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4-HYDROXY-FURANONE DERIVATIVES AS NEW INDICATORS OF MALT QUALITY

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by

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ABSTRACT

In 1991, work in Germany using aroma dilution analysis showed that 2,5-dimethyl-4-hydroxy-3(*2H*)-furanone (DMHF) was an important contributor to the sweet/caramel flavour of dark lager. In 1996 Japanese workers found that the concentration of this compound correlated well with the perceived sweetness of a series of experimental light lagers. The aim of this project was to establish the occurrence of DMHF, and the closely related methyl (MHF) and ethyl methyl (EMHF) derivatives, in British malts and determine the important factors influencing their formation. The overall aim was to establish the connection between the concentration in malt and beer so that malt furanone concentration could be used as a quality indicator.

From a chemical viewpoint, the furanones are typical intermediate products of the Maillard reaction which occurs on heating sugars and amino acids together and we found, not too surprisingly, that the compounds could not be detected in dry barley, soaked barley or green malt but that kilning was essential for their formation. Both MHF and DMHF were formed but never EMHF. At this point, the degree of heating was critical. Lengthy drying in the 50-60^{0}C range did not produce furanones but in the 90-110^{0}C range easily detectable amounts formed within a few hours although only DMHF reached concentrations above the aroma threshold in water. The highest concentrations were obtained with malts that had been stewed at 70^{0}C before drying. The same relationship to heat input was seen on examination of commercial malt samples; lager malt and high enzyme malt were free of furanones, ale malt contained a little but crystal malt had a high concentration of DMHF. Significant amounts were found in a sample of wheat malt. The formation pattern of furanones was more complicated than originally expected. At the final stage of drying after an initial increase, the concentration of furanones declined and then rose again to peak after a few hours. Examination of ten barley varieties under identical malting conditions yielded a six fold variation in DMHF concentration.

Analysis of ten commercial beers showed that all contained MHF and DMHF but only one had significant amounts of EMHF. With one exception, the five beers having more than two flavour units of DMHF were reported by a taste panel to have a sweet/caramel aroma. In laboratory fermentations crystal malt contributed directly to beer DMHF concentration but fermentation of both ale and lager malts resulted in beers with much higher concentrations than predicted. These malts appear to contain compounds, as yet unidentified, which can be converted into both DMHF and EMHF by yeast and this is particularly true for ale malt.

The compound, DMHF, has been shown to contribute a sweet/caramel/malty/fruity note to the aroma of some malts and beers. Controlling aspects of the malting process have been identified and it is now possible to set, empirically, a malt DMHF specification for a particular beer. However, more research is needed into the final kilning and fermentation conditions to understand formation and transformation of furanone precursors to allow a generalised, theoretical approach to quality assurance.

4-Hydroxy-furanone derivatives as new indicators of malt quality

J C Slaughter, ICBD, Department of Biological Sciences, Heriot-Watt University

SUMMARY

Introduction and Aims

Three 4-hydroxy-3(2*H*)furanone derivatives, 5-methyl-4-hydroxy-3(2*H*)furanone (MHF), 2,5-dimethyl-4-hydroxy-3(2*H*)furanone (DMHF) and 2(or 5)-ethyl-5(or 2)-methyl-4-hydroxy-3(2*H*)furanone (EMHF) have been identified as flavour compounds in foods such as fruits, cheese, cooked meats, seeds and cereals, bread, yeast extracts, coffee, wines, malt, beer and fermented soy products. MHF is associated with meaty and brothy flavour notes and has a flavour threshold value of 8.3 mg/l in water. The other two compounds have a sweet, caramel flavour with much lower flavour thresholds, 0.16 mg/l for DMHF and 0.02 mg/l for EMHF. In most foods investigated so far, DMHF is the most important compound, but in a few situations, such as fermented soy bean products, EMHF provides the major flavour note.

The Maillard reaction provides a theoretical understanding of the way in which the furanones arise spontaneously in cooked foods. The details of the reactions are very complex but the overall situation is well understood. On heating with amino acids, sugars give rise first to Amadori compounds which can lead to 1-deoxydiketoses. C_6 compounds can cyclise to give DMHF directly and similar reaction of the C_5 compounds results in MHF production. Addition of a C_2 fragment, probably derived from alanine through Strekker degradation, results in EMHF formation from pentoses. In a similar reaction, addition of a C_1 fragment derived from glycine, provides an alternative route to DMHF. At higher temperatures direct thermal degradation of monosaccharides can give rise to furanones. In practice, MHF and DMHF both arise spontaneously in many heated foods but this does not seem to be true for EMHF. This compound appears to always be a product of yeast fermentation and, in some situations, additional amounts of DMHF can also be produced by fermentation.

The importance of DMHF in the flavour of lager, particularly dark lager, was established in 1991 using the aroma extract dilution technique. In 1996 both DMHF and EMHF were reported in Japanese beers but, on the basis of taste panel work, DMHF appeared to be the main contributer to the sweet flavour in the beers tested. MHF has not been reported at significant levels in malt or beer. These results indicate a role for DMHF, and possibly EMHF, in the flavour of beer in general and it appears likely that they may be particularly significant in UK ales where a sweet/caramel note is often a major aspect of the overall flavour.

The present work was undertaken to clarify the significance of 4-hydroxyfuranones in the flavour profile of commercial British malts and beers and to understand the relationship between malt and beer with a view to being able to set quality specifications for malt.

Methods

Germination

Malts were prepared using a Seeger Micro-maltings unit. Barley, Prisma 1.8%N (Triumph x Cambinus) x Piccolo) was steeped at 16°C for 8 hr then 24 hr following a 16 hr air rest. Germination was then carried out at 16°C with daily turning of the grain to ensure even development. Samples were taken and kilned as appropriate.

Kilning procedures

Temperature programmes are described in the text. Stewing was carried out by sealing the green malt in an airtight vessel and heating in an oven at 67-71°C for 4 hr. The malt was then dried by kilning with a free air flow through the grain bed. Once kilned, all samples were derooted by hand and stored in airtight containers until required for extraction.

Preparation of malt hot water extract.

