THE QUALITY OF BITTERNESS IN BEER

By

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DEDICATION

Dedicated to Dad, Mum, Daré, Seun, Bimbo, Emmanuel and my lovely partner Saskia Hofmann for all their love, support and understanding – now and in the future.

Abstract

The bitterness of beer remains one of its most important flavour attribute. However, complexity surrounding bitterness perception alone represents a significant challenge in its understanding, in addition to other factors relating to production processes and raw materials used in making beer. The aim of this research was to better understand beer bitterness in terms of its intensity and quality, and how hopping technology (e.g. quantity and time of hop addition during the brewing process), hop aroma and variety may effect perceived bitterness intensity and quality. This project combined both analytical and sensorial analysis, and used statistical techniques to identify the key compounds driving bitterness perception. Analytical techniques were employed to quantify bitterness-contributing compounds and analytical bitterness units in beers alongside descriptive analysis of perceived sensory bitterness. Perceived sensory bitterness (both qualitative and quantitative) was assessed using a purpose developed list of well-defined bitterness attributes, to aid the characterisation of bitterness quality in beers. The impact of cross-modal flavour interactions on the perception of beer bitterness intensity and qualities was also investigated at varying analytical bitterness levels. Lastly, the effect of hop variety, and how this significant raw material affects perceived bitterness quality was also investigated using three distinctively different hop varieties (Hersbrucker, East Kent Goldings and Zeus).

Results revealed a significant impact of the analytical profiles of beers, derived from the type of hop products and hopping technology adopted in beer production, on the perceived bitterness quality of beer. In general, beers high in hop acid compounds and polyphenols were, as expected, perceived to be higher in bitterness intensity. The results further showed that bitterness intensity also played a role in the perception of bitterness quality i.e. whether the beer was 'harsh', 'lingering' or 'round'. However, beers high in these compounds were not always of 'negative' bitterness quality, and correspondingly lower amounts of these compounds did not always result in 'positive' bitterness qualities in beer. Results on the impact of cross-modal flavour interactions showed that hop aroma plays a crucial role in beer bitterness perception, and can modify the perception of bitterness intensity and quality as well as temporal profiles of bitterness in beer depending on analytical bitterness levels. The investigation into the impact of hop variety on perceived bitterness quality revealed hop-derived bitterness profiles relating to individual hop varieties, and further suggests that careful selection of the hop variety used for brewing is important for controlling perceived bitterness quality.

This research provides a comprehensive interdisciplinary approach to understanding perceived beer bitterness and its associated qualities, with findings suggesting that besides bitterness intensity, the quality and overall impression of bitterness as perceived by consumers are significantly influenced by raw materials, production processes and other factors such as aroma which might be overlooked by brewers - but are likely to be significant for consumer preference and the success of a brand.

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LIST OF ABBREVIATIONS

BU	Bitterness Units
IBU	International Bitterness Units
TI	Time Intensity
TDS	Temporal Dominance of Sensations
HPLC	High Performance Liquid Chromatography
RP-HPLC	Reverse Phase- High Performance Liquid Chromatography
LC-MS	Liquid Chromatography-Mass Spectrometry
IKE	Isomerised Kettle Extracts
PIKE	Potassium-Form Isomerised Kettle Extracts
α-acid	alpha-acid
Rho	Rhodihydroiso-alpha-acid
Tetra	Tetrahydroiso-alpha-acid
Hexa	Hexahydroiso-alpha-acid
PHA	Pure Hop Aroma
SPE	Solid Phase Extraction
SFE	Supercritical Fluid Extraction
TRC	Taste Receptor Cells
PROP	6-n-propylthiouracil
PTC	Phenylthiocarbamide
OE	Olfactory Epithelium
TT	Thermal Taster
TnT	Thermal Non-Taster
FCP	Free Choice Profiling
CATA	Check-All-That-Apply
DA	Descriptive Analysis
QDA	Qualitative Descriptive Analysis
TPC	Total Polyphenol Content
CV	Coefficient of Variance
AHC	Agglomerative Hierarchical Clustering
PCA	Principal Component
PLSR	Partial Least Square Regression
CA	Correspondence Analysis
VIP	Variables Important for Projection
ANOVA	Analysis of Variance
T/C	Trans/Cis
PA	Phenolic Acids
mL	Millilitre
mg	Milligram
g	Gram
L	Litre
nmoL	Nanomole
%	Percent
°C	Degree centigrade
Sec	Second
h	Hour

1 Introduction

The five taste modalities - sour, salty, umami, sweet and bitter, in combination with other factors such as aroma and visual cues aid our evaluation of daily dietary choices (Sørensen, Møller, Flint *et al.*, 2003). Bitterness remains a key organoleptic attribute of several beverages such as coffee, grapefruit juice and beer, despite the evolutionary development of human sensory systems to reject ingestion of bitter substances due to fear of toxicity (Drewnowski, 2001). In beer, bitterness is a key component of flavour and aftertaste, which significantly impacts consumer choice and brand success. Conventionally, bitterness is achieved by the addition of hops (in various forms) to generate isohumulones, beer's major bittering principles during the boiling stage of the brewing process (Jaskula, Goiris, De Rouck *et al.*, 2007).

Remarkably, perceived bitterness can be of varying sensorial quality, as evidenced by reports for several bitter tastants, e.g. the bitterness of caffeine and quinine were perceived to be 'medicinal', 'chalky' and 'metallic' in comparison to isohumulones in beer (Fritsch & Shellhammer, 2009). Many in the brewing industry also comment anecdotally on the 'Quality of Bitterness' of a beer e.g. whether it is 'harsh', 'smooth', 'clinging' or 'harmonious' with respect to bitterness. However, what is meant by these terms remains far from clear and is subject to individual interpretation, which doubtless reflects the diversity represented by bitterness as a sensory attribute. Presently not all aspects of bitter taste sensation are fully understood. Bitterness sensation can be elicited by over a thousand compounds of extensively different molecular size and structures, consisting of numerous chemical functional groups, at

extremely low thresholds (Drewnowski & Gomez-Carneros, 2000). Bitter tasting congeners include organic acids, peptides, amino acids, polyphenols, hop acids, acyl sugars, caffeine and sulfimides such as saccharin (A. Drewnowski, 2001; Rouseff, 1990). The perception of bitterness is further complicated by significant variations amongst the population, with varying sensitivities to bitterness largely dictated by the genetic make-up of individuals (Bartoshuk, 2000; Mennella, Pepino, & Reed, 2005; Prutkin, Duffy, Etter *et al.*, 2000).

Instrumental measurements of bitterness in beer are mostly based on the spectrophotometric International Bitterness Units (IBU) method, or high pressure liquid chromatography (HPLC) analysis (ASBC Method of Analysis, 1978). However, such measurements do not always correlate with the overall impression of bitterness perceived by consumers. This is due in part to the lack of an agreed bitterness lexicon with which to characterise subtle differences perceived by the consumer during beer consumption. Additionally, the perception of bitter tastants (caffeine, quinine) including those found in beer (isohumulones), exhibits a time-course - i.e. the perceived bitterness intensity varies over time, and is thus best measured sensorially using techniques such as time intensity (TI), or temporal dominance of sensation (TDS) (Leach & Noble, 1986; Sokolowsky & Fischer, 2012).

The brewing industry has seen an increase in consumer preference for light, low bitterness lager beers in more recent years (Schönberger & Kostelecky, 2011). Consequently, the average levels of bitterness in lager beers have reduced; placing greater emphasis on the qualitative aspects of perceived bitterness, rather than JUST intensity, as an indicator of brand

success. Our knowledge of beer bitterness perception will undoubtedly be enhanced further by research targeted at developing well-defined descriptive lexicons to differentiate between various qualities of bitterness in beer, and the identification of the key drivers of different bitterness qualities – derived either as a result of the raw materials used in brewing, or in the adopted brewing process. Furthermore, the multifaceted nature of bitterness sensation necessitates that interactions between beer bitter tastants and the effects of cross-modal interactions be thoroughly investigated.

The following literature review provides a brief overview of the brewing process, hops (one of beer's four major ingredients) and hop-derived bitterness in beer. This is followed by consideration of what is known about the major factors affecting hop-derived bitterness in beer, other sources of bitter tastants in beer, hop products and the perception of bitterness in beer. Common analytical and sensory methods used for investigating bitterness are also included, as well as an overview of 'Quality of Bitterness' as defined for this project.

The research objectives and thesis structure are presented in the last section of Chapter 1.

Due to confidentiality reasons, the brand names of the commercial beers used in parts of this study were anonymised.

1.1 Beer production

Four major ingredients are needed for the production of beer – malted barley, hops, water and yeast (Wunderlich & Back, 2009). An outline of the brewing process is depicted in Figure 1.1, showing the key brewing stages – 'mashing', 'lautering', 'boiling', 'cooling', 'fermentation' and 'conditioning'. The production of malted barley is usually carried out by maltsters outside the brewhouse but it is not uncommon for big breweries to have their own malting plant adjacent to the brewery. The malting process involves hydrating pre-selected barley seeds by steeping them in water followed by a regime of controlled germination under conditions where temperature, oxygen, water content, and germination time are closely monitored (Aniche & Palmer, 1990). Controlled germination initiates increased enzymatic activity within the grain, resulting in partial degradation of the starchy endosperm and consequently the availability of short chain starches for the latter stages of beer production (Burger & LaBerge, 1985). The resultant 'green malt' is kilned at temperatures determined by the type of malt to be produced. Pale malts, commonly used in lager beers are kiln dried at lower temperatures (85°C) while ale malts require relatively higher kilning temperatures (Agu & Palmer, 1997). Dark malts, commonly used for brewing dark-coloured beers such as stouts, are acquired by kilning or roasting at very high temperatures (~180°C) (Coghe, Gheeraert, Michiels *et al.*, 2006).

The primary purpose of 'mashing' is to initiate enzymatic activity in the malt to break down starch molecules into fermentable sugars, thereby making them available for fermentation in the latter stages of the brewing process. This is done by combining milled malted grains with hot brewing

liquor (water) at a set temperature of 65°C (infusion mashing) or at variable temperatures, in the mash tun, to ensure maximum extraction of sugars from the grain (Briggs, Brookes, Stevens *et al.*, 2004). A proportion of adjuncts (maize, wheat and rice) can be added to barley malt as a secondary source of starch or to impart certain flavour characters in beer at this stage (Agu, 2002). The mixture is subsequently 'lautered' in a lauter tun by separating the spent grains from the liquid commonly known as 'sweet wort'.

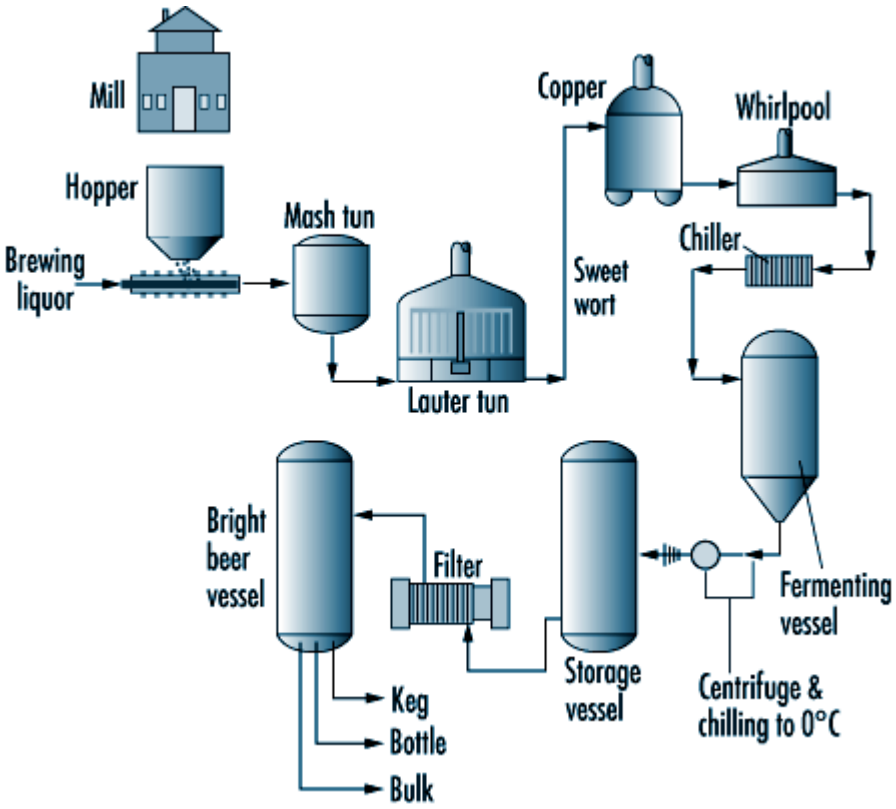


Figure 1.1: An outline of the brewing process (from the Encyclopaedia of Occupational Health and Safety, www.ilocis.org).

The sweet wort is subsequently transferred into another vessel commonly known as the 'copper' and boiled with hops. Bittering hops are typically added at the start of the boil to allow an hour or longer for the thermal isomerisation of hop acid precursors (α-acids) to their bitter tasting derivatives (iso-α-acids or isohumulones) (Hudson & Birtwistle, 1966). Brewers also commonly add aroma hop varieties in the last 15 minutes of the boil to give the beer extra hop flavour, in a practice known as 'late hopping'. The spent hop matter and trub are separated from the boiled wort in the whirlpool (Hieronymus, 2012).

The boiled wort, now also sterilised, is quickly cooled in preparation for 'fermentation' which commences upon the addition of a culture of lager (*Saccharomyces pastorianus*) or ale yeast (*Saccharomyces cerevisiae*) depending on the required beer style (Erbe & Brückner, 2000). The wort is kept at the optimal fermentation temperature for the yeast strain added for the duration of primary fermentation. The optimal fermentation temperature for ale yeast is between 10 - 25°C and 7 - 15°C for lager yeast (Perpète, Santos, Bodart *et al.*, 2005). During fermentation fermentable sugars are converted into ethanol, carbon dioxide and an array of aroma compounds are also formed as by-products of yeast metabolism (De Keukeleire, 2000). The resultant 'young beer' subsequently undergoes 'maturation' to allow off-flavours e.g. diacetyl to dissipate, while other desirable flavours generated during fermentation mature to give a more rounded flavour in the finished beer (Pascoe, Ames, & Chandra, 2003). The matured beer is subsequently 'finished' (e.g., centrifuged, stabilised, filtered….), carbonated and packaged into cans, bottles or kegs (Lowe & Elkin, 1986).

1.2 Hops

Hops (*Humulus lupulus L.*) are dioecious (separate male and female) climbing plants belonging to the Cannabaceae family and are a key ingredient in the production of beers (Shephard, Parker, Darby *et al.*, 2000). Only the female plants are cultivated and of commercial relevance as they contain the compounds of interest to brewers.

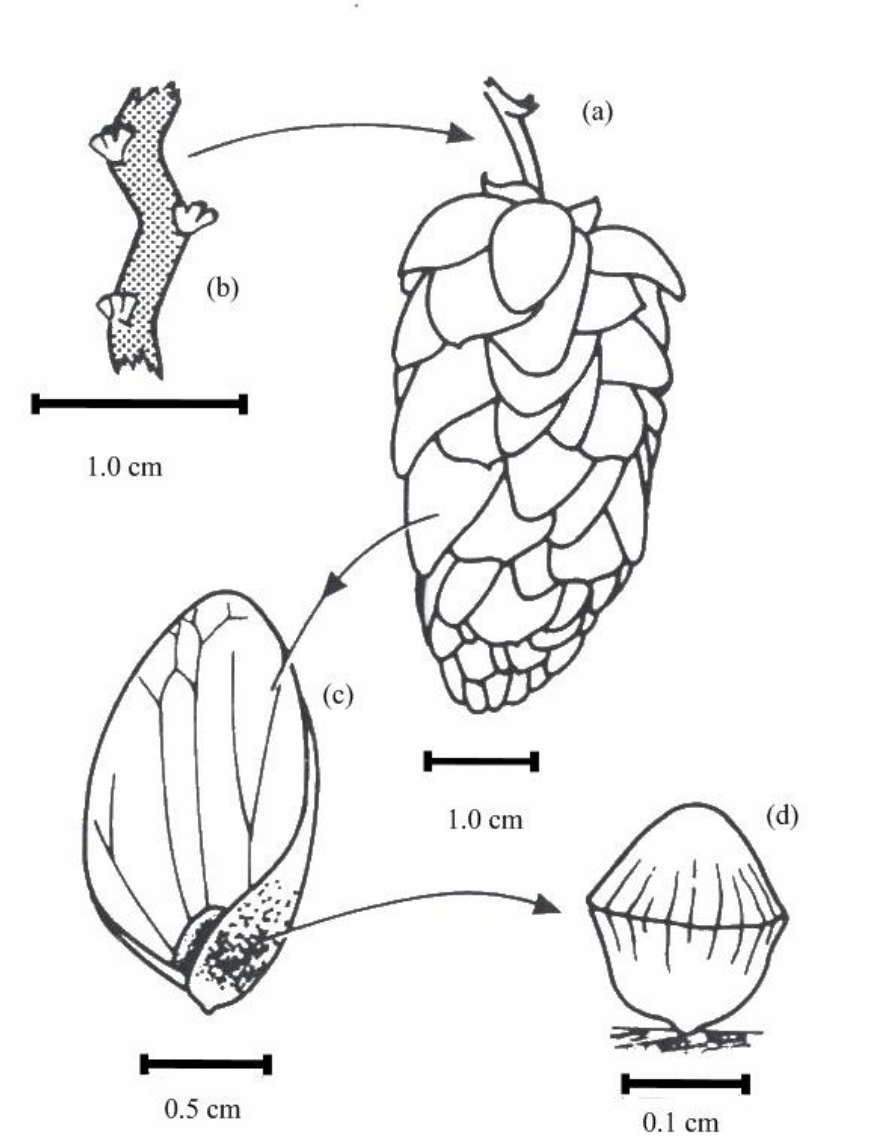


Figure 1.2: A hop cone and its key segments; (a) hop cone, (b) hop cone strig, (c) bracteoles and (d) lupulin glands (Hough, Briggs, Stevens *et al.*, 1982).

A hop cone, shown in Figure 1.2a is made up of several bracteoles (c) consisting of lupulin glands (d) at the inner and outer base of each bracteole. The sticky lupulin glands are yellowish-green in colour, and houses hundreds of secondary metabolites, including the soft and hard resins as well as volatile hop essential oil compounds (Almaguer, Schönberger, Gastl *et al.*, 2014; Neve & Neve, 1991). The relative concentrations of these metabolites serve as markers for varietal characterisation of hops as well as the source of flavour-imparting compounds of importance to brewers (Jaskula, Goiris, Opstaele *et al.*, 2009). Within the soft resin fraction, the α- & β-acids are present as a mixture of closely related congeners, derived from the same biosynthetic pathway via a 4-deoxy-α-acid precursor (Stewart & Priest, 2006). The major α-acids isomers present include cohumulone (1a), humulone (1b), adhumulone (1c), whilst the main β-acids include colupulone (2a), lupulone (2b) and adlupulone (2c). The minor constituents of hop α-acids are prehumulone (1d) and posthumulone (1e), with the β-acid equivalents – prelupulone (2d) and postlupulone (2e) (Benitez, 1997; Verzele, 1958). Although the α- and β-acid isomers only differ structurally with regard to the alkyl side chain ('R' group), this causes variations in polarity, which affects their behaviour during the brewing process as well as their sensory perception in beer (Hughes & Simpson, 1996).

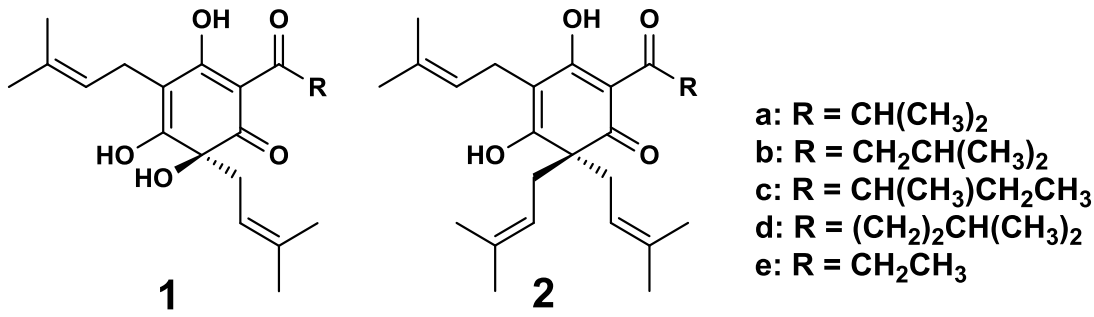


Figure 1.3: Hop α-acids (1) & β-acids (2): cohumulone (1a), humulone (1b), adhumulone (1c), prehumulone (1d), posthumulone (1e); β-acids: colupulone (2a), lupulone (2b), adlupulone (2c) prelupulone (2d) and postlupulone (2e).

In contrast to the soft resins which are generally characterised by their solubility in *n*-hexane, the hard resin fraction of hops are only soluble in ether and cold methanol (Stevens, 1967). Some of the compounds within the latter fraction of hops remain unidentified, and their contribution to beer bitterness quality is unclear. The major constituents of hard resins identified so far are prenyl flavonoids such as xanthohumol and desmethyl-xanthohumol. The oxidation of hop α- and β-acids, due to poor hop storage also contributes to an increase in the hard resin fraction of hops, and a concomitant reduction in the soft resin fraction of hops (Taniguchi, Matsukura, Ozaki *et al.*, 2013).

The volatile essential oil component of hops represents only a small proportion (0.5% - 3.0% v/w) of a dried hop cone, although their flavour contribution, i.e. distinct 'hoppy' aroma and flavour, and enhancement of beer mouthfeel is significant (Steinhaus & Schieberle, 2000; Van Opstaele, Goiris, De Rouck *et al.*, 2012a). Hop essential oil consists of a complex combination of over a thousand mostly volatile compounds from which

only a few hundred have been conclusively identified to date (Roberts, Dufour, & Lewis, 2004). The compounds within this fraction can be separated into three groups, consisting of simple terpene hydrocarbons, oxygenated sesquiterpenes and sulfur containing compounds (Schönberger & Kostelecky, 2011; Sharpe & Laws, 1981). Varietal classifications are often derived from the ratio of hop essential oil to soft resin content. Hops with high levels of α-acids to essential oil are primarily preferred for imparting bitterness (bittering hops), whilst those with a higher level of aroma to hop α-acids are preferred for imparting hop flavour in beer (aroma hops). However, certain varieties are known as dual-purpose hops since they contain relatively balanced levels of essential oil and α-acids can be used for either purpose (Neve & Neve, 1991; Schönberger & Kostelecky, 2011). Besides their flavour contributions to beer, hops also possess preservative properties, helping to protect beer against spoilage due to their their resistance to Gram-positive bacteria like *Pediococcus* and *Lactobacillus* (Rückle & Senn, 2006; Sakamoto & Konings, 2003; Van Cleemput, Cattoor, De Bosscher *et al.*, 2009). The inhibition of Gram-positive bacteria is reported to be stronger for β-acids compared to α-acids (Kramer, Thielmann, Hickisch *et al.*, 2015). Certain health benefits have also been reported for some of the compounds found predominantly in hops; in particular, hop prenylflavonoids have been shown to exhibit anticancer properties (Albini, Dell'Eva, Vené *et al.*, 2006; Siegel, Miternique-Grosse, Griffon *et al.*, 2008).

1.3 Hop products

Hop products are extensive in range, fitting broadly into three groups consisting of non-isomerised, isomerised and hop oil products. They can however be further classified based on the point at which they are added in the brewing process e.g. in the kettle or further downstream after fermentation. A simple breakdown of these products according to the European Brewing Convention (EBC) manual of good practice is presented in Figure 1.4 (Benitez, 1997).

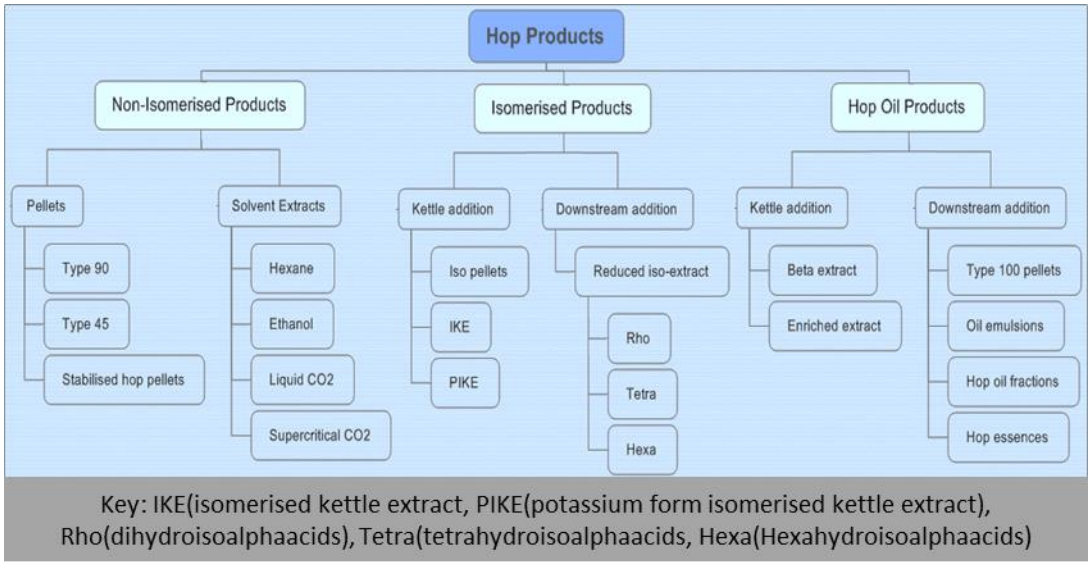


Figure 1.4: A classification of commonly available hop products for brewing.

Hop products are also available in different forms, as compressed hop cones, pellets or liquid extracts as shown in Figure 1.5. Once hops are harvested, their moisture content is reduced from around 80% to 10% by kiln drying to prevent spoilage and oxidation of hop compounds (Esslinger, 2003). The resulting dried flowers can be compressed and packaged as compressed hop cones, or further processed to give pellet hop products

which are easily stored and handled in the brewhouse (Clarke, 1986) (Figure 1.5). Heavy metals and other chemical contaminants within the vegetative segment of hops (from pesticides etc.) are also reduced as a result of hop processing (Stewart & Priest, 2006).

Hop extracts are nowadays acquired using either ethanolic extraction, liquid CO_2 or supercritical CO_2 rather than solvents such as ether or hexane which were previously used in their preparation (Esslinger, 2003). Hop extracts can vary in composition depending on the extraction technique, potentially effecting the bitterness quality achieved in beer. Beers bittered with CO_2 extracts were reported to lack certain flavour character e.g. 'kettle flavour', perhaps due to the absence of hop tannins and proteins which are lacking in these extracts (Imbert, 2009).

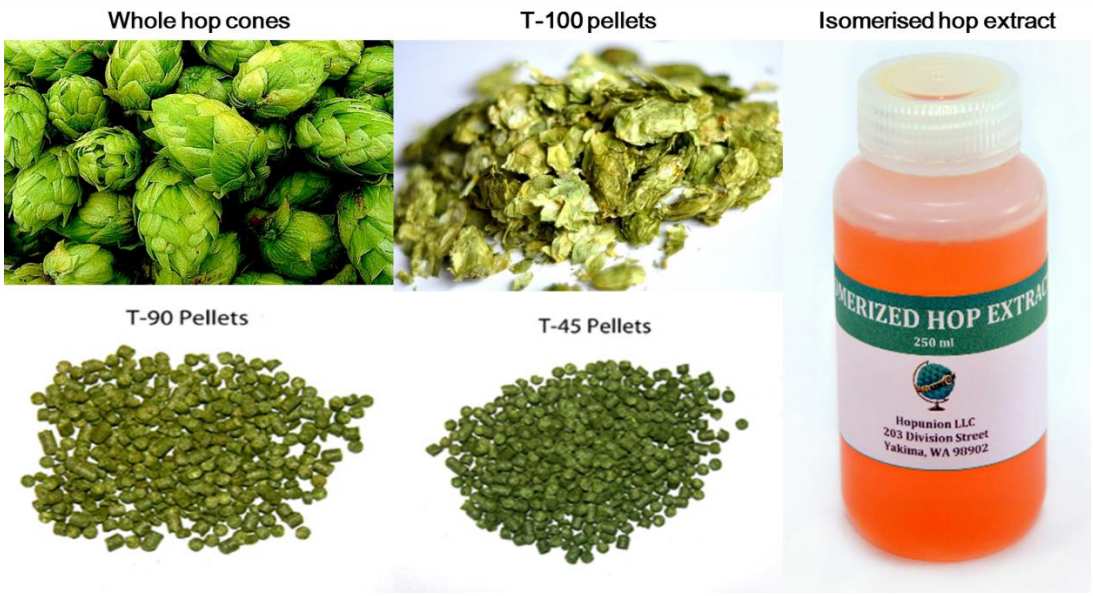


Figure 1.5: Different hop forms; whole hop cones, pellets and aqueous hop extracts.

Nonetheless, hop products have gained in popularity in recent years because they offer brewers added flexibility, increased efficiency and reduced batch to batch variation which would otherwise occur in conventionally hopped beers bittered with whole hop cones (Laws, 1981; Moir, 2000). Hop utilisation, a term frequently used in the industry to represent the amount of iso-α-acids present in wort or beer relative to the amount of α-acids added through conventional addition of hop cones or pellets is also improved when hop products are used (Jaskula-Goiris, Aerts, & De Cooman, 2010). This is so because hop products are concentrated, more soluble and in some cases can be added further downstream in the beer production process, thus reducing losses of bittering principles during other stages of the brewing process (Wilsoh, Roberts, Smith *et al.*, 2001).

Importantly, to impart bitterness to beer when non-isomerised hop products are used, regardless of the type or form, they must be added at the onset of wort boiling in order to ensure adequate isomerisation of hop α-acids to iso-α-acids. Hop addition at the onset of boiling and long contact times (~60 min) give good α-acid utilisation (Jaskula, Goiris, Opstaele *et al.*, 2009).

1.3.1 Pre-isomerised hop products

Due to the slow rate of α-acid solubilisation, inconsistent isomerisation rates, as well as extended boil times required to transform α-acids into iso-α-acids during the brewing process (Jaskula, Goiris, Opstaele *et al.*, 2009); it has become advantageous for brewers to use pre-isomerised hop products which have been produced outside the brewhouse (Weiss,

Schönberger, Mitter *et al.*, 2002). Pre-isomerised hop products include isomerised pellets, isomerised kettle extracts (IKE) and isomerised liquid extract such as Isohop®, an aqueous solution of iso-α-acids widely available at a concentration of 30% w/w for addition to beer post fermentation. The latter product is the most common because it is readily soluble in beer, thus its addition at the latter stages of the brewing process allows complete dissolution, and therefore higher utilisation values of around 70 – 95% (Benitez, 1997; Stewart & Priest, 2006). A consistent product in relation to bitterness flavour is consequently attained with Isohop® in comparison to hop cone usage for example (Stewart & Priest, 2006).

The production of isomerised hop pellets involves adding approximately 3% of the total hop weight in magnesium oxide (MgO) to hop pellets before mixing and packaging. The packaged hops are then conditioned in a hot room at approximately 55°C for up to 14 days depending on hop variety (Hughes & Simpson, 1996). Beers brewed with pre-isomerised products tend to have higher cis/trans ratios (impact on bitterness quality discussed further in Section 1.4) than those obtained in conventionally hopped beers (70:30%), because the magnesium oxide used to catalyse the isomerisation reaction strongly favours the formation of the cis- isomers (Stewart & Priest, 2006).

Isomerised kettle extracts can be produced from magnesium salt (IKE) or the potassium salt (PIKE); in the former, magnesium is used to catalyse the isomerisation reaction from a CO_2 extract containing α-acids, whilst the latter is afforded by the stoichiometric addition of potassium hydroxide to the product of the magnesium salt extract (Esslinger, 2003). Of the

resultant products, PIKE is usually more viscous than IKE and tends to separate into resin and waxy layers; this creates handling issues that limits its use in the brewery (Stewart & Priest, 2006).

1.3.2 Light-stable hop products

In addition to aqueous hop extracts, light-stable hop products consisting of rho-dihydroiso-α-acid (Rho), tetrahydroiso-α-acid (Tetra) and hexahydroiso-α-acid extracts (Hexa) are commonly used by brewers as downstream products. These light stable hop-derived bittering products offer brewers improved light and foam stability in beer (Bolívar, Gasparri, & Zufall, 2006). The stability to light in beer is imperative because of the sensitivity of iso-α-acids to UV and light. In the presence of sunlight, conventionally hopped beers if bottled in clear/white or green bottles can rapidly develop a 'skunky' flavour, caused by the degradation of iso-α-acids in a reaction catalysed by UV light (Moir, 2000). This reaction can be prevented by chemically modifying the side chains of the iso-α-acid five-membered ring compound, thereby altering the chemistry of the side chains to avoid the production of 3-methyl-2-butene-1-thiol (MBT); the compound responsible for skunky flavour. Light-stable hop extracts are produced by the reduction of iso-α-acids with a solution of sodium borohydride to give Rho, by hydrogenation of iso-α-acids to give Tetra, and the combination of both processes to give Hexa (De Keukeleire, 2000; Oladokun, Smart, & Cook, 2016). It is also common for brewers to use a blend of bittering products e.g. iso-α-acid extract and a reduced derivative such as Rho or Tetra to achieve the desired level of bitterness in beer; or to boost the foam properties of beer, since reduced hop products possess stronger foam enhancing properties (De Keukeleire, 2000). The relative

bitterness ratios and foam enhancement capacity of iso-α-acids and their reduced derivatives are presented in Table 1.1. The intensity of bitterness of Tetra is perceived to be almost twice as bitter as iso-α-acids whilst Rho is 0.6 times, and Hexa agreed to be of approximately the same bittering capacity as iso-α-acids. The use of pre-isomerised hop products has been reported to give beers sensorially described as having 'harmonic', and 'non-lingering' bitterness when compared to beers brewed with non-isomerized hop products added conventionally (Jaskula, Syryn, Goiris *et al.*, 2007).

Table 1.1 - Relative bitterness and foam enhancement capabilities of light-stable hop products (De Keukeleire, 2000).

Compounds	**Relative Bitterness**	**Foam Enhancement**
Iso-α-acids	1	xx
Rho	0.6 - 0.7	xx
Tetra	1.5 - 1.9	xxx
Hexa	1.0 - 1.2	xxxx

1.3.3 Special hop products

This category of hop products includes essential oil fractions in the form of emulsions, essences or enriched extracts typically acquired by way of selective CO_2 extraction followed by repeated distillation to achieve high purity volatile hop fractions (Briggs, Brookes, Stevens *et al.*, 2004). Van Opstaele described a method consisting of supercritical fluid extraction (SFE) followed by solid phase extraction (SPE) for the preparation of

distinct fractions with 'floral', 'citrus' and 'spicy' character from the total hop essential oils of a single hop variety (Van Opstaele, Goiris, De Rouck *et al.*, 2013). The popularity of hop oil products with brewers is in part due to alterations that occur to labile hop aroma compounds in the wort boiling stage of the brewing process. Elevated boil temperatures result in the loss of these compounds due to evaporation, thus reducing the characteristic 'hoppy' aroma that they impart in beer. Many brewers use hop oil products in the latter stages of brewing to compensate for this loss, and also to impart characteristic flavour notes in beer e.g. hop oil fractions can be added to impart 'hoppy', 'citrusy', 'floral' and 'spicy' aroma notes in beer (Bernotiene, Nivinkiene, Butkiene *et al.*, 2004).

1.4 Hop-derived bitterness

In a conventional brew, hops in the form of cones, pellets or extracts are added to the wort in the kettle and boiled for around 60 - 90 minutes. This process results in the thermal isomerisation of about 50-70% of hop α-acids via an acyloin-type ring contraction reaction to give iso-α-acids (collectively known as isohumulones), which are regarded as beer's major bittering principles (De Keukeleire, 2000; Haseleu, Lagemann, Stephan *et al.*, 2010; Jaskula-Goiris, Aerts, & De Cooman, 2010; Jaskula, Goiris, Opstaele *et al.*, 2009). Each major α-acid congener – cohumulone (1a), humulone (1b) and adhumulone (1c) subsequently becomes iso-cohumulone, iso-humulone and iso-adhumulone, respectively (See Figure 1.6). Additionally, the isomerisation reaction creates a chiral centre within the acyloin-type five-membered ring product at C4 and C5, such that each

of the isomerised products is now present as a cis/trans isomeric mixture i.e. cis-isohumulone and trans-isohumulone, at a ratio of approximately 70:30% (Figure 1.6, 2a-c and 3a-c) (Steenackers, De Cooman, & De Vos, 2015).

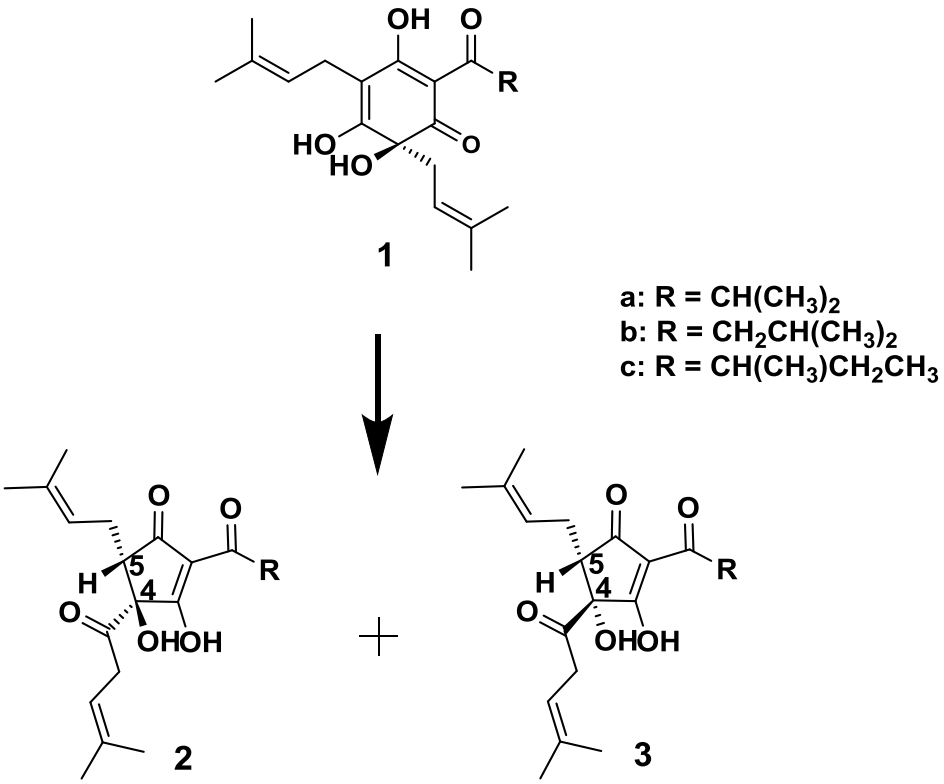


Figure 1.6: *Hop α-acids (1); cohumulone (1a), humulone (1b), adhumulone (1c) and iso-α-acids, (2&3): trans-iso-α-acids; trans-isocohumulone (2a), trans-isohumulone (2b), trans-isoadhumulone (2c), cis-iso-α-acids; cis-isocohumulone (3a) cis-isohumulone (3b) and cis-isoadhumulone (3c).*

The cis-isomers of all three α-acid congeners have been shown to elicit greater bitterness intensity relative to the trans-isomers in beer (Hughes & Simpson, 1996). Cis-isohumulone in particular has been described as the most bitter of all the isomerised α-acid congeners (Hughes, 2000), and the most stable bitterness contributor in beer (Schönberger & Kostelecky,

2011). The cis-isomers are also more thermodynamically stable than their trans- counterparts (Jaskula, Goiris, De Rouck *et al.*, 2007), due largely to entropic differences pertaining to structural geometry, which favours the cis-isomer configuration over the trans-isomer (De Cooman, Aerts, Overmeire *et al.*, 2000).

Consequently, both trans/cis- isomers have been noted to partition into beer foam at different rates; the trans-isomers have greater foam activity compared to the cis-isomers, hence cis-isomers are observed in greater quantities in beer (Hughes, 2000). More importantly, the instability of the trans-iso-α-acids means that they are susceptible to degradation during storage, especially at low beer pH and high temperatures (De Cooman, Aerts, Overmeire *et al.*, 2000; Intelmann, Haseleu, Dunkel *et al.*, 2011; Jaskula, Syryn, Goiris *et al.*, 2007) . A number of trans- degradation products, formed via proton-catalysed reactions of trans-iso-α-acids during beer storage e.g. tri- and tetra-cyclic compounds, including tricyclohumol, tetracyclocohumol, epitetracyclocohumol, and tricyclocohumene have been assessed sensorially to elicit 'harsh' and 'lingering' bitterness qualities (Intelmann, Kummerlöwe, Haseleu *et al.*, 2009).

A list of hop-derived bitter tastants in beer, taken from Schonberger's review of the role of hops in the brewing industry, is presented in Table 1.2 (Schönberger & Kostelecky, 2011). Whilst the contribution of some of these compounds to the quality of bitterness remains unclear, the noted high concentrations of iso-α-acids and their corresponding low taste thresholds further affirm their status as beer's major bitterness contributors.

