Control of Malt Roasting Operations for Consistent Delivery of Desired Product Flavour

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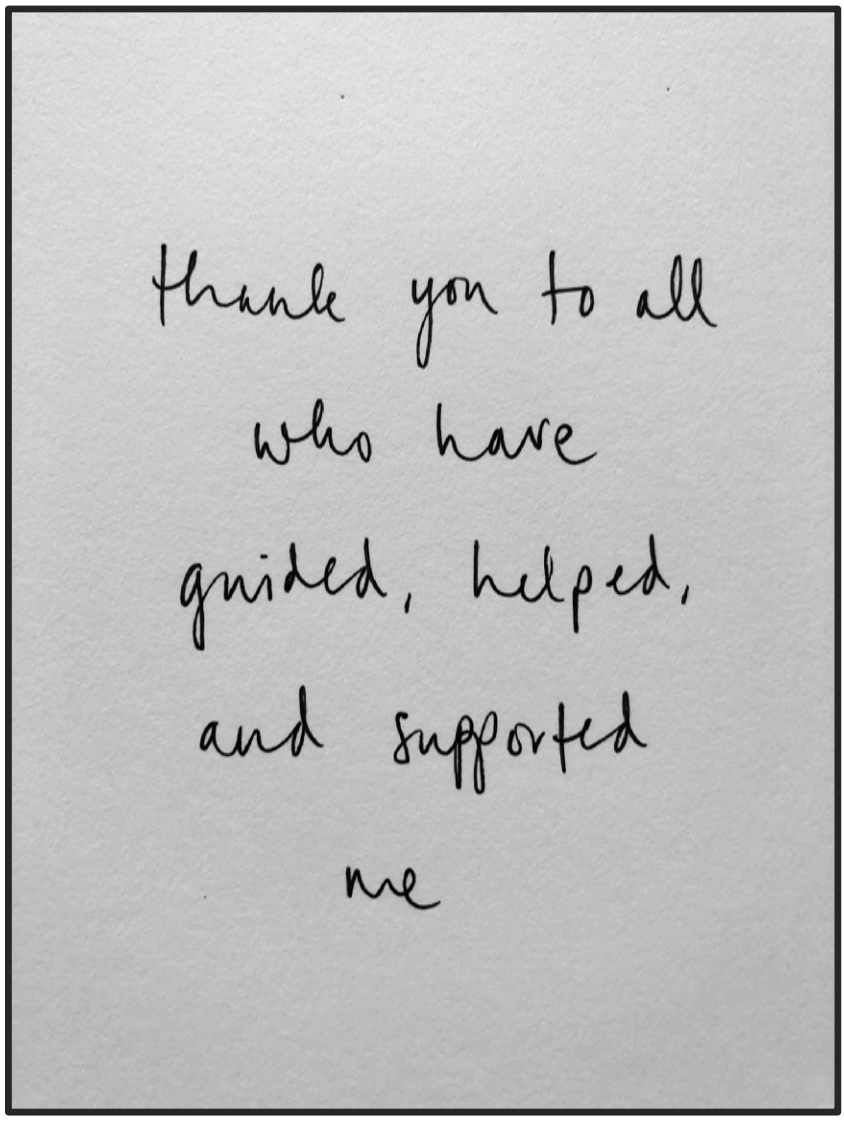

Abstract

Roasted malts are used in the brewing industry to impart colour and flavour to beers. The diversity of colours and flavours roasted products can provide are dependent upon a variety of factors: roasting time and temperature, initial moisture content, roasting substrate type (i.e. green malt, pale malt, or unmalted barley), and roasting sequence (i.e. stewing, kilning, and/or roasting). The aim of this research was to improve understanding of thermal flavour generation in roasted malt and barley, and thereby improve understanding of roasting process control. In this research, instrumental analysis was employed to positively identify and quantify thermally generated volatile compounds in roasted products, while sensory analysis evaluated the odour activity of identified volatiles, and quantitatively described aroma qualities of roasted products.

Gas Chromatography-Olfactometry (GC-O) identified 45 odour active compounds across a range of six commercially available roasted malts. Of these, formation of 20 key compounds were monitored as a function of roasting time and temperature across the three roasting substrate types, using a custom-built laboratory-scale roaster for precise time/temperature control. 3D response surface models were produced showing compound concentrations as a function of roasting time and temperature in each roasted substrate. The roasted product 'flavour space' (depicted via principal component analysis (PCA)) visualised formation of 20 compounds over the course of roasting conditions for each roasted substrate, in addition to the six previously examined commercially roasted products. The identification of volatile sulphur compounds (VSCs) by GC-PFPD (Pulsed Flame Photometric Detection) in roasted products presented novel detail of VSCs in roasted products, showing the highest numbers of VSCs in products roasted between 200 °C and 230 °C, however, prolonged roasting times reduced concentrations. Quantitative Descriptive Analysis (QDA) of the aroma of selected roasted products identified roasting temperature as the major significant factor in the aroma characteristics of roasted products.

This research provides comprehensive understanding of thermal flavour generation in roasted malts and barley by the interdisciplinary approach of combining instrumental and sensorial analysis. Awareness of volatile formation as a function of process conditions has potential to improve process control and product quality for commercial roasting.

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Table 0.1: List of abbreviations

°C	Degrees Celsius
µg	Microgram(s)
%	Percent
AHC	Agglomerative Hierarchical Clustering
ANOVA	Analysis of Variance
CO_2	Carbon Dioxide
EBC	European Brewery Convention
g	Gram(s)
ga	Gauge
GC	Gas Chromatography
HMF	Hydroxymethylfurfural
HPLC	High-Performance Liquid Chromatography
kDa	Kilodalton(s)
kg	Kilogram(s)
LRI	Linear Retention Index (Kovat's)
m/z	Mass-to-charge-ratio
min	Minute(s)
mL	Millilitre(s)
MS	Mass Spectrometry
ms	Millisecond(s)
mV	Millivolt(s)
ng	Nanogram(s)
PCA	Principal Component Analysis
PFPD	Pulsed Flame Photometric Detection
psi	Pounds per Square Inch
PTV	Programmed Temperature Vaporiser
QDA	Quantitative Descriptive Analysis
rpm	Rotations per Minute
s	Second(s)
SPME	Solid Phase Microextraction
V	Volt(s)
VSC	Volatile Sulphur Compound
x g	Times Gravity
α	alpha

Publications

Conference Proceedings:

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The flavour properties of roasted malts: a gas chromatography-olfactometry study.

H. Parr, I. Bolat, P. Miller, S. Clegg, D. Cook

(Parr et al., 2018)

Journal Article:

Journal article, Journal of Food Chemistry, 2020.

Modelling flavour formation in roasted malt substrates under controlled conditions of time and temperature.

H. Parr, I. Bolat, D. Cook

(Parr et al., 2020)

Chapter 1 - Introduction and Literature Review

Roasted products are used to impart flavour and colour to beers. Within the family of roasted products originating from barley, three major roasting substrates can be used: green malt, the product of germination; pale malt, kilned malted barley; and unmalted barley. The variety of process conditions employed (i.e. roasting time, temperature, and moisture content) directly influence the extent to which thermal flavour generation reactions occur. The Maillard reaction, caramelisation, lipid degradation, and pyrolysis are the core volatile compound formation pathways that occur during roasting. The variety of available products are expected by brewers to contribute specific characteristics when used in production.

Commercially available products provide a spectrum of colours and a variety of expected characteristics, according to the specifics of the processes employed. Present roasting operations rely on colour development as an indicator of process control. This results in batch-to-batch variation, particularly regarding consistency of flavour contribution. Improved understanding of thermal flavour generation in roasted products will provide further accuracy in roasting operations. The following literature review provides a summary of the mechanisms that contribute to the variety of functional properties within the range of roasted malts, and the analytical techniques that can be used to assess the qualities they exhibit.

1.1 Malt

Barley malt is a major raw material in the production of roasted malts. Malting is the process of deliberate germination of cereal grains under controlled conditions, and the subsequent drying of these grains.

The most commercially significant of the crops for the process is barley. Malted barley is not usually commercially available as a food product in its own right, but is used extensively as an ingredient in many foods and beverages. Malted barley is a major ingredient in beer (the global brewing industry accounts for the majority of worldwide barley malt usage), whisky, and malt vinegar; and is also used as coffee extender, and included in brown breads (Bamforth, 1993). Barley malts are also key ingredients in malt extract drinks, namely Ovaltine, and Horlicks.

Regarding malts' use in the brewing industry, after the malt has been produced it undergoes further processing to form grist, which is the combination of milled malts

that are used in a brew; and then wort, the fermentation medium. The role of malt in brewing beer is to provide the fermentation medium for the anaerobic respiration of yeast to form ethanol and CO_2, along with additional flavour compounds as a result of fermentation.

1.1.1 The Industrial Malting Process

Prior to the malting process, intake of raw barley is evaluated by rapid analytical methods, namely assessing moisture and protein content. Visual inspection upon intake determines the presence of pre-germination, insects, fungal infestation, and general abnormalities (Esslinger, 2009). Accepted intake of raw barley is then pre-cleaned (removal of coarse impurities (i.e. straw, stones, metallic contamination etc.)) and stored in silos with aeration, drying and cooling facilities, temperature control, and pest control (Esslinger, 2009).

Typically, the modern malting process takes approximately seven days to complete. Raw barley is used as the malting substrate, and undergoes three major processing stages: steeping, germination, and kilning (discussed below), resulting in the production of barley malt, also referred to as pale malt, or white malt.

1.1.1.1 Steeping

Steeping ensures the grain is suitably hydrated by taking up water, which allows the grain to modify and germinate uniformly (Briggs, 1998b). The grains must reach a moisture content of approximately 40 - 46 % in order for the subsequent germination step to be carried out (Gruber, 2001, Bamforth, 1993).

Steeping vessels are typically cylindrical in shape with either a conical or flat bottom. The steeping process is carried out in cycles of wet and dry stands, with the addition of air from the base of the steeping vessel to add oxygen and aid mixing. Kernel water uptake is dependent upon water temperature and wet stand duration. Higher water temperature and greater duration of wet stands results in accelerated kernel water uptake (Esslinger, 2009). However, this increases the risk of microbial growth and over-exposure to water resulting in drowning of the kernels.

During dry stands, humidified air passes through the bed of kernels from the top of the bed downwards. This provides oxygen, while reducing CO_2 and heat (generated by the germinating grains). Typically, two alternating wet and dry stands are

employed for adequate steeping, with a total duration of around 48 h (Esslinger, 2009).

1.1.1.2 Germination

Germination is necessary to generate the maximum extractable material from the barley grain. This process can take around 4 - 5 days in modern maltings. To ensure the production of the maximum amount of extractable material, the modification of the grain via the action of enzymes must be promoted. Modification indicates the beneficial changes that occur within the cereal grains, namely the accumulation of hydrolytic enzymes, and the physical softening of the grain (Briggs, 1998b). The accumulation of hydrolytic enzymes enables the production of fermentable sugars from the starch granules within the endosperm component of the grain.

The individual roles of the hydrolytic enzymes found in malt are as follows: α-amylase and β-amylase catalyse the hydrolysis of the α(1→4) linkages in amylose and amylopectin. Limit dextrinase hydrolyses the α(1→6) side chains, and α-glucosidase (maltase) hydrolyses either α(1→4) or α(1→6) linkages, and therefore hydrolyses maltose (Briggs, 1998b, Bamforth, 1993).

Following steeping, the grains are transferred to a germination vessel, which are typically cylindrical in modern maltings. The cylindrical shape allows for thorough mixing by a rotating mechanical arm, mixing the radius of the bed of germinating grains cyclically with vertical turner screws. This prevents the matting of rootlets over the period of germination. Temperature controlled humidified air is circulated through the bed of grains throughout germination. Visual inspection is commonly used to determine the extent of germination. The establishment of rootlets on each grain will indicate that germination has taken place successfully.

1.1.1.3 Kilning

Kilning is necessary to arrest germination. When kilning to produce regular base malts, dehydration must be carried out until the moisture content of the malt has been reduced to 3 - 5%. Kilning makes the malt stable, ready for storage, and allows the now brittle rootlets to be removed by ‘deculming’. Kilning is also crucial for the development of colour, and initial thermal flavour generation. Drying the grain and

halting germination is also important in the removal of undesirable green-grain off-flavours (Briggs, 1998a).
Germinated grains are transferred to kilning vessels, which allow large quantities of heated air to pass through the base of the bed of grains by a false bottom. The temperature of the heated air should be gradually increased over the course of kilning in order to minimise enzyme denaturation. The relative humidity of exhaust air indicates the extent of drying that has occurred.
The products of kilning alone are base malts, low in their range of flavours and characteristics. Subsequent thermal processing is required to enable the development of additional thermal flavour generation.

1.2 Roasted Malts

Speciality malts, including roasted malts, typically form up to 5 % of the total grist (Walker and Westwood, 1992). However, this may be increased to yield pronounced speciality malt characteristics if desired by the brewer. Roasted malts are considered to be speciality malts, as they are often used solely to provide colour and flavour to beers. Whereas base malts are used to provide the majority of extract and the diastatic enzymes to break down starch to fermentable sugars. Roasted malts have a lack of enzyme activity, due to the high temperatures they are subjected to during their processing, which causes the denaturation of the enzymes within the grains (Samaras et al., 2005).
In the roasting of barley (and barley malt) products, roasting techniques can be applied to raw barley; green malt, the product of germination, which has not undergone any drying processes; and pale malt, germinated malt that has been kilned. The roasted products of these malt raw materials can be used by brewers to adjust the final flavour and colour of beers (O'Shaughnessy, 2003). Therefore, the variety of beer styles available within the brewing industry is greatly expanded by the contributions of combinations of speciality malts, due to their ability to impart specific flavours and colours in differing intensities (Coghe et al., 2003a).
As roasted malts are a component of the grist, they undergo the subsequent steps of the brewing process, and are therefore subjected to the variability that may occur during the process steps (Walker and Westwood, 1992). Consequently, the exact colours and flavours that are present in the roasted malt may not be present in the

final beer. Channell et al. (2010) noted that roasted malts have a direct effect on the flavour of the final beer, as the roasted flavours remain in the beer throughout processing, including fermentation. In contrast to this, base malts typically have an indirect effect on the final organoleptic qualities of the beer. This is because the base malts provide the chemical feedstock from which the yeast may generate the flavour of the beer (Channell et al., 2010).

The typical parameters (extract, colour, and moisture content) that are observed or measured during the production of roasted malts are insufficient to accurately determine the flavour compounds present, and therefore the brewing impacts of the roasted malts in question. A more complete understanding of the potential applications of the roasted malts is required (Coghe et al., 2003a).

1.2.1 Malt Roasting Operations

Commercial malt roasting drums share many similarities to commercial coffee roasting drums. Figure 1.1 shows commercial scale malt and coffee roasting operations, displaying the typical cooling mechanism. The specifications of roasting drums vary with differing manufacturers.

Figure 1.1: Commercial scale roasting drums.

Removal of roasted malt from commercial roasting drum (left), and removal of roasted coffee from commercial roasting drum, showing typical cooling mechanism (right).

Malt roasting operations differ from modern base maltings in scale, as lower volumes of speciality malts are required to contribute to a brew. Typically, roasted malts are produced in batches of around one to two tonnes. Although the roasting drums used are controlled by pre-set conditions, this does not allow for differences in the individual batch composition and specification (Briggs, 1998b). Malt roasting is a labour intensive process, and requires the focus of experienced maltsters to monitor

the development of the product throughout its processing (Gruber, 2001). These individuals may alter the conditions in the drum if necessary, according to the results of the analysis.

While necessary to include the kilning step in the production of base malts, roasted malts can be dried in a variety of combinations. The green malt, the product of germination, can be kilned, roasted, or kilned and roasted. The use of each technique yields a range of resultant products. In addition, unmalted barley is roasted to high temperatures in commercial malt roasting operations.

The whole roasting process can take up to 2 hours and 30 minutes (Blenkinsop, 1991). Blenkinsop (1991) noted that the manufacture of darker roasted products, by more intense roasting, results in a decrease in yield. This is because the higher processing temperatures used cause an increased loss of water by evaporation, which decreases the mass of the grain, and consequently the yield.

In the very darkest roasted products, it is common to quench the roasting grains within the vessel. Quenching is the action of spraying the roasting malt with water. Due to the high temperatures reached, the evaporative cooling of the water on the hot malt minimises the risk of fire; and enables the malt to achieve the target colour and flavour (Yahya et al., 2014). Yahya et al. (2014) found that quenching the malt had no effect on the final moisture content of roasted malts, but does influence the final colour and flavour. After the malt has been roasted, it is necessary to cool the product, which can be carried out by transferring the malt to a circular cooling vessel with a perforated floor (Blenkinsop, 1991). Mechanical rakes help to ensure the product cools to room temperature as evenly as possible (Figure 1.1).

1.2.1.1 Common Commercially Available Roasted Malts

Table 1.1 lists publicly available information on roasted products available within the current commercial range (either commonly available, or specifically available from Boortmalt). Listed is an indication of the varying roasting parameters and substrates used to achieve the desired colour and moisture content of the final products. The three major roasting substrates are used: pale malt, green malt, and unmalted barley, discussed in Sections 1.2.1.1.1, 1.2.1.1.2, and 1.2.1.1.3, respectively. It should be noted that the true commercial range of roasted malt products undoubtedly exceeds the range listed in

Table 1.1. In addition to this, the given names of the products, and the colour ranges used to define the malts produced will differ between manufacturers (Briggs, 1998b).

Table 1.1: A typical range of commercial roasted products based on barley.

Malt Type	Roasting Substrate	Finishing Temperature (° C)	Colour (° EBC)	Maximum Moisture (% w/w)
Amber Malt	Pale Malt	100 – 150	45 – 85	<3.5 – 4.0
Caramalt	Green Malt	-	30	3.0 – 7.5
Light Crystal Malt	Green Malt	-	90 – 140	6.0
Medium Crystal Malt	Green Malt	-	160	5.5
Dark Crystal Malt	Green Malt	-	200	5.0
Chocolate Malt	Pale Malt	225	1200	3.5
Black Malt	Pale Malt	230	1450	3.5
Roasted Barley	Unmalted Barley	230	1500 – 1700	<2.0 – 3.5

(Briggs, 1998b, Boortmalt, 2010j, Blenkinsop, 1991)

1.2.1.1.1 Roasted Pale Malts

Roasted pale malts yield the widest range of colours and flavours within their category of roasted substrate. Roasted pale malts are essential in the brewing of porters and stouts, but can also be used in the preparation of dark lagers, and in mild, brown, and old ales (Gruber, 2001). The products of roasting pale malt range from amber malt (45 – 85 °EBC) to black malt (1500 °EBC) (
Table 1.1).

As discussed in Section 1.1.1.3 above, pale malts have been kilned, and therefore have a low moisture content (4-6 % w/w (Blenkinsop, 1991)). Subtle differences in roasting parameters have a marked effect on the characteristics of the roasted product. For example, chocolate malt is roasted up to 225 °C, whereas black malt is roasted up to 230 °C (
Table 1.1). Only 5 °C difference in roasting temperature is noted, yet chocolate malt yields a nutty, coffee, smoky, chocolate, burnt flavour while avoiding the bitter

astringency of black malt (Gruber, 2001, O'Shaughnessy, 2003). Like all roasted pale malts, chocolate malt ought to have a uniformly friable endosperm, which should be free from shine. Scorching or charring of the grains during the roasting of pale malts should be avoided, as this results in excessive loss in extract, and loss of extractable colour (Boortmalt, 2010c). On the opposite end of the scale, amber malt provides a dry, biscuit characteristic to beers. Winter malting barley varieties with a high modification level are regularly used for the production of amber malt. The enzyme content of the final roasted malt is typically very low (Boortmalt, 2010a).

1.2.1.1.2 Roasted Green Malts

Crystal malts are produced from green malts, achieved by a three-step roasting process. Indirect heating (stewing) then direct heating (drying and roasting) are required (Vandecan et al., 2011). Green malt, which is high in moisture (40-44 % w/w (Gruber, 2001)), must be stewed in the initial stage of roasting. During stewing, the grain is sealed in the roasting vessel, in which heated air circulates around the sealed vessel heating the green malt to 65 °C. The moisture content of the grains in this stage remains high. Under these conditions, the endosperm liquifies, as amylolysis and proteolysis take place (Blenkinsop, 1991).

After stewing, heated air flows through the roasting vessel, which firstly dries then roasts the stewed grains. Crystal malts are easily distinguished from other roasted products by their glassy endosperm if examined using a farinator (see Section 1.5.1). In addition to flavour and colour, crystal malts will contribute to the mouthfeel, foam, and foam retention when used in a brew (Gruber, 2001). Although typically forming 3-5 % of the total grist, crystal malts can comprise up to 10 % of the grist in some brewing operations (Jackson and Hudson, 1978).

Table 1.1 shows the range of final colours of crystal malts that can be produced. This can be achieved by introducing variations in the roasting times and finishing temperatures employed. For example, caramalt has the lowest colour of the roasted products listed and is produced in a similar way to the standard crystal malts. The roasting is carried out at a lower temperature, for a longer period of time, which results in the final roasted malt product being lower in sweetness, with a dry flavour. Therefore, caramalt is used most commonly in lager beers (Boortmalt, 2010d).

As an alternative to roasting green malts to produce crystal malts, caramel malts can be produced by altering the kilning process, and therefore removing the need for roasting. Although this method of producing caramel malts can be employed successfully, the green malt products in this thesis refer to stewed and roasted variation.

1.2.1.1.3 Roasted Unmalted Barley

The roasting of unmalted (raw) barley yields a product distinct from its malted equivalents (i.e. black malt). Roasted barley is characterised by its strong coffee flavour, and imparts unique dryness to a beer (Gruber, 2001). Beers produced using roasted barley have a lighter coloured head than those produced using black or chocolate malts (Boortmalt, 2010j). During roasting, charring must be avoided during the processing of roasted barley, as this results in loss of extractable colour. Roasted barley forms the smallest contribution to the range of available roasted products. Typically, roasted barley products are roasted to high temperatures, and are very dark in colour. Barley roasted to low temperatures (i.e. 130 – 160 °C) is not currently available commercially.

1.2.1.2 Roasted Malt Storage

After the malt is roasted and is delivered to the brewer, the conditions in which the malt is stored, and the length of the storage time to which the malt is exposed will have a direct effect on the quality of the final beer. Roasted malts can lose their characteristic flavours and aromas upon storage, so should be used soon after being produced (Briggs, 1998a). If roasted malts are used after they begin to deteriorate, the quality of the final product will be reduced, and batch to batch variation of the final beer will be introduced. This variation is not a result of a maltings' processes, but is due to the brewers' stock management of raw materials.

1.3 Thermal Flavour Generation

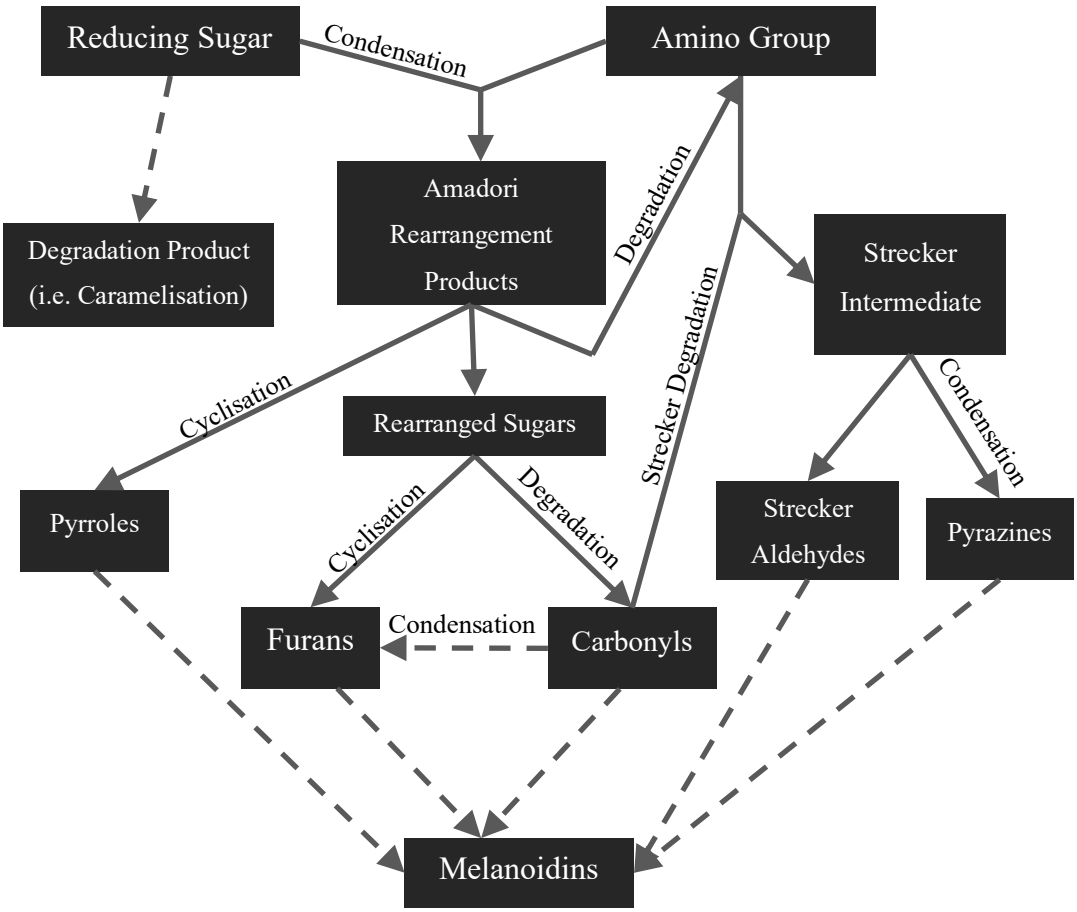
Thermal flavour generation is responsible for the development of both flavour and colour during the roasting of malts and barley. Although the results of the flavour generation reactions are not visible, the reaction pathways that are typically

characterised by the development of colour in foods are also responsible for the development of flavour.

1.3.1 The Maillard Reaction

The Maillard reaction is a non-enzymatic browning reaction, which consists of a complex sequence of interactions, comprising of three key stages (Echavarría et al., 2012, Mlotkiewicz, 1997, Yoo, 1997). The reactions are dependent upon factors including the pH, time, temperature, concentration of reactants, and reactant type (Ames, 1998). In addition to these factors, some reactions of the Maillard pathway take place at a faster rate in the absence of free water, while others are inhibited (Adams and Kimpe, 2009). The pH optimum for the Maillard reaction is between 6 and 8, and can occur at temperatures above 50 °C, but can also take place biologically at temperatures of 37 °C at a much slower rate (Blenkinsop, 1991, Kroh, 1994). The pH of green malt and pale malt is 5.8-6.2, and 5.6-5.8 respectively (Bamforth, 2001). During processing at progressively higher temperatures, the pH of the malt can decrease further by 0.2-0.3 (Bamforth, 2001). The pH of malt generally falls below that for optimum rate of the Maillard reaction. However, the reaction is carried out, along with other thermal flavour generating reactions.
Sections 1.3.1.1, 1.3.1.2, and 1.3.1.3 below describe the three major stages of the Maillard reaction pathway. In order to aid these descriptions, Figure 1.2 below is a reaction scheme for the pathway as a whole.

Figure 1.2: Simplified kinetic scheme of flavour generation by the Maillard reaction



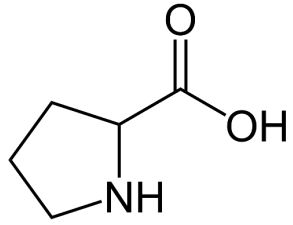
(Jousse et al., 2002)

1.3.1.1 Initial Stage

The early stages of the Maillard reaction consist of a complex network of reactions, involving carbonyl compounds, in the form of reducing sugars; and compounds with a free amino group, which could be present in an amino acid, peptide, or protein (Echavarría et al., 2012, Yoo, 1997). The Maillard reaction begins with the condensation of the sugar and amine in an aqueous solution (Hodge, 1953). The specific reactions that are carried out during the Maillard reaction depend upon the reagents within the raw material foodstuff being thermally processed (Yoo, 1997). In malted barley, the main reducing sugar present is maltose, which is a disaccharide consisting of two glucose molecules which are bound together by a glycosidic bond (Channell et al., 2010). Sucrose is also present in notable amounts in malt. The main

amino acid in malt is proline (Channell et al., 2010). Proline is a cyclic, non-polar amino acid, the structure of which is shown in Figure 1.3. This amino acid commonly gives rise to bready, popcorn flavours when used during the Maillard reaction (van Boekel, 2006).

Figure 1.3: Structure of proline.



The N-glycosylamines that are formed as a result of the condensation reaction are isomerised to form the Amadori or Heyns products (Echavarría et al., 2012). Amadori and Heyns intermediates are thermally unstable, but are more stable than the preceding N-glycosylamines. If the reducing sugar in the reaction is a ketose, the Heyns product will be produced (van Boekel, 2006). The Amadori and Heyns products can then either undergo dehydration or fragmentation. Dehydration results in the formation of furfurals and reductones, whereas fragmentation causes the formation of dicarbonyl compounds (Yoo, 1997). The products of the early stage of the Maillard reaction do not contribute directly to the flavour of the malt. However, the subsequent rearranged intermediates are key precursors of flavour compounds (Yoo, 1997).

1.3.1.2 Intermediate Stage

The Amadori and Heyns products are decomposed in this stage of the Maillard reaction. The amino group is released, and sugar fragmentation products are formed during the intermediate stage of the Maillard reaction (van Boekel, 2006). The Amadori products in the case of malt are maltulose and proline-maltulose (Mori, 2004). The degradation of the Amadori or Heyns products can be carried out by four possible mechanisms, which can involve deoxyosones, fission, or the Strecker degradation (Mlotkiewicz, 1997). The Strecker degradation is discussed in the following section.

1.3.1.2.1 Strecker Degradation

Both the Strecker degradation and the Amadori rearrangement serve the same purpose, which is to reductively aminate different dicarbonyl sugar fragments by the action of amino acids (Yaylayan, 2003). The Strecker degradation is the primary route for the conversion of amino acids to structurally related aldehydes which have significant flavour value (Rizzi, 1999, van Boekel, 2006). Also, the Strecker degradation results in the formation of 2-aminocarbonyl compounds (Yaylayan, 2003). These compounds are reactive intermediates which facilitate aroma generation (e.g. pyrazines) during the Maillard reaction (Yaylayan, 2003). 1-pyrroline, 2-acetyl-1-pyrroline (2-AP), and hydroxyacetone are formed during the Strecker degradation of proline (Rizzi, 1999). 2-AP is characterised by its popped corn and bread crust aroma, which is present in some roasted malts (Rizzi, 1999, Buttery et al., 1997). The Strecker aldehyde 3-methylbutanal is present in roasted malts, and exists in highest amounts in malts of 150 °EBC (Coghe et al., 2004). This is due to its volatility, so is driven off at higher temperatures during processing. The Strecker degradation involves the release of CO_2, and many of the heterocyclic compounds that are responsible for the flavour and aroma of foods are formed during the reactions involved (Echavarría et al., 2012).

1.3.1.3 Final Stage

The final stage of the Millard reaction concerns the formation of nitrogenous polymers and co-polymers, which are brown in colour (Mlotkiewicz, 1997). The majority of the products of the final stage of the Maillard reaction are responsible for the colour of the roasted malts. Low molecular weight (<1 kDa) chromophores, and high molecular weight (>100 kDa) melanoidins are produced. Melanoidins are the dominant colourants in the majority of roasted malts, but can also contain chromophores in equal measure in some roasted malts (Coghe et al., 2006). Colour formation during the Maillard reaction increases with increasing temperature and pH (Mlotkiewicz, 1997).

1.3.1.4 Notable Products of the Maillard Reaction in Roasted Malts

Mori (2004) identified the main volatile products of the Maillard reaction concerning maltose and proline as being DDMP (2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-

pyran-4-one), 2-furanmethanol, 2-furfural, and maltol. This, however, is the case when the Maillard reaction between these two molecules is carried out under controlled conditions, at a pH of 8. During the roasting of malts, this is unlikely to be the case, due to the natural variation within the raw materials used, and the processing conditions themselves. The reducing sugar and amino acid contents of malt does not comprise of maltose and proline alone, as a variation of reducing sugars and amino groups are present.

1.3.2 Caramelisation

Caramelisation is the process of thermal degradation of sugars. Caramelisation is similar to the Maillard reaction, in the respect that it is an example of non-enzymatic browning (Quintas et al., 2007). In addition to this, caramelisation and the Maillard reaction can take place simultaneously (Purlis, 2010). Caramelisation can be used as a method of providing colour to food products, while enabling the development of flavour. The process involves the partial thermal decomposition of carbohydrates (Birch, 1977). This includes complex reactions, which require the presence of reducing carbohydrates, and the absence of nitrogenous compounds (Purlis, 2010). Caramelisation is partially responsible for caramellic flavours in foods and beverages (Kroh, 1994). The reaction leads to the formation of similar products to the Maillard reaction. However, the amino group acts as a catalyst in the Maillard reaction, which causes the formation of the products to occur at higher rates and at higher concentrations in comparison to those during caramelisation (van Boekel, 2006). In addition to this inefficiency, caramelisation occurs at temperatures exceeding 120 °C (Purlis, 2010), above the minimum temperature for the Maillard reaction to occur. The sugar degradation that is carried out during caramelisation includes the initial enolisation reaction, then dehydration, dicarboxylic cleaving, retro-aldol reaction, aldol condensation, then finally a radical reaction (Kroh, 1994). The enolisation reaction is of greatest significance with regard to caramelisation as a whole, due to this reaction initiating the subsequent sugar degradation reactions.

The concentrations of aroma compounds that are formed during caramelisation can be influenced by the temperature and the pH of the surroundings during the reaction (Kroh, 1994). The compounds that typically impart a caramellic aroma in a food or

beverage are present as a result of caramelisation are furans, furanones, pyrones, and carbocyclic compounds (Kroh, 1994).

1.3.3 Lipid Degradation

The lipid component of barley forms 2-4% of the dry weight of the kernel (Arendt and Zannini, 2013). The majority of the lipid distribution in the barley kernel is in the endosperm (~70 %), with the remaining proportion (~30 %) in the embryo (Arendt and Zannini, 2013). The composition of the lipids within the grain will be influenced by barley variety and nitrogen fertilisation, while major differences will be caused by environmental conditions. Ultimately, however, linoleic acid is the main fatty acid in barley, forming 52.4-58.3 % of the grains' lipid composition (Arendt and Zannini, 2013).

Lipid oxidation is a primary source of lipid degradation products which, like the majority of thermal flavour generation reactions, involves a complex chain of reactions (Zamora et al., 2015). As a result of lipid oxidation, alkanes can be formed, which are relatively stable compounds. In addition to these stable alkanes, unstable products are also formed which take place in subsequent reactions. This includes aldehydes, which can degrade as a result of thermal processing (Zamora et al., 2015). However, in dry roasting conditions, the degradation of aldehydes as a result of lipid degradation may be reduced in comparison to when degrading in aqueous solutions. Lipid derived aldehydes are known to contribute to the aroma of foods, in addition to their tendency to take place in further thermal flavour generation reactions (Zamora et al., 2015). The extent to which lipid degradation products can be formed, and the products of the potential reactions is ultimately dependent upon the structure of the lipids, the composition of the matrix, and the temperature at which the roasting occurs (Santos et al., 2013).

1.3.4 Pyrolysis

Pyrolysis is defined as the air-free thermal decomposition of a substance, which typically results in the formation of a combination of gases, liquids, and solids (Lin et al., 2009). The solid product of the reaction is referred to as 'char'. The reaction shares similarities with the Maillard reaction. Wnorowski and Yaylayan (2000) found that many key Maillard products can be formed either as a result of pyrolysis

or by aqueous Maillard systems. The mechanisms by which the compounds are formed are shared in both systems.

Pyrolysis of malt results in the formation of a variety of aroma compounds. During the roasting of barley, the granular starch becomes strongly pigmented and 1,6-anhydroglucose is formed as a result of the reaction (Briggs, 1998a). Yahya et al. (2014) noted that maltol is produced by the pyrolysis of malts, and that pyrrole is formed by the pyrolysis of serine. In addition to this, furfural can be formed as a result of the pyrolysis of glucose (Paine Iii et al., 2008). The formation of furfural as a result of this reaction is most likely to occur during the manufacture of the darkest roasted malts, namely black malt and roasted barley (Yahya et al., 2014). Wnorowski and Yaylayan (2000) found that when a pyrolysis reaction was carried out, it was possible to detect the presence of carbon dioxide, water, methylamine, and acetic acid. They noted that this indicated that pyrolytic conditions of the reaction caused the facile decarboxylation and deamination of amino acids, and the dehydration of sugar.

Pyrolysis of biomass typically occurs at temperatures between 377 °C and 527 °C, whereas the pyrolysis of malts occurs at lower temperatures (Demirbaş, 2000, Tehrani et al., 2002). Temperatures higher than 250 °C are rarely achieved in roasting operations. Pyrolysis during the roasting of malt occurs at the lowest moisture contents and highest processing temperatures, in excess of 200 °C (Yahya et al., 2014). It can therefore be assumed that roasted malts produced using green malt undergo a reduced rate of pyrolysis in comparison to roasted malts produced using pale malt.

There are different classifications of pyrolysis, based upon the heating rate and temperature of the reaction. ‘Slow’ pyrolysis, for example, occurs at lower heating rates in comparison to ‘fast’ pyrolysis (Lin et al., 2009). As pyrolysis occurs at high temperatures, close to the combustion point of malt, it is necessary to quench the roasting malt during the final stages of roasting by spraying water on the hot malt. As discussed above in Section 1.2.1, this acts to reduce the temperature of the malt enough to prevent the combustion of the malt, and thus the development of excessive burnt flavours.

1.3.5 Thermal Flavour Generation in Roasted Malts

The flavour compounds that may be formed during the roasting of malts occur in various combinations. This is dependent on the processing conditions that are used, and the raw material being roasted. Coghe et al. (2006) identified that light coloured roasted malts (i.e. caramalt) contained higher proportions of volatile Maillard products in comparison to darker roasted malts. Darker roasted malts are more likely to contain higher proportions of caramelisation and pyrolysis products. Roasted barley, and chocolate and black malts have high concentrations of melanoidins and heterocyclic compounds, which results in their characteristic bitter taste. This is exaggerated due to the relative lack of reductones and aldehydes, as the preceding saccharification stage is not carried out (Blenkinsop, 1991). This gives the darker roasted products their characteristic colour, and astringent, bitter, and bunt sugar or caramel flavours. Therefore, different malts contain different concentrations of flavour compounds due to the extent to which the various thermal flavour generation pathways have taken place, which is dependent on the raw materials used, and the processing conditions employed.

Blenkinsop (1991) noted that the favourable flavour profiles of crystal malts have enabled the development of new brands of beer with specific flavour profiles. This supports the hypothesis that improved understanding of how flavour can be manipulated through thermal reactions can be used to develop products with specific flavour profiles, tailored to particular products, and the desirable sensory qualities they should possess. In addition to the possible alterations that can be made regarding the process conditions, the combinations in which the roasted malts are used within the grist, and therefore a beer, can also be used to alter the final flavour profile of the product. The number of possible combinations of raw materials, processing conditions, and grist formulations is vast.

Alkylated pyrazines contribute to the roasted aroma of roasted malts (Adams and Kimpe, 2009). Regarding roasted barley and malt products, roasted barley has a higher pyrazine content in comparison to the lighter products that can be produced by roasting (Blenkinsop, 1991). This is due to the high temperatures the roasted barley is subjected to during its roasting. Different pyrazines have different flavour detection thresholds. For example, dimethyl pyrazine has an extremely low flavour threshold, making it easily detectable by consumers (Collins, 1971).

The various malt and barley products of roasting deliver different qualities to beers. For example, roasted products produced from pale malt provide a dry flavour in the beers they are added to (Blenkinsop, 1991). This is related to the biochemical contents of the malt raw material. Pale malt is dried, so will have a low moisture content. The low moisture content will result in the Maillard reaction, caramelisation, and pyrolysis occurring readily upon exposure to the roasting process. The typical flavours of roasted malts produced with pale malt are due to the Maillard heterocyclic compounds characterised by nitrogenous pyrroles and pyrazines, whereas the flavours from roasting green malts are from the oxygen containing furan and pyran types of Maillard heterocyclic compounds (Blenkinsop, 1991).
It is difficult to gauge the effect of specific flavour and aroma compounds in the brewing process, as these compounds may volatilise, whereas others may be formed in reactions during the wort boiling stage of brewing. In addition to this, many substances are metabolised by yeast, so can form alternative substances during the fermentation stage. Flavour and aroma compounds can be lost, created, and altered throughout the brewing process (Briggs, 1998a, Walker and Westwood, 1992).

1.4 Colour Development During Roasting

The colour of beer products is to a large extent determined by the malt used in the brew. This can be provided by pigments produced as a result of the roasting of the malt, and additionally during the wort boiling stage (Nøddekær and Andersen, 2007). Methods of colour determination of wort are discussed in Section 1.8.4. Colour development during the roasting process is influenced by the extent of proteolysis and amylolysis which takes place before the roasting has begun (Blenkinsop, 1991). Following this, thermal reactions physically develop the colour of the malts during roasting. For example, melanoidins with molecular weights above 300 kDa, which are formed in the late stages of the Maillard reaction, are the major sources of colour in malts (Coghe et al., 2003b, Carvalho et al., 2016). These melanoidins have been observed to form at their greatest concentrations in systems roasted at 200 to 220 °C (Tehrani et al., 2002). The colour of a malt can also develop as a result of caramelisation. Quintas et al. (2007) observed colour changes during caramelisation, and noted that the colour development of a substance due to caramelisation occurs as a result of multiple reactions, and not only due to sucrose degradation.

There are a number of factors that affect the final colour of a roasted malt product. For example, Blenkinsop (1991) found that the degree of colour development is dependent upon the surface-area-to-volume ratio of the malt grains. For example, a smaller sized malt grain will have a higher degree of colour formation than a larger grain. In addition to this factor, the final intensity of the colour achieved in coloured malts is also affected by the degree of modification of the raw material, moisture content of the raw material, the time the raw material is held in this condition, and the temperatures the malt is exposed to during roasting (Blenkinsop, 1991).

Coghe et al. (2003b) found that extract content, pH, and moisture content decreased with increasing malt colour. The low moisture content of dark malts would be expected, as Coghe et al. (2006) also found that the rate of colour development of roasted malts is dependent on the time and temperature of the roasting process. For example, it was found that if mild roasting at 120 °C was carried out, the colour development of the malt would increase with time (3.5 °EBC/minute). However, after a colour of 270 °EBC was achieved, this colour would remain with increasing time. This indicated that an increase in temperature would be required to develop the colour of the malt further. In addition to this, Coghe et al. (2006) also established in the same study that colour development in darker roasted malts was much faster than in mild roasting processes, as the colour of these malts formed at a rate of 15 °EBC/minute. Vandecan et al. (2011) found that upon malt roasting, the initial rate of colour development was highest at temperatures between 140 and 180 °C. However, after the initial linear increase in colour at these temperatures, stagnation or even a decrease in colour values was observed (Vandecan et al., 2011). During mild roasting processes, high molecular weight melanoidins are not formed (Coghe et al., 2006). High temperatures are required to stimulate the formation of these Maillard colour compounds.

During the roasting process, visual comparisons with existing malts samples of a known colour will be made. This is done by removing small samples of the malt during its roasting, at regular and frequent intervals, to examine them by cutting or grinding the samples, then comparing them to existing standards (Blenkinsop, 1991). This subjective method of quality control can result in batch-to-batch variation. Coghe et al. (2003b) commented that the colour of malt was the most important factor in dark roasted malts for their differentiation and characterisation. The colour

of malt is the easiest method to differentiate between the various products of roasting malts. A simple visual inspection will indicate the extent to which the malts have been roasted. From this, the general flavours present in the malt can be assumed. For example, if a roasted malt sample has a dark colour, it can be assumed that the sample will have a dry flavour, with burnt, harsh, or bitter taste. However, the colour of the malt will not give an accurate indication of the flavour profile the roasted malt possesses. In order to determine the flavour compounds present in the malt, and therefore its processing potential as a component of grist, further analysis is required.

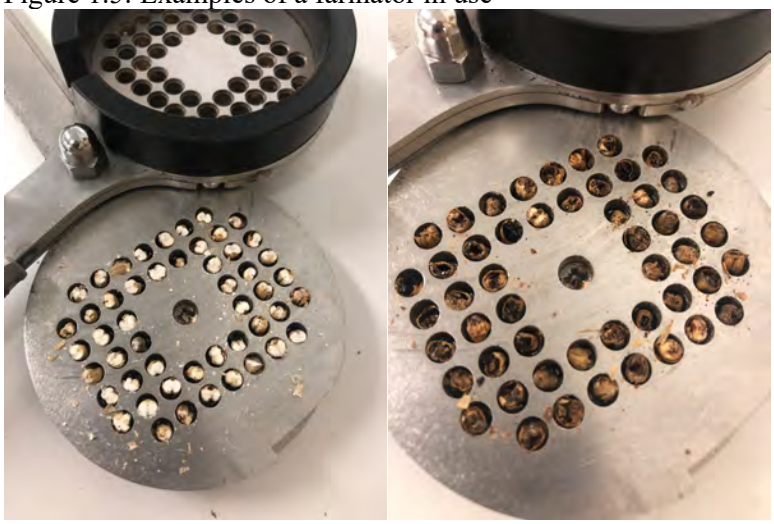
1.5 Visual Analysis of Roasted Malts

1.5.1 Farinator

A farinator is a specialist piece of equipment, used by maltsters to visually assess grains. The farinator cuts a small sample size (usually 50 grains) transversely to allow for the inspection of the endosperm (Miles et al., 2013). An example of a farinator is shown in Figure 1.4, an in use in Figure 1.5.

Figure 1.4: A farinator

Figure 1.5: Examples of a farinator in use



Pale malt (left), and medium crystal malt (right) cut by farinator.

In pale malt, ideal endosperm will appear white, opaque, mealy, and chalky (Briggs, 1998b). Whereas in crystal malts, the ideal endosperm is vitreous, or glassy. This gives an indication of the extent of amylolysis and proteolysis that has taken place in the stewing step (Section 1.2.1.1.2).

1.6 Sensory Analysis Techniques for Roasted Malt Evaluation

The sensory analysis of a product is defined as the quantification of human responses to stimuli perceived by the five senses. Different sensory evaluation techniques may be used by a researcher in order to maximise valuable data. For example, discrimination tests (for example, paired comparison, or triangle tests) are used to identify perceivable differences between products. Whereas descriptive testing yields descriptive lexicons for products, in addition to a basis for comparisons between products, and potential to identify sensory attributes that impact preferences (Stone, 2012).

Sensory evaluation techniques are designed to minimise potential sources of bias in determining consumer perception (Lawless and Heymann, 2010, d'Acampora Zellner et al., 2008). The reduction of bias may be achieved in different ways, depending on the sample being assessed, and also the sensory qualities being evaluated. For example, if a roasted malt was to be assessed concerning only the flavours present in a wort prepared from the roasted malt, it would be necessary to

conceal the true colour of the sample. This is because the colour range of roasted malts varies widely, and the colour of the sample would indicate the flavours to be expected by the panellist. This may result in the panellists' perception of certain flavours without their presence in the sample being assessed, due to the bias introduced by the panellist seeing the colour of the sample. This influence of the colour of a sample on the perceived flavour was noted by Auvray and Spence (2008), stating that the increasing intensity of colour in a sample causes the perceived taste and flavour of the sample to increase in intensity also.

Palate cleansing is essential during sensory analysis. Typically, water and plain crackers are provided, and should be taken between samples. This allows the flavours of the previous sample to be removed from the palate, reducing the risk of their being confused with the detectable flavours in the next sample.

Sensory lexicons have been developed as a descriptive vocabulary for various food types in order to increase the ease of describing the organoleptic qualities being experienced (Limpawattana and Shewfelt, 2010). When developing sensory lexicons, it must be ensured that the descriptive vocabulary of the sensory panel is not restricted, as this can occur if too few descriptors are provided when analysing samples with complex organoleptic qualities. In addition, the use of different languages may become problematic when attempting to translate the chosen descriptors. It is often the case that words do not have a direct equivalent in another language when translated, which would require the use of alternative words, causing differences in results in various languages (Nursten and Reineccius, 1996).

A lexicon for malts was developed by Murray (1999) and included the following descriptors: cereal, sweet, burnt, nutty (green, or roast), sulphur, harsh, toffee, caramel, coffee, chocolate, treacle, smoky, phenolic, fruity, bitter, biscuit, bran, and astringent. Panellists would also have the option of including additional descriptors during sensory analysis, if they believed the existing words did not fit the flavour they experienced.

In contrast to the method of developing an established lexicon for panellists to use, free choice profiling can also be used during sensory analysis. This method allows panellists to carry out descriptive analysis without having a to reach a consensus regarding the list of descriptors (Beal and Mottram, 1993). This method can also be used when a sensory panel is not trained in assessing food samples.

Coghe et al. (2004) found that sensory analysis of malts could be carried out effectively by training sensory panellists to recognise characteristic flavours associated with specific compounds, namely dimethyl sulphide, 3-methyl-butanal, 2-methylpyrazine, 2-acetylthiazole, 2-acetyl-pyrrole, 2-acetylpyridine, maltol, 4-vinylguaiacol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. This was done in order to familiarise the panellists with the typical flavours that may be present in a malt sample, so they may identify them with ease when they occur naturally within a malt wort sample. Then, the panellists were encouraged to develop a descriptive vocabulary based upon the wort samples prepared from the malts. This method is potentially more accurate than providing a prepared lexicon, because the panellists were encouraged to describe the flavours they experienced, rather than to match them with existing descriptors, which may not fit the panellists' experience exactly. Cartier et al. (2006) identified the method of sorting samples into categories. It was noted that this method could be carried out by an untrained panel, in addition to a trained panel. The study involved the sorting of fourteen different breakfast cereals into a minimum of two categorised groups by trained and untrained panels, consisting of 12 and 24 members, respectively. The investigation showed that untrained panellists can be used when carrying out specific methods of sensory analysis. In addition, the study also showed that in comparison to quantitative descriptive analysis, sorting prevents bias by avoiding an overwhelming list of potential attributes.

1.6.1 Investigations into the Sensory Properties of Roasted Malts

Cook et al. (2018) conducted highly relevant sensory evaluation of roasted malt products (sourced from Boortmalt, as in this thesis) and their effects on the sensory qualities of a variety of high grist beers (8-20 % of the grist). Sensory evaluation in this study was carried out by quantitative descriptive analysis. Sensory lexicons were generated for both the roasted products themselves, in addition to the high roasted malt grist lager beers. Comparisons between attribute scoring were made concerning the roasted products and the finished beers. The study identified highly intense flavour characteristics (i.e. burnt, coffee, smoky) transferred from the roasted products to finished beers, imparting similar characteristics. Whereas subtle characteristics (i.e. nutty, biscuit, malt loaf) were not retained in the finished beers.

The study identified the necessity for further examination in order to increase understanding of the origins of the identified sensory effects exhibited by roasted products.

Coghe et al. (2004) also conducted detailed sensory analysis of speciality malts, which included roasted malts. The study identified the flavours present in unboiled worts prepared from dark malts. The method of the sensory analysis is discussed previously in Section 1.6, and involved the training of the panel to a sufficient level to detect flavours, and describe them as they believed appropriate. It was found that the identification of flavours such as 'bitter' and 'burnt' increased with increasing malt colour, whereas the malts with moderate colours were identified as having the most 'bready' and 'caramel' flavours.

Sensory analysis of non-roasted kilned malts was investigated by Beal and Mottram (1993) using free choice profiling. The analysis was carried out on the malt itself, rather than beer produced using the malts. It was noted that this was done in order to assess how the odour of the malts changed during kilning, because creating a beer from these samples would alter the organoleptic properties of the malts as a result of the further processing the samples would undergo. During the study, the panellists were trained to describe defined odours given as an unidentified sample. Those who could not describe these standard odours were omitted from the panel. The panellists were given the opportunity to reassess the samples in order to add further comments. Finally, redundant terms provided by the panel were discarded. This sensory study on the odour of kilned malts showed that increased kilning resulted in the detection of different odours of samples of malt taken throughout the kilning process (Beal and Mottram, 1993). It was shown that the odour of the malt samples began as being fruity and hay-like, then progressed to dried grass aromas in malts kilned to higher temperatures. As kilning continued, the malt was described as having chocolate, bready, malty, roast, and burnt aromas. These results highlight the importance of the influence of the kilning process on the development of odour active compounds.

1.7 Flavour and Odour Threshold

The flavour or odour threshold of a compound is the lowest concentration at which it becomes detectable to humans by sense of taste or smell. Briggs (1998b) noted that many aroma compounds occur in malts below their flavour threshold concentrations,

so are present but undetectable. In addition to this, many of the key odorants in roasted malts are reduced to below their flavour threshold during fermentation, or evaporate during production processes (Vandecan et al., 2010). It has also been noted that macromolecules within a food matrix can interact with aroma compounds, which further affects the release of these compounds during eating (Mistry et al., 1997). This may cause an apparent aroma or flavour caused by the presence of a compound to be reduced, irrespective of its potential abundance within a food matrix. For example, aldehydes often bind to protein side-chains, reducing their sensorial value (Spears and Fascione, 2016). Potentially, many of the aroma compounds present in roasted malts, and the sensory characteristics they have the potential to impart go undetected by the consumer due to a number of factors. Namely the low concentrations at which they are present in the malts, their low detection thresholds, and the subsequent loss of particularly volatile compounds throughout processing. Despite this, in complex mixtures of aroma compounds, compounds present at sub-threshold concentrations can remain influential on the perceived aroma of a product. Interactions between volatile compounds in this manner can enhance or reduce aroma intensity (Zhu et al., 2018).

1.8 Instrumental Flavour Analysis of Roasted Malts

This section discusses the most significant methods of instrumental flavour analysis of roasted malts, including the sample preparation techniques used to optimise analysis.

1.8.1 Sample Preparation Techniques for Flavour Analysis of Roasted Malts

1.8.1.1 Solvent Extraction

Liquid-liquid, or solid-liquid extraction can be carried out by the use of a solvent. The resultant extract can be injected directly as a liquid for analysis by gas chromatography (GC). Solvent extraction is used as a method of separation or purification based upon a number of potential factors: if heat distillation would damage the product, if the solute is present in low concentrations, or if overlapping of volatiles occurs (Todd, 2014).

Typically, the ‘feed’ is the substance containing the soluble compounds of interest, which is combined with the solvent. The choice of solvent should be influenced by such factors as the sample matrix, the solvent’s interaction with analytes, and chromatographic performance (International Oragnisation of the Flavour Industry, 2011). The resultant solvent rich liquid is referred to as the extract, and the insoluble component of the feed is then discarded (Todd, 2014). Solvent extracts can be diluted with additional solvent, or can be analysed undiluted in the case of lower concentrations of volatiles.

Yahya et al. (2014) carried out the most relevant investigation into the flavour generation of commercial barley and malt roasting, using solvent extraction of volatiles from milled samples taken throughout commercial roasting. Using the method of solvent extraction, it was possible to track the formation of specific volatiles over the course of roasting operations across a range of roasted products (Yahya et al., 2014).

Special care should be taken when preparing the isolation of compounds by extraction techniques, as some flavour compounds are very reactive, whereas others readily degrade. Lack of care during sample preparation could result in some of these flavour compounds being lost, and therefore not being detected by instruments and participants alike (Paré and Yaylayan, 1997).

1.8.1.2 Headspace Analysis by Solid Phase Micro-Extraction (SMPE)

Solid Phase Micro-Extraction (SPME) is an advantageous sample preparation technique, as it allows for minimal disruption of the samples being analysed. It is used in combination with gas chromatography (GC). The sample can be analysed in its natural state, with minimal treatments. SPME gives a realistic measurement of the volatiles that are released from the sample, as only the headspace of the sample vial is analysed, and therefore only the volatiles naturally released from the sample matrix are considered (Caporaso et al., 2018). SPME can be applied to analyse almost any food system.

SPME uses a fibre which is coated with a liquid polymer, solid sorbent, or a combination of both (KGaA, 2020). The choice of fibre coating used depends upon the nature of the sample, and the affinity of the volatiles of interest with the coating of the fibre. The fibre coating is responsible for the extraction of the volatiles from the sample by adsorption (regarding headspace analysis).

Typically, the sample vial will be heated and/or agitated for a period of time (for example at 40 °C for 10 minutes (Caporaso et al., 2018)) to equilibrate, encouraging the release of volatiles from the sample matrix. Then the SPME fibre is inserted into the sample vial headspace for adsorption to take place (for example for 32 min (Hill and Smith, 2000)), while the sample vial continues to be heated. After the specified period of adsorption, the fibre is transferred to the injector port of the GC, where desorption of the volatiles takes place. Desorption typically occurs at a higher temperature than adsorption (250 °C for 3 min, for example (Hill and Smith, 2000)), in order to encourage the volatiles to desorb quickly from the fibre to ensure retention times of the eluting compounds are as accurate as possible.

1.8.1.3 Thermal Desorption

Thermal desorption enables the detection of compounds at lower concentrations than GC or MS alone (Grimm et al., 1997), due to a sample enrichment step. Compounds that may be flavour active, but present within a sample in minute quantities may be detected using this method.

Thermal desorption is often coupled with GC in order to analyse volatile organic compounds (Ou-Yang et al., 2016). The volatiles that are extracted from the sample matrix can either be trapped, or direct thermal desorption can be employed. Direct thermal desorption involves sparging volatiles from a sample, then transferring them directly to the head of a chromatographic column, whereas trapping the volatiles with the use of a sorbent trap concentrates the volatiles that are extracted from a sample. Trapping is carried out independently of the GC column, then the sorbent trap is connected to the GC, where the trap is heated during GC elution to remove residual compounds with high boiling points (Ou-Yang et al., 2016).

The use of thermal desorption in combination with GC techniques in order to identify low levels of aroma compounds present in roasted malts has not yet been reported.

1.8.2 Gas Chromatography

Gas chromatography (GC) has been instrumental in advancing the knowledge of food flavour (Delahunty et al., 2006). It allows the separation, identification, and quantification of the compounds which comprise an overall aroma or flavour. GC

has been used extensively in the analysis of beer to aid the identification and quantification of over 600 volatile compounds in different beer types (Castro and Ross, 2018).

A column is used in all GC configurations. Modern GC columns are relatively flexible yet fragile lengths of hollow fused silica which is coiled and held with a metal frame. The column is internally coated with a stationary phase, the polarity of which will be selected based upon the polarity of compounds being separated. The column is housed within an oven with accurate temperature control, securely connected to the injector port and detector by the use of ferrules and nuts.

The mobile phase is the carrier gas, which should be highly purified and free from oxygen, water, and hydrocarbons (International Oragnisation of the Flavour Industry, 2011). Typically, helium or hydrogen are used.

As a mixture of compounds are injected into the column, each interacts with the column in a different way while also being carried along by the carrier gas, resulting in each compound's differing retention time (the time after injection when a compound elutes from the column). The temperature ramp within the oven also influences the retention time of a compound on the basis of boiling point and compound volatility.

1.8.3 Detection Methods

Gas Chromatography can be coupled with a wide variety of detection methods, each favouring the detection of components of interest within a mixture. Detailed below are the relevant detection methods used throughout this research.

1.8.3.1 Gas Chromatography-Olfactometry

Gas Chromatography-Olfactometry (GC-O) involves the simultaneous use of instrumental detection and a sniffing port. The compounds present in a sample are separated via the GC column, and are then delivered through an outlet for a sniffing port (d'Acampora Zellner et al., 2008). When using GC-O in combination with instrumental detection (for example, mass spectrometry), a splitter is used to separate the flow of GC effluent equally between both detectors.

GC-O is a method of sensory analysis which combines instrumental analysis with the use of human participants. The sensory impact of the compounds identified by the

analytical chemistry component of the method must be assessed by a human (Lawless and Heymann, 2010). The human nose is used during GC-O due to its increased sensitivity in comparison to instrumental detectors. This is beneficial, as it has been reported that many odour active volatiles are present at very low concentrations within a food or sample (van Ruth, 2001). By carrying out this method of analysis, the potential aroma compounds that contribute to the flavour of a product can be identified (Lawless and Heymann, 2010).

The methods that can be employed to quantify the potency of the odour identified can be dilution analysis, which may be Aroma Extract Dilution Analysis (AEDA); detection frequency methods; and intensity methods (van Ruth and O'Connor, 2001). During the AEDA method, a sample extract is sniffed at multiple dilutions until the odours are no longer detected. The dilution factor is the last dilution at which the volatiles can be detected (van Ruth and O'Connor, 2001). Detection frequency requires 6-12 participants, who sniff the undiluted extract, while recording the start and end times of the odours. The number of assessors that detect the odour simultaneously is used as a measure of intensity of the compound (van Ruth and O'Connor, 2001). Whereas in intensity methods, the perceived intensity of a compound may be recorded by the participant in the form of a scale.

The discomfort experienced by panellists at the sniffing port is a result of the requirement to remain seated in an uncomfortable position for long periods of time. Panellists are asked to sniff dry, hot air for sessions often exceeding 30 minutes. Sniffing dry air reduces the panellists' sensitivity, causing nasal dehydration over long periods of analysis. In order to combat these discomforts, the air should ideally be humidified to reduce nasal dehydration, and sessions should not exceed 25 minutes to attempt to reduce nasal fatigue (Delahunty et al., 2006). The sniffing port should also be isolated from any ambient odours, as these may interfere with the detection of volatiles (d'Acampora Zellner et al., 2008). As with any sensory analysis, panellists and panel leaders should refrain from wearing perfume on the day of analysis, and avoid strong smelling food or drink at least two hours before the analysis.

GC-O enables the identification of the odour active volatiles in a sample, and the degree to which those compounds are detectable. However, panellists can only describe an aroma when it occurs, rather than identify and quantify that compound.

When used in combination with other detection techniques (MS, for example), the odour active compounds underlying an aroma can be assigned. It has been noted that of the great number of volatiles present in a food, very few contribute directly to the flavour and aroma of the food (van Ruth, 2001). For example, Coghe et al. (2004) identified that of the 250 volatile compounds identified in dark malts, not all of them contribute to flavour. In addition, GC analysis alone does not give an indication of an aroma's significance within a sample as a whole. The peak area of a compound is not directly proportional to the intensity of its aroma within the sample (d'Acampora Zellner et al., 2008). However, GC-O utilises the human nose as a detector, identifying compounds that are likely to be significant sensorially, particularly in combination with techniques such as AEDA, to identify those which remain odour active at high dilution factors.

Despite this, some of the compounds identified by panellists during GC-O work may not be flavour active when within the original food material (Mistry et al., 1997). The compounds present within the flavour matrix of the food may interact with each other when in a solution as a whole, but can appear to have a different aroma when isolated as a single compound.

1.8.3.1.1 Aroma Recombination Studies

GC-O analysis can be followed, in some cases, by aroma recombination studies. Aroma recombination is a method of verification of the previous GC-O results. The method of analysis uses the data obtained from GC-O to recombine the key odorants that were identified, in specific combinations in an attempt to mimic the odour of the original food (van Ruth, 2001). Aroma recombination studies involve the use of a sensory panel, who determine how close the reconstituted product aroma is to that of the authentic sample.