Malt (75g) was finely ground in a Buhler Universal Laboratory disc mill (type DLFU) set with a 0.2mm disc gap. The grist was stirred into 350ml distilled water at 65°C, and this temperature was maintained for 60 minutes, stirring every 20 minutes. The mash was then filtered using Whatmann No.2 paper and boiled for 50 minutes. Once cooled the extracts were cleared of precipitate by centrifugation at 3000 xg for 10 minutes. The hot water extracts (HWE) were stored at 4°C and analysed within 24 hr.

Aroma assessment

A panel of four assessors was asked to give descriptions of the aroma of freshly poured beers without any terminological prompting. The panel members had a general training in beer tasting and flavour description but had no prior knowledge of the 4-hydroxfuranones or their characteristic flavours.

GC-MS Analysis.

To extract the furanones from the malt extract or degassed beer, a 20 ml aliquot 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 g for 10 minutes, the organic layer was rotary evaporated to 1ml and decanol was added to a concentration of 10mg/l. 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 40°C, with a 2 minute hold, to 200°C at a rate of 5°C/min. The final temperature of 200°C 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.

Coefficients of variation for MHF (13.0%) and DMHF (5.4%) in malt extracts, DMHF (13.4%) and EMHF (11.8%) in beer, and DMHF (1.5%) and EMHF (3.4%) in standard solutions were calculated on the basis of six repeat determinations on a single sample of malt, beer or standard solution. The recoveries of DMHF from malt extract and from beer, based on addition of 1mg of the standard compound per litre, were the same at 53.9% (cv 6.4%). Recovery of EMHF from beer was 61.7% (cv 13.3%). The lowest concentration of furanones quantifiable by this method is 0.01 mg/L of the aqueous phase.

Key Results

DMHF occurs at significant levels in certain types of commercial malts

Water extracts of commercial lager malt and high enzyme malt did not contain furanones whereas small amounts equivalent to about 1 Flavour Unit were found with ale malts. Crystal malt yielded DMHF concentrations up to 26 times the aroma threshold value.

DMHF occurs in beers at up to nine time its aroma threshold value

A survey of ten commercial beers showed that all contained DMHF, in five cases at concentrations equivalent to more than 2 Flavour Units. Four of these beers were identified as having a sweet aroma note by an unprompted flavour panel. Neither of the other two furanones seem likely to be important in beer aroma.

Kilning is essential for formation of furanones in malt

Furanones were not found in barley or green malt; MHF and DMHF occurred in germinated grain only after kilning at sufficiently high temperatures. EMHF was never found in malt. Germinated grain dried in the laboratory at 50-60^{0}C did not develop furanones even after 18h whereas at 95-110^{0}C furanones appeared within a few hours. For given kilning conditions, stewed grain developed higher furanone concentrations than unstewed. The relationship between furanone concentration and time in the kiln was complex. Furanones appeared as the grain became dry, reached a peak and after declining for a while rose to a second , higher peak after a number of hours at a rate depending directly on the temperature. During this time colour rose smoothly to reach a plateau at the same time as the second furnaone peak.

Length of germination and barley variety both affect furanone levels

Prisma barley was germinated under standard conditions and samples taken daily. These were stewed at 70^{0}C for 4h and then kilned at 110^{0}C for 2h. The furanone content of water extracts peaked at 66h-72h. The DMHF content at 66h was approximately twice that at 24h and 120h. The colour of the extracts

reached a maximum at 48h so over the period of germination there was not a constant relationship between colour and furanones.

Nine varieties of barley were germinated and kilned under standard conditions. The DMHF content of water extracts varied six-fold from about 0.5 to 3 Flavour Units. The source of the variation is not currently understood and more research is needed to discover whether it is intrinsic to the varieties themselves or in their response to malting conditions.

Yeast can produce furanones during fermentation but the amount depends on the malt

Worts made from single types of commercial malts were fermented in the laboratory. The concentration of DMHF in beer produced from crystal malt was much the same as in the wort. However, for both lager and ale malts the concentration was increased by fermentation and this was particularly so for the ale malt. The beers from all three malt types contained EMHF at relatively similar concentrations.

Conclusions

DMHF, but not MHF or EMHF, may contribute to the aroma of malt.

The amount of DMHF present depends on the barley variety, extent of germination, stewing and the degree of heating.

Under standard conditions there is a good relationship between malt DMHF and colour for a single barley variety but the two qualities are not tightly connected over a wider range of production techniques.

Both DMHF and EMHF may contribute to beer flavour although DMHF appears to be much the more important. DMHF arises directly from the malt and from fermentation. EMHF results entirely from fermentation. The amounts formed depend on the malt type. The furanone precursors appear to be highest in malts exposed to intermediate levels of heating in their preparation.

Implications

1. Beer quality assurance could be improved in defined cases by specifying the DMHF content of the malt.
2. Malts with varying ratios of furanone content to colour could be produced so widening the range of beer types which can be produced.
3. Although the simplest relationship between malt and beer furanone content occurs with crystal malts, the skills could be developed to allow beers of a given furanone content to be made from less highly kilned malt by taking advantage of the ability of yeast to produce furanones from precursor compounds in the malt. This would have both economic and environmental advantages by reducing fuel demand in maltings.

TECHNICAL DETAILS

PAPERS ACCEPTED FOR PUBLICATION

1. Key Steps during Barley Malting that Influence the Concentration of Flavour Compounds.

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ABSTRACT

The production of the three potential 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), during the malting of barley was investigated. Malts were germinated for different lengths of time and kilned using a variety of regimes. The hot water extracts of the malts were extracted with methyl acetate and analysed by gas chromatography-mass spectrometry. It was demonstrated that both germination and kilning were essential for the formation of MHF and DMHF as neither green malt, raw barley nor kilned raw barley contained these flavour compounds. EMHF was not detected in any of the samples tested. The levels of MHF and DMHF detectable in the malt were shown to increase as the length of germination increased up to 66 hr after which time the furanone content declined. Low temperature kilning gave rise to little or no furanones as did roasting over short periods. However if the green malt was stewed prior to roasting then the levels of DMHF in the hot water extracts increased to above that of the aroma threshold in water. The highest concentration of MHF was 19% of its aroma threshold.