Table 1.2: A list of hop-derived bitter compounds in beer including threshold and usual concentration (Schönberger & Kostelecky, 2011).

Bitter Compounds	Threshold (μM/L)	Concentration in lager beer	Concentration in US craft beer
Cohumulone	17	1	10
Humulone	21	2	10
Adhumulone	21	1	2
cis-Isocohumulone	7	31	15
cis-Isohumulone	10	33	18
cis-Isoadhumulone	8	11	10
trans-Isocohumulone	19	15	9
trans-Isohumulone	20	14	12
trans-Isoadhumulone	15	5	2
Colupulone	39.3	0	1
Lupulone	35	0	Unknown
Adlupulone	37	0	Unknown
Cohulupone	7.9	7	7
Hulupinic acid	68.5	0	Unknown
Notricyclocohulupone	90.3	0	Unknown
Dehydrotricyclolupulone	40.6	0	Unknown
Tricyclocolupulone	37.9	0	Unknown
Hydroperoxytricyclcolupulone	20.7	3	Unknown
Hydroxytricyclocolupulone	14.7	8	Unknown
Xanthohumol	10	0	2
Isoxanthohumol	16	2	5
8-Prenylnaringenin	7.5	0	Unknown

1.4.1 Other sources of bitterness in beer

Approximately 70% of beer bitterness is thought to be derived as a direct addition of hops (Techakriengkrai, Paterson, Taidi *et al.*, 2004), but this value may now be much higher due to a better understanding of hops and the ability to store them more appropriately to prevent oxidation (Hieronymus, 2012). Nevertheless, there are other contributions to beer bitterness from other compounds e.g. oxidized hop compounds and polyphenols.

1.4.1.1 Oxidized hop acids

The oxidation of hop acids results in the formation of humulinones from α-acids and hulupones from β-acids, as is depicted in Figure 1.7, 3a-c and 4a-c.

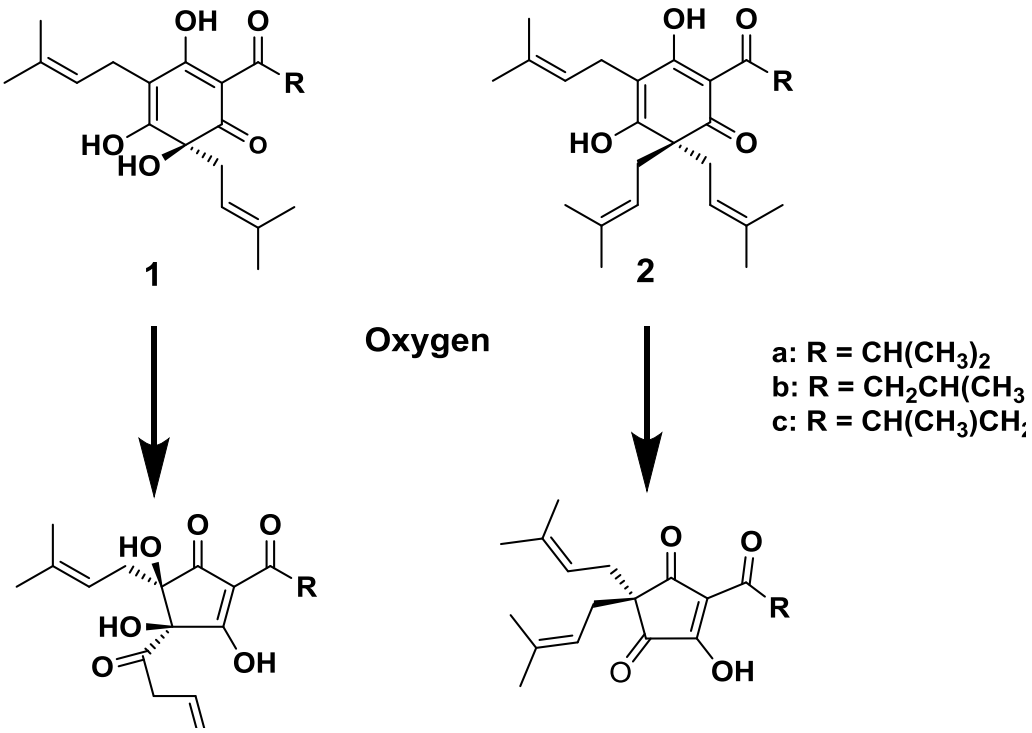


Figure 1.7: The conversion of α- (1) and β- acids (2) to Humulinones (3) and Hulupones (4) via oxidation reaction. α-acids: cohumulone (1a), humulone (1b), adhumulone (1c); β-acids: colupulone (2a) lupulone (2b), adlupulone (2c); Humulinones: cohumulinone (3a), humulinone (3b), adhumulinone (3c); Hulupones: cohulupone (4a), hulupone (4b) and adhulupone (4c).

Both groups of compounds are generated primarily during hop storage and transferred into beer when these hops are used (Haseleu, Lagemann, Stephan *et al.*, 2010). Recent findings have shown that oxidized hop acids are also formed during wort boiling, as well as in dry-hopped beers (Maye & Smith, 2016; Taniguchi, Matsukura, Ozaki *et al.*, 2013). Reports on the

impact of humulinones (Figure 1.7, 3a – c) on beer bitterness are contradictory; While Verzele (1986) reported small bitterness for humulinones in beer (Verzele, 1986), McLaughlin (2008) suggested that humulinones might be the source of harsh bitterness in beers (McLaughlin, Lederer, & Shellhammer, 2008). More recently, humulinones were associated with an increased perception of bitterness usually observed in dry-hopped beers (Parkin, 2014). Humulinones have been detected in hop cones and pellets at around 0.2 – 0.5% w/w (Maye, Smith, & Leker, 2016). The bitterness intensity of humulinones was originally thought to be about 40% that of iso-α-acids according to Whitear (Whitear & Hudson, 1964), but recent findings have suggested a higher value of about 65% (Algazzali, 2016; Steiner, 2015). These reports suggest an increasing awareness and acceptance of humulinones as a major source of additional bitterness, especially in dry-hopped beers (Maye, Smith, & Leker, 2016).

β-acids have historically been thought to contribute very little to beer bitterness and quality, due to their markedly lower solubility in water relative to α-acids (Moir, 2000). The relatively low amounts solubilised into beer are easily adsorbed to filter material and yeast trub during beer production (Intelmann, Batram,. 2009). However, their oxidation products, afforded during wort boiling are much more soluble and present in beer in moderate concentrations; the so called hulupones (Figure 1.7, 4a – c) account for about 10% of the initially added β-acids from hops (Haseleu, Intelmann, & Hofmann, 2009b). Other tricyclic β-acid transformation products have also been reported by Haselau *et al* (2009) to be present in beer at very low concentrations, and thus are suggested to contribute minimally to bitterness taste of beer (Haseleu, Intelmann, & Hofmann,

2009b; Intelmann, Haseleu, Dunkel *et al.*, 2011; Schönberger & Kostelecky, 2011).

The isomerised derivatives of the major hard resin compounds of hops (xanthohumol and desmethylxanthohumol, Section 1.2) also contribute to beer bitterness (Wunderlich, Zürcher, & Back, 2005). Xanthohumol undergoes transformation during the wort boiling process to give isoxanthohumol, while desmethylxanthohumol is converted into 6-prenylnaringenin and 8-prenylnaringenin (Stevens, Taylor, Clawson *et al.*, 1999). Brewing trials with xanthohumol-enriched hops achieved beers with comparable bitterness to normally hopped beers as well slightly better flavour stability (Wunderlich, Zürcher, & Back, 2005). More recently, Almaguer *et al.* (2015) reported a positive impact on foam and a pleasant bitterness, described to be 'sharper' than that perceived from iso-α-acids in beers brewed with hard resin extracts of hops. Improved bitterness stability was also noted in that study (Almaguer, Gastl, Arendt *et al.*, 2015).

1.4.1.2 Polyphenols

Polyphenols contribute bitterness and astringency to beer, as well as adding 'a quality of dryness' (Oliver & Colicchio, 2011). Their contribution to bitterness in beer can be achieved alone or in synergy with other bitter tastants such as iso-α-acids (Peleg, Gacon, Schlich *et al.*, 1999; Wolfe, 2012). Polyphenols can also modify perceived bitterness quality, giving it a 'harsh' and 'lingering' quality (McLaughlin, Lederer, & Shellhammer, 2008). The perception of astringency from polyphenols is based on the

level of polymerisation and concentration; a higher molecular weight and degree of polymerization can result in a stronger perception of astringency (Peleg, Gacon, Schlich *et al.*, 1999). About 80% of polyphenols in beer are derived from malt whilst those derived from hops account for about 20% (Aron and Shelhammer, 2010). Hop polyphenols include proanthocyanidins, phenolic acids, stilbenes, flavanols, flavonoids and prenylated flavonoids which constitute about 3 - 6% of the dried hop cone (Goiris, Jaskula-Goiris, Syryn *et al.*, 2014; Schönberger & Kostelecky, 2011). According to Mikyska *et al*. (2002), both malt and hop polyphenols can affect the 'harsh' character of beer bitterness (Mikyška, Hrabak, Hašková *et al.*, 2002). Therefore, the contribution of polyphenols to perceived beer bitterness and quality is thought significant.

1.4.1.3 Amino acids

Both L- and D- amino acids represent another group of compounds that contribute to the flavour of food and beverages (Kirimura, Shimizu, Kimizuka *et al.*, 1969). Of particular interest in beer are the predominantly malt-derived L- enantiomeric forms which include L-leucine, L-tyrosine, L-phenylalanine and L-tryptophan that have all been identified to elicit bitter taste (Schiffman, Sennewald, & Gagnon, 1981; Schönberger, 2006; Solms, 1969). The L-enantiomeric forms of amino acids were generally considered to be tasteless or bitter whilst, the D-enantiomers were considered to taste sweet (Solms, Vuataz, & Egli, 1965). However, research by Schiffman & Sennewald (1981) showed that this generalisation of amino acid taste based on chirality is not necessarily always correct (Schiffman, Sennewald, & Gagnon, 1981). As an example, the authors

found that in contrast to convention both L- and D-enantiomeric pairs of alanine and serine elicited sweet taste, whilst L-proline was found to elicit sweet taste and D-proline tasted bitter (Schiffman, Sennewald, & Gagnon, 1981). Some of the bitter tasting amino acids (L-tryptophan, L-phenylalanine) also reportedly share the same peripheral bitter taste mechanisms with bitter-tasting urea (Keast & Breslin, 2002; Schönberger, 2006). With the use of various yeast strains, different malt and mashing protocols, and fermentation practices adopted in the brewing industry today - the contribution of these amino acids could prove significant for the overall impression of bitterness in beer. There are currently no reports on the contribution of amino acids to beer bitterness quality, thus the contribution of these compounds clearly requires further investigation.

1.5 Analytical measurement of beer bitterness

The most common way for brewers to determine the bitterness level of their beers is by the International Bitterness Unit (IBU) method (ASBC Method of Analysis, 1978). This is based on a spectrophotometric method where 1 BU is assumed to equal 1mg/L of isohumulones (Oliver & Colicchio, 2011). However, the IBU method has been shown not to correlate well with perceived bitterness quality (Schönberger, 2006). This is partly down to the non-specific nature of the method which is based on the absorbance reading of an extract of beer taken at 275 nm; at this wavelength numerous other compounds absorb, some of which contribute to bitterness to differing extents (e.g. humulinones and polyphenols) and some of which do not. This can result in an inflated estimation of beer bitterness relative to consumer perception (Christensen, Ladefoged, & Nørgaard, 2005). This

method is also not recommended for analysis of beer bittered with reduced isohumulones, and there is no way of identifying the presence of advanced hop products in beer prior to analysis (Bolívar, Gasparri, & Zufall, 2006). Generally, the higher the level of iso-α-acids, the higher the perceived bitterness intensity. Lager beers today are reported to range typically from 6 – 30 BU although much more bitter beers (>35 BU) are also widely available commercially (Kolpin & Shellhammer, 2009; Schönberger & Kostelecky, 2011). For more precise measurement of bitterness in beer High Performance Liquid Chromatography (HPLC) coupled with Ultra Violet (UV) and Mass Spectrometer (MS) detectors are preferred because they allow for the selective quantification of isohumulones in beer (Haseleu, Intelmann, & Hofmann, 2009a; Jaskula, Goiris, De Rouck *et al.*, 2007; Taniguchi, Matsukura, Ozaki *et al.*, 2013). Furthermore, with an enhanced method the determination of several hop bittering products in beer in a single run is possible (Figure 1.8) (Oladokun, Smart, & Cook, 2016).

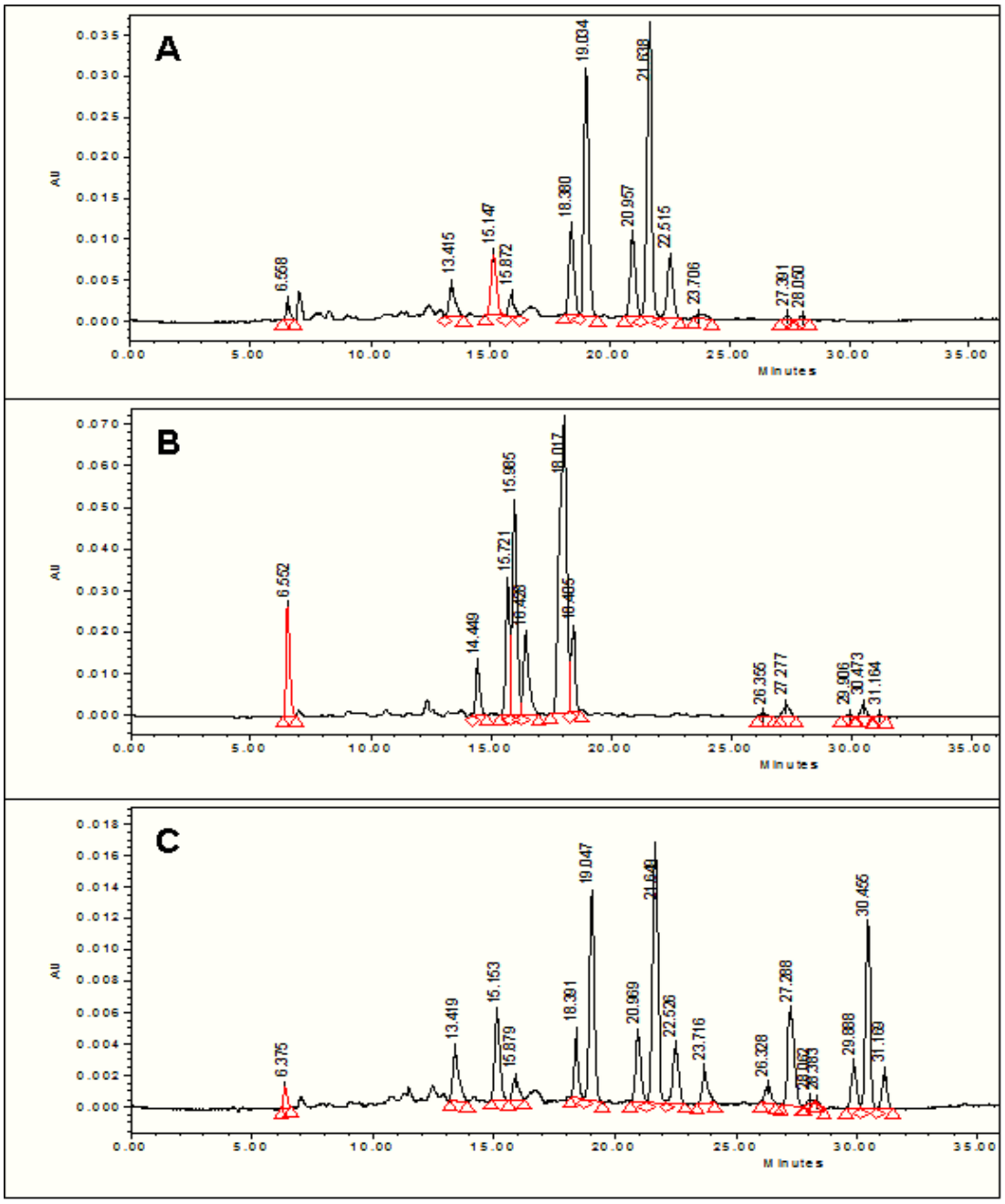


Figure 1.8 – A: HPLC separation of lager beer bittered with iso-α-acids; Internal standard (IS) (6.5), cohumulinone (13.4), humulinone (15.1), adhumulinone (15.8), trans-isocohumulone (18.3), cis-isocohumulone (19.0), trans-isohumulone (20.9), cis-isohumulone & trans-isoadhumulone (21.6), cis-isoadhumulone (22.5), cohumulone (23.7), humulone (27.4), adhumulone (28.1). B: separation of a lager beer bittered with Rho & Tetra; (IS) (6.5), trans-ρ-isocohumulone (14.4), cis-ρ-isocohumulone (15.7, 15.9), trans-ρ-isohumulone (16.4), cis-ρ-isohumulone (18.0), trans-ρ-isoadhumulone (18.4) & trans-tetrahydroisocohumulone (26.4), cis-tetrahydroisocohumulone (27.3), trans-tetrahydroisohumulone (29.9), cis-tetrahydroisohumulone & trans-tetrahydroisoadhumulone (30.5), cis- tetrahydroisoadhumulone (31.2); C: separation of a lager beer bittered with iso-α-acids & Tetra; IS (6.4), cohumulinone (13.4), humulinone (15.5), adhumulinone (15.8), trans-isocohumulone (18.07), cis-isocohumulone (18.3), trans-isohumulone (19.0), cis-isohumulone & trans-isoadhumulone (20.9), cis-isoadhumulone (22.5), cohumulone (28.0), humulone (28.3), adhumulone (28.1), trans-tetrahydroisocohumulone (25.71), cis-tetrahydroisocohumulone (26.3), trans-tetrahydroisohumulone (27.3), cis-tetrahydroisohumulone & trans-tetrahydroisoadhumulone (30.4), cis- tetrahydroisoadhumulone (31.1) (Oladokun, Smart, & Cook, 2016).

1.6 Factors affecting hop-derived bitterness

Besides variations associated with hop varieties, provenance, crop year etc., achieving consistently high levels of hop utilisation based on the levels of α-acids converted to iso-α-acids and retaining them in beer is of paramount importance to brewers, both from a financial and quality point of view. However, this is often a big challenge for brewers because α-acid isomerisation is inconsistent, time consuming and inefficient (Jaskula, Syryn, Goiris *et al.*, 2007). Upon their generation, losses of iso-α-acids can occur in the trub during fermentation, filtration and maturation, leading to inconsistent concentration of bitter congeners in beer - the perceived intensity and quality of bitterness is consequently altered (Hieronymus, 2012; Jaskula, Syryn, Goiris *et al.*, 2007). Brewing low wort gravity can reduce the likelihood of iso-α-acids being lost in the trub during beer production, because more bittering principles are retained in the wort and consequently in beer (Stewart & Priest, 2006). The loss of iso-α-acids during fermentation can be as high as 20% due to a drop in pH as wort is gradually converted into beer (pH 5.3 to 4.5); iso-α-acids consequently precipitate out of solution and are lost by adsorption onto yeast cells, filter materials or storage containers (Benitez, 1997; Jaskula, Goiris, Opstaele *et al.*, 2009). The boil time is also crucial since isomerisation is temperature dependent, longer boil times of up to about 90 minutes usually increase efficiency of isomerisation but the majority of hop essential oils and other volatiles which are responsible for aroma and flavour in beer are lost to evaporation after about 30 minutes (Verzele, 1965). Nonetheless, the relationship between boil time and utilisation is not linear and boil times in

excess of 90 minutes can lead to the generation of unwanted hop acid breakdown products (Hieronymus, 2012).

1.6.1 Hopping technology

The hopping technology adopted for brewing refers to the type, form, levels and the respective addition points of hops during the brewing process (Van Opstaele, Praet, Aerts *et al.*, 2013). Hopping technology can impact significantly on the flavour profile and the quality of bitterness achieved in beer as a result of the final bittering principle achieved. Whilst conventional hopping practices involve the addition of hops (in various forms) at the start of boil for about an hour and in the last fifteen minutes, brewers now regularly add hops at several stages of the brewing process. It is also common for brewers to add other bittering or flavouring hop products to beer just before bottling. As for gradual introduction of hops into beer at various stages of the brewing process, techniques such as 'first-wort hopping' are common and involve adding ~30% of the total aroma hop required into the 'kettle' during the transfer of sweet wort from the lauter tun into the kettle. In the transfer time, hop essential oils diffuse into the wort as well as oxidise. This method is believed to improve the so called 'kettle flavour' character of beer, yielding a pleasant tasting beer with increased bitterness and 'crisper' mouthfeel (Hieronymus, 2012). However, the attainment of 'kettle flavour' in beer is not solely down to hopping technique, but has been attributed to biotransformation reactions between yeast and hop oil compounds (Takoi, Koie, Itoga *et al.*, 2010). Another hopping technique known as 'dry hopping' involves steeping hops into beer during fermentation or conditioning for a few days to weeks,

during which time most of the hop essential oils diffuse into the beer to give a characteristic hoppy aroma and flavour in beer (Cocuzza & Mitter, 2008; Schönberger & Kostelecky, 2011). The process of dry-hopping is now very popular, and is partly responsible for the boom in craft beer popularity. There is however a downside to this technique with respect to bitterness flavour, the 'hoppy' character achieved as a result of this process is extremely sensitive to factors such as hop variety, growth area and picking date - factors which are mostly out of the brewer's control. Furthermore, in high alcohol beers, compounds within the vegetative matter of hops are easily extracted into the beer, due to the solvating power of ethanol (Schönberger & Kostelecky, 2011). This is perhaps one of the reasons for the noticeable increase in bitterness intensity and 'harsh' bitterness quality observed in dry-hopped beers (Todd, Johnson, & Worden, 1972).

1.7 Taste perception

For a long time researchers have attempted to fully understand how a few taste receptors could suffice to detect several thousands of bitter molecules we encounter on a daily basis and how bitter taste information in general is encoded. As a result, different models were proposed to account for the transduction of taste - the first, commonly referred to as the 'labelled line' model suggests that groups of taste receptor cells (TRCs) are selectively tuned to a specific taste modality or tastants, with the response being sent through afferent nerves specifically tuned to that response only (Lindemann, 2001). The second model known as the 'across-fibre pattern'

model involves two possible pathways where taste receptor cells (TRCs) can be broadly tuned, thereby allowing for a group of TRCs to encode for several tastants, with the afferent fibres carrying information for all taste modalities; or - that the TCRs are still tuned to individual taste modalities but the same afferent fibre carries information for numerous taste modalities (Chandrashekar, Hoon, Ryba *et al.*, 2006). Recent studies by Alder et al. (2000), Chandrashekar et al. (2000) and Nelson et al. (2001) all provided evidence that the receptors cells of bitter, sweet and umami tastants were expressed in distinct taste cell populations only, thus demonstrating that the encoding of bitter, sweet and umami taste modalities fits the labelled-line model (Adler, Hoon, Mueller *et al.*, 2000; Chandrashekar, Mueller, Hoon *et al.*, 2000; Nelson, Hoon, Chandrashekar *et al.*, 2001). Zhang et al. (2003) found that mice genetically modified to lack the ability to perceive bitter, umami and sweet tastes still responded to sour and salty tastes, they therefore concluded that both sour and salty tastes are detected independently of the other three modalities, and possibly through a model involving a different population of cells specifically tuned to ionic tastes (Zhang, Hoon, Chandrashekar *et al.*, 2003). Nonetheless, the observation in several studies that multiple bitter taste receptors were activated, albeit to varying degrees by a wide range of chemically diverse bitter tasting compounds provides some evidence for 'across-fibre patterning model'. For example, the three bitter taste receptors (TAS2R1, TAS2R14, and TAS2R40) identified by Intelmann *et al.* (2009) were activated by a combination of hop bitter acids (Intelmann, Batram, Kuhn *et al.*, 2009). For this reason, it is possible that some level of across-fibre patterning occurs in the taste receptor cells at the

periphery, even if most of the evidence presented to date is highly in favour of the labelled-line model of taste perception.

1.7.1 Bitterness perception

The complexity of bitterness sensation often results in individuals perceiving the same level of bitterness differently or describing the same bitter tastants with different terms. For example, a bitter solution of urea may be described as 'harsh' and 'medicinal' by an individual, while another individual might describe the same bitter solution as 'chalky' and 'lingering'. The lack of a common language and variation between individuals remain some of the major challenges to our understanding of bitterness perception. Bitter taste perception occurs as a result of the activation of receptors present on the exterior of receptor cells in the oral cavity by bitter tasting compounds (Schönberger, 2006). The human taste receptors are located mainly on the surface of the tongue but may also be present in the soft palate, pharynx, epiglottis and even in the larynx (A. Drewnowski, 2001). They are comprised of hundreds of epithelial cells located within taste buds, with the taste buds in turn aggregating to form gustatory papillae which are visible as little red dots on the surface of the tongue (Figure 1.9). The three major different types of papillae (fungiform, foliate and circumvallate papillae) are all located in various parts on the surface of the tongue (Rouseff, 1990). The reception of bitter taste is particularly concentrated but not limited to the back of the tongue (Jackson, 1994).

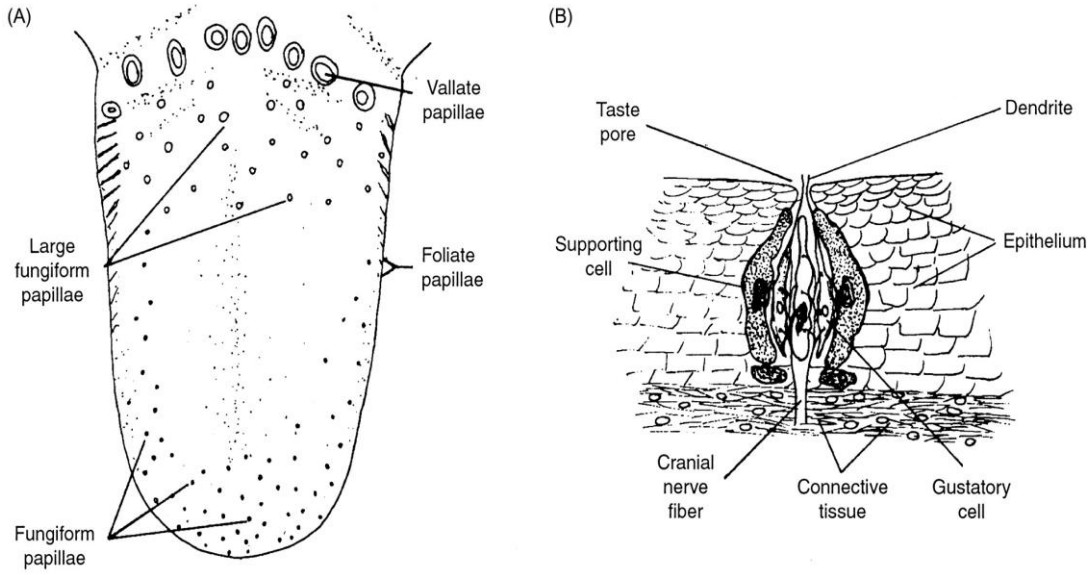


Figure 1.9: The taste receptors. A; The tongue and gustatory papillae, B; taste bud (Jackson, 1994).

A group of G-protein coupled receptors which are encoded by the TAS2R gene family mediate the perception of bitterness in humans (Chandrashekar, Mueller, Hoon *et al.*, 2000; Intelmann, Batram, Kuhn *et al.*, 2009; Maehashi, Matano, Wang *et al.*, 2008) Critically, each TAS2R receptor can be activated by various bittering compounds and most bitter compounds are capable of activating several TAS2R receptors at once, hence the recognition of numerous bitter tastants is possible and has been widely reported (Behrens, Reichling, Batram *et al.*, 2009). However, this has also caused complications in the continuing research to identify the bitter tastants which bind to these receptors. About 20 of the known 25 TAS2R receptors have had their cognate bitter molecules assigned, with three receptors in particular (hTAS2R1, hTAS2R14, and hTAS2R40) found to be activated by different hop bitter acids (Intelmann, Batram, Kuhn *et al.*, 2009). Some polyphenols, including epicatechin have also been reported to activate TAS2R4, TAS2R5 and TAS2R39 receptors (Soares,

Kohl, Thalmann *et al*. 2013). The findings of the adaption of TAS2R receptors to activation by numerous agonist bitter compounds is further reinforced by other research which has indicated that both TAS2R1 and TAS2R14 can be activated by other bitter compounds such as picrotin and bitter peptides of casein hydrolysates (Maehashi, Matano, Wang *et al.*, 2008).

1.7.2 Taste sensitivity

Inherent genetic traits can result in amplified sensitivity to certain tastants or specific taste deficiencies e.g. ageusia. It is widely accepted that ~25% of the population have an increased density of fungiform papillae and taste pores on their tongue which causes an increased aversion to bitter compounds such as 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC) (Bartoshuk, Duffy, & Miller, 1994). This sensitivity is a heritable trait which places the population into three categories based on their ability to taste PROP; supertasters (~25%), medium tasters (~50%) and non-tasters (~25%) (Bartoshuk, 1993). Supertasters, a majority of which are women, demonstrate a strong dislike for bitter tasting compounds regardless of the source (Dinehart, Hayes, Bartoshuk *et al.*, 2006; Drewnowski & Gomez-Carneros, 2000). Therefore, an individual's PROP status can reasonably be expected to strongly influence how they rate and judge both the intensity and quality of bitterness in beer. The gender effects in perceived bitterness observed by Bartoshuk et al. (1994) correlate well with the fact that the majority of PROP supertasters are women, but the author also cautioned that this does not necessarily make female panellists better assessors of bitterness or indeed bitterness quality

(Bartoshuk, Duffy, & Miller, 1994). PROP status reportedly had no effect on the perception of wine bitterness and astringency temporality (Thorngate & Noble, 1995). In 2000 Cruz and Green identified another genetic marker of individual taste variation, involving the perception of a 'phantom' taste by certain individuals; as a direct result of temperature stimulation of the tongue in the absence of a stimuli (Cruz & Green, 2000). These individuals are known as Thermal Tasters (TT) and have been found to display a high level of sensitivity at supra-level to aroma, taste and other trigeminal sensations (Clark, 2011; Green & George, 2004; Yang, Hollowood, & Hort, 2014). Studies into how the flavour of wine and beer are perceived by thermal and thermal non-tasters (TnT) status have shown that TTs tend to rate sweetness, sourness, bitterness, astringency and overall flavour intensity higher than TnTs (Pickering, Moyes, Bajec *et al.*, 2010; Pickering, Bartolini, & Bajec, 2010).

1.8 Sensory analysis of bitterness in beer

In order to better understand the perception of bitterness in beverages such as wine, coffee and beer, several sensory techniques have been employed as they better characterise human psychophysical response to tastants in beverages and foods (Murray, Delahunty, & Baxter, 2001). For qualitative sensory analysis of beer bitterness, Free Choice Profiling (FCP) or other forms of descriptive sensory methods such as Qualitative Descriptive Analysis (QDA), Check-All-That-Apply (CATA) are commonly used (Adams, Williams, Lancaster *et al.*, 2007; Fritsch & Shellhammer, 2009; McLaughlin, Lederer, & Shellhammer, 2008). For intensity and magnitude differentiation rating techniques such as rank-rating or intensity

rating methods can be used (Kim & O'Mahony, 1998; Lawless & Heymann, 2010). However, since the temporal properties of bitterness are not adequately captured by descriptive sensory techniques - Time Intensity (TI) measurements or other temporal techniques such as Time Dominance of Sensation (TDS) are often used to evaluate the temporal properties of bitterness in beer (Cliff & Heymann, 1993; King & Moreau, 1996; Labbe, Schlich, Pineau *et al.*, 2009; Noble, 1995). TI analysis of bitterness allows the temporal facet of bitterness to be captured via the extraction of parameters such as maximum perceived intensity (Imax), total duration of taste perceived (Tdur), time taken to reach maximum intensity (Tmax) and area under the curve (AUC), which can be acquired from trapezoid calculations for bitterness intensity (Figure 1.10) (Cliff & Heymann, 1993; Fritsch & Shellhammer, 2009).

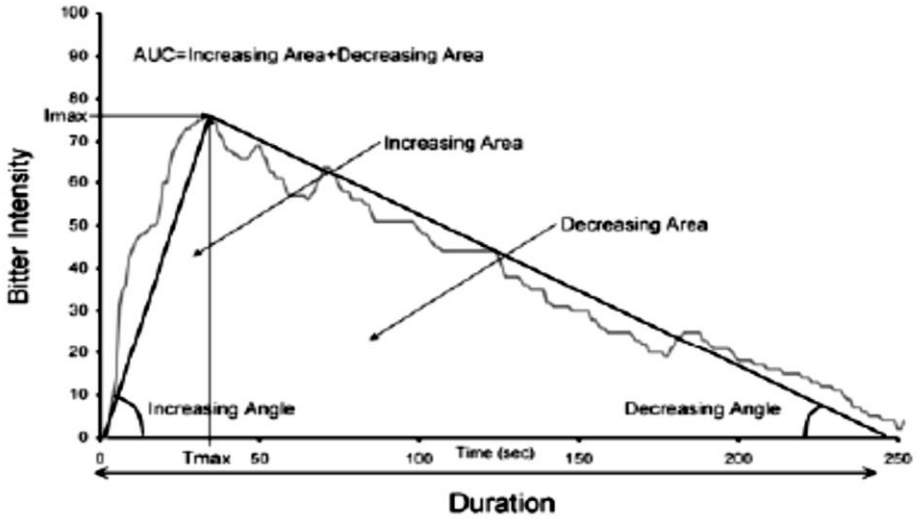


Figure 1.10: Parameters extracted from a time-intensity curve (Fritsch & Shellhammer, 2009).

Hughes and Bolshaw (1995) first evaluated temporal variations between reduced-iso-α-acids and iso-α-acids at equimolar concentrations in water before further studies revealed that the intensities of iso-α-acids relative to that of the reduced hop acids show a differential bitterness time-course (Hughes & Bolshaw, 1995; Weiss, Schönberger, Mitter *et al.*, 2002). More recently, temporal differences between the bitterness profiles of iso-α-acids and their reduced derivatives in an unhopped beer were investigated; Tetra was found to exhibit a linger, and create a greater bitterness impact when compared to Rho for example, whereas there was no difference in the temporality of Iso, Rho and Hexa (Fritsch & Shellhammer, 2009). Qualitative data generated by FCP for these hop-derived bitter tastants found Hexa to be more 'medicinal', 'metallic' and 'sharp' whilst, Rho and Iso were reported to exhibit positive bitterness qualities, described as 'smooth' in comparison to Hexa and Tetra. Tetra was also found to be 'harsher', 'medicinal' and 'aspirin-like', although the same study found little correlation between the hop bitter acids described as being caffeine or quinine-like and these bitter compounds themselves (Fritsch & Shellhammer, 2009). These studies provide more evidence of temporal and qualitative differences between the various hop-derived bittering products available to brewers to bitter beer.

The study presented in this research project improves on the current knowledge in the scientific literature regarding the perception of bitterness quality, in terms of impact of brewing practices allied to hopping technology, hop aroma and hop variety.

1.9 What is 'Quality of Bitterness'?

The meaning of the term 'Quality of Bitterness' remains unclear even to many in the brewing industry who often use the term! Besides the aforementioned challenges that bitterness sensation and variation in its perception pose, bitterness sensation is clearly multifaceted. The proof for this can be seen in some of the attributes commonly used to describe the perceived 'Quality of Bitterness' in beer. These terms include attributes such as 'harsh', 'smooth', 'lingering', 'harmonious', 'astringent' and 'metallic' - terms which capture, in part, key properties of taste such as time-course ('lingering') and mouthfeel ('astringent'). Furthermore, it is clear that some of these bitterness attributes are in normal usage considered positive ('harmonious') whilst others (e.g. 'metallic') might be considered less desirable. In addition, the hedonic effect of these qualitative terms is doubtless context dependent – i.e. varies with the sensory properties of a particular beer. The word 'quality' in 'Quality of Bitterness' can be a source of confusion, because this descriptive noun is also commonly used interchangeably to describe the five taste modalities that humans recognize, i.e. sour, sweet, bitter, salty and umami. An alternative term to aid our approach to understanding bitterness, in relation to the aims of this research is 'character'. Character is defined in the English dictionary as 'the combination of traits and qualities distinguishing the individual nature of a person or thing'. As such, this definition allows us to investigate bitterness as a wholesome flavour percept, allowing us to look at bitterness perception based on multiple facets – including its intensity, temporal and spatial characteristics (McBurney, 1976). In this regard, the intensity of bitterness refers to the

magnitude of the bitterness sensation elicited by bitter tastants at a point in time. Temporal facets cover the time-course of bitterness intensity elicited by bitter tastants as perceived over a period of time, while the spatial pattern of bitterness sensation refers to the location of bitterness sensation on the tongue and in the oral cavity i.e. at the tip of the tongue or at the back of the throat (Keast & Breslin, 2003; McBurney, 1976). This combination of bitterness facets, which are best captured with a combination of sensory methods, constitute 'Quality of Bitterness'. A good example of quality in relation to taste can be found in sweet sensation (O'Donnell, 2012), where sweeteners – both non-calorific (saccharin, aspartame, cyclamate) and carbohydrate sweeteners (sucrose) when presented at appropriate concentrations all elicit a sweet taste. However, this sweet taste is often closely accompanied by other taste characters which have been described variously as 'metallic', 'bitter', 'cooling' or 'liquorice-like' (O'Donnell, 2012). Furthermore, artificial sweeteners also exhibit differential temporal profiles in comparison to sucrose, thus affecting the quality of sweet taste perceived in the products they are sweetened with (Portmann & Kilcast, 1996; Schiffman, Crofton, & Beeker, 1985).

1.10 Research objectives and thesis layout

This research project on the quality of bitterness in beer used a multidisciplinary approach involving both analytical and sensory science. A series of analytical techniques were used to quantify several known, and some potential, bitter tastants in beer (Chapter 2). In Chapter 3, sensory techniques including Descriptive Analysis (DA) and intensity ratings were used to investigate the qualitative facets of selected exemplar beers based on the analytical measures presented in Chapter 2. One of the key aims of the sensory study presented in Chapter 3 was to develop a bitterness lexicon with which to characterise qualitative (both positive and negative) aspects of bitterness character. In Chapter 4, findings on the impact of hop aroma (in the form of hop essential oils) at different BU levels on bitterness quality of beers are presented - in order to shed light on the effect of cross-modal flavour interactions on the perception of beer bitterness quality. In Chapter 5, bitterness quality in relation to three distinctively different hop varieties (Hersbrucker, East Kent Goldings and Zeus) and the impact of hop aroma extracts is presented. The last study was designed to determine if the use of distinctively different hop varieties for beer bittering confers the attained beer (at equibitter intensity levels) with specific bitterness quality as a result of the hop used in producing the beer.

Overall conclusions and suggestions for further work are presented in Chapter 6.

The study presented in Chapters 2 & 3 has been published and correspond to:

Oladokun, O., Tarrega, A., James, S., Smart, K., Hort, J., & Cook, D. (2016). The impact of hop bitter acid and polyphenol profiles on the perceived bitterness of beer. *Food chemistry*, *205*, 212-220.

The study presented in Chapter 4 has been published and correspond to:

Oladokun, O., Tarrega, A., James, S., Cowley, T., Dehrmann, F., Smart, K., Cook, D. and Hort, J., (2016). Modification of perceived beer bitterness intensity, character and temporal profile by hop aroma extract. *Food Research International*, *86*, pp.104-111.

The study presented in Chapter 5 has been published and correspond to:

Oladokun, O., James, S., Cowley, T., Dehrmann, F., Smart, K., Cook, D. and Hort, J., (2017). Perceived bitterness character of beer in relation to hop variety and the impact of hop aroma. *Food chemistry*, 230, 215 – 224.

In all cases the first author conducted, analysed and drafted the published manuscript under the guidance and with critical input from co-authors.

2 ANALYTICAL CHARACTERISATION OF COMMERCIAL BEERS FOR BITTER-TASTING CONGENERS

2.1 INTRODUCTION

Beer is composed of several chemical constituents including sugars, nitrogenous materials such as amino acids, vitamins; proteins, lipids, organic acids, sulfur compounds, polyphenols and hop acids (MacWilliam, 1968). Of particular interest in this study are hop-derived compounds, amino acids and polyphenols which, as discussed in Chapter 1 contribute to perceived bitterness in beer. Bitterness is enjoyed in foods and beverages such as coffee, nuts, fruits and beer (Lesschaeve & Noble, 2005). Whereas the bitterness characters of tea and red wine have been attributed mainly to flavonoid phenols, approximately 70% of beer bitterness is typically derived from the addition of hops (*Humulus lupulus*) during the boiling stage of the brewing process to yield iso-α-acids from α-acids (Arrieta, Rodríguez-Méndez, De Saja *et al.*, 2010; Caballero, Blanco, & Porras, 2012). In recent years beer-bittering practice has diversified, with the development and usage of hop products in a variety of different forms, and with varied points of addition to the brewing process (e.g. kettle addition, post-fermentation bittering products, or 'dry hopping', which is also feasible at a number of different points).