1.8.3.2 Mass Spectrometry

Mass spectrometry (MS) is a destructive method of analysis: the reaction cannot be reversed. MS has three main stages: the ionisation of molecules, the separation of the resultant ions according to their mass-to-charge-ratio, and the determination of the abundance of each of these ions (Paré and Yaylayan, 1997). The results of MS allow the researcher to identify and quantify compounds present within a complex mixture.

The principle of electron impact (EI) MS involves the use of a high-energy electron beam (e^-), which is directed towards a subject of interest (M). This causes an outer electron from M to be removed, resulting in the formation of an ion-radical ($M^{+\cdot}$). This ion-radical is often referred to as a 'molecular ion' (Paré and Yaylayan, 1997). This basic principle of MS can be simplified in the form of an equation, as follows:

$$M + e^- \rightarrow M^+ + 2e^-$$

(Paré and Yaylayan, 1997).

The ion-radical commonly undergoes cleavage to form fragment ions (MacLeod, 1973). The fragmentation is specific to the structure of the molecule. The fragment ions are transferred to a mass analyser, in which they are subjected to electromagnetic forces which separate the ions according to their mass-to-charge-ratio (*m/z*) (Paré and Yaylayan, 1997).

MS is commonly combined with gas chromatography (GC-MS), as the effluent from the chromatograph, which contains separated gaseous solutes, is passed directly into the mass spectrometer (MacLeod, 1973). This method is particularly valuable when used during food flavour analysis (MacLeod, 1973, Paré and Yaylayan, 1997). Channell et al. (2010) investigated the possibility of monitoring flavour development during the roasting of the malts by the use of on-line mass spectrometry. It was found that on-line MS (by atmospheric pressure chemical ionisation (APCI)-MS) could be used to effectively study flavour generation in roasted malts. The high temperatures that are reached during the roasting of malts results in the volatility of significant flavour compounds (Channell et al., 2010). These high processing temperatures cause small volatile aldehydes, which are key flavour compounds in kilned malts, to be driven off. The on-line MS analysis facilitated real time analysis, as the thermally generated flavour compounds were carried into the mass spectrometer as they were formed and volatilised. The use of APCI-MS allowed for atmospheric pressure to carry volatile compounds to the MS, as opposed to the use of vacuum as in EI-MS. This study did not consider the flavour active compounds that were formed within the roasted malt grain during roasting, as only the aromas released from the roasting malt were analysed. However, Yahya et al. (2014) considered this aspect of flavour formation during malt roasting. In the study, malts were roasted in a commercial plant, while samples of the roasting malts were removed at various stages throughout the roasting process, immediately frozen in liquid nitrogen and stored for subsequent analysis. The samples were extracted in methanol (discussed above in Section

1.8.1.1) and analysed by GC-MS, identifying the flavour compounds present and the time course of their production during roasting within the roasted products. The study provides the foundation of analysis into the formation of odour active volatiles in roasted malts, expanded upon in the series of investigations carried out over the course of this research.

1.8.3.3 Pulsed Flame Photometric Detection (PFPD)

Pulsed flame photometric detection (PFPD) is another destructive method of compound detection. PFPD can be used as either sulphur specific or phosphorous specific detection methods (detection limits 180 fg/s and 7 fg/s, respectively) (Amirav and Jing, 1995). In this research, PFPD was used as sulphur specific detection. PFPD can be used when Mass Spectrometry is insufficiently selective regarding the detection of the low concentrations of sulphur containing compounds (Li et al., 2008).

PFPD functions by combining the flow of a mixture of hydrogen gas and air with the compounds that elute from the GC analysis column, both of which are continuously fed to a pulsed flame chamber (Amirav et al., 2015). Within the chamber there is another flow of hydrogen and air mixture, which ignites a flame from a light-shielded wire, which is continuously heated. The flame is extinguished after a few milliseconds. Due to the continuous flow of hydrogen and air, the flame is reignited after a few hundred milliseconds, creating the pulsed periodic flame. To detect the compounds being ignited, the light emitted from the flame is detected with a photomultiplier (Amirav et al., 2015).

PFPD is usually used in combination with headspace SPME (see Section 1.8.1.2) as pre-concentration of volatile sulphur compounds is usually necessary due to the low concentrations present in samples (Bravo et al., 2005, Hill and Smith, 2000, Fang and Qian, 2005). However, analysis of the sulphur containing volatile compounds in roasted malts using PFPD has not previously been reported.

1.8.4 Methods of Colour Determination of Wort

The determination of the colour of a roasted malt is the simplest method of differentiation between the various products of roasted malts. The colour of the

product will indicate the extent to which the malts have been roasted, and the flavours to be expected from the malt being assessed.

1.8.4.1 International Commission on Illumination (CIE) L*a*b* Method

Coghe et al. (2003b) found that the CIE *L*a*b** method for the examination of darkness and colour shades in wort is effective in enabling the differentiation between wort samples containing different malt types. This method is also successful when determining the colours of hazy beers and worts (Sharpe et al., 1992). It involves a tristimulus measurement, assessing the hue, value, and chroma of a colour. *L** indicates the value (lightness or darkness) of a colour, and the a* and b* values describe the hue (the actual colour). The chroma (vividness or dullness) can be calculated using the following equation:

$$\text{Chroma C}^* = \sqrt{a^{*2} + b^{*2}}$$

(Sharpe et al., 1992).

The results of the method can be converted to °EBC (Sharpe et al., 1992). The CIE *L*a*b** method is also used during the determination of the colour development of coffee during roasting (Santos et al., 2016). As the methods of coffee roasting and malt roasting share similarities, it is reasonable to assume that the methods of analysis of the roasted products could also be shared.

1.8.4.2 Spectrophotometric Method

Spectrophotometric methods of colour analysis were carried out in Chapter 3. Detail of colour determination according to this method are specified in Section 2.6.

In this method of colour determination, a single 'slice' of light is assessed, and is therefore unable to assess the 'true' colour of the sample. In addition, hazy samples are likely to cause back scattered light due to the suspended particles within the sample (Sharpe et al., 1992).

1.8.4.3 Coloured Glasses

This method is the least reliable of all the methods of malt colour determination. It relies on the subjective judgement of an individual, as they make visual comparisons of the colour of Congress wort with coloured glasses. The glass discs have colours ranging from 2 to 27 °EBC (Coghe et al., 2003a). Issues with this method include the

ageing of the coloured glass discs over time if stored incorrectly, and the operator of the system becoming optically fatigued, making them unable to compare colours effectively (Sharpe et al., 1992). In addition to this, the coloured discs may not match the exact colour of the sample being assessed.

1.9 Summary

Understanding thermal flavour generation in roasted products is fundamental in implementing process control and precision in the final products' flavour contribution. This research will build upon previous extensive research into thermal flavour generation by the use of sensory and instrumental analysis techniques. The analysis of commercially available roasted malts will form the basis of the following investigations, ultimately providing detailed knowledge of product flavour to roasters. This will include focus on key odour active volatile compounds, and their formation throughout roasting across the product range. The identification of processes by which novel products can be generated may be possible from this research by the increased understanding of thermal flavour generation across a variety of roasting conditions.

The following thesis examines and discusses four major investigations, firstly considering odour active volatile compound generation in roasted products (Chapter 3, Chapter 4, and Chapter 5), followed by the observation of the effects of these volatile compounds on the aroma qualities of the products themselves (Chapter 6).

Chapter 2 - General Materials and Methods

2.1 Commercial Roasted Samples

Nine commercial roasted malt samples were sourced from Boortmalt (Pauls Malt Ltd., Knapton, UK): roasted barley, black malt, chocolate malt, pale chocolate malt, dark crystal malt, medium crystal malt, light crystal malt, caramalt, and amber malt (20 kg sacks of each roasted product).

Portions of the samples were milled to produce a fine powder, according to Section 2.5, then vacuum-sealed in foil-lined pouches and stored at -80 °C ready for subsequent analysis. Small quantities of each unmilled roasted product were also vacuum-sealed in foil-lined pouches at -80 °C for later use. These commercial samples were used throughout the studies reported in this thesis.

2.2 Laboratory Malting Materials

All laboratory malted products were produced from the same batch of a winter variety of malting barley (Flagon) provided by Crisp Malt Ltd (Fakenham, UK). Following intake, the 20 kg sack of raw barley was stored in a dark ambient dry store, and used within one month.

2.3 Laboratory Scale Malting

Barley was micromalted using a Custom Lab Micromaltings K steep-germinator and kiln (Custom Laboratory Products, Keith, UK). The steep germinator housing four drums (500 g barley per drum) was used to produce the green malt and pale malt for analysis. Malting was carried out under the following conditions: Steeping (16 °C) 7 h wet, 12 h dry, 8 h wet, 12 h dry, 4 h wet. Germination: 5 days at 16 °C. The drums were mechanically rotated every 10 minutes to prevent matting of rootlets. The green malt produced was then either refrigerated (0-5 °C) and roasted within the next 12 h, or kilned to produce pale malt. Kiln programming to produce pale malt was as follows: 55 °C for 12 h, followed by 65 °C for 6 h, then 85 °C for 2 h, and finally 95 °C for 2 h. The pale malt was then cooled to ambient temperature before removing rootlets manually over a grain sieve. Samples were vacuum packed in foil-lined pouches, and stored at -80 °C for use within one month.

2.4 Laboratory Scale Roasting

2.4.1 Laboratory Scale Roaster

A purpose built laboratory scale roaster, capable of precise time-temperature control, was fabricated with the assistance of the University Engineering faculty workshops. It comprised a GC oven (Hewlett Packard (HP) 6890 Series GC System) which was modified to accommodate a mini roasting vessel fabricated from stainless steel mesh (drum dimensions: 8 cm diameter x 15 cm length. Mesh: 2×2 mm) which could be rotated during heating at speeds relevant to commercial roasting. A diagram of the laboratory scale roaster is shown in Figure 2.1.

Figure 2.1: Diagram of Laboratory Scale Roaster

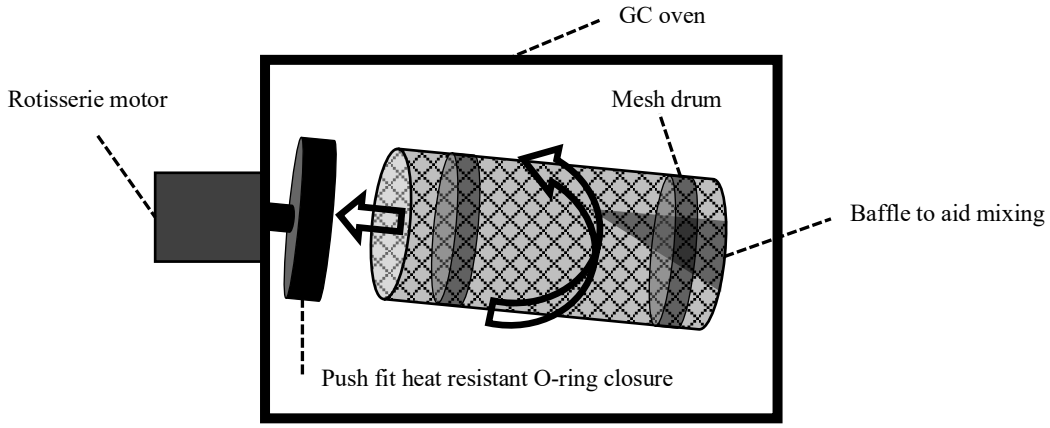


Diagram not to scale.

The roasting substrate sample (~100 g batch size) was filled into the mesh drum and then attached to a rotating shaft via a push fit closure sealed with a heat resistant O-ring to secure the drum, while allowing easy release from the rotating component when roasting was complete. A barbecue rotisserie motor (GM012 model, BBQ Foukou, Korakas, Cyprus) was used to rotate the mesh drum (at 43 RPM) within the GC oven. The use of the GC oven allowed accurate temperature control during roasting.

2.4.2 Production of Laboratory Roasted Products

Preliminary experiments were conducted using the laboratory scale roaster to determine the time ranges within which each substrate could be heated at

temperatures between 100-230 °C to achieve representative roasted products. These ranges of time-temperature encompassed the realm of normal roasted products and also some additional extremes such that at the edges of design spaces some samples were not dried down to typical finishing moisture content or at the top end some samples bordered on the 'burnt toast' end of roasting.
Roasting parameters (isothermal in each case) for the roasting substrates were selected as follows:

- Pale malt and raw barley:
    - Time: 10 min - 30 min
    - Temperature: 100 °C - 230 °C
- Green malt:
    - Time: 20 min - 50 min
    - Temperature: 135 °C - 165 °C

Green malt was first 'stewed' in a sealed glass bottle at 65 °C for 1 hour in a laboratory oven (Genlab Ltd., Cheshire, UK) before being transferred to the roasting drum and roasted under a series of time-temperature conditions within the above boundaries and as determined using experimental design software.
Design Expert (Version 11, StatEase, Minneapolis, USA) was used to create a 24 point D-optimal response surface design based on the above ranges of time and temperature for each of the three roasting substrates. Each substrate was then roasted using the 24 different combinations of time and temperature according to the D-optimal design. Run order was fully randomised within the design. After roasting, the products were immediately removed from the drum, and frozen in liquid nitrogen (-196 °C). At this stage, the rootlets of frozen roasted green malt products were removed whilst brittle. The roasted products were vacuum packed, and stored at -80 °C prior to analysis.

2.5 Milling

Malt samples were milled using a Buhler Miag disc mill (Uzwil, Swizerland), to a fine powder (0.2 mm). The mill was vacuum cleaned between samples, to avoid cross-contamination.

2.6 Colour Analysis

Colour was evaluated according to the standard EBC Analytica methodology, analysed by spectrophotometric methods (EBC-Analytica, 2000b).

2.6.1 Mashing Parameters

Firstly, a congress mash of each sample being analysed was produced according to Method 4.5.1 of EBC Analytica (available online) (EBC-Analytica, 2004) using a 1-Cube Mash Bath (Havlíčkův Brod, Czech Republic). For the preparation of roasted malt mash, the milled roasted sample (25 g) was combined with pale malt (25 g). The malts were mixed together with 200 g water (at 45 °C). The beaker containing the malt water mixture was then stirred in the mash bath for 30 min at 45 °C. After which, a temperature increase of 1 °C/min was introduced for 25 min reaching 70 °C, which was held for 60 min. The mash was then cooled to 20 °C. Water was then added to reach 450 g beaker contents.

2.6.2 Filtration

The contents of the beaker were emptied fully into a filter paper above a conical flask. The first 100 mL filtrate were returned to the funnel. 50 mL of the filtrate was immediately passed through a Millipore Millex HA 0.45 μm membrane filter (Merck KGaA, Darmstadt, Germany). The wort was established as clear by <0.02 absorbance at 700 nm when compared to water.

2.6.3 Colour Determination by Spectrophotometric Methods

A Biochrom Ultrospec 2100 pro Spectrophotometer (Biochrom, Holliston, MA, USA) was used to analyse the filtered wort. Absorbance was measured at 430 nm. The wort was diluted as appropriate to allow for absorbance readings within the limits of the spectrophotometer.

To calculate the colour units in °EBC, the following equation was used:

$$°EBC = (25 \times A_{430} \times DF) \times 2$$

Where:
A_{430} = Absorbance at 430 nm
DF = dilution factor

2.7 Preparation of Flavour Extracts by Solvent Extraction

Methanol (16 mL) containing an internal standard (5-nonanone, 5 µg/mL) was added to 8 g of milled roasted malt sample in a sealable glass vial and mixed on a roller bed for 30 min, then transferred to a centrifuge tube by Pasteur pipette and centrifuged at 4000 *g* for 10 min. The supernatant was then transferred to 2 mL GC vials and stored at -80 °C prior to analysis. Methanol (HPLC/ LC-MS grade) used for solvent extraction of volatile compounds was sourced from VWR International Ltd.

2.8 Gas Chromatography Operating Conditions

The volatile compounds within the samples were separated using a Gas Chromatograph (details of specific GC models in individual Chapters) fitted with a ZB-Wax column (30 m × 0.25 mm ID × 1.0 µm film thickness; Phenomenex, Macclesfield, UK). The oven temperature was programmed as follows: 40 °C for 1 min, then a temperature ramp at 4 °C/min to 220 °C, holding for 10 min. Helium carrier gas was used at 18 psi. The injector was operated at 240 °C, 1 min, in splitless mode.

2.9 Selected Ion Methods (SIMs) of Targeted Detection

The MS was run in selected ion mode (SIM) to identify specific compounds of interest. *m/z* values monitored in each method are detailed in Sections 2.9.1 and 2.9.2. The selected ions were monitored only for the corresponding time window in which the compound would elute from the column to prevent overburdening the method.

A guard column was used to prevent the impurities within the flavour extracts degrading column performance, that would otherwise have resulted in reducing the accuracy of the peak areas recorded. The guard column and injector liner were changed after every 24 injections of samples to retain accuracy of data.

2.9.1 *m/z* values monitored in selected ion method 1

The *m/z* values monitored of the compounds of interest in SIM1: 2-methylfruan (81, **82**), hexanal (**56**, 82), pyrazine (53, **80**), 2,3-dimethylpyrazine (67, **108**), furfural (95,

96), 2-n-pentylpyridine (**93**), methyl-2-furoate (95, **126**), phenylacetaldehyde (91, **120**), 2-(5H)-furanone (55, **84**), furaneol (85, **128**), hydroxymethylfurfural (97, **126**).

2.9.2 *m/z* values monitored in selected ion method 2

The *m/z* values monitored of the compounds of interest in SIM2: pentanal (**58**, 86), 1-methylpyrrole (80, **81**), 2-pentylfuran (81, **138**), acetic acid (45, **60**), 5-methylfurfural (109, **110**), 2-acetyl-5-methylfuran (109, **124**), 2-furanmethanol (**97**, 98), maltol (71, **126**), 2-formylpyrrole (94, **95**).

2.10 Moisture Content Determination

The moisture content of roasted samples was determined according to EBC Analytica Method 4.2 (available online) (EBC-Analytica, 2000a). For each sample, three replicate determinations were carried out. 5 g of milled malt was weighed into a lidded metal pot, then placed in a laboratory oven (Genlab Ltd., Cheshire, UK) at 105 °C for 3 hours. After which, the loss in mass was calculated as a percentage. Samples with moisture content <5% were considered to be 'finished' roasted products.

Chapter 3 - The identification of odour active volatiles in roasted malts and roasted barley by Gas Chromatography-Olfactometry (GC-O)

3.1 Introduction

Commercial roasting operations produce a variety of roasted products, each generated from a particular roasting substrate, to varying roasting times and temperatures. The brewers' choice of roasted malts used in a brew will impact the final flavour and colour of a beer. It is important to understand the aroma contribution of the range of roasted products in order to accurately predict the impact they will have on the final product. The use of flavour analysis by gas chromatography is a useful tool in the separation, identification, and quantification of volatile compounds. However, the concentration of a compound alone does not give an accurate representation of its contribution and influence on the sensorial qualities of a product. In addition, many odour active volatiles are present at very low concentrations within a sample (van Ruth, 2001).

As described in detail in Chapter 1 Section 1.8.3.1, gas chromatography-olfactometry (GC-O) gives an indication of the predicted impact of each compound within the sample mixture. The olfactory bulb is more sensitive than an instrumental detector, therefore the aroma qualities of a sample must be assessed by a human participant (Lawless and Heymann, 2010). The GC-O technique allows the identification of the aroma compounds that contribute to the flavour of a product (Lawless and Heymann, 2010).

Considerable research has been carried out to determine which compounds are formed as a result of thermal flavour generation (Hwang et al., 1993, Mori, 2004, Rizzi, 1999, Smit et al., 2009, van Boekel, 2006). Whereas relatively little has been done to determine which of these compounds are flavour active, and furthermore which are the most influential in their impact on the aroma across the variety of roasted malts. The significance of each compound within the range of roasted products must be considered, due to the diversity in product characteristics.

This study considers the odour activity of the compounds detected by a GC-O panel in addition to instrumental identification, combining both methods of analysis. This allows for a comprehensive evaluation of the range of roasted malts, including their

specific aroma qualities that are a direct result of the presence and odour activity of compounds due to thermal flavour generation reactions.

3.2 Materials and Methods

3.2.1 Samples

Six of the nine available commercial samples were analysed in this study, as detailed in Section 2.1: amber malt, caramalt, medium crystal malt, chocolate malt, black malt, and roasted barley. The reduced range of six products were selected to examine due to time limitations, as GC-O is a highly time-consuming form of analysis.

3.2.1.1 Sample Roasting Substrates

3.2.1.1.1 Pale Malt

Of the six analysed samples, amber malt, chocolate malt, and black malt are produced from the roasting of pale malt. Pale malt is the kilned (dried) product of the malting process. For more details, see Chapter 1 Section 1.2.1.1.1.

3.2.1.1.2 Green Malt

The roasted green malt products analysed in this study were caramalt and medium crystal malt. These malts are often referred to as crystal malts. The production of crystal malts is detailed in Chapter 1 Section 1.2.1.1.2.

3.2.1.1.3 Raw Barley

Roasted barley is the only unmalted barley sample analysed in this study. When roasted, it has not undergone any of the three stages of malting, as detailed in Chapter 1 Section 1.2.1.1.3.

3.2.2 Colour Analysis

The EBC Analytica standard method of determining colour was used, as detailed in Section 2.6.

3.2.3 Chemicals

Authentic analytical standards (>90% purity) were purchased to confirm the identify the odour active compounds within the roasted samples. Suppliers of chemicals are displayed in Table 3.1.

Table 3.1: Source of authentic analytical standards.

Compound	CAS	Purity (%)	Source
1-methylpyrrole	96-54-8	>99	Sigma Aldrich
1-octen-3-ol	3391-86-4	>98	Sigma Aldrich
1-pentanol	71-41-0	>99.8	Sigma Aldrich
2-acetyl-5-methylfuran	1193-79-9	98	Sigma Aldrich
2-acetylfuran	1192-62-7	>98.5	Sigma Aldrich
2-acetylpyrrole	1072-83-9	>98.5	Sigma Aldrich
2-acetylthiazole	24295-03-2	99	Fisher Scientific
2-ethoxythiazole	15679-19-3	>99	Sigma Aldrich
2-ethylfuran	3208-16-0	>99	Sigma Aldrich
2-formylpyrrole	1003-29-8	99	Fisher Scientific
2-furanmethanol	98-00-0	98	Sigma Aldrich
2-methyl-5-(methylthio)furan	13678-59-6	99	Fisher Scientific
2-methylbutyraldehyde	96-17-3	>95	Sigma Aldrich
2-methylbutyric acid	116-53-0	>97	Sigma Aldrich
2-methylfuran	534-22-5	99	Sigma Aldrich
2-methyltetrahydro-3-furanone	3188-00-9	98	Sigma Aldrich
2-methylthiophene	554-14-3	>98.5	Sigma Aldrich
2-n-pentylpyridine	2294-76-0	98	Fisher Scientific
2-pentylfuran	3777-69-3	>97	Sigma Aldrich
2,3-dimethylpyrazine	5910-89-4	>98	Sigma Aldrich
2,4-nonadienal	5910-87-2	>90	Sigma Aldrich
2,5-dimethylpyrazine	123-32-0	>98.5	Sigma Aldrich
2,6-dimethylpyrazine	108-50-9	98	Sigma Aldrich
2(5H)-furanone	497-23-4	98	Sigma Aldrich
4-cyclopentene-1,3-dione	930-60-9	95	Sigma Aldrich
4-ethylguaiacol	2785-89-9	>98	Sigma Aldrich
HMF	67-47-0	99	Sigma Aldrich
5-methyl-2-furanmethanol	3857-25-8	97	Fisher Scientific
5-methylfurfural	620-02-0	>98.5	Sigma Aldrich
Acetic Acid	64-19-7	>99.8	Sigma Aldrich
Cyclopenten-1-one	21835-01-8	97	Sigma Aldrich
Ethylpyrazine	13925-00-3	98	Sigma Aldrich
Furaneol	3658-77-3	>99	Sigma Aldrich
Furfural	98-01-1	>97.5	Sigma Aldrich
Hexanal	66-25-1	>95	Sigma Aldrich
Hydroxyacetone	116-09-6	90	Sigma Aldrich
Maltol	118-71-8	99	Fisher Scientific
Methyl-2-furoate	611-13-2	98	Sigma Aldrich
Pentanal	110-62-3	>97.5	Sigma Aldrich
Phenylacetaldehyde	122-78-1	95	Sigma Aldrich
Pyrazine	290-37-9	>99	Sigma Aldrich
Pyrrole	109-97-7	>98	Sigma Aldrich
Sotolone	28664-35-9	>97	Sigma Aldrich
Thiophene	110-02-1	>99.5	Sigma Aldrich
Undecanal	112-44-7	>98	Sigma Aldrich

3.2.4 Flavour Extract Preparation

Flavour extracts of the six malt samples were prepared according to Section 2.7. Methanol with 5 μg/mL 5-nonanone (internal standard) was used to dilute the flavour extracts to the following dilution factors (DF): 10, 100, and 1000. The diluted flavour extracts of the samples were then transferred to 2 mL gas chromatography vials and stored at -80 °C ready for analysis.

3.2.5 Panellist Recruitment

Following ethical approval (approval code SBREC160125A), four female panellists were recruited using posters, which were displayed around the University campus (Sutton Bonington, The University of Nottingham, UK); and via e-mail to postgraduate students within the Division of Food Sciences. Financial gratuity (£125) was provided to each panellist upon completion of all required sessions. The commitment of each panellist to complete the full series of GC-O sessions was essential.

3.2.6 GC-MS Operating Conditions

The volatile compounds within the flavour extracts were separated using a Trace GC Ultra Gas Chromatograph (Thermo Scientific, Waltham, MA, USA), according to Section 2.8.

3.2.7 Gas Chromatography-Olfactometry

The GC effluent was split equally between the MS (Thermo Scientific, Waltham, MA, USA) and odour port by the use of an SGE Silflow three port column splitter (Trajan, Ringwood, Victoria, Australia). The portion of column leading to the odour port was fed out of the GC oven and maintained at 200 °C using a heated transfer line to maintain volatility and peak integrity at the odour port. The sniffing port was cleaned with methanol between samples to prevent the false detection of residual aroma compounds. GC-O sessions were limited to a maximum of 30 min, with a minimum of a 30 min break between sessions, in order to reduce olfactory fatigue (Feng et al., 2015). During the GC-O sessions, panellists were required to describe aromas immediately as they eluted from the column (free generation of personal aroma descriptors) and score the perceived intensity of each aroma on a scale of 1 to

3 (low to high). The retention time of each reported aroma was recorded. The GC-O panel analysed the flavour extracts in the following order: caramalt, chocolate malt, black malt, amber malt, medium crystal malt, and finally roasted barley.
The panel (n=4) was not trained to describe the aromas they detected in a specific way. This was done in order to ensure that the panelists were efficient with their descriptions and would report the detection of aromas as soon as possible during analysis sessions. The panel described aromas using their own vocabulary, which could be interpreted from their personal descriptions (e.g. 'bovril'), to more standard descriptors (i.e. 'meaty, savoury').

3.2.7.1 Aroma Extract Dilution Analysis (AEDA)

When processing the data, the presence of an aroma was reported when at least 50% of the panellists detected a similar aroma quality within the same 30 second window during the analysis of a sample. The flavour dilution (FD) factor of a compound was defined as the maximum dilution at which the aroma could be detected by at least 50% of the panel (Kaneko et al., 2012). The FD factor of each compound within each roasted sample was determined.

3.2.8 Compound Identification

Compounds were identified based upon five levels of validation: linear retention index (LRI) against alkanes (C8-C22) when compared to literary sources on the same WAX phase; LRI comparison with authentic standards when assessed under the same chromatographic conditions; LRI comparison with compounds identified in the same samples by thermal desorption (detailed in Section 3.2.9); EI-MS library matching; and aroma description in comparison with online reference aroma descriptions.

3.2.9 Thermal Desorption (TD)

Compound identification was also derived from thermal desorption (TD) data, in which the same roasted products were analysed more comprehensively using TD-GC-MS-MS than was possible during the relatively short GC-O separation/ run time. Kovat's linear retention indices (LRI's) were used to cross correlate the two data sets.

3.2.9.1 TD-GC-MS Operating Conditions

Volatile compounds were firstly trapped onto a Tenax TD tube (Buchem B.V., Apeldoorn, The Netherlands) by a flow of N_2 gas (45 mL/min) which passed over the milled malt sample (20 g dry, or 10 g with 25 mL H_2O), which was stirred in a sealed vessel, held in a water bath (35 °C). The malt was warmed and stirred for 5 minutes without N_2 flow, then with N_2 for 30 minutes. A TD-100 autosampler was used (Markes International Ltd, Llantrisant, UK), allowing the analysis of the contents of the TD tubes. The volatile compounds within the TD tubes were separated using a Trace 1300 Gas Chromatograph (Thermo Scientific, Waltham, MA, USA) fitted with a ZB-Wax column (30 m × 0.25 mm ID × 1.0 μm film thickness; Phenomenex, Macclesfield, UK), and TSQ 8000 Evo triple quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). The helium carrier gas was at a constant pressure (14.5 bar). The oven temperature was programmed as follows: 30 °C for 15 min, then an initial temperature ramp of 1 °C/min to 40 °C, held for 15 min, then a final temperature ramp of 5 °C/min to 220 °C, held for 2 min.

3.2.9.2 TD Compound Identification

The LRI values for the EI-MS library identified compounds from the TD data were calculated and compared with the LRI values reported in the literature on a WAX column. Compounds confirmed to be present in the roasted product samples from this highly sensitive analysis method were used to identify candidate compounds giving rise to odour active peaks at similar LRI values in the GC-O study.

3.2.10 Compound Quantification

After identification of odour active peaks by GC-O, quantification of 20 compounds was carried out across the commercial sample set. The undiluted flavour extracts of the roasted samples were re-analysed using GC-MS. A Trace 1300 Gas Chromatograph (Thermo Scientific, Waltham, MA, USA) was used with an ISQ QD single quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) to analyse 100 % of the effluent. GC-MS operating conditions were as described in Section 2.8.

Compound concentrations were calculated after correction against the internal standard (5 μg/mL 5-nonanone) using external standards of the 20 selected compounds, each at the following concentrations: 0.5, 1, 5, 10, and 25 μg/mL. The MS was run in selected ion mode (SIM) to identify the specific compounds of interest. This method of compound identification and quantification is detailed in Section 2.9.

3.2.11 Data Analysis

Principal component analysis (PCA) was carried out using XLSTAT software (Addinsoft, SARL, Paris), in order to depict the relationship between the FD factors of the identified compounds and the range of roasted products.

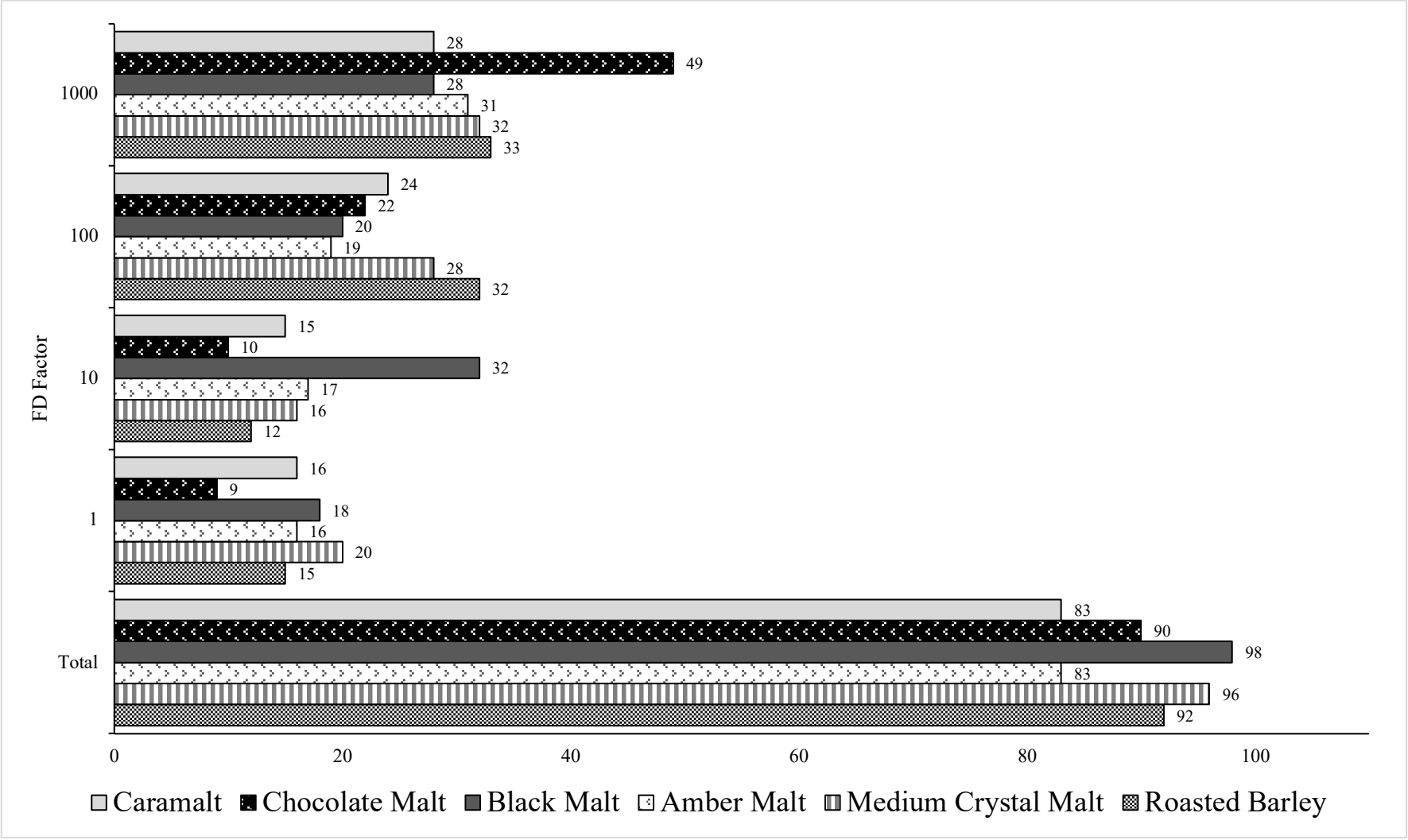
3.3 Results and Discussion

3.3.1 Aroma Detection by the GC-O Panel

During the initial stage of analysis, odour active peaks were identified by the panel using Aroma Extract Dilution Analysis (AEDA). As detailed in Section 3.2.7.1, the presence of an aroma was confirmed when ≥50 % of the panel detected the aroma in the dilution of the sample being analysed. For each dilution (DF 1, 10, 100, and 1000), this process was repeated. From this, it could be determined how many aromas remained detectable throughout the dilutions, and therefore identify the FD factor of the compounds being detected. The FD factor of a compound was defined as the maximum dilution at which the aroma could be detected by ≥50% of the panel (Kaneko et al., 2012). The FD factor indicates an aroma's likely impact on the sample aroma as a whole.

Figure 3.1 shows the number of odour active peaks detected by the panel. At this stage, the compounds causing the peaks were unidentified, other than by their detected aroma. The figure illustrates the total number of odour active compounds detectable in each sample, and the contribution of these compounds by the proportions of the total number of detectable compounds in the range of FD factors.

Figure 3.1: The number of detected odour active peaks with a specified flavour dilution (FD) factor for each of the roasted samples.



The chocolate malt sample exhibited the greatest number of detected compounds with the highest FD factor of >1000 (n=49), 16 more than in the next ranked sample, roasted barley (n=33) (Figure 3.1). However, chocolate malt did not yield the highest number of detectable odour active peaks overall (n=90 compared to n=98 in black malt). Despite this, the highest proportion of its detected compounds of all the samples remained detectable to the highest dilution (DF1000). This indicates that these detected compounds at DF1000 have the greatest impact on the aroma of the chocolate malt sample in comparison to the other analysed samples. Other samples may have more detectable aromas, but these have reduced influence on the overall aroma of the sample, as they are not detectable when diluted to such a degree.

The high frequency of detection of high impact aromas in the chocolate malt sample is likely to be a direct result of the roasting conditions used to manufacture this product. The finishing temperature achieved during the roasting of chocolate malt is 225 °C (Boortmalt, 2010e). Throughout roasting, care is taken to avoid the scorching of the grains by excessive or prolonged roasting. This may result in the enhanced retention of odour active volatiles. Prolonged roasting could result in the loss of odour active volatiles by volatilisation; degradation; or use as precursors to subsequent reaction pathways.

The two samples roasted to the lowest temperatures for the shortest times (caramalt and amber malt) yielded the fewest number of detectable compounds in total (n=83). However, all of the samples were striking for the sheer complexity of aroma and this high number (83-98) of odour active peaks.

3.3.2 Identification of Odour Active Compounds

Wherever possible, compounds were assigned to each of the GC-O odour active peaks using the five identification criteria listed in Section 3.2.8. In this way, 45 odour active peaks were firmly assigned to compounds using the identification methods indicated in Table 3.2. Naturally this implies that a further 52 odour active regions could not be assigned to compounds – usually due to a lack of sensitivity in the GC-MS analysis (i.e. peaks could not always be identified that were coincident with noted aromas). Volatile sulphur compounds (see Chapter 5) are a group of compounds likely responsible for many such odour active regions in the GC-O olfactograms which were not able to be sensitively analysed under present

chromatographic conditions. Table 3.2 shows the 45 odour active compounds which were assigned to odour active peaks. The table provides relevant odour descriptors from the panel, evidence on which assignments were based, and the measured FD factors for each compound in each roasted product.

Table 3.2: Identification details and flavour dilution (FD) factors for 45 odour active compounds in six roasted product flavour extracts.

Compound	Compilation of Reported Aromas	LRI ZB-WAX[A]	Identification Method [B]	Roasted Sample [C]					
				AM	CA	MC	CH	BL	RB
2-methylfuran	*Sweet, Baked, Roasted*	889	*LRI + STD + TD + MS + A*	0	1	100	100	100	0
2-methylbutanal	*Burnt, Nutty, Fruity, Musty*	936	*LRI + STD + TD + A*	1	1	100	1000	100	1
2-ethylfuran	*Musty, Toast, Roasted, Bread*	967	*STD + TD + A*	10	1000	1000	1000	10	100
Pentanal	*Fermented, Sweet, Baked, Roasted*	1031	*LRI + STD + TD + MS + A*	1000	1000	100	10	100	100
Thiophene	*Sweet, Sour, Musky, Nutty*	1056	*LRI + STD + TD + A*	0	0	0	0	0	10
Hexanal	*Fermented, Floral, Vegetable, Cheese, Sweet*	1135	*LRI + STD + TD + MS + A*	1000	0	0	1000	10	100
2-methylthiophene	*Roasted, Sawdust, Musty*	1149	*LRI + STD + TD + A*	100	100	1	1000	1	1000
1-methylpyrrole	*Roasted, Nutty, Sweet*	1209	*LRI + STD + MS + A*	1000	100	0	100	1000	100
Pyrazine	*Sweet, Fruity, Baked, Vegetable*	1272	*LRI + STD + TD + MS + A*	1000	100	100	1000	1	100
2-pentylfuran	*Sweet, Fruity, Baked, Vegetable*	1280	*LRI + STD + TD + MS + A*	1000	100	100	1000	1	100
1-pentanol	*Musty, Fermented, Sweet, Baked*	1290	*LRI + STD + TD + A*	1	1000	100	1	10	10
2-methyltetrahydro-3-furanone	*Fermented, Sweet, Musty*	1320	*LRI + STD + TD + A*	0	100	1000	0	1000	100
Hydroxyacetone	*Musty, Fermented, Woody*	1365	*STD + TD + MS + A*	100	1000	100	1000	1	1000
2,5-dimethylpyrazine	*Musty, Fermented, Woody*	1370	*LRI + STD + TD + A*	100	1000	100	100	1000	1000
Ethylpyrazine	*Grain, Nutty, Fermented, Fruity*	1384	*LRI + STD + TD + A*	100	1000	100	100	1000	1000
2,6-dimethylpyrazine	*Grain, Nutty, Fermented, Fruity*	1385	*LRI + STD + TD + A*	100	1000	100	100	1000	1000
2,3-dimethylpyrazine	*Sweet, Baked, Chocolate, Nutty*	1397	*LRI + STD + TD + MS + A*	100	1	100	1000	1000	1
2-methyl-5-(methylthio)furan	*Sweet, Musty, Roasted, Grain*	1424	*LRI + STD + TD + A*	1000	1000	1000	1000	10	0
2-ethoxythiazole	*Sweet, Baked, Nutty*	1433	*LRI + STD + A*	10	100	1	1000	10	100
Furfural	*Sweet, Baked, Nutty*	1433	*LRI + STD + TD + MS + A*	10	100	1	1000	10	100
1-octen-3-ol	*Musty, Fruity, Baked*	1461	*LRI + STD + TD + A*	1000	100	10	0	1	10
Acetic Acid	*Musty, Fruity, Baked*	1477	*LRI + STD + TD + MS + A*	1	0	1	10	10	1000
Pyrrole	*Sour, Sweet, Baked, Burnt, Green*	1525	*LRI + STD + TD + A*	1	10	0	100	1	1000
2-acetylfuran	*Sour, Sweet, Baked, Burnt, Green*	1530	*LRI + STD + TD + A*	1	1000	0	1000	100	100
2-n-pentylpyridine	*Baked, Roasted, Caramel, Musty*	1574	*LRI + STD + TD + MS +A*	10	0	1000	1000	1000	100
Methyl-2-furoate	*Musty, Roasted, Fermented*	1581	*LRI + STD + TD + MS + A*	1000	100	1000	100	10	100
Undecanal	*Fermented, Green, Cocoa*	1592	*LRI + STD + A*	1	100	100	1000	0	10
5-methylfurfural	*Fermented, Green, Cocoa*	1593	*LRI + STD + TD + MS + A*	1	100	100	1000	10	10
2-acetyl-5-methylfuran	*Musty, Fermented, Roasted*	1632	*STD + TD + MS + A*	10	1	1000	1000	10	1000
2-furanmethanol	*Chocolate, Baked, Sweet, Musty*	1659	*LRI + STD + TD + MS + A*	10	1000	1000	1000	1000	1000
4-cyclopentene-1,3-dione	*Baked, Sweet, Chocolate, Liquorice, Fermented*	1667	*LRI + STD + TD + A*	1000	0	100	1000	10	1
2-methylbutyric acid	*Baked, Sweet, Chocolate, Liquorice, Fermented*	1670	*LRI + STD + TD + A*	1000	10	0	1000	10	1
Phenylacetaldehyde	*Chocolate, Fermented, Dairy, Sweet, Musty*	1674	*LRI + STD + MS + A*	10	1000	1000	1000	1000	1000
2-acetylthiazole	*Baked, Sweet, Chocolate, Liquorice, Fermented*	1679	*LRI + STD + A*	1	1000	1000	10	10	1

5-methyl-2-furanmethanol	*Burnt, Musty, Buttery, Fermented, Cocoa*	1733	*LRI + STD + A*	0	0	1000	10	10	10
2,4-nonadienal	*Burnt, Musty, Buttery, Fermented, Cocoa*	1736	*STD + A*	0	0	0	10	10	10
2(5H)-furanone	*Musty, Baked, Green, Sweet*	1800	*LRI + STD + TD + MS + A*	1000	10	0	0	0	0
Cyclopenten-1-one	*Roasted, Sweet, Baked*	1920	*STD + A*	10	100	1	1000	100	0
2-acetylpyrrole	*Roasted, Meaty, Salty, Burnt, Fermented*	2012	*LRI + STD + A*	10	0	100	10	1000	10
Maltol	*Roasted, Meaty, Salty, Burnt, Fermented*	2019	*LRI + STD + TD + MS + A*	10	0	100	10	1000	10
Furaneol	*Burnt, Baked, Musty, Sweet*	2063	*LRI + STD + MS + A*	1000	1000	1	1	100	1000
4-ethylguaiacol	*Burnt, Baked, Musty, Sweet*	2069	*LRI + STD + A*	1000	1000	1	1	100	1000
2-formylpyrrole	*Musty, Baked, Burnt, Sweet*	2081	*LRI + STD + MS + A*	1000	0	1000	1000	1000	100
HMF	*Caramel, Roasted, Fermented, Burnt*	2246	*STD + TD + MS + A*	1000	1000	100	1000	100	100
Sotolone	*Fermented, Baked, Cocoa, Caramel, Meaty*	2288	*LRI + STD + A*	100	1000	10	1000	1000	1000

[A] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.

[B] Identification based upon LRI comparison with authentic standards (STD), LRI comparison with LRI from literature on a WAX column (LRI), thermal desorption (TD) data, EI-MS library matching (MS), and aroma description in comparison with online reference aroma description (A).

[C] The roasted sample is abbreviated as follows: amber malt (AM), caramalt (CA), medium crystal malt (MC), chocolate malt (CH), black malt (BL), and roasted barley (RB).

Compounds with FD factors listed as zero in particular samples in Table 3.2 were not detected in the sample by the panel. The aroma descriptors listed are a combination of those reported by each of the four panelists, across all six of the roasted malts, at each dilution of the flavour extracts. This provided a general aroma description which was confirmed to correspond with reference aroma descriptors for the underlying compound (based on descriptors from Good Scents (2018a)). Compounds with identical aroma descriptors eluted from the column within the same 30 second window. Consequently, the panelists may have detected a combination of the compounds within the 30 second window whilst describing them. These compounds therefore share aroma descriptors in Table 3.2. Due to the sheer aroma complexity of roasted malt samples, not all aroma compounds could be adequately separated in a timescale suited to GC-O analysis (even with shared sniffing duties splitting the chromatogram into two halves). Thus some compounds overlapped at the sniffing port and reported aromas may result from sensory interactions between 2 or more underlying compounds. The interactions between compounds within a mixture has an effect on the aroma qualities of a roasted malt as a whole. The way in which an odour active compound is perceived can be affected in a number of ways: interactions between each other within the matrix can lead to certain compounds being masked, detected simultaneously, or distorting the expected aroma of certain compounds to result in the perception of a different aroma.

The compounds identified in Table 3.2 are listed by increasing linear retention index. To summarise the identified compounds according to chemical class, oxygen containing heterocyclics were the largest group (n=17), then nitrogenous heterocyclics (n=10). Other identified compound classes included aldehydes, alcohols, ketones, carboxylic acids, and sulphur containing compounds. The compounds listed in the table are not the only compounds present within the range of roasted samples, but were identified in the present study as being associated with key odour active peaks on the GC-O olfactograms of commercially available roasted malts. By way of comparison, when analysing beer, over 600 compounds have been identified over many years of investigation (Castro and Ross, 2018). Not all of these are odour active and to provide any detailed explorations into the effects or formation of these compounds, the list must be reduced to focus the study.

3.3.2.1 Formation of Volatile Aroma Compounds in Roasted Malts

The most abundant amino acid in malt is proline, which is cyclic, non-polar, and can provide 'popcorn' and 'bready' aromas when used as a substrate in the Maillard pathway (Channell et al., 2010, van Boekel, 2006). Maltose is the most abundant reducing sugar in malt, which may take part in the Maillard reaction, along with proline (Channell et al., 2010). Maltose can also take part in caramelisation (Purlis, 2010). Baked aromas were consistently detected by the panel throughout analysis of the range of roasted samples, also being described as 'bready' (namely 2-ethylfuran, compounds described as 'baked' (n=23) included 2-methylfuran, pentanal, 1-pentanol, furfural, 2-n-pentylpyridine, 2-furanmethanol, and furaneol (Table 3.2)).
In 24 of the 45 identified compounds, chocolate malt had a FD factor >1000, the most of all the samples. This is consistent with this sample having the greatest number of aromas detected at FD>1000 (Figure 3.1). The reported aroma of 'chocolate' or 'cocoa' occurs in 8 of the 24 compounds detected at FD>1000 in chocolate malt (2,3-dimethylpyrazine, undecanal, 5-methylfurfural, 2-furanmethanol, 4-cyclopnetene-1,3-dione, 2-methylbutyric acid, phenylacetaldehyde, and sotolone). In addition, the remaining compounds with these reported aromas in Table 3.2 were detected in the chocolate malt sample, but to lower FD factors (2-acetylthiazole, 5-methyl-2-furanmethanol, and 2,4-nonadienal (all FD10 in chocolate malt)). This supports the expected sensory attributes of this sample to contribute chocolatey characteristics as its name suggests.
The sample with the lowest number of identified compounds at FD>1000 was medium crystal malt (n=10). However, at FD100, 18 compounds were identified. This indicates that in medium crystal malt, there was a reduced range of odour active compounds which contributed heavily to the overall sensory characteristics of this product. Crystal malts are predominantly caramellic, or toffee like in their aroma, and this is therefore the major contribution they make to a brew (Blenkinsop, 1991). Compounds likely to contribute to this in the caramalt and medium crystal malt samples were: 2,3-dimethylpyrazine, 2-methyl-5-(methylthio)furan, 2-n-pentylpyridine, 2-furanmethanol, 2-formylpyrrole, HMF, and sotolone. The degree to which a crystal malt is roasted (namely roasting time and temperature) influences the formation of flavour and colour in the product. Crystal malts include caramalt; and light, medium, and dark crystal malts. The range of crystal malt products contribute a range of caramel flavours, to varying degrees of 'burnt' (Blenkinsop, 1991, Gruber,

2001). In addition to caramellic flavour, crystal malts provide textural enhancement to beers when used in the grist. The mouthfeel, foam, and foam stability can be improved when crystal malts are used in a brew (Gruber, 2001).

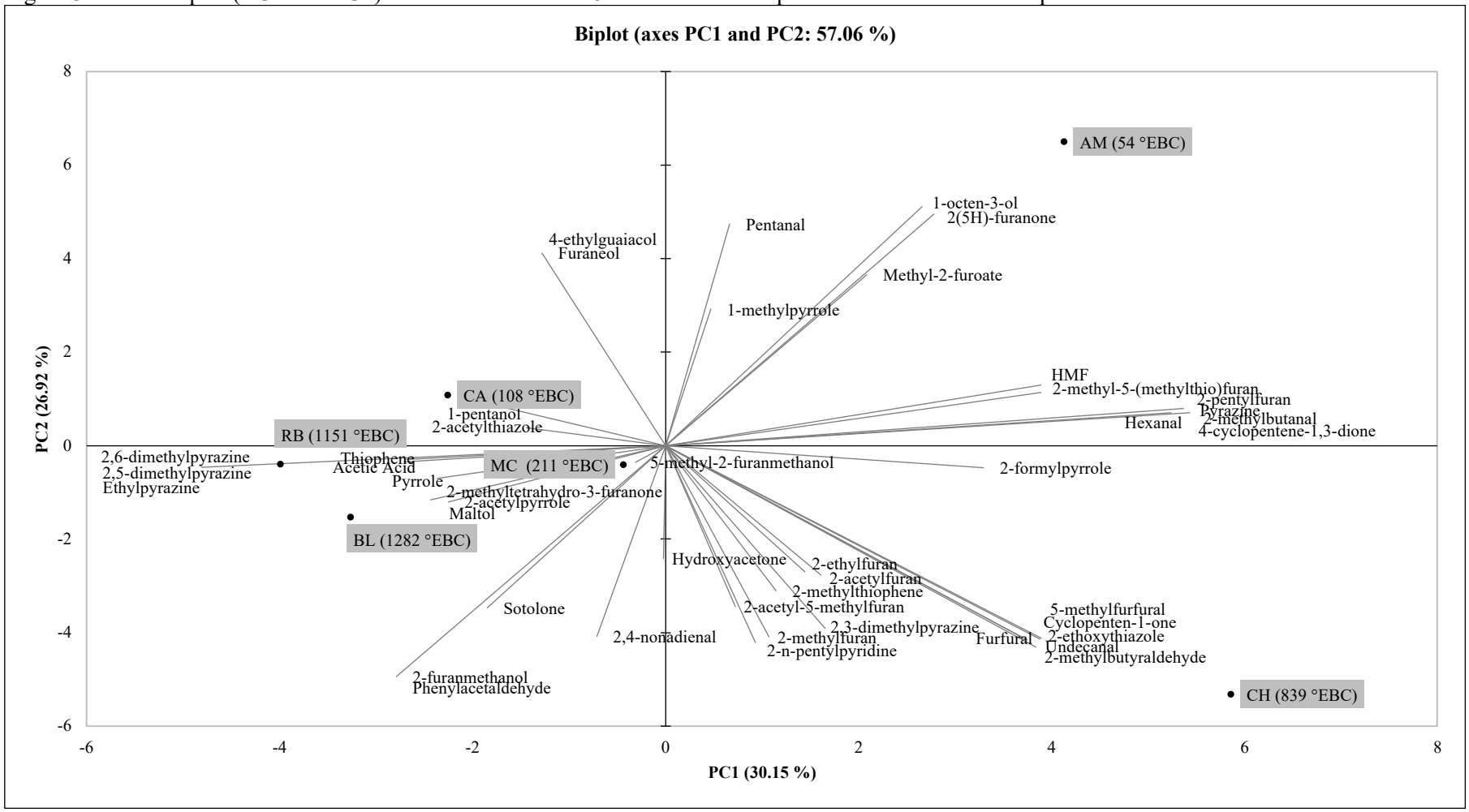
All of the sulphur containing compounds assigned in Table 3.2 (n=5) could not be firmly identified using EI-MS library matching (indicated by 'MS' in the table). This is principally because of the moderate sensitivity of the detector to sulphur compounds, the complexity and lack of full separation of aroma compounds in GC-O chromatograms and the fact that odours for this group of compounds are detected even when present in the ppb (µg/L) range. VSCs can be present at very low concentrations, but remain flavour active (Hill and Smith, 2000). The receptors of the human nose can detect the aroma of these compounds when instrumental methods of detection are unsuccessful. This highlights the importance of the GC-O method as a crucial component of the identification of odour active compounds in flavour analysis. Thermal desorption-GC-MS/MS data were more successful at identifying the sulphur compounds present due both to the pre-concentration step and the use of more resolving chromatographic conditions applied in this analytical study without concurrent olfactometry. Four of the five identified sulphur compounds yielded FD factors >1000 in at least one of the roasted samples (Table 3.2). Without the use of GC-O, the aroma impact of these VSCs would have been overlooked. Combining the data from Figure 3.1 and Table 3.2 highlights that there are a number of detected compounds at FD>1000 that remain unidentified by this study. It is possible that a proportion of these compounds are VSCs that were detectable in this study by only the panellists, and not by instrumental methods of detection. It is known that the key raw materials used in the brewing process (malt, roasted malt, and hops) contain a range of VSCs, some of which may remain throughout the entire brewing process (Lermusieau and Collin, 2003). This highlights the importance of identifying the VSCs in roasted malts and roasted barley, and how they may be responsible for the detection of many aromas at high FD factors across the range of products. Further investigation into the possible VSCs present in the range of roasted malt and roasted barley is necessary by more sensitive and selective methods of identification (as carried out and detailed in Chapter 5).

The FD factor data for the 45 compounds in Table 3.2 were analysed using Principal Component Analysis (PCA). The PCA biplot in Figure 3.2 shows the relationship between the FD factors of the 45 identified odour active compounds and the six

roasted samples. The variation in the data set is explained partially by PC1 (30.15 %) and PC2 (26.92 %).

In the Principal Component Analysis of the same data (FD factors of the 45 identified compounds in the six samples), PC3 accounted for 19.26 % of the variation in the data set. A biplot examining this principal component is not included in the current discussion. The consideration of multiple principal components was excluded due to the minimal proportion of the variation in the data accounted for by the following principal components (PC3, PC4 etc.).

Figure 3.2: PCA Biplot (**PC1** and **PC2**) of the FD factors of 45 odour active compounds and the roasted samples.



The roasted sample is abbreviated as follows: amber malt (AM), caramalt (CA), medium crystal malt (MC), chocolate malt (CH), black malt (BL), and roasted barley (RB).

PCA biplots are useful in visualising large data sets. Particularly in this study, large volumes of data were gathered, with FD values for each compound in each roasted sample. The biplot in Figure 3.2 shows the association of each compound to the sample in which it had the highest sensorial impact (by way of its FD factor). The most polarising samples are plotted on the extremes of the biplot, whereas those plotted relatively closely together showed minimal differences in identified compound FD factors.

PC1 separates samples according to the proportion of compounds in the samples that were detected at high FD factors. Samples with the highest number of compounds that were detected at high FD factors load positively on PC1, whereas samples with lower FD factors or fewer identified compounds load negatively on PC1. On PC1, chocolate malt, roasted barley, and amber malt account for the majority of the variation in the plotting of the samples (41 %, 19 %, and 20 %, respectively). This effect is visible, as these samples are plotted the furthest away from each other. Plotted positively on PC1 are amber malt and chocolate malt (Figure 3.2). The compounds plotted most positively along PC1 have high FD factors in both of these malts. For example, Table 3.2 shows that pyrazine has FD>1000 in only the amber and chocolate malt samples, causing it to be plotted positively on PC1. Pyrazine was identified in all of the analysed samples, with FD100 in caramalt, medium crystal malt, and roasted barley. However, as it was more influential to the sensory properties of amber malt and chocolate malt, pyrazine is plotted positively on PC1 between the two samples.

Where compounds were identified as having FD>1000 in the majority of the samples, the compound is not plotted extremely closely to any particular sample. For example, phenylacetaldehyde and 2-furanmethanol were detected at FD>1000 in all the samples except for amber malt, in which they were both detected at FD10. These compounds are plotted on the same vector, at a polar opposite to the amber malt sample (Figure 3.2). To illustrate this further, 2-formylpyrrole was detected at FD>1000 in amber malt, medium crystal malt, chocolate malt, and black malt. The compound was detected to FD100 in roasted barley, and not identified in caramalt. In Figure 3.2, 2-formylpyrrole is plotted positively on PC1, between amber malt and chocolate malt but closer to zero than those compounds detected at FD>1000 in those samples.

As discussed above, the roasting conditions (of time, temperature and moisture) of chocolate malt appear to favour the retention of odour active volatiles that remain highly impactful on the aroma qualities of this product. Figure 3.2 highlights this, as many compounds are plotted closely to this sample, and are associated with high FD factors in this sample above others. Regarding their processing, black malt shares similarities with chocolate malt. Chocolate malt is roasted to a finishing temperature of 225 °C, whereas black malt is finished at 230 °C (Boortmalt, 2010e, Boortmalt, 2010c). Both are produced by the roasting of pale kilned malt. Despite these similarities, the black malt sample yielded fewer identified compounds with high sensory impact (n=13 FD>1000). The fine details of roasting procedures for chocolate and black malt manufacture are proprietary and not publicly available. It can be assumed that the roasting of black malt is prolonged in order to achieve its darker colour (1282 °EBC compared with chocolate malt's 839 °EBC). In addition, quenching by the use of water sprays is used in some commercial roasting operations, and is known to be used in the roasting of black malt (Yahya et al., 2014). Quenching is used to minimise the risk of fire, and to achieve target colour and flavour (Yahya et al., 2014). These key processing differences may have resulted in the loss of odour active volatiles in the black malt sample, reducing their sensory impact in the sample. When added to beers, chocolate malt can add a nutty, roasted flavour without the dry astringency that can be achieved by the addition of black malt (Gruber, 2001). Astringency is a textural quality, rather than flavour or aroma contribution.

Consistent with the results shown in Table 3.2, medium crystal malt is located closely to the center of the biplot in Figure 3.2. The majority of the compounds identified in this sample were at FD100 (n=18), not of sufficient significance in this sample when compared to their impact in the other samples.

Roasted barley and black malt are plotted closely together within Figure 3.2 due to their similarities, which are caused by the processing temperatures achieved during their processing. Both roasted products can be processed up to 230 °C (Boortmalt, 2010j, Boortmalt, 2010c).

Although black malt and roasted barley are expected to provide relatively similar aroma contributions to a beer (as a result of their finishing temperatures), they both have different uses when included in the grist. Roasted barley is commonly used to

provide a lighter colour head than can be produced when using chocolate or black malts (Boortmalt, 2010i), whereas black malt is used to impart more astringency than can be achieved by using other roasted malts (Boortmalt, 2010b, Gruber, 2001). In addition to the physical effects of these particular products, there are specific differences regarding the presence of particular compounds and their FD factors in both samples. Figure 3.2 shows the roasted barley is characterised by high FD factors in alkylated pyrazines (2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and ethylpyrazine) in addition to thiophene and pyrrole. Whereas black malt is plotted closest of both samples to intermediate Maillard products including maltol, 2-furanmethanol, phenylacetaldehyde, and sotolone, which indicates their higher FD factors in black malt.

3.3.3 Quantification of Odour Active Compounds

From the data in Table 3.2 (45 key odour active peaks and associated compounds in roasted malts), we selected a sub-set of 20 compounds to quantify across the range of commercial samples. These were selected on the basis of i) their aroma significance across the range of samples ii) including compounds from different chemical classes and different pathways of thermal flavour generation and iii) their ease of quantification by GC-MS (including factors such as resolution using the applied method and presence of characteristic ions for quantitation). Thus, since alkylated pyrazines share common pathways of formation we elected to analyse 2,3-dimethylpyrazine as representative of this group and did not similarly quantify 2,5-dimethylpyrazine, 2,6-dimethylpyrazine or ethylpyrazine. Volatile sulphur compounds were also eliminated in this investigation, due to their lack of sensitive detection by mass spectrometry as discussed in Section 3.3.2. Alternative, more sensitive, detection methods were required to identify the range of volatile sulphur compounds in the range of roasted malts (see Chapter 5). The 20 selected compounds included oxygen containing heterocyclics (n=11), and nitrogenous heterocyclics (n=5) in addition to small aldehydes (one Strecker degradation product and others derived principally from lipid degradation) and acetic acid.

In order to quantify the selected compounds, authentic external standard calibration was required, as detailed in Section 3.2.10. Table 3.3 shows the 20 compounds

selected, and the calibration R^2 values used for quantification. The high correlation coefficients demonstrate good linearity for the majority of compounds.

Table 3.3: Authentic external standard calibration R^2 values and aroma descriptors for 20 odour active compounds identified in the range of roasted samples.

Compound	Aroma [A]	R^2
1-methylpyrrole	*Smoky, woody, herbal*	0.9995
2-(5H)-furanone	*Buttery*	0.9997
2-acetyl-5-methylfuran	*Musty, nutty, hay, coconut, milky*	0.9993
2-formylpyrrole	*Musty, beefy, coffee*	0.9998
2-furanmethanol	*Musty, sweet, caramellic, bready, coffee*	0.9993
2-methylfuran	*Ethereal, acetone, chocolate*	0.9996
2-n-pentylpyridine	*Fatty, green, bell pepper, mushroom, herbal*	1
2-pentylfuran	*Fruity, green, earthy, beany, vegetable, metallic*	1
2,3-dimethylpyrazine	*Nutty, cocoa, peanut butter, coffee, walnut, caramellic*	0.9999
5-methylfurfural	*Spicy, caramellic, maple*	0.9999
Acetic Acid	*Sharp, pungent, sour, vinegar*	0.9918
Furaneol	*Sweet, candy floss, caramellic, strawberry, sugar*	0.9929
Furfural	*Sweet, woody, almond, bread, baked*	0.9999
Hexanal	*Fresh, green, leafy, vegetal, fruity*	0.9992
HMF	*Fatty, buttery, musty, waxy, caramellic*	0.9987
Maltol	*Sweet, caramellic, candy floss, jammy, fruity, bread*	0.9965
Methyl-2-furoate	*Fruity, mushroom, fungal, tobacco, sweet*	0.9999
Pentanal	*Fermented, bready, fruity, nutty, bready*	0.8178
Phenylacetaldehyde	*Green, sweet, floral, clover, honey, cocoa*	0.9995
Pyrazine	*Pungent, sweet, roasted, hazelnut, roasted barley*	0.9997

[A] Aroma descriptors from Good Scents Company, available online (Scents, 2018a).