Keywords: barley, malt, flavour, hydroxyfuranones, kilning

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 foods such as roasted sesame (16), popcorn (6), strawberry (17), pineapple (13), tomato (5), honey (7) and in fermented products, for example beer (15), soy sauce (12) and Japanese miso (10). These compounds are known to be products of the heat driven Maillard reaction (2) which requires sugar and amino acid as substrate and catalyst for furanone formation. Due to the nature of the production of these compounds they are found to flavour many heat processed foods as well as untreated and fermented products. Both DMHF and EMHF have distinctive sweet/caramel like flavours and aroma thresholds in water of 0.16mg/l and 0.02mg/l respectively, whereas MHF has a meaty/brothy flavour and a much higher aroma threshold of 8.3mg/l.

DMHF and MHF, but not EMHF have been found in barley malts with concentrations of DMHF in the hot water extract exceeding the aroma thresholds. This was particularly the case in malts of high colour which had therefore undergone a more rigorous kilning (9). Although the spontaneous production of EMHF in model Maillard systems has been demonstrated (3), it has not been found in malt (9; Mackie & Ray, unpublished). However it seems that a precursor for EMHF is present in some malts as, during fermentation of vigorously kilned malts, EMHF is produced at levels much greater than the aroma thresholds (9).

As the malting process can influence the three necessary components for the Maillard reaction, heat, sugar and amino acids, it is likely that variation in the conditions of germination, which releases sugars and amino acids into the grain, and kilning, which applies heat to the grain, will determine the concentration of the furanones found in the final product.

The aim of this study was to identify the key steps during malting which influence furanone formation and thus open the way to control the concentration of the compounds in commercial malts.

EXPERIMENTAL

Germination

Malts were prepared using a Seeger Micro-maltings unit. Barley, Prisma 1.8%N (Triumph x Cambinus) x Piccolo) was steeped at 16°C for 8 hr then 24 hr following a 16 hr air rest. Germination was then carried out at 16°C with daily turning of the grain to ensure even development. Samples were taken and kilned as appropriate.

Kilning procedures

To investigate the effect of length of the germination period on furanone content, samples were taken at 24, 48, 66, 72, 96 and 120 hr post-steep and stewed by sealing the green malt in an airtight vessel and heating in an oven at 67-71°C for 4 hr. The malt was then dried by kilning at 110 °C for 2 hr with a free air flow through the grain bed(kiln E). In addition, to evaluate the effect of kilning on furanone content, green malt

sampled after 96 hr germination was kilned using one of the following temperature regimes with the air allowed to flow through the grain bed:

A: 50°C (16 hr)

B: 52°C (2.7 hr), 56°C (4 hr) & 84°C (2.5 hr)

C: 60°C (16 hr) & 85°C (2 hr)

D: 95°C (4 hr)

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

Preparation of malt hot water extract.

Malt (75g) was finely ground in a Buhler Universal Laboratory disc mill (type DLFU) set with a 0.2mm disc gap. The grist was stirred into 350ml distilled water at 65°C, and this temperature was maintained for 60 minutes, stirring every 20 minutes. The mash was then filtered using Whatmann No.2 paper and boiled for 50 minutes. Once cooled the extracts were cleared of precipitate by centrifugation at 3000 xg for 10 minutes. The hot water extracts (HWE) were stored at 4°C and analysed within 24 hr.

GC-MS Analysis.

To extract the furanones from the malt, 20 ml of HWE 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 1ml and decanol was added to a concentration of 10mg/l. 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 40°C, with a 2 minute hold, to 200°C at a rate of 5°C/min. The final temperature of 200°C 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 performing a HWE, solvent extraction and GC-MS analysis on the same malt sample as 5 separate experiments. The coefficient of variation for DMHF was 5.4% and 13% for MHF. The recovery rate of DMHF from a malt HWE was also established. The furanone was added to a HWE to increase the concentration by 1mg/l. This sample was then solvent 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. Therefore the concentrations for both furanones in the text are the experimentally derived values unadjusted for recovery rates.

RESULTS AND DISCUSSION

Essential conditions for furanone formation.

Hot water extracts were prepared from raw barley, steeped but non-germinated barley before and after kilning at 95°C (kiln D), green malt, which had been germinated for 3 days, and kilned malt prepared by kilning the green malt at 95°C for 4 hr (kiln D). Furanones were found only in the grain which had been both germinated and kilned: MHF 1.14mg/l and DMHF 0.04mg/l. EMHF was not detected in any of the samples analysed.

Effect of length of germination period.

A series of malts were prepared as described in the methods section to examine the effect of the length of germination period on furanone content. Levels of DMHF and MHF in kilned malts of differing germination time increased up to 66 hr germination after which levels of both compounds decreased as germination progressed (Fig. 1). The increase in furanone concentrations over the first 3 days of germination seems most likely to result from increasing levels of free sugars and amino acids in the green malt prior to kilning. As this malt was stewed before kilning the levels of these compounds are likely to be influenced by the concentration of hydrolytic enzymes present in the endosperm which continue to break down starch and protein during stewing. The activity of the two major enzymes involved in the release of free sugars, α- and β-amylase, are known to increase dramatically during germination until approximately 70 hr after which time they remain constant (8, 1). These increases in the potential furanone precursors, free sugars and amino acids, are reflected in the rise of furanone concentrations seen over the first 3 days of germination. The decrease in furanones after 66 hr germination, when the root and ascospire growth are clearly evident and the grain could be considered to be "overgrown" (4), could result from increased embryonic metabolism utilising the free sugars and amino acids thus depleting the endosperm store.

The results suggested that MHF was unlikely to contribute to malt flavour as it was never found at a concentration greater than 19% of the flavour threshold value and even if the data were adjusted to account for the recovery rate established for DMHF this would only increase the concentration of MHF to 38% of its flavour threshold (8.3mg/l). On the other hand, DMHF varied from 22% to 369% of its flavour threshold value (0.16mg/l) and so could contribute significantly to the flavour of some malts. Regardless of germination length or kilning regime, no EMHF was detectable in any sample. This confirms previous reports which suggested that the Maillard reaction in malts does not produce this furanone and its appearance in beers is a result of yeast fermentation (14, 9).