In addition to α-acids, hops are also a source of polyphenols in beer and the amount of polyphenols derived from hops will depend on hop variety, form and the point at which the hops are added during the brewing process. However, depending on hopping levels, brewing malt usually represents the major source of polyphenols in beer (Aron & Shellhammer, 2010; Callemien & Collin, 2009). Hop polyphenols are a class of compounds

with diverse chemical structures and can be divided into groups consisting of simple hydroxycinnamic and hydroxybenzoic acid derivatives (phenolic acids), flavanols, flavonol glycosides and prenylated flavonoids (Goiris, Jaskula-Goiris, Syryn *et al.*, 2014). Polyphenols contribute to bitterness, colour, body, and astringency in beer and other beverages such as tea and wine, (Collin, Jerkovic, Bröhan *et al.*, 2013) and have been recognised to influence the acceptance of beverages (Drewnowski & Gomez-Carneros, 2000). Several phenolics including ferulic acid, *p*-coumaric acid and protocatechuic acid have been noted to elicit astringency (Callemien & Collin, 2009) - the oral sensation perceived as a drying, puckering or rough mouth-feel and resulting from the precipitation of proline-rich proteins in saliva by polyphenols (McLaughlin, Lederer, & Shellhammer, 2008). Flavanol monomers such as catechin and epicatechin were found to be more bitter than astringent (Drewnowski & Gomez-Carneros, 2000; Peleg, Gacon, Schlich *et al.*, 1999). Several non-hop derived compounds e.g. amino acids (tyrosine & tryptophan) and brewing parameters such as pH, gravity, density and alcohol content, which are key indicators of brewing processes, all contribute to the overall body and mouthfeel of beer e.g. whether 'thin' or 'full' and can consequently impact the perceived quality of bitterness in beer (Guinard & Mazzucchelli, 1996; Langstaff & Lewis, 1993).

It is widely accepted within the brewing industry that the bitterness characteristics of beers differ due to factors not determined using the simplistic analytical measurement of bitterness units (BU). It is anticipated that this might relate to the diversity and varying concentrations of hop-derived and non-hop-derived bitter tasting compounds found in beer as a

result of hop products, hopping strategies, and overall brewing practice employed across the industry in bittering beer. Whilst there is some knowledge of the individual bitterness qualities which hop acid isomers impart to beer (Fritsch & Shellhammer, 2009), the links between hopping practice, bittering congener profile and the perceived bitterness characteristics of beers remains poorly understood.

The research reported in this chapter is based on a sample set of 34 commercial lager beers which were sourced from around the world. Beers were analysed for a wide array of compounds with the potential to impact on bitterness perception, namely: hop-derived bitter compounds, polyphenolic compounds and amino acids. Furthermore, the beers were characterised in terms of their pH, gravity, density and alcohol contents.

2.2 MATERIALS

2.2.1 Beer samples

34 fresh commercial lager beers were sourced from 17 countries over 4 continents and analysed within 8 weeks of production. For reasons of confidentiality the beers are not identified but the countries from which they were sourced are as follows: Australia (2), Belgium (1), Cuba (1), Czech Republic (6), Denmark (1), France (1), Germany (2), Hungary (1), Italy (2), Netherlands (3), Poland (2), Peru (1), Romania (1), South Africa (3), Turkey (1), UK (2) and USA (4).

2.2.2 Chemicals

2.2.2.1 Phenolic acid standards

Hydroquinone (99%), catechin (99%), epicatechin (98%), 4-hydroxybenzoic acid (99%), caffeic acid (95%), vanillic acid (97%), syringic acid (95%), *p*-coumaric acid (98%), sinapic acid (98%), ferulic acid (99%), 2,5-dihydroxybenzoic acid (98%), gallic acid (98%), cinnamic acid (98%), phenyl acetic acid (99%), 1,2-dihydroxybenzene (99%), hydroxymethylfurfural (99%), 2-phenylethanol (99%) were all purchased from Sigma Aldrich (UK). 4-hydroxyphenylacetic acid (99%) was acquired from Alfar Aesar and protocatechuic acid (99.6%) from HWI analytics.

2.2.2.2 Extraction chemicals

Ethyl benzoate, isooctane and methanol (all HPLC grade) as well as orthophosphoric acid 85% (RPE grade) were purchased from VWR (UK). Reverse osmosis (RO) water prepared with an Omega filtration system was used for the preparation of phenolic and external standards, hop acid standards, HPLC mobile phase and for the extraction of polyphenols from beer.

2.2.2.3 Total polyphenol content

Carboxymethylcellulose (CMC), ethylenediamine tetraacetic acid (EDTA), ammonia and ferric reagent solutions were all analytical grade chemicals purchased from VWR (UK).

2.2.2.4 Humulinone synthesis

CO_2 extract of α-acid resin (86%) was kindly donated by Botanix (Paddock Wood, Kent, UK.) Cumene hydroperoxide, diethyl ether, sodium bicarbonate, hexane, phosphoric acid and hydrochloric acid (HCl) were all purchased from Sigma and of analytical grade.

2.2.2.5 Hop acid standards

International Calibration hop Standards (ICS) used for HPLC calibration were acquired from Labor Veritas (Zurich, Switzerland). The International Calibrant Extract 3 (ICE-3) contained α- and β-acids [44.64% (w/w) α-acids, 24.28% (w/w) β-acids]. The dicyclohexylamine salt of iso-α-acids (DCHA-ISO) was 62.30% (w/w), containing only trans-iso-α-acids. For reduced iso-α-acids, the DCHA salt of Tetra was 99.40% (w/w).

2.2.3 Instrumentation

2.2.3.1 HPLC

HPLC analyses were carried out on a Waters Alliance 2695 instrument equipped with a column heater and a membrane degasser. Detection was achieved with a UV detector and peak areas were processed with the operating HPLC software (Empower 2). Separation of phenolics and hop acids was achieved with a Purospher STAR rp-18 end-capped column (250 X 4.6 mm, 3 µm) from Merck Millipore (UK) coupled with a C18 guard cartridge. A roller bed (Thermo Scientific, UK) was utilised for the purpose of extraction.

2.2.3.2 GC-MS

GC-MS analysis was used in combination with the EZ:FAAST amino acid assay kit to determine the amino acid concentrations of the beers. A trace DSQ II GC-MS (Thermo Fischer, Manchester, UK) fitted with an AAA column (10 m x 0.25 mm ID, Phenomenex, UK) was used.

2.2.3.3 DMA 4500

Beer brewing parameters including density, specific gravity (SG), present gravity (PG), original gravity (OG) and percent alcohol content (ABV) were analysed using an Anton Paar DMA 4500 coupled to an Alcolyzer Plus

(Anton Paar, Graz, Austria). A Jenway 3510 pH meter, calibrated before use each time was used for the determination of beer pH.

2.3 METHODS

2.3.1 Determination of beer phenolic acid profile

2.3.1.1 External standard preparation of phenolic standards

A stock solution (20 mg/L) of standard grade phenolic acids was prepared by dissolving (0.01 g) of the standards in a 500 mL volumetric flask containing a mixture of RO water and methanol (~200 mL) before making up to mark with the same solvent mixture. Serial dilutions were made from the stock solution to achieve external standard concentrations of 10, 5, 2.5 and 1 mg/L.

2.3.1.2 Extraction of phenolic acids from beer

Phenolic acids were extracted from beer by a newly developed double solvent extraction process. Degassed beer (10 mL) was transferred into a 50 ml centrifuge tube before acidification with orthophosphoric acid (500 µL). Ethyl acetate (20 mL) was then added before extraction on a roller bed for 1 h. After extraction, the residual beer from the bilayer mixture was discarded before RO water (10 mL) was added and the tube contents mixed on the roller bed for another 30 min. The water layer was subsequently removed and discarded whilst the extracted ethyl acetate layer was transferred into a glass tube and dried down under a controlled flow of Nitrogen using a Visidry attachment coupled to a SPE manifold (Supelco). The residue was reconstituted in a fixed volume of methanol (2 mL) and transferred into glass vials ready for HPLC analysis.

2.3.1.3 HPLC determination of phenolic acids in beer

The chromatographic method consisted of a binary solvent system of (A) 1.25 % v/v acetic acid and (B) 0.1% v/v orthophosphoric acid in acetonitrile. The gradient elution protocol was as follows: 0-25 min: 98% A, 2% B; 25-30 min: 76% A, 24% B; 35-40 min: 55% A, 45% B; 45 min: 15% A, 85% B; 50 min: 0% A, 100% B; 55-65 min: 98% A, 2% B. Injection volume was 10 µL with a flow rate of 0.5 mL/min and a run time of 65 min. Column temperature was set at 30°C and the UV spectra of the phenolic acids were recorded in the range of 220 – 330 nm, the phenolic acids peak areas were extracted at 280 nm.

2.3.2 Determination of beer total polyphenolic content

Beer total polyphenolic content was determined by the international method proposed by the American Society of Brewing Chemists (ASBC) (Beer- 35). This method is based on the reaction of polyphenols with ferric iron in alkaline solution. Beer (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1%) and ethylenediaminetetraacetic acid (EDTA, 0.2%, 8 mL) in a volumetric flask (25 mL). A solution 3.5% ammonium ferric citrate (0.5 mL) was added, followed by ammonium hydroxide (33% in H_2O, 0.5 mL) with mixing after each addition before making up to mark with RO water. The solution was left to stand at room temperature for 10 min before measuring absorbance at 600 nm in a 2.5 mL cuvette; the recorded absorbance was multiplied by 820 to yield the total polyphenolic value reported in mg/L.

2.3.3 Determination of beer hop acid profile

2.3.3.1 Preparation of hop acid standards

External standard solutions of α-acids (0.5, 1, 2, 4 and 8 mg/L), Iso (1, 10, 20, 40 and 60 mg/L), Tetra (0.5, 1, 2, 4 and 8 mg/L) and Humulinones (0.5, 1, 2, 4 and 8 mg/L) were all prepared in 5% acetonitrile in water and extracted similarly to beer samples as described in Section 2.3.3.3. These standards were used to plot calibration curves for the quantification of hop bitter acids in the beer samples.

2.3.3.2 Organic synthesis of Humulinones from Humulones

Due to the commercial unavailability of humulinones a pure sample was synthesised in the laboratory from humulone resin, a CO_2 extract of hops (86.3% α-acids), using a modified version of the method described by (Taniguchi, Matsukura, Ozaki *et al.*, 2013). Humulone (1.41 g) and cumene hydroperoxide (0.7 mL) were dissolved in diethyl ether (7 mL). A solution of saturated sodium bicarbonate ($NaHCO_3$, 6g dissolved slowly in 40 ml RO water) was added to the solution and kept at room temperature in a sealed vessel for 5 days, after which the sodium salts of humulinones were generated. The salts were filtered and washed with water (150 mL) and diethyl ether (150 mL) twice under vacuum in a Buchner flask and funnel. The crude extract (1.45 g) was subsequently dissolved in methanol (100 mL) containing 1% v/v phosphoric acid before the addition of a 0.5N HCl (800 ml) solution. The solution was partitioned with hexane (1000 mL x 2) before evaporating the hexane layer to dryness to yield humulinones (99% purity by HPLC).

2.3.3.3 Extraction of hop bitter acids from beer

Cold beer was degassed with a Thermo scientific magnetic stirrer for 1 h followed by the transfer an aliquot (10 mL) into a 50 mL centrifuge tube. The beer sample was acidified by the addition of orthophosphoric acid (100 µL) and an internal standard (benzoic acid) was added (0.003 mg/L). The mixture was then extracted into isooctane (20 mL) 1 h on a roller bed. The isooctane extract was transferred into a glass tube and evaporated under a controlled flow of Nitrogen with a Visidry attachment coupled to a solid phase extraction manifold (Supelco). The residue was dissolved in acetonitrile (2 mL) and a second internal standard of ethyl benzoate (0.011 mg/L) added to give the HPLC sample.

2.3.3.4 HPLC determination of hop bitter acids in beer

The mobile phase consisting of a binary solvent system of (A) 1% v/v acetic acid and (B) acetonitrile acidified with orthophosphoric acid (0.1%) v/v was degassed with the system degasser unit. The gradient elution protocol for the method was as follows: 0-5 min: 30% A, 70% B; 15 min: 20% A, 80% B; 20 min: 20% A, 80% B; 25 min: 10% A, 90% B; 30 min: 10% A, 90% B; 35 min: 0% A, 100% B; 44 min: 0% A, 100% B; 46 min: 30% A, 70% B; 55 min: 30% A, 70% B. The injection volume was 10 µL with a flow rate of 0.5 mL/min over a 55 min run time.

Column temperature was maintained at 25°C (ambient temperature). UV spectra of eluting compounds were recorded in the range 220 – 330 nm. Iso-α-acids and Tetra chromatograms were extracted at 270 nm whilst α- and β-acid chromatograms were extracted at 310 nm.

2.3.4 Determination of bitterness units (BU)

Bitterness units were determined using the ASBC international method Beer-23A. Beer (5 mL) was transferred into a 50 mL centrifuge tube and acidified with 3N HCl (0.5 mL). Isooctane (10 mL) was added and the mixture was shaken by hand three times before extraction on a roller bed for 15 min. The mixture was subsequently centrifuged at 400 x g twice for 5 min each time to ensure complete phase separation. An aliquot of the clear isooctane layer was transferred into a cuvette and absorbance was measured with a spectrophotometer at 275 nm. The recorded absorbance was multiplied by an empirical factor of 50 to give BU values in mg/L.

2.3.5 Determination of beer amino acid content

Beer amino acids were extracted and derivatized with the EZ:FAAST amino acid kit (Phenomenex, Macclesfield UK). Beer (25 µL) was added to an internal standard solution of norvaline (100 µL) before slowly drawing the mixture through a sorbent tip. This was followed by washing of the sorbent bed with a solution of propanol (200 µL), before the sorbent bed was eluted into an eluting solution (200 µL) consisting of propanol and sodium hydroxide. Chloroform (50 µL) and isooctane (100 µL) were sequentially added, with vortexing after each addition to derivatize the amino acids in solution. The derivatized amino acids were recovered in the organic layer, transferred into a GC vial using a glass pipette and purged down with nitrogen. The derivatized amino acids were reconstituted in a mixture of isooctane: chloroform (80:20, v/v) (100 µL) and transferred into the GC vial insert for GC-MS analysis.

2.3.5.1 GC-MS analysis of amino acids in beer

Derivatized amino acids were analysed with a Trace DSQ II GC-MS (Thermo Fisher, Manchester, UK). Samples (1 uL) were injected in splitless mode using an AS3000 autosampler (Thermo Fisher, Manchester, UK) onto an AAA column (10 m x 0.25 mm ID; Phenomenex, Macclesfield, UK). GC carrier gas was Helium at a pressure of 8 psi. Initial oven temperature was set at 90°C and this was ramped to 320°C at a rate of 20°C per min. For detection, the mass spectrometer was set to selected ion mode (sim) to monitor ions of m/z 84, 101, 114, 116, 130, 144, 146, 155, 156, 158, 172, 180, 184, 243 and 244 with a 0.03 sec dwell time. Samples were analysed in triplicate and calibration was achieved by comparison of peak areas of the amino acid in the external standard to those in the samples after normalization to the norvaline internal standard.

2.3.6 Statistical analysis

The XLSTAT package version 2015.6 (Addinsoft, Paris) was used for statistical analysis. Beer amino acids were grouped based on the total sum of these compounds in the beers using Algometric Hierarchical Clustering (AHC) with Ward's method. Significance between clusters was determined by simple means test of the sum of amino acids in each cluster. Pearson R correlation Principal Component Analysis (PCA) was used for grouping of beers selected for sensory evaluations.

2.4 RESULTS AND DISCUSSION

2.4.1 Phenolic profile of beers

The liquid-liquid extraction protocol using ethyl acetate and water enabled the effective analysis of quantitatively significant phenolic compounds in beer. An example of the chromatographic separation achieved with the extraction protocol and HPLC method for the Czech lager beer (L) is provided in Appendix 1. The HPLC method enabled the simultaneous separation and quantification of several phenolics in beer. Where possible, the phenolic compounds were identified based on both prior knowledge and by matching peaks against authentic standards run separately, and with regard to both retention time and UV absorbance spectrum. Whilst not all peaks on the trace could be identified, unknown peak areas were also integrated and are included in the analytical profiles of the beers, labelled as unknown 1, 2….etc. The elution pattern of phenolic acids in beer followed an order of decreasing polarity under RP-HPLC conditions, thus phenolic acid derivatives of benzoic acid were eluted before the hydroxycinnamic acid derivatives. The polarity of phenolic acids is increased mostly by the hydroxyl group at the para-position, followed by the ortho- and meta-positions of the benzene ring (Torres, Mau-Lastovicka, & Rezaaiyan, 1987). To illustrate the variability present in the data set for the phenolic profile of each of the 34 beers, the total contents of the quantified phenolic compounds in each beer was summed (Table 2.1) - and shows a substantial range of concentrations (3.9 to 21.2 mg/L).

Table 2.1: Variation in the total analysed concentrations of phenolic compounds (by HPLC) in 34 commercial lager beers.

		TPCC* (mg/L)	
BEER	**COUNTRY**	**SUM**	**SE**
O	USA	21.17	1.5
Q	USA	13.12	0.8
HH	Czech Republic	18.25	1.5
E	Czech Republic	17.73	1.8
L	Czech Republic	13.87	1.1
AA	UK	12.26	1.1
T	Czech Republic	12.73	0.6
I	Czech Republic	12.05	1.0
W	Belgium	11.39	0.4
G	Hungary	11.26	0.8
X	Czech Republic	9.61	0.2
M	Italy	9.17	1.0
K	Czech Republic	9.28	1.5
Y	Netherlands	8.45	0.7
Z	Turkey	8.18	0.4
GG	Romania	8.89	0.5
A	Poland	8.47	0.4
EE	Peru	8.26	0.8
N	France	7.44	0.7
CC	Italy	7.30	0.5
B	Poland	7.67	0.5
R	USA	6.55	1.0
DD	Australia	5.75	0.5
V	UK	6.45	0.5
FF	Cuba	6.32	0.7
S	Denmark	5.12	0.2
P	Germany	6.10	0.2
J	Germany	5.75	0.6
H	Netherlands	4.79	0.5
U	Netherlands	5.84	1.0
C	South Africa	5.59	0.2
BB	Australia	4.21	0.5
D	South Africa	4.42	0.3
F	South Africa	3.91	0.1

* TPCC = total phenolic compound concentration of gallic acid, hydroquinone, protocatechuic acid, catechin, epicatechin, 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, caffeic acid, vanillic acid, sinapic acid, syringic acid, p-coumaric acid, ferulic acid and cinnamic acid quantified in beer by HPLC. SE is standard error of three independent replicate analyses.

Ferulic acid was the most abundant phenolic acid present in the beers, with a concentration ranging from 0.98 mg/L in the Australian lager (BB) to 7.61 mg/L in the American lager beer O (data not shown). *p*-coumaric acid is the precursor compound to ferulic acid and is formed via the shikimic acid reaction pathway, therefore the concentration of ferulic acid is usually greater than that of *p*-coumaric acid in beer (Garcia, Grande, & Gándara, 2004). The concentrations of *p*-coumaric acid across the beers followed a similar pattern as observed for ferulic acid, with beers BB and O containing 0.37 mg/L and 3.07 mg/L respectively (data not shown). Beer O was also found to contain the highest amount of phenolic compounds overall (21.17 mg/L) (Table 2.1); whilst beer F, a South African lager beer had the lowest concentration at 3.91 mg/L. Beers brewed in Germany (J, P), South Africa (F, C, D), Denmark (S) and Australia (BB, DD) all had phenolic compound concentrations below 8 mg/L whilst the Czech beers (I, E, L, HH) and American lagers (O, T, Q) all had phenolic compound concentrations of >10 mg/L. These values represent the total free phenolic acid content of beer which is reported to be approximately 10 - 20% of total beer polyphenol content, since a significant portion of beer phenolics are suggested to exist in bound form (Floridi, Montanari, Marconi *et al.*, 2003). The Czech beer (HH) was the only sample that contained gallic acid, whilst catechol, chlorogenic, salicylic, homovanillic and gentisic acids were not detected in the beers, in agreement with reports from other studies (Garcia, Grande, & Gándara, 2004; Jandera, Škeříková, Řehová *et al.*, 2005).

2.4.2 Correlation between Total Polyphenol (ASBC) and phenolic acid contents by HPLC

The TPC of the beers was found to range between 74 and 256 mg/L. Similar values (70 – 240 mg/L) were reported by Dvorakova et al. (2007) (Dvorakova, Hulín, Karabin *et al.*, 2007). The lowest concentration was found in the Hungarian lager beer (G) whilst beers O and T, both American lagers and the British lager beer (AA) all had polyphenol contents greater than 250 mg/L. Beer O contained the highest amount of phenolic compounds and total polyphenol content. A plot of total phenolic compound concentration versus TPC is displayed in Figure 2.1.

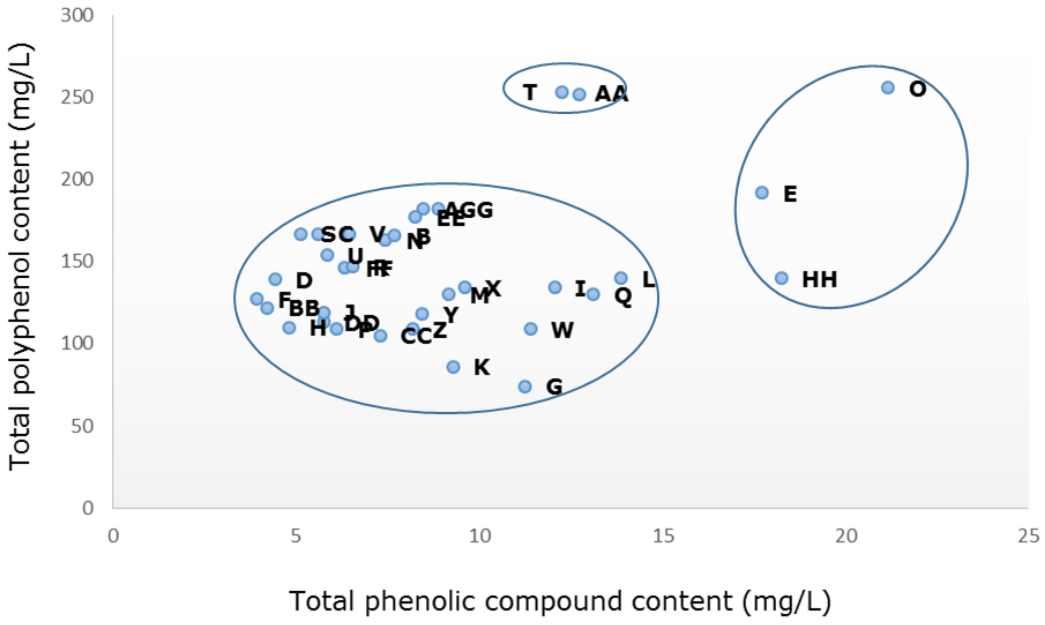


Figure 2.1: Plot of total phenolic compound concentrations (HPLC) versus total polyphenol content (according to ASBC method Beer-35) for 34 commercial lager beers.

The plot has been annotated to show three main clusters. The first cluster is of beers characterised by TPC values of approximately 74 – 180 mg/L and phenolic compound contents ranging between 3 and 15 mg/L. Beers AA and T formed a separate cluster, due to the high TPC in these beers (>250 mg/L). In the last cluster, consisting of beers E, O and HH, TPC ranged from 145 – 253 mg/L and phenolic compounds were in excess of 15 mg/L. Interestingly, this data shows that there was no linear relationship between TPC and the sum of phenolic compounds quantified. This is most likely due to the highly-varied brewing techniques and ingredients employed in the industry. The observed higher TPC concentrations (>250 mg/L) in beers T, AA and O suggests that these beers were dry-hopped products.

2.4.3 Hop bitter acid profile of beers

For the bitterness profiles of these beers, the bitter tasting hop acids present in the lager beers were evaluated using two separate analytical methods; firstly, by HPLC as described in section 2.3.3.4 and secondly by the common international bitterness unit (IBU) method described in section 2.3.4. The latter method has been suggested to give inflated bitterness values due its susceptibility to interference from other compounds present in beer that absorb light at the wavelength of measurement (Schönberger, 2006; Tomlinson, Ormrod, & Sharpe, 1995). In contrast, HPLC measurements are agreed to provide a better assessment of beer bitterness because they allow for the selective quantification of iso-α-acids, the major bittering principles in beer (Ting, Kay, & Ryder, 2007). A comparison of the hop bitter acid concentrations

in the beers by BU and HPLC methods is presented in Figure 2.2. The results showed that the BU of the lager beers ranged from 8 - 36 mg/L, although bitterness was overestimated by the BU method in comparison to HPLC values in around 60% of the samples. The sum of analysed iso-α-acid hop acid concentrations (HPLC) was between 8 and 41 mg/L. The average bitterness across all 34 beers in both methods was ~23 mg/L and the most bitter beers had concentrations >30 mg/L (GG, AA and L). The latter beers each had lower BU values in comparison to HPLC values, e.g. beer AA had a BU value of 34 mg/L but the HPLC value was 41 mg/L. This could potentially be explained by extraction variation in the BU method, resulting from the complexity of these beers. Around 7 of the beers including U, J, I, E, C and T had similar bitterness concentrations according to both methods. The American light lager beer R contained the lowest amount of hop acids (8 mg/L) and did not contain any reduced iso-α-acid products.

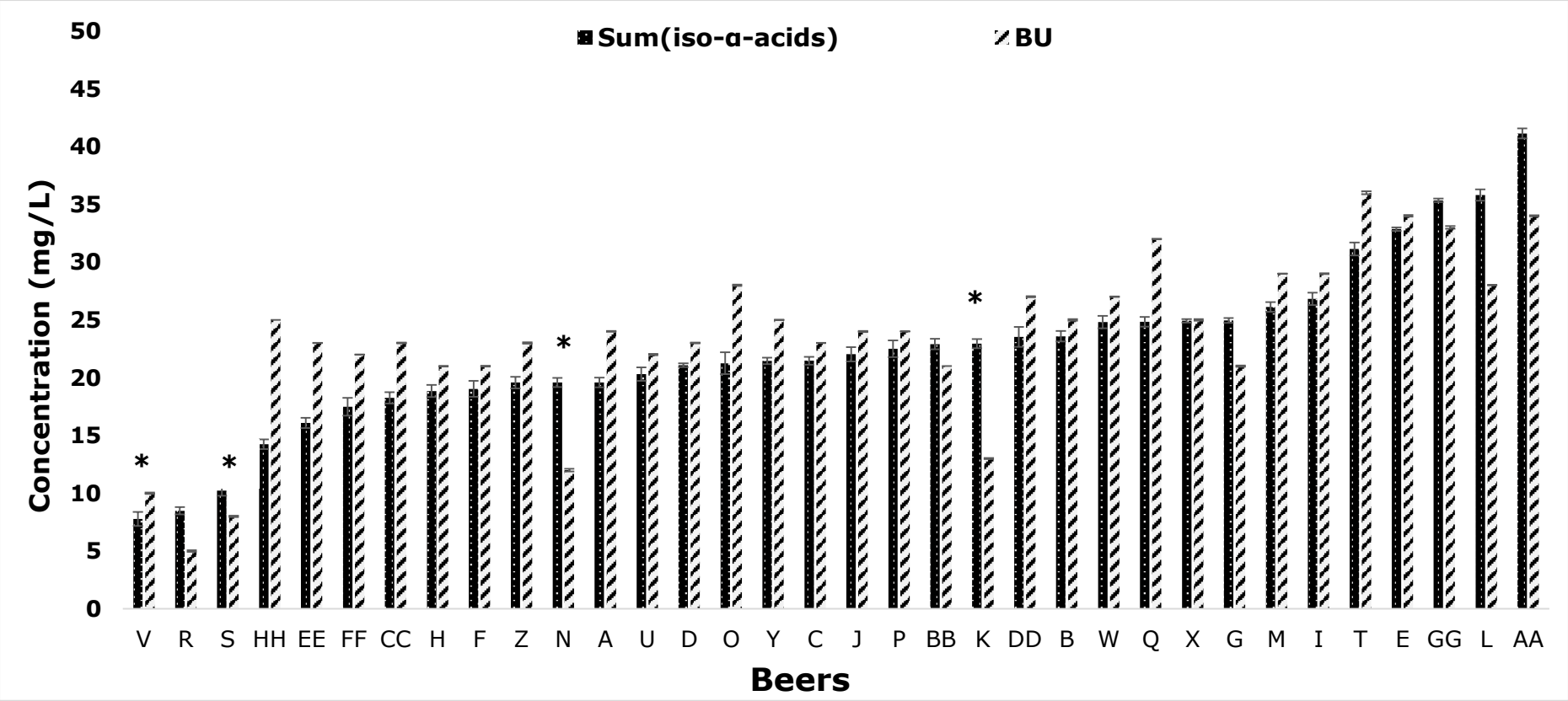


Figure 2.2: A comparison of the bitterness of 34 commercial lager beers as determined both by spectrophotometric BU values (striped bar) and the sum of iso-humulones determined by HPLC analysis (black bars). Data are ordered by increasing sum of iso-α-acids; error bars represent standard error values of 3 independent replicate analyses. *****Signifies beers containing tetrahydro-iso-α-acid.

Inspection of the HPLC chromatograms revealed that both iso-α-acids and tetrahydro-iso-α-acids were present in beers K, S, V & N, as well as the presence of humulinones at up to 3 mg/L in beers O, Q, AA, T and V. The presence of tetrahydro-iso-α-acids in beers K, S, V & N explains the lower BU values attained in these beers relative to the values determined by HPLC, since BU absorbance is taken at a lower wavelength (275 nm) to the absorption maximum of tetrahydro-iso-α-acids (310 nm). Significantly, of these four beers the BU value of beer V, which contained humulinones was the only one greater than the attained HPLC value. This was also the case in the other beers containing humulinones (O, Q and T) except for beer AA. This suggests that humulinones contribute to the bitterness values attained with BU method as has been previously observed (Parkin, 2014), although the contribution of beer polyphenols to BU values in this case also cannot be discounted either. The presence of humulinones as well as relatively high polyphenol content in beers O, Q, AA, T and V further supports the hypothesis that these beers were dry-hopped. The low TPC observed in beer Q in comparison to the other dry-hopped beers could be explained by different rates of dry-hopping. Beers that contained Tetra hop products (K, S, N & V) and those dry-hopped (Q, AA, T & O) both displayed distinctive polyphenol and bitter acid profiles. The highly dry-hopped beers (T, AA, Q) and high bitterness Czech lagers (E and L) all had correspondingly high contents of phenolic compounds (see Table 2.1 and Figure 2.2).

2.4.4 Amino acid profile of beers

The uptake of nitrogen in the form of amino acids during fermentation forms the basis of yeast growth; with amino acids representing the major source of nitrogen for yeast metabolism (Lekkas, Stewart, Hill *et al.*, 2005). Amino acids are also important for beer flavour since they serve as precursor compounds to the generation of esters and higher alcohols, e.g. ethyl acetate, isobutanol and amyl alcohols - via the Ehrlich chemical pathway (Engan, 1970; He, Dong, Yin *et al.*, 2014). Amino acids and small peptides are considered to have sweet, umami, kokumi, sour and bitter taste (Soares, Kohl, Thalmann *et al.*, 2013). A total of 20 amino acids were quantified in the beers and the amino acid profile of each of the 34 beers is presented in Figure 2.3. Based on the total sum of amino acids measured, Algometric Hierarchical Clustering (AHC) dissimilarity grouping placed the beers into 3 clusters as presented in Figure 2.3 and Figure 2.4. Of the 20 amino acids measured, proline was found to be the most abundant, with a residual concentration of approximately 565 nmol/mL in beer. This is in line with several findings in the literature which have found proline to be the most abundant amino acid in wort and the least utilised amino acid by yeast (He, Dong, Yin *et al.*, 2014; MacWilliam, 1968). Amino acids including alanine, valine, leucine, glycine and phenylalanine were also observed in higher concentrations relative to the other amino acids quantified (>50 nmol/mL) in these beers. This may suggest that the wort in this case contained more of these amino acids than was required for yeast growth and assimilation during fermentation. The uptake of amino acids by both ale and lager yeasts during fermentation has been reported to follow a distinct order or pattern which places the amino acids into four

groups (Group A, B, C and D) based on how quickly they are assimilated by yeast during the fermentation cycle (Perpète, Santos, Bodart *et al.*, 2005). The amino acids in these groups are very similar for both lager and ale yeast strains. Group A amino acids such as glutamine and serine are taken up quickly during the early stages of fermentation relative to group C amino acids such as valine, leucine and tryptophan. According to the present set, greater residual amino acids were recorded for amino acids in Group C and D relative to amino acids reported in the literatures to be in Group A and B, which is consistent with the above assimilation order.

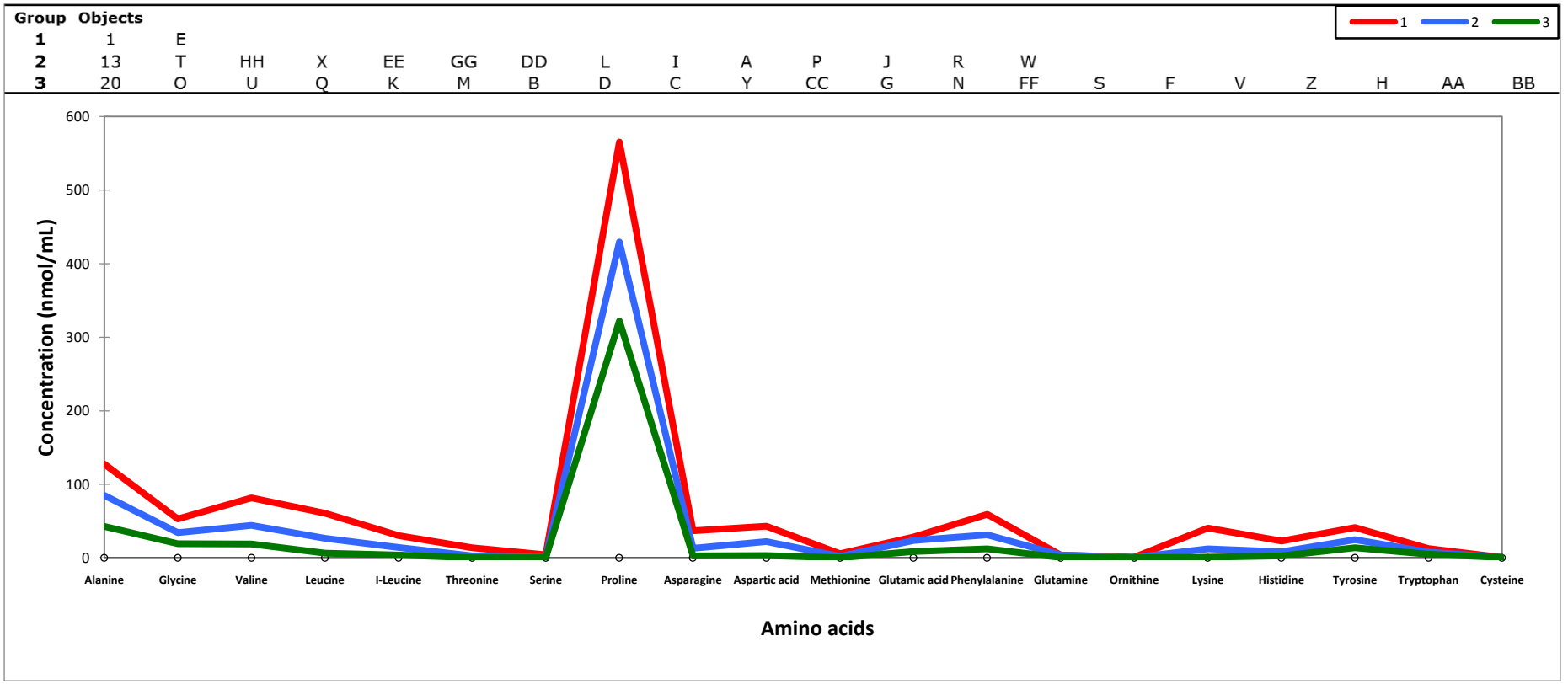


Figure 2.3: The amino acid profile of beers showing the individual amino acids measured. The groups were obtained by AHC using Ward's method and based on the total concentration of amino acids in beer; Group 1 in red: beers with the highest concentration of amino acids, Group 2 in blue: beers with intermediate levels of amino acids and Group 3 in green: beers with the lowest concentrations of measured amino acids.

Beer E, a Czech lager, was the single beer represented in Group 1 of the AHC profile clustering, reflecting the fact that it contained the highest level of every single amino acid measured. This beer was significantly different in its amino acid profile in comparison to beers in Group 2, which consisted of 13 beers with relatively high amounts of amino acids but lower than for beer E in Group 1. Some of the beers in this group included T, X, GG, L, A, R and J. The third group consisted of beers which were lowest in the overall content of amino acid measured. Beers in this group include O, K, N, AA, BB and CC. The total sum of amino acids in the 34 beers, upon which the AHC categorization was based is further presented in Figure 2.4. Beer E contained the highest levels of amino acids - with a total concentration of 1235 nmol/mL whilst beer BB contained the least amount of amino acids at just 308 nmol/mL. This significant difference in amino acid concentrations of the beers could be attributed to several factors including, the protein contents of raw materials utilised in brewing, the malting and mashing processes, as well as yeast strains used for fermentation (Erbe & Brückner, 2000; He, Dong, Yin *et al.*, 2014; MacWilliam, 1968). In this regard, all-malt beers, i.e. those brewed only with malted barley without the addition of adjuncts such as rice or maize, usually retain greater amounts of residual amino acids, since the presence of adjuncts usually leads to the dilution of wort nitrogen (Mathias dos Santos & de Mello, 2014). Furthermore, beers made with well-modified lager malt and subsequently mashed at a low temperature stand (48°C) have been reported to retain high levels of nitrogen and subsequently, residual amino acids (Crabb & Hudson, 1975). The use of different adjuncts i.e. rice or maize only results in minor changes in the amino acid

composition of wort, with residual amino acid content more dependent on the proportion of malt to adjunct in the brew (Meilgaard, 1976).

The mashing process adopted during beer production, i.e. whether infusion mashing at a single temperature (68°C) or one involving several temperature ramps can also affect the residual levels of nitrogen in wort and amino acids in beer (Crabb & Hudson, 1975; MacWilliam, 1968).

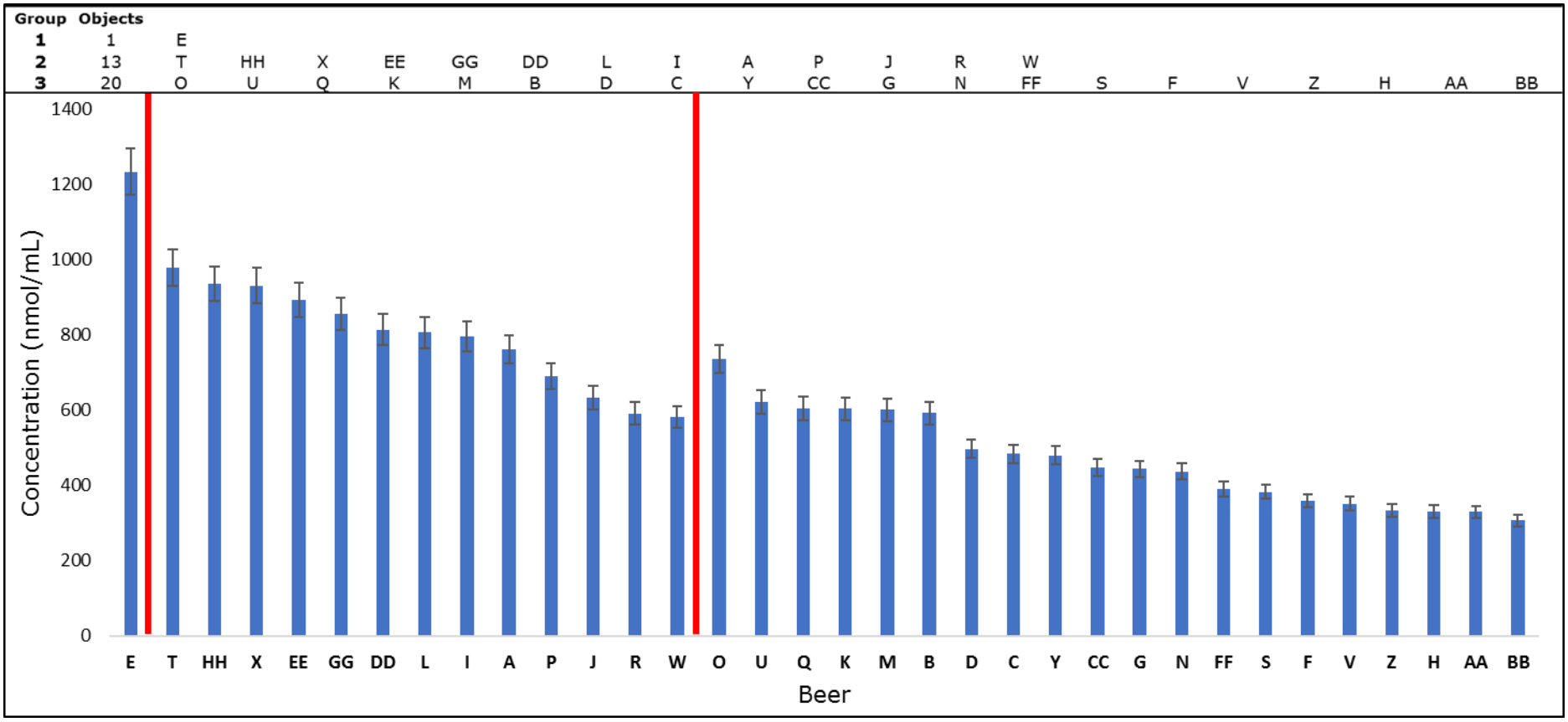


Figure 2.4: The total concentration of amino acids measured in the beers. Groupings as illustrated by the red lines are based on the relative concentrations of amino acids in the beers. Error bars represent the standard deviation of triplicate analysis.

