PROJECT REPORT No. 242

FACTORS AFFECTING THE DEVELOPMENT OF ANTIOXIDANT PROPERTIES OF MALTS DURING THE MALTING AND ROASTING PROCESS

MARCH 2001

Price £3.90

PROJECT REPORT No. 242

FACTORS AFFECTING THE DEVELOPMENT OF ANTIOXIDANT PROPERTIES OF MALTS DURING THE MALTING AND ROASTING PROCESS

By

C S CHANDRA, L A BUGGEY, S PETERS, C CANN, C LIEGEOIS

Brewing Research International,
Lyttel Hall, Nutfield, Surrey RH1 4HY

This is the final report of a two-year project which started in October 1998. The work was funded by a grant of £60,000 from the Home-Grown Cereals Authority (project no. 1184).

CONTENTS

Abstract		**1**
Summary		**2**
The following parts of this report are available on request from HGCA		
I.	**Introduction**	**5**
II.	**Materials and Methods**	**12**
	- Malt extraction	**12**
	- Malt analyses	**12**
	- Antioxidant assays:	**12**
	a) Determination of total antioxidant activity by ABTS radical method	**12**
	b) Determination of antioxidant composition by HPLC	**13**
	- Polyphenol assay	**14**
	- Malting:	**14**
	a) Pale malts	**14**
	b) Roasted malts	**15**
	- Production of pilot brews	**15**
III.	**Results and Discussion**	**16**
	- Polyphenols and antioxidants in malts	**17**
	- Malts and antioxidants	**19**
	- Pale malts	**19**
	- Roasted malts	**25**
	- Crystal malts	**27**
	- Black malts	**30**
	- Malt antioxidant profiles	**32**
	- Reduction potential throughout brewing	**33**
V.	**References**	**38**

ABSTRACT

The total antioxidant activities (taa) of pale and speciality malts were determined. Varieties grown in the U.K. (1998 harvest) did not show differences in the taa when malted under similar conditions. Both the winter and the spring barleys showed similar antioxidant potential.

The processing methods employed during the malting of pale malts were shown to affect the antioxidant yield. Standard malt kilning regimes, with proteolytic and amylolytic rests, did not increase the taa. Under the constraints of the standard kilning regimes, higher modification with quicker drying after the 'break period' shows an increase in taa. The antioxidant profile of the barley was very similar to that of the malt, suggesting a major contribution from the natural antioxidants found in barley. The contribution from heat-induced malt antioxidants formed during kilning is minor.

In the case of coloured malts, the taa was found to correlate with colour up to a malt colour of 400°EBC. Above this limit, no further increase in total antioxidant activity was observed. A relationship between temperature and moisture levels during the roasting process and the production of antioxidants was noted. This association was tested for a low colour crystal malt (39°EBC) and a high colour crystal malt (220°EBC). The antioxidant yield dramatically increased at moisture levels below 5%, suggesting that a quicker drying period after the stewing stage would be more favourable for increasing taa. For black malts (1750°EBC), the higher grain temperatures employed during roasting resulted in a higher taa in the malt. Coloured malt antioxidant profile showed that both natural and heat induced antioxidants are present. However the level of antioxidants vary in different malt types.

Limiting the contact with oxygen during mashing and throughout brewing was achieved using a CO_2 blanket. This had the effect of reducing oxidation, thus maintaining antioxidant concentration and reduction potential in the final beer. The use of reducing agents in the brew also helped to maintain taa levels throughout the brewing process. A comparison of antioxidant profiles also showed that most of the antioxidants found in malt were also present in beer. Malt was also found to be the main source of antioxidants in beer.

SUMMARY

Oxidation reactions are a major problem for beer flavour stability. Antioxidants, which prevent or retard these reactions, are therefore crucial in extending the lifetime of a beer and enhancing flavour stability. Antioxidants are compounds that limit reactions caused by reactive oxidising species such as oxygen-containing free radicals, and act either by slowing the rate of reaction or preventing oxidative damage by removing the reactive oxygen species. Antioxidants may be added to the beer extraneously, but many brewers prefer a natural source by utilising the antioxidants inherent within the brewing raw materials. The use of high antioxidant malts not only provides protection in the final beer, but also guards against oxidative damage in the brewhouse. In addition, the use of high antioxidant malts could also contribute to the perception of wholesomeness that is associated with cereal-based foods and beverages.

The aim of this project, therefore, is to enable British maltsters to produce malts with a greater antioxidant potential. Better control over malt antioxidant content and the ability to increase this concentration in different products will add value to British malts. There are also possible health benefits and enhanced stability of both the raw materials and the final products. This could lead to an increase in the demand for high antioxidant malts made from home-grown cereals, particularly when export markets are taken into account.

Certain barley varieties are already considered to be particularly effective as a good source of antioxidants and are specified by some brewers. The methods of malting and kilning that can enhance antioxidant content are not fully understood, therefore these were key areas of investigation in this research.

Qualitative determination of antioxidants was achieved using high performance liquid chromatography (HPLC). This was coupled with a post-column peak height suppression chemiluminescence method, which allowed the detection of antioxidants. Determination of total antioxidant activity (taa) was achieved using a 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolourisation assay. This method employed the generation of a long-lived ABTS radical cation chromophore, and measured the relative abilities of antioxidants to quench the radical in relation to relevant antioxidant standards. The determination of polyphenols was achieved *via* a UV spectroscopic assay.

Spring and winter varieties from the 1998 U.K crop, were malted using standard conditions to produce pale malts. These malts showed no appreciable differences in taa.

The processing conditions used during malting were found to have a profound effect on the antioxidant yield. The germination and kilning regimes used had a particular effect on the taa of pale malts. Under the restriction of standard malting practices, the degree of modification is a very important factor and this was found to have a positive correlation with taa. Malting trials for highly modified malts showed an increase in taa at the end of kilning of 35-40%, compared with low modified and standard malt. The high modification levels favoured the development of Maillard reaction products, some of which had antioxidant activity and hence increased the taa. The drying regime used during the kilning of pale malt needs to be considered when attempting to improve the taa.

The use of amylolytic (2hrs at 65°C) and proteolytic (2hrs at 35°C) breaks were investigated and were shown to have no appreciable effect on the taa. Similarly, the use of quicker drying periods after the breaks did not appear to alter the taa. This was thought to be because the regimes utilised resulted in no significant differences in the relative humidity, and at no time was the relative humidity in the kiln low enough to yield a grain moisture content of below 5%. Such kilning regimes were found to have no significant contribution to the taa (see results for crystal malts).

Comparisons were made between the different antioxidant species present in barley and in pale malts prepared from the barley, using the HPLC method described. This showed that most of the antioxidant peaks present in the barley were also present in the malt samples. It was therefore concluded that the natural antioxidants present in pale malts, e.g. the polyphenols, provided a large contribution to antioxidant activity. One of the peaks found in barley was not present in the malt however, which suggested that some effect of the processing resulted in the loss of this particular compound. One peak present in the malt was not present in the original barley. This implied that this compound originated during processing. The taa was found to be higher in the malts than in the barley, which was thought to be due to increases in the levels of heat-produced antioxidants.

Taa levels were found to rise with an increase in the colour up to 400°EBC. This increase was observed regardless of whether the malts were produced from single varieties or from different

varieties. An increase in colour beyond 400°C resulted in no further effect on antioxidant yield however. The levels of Maillard reaction products increase with temperature, therefore a relatively higher percentage of these compounds were believed to be present in the coloured malts. As the kilning temperature increased and malts with a colour of over 400°C were produced, the production of further coloured compounds was not accompanied by an enhancement in the taa levels. The polyphenol levels were also observed to rise as colour increased.

The temperature, moisture and taa profiles produced during roasting of a low colour (39°EBC) and a high colour (220°EBC) crystal malt were investigated. It was found that the taa increased once the moisture levels had decreased to below 5%. The taa levels were independent of temperature at high moisture levels, but dependent upon temperature at low moisture levels. This implied that a more rapid drying period after the stewing stage would be more favourable for increasing the taa of a malt. For the production of black malt (1750°EBC), higher grain temperature was required during roasting, which leads to higher taa levels in the malt.

The effects of reducing contact with oxygen in the brewhouse and the addition of reducing agents were investigated, to compare the effects of natural antioxidants in malt with those added during brewing. Beers brewed under a CO_2 blanket showed higher taa levels throughout most of the brewing process than those of a control brew. Brews produced with the addition of known reducing agents such as potassium metabisulphite and ascorbic acid showed higher antioxidant activity in the brewhouse prior to boiling. Little taa was measured in the last runnings for all the brews except for the potassium metabisulphite brew, where the taa was observed to be higher than that of the control brew. The last runnings stage of brewing might therefore be prone to oxidation, however this could feasibly be counteracted by the use of potassium metabisulphite. The HPLC antioxidant profile for the control beer was similar to that of the malt, which implied that the most significant contribution to the antioxidant activity in beer was from the malt.

INTRODUCTION

The deterioration in the flavour of stored beer is influenced by both enzymatic and non-enzymatic oxidation during the malting and brewing process, and by longer-term oxidation processes during beer storage. Delaying flavour staling is one of the greatest challenges for brewers, however it can be partially achieved by choosing suitable raw materials, particularly malt[1].

The development of oxidised flavour in beer has been associated with the formation of volatile carbonyl compounds. Of these, *trans*-2-nonenal is thought to be one of the major causes of stale flavour in aged beer. Different mechanisms of *trans*-2-nonenal formation have been proposed: Strecker degradation of amino acids, melanoidin mediated oxidation of higher alcohols, oxidative degradation of isohumulones, aldol condensation of short chain aldehydes, and enzymatic or non-enzymatic oxidation of fatty acids. It is generally accepted that the enzymatic degradation of lipids during malting and mashing, followed by autooxidation of intermediates in the brewhouse and in the final packaging, is the main mechanism of beer oxidation[1].

Antioxidants are compounds capable of delaying, retarding or preventing oxidation processes, and are therefore thought to have a significant effect in malting and brewing as inhibitors of oxidative damage.

Antioxidants such as sulphites or ascorbic acid can be added during the brewing process[2]. In recent years however, there has been a general trend towards minimising the use of added 'artificial' antioxidants in beer production. Also, the effectiveness of some of these antioxidant compounds is in doubt, particularly as the oxidising potential is not removed from solution in the way that oxidised polyphenolic material, for example, would precipitate. Furthermore, the use of additives in brewing is becoming increasingly restricted and is not permitted in some countries[3].

Consequently, attention is now increasingly directed towards the brewing process and the properties of the raw materials. For example, protecting the endogenous antioxidants present in the barley during malting could increase the reduction potential of the brew, thus helping to inhibit oxidative processes detrimental to flavour stability and thereby avoiding the use of exogenous antioxidant compounds[4].

Reducing agents that are known to be important in malting and brewing include[5].

1. Melanoidins and reductones.

   These are formed in the malt by the thermal degradation of sugars and by Maillard (non-enzymatic browning) reactions that occur during the heating of reducing sugars with amino acids. Maillard reaction products act as scavengers for reactive oxygen species such as superoxide, peroxide and hydroxyl radicals. They are known to be highly efficient antioxidants in the production and storage of food.

2. Phenolic substances, including phenolic acids and various polyphenol compounds.

   These compounds also act as radical scavengers, and are highly effective in inhibiting non-enzymatic lipid peroxidation. Both malt and hops contribute these substances to the wort and beer, however the majority originates from the malt. About 70-80% of the polyphenols in wort are malt-derived and 20-30% are from the hops. It is likely that the polyphenols from these two sources are different, and the specific properties are highly dependent upon the degree of polymerisation. Lower molecular weight polyphenols are, in particular, excellent antioxidants. With increasing molecular weight, the reducing power and the solubility of the polyphenols decreases.