Effect of kilning procedure.

Green malts, germinated for 96 hr, were kilned using a variety of procedures ranging from slow drying with relatively cool air (50°C) to fast drying with hot air (110°C) after stewing. The levels of furanones found in these malts (Table I) indicated the importance of kilning on the final concentration of MHF and DMHF in malts. The lowest levels of both furanones were evident with the lowest kiln temperature (kiln A), and is

DMHF was detected in the malt kilned at the highest temperatures (kiln E). This malt was initially kept out of the air flow maintaining the green malt in a warm and wet condition for four hours to stew, before finishing at a high temperature using a direct airflow. This regime produced a malt of considerably higher colour and furanone content than any of the others and is typical of the type of process used to manufacture crystal malts. During stewing, the enzymatic activity of the grain continues and the content of the endosperm becomes liquefied, dramatically increasing the levels of free sugars and amino acids(4), which may then be used in the Maillard reaction accelerated by the high finishing temperatures. However, confirmation of the exact mechanism is required as it unclear whether the boosted levels of furanones seen with kiln E, are a direct result of the stewing of the grain or the higher final temperature or a combination of the two.

The results (Table I) certainly indicate that kilning temperature alone is not a good indicator of the amount of furanones produced. Although a high temperature was employed throughout kiln D, which should in theory stimulate the Maillard reaction and thus boost furanone levels, the furanone content of this malt type was lower than that of kiln B where the malt was finished 10°C lower. This may be linked to the levels of moisture in the grain as previous work has stated that dry-roasting conditions do not favour the production of these furanones in model systems (11). It is likely that the high initial temperature causes moisture levels in kiln D to drop more quickly to below the amount required for furanone production than in kiln B. This hypothesis may also help to explain why the furanone content in kiln B is higher than kiln C despite near identical finishing temperatures. The cooler start in kiln B would result in a lower rate of drying during the first 4 hr thus allowing a greater extent of furanone formation during this period. However this suggestion does require verification by monitoring moisture levels during the kilning process.

In general the colour of the malt also increased with increasing kiln temperatures, and the darkest malt had the highest furanone content. This is in agreement with other authors who stated that the furanone content of a dark malt was considerably higher than a pale (15, 9). However, using specific kilning regimes it was possible to disrupt this relationship and produce a pale malt of 6.0 EBC, (kiln B), which contained a higher concentration of MHF and DMHF than in a slightly darker malt of 10 EBC (kiln D): 1.57mg/l MHF and 0.12mg/l DMHF in kiln B as against 1.14mg/l MHF and 0.04mg/l DMHF in kiln D. Furthermore, altering the length of the germination period appeared to enhance these anomalies in the relationship between malt colour and furanone content (Fig.1.). For example the extracts of malts made from grain germinated for 66 hr and 120 hr (kiln E) both had a colour of 54 EBC, but the 66 hour malt contained over double the concentration of DMHF as compared to the 120 hour malt. This ability to change the relationship between malt colour and the content of an important flavour compound may prove to be a useful technique in the production of specialty malts but does require further investigation into the mechanism and controlling influences of furanone synthesis during kilning by monitoring enzymatic activity, carbohydrate and amino acid profiles during malting.

CONCLUSIONS.

The results clearly indicate that the furanone levels in malt, produced on a laboratory scale, were directly affected by the method of malting employed. Both steps of the malting process investigated, germination period and kilning, were seen to influence the furanone content of the final product. The highest furanone levels occurred when modification had progressed to its fullest but before the grain had become overgrown and overmodified, and then kilned using a low temperature stew followed by a high temperature finish. It was further demonstrated that, although as a general rule, higher kilning temperatures have been associated with higher concentrations of furanones, it was possible to produce malts with similar colours but different furanone levels depending on the length of the germination period and the kilning regime. Understanding how the malting procedure influences DMHF levels in malt will allow control over the level of this flavour compound. However a more detailed examination of production during malting and also fermentation is required before the furanone content of beers can be related to the techniques of malt production.

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Table I. Colours (EBC) and levels of furanones, MHF and DMHF (mg/l), detected in HWEs of malts germinated for 96 hr then kilned using one of the following regimes: A: 50°C (16 hr); B: 52°C (2.7 hr), 56°C (4 hr) & 84°C (2.5 hr); C: 60°C (16 hr) & 85°C (2 hr); D: 95°C (4 hr); E: 67-71°C indirect heating to stew (4 hr) & 110°C (2 hr). nd-not detected.

Kilning regime	MHF (mg/l)	DMHF (mg/l)	Colour (EBC)
A	0.24	nd	5.5
B	1.57	0.12	6
C	0.89	0.03	8
D	1.14	0.04	10
E	0.72	0.43	56

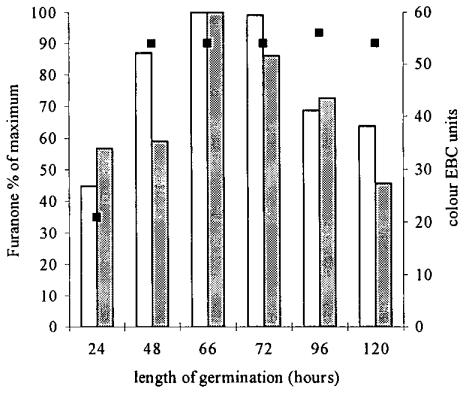

Fig. 1. Colours (■) and relative furanone levels, MHF (clear bars) and DMHF (filled bars), detected in HWEs of malts germinated at 16°C for increasing lengths of time and kilned at 110°C for 2 hr after stewing the green malt at 67-71°C for 4 hr. Maximum content of both furanones occurred at 66 hr and the values were 1.04mg/l MHF and 0.59mg/l DMHF in the HWEs.