2.4.5 Brewing parameters of beers

Brewing parameters such as pH, gravity, density and alcohol levels are important for the perception of bitterness and mouthfeel properties of beer (Langstaff & Lewis, 1993). These parameters were measured for the 34 beers in the present study and the results are presented in Table 2.2. Beer present gravity (PG), which is related to the level of residual carbohydrates such as unfermentable dextrins, ranged from 2.96 in beer F to 13.80 in beer Q. The original gravity (OG) value is related to the original wort composition before fermentation, this value was lowest for the beer which had the lowest PG (beer F) and highest for beer E with a value of 49.81. The very low PG and OG recorded for beer F and others such as D and S suggests the use of enzymes during the mashing process. These beers were 'Lite beers' which are often produced from high gravity wort with the addition of enzymes during the mash also employed to convert unfermentable dextrins into sugars that are fermentable by the yeast during fermentation, thus achieving complete attenuation. This subsequently results in very little residual carbohydrates in the beer, and thus the low PG recorded. Beer S was the least alcoholic beer with an ABV (%) of 3.52 whilst beer O was the most alcoholic beer with an ABV (%) of 4.90. pH values for the beers ranged from 3.94 as the lowest value in beer BB to 4.73 as the highest in beer T. Beers with higher pH values were generally also higher in amino acid contents and vice versa, as can be seen for beers T and BB on the opposite ends of the scale. Wort pH is affected by degree of malt kilning, with kilning decreasing wort pH as a result of the generation of acidic melanoidin compounds in the malt (Crabb & Hudson, 1975). The use of less-modified malt for brewing depending on

the adopted mashing protocol can also lead to higher wort pH (Crabb & Hudson, 1975), and more importantly the selected yeast strain will also have a substantial influence on finished beer pH (Coote & Kirsop, 1976).

Table 2.2: Gravity, alcohol and pH data for the trial beers ordered from high to low pH values. PG is present gravity, OG is original gravity, SG is Standard gravity and ABV is alcohol by volume.

BEER	PG	OG	ABV (%, V/V)	pH	SG	DENSITY(G/CM^3)
T	7.52	41.30	4.36	4.73	1.0075	1.00571
X	9.35	41.39	4.13	4.68	1.0094	1.00754
HH	9.15	46.33	4.81	4.61	1.0091	1.00734
U	7.89	42.72	4.49	4.56	1.0079	1.00608
L	12.38	44.02	4.08	4.55	1.0124	1.01056
K	8.03	39.30	4.03	4.52	1.0080	1.00622
Y	7.68	42.30	4.47	4.51	1.0077	1.00587
I	9.51	38.34	3.71	4.50	1.0095	1.00770
EE	8.93	42.35	4.31	4.46	1.0089	1.00712
A	6.05	42.74	4.75	4.46	1.0061	1.00424
E	12.82	49.17	4.69	4.44	1.0128	1.01100
FF	7.16	38.25	4.00	4.41	1.0072	1.00535
GG	9.14	40.03	3.98	4.41	1.0091	1.00733
B	6.08	41.48	4.57	4.39	1.0061	1.00427
D	3.61	36.04	4.18	4.36	1.0036	1.00181
Q	13.80	45.91	4.14	4.35	1.0138	1.01197
R	5.91	35.01	3.74	4.33	1.0059	1.00410
F	2.96	31.52	3.67	4.30	1.0030	1.00116
P	6.24	38.92	4.21	4.30	1.0062	1.00443
J	6.23	34.66	3.66	4.29	1.0062	1.00440
H	6.23	38.91	4.21	4.28	1.0062	1.00442
N	8.65	42.10	4.28	4.28	1.0089	1.00711
DD	6.26	41.71	4.58	4.28	1.0063	1.00445
M	7.33	44.49	4.80	4.25	1.0073	1.00552
G	6.77	40.14	4.30	4.24	1.0068	1.00496
Z	8.60	44.89	4.69	4.24	1.0086	1.00679
S	4.33	31.82	3.52	4.23	1.0043	1.00253
C	5.47	43.47	4.92	4.23	1.0055	1.00366
O	11.47	49.81	4.96	4.22	1.0114	1.00965
AA	8.86	38.34	3.79	4.21	1.0089	1.00705
W	7.89	42.11	4.41	4.18	1.0079	1.00608
CC	7.28	40.86	4.33	4.16	1.0073	1.00547
V	7.28	40.24	4.25	4.07	1.0073	1.00547
BB	6.52	42.01	4.58	3.94	1.0065	1.00471

2.4.6 Selection of exemplar beers for sensory evaluation

Based on the foregoing analysis of bitter congeners and brewing parameters, substantial variation was evident within the sample set. In order to understand how the varying contents of hop acids, phenolic compounds, amino acid content and brewing parameters observed in these beers impact on their perceived sensory bitterness, a sub-set of 'exemplar beers' were selected with the aid of a PCA. The two PCA bi-plots shown in Figure 2.5A and B together accounted for about 71% of variation within the analytical data set. In Figure 2.5A the loadings along PC 1 (42% of variation) in quadrant 3 was associated with beers low in phenolic acids, PG, humulinones, iso-α-acids and amino acids while the opposite quadrant (2), relating to positive loading along PC1 correlated with beers high in these compounds as well as high PG. The negative loadings along PC2 (18%), in quadrant 1 identified beers bittered with Tetra and other advanced hop products such as pre-isomerised hop products, which were negatively correlated with beers in quadrant 4. Beers in this quadrant (4) had relatively higher trans/cis ratios and residual α-acids – i.e. those which had used conventional hopping practice as opposed to pre-isomerised or light stable products. Beers in quadrant 1 had lower T/C ratios, further indicative of the use of pre-isomerised hops as well as containing Tetra. Beers in quadrant 2 were generally higher in hop acids, polyphenol contents, PG and amino acids compared to beers in quadrant 3 which were characterised by lower levels of these compounds. The beers in quadrant 4 were correlated with high T/C ratio and residual α-acids which, as noted, is indicative of conventional hopping practice.

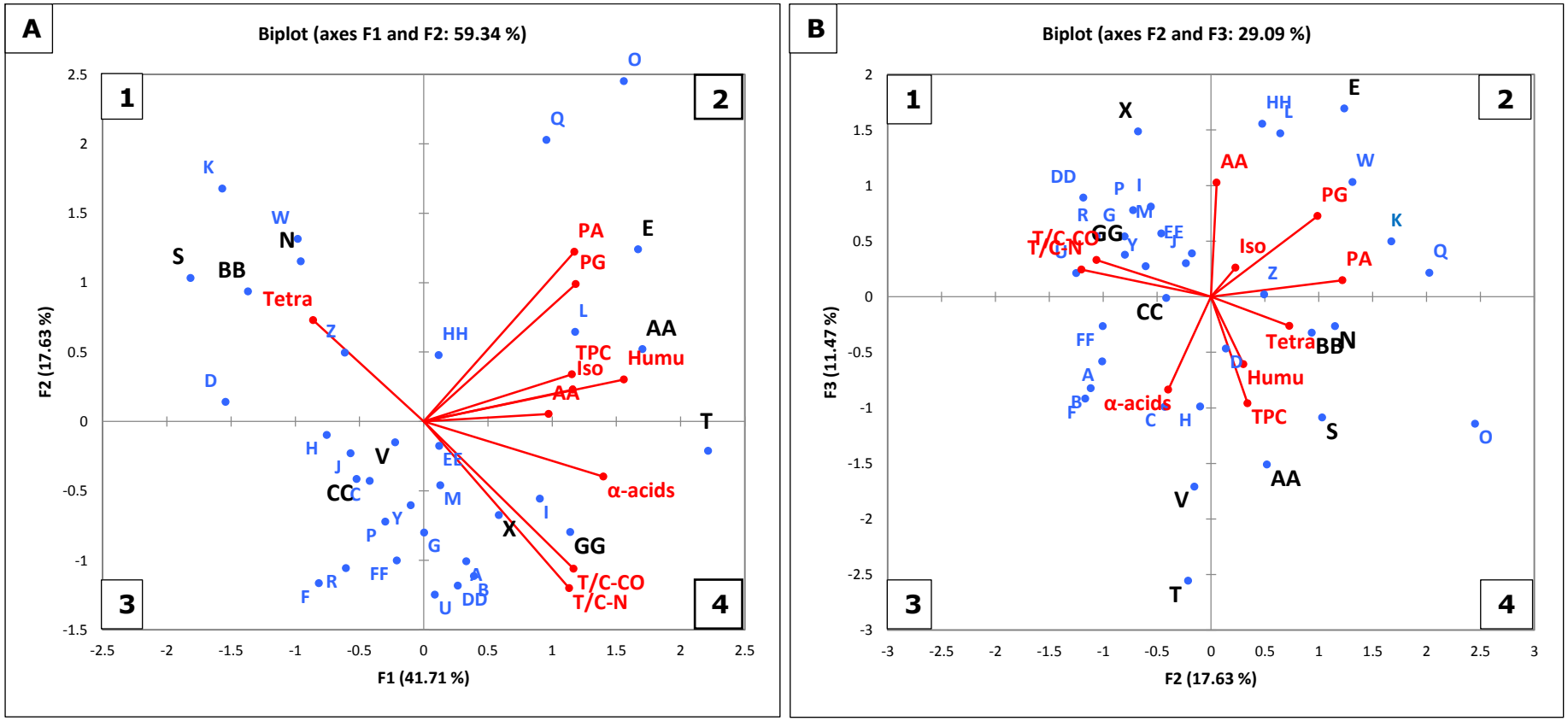


Figure 2.5: PCA plot of the beers in relation to the analytical measures. (A); Biplot of PC 1 and 2 accounting for 60% of variation in the data and (B); Biplot of PC 2 and 3 accounting for an additional 30% of variation in the data. In red: Tetra is tetrahydro-iso-α-acid, TPC is total polyphenol content, Iso is iso-α-acids, AA is amino acids, PA is phenolic acid content, Humu is humulinones, PG is present gravity. Letters in black are the selected beers and in blue, the remaining beers.

The key drivers of the quadrant groupings of the beers is further confirmed on PC2 and 3 which accounted for 30% of variation within the data set (Figure 2.5B). Beers GG and X were correlated with high T/C ratios and in the opposite quadrant to beers S, N and BB which were correlated with tetrahydro-iso-α-acid and other advanced hop products. Beers E and K were correlated with high levels of phenolic acids and amino acids and iso-α-acids.

A total of 10 beers were selected from the 4 PCA quadrants in Figure 2.5A to represent the diversity amongst the 34 beers: beers CC and V were selected from quadrant 3, X and GG from quadrant 4, S, N and BB from quadrant 1 and beers E, AA and T from quadrant 2. The sensory analysis of these beers is reported in the following chapter.

2.5 CONCLUSIONS

The analytical assessment of 34 selected beers was conducted based on compounds and brewing parameters which are known to either contribute to or affect perceived beer bitterness intensity and quality. Based on both polyphenolic and hop acid profiles, some beers (Q, O, AA, and T) were identified to have been dry hopped. Some of the beers were also found to contain different analytical bitterness profiles as a result of the hopping technology adopted. For example, beers K, S, N and V were found to contain a mixture of Tetra as well as iso-α-acids based on the lower T/C ratios and residual α-acid concentrations measured. Beer V in particular was unique amongst the 34 beers in its bitterness profile, containing a complex mixture of bittering compounds (humulinones, iso-α-acids, α-acids and Tetra). Beers (GG, I and X) were most likely conventionally

bittered, with the PCA showing a strong correlation between these beers and high T/C-ratios and residual α-acid contents. Beer E was found to contain the highest concentration of amino acids (1235 nmol/mL) whilst beers BB, V, and AA were noted to contain <400 nmol/mL of amino acids; these differences in amino acid concentrations were attributed to varying fermentation processes, yeast strains and raw material usage. From the measured brewing parameters, the 'Lite' lager beers were found to have the lowest OG, PG and alcohol content (indicative of enzyme use during mashing) whilst beer O was the most alcoholic and highest in OG, PG and Density. Having thus established the analytical profiles of these beers in relation to bitter tasting congeners and brewing parameters in this chapter, 10 beers representative of the diversity present in the data set (V, X, N, S, BB, AA, GG, T, E and CC) were selected for sensory evaluation in Chapter 3.

3 DEVELOPMENT OF A BEER BITTERNESS LEXICON AND SENSORIAL EVALUATION OF SELECTED BEERS BASED ON ANALYTICAL MEASURES OF BITTER CONGENERS AND BREWING PARAMETERS

3.1 INTRODUCTION

The use of sensory techniques to measure bitterness flavour in beverages as is perceived by consumers is preferable to just analytical measures. Sensory science as a discipline allows for the measurement of human responses to psychophysical stimuli, and involves the measurement, interpretation and understanding of human response to food and drink products as perceived by the senses of sight, taste, touch, smell and hearing (Martens, 1999). Both bitterness intensity, which corresponds to the magnitude of bitterness perceived, and the quality of bitterness (i.e. whether it is considered to be 'harsh', 'lingering', 'astringent' or 'rounded' in nature) are key indicators of brand success and consumer satisfaction (Keast, Bournazel, & Breslin, 2003; Schönberger, 2006). For the evaluation of bitterness in beer, descriptive sensory techniques such as QDA, and FCP have been used (McLaughlin, Lederer, & Shellhammer, 2008; Reinbach, Giacalone, Ribeiro *et al.*, 2014) as discussed in Section 1.8. These methods usually involve the generation and definition of a list of attributes with which bitterness can be evaluated by a trained panel. The aim of the study reported in this chapter was firstly to develop a sensory bitterness lexicon (using Descriptive Analysis (DA)), which allowed for adequate differentiation of perceived beer bitterness qualities, and to further determine the bitterness qualities, using a CATA sensory method of the 10 beers selected for sensory evaluation based on the analytical measures presented in Chapter 2. Further sensory evaluation of the beers

using quantitative analysis such as rank-rating was conducted; based on a sub-set of 6 of the 10 beers for a selected number of bitterness quality attributes. Correlation between the perceived sensorial bitterness qualities of the beers and the previously determined analytical profiles in relation to hop-related bitter compounds, polyphenols and brewing parameters was further determined (Chapter 2).

3.2 MATERIALS AND METHODS

3.2.1 Sensory panel selection

Ethical approval for the sensory element of this investigation was obtained from the University of Nottingham Medical Ethics Committee (Approval number: J12022015) and all participants gave written informed consent to participate in the study. Trained panellists from the University of Nottingham beer panel were selected for this study principally on their availability to attend all the study sessions, and were given a disturbance allowance for participating. Both qualitative and quantitative aspects of bitterness were evaluated by experienced panellists from the University of Nottingham trained beer panel (n=6) using descriptive analysis.

3.2.2 Bitterness lexicon generation

Panellists used the subset of 10 selected beers (Section 2.4.6) for generating and defining a bitterness lexicon. These beers represented extreme variation in analytical variables and brewing parameters measured as previously discussed (Chapter 2). The beers were presented to the panellists in pairs (N & E, AA & CC, GG & V, BB & X and T & S) from

opposite PCA quadrants (Figure 2.5A) shown to be different in their analytical profiles. Panellists then attended a further 2 x 2 h sessions during which they tasted and described the bitterness of 10 mL samples of each beer and participated in group discussions to agree a final list of clearly defined bitterness-related terms. Where necessary reference standards e.g. citric acid/vinegar/lime for 'acidic' attribute, tannic acid solution for 'astringent' and ferrous sulfate for 'metallic' attribute was prepared in Evian spring water (1 mg/L) (Danone, France) and given to panellists to aid attribute definition.

3.2.3 Evaluation of bitterness quality by CATA

For the evaluation of bitterness quality, samples labelled with 3-digit random codes (10 mL) of each of the 10 selected beers were evaluated twice in 2 additional sessions using a Check–All-That-Apply (CATA) technique, (Dooley, Lee, & Meullenet, 2010) where panellists were asked to indicate the bitterness terms in the lexicon which were relevant to each beer sample. Samples were served in a 50 mL glass vial. A list of the agreed bitterness attributes was presented (randomised per subject) to the panellists who were asked to tick all the attributes that best described the sample ingested. Data were collected with Fizz software (Biosystèmes, France). Each sample was presented individually and assessed in triplicate following a randomised balanced order based on a Partial Latin Square design and served at 4±2°C. During sample evaluation, panellists were given 5 min to evaluate each sample followed by a 3 min break to minimise bitterness carry over and palate saturation. Evian water (Danone, France) and crackers (Rakusen's, UK) were provided for palate cleansing.

3.2.4 Quantitative evaluation of bitterness intensity and selected bitterness attributes in a sub-set of 6 beers

The intensity of selected bitterness quality attributes was determined using a rank-rating technique for 6 out of the 10 beers, since the evaluation of all 10 beers by this method would have been arduous. The following 6 beers (N, V, BB, GG, E, and AA), which were well differentiated according to the CATA results were rank-rated for the intensity of the following significantly different attributes: 'harsh', 'round', 'progressive', 'metallic', 'smooth', as well as overall bitterness intensity. Rank-rating is a combined technique which allows for an initial evaluation of whether samples are different from each other from the ranking data, and secondly a measure of the magnitude of the difference, if it exists, which can be ascertained from the intensity rating scores (Kim & O'Mahony, 1998). Panellists were trained to use the rank-rating technique for bitterness intensity and this training was applied for the evaluation of the other 5 bitterness attributes. No training was needed for ranking per se other than the instruction to rank the samples presented from low to high intensity of the attribute in question. For rating, panellists were trained in the use of a scale anchored from 0 – 10, with 0 representing low and 10 representing high intensity of an attribute. To familiarise the panel with the range of intensity represented by the scale, subjects were presented with beers of differing BU levels and they discussed where they should be placed on the scale.

Samples were served at 4°C and 30 mL samples were used for the rank-rating evaluation of 3 of the selected bitterness attributes. A 5 min break followed, during which time panellists cleansed their palates with Evian water (Danone, France) and crackers (Rakusen's, UK). Panellists also took

an enforced 2 min break between each of the evaluated bitterness attributes. A further 30 mL sample was then provided to the panellists after the 15 min break to evaluate the remaining 3 bitterness attributes. Samples were evaluated in duplicate over two sessions and presented according to a Partial Latin Square design. Data was collected with Fizz software (Biosystèmes, France).

3.2.5 Data analysis and processing

Statistical analyses were performed with XLSTAT v2015 package (Addinsoft, Paris). Significance was set at α = 0.05. The binary data from CATA was first tested for significance by Cochran's Q test which allowed for significantly different attributes between the beers to be determined, followed by Correspondence Analysis (CA). This method is particularly useful in understanding relationships between rows and columns populated by binary data like those acquired from rank analysis. The rank data were analysed using Friedman's test and Nemenyi's multiple pairwise comparison to determine the pairs of samples that were significantly different from each other for the attribute in question. The intensity data from rating scores were analysed by 2 (subjects and samples) way analysis of variance (ANOVA) with Tukey's post-hoc test. Furthermore, to relate both analytical and sensory data Partial Least Square Regression (PLSR) models were conducted using both analytical and sensory data from the 6 beers subjected to rank-rating. Predictive models were developed and used to predict the perceived bitterness qualities of the remaining 4 beers. The model was built from explanatory variables consisting of 10 measured analytical variables (X) which contributed significantly to the model,

insignificant variables – those having a Variable Important for the Projection (VIP) less than 1, were removed from the model. The significant model variables were the sum of TPC, PA, iso-α-acid, α-acid, humulinones, Tetra, density as well as the concentration of 3 individual amino acid compounds which have been previously identified to contribute to beer bitterness (phenylalanine, tyrosine and tryptophan) (Section 1.4.1.3). Sensory data consisting of the mean intensity scores for bitterness attributes 'harsh', 'progressive', 'metallic', 'round', 'smooth' and bitterness intensity of the 6 beers formed the dependent variable (Y) of the model. R^2 values were calculated – indicating goodness of fit.

3.3 RESULTS AND DISCUSSION

3.3.1 Bitterness attributes

A total of 20 bitterness attributes were initially attained following beer tasting. These attributes were condensed down to 13 upon discussion and training to remove redundant bitterness and non-bitterness related attributes (Table 3.1). For example, both 'drying' and 'astringent' were agreed to represent the same bitterness attribute and as a result 'drying' was removed from the bitterness lexicon; this was also the case for both 'spiky' and 'sharp' bitterness, with the former removed upon agreement that both attributes were the same.

The 13 bitterness attributes agreed on by the tasting panel to fully describe the range of bitterness characteristics presented by the samples and their definitions are presented in Table 3.1, including attributes such as 'astringent', 'metallic', 'harsh' and 'sharp' which were the same as those

presented by McLaughlin et al (2008), in their study on the impact of hop polyphenols on beer bitterness (McLaughlin, Lederer, & Shellhammer, 2008), using the FCP method.

Table 3.1: Bitterness quality attributes and their definition, as agreed by a trained sensory panel.

Attributes	**Definition**
Harsh	Tingly, irritating and raspy bitterness
Acidic	Vinegary, fruit-like acidity
Tart	Acidic with sour notes
Round	Pleasant, not spiky
Metallic	Tin/metal taste, silver coin taste
Sharp	Instant, bitterness taste at the tip of the tongue
Smooth	Velvety
Astringent	Dry, causing drying of the mouth
Artificial	Chemically, unnatural beer taste
Vegetative	Cabbage, sprout-like bitterness, hop tea
Progressive	Gradual increase in bitterness perception
Instant	Instantaneous bitterness
Diminishing	Rapid decrease of bitterness taste after ingestion

Some of the attributes in Table 3.1 e.g. 'smooth' and 'round' represent what brewers might deem as positive bitterness attributes (depending to some extent on beer style), whilst others including 'harsh', 'sharp' and 'metallic' bitterness attributes are potentially negative attributes. It is also clear that the agreed lexicon represents key facets of bitterness perception

as discussed in Section 1.9. Attributes such as 'progressive' and 'lingering' allude to the temporal properties of perceived bitterness, 'astringent' and 'harsh' are potentially linked to trigeminal sensations and oral irritation in relation to perceived bitterness. The 'sharp' bitterness attribute described as bitterness taste at the tip of the tongue represents oral spatial facets of bitterness perception, where the perception of bitterness is concentrated in certain locations within the oral cavity (Keast & Breslin, 2003). In general, the agreed attributes were well understood by the panellists although there were some difficulties in defining certain bitterness attributes, as can be seen in the case of 'smooth' bitterness which was defined as 'velvety'. This is rather problematic in that it makes training for this particular attribute difficult, because the definition has no defined example by which it can be communicated to an ordinary person and there is no reference material for this particular attribute. Consequently, it can be difficult to fully verify that the panellists are describing and rating this same attribute in the same manner in beer.

3.3.2 Bitterness qualities of selected beers

The bitterness qualities of the 10 selected beers, as determined initially by CATA, revealed significant associations between certain beers and particular bitterness attributes. The Cochran's Q test of independence which was used to compare the beers based on the binary CATA data was significant ($p < 0.05$). Of the 13 attributes, 9 were significantly different across the beers evaluated, as is shown in Table 3.2. The significant attributes distinguishing the bitterness characters amongst the beers were 'harsh', 'round', 'smooth', 'sharp', 'metallic', 'vegetative', 'progressive',

'instant' and 'diminishing'. The results further showed that beer E was significantly associated with 'harsh', 'metallic' and 'progressive' bitterness qualities compared to beer V which was not associated with these attributes. For the attribute 'round' beers AA, CC, E, GG were least associated with this attribute in comparison to beer V which was most correlated with 'round' bitterness character. Beer GG was associated with 'sharp' bitterness in contrast to beer V and BB. For 'smooth' bitterness beer V was strongly correlated with this attribute but not AA. Beer BB was mostly correlated with 'vegetative' bitterness unlike CC, N, GG, and S which were less 'vegetative' in bitterness character. 'Instant' bitterness was least correlated with beer X out of the 10 beers and 'diminishing' bitterness was mostly correlated with beers V and S. Although 'instant' bitterness was a significant attribute, no significant differences were found amongst the beers for this attribute.

Table 3.2: Cochran's Q test of independence for the 10 lager beers (based on CATA data).
p-values represents bitterness attributes (in red) that were significantly differently amongst the 10 lager beers. Beers with different sub-group codes (a), (b), (c) are significantly different ($p < 0.05$).

Attributes	p-values	**AA**	**BB**	**CC**	**E**	**GG**	**N**	**S**	**T**	**V**	**X**
Harsh	**0.00**	0.67 (bc)	0.28 (abc)	0.22 (ab)	0.78 (c)	0.72 (bc)	0.28 (abc)	0.11 (a)	0.50 (abc)	0 (a)	0.39 (abc)
Acidic	0.49	0.56 (a)	0.44 (a)	0.39 (a)	0.33 (a)	0.33 (a)	0.33 (a)	0.56 (a)	0.39 (a)	0.33 (a)	0.28 (a)
Tart	0.22	0.56 (a)	0.44 (a)	0.28 (a)	0.39 (a)	0.28 (a)	0.22 (a)	0.33 (a)	0.50 (a)	0.39 (a)	0.17 (a)
Round	**0.00**	0.17 (a)	0.28 (ab)	0.17 (a)	0.11 (a)	0.11 (a)	0.33 (ab)	0.28 (ab)	0.44 (ab)	0.78 (b)	0.28 (ab)
Metallic	**0.04**	0.50 (ab)	0.33 (ab)	0.56 (ab)	0.61 (b)	0.50 (ab)	0.50 (ab)	0.44 (ab)	0.33 (ab)	0.06 (a)	0.56 (ab)
Sharp	**0.01**	0.33 (ab)	0.11 (a)	0.22 (ab)	0.17 (ab)	0.61 (b)	0.33 (ab)	0.33 (ab)	0.39 (ab)	0.06 (a)	0.17 (ab)
Smooth	**0.01**	0.17 (a)	0.39 (ab)	0.28 (ab)	0.22 (ab)	0.17 (a)	0.39 (ab)	0.22 (ab)	0.17 (a)	0.72 (b)	0.44 (ab)
Astringent	0.66	0.56 (a)	0.33 (a)	0.33 (a)	0.33 (a)	0.33 (a)	0.33 (a)	0.22 (a)	0.39 (a)	0.39 (a)	0.28 (a)
Artificial	0.52	0.39 (a)	0.17 (a)	0.33 (a)	0.39 (a)	0.28 (a)	0.33 (a)	0.39 (a)	0.28 (a)	0.11 (a)	0.22 (a)
Vegetative	**0.00**	0.17 (ab)	0.50 (b)	0 (a)	0.39 (ab)	0.06 (a)	0.06 (a)	0.06 (a)	0.22 (ab)	0.33 (ab)	0.17 (ab)
Progressive	**0.01**	0.61 (ab)	0.67 (ab)	0.67 (ab)	0.83 (b)	0.50 (ab)	0.50 (ab)	0.44 (ab)	0.56 (ab)	0.17 (a)	0.67 (ab)
Instant	**0.02**	0.50 (a)	0.06 (a)	0.33 (a)	0.50 (a)	0.50 (a)	0.22 (a)	0.28 (a)	0.50 (a)	0.28 (a)	0.17 (a)
Diminishing	**0.00**	0.17 (a)	0.17 (a)	0.17 (a)	0.11 (a)	0.28 (a)	0.17 (a)	0.56 (b)	0.17 (a)	0.56 (b)	0.22 (a)

The association between the beers and certain bitterness character attributes is further confirmed by the correspondence analysis plot shown in Figure 3.1 which explains over 72% of variation within the dataset. In agreement with the Q test results, both beer S and V were strongly associated with 'diminishing' and 'round' and 'smooth' bitterness qualities, whilst beer BB was 'vegetative', and X 'astringent' and 'tart'. Beers E and AA were mainly characterised by 'harsh' and 'progressive' bitterness qualities. Both beers GG and CC were correlated with 'sharp', 'instant' and 'artificial' bitterness qualities. The correspondence analysis plot of the CATA data confirms significant correlations between some of the bitterness attributes and the different beers. These differences are largely due to the different analytical profiles of the beers as has been presented in Chapter 2.

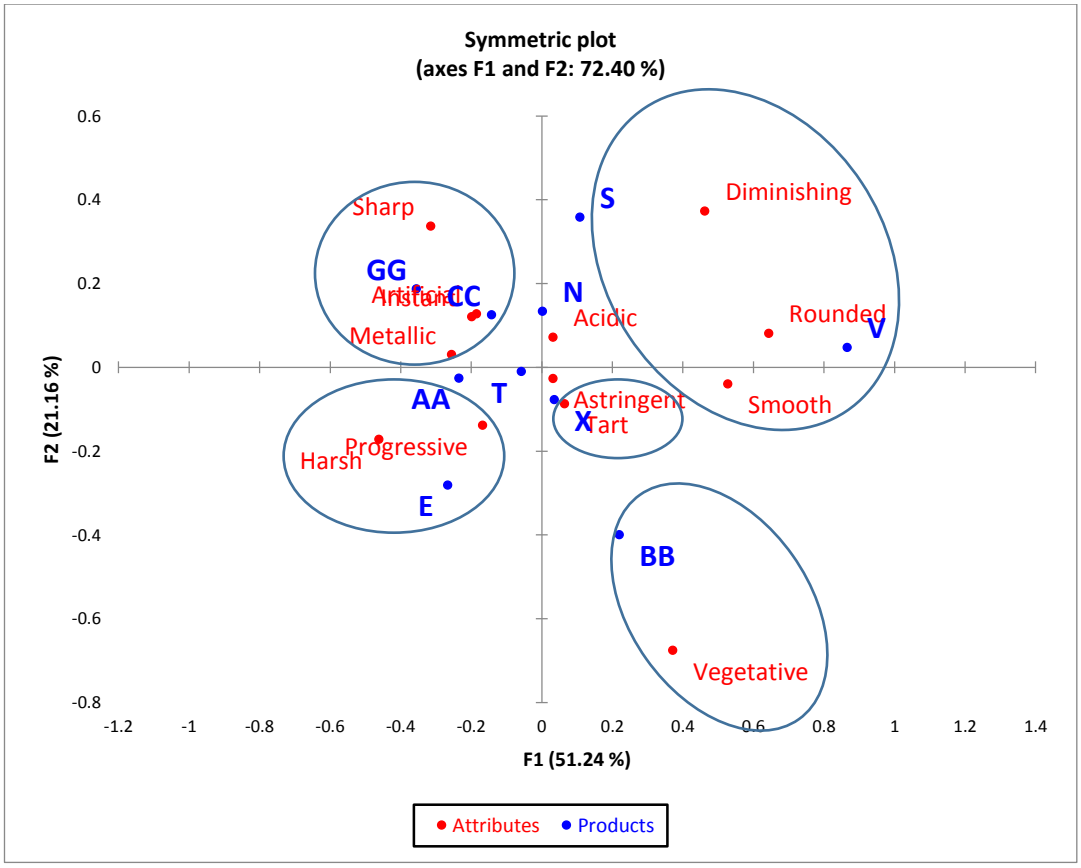


Figure 3.1: Correspondence analysis symmetric plot of bitterness attributes, showing beers and bitterness quality attributes closely related to them.

3.3.3 Intensity ratings of bitterness and related quality attributes

The CATA results merely indicate the bitterness quality attributes present in the beers, as well as significant correlations between the beers and certain bitterness qualities; with no indication of the magnitude of difference in intensities of the bitterness attributes in the beers. As such, whilst the 10 beers were found to vary in their perceived bitterness qualities based on this result, quantitative analysis allowing for the magnitude of difference for each attribute between the beers to be determined is essential to further understand the perception of beer bitterness quality. The rank-rating evaluation results for the 6 selected

beers (N, V, BB, GG, E, and AA) and selected bitterness character attributes are shown in Table 3.3. The results show good agreement in both the sum of rank scores and intensity ratings. Beers GG, E and AA were rated highest, respectively for bitterness intensity and harsh bitterness quality. Beer AA was the most bitter and harshest in both instances whilst beer V was least bitter and harsh. The fact that the beers rated highest in bitterness intensity were also perceived to have a 'harsh' bitterness quality could indicate an association between beer bitterness intensity and perceived bitterness quality. For the intensity of 'round' bitterness beer V was ranked and rated to be most 'round' of all the beers, beer AA was least 'round'. Beer V was least metallic compared to beer E which was ranked to be the most 'metallic' beer in bitterness character. For 'smooth' bitterness character, there was no significant difference between the six beers based on the intensity rating scores, although the rank data revealed that beer N was perceived to be significantly 'smooth' in bitterness character compared to beer E and AA.

Table 3.3: Sum of ranks and mean intensity ratings for selected beers and bitterness quality attributes. High rank scores indicate that samples were placed last, and therefore higher in the intensity of the attribute in question relative to the sample placed first.

	Sum of ranks					
	N	**V**	**BB**	**GG**	**E**	**AA**
Bitterness intensity	24a	29ab	34abc	51bc	55bc	59c
Harsh	34ab	22a	33ab	44abc	51bc	68c
Round	43abc	63c	56bc	36ab	31ab	23a
Metallic	48b	20a	30ab	50b	53b	51b
Smooth	52b	50b	50b	39ab	31a	30a
Progressive	35ab	25a	32ab	53b	50ab	57b
	Mean intensity scores					
	N	**V**	**BB**	**GG**	**E**	**AA**
Bitterness intensity	2.46a	3.54ab	4.08abc	5.83abc	6.51bc	7.78c
Harsh	3.95ab	2.58a	2.73a	4.67abc	7.20bc	8.93c
Round	5.12ab	6.41b	6.29ab	4.55ab	3.41ab	2.82a
Metallic	4.00ab	0.842a	2.97ab	6.13b	5.00ab	4.88ab
Smooth	5.62a	4.68a	6.13a	5.19a	3.40a	3.08a
Progressive	4.03ab	3.08a	3.02a	6.34ab	6.28ab	7.13b

a, b, c – sample with different letter codes in a row are significantly different ($p<0.05$).

For the last bitterness character attribute assessed (progressive), based on the rank scores - it was observed that of the three beers that were highest in bitterness intensity (GG, E, AA) only GG and AA were also perceived to be significantly 'progressive' in bitterness character relative to beer V. Furthermore, a significant difference was only observed for this attribute, (based on the intensity ratings) between beers V and BB compared to beer AA which was rated to be most progressive in bitterness.

In general these results show good agreement between the measured analytical bitterness levels (BU) of these beers (Section 2.4.3) and their perceived bitterness intensities, as has been suggested in the literature (Techakriengkrai, Paterson, Taidi *et al.*, 2004). Beers GG, E, and AA had iso-α-acid concentrations of 35, 32 and 41 mg/L respectively. It appears that perceived bitterness intensity which is concomitant with iso-α-acid concentrations plays a big role in the overall impression of bitterness and its perceived qualities. However, it is unlikely that bitterness intensity is the only driver of bitterness quality, since beers that were high in bitterness (analytically) were not all automatically perceived to be just 'harsh', 'metallic' and 'progressive' in bitterness character based on the intensity ratings observed. As an example, beer E was lower in analytical bitterness than GG and AA but was perceived to be as intense in bitterness and of 'metallic' and 'harsher' in bitterness character like beer GG and AA. As such, there must be factors other than bitterness intensity which are responsible for perceived differences in beer bitterness quality.

This quantitative analysis of bitterness quality attributes further complements the CATA results and provides additional intensity information concerning the bitterness qualities of the selected beers.

3.3.4 Prediction of bitterness quality based on analytical variables

Using techniques such as PLSR it is possible to relate both analytical and sensory data by a linear multivariate model. In total, 10 significant variables (see section 3.2.5) were used to generate predictive models for perceived beer bitterness quality. The Q^2 cumulative index value of the model, indicative of the model quality and predictive power was good, with a value of 0.661 after only 3 components. The cumulative index accounted for 94 and 84% variation in the dependent and explanatory variables, respectively. A correlation map generated as part of the model of the explanatory and dependent variables in relation to the 6 beers is presented in Figure 3.2.

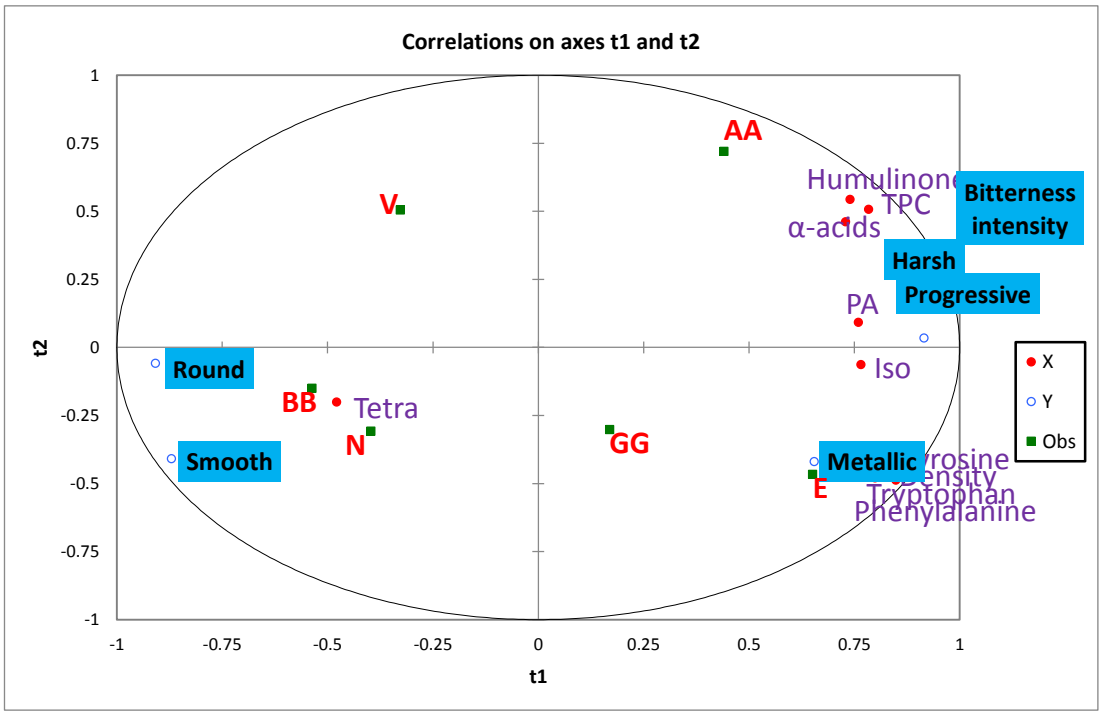


Figure 3.2: Correlation map of analytical and sensory data, with the spatial association of the beers in relation to the variables.

The correlation map shows significant differences in bitterness quality for the 6 beers and the analytical variables driving this perceived difference in bitterness quality. In agreement with the previous findings from CATA and rank-rating assessment, beer AA, which was strongly correlated with high levels of polyphenols, iso-α-acids, residual α-acids and humulinones, was perceived to be high in bitterness intensity, and 'progressive' and 'harsh' in bitterness character. The analytical profile of this beer strongly suggests that this beer was dry-hopped, as previously discussed in section (2.4.3). Beer AA was negatively correlated with beers BB and N which were low in these compounds and contained a blend of Tetra or pre-isomerised hop acids; these beers were relatively lower in bitterness intensity and perceived to have 'smooth' and 'round' bitterness characters. Beer V was negatively correlated with beers G and E based on low density/gravity and low concentrations of the amino acids tryptophan, tyrosine and phenylalanine. This beer shared similar bitterness characteristics with beers BB and N which were 'round' and 'smooth'. Beer E was high in density and amino acids and the perceived bitterness of this beer was significantly different from that of beer V, beer E was strongly correlated with 'metallic' bitterness character. Interestingly, this correlation map further shows that whilst beer E was still associated with high bitterness intensity and 'harsh' and 'progressive' bitterness character, the major driver of the 'metallic' bitterness quality of this beer was due to its relatively higher residual amount of amino acids and density/gravity. However, it is possible that a high metal content in this particular beer may be a contributory factor in its perceived 'metallic' bitterness character. Like

beer E, beer GG was associated with 'metallic', 'progressive' and high bitterness intensity as compared to beers V, BB and N.

The equation of the predictive model for each bitterness attribute is presented along with attained R^2 values in Table 3.4.

Table 3.4: Predictive model equations and goodness of fit coefficient (R^2) values showing goodness of fit.