   A well-established property of certain polyphenols is their propensity to form stable, insoluble complexes with proteins, which precipitate during brewing. Higher molecular weight polyphenols have an increased tendency to form these insoluble complexes, and at any one time the reducing power of these compounds depends upon their equilibrium with the proteins present.

3. Thiol compounds.

   These are barley-derived, however their concentrations are highly variety-dependent. Oxidation results in the loss of free thiols by the formation of disulphide bridges in proteins, resulting in agglomeration and a decrease in reducing power.

4. Non-fermentable reducing sugars.

   These are inherent in the barley, and also formed during germination from the starch.

5. Carotenoid compounds and vitamins.

   These are present in variable, minor quantities in barley. Whilst they can be effective antioxidants, they are often lost during kilning due to the high temperatures involved.

Of these, melanoidins and polyphenols are the most significant sources of natural antioxidants (Figure 1).

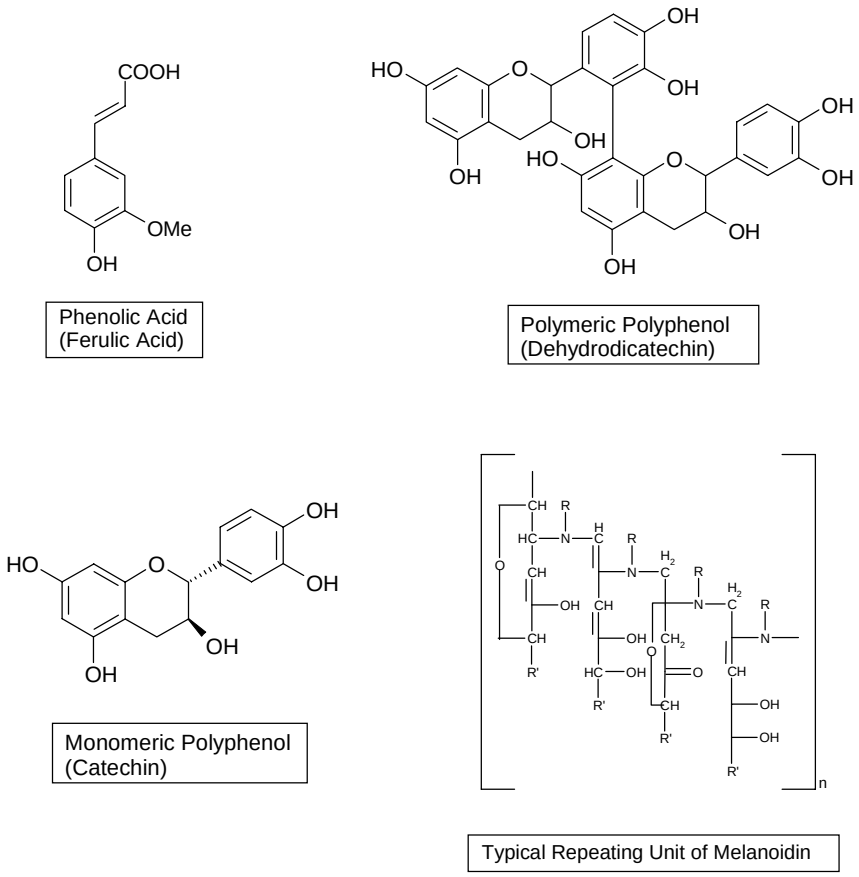


Figure 1: Examples of important reducing species.

The net contribution from the malt to the redox potential and its status during the course of the brewing and in the final beer is dependent upon the interactions of the individual reducing agents with each other and various other factors. In some situations reducing agents may become prooxidant. Thus the oxidation/reduction state is very important in deterioration, and an elevation in the reducing activity is favourable for good flavour stability.

Malt

Malt has pro-oxidant activity from species including oxidases and oxidised lipids, and antioxidant activity from species such as polyphenols and Maillard reaction products[1]. The contribution of malt to the oxidation/reduction status throughout brewing and in the beer is therefore very important.

Whilst lipids are essential for good fermentation, they are detrimental to the maintenance of flavour. Their negative effect on flavour is due to the metabolism of lipid precursors into flavour active compounds. One such route is the lipoxygenase pathway. This involves the release of fatty acids *via* the hydrolytic action of lipases and the subsequent lipoxygenase-catalysed oxidation of the unsaturated fatty acids to produce hydroperoxides that, under the action of further enzymes, form staling aldehydes[8]. Phenolic compounds have an inhibiting effect on the two lipoxygenases found in barley (LOX1 and LOX2), and also on the autooxidation pathway[6].

Malt contains natural antioxidants originating either from:

i) Barley: This is mainly due to polyphenols and varies between varieties. The selection of barley variety is therefore the first stage in maximising the reduction potential.

ii) Malting process (mainly Maillard reaction products): The first two stages of the malting process are steeping and germination. Phenolic acids bound to the cellular walls of the germinated barley have significant potential as antioxidants, indeed it has been reported that the antioxidant activity of these bound compounds is approximately twice that of the activity due to the free phenolic compounds[6]. Many compounds that are important to the reducing power of the final malt are produced during endosperm modification, and the degree of modification may have the greatest influence on malt antioxidants. The formation of important water-soluble polyphenols (some of which are however lost through leaching of the grain during steeping), Maillard reaction precursors and non-fermentable reducing sugars occurs during these stages. Highly modified malt contains more polyphenols, anthocyanogens, catechins, tannoids and other reducing substances then less modified malts[10,11,12,13,14].

The kilning stage of malt production also has a very significant effect on antioxidant levels and thus reduction potential. Melanoidins and reductones are formed by the thermal degradation of sugars and by Maillard reactions (non-enzymatic browning reactions) during the heating of reducing sugars with amino acids (figure 2). The high temperatures in kilning also have the effect of deactivating enzymes present in the barley. Some of these enzymes have an oxidising effect, therefore this will further benefit the reduction potential, however reducing enzymes are also deactivated by the heat.

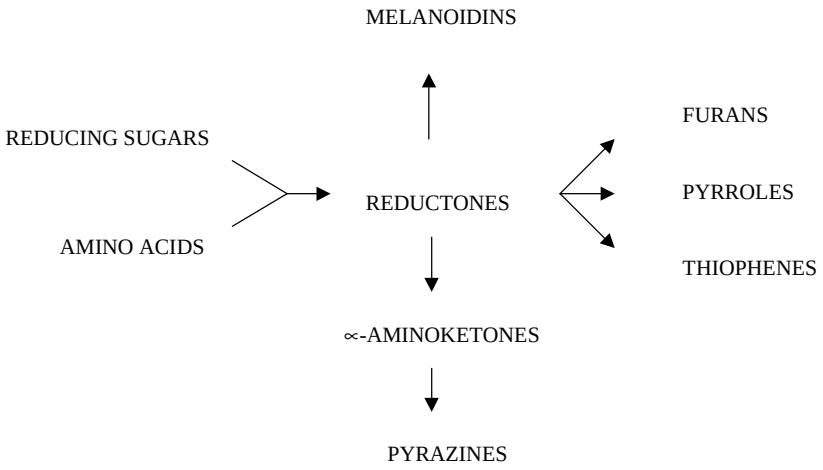


Figure 2: Maillard reaction scheme.

Roasted malts

Coloured and roasted malts have traditionally been used by the brewer as a source of natural colours and flavours. Their positive effect on beer flavour stability has long been noted. The reduction potential of the brew can be increased through the use of coloured malts. Coloured malt production involves higher temperatures than those encountered during the kilning of pale malts, and it is these elevated temperatures which enhance the formation of colour and flavour compounds. The increased yield of Maillard reaction products at higher temperatures could also cause the increase in the reduction potential of the malt and hence in the brew[15,16].

Brewing

Oxidation during the brewing process has an important influence on a number of beer parameters such as wort separation, colour, polyphenol content, beer flavour and stability. The antioxidant content during the various stages of brewing is therefore highly important. The chemistry of brewing is highly complex, and the overall reduction potential of the brewing liquor throughout the different stages is dependent upon a number of variables. The antioxidant content of the malt used will obviously have a major effect. The contribution from the malt to the wort depends upon the amount of antioxidant species extracted during mashing, which is obviously dependent upon

their solubility. Maximising the antioxidant content of the malt and, in particular, the level of soluble antioxidant species could therefore increase the reduction potential of the brewing liquor throughout brewing.

The antioxidant profile of the wort does not remain constant during brewing. The composition and concentration of antioxidant species constantly changes due to various chemical and physical processes. Maximising the taa whilst also reducing the factors that cause a decrease in reducing power during brewing would serve to increase the reduction potential of the final beer.

The constituents of beer have a tendency to become oxidised during brewing. The greatest changes occur during mashing. The polyphenols, melanoidins and the reduced thiol groups of proteins are all readily oxidised during mashing. Lipid oxidation, which has a major negative effect on beer flavour stability, is also a problem at this stage. This factor is related to the husk integrity and the avoidance of acrospire damage, due to the large proportion of fatty acids contained within this part of the grain. The degree of acrospire damage in the malt therefore directly influences the leaching of lipids into the brew during mashing[20].
Oxygen absorbed by mash or by the boiling wort decreases the reducing power, and the avoidance of oxygen pick-up at these stages could help prevent oxidation of the lipids and the polyphenols. Polyphenols have a pronounced tendency to form complexes with proteins in the brewing liquor, and at any one time the availability of the polyphenol antioxidants is dependent upon this equilibrium process. Oxidative polymerisation of polyphenols, and the increased propensity of these larger, polymerised species to form such complexes, results in the formation of insoluble species. These are lost due to precipitation and filtration, which can lead to a decrease in the reducing power of the wort. Conditions during mashing can also result in the breakdown of dimeric polyphenols, releasing more soluble, smaller polyphenols into the wort. The overall reducing power in the wort is therefore dependent upon the balance of these effects[5].

Beer

The oxidation/reduction state of the final beer will have a very important role in the flavour stability. This depends upon the net contribution of the various compounds in the beer, which will be directly influenced by the redox reactions and the reduction potential throughout the malting and brewing stages. The reducing power of the final beer is mainly due the presence of melanoidin compounds, polymerised polyphenols and unfermented reducing sugars. Oxygen in

the headspace of the packaging is consumed during storage, and the deterioration is greater when there is more air in the head space[18]. The reduction potential of the stored beer depends upon the balance between the various processes and reactions that occur over time, however there is generally a gradual decrease as the oxygen in the headspace and dissolved within the beer is consumed by reactions with reducing species. The greater the initial reduction potential of the beer on packaging, and the less the promoters of oxidation that are present, the more stable the beer should be.

MATERIALS AND METHODS

Malt extraction

Malt samples (7-8g) were frozen in liquid nitrogen prior to milling in a coffee grinder for 30 seconds. 5g of the milled malt was added to 40ml of acetate buffer (pH 5.4) and ground vigorously at 0°C with a pestle and mortar for 1 minute. The extract was centrifuged at 5,000g for 5 minutes, before filtration at 0°C through a Whatman No. 1 filter. The extract was then rapidly frozen in liquid nitrogen, before being stored at -18°C until required.

Malt analyses

The moisture content of the malts was estimated by the loss in mass on drying under specified conditions (IOB Methods of analysis, 1997:2.2 and 3.2). The determination of Hot Water Extract (HWE) of ale, lager and distilling malt used a constant temperature infusion mashing procedure of a coarsely ground sample (0.7mm) (IOB methods of analysis, 1997: 2.3). The determination of HWE of coloured malt was based on a mashing procedure and mixed grist (IOB Methods of analysis, 1997: 3.3). The colour was measured in EBC units using a colour comparator (IOB Methods of analysis, 2.5 and 3.4).

Antioxidant assays:

a) Determination of taa using the ABTS radical assay.

The basis of the method was the generation of a long-lived ABTS radical cation chromophore and measurement of the relative abilities of antioxidants in a sample to quench the radical in comparison to catechin or other relevant antioxidant standard.