Table 3.3 also lists reference aroma descriptors of the compounds from an online source (Scents, 2018a). These descriptors differ somewhat from those reported by the panel in Table 3.2. The panel assessed the aromas as they eluted from the column, which may have included the assessment of multiple aromas if the retention times of the compounds were extremely close. The descriptors given by the panel were matched with those available online by interpreting the panel's lexicon, given their lack of training.

Table 3.4 shows the calculated concentrations of the 20 selected compounds across the range of six roasted products. The table shows that the identification of the compounds from GC-O analysis was successful, i.e. all of the compounds selected for quantification were found to be present in those samples, apart from 2-n-

pentylpyridine which was not identified in medium crystal malt, in spite of the FD factor of 1000 noted in this sample at the corresponding retention index. It is thus doubtful that 2-n-pentylpyridine was solely responsible for the odour active peak noted by the panel in that region.

Table 3.4: Calculated concentrations (µg/g) of 20 odour active compounds in six roasted product flavour extracts.

Compound	LRI ZB-WAX[A]	EI-MS Quantitation Ion (*m/z*)	Concentration in Roasted Sample (µg/g)[B]					
			AM	CA	MC	CH	BL	RB
2-methylfuran	*888*	*82*	0.15	0.31	0.47	0.06	0.72	0.11
Pentanal	*1010*	*58*	0.32	0.48	0.55	0.22	0.19	0.29
Hexanal	*1103*	*56*	0.26	0.63	0.17	0.22	0.12	1.02
1-methylpyrrole	*1163*	*81*	0.18	0.12	0.10	0.06	0.06	0.13
Pyrazine	*1233*	*80*	0.72	0.51	0.29	3.99	4.00	13.51
2-pentylfuran	*1248*	*138*	1.43	1.64	0.70	0.40	2.72	1.29
2,3-dimethylpyrazine	*1365*	*108*	0.44	0.51	0.30	1.18	1.55	2.56
Acetic Acid	*1488*	*60*	183.07	188.71	282.31	63.75	53.43	92.87
Furfural	*1490*	*96*	31.38	140.52	179.61	72.88	100.00	78.05
2-n-pentylpyridine	*1592*	*120*	0.39	6.41	-	5.79	8.58	2.73
5-methylfurfural	*1600*	*110*	7.03	17.30	21.86	13.05	22.70	22.67
Methyl-2-furoate	*1603*	*120*	0.50	1.81	1.35	7.33	7.28	8.74
2-acetyl-5-methylfuran	*1641*	*124*	0.15	0.30	0.45	1.08	1.29	1.20
Phenylacetaldehyde	*1674*	*120*	12.79	28.25	15.29	6.06	5.70	6.17
2-furanmethanol	*1682*	*98*	30.03	201.83	276.75	1.87	2.68	2.99
2-(5H)-furanone	*1782*	*84*	7.55	23.11	30.54	8.25	8.69	7.07
Maltol	*2000*	*126*	88.25	365.11	510.46	122.99	146.69	213.67
Furaneol	*2063*	*128*	3.47	4.60	7.12	1.65	2.22	2.13
2-formylpyrrole	*2065*	*95*	2.40	2.00	2.86	14.18	14.44	16.13
HMF	*2246*	*126*	335.97	971.90	1211.47	115.14	175.23	118.51

[A] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.
[B] The roasted sample is abbreviated as follows: amber malt (AM), caramalt (CA), medium crystal malt (MC), chocolate malt (CH), black malt (BL), and roasted barley (RB).

The final concentration of compounds present is balanced between the extent of formation; the amount of stripping due to the volatility at process temperatures; and the depletion due to subsequent chemical reactions. It is known that the Maillard reaction takes place during the roasting of malts and barley, along with other thermal flavour generation pathways including caramelisation, lipid degradation, and pyrolysis (Yahya et al., 2014, Jehle et al., 2011). During the Maillard reaction pathway, reductones are formed as intermediate products, which can be converted in later stages of the pathway to form oxygen or nitrogen containing heterocyclic compounds (Gretenhart, 1997). Oxygen heterocyclics have malty, caramel, toffee aromas, whereas nitrogen heterocyclics are characteristically nutty, with stronger coffee aromas (Gretenhart, 1997).

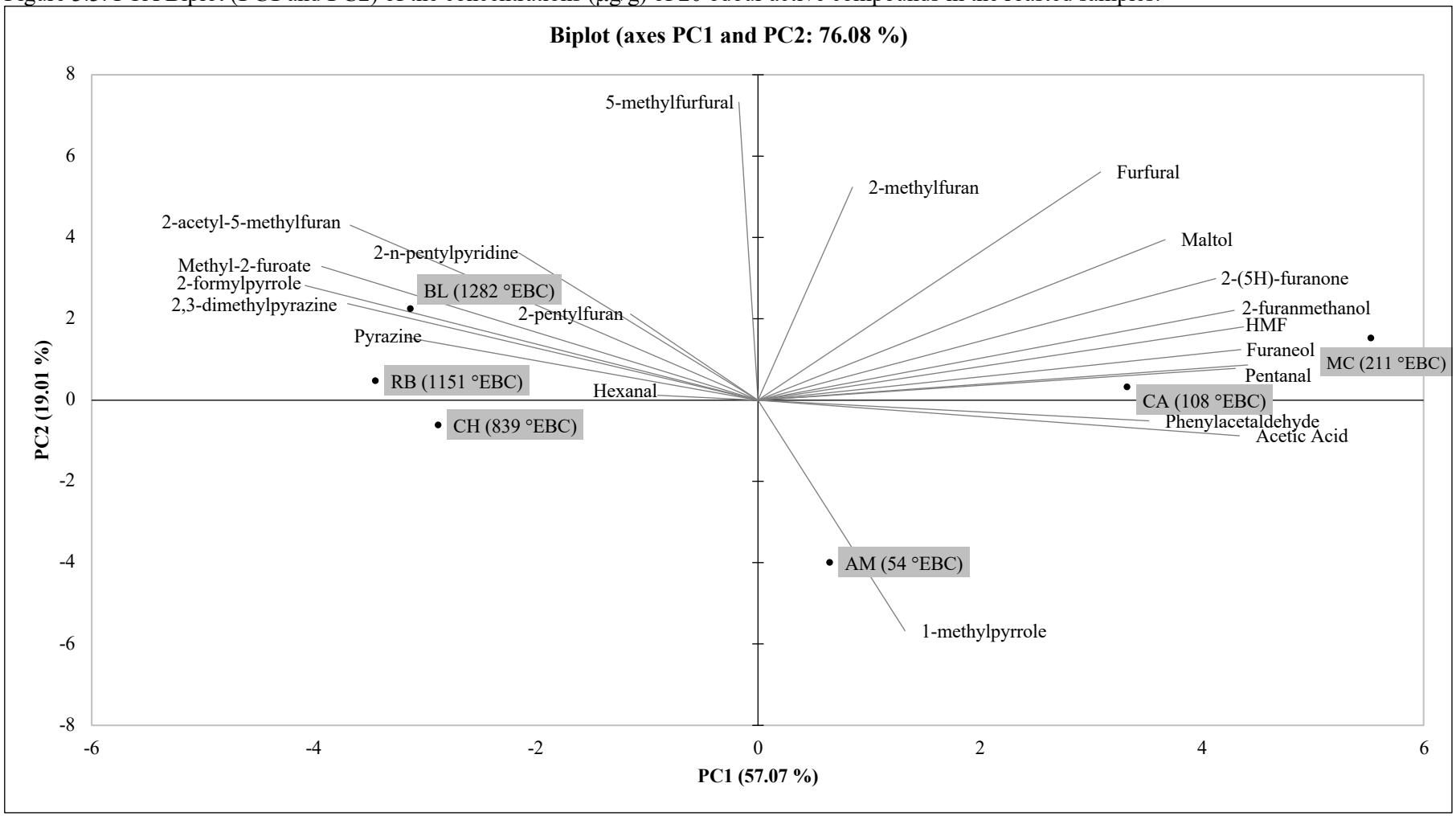
Nitrogen heterocyclics are more common in malts exposed to higher roasting temperatures (Gretenhart, 1997). Pyrazine itself, and 2,3-dimethylpyrazine are both nitrogenous heterocyclics, and were quantified in this study. Pyrazines are responsible for nutty, coffee, roasted, and bitter flavours, and are more concentrated in darker roasted products (O'Shaughnessy, 2003). Pyrazines are formed as a result of the Strecker degradation within the Maillard reaction, but can also be formed during caramelisation by reactions between ammonia and carbon fragments (Vandecan et al., 2010, Adams and Kimpe, 2009). Some pathways of the Maillard reaction occur faster in the absence of water, which corresponds to the pyrazines' increased influence on the aroma qualities of the darkest roasted products, due to the high temperatures achieved during roasting which result in low moisture (Adams and Kimpe, 2009). However, the concentration of pyrazines is reduced towards the end of roasting (O'Shaughnessy, 2003). This may be because the Maillard pathway provides a rich source of intermediates for subsequent reactions, including pyrolysis, which occurs at conditions near the combustion point of the roasting malt, and results in the formation of, for example, furans (Paine Iii et al., 2008, Mottram, 1998).

Amber malt, caramalt, and medium crystal malt all had higher concentrations of acetic acid (183.07 μg/g, 188.71 μg/g, and 282.31 μg/g, respectively) than the remaining samples (63.75 μg/g in chocolate malt, 53.43 μg/g in black malt, and 92.87 μg/g in roasted barley). Acetic acid eluted from the column closely to furfural (1488 LRI, and 1490 LRI, respectively). The detection of the two compounds by the panel may have been influenced by the elution of both compounds at similar

retention times. Furfural and acetic acid yielded separate peaks necessary for quantification, but may have overlapped during the GC-O analysis. This may have resulted in combined detection of aromas during this busy section of the chromatogram during GC-O analysis, but detection of separate peaks by GC-MS analysis. The FD factor of acetic acid across all of the samples remained relatively low despite its high concentration (excluding FD>1000 in roasted barley).

Figure 3.3 shows a PCA biplot of the concentrations of the 20 compounds within the range of six analysed roasted samples. The biplot indicates which samples are associated with having higher concentrations of the quantified compounds.

Figure 3.3: PCA Biplot (**PC1** and **PC2**) of the concentrations (µg/g) of 20 odour active compounds in the roasted samples.



The roasted sample is abbreviated as follows: amber malt (AM), caramalt (CA), medium crystal malt (MC), chocolate malt (CH), black malt (BL), and roasted barley (RB).

The biplot in Figure 3.3 enables alternative visualisation of the data from Table 3.4. It shows the clear differences between the samples and segregates them according to the samples' association with high concentrations of particular compounds.
PC1 accounts for the majority of the variation in this biplot (57.07 %). From the concentration data in Table 3.4, PC1 clearly separates the samples according to the differences in odour active compound concentrations between roasted green malts (caramalt and medium crystal malt) and the 'dry roasted' products (chocolate malt, black malt, and roasted barley). Amber malt, a pale malt roasted to temperatures between 100 °C and 150 °C (Boortmalt, 2010a), falls between the two groups hence its position closest to zero on PC1.
The crystal malt samples are produced from the roasting of green malt, the product of germination. As green malt is not kilned (dried) before it is roasted, it is high in moisture (40-44 %), around four times that of raw barley (Arendt and Zannini, 2013, Gruber, 2001). Light coloured malts contain the highest proportions of volatile Maillard products, whereas darker roasted products contain fewer of these flavour volatiles (Coghe et al., 2006). Darker roasted malts are more likely to contain higher proportions of caramelisation and pyrolysis products. Roasted barley, chocolate, and black malts have high concentrations of melanoidins and heterocyclic compounds which results in their characteristic bitter taste. Melanoidins also contribute to the colour of the product (Echavarría et al., 2012). The bitter characteristic of those darker products is exaggerated due to the lack of reductones and aldehydes, as the preceding saccharification step is not carried out during the roasting of kilned malt and unmalted barley (Blenkinsop, 1991).
The concentrations of thermally generated volatiles are dependent upon a number of contributing factors. For example, adequate activation energy must be achieved in order for each particular reaction to occur, in addition to sufficient pooling of reactants. Furthermore, decreasing concentrations of certain compounds may occur, as a result of their use in subsequent reactions at higher activation energy. The characteristic high concentrations of intermediate stage Maillard products in the crystal malt samples indicates the absence of further thermal flavour generation reactions in these samples. Contrasting this, the lower concentrations of those compounds in the products roasted to higher temperatures and produced from different substrates (i.e. chocolate malt, black malt, and roasted barley) does not indicate the lack of the intermediate stages of the Maillard reaction occurring, but

that subsequent flavour generation was facilitated by the roasting conditions, likely using the intermediate products in the process.

Maltol, for example, is formed in the intermediate stages of the Maillard reaction (Vandecan et al., 2011). Maltol has a higher concentration in the crystal malt samples, perhaps due to the extent to which the intermediate stage of the Maillard reaction occurs during the moderate temperature roasting of these samples. Higher concentrations of maltol can be formed and maintained in these samples, rather than taking part in subsequent reactions. Maltol can be formed in a number of additional different thermal pathways: including from disaccharides, or from proline-amadori products (Yaylayan and Mandeville, 1994). This complexity results in the distinct concentrations of maltol across the range of roasted products.

The amber malt sample yielded low concentrations of many of the compounds quantified, so falls the closest to zero on PC1. The differences between the crystal malts and amber malt in Figure 3.3 may be due to the roasting substrate used, in addition to the roasting temperatures. Amber malt is produced from pale malt, roasted to a maximum of 100 to 150 °C. The development of colour in the grains throughout roasting is limited to 45 to 60 °EBC. It can be used to contribute depth of colour in some beers where the flavour contribution should be minimal (Boortmalt, 2010a).

Although the concentrations of the compounds in Table 3.4 are low in the amber malt sample, the FD factors of these compounds are high. Ten of the 20 quantified compounds yielded FD>1000 in the amber malt sample, despite the low concentrations (Table 3.2). This indicates that the odour activity of these compounds in amber malt is high.

In addition, 1-methylpyrrole is plotted closely to amber malt in Figure 3.3. 1-methylpyrrole is a nitrogenous heterocyclic, the aroma of which is smoky, woody, and herbal (Table 3.3), reported by the panel as roasted, nutty, and sweet (Table 3.2). The concentration of 1-methylpyrrole is relatively low in all of the samples (from 0.06 μg/g to 0.18 μg/g). The highest of these low concentrations caused this compound to be plotted closely to amber malt.

It is clear that the raw materials and the roasting conditions employed during the production of the samples influenced the formation of odour active compounds. The range analysed in this study formed two clear poles of the scale of roasted malts and

roasted barley samples, visible in Figure 3.3. This is predominantly caused by the variation of the loading on PC1. On PC2 of Figure 3.3, the majority of the variation is determined by the amber malt sample (66 %, sharing similarities with Figure 3.2 (57 %)). The compounds in Figure 3.3 load positively on PC2, and samples plotted more positively on PC2 are more likely to have a higher concentration of the compounds investigated. Lower concentrations in the amber malt sample result in it loading negatively on PC2, while the most positively loaded sample, black malt, is in the opposing quadrant of the Figure.

3.4 Conclusions

This study highlights the odour activity of 45 compounds present in a range of six commercially available roasted products. The FD factors of each compound regularly differed between the samples, indicating their specific influence on the aroma qualities of each sample. Chocolate malt was identified as having the highest number of highly odour active compounds (n=49 unidentified compounds at FD>1000). After identification, 24 of the 45 identified compounds in chocolate malt were detected at FD>1000. The high odour activity of the compounds detected in chocolate malt were partially due to the specific thermal processing used to produce the sample. The effects of roasting time and temperature influence the aroma impact of the range of compounds within a roasted product, in addition to the roasting substrate.

The correlation between the GC-O FD factors and reported concentration data was largely unsuccessful due, in part, to the complexity of the olfactograms of the roasted samples. In some cases, successful correlations between both sets of data were identified (particularly regarding 2,3-dimethylpyrazine and 2-(5H)-furanone), whereas in others (for example, phenylacetaldehyde, pyrazine, and 2-furanmethanol) there was not a good correlation. Co-elution of compounds due to the complexity of the chromatograms may have reduced the reliability of the FD factor data, as panellists may have detected multiple compounds at a time. This would result in confusion of specific aroma volatile significance (by inaccurate recording of compounds' FD factors). In addition to this, it is likely that a number of the highly odour active compounds detected by the panel were not identifiable by mass spectrometry due to their low odour thresholds. In particular, it is believed that a

number of the unidentified compounds are sulphur containing volatiles. The high odour activity of these compounds in the range of samples indicates the need for identification, as they have such a great impact on the aroma qualities of the roasted products. Due to their low concentrations but high odour activity, volatile sulphur compounds must be detected by highly sensitive and selective methods of sample preparation and detection. This is investigated further in Chapter 5.

With additional analysis, the concentrations of 20 of the 45 odour active compounds were determined in each of the roasted samples. The use of principal component analysis displayed the association of samples with high concentrations of particular compounds. A clear difference was identified between the paler samples (amber malt, caramalt, and medium crystal malt), and the darker roasted samples (chocolate malt, black malt, and roasted barley). In addition to this difference, the crystal malts (produced by the roasting of green malt) were separated further on the biplot, indicating the significance of the roasting substrate in the formation of particular compounds over the course of roasting. The use of different roasting substrates yields a range of aroma qualities that cannot be achieved by altering the roasting time and temperature alone. Upon the examination of the concentrations of 20 selected compounds, crystal malts were found to contain higher concentrations of particular compounds (i.e. maltol, furaneol, pentanal, and 2-furanmethanol) in comparison to those samples produced by the roasting of pale malt or unmalted barley. These effects were also contributed to by the moderate temperatures used to produce the paler roasted products.

The effects of process conditions and roasting substrate influence the formation and depletion of compounds as a result of thermal flavour generation reactions, namely the Maillard reaction, caramelisation, lipid degradation, and pyrolysis. Further examination is required to define the effects of roasting substrate, and roasting time and temperature on the development of the 20 selected odour active compounds throughout the roasting process.

Chapter 4 - Modelling flavour formation in roasted malt substrates under controlled conditions of time and temperature

4.1 Introduction

The factors contributing to the spectrum of flavours that are available from roasted or kilned speciality malts include: the cereal and whether malted or unmalted, variety, malting parameters/degree of modification, and the thermal processing steps; namely kilning, stewing, and roasting (Coghe et al., 2004). Thermal processing steps have the greatest influence on the final flavour attributes of roasted malt products (Yahya et al., 2014).

Roasted malts can be separated into three main categories, due to the roasting substrates that are used: colour malts, caramel/crystal malts, and roasted barley (Coghe et al., 2004, Gretenhart, 1997). The substrates are, respectively: pale malt, green malt, and raw barley. These raw materials are all taken from various stages of the malting process, as detailed in Chapter 1 Section 1.2.1.

An investigation, studying the formation of flavour and colour of dark speciality malts by Vandecan et al. (2011) noted the importance of the moisture content of the malt during the roasting process. The malts in the study were processed up to 180 °C, which does not include the very highest temperatures used to commercially produce speciality malts (O'Shaughnessy, 2003). The laboratory roasted products produced in the current study were intended to cover the full range of conditions employed to produce speciality malts, proportionate to the reduced batch size of a laboratory scale roaster.

Previous GC-Olfactometry studies (Chapter 3) identified 45 odour active compounds across a range of six commercially available roasted products. Following the quantification of 20 key odour active compounds (selected from the original 45 compounds), clear differences between the products were identified. In addition to the importance of thermal processing, the influence of roasting substrate was highlighted. In this study, instrumental analysis was used exclusively to quantify and model the formation of the 20 key odour active compounds in roasted products produced from three different and commonly used roasting substrates (barley, green malt, and pale malt). The objective was to model the formation of key roasted

product flavour compounds across a range of process times, temperatures and initial moisture contents in order to map the flavour space of potential roasted products prepared from the three basic substrates. To do this, a laboratory scale roasting drum was designed, featuring a cylindrical mesh cage which was rotated inside a Gas Chromatograph oven, used for precise time-temperature control (Chapter 2 Section 2.4.1). The laboratory scale roaster was used to accurately control the roasting conditions in small batches (100 g) of substrate so that flavour formation could be accurately modelled relative to those conditions. It is acknowledged that further work would then be required to translate these findings to commercial roasting drum operations where bulk effects and differences in power input per tonne of substrate would impact on flavour formation. However, the present approach enables a deeper understanding of how variation of the thermal processing conditions impacts on the formation of key groups of flavour compounds. The flavour volatile profiles of roasted malt products are complex. In this study, the formation of 20 key compounds were monitored, which were selected based on their known aroma impacts, from prior GC-Olfactometry studies (See Chapter 3) and which were representative of different thermal flavour generation chemistries.

4.2 Materials and Methods

4.2.1 Chemicals

Authentic analytical standards (>95% purity) were purchased to identify and quantify the 20 aroma compounds within the roasted samples. Suppliers of chemicals were as follows: Sigma Aldrich: 2-methylfuran, pentanal, hexanal, 1-methylpyrrole, pyrazine, 2-pentylfuran, 2,3-dimethylpyrazine, furfural, acetic acid, methyl-2-furoate, 5-methylfurfural, 2-acetyl-5-methylfuran, phenylacetaldehyde, 2-furanmethanol, 2-(5H)-furanone, furaneol, and hydroxymethylfurfural. Fisher Scientific: 2-n-pentylpyridine, maltol, and 2-formylpyrrole.
Methanol (HPLC/ LC-MS grade) used for solvent extraction of volatile compounds was sourced from VWR International Ltd.

4.2.2 Sample Preparation

All laboratory malted products were produced from the same batch of a winter variety of malting barley (Flagon) provided by Crisp Malt Ltd, as described in Chapter 2 Section 2.3.

Malted and unmalted barley was roasted using the laboratory scale roaster (Section 2.4.1) to the parameters specified in Section 2.4.1. Flavour extracts were prepared from each of the roasted samples, according to the protocol described in Section 2.7.

4.2.3 Sample Analysis

4.2.3.1 Gas Chromatography-Mass Spectrometry Operating Conditions

The volatile compounds within the flavour extracts were separated using a Trace 1300 Gas Chromatograph (Thermo Scientific, Waltham, MA, USA) according to the GC operating conditions detailed in Section 2.8. GC effluent was analysed by the MS (Thermo Scientific, Waltham, MA, USA).

The MS was run on selected ion methods (SIMs) to identify the specific compounds of interest, details of which are described in Section 2.9.

A guard column was used to prevent the impurities within the flavour extracts degrading the column itself, that would otherwise have resulted in reducing the accuracy of the peak areas recorded. The guard column and injector liner were changed after every 24 injections of samples to retain accuracy of data.

4.2.3.2 Peak Identification and Quantification Against External Standards

Compounds were identified based upon three levels of validation: linear retention index (LRI) against alkanes (C8-C22) when compared to literary sources on the same WAX phase; LRI comparison with authentic standards when assessed under the same chromatographic conditions; and by EI-MS library matching. These methods of identification were carried out in addition to the previous identification of the 20 compounds' known aroma impact on the range of commercial roasted products in the previous GC-Olfactometry studies (Chapter 3).

Authentic analytical standards of the 20 selected flavour volatiles were analysed by GC-MS at the following concentrations to give a calibration curve, from which concentrations could be calculated in the samples: 0.5 ppm, 1 ppm, 5 ppm, 10 ppm,

25 ppm. An internal standard (5-nonanone, 5 μg/mL) was used in each standard solution.

4.2.3.3 Moisture Content Determination

The moisture content of each of the 24 roasted samples for each substrate was determined as described in Section 2.10.

4.2.4 Data Modelling and Statistical Analysis

Following GC-MS analysis, the concentrations of each compound in each of the three substrates were modelled against the factors of time and temperature using the Design Expert software. Factors which were non-significant ($p > 0.05$) were removed from models until a significant model resulted with factors each of which were significant ($p < 0.05$), and the model R^2 was maximised. Interaction terms between factors were included in models where significant. Statistical details of the models derived for the production of each compound in each roasting substrate are detailed in the - Appendices Section 8.1, displayed in Table 8.1 and Table 8.2. Principal Component Analysis (PCA) was carried out using XLSTAT software (Addinsoft, SARL, Paris, France), in order to depict the relationship between the concentrations of the 20 compounds over the range of roasted products' roasting time, temperature, and substrate.

4.3 Results and Discussion

The moisture content of each laboratory prepared sample, and the concentrations of each of the 20 key odour active compounds in samples <5 % moisture (w/w) are shown in Table 4.1. To facilitate interpretation of this large amount of data, Principal Component Analysis (PCA) will firstly be used to visualise the variation in the data set. Secondly, the response surface models showing the trend in volatile formation as a function of roasting time and temperature for 10 volatiles will be presented. These 10 compounds were selected to be representative of different thermal flavour generation chemistries; namely: pyrazine, 2-acetyl-5-methylfuran, methyl-2-furoate, 2-methylfuran, maltol, 2-furanmethanol, acetic acid, phenylacetaldehyde, 2-pentlyfuran, and 2-n-pentylpyridine.

Table 4.1: Concentrations (µg/g) of 20 odour active compounds in laboratory roasted and commercially roasted samples.

Roasted Sample [A]	Temperature (°C)	Time (min)	Final Moisture (% w/w)	2-methylfuran	Pentanal	Hexanal	1-methylpyrrole	Pyrazine	2-pentylfuran	2,3-dimethylpyrazine	Furfural	Acetic Acid	2-n-pentylpyridine	Methyl-2-furoate	5-methylfurfural	2-acetyl-5-methylfuran	Phenylacetaldehyde	2-furanmethanol	2-(5H)-furanone	Maltol	Furaneol	2-formylpyrrole	HMF
				LRI ZB-WAX [B]																			
				894	1003	1107	1167	1239	1254	1373	1498	1534	1609	1615	1615	1656	1688	1697	1798	2019	2081	2081	2558
Raw Barley	135	25	4.41	-	0.0438	0.0839	0.0567	0.626	0.987	0.561	6.46	91.5	-	0.188	2.37	0.0554	6.93	2.46	1.76	6.50	1.86	2.50	85.7
	135	30	3.72	-	0.0410	0.0792	0.0530	0.576	1.07	0.519	6.06	95.0	-	0.129	2.41	0.0803	6.64	2.24	1.96	6.46	1.39	1.24	82.1
	165	10	4.69	-	0.0355	0.0759	0.0628	0.526	1.05	0.663	5.10	93.4	-	0.242	2.38	0.0891	6.23	2.49	3.17	7.21	2.43	2.67	73.5
	165	20	2.60	-	0.0335	0.0690	0.0594	0.564	1.13	1.32	6.76	115	0.140	0.261	2.77	0.112	2.79	3.48	3.37	7.62	4.36	3.17	72.7
	165	30	2.02	0.129	0.0357	0.0779	0.0561	1.00	1.22	2.02	13.1	130	0.201	0.241	4.13	0.144	1.87	5.25	3.36	7.02	4.14	4.34	71.9
	200	10	1.55	0.201	0.0325	0.0835	0.0547	1.96	1.06	2.84	23.5	137	0.433	0.384	6.40	0.247	2.84	8.29	4.04	8.82	5.73	4.88	93.9
	200	15	0.838	0.756	0.0424	0.0979	0.0418	3.08	1.09	2.37	76.7	148	0.276	0.996	27.4	0.336	1.92	16.8	9.25	11.6	5.79	19.1	164
	200	20	0.578	1.21	0.0512	0.106	0.0431	3.21	0.955	2.48	83.1	156	0.577	1.63	36.6	0.541	2.16	12.8	10.9	14.8	5.03	34.1	142
	200	25	0.307	1.88	0.0435	0.102	0.0351	3.34	1.46	2.84	80.1	127	1.33	2.16	26.5	0.757	1.72	9.05	7.34	13.7	3.08	37.9	104
	230	10	0.297	2.62	0.0415	0.104	0.0467	5.89	1.10	3.63	96.8	142	0.564	2.58	32.3	0.946	2.52	12.9	10.4	14.2	5.00	41.1	98.2
	230	20	0.117	7.45	0.0445	0.0888	0.101	9.84	1.99	4.73	78.5	135	4.47	7.93	24.3	3.02	6.49	5.52	12.9	46.0	3.34	38.5	56.1
	230	30	0.107	10.0	0.0472	0.103	0.176	21.5	3.09	6.84	84.3	119	11.4	10.1	37.6	3.79	8.79	4.58	12.8	93.8	3.82	22.2	89.1
Green Malt	135	50	4.64	0.748	0.0665	0.0976	0.374	0.347	1.47	0.435	127	464	-	2.69	38.8	0.778	35.1	360	76.7	613	18.8	5.08	897
	143	43	4.06	0.625	0.0665	0.0879	0.272	0.393	1.32	0.537	123	406	-	2.97	33.8	0.715	23.7	265	71.2	513	16.5	4.39	839
	150	35	4.14	1.68	0.0776	0.0945	0.294	0.452	1.32	0.457	122	392	-	3.07	34.3	0.730	22.7	280	64.1	524	17.0	5.12	865
	150	50	2.33	0.598	0.0590	0.112	0.215	0.430	1.54	0.409	118	425	-	1.93	42.2	0.825	9.99	233	56.0	614	14.0	6.51	1006
	158	28	4.11	0.619	0.0707	0.0883	0.295	0.529	1.32	0.525	113	348	-	2.89	31.1	0.695	21.7	251	63.0	466	16.3	4.56	771
	158	43	2.02	0.606	0.0781	0.129	0.198	0.574	1.94	0.455	96.1	369	-	3.42	46.1	0.798	9.12	184	48.4	546	13.2	7.58	915
	165	35	2.07	0.671	0.115	0.134	0.255	0.605	2.15	0.620	114	451	-	3.34	62.0	1.08	11.4	217	53.5	656	14.1	8.72	945

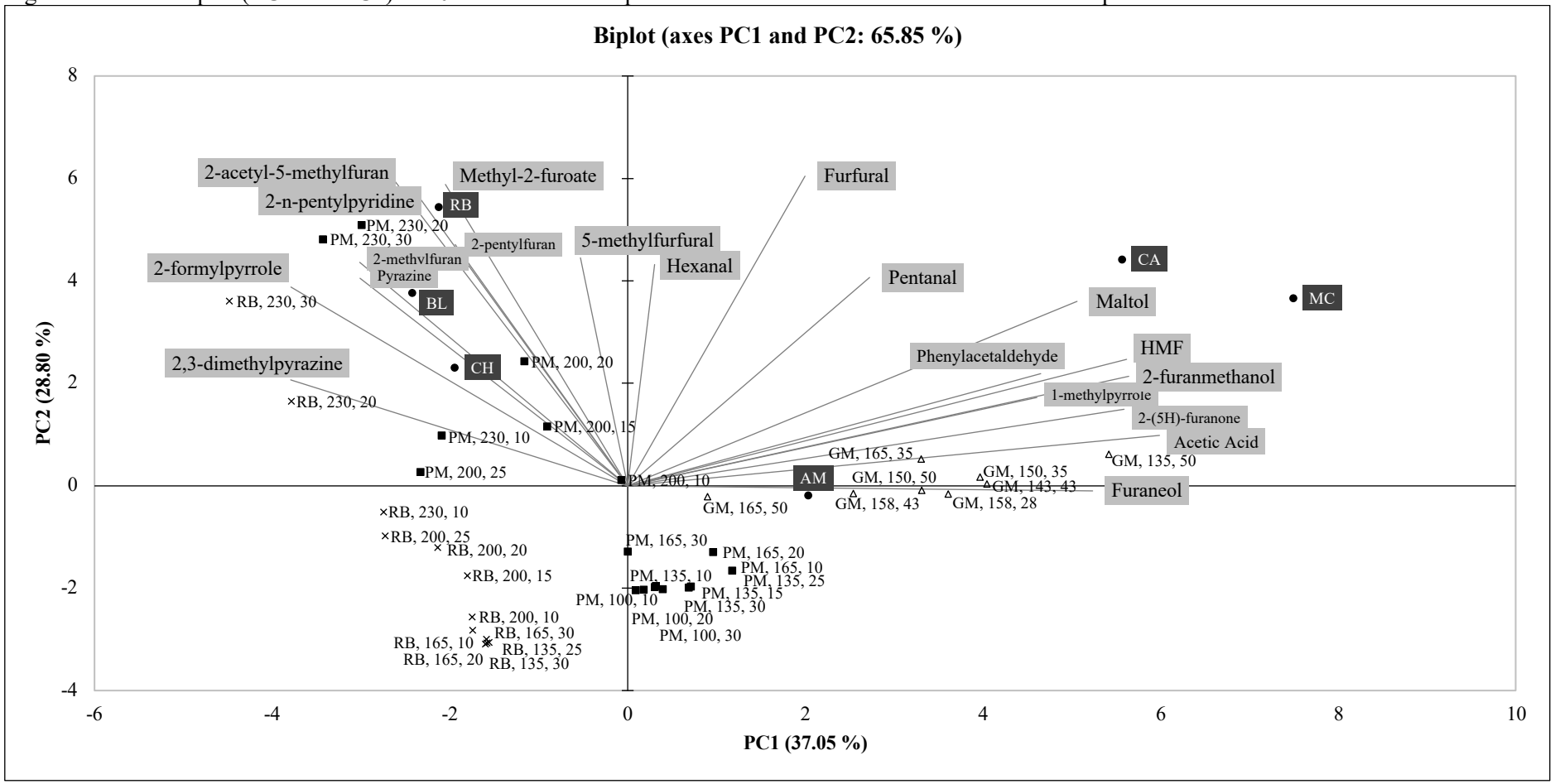
	165	50	1.31	0.577	0.0535	0.129	0.111	1.01	1.62	1.36	174	233	-	4.24	49.0	0.791	10.9	75.2	47.0	295	8.57	6.07	747
Pale Malt	100	10	2.80	0.221	0.0435	0.129	0.161	1.73	0.746	2.32	26.0	255	-	1.14	10.9	0.181	20.4	12.0	20.0	27.4	8.02	3.27	349
	100	20	2.45	0.246	0.0649	0.132	0.180	1.96	0.875	1.76	26.7	235	0.199	0.486	9.88	0.192	23.1	10.2	19.9	30.0	7.44	4.16	318
	100	30	2.38	0.240	0.0682	0.141	0.170	1.76	0.856	1.85	29.4	253	0.0780	1.02	10.7	0.193	22.1	11.7	22.0	28.1	8.51	3.40	362
	135	10	2.23	0.253	0.0430	0.158	0.163	1.75	1.06	2.15	29.0	220	0.421	0.806	9.32	0.168	23.1	9.84	21.5	31.9	11.2	3.62	386
	135	15	1.99	0.235	0.0737	0.158	0.233	1.18	1.10	1.03	27.6	279	0.206	0.495	11.1	0.230	17.5	11.8	23.5	48.7	10.4	5.78	333
	135	25	1.60	0.263	0.0555	0.140	0.214	1.04	1.46	0.863	28.0	293	-	0.532	12.4	0.252	13.1	14.3	25.9	56.5	11.3	7.21	358
	135	30	1.39	0.235	0.0551	0.129	0.192	1.20	1.47	1.17	27.7	261	0.231	0.824	11.6	0.252	12.5	12.2	22.4	50.4	10.9	5.39	380
	165	10	0.779	0.379	0.0743	0.157	0.259	0.901	1.89	0.883	33.6	340	0.304	0.783	18.3	0.435	8.58	17.2	28.6	80.2	15.7	7.39	421
	165	20	0.674	0.424	0.0713	0.194	0.216	0.946	2.78	1.17	53.4	317	0.651	1.17	27.2	0.459	7.58	20.1	29.7	79.9	16.3	8.93	507
	165	30	0.225	0.545	0.0448	0.203	0.171	0.977	3.42	1.29	77.7	225	0.928	1.10	30.1	0.397	6.45	17.2	20.3	62.3	11.0	7.13	491
	200	10	0.301	0.998	0.0387	0.166	0.138	1.63	2.63	1.56	174	243	1.80	2.62	97.0	0.924	7.47	28.0	12.6	58.2	12.0	13.0	780
	200	15	0.119	3.08	0.0761	0.268	0.119	1.79	5.18	0.808	162	252	2.42	2.17	136	1.04	7.25	18.8	17.5	86.3	8.68	30.9	466
	200	20	0.023	4.73	0.198	0.431	0.111	2.21	6.47	1.02	166	268	5.60	2.89	148	1.28	9.76	14.1	22.2	107	9.34	43.4	458
	200	25	0.022	3.39	0.0679	0.263	0.0631	1.53	6.16	0.912	116	119	4.58	2.71	60.4	0.942	3.52	5.59	10.0	54.2	3.56	27.5	181
	230	10	0.077	5.79	0.0848	0.205	0.107	2.33	3.49	0.804	179	148	3.12	3.69	70.5	1.34	6.28	6.90	10.5	62.2	5.82	41.4	180
	230	20	0.045	11.2	0.102	0.345	0.152	4.34	9.96	0.904	225	188	14.8	9.99	108	3.22	11.3	7.67	17.7	138	5.83	44.0	196
	230	30	0.103	8.57	0.0742	0.336	0.124	5.14	11.0	1.56	177	114	22.8	10.5	86.9	3.45	11.5	3.25	8.53	157	5.21	21.4	212
Commercial	AM		1.67	0.300	0.640	0.520	0.360	1.44	2.86	0.880	62.8	366	0.780	1.00	14.1	0.300	25.6	60.1	15.1	177	6.94	4.80	672
	CA		5.06	0.620	0.960	1.26	0.240	1.02	3.28	1.02	281	377	12.8	3.62	34.6	0.600	56.5	404	46.2	730	9.20	4.00	1944
	MC		2.84	0.940	1.10	0.340	0.200	0.580	1.40	0.600	359	565	-	2.70	43.7	0.900	30.6	554	61.1	1021	14.2	5.72	2423
	CH		2.98	0.120	0.440	0.440	0.120	7.98	0.800	2.36	146	128	11.6	14.7	26.1	2.16	12.1	3.74	16.5	246	3.30	28.4	230
	BL		1.97	1.44	0.380	0.240	0.120	8.00	5.44	3.10	200	107	17.2	14.6	45.4	2.58	11.4	5.36	17.4	293	4.44	28.9	350
	RB		2.12	0.220	0.580	2.04	0.260	27.0	2.58	5.12	156	186	5.46	17.5	45.3	2.40	12.3	5.98	14.1	427	4.26	32.3	237

[A] Laboratory roasted samples: roasting substrates were raw barley, green malt, and pale malt. Commercial roasted samples are: amber malt (AM), caramalt (CA), medium crystal malt (MC), chocolate malt (CH), black malt (BL), roasted barley (RB).
[B] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.

4.3.1 Principal Component Analysis (PCA)

PCA was used to analyse the variation in concentrations of the 20 volatile compounds across 37 laboratory roasted samples and the sample set of commercial roasted products. The biplot for principal components 1 and 2 is shown in Figure 4.1 and accounts for almost 66% of the variation in the data set. The number of laboratory roasted samples used for PCA analysis was narrowed down by including only those (n=37) which were deemed to be 'finished' products after roasting, i.e. had a moisture content of less than 5 % w/w (Table 4.1).

Figure 4.1: PCA bi-plot (**PC1** and **PC2**) of 20 odour active compounds and the concentrations in the roasted samples.



Lab roasted samples are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min). Commercial roasted malt samples are: roasted barley (RB), black malt (BL), chocolate malt (CH), medium crystal malt (MC), caramalt (CA), and amber malt (AM). Samples plotted are <5% moisture.

PC1 accounts for 37.05 % of the variation in the data set, whereas PC2 accounts for 28.80 %. PC1 mainly separates the samples according to roasting substrate, and the degree to which those substrates were roasted. Green malt samples load positively on PC1, as to a lesser extent do pale malt samples that were roasted for relatively short times at lower temperatures. Samples that project more negatively on PC1 have been roasted at higher temperatures, and for longer times. This trend is exhibited within each substrate group of the roasted samples. PC2 is largely driven by the concentration of volatile compounds in the samples, which is why all of the volatile loading vectors project upwards in Figure 4.1. Samples plotted more positively on PC2 are more likely to have a higher concentration of the compounds investigated. The upper right quadrant of Figure 4.1 represents volatile compounds which are maximised in green malt products, most typically as a result of Maillard chemistry. Diagonally opposite, the bottom left hand quadrant plots samples which contrast these qualities: i.e. samples which are lowest in these Maillard products. Roasted barley samples that were less intensively roasted are loaded in this quadrant; these have been neither malted nor stewed and thus contained particularly low concentrations of Maillard reaction precursors. The top left sector defines the heavily 'dry roasted' sector of products typified by black malt and chocolate malt commercial products. The volatile compounds likely to be present at higher concentrations in these 'dry roasted' products are heterocyclic compounds such as pyrazines, substituted furans, and pyrroles. Laboratory roasted samples loading in this quadrant typically were the roasted samples of raw barley and pale malt, roasted for the longest times at the highest temperatures (200-230 °C). The diagonally opposite quadrant (bottom right) features the lower temperature treated pale malt samples, which yielded the lowest concentrations of heterocyclics.

The importance of the inclusion of all three roasting substrates is highlighted by their locations in Figure 4.1. The three substrate types of laboratory roasted samples cluster and exhibit individual trends within those groups, emphasising the effect of substrate on roasted product flavour development. In addition to the chosen substrate (i.e. if the barley is malted), the moisture content at the beginning of roasting, and whether or not stewing is used, have considerable influences on the formation of odour active volatiles in the samples.

Whilst forming distinct clusters under less intense roasting conditions, roasted barley and pale malt samples locate in the upper left-hand sector of Figure 4.1 and become

more similar to one another in their volatile composition as they are roasted at the highest temperatures. Therefore, barley and pale malt have a more similar volatile composition when pyrolyzed at higher temperatures and low moisture contents, but are distinct from one another when more delicate roasting processes are applied. Less severe roasting conditions (≤165 °C) enable the pale malts to generate and retain some characteristic Maillard pathway intermediates and products, which is why those samples load more positively on PC1 in comparison to the roasted pale malt samples roasted above 200 °C. In these samples, roasting temperatures exceeding 200 °C result in further volatile generation, namely heterocyclics as a result of pyrolysis.

Commercially available samples were analysed in this study to show where the laboratory roasted samples fell within the commercial range of products. Proximity of samples within Figure 4.1 indicates similarity in volatile composition. Samples plotted closely to specific volatile loading vectors contain high levels of those particular compounds, whilst samples positioned diametrically opposite a volatile compound contain the lowest levels. For example, [RB, 230, 20] is plotted closely to 2,3-dimethylpyrazine, which indicates its relatively high concentration (4.7 μg/g) in this sample. 2-furanmethanol is plotted closely to medium crystal malt and caramalt commercial samples, which contained 553.5 μg/g and 403.7 μg/g respectively. In comparison, [RB, 230, 20] contained just 5.5 μg/g of 2-furanmethanol.

The commercially available crystal malt samples (caramalt and medium crystal malt), were more closely associated with higher concentrations of the green malt odour active compounds than were the laboratory roasted green malt samples. In comparison to this, the highest roasting temperatures of both pale malt ([PM, 230, 20] and [PM, 230, 30]) and raw barley ([RB, 23, 30] and [RB, 230, 20]) resulted in these samples being plotted outside the range of the commercial samples of roasted pale malt and barley, in relation to having higher volatile concentrations than the commercially available samples (commercial roasted barley, black malt, and chocolate malt). These differences reveal the effects of the differing roasting conditions between commercial drum roasting, and laboratory scale roasting. The differences in the rates of heat transfer and volatile stripping would have contributed to this, in addition to the uncontrollable factors of the study, namely barley variety, and precision of replicating stewing conditions for green malt samples.

While the biplot in Figure 4.1 shows the results of monitoring the variation in concentrations of 20 key odour active compounds across the variety of analysed samples, the complexity of roasted product flavour should be acknowledged. Thus, more nuanced differences between sample groups might be identified by increasing the number of compounds quantified. However, by investigating the formation of 20 volatiles as a function of roasting time and temperature, the similarities in volatile composition of the samples could be identified, in addition to the suggestion of gaps in the PCA roasted malt 'flavour space' where there are currently no commercial products.

4.3.2 Modelling Flavour Formation: The Interaction Between Roasting Time and Temperature

For each roasting substrate, 24 laboratory roasted samples were prepared at different temperatures and times according to a D-optimal design (Design Expert software, StatEase, Minneapolis). Concentration data for each of the 20 volatiles (Table 4.1 full data set including samples with a final moisture content >5%) were then modelled as a function of process time and temperature. Thus, three models were produced for each compound, depicting the formation of the compound in each roasted substrate across the range of roasting times and temperatures. Table 4.2 and Table 4.3 summarise the model fit data for each investigated compound. The interactions between the two factors of roasting temperature (factor A) and time (factor B) are stated, in addition to the significance of the overall model, and the R^2 values of the data as plotted in the model. The statistical significance of individual factors (A and B), including all other factors for model hierarchy are displayed in - Appendices Section 8.1 Table 8.1 and Table 8.2.

Table 4.2: Model fit data for the predictive models for compound concentration (compounds 1-10).

Roasting Substrate		2-methylfuran	Pentanal	Hexanal	1-methylpyrrole	Pyrazine	2-pentylfuran	2,3-dimethylpyrazine	Furfural	Acetic Acid	2-n-pentylpyridine
Raw Barley	Model	Cubic	Quadratic	Quadratic	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Cubic
	Model p Value	<0.0001±	0.0017±	0.0012^	0.0003±	<0.0001^	<0.0001±	<0.0001±	<0.0001^	<0.0001±	<0.0001±
	Model R^2	**0.991**	**0.581**	**0.475**	**0.656**	**0.992**	**0.968**	**0.973**	**0.872**	**0.947**	**0.94**
	AB p Value	<0.0001	-	-	0.0038	<0.0001	<0.0001	<0.0001	-	0.0377	<0.0001
	A^2B p Value	<0.0001	-	-	-	<0.0001	0.0007	-	-	0.0007	0.0013
	AB^2 p Value	-	-	-	-	0.0017	-	-	-	-	-
Green Malt	Model	Mean	Linear	Cubic	2Fl	Quadratic	2Fl	Quadratic	Linear	Quadratic	NF
	Model p Value	-	0.0008±	<0.0001±	<0.0001±	<0.0001±	<0.0001±	0.0124±	0.004±	0.0002±	-
	Model R^2	**0**	**0.526**	**0.851**	**0.778**	**0.934**	**0.784**	**0.445**	**0.346**	**0.714**	**-**
	AB p Value	-	-	0.0684	0.0012	<0.0001	0.0341	-	-	<0.0001	-
	A^2B p Value	-	-	-	-	-	-	-	-	-	-
	AB^2 p Value	-	-	-	-	-	-	-	-	-	-
Pale Malt	Model	Cubic	Linear	Quadratic	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Quadratic	Cubic
	Model p Value	<0.0001±	0.0322±	<0.0001±	0.006^	<0.0001^	<0.0001±	0.0008±	<0.0001^	0.0002±	<0.0001^
	Model R^2	**0.979**	**0.21**	**0.873**	**0.411**	**0.963**	**0.991**	**0.598**	**0.917**	**0.583**	**0.982**
	AB p Value	0.001	-	0.0064	-	<0.0001	<0.0001	-	-	-	<0.0001
	A^2B p Value	-	-	-	-	0.0006	<0.0001	-	-	-	<0.0001
	AB^2 p Value	0.001	-	-	-	-	0.0066	-	-	-	-

± Non-significant lack of fit for model. ^ Significant lack of fit for model.
Table shows statistical information for interaction of factors A (temperature) and B (time). Additional factors' statistical information is shown in - Appendices Table 8.1.
Shading indicates significant interaction of factors A and B.

Table 4.3: Model fit data for the predictive models for compound concentration (compounds 11-20).

Roasting Substrate		Methyl-2-furoate	5-methylfurfural	2-acetyl-5-methylfuran	Phenylacetaldehyde	2-furanmethanol	2-(5H)-furanone	Maltol	Furaneol	2-formylpyrrole	HMF
Raw Barley	Model	Cubic	Quadratic	Cubic	Cubic	Cubic	Quadratic	Cubic	Cubic	Quadratic	Cubic
	Model p Value	<0.0001±	<0.0001^	<0.0001^	<0.0001^	<0.0001^	<0.0001±	<0.0001±	<0.0001±	<0.0001^	>0.05^
	Model R^2	**0.986**	**0.84**	**0.986**	**0.966**	**0.751**	**0.94**	**0.985**	**0.786**	**0.778**	**0.383**
	AB p Value	<0.0001	-	<0.0001	<0.0001	0.0193	-	<0.0001	-	-	-
	A^2B p Value	<0.0001	-	<0.0001	<0.0001	-	-	<0.0001	-	-	-
	AB^2 p Value	-	-	-	-	-	-	0.0419	-	-	-
Green Malt	Model	Linear	Linear	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Linear	Quadratic
	Model p Value	>0.05±	0.0003±	<0.0001±	<0.0001±	<0.0001±	0.0005±	<0.0001±	<0.0001±	<0.0001±	0.0003±
	Model R^2	**0.127**	**0.568**	**0.856**	**0.969**	**0.926**	**0.674**	**0.774**	**0.883**	**0.747**	**0.695**
	AB p Value	-	-	0.0034	0.0013	<0.0001	0.0001	<0.0001	<0.0001	-	0.0274
	A^2B p Value	-	-	-	-	-	-	-	-	-	-
	AB^2 p Value	-	-	-	0.003	<0.0001	-	-	0.0013	-	-
Pale Malt	Model	Cubic	Cubic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Quadratic	Quadratic	Cubic
	Model p Value	<0.0001^	<0.0001^	<0.0001±	<0.0001±	0.0044±	0.0002±	<0.0001±	<0.0001±	<0.0001±	0.0027^
	Model R^2	**0.959**	**0.868**	**0.949**	**0.928**	**0.436**	**0.586**	**0.862**	**0.629**	**0.892**	**0.536**
	AB p Value	0.0009	-	0.0001	0.3395	-	-	0.0015	-	0.0363	-
	A^2B p Value	0.0258	-	0.014	0.0056	-	-	0.0314	-	-	-
	AB^2 p Value	0.0059	-	-	-	-	-	-	-	-	-

± Non-significant lack of fit for model. ^ Significant lack of fit for model.
Table shows statistical information for interaction of factors A (temperature) and B (time). Additional factors' statistical information is shown in - Appendices Table 8.2.
Shading indicates significant interaction of factors A and B.

The complexity of the interaction is indicated by the nature of the model. Cubic models indicate the most complex interactions, followed by quadratic models. The R^2 values indicate how closely the inputted data fits the model.

Of the 20 odour active compounds modelled in this study, 10 are discussed further in this Chapter, chosen to be representative of particular thermal flavour generation pathways: pyrazine, 2-acetyl-5methylfuran, methyl-2-furoate, 2-methylfuran, maltol, 2-furanemthanol, acetic acid, phenylacetaldehyde, 2-penylfuran, and 2-n-pentlypyridine. Differences in the generation of compounds across the three roasted substrates will be examined.

It is a feature of the response surface models that the stewed roasted green malt samples exhibit visibly different trends to the roasted raw barley and pale malt samples. A key reason for this (in addition, for example, to the high starting moisture content, length of stewing period, or chosen barley variety) is because green malt has higher levels of hydrolytic enzymes in the endosperm. As a result of the additional stewing step, these enzymes continue to break down starches and proteins in the kernel. Consequently, there are dramatically different concentrations of precursors to thermal flavour generation reactions in the stewed green malt which results in higher concentrations of, for example, furanones (Mackie and Slaughter, 2000). In contrast, the models for the formation of the compounds in roasted raw barley and pale malt are visibly similar in response surface shape, but with differences on the concentration axis.

The main reactions that occur during roasting (the Maillard reaction, caramelisation, lipid degradation, and pyrolysis) can occur under a range of conditions (i.e. moisture content, temperature, concentrations of reactants etc.). The precise combination of conditions favour particular reactions through the time course of roasting. The resultant concentration of a compound is an outcome of its rate of formation minus the rate of its loss. Losses can be due to volatility, or through conversion to subsequent products as a result of additional thermally induced reactions. There are a number of compounds that can be formed by multiple routes, under varying conditions. For example, maltol (discussed in Section 4.3.2.2.1 below) can be formed by both the Maillard reaction, in addition to pyrolysis when roasting occurs at high temperatures with low moisture. The compounds discussed below are categorised by their favoured formation conditions, and therefore the likely reactions that occur to facilitate their formation.

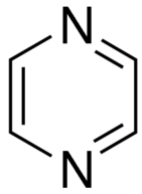
4.3.2.1 Compounds formed at greater concentrations under conditions of high temperature and low moisture

Increased concentrations of pyrazine, 2-acetyl-5-methylfuran, methyl-2-furaote, and 2-methylfuran were favoured under high temperature, low moisture conditions. Each of these compounds is discussed individually below.

4.3.2.1.1 Pyrazine

Pyrazine is characterised by its pungent, roasted hazelnut, roasted barley, sweet aroma (Scents, 2018b). It is a nitrogen containing heterocyclic compound (Figure 4.2) formed via the Maillard pathways: the nitrogen coming from the amino group, and the carbon from the reducing sugars that take part in the reaction pathway (Müller and Rappert, 2010).

Figure 4.2: Structure of pyrazine

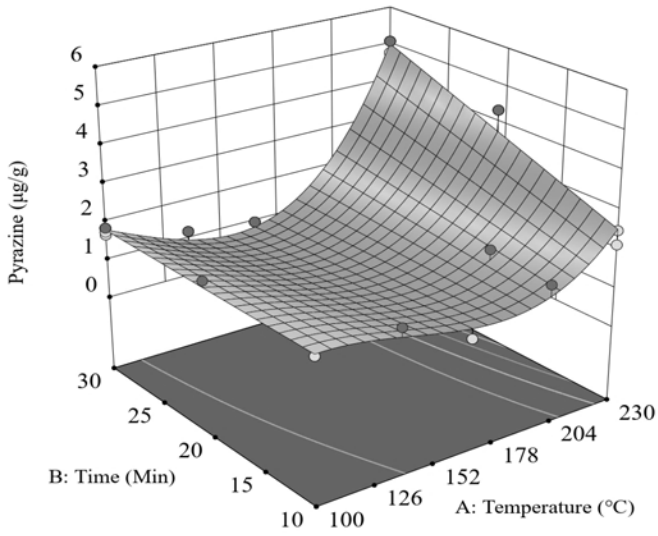


Pyrazine is typically found in products that are processed to high temperatures (>180 °C) (Vandecan et al., 2011). Pyrazine formation through thermally induced reactions has at least two major known pathways. Firstly, the aminocarbonyl compounds produced via Strecker degradation of amino acids can condense to form pyrazines. Secondly, small carbon fragments generated through sugar degradation can react with ammonia generated from the pyrolysis of compounds such as cysteine to produce the pyrazine ring structure. This second pathway is likely to be responsible for the much higher production of pyrazine at 230 °C in roasted pale malt and roasted barley (Figure 4.3a and b, respectively), whereas Strecker degradation reactions probably predominated in the roasted green malt system where much lower levels of pyrazine were generated (Figure 4.3c).

Figure 4.3: The concentrations of pyrazine (μg/g) modelled as a function of roasting time (min) and temperature (°C).

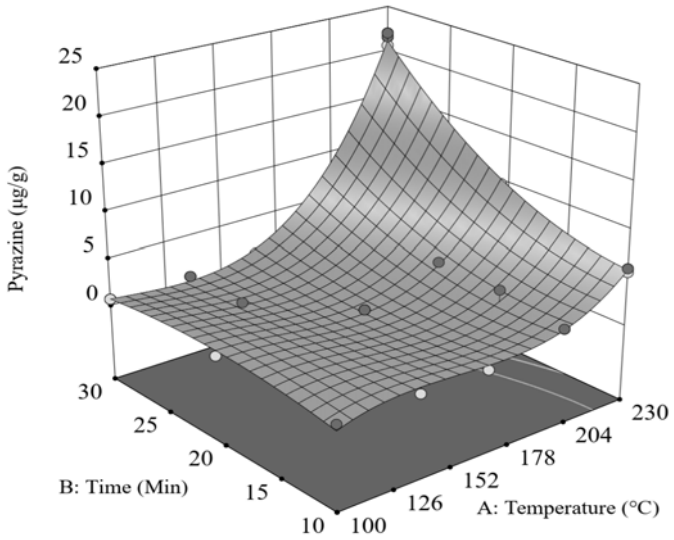
A

Roasted Pale Malt



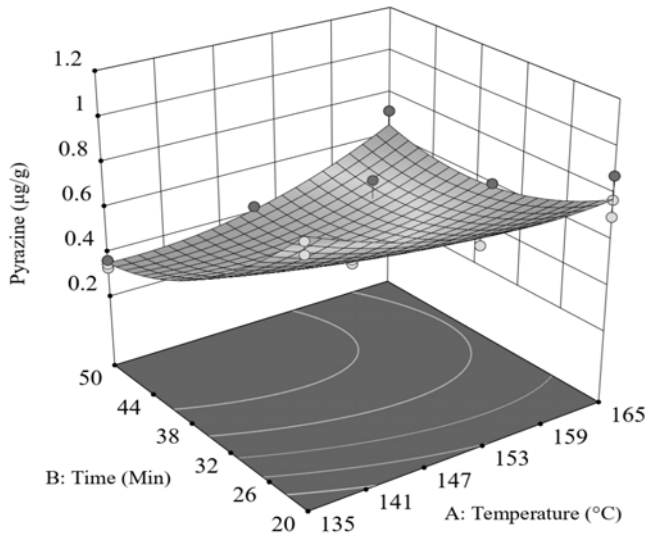
B

Roasted Unmalted Barley



C

Roasted Green Malt



Previous research reported pyrazine concentration increased in speciality malts that were roasted to 180 °C (Vandecan et al., 2011). The response surface models in Figure 4.3a and b show the marked increase of pyrazine in roasted raw barley and pale malt after roasting temperatures exceeded 200 °C.

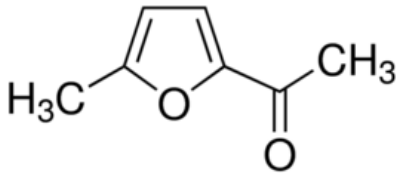
Roasted raw barley yielded the highest concentrations of pyrazine at the highest roasting temperatures and times (Figure 4.3b), particularly [RB, 230, 30] at 22.1 μg/g, whereas [PM, 230, 30] reached 5.6 μg/g. This supports the fact that the aroma descriptor 'roasted barley' is often assigned to pyrazine (Scents, 2018a).

Pyrazine can also be formed by heating serine or threonine in the absence of sugars (Hwang et al., 1993).When forming pyrazine from reactions involving serine, it was found that pyrazine is formed to a higher concentration when heating under high temperature-short time conditions (300 °C for 7 min) as opposed to low temperature-long time conditions (120 °C for 4 h) (Shu, 1999).

4.3.2.1.2 2-acetyl-5-methylfuran

2-acetyl-5-methylfuran is characterised by its musty, nutty, hay-like, caramellic aroma (Scents, 2018a). It is an oxygen containing heterocyclic compound, known to be formed during the Maillard reaction, displayed in Figure 4.4 (Nikolov and Yaylayan, 2011).

Figure 4.4: Structure of 2-acetyl-5-methylfuran

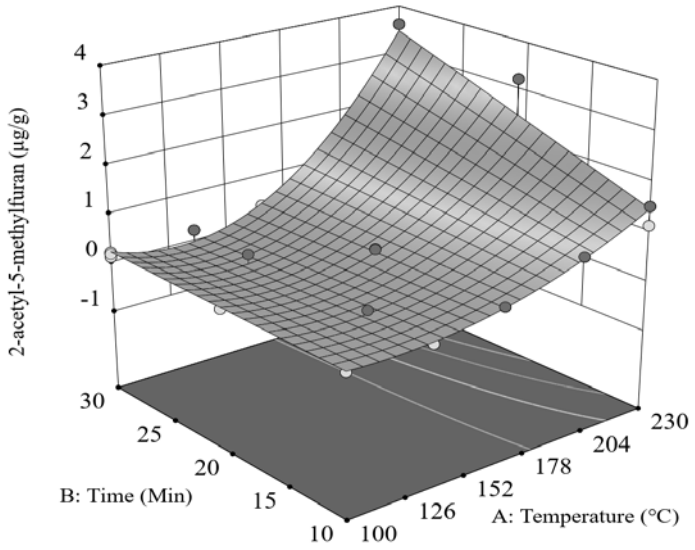


The response surface models for 2-acetyl-5-methylfuran indicate that this compound was formed at much higher concentrations in the dry roasted high temperature laboratory roasted samples. Models were remarkably similar when comparing pale malt and raw barley (Figure 4.5a and b, respectively), suggesting that the prior germination and kilning applied to pale malt had little influence on formation of this compound. The highest concentration of 2-acetyl-5-methylfuran in roasted raw barley was in [RB, 230, 30] at 4.0 μg/g, and in roasted pale malt sample [PM, 230, 30] at 3.6 μg/g.

Figure 4.5: The concentrations of 2-acetyl-5-methylfuran (µg/g) modelled as a function of roasting time (min) and temperature (°C).