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2. The contribution of 4-hydroxyfuranone derivatives to the aroma of commercial beers and malts

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ABSTRACT

Analysis of hot water extracts prepared from a range of commercial malts showed that 2,5-dimethyl-4-hydroxy-3(2*H*)furanone (DMHF) varied from undetectable (lager malt) to 4.2 mg/l (crystal malt), a concentration twentysix times the flavour threshold in water. 5-Methyl-4-hydroxy-3(2*H*)furanone (MHF) was detected in all samples except one but was always well under its flavour threshold value. 2(or 5)-Ethyl-5(or 2)-methyl-4-hydroxy-3(2*H*)furanone (EMHF) was not detected in any of the samples. Fermentation of lager, ale and crystal malt extracts with an ale strain of yeast led to the appearance of EMHF in all cases as well as additional DMHF. The greatest increases in both compounds was with the ale malt. Both increases and decreases occurred in MHF concentration as a result of fermentation but final levels were always well below the flavour threshold value. Analysis of ten commercial beers found DMHF in all the samples and in five cases levels exceeded twice the flavour threshold value in beer with flavour units from 2.4 to 9.1. A flavour panel noted that in four of these cases the beer had a distinctly sweet/caramel aroma which is typical of DMHF. EMHF was undetectable in six samples, detectable, but unquantifiable, in three cases but at about 80% of the flavour threshold value in the remaining sample. MHF was found in all samples but at insignificant levels. The results show that DMHF is an important flavour compound in British ales and EMHF may make a contribution in a limited number of situations. The contribution of malt type, brewery processing and yeast strain in determining the concentration of the two 4-hydroxyfuranones in beer remains uncertain.

Key words: malt, flavour, 4-hydroxyfuranones, beer

INTRODUCTION

Three 4-hydroxy-3(*2H*)furanone derivatives, *5*-methyl-4-hydroxy-3(2*H*)furanone (MHF), 2,5-dimethyl-4-hydroxy-3(2*H*)furanone (DMHF) and 2(or 5)-ethyl-5(or 2)-methyl-4-hydroxy-3(2*H*)furanone (EMHF) have

been identified as flavour compounds in foods such as fruits[4,14,23,25,38], cheese[20], cooked meats[7,17,34,36], seeds[3,33] and cereals[5,6,8], bread[30], yeast extracts[21], coffee[37], wines[9,24], malt[19,32], beer[10,27,31] and fermented soy products[11,22,28]. The occurrence and formation of furanones in foods and their more general biological significance have been reviewed recently[35,38]. MHF is associated with meaty and brothy flavour notes and has a flavour threshold value of 8.3 mg/l in water. The other two compounds have a sweet, caramel flavour[15] with much lower flavour thresholds, 0.16 mg/l for DMHF and 0.02 mg/l for EMHF. In most foods investigated so far, DMHF is the most important compound, but in a few situations, such as fermented soy bean products, EMHF provides the major flavour note[11,22].

The Maillard reaction provides a theoretical understanding of the way in which the furanones arise spontaneously in cooked foods. The details of the reactions are very complex but the overall situation is well understood[1,2,18]. On heating with amino acids, sugars give rise first to Amadori compounds which can lead to 1-deoxydiketoses. C_6 compounds can cyclise to give DMHF directly and similar reaction of the C_5 compounds results in MHF production. Addition of a C_2 fragment, probably derived from alanine through Strekker degradation, results in EMHF. In a similar reaction, addition of a C_1 fragment derived from glycine, provides an alternative way to DMHF from pentoses. At higher temperatures direct thermal degradation of monosaccharides can give rise to furanones. In practice, MHF and DMHF both arise spontaneously in many heated foods but this does not seem to be true for EMHF. This compound appears to always be a product of yeast fermentation and, in some situations, additional amounts of DMHF can also be produced by fermentation[10,11].

The importance of DMHF in the flavour of lager, particularly dark lager, has been established using the aroma extract dilution technique[31]. Both DMHF and EMHF have been found in Japanese beers but, on the basis of taste panel work, DMHF appeared to be the main contributer to the sweet flavour in the beers tested[27]. MHF has not been reported at significant levels in malt or beer. These results indicate a role for DMHF, and possibly EMHF, in the flavour of beer in general and it appears likely that they may be particularly significant in UK ales where a sweet/caramel note is often a major aspect of the overall flavour.

The present work was undertaken to clarify the significance of 4-hydroxyfuranones in the flavour profile of commercial British malts and beers.

METHODS AND MATERIALS

Preparation of malt hot water extracts

The malts used, ale, lager, high enzyme, wheat and crystal were obtained from a commercial maltings. Malt (75g) was finely ground in a Buhler Universal Laboratory disc mill (type DLFU) set with a 0.2mm disc gap.

The grist was stirred into 350ml distilled water at 65°C, and this temperature was maintained for 60 minutes, stirring every 20 minutes. The mash was then filtered using Whatman No.2 paper and boiled for 50 minutes. Once cooled the extracts were cleared of precipitate by centrifugation at 3000 x g for 10 minutes. Samples of hot water extracts (HWE) were also taken before boiling. All extracts were stored at 4°C and analysed within 24 hours.

Yeast strain and fermentation conditions.

Saccharomyces cerevisiae (strain NCYC 1108, from the ICBD culture collection) was grown aerobically in 250ml conical flasks containing 1.5% malt extract medium (Oxoid) in an orbital incubator at 25 °C. For the experimental fermentations, cells were inoculated into 100ml of the HWE at 10^7 cells/ml and incubated at 25°C for 3 days when fermentation was complete.

Measurement of colour

The colour of both hot water extracts and beers was measured spectrophotometrically using the IoB recommended method[16].

Preparation of organic extract for GC MS analysis

Hot water extracts To extract the furanones, 20 ml of test 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 800 osc/min. The organic layer was retained after centrifugation at 2000 x g for 10 minutes and rotary evaporated to 1ml for GC-MS analysis. 10 µl n-Decanol solution (1.0 g/L in ethanol) was added to act as internal standard.

Beers For the commercial beers, the bottles or cans were opened, 20ml was decanted into a glass for aroma assessment and the remaining beer left overnight, loosely covered, to de-gas. For samples produced in the laboratory, yeast was removed by centrifugation and the samples again left covered, but unsealed, overnight. In both cases, methyl acetate extraction was carried out as described for the malt HWEs.