Attribute	Equation	R^2
Bitterness intensity	-28.60+1.30E-02*TPC+9.089E-02*PA+4.83E-02*Iso+0.26*Humulinones+1.25E-03*Phenylalanine+8.95-03*Tyrosine-2.03E-02*Tryptophan-9.59E-02*Tetra+27.49*Density+0.21*α-acids	**0.90**
Harsh	-34.82+0.017*TPC+0.11*PA+7.92E-02*Iso+7.34E-02*Humulinones+1.97E-03*Phenylalanine+1.37E-02*Tyrosine-4.06E-02*Tryptophan-7.81E-02*Tetra+31.74*Density+0.26*α-acids	**0.93**
Smooth	31.29-6.40E-03*TPC-6.52E-02*PA+6.01E-03*Iso-0.68*Humulinones-4.47E-06*Phenylalanine-6.54E-04*Tyrosine-1.73E-02*Tryptophan+0.14*Tetra-23.41*Density-0.12*α-acids	**0.95**
Round	48.18-8.80E-03*TPC-5.13E-02*PA-5.32E-02*Iso+0.15*Humulinones-4.01E-03*Phenylalanine-1.23E-02*Tyrosine+1.37E-02*Tryptophan+1.36E-02*Tetra-38.84*Density-0.13*α-acids	**0.95**
Metallic	-81.22+5.81E-03*TPC-3.47E-03*PA+0.10*Iso-1.29*Humulinones+1.21E-02*Phenylalanine+2.79E-02*Tyrosine-0.01*Tryptophan+0.17*Tetra+81.03*Density+6.06E-02*α-acids	**0.96**
Progressive	-51.78+0.01*TPC+6.12E-02*PA+6.87E-02*Iso-0.24*Humulinones+5.46E-03*Phenylalanine+0.01*Tyrosine-1.68E-02*Tryptophan-8.13E-03*Tetra+51.09*Density+0.16*α-acids	**0.97**

The attained predictive models were well fitted with goodness of fit coefficient (R^2) values no less than 0.90. The model was used to predict the intensity of perceived bitterness and character attributes (harsh, smooth, round, progressive, metallic), for the remaining four samples (X, CC, T and S), as well as the position of these beers in relation to the other six beers in the new space of components. According to the attribute intensity predictions shown in Table 3.5, of the four beers, beer T would be the most bitter and of 'harsh' and 'progressive' bitterness character. In contrast, both beers CC and S were predicted to be relatively less bitter and of 'round' and 'smooth' bitterness characters. Beer X was predicted to be the most 'metallic' in bitterness character, placing it closely with beer E according to the scores of this beer in Table 3.5. The predicted value of beer CC and S indicate similar bitterness characters with beers BB and N, whilst beer AA was predicted to be similar to beer T.

Table 3.5: Model prediction values for bitterness intensity and related character attributes.

Beer	Bitterness intensity	Std. dev. (Mean)	Harsh	Std. dev. (Mean)	Round	Std. dev. (Mean)	Metallic	Std. dev. (Mean)	Smooth	Std. dev. (Mean)	Progressive	Std. dev. (Mean)
X	4.24	0.87	3.98	0.87	5.12	0.43	4.28	0.48	5.11	0.36	4.57	0.38
T	8.17	1.16	8.80	1.17	2.97	0.57	3.90	0.65	2.36	0.49	7.12	0.51
S	2.48	0.76	1.84	0.76	6.43	0.37	3.24	0.42	6.41	0.32	2.95	0.33
CC	3.09	0.64	2.43	0.64	6.19	0.31	2.74	0.35	5.66	0.27	3.22	0.28

Std. dev. represents standard variation on mean predicted values.

To confirm these predictions the spatial arrangement of the beers in the coordinates space was plotted as a scatter plot and is presented in Figure 3.3A and B. The clustering of the 6 beers upon which the predictive models were built is shown in Figure 3.3A, and the relationships between them is as previously discussed in Section 3.3.3 and Figure 3.2. The position of the remaining 4 beers in relation to these 6 beers is shown in Figure 3.3B and is as expected, based on the analytical profiles of these beers and the sensory data attained from the CATA and rank-rating results. This suggests that the prediction of the model for each bitterness attribute is reliable. Beer T was clustered closely to AA based on the model, and as expected based on the analytical profile of the beers which showed strong correlations with high concentrations of the measured bitter-tasting variables. Therefore, the position of beer T close to AA in the model is logical and correct. The clustering observed in the opposite quadrant is also very good, with beer S which contained Tetra and CC (both low in measured analytical variables) closely clustered together, and in close proximity with the other beers (BB and N) that shared the same analytical profiles. Beer X was clustered with GG according to the prediction of sensory bitterness character based on the analytical profile of high residual levels of α-acids, and high T/C ratio which denotes conventional hopping in these beers. In the opposite quadrant beer V is present alone with none of the four beers predicted clustered with it. This data shows that reliable prediction of perceived bitterness quality attributes based on the selected analytical variables is possible.

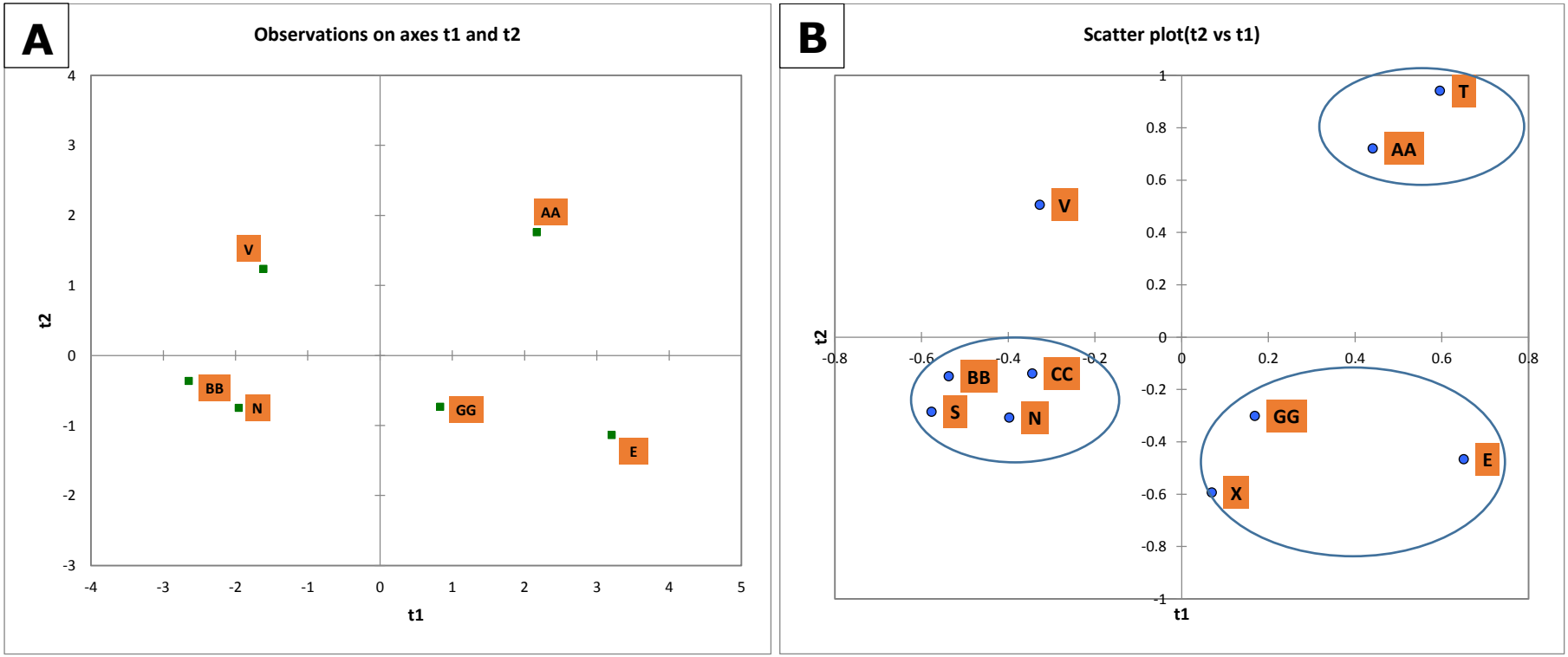


Figure 3.3: (A) Scatter plot of the position of the 6 beers in t coordinate space. (B) Scatter plot of the position of the predicted 4 beers in relation to the 6 beers used to build the model.

3.3.5 Perceived bitterness character and correlation to analytical measures

In order to better understand beer bitterness qualities and potential key analytical markers of some of the observed bitterness characters, correlations between the previously determined analytical profile of the selected beers and the sensory data was explored. For this purpose, both the PCA plot used to select the beers in Chapter 2, as well as the CA symmetric plot showing significant association between the beers and their perceived bitterness qualities are presented in Figure 3.4A & B. This revealed that beer CC, selected from quadrant 3 of the PCA with relatively low hop bitter acid, amino acid and polyphenol contents, was perceived as having an 'sharp', 'metallic' and 'instant' bitterness qualities; alongside the conventionally bittered beer GG with high T/C ratios (selected from quadrant 4) of the PCA. Beer X from the same PCA quadrant as GG was perceived to have an 'astringent' and 'tart' bitterness character. Interestingly, beer V which was selected from the same quadrant as CC for having low concentrations of iso-α-acids, amino acids, polyphenols and also deduced to have been dry-hopped from the presence of humulinones, had 'round' and 'smooth' bitterness characters; temporally this beer was 'diminishing' in bitterness. This finding further suggests that low bitterness intensity does not necessarily result in 'round', 'smooth' or 'diminishing' bitterness qualities in beers. One could reasonably expect beer CC to have the same bitterness characteristics as V if that were the case. Beers N and S from quadrant 1 of the PCA, which were bittered with a blend of Tetra as well as iso-α-acids, were perceived as 'diminishing' and somewhat 'acidic' in bitterness. Considering the trained panel was not aware of the analytical bitterness fingerprint of these beers, it is interesting that all of

the beers containing Tetra (S, N, and V) were grouped together based on their sensory bitterness character in the upper right quadrant of Figure 3.4B. Furthermore, they were negatively correlated in this plot with beers AA, T and E which were relatively high in levels of humulinones, iso-α-acids and polyphenols; and perceived to have 'harsh' and 'progressive' bitterness qualities. This supports the concept that besides the intensity of bitterness, the character of bitterness in beer is also impacted by the type of hop product used for bittering. Although beers S, N and BB were clustered together in quadrant 1 of the PCA plot (based on the presence of Tetra, lower T/C ratios and residual α-acids) in Figure 3.2A; beer BB was actually exclusively bittered with pre-isomerised hop products, and interestingly perceived to have a different bitterness quality in comparison to the other two beers (S and N). Beer BB was perceived to be predominantly 'vegetative' in bitterness character as is shown in Figure 3.4B, further lending weight to the notion that the use of certain hop-derived bittering compounds in beer can result in different bitterness qualities.

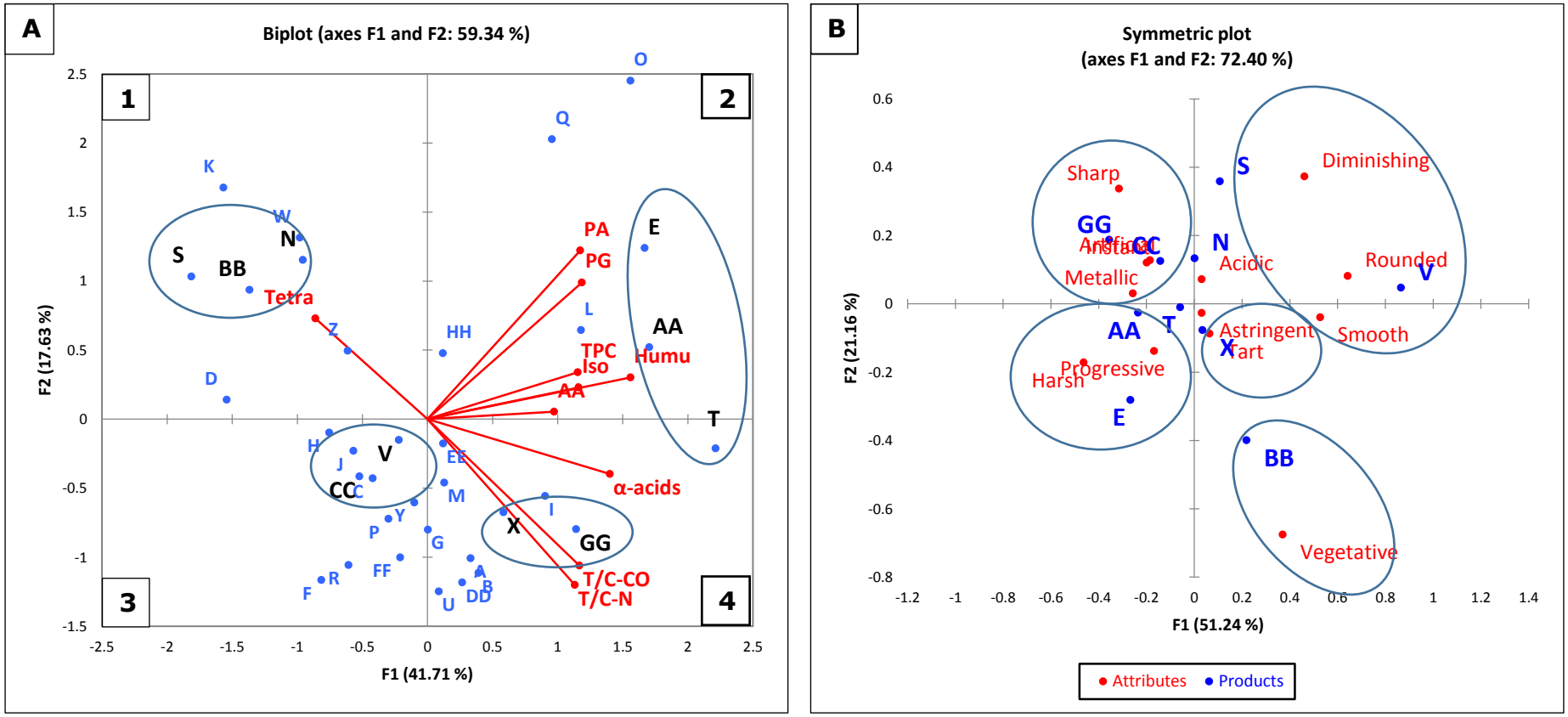


Figure 3.4: (A) PCA clustering of beers based on their analytical profiles, circled beers are those selected for sensory evaluation. Letters in black are the selected beers and in blue, the remaining beers. (B) CA symmetric plot of the 10 beers based on CATA evaluation.

Beers AA, E and T from quadrant 2 of the PCA, contained the highest concentrations of the measured variables of hop acids, humulinones, polyphenols, amino acids and present gravity, they were described as having a 'harsh' and 'progressive' bitterness characters. A study of the interaction between iso-α-acids and hop polyphenols by TI and FCP as reported by McLaughlin et al. (2008) found a significant effect of polyphenols on perceived intensity, as well as character of bitterness. In that study, samples high in polyphenols were higher in intensities of 'harsh', 'medicinal', and 'metallic' bitterness (McLaughlin, Lederer, & Shellhammer, 2008). The stated findings for the bitterness characters of beers AA, E and T in particular are in agreement with those findings.

The contribution of humulinones to beer bitterness character is gaining increasing attention due to the popularity of dry-hopping. Recent studies have now shown that humulinones have a 'smooth' bitterness quality and are approximately 65% as bitter as iso-α-acids in terms of intensity, thus representing a significant additional source of bitterness in beer which brewers must carefully consider (Algazzali, 2016; Maye, Smith, & Leker, 2016). However, the nature of the contribution of humulinones to beer bitterness quality cannot be deduced from this study since none of the beers investigated were found to exclusively contain humulinones for bittering purpose - the beers that contained humulinones also had other hop-derived bitter compounds such as iso-α-acids and Tetra in them, e.g. beer V.

3.4 CONCLUSIONS

In this chapter, 10 exemplar beers, varying significantly in analytical bitterness profiles as a result of hopping technology (selective usage of various hop products, points of addition) and brewing parameters, were used to develop and define a bitterness lexicon consisting of 13 bitterness attributes, which allowed for differentiation in perceived beer bitterness qualities. Upon evaluation of the 10 beers by CATA, results revealed that these beers were distinctive in their perceived bitterness qualities. Beers (AA, T) which were generally high in the measured analytical variables including polyphenols, hop acids and humulinones as a result of dry-hopping were perceived to be 'harsh', 'progressive' and expectedly of high bitterness intensity. In contrast, beers low in these compounds were generally associated with low perceived bitterness intensity but not always with positive bitterness character; beer CC in this category was described as having an 'artificial' and 'instant' bitterness character, whilst beer V although also dry-hopped was associated with 'round', 'smooth' and 'diminishing' bitterness qualities. Conventionally bittered beers (GG, X) had 'sharp', 'instant' and 'metallic' in bitterness character. Beers bittered with Tetra were perceived to be different in their bitterness qualities, with all the beers (S, N, V) containing a blend of both iso-α-acid and Tetra associated with 'acidic', 'diminishing' and 'round' bitterness qualities. One of the beers (BB), similar in analytical profile to S, N and V was bittered with pre-isomerised hop acids and perceived to have a 'vegetative' bitterness character. The rank-rating evaluation that followed for a sub-set of 6 beers for 6 bitterness attributes showed agreement between analytical bitterness and perceived bitterness intensity. Furthermore,

perceived bitterness intensity was also found to be associated with, but not the sole driver of perceived beer bitterness quality. 10 selected analytical variables in combination with the mean intensity scores were used to generate a PLSR model for the prediction of bitterness intensity as well as other bitterness quality attributes. The biplot of the analytical and sensory data was largely in agreement with the rank-rating results. However, this analysis also showed separation between the clustering of beers E and AA and suggested that the key analytical drivers of the perceived bitterness qualities in these beers were different – perceived high bitterness intensity, 'harsh' and 'progressive' bitterness character were driven by high hop-related bitter compounds and polyphenols in beer AA; whilst these attributes and perceived 'metallic' qualities of beer E was strongly related to high beer density as well as high concentrations of tyrosine, tryptophan and phenylalanine. The generated model was further used to predict the sensory bitterness qualities of the 4 beers excluded from the rank-rating evaluation, with results demonstrating logical clustering based on the analytical and sensory profiles of these beers. These results show that the bitterness quality of beer can be different and is largely driven by the production processes employed by brewers in terms of hopping strategy, and the raw materials used. These factors give beers a distinct analytical fingerprint in terms of polyphenols, amino acids, beer quality parameters and hop-derived bitter compounds which all influences the overall impression bitterness quality in beer.

4 THE IMPACT OF HOP AROMA ON PERCEIVED BEER BITTERNESS INTENSITY, QUALITY AND TEMPORAL PROFILES

4.1 INTRODUCTION

The flavour of food and beverages is multifaceted - involving taste, smell, texture, visual appearance, sound and even trigeminal sensations; all of which are key for consumer satisfaction (Clark, Hewson, Bealin-Kelly *et al.*, 2011; Hewson, Hollowood, Chandra *et al.*, 2009; Sørensen, Møller, Flint *et al.*, 2003). Of the four main brewing ingredients (water, malted barley, yeast and hops), hops (*Humulus lupulus L.*) remain an essential flavour ingredient in beer (Schönberger & Kostelecky, 2011). Hop resins and essential oils, located within the lupulin glands of the female hop flowers are the sources of bitterness and aroma characters in beer, respectively (De Keukeleire, 2000; Van Opstaele, Goiris, De Rouck *et al.*, 2012b). For bitterness, hop α-acids found within hop resins are thermally isomerised to bitter tasting iso-α-acids during the boiling stage of the brewing process (De Keukeleire, 2000).

Hop essential oils contain several volatile aroma compounds which are the source of desirable 'hoppy' character, often sensorially characterised using descriptors such as 'floral', 'fruity', 'spicy', 'herbal' or 'woody' in beer (Eyres, Marriott, & Dufour, 2007; Eyres, Marriott, Leus *et al.*, 2015). These complex oils contain numerous odour-active compounds (most of which are yet to be identified) which, significantly contribute to their aroma profile (Eyres, Marriott, & Dufour, 2007). The total essential oil constituent of hops is typically isolated by a combination of CO_2 extraction and distillation processes, with fractions of individual odour characters such as 'floral', 'citrus' and 'spicy' obtained from the total hop essential oil by

chromatography and further distillation (Van Opstaele, Goiris, De Rouck *et al.*, 2012a, 2012b). The 'spicy' fraction of hop essential oils is currently the subject of intense research to identify the compounds responsible for this particular hop character in beer (Van Opstaele, Praet, Aerts *et al.*, 2013). Significantly, the use of the descriptive term 'spicy' to describe certain hop flavour impressions in beer may indicate the activation of trigeminal receptors in the oral and nasal cavities by aroma compounds present within this fraction of hop essential oil.

In a bid to achieve desirable 'hoppy' characters and enhanced flavours in beer, brewers now regularly add hops at numerous stages of the brewing process, including the latter stages during fermentation or maturation in a process known as 'dry-hopping' (See Section 1.6.1). Alternatively, hop essential oils from selected varieties are available commercially as hop aroma extracts and can be added to beer post-fermentation for flavour intensification and product differentiation (Eyres & Dufour, 2009). The addition of hop aroma extracts to unhopped beer has been previously reported to contribute to an improved mouthfeel, fullness and increased bitterness perception (Goiris, Ridder, Rouck *et al.*, 2002; Van Opstaele, Goiris, De Rouck *et al.*, 2012a, 2012b; Van Opstaele, Praet, Aerts *et al.*, 2013). In this regard, the phenomenon of taste-aroma and taste-trigeminal interactions should be considered since many reports in the literature have shown a strong relationship between the human sense of taste and olfaction (Pfeiffer, Hollowood, Hort *et al.*, 2005; Small & Prescott, 2005; Stevenson, Prescott, & Boakes, 1999). The perception of flavour during food consumption usually involves the concurrent stimulation of the olfactory epithelium (OE) in the nasal cavity by volatile compounds/odours

via sniffing (orthonasal); and during oral processing/swallowing, which forces volatiles into the OE via the back of the throat (retronansal) (Hummel, 2008; Visschers, Jacobs, Frasnelli *et al.*, 2006). Some examples of this phenomenon include the common attribution of perceived taste qualities to odours e.g. the description of vanilla as having a 'sweet' smell, and the perceptual increase in intensity ratings of samples containing congruent odours and tastants (Dalton, Doolittle, Nagata *et al.*, 2000; Murphy, Cain, & Bartoshuk, 1977; Pfeiffer, Hollowood, Hort *et al.*, 2005). Other examples, based on detection threshold experiments (controlled for physiochemical interactions) between taste and odour compound pairs have revealed that subthreshold concentrations of odour compounds are more easily detected orthonasally when presented together with a subthreshold concentration of a taste compound, than when it is presented alone (Dalton, Doolittle, Nagata *et al.*, 2000). The role of familiarity on the observed level of taste-aroma interactions is inconsistent; some researchers only observed additivity in taste-aroma pairs which are usually encountered together (Dalton, Doolittle, Nagata *et al.*, 2000; Labbe, Damevin, Vaccher *et al.*, 2006), while others have reported additivity in taste-aroma pair irrespective of familiarity (Delwiche & Heffelfinger, 2005).

Although both taste and trigeminal sensations are sensed by distinct sensory systems, interactions exist between them which can also affect the perception of flavour in foods (Hewson, Hollowood, Chandra *et al.*, 2009). Trigeminal sensations involve the perception of texture, pungency and temperature within the oral cavity, nasal cavity or on the tongue (Cullen & Leopold, 1999). Oral irritation can reduce perceived intensity of taste and odour (Prescott, Allen, & Stephens, 1993). Lawless et al. (1985)

also demonstrated the masking of both olfactory and gustatory sensations by oral capsaicin (Lawless, Rozin, & Shenker, 1985).

The aim of this study was to investigate the impact of hop aroma on the perceived intensity, quality and temporal profile of bitterness in beer. Based on the finding in Chapter 3, which indicated that beer bitterness intensity can affect perceived bitterness quality, the impact of hop aroma on perceived bitterness quality in this study was considered at three different BU levels. The Pure Hop Aroma (PHA) extracts of Hersbrucker and East Kent Goldings (EKG) hop varieties were selected for the addition of hop aroma to the beers, and also added at different concentration levels to investigate potential interactions between the level of bitterness (BU) and hop aroma. The Hersbrucker hop variety has previously been reported to impart a 'hoppy', 'green'/'herbal' aroma as well as a 'spicy' mouthfeel to beer (Van Opstaele, Goiris, De Rouck *et al.*, 2012a). The essential oil extract of this particular variety was also found to contain higher levels of oxygenated sesquiterpenes, the compounds implicated in the generation of 'spicy' hoppy aroma character in beer. (Peacock, Deinzer, Likens *et al.*, 1981; Tressl, Engel, Kossa *et al.*, 1983; Van Opstaele, Goiris, De Rouck *et al.*, 2012b). For completeness and to deduce how comparable the base beer used in this study is to some of the beers studied in previous chapters, the analytical assessment of the base beer was conducted to determine the analytical profile of the beer in relation to polyphenol content and other brewing parameters (pH and alcohol levels etc.). The volatile chemical composition of the two selected hop aroma extracts was also evaluated by GC-MS to determine similarities/dissimilarities in their chemical constituents.

4.2 MATERIALS AND METHODS

To investigate the impact of hop aroma compounds on bitterness perception, an unhopped base lager beer was brewed, to which pre-isomerised iso-α-acid and hop aroma extracts were added, to produce a two-factorial design of samples at different BU levels and hop aroma concentrations. Various aspects of perceived sample bitterness were then assessed by a trained sensory panel using a combination of descriptive, discrimination and time-intensity techniques. Notably, to enable the effects of olfactory components of perception to be decoupled from oral (taste and mouthfeel) components, sensory tests were performed with and without nose clips for the Hersbrucker aroma addition only.

4.3 Base beer production

The unhopped lager base beer used in this study was prepared at the 10 hL SABMiller research brewery at the Sutton Bonington campus of the University of Nottingham. The standard brew was prepared from a grist composition of 70% pilsner malt and 30% dextrose adjunct. Mash-in temperature was 48 °C with addition of $CaCl_2$ at a rate of 100 mg/L. This was followed by wort boiling for 60 min (5% evaporation) and a 15 min trub stand time. The wort was cooled and fermented with a standard SABMiller lager yeast for 10 days, and maturation followed for 4 days. The beers were packaged in 330 mL brown bottles and stored at 3 °C until their preparation for sensory appraisal. The original gravity and pH of the base beer were 1.044 and 4.23, respectively. The measured brewing parameters of the base beer was 6.56 (PG), the SG was 1.0066 and OG was 45.55. Attained density was 1.00253 and beer alcohol by volume was

5%. Lastly, the total polyphenol content measured was low at an average of 136 mg/L, and pH 4.23. The measured variables are in line with some of the values recorded for commercial lager beers studied in Chapter 2.

4.3.1 Pre-isomerised iso-α-acid extract (Isohop)

Different BU levels (Low 13 BU, Medium 25 BU, High 42 BU) were achieved by the addition of a commercially available food grade standardised solution of iso-α-acids (30% w/w, density = 1.075 g/ mL), kindly provided by Botanix Ltd. (Kent, UK).

4.3.2 Hop aroma extract product

A commercial pure hop aroma extract of Hersbrucker and EKG hop varieties (60% w/w, density = 1.020 g/mL) were used to add and vary the level of hop aroma compounds in the base beer, by addition at the following levels – L0, L1 and L2, corresponding to 0, 245, 490 mg/L of beer respectively. These varieties were selected primarily based on differences relating to their origin, with Hersbrucker being a German hop variety and EKG an English seeded hop variety. The hop aroma extracts were supplied as a food grade solution, and were kindly provided by Botanix Ltd. (Kent, UK). These commercial products contain hop aroma compounds blended into propylene glycol for easy dissolution into beer. They are acquired by a combination of CO_2 extraction and distillation, and do not contain hop acids or other bitter-tasting congeners known to contribute to beer bitterness.

4.3.3 Sample preparation

Beer samples were prepared from the base beer 48 h in advance of sensory evaluation. Preparation involved uncapping the bottled base beer, followed by the addition of the respective level of Isohop (for bitterness) and hop aroma extracts (for aroma). For 13, 25 and 42 BU levels, Isohop was added at 13, 26 and 43 µL per 330 mL of beer. For the three hop aroma levels (L0, L1 and L2) hop aroma extracts were added at 0, 132 and 264 µL per 330 mL of beer, respectively. Both solutions were accurately added to the beer using Rainin pipettes fitted with sterilised graduated pipette tips (Mettler Toledo, US). After addition, the bottles were recapped with sterilised bottle caps and gently mixed by inverting the bottle at a rate of one inversion per second for 10 s. The beers were immediately transferred to cold storage (3 °C) until sensory testing.

Table 4.1: Experimental design showing the different levels of hop aroma and analytical bitterness units.

	Hersbrucker (mg/L)			**East Kent Goldings (mg/L)**		
Bitterness	***L0***	***L1***	***L2***	***L0***	***L1***	***L2***
Low (13 mg/L)	0	245	490	0	245	490
Medium (25 mg/L)	0	245	490	0	245	490
High (42 mg/L)	0	245	490	0	245	490

4.4 Analytical measures

4.4.1 Determination of total polyphenol content

Beer TPC was determined according to the ASBC Beer-35 polyphenol method (ASBC Method of Analysis., 2011). The full procedure of the assay is as previously presented in Section 2.3.2 . Triplicate measurements were conducted.

4.4.2 Measurement of brewing variables and pH

Specific gravity (SG), original gravity (OG), density and alcohol content of the base beer were determined using an Anton Paar DMA 4500 and Alcolyzer Plus (Anton Paar, Graz, Austria). The pH of the beers was measured with a calibrated standard pH meter (Metler Toledo). Measurements were carried out in triplicate.

4.4.3 GC-MS analysis of hop aroma extracts

The volatile chemical compounds constituting the hop aroma extracts were analysed by GC-MS using a Trace GC Ultra chromatograph with a DSQ I mass spectrometer (Thermo Fisher, Manchester, UK). A solution of the hop aroma extracts were prepared in methanol at a concentration of 490 mg/L, an aliquot of 1 uL was injected into the GC-MS for analysis. The GC ran in splitless mode with a splitless time and split flow of 1 min and 25 mL/min, respectively. Analytes were separated on a ZB-WAX column (30 m x 0.25 mm ID, 1 μm) with the aid a helium carrier gas set at 8 psi. The initial oven temperature was 28°C, held for 3 min and increased at a rate of 4°C/min to 250°C. The mass spectrometer was operated in full scan mode

and scanned for ions of *m/z* 28 – 250. The average peak areas of the volatile compounds from three replicate injections was used to calculate a relative (%) value in both hop aroma extracts.

4.5 Sensory evaluation

Ethical approval for the sensory aspect of this study was obtained from the University of Nottingham Ethics Committee (Approval number D14052015). All participants gave informed consent to participate in the study and were given a disturbance allowance for their participation.

4.5.1 Subjects

Experienced subjects (5 male, 2 female) from the University of Nottingham trained beer panel took part in each element of this study, they were selected for this study principally on their availability to attend all the study sessions. They attended 12 sessions each lasting 2 h per session. A further 8 assessors (1 male & 7 female, mean age 40 years) also experienced in sensory testing of beer participated in the triangle test.

4.5.2 Sensory properties of the hop aroma extract

To determine the sensory character of the hop extracts, a solution of the hop aroma extracts was prepared at a concentration of 490 mg/L (L2) in Evian water (Danone, Paris). The panel were instructed to cleanse their palate with mineral water (Evian, Danone, Paris) before smelling and tasting the hop aroma extract solutions (EKG, Hersbrucker) and describing its sensory properties.

To further determine if the hop aroma extracts possessed any taste or mouth-feel properties, a triangle test (ISO 4120, 2004) comparing the hop extract solution sample with water was carried out whereby subjects wore nose clips to prevent any olfactory stimulation. This test was only carried out for the Hersbrucker hop aroma due to time restrictions (to ensure the beers were tasted while still fresh), where subjects were presented with three 10 mL solutions, according to a randomised partially balanced design and asked to pick out the different sample. They were also asked to indicate why they thought it was different. They were instructed to use a palate cleanser of mineral water (Evian, Danone, Paris) and crackers (Rakusen's, UK) prior to assessing each sample.

4.5.3 Refined bitterness lexicon

The previously developed and defined bitterness lexicon consisting of 13 bitterness character attributes (Section 3.3.1) was refined to 12 attributes in this study. The panel recommended that the attributes 'round' and 'smooth' be combined and redefined. Therefore the 12 final attributes used here were *harsh* (tingly, raspy, irritating); *citric* (fruit-like acidity); *round* (smooth, pleasant, not spiky or harsh); *metallic* (taste of tin/metal, silver coin taste); *sharp* (instant bitterness taste on the tip of the tongue); *astringent* (drying, causing drying of the mouth); *artificial* (chemically, unnatural beer flavour); *vegetative* (cabbage, sprout-like bitterness, hop tea taste); *progressive* (increasing bitterness perception); *lingering* (bitterness intensity perceived after seconds of beer consumption); *instant* (instantaneous bitterness perception); *diminishing* (rapid decrease in bitterness perception upon ingestion).

4.5.4 Perceived bitterness intensity and character

The impact of hop aroma on both perceived bitterness intensity and the intensity of selected bitterness character attributes upon the addition of both EKG and Hersbrucker hop aroma were assessed using the rank-rating technique (Kim & O'Mahony, 1998). This combined technique was selected as it allows an initial evaluation of whether samples could be discriminated from each other from the ranking data, and a measure of the magnitude of the difference, if it exists, from the intensity rating scores. The selected bitterness character attributes, representing different facets of bitterness perception in beers were 'round', 'harsh' and 'lingering'.

Subjects were trained to use the rank-rating technique for each attribute. No training was needed for ranking per se other than the instruction to rank the samples presented from low to high intensity of the attribute in question. For rating, subjects were instructed that the scales presented were anchored from 0 to 10, with 0 representing low and 10 representing high intensity of an attribute. To familiarise the panel with the range of intensity represented by the scale, subjects were presented with beers of differing BU levels (13, 25 and 42 BU) and they discussed where they should be placed on the scale. To reinforce and evaluate panellist scale use, they were also provided with commercial beers assessed by High Performance Liquid Chromatography (Oladokun, Smart, & Cook, 2016) to be within a similar range of BU selected for this study. To re-familiarise the panel with the specific character attributes of 'round' and 'harsh' the subjects were given commercial beers, appraised to be of said bitterness characters as in Chapter 3, as reference. The attribute 'linger' was assessed as the intensity of bitterness perceived after 10 secs upon

swallowing the sample. The panellists were trained to use a stopwatch to assess this. Panel performance was monitored based on their ability to consistently identify the selected bitterness attributes in different samples in different sessions.

4.5.5 Temporal profile of bitterness

In order to understand how hop aroma affected the time course of bitterness intensity, TI measurements were conducted for the beers with Hersbrucker hop aroma added only – due to time restrictions. Before TI evaluation, the panellists underwent further training to ensure that they were still comfortable with the use of the scale (for rating intensities) and the TI data collection set-up. The panel had considerable previous experience with the TI technique. Beer samples at 13, 25 and 42 BU (with no hop aroma extract added) were re-introduced to the panellists as standards to practice intensity ratings on the scale.

4.5.6 Sample evaluation

In all cases samples were served at 4 ± 2 °C in a 50 mL glass vial which was labelled with a 3-digit random code. Subjects cleansed their palates with Evian water (Danone, France) and crackers (Rakusen's, UK) before evaluating each sample. Appropriate breaks (3 min between attribute) were built into the design of the evaluation sessions to ensure that bitterness carry-over and palate saturation was kept to a minimum. All data were collected with Compusense Cloud (Compusense, Canada).

For rank-rating evaluations subjects were presented with sets of 3 samples representing different levels of Hersbrucker and EKG hop aroma extract addition (L0, L1, and L2) at one BU level, although this relationship between the three samples for each hop aroma was not disclosed to the subjects to avoid bias. Subjects were first asked to rank the samples for bitterness intensity. They were then asked to retaste the samples and rate them on the intensity scale. Subjects followed the same protocol to assess the bitterness character attributes 'round', 'harsh' and 'linger', at each of the 3 BU levels. Three replicate assessments were carried out at each BU level, and the experimental design was balanced to moderate inter-session variation. The above evaluations were then repeated with the use of nose clips for the Hersbrucker hop aroma, to isolate the impact of the oral stimulation by the hop aroma extract from its olfactory component for the low and high BU samples only. Subjects were only allowed to remove the nose clips during the break period (between attribute evaluations). A sample volume of 30 mL was used for all rank-rating evaluations.

TI evaluation was carried out on low and high BU for Hersbrucker samples only. Subjects evaluated samples selected according to a randomised balanced design. Evaluations were performed with and without nose clips over three replicate assessment. 10 mL samples were used for all TI evaluations which lasted for a time period of 60 s. The following TI parameters were extracted from the TI curves: maximum intensity (IMax), time to maximum intensity (TMax), area under the curve (AUC) and increasing angle (IAngle) (Duizer, Bloom, & Findlay, 1997), using an Excel Macro provided by Compusense.

4.6 Statistical analysis

Statistical analyses were carried out with XLSTAT version 2015.6 (Addinsoft, Paris) and STATGRAPHICS Centurion XVII statistical software (Statpoint Technologies, Virginia), significance was derived at $\alpha = 0.05$. Rank data was analysed using Friedman's test followed by Nemenyi's pairwise comparison test. Attribute intensity ratings were analysed using a three-factor (BU level, Hop aroma level and subjects) analysis of variance (ANOVA) to identify if differences existed between samples and if interactions between BU and Hop aroma levels were evident. Where significance was established, a Tukey's HSD post hoc test was used to identify which specific samples were discriminated from each other. STATGRAPHICS Centurion XVII was used to generate interaction plots between hop aroma and BU levels. The impact of hop aroma on TI parameters was analysed using a two factor (sample, subject) ANOVA and subsequent Tukey's post hoc tests. A PCA was used to characterise the variance within the identified volatile hop aroma compounds with respect to each hop variety.

4.7 RESULTS

Due to the length of this chapter the results and discussions sections are presented separately.

4.7.1 Chemical characterisation of hop aroma compounds

The GC-MS analysis of the hop aroma extracts based on the chemical constituents of these extracts is shown below as a PCA in Figure 4.1. Several volatile aroma compounds were detected as shown below, based on these compounds the selected varietal hop aroma extracts were

significantly different from each other. The PCA, accounting for 100% of variation in the data set shows separation for the hop aroma extracts along PC1 (87%). The EKG hop aroma extract was strongly correlated with oxygenated compounds while Hersbrucker was strongly correlated with terpene hydrocarbons, including mono- and sesquiterpene hydrocarbon compounds. Sulfur compounds were not detected in the essential oil of these hops.

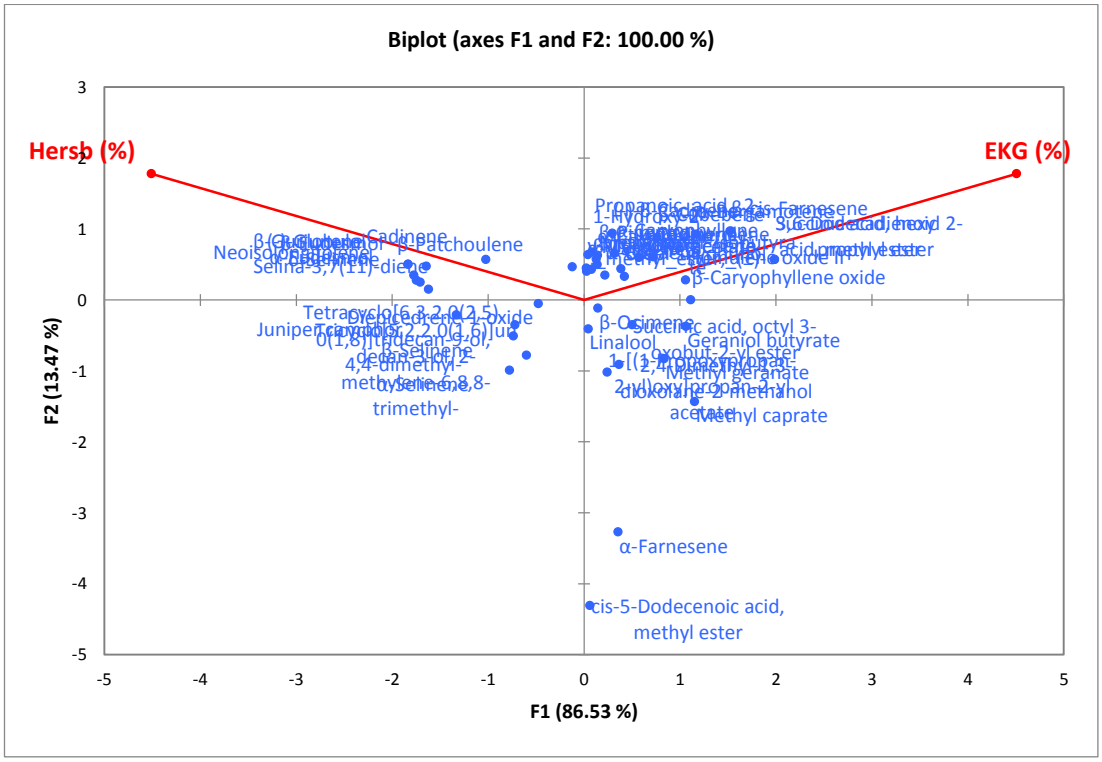


Figure 4.1: PCA plot of Hersbrucker (Hersb) and EKG hop aroma extracts based on the relative concentrations of their volatile chemical constituents (also see Appendix 3 for a full list of compounds).