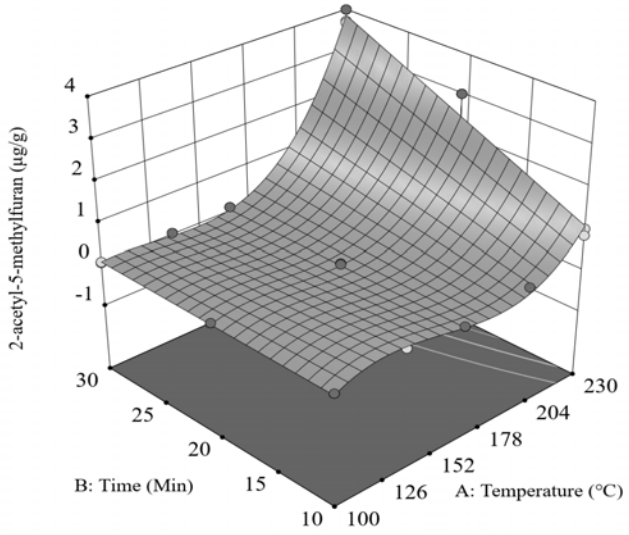
A

Roasted Pale Malt



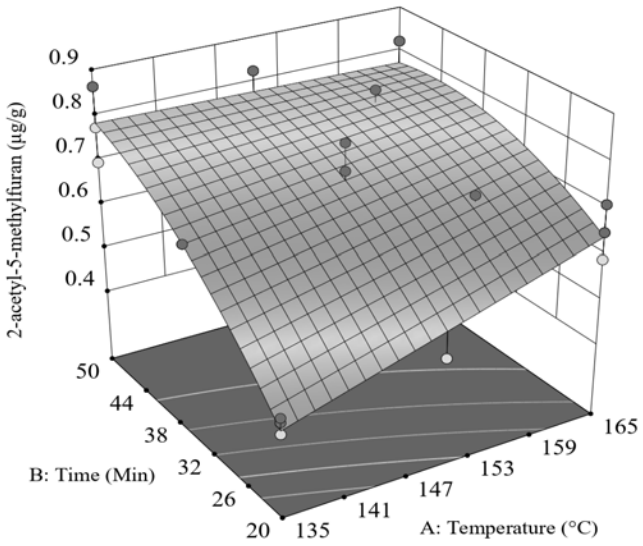
B

Roasted Unmalted Barley



C

Roasted Green Malt



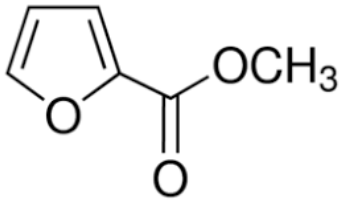
In roasted stewed green malt, the concentration of 2-acetyl-5-methylfuran was notably lower than for the other two roasted raw materials, remaining below 1.0 μg/g in all roasted samples (Figure 4.5c). Despite this, the individual factors of roasting time and temperature had a significant effect on the concentration of 2-acetyl-5-methylfuran in roasted stewed green malt ($p<0.0001$ and $p=0.0035$ respectively), as did the interaction between those two factors ($p=0.0034$).

The models presented for this compound suggest that high roasting temperatures are required in order to produce the highest levels of this compound; the green malt samples were not finished at temperatures above 165 °C, at which temperature concentrations in the product plateaued at around 0.8 μg/g. In the roasted barley and pale malt models, 2-acetyl-5-methylfuran production clearly increased steeply at process temperatures above 180 °C. In a study monitoring flavour compound formation in commercially roasted chocolate malt, O'Shaughnessy (2003) found that 2-acetylfuran (a volatile compound very similar to 2-acetyl-5-methylfuran) was formed to the highest concentrations when the product was roasted from 75 min to 97 min when the final chocolate malt product was discharged. This supports the model that extended periods of roasting (at high temperatures) results in higher concentrations of acetyl furans.

4.3.2.1.3 Methyl-2-furoate

Methyl-2-furoate is the methyl ester of 2-furoic acid, as displayed in Figure 4.6, and is commonly used as a fragrance to contribute to coffee, tobacco, nut, and mushroom aromas and flavours (Fu and Doucet, 2011, Scents, 2018a).

Figure 4.6: Structure of methyl-2-furoate

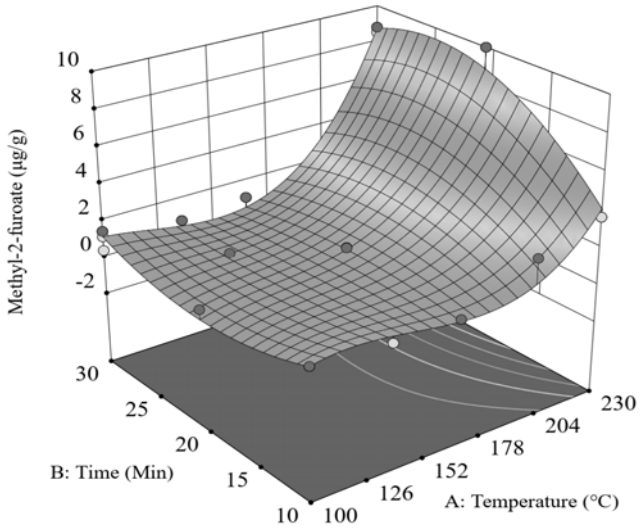


Both roasted pale malt and roasted unmalted barley samples (Figure 4.7a and b) yielded a steep increase in methyl-2-furoate concentration in samples roasted at the highest temperatures.

Figure 4.7: The concentrations of methyl-2-furoate (µg/g) modelled as a function of roasting time (min) and temperature (°C).

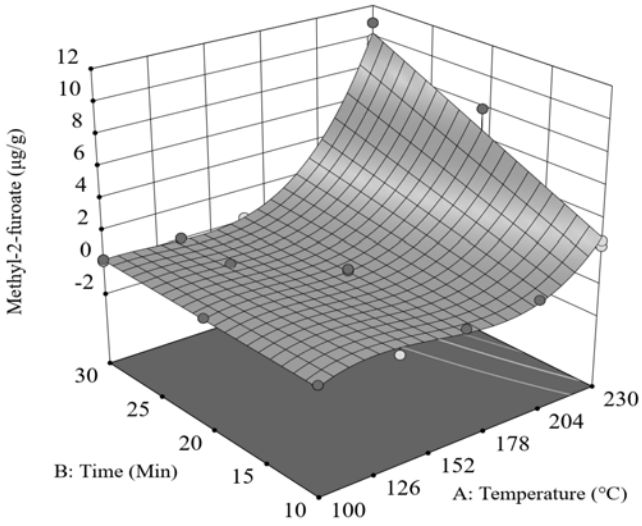
A

Roasted Pale Malt



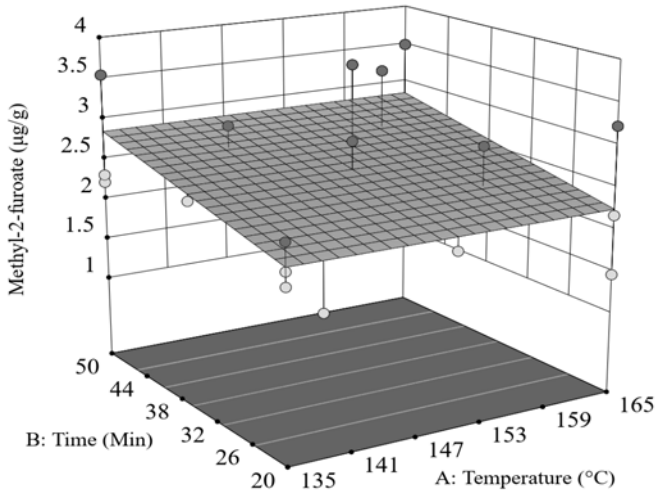
B

Roasted Unmalted Barley



C

Roasted Green Malt



The sample with the highest concentration was [PM, 230, 30] at 10.5μg/g, and was the highest concentration of all the analysed samples. However, the roasted barley sample with the highest concentration was also [RB, 230, 30] with 10.1 μg/g methyl-2-furoate.

The model for the formation of methyl-2-furoate in stewed and roasted green malt samples showed a linear trend, with no interaction between the factors of roasting time and temperature (Figure 4.7c).The R^2 value for this model (Figure 4.7c) indicates how loosely the concentration data fit the model. However, the concentration of methy-2-furaote in the stewed and roasted green malt samples increased slightly with increasing roasting time, peaking at 4.24 μg/g in [GM, 165, 50]. It is likely that sufficient temperatures were not achieved in order to form larger quantities of the compound.

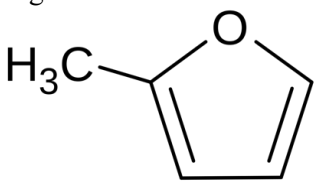
In addition to being an odour active volatile, methyl-2-furoate has an alternative role in the search for sustainable fuels (Khusnutdinov et al., 2013). It can be produced on an industrial scale from furfural (obtained from renewable bioresources, namely sugar cane) by oxidative esterification (Fu and Doucet, 2011, Khusnutdinov et al., 2013). Although methyl-2-furaote is synthesised under different conditions to those in the roasting of malts, parallels can be drawn between the two reactions.

The PCA biplot of Figure 4.1 shows furfural to be present at higher concentrations in the roasted green malt samples, and Table 4.1 also shows the roasted pale malt samples roasted above 200 °C have increasing concentrations of furfural. Figure 4.7 shows the increased concentration of methyl-2-furoate in both roasted pale malt and roasted unmalted barley samples at the highest roasting temperatures. This lack of correlation between the two models of formation of furfural and methyl-2-furoate suggests that an alternative mechanism occurs in the malt roasting process leading to the formation of methy-2-furoate, as opposed to that of industrial scale synthesis.

4.3.2.1.4 2-methylfuran

2-methylfuran is an alkylated furan, with an ethereal, acetone, chocolate aroma, the structure of which is shown in Figure 4.8 (Scents, 2018a). In addition to being found in roasted malts, 2-methylfuran is also found in roasted coffee, a roasted product which shares many similarities regarding thermal processing (Lee et al., 2017).

Figure 4.8: Structure of 2-methylfuran



A number of different pathways facilitate the formation of 2-methylfurn during roasting. Caramelisation can result in the formation of furans, in addition to pyrolysis reactions with the aid of redox chemistry (Paine Iii et al., 2008). Another possible route for the formation of 2-methylfuran is by the degradation of HMF (also found in roasted malts (Table 4.1) via caramelisation, which acts as a precursor to the formation of a number of furans (Kroh, 1994).

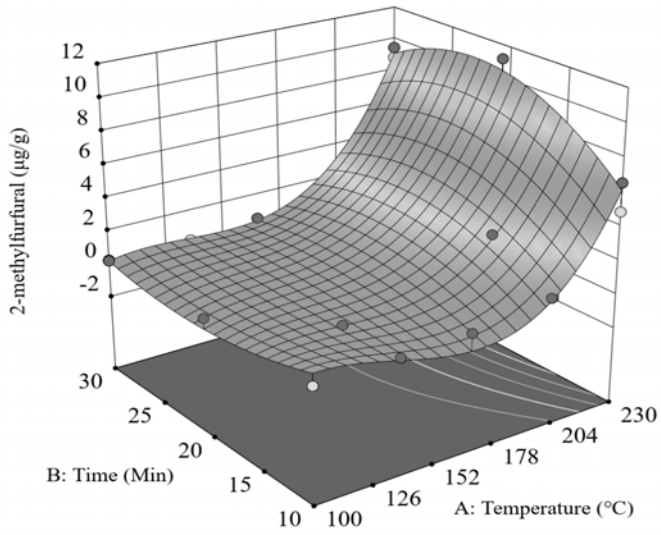
The highest concentrations of 2-methylfuran in the roasted samples were found in those that were 'dry roasted', particularly those roasted to temperatures >200 °C for a minimum of 15 min. This favours the possible route of caramelisation to the formation of 2-methylfuran, as temperatures exceeding 120 °C are required for caramelisation to take place (Kroh, 1994).

Roasted samples of pale malt yielded a maximum of 11.2 μg/g 2-methylfuran in [PM, 230, 20] (Figure 4.9a), reducing to 8.57 μg/g in [PM, 230, 30]. In addition to its formation, it is also possible that 2-methylfuran can undergo subsequent reactions with HMF to form polymeric structures via the Maillard reaction (Nikolov and Yaylayan, 2011). This may explain the decrease in concentration of 2-methylfuran in the roasted pale malt sample roasted for an extended period at 230 °C (8.57 μg/g in [PM, 230, 30]).

Figure 4.9: The concentrations of 2-methylfuran (µg/g) modelled as a function of roasting time (min) and temperature (°C).

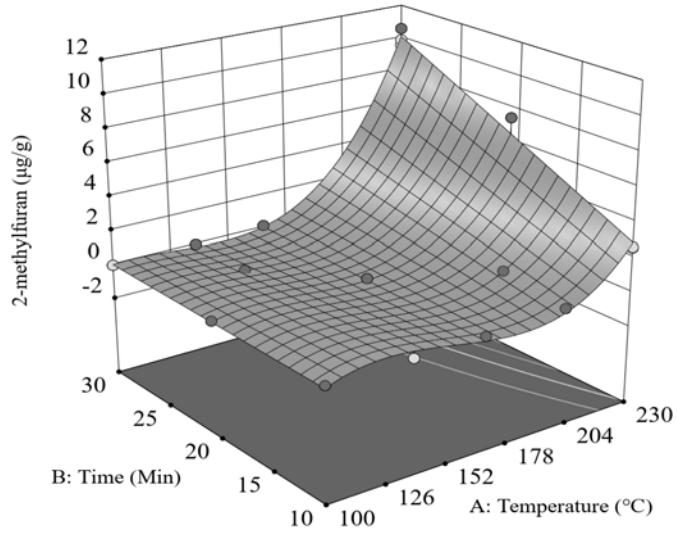
A

Roasted Pale Malt



B

Roasted Unmalted Barley



The roasted raw barley samples exhibited a similar trend to that observed for the roasted pale malt samples, with dramatic increases in 2-methylfuran concentration in samples roasted at >200 °C (Figure 4.9b). The highest concentration was achieved in [RB, 230, 30], at 10.0 μg/g. The barley samples roasted to 230 °C exhibited a sharper increase in 2-metylfuran concentration with longer roasting times at this temperature: from 2.62 μg/g in [RB, 230, 10], to 7.45 μg/g in [RB, 230, 20], then finally to 10.0 μg/g in [RB, 230, 30].

Despite the differences in models between the two substrates, the significance of the interaction of roasting time and temperature remains (Table 4.2). Regarding the interaction between the two factors in the roasted raw barley model (Figure 4.9b), the factor of temperature within the interaction is more influential, as the p value of the A^2B interaction is <0.0001. Whereas in the pale malt model (Figure 4.9a), the factor of time is more influential, as the AB^2 factor's p value = 0.001.

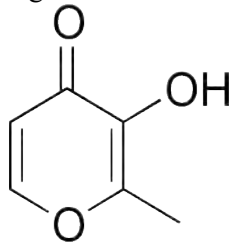
The formation of 2-methylfuran in the roasted and stewed green malt samples had no correlation, therefore the model for the formation of this compound in this substrate is not shown.

4.3.2.2 Compounds formed at greater concentrations under conditions of low temperature and high moisture

4.3.2.2.1 Maltol

Maltol is formed in the intermediate stages of the Maillard reaction pathway (Vandecan et al., 2011). It has an oxygen containing heterocyclic structure (Figure 4.10), and is characterised by its sweet, jammy, baked aroma (Pittet et al., 1970, Scents, 2018a).

Figure 4.10: Structure of maltol



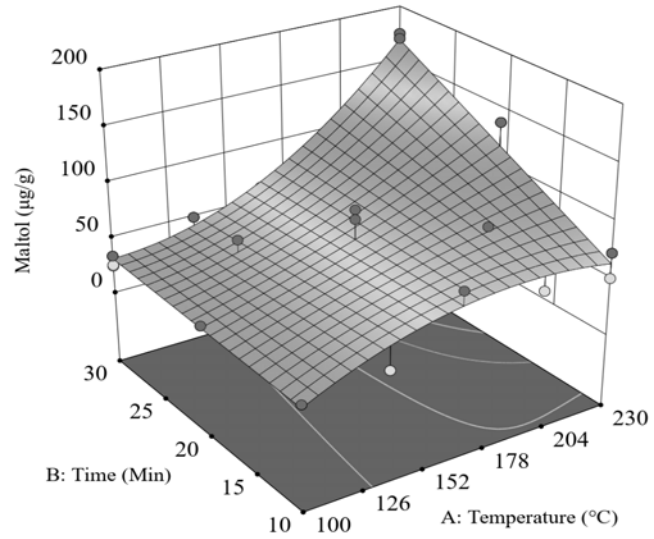
Roasted green malt samples contained the highest concentrations of maltol (from 226.1 μg/g to 972.0 μg/g) (Figure 4.11c), as compared with 24.7 μg/g to 175.1 μg/g

for roasted pale malts (Figure 4.11a) and 5.1 μg/g to 100 μg/g for roasted barley samples (Figure 4.11b).

Figure 4.11: The concentrations of maltol (μg/g) modelled as a function of roasting time (min) and temperature (°C).

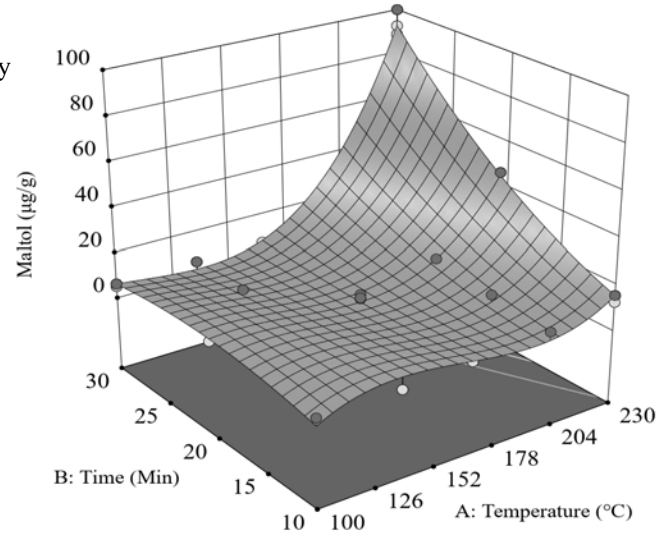
A

Roasted Pale Malt



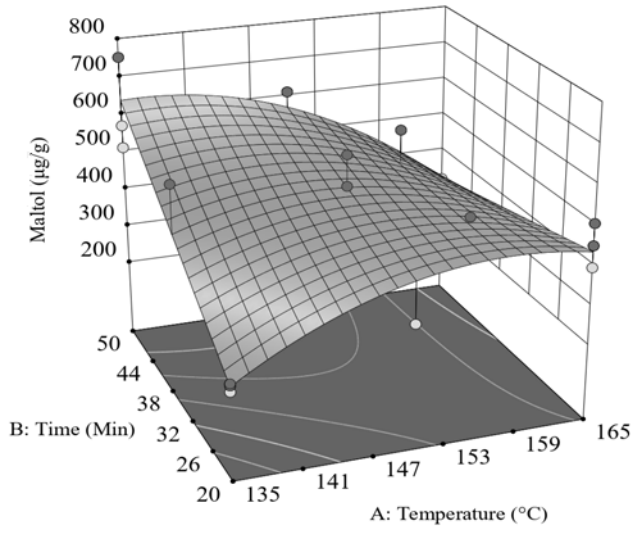
B

Roasted Unmalted Barley



C

Roasted Green Malt



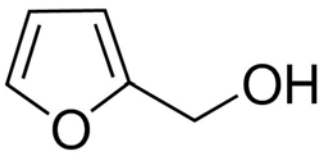
With the ‘dry roasted’ (pale malt/ barley) samples it was evident that maximal levels of maltol were obtained in samples treated at the highest roasting temperatures for the longest time. This strongly suggests a pyrolytic route to maltol in addition to its production via classic Maillard chemistry; the model for raw barley clearly shows this effect at temperatures in excess of 200 °C and at longer process times (Figure 4.11b). Under green malt processing conditions the model indicates that maltol formation was favoured by higher temperatures (165 °C) at the shortest process time (20 min) or for maximum concentration, lower temperature (135 °C) and the longest process time (50 min) (Figure 4.11c).

Maltol can be formed through a number of different pathways (e.g. from disaccharides, or from proline-amadori products) during thermal processing, which lead to its distinct concentrations in roasted products (Yaylayan and Mandeville, 1994). This is also influenced by the availability of precursors in the raw materials. Yahya et al. (2014) also showed that maltol concentrations in roasted products increased steeply during the late, high temperature-low moisture stage of roasting. This suggests, as noted here, that there are routes to maltol formation via pyrolysis in addition to Maillard reactions. An earlier study conducted by O'Shaughnessy (2003) monitored flavour formation in a range of three malts and barley in commercial roasting operations. In chocolate malt, which is a highly coloured roasted pale malt, the concentration of maltol increased over time, then decreased (O'Shaughnessy, 2003), which is not in accordance with this investigation. The maximum product temperature was 230 °C, roasted for up to 97 min. The details of temperature ramping during the commercial production of chocolate malt were not reported.

4.3.2.2.2 2-furanmethanol

2-furanmethanol is a furan ring with a substituted hydroxymethyl group (Figure 4.12), the aroma of which is described as being musty, sweet, caramellic, bready, and coffee-like (Scents, 2018a).

Figure 4.12: Structure of 2-furanmethanol



2-furanmethanol can be formed as a result of the Maillard reaction, in the presence of maltose and proline (Mori, 2004). It can also be formed from maltose by caramelisation in the absence of proline (Mori, 2004). Wnorowski and Yaylayan (2000) proposed a mechanism for the production of 2-furanmethanol, in which the Amadori product forms a pentose intermediate through a retro-aldol reaction.

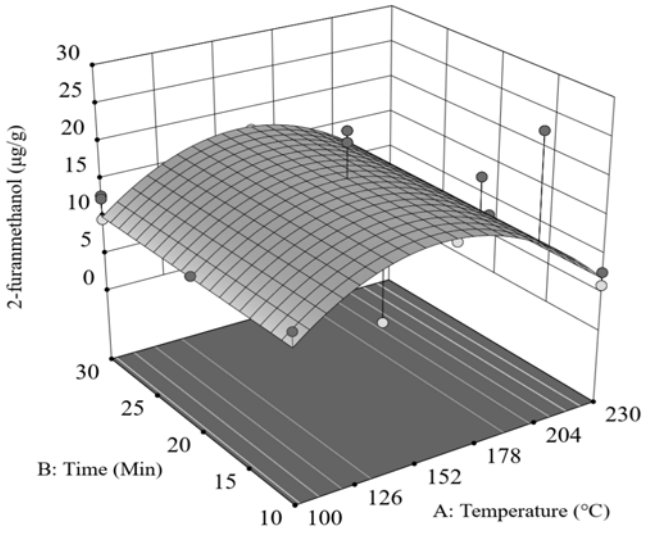
In Chapter 3, 2-furanmethnol was found to be highly odour active (FD>1000) in the majority of the commercial samples analysed. The samples in which 2-furanmethanol was highly influential on the aroma included the crystal malt samples (caramalt and medium crystal malt), and the darkest dry roasted products (chocolate malt, black malt, and roasted barley).

The reaction between maltose and proline by the Maillard reaction is known to be favoured by heating within an aqueous solution, but can also occur in pyrolytic conditions (Mori, 2004, Wnorowski and Yaylayan, 2000). A consequence of this are the higher concentrations of 2-furanmethanol in the stewed and roasted green malt samples in Figure 4.13c. The highest concentration of 2-furanmethanol was achieved in [GM, 135, 50] at 360 μg/g. This highlights the favourable conditions under which 2-furnamethanol is formed to the highest concentrations, in aqueous conditions supported by milder temperature roasting.

Figure 4.13: The concentrations of 2-furanmethanol (µg/g) modelled as a function of roasting time (min) and temperature (°C).

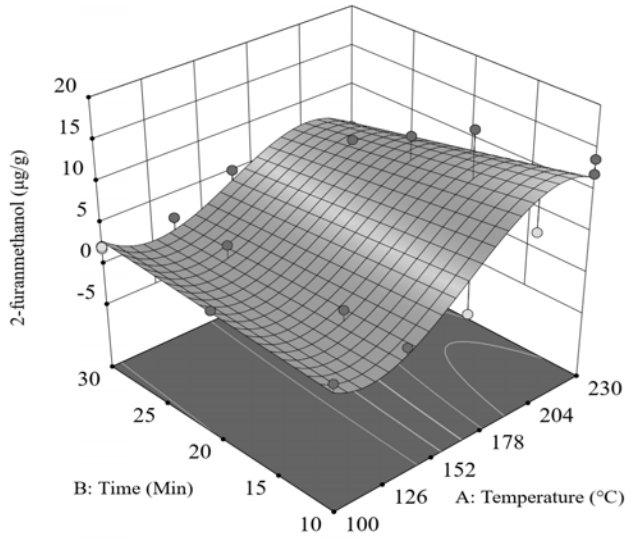
A

Roasted Pale Malt



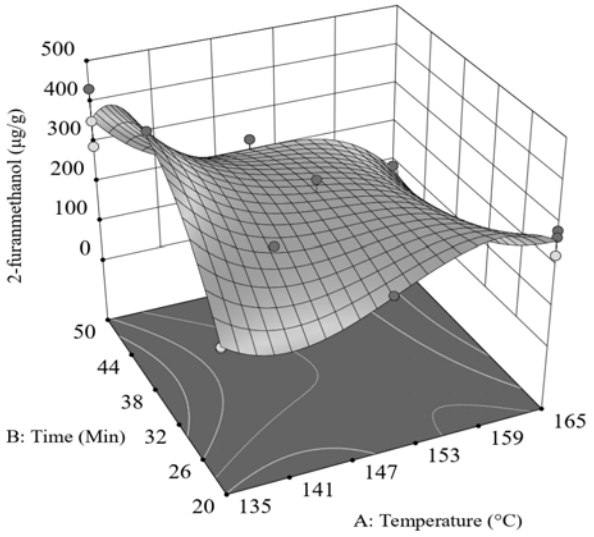
B

Roasted Unmalted Barley



C

Roasted Green Malt



There is a visibly clear difference in the concentration of 2-furanmethanol between the roasted green malt samples (Figure 4.13c), and the roasted pale malt and roasted barley samples (Figure 4.13a and b, respectively). The greatest concentration of 2-furnamethanol in the latter roasted substrates was 28.0 μg/g in [PM, 200, 10]. However, for the roasted pale malt model (Figure 4.13a), there was no interaction between the factors of roasting time and temperature and the R^2 value was poor (Table 4.3). The data points plotted in the Figure do not align well with the model. The model data for 2-furanmethanol production in roasted unmalted barley samples indicates an interaction between the factors of time and temperature in the formation of 2-furanmethanol. The complexity of the interaction is indicated by the cubic model (Table 4.3). The greatest concentration was achieved in the [RB, 200, 15] sample (16.8 μg/g), then decreased with increasing roasting time and temperature. This further demonstrates that milder roasting conditions favour higher concentrations of 2-furnamethanol accumulating.

In a previous investigation into the formation of key volatiles throughout commercial roasting by Yahya et al. (2014), 2-furanmethanol was suggested to be formed as a thermal degradation product of melanoidins, in addition to its formation under milder roasting temperatures. This may be the cause of the relatively high concentrations of 2-furanmethanol in Figure 4.13a and b under dry roasting conditions at temperatures exceeding 200 °C.

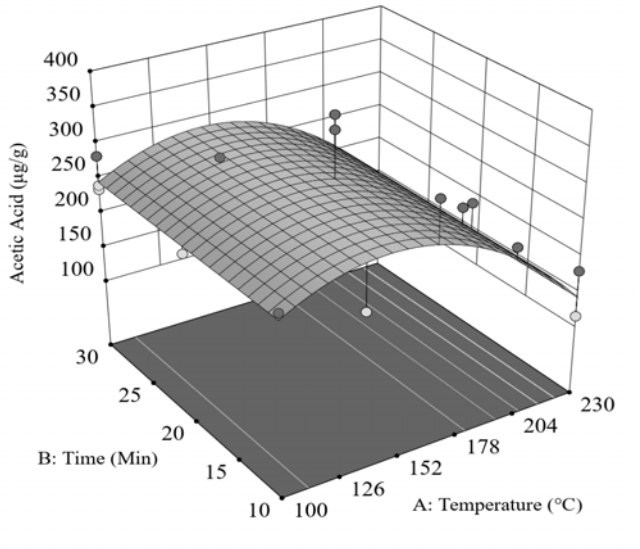
4.3.2.2.3 Acetic Acid

Acetic acid was the only carboxylic acid monitored in this investigation, and has a sharp, pungent, sour, and vinegar-like aroma (Scents, 2018a). It is known to be an intermediate product of the Maillard reaction, both in model systems, and in malts (Coghe et al., 2006). Acetic acid was found to be present at high concentrations in all three roasted substrates, but was highest in the roasted green malt samples, in which the interaction between roasting time and temperature was the most significant ($p<0.0001$ (Table 4.2)) (Figure 4.14c). The concentration of acetic acid in the stewed and roasted green malt samples ranged from 233 μg/g in [GM, 165, 50], to 464 μg/g in [GM, 135, 50]. Milder roasting temperatures favoured the formation of acetic acid in this substrate.

Figure 4.14: The concentrations of acetic acid (µg/g) modelled as a function of roasting time (min) and temperature (°C).

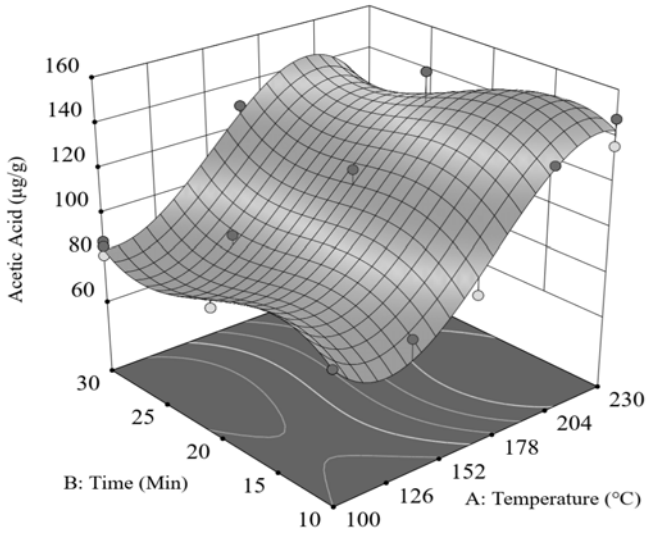
A

Roasted Pale Malt



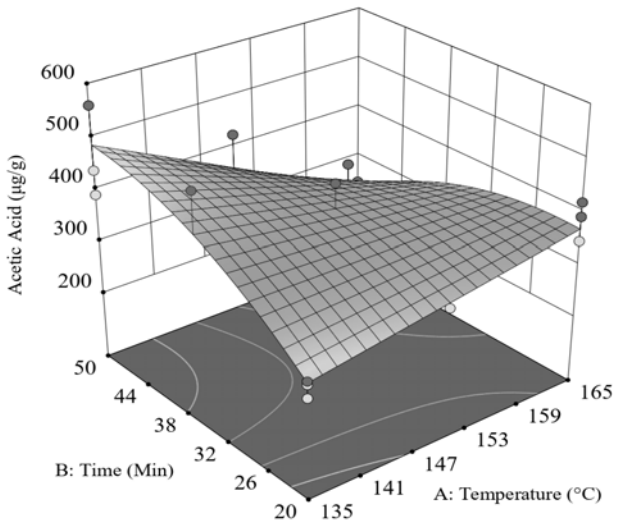
B

Roasted Unmalted Barley



C

Roasted Green Malt



The interaction between roasting time and temperature was more complex in the roasted unmalted barley samples (Figure 4.14b) compared to this interaction in the roasted green malt samples, indicated by its cubic model (as opposed to the quadratic model in Figure 4.14c). The interaction between the two factors in the roasted unmalted barley samples showed temperature to be more influential, over time (Table 4.2), with the highest concentrations achieved in the samples roasted at the highest temperatures.

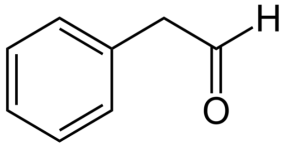
The model for the roasted pale malt samples (Figure 4.14a) does not have a strong R^2 value (weak predictive power), however, does indicate a clear visual trend, showing decreasing acetic acid concentration with increasing roasting time. This may be due to volatilisation, conversion to other compounds, or incorporation into the melanoidin skeleton (Coghe et al., 2006, Vandecan et al., 2011).

4.3.2.3 Product of Strecker Degradation

4.3.2.3.1 Phenylacetaldehyde

Phenylacetaldehyde has a floral, honey, green, cocoa, sweet aroma (Scents, 2018a). It is a Strecker aldehyde formed in thermally treated foodstuffs through the Strecker degradation of phenylalanine (Channell et al., 2010, Farmer, 1994, Rizzi, 1999, Smit et al., 2009). Phenylacetaldehyde was the only Strecker aldehyde monitored in this study, so was included in this section for further examination. The structure of phenylacetaldehyde is shown in Figure 4.15.

Figure 4.15: Structure of phenylacetaldehyde



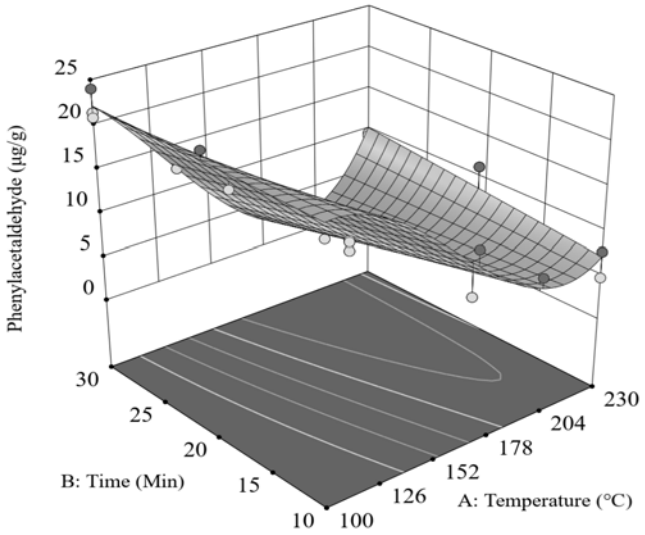
Strecker degradation reactions require dicarbonyl compounds in addition to an amino acid. Small and reactive dicarbonyl compounds are generated from sugar degradation reactions, which may result from either Maillard chemistry or caramelisation reactions. The gross trends in phenylacetaldehyde production across the roasted substrates indicate that much higher levels were generated in the roasted green malt products (Figure 4.16c). This overall trend likely results from a combination of enhanced Maillard reactivity brought about by the stewing process in roasted green

malt production, and the lower losses due to volatilisation of phenylacetaldehyde at the lower green malt finishing temperatures.

Figure 4.16: The concentrations of phenylacetaldehyde (µg/g) modelled as a function of roasting time (min) and temperature (°C).

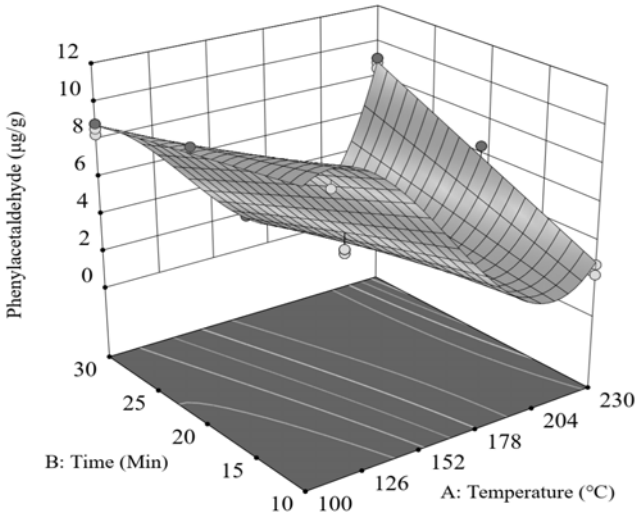
A

Roasted Pale Malt



B

Roasted Unmalted Barley



C

Roasted Green Malt

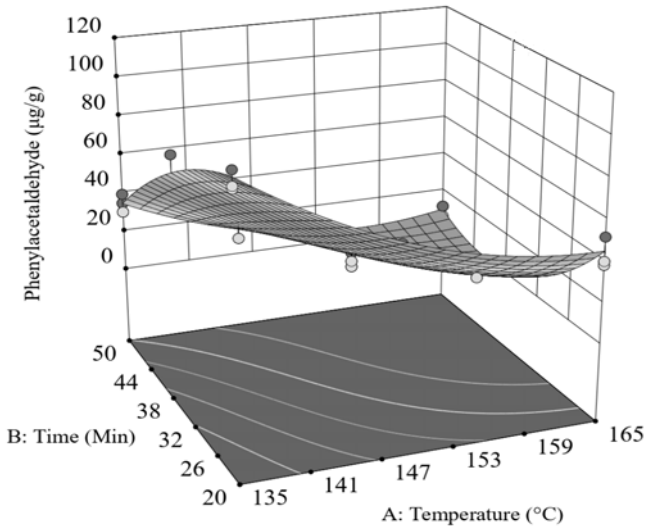


Figure 4.16a and b show that the response surface models for phenylacetaldehyde formation in roasted samples of pale malt and unmalted barley (respectively) were of similar form, but with differences in concentration. The highest concentrations were found in the samples roasted at the lowest temperatures ([PM, 100, 30] at 24 μg/g, for example (Figure 4.16a)). As the roasting temperature increased, the concentration of phenylacetaldehyde initially dropped ([PM, 200, 25] at 3.5 μg/g), then increased again at the very highest roasting temperatures ([PM, 230, 30] at 9.6 μg/g).
This trend suggests that in very dry, high temperature roasted systems another pathway to phenylacetaldehyde may exist; for example via pyrolysis of phenylalanine as opposed to Strecker degradation. This theory for the formation of phenylacetaldehyde is not proven by the current experiments, but the shapes of the models for both raw barley and pale malt are consistent with there being a secondary route to the production of phenylacetaldehyde at high roasting temperatures. This trend was not seen in the roasted stewed green malt samples where treatments did not include a high enough roasting temperatures to exhibit the final increase of phenylacetaldehyde.

4.3.2.4 Products of Lipid Degradation

Lipid oxidation is one of the many thermal flavour generation reactions that occur during the roasting of malts and barley. As discussed in Chapter 1 Section 1.3.3, lipids form 2-4 % of the dry weight of the barley kernel, with linoleic acid forming 52.4-58.3 % of the grains' lipid composition (Arendt and Zannini, 2013). Both lipid degradation products discussed below originate from linoleic acid.

4.3.2.4.1 2-pentylfuran

2-pentylfuran is an oxygen containing heterocyclic compound, with aroma attributes including: fruity, green, earthy, beany, vegetal, and metallic (Scents, 2018a). While containing a furan ring (Figure 4.17), 2-pentylfuran is not formed via the Maillard reaction, but by singlet oxygen from linoleic acid (Min et al., 2005).

Figure 4.17: Structure of 2-pentylfuran

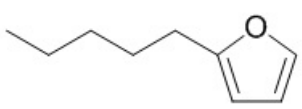
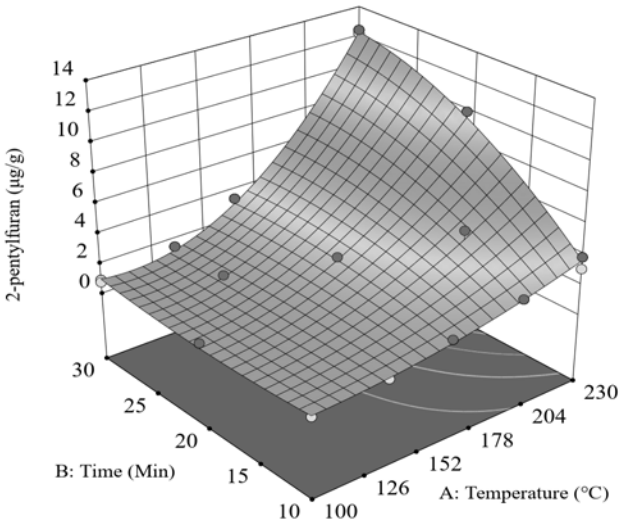


Figure 4.18: The concentrations of 2-pentylfuran (μg/g) modelled as a function of roasting time (min) and temperature (°C).

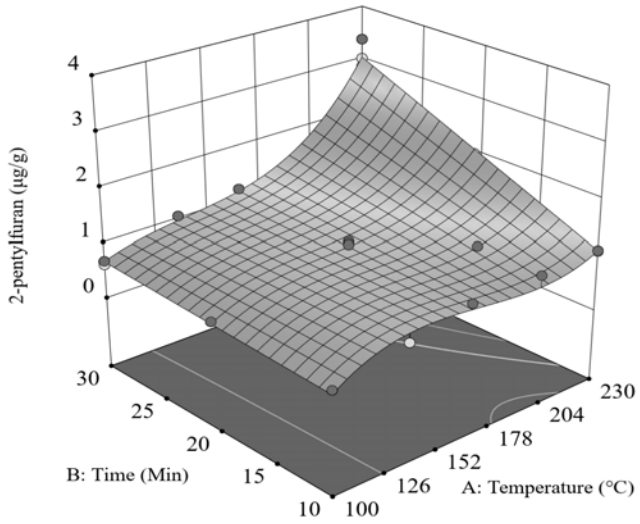
A

Roasted Pale Malt



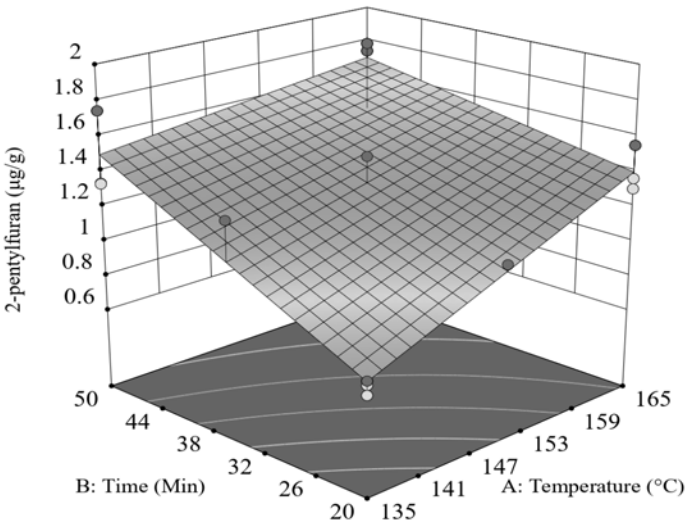
B

Roasted Unmalted Barley



C

Roasted Green Malt

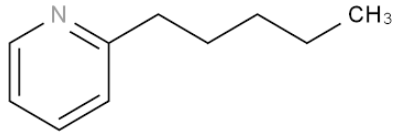


The roasting of pale malt yielded the highest concentrations of 2-pentylfuran of the three substrates, with 12.4 μg/g in [PM, 230, 30]. Germination and kilning may have influenced the availability of linoleic acid as a precursor to 2-penylfuran in the pale malt as a roasting substrate. The lack of formation of 2-pentylfuran to such a degree as in the roasted pale malt samples (Figure 4.18a) is apparent in Figure 4.18b and c.

4.3.2.4.2 2-n-pentylpyridine

2-n-pentylpyridine is a nitrogen containing heterocyclic compound (Figure 4.19), with a fatty, green, bell pepper, mushroom, herbal aroma (Scents, 2018a). It is formed as a result of lipid degradation from linoleic acid (Kim et al., 1996).

Figure 4.19: Structure of 2-n-pentylpyridine

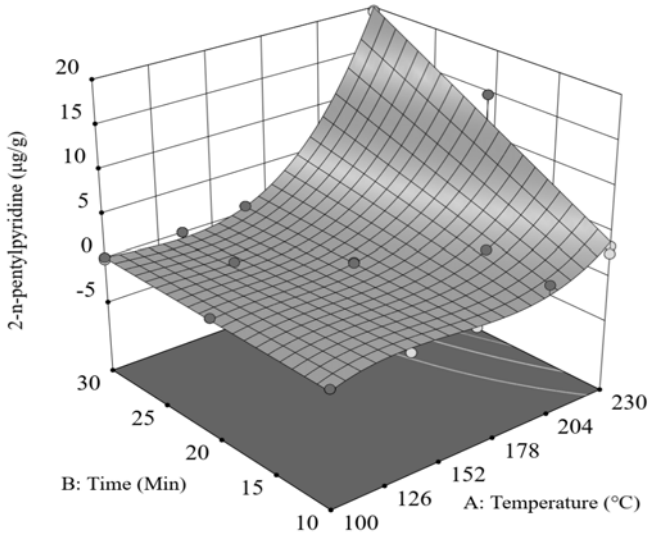


2-n-pentylpyridine has a low odour detection threshold (0.2 ng/L in air (Kim et al., 1996)), and was detected by the panel in previous GC-O studies (Chapter 3) at FD>1000 in three of the six analysed commercial samples (medium crystal malt, chocolate malt, and black malt). Also, in the commercial roasted barley sample, 2-n-pentylpyridine was detected at FD 100. The compound was therefore found to be more influential on the sensory characteristics in the darker roasted commercial samples. This trend is supported by the models of 2-n-pentylpyridine formation in the laboratory roasted samples. The roasted pale malt samples, for example, have a low concentration <200 °C (0 μg/g to 0.928 μg/g), then a dramatic increase in concentration to 22.8 μg/g in [PM, 230, 30] (Figure 4.20a). This trend is similar in the laboratory roasted unmalted barley samples (Figure 4.20b), but to a lower concentration (11.4 μg/g), consistent with the FD factor of 100 in the commercial roasted barley sample in the previous GC-O study.

Figure 4.20: The concentrations of 2-n-pentylpyridine (μg/g) modelled as a function of roasting time (min) and temperature (°C).

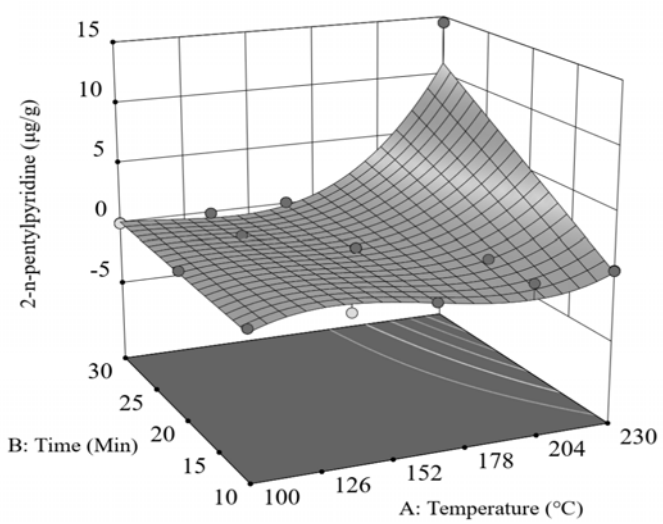
A

Roasted Pale Malt



B

Roasted Unmalted Barley



The interaction of factors of roasting time and temperature were highly significant in both models (AB: $p<0.0001$). Each model was also highly significant ($p<0.0001$), both fitted to cubic models, indicating a complex interaction between the factors. 2-n-pentylpyridine was not detectable in the laboratory stewed and roasted green malt samples.

4.3.3 Selection of Samples for Further Analysis

As discussed in Section 4.3.1, the range of six commercially available samples were analysed in order to show where the laboratory roasted samples fell within the commercial range of products. In order to identify laboratory roasted samples with distinctive sensory characteristics when compared to the equivalent commercially available samples, additional analysis is required. In Chapter 6 sensory analysis by partial Quantitative Descriptive Analysis (QDA) by a trained panel is carried out, analysing the aroma profiles of a selection of the laboratory roasted samples in addition to a selection of commercially available samples to identify any notable differences.

Seven laboratory roasted samples were selected to examine further using sensory analysis by a trained panel. The seven selected laboratory roasted samples and their roasting parameters are listed in Table 4.4.

Table 4.4: Laboratory roasting parameters for samples selected for further analysis.

Roasting Substrate [A]	Temperature (°C)	Time (min)
RB	135	30
	200	25
PM	165	20
	200	25
GM	135	50
	158	43
	165	35

[A] Roasting substrates are abbreviated as follows: raw barley (RB), pale malt (PM), and green malt (GM).

Selection of these samples was primarily based upon their position within the PCA biplot, shown in Figure 4.1 (Section 4.3.1) together with informal tasting of the samples to provide a preliminary check on their sensory properties. Small quantities of the dry milled laboratory roasted samples were tasted by the investigators,

identifying notable characteristics. Those with distinctive characteristics were selected for additional analysis.

Only samples with <5 % moisture (w/w) were considered, in order to represent 'finished' products. From this, samples that presented potential gaps in the commercial range, in addition to samples that appeared to be particularly novel were reviewed further. For example, [RB, 135, 30] was selected as a result of its novelty, as currently the only commercially available roasted unmalted barley products are roasted at high temperatures, and provide a strong coffee flavour (Gruber, 2001). The pair of [-, 200, 25] samples, both from unmalted barley and pale malt, were selected to compare the sensory qualities of the two substrates when roasted under identical conditions. When monitoring the formation of odour active compounds favoured under dry roasting conditions (i.e. pyrazine, 2-acetyl-5-methylfuran, methyl-2-furotae, and 2-methylfuran), concentrations typically increased sharply in roasting temperatures exceeding 200 °C. In addition, these samples were not plotted closely to any commercially roasted samples in Figure 4.1, indicating their potential novelty.

Three stewed and roasted green malt samples were selected from the range displayed in Figure 4.1. The selected green malt samples represent two extremes of the green malt products ([GM, 165, 50] and [GM, 135, 50]), in addition to a moderately roasted green malt sample from the centre of the green malt experimental design ([GM, 158, 43]). All the laboratory produced green malt samples were plotted distinctly from the commercial crystal malts (caramalt, and medium crystal malt) in Figure 4.1. Therefore, the variety within the laboratory produced range should be examined further.

4.4 Conclusions

Modelling odour active compound formation over a range of roasting parameters has enabled better understanding of how the substrate and roasting conditions combine to generate the volatile aroma composition of roasted malt and barley products. This study also compared laboratory-roasted samples to commercial samples in terms of their concentrations of 20 key odour active compounds. The PCA plot of the resulting data depicts a 'flavour space' for roasted products produced from these three substrates, indicating how control of time, temperature and initial moisture

content during roasting determined product volatile aroma characteristics. Compounds such as maltol, phenylacetaldehyde, 2-furanmethanol, HMF and acetic acid were formed at highest concentrations in roasted green malt products, indicating greater formation via Maillard chemistry in the liquid phase and/or lower losses of volatiles at the more moderate green malt roasting temperatures. In contrast, odour-active compounds such as pyrazines, pyrroles, pyridines, 2-methylfuran, 2-pentylfuran, methyl-2-furoate and 2-acetyl-5-methylfuran were formed at greater concentrations in the 'dry roasted' products, indicative of greater formation via Maillard chemistry in the solid phase or pyrolysis at higher temperatures and very low moisture contents.

The development of response surface models for the formation of each of the 20 compounds as a function of time and temperature in each roasting substrate clearly demonstrated the complexity of thermal flavour generation which results from factors such as there being multiple pathways to individual compounds which have different activation energies or temperature ranges at which they become active. Furthermore, differences in compound volatility and the potential for onwards thermal reactions in some cases further complicate the form of models. Thus the predictive power of cubic models fitted to some compound concentration data was still low and some models had a significant lack of fit indicating that the trends in data across the design space were too complex to accurately model without 'over-fitting' the data (e.g. pentanal, hexanal, 2-furanmethanol, 2-formylpyrrole, hydroxymethylfurfural). Several of the models derived indicate that concentrations of volatile compounds increased steeply at very high temperature (>180 °C) under low moisture conditions. Since these conditions prevail at the end of pale malt or roasted barley production this indicates how difficult precise flavour control is for these product-types. Traditionally colour is used as the yardstick for process control, but brewers recognise that there can be substantial differences in flavour attained from different batches of the same roasted product.

This study highlights the range of chemistries that occur during thermal processing, and how specific alterations in roasting conditions can influence the production of particular odour active compounds. Opportunities for additional analysis have also been identified, which can facilitate the understanding of the formation of flavour by roasting. Low flavour threshold compounds (e.g. odour active volatile sulphur compounds) should be analysed in a proportion of the laboratory roasted samples in

addition to the commercial range (as previously recognised), in order to increase understanding of the conditions under which a more representative range of odour active compounds are formed. In addition, the aroma qualities of the samples as a whole must be examined in order to understand the overall impact of particular roasting parameters on the sensory properties of roasted products. Understanding the formation of flavour by roasting is an essential step in product development, as this information may be used by maltsters to engineer roasted products with specific desirable characteristics.

Chapter 5 - Volatile sulphur compounds in roasted products: semi-quantitative identification using SPME-GC-PFPD

5.1 Introduction

Volatile sulphur compounds (VSCs) are found in a variety of foods, for example coffee, beer, wine, meat, cheese, onion, and garlic (Jiemin et al., 2004, Fuchsmann et al., 2015, Fang and Qian, 2005). In each of these products, VSCs contribute a range of flavour characteristics, depending on the combination of VSCs found, and their concentrations.

Sulphur aroma compounds typically have low sensory detection thresholds, and so can be present in foods at very low concentrations whilst remaining flavour active (Hill and Smith, 2000). As a result of this trait, VSCs contribute significantly to the overall aroma of many products. The off-putting reputation of VSCs (i.e. 'rotten egg' 'onion', 'cabbage', etc.) often results in the positive contribution of these compounds being overlooked, as sulphur compounds can contribute to the aroma and flavour of foods positively (Hill and Smith, 2000).

The nature and varying concentrations of different volatile sulphur compounds can result in a range of aroma characteristics (Hill and Smith, 2000, Fang and Qian, 2005). Typically, high concentrations of VSCs result in the most unpleasant, overpowering off-flavours and aromas. Whereas low concentrations of a combination of compounds can impart pleasing savoury characteristics to the overall aroma of a product. Specifically, in parallel systems to malt roasting, coffee aroma is associated with sulphur volatiles and their leading contribution to the overall roasted aroma of the product (Yu et al., 2012).

In beer, VSCs are typically associated with off-flavours and spoilage, as bacterial infection can lead to the production of sulphur volatiles. The major provider of sulphur compounds found in beer is the fermentation process, rather than arising directly from the raw materials themselves (Hill and Smith, 2000). However, the key raw materials used in the brewing process (malt, roasted malt, and hops) contain a range of VSCs, some of which may remain throughout the entire brewing process (Lermusieau and Collin, 2003).

In Chapter 3 previously, gas chromatography-olfactometry (GC-O) was used to identify the odour active compounds of interest in a range of six commercially available roasted malts. Particularly, the chocolate malt sample proved to be of interest, yielding a greater number of detected aromas than the number of peaks detected by mass spectrometry (MS). A more sensitive and selective method of compound identification was required to shed light on the highly odour active nature of this roasted sample, among others. This Chapter examines the commercial and laboratory produced range of roasted malted and unmalted barley, identifying and quantifying the range of VSCs in the samples.

Conventional methods of liquid injection with GC-MS are insufficiently sensitive to detect the presence of low-level VSCs. Preconcentration of the volatiles in the sample is common in VSC analysis (Jiemin et al., 2004). In this investigation, the preconcentration step of headspace solid-phase microextraction (SPME) was used in combination with gas chromatography, coupled with a pulsed flame photometric detector (PFPD). This detector is sulphur specific. Fang and Qian (2005) found the quantification limits of SPME-GC-PFPD could be 0.5ppb for the most volatile sulphur compounds in wines, which is below, or in the range of most sensory detection limits. The quantification of VSCs is particularly challenging due to their high reactivity and low concentrations in products (Vazquez-Landaverde et al., 2006). In comparison to other sample analysis techniques, headspace SPME reduces analysis duration and sample manipulation (Vazquez-Landaverde et al., 2006). Hill and Smith (2000) developed a successful analysis method (on which the one in this investigation is based) by using SPME-GC-PFPD for the analysis of beer. Necessary alterations to this method were made, as detailed in Section 5.2 below.

The VSC contents of the comprehensive range of roasted malts has not yet been characterised in detail. Therefore, this investigation highlights new findings of the proportions of VSCs in roasted malts (roasted pale and green malt) and roasted barley, and suggests the possible pathways for their formation during the roasting process.

5.2 Materials and Methods

5.2.1 Samples

5.2.1.1 Commercial Samples

The full range of nine commercial samples were analysed in this study, as detailed in Section 2.1: Roasted Barley, Black Malt, Chocolate Malt, Pale Chocolate Malt, Dark Crystal Malt, Medium Crystal Malt, Light Crystal Malt, Caramalt, and Amber Malt.

5.2.1.2 Laboratory Roasted Samples

As detailed in Chapter 4 Section 4.3.3, a range of seven laboratory roasted samples were selected from the range of 37 laboratory roasted samples.

The roasted substrate, roasting times, and temperatures of each sample examined in this investigation are detailed in Table 5.1 below. Laboratory roasted samples were prepared as described in Section 2.3.

Table 5.1: Details of laboratory roasted samples analysed in Quantitative Descriptive Analysis

Abbreviation	Roasting Substrate	Roasting Temperature (°C)	Roasting Time (Min)
GM 135 50	Green Malt	135	50
GM 158 43	Green Malt	158	43
GM 165 35	Green Malt	165	35
PM 165 20	Pale Malt	165	20
PM 200 25	Pale Malt	200	25
RB 135 30	Raw Barley	135	30
RB 200 25	Raw Barley	200	25

5.2.2 Chemicals

Authentic analytical standards (>90% purity) were purchased to identify the sulphur containing compounds within the roasted samples. Suppliers of chemicals are displayed in Table 5.2.

Due to the lack of prior literature reporting the VSC contents of roasted malts, parallel products were considered (e.g. roasted coffee) to provide likely candidates for the identification of VSCs in the samples analysed in this investigation. The contents of Table 5.2 include all purchased compounds, not only those identified in the range of samples.

Table 5.2: Source of authentic analytical standards of purchased volatile sulphur compounds of interest.

Compound	CAS	Purity (%)	Supplier
1-heptanethiol	1639-09-4	97	VWR International
2-acetyl-2-thiazoline	29926-41-8	>96	Sigma Aldrich
2-acetyl-3-methylthiophene	13679-72-6	98	Sigma Aldrich
2-Acetyl-5-methylthiophene	13679-74-8	98	Fisher Scientific
2-acetylthiazole	24295-03-2	99	Sigma Aldrich
2-acetylthiophene	88-15-3	95	Sigma Aldrich
2-ethoxythiazole	15679-19-3	>95	Sigma Aldrich
2-ethyl-4-methyl thiazole	15679-12-6	98	VWR International
2-formyl-5-methylthiophene	13679-70-4	98	VWR International
2-formylthiophene	98-03-3	98	Sigma Aldrich
2-furfurylthiol	98-02-2	98	Sigma Aldrich
2-methyl-3-(methylthio)furan	63012-97-5	>98	Sigma Aldrich
2-methyl-3-furanthiol	28588-74-1	95	Sigma Aldrich
2-methyl-5-(methylthio)furan	13678-59-6	>95	Sigma Aldrich
2-methyl-5-ethyl thiophene	40323-88-4	99	Fisher Scientific
2-methylthiophene	554-14-3	>98.5	Sigma Aldrich
2-propyl thiophene	1551-27-5	97	VWR International
2-propyl-2-thiazoline	17626-75-4	99	VWR International
2,4,5-trimethylthiazole	13623-11-5	98	Sigma Aldrich
2,5-dimethylthiophene	638-02-8	98	Sigma Aldrich
3-mercapto-2-pentanone	67633-97-0	95	Fisher Scientific
3-methylthiophene	616-44-4	98	Sigma Aldrich
4-methylthiazole	693-95-8	99	Sigma Aldrich
4,5-dihydro-2-methyl-3-thiophenone	13679-85-1	97	Fisher Scientific
4,5-dihydro-3(2H)-thiophenone	1003-04-9	98	Sigma Aldrich
4,5-dimethylthiazole	3581-91-7	98	VWR International
Diallyl sulfide	592-88-1	97	Fisher Scientific
Dimethyl disulfide	624-92-0	>99	Sigma Aldrich
Dimethyltrisulfide	3658-80-8	98	Fisher Scientific
Methional	3268-49-3	98	VWR International
Methyl allyl sulfide	10152-76-8	98	VWR International
Methyl propyl disulfide	2179-60-4	90	Sigma Aldrich
Methyl-2-(methylthio)acetate	16630-66-3	98	Sigma Aldrich
Methyldithiolane	5616-51-3	99	VWR International
Pentylthiophene	4861-58-9	98	VWR International
Thiophane	110-01-0	99	Sigma Aldrich
Thiophene	110-02-1	>99	Sigma Aldrich

Compounds are listed alphabetically.

5.2.3 Sample Preparation

Standard glass headspace vials (20 mL capacity) were used in this study. Within each vial, 4 g of milled malt sample was mixed with 8 mL water containing 5 μg/g benzothiazole as an internal standard. External standard calibrations for every concentration for each compound, and each sample were carried out in duplicate.

5.2.4 SPME Operating Conditions

A 23 ga divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (StableFlex, Sigma Aldrich, Dorset, UK) was used for the SPME analysis of the samples. Upon analysis, each vial was incubated for 30 min at 30 °C. The vial was agitated at 500 rpm on a 2 s on, 4 s off sequence. The SPME fibre was then inserted into the vial through the septum of the vial lid. Adsorption of headspace volatiles would then take place for 30 minutes while remaining incubated at 30 °C, without agitation. Desorption of the volatiles was carried out in the PTV (Programmed Temperature Vaporiser) injector for 5 min at 240 °C.

5.2.5 GC-PFPD Operating Conditions

The GC operating conditions were as described in Section 2.8 using a Bruker Scion 456-GC (Scion Instruments, Livingston, Scotland, UK) fitted with a PTV injector set to 240 °C for 5 min. All GC effluent was fed to a PFPD set to 210 °C. The PFPD settings were as follows: multiplier voltage: 600 V; trigger level: 200 mV; gate delay: 6.0 ms; gate width: 20.0 ms; range: 9. Range 9 was chosen to reduce the noise on the baseline of the chromatographs, to give increased confidence in peak detection. The decline in range has reduced the area and number of the peaks detected slightly, in comparison to running the PFPD at range 10.

5.2.6 Peak Identification and Quantification by External Standards

Compounds were identified based upon two levels of validation: linear retention index (LRI) against alkanes (C8-C22) when compared to literary sources on the same WAX phase; and LRI comparison with authentic standards when assessed under the same chromatographic conditions.

For calibration of authentic standards, stock solutions of each compound were made at 1000 μg/g in methanol (HPLC/ LC-MS grade, VWR International Ltd.) with 5 μg/g benzothiazole as the internal standard. Stock solutions of the compounds were then diluted in water with internal standard (5μg/g benzothiazole) to the following concentrations: 0.25, 0.5, 1, 5, and 10 μg/g. Standard solutions were added to 4 g milled raw barley, milled according to Section 2.5. The raw barley was used to attempt to replicate the compounds' interactions within the matrix of the roasted samples when occurring naturally, as opposed to when being inoculated in the raw barley standard solutions.

5.2.7 Principal Component Analysis

Principal Component Analysis (PCA) was carried out using XLSTAT software (Addinsoft, SARL, Paris, France), examining PC1, PC2, and PC3.

5.3 Results and Discussion

Example chromatographs of roasted barley, pale chocolate malt, medium crystal malt, [GM, 135, 50], [RB, 200, 25], and [PM, 200, 25] are shown in Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4, Figure 5.5, and Figure 5.6, respectively. Examples of the raw data chromatographs of the remaining analysed samples are displayed in the - Appendices Section 8.2. The identified peaks are labelled with their corresponding compound, excepting in busy areas to avoid overcrowding of labels. From the raw chromatographic data, peak areas were noted, from which concentrations were calculated using calibration data from authentic external standards. The R^2 values of the linear calibrations of these external authentic standards were essential in calculating the concentrations of the compounds semi-quantitatively. The R^2 values are quoted in Table 5.3. Regarding eight of the 30 authentic standards, no linear calibration could be identified. This may be due to the authentic standard compounds' interaction within the matrix of milled raw barley upon mixing, causing the compound to adhere to the sample rather than volatilising at a linear rate to increased concentration.

