager malt adjunct worts

	Beer EMHF concentration (mg/litre)	
adjunct	Ale malt	Lager malt
none	0.09	0.09
barley	0.08	0.07
maize	0.08	0.10
wheat	0.10	0.09
stewed malt	0.13	0.08
syrup (40%)	nd	nd
syrup (20%)	nd	nd

nd: none detected.

Table IX. The effect of fermentation temperature on furanone content of beer

temperature	fermentation length (h)	DMHF mg/litre	EMHF mg/litre	final gravity
25ºC	72	0.74	0.12	1.0044
20ºC	72	0.50	0.09	1.0043
13ºC	96	0.40	0.10	1.0042

Furanones (mg/litre) detected in green beer prepared by fermenting ale malt wort for up to 96 hours in an orbital shaker at 25º, 20º or 13ºC.

Table X. Treatment of green beers with stabilising agents

treatment	DMHF mg/litre	MHF mg/litre	EMHF mg/litre
None	0.40	0.35	0.12
PVPP	0.45	0.32	0.14
Lucilite PC 5	0.41	0.33	0.13

Table XI. Prediction of beer DMHF content using IOB fermentability test

Sample	DMHF	Gr.Xs°	DMHF/Gr.Xs°
Ale malt			
HWU	0.25	58.6	0.004
IOB	0.12	28.6	0.004
Lager malt			
HWU	0.20	57.4	0.003
IOB	0.09	28.3	0.003

DMHF content (mg/litre) of ale and lager malt worts fermented using the IOB fermentability test and the fermentation method described in methods (HWU).

Table XII. 4-Hydroxyfuranone content at the different stages of commercial scale ale production

Sample	Gravity	colour	DMHF	MHF	EMHF
Lauter tun	1.0397	18.0	0.21	0.39	nd
Copper boil	1.0605	83.8	0.36	0.89	nd
Fermentation	1.0131	89.5	1.14	0.19	0.19
Cold conditioning tank	1.0089	87.2	0.69	nd	0.23
Bright beer tank	1.0049	45.3	0.27	nd	nd

Properties of samples donated from the production line of a U.K. manufactured ale. nd = not detected.

FIG. 1. Schematic representation of the effect of increased kilning on the levels of furanones and heat and yeast precursors in malt