Aroma assessment

A panel of four assessors was asked to give descriptions of the aroma of freshly poured beers without any terminological prompting. The panel members had a general training in beer tasting and flavour description but had no prior knowledge of the 4-hydroxfuranones or their characteristic flavours.

GC-MS analysis of 4-hydroxyfuranones.

Analysis was carried out using a Hewlett Packard HP6890 Series GC and Mass Selection System fitted with an HP-5MS column (ext diam, 0.25mm, int diam, 0.25µm, length, 30m)[10]. 1µl of organic extract was injected using the splitless mode at 1.5ml/min for 1 min, temperature 220°C. The column temperature ran

from an initial 40°C, with a 2 min hold, to 200°C for 40 min with a ramp rate of 5 ^{0}C per min, using Analytical grade helium as the carrier gas at a flow rate of 1.5ml/min. The MS detector was used in the SIM mode and 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). The curve for DMHF was also used for MHF in the absence of commercially available standard material. Solvent extraction and GC-MS analysis was repeated twice for each sample.

Coefficients of variation for MHF (13.0%) and DMHF (5.4%) in malt, DMHF (13.4%) and EMHF (11.8%) in beer, and DMHF (1.5%) and EMHF (3.4%) in standard solutions were calculated on the basis of six repeat determinations on a single sample of malt, beer or standard solution. The recoveries of DMHF from malt extract and from beer, based on addition of 1mg of the standard compound per litre, were the same at 53.9% (cv 6.4%). Recovery of EMHF from beer was 61.7% (cv 13.3%). Data in the text have been adjusted for these recovery values. The lowest concentration of furanones quantifiable by this method is 0.01 mg/L of the aqueous phase.

RESULTS AND DISCUSSION

Analysis of commercial malts

Hot water extracts were prepared in duplicate from a range of commercial malts on two separate occasions and the 4-hydroxyfuranone content measured (Table 1). In agreement with previous work, MHF and DMHF, but not EMHF, were found and, despite batch to batch variation, the concentration of DMHF was in proportion to the extent of heating during preparation of the malts from the barley[10]. The lager and the high enzyme malt, which would have been dried in the kiln at relatively low temperatures to avoid development of colour and flavour, did not contain any detectable amount of the compound but it was easily measurable in the ale malt extract at near the flavour threshold value. This malt would have experienced higher kilning temperatures as evidenced by the higher colour. The crystal malt, a speciality malt produced by stewing the grain and then roasting at high temperatures specifically to increase colour and flavour, yielded the highest concentrations of furanones with DMHF present at between ten and twentysix times the flavour threshold in water (0.16 mg/l). The wheat malt, with a colour two to three times that of the ale malt, contained DMHF at ten times the flavour threshold. The effect on DMHF content of boiling the extracts for 50 min was very slight.

MHF was detected in all the hot water extracts except in one batch of lager malt. Boiling appeared to cause a rise in MHF in about half of the samples tested but the compound is very unlikely to contribute to flavour in any situation; the highest concentration found, which was in one of the crystal malt extracts, represents only about 8% of the flavour threshold value in water (8.3 mg/l).

Analysis of commercial beers

Ten samples of beer were purchased from an off-licence and analysed (Table 2). Colour, pH and 4-hydroxyfuranone content were measured and a taste panel was asked to identify the flavour notes in the beer aroma without any terminological prompting. %Abv was taken from the label. All the beers contained MHF, but at much lower levels than the threshold value of 8.3 mg/l in water, so this compound seems not to be important in flavour unless major, currently unknown, additive or synergistic effects occur. EMHF was detected in the stout and both lagers but at too low a concentration to be quantifiable. The compound occurred in the barley wine in easily measureable amounts and would provide about 0.8 Flavour Units using the threshold value of 0.7 mg/l reported for beer[27]. In contrast, DMHF occurred in every one of the beers and at levels likely to be significant in flavour. The largest amount was found in the barley wine where it is clearly a primary flavour note. This was borne out by the comments of the taste panel that this beer had a strong, sweet, sugary note reminiscent of candy floss which is the typical aroma of pure DMHF. Four other beers had DMHF concentrations equivalent to more than two Flavour Unit and in three of the cases the taste panel reported a sweet note in the aroma. The exception was the brown ale which was reported as "yeasty", an aroma which could easily have masked a sweet note. No sweet note was detected in the aroma of the remaining beers where the Flavour Units varied from 0.6 to 1.6 and comments from the panel did not suggest that the beer aroma was disguising this characteristic. Thus, even where a distinct furanone flavour note is not obvious, DMHF fell into the secondary rank of flavour compounds in the beers tested and so may still be an important contributor, though not a distinctive one, to the flavour of a wide range of beers. These results agree with earlier work that perceived sweetness in a pale lager beer was strongly correlated to DMHF content[27] and, furthermore, suggest that there may be situations where EMHF is a significant contributor. Any interaction between DMHF and EMHF in flavour perception remains to be determined.

Fermentation of boiled malt extracts

The boiled malt extracts derived from the ale, lager and crystal malts referred to in Table 1 were fermented as described in the Methods Section and the concentration of the 4-hydroxyfuranones determined after three days when fermentation was complete. The effect of fermentation on MHF concentration was variable. In some cases the compound increased in concentration whilst in others it declined but final amounts were still always well below the flavour threshold value so, as with the commercial beers, MHF seems unlikely to contribute to the flavour of laboratory beers. DMHF concentration showed an increase in five of the six fermentations but a decline in one of the crystal malt experiments. In the "beers" from ale and crystal malts, DMHF was mainly well above its flavour threshold value and so would be expected to make a major contribution to flavour. The lager malt fermentations produced values from about 0.7 to 3.5 Flavour Units so a contribution from slight to distinct would be expected. These results suggest that production of lager malts needs to be controlled particularly carefully as, although the malts do not contain DMHF itself, precursors are formed and the compound can then be produced during fermentation to a level very critical in the