It is clear from the raw data shown that samples roasted at higher temperatures for longer times resulted in a greater number of detectable VSC peaks. There is a clear visible difference between the number of peaks detected in the commercial samples

in Figure 5.1 and Figure 5.2 (RB and PC, respectively) (many), in comparison to Figure 5.3 (MC) (few). Also reflecting this trend, the laboratory roasted samples in Figure 5.5 and Figure 5.6 ([RB, 200, 25], and [PM, 200, 25], respectively) yielded a greater number of detected VSC peaks than in Figure 5.4 ([GM, 135, 50]). It is a clear result of differences in roasting temperatures and times in these groups of samples that cause the differences between the number of detected VSC peaks.

Figure 5.1: PFPD chromatograph of a sample of roasted barley from Boortmalt.

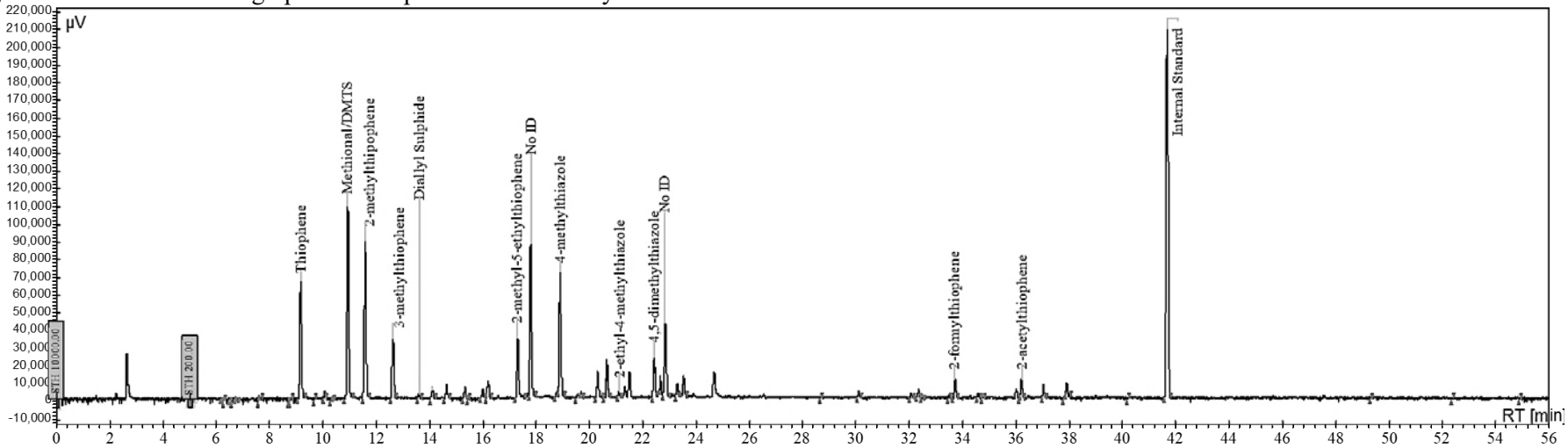


Figure 5.2: PFPD chromatograph of a sample of pale chocolate malt from Boortmalt.

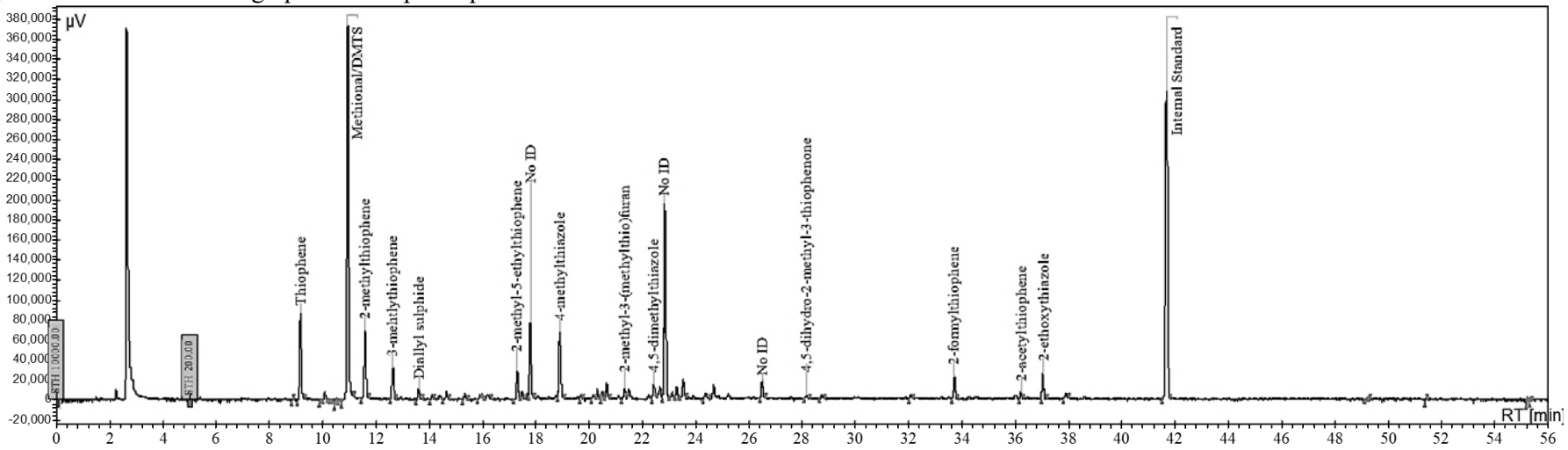


Figure 5.3: PFPD chromatograph of a sample of medium crystal malt from Boortmalt.

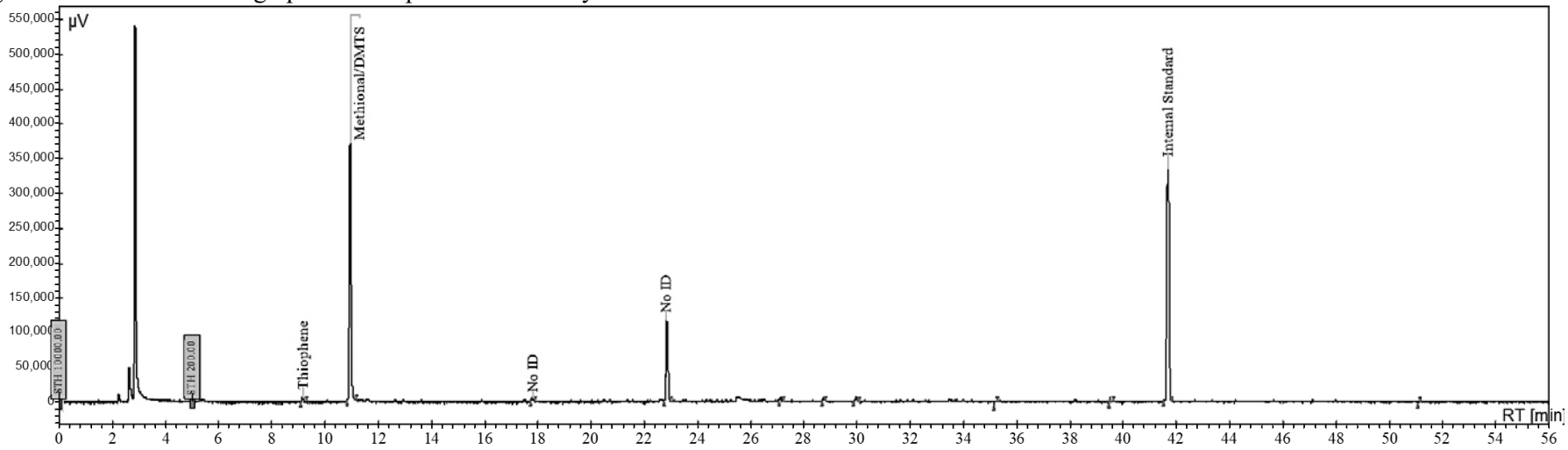


Figure 5.4: PFPD chromatograph of a sample of laboratory roasted green malt (135 °C for 50 min).

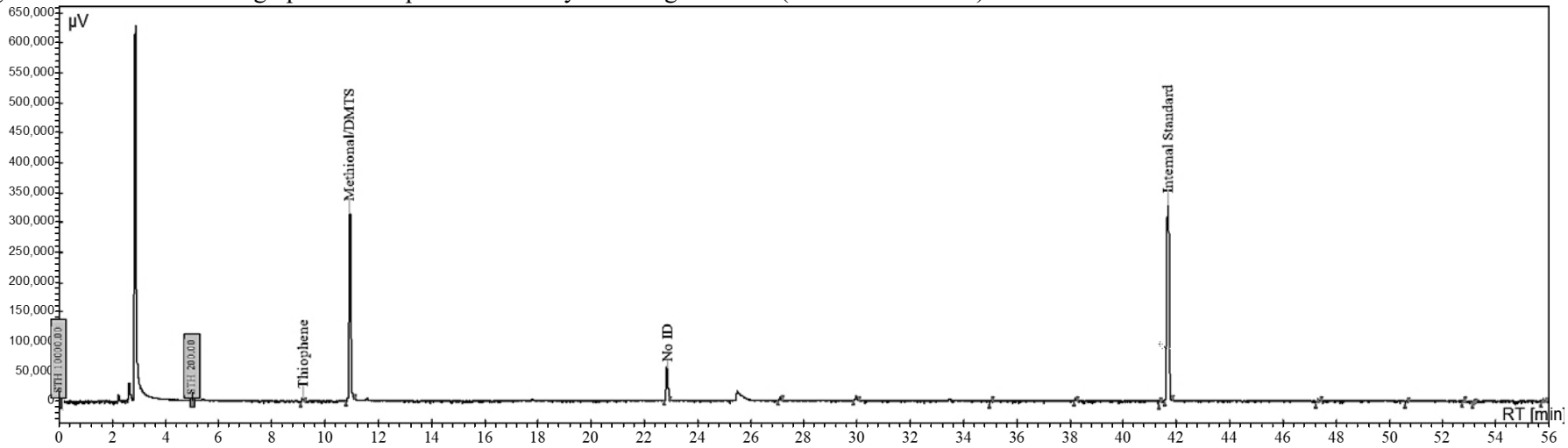


Figure 5.5: PFPD chromatograph of a sample of laboratory roasted barley (200 °C for 25 min).

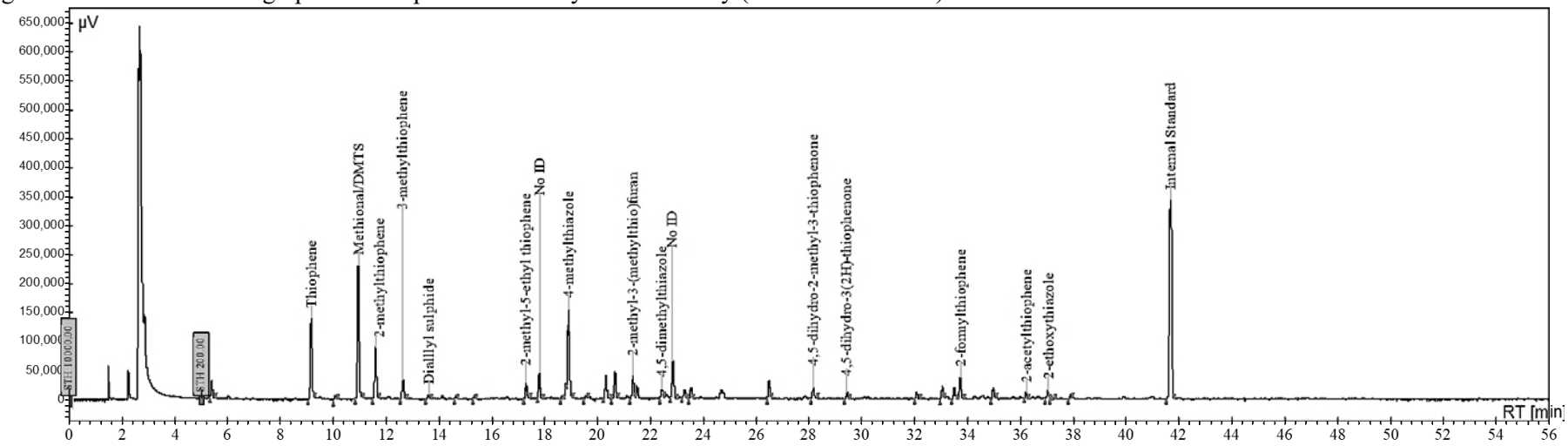


Figure 5.6: PFPD chromatograph of a sample of laboratory roasted pale malt (200 °C for 25 min).

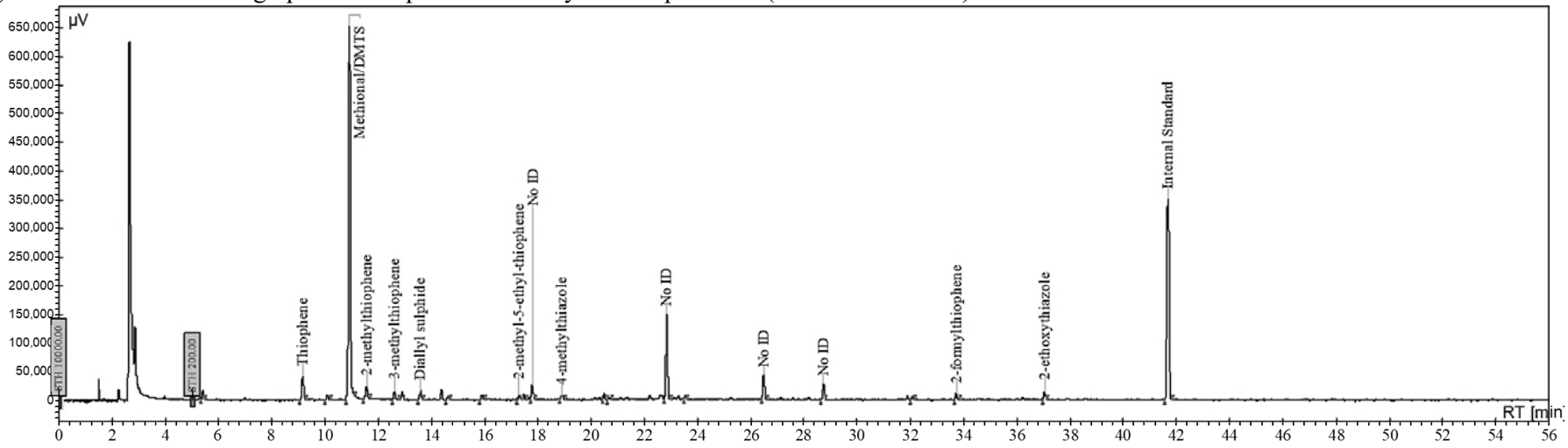


Table 5.3: Authentic external standard calibration R^2 values and aroma descriptors for 30 sulphur containing compounds identified in the range of roasted samples.

Compound	LRI (ZB-WAX) [A]	Aroma [B]	R^2
Methyl allyl sulphide	1027	*Alliaceous, garlic, onion*	0.973
Thiophene	1069	*Alliaceous, garlic*	§
Methional	1106	*Musty, potato, tomato, earthy, vegetal*	0.970
Dimethyl trisulphide	1106	*Onion, cooked onion, savoury, meaty*	0.862
2-methylthiophene	1125	*Alliaceous, onion, green, roasted*	§
Thiophane	1145	*Cabbage*	0.838
3-methylthiophene	1157	*Fatty, wine*	0.986
Diallyl sulphide	1187	*Onion, garlic, horseradish, metallic*	§
2,5-dimethylthiophene	1204	*Nutty, sulphurous*	§
Methyl propyl disulphide	1272	*Onion, radish, mustard, tomato*	0.966
1-heptanethiol	1278	*Sulphurous, onion*	0.934
2-methyl-5-ethyl thiophene	1281	*Sulphurous, solvent, fruity, burnt*	0.997
2-propyl thiophene	1291	*Chemical*	0.918
4-methylthiazole	1323	*Nutty, green, vegetal, tomato*	0.991
2-ethyl-4-methyl thiazole	1381	*Nutty, green, pistachio*	0.858
2-methyl-3-(methylthio)furan	1386	*Beef, cheese, coffee, minty, spicy*	0.866
4,5-dimethylthiazole	1416	*Roasted, nutty, fish, green, shellfish*	§
2-propyl-2-thiazoline	1420	*Meaty, nutty, cooked, roasted*	§
2-methyl-5-(methylthio)furan	1422	*Onion, garlic, coffee, mustard*	0.897
Pentylthiophene	1494	*Fatty, hazelnut, roasted barley, fermented*	0.833
Methyldithiolane	1502	*Onion, rooty, vegetal*	0.872
4,5-dihydro-2-methyl-3-thiophenone	1579	*Sulphurous, fruity, berry*	0.861
4,5-dihydro-3(2H)-thiophenone	1617	*Garlic, meaty, green, vegetal, buttery*	0.876
2-formylthiophene	1753	*Almond, bitter almond, cherry*	0.882
2-acetyl-2-thiazoline	1818	*Corn chip, toasted, bread, nutty*	0.996
2-acetyl-3-methylthiophene	1824	*Phenolic, almond, floral, sweet, honey*	0.942
2-acetylthiophene	1836	*Nutty, hazelnut, walnut*	§
2-formyl-5-methylthiophene	1845	*Almond, sweet, cherry, bready, woody*	0.910
2-ethoxythiazole	1864	*Musty, vegetal, roasted, nutty, burnt*	0.994
2-acetyl-5-methylthiophene	1933	*Sweet, spicy, honey, almond, cracker*	§

[A] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.
[B] Aroma descriptors from Good Scents Company, available online (Scents, 2018a).
§ No linear calibration was obtained.

The peak detected at 10.92 min (LRI 1106) was consistently found across all the samples (Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4, Figure 5.5, and Figure 5.6), Confirmation of the identification of this peak of interest by use of external standards was unsuccessful, due to the elution of two potential compounds at this exact retention time: methional, and dimethyl trisulphide (DMTS). As mass spectrometry was not used as a method of compound detection, EI-MS library matching could not

be used as a method of assisting peak identification to distinguish between these two possible compounds.

Considering the GC-O (Gas Chromatography-Olfactometry) results of Chapter 3 the elution of either compound (methional or DMTS) can be deduced. As stated in Table 5.3, the aroma of methional is predominantly 'musty, potato, tomato, earthy, and vegetal', whereas the aroma of DMTS is described as 'onion, cooked onion, savoury, and meaty' (Scents, 2018a). Within the GC-O analysis of the roasted malts in Chapter 3 (roasted barley, black malt, chocolate malt, medium crystal malt, caramalt, and amber malt), the relevant retention time window (11.00 min) yields a range of panel generated aroma descriptors at this particular time (listed in Table 5.4). Among these, 'musty' is predominantly detected across the range of analysed samples. This strongly suggests the presence of methional over the presence of DMTS. 'Onion', or 'meaty' aromas were not detected by the panel at this retention time. Suggestions of 'savoury' aromas were present within the range of descriptors used, however this was not adequately explicit in order to confirm the presence of DMTS within the samples (Table 5.4). It can therefore be concluded that methional is most likely the VSC identified in the samples, as opposed to DMTS. Nevertheless, this identification remains tentative, and the discussion of the potential presence of both compounds remains throughout this discussion.

Table 5.4: Compilation of all detected aroma descriptors at 11.00 min across the range of roasted malts analysed by gas chromatography-olfactometry.

	All Detected Aroma Descriptors at 11.00 min
RB	*Sweet, baked, salty, pine, vegetal, musty, floral, metallic, baked*
BL	*Roasted, fruity, fermented, baked, sweet, dairy*
CH	*Musty, floral, caramellic, fruity, nutty, musk, fruity, sour, dairy*
MC	*Musty, dairy, nutty, sweet*
CA	*Sweet, marshmallow, burnt, baked, toffee, dairy, mould, musty, bread, rotting apple, chocolate, fruity, yeasty*
AM	*Toffee, pine, hay, toast, gingerbread, sweet, malty, sour*

Roasted malt samples are as follows: roasted barley (RB), black malt (BL), chocolate malt (CH), medium crystal malt (MC), caramalt (CA), and amber malt (AM). Aroma descriptors are a combination of all panel (n=4) data across all dilutions (dilution factor 1, 10, 100, and 1000) of flavour extracts analysed for each roasted malt sample.

5.3.1 Volatile Sulphur Compound Quantification in Roasted Samples

Calculated concentrations of 22 of the 30 identified VSCs are displayed in Table 5.5. The remaining eight identified compounds' volumes within each sample are quoted as peak areas in Table 5.6, due to the compounds' lack of linear calibration (Table 5.3). Due to this, calculating the concentration of these compounds would have been inaccurate. The peak areas give an indication as to the relative amounts of these identified compounds across the various roasted samples.

Table 5.5: Mean concentrations of 22 volatile sulphur compounds as in 1g of malt sample (ng/g) (n=2).

Roasted Sample [A]	Methyl allyl sulphide	Methional	Dimethyl trisulphide	Thiophane	3-methylthiophene	Methyl propyl disulphide	1-heptanethiol	2-methyl-5-ethyl thiophene	2-propyl thiophene	4-methylthiazole	2-ethyl-4-methyl thiazole	2-methyl-3-(methylthio)furan	2-methyl-5-(methylthio)furan	Pentylthiophene	Methyldithiolane	4,5-dihydro-2-methyl-3-thiophenone	4,5-dihydro-3(2H)-thiophenone	2-formylthiophene	2-acetyl-2-thiazoline	2-acetyl-3-methylthiophene	2-formyl-5-methylthiophene	2-ethoxythiazole
	LRI (ZB-WAX) [B]																					
	1027	1106	1106	1145	1157	1272	1278	1281	1291	1323	1381	1386	1422	1494	1502	1579	1617	1753	1818	1824	1845	1864
RB	111	25733	13888	-	22.5	-	-	1194	11.5	116	15.3	1618	5087	-	-	-	-	1044	102	-	420	2558
BL	-	72818	39299	-	15.1	-	-	675	57	31.6	19.2	1327	4870	19.3	-	-	-	860	-	-	-	3187
CH	-	99151	53510	-	17.2	-	-	938	38.8	66.9	23.5	2037	7223	-	-	-	-	1269	-	-	-	6047
PC	-	57352	30952	-	14.6	-	2945	667	23.6	65.8	-	2107	4676	-	-	162	-	1470	-	-	-	5496
DC	-	81084	43760	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MC	-	47958	25882	-	-	-	-	-	-	-	-	-	-	8.49	-	-	-	-	-	-	-	-
LC	24.4	19577	10565	-	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CA	-	28815	15551	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60.1	-	-	-	-
AM	-	57681	31129	-	-	69.2	1773	236	-	-	-	-	-	-	30.8	-	-	-	-	-	-	-
RB 200 25	-	25535	13781	-	12.1	-	-	396	-	119	-	5104	-	-	-	631	126	1667	-	-	-	2194
RB 135 30	66.9	3404	1837	78	-	-	-	-	-	1.09	-	-	-	-	-	-	-	-	-	-	-	445
PM 200 25	-	95816	51710	-	5.83	-	-	134	-	6.23	-	-	1956	-	-	-	-	545	-	-	-	2320
PM 165 20	-	95247	51403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	345	-	-	-	-

GM 165 35	-	73872	39867	-	-	-	-	-	-	-	-	-	-	-	-	-	-	259	-	-	-	-
GM 158 43	-	70340	37961	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM 135 50	-	42009	22671	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.0	-	-

[A] Commercial roasted malt samples (n=9) are: roasted barley (RB), black malt (BL), chocolate malt (CH), pale chocolate malt (PC), dark crystal malt (DC), medium crystal malt (MC), light crystal malt (LC), caramalt (CA), and amber malt (AM). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
[B] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.

Table 5.6: Mean peak areas (μV) of 8 volatile sulphur compounds (n=2).

Roasted Sample [A]	Thiophene	2-methylthiophene	Diallyl sulphide	2,5-dimethylthiophene	4,5-dimethylthiazole	2-propyl-2-thiazoline	2-acetylthiophene	2-acetyl-5-methylthiophene
	LRI (ZB-WAX) [B]							
	1069	1125	1187	1204	1416	1420	1836	1933
RB	5033.5	6526.1	350.7	-	1786.8	-	1008.9	-
BL	3339.0	3179.1	696.8	-	638.6	-	304.9	-
CH	4942.1	3966.6	1513.6	-	1164.5	-	636.2	-
PC	6693.6	5254.4	1217.1	123.7	1354.2	-	772.0	-
DC	1223.4	-	-	-	-	-	-	-
MC	554.2	-	-	-	-	-	-	-
LC	660.2	-	-	-	-	-	-	-
CA	548.8	-	-	-	-	-	-	-
AM	1478.6	-	-	-	-	-	-	-
RB 200 25	10882.5	7545.3	588.9	-	1627.1	892.9	764.0	-
RB 135 30	1176.6	649.5	-	-	-	-	-	177.7
PM 200 25	3220.4	1821.2	1275.0	-	-	300.7	-	-
PM 165 20	2059.2	654.3	-	-	-	-	-	-
GM 165 35	1040.5	360.9	-	-	-	-	-	-
GM 158 43	872.3	397.4	-	-	-	-	-	-
GM 135 50	521.1	-	-	-	-	-	-	-

[A] Commercial roasted malt samples (n=9) are: roasted barley (RB), black malt (BL), chocolate malt (CH), pale chocolate malt (PC), dark crystal malt (DC), medium crystal malt (MC), light crystal malt (LC), caramalt (CA), and amber malt (AM). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
[B] Linear retention index against alkanes (C8-C22) on a ZB-WAX column.

Many of the sulphur compounds identified across the 16 samples analysed were forms of thiophenes (n=12), including thiophene itself. The structure of thiophene is shown in Figure 5.7. The branched forms of thiophene found in the roasted samples are namely alkylated, formylated, and acetylated (Table 5.5 and Table 5.6).

Figure 5.7: Structure of thiophene

The remaining groups of heterocyclic VSCs identified include: thiazoles (n=4), thiophenones (n=2), thiazolines (n=2), thio-furans (n=2), thiophane, and methyldithiolane. Non-cyclic VSCs were also identified across the samples: methyl allyl sulphide, methional, DMTS, 1-heptanethiol, methyl propyl disulphide, and diallyl sulphide.

Both the thiophene, and methional/DMTS peaks were consistently detected in all the samples analysed. The peak area of thiophene ranged from 10882.5 μV in [RB, 200, 25], to 548.8 μV in caramalt (Table 5.6). The peak area of thiophene, and therefore it's concentration, was highest in samples treated to high roasting temperatures and times. This trend does not translate to the methional/DMTS peak. The concentration of methional/DMTS is highest in chocolate malt, followed by the laboratory roasted pale malts [PM, 200, 25] and [PM, 165, 20]. The roasted raw barley samples are within the lowest four concentrations of methional/DMTS. The Boortmalt roasted barley sample is roasted to 230 °C, higher than the chocolate malt sample (225 °C) (Boortmalt, 2010j, Boortmalt, 2010e).

Methional can be formed by Strecker degradation of methionine (sulphur containing amino acid) during barley malting (Mikulíková et al., 2009). DMTS can be formed via methanethiol with methional as a precursor (Prentice et al., 1998). It is known that the Strecker degradation forms a component of the Maillard reaction: a pathway of reactions starting with the condensation of an amino group and a reducing sugar (Yoo, 1997, Echavarría et al., 2012, Hodge, 1953). This reaction occurs during thermal processing, which would therefore favour the formation of both methional and DMTS across the processes of barley malting, unmalted barley roasting, and malted barley roasting.

The relatively mild thermal processing that occurs during malting and kilning (to produce pale malt) favours the conversion of the sulphur containing amino acid methionine to simpler, more volatile VSCs (Mikulíková et al., 2009). As a result of this prior thermal processing to roasting, roasted pale malts in this study contain

higher concentrations of methional/DMTS. Whereas roasted raw barley does not undergo any processing prior to roasting, so the majority of the methionine present in the grain remains, and little is converted to these subsequent volatiles. The volatiles formed during the roasting of raw barley are as a result of the severe roasting conditions employed, rather than the preconcentration of volatiles during milder thermal processing (malting and kilning).

The commercial roasted barley sample contained the greatest number of identified peaks (n=18), and dark crystal malt was the sample with the least identified peaks (n=2) (Table 5.5 and Table 5.6). Commercial roasted barley also exhibited the highest number of VSCs with the greatest concentrations, in eight of the 19 peaks identified in this sample. The eight peaks, largest in roasted barley of all the samples, are: methyl allyl sulphide, 2-methylthiophene, 3-methylthiophene, 2-methyl-5-ethyl thiophene, 4,5-dimethylthiazole, 2-acetyl-2-thiazoline, 2-acetylthiophene, 2-formyl-5-methylthiophene (Table 5.5 and Table 5.6). Following commercial roasted barley, [RB, 200, 25] was the next sample with the greatest number of identified peaks at the highest concentrations of all the samples, seven of 15 identified peaks in the sample: thiophene, 4-methylthiazole, 2-methyl-3-(methylthio)furan, 2-propyl-2-thiazoline, 4,5-dihydro-2-methyl-3-thiophenone, 4,5-dihydro-3(2H)-thiophenone, and 2-formylthiophene. [RB, 200, 25] was the laboratory roasted sample that shared the most similarities with the commercial roasted barley (230°C (Boortmalt, 2010j)). It is a positive reflection of the laboratory roasting practices that this sample should share similarities in its VSC contents with its commercially produced counterpart. Finally, the chocolate malt sample exhibited the highest concentration of five VSCs, the most prominent of all the roasted pale malt samples. Chocolate malt was highest of all the samples in: methional/DMTS, diallyl sulphide, 2-ethyl-4-methyl thiazole, 2-methyl-5-(methylthio)furan, and 2-ethoxythiazole. The malt samples with the highest concentrations of the most compounds are all treated to high temperatures and/or for long roasting times. Increased roasting temperature and roasting time increases the formation of VSCs during roasting. However, if excessive thermal flavour generation is supported in the roasting vessel for a prolonged period of time, this can result in the loss of VSCs. This occurs during the roasting of black malt, discussed below.

5.3.2 Volatile Sulphur Compound Formation During Malting and Roasting

This section will discuss the conditions required to form sulphur volatiles of interest, by considering the precursors required, and mechanisms by which those precursors are used to form VSCs.

VSCs are mainly produced through thermal degradation of sulphur containing amino acids and Maillard reactions (Zheng et al., 1997). The composition of barley ultimately influences the reactions that may occur throughout its processing, including malting, kilning, stewing, and roasting. The protein composition of barley includes hordeins: major storage proteins which form 30-50 % of the total protein content of a barley grain (Arendt and Zannini, 2013). Within these, sulphur containing amino acids are found. Cysteine and methionine are the two sulphur containing amino acids found in proteins. Cysteine forms 0.28 g/kg and methionine forms 0.20 g/kg in hulled barley (% w/w) (Arendt and Zannini, 2013).

The differences between malted barley and unmalted barley regarding the changes in proportions of sulphur containing amino acids are noted only regarding methionine. Singh and Sosulski (1986) investigated the amino acid composition of hulled barley over 8 days germination (the second stage of malting). Amino acid composition was determined by analysing the component amino acids within the total protein content of the grain. Cystine and methionine were present in hulled barley (2.2 and 2.0 g/16 g Nitrogen, respectively). Over 8 days germination, cysteine remained at 2.3, 2.2, and 2.2 g/16g Nitrogen over 2, 5, and 8 days respectively. Whereas methionine reduced from 2.0 to 1.4 g/16g Nitrogen after 2 days germination, which then remained over the full 8 days. The effect of kilning on the concentrations of these amino acids was not investigated. Likewise, Mikulíková et al. (2009) found that the methionine concentration in malt kilned at over 50 °C decreased dramatically from 180 μg/g (at 50 °C) to less than 10 μg/g with increasing kilning/roasting temperatures above 120 °C. These studies highlight the readiness of methionine to degrade to form VSCs over the course of malting. Due to the absence of the malting step in unmalted barley, this degradation of methionine does not occur in the roasted unmalted barley samples. Perhaps because of this lack of initial methionine degradation to the most volatile VSCs, the commercial roasted barley sample yielded the highest number of identified VSCs of all the analysed samples (n=18), the

majority of which were to the highest concentrations of all the samples analysed (n=8) (Table 5.5 and Table 5.6).

In addition, the sustained concentrations of cysteine and methionine in unmalted barley may result in the sulphur containing amino acids reacting differently under harsh roasting conditions, as the degradation to smaller, more volatile compounds is not favourable under these conditions.

Due to the concentration of cysteine remaining relatively constant throughout the malting process, almost the same as in unmalted barley, the thermal flavour generation reactions of cysteine must be considered as a route of formation of VSCs in the roasted samples (Singh and Sosulski, 1986). Hofmann and Schieberle (1998) investigated the key aroma compounds formed by reactions between cysteine and various carbohydrates (ribose, glucose, and rhamnose). It was discovered that up to four times the flavour dilution (FD) factor of 2-acetyl-2-thiazoline was detected when formed under dry heating conditions in comparison to when formed in an aqueous solution (Hofmann and Schieberle, 1998). 2-acetyl-2-thiazoline was found only in the commercial roasted barley sample (102 ng/g) of all 16 analysed samples (Table 5.5). The dry roasting conditions favoured the formation of 2-acetyl-2-thiazoline during the roasting of this barley sample. It is likely that the dry roasting conditions, which are essential for the roasting of raw barley and pale malts, encourage the formation of greater quantities of VSCs in these samples. In addition, the supplementary evidence of the lack of formation of large quantities of a variety of VSCs in the crystal malt samples (roasted green malts) also supports this trend. Green malts contained 40-44 % moisture, around four times the moisture content of raw barley (Arendt and Zannini, 2013, Gruber, 2001).

The main compositional difference between malted and unmalted barley is that the malting process results in the preconcentration of Maillard reaction precursors and intermediate compounds, which results in the formation of a readily available feedstock of reactants set to take part in thermal flavour generation reactions upon roasting, thus forming volatile heterocyclic compounds (Yahya et al., 2014). This is likely to contribute to the major differing concentrations of VSCs between roasted pale malts and roasted unmalted barley.

There were a number of compounds that exhibited higher concentrations in roasted malted barley samples (pale chocolate malt, chocolate malt, and black malt) rather than in roasted unmalted barley samples (roasted barley, and [RB 200, 25]). Namely:

2-propyl thiophene, diallyl sulphide, 2-methyl-5-(methylthio)furan, 2-ethyl-4methyl thiazole, and 2-ethoxythiazole. The chocolate malt sample yielded the highest number of VSC peaks at the highest concentrations of all the analysed roasted pale malt samples (n=5), above black malt (n=2), which is roasted to a higher temperature for a longer time (Boortmalt, 2010e, Boortmalt, 2010c). During roasting of chocolate malt, temperatures up to 225 °C are reached. Care is taken throughout roasting to avoid scorching of the grains, and the colour development is closely monitored (Boortmalt, 2010e). This will prevent excessive roasting, thereby retaining the key flavour compounds formed during thermal flavour generation that would otherwise react further to form colour rich melanoidins (Echavarría et al., 2012). This is likely to be the cause of the relatively low concentrations of the VSCs in the black malt sample in comparison to the chocolate and pale chocolate malts. Black malt is roasted to 230 °C, for a longer period of time. Prolonged roasting would result in the loss of volatiles due to volatilisation, or by taking part in further reactions.

It has been previously noted in other roasted materials (sesame seeds microwaved for 8 min, and rapeseeds roasted at 140 °C for 60 min) that increasing thermal processing parameters increases the concentrations of some VSCs, namely DMTS (among others) (Jia et al., 2019, Ortner et al., 2016). In addition, VSCs are known to form as a result of Strecker degradation as a component of the Maillard pathway (Jia et al., 2019). Zheng et al. (1997) investigated the reactions between furaneol and cysteine to form VSCs. Furaneol is a known component of roasted malts and barley (see Chapter 3 and Chapter 4). It is known that at 160 °C, reactions between furaneol and cysteine can form a range of thiophenes, thiophenones, and thiazoles (Zheng et al., 1997, Shu et al., 1986, Jia et al., 2019). Also, at 130 °C, furaneol can act as a precursor in the formation of thiophene by the exchange of the oxygen atom for sulphur from hydrogen sulphide (Zheng et al., 1997). The highest concentrations of thiophene were achieved in [RB, 200, 25], roasted barley, and pale chocolate malt, but it was identified in all 16 roasted samples analysed (Table 5.5). This may be because all of these samples were roasted at temperatures exceeding 130 °C. It is possible, however, that amber malt was processed to lower temperatures (finishing temperatures quoted as 100 - 150 °C (Boortmalt, 2010a)). As amber malt's thiophene peak area falls in the very centre of the sample range (Table 5.5), it can be assumed

that the roasting conditions of this sample supported adequate formation of thiophene in the sample.

The majority of the sulphur volatiles identified in this study were formed as a result of Maillard chemistry, and are therefore more numerous and present in higher concentrations in samples roasted to higher temperatures for longer times. Under these conditions, Maillard chemistry readily occurs. Cysteine will readily take part in thermal flavour generation (by Maillard reaction or Strecker degradation), rather than thermal degradation (pyrolysis) (Zheng et al., 1997). During thermal flavour generation reactions, larger compounds fragment to form smaller, more readily reactive molecules. Hydrogen sulphide can be formed as a fragment of larger sulphur containing compounds (i.e. sulphur containing amino acids or vitamins). Hydrogen sulphide is a common sulphur containing fragment, and can contribute to the formation of sulphur containing heterocyclics by combining with carbon containing fragments which can form as a result of caramelisation, the Maillard reaction, or pyrolysis (Zheng et al., 1997).

In addition to Maillard chemistry, a number of other thermal flavour generation reactions result in the formation of VSCs during roasting. For example, thiamine (vitamin B1) forms 0.646 mg/100g of hulled barley (Arendt and Zannini, 2013). Thiamine is a sulphur-containing water soluble vitamin, the structure of which is shown in Figure 5.8. Under thermal processing, thiamine can degrade to form hydrogen sulphide (Arnold and Dwivedi, 1971). As discussed above, these fragments of thiamine, including hydrogen sulphide, may react with carbon fragments from thermal flavour generation reactions to form a range of potential VSCs. It is known that cornmeal with 0.5 % thiamine content produces 4-methylthiazole as a key volatile when extruded at 180 °C (Nursten, 1991). The structure of 4-methylthiazole is shown in Figure 5.9. It is clear that 4-methylthiazole is a fragment of thiamine, given the structure of the alkylated heterocyclic compound (Figure 5.8 and Figure 5.9). The commercial roasted barley sample and [RB, 200, 25] yielded the highest concentrations of 4-methylthiazole in the analysed samples in this investigation (116.2 ng/g and 119.2 ng/g, respectively) (Table 5.5). The [RB, 135, 30] sample yielded an extremely low concentration of 4-methylthiazole (1.1 ng/g). These results suggest the formation of 4-methylthiazole is favoured by thermal processing (above 180 °C) of raw cereals, particularly, by the thermal degradation of thiamine.

Figure 5.8: Structure of thiamine

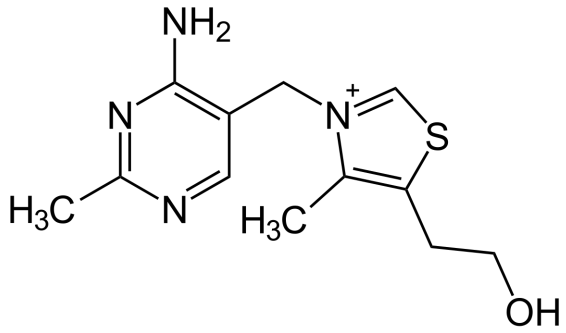
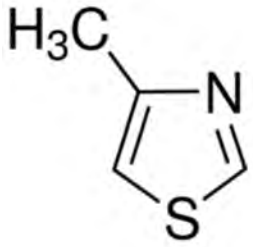


Figure 5.9: Structure of 4-methylthiazole



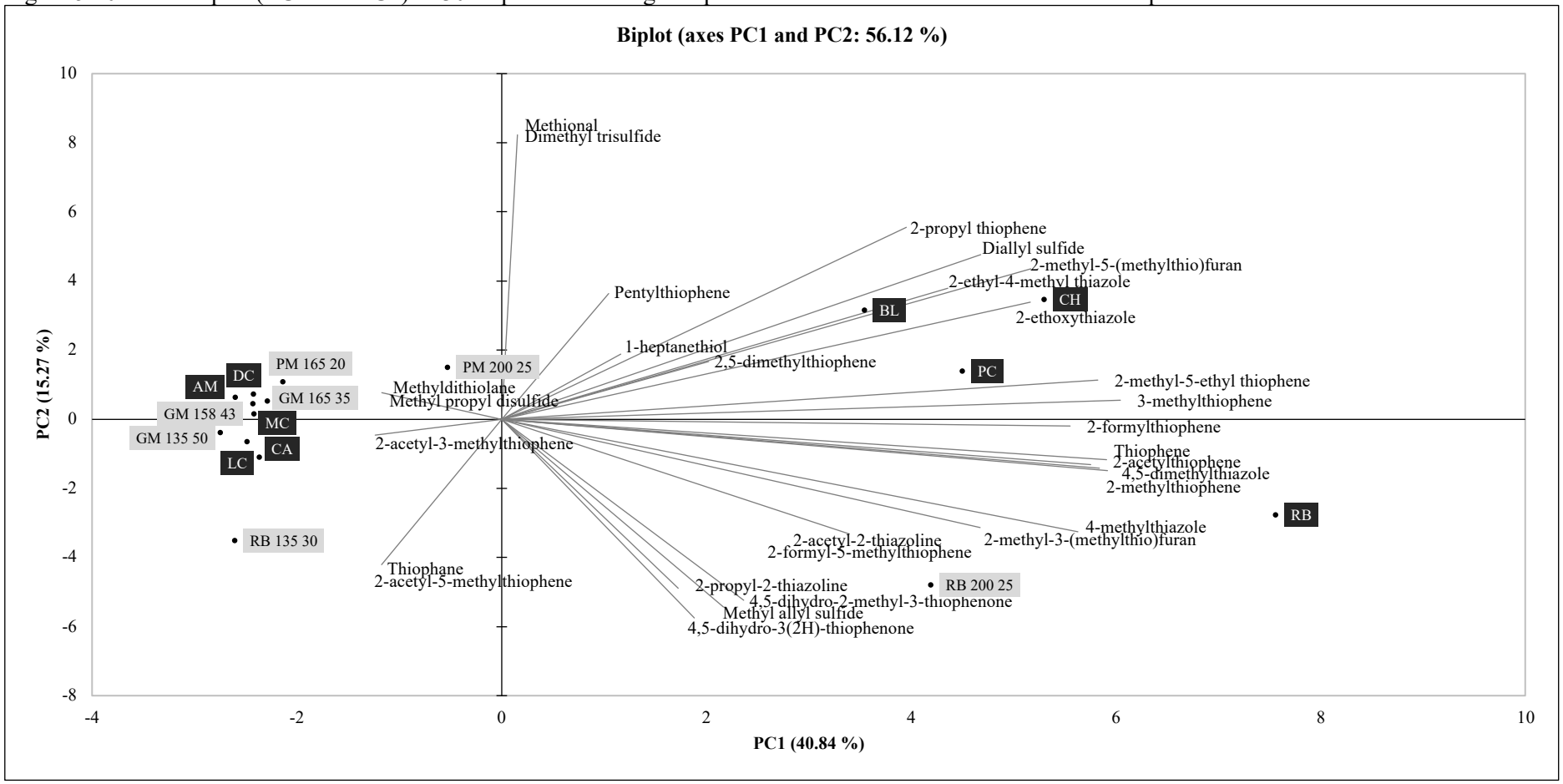
Crystal malts share similarities with pale malts, as they are produced from a malted barley. However, crystal malts begin their three stages of roasting (stewing, drying, and roasting) from an earlier point. Throughout the roasting stages of crystal malts, the quantity of maltose, glucose, maltotriose, and fructose decrease (Blenkinsop, 1991). This indicates their use in thermal flavour generation reactions to form subsequent compounds, which includes the limited number of VSCs identified in the crystal malt samples. A total of eight peaks were identified across the range of crystal malts analysed in this investigation, with as few as two peaks identified in dark crystal malt (Table 5.5 and Table 5.6). The processing methods applied to produce crystal malts do not support the formation of sulphur volatiles in comparison to the roasting of the drier roasting substrates (unmalted barley and malted barley). The VSCs that are present in the roasted samples analysed may not be present in the final beers that are produced using them. The volatility of the compounds may cause them to be lost during the thermal processing steps of brewing, or they may be converted to increasingly volatile compounds (Hill and Smith, 2000). For example, dimethyl sulphide (DMS) and methionol are the main volatile sulphur compounds in beer (Hill and Smith, 2000), and were not identified in this investigation. However, the peaks consistently detected before 5.00 min retention time were not investigated due to their elution before the first alkane in the series (hence lack of an accurate retention index). The peak at ~3.00 min retention time is speculated to be DMS, due

to its prevalence within all the samples analysed (Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4, Figure 5.5, and Figure 5.6). DMS is known to form as a result of the thermal decomposition of S-methylmethionine, and commonly occurs in malted barley (Nanamori et al., 2011).

5.3.3 Principal Component Analysis

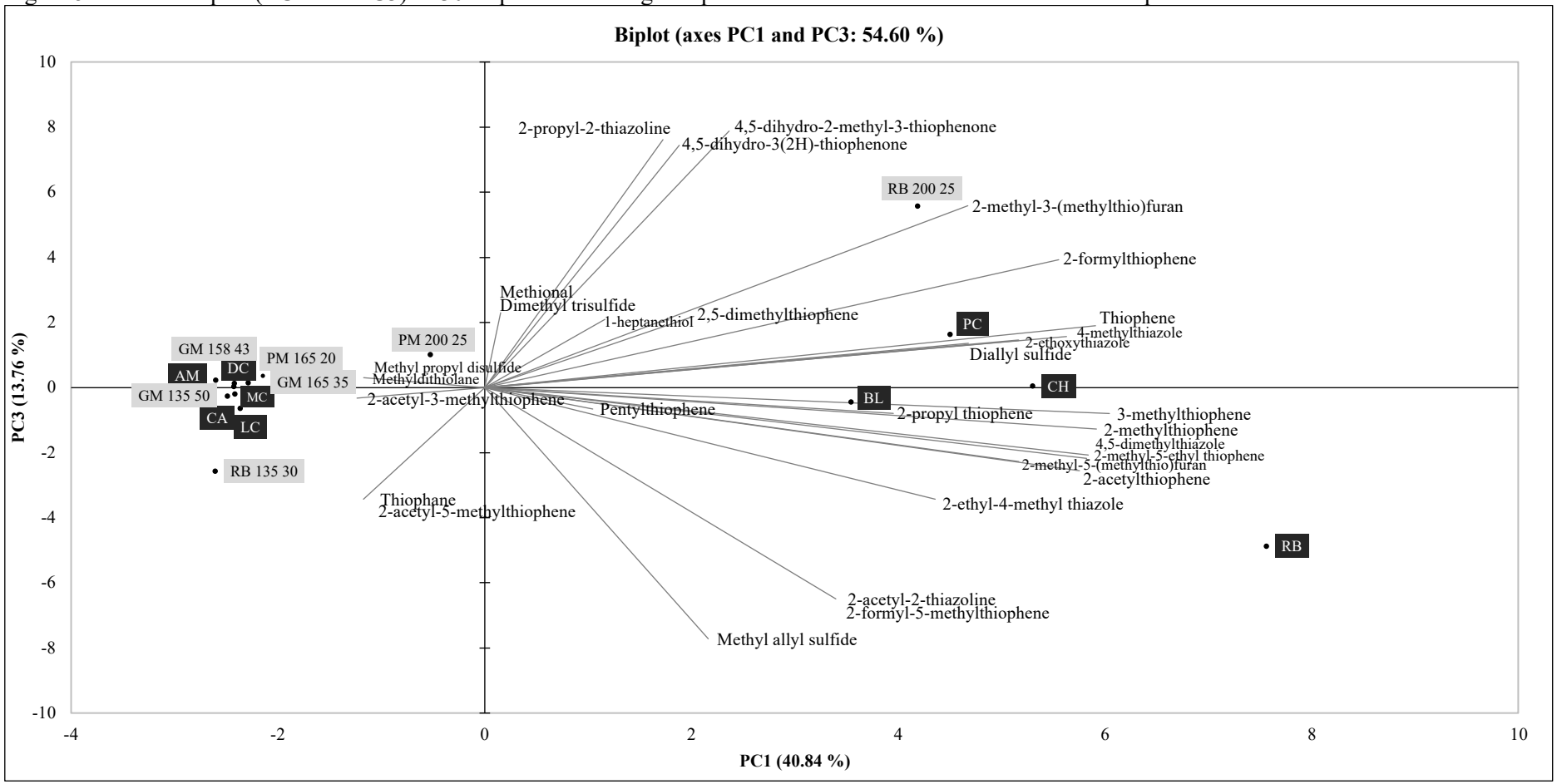
Principal component analysis is a useful tool for visualising the trends in data sets. Each principal component, a combination of factors, accounts for a proportion of the variation in the data set being analysed. Analysing multiple combinations of principal components gives an increasingly thorough visualisation of the loading of the data within the 3-dimensional matrix in which the data is plotted. In this investigation, PC1, PC2, and PC3 each contributed significantly to explaining the variation in the data set (Figure 5.10 and Figure 5.11). PC1 accounts for the majority of the variation in the data (40.84 %) and PC2 and PC3 are responsible for 15.27 % and 13.76 % respectively. As these two components each account for similar proportions of variation, they have both been included here for data analysis. Between Figure 5.10 and Figure 5.11 the data loading on PC1 remains the same. This makes comparisons between PC2 and PC3 simpler, as only the data loading on the *y*-axis, and the differences between the two figures is relevant in this respect. Each of the three principal components are discussed individually below.

Figure 5.10: PCA bi-plot (**PC1** and **PC2**) of 30 sulphur containing compounds and the concentrations in roasted samples.



Commercial roasted malt samples are: roasted barley (RB), black malt (BL), chocolate malt (CH), pale chocolate malt (PC), dark crystal malt (DC), medium crystal malt (MC), light crystal malt (LC), caramalt (CA), and amber malt (AM). Laboratory roasted samples are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).

Figure 5.11: PCA bi-plot (**PC1** and **PC3**) of 30 sulphur containing compounds and the concentrations in roasted samples.



Commercial roasted malt samples are: roasted barley (RB), black malt (BL), chocolate malt (CH), pale chocolate malt (PC), dark crystal malt (DC), medium crystal malt (MC), light crystal malt (LC), caramalt (CA), and amber malt (AM). Laboratory roasted samples are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).

5.3.3.1 Principal Component 1: Roasting Conditions

As PC1 accounts for the largest proportion of the variation in the data (40.84 %), its loading of the data is important to recognise. PC1 separates the samples according to the degree to which they have been roasted; and the number of, and concentration of identified VSCs in each sample (Figure 5.10 and Figure 5.11). Each sample's position within the bisectors of PC1 depends on the identified compounds' concentration within each sample. A compound's highest concentration is in the samples plotted closest to it. For example, chocolate malt is plotted the most positively of all the roasted pale malt samples on PC1. The chocolate malt sample has the highest concentration of all the samples in the VSCs plotted closest to it, i.e: 2-ethoxythiazole, 2-methyl-5-(methylthio)furan, diallyl sulphide, and 2-ethyl-4-methylthiazole (Table 5.5).

Regarding both the roasted raw barely samples and roasted pale malt samples, samples are plotted increasingly positively on PC1 as their finishing temperatures and roasting times increase. However, black malt is plotted the closest to zero of the three samples: black malt, pale chocolate malt, and chocolate malt. The black malt sample typically contained lower concentrations of the compounds in comparison to the other samples plotted closest to it: chocolate malt and pale chocolate malt (Table 5.5). Black malt undergoes the highest finishing temperatures of all the roasted pale malt samples analysed in this study (Boortmalt, 2010c). As discussed above, higher processing temperatures may cause the volatile compounds formed during the roasting of black malt to volatilise during the final stages of roasting, or may feed into the formation of high molecular weight melanoidins at the final stages of the Maillard reaction (Echavarría et al., 2012). As a result of this, the aroma of black malt is diminished in comparison to malts roasted to a lesser degree, but can still impart a smoky characteristic to beer (Gruber, 2001). The finishing temperatures of chocolate malt and black malt differ by only 5 °C (225 °C and 230 °C, respectively) (Boortmalt, 2010e, Boortmalt, 2010c). It can be assumed that these finishing temperatures are employed for different lengths of time, as details on this are not publicly available. Roasting a product at a certain temperature for a given time has an effect on the extent of thermal flavour generation reactions, depending on the compounds in question (Vandecan et al., 2011). In addition to this, the use of water sprays is commonly used in commercial roasting at severe roasting conditions in order to prevent charring, and to encourage colour development (Boortmalt, 2010c).

Yahya et al. (2014) found that upon monitoring the formation of key thermal flavour generation products during commercial roasting operations, the addition of water sprays resulted in increased production of some compounds (2-cyclopentene), and a decrease in others (pyrrole, for example). It is likely that the use of quenching in the production of the very darkest roasts (i.e. black malt, and roasted barley) results in the same effects, regarding the formation of particular VSCs.

Amber malt is the most negatively plotted commercial roasted pale malt sample on PC1. Amber malt is characterised by the dry, biscuit flavour it imparts to a brew, and is typically roasted up to temperatures from 100 °C to 150 °C (Boortmalt, 2010a). Seven VSCs were identified in the amber malt sample, two of which were exclusively in this sample: methyl propyl disulphide ('onion, radish, mustard, tomato' aroma), and methyldithiolane ('onion, rooty, vegetal' aroma) (Table 5.5). In Figure 5.10 and Figure 5.11, these compounds share the same point on the biplots due to their similarity in concentration and occurrence within the sample set. Excepting these two compounds, the concentrations of the VSCs identified within the amber malt sample were rarely at high concentrations relative to the other analysed samples.

The three roasted barley samples ([RB,135, 50], [RB, 200, 25], and Boortmalt roasted barley) are plotted increasingly positively on PC1 with increasing roasting temperature (Figure 5.10 and Figure 5.11). The inherent compositional difference between malted barley and unmalted barley causes the difference exhibited between the samples [RB, 200, 25] and [PM, 200, 25] in Figure 5.10 and Figure 5.11, and in the raw data exhibited in Figure 5.5 and Figure 5.6. These samples are processed to the same roasting times and temperatures, yet Figure 5.10 highlights the clear association of [RB, 200, 25] with its fellow Boortmalt roasted barley, while [PM, 200, 25] is plotted in the opposing quadrant. [PM, 200, 25] is associated with the lighter roasted products, closer on PC1 to the crystal malts than the chocolate and black malts (Figure 5.10). [RB, 200, 25] and [PM, 200, 25] contain 2-propyl-2-thiazoline, found in only these two samples (peak areas of 892.9 μV and 300.7 μV respectively) (Table 5.6). 2-propyl-2-thiazoline was not identified within the commercial range of roasted samples. This highlights a difference between the results achieved from laboratory-scale production compared to commercial scale roasting operations. Laboratory-scale roasting assured homogeneity within the roasted batches, whereas industrial roasting drums are equipped with specialist

apparatus to allow for additional processing steps. Namely, quenching the grains with water sprays in the laboratory-scale roasting drum was not possible. This step, which would have been used at high roasting temperatures exceeding 200 °C in commercial operations, may have contributed to the formation and persistence of 2-propyl-2-thiazoline in the laboratory roasted samples.

All the crystal malt samples in this study (laboratory roasted and Boortmalt) are plotted negatively on PC1, and contained lower concentrations of VSCs in comparison to the samples plotted in the positive bisector of PC1 (Figure 5.10 and Figure 5.11). The roasted pale malt samples that load similarly to the roasted crystal malts (amber malt, and [PM, 165, 20]) in Figure 5.10 and Figure 5.11 are roasted to relatively low temperatures. They load similarly to the crystal malt samples due to their similarities of final roasting temperatures, in addition to their similarities in concentrations of VSCs. In amber malt and [PM, 165, 20], six and four VSCs were identified, respectively (Table 5.5 and Table 5.6). The VSC contents of these samples are therefore appropriately grouped without distinction between crystal malt and lightly roasted pale malts. This is however, only considering their VSC contents. In other respects, these samples exhibit clear differences (see Chapter 3, Chapter 4, and Chapter 6).

5.3.3.2 Principal Component 2: Methional/Dimethyl trisulphide

Concentrations of the peak identified as methional/dimethyl trisulphide (DMTS) had a major loading on PC2. The contribution of this peak in PC2 is 15 %, in comparison to the mean of 3 % contribution of the other 28 variables (data not shown). As discussed above, absolute identification of either methional or DMTS could not be confirmed by the two levels of validation in this investigation. PC2 of Figure 5.10 separates the samples according to their concentrations of methional and/or DMTS. All the roasted pale malt samples analysed are plotted positively on PC2, indicating higher concentrations of the methional/DMTS peak. Whereas the three barley samples consistently load negatively on PC2, and are distinct from the loadings of the other roasted green and pale malt samples. The three roasted unmalted barley samples have similar negative loadings on PC2, irrespective of their position on PC1. With regard to the crystal malt samples, increasing degree of roasting (roasting time and roasting temperature) results in these samples being plotted more positively on PC2. Therefore, increasing roasting conditions causes the concentration of

methional/DMTS in crystal malts to increase. Light crystal malt is the most negatively plotted crystal malt sample, followed by caramalt, [GM, 135, 50], medium crystal malt, [GM, 158, 43], [GM, 165, 35], and finally dark crystal malt being plotted most positively on PC2. This indicates the selected laboratory roasted green malt samples fall within the range of commercial products regarding the concentration of methional/DMTS. Dark crystal malt contained only two identified VSC peaks, the least of all the samples: thiophene, and methional/DMTS. However, of all the crystal malt samples, dark crystal malt contained the highest concentrations of all three compounds. For example, the peak area of thiophene was 1223.4 μV in dark crystal malt, compared to 548.8 μV in caramalt (Table 5.6). Therefore, the increased intensity of roasting (higher roasting temperature, for longer time) in comparison to the other crystal malts in this study, results in the formation of higher concentrations of VSCs during the roasting of dark crystal malt.

5.3.3.3 Principal Component 3: Separating Roasted Barley Samples

PC3 accounts for 13.76 % of the variation in the data, and mainly separates the data according to sample concentrations of methyl allyl sulphide, 2-propyl-2-thiazoline, 4,5-dihydro-2-methyl-3-thiophenone, and 4,5-dihydro-3(2H)-thiophenone (Figure 5.11). These four compounds each account for 13 % (mean) of the contribution of the 30 variables, the remainder of which account for 2 % (mean) each. Methyl allyl sulphide is the most negative point on PC3, and the remaining three major compounds on PC3 are plotted at the most positive points on this axis.

PC3 is most useful in displaying the differences between the roasted barley samples. In comparison to PC2, the three roasted barley samples are distributed both positively and negatively on PC3, which illustrates differences between the samples that were not previously identified in PCs 1 and 2. The laboratory roasted sample [RB, 200, 25] is the sample most closely associated with the three maximum positively loaded compounds: 2-propyl-2-thiazoline, 4,5-dihydro-2-methyl-3-thiophenone, and 4,5-dihydro-3(2H)-thiophenone. As PC2 does not illustrate the differences within the range of roasted raw barley samples, PC3 is necessary to do this. Methyl allyl sulphide was identified in three samples only: roasted barley (100 ng/g), [RB 135, 30] (66.9 ng/g), and light crystal malt (24.4 ng/g) (Table 5.5). The lack of identification of methyl allyl sulphide in [RB, 200, 25] differentiates the

loadings on PC3. In addition, this principal component highlights [RB, 200, 25]'s relative similarities with its pale malt equivalent: [PM, 200, 25]. These samples are both plotted positively on PC3.

While PC2 separated the crystal malt samples the most effectively, PC3 condensed the loadings of the crystal malt samples. The range in concentrations of the four VSCs of interest on PC3 (methyl allyl sulphide, 2-propyl-2-thiazoline, 4,5-dihydro-2-methyl-3-thiophenone, and 4,5-dihydro-3(2H)-thiophenone) is not sufficient to separate the crystal malt samples. Methyl allyl sulphide was identified in light crystal malt only, and none of the other 3 compounds of interest in PC3 were identified in any of the crystal malt samples (Table 5.5).

5.3.4 Volatile Sulphur Compound Identification: Suggested Improvements

As discussed above, distinguishing between methional and DMTS was speculative, due to the raw data available from this investigation. To aid the positive identification of the peak of interest, it would be necessary to include a steady hold, or to reduce the temperature ramp rate within the retention times of interest (between 10 min and 12 min). This would change the retention times of the analytical standards of methional and DMTS, enabling confident identification.

A number of significant peaks within the samples remain unidentified. Particularly at the following retention times: 17.79 min (LRI 1294) (Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.5, and Figure 5.6), and 22.85 min (LRI 1428) (Figure 5.1, Figure 5.2, Figure 5.3, Figure 5.4, Figure 5.5, and Figure 5.6). Looking at the order of elution of VSCs under chromatographic conditions it is tentatively suggested that these are a different positional isomer of 2-methyl-5-ethyl thiophene (17.79 min) and a dimethyl thiazole (22.85 min). Firm identification of these additional compounds would help to give an increasingly thorough assessment of the formation of VSCs in roasted malt and roasted barley. However, the characterisation of VSCs in roasted malt samples reported in this Chapter contributes new and previously unpublished knowledge on the flavour composition of roasted malts.

5.4 Conclusions

The majority of the sulphur volatiles identified in this study were formed as a result of Maillard chemistry (including Strecker degradation) and/or pyrolysis reactions originating from sulphur contained in the amino acids methionine and cysteine, or other organic forms such as thiamine. Roasting parameters (roasting temperature and roasting time) had the greatest influence on the occurrence and concentration of VSCs in the samples, in addition to the substrate being roasted. Higher roasting temperatures, and for longer periods of time, resulted in more VSCs being identified overall, and typically in higher concentrations. The major exception to this general trend was the black malt sample, which contained lower concentrations of compounds identified in similar samples roasted to a slightly lower temperature, and possibly for a shorter period of time (chocolate malt and pale chocolate malt). It is likely that prolonged roasting at high temperatures may result in the loss of aroma volatiles due to the formation of melanoidins as a product of the final stage of the Maillard reaction.

In addition to the differences created by a variety of roasting parameters, an inherent compositional difference between malted barley and unmalted barley was highlighted. The conversion of methionine over the course of malting to more volatile VSCs may be a major source of the differences in identified VSCs in roasted malts and roasted barley. The highest number of VSCs of all the samples (n=18) in roasted barley may be explained by the lack of extremely volatile VSCs that are formed during malting, allowing the reactions of methionine under harsher conditions to form more complex VSCs.

Dark crystal malt exhibited the highest concentrations of identified VSCs of all the crystal malt samples, confirming that increased roasting parameters favour the formation of VSCs to higher concentrations. Although a total of eight VSC peaks were identified across the range of crystal malts, it is clear that the roasting of green malt does not facilitate the formation of VSCs to high concentrations. The sensory characteristics of these crystal malts do not correlate with the expected savoury, meaty aromas of sulphur volatiles, indicating their low sensory activity in crystal malt products.