4.7.2 Panel performance

The analysis of the discriminative and repeatability performance of the panellists for two beers with different bitterness intensity and character attributes is shown in Figure 4.2. Beer E, previously determined (in Chapter 2) to be of high bitterness intensity was rated with an average score of 7.81 on a scale of 0 – 10 over 3 sessions. In contrast and as expected, beer V was appropriately rated with an average score of 3.70. Beer E was also rated highly for harsh, lingering and astringent bitterness characters compared to beer V which was rated highly for round bitterness character. The low mean coefficient of variance (CV) values calculated for each attribute for both beers over the three sessions further confirm that the panel were consistent and repeatable in their discrimination and rating of these bitterness attributes.

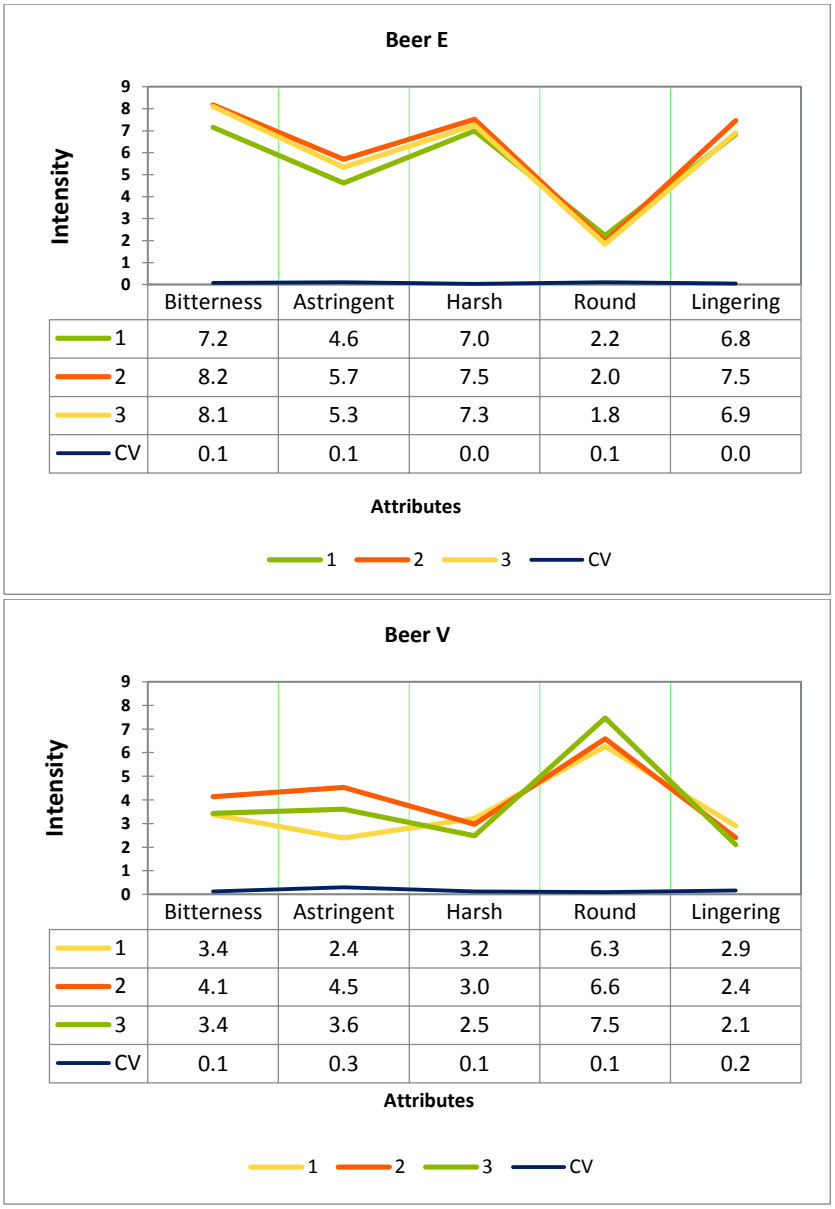


Figure 4.2: Panel performance assessment for the key bitterness quality attributes in three sessions (1, 2, and 3) for Beer E and V. CV represents the coefficient of variance.

The panel performance data showed that all panellists were able to both discriminate and consistently rate the bitterness attributes of interest in different beers. Consistent bitterness attribute identification and ratings was also demonstrated over replicate sessions, confirming that the panellists were reproducible and reliable for the evaluation of bitterness intensity and its quality attributes in beer.

4.7.3 Sensory properties of hop aroma extract

The sensory qualities of the hop aroma extracts in water as perceived by the panel are listed in Table 4.2 for both EKG and Hersbrucker. Some of the terms used to describe the solution of Hersbrucker include 'herbal', 'woody', 'hoppy' and 'orange peel'. The taste/ mouthfeel properties of the solution were described as 'gingery', 'spicy', 'mouth coating', 'tingly' and 'peppery', with no notable mention of 'bitter' (Table 4.2). For EKG, terms such as 'musty', 'citrusy' and 'melon' were used. There was also an astringent mouthfeel to the extract solution of this particular hop variety. The triangle test for the Hersbrucker solution, where nose clips were used, revealed only 4 of 15 people correctly identified the odd sample implying no significant difference was perceived ($p = 0.74$). However, panellists who discriminated correctly between the hop aroma and water solution described it as 'peppery' and 'soapy', indicating the presence of trigeminal-type sensations for these panellists in the Hersbrucker solution.

Table 4.2: Odour and mouthfeel properties of hop aroma extract of Hersbrucker Spät and EKG (without and with nose clips).

Hersbrucker Spät		***East Kent Goldings***	
Odour descriptors	**Mouthfeel descriptors**	**Odour descriptors**	**Mouthfeel descriptors**
Herbal	Gingery	Melon	Astringent
Lime	Mouth coating	Musty	Soapy
Orange peel	Spicy	Orange	
Piney/nutty	Tingly	Citrusy (artificial)	
Hoppy	Peppery		
Woody	Medicinal		

4.7.4 Effect of EKG hop aroma addition on perceived bitterness intensity and character

The mean sample rank scores for bitterness intensity and bitterness character attributes, across the three BU levels, upon the addition of the EKG hop aroma extract to the base beer are presented in Table 4.3. The mean sample rating scores for these bitterness attributes, across the three BU levels are also presented in Figure 4.3 as spider plots.

4.7.4.1 Sum of ranks

According to the Friedman's test the addition of EKG hop aroma significantly impacted on perception of bitterness intensity. At low and medium BU levels, samples with hop aroma addition at L2 were ranked to be significantly more bitter than L0. At the high BU level the addition of hop aroma had no significant impact on the perception of bitterness intensity. For bitterness character attributes, at low BU only harsh bitterness character was significant for the L2 samples compared to L0, with the remaining bitterness attributes insignificant. At medium BU, round bitterness attributes was not significant but both harsh and lingering bitterness characters were significant for samples L2 compared to L0. At high BU, only round bitterness character was significant for L0, i.e. the samples at this BU level with no hop aroma added were ranked to be significantly round in bitterness character compared to both L1 and L2 samples.

Table 4.3: Sum of ranks for beer samples and results of Nemenyi's multiple pairwise comparison test by BU level for EKG hop aroma addition (L0, L1 and L2 correspond to 0, 245 and 490 mg/L addition of EKG hop aroma extract).

	Hop aroma extract level		
BU Level	***L0***	***L1***	***L2***
Low (13BU)			
Bitterness intensity:	29[a]	46[b]	51[b]
Harsh:	32[a]	46[ab]	48[b]
Round:	46[a]	39[a]	41[a]
Lingering:	36[a]	43[a]	47[a]
Medium (25BU)			
Bitterness intensity:	32[a]	38[ab]	50[b]
Harsh:	31[a]	39[ab]	50[b]
Round:	46[a]	41[a]	33[a]
Lingering:	33[a]	39[ab]	48[b]
High (42BU)			
Bitterness intensity:	36[a]	38[a]	40[a]
Harsh:	36[a]	39[a]	39[a]
Round:	45[b]	37[ab]	32[a]
Lingering:	38[a]	34[a]	42[a]

[ab] samples with same letter code in a row are not significantly different according to Nemenyi's test ($p < 0.05$).

4.7.4.2 Intensity ratings

The analysis of the intensity scores by ANOVA is presented as spider plots in Figure 4.3. The ANOVA analysis revealed no significant effect of judges ($p>0.05$) and also confirmed the significant impact of EKG hop aroma on bitterness intensity and its quality attributes, generally in line with the rank scores. In agreement with the mean rank scores, both L2 samples were significantly harsher and perceived to be more bitter than L0 samples at Low BU. At medium BU the intensity scores were completely in agreement with the rank scores for bitterness intensity, harsh and lingering bitterness

characters. However, there was a significant effect of hop aroma on round bitterness character based on the intensity scores, with both samples (L1, L2) perceived to be less round in bitterness character than L0. At high BU, none of the bitterness character attributes were significantly different between the samples.

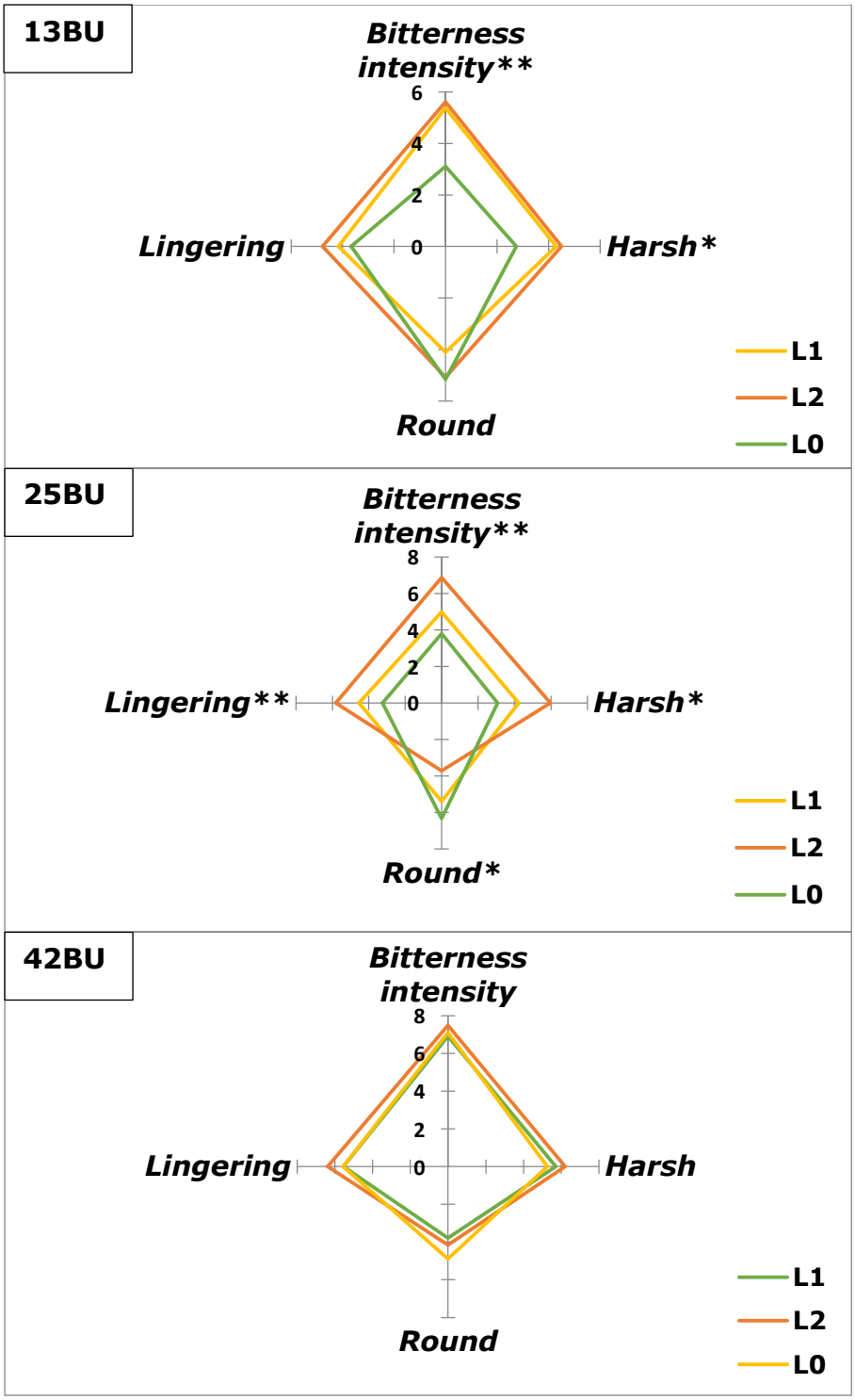


Figure 4.3: Spider plots of mean bitterness intensity and bitter character attributes based on intensity ratings for EKG hop aroma addition. Low: (13BU) beer, Medium: (25BU) beer and High: (42BU) beer. L0, L1 and L2 at each BU level corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance derived at *5% and **1% level.

4.7.5 Effect of Hersbrucker hop aroma extract on perceived bitterness intensity and character

The result of the impact of the addition of Hersbrucker hop aroma to the beers at each BU level, as determined by mean rank scores is presented in Table 4.4. The mean sample ratings for bitterness intensity and bitterness character attributes, across the three BU levels are also presented in Figure 4.4 as spider plots.

4.7.5.1 Sum of ranks

In line with observations for the EKG hop aroma, the addition of this extract to beer resulted in an increased perception of bitterness intensity. Samples with hop aroma addition at L1 and L2 were perceived to be more bitter than sample L0 as shown in Table 4.4. In contrast to the findings at high BU for EKG where the rank data showed no significance for bitterness intensity, the addition of Hersbrucker hop aroma at L1 was ranked to be significantly more bitter than L0 at high BU level. For harsh bitterness character, there was no significance at low and high BU levels but sample L1 was significantly harsher in bitterness character at medium BU. Samples L1 relative to L2 & L0 was significantly round in bitterness character at low BU, whilst samples L0 were of the same character at medium and high BU levels. The mean rank scores revealed that the attribute 'lingering' was only affected by hop aroma addition at high BU levels, sample L2 was ranked to have a more lingering bitterness compared to L0 and L1.

Table 4.4: Sum of ranks for beer samples and results of Nemenyi's multiple pairwise comparison test by BU level for Hersbrucker hop aroma addition (L0, L1 and L2 correspond to 0, 245 and 490 mg/L addition of hop aroma extract).

	Hop aroma extract level		
BU Level	***L0***	***L1***	***L2***
Low (13BU)			
Bitterness intensity:	30^{a}	35^{ab}	52^{b}
Harsh:	36^{a}	38^{a}	46^{a}
Round:	34^{a}	50^{b}	36^{a}
Lingering:	41^{a}	38^{a}	41^{a}
Medium (25BU)			
Bitterness intensity:	28^{a}	42^{b}	44^{b}
Harsh:	30^{a}	44^{b}	40^{ab}
Round:	44^{b}	38^{a}	32^{a}
Lingering:	36^{a}	37^{a}	41^{a}
High (42BU)			
Bitterness intensity:	32^{a}	51^{b}	43^{ab}
Harsh:	41^{a}	40^{a}	45^{a}
Round:	51^{b}	39^{a}	36^{a}
Lingering:	34^{a}	43^{ab}	49^{b}

^{ab} samples with same letter code in a row are not significantly different according to Nemenyi's test ($p < 0.05$).

4.7.5.2 Intensity ratings

ANOVA of the bitterness intensity rating data also indicated that significant differences were evident between samples at each BU level ($p < 0.05$) as shown in Figure 4.4. These results largely confirm the observations from the rank data. For example, at low BU, samples with hop aroma levels L0, L1 and L2 were given a mean score of 3.47, 3.83 and 5.71 for bitterness intensity respectively. Although no significant interaction was evident between BU and hop aroma level ($p = 0.22$) the impact of hop aroma addition did appear to change at different BU levels. L2 samples were rated to be significantly more bitter than L0 and L1 at low BU, while at medium bitterness, samples L1 and L2 were rated to be significantly more bitter than L0. No significant difference in bitterness intensity ratings were observed at the high BU level but sample L1 was rated highest (6.41) for this attribute. Both Friedman and ANOVA analyses revealed no significant difference in the scores of 'harshness' between the samples at low and high BU levels. However, at medium BU, both samples L1 and L2 were ranked and rated to be significantly harsher in bitterness character relative to L0. The differential effect at the medium BU level was highlighted as a significant BU*Hop aroma interaction in the ANOVA ($p = 0.05$) and is evident in the interaction plot shown in Figure 4.5.

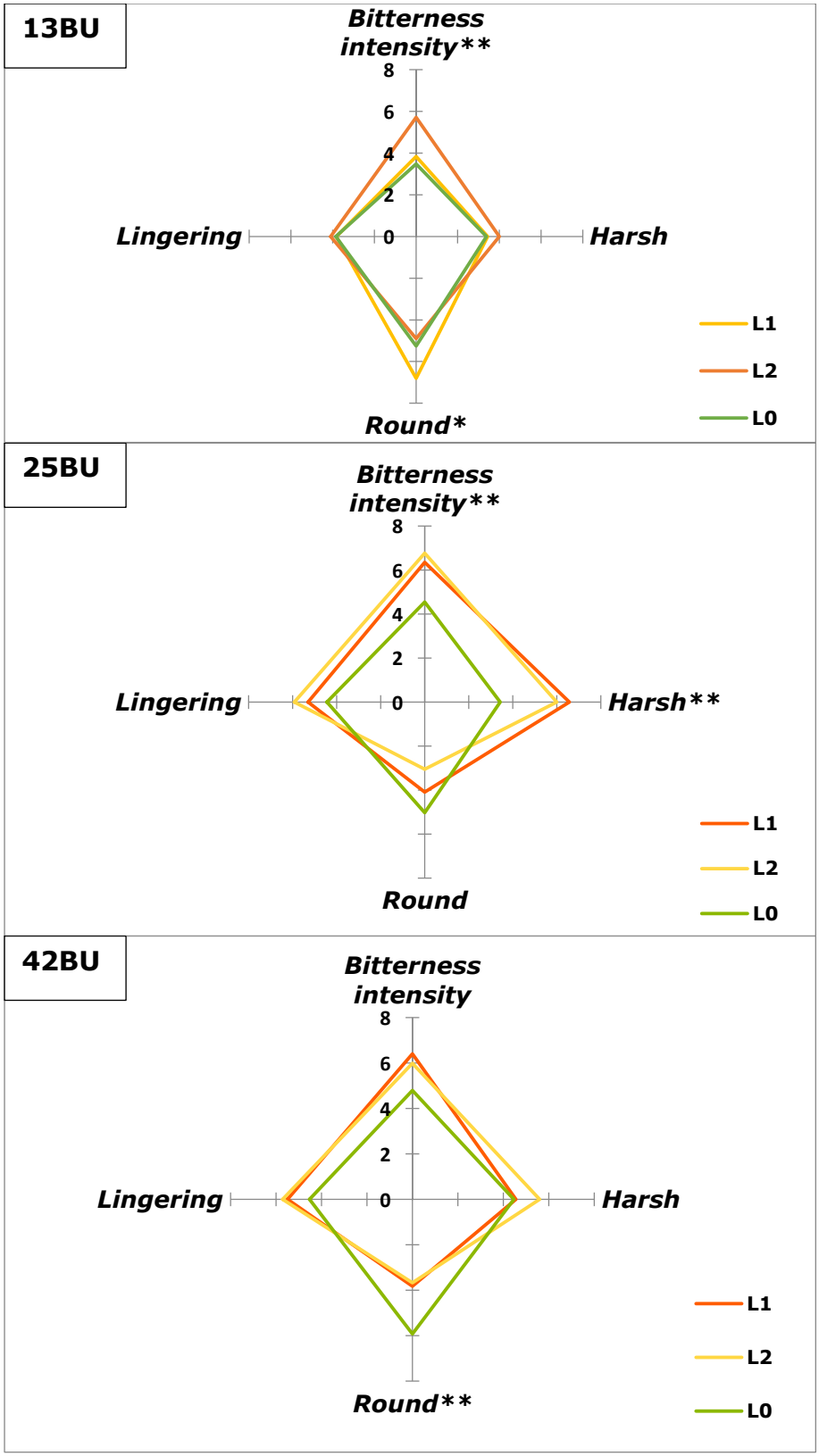


Figure 4.4: Spider plots of mean bitterness intensity and bitter character based on intensity ratings for Hersbrucker hop aroma addition. Low: (13BU) beer, Medium: (25BU) beer and High: (42BU) beer. L0, L1 and L2 at each BU level corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance derived at *5% and **1% level.

For round bitterness character, although L1 samples were ranked as rounder in bitterness character relative to L0 and L2 the rating data revealed a slightly different result, with L1 rated as significantly rounder than L2, but not L0, signifying that although perceptible, the magnitude of the difference in roundness of bitterness was bigger between L1 and L2 than L1 and L0 at low BU. At medium and high BU levels, samples with no hop aroma added (L0) were generally ranked and rated to have a rounder bitterness character relative to those with hop aroma added. This observation was significant in intensity rating at high BU level ($p < 0.05$) as can be seen in Figure 4.4. The interaction between BU level and hop aroma levels in the rating data for round bitterness character is also apparent in Figure 4.5B, where unlike at medium and high BU levels the addition of hop aroma at L1 resulted in a much rounder bitterness rating at low BU level.

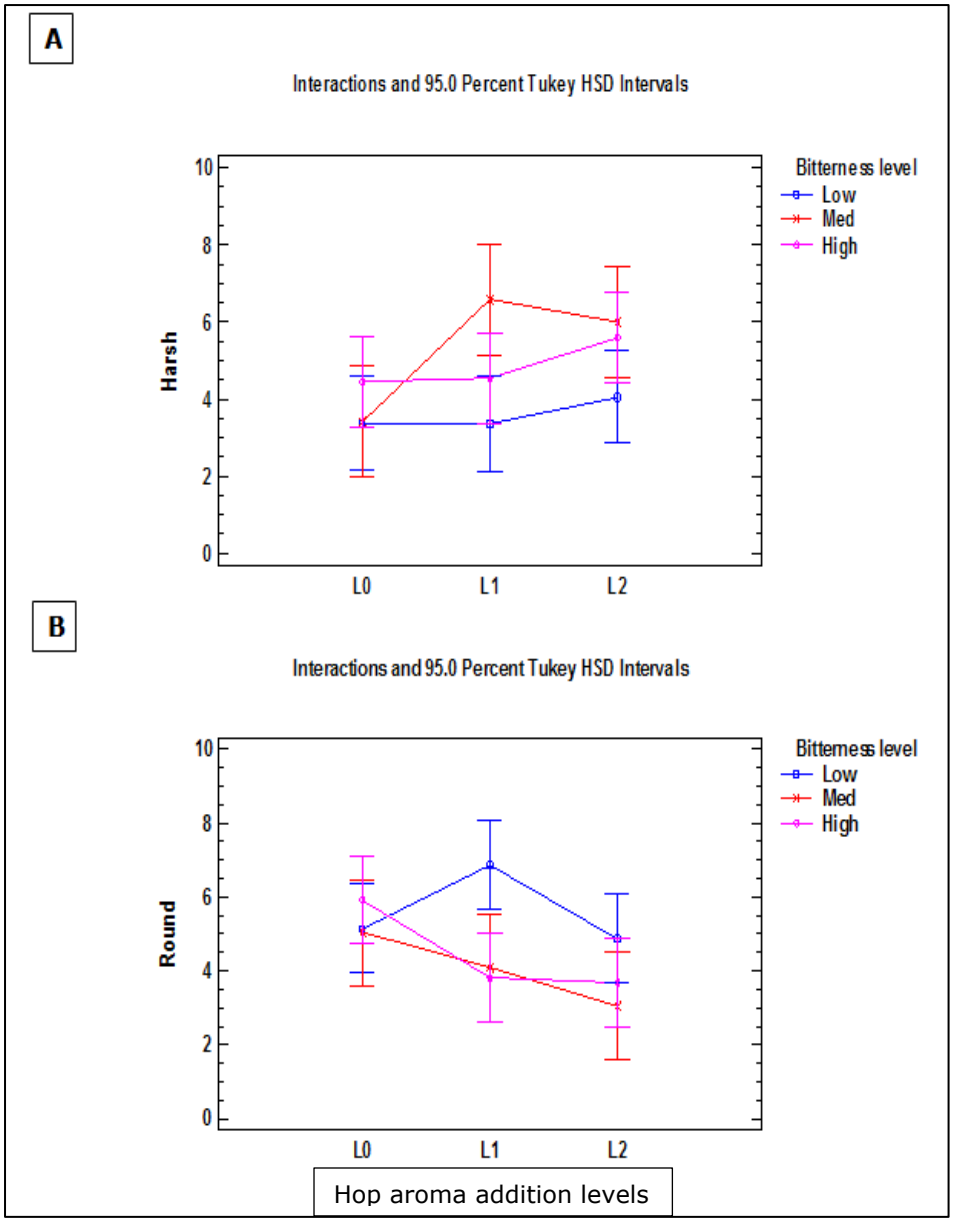


Figure 4.5: Interactions between BU and aroma levels for Hersbrucker hop aroma addition. A: interactions for intensity of harsh bitterness character and B: interactions for intensity of round bitterness character. L0, L1 and L2 corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance derived at 5% level.

4.7.6 Effect of Hersbrucker hop aroma extract on perceived bitterness intensity and character with nose clips on

The impact of occluding olfaction on perception of bitterness in the beers was determined by subjects whilst wearing nose clips. The findings in this regard for sample rank scores for bitterness intensity and bitterness character attributes, at low and high BU levels is presented in Table 4.5. The mean sample rating scores for bitterness intensity and bitterness character attributes, at low and high BU levels are presented in Figure 4.6 as spider plots.

4.7.6.1 Sum of ranks

With nose clips on at low BU only harsh bitterness character was significant upon the addition of Hersbrucker hop aroma. At high BU, even with nose clips on sample L2 was ranked to be more bitter. This was the case for harsh bitterness character as well, and for round bitterness character – beer L0 was perceived to be of round bitterness character compared to L1 and L2. Lingering bitterness character was not significant at high BU levels with nose clips on.

Table 4.5: Sum of ranks for beer samples and results of Nemenyi's multiple pairwise comparison test by BU level, with nose clips for Hersbrucker hop aroma addition (L0, L1 and L2 correspond to 0, 245 and 490 mg/L addition of hop aroma extract).

	Hop aroma extract level		
BU Level	***L0***	***L1***	***L2***
Low (13BU)			
Bitterness intensity:	41^{a}	40^{a}	45^{a}
Harsh:	38^{ab}	35^{a}	53^{b}
Round:	44^{a}	43^{a}	39^{a}
Lingering:	39^{a}	41^{a}	46^{a}
Medium (25BU)			
Bitterness intensity:	-	-	-
Harsh:	-	-	-
Round:	-	-	-
Lingering:	-	-	-
High (42BU)			
Bitterness intensity:	33^{a}	44^{ab}	49^{b}
Harsh:	33^{a}	44^{ab}	49^{b}
Round:	49^{b}	43^{ab}	34^{a}
Lingering:	37^{a}	46^{a}	43^{a}

^{ab} samples with same letter code in a row, per nose clip condition, are not significantly different according to Nemenyi's test ($p < 0.05$). (-) Not evaluated.

4.7.6.2 Intensity ratings

The rating data for bitterness intensity and the selected bitterness attributes at low BU was in total agreement with the rank data. When nose clips were used at low BU, there were no significant differences observed between samples based on rank or rating scores for bitterness intensity (Figure 4.6 Low). However, at high BU, sample L2 was ranked as significantly more bitter than L0 and L1 (Figure 4.6 High). This effect was not significant in the rating data indicating that the perceived increase in bitterness intensity was likely to be subtle, but nevertheless perceptible. There was no significant interaction between BU and hop aroma level for bitterness intensity with nose clips on ($p = 0.96$).

For round bitterness at high BU, the significant difference based on rank scores was not picked up in the rating data, again indicating that the difference was subtle, but nevertheless perceptible. When rating with nose clips on, very few differences in bitterness character were observed indicating that the differences in rankings above were fairly subtle. No significant interactions between BU and Hop aroma level were evident. The only significant difference observed confirmed that, at low BU, L2 hop aroma addition resulted in a harsher bitterness character increasing from a score of 2.6 and 2.5 for L0 and L1 respectively, to 3.8 at L2.

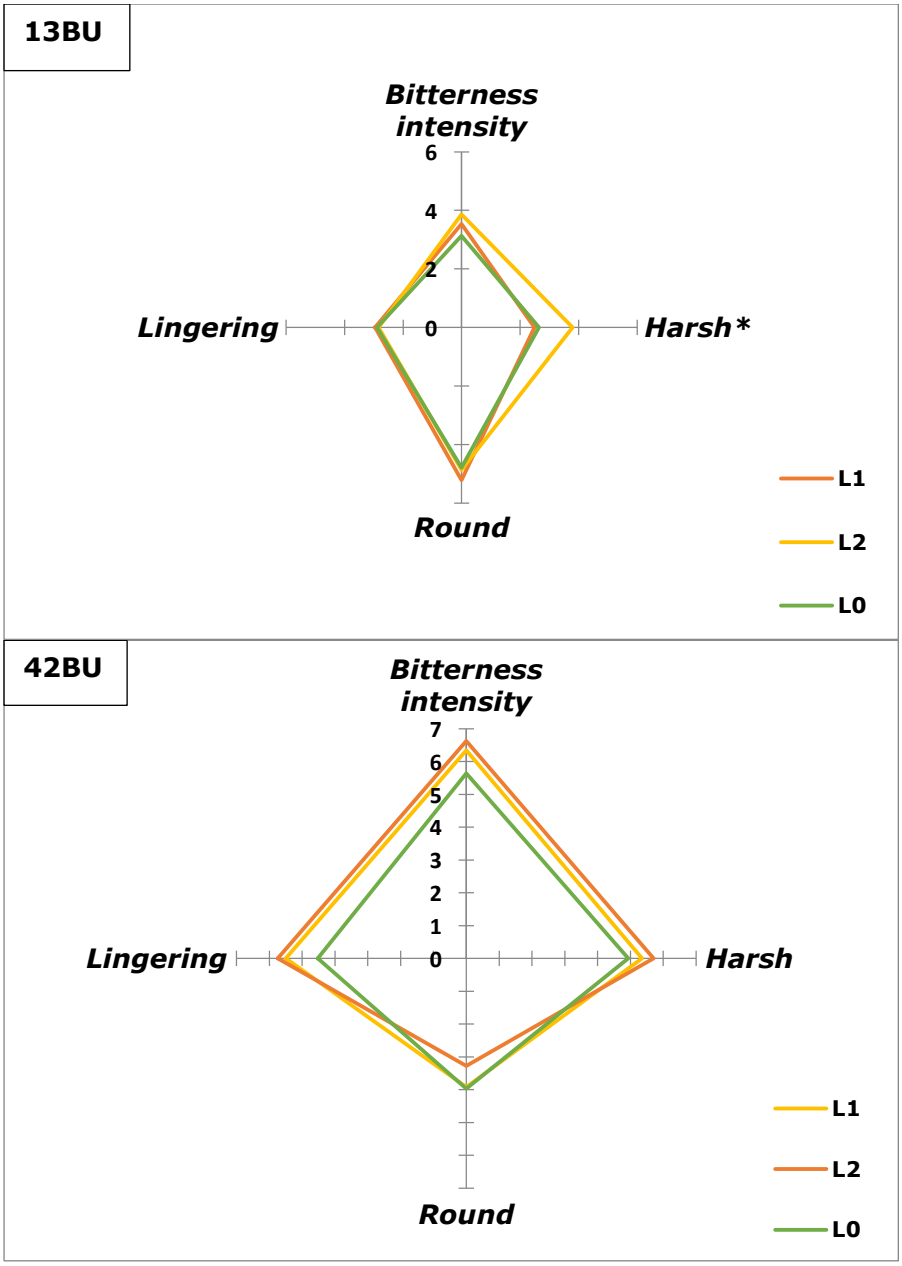


Figure 4.6: Spider plots of mean bitterness intensity and bitter character based on intensity ratings with nose clip on for Hersbrucker hop aroma. Low: (13BU) beer and High: (42BU) beer. L0, L1 and L2 at each BU level corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance derived at *5% and **1% level.

4.7.7 Time-intensity results for Hersbrucker hop aroma addition

The average TI curves (n = 21 (7 subjects $*$ 3 replicates)) from evaluations performed without and with nose clips are presented in Figure 4.7 and Figure 4.8, respectively. Without nose clips, according to the ANOVA and subsequent Tukey multiple comparison tests, at low BU level the temporal profile of L2 upon Hersbrucker hop aroma addition was significantly different for three TI parameters – IMax, TMax and AUC in comparison to L0. Imax, which corresponds to the maximum intensity perceived, was greater for L2, while TMax, the time it took to reach maximum intensity was shorter compared to L0. AUC, which represents an overall integration of bitterness intensity, was greater for L2 than L0. At high BU, IAngle, the parameter denoting the rate of onset of bitterness sensation was the only TI parameter discriminating the samples. This angle was smaller for L2, denoting a faster rate of bitterness onset.

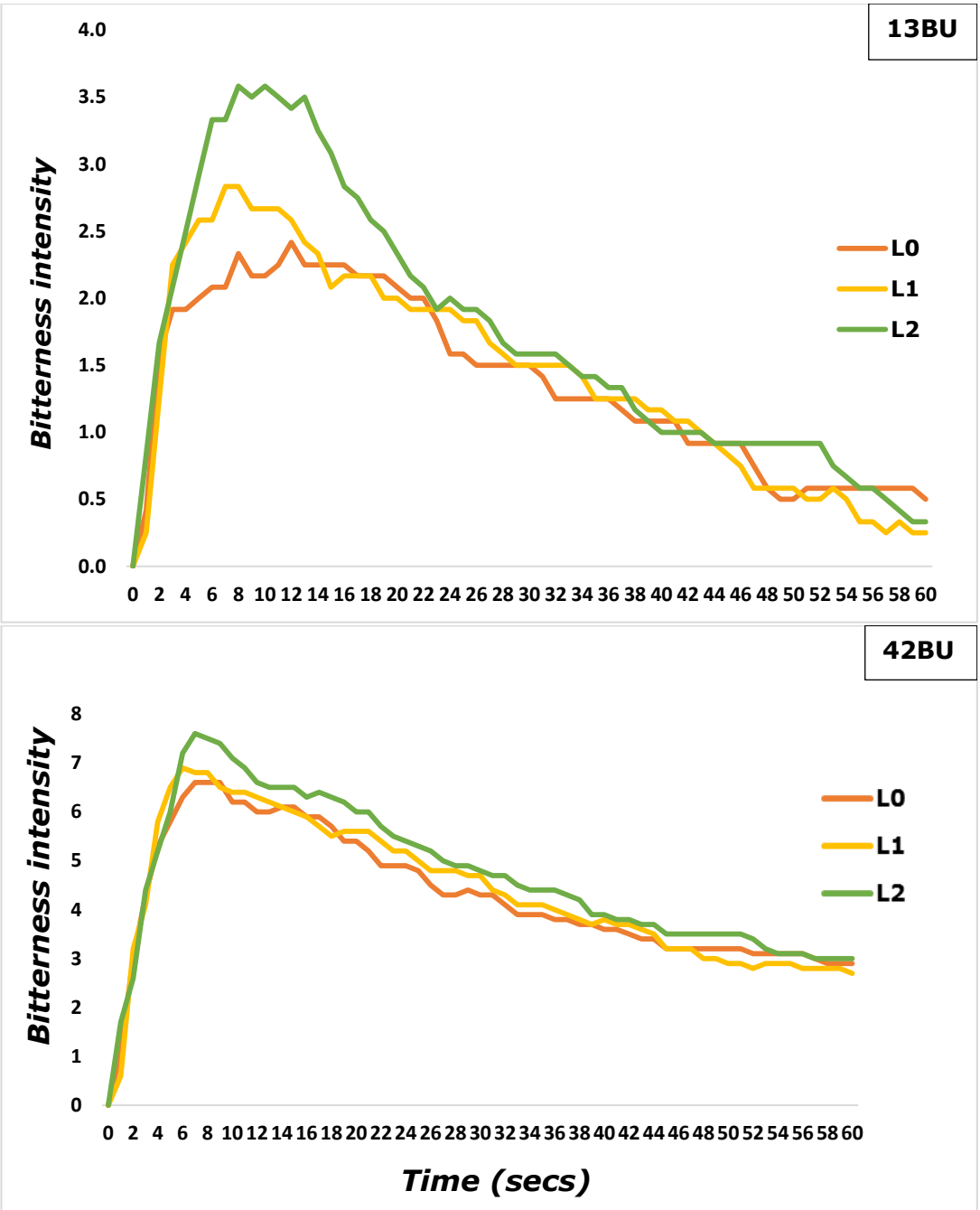


Figure 4.7: Average time-intensity curves for Hersbrucker hop aroma addition without nose clip on. Low: (13BU) beer and High: (42BU) beer. L0, L1 and L2 correspond to hop aroma extract addition levels of 0, 245 and 490 mg/L respectively. Significance derived at 5% level. (n= 21 based on 7 panellists x 3 replicate measurements).

When nose clips were used, no significant differences were revealed for any of the parameters, indicating no significant difference between the samples in terms of their temporal profiles.

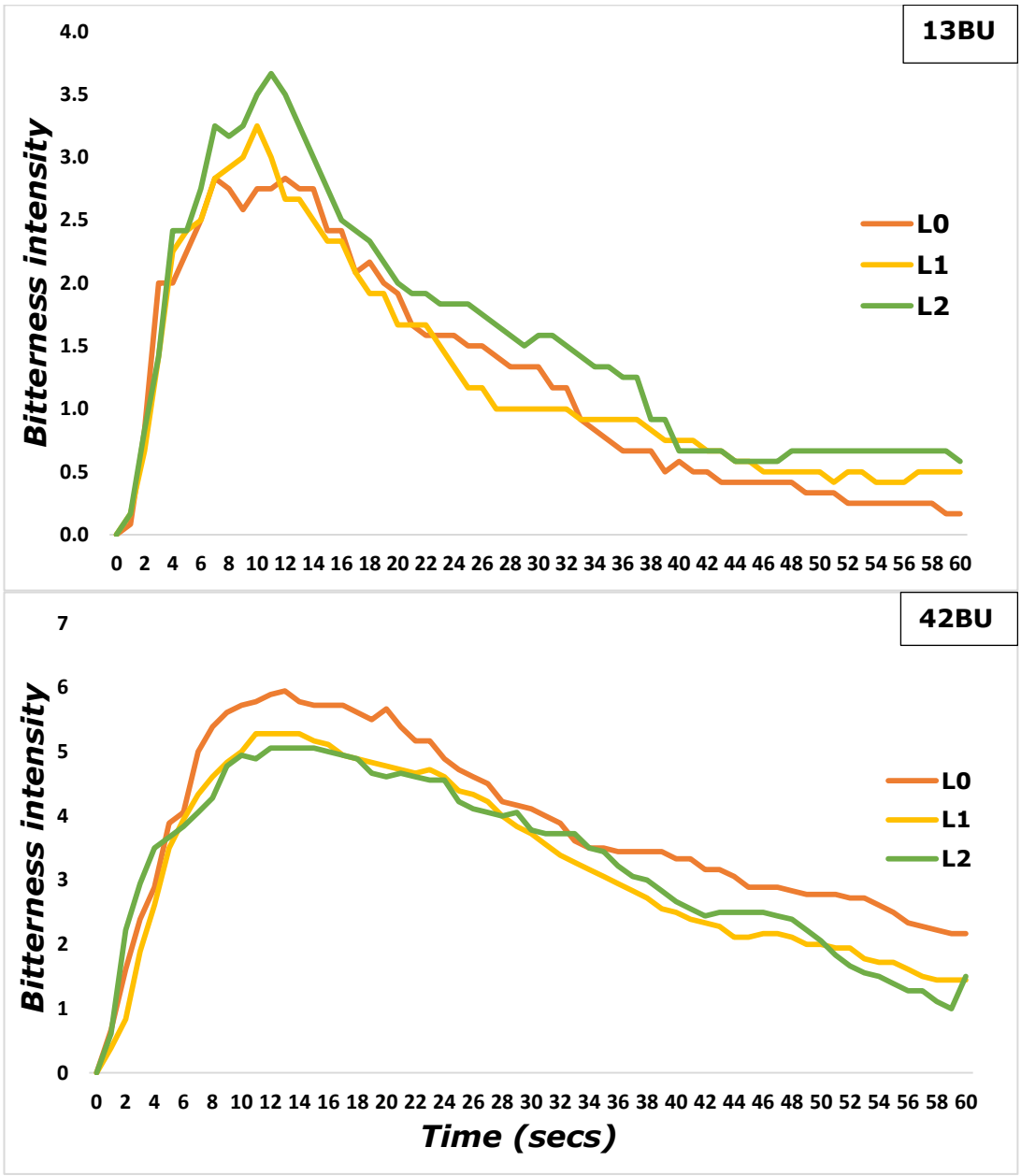


Figure 4.8: Average time intensity curves for Hersbrucker hop aroma addition with nose clip on. A: low (13BU) beer and B: high (42BU) beer. L0, L1 and L2 correspond to hop aroma extract addition levels of 0, 245 and 490 mg/L respectively. Significance derived at 5% level. (n= 21 based on 7 panellists x 3 replicate measurements).