A chromophore solution (3.125ml) containing the ABTS radical cation was added to 25µl of sample and the absorbance (abs.) at 734nm recorded up to 14 minutes after initial mixing, against a solvent blank. All determinations were carried out in triplicate, at 25°C.

The malt extracts were diluted with acetate buffer such that, after the introduction of a 25µl aliquot into the assay, they produced between 15-80% inhibition of the blank absorbance. The percentage inhibition of absorbance at 734nm was calculated using the following equation:

% Inhibition = 100 x (abs. (blank) – abs. (sample or standard)) / abs. (blank)

The taa was calculated as a function of the concentration of catechin standard reference data, and as a function of the final concentration for the malt extract. The value was expressed in mM/catechin equivalence units (defined as the concentration of catechin with equivalent activity to a 1g/l malt extract under investigation) using the following equation:

taa (mM/catechin equivalence) = (% inhibition $_{\text{sample of 1g/l}}$) / (% inhibition $_{\text{standard}}$) x C $_{\text{standard}}$

NB *abs. was measured as the absorbance at 734nm after 14 minutes*
$C_{standard}$ was the concentration of the standard in mM

b) Determination of antioxidant composition by HPLC.

This chromatographic method gave a measurement of some of the individual antioxidant species contained within a given sample and allowed the antioxidant potential of each individual component within a complex mixture to be estimated by a post-column chemiluminescence detection method.

The chemiluminescent assay was carried out using a Merck LaChrom HPLC coupled to a Lumac Biocounter luminometer. A mixture of hydrogen peroxide and luminol was introduced into the luminometer and the degree of chemiluminescence was measured. Any antioxidants present within a sample reduced the degree of luminescence. The assay measured the degree of suppression of various antioxidant compounds.

A buffer system was used to carry the reagents for generation of the chemiluminescence. This was prepared using tris (hydroxymethyl) aminomethane, sodium dihydrogen orthophosphate and diethylenetriamine pentaacetic acid. The pH of this buffer was adjusted to give a pH of 7.75 using 10M sodium hydroxide. The buffer was separated into two equal aliquots. Hydrogen peroxide solution was added to the first aliquot (***Solvent 1***), and luminol and peroxidase were added to the second (***Solvent 2***). The two solvent systems were mixed *via* a post-column HPLC reaction pump and the chemiluminescence generated was measured using a luminometer.

Filtered samples were injected using a Merck LaChrom L-7200 autosampler on to a Phenomenex Primesphere C18 HC column. The eluted antioxidants were detected using a Merck LaChrom L-7450 diode array detector set at 254, 280, 320, 430nm, a Merck LaChrom L-7450 fluorescence detector set at an excitation wavelength of 270nm and an emission wavelength of 323nm, and a Lumac/3M biocounter M2010.

Polyphenol assay

A CMC-EDTA solution was prepared using carboxymethyl cellulose (CMC) containing 2% ethylenediamine tetraacteic acid (EDTA). 0.8ml of this reagent and 50µl of green ammonium iron citrate solution (containing 16% iron) were mixed with the sample or a blank (1ml) in a cuvette.. 50µl of concentrated ammonia solution was then added, and the mixture was left at room temperature for 10 minutes before reading the absorbance at 600nm.

Malting:

Barley cultivars were obtained from Crisps Maltings UK.

a) Pale malts

4 spring (Chariot, Alexis, Century and Optic) and 3 winter (Pearl, Fanfare and Halcyon) barleys (table 1) were malted in a 50kg capacity pilot malting under standard conditions: three steep schedule, ~46 hours in total, at 15-18°C, followed by four days germination at 15-18°C. A standard malting kilning regime took place in a realistic model of a commercial kiln.

Two extra malts were produced using the Optic barley. One had low modification (two steep schedule, ~44 hours, at 15-18°C, followed by four days germination at 15-18°C), the other had high modification (two steep schedule, ~50 hours, at 16-19°C with the addition of 0.25ppm giberellic acid at cast, followed by a four day germination at 15-18°C). The kilning was programmed to dry the malts more rapidly than is usual. The moisture levels reduced very rapidly, causing a faster increase in air-off temperature.

Two extra white malts were produced using the Chariot barley. One incorporated a 2hr amylolytic stand (17 hours into kilning the air-on temperature was held at 75°C for 2 hours with 100% recirculation and 40% fan speed), and the other incorporated a 2hr proteolytic stand (4 hours into kilning the air-on temperature was held at 60°C for 2 hours with 100% recirculation and 40% fan speed).

b) Roasted malts

Four coloured products were produced using the same Fanfare barley (table 2). Green malts were prepared by steeping at 16°C followed by four days germination at 18°C in an 8x1 kg capacity micro-malting pilot. Chit malts were obtained after only one day of germination. Roasting was carried out in a 2kg pilot roaster. Two crystal malts (39°EBC and 220°EBC) were produced from the same green malt stewed in the roaster for 40 minutes. During this time, the temperature was raised from 20°C to 102°C whilst minimising moisture loss. At the end of stewing, the roaster flap was opened to allow drying. Heat input was set to allow evaporative cooling. Drying was continued until the temperature reached 127°C and 153°C respectively, producing two different coloured malt products.

Black malts (1750°EBC) were produced from the dried chit malt. Roasting was carried out by raising the product temperature to 228°C.

Production of pilot brews

Pilot brewing was carried out using the BRI 1hl plant. Four batches of 11°P lager were produced, using 13.5kg Optic malt and 1.6kg Cara malt. The four brews consisted of a control lager brewed using Lyttel Hall lager recipe, a batch brewed under a carbon dioxide blanket (to minimise the presence of oxygen), a brew with ascorbic acid added to the mash and a brew with potassium metabisulphite added during milling.

RESULTS AND DISCUSION

Malt contains several types of antioxidants, which can be classified as:

1. Natural, including polyphenols and reduced proteins of barley
2. Heat induced e.g. melanoidins

These malt antioxidants combine to reduce or delay the action of reactive oxygen species, which can cause oxidative damage and lead to undesirable oxidised flavours in malted products. The effectiveness of these antioxidants largely depends upon the specific reactive oxygen species present.

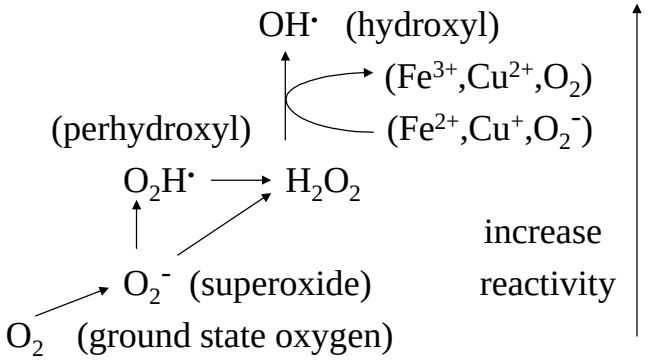


Figure 3: Formation of reactive oxygen species

Figure 3 illustrates the formation of reactive oxygen species. Oxygen in its ground state (O_2) is relatively unreactive, however it can become activated to a higher, more reactive, energy state. Addition of an electron produces a superoxide radical O_2^-, which can then become converted to the more reactive perhydroxyl radical ($O_2H^{\cdot}$). Hydrogen peroxide can be formed from both superoxide or perhydroxyl radicals. Transition metal ions such as Fe^{n+} and Cu^{n+} can catalyse hydrogen peroxide breakdown, generating hydroxyl radicals, which are the most reactive of the oxygen species shown.

Three antioxidant assays were selected to study the effect of malt antioxidants on different reactive oxygen species. These were:

1. Superoxide radical assay – to measure the effectiveness of malt antioxidants against the superoxide radical
2. ABTS radical assay – to measures effectiveness of malt antioxidants against $H^{\cdot}$-donating species.
3. HPLC post column chemiluminescence assay – to measures the profile of malt antioxidants which are effective against $H^{\cdot}$-donating species.

Figure 4: Comparison of antioxidant assays (HPLC and ABTS) for crystal malt

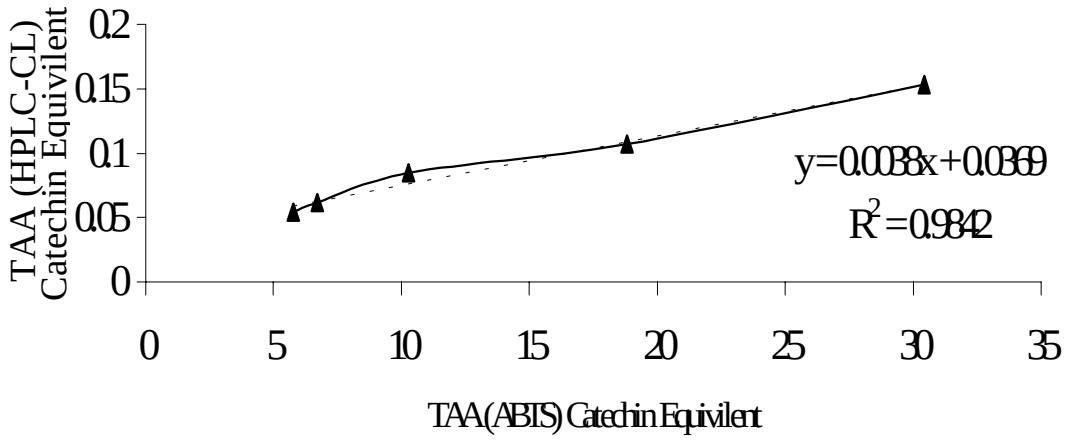


Figure 4, which shows the result of an analysis of crystal malt, illustrates that there is a good correlation between the antioxidant activities measured by methods 2 and 3.

Polyphenols and antioxidants in malts

Polyphenols are a major source of antioxidants in malts, which account for much of the antioxidant activity in beer. Many of these compounds scavenge oxygen-containing free radicals, including superoxide, hydroxyl and peroxyl radicals.

Flavonoid polyphenols account for 70-80% of polyphenols in barley and include (+)-catechin, ferulic acid, (-)-epicatechin, (+)-gallocatechin, coumaric and vanillic acids. This group of antioxidants plays an important role in reducing the damaging effect of oxidation. It is possible to identify some of these compounds *via* HPLC post column chemiluminescence assay. A qualitative examination of different malt types using this assay showed that the number of

polyphenolic flavonoid peaks identified in each malt type was similar, whereas the overall number of antioxidant peaks increases as the malt colour increases (Table 1). This implies that the concentration of polyphenol antioxidant species is relatively constant in these malts, irrespective of colour and hence kilning temperatures. The increase in antioxidants that is observed for higher colour malts must, therefore, be due to other species i.e. Maillard reaction products. This is understandable as these compounds are formed at higher temperatures, and they are known to be responsible for both colour and antioxidant ability. Therefore, as colour increases due to higher kilning temperatures, so does the number of Maillard compounds in the malt, which give rise to a greater number of antioxidant peaks in the HPLC assay.

Table 1: HPLC post column chemiluminescence analysis of various malt types

Sample	Total number of antioxidant peaks	Number of polyphenolic flavonoids
Lager	7	4
Ale	7	4
Amber	7	3
Cara	6	3
Crystal	7	3
Chocolate	13	4
Black	17	3
Roasted	15	3

Table 2: Total polyphenols and antioxidant polyphenols for various malts

Sample	Total Polyphenol levels (mg/kg)	Antioxidant polyphenol mg/kg (%)
Lager	8.63	1.3 (15)
Ale	8.17	1.3 (15)
Amber	11.76	0.1 (0.5)
Cara	23.82	1.3 (5)
Crystal	46.26	4.6 (10)
Chocolate	157.7	6.5 (4)
Black	396.95	20.6 (5)
Roasted	366.41	27.9 (7)

Table 2 shows the total polyphenol levels for various malts, and the percentages of these compounds that have antioxidant capabilites. The levels of polyphenols increase dramatically

with colour, however it can be seen that relatively few of these species are actually antioxidant in nature.