perception of flavour. EMHF was synthesised during fermentation in all three cases to a surprisingly consistent extent given the wide range of heat treatments used in production of the malts; a nearly 40 fold variation in colour occurred whilst the range for EMHF was only just over two fold. Whilst most EMHF was produced from the ale malt (about 1.6 Flavour Units) the increase was not much less for both the other malts (about 0.6 to 1.4 Flavour Units). The experiment confirms the synthesis of both DMHF and EMHF during fermentation with the extent dependent on the malt type although not in exactly the way which would be predicted from the amounts of the 4-hydroxyfuranones found in hot water extracts of the malts. Lager malt had no measureable amounts of DMHF or EMHF and, although both were formed during fermentation, the resultant "beer" also had the lowest concentrations of the two compounds of all three malt types. However, fermentation of ale malt resulted in a greater production of both DMHF and EMHF than did fermentation of the crystal malt extract so that the final "beer" concentration of furanones was higher with the ale malt than with the crystal malt although the indication from HWE values had been very much the opposite. This suggests that, whilst some heating is necessary to form the precursors of the 4-hydroxyfuranones, more extensive heating regimes actually destroy the compounds. It also indicates that, when the malting and kilning procedures are investigated more closely, the connection between colour and flavour in the resultant beers is not a rigorous one. The results suggest that beer 4-hydroxyfuranone concentration will depend on the exact grist composition in addition to the previously noted factors of yeast strain and wort strength[27] and that it should be possible to separate colour and flavour to produce pale beers with distinctive aspects of typical ale aroma.

The malt compounds used by yeast as precursors for synthesis of DMHF and EMHF are unknown. There have been suggestions that pentose phosphate pathway intermediates can be metabolised by yeasts to furanones[13,28,29]. Other workers have shown that unidentified thermal products of sugars can be similarly metabolised but there is no agreement on the nature of the compounds involved in furanone formation during beer production[12,26]. In addition, it has been claimed that production of EMHF in beer is yeast strain specific[27] and it is interesting in this context that EMHF was absent from all the regular ales analysed but present to some extent in both lagers, the stout and the strong ale (Table 2). However, whether this is truly a reflection of the properties of the yeast strains used commercially or is more related to the exact nature of the different brewing processes is unknown. A further question is thus raised over the source of DMHF in these beers. If the yeasts used in the breweries cannot produce EMHF, is this also true for DMHF? In this case the yeast would make no contribution to the beer furanone content at all and DMHF would then be derived entirely from the malt. On the other hand in other circumstances, yeast may synthesise significant amounts of both DMHF and EMHF during fermentation and so contribute to a flavour note currently associated with malt. This area clearly requires more extensive investigation to clarify the exact relationships between malt processing, wort composition and beer 4-hydroxyfuranone levels.

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Table 1 4-Hydroxyfuranones in Hot Water Extracts of commercial malts

Malt type	Colour (EBC units)	Sp Gr (excess degrees)	MHF (mg/l)	DMHF (mg/l)
Ale (1)	16 (17)	58.3 (59.1)	0.31 (0.33)	0.07 (0.15)
(2)	15 (15)	58.1 (58.2)	0.54 (0.97)	0.19 (0.11)
Lager (1)	10 (16)	57.3 (57.7)	nd (nd)	nd (nd)
(2)	10 (14)	55.3 (54.7)	0.41 (0.66)	nd (nd)
Crystal(1)	327 (351)	39.5 (39.3)	0.67 (0.63)	4.22 (4.15)
(2)	384 (399)	31.9 (32.4)	0.24 (0.42)	1.58 (2.36)
High Enzyme (1)	15 (15)	56.5 (56.3)	0.05 (0.16)	nd (nd)
Wheat (1)	36 (36)	56.5 (56.3)	0.67 (0.63)	1.65 (1.73)

Two separate sets of analyses were carried out at different times as indicated by (1) or (2) after the malt type except for the high enzyme and wheat malts which were examined on one occasion only. All data are averages of two determinations (nd – not detectable). Figures in parenthesis show the values after boiling the HWE for 50 min. EMHF was not detected in any sample.

Table 2 4-Hydroxyfuranones in commercial beers

Beer type	**Colour** (EBC units)	**pH**	**%abv**	**MHF** (mg/l)	**DMHF** (mg/l)	**EMHF** (mg/l)
Stout [s]	116	4.02	4.3	0.48	0.71 (*2.4*)	nq
Barley wine [s]	38	4.26	9.1	0.86	2.73 (*9.1*)	0.55 (*0.8*)
Brown ale	60	4.07	5.0	0.26	1.64 (*5.5*)	nd
Ale 1 [s]	35	4.12	4.7	0.77	1.67 (*5.6*)	nd
2	14	4.36	4.4	0.43	0.48 (*1.6*)	nd
3	18	4.07	4.2	0.41	0.24 (*0.8*)	nd
4	57	4.43	6.5	0.12	0.37 (*1.2*)	nd
5 [s]	33	4.2	4.1	0.55	1.06 (*2.5*)	nd
Lager 1	10	3.97	4.1	0.22	0.19 (*0.6*)	nq
2 *	14	4.57	4.4	0.33	0.30 (*1.0*)	nq

%abv: % Alcohol by volume

*European origin

[s] Reported by the taste panel as having a sweet aroma note

Figures in parenthesis are Flavour Units calculated using the threshold values in beer[27]; 0.3mg /l for DMHF and 0.7 mg/l for EMHF.

Nd – not detectable; nq – detectable visually on the GC trace but not quantifiable.

Table 3 4-Hydroxyfuranone content of fermented, boiled malt extracts

Malt type	MHF (mg/l)	DMHF (mg/l)	EMHF (mg/l)
Ale (1)	0.26 (-0.07)	3.59 (3.44)	1.16 (1.16)
(2)	1.82 (0.85)	2.14 (2.03)	1.07 (1.07)
Lager (1)	0.08 (0.08)	0.22 (0.22)	0.43 (0.43)
(2)	1.26 (0.60)	1.04 (1.04)	0.87 (0.87)
Crystal(1)	0.14 (-0.49)	1.36 (-0.56)	0.55 (0.55)
(2)	0.37 (-0.04)	3.64 (1.28)	0.98 (0.98)

Two separate sets of analyses were carried out at different times as indicated by (1) or (2) after the malt type. The data in parenthesis show the change in concentration of the furanones from boiled malt extract (Table 1) to "beer".