4.8 DISCUSSION

4.8.1 Analytical profile of base beer and aroma profile of hop extract

The results of the analytical profile (Section 4.4.1) of the base beer as well as the chemical composition of the hop aroma extracts (Section 4.4.3) utilised in this study were determined in order to allow for potential correlations with attained sensory data. The quality parameters measured for the base beer showed that it was fit for purpose in terms of its use as a base beer. The low PG observed in the base beer can be explained by the fact that this was not an all-malt brew, with the use of a 30% glucose adjunct contributing to the observed low PG. Furthermore, the lower TPC value recorded (132 mg/L) compared to the average value for lager beers as reported in Section 2.4.2 is acceptable since the base beer was not hopped. Beer polyphenols are derived majorly and primarily from malt and then hops (Aron & Shellhammer, 2010), so the absence of hops as well as the use of adjunct in the base beer explains the low TPC content measured. The analytical assessment of the hop aroma extracts by GC-MS showed that these varietal extracts were different in terms of their volatile chemical composition. The chemical constituents of hop oil are divided into 3 main groups consisting of hydrocarbons (monoterpenes, sesquiterpenes, and aliphatic hydrocarbons), oxygenated compounds (terpene alcohols, sesquiterpene alcohols, others e.g. alcohols, epoxides & ketones) and sulphur-containing compounds (thioesters, sulphides) (Almaguer, Schönberger, Gastl *et al.*, 2014). The differences found in the hop aroma extracts were associated with the levels of non-polar, volatile hydrocarbons and polar oxygenated compounds (Sanchez, 1990). The EKG hop aroma extracts contained mostly oxygenated oil compounds in

comparison to Hersbrucker, which contained significant amounts of terpene hydrocarbon compounds. Examples of these compounds in the former include linalool, methyl caprate, β-caryophyllene oxide, geraniol butyrate and β-ocimene. In the latter, compounds such as juniper camphor, cadinene, selinene, and β-patchoulene were highly prevalent. The none detection of sulfur compounds in the hop oils was due to the low sensitivity of the detector used for sulfur compounds, as well as the fact that sulfur compounds, although potent in aroma and with low odour thresholds are only present in hop oils in trace amounts (Almaguer, Schönberger, Gastl *et al.*, 2014). These results show clearly that the chemical constituents of these varietal aroma extracts are different.

4.8.2 Taste and mouthfeel qualities of hop aroma extract solutions

After establishing that the analytical composition of the selected hop aroma extracts were different, their smell and taste properties were determined in water and further confirmed by a triangle test for the Hersbrucker hop aroma extracts. The descriptive terms used to characterise the Hersbrucker hop aroma extract solution included 'herbal', 'woody' and 'lime'; and for the EKG hop aroma extract – 'melon', 'musty' and 'citrusy'. These terms consisted mainly of those derived as a result of orthonasal aroma perception although the use of attributes such as 'tingly', 'spicy' and 'peppery' in Hersbrucker and 'astringent' in EKG also suggests some element of trigeminal or tactile sensations being elicited by hop aroma compounds present within the hop aroma extracts. The results also suggest that at the levels used in this study, the hop aroma extracts of the selected varieties were not perceived as bitter so any change to bitterness

measures must be due to some form of perceptual interactions. Furthermore, these findings support previous reports of a 'spicy' character or impression commonly associated with the Hersbrucker hop variety in the literature (Goiris, Ridder, Rouck *et al.*, 2002; Van Opstaele, Goiris, De Rouck *et al.*, 2012a, 2012b), and further show that hop aroma extracts can elicit mouthfeel properties of a trigeminal nature e.g. 'peppery' that are not just associated with olfactory stimulation, as this was reported even when nose clips were worn.

4.8.3 Impact of hop aroma on perceived bitterness intensity

Based on the observed intensity rating scores presented in Figure 4.3 and Figure 4.4 for EKG and Hersbrucker respectively, the addition of both hop aroma extracts caused a significant increase in perception of bitterness intensity at low and medium BU levels in beer. The mean rank scores presented in the same order in Table 4.3 and Table 4.4 support these interpretations. Aroma addition at L2 had the most significant impact on perceived bitterness intensity for both hop aroma extracts. These findings also show that the effect of hop aroma on bitterness intensity is dependent on the inherent analytical level of bitterness (i.e. BU), the level of aroma addition as well as the hop aroma extract used. While both hop aroma extracts increased perceived bitterness intensity at low and medium BU levels, this increase varied depending on the BU level and level of aroma addition. For example, at low BU with EKG, both levels of addition (L1, L2) were perceived to be more bitter compared to medium BU level, where only addition at L2 gave a significantly more bitter beer. In contrast, upon the addition of Hersbrucker at low BU, sample L2 was perceived to be more

bitter while at medium BU both addition levels resulted in beers with increased bitterness intensity. Furthermore, the addition of EKG hop aroma at high BU had no significant impact on perceived bitterness intensity whilst the addition of Hersbrucker, based on the rank data showed that L1 samples were perceived to be more intense in bitterness. Interestingly, according to the rank and rating data at low BU, the observed increase in bitterness intensity upon the addition of Hersbrucker was eliminated when nose clips were used (Table 4.5, Figure 4.6), suggesting that this effect was driven by volatile hop aroma compounds stimulating receptors via the retronasal route. It is likely that this is due to the aroma compounds stimulating olfactory receptors although such compounds may also be able to stimulate trigeminal receptors in the nasal passages. These observations are characteristic of perceptual taste-aroma interactions, where the combined input from the sense of smell and taste gives the overall impression of flavour (Auvray & Spence, 2008; Small & Prescott, 2005). The effect is further reinforced by cognitive association, where association between two stimuli is learned, here, the congruency between beer and 'hoppy' aroma. It would seem that the samples with hop aroma added were perceived as more bitter due to a cognitive association between their elevated 'hoppy' aroma and levels of bitterness that more typically accompany this e.g. bitter ale beers (Steele, 2013). At high BU, with nose clips on, in contrast to the findings at low BU, panellists still perceived the sample with hop aroma addition at L2 as having a higher bitterness intensity based on the ranking evaluations (Table 4.5, Figure 4.6). This observation suggests that there must be another factor accounting for the increased perception of bitterness. This could be due

to further perceptual interactions between bitterness and trigeminal sensations in the oral cavity associated with the hop aroma, since the perceived increased bitterness intensity at this level cannot be due to olfaction. Taste–trigeminal interactions have been reported previously in beverages (Hewson, Hollowood, Chandra *et al.*, 2009).

4.8.4 Impact of hop aroma on perceived bitterness character

The intensity and the character of bitterness perceived e.g. whether lingering or harsh bitterness, are key indicators of beer quality (Meilgaard, 1976). This study has provided evidence for the first time that the character of bitterness in beer can be modified by the addition of hop aroma compounds. Upon the addition of EKG hop aroma at low and medium BU, L2 samples were perceived to be significantly harsh in bitterness character, whilst the same level of hop aroma addition caused a lingering bitterness at medium BU. Beers with no aroma addition were perceived to be of round bitterness character at medium and high BU levels. Both harsh and lingering bitterness characters were not significant based on the results at high BU for EKG hop aroma addition. The results, based on the Hersbrucker hop aroma addition show that the 'roundness' or 'harshness' of the bitterness experienced in beer is affected by both BU and hop aroma levels (Figure 4.5). Notably, the beer with Hersbrucker hop aroma addition at L1 was described as 'round' in bitterness character, whereas the addition of the same level of hop aroma did not result in 'round' bitterness at medium and high BU levels. The sensory data obtained with nose clips also highlighted a key role for the olfactory component of hop aroma in determining the character of bitterness. When

nose clips were used (occluding olfaction) at low BU, the bitterness character of the beers was reported as harsh, as opposed to round, for the same level of hop aroma addition (Figure 4.6 Low), further suggesting that the hop volatile constituents of Hersbrucker also affects perceived bitterness. At high BU levels, the occlusion of olfaction resulted in sample L2 being perceived as significantly harsher than sample L0 (Table 4.5). This suggests that some level of trigeminal sensation perceived in the mouth is contributing to the perception of harsh bitterness, and indeed this is borne out in the panel's definition of harsh bitterness which includes sensations described as 'tingly', 'painful', 'irritating' and 'raspy'. Similarly, Gawel, Oberholster, and Francis (2000) defined harshness in red wine as 'a negative hedonic grouping suggesting aspects of excessive unbalanced astringency, excessive roughness and/or bitterness'. The non-significant rating of harshness in the low BU beer when nose clips were not used (Figure 4.4 Low) suggests that volatile hop aroma compounds can modulate harshness by reducing perceived trigeminal sensations. These findings, in combination with the greater perceived bitterness intensity in L2 samples at high BU (in the absence of olfaction), further support the stimulation of trigeminal receptors by hop aroma extracts in the oral cavity. To further confirm this, the panellists were invited to an additional session to discuss what they perceived and how they evaluated the samples when nose clips where used. Panellists revealed that at low BU levels, they found it difficult to determine the most bitter sample of the three presented when nose clips were worn. This supports the importance of the olfactory component on bitterness perception at low BU. Furthermore, they described high BU samples as having a 'drying

sensation on the tongue as well as the back of the throat' which contributed to their assessment of bitterness intensity and character at this BU level. Tactile and trigeminal sensations such as 'astringency' and 'drying', as well as information regarding nociception, irritation and consistency all influence the overall perception of flavour; they are sensed during food consumption and processed by the trigeminal system (Auvray & Spence, 2008; Delwiche, 2004).

There are two possibilities in relation to our findings that even when nose clips were used at high BU, beers with L2 hop aroma addition were perceived to be more bitter and harsher than L0. Firstly, it is possible that the 'drying' sensation described by subjects on the tongue and back of the mouth is confused or misinterpreted for bitterness sensation. The perception of bitterness and mouth dryness has been referred to as a 'twin sensation', with taste (bitterness) and tactile (dryness) sensations often difficult to perceptually separate in compounds exhibiting both qualities (Lyman & Green, 1990). Secondly, tactile sensations in the mouth could potentially accentuate the perception of bitterness at high concentrations, since this effect was found to be significant (for bitterness intensity) only at high BU levels. The hop aroma solution in itself was also not perceived to be harsh. These results show that both the perceived intensity of bitterness and character in beer can be modified by the addition of hop aroma compounds in the form of hop aroma extracts, with the effect on bitterness intensity being more prominent in beers of low and medium analytical bitterness concentrations (BU). While the levels of acidity (pH), sweetness or alcohol level, known to affect bitterness, may also play a

role, these variables were kept constant in this experiment and as such are not expected to have skewed the findings.

4.8.5 The impact of hop aroma on the temporal profile of bitterness

The dynamic nature of flavour perception means that its experience during food and drink consumption changes from time to time, especially in the perception of beer bitterness which has been shown to exhibit a time course (Fritsch & Shellhammer, 2009; Pangborn, Lewis, & Yamashita, 1983). Here, although somewhat limited and only based on the Hersbrucker hop aroma the results indicate that the temporal profile of bitterness is affected by retronasal perception of hop aroma. At low BU, a significantly higher IMax for sample L2 relative to L0 meant that the former was perceived to be more bitter than the latter not just overall (as shown by rank-rating results) but that this is constantly the case over time as shown in the TI curves. The observed shorter TMax for L2 relative to L0 suggests that in the presence of hop aroma maximum bitterness was also reached more quickly. The significantly greater AUC parameter also concurs with the overall rank and rating data that beers with added hop aroma had a significantly greater overall impression of bitterness in comparison to L0, despite the fact it was clear that the hop aroma extract itself is not bitter. The significant difference in only the IAngle at high BU suggests minimal impact of olfaction on bitterness time course at high bitterness concentrations. This difference compared to what was observed in the rank-rating evaluation could be due to the duration of assessment, which was instantaneous for rank-rating but over a duration of time (1 min) for the time intensity evaluation.

An inspection of the average time-intensity curves from individual panellists when nose clips were used (at low BU) revealed that a selected number of the panellists (4) still perceived the samples with hop aroma added as more bitter (See Appendix 2). This may indicate differences in trigeminal perception amongst individuals and further support evidence from the rank-rating results that hop aroma extracts add a noticeable trigeminal mouthfeel component which affect the time course of beer bitterness. Both IMax and AUC parameters extracted from the average curves of these selected panellists showed that they perceived sample L2 to be significantly more 'bitter' than L0 at low BU. These results, albeit with a limited number of panellists, suggest further research is warranted to investigate the impact of different hop aroma compounds on the temporal profile of bitterness and more importantly how this relates to consumer acceptance.

4.9 CONCLUSIONS

The study in this chapter investigated the impact of hop aroma on perceived bitterness intensity and character attributes by varying both the level of hop aroma as well as analytical bitterness (BU). Two varietal hop aroma extracts from EKG and Hersbrucker were used to add hop aroma to the base beers at different BU levels (low, medium and high). Analytical assessment of the hop aroma extracts by GC-MS revealed significant differences between the two selected hop aroma extracts, with EKG found to be majorly characterised by volatile oxygenated hop oil compounds, whilst Hersbrucker was high in terpene hydrocarbon compounds. These differences were perceptible in the aroma profile of the hop aroma extracts when assessed by a trained panel. The Hersbrucker hop aroma was described as 'hoppy', 'piney/nutty' and 'herbal' whilst the EKG hop aroma extract was described as 'citrusy', 'musty' and 'melon'. This assessment further revealed some element of trigeminal sensation to the hop aroma extract, mouthfeel attributes such as 'mouth coating', 'tingly', 'peppery' and 'astringent' were used to describe the mouthfeel properties of the aroma solutions. Upon addition to beer, both hop aroma extracts significantly impacted perceived bitterness intensity especially at low and medium BU levels. Only the Hersbrucker hop aroma had an impact on perceived bitterness intensity at high BU. Observed differential effect of hop aroma on perceived bitterness character was attributed to the different varietal hop aroma extracts, as well as BU and hop aroma addition levels. When beers with Hersbrucker hop aroma added at low and high BU levels were evaluated whilst wearing nose clips, the perceptual increase in bitterness intensity was eliminated at low BU but not at high BU, with

addition at L1 still perceived as more bitter at high BU. At low BU the occlusion of olfaction also caused the bitterness character of the beer to change from ‘round’ to ‘harsh’. These findings suggest that at low BU the increase in perceived bitterness intensity is mainly driven by olfaction via the retronasal route, whilst interactions between the high analytical bitterness and certain trigeminal sensations in the mouth are the key drivers of these perceptual changes in bitterness in high BU beers. The effect of hop aroma on the time course of bitterness was also shown to be significant at low BU levels for the highest level of hop aroma addition (L2). This study provides evidence that the perceived intensity, character and temporal profile of hop-derived bitterness in beer is a multi-modal sensation which is significantly influenced by hop aroma.

5 PERCEIVED BITTERNESS CHARACTER OF BEER IN RELATION TO HOP VARIETY AND THE IMPACT OF HOP AROMA

5.1 INTRODUCTION

Both the bitter taste and aroma of beer are important flavour attributes that consumers expect and enjoy to a varying degree during consumption (Hough, Briggs, Stevens *et al.*, 1982). The addition of hops (*Humulus lupulus L.*) to wort during the boiling stage of the brewing process is key to these beer flavour attributes. For bitterness, the conversion of α-acid congeners to their bitter tasting iso-α-acids takes place at boiling temperature (De Keukeleire, 2000). For hop aroma, brewers often 'late hop' beer by adding a small amount of the overall hop weight required for the beer recipe in the last fifteen minutes of the boil (Schönberger & Kostelecky, 2011). This short boil time ensures the preservation of hop essential oil compounds which are responsible for hop aroma character in beer. Alternatively - to increase the 'hoppy' aroma of beer brewers can add hops further downstream in the brewing process, or they can add commercially PHA extracts to create 'hoppy' flavours often described as 'floral', 'herbal' or 'woody' (Eyres, Marriott, Leus *et al.*, 2015).

Analysis of beer bitterness by spectrophotometric assay (BU) or by more precise techniques such as High performance Liquid Chromatography (HPLC) methods have been shown to agree with perceived bitterness intensity in beer (Techakriengkrai, Paterson, Taidi *et al.*, 2004) but this is not the case for bitterness quality or bitterness time-course which are better captured by descriptive sensory techniques such as QDA, FCP or CATA (McLaughlin, Lederer, & Shellhammer, 2008; Reinbach, Giacalone, Ribeiro *et al.*, 2014; Sokolowsky & Fischer, 2012).

The perception of beer bitterness is multifaceted and features temporal changes in both the perceived intensity and character of bitterness (Section 1.9). While the intensity of bitterness corresponds to the magnitude of bitter taste sensation perceived, the temporal profile represents the time-course of bitterness intensity over a period of time (Keast & Breslin, 2003). Lastly, as discussed in Chapter 1 the 'character' or 'quality' of bitterness refers to qualitative aspects of bitterness as it is perceived in the mouth; described by brewers with attributes such as 'harsh', 'smooth', 'astringent', 'lingering', 'round' or 'sharp' (McLaughlin, Lederer, & Shellhammer, 2008). These bitterness facets, in addition to values acquired by analytical measures, provide a better picture to brewers of the overall impression of beer bitterness as perceived by consumers.

This research has shown as presented in Chapters 2 and 3 that the type of hop products used, hopping regime and adopted brewing practice give beers a distinct analytical profile, which in turn impacts on perceived bitterness quality. The findings in relation to the impact of hop aroma on perceived beer bitterness has also been shown in Chapter 4, with findings revealing that hop aroma significantly impacts on both perceived bitterness intensity and character. Such effects on bitterness quality are principally due to taste-aroma interactions, and potentially trigeminal sensations elicited in the mouth by hop aroma extracts.

Both the time of hop addition and hop variety used for beer production have been suggested as factors that may impact bitterness quality (Hieronymus, 2012). Aroma hop varieties i.e. those used predominantly by brewers to impart hop aroma and flavour are also thought to contain 'unspecific bitter substances' which contribute positive bitterness quality

when added at the onset of the boil (Hieronymus, 2012). However, to date there is no scientific study designed to investigate the impact of hop variety in relation to perceived bitterness character in beer. Consequently, the aim of this chapter was to investigate the perceived bitterness intensity and character of beers hopped with distinctively different hop varieties using both analytical and sensory measures, in a bid to determine if certain hop varieties confer beer with certain bitterness qualities. The impact of hop aroma on the distinct hop-derived bitterness qualities was also investigated.

5.2 MATERIALS

A liquid malt extract was used to brew beers individually hopped with Hallertau Hersbrucker, East Kent Goldings (EKG) or Zeus hop varieties. The former two hop varieties were chosen for continuity with the previous chapter where both varieties were studied. A set of the three hopped beers also had hop aroma extracts (Hersbrucker and EKG) added after bottling to investigate the impact of these different hop aromas on the hop-derived bitterness in the singly-hopped beers. Analytical measurements of iso-α-acid and polyphenol contents of the beers were conducted, as well as sensory measures of perceived bitterness intensity and character attributes. The bitterness character profile of each singly-hopped beer and those with hop aroma extract added was determined by CATA. Rank-rating sensory methodology was used to acquire quantitative differences in perceived bitterness intensity as well as selected bitterness character attributes in the beers.

5.2.1 Malt extract

A liquid malt extract (Cedarex light) supplied by Muntons plc (UK) was used to brew the singly-hopped beers in this study.

5.2.2 Hops

Fresh hops in T90 pellet form (Hallertau Hersbrucker and Zeus) from the 2015 crop year were purchased from SimplyHops, Kent, UK. Vacuum packed T90 pellets of East Kent Goldings (EKG) hops, also 2015 crop year was purchased from BrewUK, Old Sarum UK.

5.2.2.1 Selection of hop varieties

The three hop varieties selected for the brewing trials differed with respect to their country of origin, level of α–acids as well as aroma profiles. Hersbrucker, a German aroma variety had the lowest α–acid content (1.5 – 4%) and is described as fragrant, floral and fruity. East Kent Goldings is a British seeded hop variety with α–acid content of (4.5 – 6.5%) and is described as spicy and citrusy. The American hop Zeus is described as aromatic and pungent, and is a common super high α–acid hop variety (15 – 17%). Specification details were obtained from (SimplyHops, 2016).

5.2.3 Hop aroma extract

Both Hersbrucker and EKG hop aroma extracts (60% w/w, density = 1.020 g/mL) were supplied as a food grade solution by Botanix Ltd. (Kent, UK) and were used for the addition of hop aroma into the beers. These varietal extracts were used because their taste and mouthfeel properties have been defined and are as presented in Chapter 4. The extracts (PHA® Varietal Topnotes) represent the total essential oil composition of the

individual hop varieties blended into propylene glycol for easy dissolution into beer.

5.2.4 Chemical and reagents

5.2.4.1 Phenolic acid standards

The following phenolic acid standards were prepared and quantified - syringic acid (95%), *p*-coumaric acid (98%), hydroquinone (99%), catechin (99%), epicatechin (98%), 4-hydroxybenzoic acid (99%), caffeic acid (95%), vanillic acid (97%), tyrosol (99.5%), sinapic acid (98%), ferulic acid (99%) and cinnamic acid (98%) were purchased from Sigma-Aldrich (UK). Protocatechuic acid (99.6%) was acquired from HWI analytic (Germany).

5.2.4.2 Hop acid standards

Iso-α-acid standard (ICE-3) containing trans-isocohumulone, trans-isohumulone, trans-isoadhumulone (62.3% w/w) were purchased from Labor Veritas Co. (Switzerland).

5.2.4.3 Other chemicals

Carboxymethylcellulose (CMC), ethylenediamine tetra acetic acid (EDTA), ammonia, ferric reagent solutions and orthophosphoric acid (85%) were all technical grade chemicals from VWR (UK). 2, 2, 4-trimethylpentane and acetonitrile (HPLC grade) were also from VWR (UK).

5.2.5 Instrumentation

A Waters Alliance 2695 HPLC instrument equipped with a column heater and a membrane degasser was used for the detection and measurement of both hop bitter acids and phenolic acids (See Section 2.3.1).

5.3 METHODS

5.3.1 Analysis of hop bitter acids in beer

5.3.1.1 Extraction of hop bitter acids from beer

The extraction of hop bitter acids from beer is as described in Section 2.4.7.

5.3.1.2 HPLC-UV analysis of hop bitter acids

Hop acid separation on the HPLC was achieved using the mobile phase and gradient method described in section 2.4.8. Iso-α-acid peak areas were extracted at 270 nm. Samples were analysed in triplicate and hop acid concentrations were acquired from calibration curves generated from external standards prepared in the range of (1, 5, 10, 20, 40 mg/L).

5.3.2 Analysis of polyphenols in beer

5.3.2.1 Extraction of beer phenolic acids from beer

The phenolic compounds listed in section 4.2.4.1 were extracted from beer by liquid-liquid extraction. The method of extraction is as described in section 2.4.2.

5.3.2.2 HPLC-UV analysis of beer phenolic acids

The chromatographic method used for the separation of beer phenolics is as presented in section 2.4.3. Samples were analysed in triplicate and

phenolic acid concentrations were determined from calibration curves generated from external standards prepared in the range of (1, 10, 20, 40 mg/L).

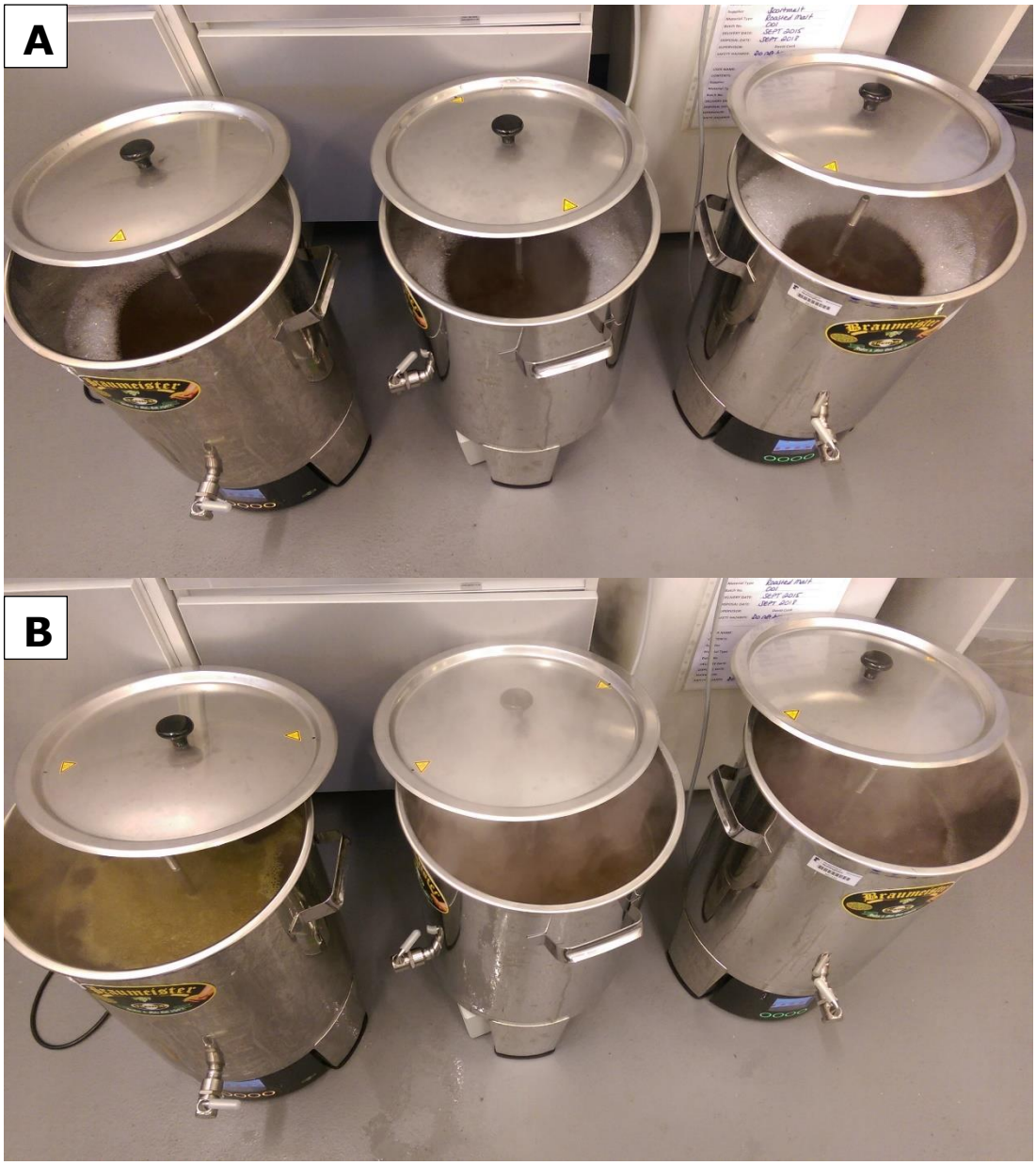
5.3.3 Determination of beer total polyphenol content

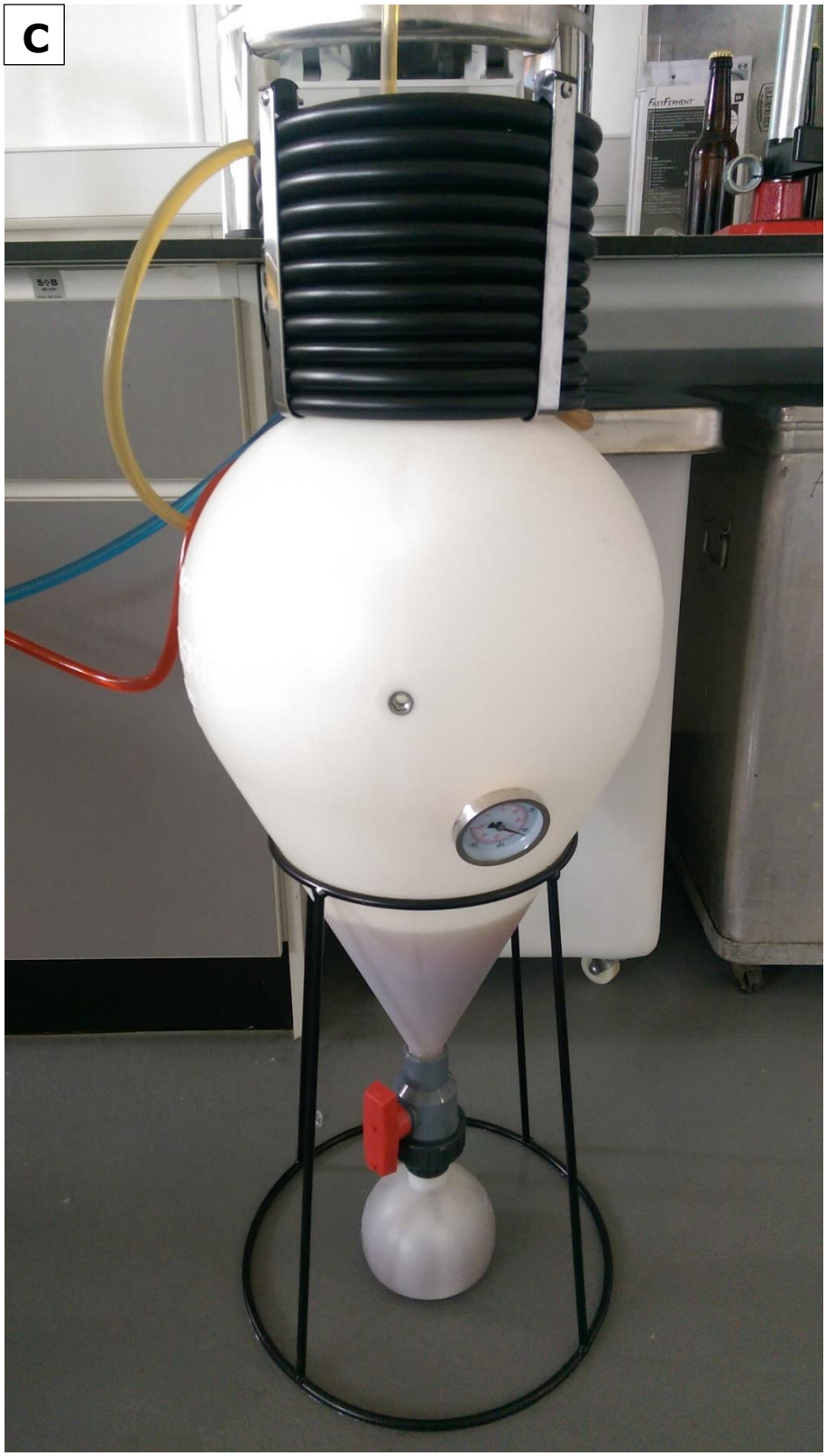
The Total Polyphenol Content (TPC) of beer was determined according to ASBC Beer-35 method (ASBC Method of Analysis, 1978). This method is as described in Section 2.4.4.

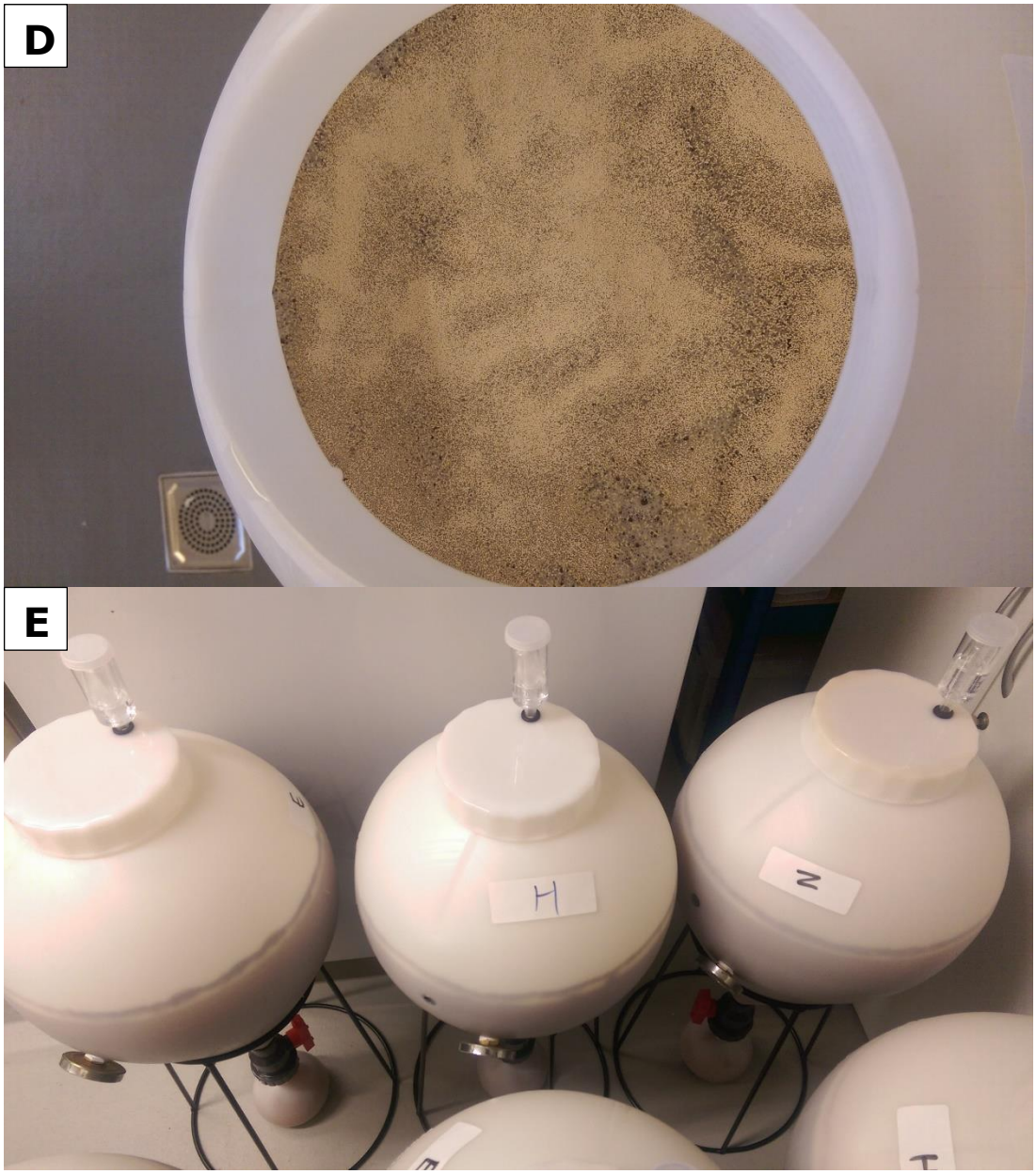
5.3.4 Production of individually hopped beers

A liquid malt extract was chosen as a suitable base for brewing the beers in order to ensure that the analytical bitterness (BU) achieved in the individually hopped beers were similar. The alternative approach, involving the malt mashing stage of the brewing process would have caused significant variations in bitterness between the beers due to mash extraction variations. Brewing was conducted in a 20 L (final beer capacity) Braumeister system (Spiedel, Germany). Some of the key stages of the brewing processes undertaken are presented in Figure 5.1 A – F. Preliminary brews were first carried out to assess the actual utilisation (i.e. the rate of conversion of α–acids to iso-α-acids) attained on the scale in which the beer was being brewed. For the actual brews, approximately 3 kg of malt extract was weighed into a Braumeister prefilled with warm brewing liquor (8 L), the mixture was made up to 28 L in total volume (Figure 5.1A). The mixture was subsequently brought to the boil after which time the hops were added (Figure 5.1B). After hop addition, the wort

was boiled for 60 min and upon completion stirred vigorously and left for 15 min to aid the coagulation and sedimentation of spent hop materials and protein. The resulting hopped wort was cooled and transferred into a fermenter for fermentation (Figure 5.1C). The wort (~24 L) was fermented with Saflager S-23 dry yeast sachets (2 x 11.5 g) from Fermentis at 15°C for 7 days (Figure 5.1D). A 30 L volume FastFerment conical fermenter was used for fermentation and fermentation was carried out in a temperature controlled room set at 15°C (Figure 5.1E). The young beer was transferred to a cold room (3°C) for another 5 days before being filtered with a HOBRACOL 200 VS sheet filter (Hobra – Školník, Czech Republic) into a Cornelius keg, and carbonated (5 g/L of CO_2) before being bottled (Figure 5.1F). Two independent brews were conducted for each of the selected hop variety studied. Beers were hopped to achieve an initial target of 20 BU in the boil, with losses during fermentation and filtration expected to bring this down to a final bitterness concentration of ~13 BU. This level of analytical bitterness was selected based on previous findings in Chapter 4 which showed significant impact of hop aroma at this bitterness concentration. For the purpose of the sensory study the beers were brewed with the additional prerequisite that the difference in BU between each singly-hopped beer and replicate brews be no more than 3 BU. The average original gravity, final gravity, ABV (%) and pH for each beer in both replicate brews was: Hersbrucker (1.008, 1.044, 4.57, and 4.30); EKG (1.008, 1.0043, 4.50 and 4.30); Zeus (1.008, 1.043, 4.50 and 4.30).


A
B


C


D
E
H
N

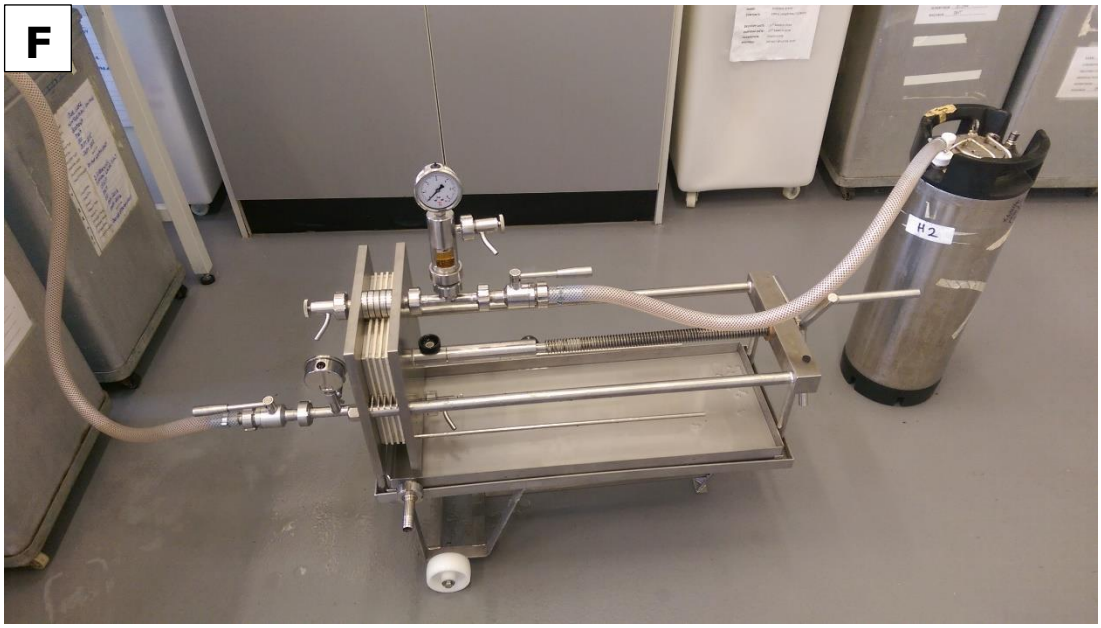


Figure 5.1: Brewing stages of the individually hopped beers. (A) Bringing the dissolved malt extract in brewing liquor (wort) to the boil, (B) imparting bitterness into the wort by hop addition and boiling, (C) the simultaneous cooling and transfer of the hopped wort into the fermentation vessel, (D) addition of dry yeast to the wort (by sprinkling) before the commencement of fermentation, (E) fermentation of the wort in temperature controlled chambers (15°C), (F) filtering the beer into Cornelius kegs before bottling.

5.3.4.1 Preparation of samples with hop aroma extract

Beers with hop aroma added were prepared 48 h in advance of tasting to allow the hop extract to fully solubilise and equilibrate with the beer medium. Hop aroma extracts were added to the base beers at a rate of 245 mg/L using a Rainin pipette (Mettler Toledo, US). This level of addition was selected based on the dosage recommendation of the supplier, and represented L1 addition level in the previous study presented in Chapter 4. Upon addition, the beer bottles were recapped with sterilised bottle caps and inverted (one inversion per second for 10 seconds) before storage in the cold room (3°C). 2 replicate samples were prepared as described for sensory evaluation.

5.3.5 Sensory evaluation of beer bitterness

The sensory aspect of this study received ethical approval from the University of Nottingham Medical Ethics Committee (Approval reference: P12042016) and all participants gave informed consent to participate in the study. Participants were given a disturbance allowance for their participation.

5.3.5.1 Subjects

8 experienced beer tasters (5 male, 3 female) from the University of Nottingham trained beer panel were selected for this study based on their performances in previous studies, as well as availability to attend all the sessions. They attended 16 sessions each lasting a minimum of 2 h.