Malts and antioxidants

The effect of variety and processing conditions on total antioxidant activity (taa) was investigated in both pale and coloured malts. To investigate which parameters had the greatest effect on the total antioxidant yield of malt, a series of experiments were carried out using the BRI pilot malting and roasting facilities. All these experiments were designed to mimic the conditions that are commonly used in the production of each malt type. For pale malts, the degree of modification, varietal effects and the kilning regimes were studied as these are the important processing parameters, whereas for coloured malts the roasting conditions were the focus of investigations.

Pale malts

Table 3 shows the standard analysis of pale malts produced by the same malting and kilning regime.

Table 3: Pale malt analyses

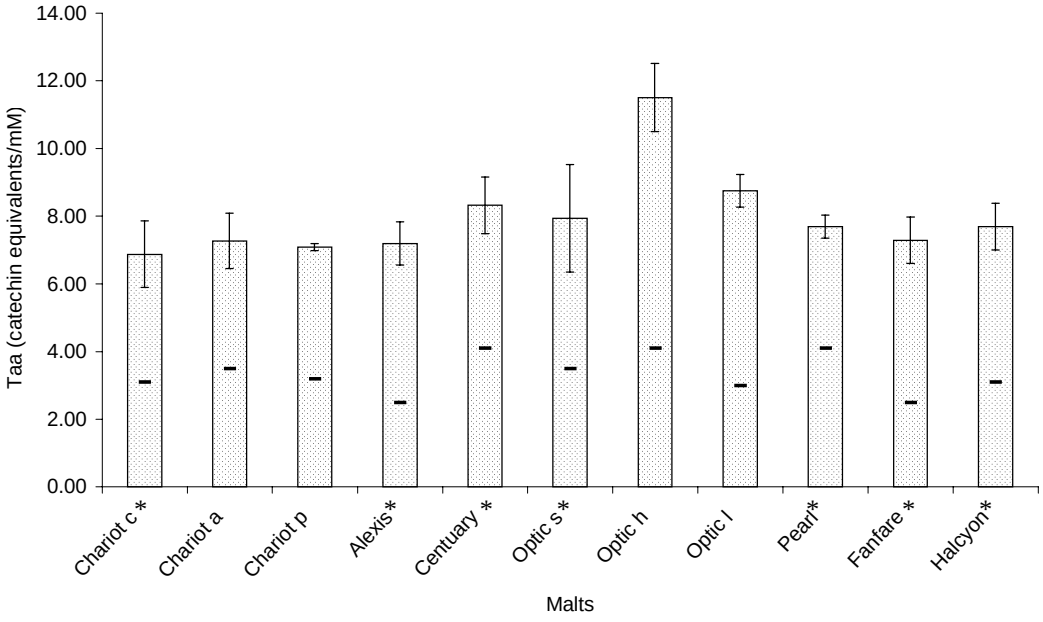
Pale malt from different barley varieties	**$HWE_{0.7}$ (L°/kg)**	**Moisture (%)**	**Colour (°EBC)**
Chariot[1] (c)	315	4.0	3.1
Chariot[1] (a)	316	3.4	3.5
Chariot[1] (p)	315	4.3	3.2
Alexis[1]	306	6.0	2.5
Century[1]	306	3.7	4.1
Optic[1] (s)	310	5.2	3.5
Optic[1] (h)	316	3.9	4.1
Optic[1] (l)	313	4.0	3.0
Pearl[2]	312	3.7	4.1
Fanfare[2]	316	6.0	2.5
Halcyon[3]	298	4.4	3.1

[1]spring or [2]winter barley; (c) control kilning regime, (a) kilning with 2 hour amylolytic stand, (p) kilning with 2 hour proteolytic stand, (s) control malting and kilning regime, (h) malt with high modification and special kilning, (l) malt with low modification and special kilning.

There was very little difference in the traditional malt analysis of these samples.

Figure 5 shows taa for the pale malts produced from barley varieties Chariot*, Alexis*, Centuary*, Optic*, Pearl*, Fanfare* and Halcyon* grown in the U.K. (1998 harvest). From this, it can be seen that most malts* did not show large differences in the taa when malted using standard conditions. Both the winter and the spring barleys showed similar antioxidant potential. The affect that the harvest year had on taa was not investigated. The influence of modified malting regimes can also be seen in figure 5. The effect of malting a Chariot barley incorporating either an amylolytic (2hrs at 60°C, Chariot a) or a proteolytic (2hrs at 35°C, Chariot p) break was investigated. These malts showed little difference compared with the Chariot barley malted using standard malting conditions (Chariot c). However high degree of modification and kilning strategy involving quick removal of water by utilising 100% fan speed seem to increase the antioxidant activity of Optic (h) malt without impacting on colour.

Figure 5: Total antioxidant activity of pale malts.



(horizontal mark = colour/°EBC)

* Malted using standard conditions.

The affect of modification levels on the taa is clearly illustrated in Figures 5 and 6 for Optic malt. Under the restriction of standard malting practices, the degree of modification is a very important factor and was found to influence the taa.

Figure 6: Comparison between the development of total antioxidant activity for high modified malt and low modified malt during kilning.

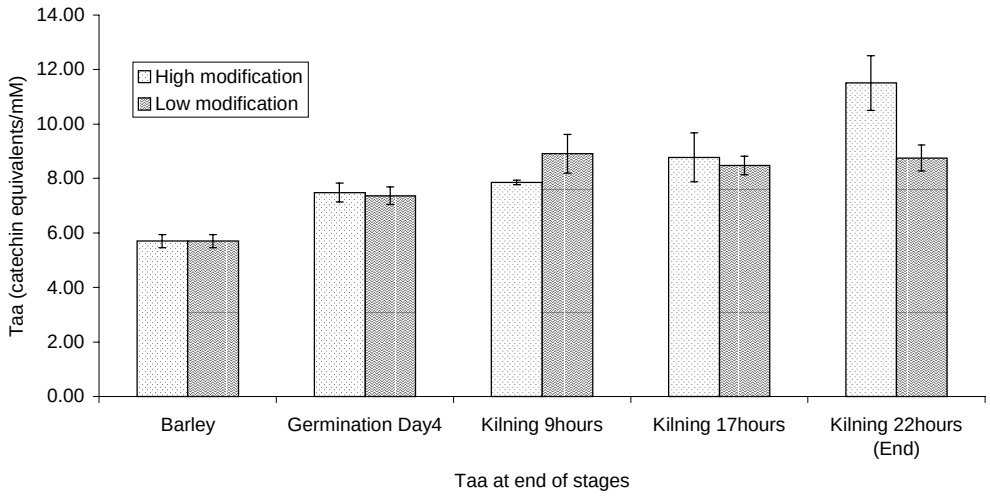
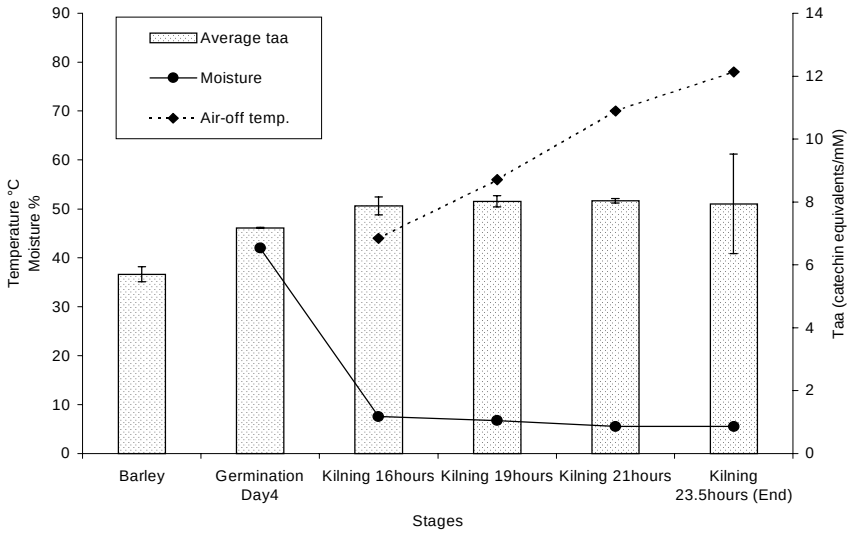


Figure 6 shows that during the malting, and throughout most of the kilning, the taa of low modified malt (Optic l) and high modified malt (Optic h) was similar, until the last stage of kilning when there was a pronounced increase in the taa of the highly modified malt. The highly modified Optic malt showed a 35-40% increase in the taa at the end of kilning compared with the low modified and a standard malt (figure 5, Optic s). In addition to the high modification, the increase in taa for Optic malt (h) can also be attributed to the kilning strategy used. Kilning for this malt involved rapid drying of the grain bed after the “break point” period to aid quick removal of moisture. Therefore, due to combination of high modification and kilning strategy Optic malt produced high taa.

Figure 7: Development of total antioxidant activity with changes in moisture and temperature levels during kilning of a lager malt.



Since amino acids and sugars are produced during modification, and these are then converted to Maillard reaction products during the later kilning would explain why combination of both modification and kilning regimes are important for antioxidant yield. Figures 8 & 9 also show that the taa in both of the malts were higher than in the barley. This is thought to be due to increases in polyphenols, tannoids, and thiols that originated during endosperm modification, as well as the formation of melanoidins and reductones due to Maillard reactions during kilning.

The kilning regimes used in pale malt production needed to be altered drastically to improve the taa of pale malts. Figures 7, 8 and 9 show the taa, temperature and moisture profiles at key stages during malting for a control malt, a low modified malt and a highly modified malt respectively. Again, it can be seen that in each of the cases, the taa increased from the barley to the malt. The taa had risen by approximately 15% by day 4 of germination. This might be attributable to changes in endosperm modification. The control and the low modified malts showed similar taa profiles during kilning.

Figure 8: Development of total antioxidant activity with changes in moisture and temperature levels during kilning of low modified malt.

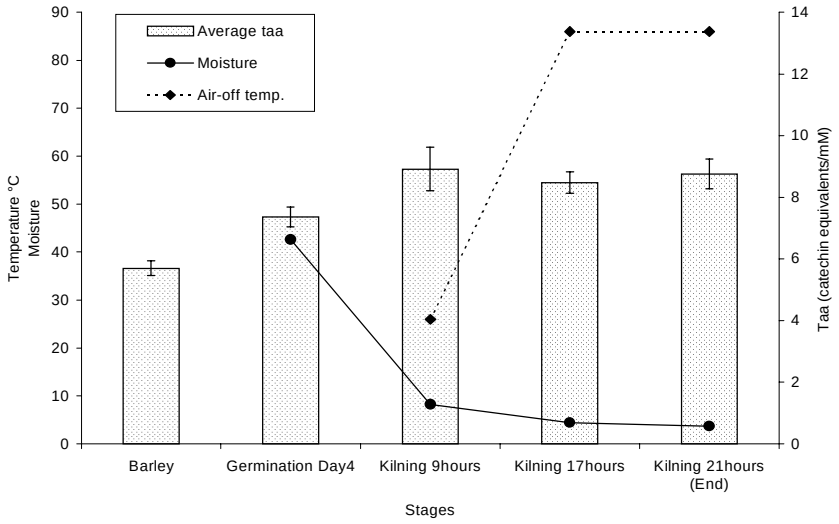
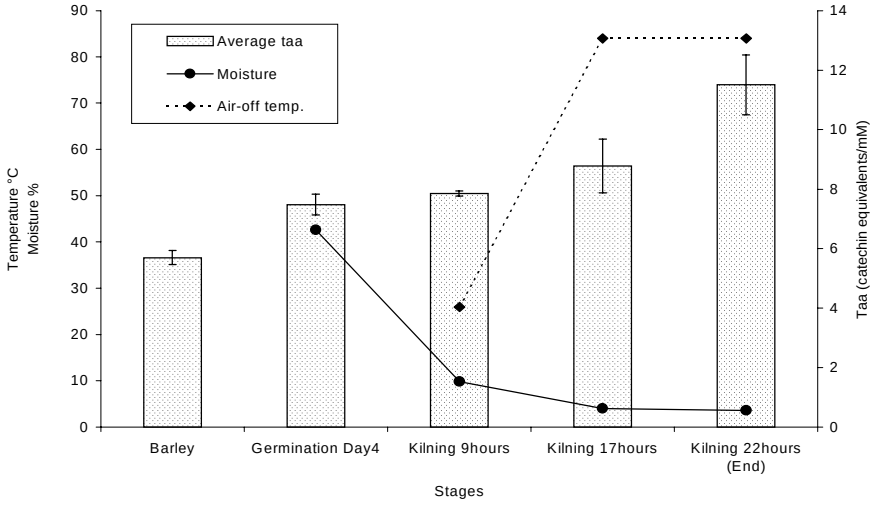


Figure 9: Development of total antioxidant activity with changes in moisture and temperature levels during kilning of high modified malt.