The sulphur volatiles identified in this investigation contribute to the overall aroma of the roasted samples (see Chapter 3 and Chapter 6). The effect of VSCs on the

pronounced aroma of roasted malts and roasted barley may be reduced upon subsequent processing steps in beer production due to volatilisation, further flavour generation reactions, or assimilation or biotransformation by yeast.

Chapter 6 - Partial Quantitative Descriptive Analysis of the aroma of commercial and laboratory roasted malt and unmalted barley

6.1 Introduction

The characterisation of desirable sensory qualities is an essential step in the development of new products. Novel products should meet consumer expectations while emphasising gaps in the current product range and thus strengthening the likely success of the introduction of new products. Quantitative Descriptive Analysis (QDA) is a recognised sensory evaluation technique that enables objective, comprehensive, and informative data to be obtained (Kemp et al., 2018). The use of QDA in sensory analysis enables the quantification and characterisation of sensory properties of products, which allows product developers to understand the sensory characteristics of the product range, and therefore identify new characteristics or areas to introduce novel characteristics (Kemp et al., 2018, Puri et al., 2016). QDA typically encompasses the analysis of aroma, flavour, and mouthfeel of products. However, in this investigation, only aroma was evaluated due to the lack of food grade preparation by laboratory-scale roasting (consequently 'partial QDA'). Commonly used in combination with QDA is the multivariate statistical analytical technique of Principal Component Analysis (PCA). PCA has been used throughout the current research in order to identify patterns of correlation within large data sets (Chapter 3, Chapter 4, and Chapter 5). In sensory analysis, QDA and PCA are used in combination, which has led to the successful characterisation of the sensorial attributes in a wide range of food products. Previously analysed products include, for example, UHT milk, cheddar cheese, soy proteins, yogurt, and polenta (Puri et al., 2016, Zeppa et al., 2012). In addition, the sensory qualities of roasted malts have previously been analysed successfully using QDA by a number of researchers in order to pursue quality control, benchmark products, and explore roasted product flavour (Coghe et al., 2004, Kim et al., 1998, Murray, 1999).

The combination and concentration of flavour and odour active compounds in a product directly affects its sensory characteristics. Although instrumental analysis (namely Gas Chromatography-Mass Spectrometry (GC-MS)) can quantify the constituent compounds within a product, this does not provide an indication of their

impact on the perception of aromas and flavours in the product itself. Knowledge of the volatile composition of roasted malts is not sufficient to assess the influence of the volatiles on the sensory perception of those products (Castro and Ross, 2018). The rate at which volatile compounds are released from the matrix and the aroma properties of the compounds has an effect on their perception by olfactory receptors (van Ruth, 2001). Consequently, sensory analysis techniques have potential to substantiate instrumental volatile identification and quantification.

Previous investigations in the current research have explored the presence, odour activity, formation, and concentrations of odour active compounds found in roasted malt and barley products. Firstly, using GC-Olfactometry (GC-O) 45 odour active compounds in a range of six commercially available roasted malts were identified (Chapter 3). From this study, 20 key odour active compounds were selected to be monitored in their formation in laboratory roasted products under controlled conditions of roasting time and temperature (Chapter 4). The large variety of laboratory roasted samples was then reduced for further analysis (details of sample selection are discussed in Chapter 4 Section 4.3.3). Initially, volatile sulphur containing compounds in the selected samples were identified based upon their suggested aroma significance from the GC-O aroma detection data (Chapter 5). In this investigation, the selected laboratory roasted samples (n=7) were analysed by a trained sensory panel along with a selection of four commercially roasted products (n=4), in order to define a lexicon for the aroma attributes of the samples in question, then determine which attributes contribute to the overall aroma within the range of roasted products. In addition to the aroma characterisation of the analysed samples, the aim of this study was to identify potentially novel products within the laboratory roasted sample range by the identification of unique aroma attribute contributions in comparison to those of existing products.

6.2 Materials and Methods

6.2.1 Samples

6.2.1.1 Commercial Samples

Four of the nine available commercial roasted samples were selected to analyse in this study, as detailed in Section 2.1: amber malt, medium crystal malt, chocolate malt, and roasted barley. These four commercial samples were selected to analyse in

this study to represent the variation in the commercial range, in addition to the represent the typical commercially available roasted products from each roasting substrate. Details of this for each sample are shown in Table 6.1.

Table 6.1: Details of commercial samples analysed in Quantitative Descriptive Analysis

Sample	Abbreviation	Roasting Substrate
Amber malt	AM	Pale Malt
Chocolate malt	CH	Pale Malt
Medium crystal malt	MC	Green Malt
Roasted unmalted barley	RB	Raw Barley

6.2.1.2 Laboratory Roasted Samples

As detailed in Chapter 4 Section 4.3.3, a range of seven laboratory roasted samples were selected from the range of 37 laboratory roasted samples.

The roasted substrate, roasting times, and temperatures of each sample examined in this investigation are detailed in Table 6.2 below.

Table 6.2: Details of laboratory roasted samples analysed using Quantitative Descriptive Analysis

Abbreviation	Roasting Substrate	Roasting Temperature (°C)	Roasting Time (Min)
GM 135 50	Green Malt	135	50
GM 158 43	Green Malt	158	43
GM 165 35	Green Malt	165	35
PM 165 20	Pale Malt	165	20
PM 200 25	Pale Malt	200	25
RB 135 30	Raw Barley	135	30
RB 200 25	Raw Barley	200	25

For laboratory roasted unmalted barley, the winter malting variety Flagon was used, as provided by Crisp Malt Ltd. (Fakenham, UK). Green malt was sourced from Soufflet Malt UK Ltd (Shobnall Maltings, Burton-Upon-Trent, UK). After collection, the green malt was processed as described in Section 2.3. For pale malt production, the same green malt from Soufflet Malt UK Ltd was kilned in the Custom Lab Micromaltings K steep-germinator and kiln (Custom Laboratory Products, Keith, UK) as described in Section 2.3. The green malt was prepared for kilning immediately upon its collection to arrest germination.

6.2.1.3 Sample Preparation

For sensory analysis, 80 g of each sample was milled (as detailed in Section 2.5) and combined with 120 mL water. After which, 15 g of the malt/water mixture was then transferred into amber glass screw top vials, marked with a three-digit sample code. Amber glass vials were used to disguise the colour of the sample, preventing panel bias. Each panel member was supplied with one vial of each sample. The malt/water mixtures were prepared on the day of each analysis or training session and stored at room temperature.

6.2.2 Sensory Analysis

Partial quantitative descriptive analysis (partial QDA) was used to evaluate the aroma of the roasted samples. Only the aroma attributes of the samples (detailed in Sections 6.2.1.1 and 6.2.1.2) were analysed in this investigation because the laboratory roasted samples were not produced in food grade conditions.

6.2.2.1 Sensory Panel Members and Recruitment

Ethical approval for this investigation was granted by the University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee (ethics reference number: 367-1907) (- Appendices Section 8.3 Figure 8.11).

Eight members of the University of Nottingham Sensory Science Centre (SSC) trained beer panel (five females, and three males) were invited to take part this study via email, after ethical approval was granted. Members of the trained beer panel were selected and trained by earlier researchers at the SSC, and are regularly screened to monitor their sensory analysis ability.

Each panel member signed a consent form prior to the first session. Minimal information was provided on the nature of the study to reduce potential bias. All training and analysis sessions were carried out in the SSC of the University of Nottingham. Each session had a maximum duration of 2 hours, for which, the panel were given £30 at the end of each session.

6.2.2.2 Panel Training

During training sessions, the panel were firstly encouraged to generate a list of aroma attributes that they perceived from the full range of samples (n=11). From discussion

throughout the session, panel consensus and frequency of use of attributes were used to limit the lexicon to 12 selected attributes. Water and damp paper napkins were provided as olfactory palate cleansers. The selected aroma attributes and definitions are detailed in Table 6.3.

Table 6.3: Aroma attributes and their corresponding definition.

Attribute	**Definition**
Burnt	Burnt toast
Smoky	Burnt wood, wood smoke
Coffee	Damp used coffee grounds
Malt loaf	Soreen original malt loaf
Black treacle	Tate & Lyle black treacle
Tea/ Leaves	Damp used tea leaves
Dark Chocolate	70% cocoa solids chocolate
Potato	Overcooked baked potato skin
Marmite	Marmite yeast extract
Horlicks	Horlicks malt drink
Digestive Biscuit	Digestive biscuit
Grainy	Dry grain, dusty, wet Weetabix

After the aroma attribute and definition were confirmed, further training was conducted to ensure the panel understood each of the attributes, and were scoring consistently through rank and rating exercises. For each attribute, three samples were selected to represent low, medium, and high intensity. The rank and rate exercises were based upon the crude results of the first lexicon development session, in which the frequency of attribute use by the panel was recorded. The panel would firstly rank the three samples from low to high intensity, then rate the same three samples on a continuous scale.

6.2.2.3 Panel Performance in Training Sessions

Within each training session, Compusense Cloud software (Guelph, Ontario, Canada) was used to collect data and provide feedback on the panel's progress. The data collected was analysed to display the mean scorings from the panel, in addition to displaying anonymised individual panel members' scorings.

Following rank and rate exercises, the results from the panel were discussed within the group. The panel were encouraged to use the full extent of the scale when necessary. After group discussion of the results, the intended ranking order of the three selected samples for the assessed attribute were revealed (using sample codes

only). Reference samples were used to help resolve disagreement in scoring from the panel in the training sessions (details in Table 6.3).
Figures displaying the panel members' scoring for each sample for individual attributes were also assessed to review panel performance. These figures provided an indication of panel agreement and discrimination ability.

6.2.2.3.1 Mock evaluation

Mock evaluations formed a component of the final three training sessions. Mock evaluations were included to familiarise the panel with the final evaluation session structure (detailed in Section 6.2.2.4), in addition to identifying attributes which required further training. The mock evaluations were shortened versions of the final evaluation sessions, by reducing the number of samples assessed. This allowed for further training in particular attributes to be included in the final training sessions. A random selection of three samples were scored in the initial mock evaluation, increasing to six samples in the final mock evaluation.

6.2.2.4 Final Evaluation Sessions

Final evaluation sessions were carried out in individual booths, with access to water and damp paper napkins as in the training sessions.
During final evaluation sessions, each of the 11 total samples were presented monadically. The aroma attributes of each sample were scored before analysing the following sample. The intensity of each aroma attribute was scored on a continuous line scale from one to ten (low to high). The 12 aroma attributes were split into two screens of six attributes on each screen. A one minute break between each group of six attributes was given to restore the headspace within the sample vial and to reduce sensory fatigue. The panel were asked to not shake the bottles before sniffing, and to remember to reseal the bottle after sniffing. Re-sniffing was permitted during final evaluation.
The order of the attributes within each section was randomised for each panellist, but remained in that order for the entirety of the session. Three replicates of the analysis session were carried out. Each repeat session would give the panellists a different randomisation of attributes within the sections. The order of samples was also randomised for each panellist in each analysis session. Between each sample, a two-

minute break was given to avoid sensory fatigue, and between samples 6 and 7, a five-minute break was given.

6.2.3 Statistical Analysis

Statistical analysis by two-way ANOVA was used to analyse the scores for each attribute across the 11 analysed samples, taking each panel member's scores into account as a variable. Tukey's HSD post-hoc analysis identified groups of statistically different and statistically similar samples within each attribute scoring. Two-way ANOVA was also used to analyse the mean scores for the samples within roasting substrates, also taking each panel member's scores into account. The same ANOVA was also used to analyse aroma score means across all samples averaged according to substrate. For both tests, Tukey's HSD post-hoc analysis identified groups of statistically different and statistically similar groups of samples or substrates.

Correlation Principal Component Analysis (PCA) was carried out using XLSTAT software (Addinsoft, SARL, Paris, France), plotting the aroma attribute scores in each of the 11 analysed samples. Further correlation PCA was used to depict the full range of data, including concentrations of key volatile compounds (from Chapter 4 and Chapter 5) across the analysed sample range, in addition to aroma attribute scores.

Agglomerative hierarchical clustering (AHC) analysis was used to identify clusters of samples by their aroma scores across the attributes. AHC was also used to identify clusters of samples with regard to the full range of volatile compound concentrations and QDA data, as specified above.

Pearson correlation coefficient matrices were created based upon the concentrations of volatile compounds (sulphur containing and non-sulphur containing) and aroma attribute scores across the analysed sample range. The resultant matrices identified positive, negative, and lack of correlation of each volatile compound across 12 aroma attributes. Significant correlations were also identified. Significance was defined as $p \leq 0.05$. All partial QDA data were analysed using XLSTAT software (Addinsoft, SARL, Paris).

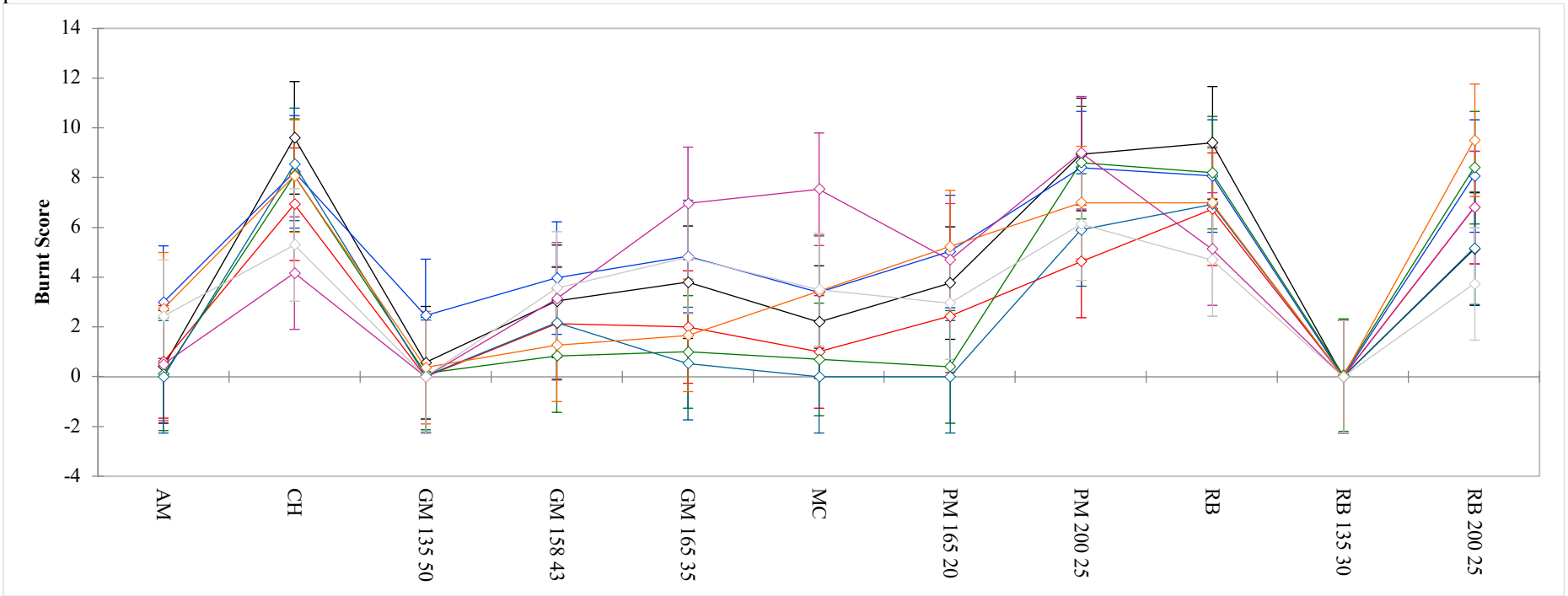
6.3 Results and Discussion

In this investigation, a trained panel (n=8) evaluated the aroma of a range of commercial and laboratory roasted products by partial QDA. Firstly, a lexicon was developed to encompass the variety of aroma qualities presented by the sample set. Using the lexicon, the component attributes of the lexicon were subsequently scored for each of the samples, in order to provide an objective measure of the aroma profile of each sample.

6.3.1 Final Evaluation Panel Performance

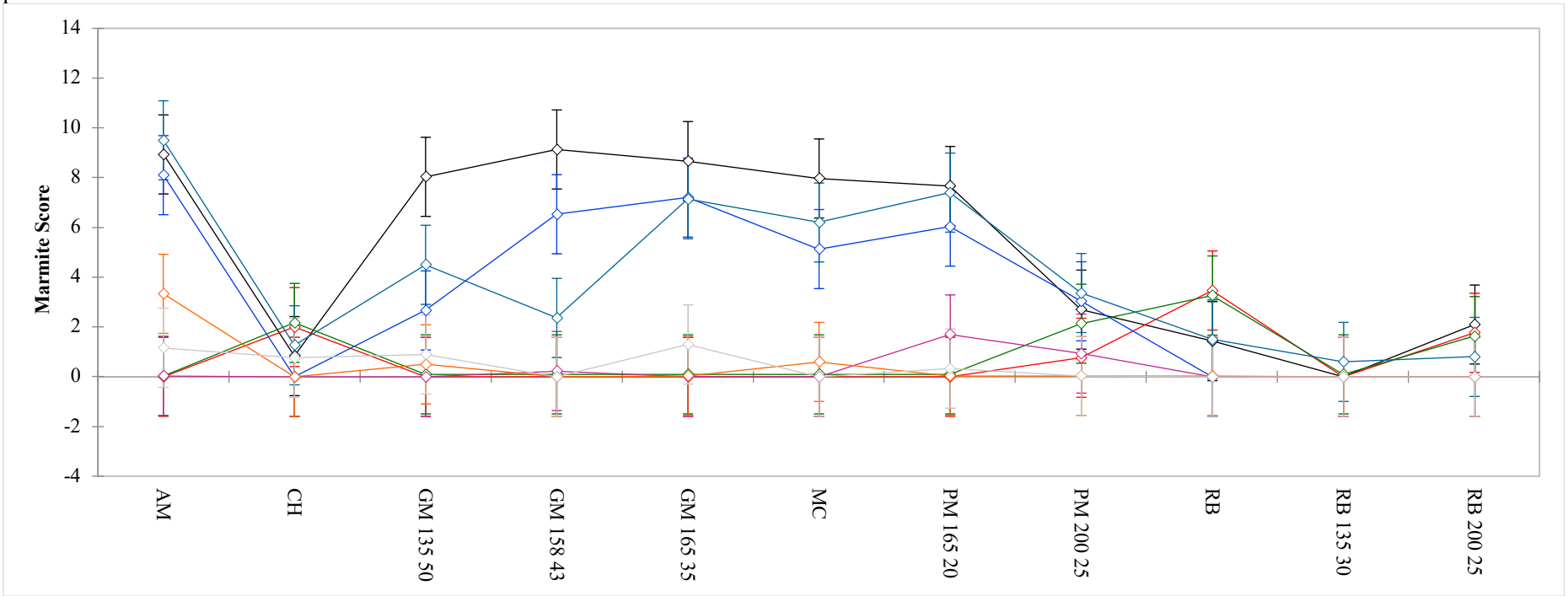
Panel performance was assessed by examining the interaction between the attribute scores for each sample provided by each panel member. Figure 6.1, Figure 6.2, and Figure 6.3 show examples of this assessment of panel performance. The Figures display an example of good, mixed, and poor panel performance, respectively.

Figure 6.1: Interaction between sample and mean aroma score for the ‘Burnt’ attribute showing scores from each panel member, indicating good panel performance.



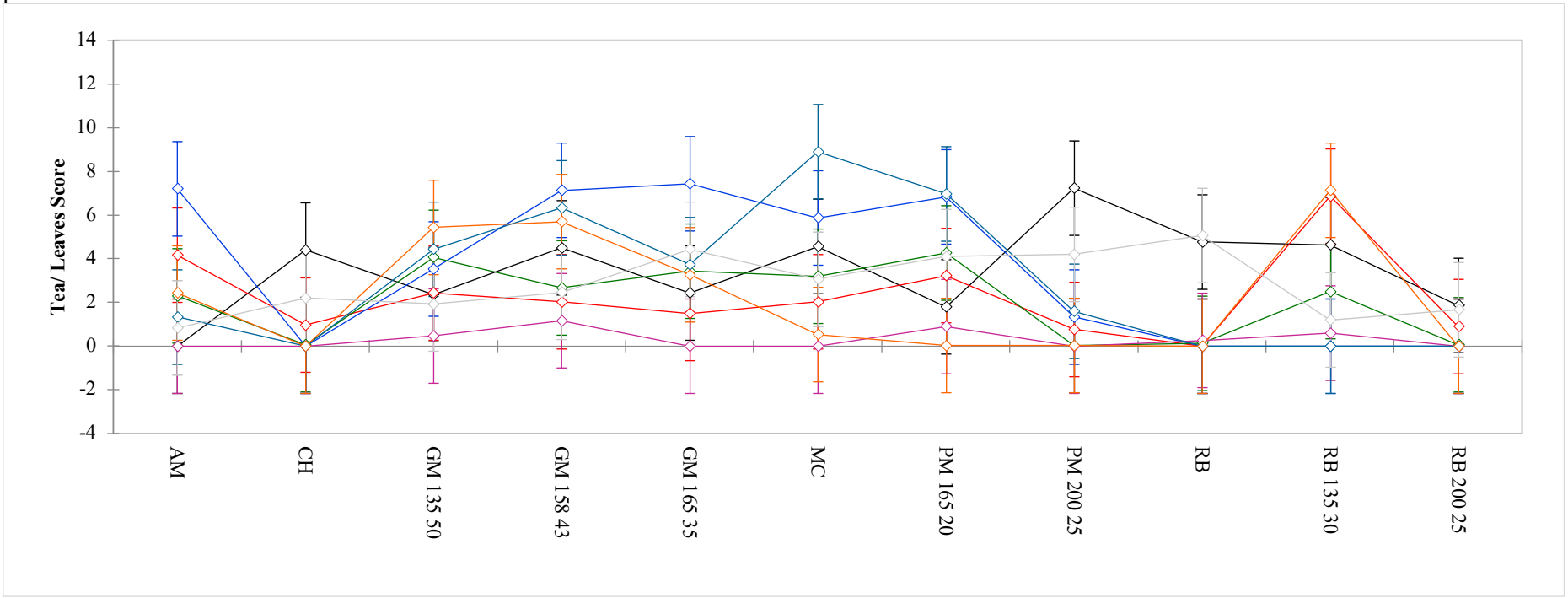
Each series indicates each panel member (n=8). Error bars are mean scores ± standard deviation (n=3).

Figure 6.2: Interaction between sample and mean aroma score for the ‘Marmite’ attribute showing scores from each panel member, indicating mixed panel performance.



Each series indicates each panel member (n=8). Error bars are mean scores ± standard deviation (n=3).

Figure 6.3: Interaction between sample and mean aroma score for the ‘Tea/ Leaves’ attribute showing scores from each panel member, indicating poor panel performance.



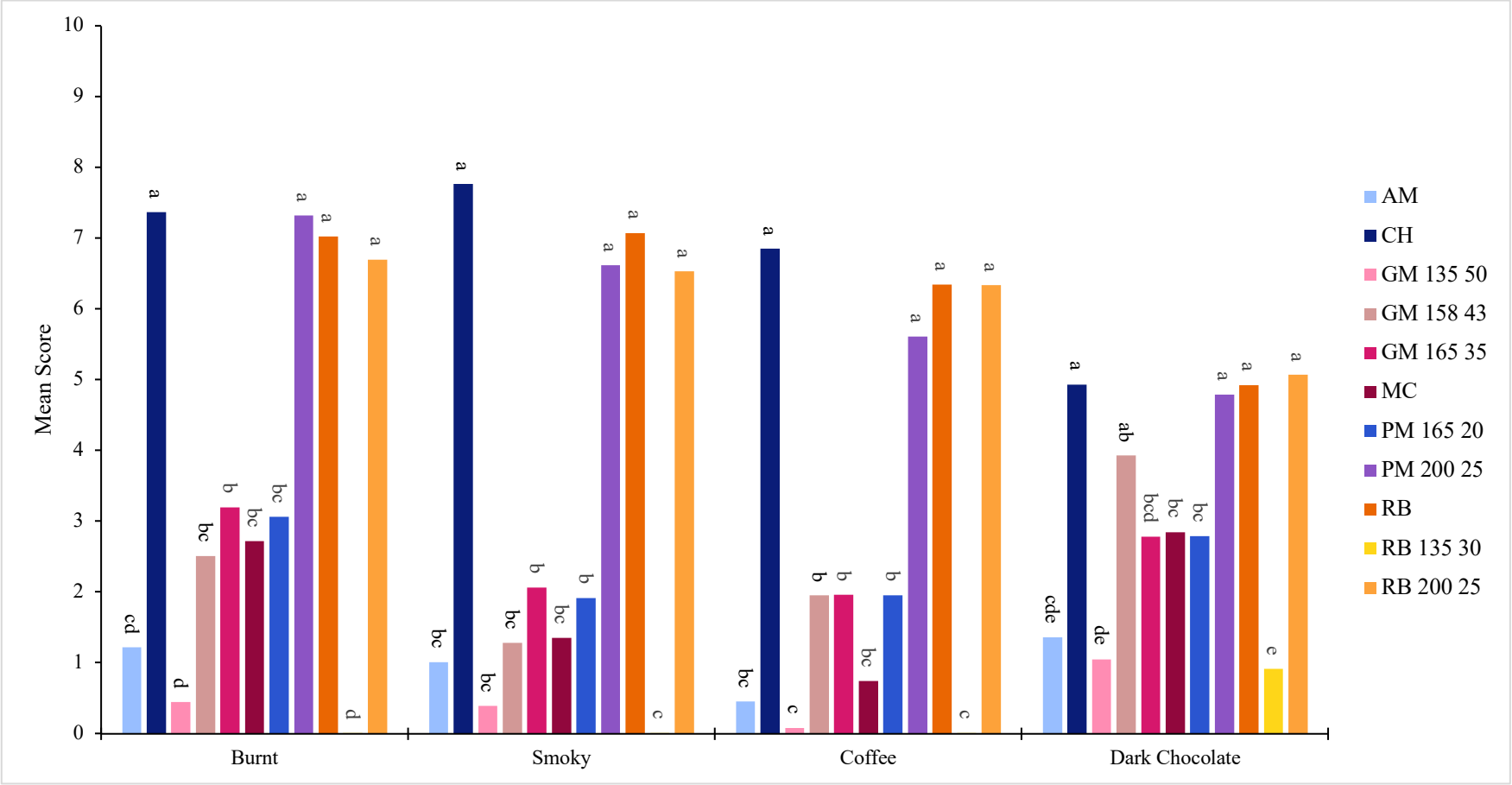
Each series indicates each panel member (n=8). Error bars are mean scores ± standard deviation (n=3).

Figure 6.1, Figure 6.2, and Figure 6.3 show panel performance by indicating panel agreement and discrimination ability. For example, Figure 6.1 shows the 'burnt' aroma scores from the panel across the 11 samples analysed. Throughout Figure 6.1, panel agreement was consistent, and discrimination ability was identifiable (i.e. the panel could recognise different samples as being low or high in the 'burnt' attribute). Figure 6.2 shows a mixed panel performance with separate groups of agreement. Three panel members were able to consistently score the 'Marmite' attribute, whereas another two panellists also scored the attribute across the samples consistently yet independently of the previously mentioned group. The remaining six panellists demonstrate poor discrimination ability regarding the 'Marmite' attribute. Figure 6.3 shows an example of poor panel performance by the panel's reduced agreement and discrimination ability. Few cases of panel agreement are visible in the scoring of the 'tea/ leaves' attribute, therefore additional training would have been beneficial in improving panel performance regarding the 'tea/ leaves' attribute.
The remaining eight Figures displaying panel performance per attribute are listed in the - Appendices Section 8.3.

6.3.2 Identification of Significant Differences Between Individual Samples Within Aroma Attributes

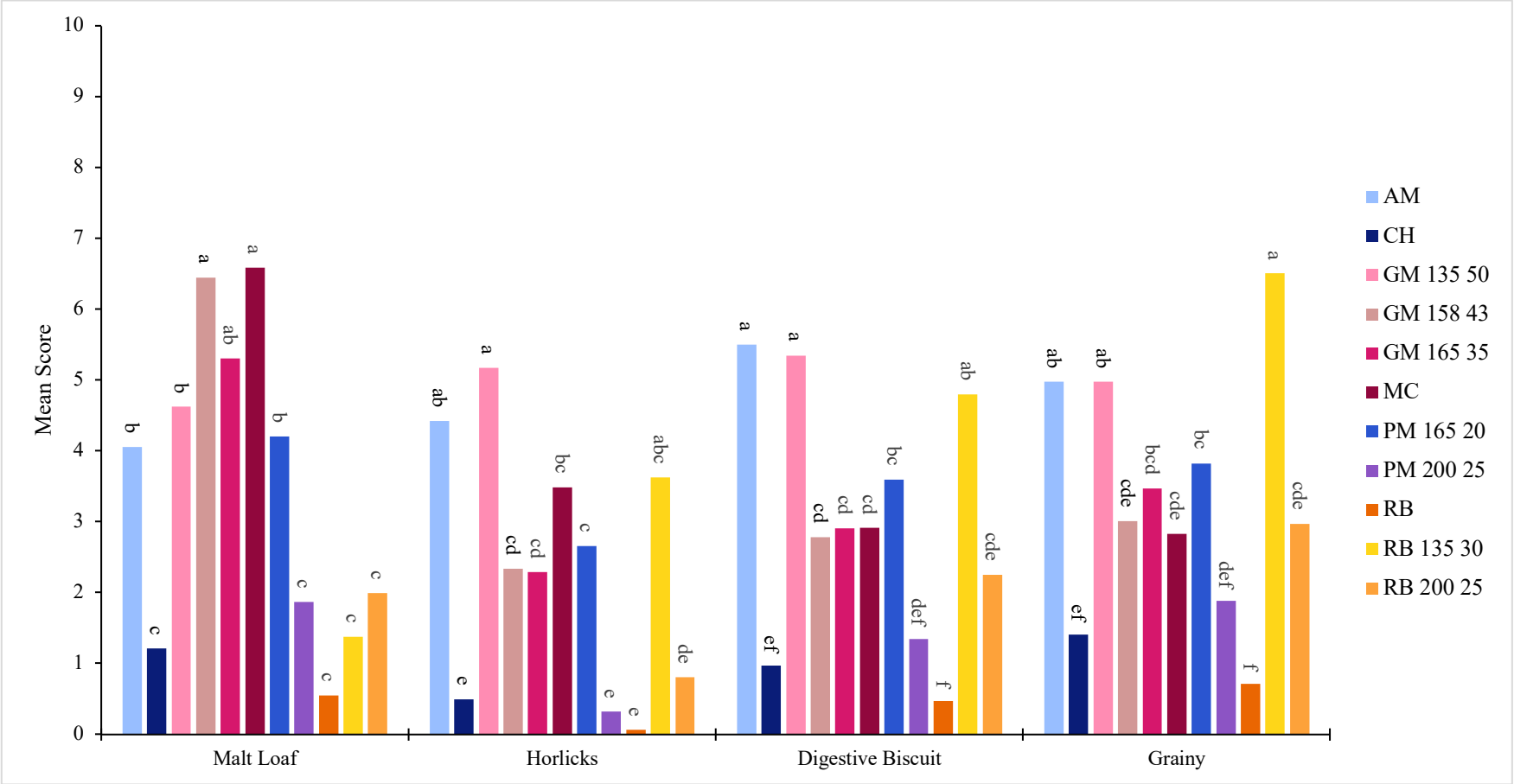
Figure 6.4, Figure 6.5, and Figure 6.6 show bar charts displaying the mean scores for 12 aroma attributes across the 11 analysed samples, over three final evaluation replicates. As detailed in Section 6.2.3, Tukey's HSD post hoc test was applied to provide pairwise comparisons for samples within each attribute (Kemp et al., 2018). Subscript lettering indicates groupings of significantly different samples.
Figure 6.4 shows mean aroma scores for the 'burnt', 'smoky', 'coffee' and 'dark chocolate' aroma attributes. Figure 6.5 shows the 'malt loaf', 'Horlicks', 'digestive biscuit', and 'grainy' aroma attributes. Figure 6.6 shows mean scores for the 'black treacle', 'potato', 'Marmite', and 'tea/ leaves' aroma attributes.
The bar charts provide a strong illustration of the scores in aroma attributes across the analysed samples. Aroma attribute score mean values for the roasted samples are reported in - Appendices Section 8.3 Table 8.3, and identifies all 12 aroma attributes as highly statistically significant ($p < 0.0001$).

Figure 6.4: Bar chart showing mean scores for the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aroma attributes across the analysed samples.



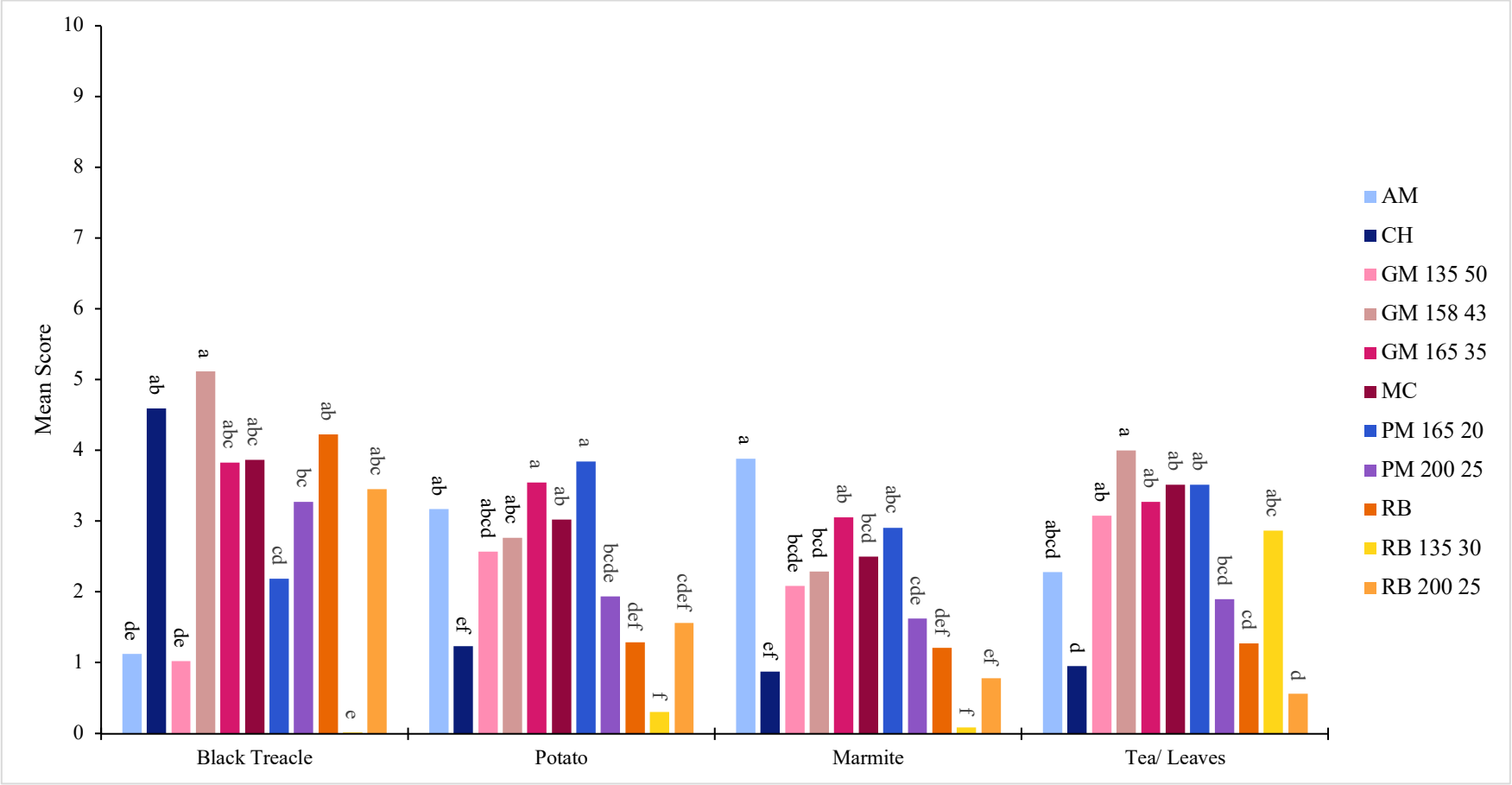
Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
Aroma scores are from 8 panellists (n=3 replicates).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey’s HSD post hoc analysis.

Figure 6.5: Bar chart showing mean scores for the ‘malt loaf’, ‘Horlicks’, ‘digestive biscuit’, and ‘grainy’ aroma attributes across the analysed samples.



Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
Aroma scores are from 8 panellists (n=3 replicates).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey’s HSD post hoc analysis.

Figure 6.6: Bar chart showing mean scores for the ‘black treacle’, ‘potato’, ‘Marmite’, and ‘tea/ leaves’ aroma attributes across the analysed samples.



Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
Aroma scores are from 8 panellists (n=3 replicates).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey’s HSD post hoc analysis.

Following two-way ANOVA, post hoc testing using Tukey's HSD by pairwise comparisons reveals statistically significant groupings of samples within each assessed aroma attribute. Greater numbers of groupings indicate greater discrimination between samples within the assessed attribute by the panel. However, frequent crossover of groupings (i.e. multiple subscript letters per sample) indicates a lack of clarity between the significantly different groups, as the sample's score is not significantly different from other samples in the same subscript letter groups. Chocolate malt, [PM, 200, 25], commercial roasted barley, and [RB, 200, 25] are typified by significantly higher scores in the 'burnt', 'smoky', and 'coffee' aroma attributes than the remaining seven samples (Figure 6.4). In the 'dark chocolate' attribute, the same four samples scored significantly higher than the remaining samples, with exception to [GM, 158, 43] (scoring 3.93).

Figure 6.5 displays the scores for the 'malt loaf' attribute, which yields distinctive scores to those of the 'Horlicks', 'digestive biscuit', and 'grainy' attributes in the same figure. Chocolate malt, [PM, 200, 25], and all three roasted unmalted barley samples (commercial roasted barley, [RB, 135, 30], and [RB, 200, 25]) were significantly lower scoring in 'malt loaf' aroma than the remaining samples. Medium crystal malt and [GM, 158, 43] were the highest scoring samples in 'malt loaf' aroma (Figure 6.5). Samples roasted below 150 °C (amber malt (Boortmalt, 2010a), [GM, 135, 50], and [RB, 135, 30]) all scored highest in the 'Horlicks', 'digestive biscuit', and 'grainy' aroma attributes.

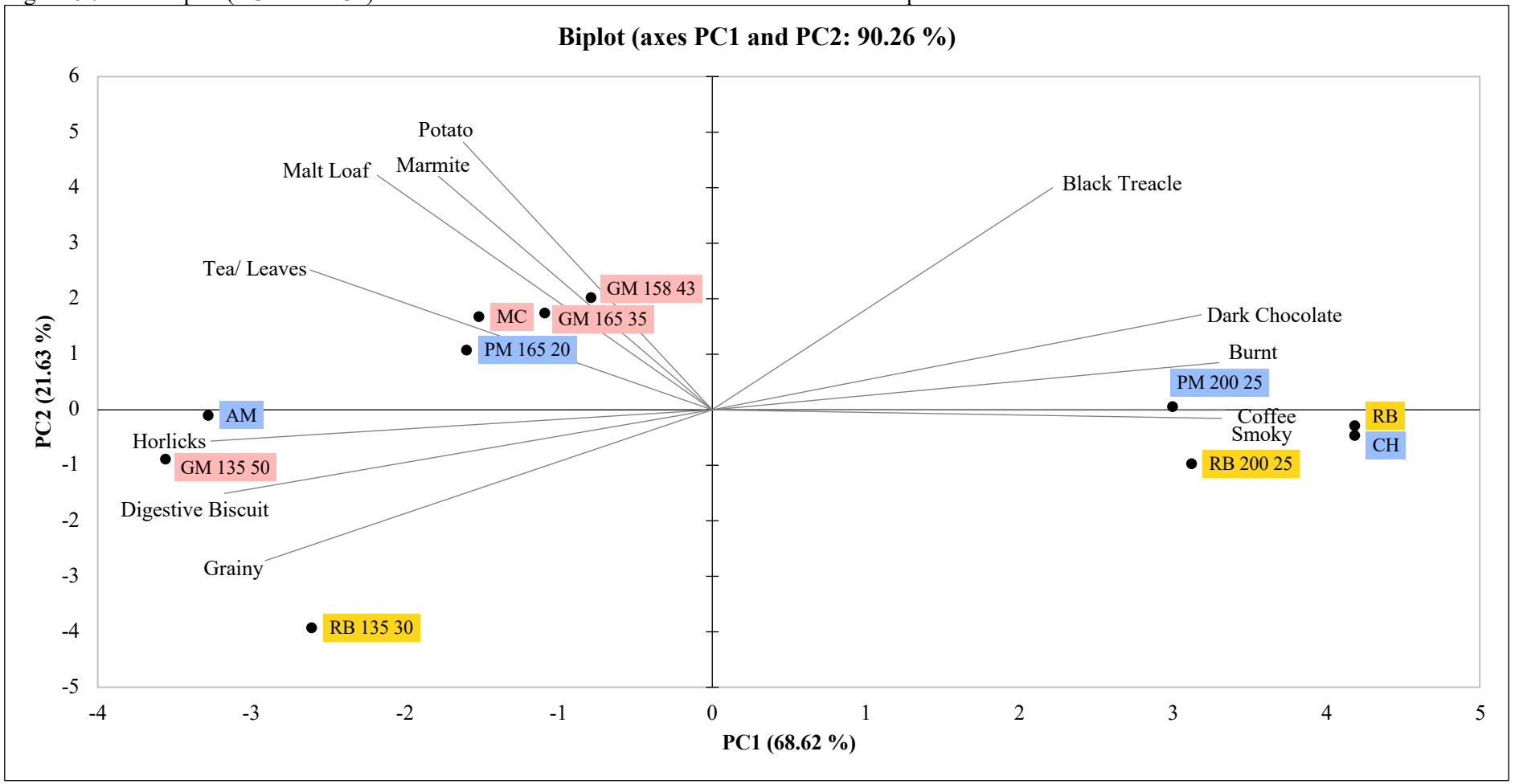
Figure 6.6 shows mean scores in the attributes with the most crossover of groupings: 'black treacle', 'potato', 'Marmite', and 'tea/ leaves'. Despite this lack of clarity between the significantly different groups of samples, each attribute is highly statistically significant ($p<0.0001$) (- Appendices Section 8.3 Table 8.3). Amber malt, [GM, 135, 50], and [RB, 135, 30] scored the lowest of all the samples in 'black treacle' aroma. There was no significant difference between [PM, 165, 20] (score: 2.19) and both amber malt and [GM, 135, 50] samples (scores: 1.13 and 1.03, respectively). However, [RB, 135, 30] was significantly lower scoring than [PM, 165, 20] ($p=0.004$ (data not shown)), yielding the 'e' post hoc grouping in the 'black treacle' attribute. The 'potato', 'Marmite', and 'tea/ leaves' attributes scored highest in amber malt, [GM, 135, 50], [GM, 158, 43], [GM, 165, 35], medium crystal malt, and [PM, 165, 20], with slight variations between the three attributes. In addition,

[RB, 135, 30] scored higher than amber malt in the ‘tea/ leaves’ aroma, in contrast to the ‘potato and ‘Marmite’ aromas.

Figure 6.7 shows a PCA biplot of PC1 and PC2 (accounting for 68.62 % and 21.63 % of the variation in the data set, respectively) of the mean aroma scores in the 12 attributes across the 11 analysed samples. For clarity, the three different roasting substrates are identified by different colour labels. Factor loadings are represented as correlations of attributes with new dimensions, and factor scores are values of analysed samples on those dimensions (Puri et al., 2016).

The use of the PCA biplot visualises the association of attributes to the sample in which they were scored highest or lowest, and provides an alternative depiction of the data presented in Figure 6.4, Figure 6.5, and Figure 6.6. Proximity of a sample to an attribute indicates its high score, and therefore prominent aroma characteristic in that particular sample. In contrast, samples plotted transversely to an attribute indicate the lack of impact of that attribute on the aroma of the sample of interest.

Figure 6.7: PCA biplot (**PC1** and **PC2**) of mean aroma scores of 12 attributes in 11 roasted samples.



Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).

Three clear groupings of samples are identifiable in Figure 6.7, closely associated with particular sensory attributes. For example, loading positively on PC1 are the following samples: commercial roasted barley, chocolate malt, [PM, 200, 25], and [RB, 200, 25]. These samples scored significantly higher than the remaining samples in the 'burnt', 'smoky', 'coffee', and 'dark chocolate' attributes (Figure 6.4). In the same four attributes, amber malt, [GM, 135, 50], and [RB, 135, 30] were consistently the lowest scoring samples. This results in their loading transversely to the burnt, smoky, coffee, and dark chocolate vectors on Figure 6.7.

The 'black treacle' attribute also loads positively on PC1, indicating its higher scores in the samples loading positively on PC1. However, as the black treacle vector loads closer to zero than the burnt, smoky, coffee, and dark chocolate vectors. This indicates its high scoring in additional samples to those loading positively on PC1, namely [GM, 158, 43], [GM, 165, 35], and medium crystal malt (Figure 6.6). The [GM, 158, 43] sample was the highest scoring sample in the 'black treacle' attribute, but was not significantly higher than the following samples: chocolate malt ($p=0.997$), commercial roasted barley ($p=0.861$), medium crystal malt ($p=0.428$), [GM, 165, 35] ($p=0.382$), and [RB, 200, 25] ($p=0.085$) (Figure 6.6). Furthermore, as discussed above, Figure 6.6 shows the lowest scores for this attribute were in the amber malt, [GM, 135, 50], and [RB, 135, 30] samples, which load in the transverse quadrant to the black treacle vector (Figure 6.7).

Loading in the same quadrant to the three samples roasted to the lowest temperatures (amber malt, [GM, 135, 50], and [RB, 135, 30]) are the following attributes: 'Horlicks', 'digestive biscuit', and 'grainy'. In these attributes, the low temperature roasted samples scored the highest of all the analysed samples (Figure 6.5). Each of the three attributes yielded a different highest scoring sample. For the 'Horlicks' attribute, [GM, 135, 50] was the highest scoring sample, but not was significantly higher than amber malt ($p=0.911$) or [RB, 135, 30] ($p=0.076$). In the 'grainy' attribute, [RB, 135, 50] was the highest scoring sample, but again was not significantly higher than amber malt ($p=0.155$) or [GM, 135, 50] ($p=0.152$). Finally, the 'digestive biscuit' attribute yielded amber malt as the highest scoring sample, although amber malt, [GM, 135, 50], and [RB, 135, 30] were not significantly different from each other. The commercial roasted barley sample was consistently the lowest scoring sample in the 'Horlicks', 'grainy', and 'digestive biscuit'

attributes, corresponding in its loading transversely to those attributes' vectors (Figure 6.5).

In the upper left-hand quadrant, the intermediate temperature roasted samples are plotted (medium crystal malt, [PM, 165, 20], [GM, 158, 43], and [GM, 165, 35]). These samples are associated with higher scores in the 'malt loaf', 'tea/ leaves', 'marmite', and 'potato' attributes.

Agglomerative hierarchical clustering (AHC) analysis was carried out, analysing the mean scores of the 12 aroma attributes across the 11 roasted samples, as detailed in Section 6.2.3. The dendrogram resulting from this analysis is displayed in - Appendices Section 8.3 Figure 8.21. AHC analysis confirms the results displayed in the biplot of Figure 6.7. Three distinct clusters of samples are identified: those roasted to temperatures exceeding 200 °C (commercial roasted barley, chocolate malt, [RB, 200, 25], and [PM, 200, 25]), intermediate roasting temperatures between 150 °C and 200 °C ([PM, 165, 20], [GM, 165, 35], medium crystal malt, and [GM, 158, 43]), and low roasting temperatures below 150 °C ([RB, 135, 30], amber malt, and [GM, 135, 50]).

6.3.2.1 Identification of Statistical Differences Between Samples Within Substrate Types

The strong influence of roasting substrate type (i.e. green malt, pale malt, or unmalted barley) on product volatile composition has been identified in previous investigations throughout this research (Chapter 4 and Chapter 5).

- Appendices Section 8.3 Figure 8.22, Figure 8.24, and Figure 8.26 show individual bar charts displaying the mean aroma attribute scores across the samples within each roasting substrate (roasted green malt, pale malt, and unmalted barley, respectively). These figures show the same data as shown in Figure 6.4, Figure 6.5, and Figure 6.6, but with simplified significantly different groupings (specific to the samples from each substrate type) and are therefore not included in the text.

Regarding roasted green malt samples, [GM, 135, 50] yielded the most significant differences compared to the other roasted green malt samples. For example, [GM, 135, 50] scored significantly lower than the other green malt samples in 'burnt', 'black treacle', and 'dark chocolate' aromas. In addition, [GM, 135, 50] scored significantly higher than the other samples in 'Horlicks' and 'digestive biscuit'

aromas than the other green malts. There were no significant differences between the roasted green malt samples in the ‘potato’, ‘Marmite’, or ‘tea/ leaves’ aromas (Figure 6.6).

Regarding the four roasted pale malts, amber malt and [PM, 165, 20] scored significantly higher than chocolate malt and [PM, 200, 25] samples in ‘malt loaf’, ‘tea/ leaves’, ‘potato’, ‘Marmite’, ‘Horlicks’, ‘digestive biscuit’, and ‘grainy’ aromas. Whereas chocolate malt and [PM, 200, 25] scored significantly higher in the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aroma attributes than both amber malt and [PM, 165, 20]. Within this, amber malt scored significantly lower than the other three pale malt samples in the ‘burnt’ and ‘dark chocolate’ aroma attributes (Figure 6.4).

The three roasted unmalted barley samples yielded significant differences commonly between [RB, 135, 30], and commercial roasted barley and [RB, 200, 25]. For example, commercial roasted barley and [RB, 200, 25] scored significantly higher than [RB, 135, 30] in ‘burnt’, ‘smoky’, ‘coffee’, ‘black treacle’, ‘dark chocolate’, potato’, and ‘Marmite’ aromas. In addition, [RB, 135, 30] scored significantly higher than both [RB, 200, 25] and commercial roasted barley in the ‘Horlicks’, ‘digestive biscuit’, ‘grainy’, and ‘tea/ leaves’ aroma attributes. In the ‘malt loaf’ attribute, commercial roasted barley scored significantly lower than in [RB, 200, 25] ($p=0.009$), with [RB, 135, 30] not significantly different to either sample.

The significant differences within substrate groups are consistent with the major trend identified in Figure 6.7, clustering samples based upon their roasting temperature, rather than their substrate type.

6.3.3 Identification of Statistical Differences Between Substrate Types

As previously identified, the strong influence of roasting substrate on product volatile composition warrants consideration. Although not the main aim of the current study, the identification of inherent differences in perceived aroma attributes between roasting substrate type is of interest.

The current study was not designed with comparisons of aroma between products from different roasting substrates in mind. In order to examine this in detail, the inclusion of equal numbers of samples from each substrate type would be required.

In addition, equivalent roasting times and temperatures across the substrate types would be essential to make valid comparisons. The following discussion provides an indication of the differences in impact of aroma qualities between the three substrate types.

Table 6.4 shows the aroma score means for each sample's roasting substrate. As detailed in Section 6.2.3, Tukey's HSD post hoc test was applied to provide pairwise comparisons for roasting substrate regarding each attribute (Kemp et al., 2018).

Table 6.4: Aroma score means across all samples averaged according to substrate.

Roasted Substrate [1]	Attribute											
	Burnt	**Smoky**	**Coffee**	**Malt Loaf**	**Black Treacle**	**Tea/ Leaves**	**Dark Chocolate**	**Potato**	**Marmite**	**Horlicks**	**Digestive Biscuit**	**Grainy**
GM	2.2 [b]	1.3 [b]	1.2 [b]	5.8 [a]	3.5 [a]	3.5 [a]	2.7 [b]	3.0 [a]	2.5 [a]	3.3 [a]	3.5 [a]	3.6 [a]
PM	4.8 [a]	4.3 [a]	3.8 [a]	2.9 [b]	2.8 [a]	2.2 [b]	3.5 [ab]	2.6 [a]	2.3 [a]	2.0 [b]	2.9 [ab]	3.0 [a]
RB	4.6 [a]	4.5 [a]	4.2 [a]	1.3 [c]	2.6 [a]	1.6 [b]	3.6 [a]	1.1 [b]	0.7 [b]	1.5 [b]	2.5 [b]	3.4 [a]
p Value (Model)	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]
p Value (Roasted Substrate)	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	0	< 0.0001[§]	0[§]	< 0.0001[§]	< 0.0001[§]	< 0.0001[§]	0[§]	0

[A] Roasted green malt (GM), n=4 samples: medium crystal malt, [GM, 135, 50], [GM, 158, 43], and [GM, 165, 35]. Roasted pale malt (PM), n=4 samples: amber malt, chocolate malt, [PM, 165, 20], and [PM, 200, 25]. Roasted raw barley (RB), n=3 samples: roasted barley, [RB, 135, 30], and [RB, 200, 25].
Subscript letters indicate significantly different groupings within each attribute from two-way ANOVA with Tukey's HSD post hoc analysis.
[§]Significant.

The effect of roasting substrate on the aroma attribute scores for the samples was identified as very highly significant ($p<0.0001$) in the majority of the attributes, with exceptions in the 'black treacle' and 'grainy' attributes which were not significant. Significantly different groups of roasted substrates identified by post hoc pairwise comparisons are denoted by subscript lettering.

The 'malt loaf' attribute identified significant differences between all three roasted substrates (Table 6.4). Roasted green malt products scored significantly higher than both roasted pale malt and roasted unmalted barley products. In addition, roasted pale malts scored significantly higher than roasted unmalted barley products.

The 'burnt', 'smoky', and 'coffee' attributes were scored significantly lower in the roasted green malt products than in both roasted pale malt and unmalted barley products, which shared no significant differences. The 'dark chocolate' attribute yielded similar results, as the roasted pale malt products were not significantly different from either the roasted green malt or roasted unmalted barley products. However, the roasted unmalted barley products scored significantly higher than the green malt products in the 'dark chocolate' attribute.

Roasted green malt samples scored significantly higher in the 'tea/ leaves' and 'Horlicks' attributes than both roasted pale malt and roasted unmalted barley products, which were not significantly different. Roasted unmalted barley products scored significantly lower in the 'marmite' and 'potato' attributes than both the roasted green malt and roasted pale malt products, which were not significantly different. In the 'digestive biscuit' attribute, roasted pale malt products were not significantly different from either the roasted green malt or roasted barley products. However, roasted green malt products scored significantly higher than the roasted barley products in this attribute.

From the statistical analysis shown in Table 6.4, it is clear that roasting substrate has a significant effect on the perception of different aroma attributes. This has potential to influence a brewer's choice of roasted product to enhance or reduce particular aroma attributes. Notably, the 'malt loaf' characteristic may be emphasised by the inclusion of roasted green malt products when included in a brew.

6.3.4 Laboratory Roasted Products with Potentially Novel Sensory Qualities

Following the discussion of aroma QDA results, laboratory roasted samples with potentially novel sensory qualities can be identified. Consideration of samples' substrate type, roasting conditions, and resultant aroma qualities allows for identification of unique characteristics in the laboratory roasted sample range.

By examining the biplot of Figure 6.7, [PM, 165, 20], [RB, 135, 30], and [GM, 135, 50] are identified as falling outside the commercial range of roasted products for the relevant substrate (in relation to the range of four commercially roasted products analysed in this investigation).

The [PM, 165, 20] sample appears to have novel aroma qualities for a roasted pale malt product. Intermediate finishing temperatures (150 °C to 200 °C) and shorter roasting times in the roasting of pale malt are not commonly used. Typically, roasted pale malts are roasted to low finishing temperatures (for example 100-150 °C for amber malt (Boortmalt, 2010a)) or high finishing temperatures (for example 225 °C for chocolate malt (Boortmalt, 2010e)). The aroma qualities of [PM, 165, 20] indicate novel characteristics as a roasted pale malt product according to its loading within Figure 6.7. Although loading closely to medium crystal malt in Figure 6.7, [PM, 165, 20] yields unique aroma qualities within the roasted pale malt products. The sample scored highest for the 'potato' attribute, but fell in the middle of the sample range for it's scoring in the remaining assessed attributes. The aroma similarities between [PM, 165, 20] and crystal malts suggest the potential to translate the roasting conditions of [PM, 165, 20] to commercial roasting to yield similar sensory qualities with reductions in processing steps, namely stewing, to produce 'imitation' crystal malts. However, these similarities are apparent regarding only the aroma of the [PM, 165, 20] sample to that of crystal malts.

The [RB, 135, 30] sample yielded differences in aroma characteristics when compared to fellow roasted unmalted barley samples (commercial roasted barley and [RB, 200, 25]). Although this low temperature roasted barley sample yielded low scores for the majority of the assessed attributes ('burnt', 'smoky', 'coffee', 'malt loaf', 'black treacle', 'dark chocolate', and 'marmite'), it was high scoring in the 'Horlicks', 'grainy', and 'digestive biscuit' attributes. These qualities are also identified in the commercially produced amber malt product (a roasted pale malt

product). The use of roasted unmalted barley in brewing is reported to provide astringency that cannot be achieved with the use of roasted pale or green malts (Gruber, 2001). In current commercial roasting, roasted unmalted barley is typically roasted to achieve a very deep red or black colour (Gruber, 2001). A low temperature/time roasted unmalted barley may contribute novel characteristics when included in the brewing process.

With regard to potentially novel roasted green malt products, it is notable that the [GM, 135, 50] yielded many similarities with the amber malt sample (Figure 6.7). This suggests that green malt can be manufactured with similar aroma characteristics to a low temperature roasted pale malt product. The [GM, 135, 50] sample's similarities to amber malt with regard to flavour and textural contribution to beers is unknown.

Although not included for analysis in this investigation, two commercially roasted products may share similarities with the [GM, 135, 50] sample. Light crystal malt yields the lowest colour in the range of Boortmalt crystal malts, whereas caramalt is described as being similar to a crystal malt, but with an extended roasting time to achieve lower colour, higher extract, and higher moisture (Boortmalt, 2010d, Boortmalt, 2010g). The exact roasting conditions of these products are not publicly available. In contrast to crystal malts, the endosperm of caramalt remains 'floury', and therefore provides reduced sweetness. In this respect, [GM, 135, 50] is likely to resemble light crystal malt. However, the 50 min roasting time of [GM, 135, 50] may have resulted in similarities to caramalt. Further investigation into the sensory qualities of the [GM, 135, 50] sample compared to additional commercial products is required to make an informed assessment of the product's novelty. In addition, due to the assessment of only the aroma of the products in this investigation, further examination of the products, considering their novel qualities regarding product flavour and textural enhancement are required.

6.3.5 Association of Results: Combining Partial QDA with Key Volatile Compound Concentrations

The previously discussed GC-O investigation identified odour active compounds in a range of six commercially roasted products (Chapter 3). Subsequently, laboratory roasted malt and barley were produced in order to monitor the formation of selected

key odour active compounds across the controlled conditions of roasting time and temperature (Chapter 4). In addition, volatile sulphur compounds have also been identified in the commercial range of roasted products and a selected range of laboratory roasted samples (Chapter 5).

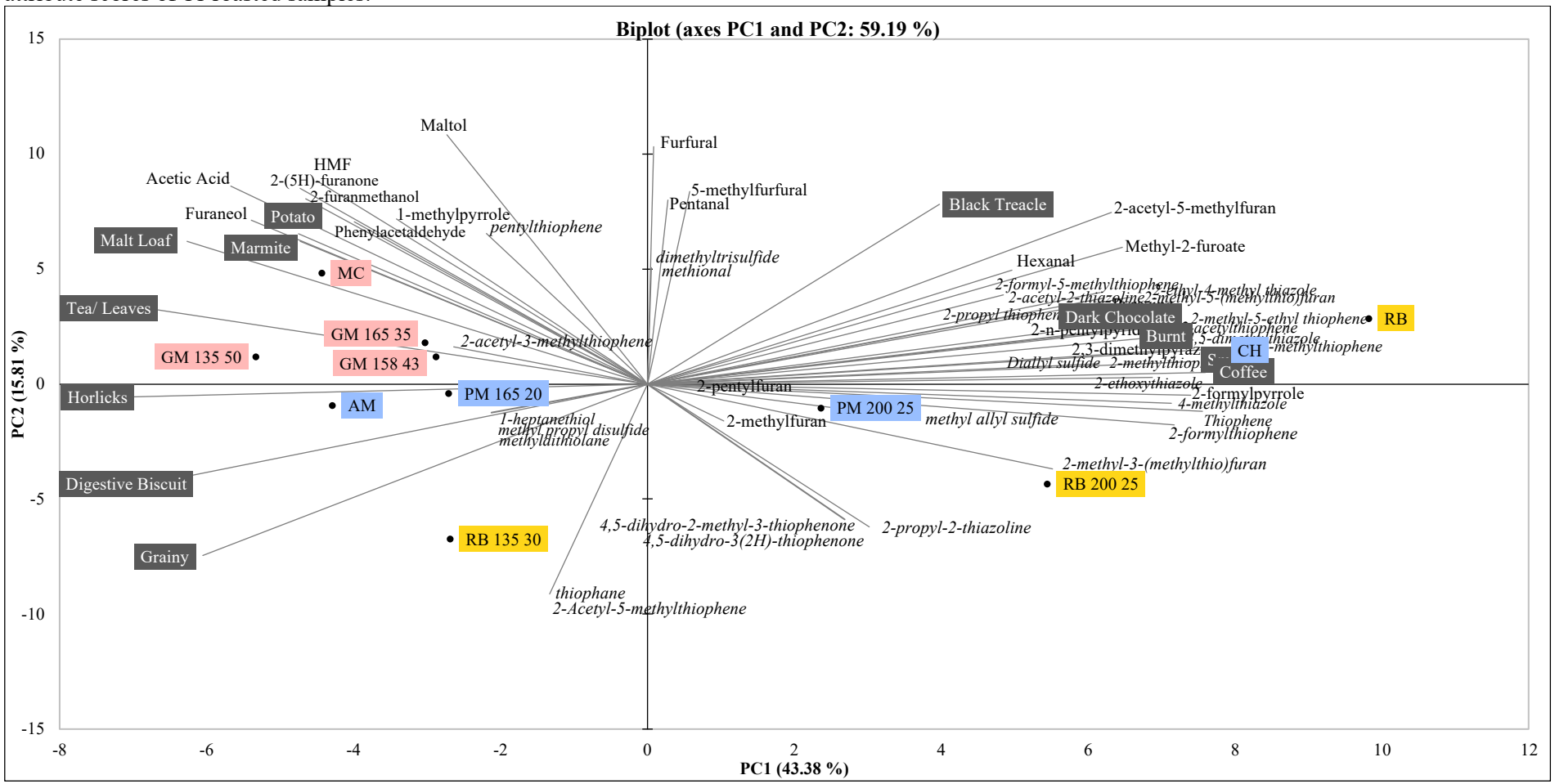
Compounds that are highly odour active and/or abundant can be considered responsible for the detection of particular attributes in the samples analysed by partial QDA. Determining which compounds cause the detection of particular aroma attributes can be attempted by re-examining the data from previous investigations in this research. It must be stressed that this method of data association is tentative, and should be investigated further to confirm the associations predicted in the following discussion.