UNPUBLISHED RESULTS

(All experimental methods are as described in the preceding published work)

Formation of furanones during kilning

A standard malt was produced, stewed for 4h at 70^0C and then dried at temperatures of either 95^0 or 110^0C. Samples were taken at intervals up to 22h and the furanone and colour of extracts measured. Both experiments showed the same trends with rate of change in direct relation to temperature. The results are shown for the 110^0C experiment (Fig A). Both loss of moisture and formation of colour followed the expected smooth curves. However, this was not the case for DMHF. Initially the compound was undetectable but began to show after 2h. The concentration rose to a peak at 3h, declined and then rose from 4h to a second, higher and broader peak around 10h. The same trends occurred with MHF but with a greater range of concentrations.

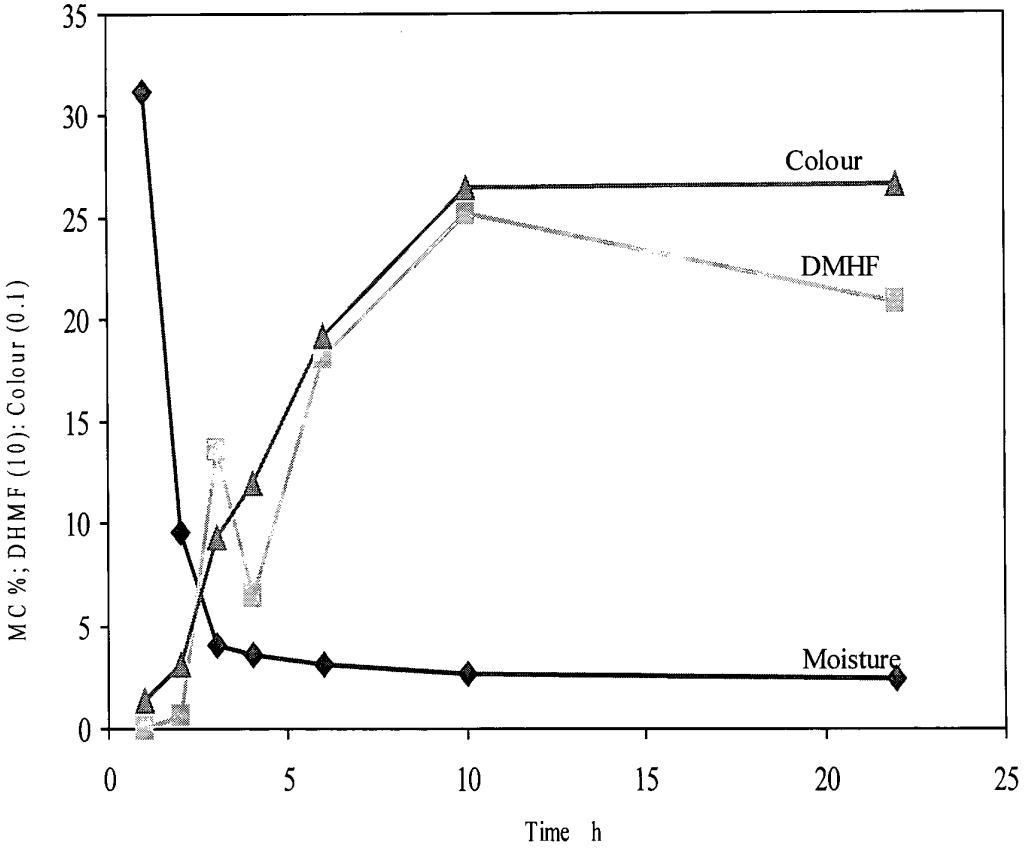
The simplest interpretation of these results is that furanones may be formed during malting by two separate mechanisms operating at different points in the process and are also being continuously converted to other compounds. The temperature of the grain bed only begins to rise towards that of the incoming air once moisture has been largely evaporated so that formation of furanones by the Maillard reaction would speed up as the grain becomes dry. As drying proceeds further, the Maillard reaction would slow down as the free water needed to allow reaction becomes limiting. After this stage the temperature of the grain rises and thermal degradation of carbohydrates would commence so resulting in a second wave of furanone production.

These results explain the fairly variable amounts of furanones found in commercial malt samples of similar colour which we have experienced as small variations in collection time around the point of reaching the required moisture could result in very different furanone concentrations. It also indicates that control of furanone concentration at a production level may be difficult. However, it does show that colour and flavour are not necessarily tightly connected in malts and this could be an area for development of new malt types with different relationships between colour and flavour from that currently accepted.

Effect of barley variety

Nine varieties of barley were malted under the standard conditions and kilned. The furanone contents of the extracts are shown in Table A. The level of DMHF ranged over six times reaching nearly three times the aroma threshold in one case. MHF was present in all cases but the range was just over two fold and the highest concentration was about 25% of the aroma threshold. MHF can be regarded as contributing to background flavour but in many cases DMHF would make a distinctive contribution. Further work is required to establish whether the variation in DMHF results from different levels of modification under the set conditions used or is intrinsic to the varieties in some other way. This is clearly an important commercial point when flavoured malts are to be produced.

Fig A Effect of Kilning at 110°C



Values for Moisture Content (MC) are given directly, values for DMHF have been multiplied by 10 and values for colour by 0.1

Table A Influence of Barley Variety

Variety	Wort Strength (Xs Degrees)	Colour (EBC)	DMHF (mg/l)	MHF (mg/l)
Halcyon	49.3	15	0.07	1.03
Gleam	43.6	16	0.15	1.18
Alexis	48.8	30	0.18	2.35
Chariot	49.6	32	0.19	2.28
Optic	49.0	28	0.23	1.46
Nevada	38.8	27	0.23	1.86
Regina	48.8	30	0.28	2.12
Esterel	38.8	32	0.44	1.96