The control malt (figure 7) was subjected to a standard kilning regime with a slow increase in air-on temperature after the break-point, at which point the moisture levels began to decrease. The results for this malt can be compared to those where a rapid increase in air-on temperature and fan speed was used to increase the rate of drying after the break-point (figure 8). The taa profiles were similar, implying that the regimes used didn't result in an appreciable difference in the relative humidity (RH) profile of the kiln and at no time RH of the kiln was the low enough to give moisture content below 5%. The results for the crystal malts (see later) showed that above 5% moisture level there was no significant contribution to the taa in the malt. The highly modified malt (figure 9) showed an increase in the taa. This was thought to be due to differences in the endosperm structure and the availability of sugars and amino acids.

Figure 10: HPLC antioxidant profile of optic barley and optic malt.

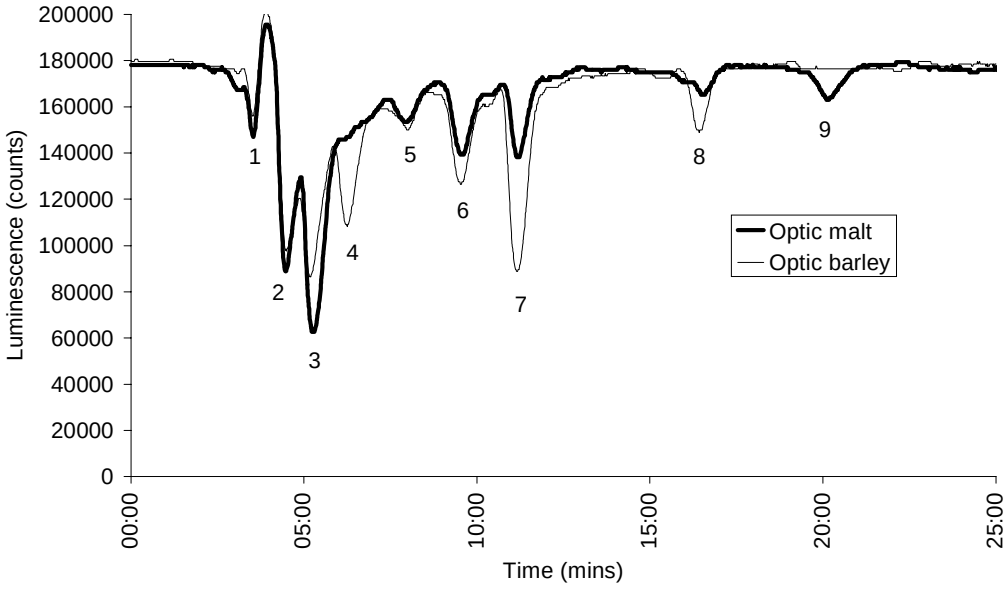


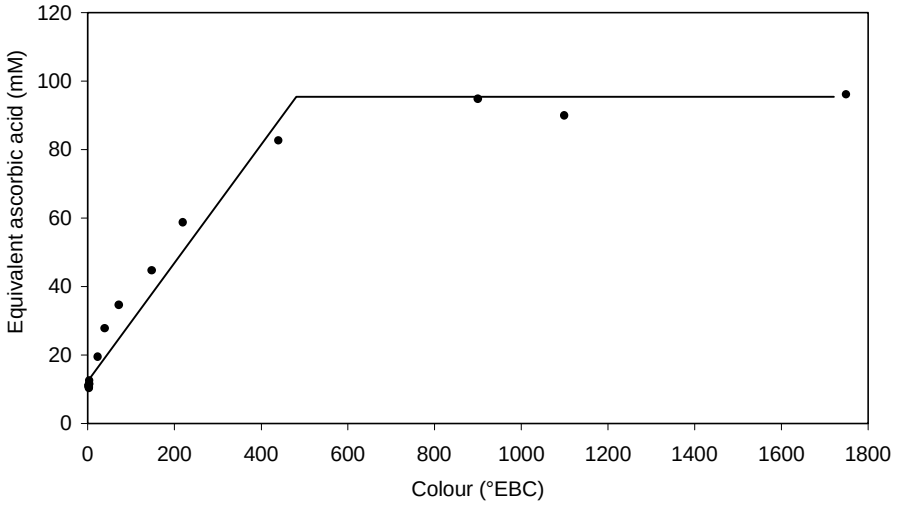
Figure 10 shows a comparison between the HPLC antioxidant profile of optic barley, and the malt prepared from it. The majority of the barley peaks were also observed in the malt, which implies that the natural antioxidants present in barley provided a large contribution to the antioxidant activity of malt. The peak labelled number 4 in the figure was found in the barley but was not present in the malt. This suggests that this particular compound modified during the malting process. Peak 9, however, was found in the malt but not in the original barley, which implied that

the compound had originated during processing. Changes in the relative amount of each compound are also occurring especially during the malting process due to differences in the phenolic compounds.

Roasted malts

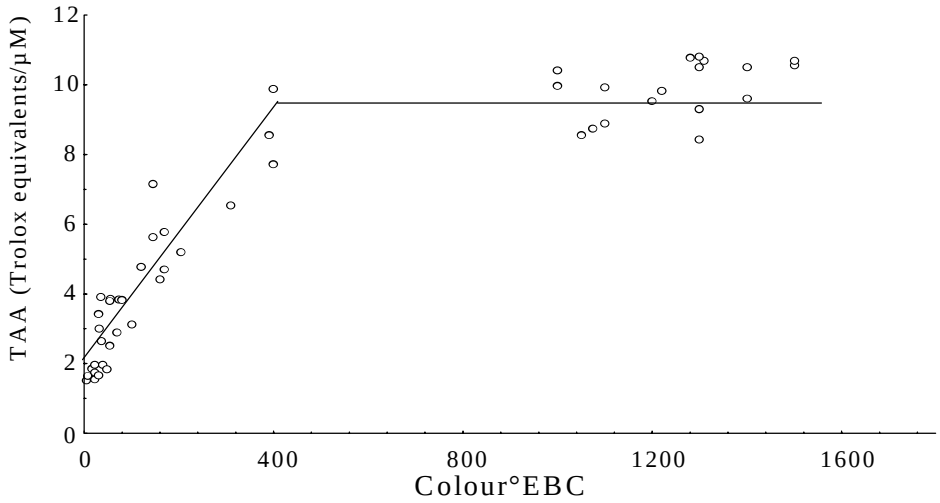
A study of total antioxidant activity of a number of commercially available coloured malts showed that the taa levels increased with the colour. This increase continued until a malt colour of approximately 400°EBC was reached, above which any further increase in colour did change the taa.

Figure 11: Total antioxidant activity of pilot malts produced from a single barley variety.



This phenomenon was observed for malts produced from both single (figure 11) and mixed (figure 12) barley varieties.

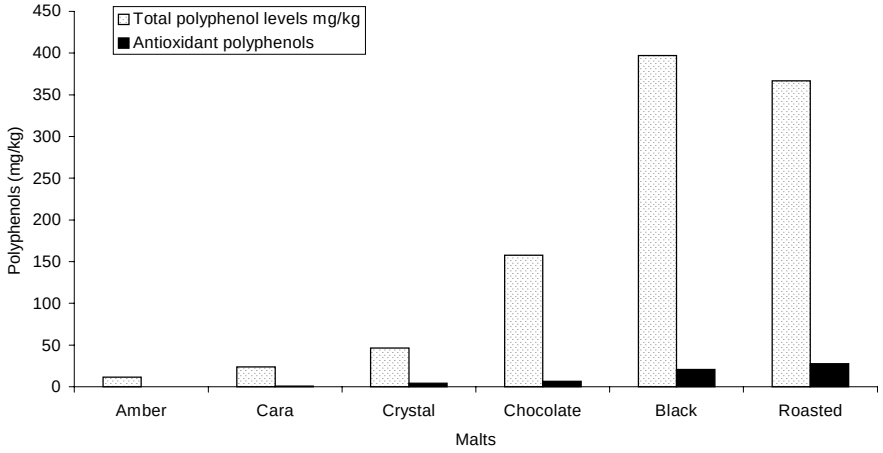
Figure 12: Total antioxidant activity of commercial malt of varying coloured produced from various barley varieties.



The formation of Maillard reaction products in coloured malts would be expected to have increased as the kilning temperature became higher, therefore the correlation between the taa and malt colour may be attributed to these compounds. As the kilning temperatures continued to rise, the production of these coloured Maillard compounds continued until a limit was reached. Maillard compounds are generally coloured, however not all of these compounds have antioxidant properties. As the colour continued to increase with temperature, beyond the point where the taa had reached a maximum level, this implied that the formation of different types of Maillard compounds was temperature dependent. The reactions that occur at lower temperatures resulted in a class of Maillard compounds that had antioxidant activity, whereas those coloured compounds produced at higher temperatures had little or no effect on the antioxidant levels. Further increases in colour were therefore not accompanied by an increase in taa.

The malt polyphenol levels, and those with antioxidant properties, were also found to rise as malt colour increased (figure 13).

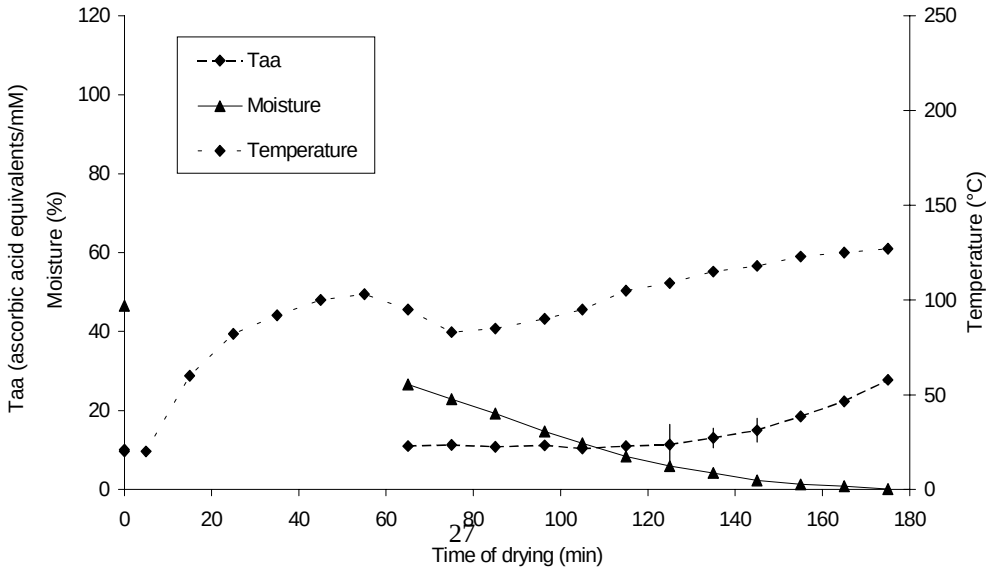
Figure 13: Total polyphenol levels and antioxidant polyphenols for malts of increasing colour.