Individual compounds are not solely responsible for the detection of attributes. When combined in the matrix, the compounds are detected as a mixture, which results in the detection of different attributes to varying degrees of prominence. The proportion of the compounds within the mixture influences the establishment of attributes in the product. This is also influenced by the detection thresholds of the compounds within the mixture (van Ruth, 2001). For example, as previously discussed in Chapter 5, volatile sulphur compounds can strongly influence the aroma of a product, but are undetectable at low levels by standard instrumental methods of detection (i.e. mass spectrometry) (Hill and Smith, 2000).

In addition to the complication of volatile/matrix interactions, the key volatile compounds which were identified and monitored in the previous investigations throughout this research do not encompass the entirety of the range of volatile compounds present in roasted products. The vast assortment of volatiles in roasted products could not be identified completely. The key volatile compounds selected present a representation of the volatiles present in the product range.

Figure 6.8 shows a correlation PCA biplot depicting PC1 and PC2 (43.38 % and 15.81 % of the variation in the data, respectively). The biplot includes the aroma QDA data of the current investigation, along with the concentrations of the quantified volatile compounds from previous Chapters (Chapter 4 and Chapter 5) across the 11 analysed samples.

Figure 6.8: PCA biplot (**PC1** and **PC2**) plotting concentrations of 29 volatile sulphur compounds (VSCs), 20 key odour active compounds, and 12 aroma attribute scores of 11 roasted samples.



VSCs indicated by *italic* font.

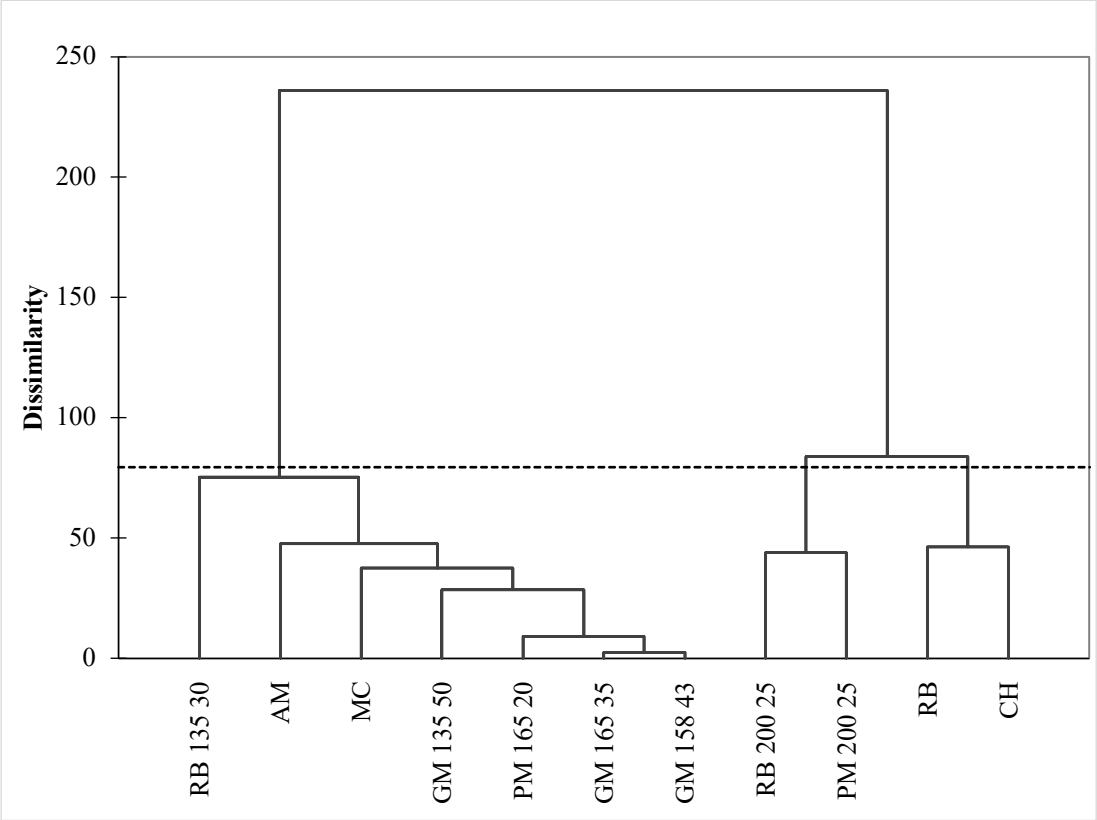
The loading of samples and aroma attribute vectors within Figure 6.8 share many similarities with the biplot of Figure 6.7. The aroma attribute vectors remain in similar locations on the biplot to those in Figure 6.7. However, as a result of adding the concentrations of key volatile compounds in the samples, the loading of the individual samples has shifted marginally. Most recognisably, the upper left quadrant of Figure 6.8 now contains volatile compounds which are maximised in the roasted green malt products, largely as a result of Maillard chemistry, along with only two VSCs. As a result, only roasted green malt products are located in this quadrant of the biplot.

Where [PM, 165, 20] shared similarities in aroma qualities to the roasted green malts in Figure 6.7, this sample is now located in the lower left quadrant of the biplot (Figure 6.8). This indicates the sample's lower concentrations of the characteristic Maillard products that are abundant in roasted green malts. This further demonstrates the strong influence of chosen substrate, the moisture content at the beginning of roasting, and whether or not stewing is used, on the considerable influences on the formation of odour active volatiles as previously identified (Chapter 4). Despite this, [PM, 165, 20] loads in close proximity to the crystal malt samples, while loading closer to amber malt than in Figure 6.7.

Also in the lower left quadrant are amber malt and [RB, 135, 30]. Only five compounds load within this quadrant (all VSCs), indicating low concentrations of the remaining volatile compounds in the three samples within this section of the biplot. The quantified compounds were selected in order to monitor thermal flavour generation in the products. Samples roasted to low and intermediate finishing temperatures therefore do not yield large quantities of the monitored volatiles. As a result, the majority of the observed compounds load positively on PC1, in close proximity to the 'dry roasted' samples, roasted to the highest finishing temperatures (principally chocolate malt, and commercial roasted barley). Also loading positively on PC1, but closer to zero, are the high temperature roasted laboratory produced samples: [PM, 200, 25] and [RB, 200, 25]. These samples' loadings in Figure 6.8 indicate the presence of the heterocyclic compounds such as pyrazines, substituted furans, and pyrroles, but to a lesser extent to those in the commercially roasted samples of chocolate malt and roasted barley. The majority of the VSCs are also more abundant in the 'dry roasted' products in Figure 6.8. These sulphur containing

compounds are associated with occurring in higher concentrations in the samples which are typified by their prominent burnt, smoky, and coffee aromas.
To aid further understanding of the biplot, AHC analysis was carried out assessing the same data examined in Figure 6.8, in order to identify clusters of samples (Figure 6.9).

Figure 6.9: Dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) analysing concentrations of 29 volatile sulphur compounds, 20 key odour active compounds, and 12 aroma attribute scores of 11 roasted samples.



Concentration values from Chapter 4 and Chapter 5.

By using AHC analysis, clusters of samples are identified by a dendrogram. In which, samples belonging to the same cluster are more similar to one another than to samples belonging to other clusters. Figure 6.9 identifies three main clusters of samples. Samples which load negatively on PC1 belong within one cluster. Samples which load positively on PC1 form the remaining two clusters: one containing [RB, 200, 25] and [PM, 200, 25], and the other containing commercially roasted barley and chocolate malt.
When comparing the final two clusters of samples, the cluster containing [RB, 200, 25] and [PM, 200, 25] indicates those two samples are more similar to each other

(within-cluster variance: 8,321) than roasted barley and chocolate malt are to each other (within-cluster variance: 22,117). This is perhaps due to their identical roasting conditions, in comparison to the 5 °C finishing temperature difference (and likely differences in roasting time) between chocolate malt and roasted barley (Boortmalt, 2010j, Boortmalt, 2010e). The 20-25 °C lower finishing temperatures in the laboratory roasted products may have resulted in lower rates of thermal volatile formation, producing the two clusters of 'dry roasted' samples in Figure 6.8.
Within the first cluster (negatively loading samples on PC1), [RB, 135, 30] is identified as dissimilar to the remaining samples. This is likely a result of the identification of two VSCs (thiophane and 2-acetyl-5-methylthiophene) which were exclusively present in this sample.
The same cluster of negatively loading samples on PC1 also identified [PM, 165, 20] as being more similar to the roasted green malt samples than to the amber malt sample (a low temperature roasted pale malt) (Figure 6.9). This is consistent with the loading of [PM, 165, 20] in the upper left quadrant of Figure 6.7, in addition to this sample's proximity to the roasted green malts in Figure 6.8.

6.3.5.1 Identification of possible volatile compounds resulting in the detection of aromas in roasted products

The use of multiple data analysis techniques enables the association of volatile compound concentrations to the detection of aroma attributes in roasted products. Along with PCA and AHC, Pearson correlation matrices strengthen these associations. Table 6.5 and Table 6.6 show the Pearson correlation matrices of the concentrations of 20 key odour active compounds, and 29 VSCs in roasted products across the 12 aroma attributes, respectively. The Tables identify where positive or negative correlations are present between each volatile compound and attribute detection. A positive correlation is indicated by '1', and '-1' represents a negative correlation, while '0' indicates the absence of a correlation. Values in bold are statistically significant correlations ($p<0.05$).

Table 6.5: Pearson correlation matrix of the concentrations of 20 key odour active compounds in roasted samples (n=11) across 12 aroma attributes.

	Attribute											
Compound [A]	**Burnt**	**Smoky**	**Coffee**	**Malt Loaf**	**Black Treacle**	**Tea/ Leaves**	**Dark Chocolate**	**Potato**	**Marmite**	**Horlicks**	**Digestive Biscuit**	**Grainy**
1-methylpyrrole	0	0	0	0	0	0	0	1	**1**	1	0	0
2-(5H)-furanone	0	-1	-1	**1**	0	**1**	0	**1**	0	1	0	0
2-acetyl-5-methylfuran	**1**	**1**	**1**	0	**1**	-1	**1**	0	0	**-1**	**-1**	**-1**
2-formylpyrrole	**1**	**1**	**1**	**-1**	1	**-1**	**1**	0	0	**-1**	**-1**	**-1**
2-furanmethanol	0	-1	-1	**1**	0	1	0	0	0	1	0	0
2-methylfuran	0	0	0	0	0	0	0	0	0	0	0	0
2-n-pentylpyridine	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	0	**-1**	**-1**	**-1**
2-pentylfuran	0	0	0	0	0	0	0	0	0	0	0	0
2,3-dimethylpyrazine	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	0	**-1**	**-1**	**-1**
5-methylfurfural	0	0	0	0	**1**	0	0	0	0	0	0	-1
Acetic Acid	-1	**-1**	**-1**	**1**	0	**1**	0	**1**	**1**	**1**	0	0
Furaneol	-1	-1	-1	**1**	0	**1**	0	**1**	**1**	1	0	0
Furfural	0	0	0	0	0	0	0	0	0	0	0	0
Hexanal	0	0	0	0	0	0	0	0	0	0	0	-1
HMF	0	0	-1	**1**	0	1	0	1	1	0	0	0
Maltol	0	0	0	**1**	0	0	0	0	0	0	0	0
Methyl-2-furoate	**1**	**1**	**1**	-1	1	-1	1	0	0	**-1**	**-1**	**-1**
Pentanal	0	0	0	0	0	0	0	0	0	0	0	0
Phenylacetaldehyde	-1	0	-1	0	0	0	-1	0	0	**1**	1	0
Pyrazine	1	1	1	-1	0	-1	0	0	0	-1	**-1**	**-1**

[A] Compound concentration values from Chapter 4.
-1= negative correlation (pale grey shading), 0= no correlation (no shading), 1= positive correlation (dark grey shading).
Values in bold are significantly different from zero ($p<0.05$).

Table 6.6: Pearson correlation matrix of the concentrations of 29 volatile sulphur compounds in roasted samples (n=11) across 12 aroma attributes.

	Attribute											
Compound [A]	**Burnt**	**Smoky**	**Coffee**	**Malt Loaf**	**Black Treacle**	**Tea/ Leaves**	**Dark Chocolate**	**Potato**	**Marmite**	**Horlicks**	**Digestive Biscuit**	**Grainy**
1-heptanethiol	0	0	0	0	0	0	0	0	1	0	0	0
2-acetyl-2-thiazoline	0	0	0	0	0	0	0	0	0	0	0	-1
2-acetyl-3-methylthiophene	0	0	0	0	0	0	0	0	0	1	0	0
2-Acetyl-5-methylthiophene	0	0	0	0	-1	0	0	-1	-1	0	0	**1**
2-acetylthiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	0	**-1**	**-1**	**-1**
2-ethoxythiazole	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	-1	**-1**	**-1**	**-1**
2-ethyl-4-methyl thiazole	1	**1**	**1**	-1	0	-1	1	0	0	-1	**-1**	**-1**
2-formyl-5-methylthiophene	0	0	0	0	0	0	0	0	0	0	0	-1
2-formylthiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	-1	**-1**	**-1**	**-1**
2-methyl-3-(methylthio)furan	**1**	**1**	**1**	0	0	**-1**	**1**	0	-1	-1	0	0
2-methyl-5-(methylthio)furan	**1**	**1**	**1**	**-1**	0	**-1**	1	0	0	**-1**	**-1**	**-1**
2-methyl-5-ethyl thiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	0	**-1**	**-1**	**-1**
2-methylthiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	-1	**-1**	**-1**	**-1**
2-propyl thiophene	1	**1**	1	0	0	-1	0	0	0	0	-1	-1
2-propyl-2-thiazoline	0	0	1	0	0	-1	0	0	0	0	0	0
3-methylthiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	-1	**-1**	**-1**	**-1**
4-methylthiazole	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	-1	**-1**	**-1**	-1
4,5-dihydro-2-methyl-3-thiophenone	0	0	0	0	0	-1	0	0	0	0	0	0
4,5-dihydro-3(2H)-thiophenone	0	0	0	0	0	-1	0	0	0	0	0	0
4,5-dimethylthiazole	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	0	**-1**	**-1**	**-1**
Diallyl sulphide	**1**	**1**	**1**	**-1**	0	**-1**	**1**	0	0	**-1**	**-1**	**-1**
Dimethyl trisulphide	0	0	0	0	0	0	0	1	0	0	0	0
Methional	0	0	0	0	0	0	0	1	0	0	0	0
Methyl allyl sulphide	0	0	0	-1	0	0	0	-1	0	0	0	0
Methyl propyl disulphide	0	0	0	0	0	0	0	0	1	0	0	0
Methyldithiolane	0	0	0	0	0	0	0	0	1	0	0	0
Pentylthiophene	0	0	0	0	0	0	0	0	0	0	0	0
Thiophane	0	0	0	0	-1	0	0	-1	-1	0	0	**1**
Thiophene	**1**	**1**	**1**	**-1**	0	**-1**	**1**	-1	-1	**-1**	**-1**	**-1**

[A] Compound concentration values from Chapter 5.
-1= negative correlation (pale grey shading), 0= no correlation (no shading), 1= positive correlation (dark grey shading).
Values in bold are significantly different from zero ($p<0.05$).

Table 6.5 and Table 6.6, in combination with Figure 6.8, are used in order to associate particular compounds with prominence of aroma attributes (below).

6.3.5.1.1 Compounds resulting in the detection of ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aromas

Significantly positive correlations were present in the following compounds in the detection of ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aromas: 2-acetyl-5-methylfuran, 2-formylpyrrole, 2-n-pentylpyridine, 2,3-dimethylpyrazine, 2-acetylthiophene, 2-ethoxythiazole, 2-formylthiophene, 2-methyl-3-(methylthio)furan, 2-methyl-5-ethylthiophene, 2-methylthiophene, 3-methylthiophene, 4-methylthiazole, 4,5-dimethylthiazole, thiophene, and diallyl sulphide (Table 6.5 and Table 6.6). In addition, methyl-2-furoate was identified as having a significant positive correlation regarding the ‘burnt’, ‘smoky’, and ‘coffee’ aroma attributes (not significant in the ‘dark chocolate’ attribute ($p=0.058$)) (Table 6.5).

Eleven of the compounds with a significant positive correlation in the four aroma attributes are VSCs, which were found to be most abundant in products roasted to the highest finishing temperatures (Chapter 5). It is known that VSCs are mainly produced through thermal degradation of sulphur containing amino acids and Maillard reactions, including the Strecker degradation (Zheng et al., 1997, Jia et al., 2019). For example, the reactions between furaneol and cysteine are known to form a range of thiophenes, thiophenones, and thiazoles (Zheng et al., 1997, Shu et al., 1986, Jia et al., 2019). It is likely that the dry roasting conditions, which are essential for the roasting of raw barley and pale malts, encourage the formation of greater quantities of VSCs in these samples. In addition, the supplementary evidence of the lack of formation of large quantities of a variety of VSCs in the crystal malt samples (roasted green malts) also supports this trend (Figure 6.8).

Regarding volatiles that do not contain sulphur, but were identified as having a significant positive correlation in the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aromas, the five identified compounds are formed to greater concentrations under low moisture, high temperature conditions (Chapter 4). At the higher extreme of the roasting parameters, moisture in the kernel is low, and as a result these factors favour slow pyrolysis reactions (Wnorowski and Yaylayan, 2000, Lin et al., 2009). Later stage Maillard products are also formed under these dry roasting conditions. For example, 2-acetyl-5-methylfuran is known to be formed during the Maillard reaction, and greater concentrations of this compound result in higher scores of the four aroma attributes (burnt, smoky, coffee, and dark chocolate) (Nikolov and Yaylayan, 2011).

6.3.5.1.2 Compounds resulting in the detection of the ‘black treacle’ aroma

The ‘black treacle’ attribute is placed between the high temperature roasted samples and the moderately roasted samples in Figure 6.8, but is closest to the samples roasted above 200 °C (seen more clearly in Figure 6.7).

In addition to its significant positive correlation in the previous aroma attributes (burnt, smoky, coffee, and dark chocolate), 2-acetyl-5-methylfuran was also identified as having a significant positive correlation in the detection of the ‘black treacle’ aroma attribute ($p=0.023$), in addition to 5-methylfurfural ($p=0.039$). Positive correlations were also identified in 2-formylpyrrole ($p=0.107$) and methyl-2-furoate ($p=0.084$), but without significance (Table 6.5).

In a study into flavour development during the commercial roasting of chocolate malt by O'Shaughnessy (2003), 5-methylfurfural was detected in the later stages of roasting (after 60 min of a total of 97 min). In addition, it has been noted that the concentration of 5-methylfurfural will only decrease in roasting temperatures exceeding 250 °C (Tehrani et al., 2002, O'Shaughnessy, 2003). The highest roasting temperature of the samples analysed in the current investigation was 230 °C, the finishing temperature of commercial roasted barley (Boortmalt, 2010j).

5-methylfurfural has a ‘sweet, caramellic, bitter’ aroma, which corresponds to the ‘black treacle’ attribute detected by the panel (O'Shaughnessy, 2003). The highest concentrations of 5-methylfurfural in the analysed samples were mixed between the moderately roasted and high temperature roasted samples. This also aligns with the scores given to the samples for the ‘black treacle’ attribute. The low temperature roasted samples (<150 °C) were scored significantly lower than the remaining eight samples (with the exception of [PM, 165, 20] (Figure 6.6)).

6.3.5.1.3 Compounds resulting in the detection of the ‘malt loaf’ aroma

The ‘malt loaf’ aroma was detected to the highest intensity in the samples roasted to intermediate finishing temperatures (medium crystal malt, [GM, 158, 43], [GM, 165, 35], and [PM, 165, 20]), in addition to amber malt and [GM, 135, 50] (Figure 6.5).

Compounds with significant positive correlations in the detection of the ‘malt loaf’ aroma were: 2-(5H)-furanone ($p<0.0001$), 2-furanmethanol ($p=0.005$), acetic acid ($p<0.0001$), HMF ($p=0.005$), maltol ($p=0.019$), and furaneol ($p=0.002$) (Table 6.5).

Acetic acid, maltol, and 2-furanmethanol were identified in Chapter 4 as being formed to the highest concentrations under low temperature, high moisture conditions (i.e. those in stewed and roasted green malts). For example, maltol is described as being 'sweet, caramellic, candy floss, jammy, fruity, and bready' in its aroma, and therefore aligns with the detection of 'malt loaf' aromas (Scents, 2018a). It is formed during the intermediate stages of the Maillard reaction, but can also be formed under pyrolytic conditions in the later stages of dry roasting (Vandecan et al., 2011, Yahya et al., 2014, Yaylayan and Mandeville, 1994). In Chapter 4, maltol was found to be formed to greater concentrations in the roasted green malt samples. The samples selected for aroma QDA exhibit the full range of maltol concentrations in the 'finished' roasted products analysed in Chapter 4 (<5% moisture w/w) (n=37 samples). From the very highest in medium crystal malt (1021 μg/g) to the very lowest in [RB, 135, 30] (6.46 μg/g).

Conversely, compounds with significant negative correlations in the detection if the 'malt loaf' aroma were: 2-formylpyrrole (p=0.027), 2-n-pentylpyridine (p=0.034), 2,3-dimethylpyrazine (p=0.027), 2-acetylthiophene (p=0.032), 2-ethoxythiazole (p=0.016), 2-formylthiophene (p=0.027), 2-methyl-5-(methylthio)furan (p=0.038), 2-methyl-5-ethylthiophene (p=0.019), 2-methylthiophene (p=0.017), 4,5-dimethylthiazole (p=0.029), diallyl sulphide (p=0.039), and thiophene (p=0.013). Many of these were found to exhibit significant positive correlations in the 'burnt', 'smoky', 'coffee' and 'dark chocolate' aroma attributes (Section 6.3.5.1.1). This further demonstrates the effect that samples with prominent 'malt loaf' aromas are also low scoring in the 'burnt', 'smoky', 'coffee', and 'dark chocolate' aroma attributes (the inverse is also the case).

6.3.5.1.4 Compounds resulting in the detection of 'Horlicks', 'digestive biscuit', and 'grainy' aromas

The aroma attributes of 'Horlicks', 'digestive biscuit', and 'grainy' were most prominent in samples roasted to low finishing temperatures (amber malt, [GM, 135, 50], and [RB, 135, 30]) (Figure 6.7). Both Table 6.5 and Table 6.6 show the identification of more significant negative correlations than positive correlations relating volatile compounds to the detection of these aroma attributes. This indicates that the lack of thermally generated volatile compounds emphasises the 'digestive

biscuit', 'grainy', and 'Horlicks' aromas in roasted products. These aroma characteristics are present as a result of the lack of, or reduced temperature of roasting conditions.

The detection of the 'Horlicks' aroma was significantly positively influenced by the concentrations of acetic acid ($p=0.035$) and phenylacetaldehyde ($p=0.014$) (Table 6.5). Phenylacetaldehyde is a Strecker aldehyde formed in thermally treated foodstuffs through the Strecker degradation of phenylalanine (Channell et al., 2010, Farmer, 1994, Rizzi, 1999, Smit et al., 2009). As an intermediate product of the Maillard reaction, phenylacetaldehyde concentrations initially decrease with increasing roasting temperature. This is likely due to its role as an intermediate product, being used in subsequent stages of the Maillard reaction. However, the results of Chapter 4 suggested a possible alternate route for the formation of phenylacetaldehyde in very dry, high temperature roasted systems via pyrolysis of phenylalanine, as opposed to Strecker degradation. Despite this, phenylacetaldehyde was found to have a significant positive effect on the detection of only the 'Horlicks' aroma (Table 6.5).

6.4 Conclusions

The use of partial QDA in this investigation identified and quantified 12 aroma characteristics across a range of commercially and laboratory roasted products. Scoring the intensity of each aroma attribute across the samples identified clusters of samples with similarities in aroma qualities. The roasting conditions and roasting substrate type were noted in order to identify samples with potentially novel aroma characteristics.

The identification of significant differences between individual samples within aroma attributes (aided by principal component analysis (PCA) and agglomerative hierarchical clustering (AHC)) yielded three distinct clusters of samples: those roasted to low finishing temperatures (<150 °C), intermediate finishing temperatures (150 - 200 °C), and high finishing temperatures (>200 °C). These clusters formed irrespective of roasting substrate type. Samples roasted to high finishing temperatures (commercially roasted barley, chocolate malt, [RB, 200, 25], and [PM, 200, 25]) scored significantly higher in the 'burnt', 'smoky', 'coffee', and 'dark chocolate' aroma attributes than the remaining samples. Samples roasted to

intermediate finishing temperatures (medium crystal malt, [GM, 158, 43], [GM, 165, 35], and [PM, 165, 20]) scored highest in the 'malt loaf', 'Marmite', 'potato', and 'tea/ leaves' aromas. Samples roasted to low finishing temperatures (amber malt, [GM, 135, 50], and [RB, 135, 30]) scored highest in the 'Horlicks', 'digestive biscuit', and 'grainy' aroma attributes. Upon identification of significant differences between roasting substrate types, the 'malt loaf' attribute yielded significant differences between all three roasting substrates, with roasted green malts scoring highest.

Laboratory roasted products were analysed in this study in order to identify samples with potentially novel aroma characteristics. Following aroma QDA, [PM, 165, 20], [RB, 135, 30], and [GM, 135, 50] were identified as falling outside the commercial range of roasted products for the relevant substrate (in relation to the range of four commercially roasted products analysed in this investigation). The [PM, 165, 20] sample yielded novel aroma qualities for a roasted pale malt product, likely to be a result of its intermediate finishing temperature and short roasting time, neither are commonly used in the roasting of pale malts. The [PM, 165, 20] sample shared similarities with commercial medium crystal malt, a roasted green malt product, therefore making this a novel product within its substrate type.

The [RB, 135, 30] sample is considered a novel roasted unmalted barley product due to its 'Horlicks', 'digestive biscuit', and 'grainy' aroma qualities. Roasted unmalted barley products are typically roasted to achieve dark colours, and as a result yield burnt aromas. In addition, roasted barley products provide astringent mouthfeel to beers that cannot be achieved with the addition of roasted pale or green malts. A low temperature/time roasted unmalted barley may contribute novel characteristics when included in the brewing process.

The [GM, 135, 50] sample yielded many aroma similarities to the amber malt sample, a low temperature roasted pale malt product. However, further investigation is required in order to confirm this sample's novelty within its substrate type.

Volatile compound concentrations from Chapter 4 and Chapter 5, in addition to partial QDA data were analysed using PCA, AHC, and Pearson correlation matrices in order to associate previously identified volatile compounds to the detection of aroma attributes in the samples. Compounds exhibiting significant positive correlations on the detection of groups of aroma attributes were identified. The largest group of volatile sulphur compounds that exhibited significant positive

correlations on the detection of aroma attributes were in the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aromas. This was in addition to a number of compounds previously identified as being more abundant in products roasted to high finishing temperatures (Chapter 4).

Regarding the ‘malt loaf’ attribute, 2-(5H)-furanone, 2-furanmethanol, acetic acid, HMF, maltol, and furaneol were identified as having a significant positive correlation on aroma detection in that attribute.

The ‘Horlicks’, ‘digestive biscuit’, and ‘grainy’ aroma attributes were characterised by their significant negative correlation from volatile compound concentrations. This indicates that the lack of thermally generated volatile compounds emphasises the ‘digestive biscuit’, ‘grainy’, and ‘Horlicks’ aromas in roasted products.

This study has highlighted the key aroma qualities of roasted products across a range of low, intermediate, and high finishing temperatures and roasting times, and across three roasting substrate types. Three laboratory roasted products with potentially novel aroma characteristics for their substrate type have been identified, in addition to the tentative association of volatile compound concentrations of previous investigations to the detection of aroma attributes in roasted samples.

With further investigation, the prominence of the identified aroma qualities of the roasted samples when subjected to the brewing process could be determined. Confirmation of the speculated results of this investigation would also require additional investigation. Namely the identification of laboratory roasted samples with potentially novel aroma characteristics, and volatile compounds that are considered to influence the detection of specific aroma attributes.

Chapter 7 - Major Findings and Future Work

7.1 Major Findings

The aim of this research was to enhance the knowledge of thermal flavour generation in roasted malts and barley, and from this, to facilitate control of roasted product flavour. The formation, concentrations and sensory properties of volatile aroma compounds were the focus of this research, both in commercially available and laboratory roasted malt and barley.

This research has delivered improved understanding of the sensory characteristics of roasted products. A range of 45 odour active compounds were identified across six commercially produced roasted malts using GC-O analysis. The likely significance of the identified compounds to the sensory properties of the products was determined by use of AEDA, providing a FD factor for each compound in each roasted product. Chocolate malt contained the highest proportion of highly sensorially influential odour active compounds (with n=49 unidentified, and n=24 identified compounds at FD>1000). The greater number of highly odour active compounds in the chocolate malt product was concluded to be a result of the finishing roasting temperature employed in its manufacture. In chocolate malt, temperatures up to 225 °C are reached, supporting thermal flavour generation reactions favoured by dry roasting conditions (namely late stage Maillard and pyrolysis), while reducing the risk of volatilisation or depletion as a result of subsequent thermal degradation.

The GC-O analysis reported in Chapter 3 provided the basis for the subsequent investigations in this research. From principal component analysis (PCA) of the FD factors of 45 identified compounds across the six samples in Chapter 3, crystal malt samples (caramalt and medium crystal malt) were plotted distinctly from the other samples, emphasising the need to fully evaluate the impacts of roasting substrate on the aroma characteristics of the final product in subsequent work. Of the 45 odour active compounds identified in the GC-O study (Chapter 3), 20 key odour active compounds were then selected to be monitored further over controlled conditions of time, temperature, and starting moisture content. In addition, the results of Chapter 3 also prompted the use of sulphur specific compound identification, due to the number of unidentified highly odour active peaks in the chocolate malt product, particularly. The 20 key odour active compounds were selected to monitor based

upon their known aroma impacts (FD factors from Chapter 3) and their representation of the different thermal flavour generation chemistries by roasting. By the selection of the 20 key odour active compounds, improved knowledge of how process conditions of time, temperature, and product moisture influence significant thermal flavour generation was established. In Chapter 4, a wide range of laboratory roasted products (n=37 'finished' products) were produced from the three major roasting substrates: unmalted barley, pale malt, and green malt. The use of experimental design software enabled the modelling of compound concentrations (n=20 compounds) in each roasted substrate as a function of roasting time and temperature. Individual models provided detail of compound formation and/or depletion in each roasted substrate, the complexity of which suggested multiple routes to formation for many compounds. The potential for particular compounds to take part in subsequent reactions, or to volatilise throughout roasting further complicated the form of the models. Significant interactions between the factors of roasting time and temperature were identified in over 50 % of the models created, across the roasted substrates of unmalted barley (n=12), pale malt (n=10) and green malt (n=12), observing the 20 odour active compounds.

PCA was used to depict a biplot of the 'flavour space', in which the formation of key compounds in each roasted substrate across the range of process conditions could be clearly visualised. Roasted green malt samples yielded the highest concentrations of compounds such as maltol, phenylacetaldehyde, 2-furanmethanol, HMF, and acetic acid, indicating their formation was favoured by the early and intermediate stages of the Maillard reaction and at higher moisture. Whereas higher concentrations of pyrazines, pyrroles, pyridines, 2-methylfuran, 2-pentylfuran, methyl-2-furoate, and 2-acetyl-5-methylfuran were formed in the 'dry roasted' products (pale malt and raw barley) particularly at temperatures exceeding 180 °C.

A selection of seven laboratory roasted samples were analysed further, firstly by volatile sulphur compound (VSC) identification, and subsequently by sensory analysis by means of aroma QDA. Laboratory roasted samples were selected according to their potential to suggest conceivable gaps in the commercial range, in addition to samples that were seemingly novel. The loading of the samples within the PCA 'flavour space' of Chapter 4 was the primary consideration for sample selection, followed by informal tasting of shortlisted samples by the researchers to preliminarily check their sensory properties.

The identification of VSCs in the range of roasted products (as detailed in Chapter 5) was novel: this form of volatile analysis by sulphur specific detection to extensively study roasted products has not yet been published. This method of analysis enabled the identification of odour active compounds that contribute to the aroma characteristics of roasted products through a variety of savoury aromas.

The identification of VSCs in roasted products accounted for many of the odour active peaks detected in Chapter 3 that were unidentifiable by mass spectrometry. 30 VSCs were identified across the analysed roasted products. The variety of VSCs identified in the samples were formed as a result of Maillard chemistry and pyrolysis reactions, originating from sulphur containing amino acids (methionine and cysteine) or from organic forms such as thiamine. Upon identification of the VSCs present in the full range of nine commercial roasted products and seven laboratory roasted products, it was concluded that those roasted to higher temperatures yielded the highest numbers of identified VSCs, and often contained the highest concentrations of these compounds. However, samples subjected to prolonged roasting at high temperatures (particularly in black malt and roasted barley) resulted in reduced concentrations of VSCs relative to chocolate malt. It was suggested that this was a result of subsequent thermal reactions, namely the formation of melanoidins as a product of the final stage of the Maillard reaction.

The effect of the malting process on VSCs in roasted products was also highlighted. Roasted unmalted barley samples contained higher concentrations of more VSCs (including for example, 2-acetyl-2-thiazoline and 2-formyl-5-methylthiophene) in comparison to roasted pale malt samples. It was suggested that the conversion of methionine over the course of malting to more volatile VSCs may account for this difference in VSC contents between the roasted substrates.

The use of sensory analysis by aroma QDA also provided enhanced understanding of the sensory characteristics of roasted products, in addition to the identification of laboratory roasted products with potentially novel aroma characteristics. The results of this study in Chapter 6 also associated the data from previous instrumental analyses namely by the use of Pearson correlation matrices to consider which volatile compounds influenced the detection of aroma attributes in the roasted products. PCA and agglomerative hierarchical clustering (AHC) analysis identified three major clusters of samples with associated aroma attributes, separated by their low (<150 °C), intermediate (150-200 °C), or high (>200 °C) finishing temperatures. Most

notably, samples roasted under ‘dry roasting’ conditions (exceeding 200 °C in this particular study but extending to 180 °C when considering the results of Chapter 4) scored significantly higher in the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aroma attributes than the other samples. Upon association of volatile concentrations to detection of aroma attributes, 11 VSCs were identified as having a significant positive correlation, along with four non-sulphur containing compounds (2-acetyl-5-methylfuran, 2-formylpyrrole, 2-n-pentylpyridine, and 2,3-dimethylpyrazine). It was concluded that the ‘dry roasting’ conditions of the samples exhibiting high scores in the ‘burnt’, ‘smoky’, ‘coffee’, and ‘dark chocolate’ aromas encourage the formation of VSCs in these samples, in addition to late stage Maillard and pyrolysis products. This is in contrast to the roasting conditions and resultant volatile compound composition of roasted green malts, for example. Following statistical analysis of the mean aroma scores averaged according to substrate type, the ‘malt loaf’ characteristic was identified as being significantly more pronounced in the roasted green malt samples than the other two roasted substrate types. This attribute was characterised by higher concentrations of 2-(5H)-furanone, 2-furanmethanol, acetic acid, HMF, maltol, and furaneol (exhibiting significant positive correlations on detection of the ‘malt loaf’ aroma).

Regarding the low temperature roasted samples analysed by aroma QDA, the lack of thermal flavour generation as a result of the low finishing temperatures resulted in their high scores for the ‘Horlicks’, ‘digestive biscuit’, and ‘grainy’ aroma attributes. Despite this, low and intermediate temperature roasted samples were identified as possessing potentially novel aroma qualities within their relevant substrate type. These samples were: [PM, 165, 20], [RB, 135, 30], and [GM, 135, 50]. However, it was noted that further investigation is required in order to confirm the novelty of these samples. The high temperature roasted samples yielded few differences from their commercially available equivalents.

Current commercial roasting operations rely on colour development as an indicator of process control, which inevitably results in batch-to-batch variation. Increased knowledge of thermal flavour generation during roasting, particularly by modelling compound formation, highlights the requirement for accuracy in manufacturing.

The current research validates the considerable variety of roasted products that are available. Commercial roasters are justified in their production of the vast range of roasted products, as each one yields slightly different aroma characteristics. When

used in combination with a variety of roasted malts in a brew, specific sensory qualities can be enhanced. The use of PCA, particularly in Chapter 4 illustrates this, as few roasted product types are identical in their odour active volatile contents and concentrations. In addition, despite similarities in aroma attributes between some roasted products, as discussed in Chapter 6, each makes a unique contribution, which is predicted to translate to a final product.

In addition to the industrial application of this research, contributions to flavour analysis of roasted malts and barley have also been made. The use of PFPD (Pulsed Flame Photometric Detection) in the identification of VSCs has contributed to the catalogue of identified compounds that are formed as a result of thermal flavour generation, particularly via the Maillard reaction involving sulphur containing amino acids or thiamine. In addition, the extensive 3-dimensional modelling of compound formation as a function of time and temperature contributes to our understanding of thermal flavour generation, particularly in roasted malt and barley.

7.2 Future Work

The following suggestions to future work are intended to build upon and improve the current research, and to provide suggestions for further detailed analysis. In order to enable the replication of the following suggestions for future work by wider industry parties, studies should include standard industry compositional data of barley and malt, namely total nitrogen content and moisture content. This could also include further detailed compositional analyses, depending on the composition detail of interest (for example, free amino nitrogen, soluble nitrogen content etc.).

7.2.1 Further Extensive Sensory Analysis

To add to the aroma analysis of Chapter 6, full flavour analysis by QDA (as opposed to aroma only) would be beneficial. This would develop the understanding of the effects of the known concentrations of key odour active compounds on the overall sensory qualities of the laboratory roasted malts.

Food grade production methods would be essential to carry out this form of analysis. The panel would assess the flavour of the roasted products as a mixture of malt and water, as in the aroma assessment. This would include additional lexicon development for flavour and mouthfeel. The inclusion of a greater sample set of

laboratory roasted samples would provide a comprehensive understanding of the effects of roasting conditions and substrate on the sensory qualities of the products. The mouthfeel, particularly astringency, of the range of laboratory roasted unmalted barley samples (roasted from 100 °C to 230 °C) would also be of interest. This is because commercially roasted barley products are typified by their astringency. Further to additional sensory analysis, the inevitable effects of wort production and fermentation on the sensory qualities imparted to beers by the laboratory roasted sample set would also be of interest. The laboratory roasted samples would be included in high proportions of the grist to exaggerate their sensory contributions for ease of analysis.

By including comprehensive analysis of the laboratory roasted range, the identification of conditions by which novel products are produced could be identified.

7.2.2 From Laboratory to Commercial Scale Production

The conversion of laboratory roasting to a commercial scale would be expected in order to successfully relate the current research to industrial production. The translation of roasting times and temperatures are known to differ greatly, considering the batch size difference from 100 g to 1-2 tonnes.

The rate of heat transfer in large scale production would be a key consideration. In the laboratory scale roasting vessel, the roasting kernels reached the target temperature quickly due to the small batch size, highly perforated mesh drum, and fan system within the adapted GC oven. In a larger scale, the volume of kernels within the roasting vessel lengthens the roasting process considerably. Therefore, translational modelling would be required to determine how findings presented in the current research under precisely controlled conditions correspond to commercial scale production.

7.2.3 The Cooling Process

In current roasting operations, the cooling process is lengthy, and can result in batch-to-batch variation. When cooled slowly, thermal flavour generation reactions can continue to take place in the hot kernels after they are discharged from the roasting

vessel. Due to the scale of industrial roasting, the large volume of kernels in the cooling bed lengthens the cooling process.

In laboratory scale production in the current research, liquid nitrogen was used to immediately freeze the kernels by pouring directly over the hot kernels to submerge them. Use of liquid nitrogen to this effect on a large scale would not be suitable. However, the use of more rapid cooling techniques and the influence of this step on product flavour is worthy of further investigation. Avoiding condensation, resulting in unwanted moisture, should be considered if this method is explored. Transfer of the hot kernels to a secondary rotating cooling vessel could potentially reduce cooling time. Innovation in this element of commercial roasting is necessary to improve consistency in roasted products by additional process control.

7.2.4 Use in Industry: A Flavour Standard

Translating the current research to direct industrial application would be beneficial. The increased understanding of thermal flavour generation in roasted malts would ultimately result in better homogeneity between and within batches, in addition to a potentially greater range of novel products.

Development of a method of creating an industrial standard for each roasted malt or roasted barley category would improve international standards of roasted products. As a result, brewers' confidence in the sensory contribution of roasted products would be improved.

Such a method of standardising roasted malts would involve the compilation of multiple sensory and instrumental analyses. Aroma, flavour and mouthfeel QDA would be included of the products, in addition to that of beers containing high grist proportions of the malts in question. Identification of key odour active marker compounds for each product category would be carried out by the methods in the current research (i.e. GC-O-MS, SPME-GC-PFPD). For example, products recognised as 'chocolate malts' would be required to contain a specific range of marker compounds, each within an individual concentration range (including, for example, 2-ethoxythiazole: 5-6.5 μg/g (Chapter 5). This specification would clearly identify products within the 'chocolate malt' category, and the expected contribution those products would make when included in a brew.

Although presenting major costly analyses, when combined with advances in production technologies, this method of standardisation would help to reduce batch-to-batch variation and increase confidence in categorising roasting products and their expected sensory characteristics.

To conclude, the research reported in this thesis has provided detailed understanding of thermal flavour generation in roasted malt and barley products, through the identification and examination of key odour active compounds across a range of roasting parameters. This research will enable commercial roasters to produce and market their products to brewers with confidence, based upon improved knowledge of their aroma characteristics. Furthermore, this research has highlighted opportunities for further innovation and improvement in roasting. As the roasting process is energy intensive, improved process control using knowledge from the present research may present opportunities to reduce primary energy consumption during processing.

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Chapter 8 - Appendices

8.1 Chapter 4 Appendices

Table 8.1: Model fit data for the predictive models for compound concentration (compounds 1-10).

Roasting Substrate	Model Component	2-methylfuran	Pentanal	Hexanal	1-methylpyrrole	Pyrazine	2-pentylfuran	2,3-dimethylpyrazine	Furfural	Acetic Acid	2-n-pentylpyridine
Raw Barley	Model	Cubic	Quadratic	Quadratic	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Cubic
	Model p Value	<0.0001±	0.0017±	0.0012^	0.0003±	<0.0001^	<0.0001±	<0.0001±	<0.0001^	<0.0001±	<0.0001±
	Model R^2	**0.991**	**0.581**	**0.475**	**0.656**	**0.992**	**0.968**	**0.973**	**0.872**	**0.947**	**0.940**
	A p Value	0.2671	0.0368	0.7562	0.0154	0.2383	0.3116	<0.0001	<0.0001	<0.0001	0.8214
	B p Value	0.3939	0.7774	-	0.0237	0.5233	0.1641	<0.0001	-	0.3312	0.9817
	AB p Value	<0.0001	-	-	0.0038	<0.0001	<0.0001	<0.0001	-	0.0377	<0.0001
	A^2 p Value	<0.0001	0.0002	0.0003	0.0692	<0.0001	0.0043	<0.0001	0.0002	0.1315	<0.0001
	B^2 p Value	-	0.0180	-	-	0.0155	-	-	-	0.4463	-
	A^2B p Value	<0.0001	-	-	-	<0.0001	0.0007	-	-	0.0007	0.0013
	AB^2 p Value	-	-	-	-	0.0017	-	-	-	-	-
	A^3 p Value	<0.0001	-	-	-	0.0012	0.0029	-	-	<0.0001	0.0365
	B^3 p Value	-	-	-	-	-	-	-	-	0.0378	-
Green	Model	Mean	Linear	Cubic	2Fl	Quadratic	2Fl	Quadratic	Linear	Quadratic	NF
	Model p Value	-	0.0008±	<0.0001±	<0.0001±	<0.0001±	<0.0001±	0.0124±	0.004±	0.0002±	-

	Model R^2	**0**	**0.526**	**0.851**	**0.778**	**0.934**	**0.784**	**0.445**	**0.346**	**0.714**	**-**
	A p Value	-	0.0069	0.0036	<0.0001	0.2748	0.0001	0.2124	-	0.0053	-
	B p Value	-	0.0016	<0.0001	0.0002	<0.0001	<0.0001	0.0260	0.0040	0.2869	-
	AB p Value	-	-	0.0684	0.0012	<0.0001	0.0341	-	-	<0.0001	-
	A^2 p Value	-	-	0.4294	-	0.0284	-	0.0251	-	-	-
	B^2 p Value	-	-	-	-	0.0039	-	-	-	-	-
	A^2B p Value	-	-	-	-	-	-	-	-	-	-
	AB^2 p Value	-	-	-	-	-	-	-	-	-	-
	A^3 p Value	-	-	0.0136	-	-	-	-	-	-	-
	B^3 p Value	-	-	-	-	-	-	-	-	-	-
Pale Malt	Model	Cubic	Linear	Quadratic	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Quadratic	Cubic
	Model p Value	<0.0001±	0.0322±	<0.0001±	0.006^	<0.0001^	<0.0001±	0.0008±	<0.0001^	0.0002±	<0.0001^
	Model R^2	**0.979**	**0.210**	**0.873**	**0.411**	**0.963**	**0.991**	**0.598**	**0.917**	**0.583**	**0.982**
	A p Value	0.0029	0.0322	<0.0001	0.0185	0.6930	<0.0001	0.0001	<0.0001	0.0019	0.0231
	B p Value	0.0071	-	0.0046	-	0.2566	0.0040	-	-	-	0.6045
	AB p Value	0.0010	-	0.0064	-	<0.0001	<0.0001	-	-	-	<0.0001
	A^2 p Value	<0.0001	-	0.0337	0.0218	<0.0001	<0.0001	-	0.0003	0.0020	<0.0001
	B^2 p Value	0.0112	-	0.0810	-	-	0.0140	-	-	-	-
	A^2B p Value	-	-	-	-	0.0006	<0.0001	-	-	-	<0.0001
	AB^2 p Value	0.0010	-	-	-	-	0.0066	-	-	-	-
	A^3 p Value	0.0009	-	-	-	0.0148	-	-	-	-	0.0076
	B^3 p Value	-	-	-	-	-	-	-	-	-	-

± Non-significant lack of fit for model.
^ Significant lack of fit for model.

Table 8.2: Model fit data for the predictive models for compound concentration (compounds 11-20).

Roasting Substrate	Model Component	Methyl-2-furoate	5-methylfurfural	2-acetyl-5-methylfuran	Phenylacetaldehyde	2-furanmethanol	2-(5H)-furanone	Maltol	Furaneol	2-formylpyrrole	Hydroxymethylfurfural
Raw Barley	Model	Cubic	Quadratic	Cubic	Cubic	Cubic	Quadratic	Cubic	Cubic	Quadratic	Cubic
	Model p Value	<0.0001±	<0.0001^	<0.0001^	<0.0001^	<0.0001^	<0.0001±	<0.0001±	<0.0001±	<0.0001^	>0.05^
	Model R^2	**0.986**	**0.840**	**0.986**	**0.966**	**0.751**	**0.940**	**0.985**	**0.786**	**0.778**	**0.383**
	A p Value	0.2190	<0.0001	0.4927	<0.0001	0.0002	<0.0001	0.8329	<0.0001	<0.0001	0.0164
	B p Value	0.5526	-	0.9021	<0.0001	0.0785	0.0465	0.8954	-	0.4496	-
	AB p Value	<0.0001	-	<0.0001	<0.0001	0.0193	-	<0.0001	-	-	-
	A^2 p Value	<0.0001	0.0006	<0.0001	<0.0001	0.9920	<0.0001	<0.0001	0.0221	0.0036	-
	B^2 p Value	-	-	-	-	-	0.0342	0.3208	-	-	
	A^2B p Value	<0.0001	-	<0.0001	<0.0001	-	-	<0.0001	-	-	-
	AB^2 p Value	-	-	-	-	-	-	0.0419	-	-	-
	A^3 p Value	<0.0001	-	<0.0001	<0.0001	0.0071	-	0.0044	0.0041	-	0.0299
	B^3 p Value	-	-	-	-	-	-	-	-	-	-
Green Malt	Model	Linear	Linear	Quadratic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Linear	Quadratic
	Model p Value	>0.05±	0.0003±	<0.0001±	<0.0001±	<0.0001±	0.0005±	<0.0001±	<0.0001±	<0.0001±	0.0003±
	Model R^2	**0.127**	**0.568**	**0.856**	**0.969**	**0.926**	**0.674**	**0.774**	**0.883**	**0.747**	**0.695**
	A p Value	-	0.0624	0.0035	<0.0001	0.2945	0.0260	0.1520	0.6015	0.0003	0.9439
	B p Value	-	0.0001	<0.0001	<0.0001	0.4678	0.1145	0.0021	0.0681	<0.0001	0.0001
	AB p Value	-	-	0.0034	0.0013	<0.0001	0.0001	<0.0001	<0.0001	-	0.0274

	A^2 p Value	-	-	-	0.0018	0.5713	-	0.0199	0.8802	-	-
	B^2 p Value	-	-	-	0.0646	0.0061	-	-	0.0440	-	0.0379
	A^2B p Value	-	-	-	-	-	-	-	-	-	-
	AB^2 p Value	-	-	-	0.0030	<0.0001	-	-	0.0013	-	-
	A^3 p Value	-	-	-	-	0.0107	-	-	0.0494	-	-
	B^3 p Value	-	-	-	-	-	-	-	-	-	-
Pale Malt	Model	Cubic	Cubic	Cubic	Cubic	Quadratic	Quadratic	Cubic	Quadratic	Quadratic	Cubic
	Model p Value	<0.0001^	<0.0001^	<0.0001±	<0.0001±	0.0044±	0.0002±	<0.0001±	<0.0001±	<0.0001±	0.0027^
	Model R^2	**0.959**	**0.868**	**0.949**	**0.928**	**0.436**	**0.586**	**0.862**	**0.629**	**0.892**	**0.536**
	A p Value	0.0150	<0.0001	<0.0001	<0.0001	0.3153	0.0014	<0.0001	0.0682	<0.0001	0.0926
	B p Value	0.8615	0.0442	0.9135	0.0030	-	-	0.9895	-	0.2048	-
	AB p Value	0.0009	-	0.0001	0.3395	-	-	0.0015	-	0.0363	-
	A^2 p Value	<0.0001	0.1520	<0.0001	<0.0001	0.0017	0.0024	0.5946	<0.0001	0.0009	0.0012
	B^2 p Value	0.1413	0.5607	-	-	-	-	-	-	0.0379	-
	A^2B p Value	0.0258	-	0.0140	0.0056	-	-	0.0314	-	-	-
	AB^2 p Value	0.0059	-	-	-	-	-	-	-	-	-
	A^3 p Value	0.0265	0.0080	-	0.0111	-	-	-	-	-	0.0355
	B^3 p Value	-	0.0386	-	-	-	-	-	-	-	-

± Non-significant lack of fit for model.
^ Significant lack of fit for model.

8.2 Chapter 5 Appendices

8.2.1 Commercially Roasted Samples

Figure 8.1: PFPD chromatograph of a sample of black malt from Boortmalt.

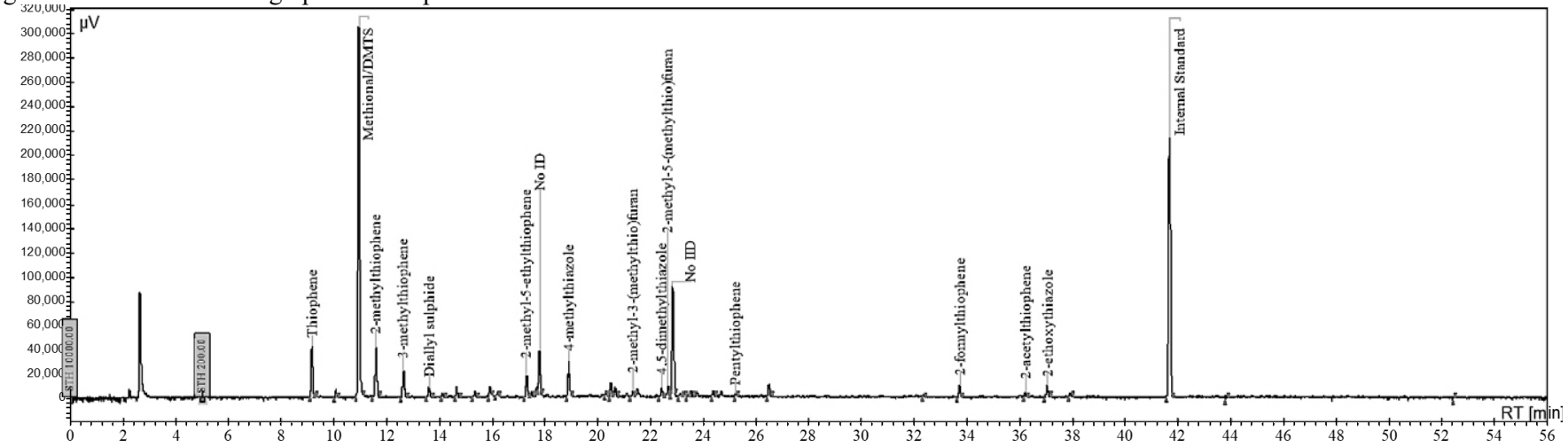


Figure 8.2: PFPD chromatograph of a sample of chocolate malt form Boortmalt.

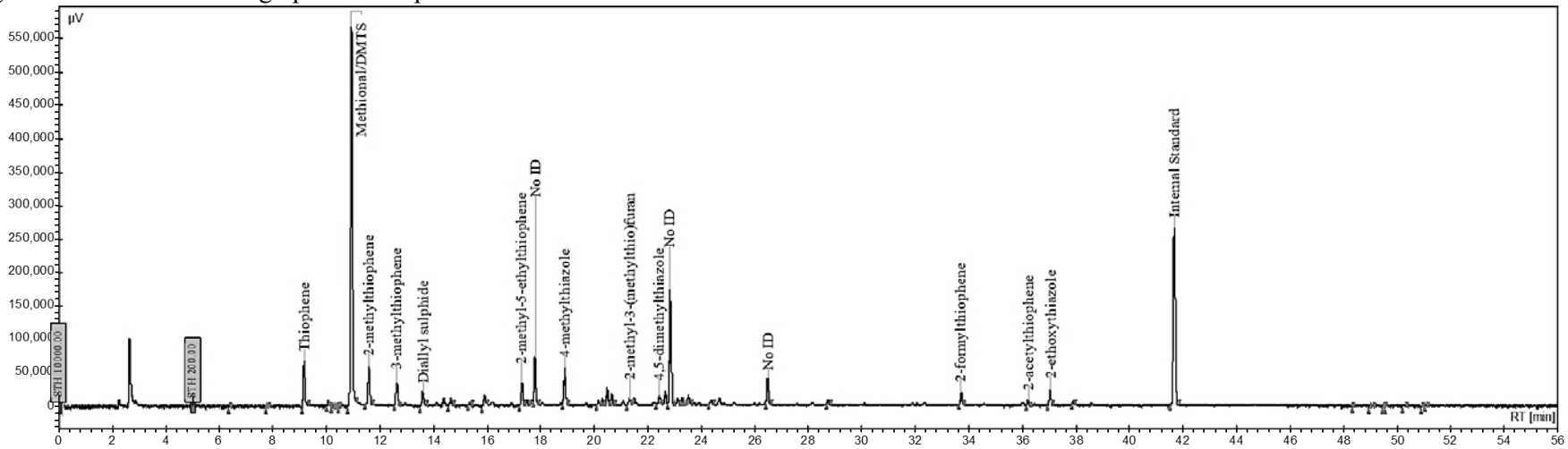


Figure 8.3: PFPD chromatograph of a sample of dark crystal malt from Boortmalt.

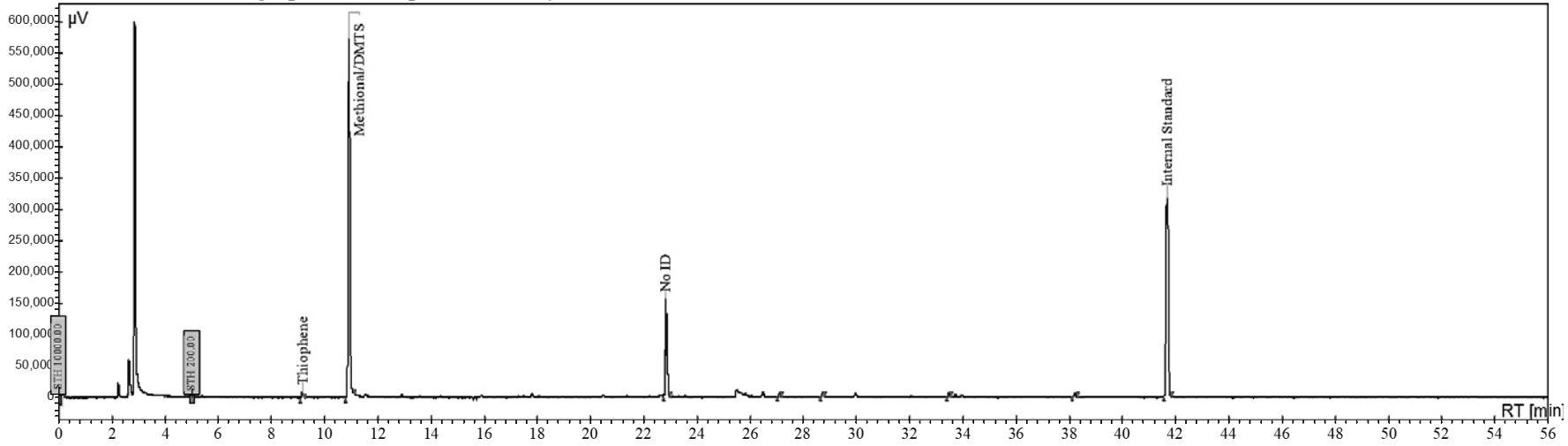


Figure 8.4: PFPD chromatograph of a sample of light crystal malt from Boortmalt.

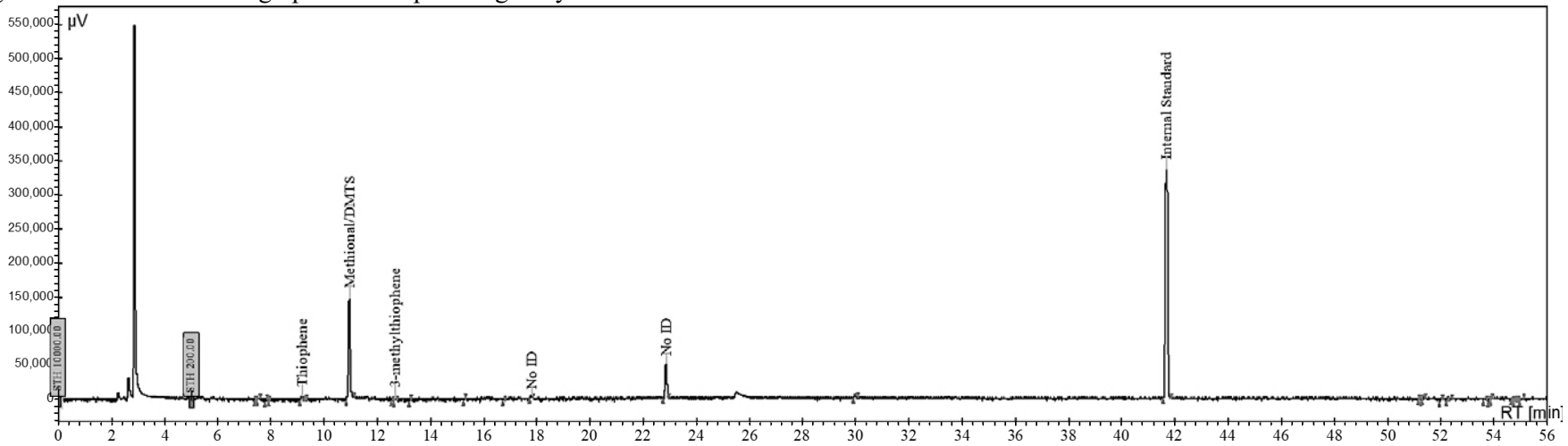


Figure 8.5: PFPD chromatograph of a sample of caramalt from Boortmalt.

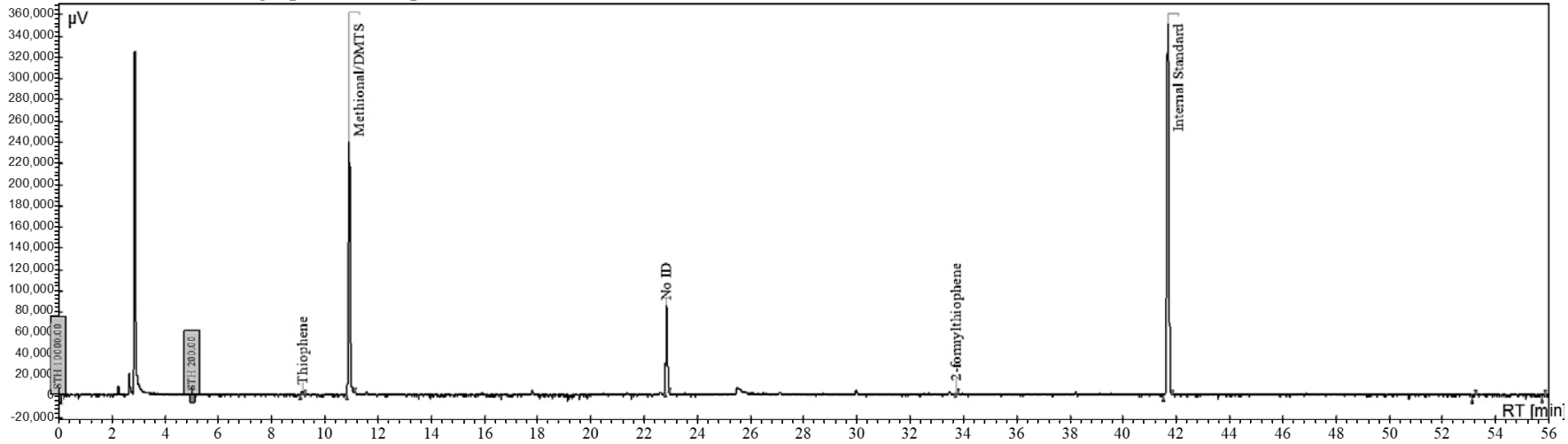
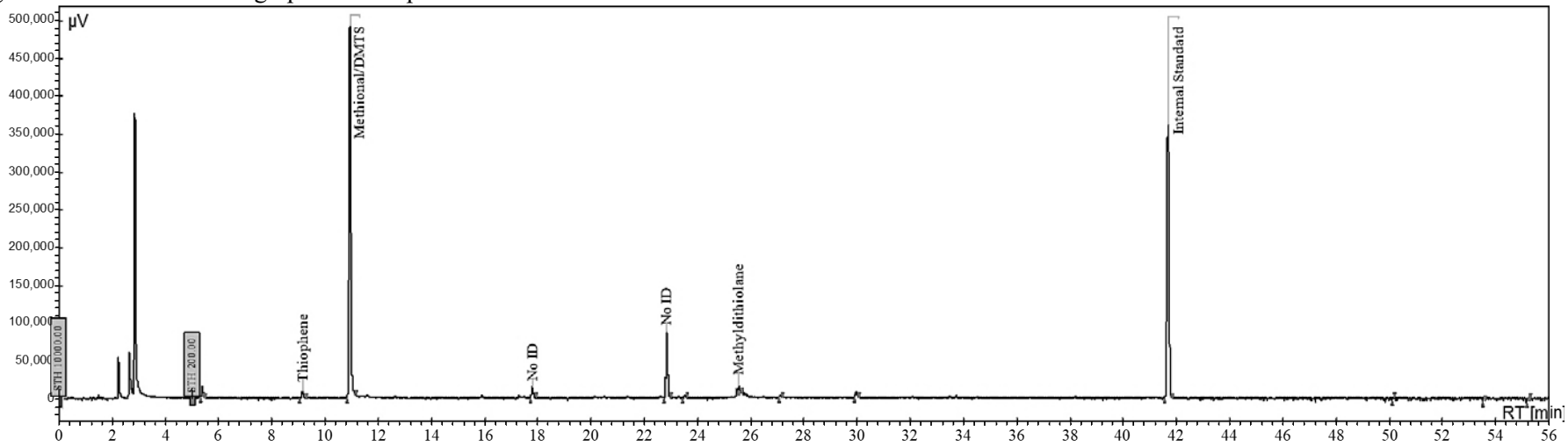


Figure 8.6: PFPD chromatograph of a sample of amber malt from Boortmalt.