It therefore appears that the formation of these compounds was also temperature-dependent. The slight drop in the polyphenol content of a roasted malt is because this product is made from barley, and therefore not malted. The lack of lack of steeping and germination during malting may possibly have resulted in a decrease in the formation (or availability) of polyphenols, or their precursors. Alternatively, higher temperatures over a period of time are required for epimerisation and polymerisation of the barley phenolic compounds when compared to those of green malt.

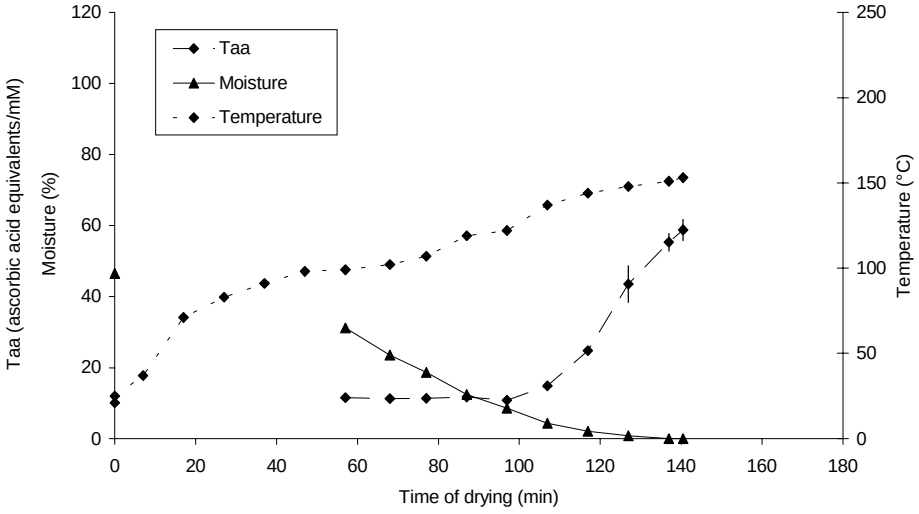
Crystal malts

Figure 14: Development of total antioxidant activity during production of low colour crystal malt (39°EBC) from green malt.



Figures 14 and 15 show the data obtained during the drying period of production of a low colour crystal malt (39°EBC) and a high colour crystal malt (220°EBC) respectively. During the production of these malts, the first phase was a stewing stage where the green malt was heated in a closed roasting drum at a temperature of 65°C, amylolytic and proteolytic processes began. The endosperm became liquefied as the starches were converted into sugars and protein breakdown occured. This stage was approximately 40 minutes long with temperature ramped to 100°C before full conversion was achieved. During the second phase (drying), the air in the roasting drum was vented to remove free moisture from the grain. The increased temperature resulted in the formation of the characteristic colour and flavour profiles due to Maillard reaction products.

Figure 15: Development of total antioxidant activity during roasting of high colour crystal malt (220°EBC) from green malt.



The taa, temperature and moisture levels were monitored during the drying period. The stewing schedule was identical for both of the malts, however the temperature attained was dependent upon the product required. Higher taa levels were obtained at higher temperatures, as previously described. Despite difference in the temperature profiles, the moisture profiles appeared to be very similar. Figures 16 and 17 illustrate in greater detail the similarity in the relationship between the taa and the moisture level for the production of both of the crystal malts.

Figure 16: Relationship between moisture, temperature and antioxidant activity for 39°EBC and 220°EBC crystal malts.

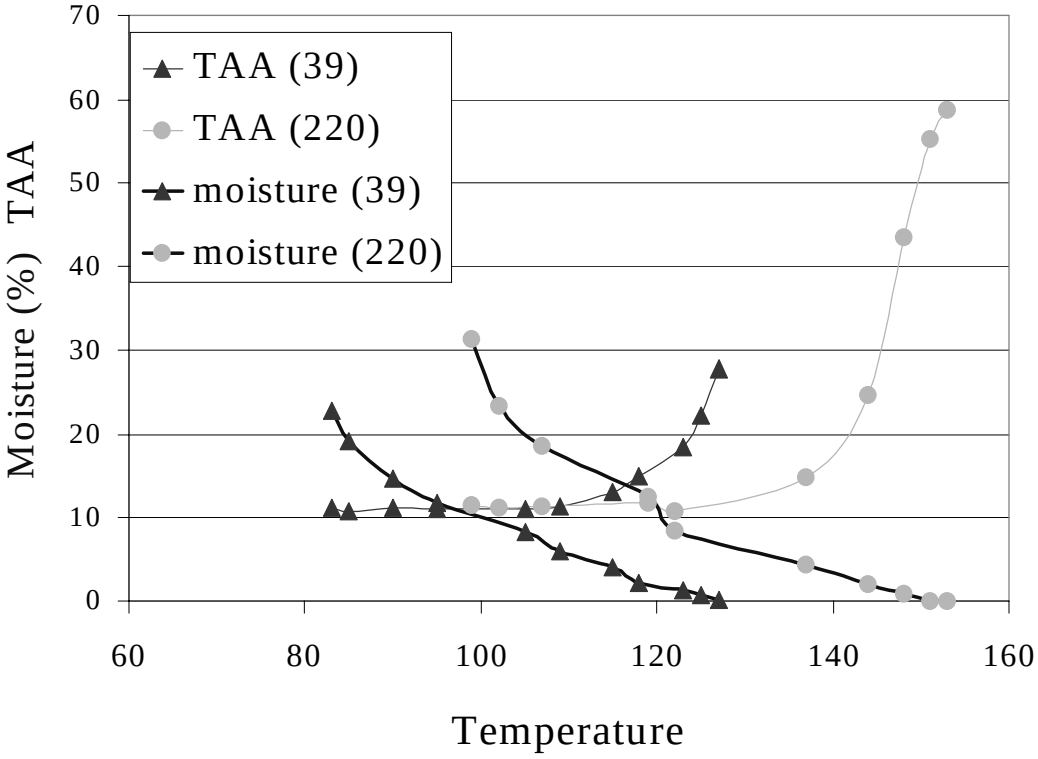
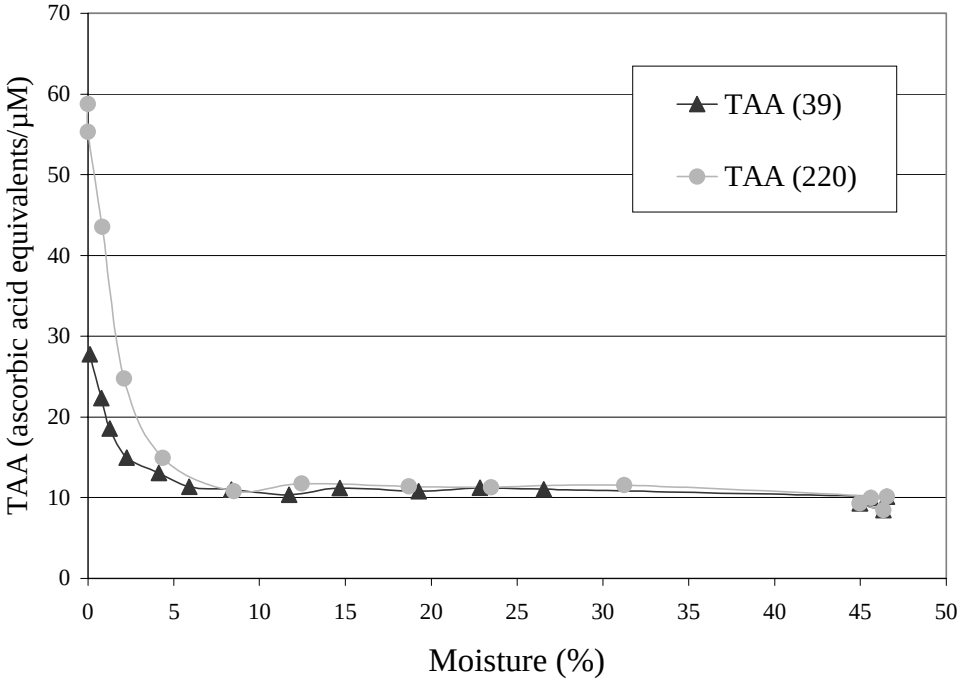


Figure 17: Relationship between moisture and antioxidant activity for 39°EBC and 220°EBC crystal malts.



It can be seen that the sharp increase in the antioxidant activity for the two malts occurs at different temperatures (figure16). A product temperature of ~140°C is required for the high colour crystal malt whereas for the low colour crystal it occurs at a temperature of ~120 °C. This difference is mainly due to the moisture of the grain. Figure 17 clearly shows that the taa for both malts was very similar and independent of temperature at higher moisture levels. An increase in the taa for both malts did not appear to occur until the moisture content was below a level of approximately 5%. Once the moisture levels had decreased below this, the production of antioxidants became dependent upon the temperature.

This implied that for crystal malts the development of antioxidant compounds was highly dependent upon moisture i.e. there was an increase in total antioxidant activity only when the moisture content had been reduced to below approximately 5%.

As the rate of drying was similar in both cases, the development of antioxidant activity depended mainly upon the airflow through the system (roaster design) rather than the heat input. Utilising a higher fan speed during drying could therefore have increased the total antioxidant activity, as this would have lead to a more rapid reduction in the moisture content.

Black malt

A black malt was produced by roasting the dried chit malt (low modified malt) from the same barley that was used for the high and low colour crystal malts. The development of total antioxidant activity and product temperature is shown in figure 18.

Figure 18: Development of total antioxidant activity during roasting of black malt (1750°EBC) from chit malt.

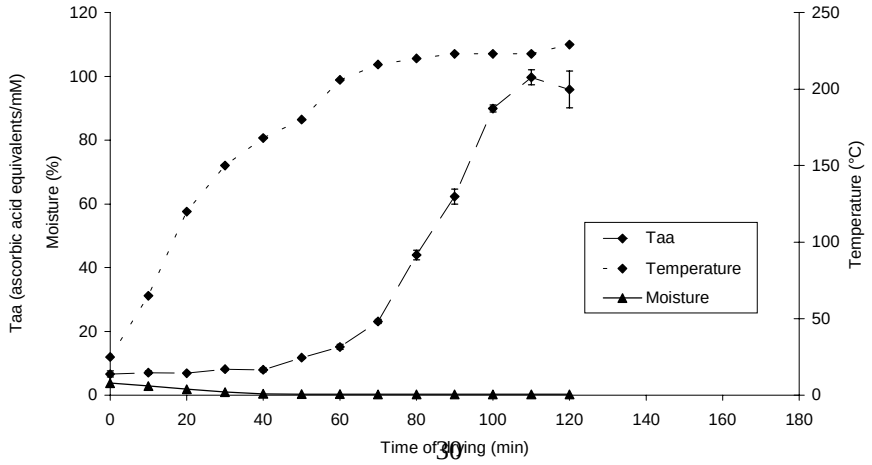
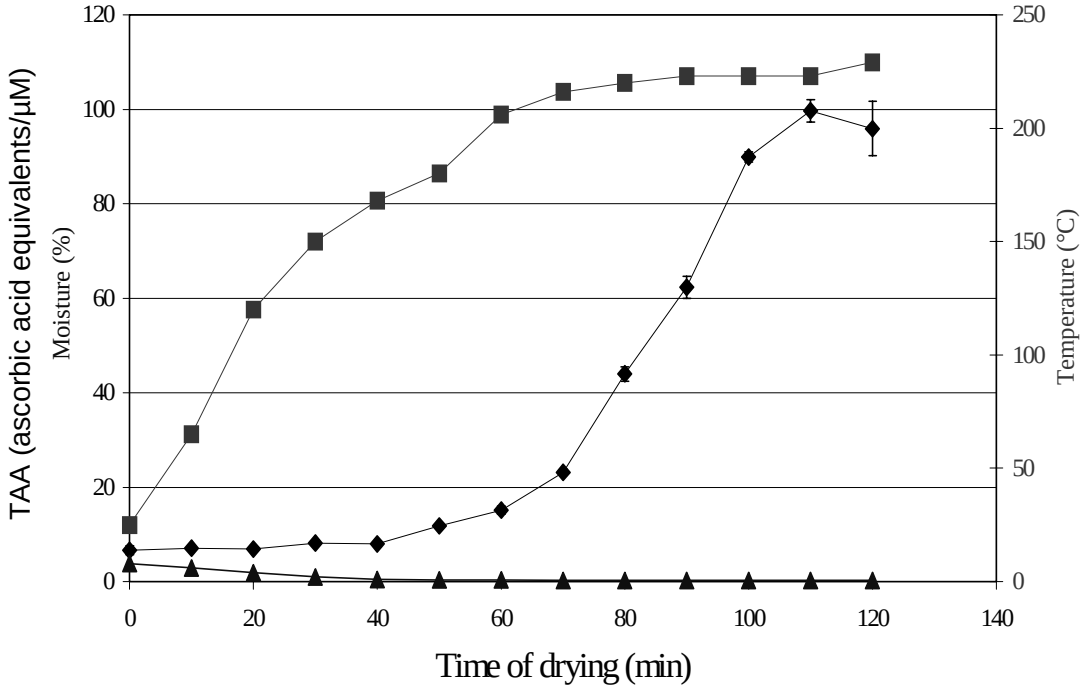


Figure 19: Relationship between moisture, temperature and antioxidant activity for black malt.



The moisture content dropped rapidly to a negligible level due to the rapid temperature rise used in the production of black malts. As the black malt is made from dried chit malt, the moisture was at the start of drying. At the very high temperatures attained during the latter stages of roasting, the product was very close to its combustion point.

Figure 19 shows that the production of taa followed the same trend as was observed for the two crystal malts i.e. taa remained constant irrespective of temperature until a specific point was reached. Beyond this, there was a rapid increase and the final level of taa was temperature-dependent. As modification was incomplete in the chit malt used for the production of the black malt, the precursors and the chemical reactions that took place during roasting were completely different than those in the crystal malts. This of course will mean that higher temperatures are required to modify the internal endosperm structure for generating the precursors for Maillard reaction to occur. Applying the roasting conditions used for black malt production to well-modified malt could confirm this difference. As with well modified and under modified pale malts, the differences that occurred in the processing of crystal and black malts may have played an important role in the final antioxidant yield.

Table 4: Roasted malts analyses

Product type from the same Fanfare barley	$HWE_{0.7}$ (L°/kg)	Moisture (%)	Colour (°EBC)
Low colour crystal	304	5.7	39
High colour crystal	301	2.8	220
Black	273	2.3	1750

Table 4 shows the malt analysis of the crystal malts and black malt produced in this pilot trial. The malt analysis shows that the products produced in this trial have similar malt analysis to those available commercially.

Malt antioxidant profiles

Figure 20: Comparison of malt antioxidants profiles for lager and crystal against known chemical antioxidant standards.

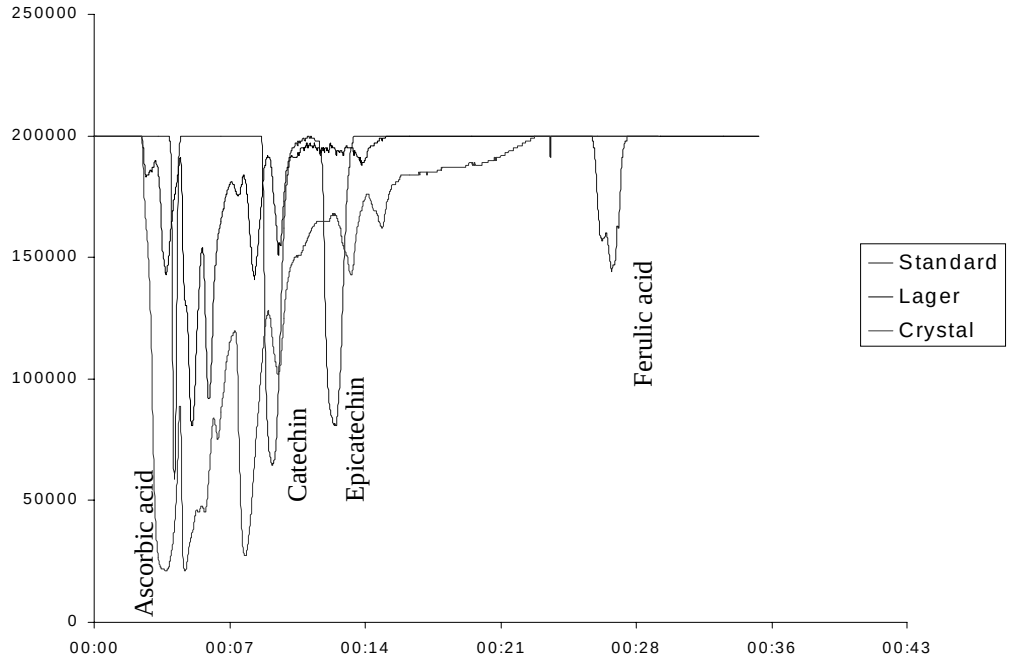
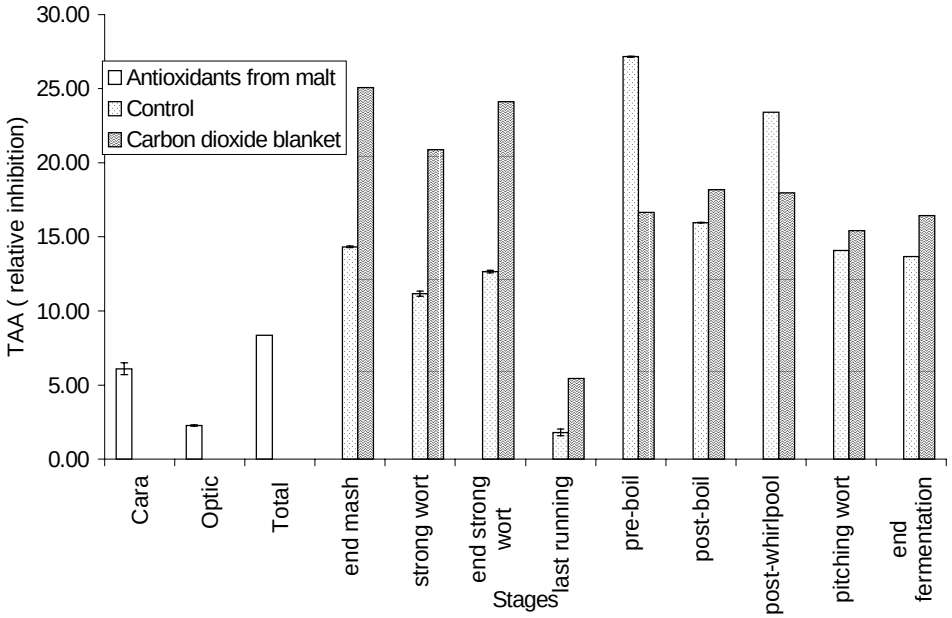


Figure 20 shows the antioxidant profiles of a lager and a crystal malt against external standards. A qualitative analysis of antioxidant profiles shows that the both pale and crystal malts have similar antioxidants profile. However, crystal has additional antioxidant compound when compared to the pale malt. The majority of the antioxidant compounds found in pale malts are phenolic in nature. In addition to these phenolic antioxidants, the coloured crystal and speciality malts have heat induced antioxidants.

Reducing power during different stages of the brewing process

The four brews produced were chosen to investigate the factors that could possible help to minimise the problems due to oxidation during brewing. It is believed to be desirable to maintain a higher reduction potential throughout the brewing process, and to have a high degree of 'carry-through' into the final beer.

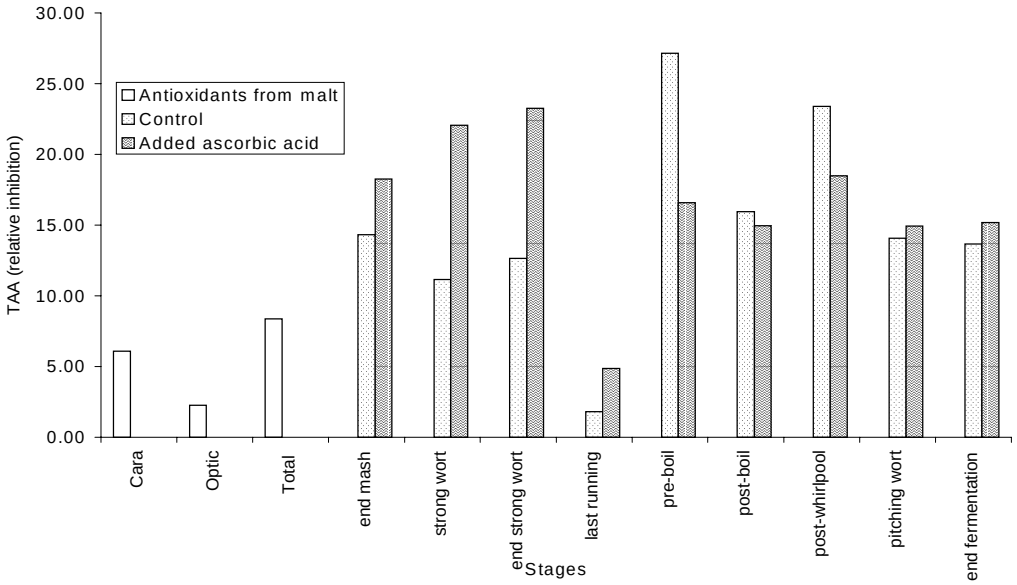
Figure 21: Comparison of total antioxidant activity at different stages of the brewing process for a control beer and beer brewed under carbon dioxide.



The effects of reducing the contact between the wort and oxygen in the brew house were studied to determine the protection malt antioxidants provide in the brewhouse. The addition of reducing agents was also investigated, to compare the effects of natural malt-derived antioxidants with those added exogenously.

To reduce oxidation reactions occurring in the brewhouse, it is crucial to have malts with high antioxidant activity and also minimise oxygen pick up at different stages of brewhouse operation. Two beers were brewed, one under CO_2 blanket and the other brewed normally, to ascertain the contribution to the total antioxidant activity at a given stage in the brewing process (figure 21). Beers brewed under a blanket of CO_2 retained higher taa levels than the control brew prior to the pre-boil stage (figure 21). However, during the boiling stage of the brewing process the antioxidant activity decreases, this may be an effect of hop addition.

Figure 22: Comparison of total antioxidant activity between control beer and the beer brewed with ascorbic acid.



The addition of known reducing agents such as ascorbic acid and potassium metabisulphite to the brew (figures 22 and 23 respectively) similarly resulted in a markedly higher antioxidant activity in the brew house prior to the pre-boil stage.

Little antioxidant activity was found in the last runnings, except for the brew with potassium metabisulphite, where the taa was noticeably higher than in the control brew. The low levels of antioxidants in the last runnings may have been a dilution effect, and the anomalously high taa noted for the potassium metabisulphite brew may have been due to a different dilution factor. However it would appear that the last runnings stage of brewing may be prone to oxidation. The

taa levels measured after the pre-boil stage were more variable for all the brews, and less distinction could be made regarding the advantages of the regimes used.

Figure 23: Comparison of total antioxidant activity between control beer and the beer brewed with potassium metabisulphite .