8.2.2 Laboratory Roasted Samples

Figure 8.7: PFPD chromatograph of a sample of laboratory roasted barley (135 °C for 30 min).

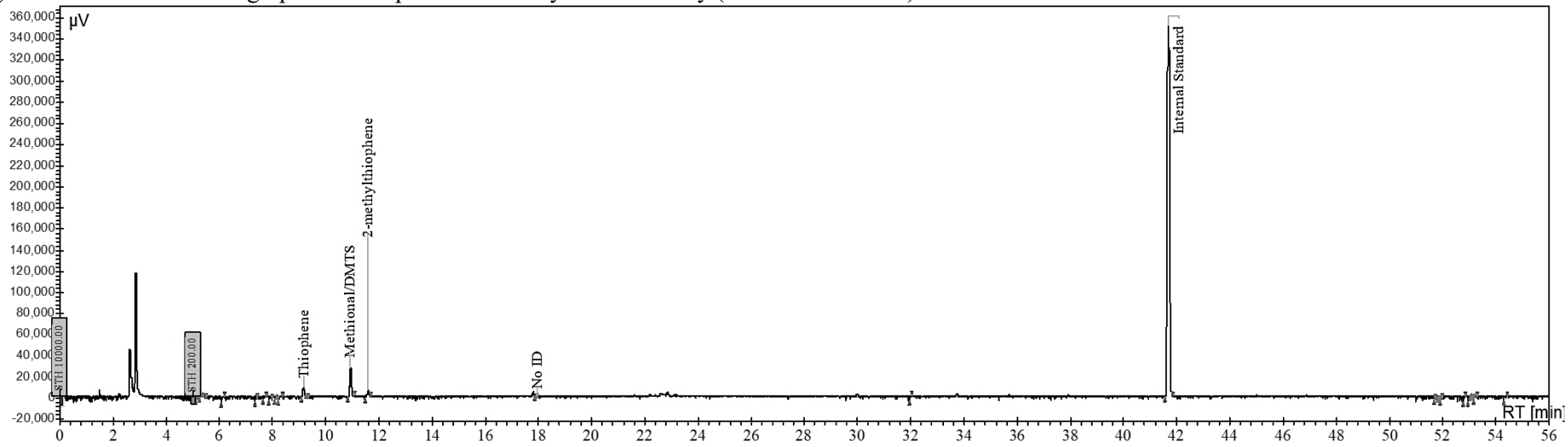


Figure 8.8: PFPD chromatograph of a sample of laboratory roasted pale malt (165 °C for 20 min).

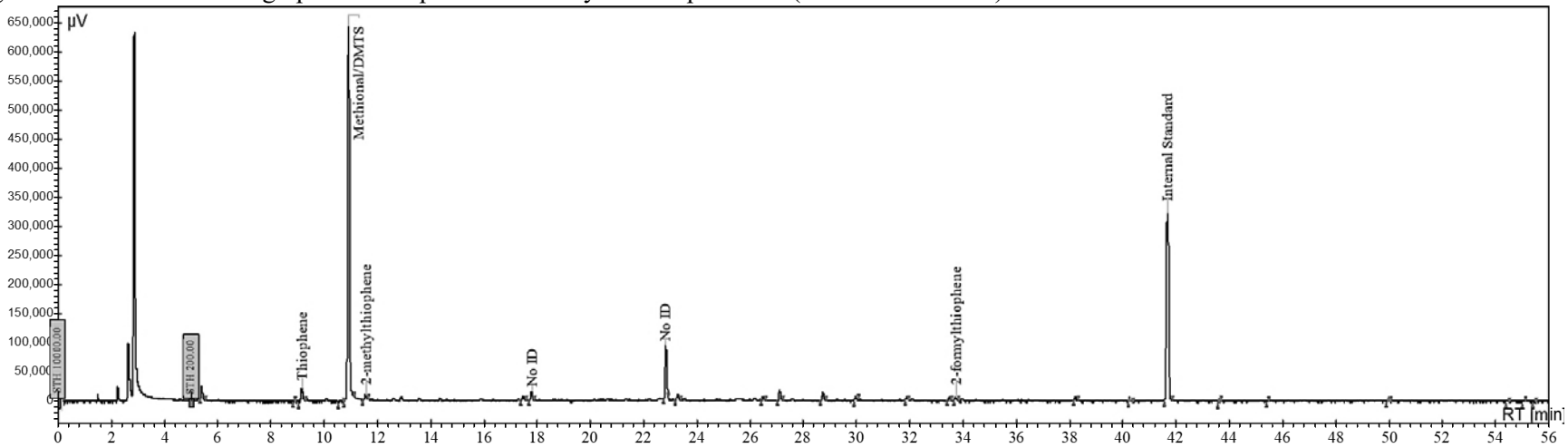


Figure 8.9: PFPD chromatograph of a sample of laboratory roasted green malt (165 °C for 35 min).

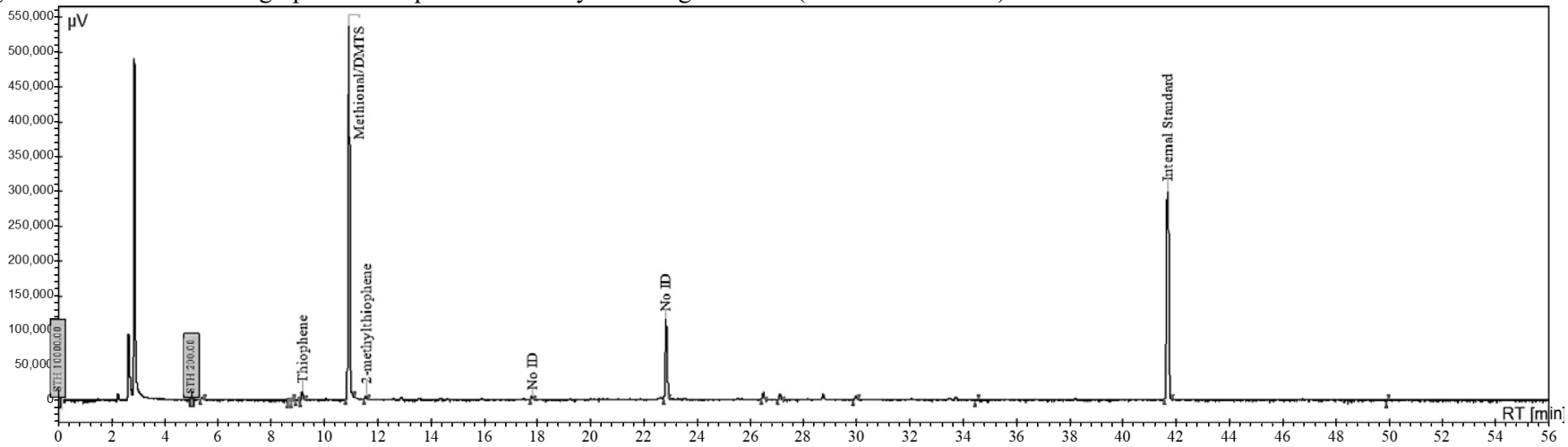
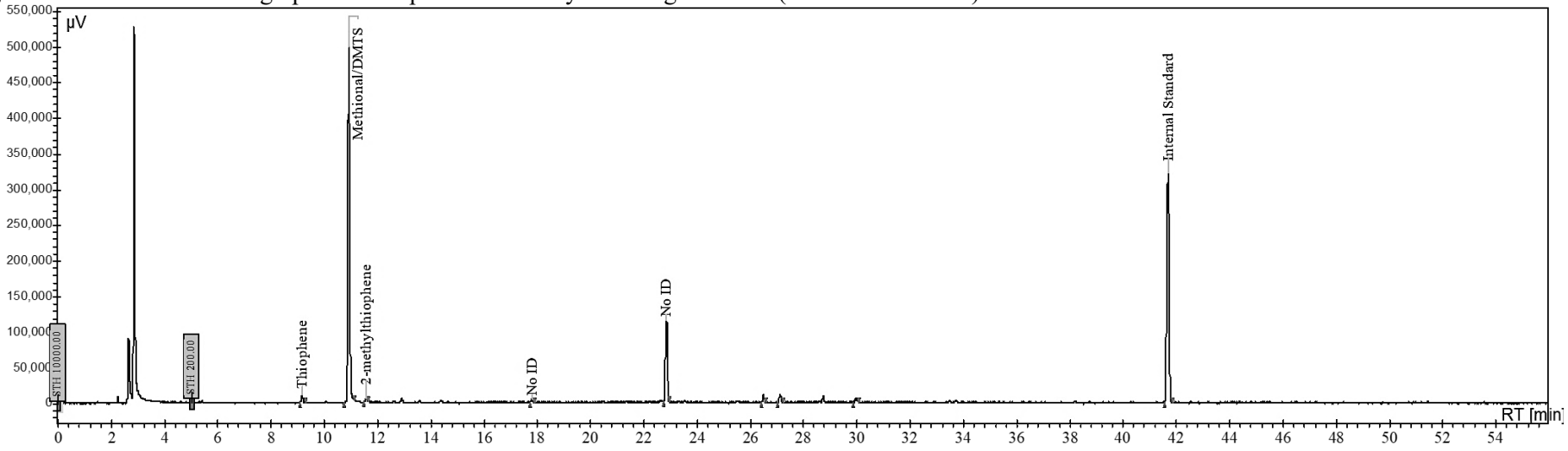


Figure 8.10: PFPD chromatograph of a sample of laboratory roasted green malt (158 °C for 43 min).



8.3 Chapter 6 Appendices

Figure 8.11: Ethical approval for the use of human participants in the assessment of the aroma of roasted products.

University of Nottingham
UK | CHINA | MALAYSIA

Faculty of Medicine & Health Sciences
Research Ethics Committee
Faculty Hub
Room E41, E Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH
Email: FMHS-ResearchEthics@nottingham.ac.uk

30 July 2019

Hebe Parr
PhD Student
c/o Professor David Cook
Professor of Brewing Science
Room C10, Bioenergy and Brewing Science
School of Biosciences
University of Nottingham
Sutton Bonington Campus
Leicestershire, LE12 5RD

Dear Ms Parr

Ethics Reference No: 367-1907 – **please always quote**	
Study Title: Sensory panel evaluation of aroma of lab roasted and commercial roasted barley malt.	
Chief Investigator/Supervisor: Professor David Cook, Professor of Brewing Science, Bioenergy and Brewing Science, School of Biosciences.	
Lead Investigators/student: Hebe Parr, PhD student, Brewing Sciences	
Other Key investigators: Dr Qian Yang, Assistant Professor in Sensory and Consumer Science, Dr Martha Skinner, Sensory Science Centre Manager, Sensory Science Department, School of Biosciences.	
Proposed Start Date: 01/08/2019	**Proposed End Date:** 01/02/2020

Thank you for submitting the above application and the following documents were received:

- FMHS REC Application form and supporting documents version 1.0: 09/07/2019.

These have been reviewed and are satisfactory and the study has been given a favourable opinion.

A favourable opinion has been given on the understanding that:

1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).
2. The Chair is informed of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Figure 8.12: Interaction between sample and mean aroma score for the ‘smoky’ attribute showing scores from each panel member.

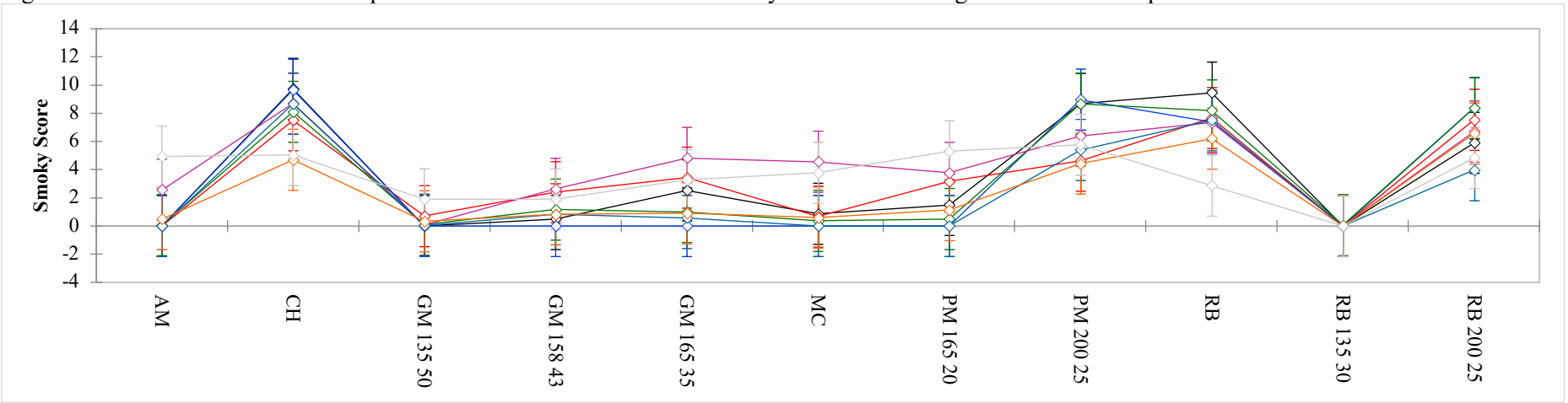


Figure 8.13: Interaction between sample and mean aroma score for the ‘coffee’ attribute showing scores from each panel member.

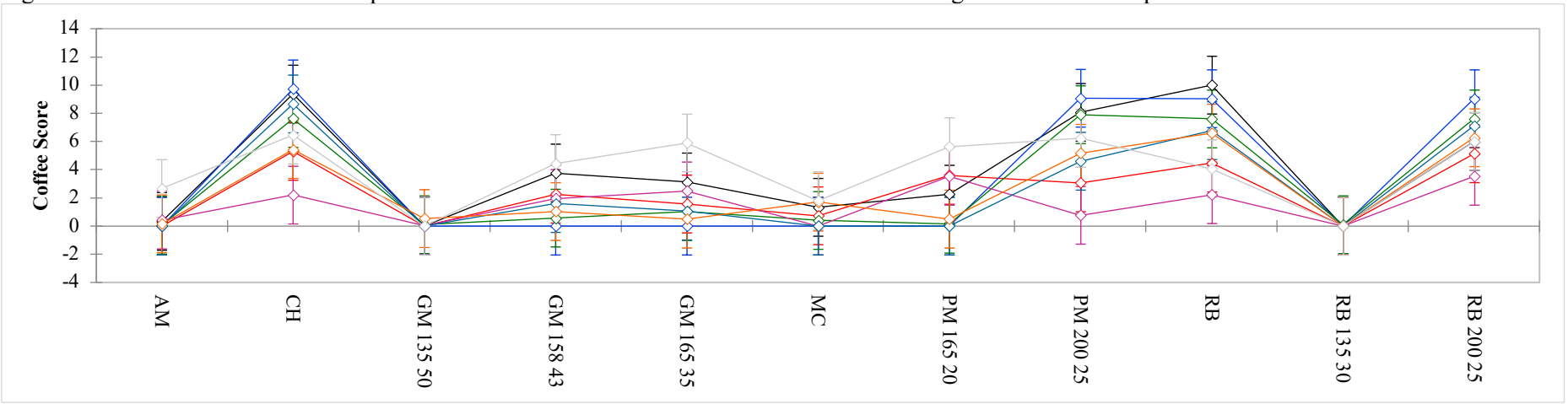


Figure 8.14: Interaction between sample and mean aroma score for the ‘malt loaf’ attribute showing scores from each panel member.

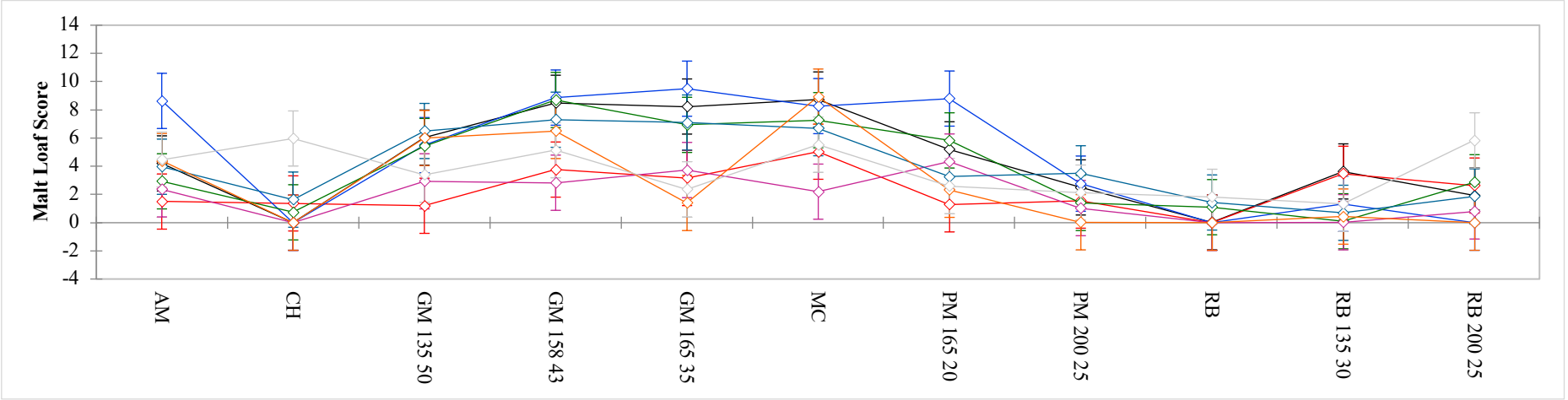


Figure 8.15: Interaction between sample and mean aroma score for the ‘black treacle’ attribute showing scores from each panel member.

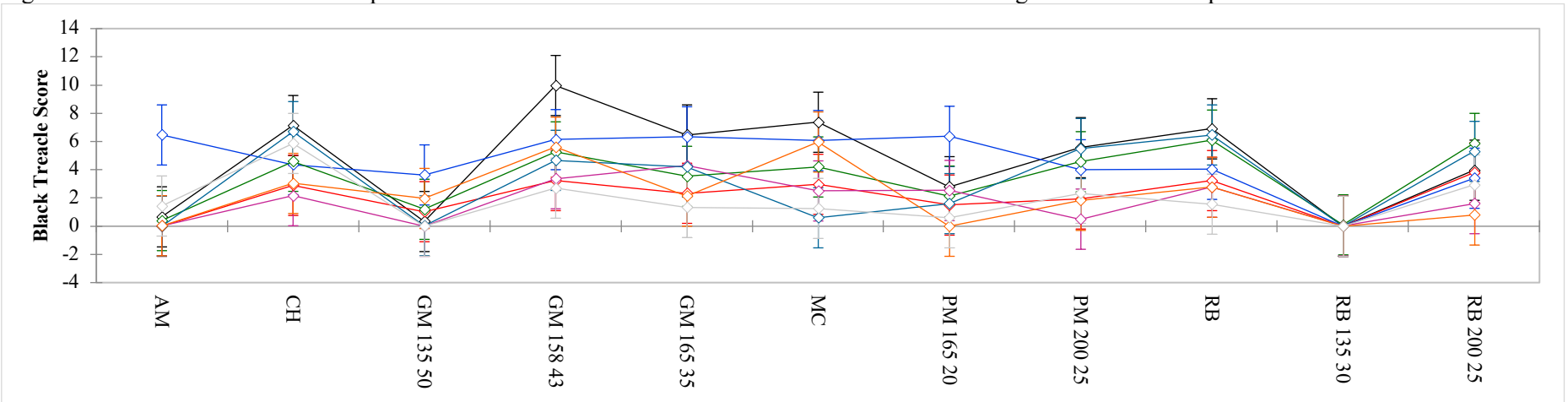


Figure 8.16: Interaction between sample and mean aroma score for the ‘dark chocolate’ attribute showing scores from each panel member.

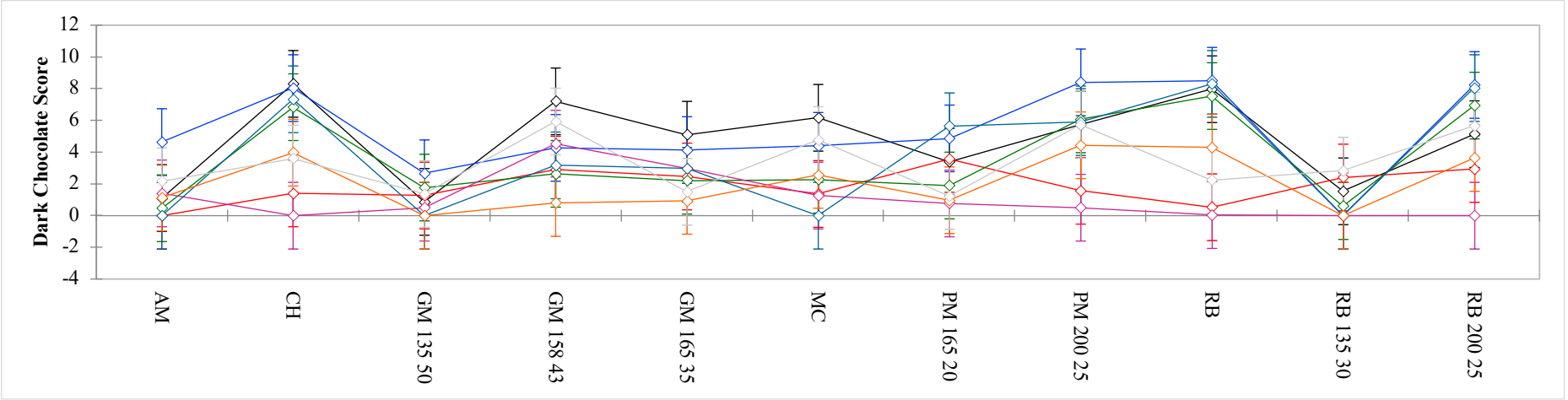


Figure 8.17: Interaction between sample and mean aroma score for the ‘potato’ attribute showing scores from each panel member.

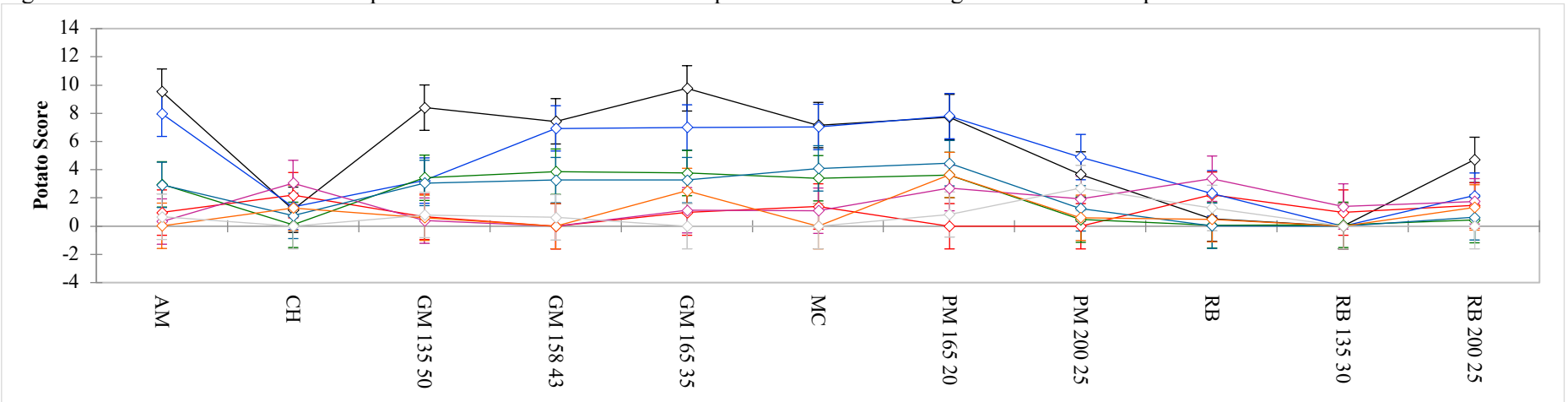


Figure 8.18: Interaction between sample and mean aroma score for the ‘Horlicks’ attribute showing scores from each panel member.

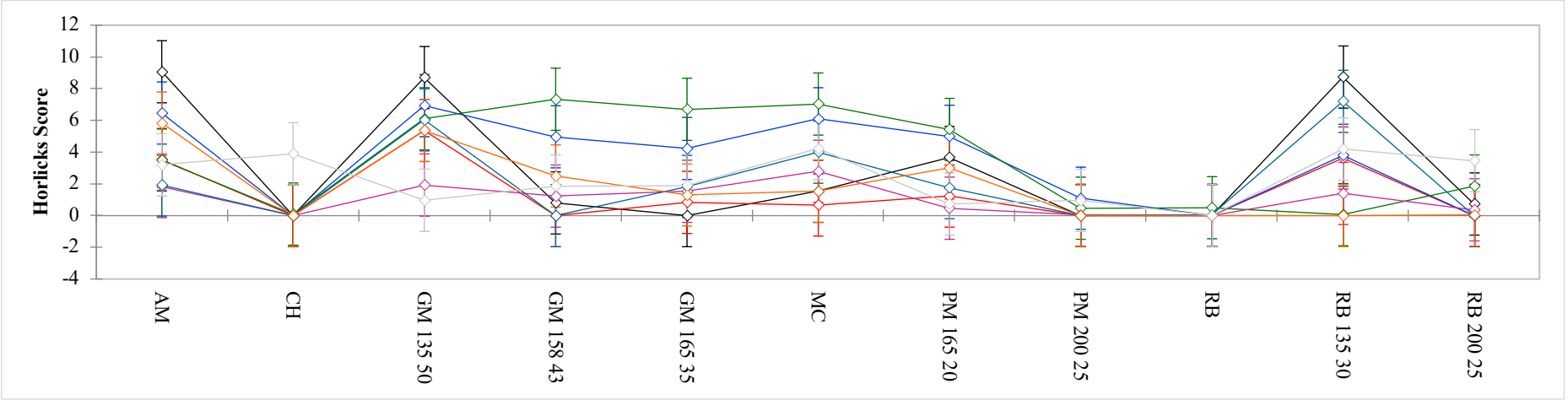


Figure 8.19: Interaction between sample and mean aroma score for the ‘digestive biscuit’ attribute showing scores from each panel member.

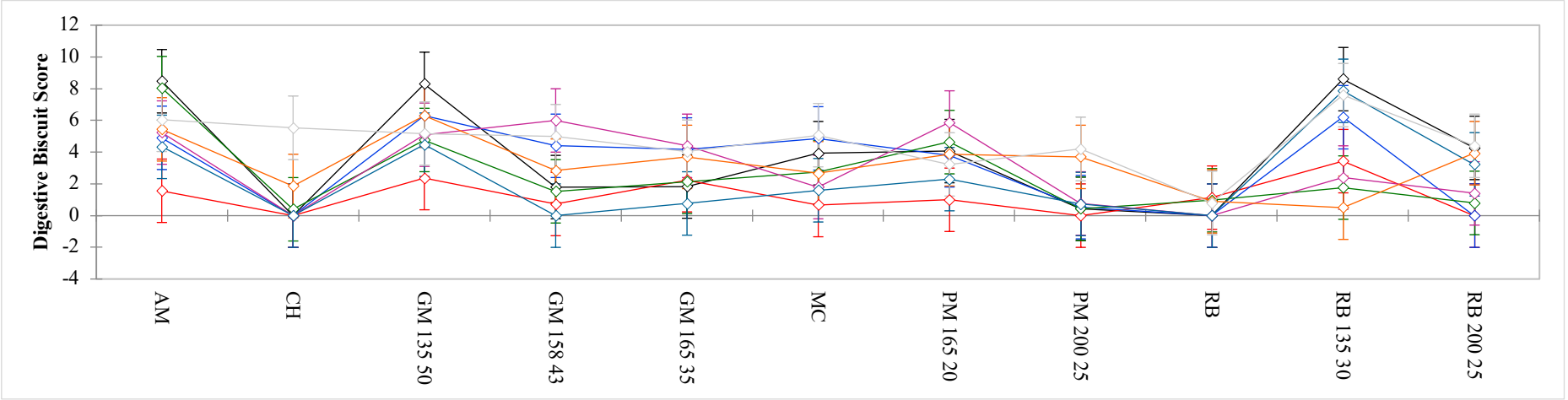


Figure 8.20: Interaction between sample and mean aroma score for the ‘grainy’ attribute showing scores from each panel member.

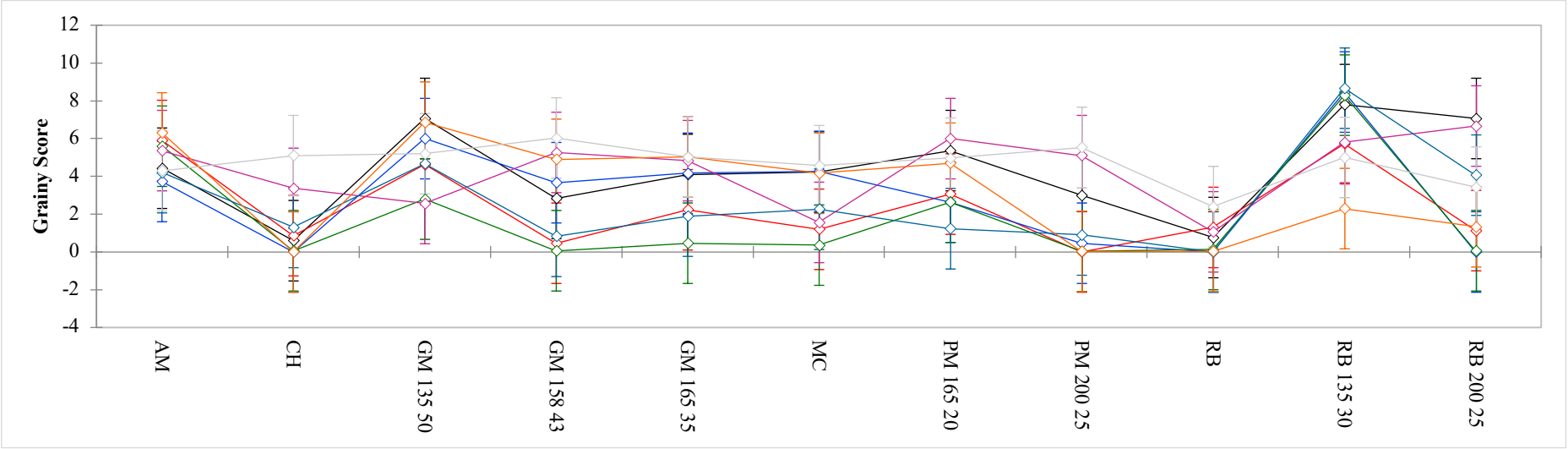
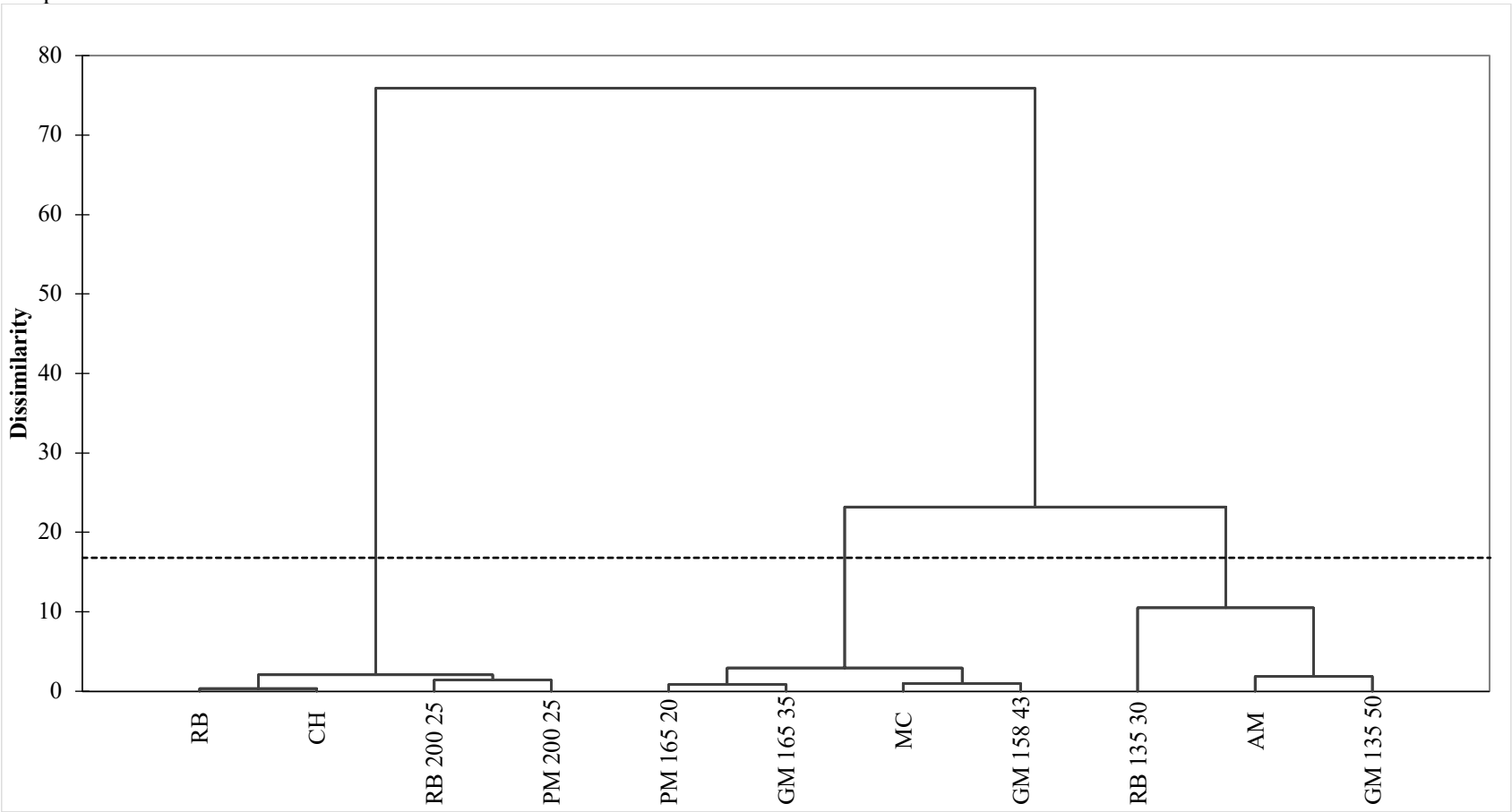


Table 8.3: Aroma attribute score means for roasted samples.

Roasted Sample[1]	Attribute											
	Burnt	**Smoky**	**Coffee**	**Malt Loaf**	**Black Treacle**	**Tea/ Leaves**	**Dark Chocolate**	**Potato**	**Marmite**	**Horlicks**	**Digestive Biscuit**	**Grainy**
AM	1.221 ^{cd}	1.008 ^{bc}	0.458 ^{bc}	4.054 ^{b}	1.129 ^{de}	2.283 ^{abcd}	1.363 ^{cde}	3.175 ^{ab}	3.888 ^{a}	4.421 ^{ab}	5.500 ^{a}	4.979 ^{ab}
CH	7.367 ^{a}	7.763 ^{a}	6.850 ^{a}	1.213 ^{c}	4.596 ^{ab}	0.954 ^{d}	4.933 ^{a}	1.242 ^{ef}	0.879 ^{ef}	0.500 ^{e}	0.975 ^{ef}	1.413 ^{ef}
GM 135 50	0.446 ^{d}	0.392 ^{bc}	0.083 ^{c}	4.625 ^{b}	1.025 ^{de}	3.083 ^{ab}	1.054 ^{de}	2.575 ^{abcd}	2.088 ^{bcde}	5.175 ^{a}	5.346 ^{a}	4.975 ^{ab}
GM 158 43	2.513 ^{bc}	1.283 ^{bc}	1.954 ^{b}	6.450 ^{a}	5.117 ^{a}	4.000 ^{a}	3.929 ^{ab}	2.767 ^{abc}	2.296 ^{bcd}	2.338 ^{cd}	2.788 ^{cd}	3.008 ^{cde}
GM 165 35	3.200 ^{b}	2.067 ^{b}	1.963 ^{b}	5.304 ^{ab}	3.829 ^{abc}	3.279 ^{ab}	2.783 ^{bcd}	3.550 ^{a}	3.054 ^{ab}	2.292 ^{cd}	2.908 ^{cd}	3.471 ^{bcd}
MC	2.721 ^{bc}	1.354 ^{bc}	0.746 ^{bc}	6.583 ^{a}	3.867 ^{abc}	3.521 ^{ab}	2.850 ^{bc}	3.025 ^{ab}	2.500 ^{bcd}	3.488 ^{bc}	2.917 ^{cd}	2.829 ^{cde}
PM 165 20	3.067 ^{bc}	1.921 ^{b}	1.958 ^{b}	4.208 ^{b}	2.192 ^{cd}	3.517 ^{ab}	2.792 ^{bc}	3.850 ^{a}	2.908 ^{abc}	2.663 ^{c}	3.596 ^{bc}	3.825 ^{bc}
PM 200 25	7.325 ^{a}	6.617 ^{a}	5.608 ^{a}	1.867 ^{c}	3.279 ^{bc}	1.900 ^{bcd}	4.792 ^{a}	1.942 ^{bcde}	1.625 ^{cde}	0.321 ^{e}	1.350 ^{def}	1.883 ^{def}
RB	7.021 ^{a}	7.075 ^{a}	6.346 ^{a}	0.550 ^{c}	4.229 ^{ab}	1.279 ^{cd}	4.925 ^{a}	1.296 ^{def}	1.213 ^{def}	0.067 ^{e}	0.475 ^{f}	0.713 ^{f}
RB 135 30	0.008 ^{d}	0.008 ^{c}	0.012 ^{c}	1.379 ^{c}	0.017 ^{e}	2.867 ^{abc}	0.921 ^{e}	0.308 ^{f}	0.087 ^{f}	3.629 ^{abc}	4.796 ^{ab}	6.508 ^{a}
RB 200 25	6.700 ^{a}	6.529 ^{a}	6.333 ^{a}	1.992 ^{c}	3.458 ^{abc}	0.562 ^{d}	5.071 ^{a}	1.563 ^{cdef}	0.788 ^{ef}	0.808 ^{de}	2.254 ^{cde}	2.971 ^{cde}
p Value (Model)	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§
p Value (Sample)	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§	< 0.0001§

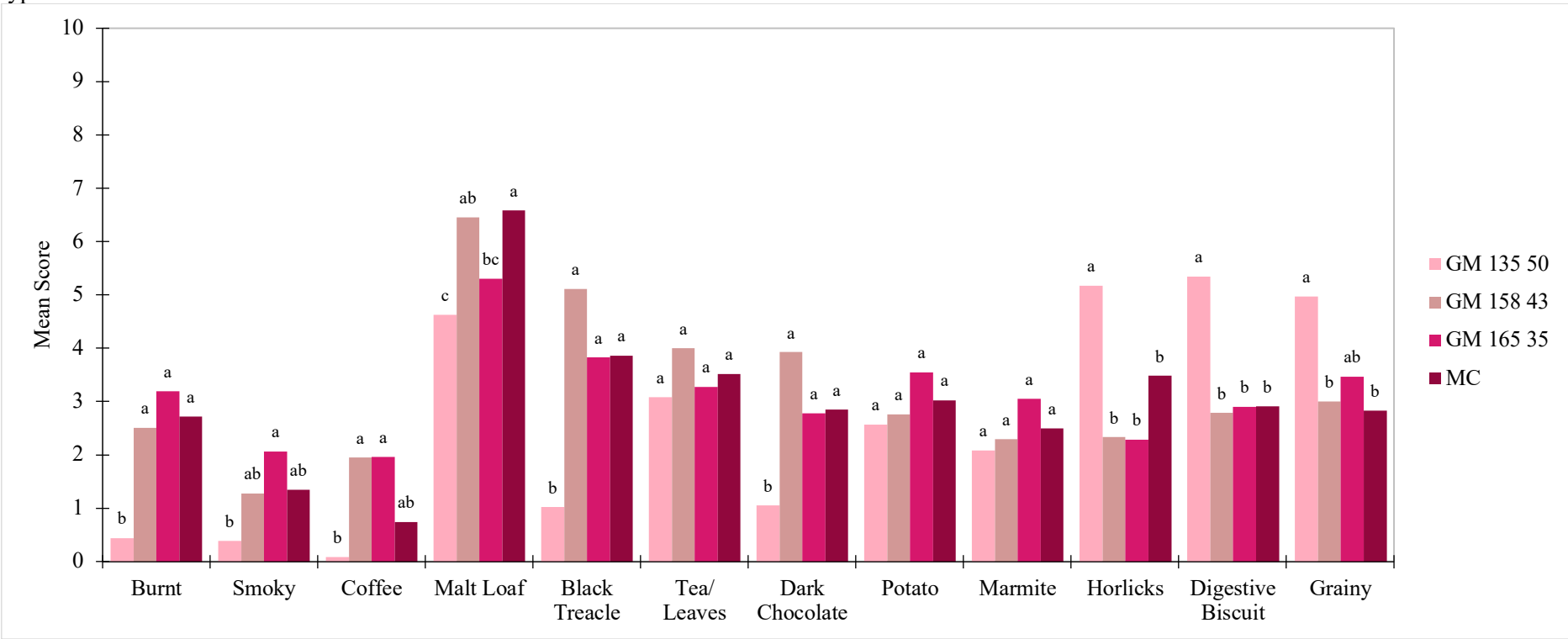
[1] Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
Aroma scores are from 8 panellists (n=3 replicates).
Subscript letters indicate significantly different groupings within each attribute from two-way ANOVA with Tukey's HSD post hoc analysis.
[§]Significant.

Figure 8.21: Dendrogram resulting from Agglomerative Hierarchical Clustering (AHC) analysing mean scores in 12 aroma attributes across 11 roasted samples.



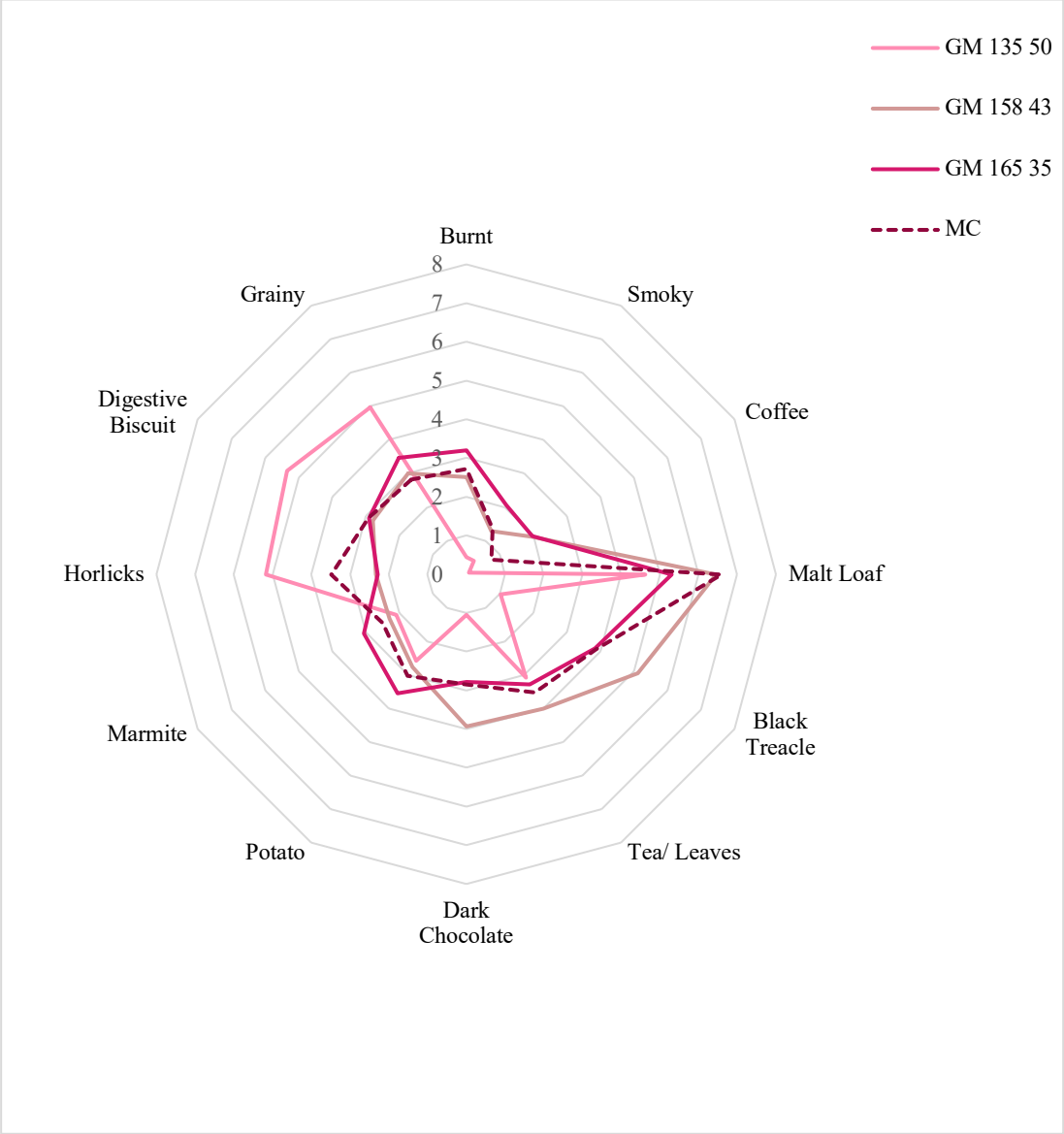
Commercial roasted malt samples (n=4) are: amber malt (AM), medium crystal malt (MC), chocolate malt (CH), and roasted barley (RB). Laboratory roasted samples (n=7) are pale malt (PM), green malt (GM), and raw barley (RB) followed by roasting temperature (°C), and roasting time (min).
Aroma scores are from 8 panellists (n=3 replicates).

Figure 8.22: Mean scores for the roasted green malt samples (n=4) across 12 aroma attributes, identifying significantly different samples within the substrate type.



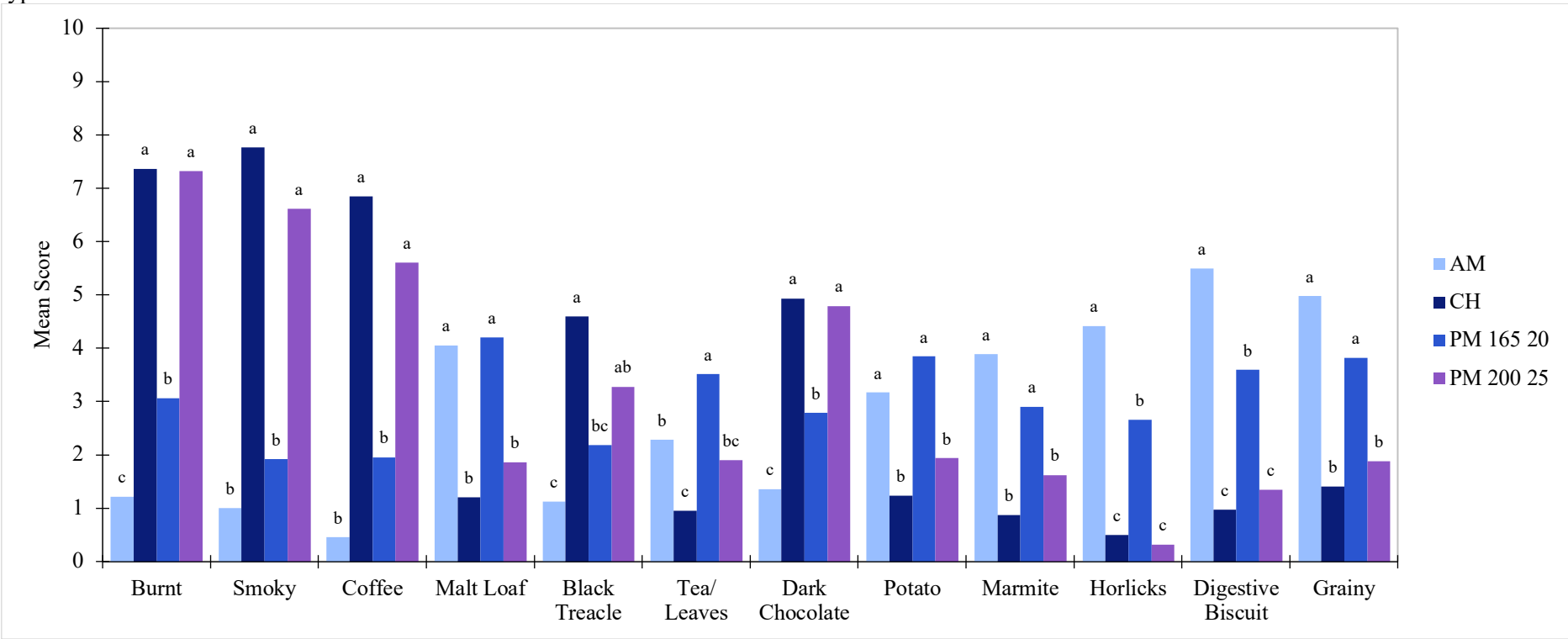
Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8).
Laboratory roasted samples are roasted green malt (GM), followed by roasting temperature (°C) and roasting time (min). The commercially available sample is medium crystal malt (MC).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey's HSD post hoc analysis.

Figure 8.23: Radar plot resulting from aroma analysis of the roasted green malt samples.



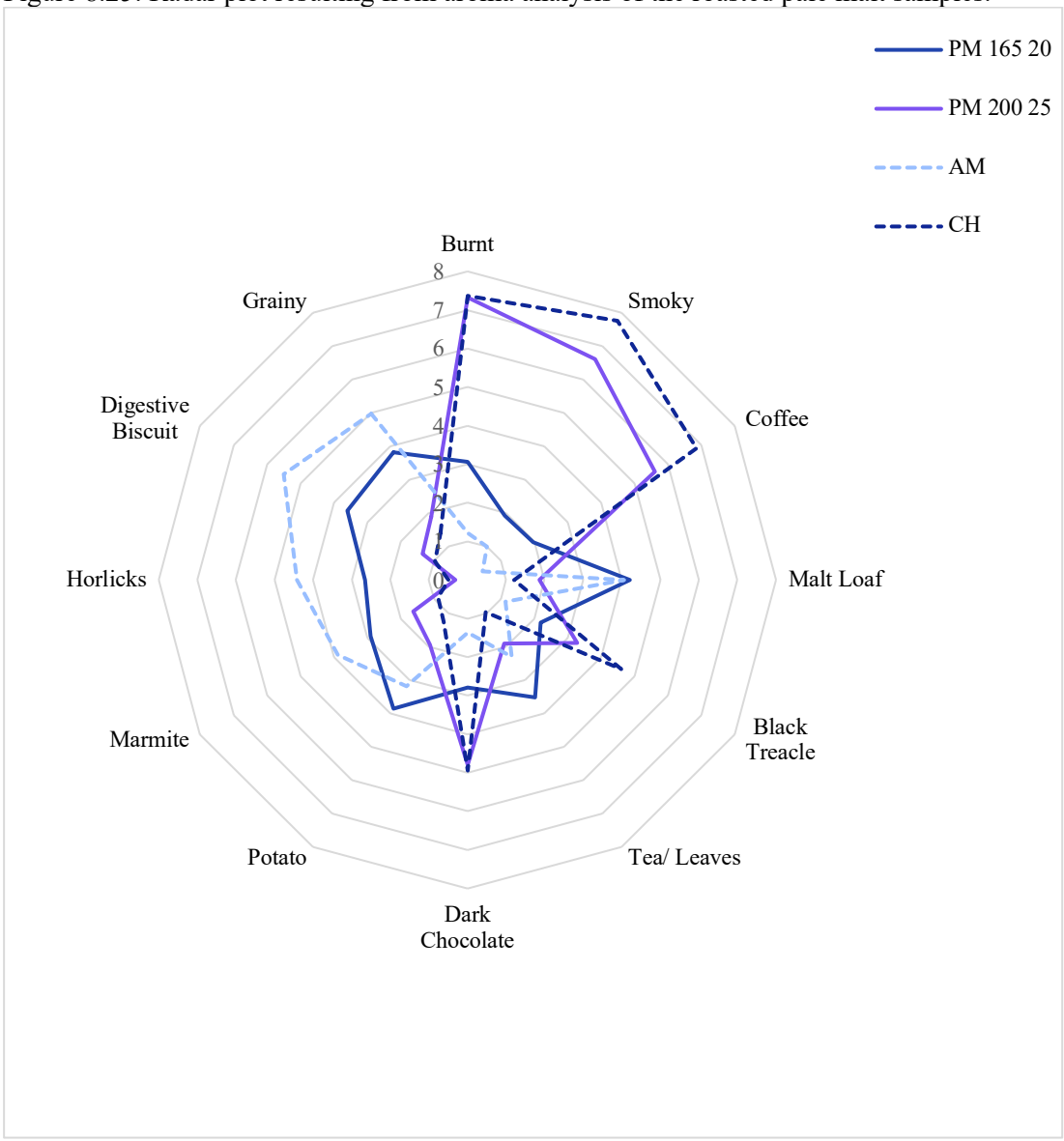
Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8). Laboratory roasted samples are roasted green malt (GM), followed by roasting temperature (°C) and roasting time (min). The commercially available sample is medium crystal malt (MC), indicated by a dotted line.

Figure 8.24: Mean scores for the roasted pale malt samples (n=4) across 12 aroma attributes, identifying significantly different samples within the substrate type.



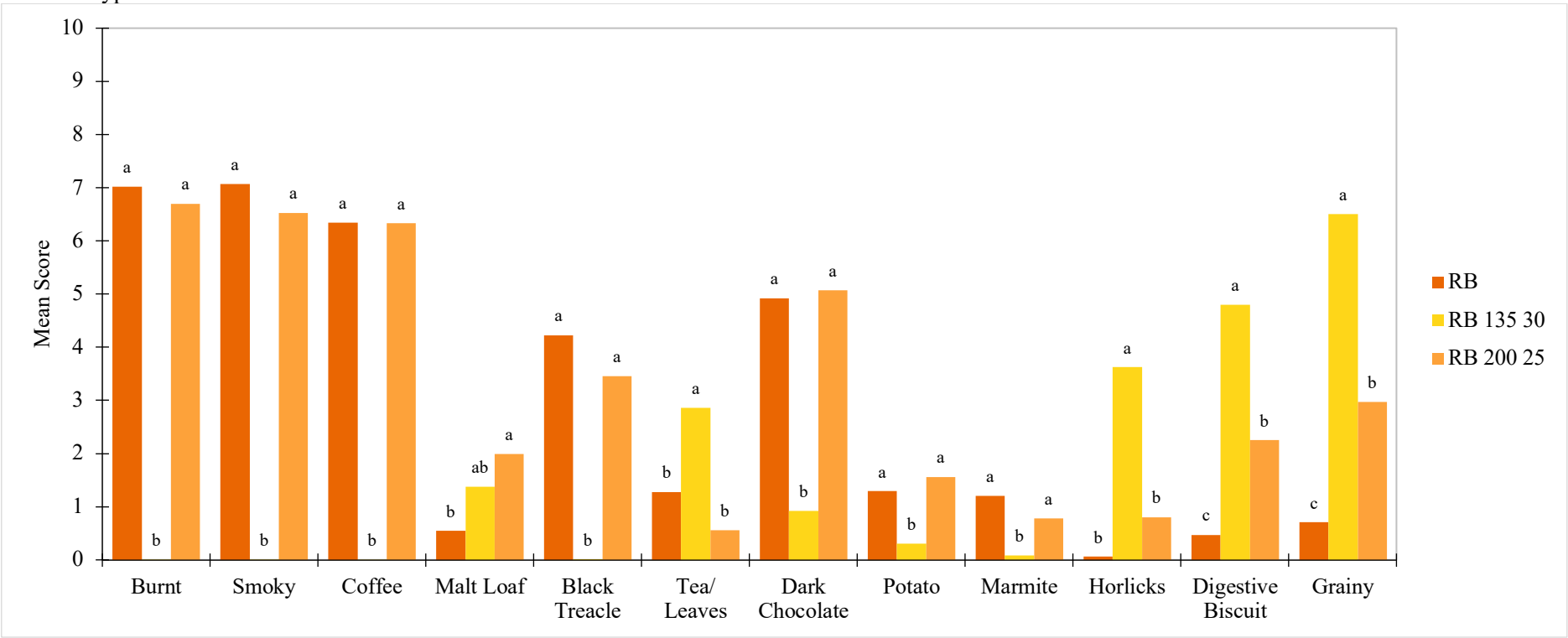
Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8).
Laboratory roasted samples are roasted pale malt (PM), followed by roasting temperature (°C) and roasting time (min). The commercially available samples are amber malt (AM), and chocolate malt (CH).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey's HSD post hoc analysis.

Figure 8.25: Radar plot resulting from aroma analysis of the roasted pale malt samples.



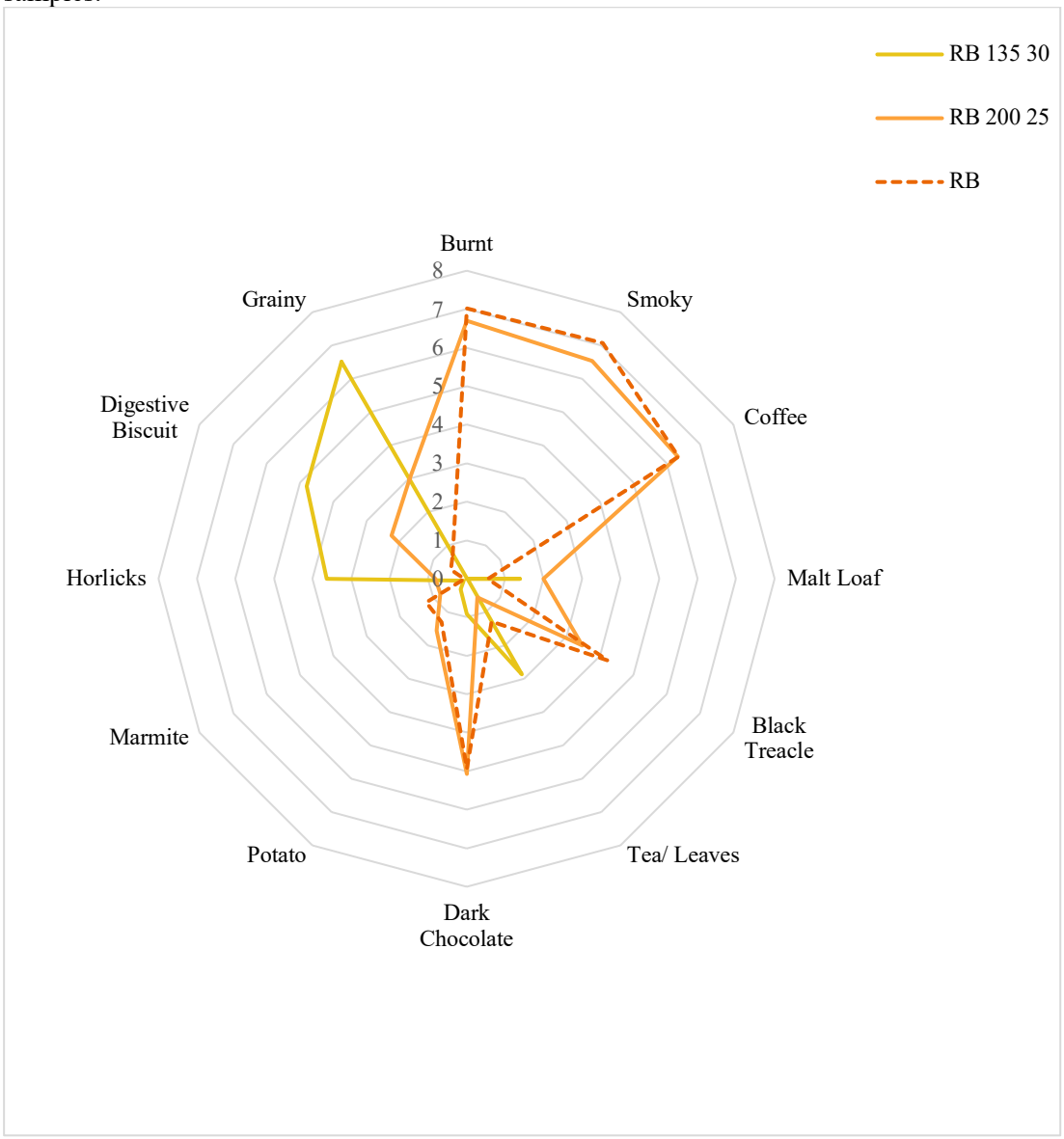
Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8). Laboratory roasted samples are roasted pale malt (PM), followed by roasting temperature (°C) and roasting time (min). The commercially available samples are amber malt (AM), and chocolate malt (CH), indicated by dotted lines.

Figure 8.26: Mean scores for the roasted unmalted barley samples (n=3) across 12 aroma attributes, identifying significantly different samples within the substrate type.



Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8).
Laboratory roasted samples are roasted raw barley (RB), followed by roasting temperature (°C) and roasting time (min). The commercially available sample is roasted barley (RB).
Lettering indicates significantly different groupings within each attribute from two-way ANOVA with Tukey's HSD post hoc analysis.

Figure 8.27: Radar plot resulting from aroma analysis of the roasted unmalted barley samples.



Scores are the mean of analysis sessions (n=3) from trained sensory panel members (n=8). Laboratory roasted samples are roasted raw barley (RB), followed by roasting temperature (°C) and roasting time (min). The commercially available sample is roasted barley (RB), indicated by a dotted line.