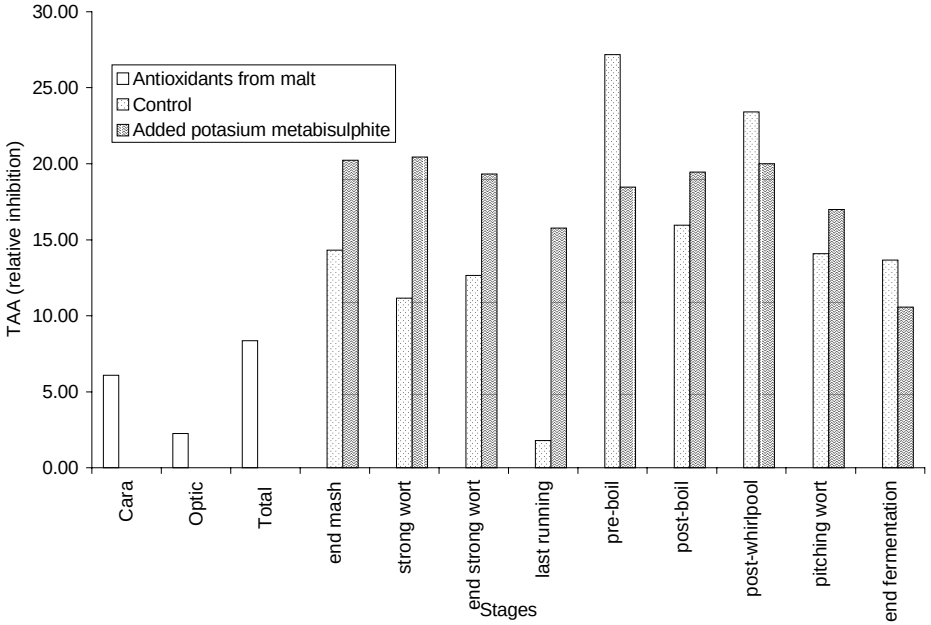
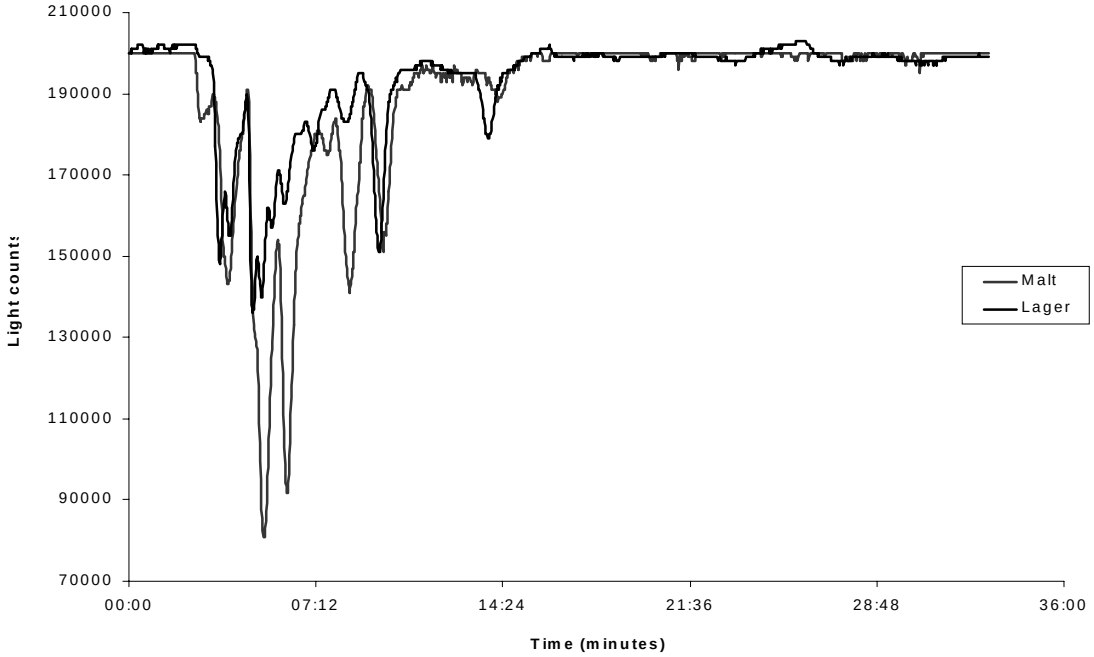


Figure 24: Comparison of total antioxidant activity between malt and lager beer.



The higher taa levels found in the antioxidant brews up to the pre-boil stage implied that this is where the added antioxidants had a protective effect, as did the CO_2 blanket. The lack of any significant differences between the control and the other brews at the end of fermentation suggested that the antioxidants found in malt carried through to the beer, whilst those added during mashing did not make any significant contribution to the beer.

Figure 25: Classification of total antioxidant activity of beer.

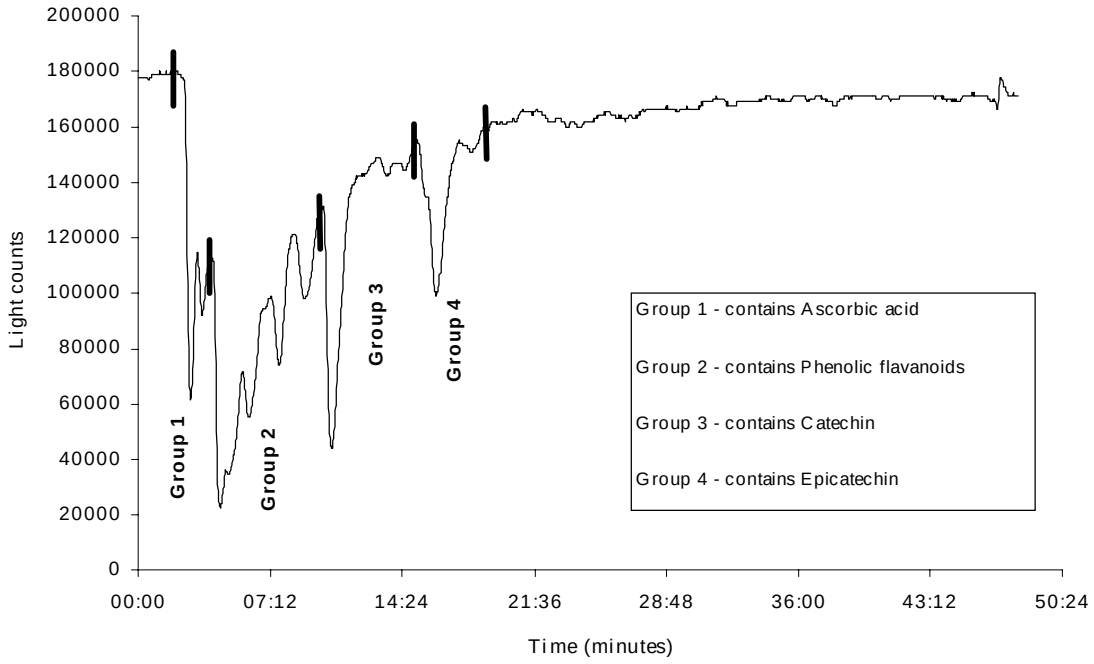
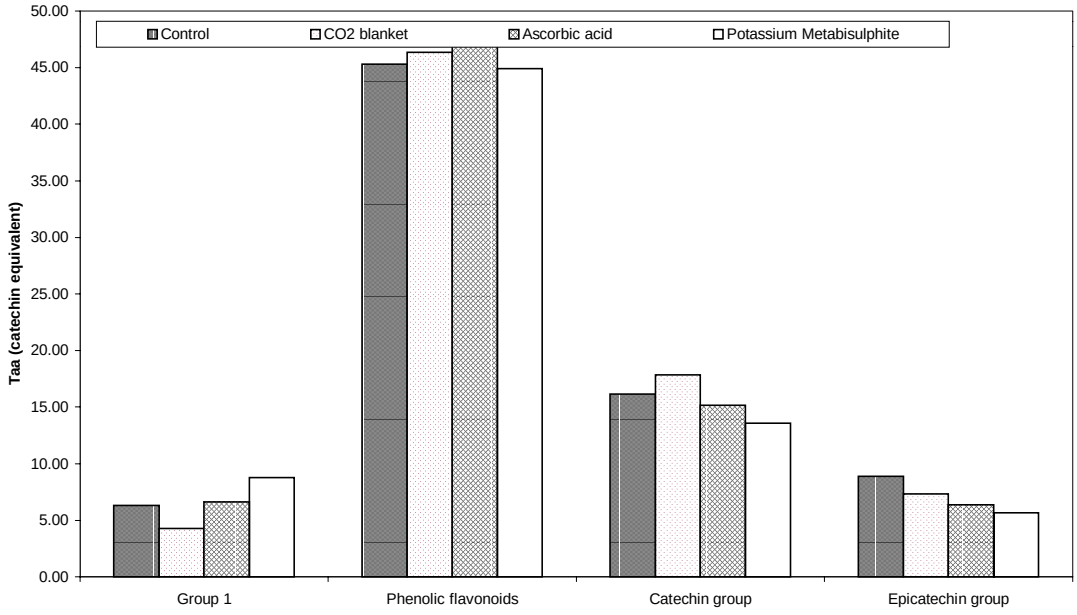


Figure 26: Comparison of total antioxidant activity between malt and lager beer.



The ‘carry-through’ was confirmed qualitatively by the HPLC antioxidant profiles for the control beer and the malt used to brew the beer (figure 24). The phenolic flavonoid group of the malts is the main contributors to the antioxidant activity of the beer followed by catechin, epicatechin and a unknown group of compounds (Group 1) which includes ascorbic acid (figure 25). The similarity in the antioxidant profiles implied that the antioxidants from malts made a significant contribution to the antioxidant activity in the beer. Some of the early antioxidant peaks in the HPLC profile for the beer corresponded to phenolic compounds and were derived from the processing, however it appeared that the main antioxidant contribution was from the malts. Figure 26 shows that the delivery of phenolic malt antioxidants to beer. The antioxidant profile is similar in all cases demonstrating that the malt antioxidants would be the major source of protection against oxidative damage in beer.

References

1. Boivin, P. *et. al., Proc. Conv. - Inst. Brew. (Asia Pac. Sect.)*, 1996, **24**, 110-115
2. Van Geluwhe, G.E.A. *et al., Brew. Digest*, 1970, **45(11)**, 70-72
3. Moll, M. *et. al.,. Brauerei Rundschau*, 1990, **101**, 2-10
4. Boivin, P. *et. al., Proc. Congr. - European Brewing Convention*, 1993, **24**, 397-404
5. Buggey, L.A. *et. al., Cervisae* (in press)
6. Boivin, P. *et. al., Proc. Congr. - European Brewing Convention*, 1995, **25**, 1599-168
7. Ohtsu, K. *et al., Brewers Digest*, 1986, **61**, 18-23
8. Scheer, F.M., MBAA *Technical Quarterly*, 1996, **33:2**, 87-90
9. Griffiths, D.J. *et. al., Proc. Congr. - European Brewing Convention*, 1997, 267-273
10. Kretschmer, K.F. *et. al., Brauindustrie*, 1984, **69:13**, 966-968, 970-972
11. Chapon, L. *et al., Eur. Brew. Conv. Monogr.*, 1980, **6**, 45-70
12. Gromus, J. *Brauwissenschaft*, 1978, **31:6**, 148-158
13. Bellmer, H.G., *Brauwelt*, 1976, **116:24**, 789-790, 792-797
14. Narziss, L. *et. al., Brauwelt*, 1975, **115:52**, 1729-1734
15. Griggs, D.L. *et. al., Proc. Int. Brew. Conf.*, 1992, **5**, 259-267
16. Cantrell, I.C. *et. al., MBAA Technical Quarterly*, 1996, **33**, 82-86
17. Van Gheluwe, G.E.A *et. al., MBAA Technical Quarterly*, 1974, **11:3**
18. Kaneda, H. *et. al., MBAA Technical Quarterly*, 1995, **32**, 90-94
19. Muller, R.E., *J. American Society of Brewing Chemists*, 1995, 53-57
20. Herrmann, H., *MBAA Technical Quarterly*, 1998, **36:1**, 49-54