5.3.5.2 Panel performance

Panel performance was monitored and assessed based on the ability of subjects to discriminate between samples; and reproducibility in different sessions. Since the panellists were already familiar with the selected bitterness attributes, this was assessed by intensity ratings (using a scale anchored form 0 – 10) for the selected attributes in beers previously determined to be significantly different in bitterness intensity and quality. Beers S (low intensity, 'acidic', and 'smooth'), V (medium intensity, 'round', 'diminishing') and E (high intensity, 'metallic', 'harsh', 'progressive') were used for this purpose, with evaluation conducted over two sessions.

5.3.6 Determination of beer bitterness character profile

The beer bitterness lexicon described in Chapter 4, consisting of 12 bitterness character attributes was used in this study for the determination of bitterness quality in the singly-hopped beers and those with hop aroma added. The bitterness character profiles of the singly-hopped beers, as well as those with hop aroma extract added, were determined using a rapid Check-All-That-Apply (CATA) method (Reinbach, Giacalone, Ribeiro *et al.*, 2014; Valentin, Chollet, Lelievre *et al.*, 2012) using the list of 12 bitterness quality attributes. In the CATA evaluation both 'progressive' and 'lingering' bitterness attributes - linked to the time-course of bitterness were grouped together as subjects agreed that these attributes were similar.

Before evaluation, panellists were re-familiarised with the bitterness attributes to ensure that they were still happy with them before participating in several tasting sessions where they were exposed to diverse exemplar beers which had bitterness characters covering all terms of the bitterness lexicon. This was followed by practice CATA sessions and then evaluation. For evaluation, panellists were given samples (10 mL), presented according to a Williams design at 4°C ± 2 and told to tick each attribute (from the list of 12) that applied to the sample. Attributes were presented in random order per panellists and 3 min breaks followed each sample evaluation, during which time panellists cleansed their palates with Evian water (Danone, France) and crackers (Rakusen's, UK) to minimise carry-over effects. Subjects tasted 6 samples per session, with samples blocked per session. The singly-hopped beer, replicates brew and those to which hop aroma extract was added (also replicated), were all tasted twice

by each panellist. Replicate beers were tasted in different sessions. Data was collected with Compusense Cloud (Compusense, Canada).

5.3.7 Evaluation of bitterness intensity and selected bitterness character attributes

For the evaluation of bitterness intensity, panellists were re-familiarised with the use of a scale anchored from 0 to 10 using commercial beers measured as differing analytically in bitterness concentration, with 0 on the scale representing low bitterness intensity and 10 representing high bitterness intensity. For bitterness character attributes, the 4 attributes representing key bitterness facets were selected (Harsh, Round, Astringent and Lingering). The attribute lingering - which was defined as the intensity of bitterness perceived after 10 seconds was chosen here instead of progressive as its definition allowed for accurate assessment of this temporal attribute and panellists used a timer for its evaluation. Before evaluation, panellists were trained in the use of the scale as for bitterness intensity for each of the bitterness character attributes with fresh exemplar beers which were predetermined to have these bitterness characters as in Chapter 3. For sample evaluation, a rank-rating technique was used since this method allows for differences between samples to be identified from rank scores, and allows the magnitude of difference between samples to be determined from the rating scores (Kim & O'Mahony, 1998). Panellists were presented with 3 samples (30 mL each at 4°C ± 2) consisting of the singly-hopped beers and were instructed to rank the samples from low to high intensity for each attribute before then rating the intensity of bitterness, harshness, roundedness, astringency and linger in the samples

on a scale from 0 - 10. This was repeated for the beers with hop aroma added. There was a 3 min break between each attribute and subjects cleansed their palates with Evian water (Danone, France) and crackers (Rakusen's, UK). As for CATA, the singly-hopped beer, replicate brews and those to which hop aroma extract were added (also replicated), were all tasted twice by each panellist. Presented samples were blocked per session and replicates were tasted in different sessions. Data was collected with Compusense Cloud (Compusense, Canada).

5.3.8 Data processing and statistical analysis

The binary data acquired from CATA was processed by taking the sum of scores for each selected bitterness attribute over the duplicate analysis and replicate brews. This value was used to generate a frequency spider plot to give an indication of the bitterness character profile of each hop variety as well as in relation to hop aroma extract addition.

Statistical analyses were conducted with XLSTAT 2016.5 (Addinsoft, Paris) and significance derived at $\alpha = 0.05$. Panellist performance was analysed by a three-factor (samples, judges & sessions) analysis of variance (ANOVA). Rank data for replicate brews were analysed using Friedman's test and Nemenyi's pairwise comparison test while the intensity rating scores of each attribute for both replicate brews were analysed using a two-factor (samples & subjects) ANOVA to identify differences between samples. A Tukey HSD post hoc test was used to identify samples that were significantly different from each other.

5.4 RESULTS AND DISCUSSION

5.4.1 Panel performance

The mean rating scores of two replicate evaluations of beers S, V and E are presented in Figure 5.2. The plot shows that panel members were able to identify and discriminate the bitterness attributes of interest in different beers. Furthermore, judges appropriately scored the beers for bitterness intensity using the full length of the scale. The intensity ratings are as expected and broadly in line with the analytical and sensory characterisation of the selected beers as presented in Chapters 2 and 3. There was agreement that Beer V was the most 'round' beer whilst beer E, the most 'harsh' in bitterness character was also rated highest for lingering and astringent bitterness characters.

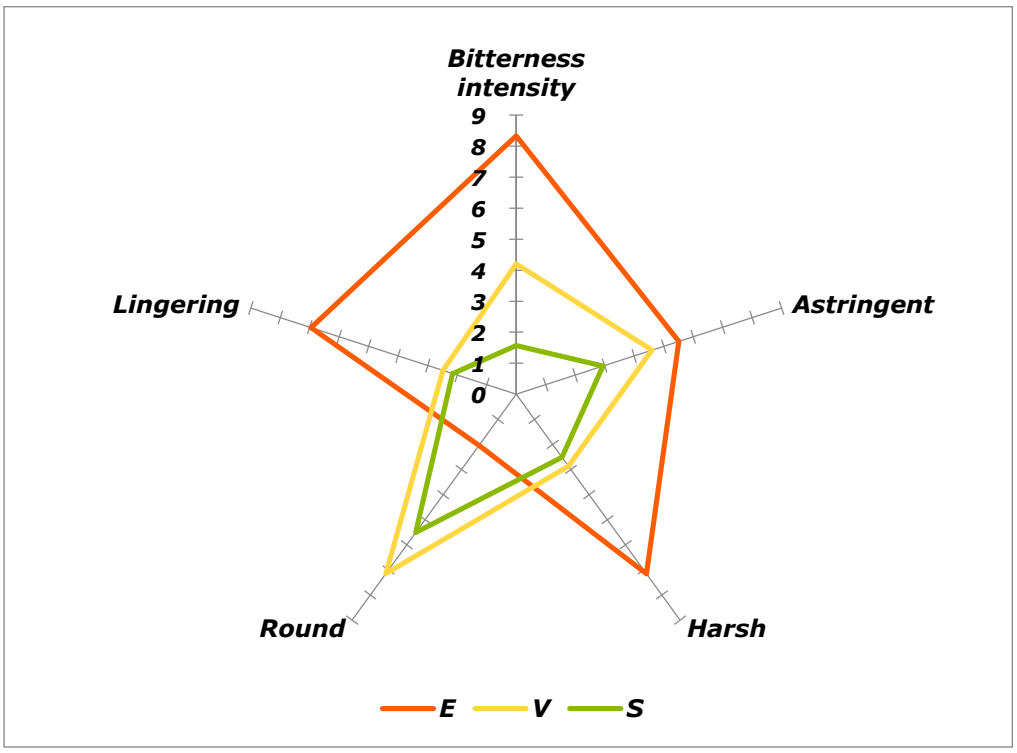
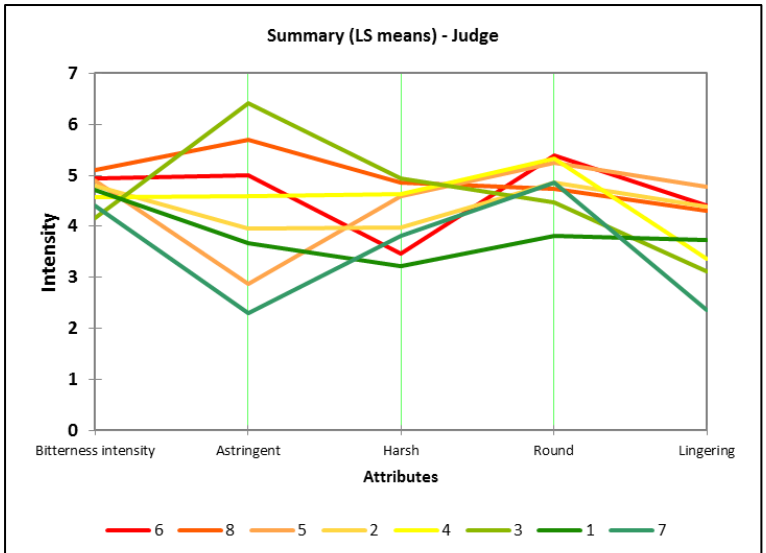


Figure 5.2: Mean intensity scores of two replicate assessments of beers S, V and E used to evaluate the ability of panel members to identify and appropriately rate the bitterness intensity as well as other related bitterness quality attributes (astringent, harsh, round and lingering).

However, the ANOVA results showed that the rating of astringent in the samples was significantly different (p=0.03) between judge 7 and 3 (Figure 5.3). Judge 7 rated the beers significantly lower than 3 for this attribute - this is likely due to individual sensitivity to this attribute as well as the samples evaluated. Whilst of the three beers evaluated, E was agreed to be the most astringent as shown in Figure 5.2 there was some astringency to the other beers assessed as well. The ANOVA analysis further revealed that beers S, V & E were significantly different for all attributes assessed based on the ratings of the panellists. The panel was reproducible as shown by the insignificance of session effect for the bitterness attributes of interest (Figure 5.3).



	Bitterness intensity	Astringent	Harsh	Round	Lingering
R^2	0.83	0.51	0.67	0.52	0.74
F	18.15	3.83	7.55	3.96	10.40
Pr > F	< 0.0001	0.001	< 0.0001	0.001	< 0.0001
Sample	89.68	9.76	35.30	18.71	46.18
	< 0.0001	0.000	< 0.0001	< 0.0001	< 0.0001
Judge	0.30	2.65	0.70	0.28	1.49
	0.95	0.03	0.67	0.96	0.20
Session	0.01	0.24	0.04	0.24	1.21
	0.93	0.63	0.85	0.63	0.28

Figure 5.3: Mean scores of bitterness attributes for beers S, V and E across replicate sessions, and summary table of significance amongst samples, judges and sessions for each bitterness attribute.

5.4.2 Analytical profile of bitterness

The analytical profile of bitterness in the individually hopped beers was assessed by measuring the concentration of iso-α-acids by HPLC. The results of the final concentrations achieved in the beers are presented in Table 5.1, a final concentration of 9, 11 and 10 mg/L of iso-α-acids (BU) were measured for the Hersbrucker, EKG and Zeus beers respectively. In the replicate brew, the concentration was 10, 12 and 10 mg/L of iso-α-acids (BU) respectively. This shows a maximum variation in the analytical bitterness concentration of 3 mg/L in the beers. It has been reported that a concentration change in the order of ±5 mg/L is required for a difference in hop bitterness to be perceived sensorially (Barnes, 2011; Scott, 1998). This observation was further confirmed upon sensory analysis in this study (Figure 5.8).

Table 5.1: Concentrations of hop iso-α-acids and total polyphenol content in the singly-hopped beers.

	mg/L			
	Iso-α-acids (BU)		**TPC***	
Brew 1	**Mean**	**± SD**	**Mean**	**± SD**
Hersbrucker	9	0.2	288	9.0
EKG	11	0.4	214	0.0
Zeus	10	0.7	209	3.3
Brew 2				
Hersbrucker	10	0.6	292	9.9
EKG	12	0.3	217	0.7
Zeus	10	1.0	205	1.9

SD - standard deviation of triplicate measurements.

*TPC = Total Polyphenol Content

As such, these beers were similar in analytical bitterness both between individually hopped beers as well as between replicate brews. This was critical for the sensory evaluation which followed, and was successfully accomplished by choosing a malt extract base upon which a consistent bitterness could be built by hop addition as well as well controlled boiling temperature and time. The final concentration achieved was close to the value of 13 mg/L which was targeted for this study.

5.4.3 Beer polyphenol profile

The polyphenolic profile of the beers was determined based on the analytical measurement of both TPC as well as selected phenolic compounds which contribute to beer bitterness (Callemien & Collin, 2009). The TPC values are also presented in Table 5.1, and they show an average TPC value of 288, 214 and 209 mg/L for Hersbrucker, EKG and Zeus beers respectively in Brew 1. In the replicate brew the average concentrations were 292, 217 and 205 mg/L respectively. The concentration of total polyphenols in the beers hopped with Hersbrucker were significantly higher than those of EKG and Zeus in both replicate brews. This is most likely explained by the greater amount of Hersbrucker hops needed to achieve the same level of bitterness in comparison to the other two varieties. For example, the amount of hops added in brew 1 to achieve the final bitterness values were 75 g, 25 g and 10 g for the Hersbrucker, EKG and Zeus brews respectively. These data further indicate that the contribution of polyphenols to beer, which is mostly credited to brewing malt (Aron & Shellhammer, 2010), is much higher when low α-acid hop varieties are

used for brewing, with potential significance for the perception of bitterness in beers.

The concentration of each of the 13 phenolic compounds as well as the average total sum of these compounds in brew 1 and 2 is presented in Figure 5.4A and B. Differences in the singly hopped beers include the presence of both catechin and epicatechin only in the Hersbrucker beer; both of these compounds were not detected in the other beers. Catechin and epicatechin are known to contribute to beer bitterness (Aron & Shellhammer, 2010; Noble, 1990). In addition, Hersbrucker was significantly higher in *p*-coumaric acid than EKG but not Zeus. EKG contained significantly higher concentrations of tyrosol than both Hersbrucker and Zeus. The average sum of phenolic acids as determined by HPLC in both replicate brews is shown in Figure 5.4B, and is significantly greater in Hersbrucker than Zeus (25.65 ± 1.3 for Hersbrucker, 24.26 ± 1.3 for EKG and 22.25 ± 1.5 for Zeus). These closer values relative to the larger difference observed in the TPC of the beers suggests that the quantified phenolic acids do not differentiate greatly between the beers. The lower values also reflect differences in the methods adopted for polyphenol quantification; the TPC values will contain both simple and complex polyphenols such as proanthocyanins which are difficult to resolve and quantify by chromatographic methods. The polyphenolic profile of beers has been previously reported to impact perceived beer bitterness character (McLaughlin, Lederer, & Shellhammer, 2008) and as previously reported in chapter 3.

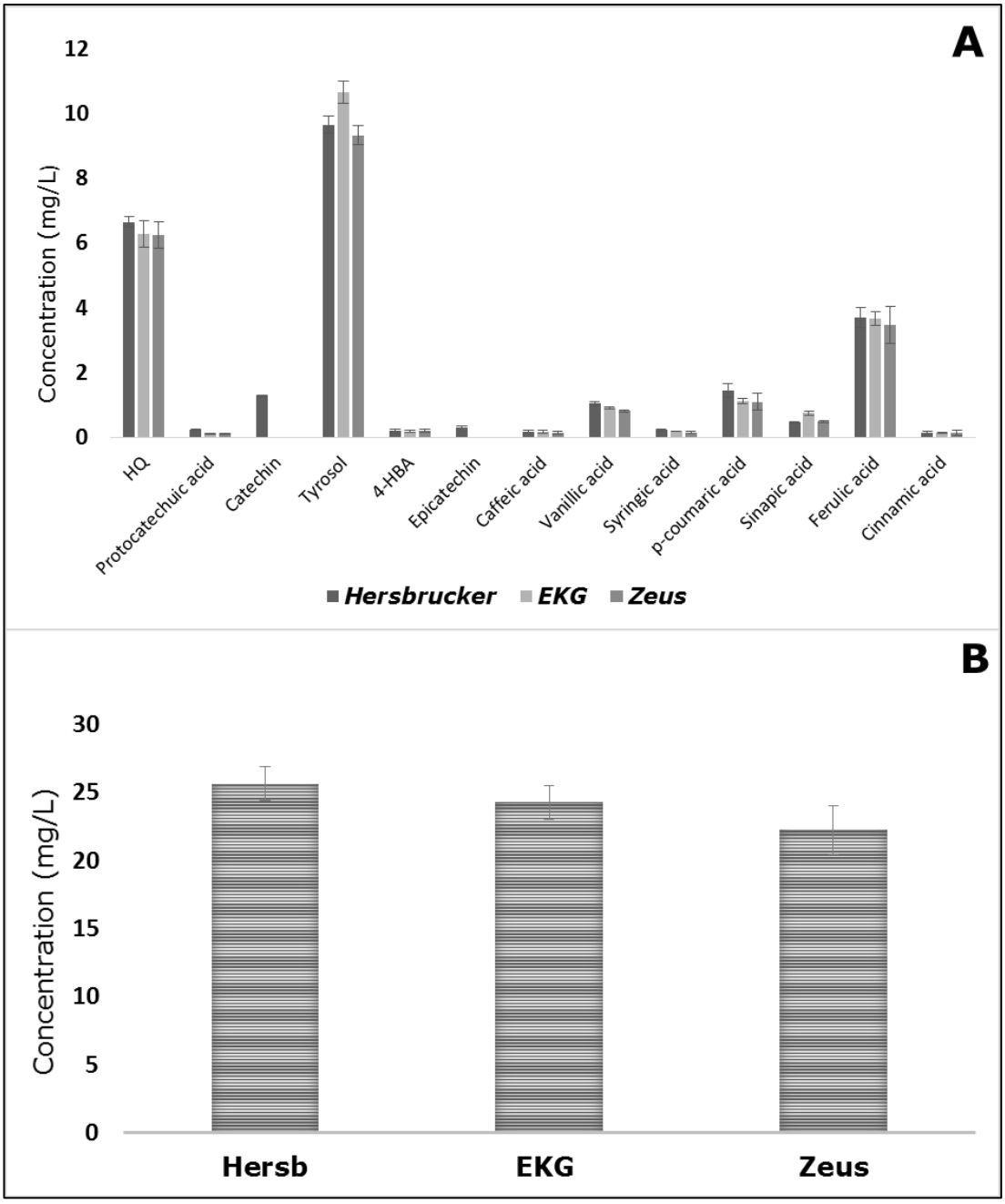


Figure 5.4: Average concentrations of selected phenolic compounds in brew 1 and 2. Error bars are standard deviation of triplicate measurements. B; Average sum of selected phenolic compounds in brew 1 and 2, errors bars represent average standard deviation of six measurements for each brew. Hersb denotes Hersbrucker.

5.4.4 Perceived bitterness profile of beers in relation to hop variety

The hop-related bitterness character profiles of the singly hopped beers are presented as CATA frequency spider plots in Figure 5.5, showing that certain bitterness character attributes were closely associated with individual hop varieties. The results show that the Hersbrucker brew was perceived to have round, diminishing, citric and astringent bitterness characters; while the bitterness attributes mostly associated with the EKG hopped beer were progressive/lingering, citric, artificial and astringent. For Zeus, the bitterness attribute mostly associated with this hop variety was diminishing, in addition to citric, metallic and astringent. These results show, for the first time, differences in the perceived character of beer bitterness as a result of the individual hop variety used.

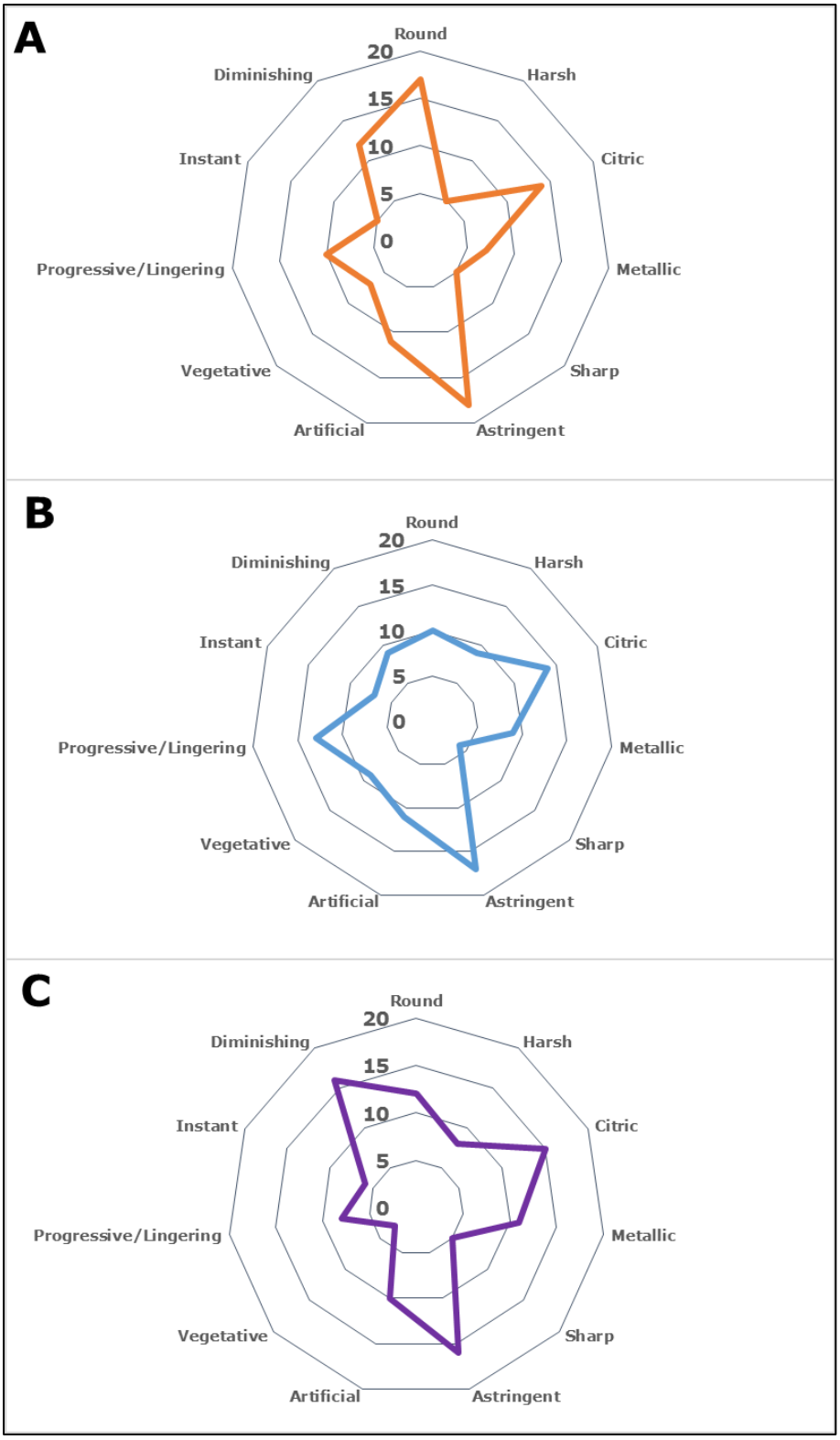


Figure 5.5: Bitterness character profile of singly-hopped beer determined by CATA evaluation (Numbers represent frequency of attribute selection). A; Hersbrucker hopped beer, B; EKG hopped beer and C; Zeus hopped beer.

5.4.5 Perceived bitterness profile of beers in relation to hop variety and hop aroma

The CATA frequency spider plots presented in Figure 5.6 and Figure 5.7 show the impact of the addition of Hersbrucker and EKG hop aroma extracts to each individually hopped beer on its perceived bitterness character profile, respectively. While lacking any perceptible taste, in water the aroma of the Hersbrucker extracts as discussed in Chapter 4 was described as 'herbal', 'orange peel', 'piney'/'nutty', 'hoppy' and 'woody' with 'mouth coating', 'spicy', 'tingly' and 'gingery' mouthfeel properties. The aroma extract of EKG in water was described as having a 'musty', 'melon', 'citrusy' and 'astringent' mouthfeel.

5.4.5.1 Impact of Hersbrucker hop aroma on bitterness quality

As shown in Figure 5.6 A, B and C the addition of Hersbrucker aroma extract had an impact on the profile of bitterness character of the beers. While addition of hop aroma did not change the frequency of round bitterness selected, there was a general increase in the frequency of harsh, lingering, citric and metallic bitterness character attributes being selected. The greatest increase in frequency of harsh and metallic bitterness characters was observed in the EKG hopped beer. The frequency of citric bitterness character increased in both Hersbrucker and Zeus hopped beers as a result of hop aroma addition. There was little increase in the frequency of astringency being selected in all beers. Interestingly, the frequency of the artificial bitterness character was reduced in all beers, indicating a masking effect of this bitterness character by hop aroma.

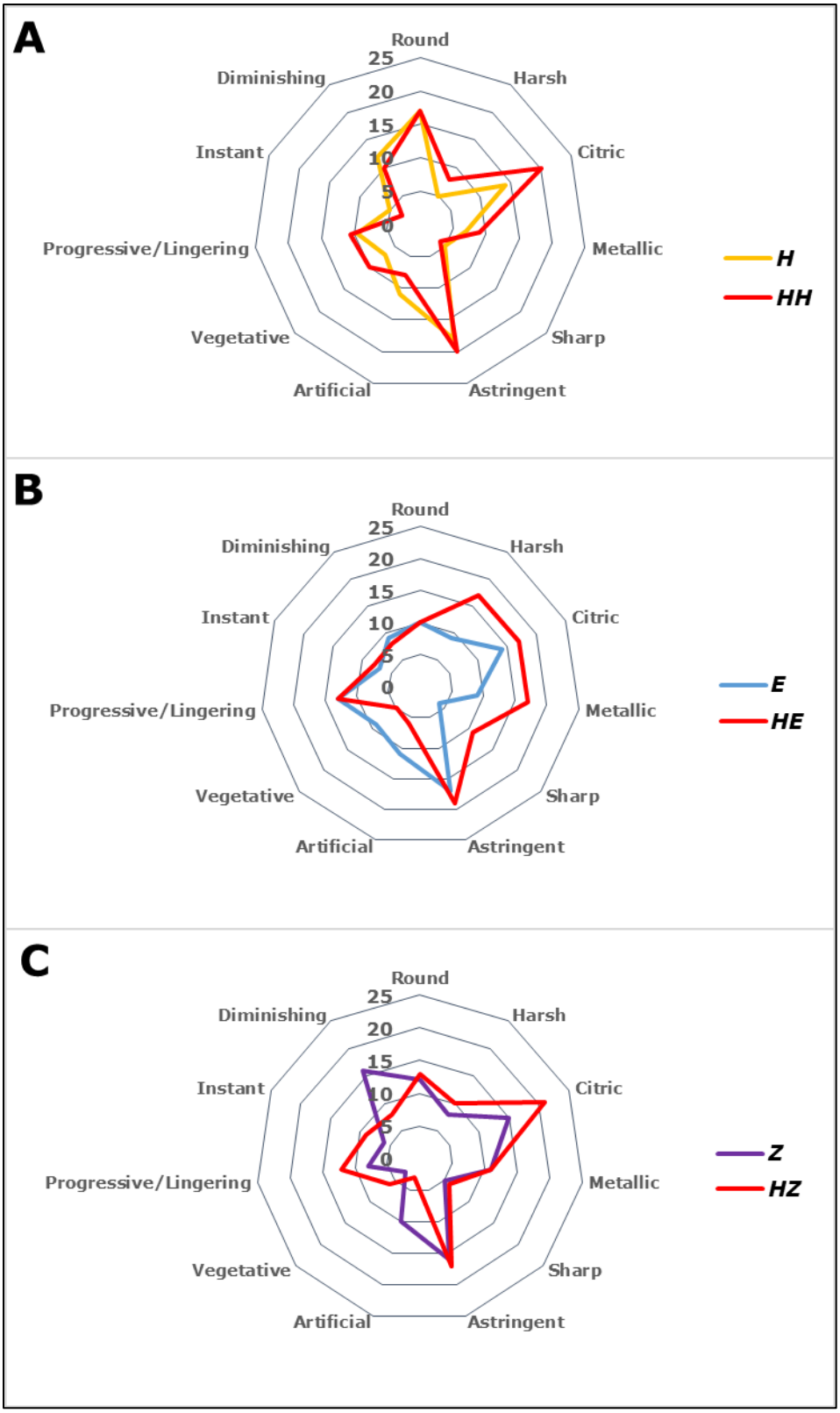


Figure 5.6: The impact on bitterness character of the addition of Hersbrucker hop aroma to the singly-hopped beers based on CATA evaluation (Numbers represent frequency of attribute selection). A; H is the Hersbrucker hopped beer, HH denotes Hersbrucker hop aroma added to the Hersbrucker beer. B; E is the EKG hopped beer, HE denotes Hersbrucker hop aroma added to the EKG beer. C; Z is the Zeus hopped beer and HZ denotes Hersbrucker hop aroma added to the Zeus beer.

For vegetative bitterness character scores, there was an increase in frequency of selection for the Hersbrucker brew, a decrease in the EKG brew and very little change in the Zeus brew. The impact of hop aroma on temporal related attributes such as diminishing, progressive/lingering was noteworthy; with hop aroma changing these bitterness attributes depending on the hop-variety derived bitterness character of the beers. For example, the Zeus and Hersbrucker hopped beers which were mostly associated with diminishing bitterness were not associated as frequently with diminishing when hop aroma was added. For progressive/lingering, there was no change for the EKG beer which was the sample already mostly associated with this bitterness character. However, with hop aroma added we see an increase in the frequency of selection of this attribute in both Zeus and Hersbrucker beers (especially Zeus), which were originally not indicated to be associated with progressive/lingering bitterness characters. The same pattern was observed for 'instant' bitterness character attribute. Frequency of selection of sharp bitterness character increased greatly in EKG but not the other two beers upon the addition of hop aroma.

5.4.5.2 Impact of EKG hop aroma on bitterness quality

In line with the findings for Hersbrucker hop aroma to the singly-hopped beers, the addition of EKG hop aroma also had an impact on the bitterness qualities of the beers. The frequency of harsh, metallic, sharp bitterness qualities increased in all beers except in the Zeus beer where the addition of EKG hop aroma led to a reduced frequency of harsh bitterness character selection. Furthermore, the combination of one of the hop aroma extract with the beer hopped with the same hop variety i.e. EE and HH led to an

increased selection frequency of harsh, metallic and sharp bitterness qualities in the beers. The addition of EKG hop aroma to beer E also led to a decrease in the frequency of selection of vegetative and round bitterness characters, with minimal changes to the frequency of selection of progressive/lingering bitterness. When the EKG hop aroma was added to the Hersbrucker beer, there was very little change in the frequencies of round, progressive/lingering, vegetative, sharp and astringent bitterness characters, whilst the frequency of selection of artificial and instant bitterness reduced. In contrast to the Hersbrucker hop aroma, there was an increase in the frequency of selection of astringent bitterness qualities in the beer upon the addition of EKG hop aroma (especially in the EKG and Zeus beer) (Figure 5.7A & C). The bitterness character of the Zeus beer changed greatly upon the addition of EKG hop aroma - with citric, metallic, sharp, astringent, vegetative and progressive/lingering bitterness characters all increasing in frequency of selection. There was no change in the frequency of selection of instant bitterness character in this beer although harsh, diminishing and artificial bitterness characters all reduced in their frequency of selection. This further supports the impact of hop aroma in masking artificial bitterness characters of beers as was observed for the Hersbrucker hop aroma addition, and further demonstrates how hop aroma can change the perceived bitterness character of beers depending, and relative to the bitterness character present in the beer as a result of the hop variety chosen. These findings further indicate that the impact of hop aroma on perceived bitterness is pertinent for beer bitterness quality.

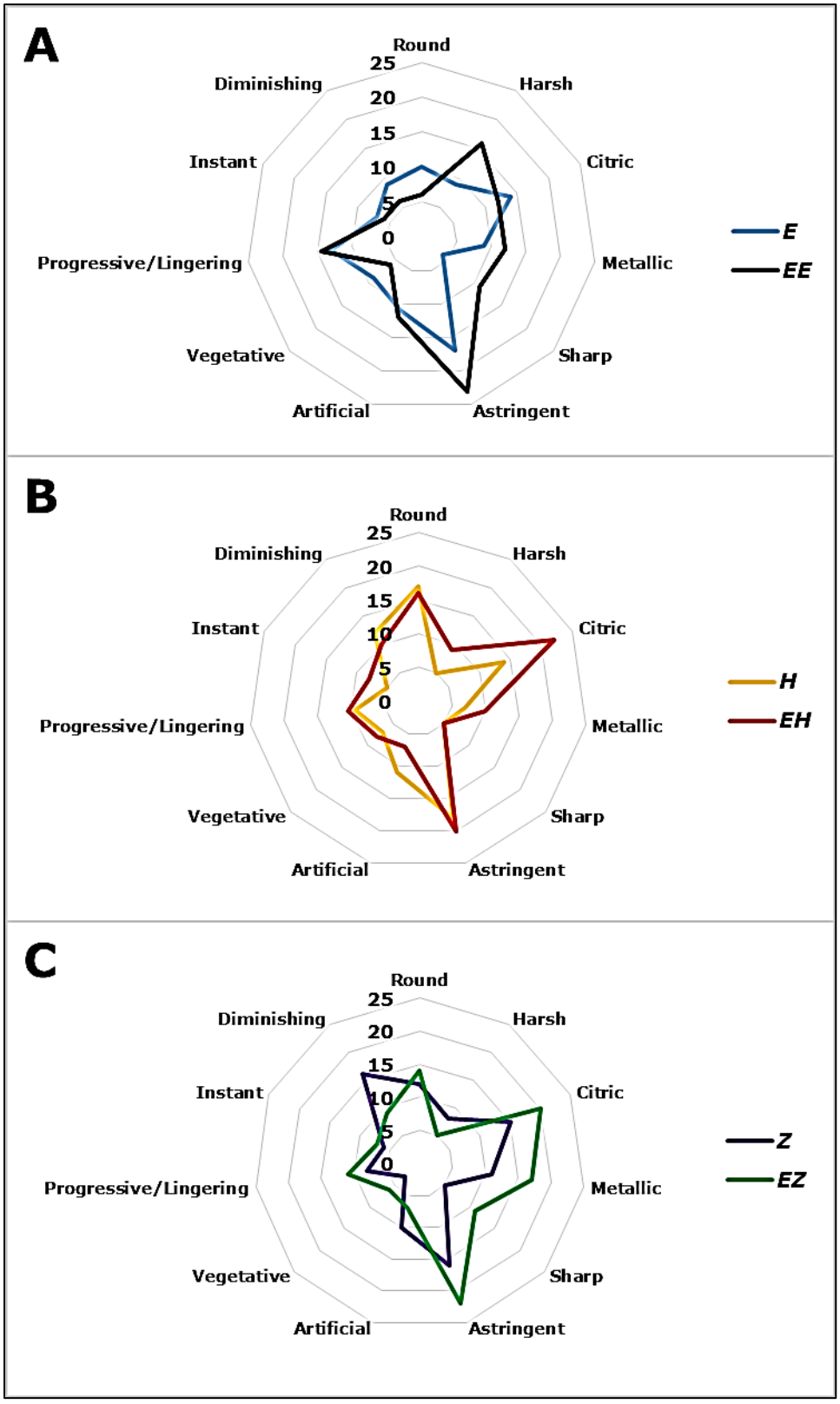


Figure 5.7: The impact on bitterness character of the addition of EKG hop aroma to the singly-hopped beers based on CATA evaluation (Numbers represent frequency of attribute selection). A; E is the EKG hopped beer, EE denotes EKG hop aroma added to the EKG beer. B; H is the Hersbrucker hopped beer, EH denotes EKG hop aroma added to the Hersbrucker beer. C; Z is the Zeus hopped beer and EZ denotes EKG hop aroma added to the Zeus beer.

5.4.6 Intensity of bitterness and selected bitterness character attributes

CATA simply indicates whether an attribute is present or not and gives no indication of intensity, however the intensity of an attribute is very likely to impact on consumer acceptance. Trends in both rank scores and intensity ratings were similar for bitterness intensity and the four selected bitterness character attributes examined, for both hop aroma additions. As such, the following results and discussions presented are based on the intensity rating scores. The intensity scores of the four selected bitterness character attributes (harsh, round, astringent and lingering) as well as perceived bitterness intensity in the three beers, with no hop aroma added are presented in Figure 5.8 as a spider plot. According to the mean rating scores, none of the bitterness attributes were significantly different amongst the beers. Based on the significantly higher levels of total polyphenols measured in the Hersbrucker beer, one would have expected this beer to be perceived as significantly more intense in bitterness but this was not the case. However, the intensity scores for this attribute in the Hersbrucker beer suggest a trend in that direction (Figure 5.8).

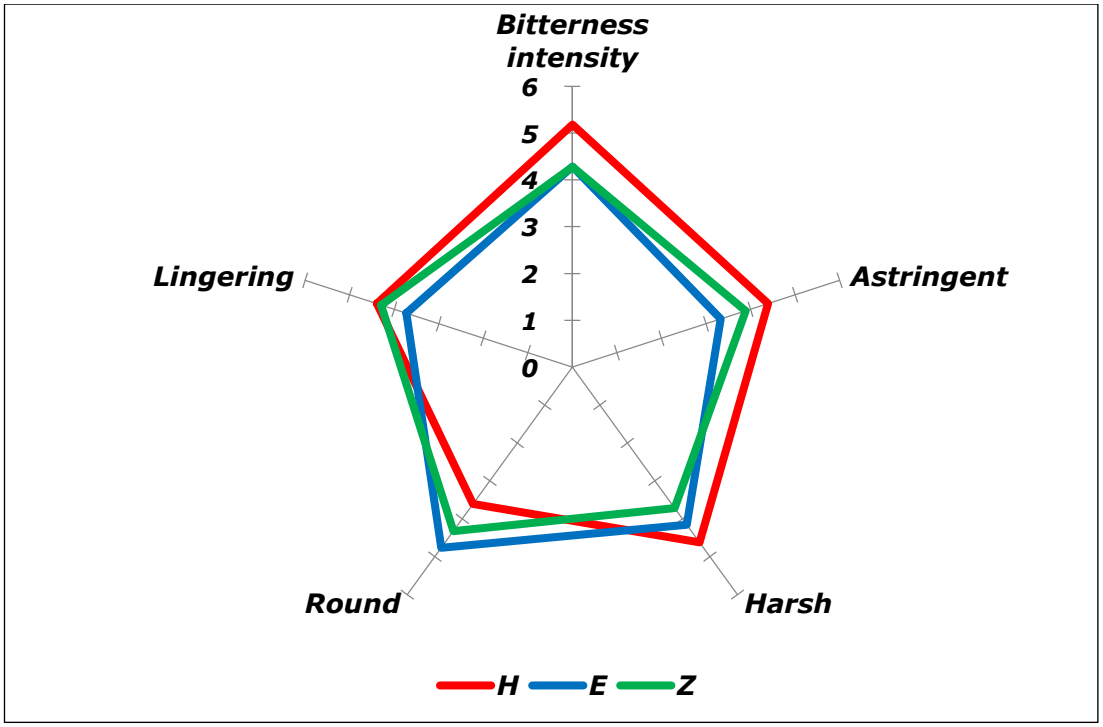


Figure 5.8: Spider plot of mean intensity scores for bitterness intensity and selected bitterness character attributes. H denotes the Hersbrucker beer, E denotes the EKG beer and Z the Zeus brew.

5.4.7 Impact of hop aroma extract on perceived bitterness intensity and selected bitterness character attributes

The impact of addition of both Hersbrucker and EKG hop aroma extracts to the singly hopped beers on selected bitterness character attributes and bitterness intensity as determined by intensity ratings is presented in Figure 5.9 A and B. For Hersbrucker addition (Figure 5.9A), the results show a significant increase in perceived bitterness intensity, astringency and lingering bitterness characters ($p < 0.05$). Notably, of the three beers, these attributes were significant for the combination of Hersbrucker aroma and the Hersbrucker hopped beer (HH). This could indicate that congruency between a hop variety and its essential oil composition may play a role in the resulting taste-aroma interaction driving the perceived

increase in bitterness intensity and character (Figure 5.9A). Both harsh and round bitterness characters did not significantly change in the beers as a result of Hersbrucker hop aroma addition. However, upon the addition of EKG hop aroma to the beers, none of the bitterness character attributes including bitterness intensity was significantly different amongst the beers (Figure 5.9B). This could be due to the difference in chemical composition of the EKG hop aroma extract, relative to the Hersbrucker hop aroma as highlighted in Chapter 4. It is important to note that differences in bitterness qualities as a result of hop aroma addition based on CATA and rank-rating analyses are not contradictory. For example, the association of beer HE in Figure 5.6B with a higher frequency of harsh bitterness character while this same beer in Figure 5.9A was rated as having a round bitterness can be explained by the fact that the two sensory methods employed measured different facets of the beer. The former results are based on CATA which simply indicates the presence or absence of an attribute in the beer, whilst the latter is based on intensity ratings of each attribute between the beers.

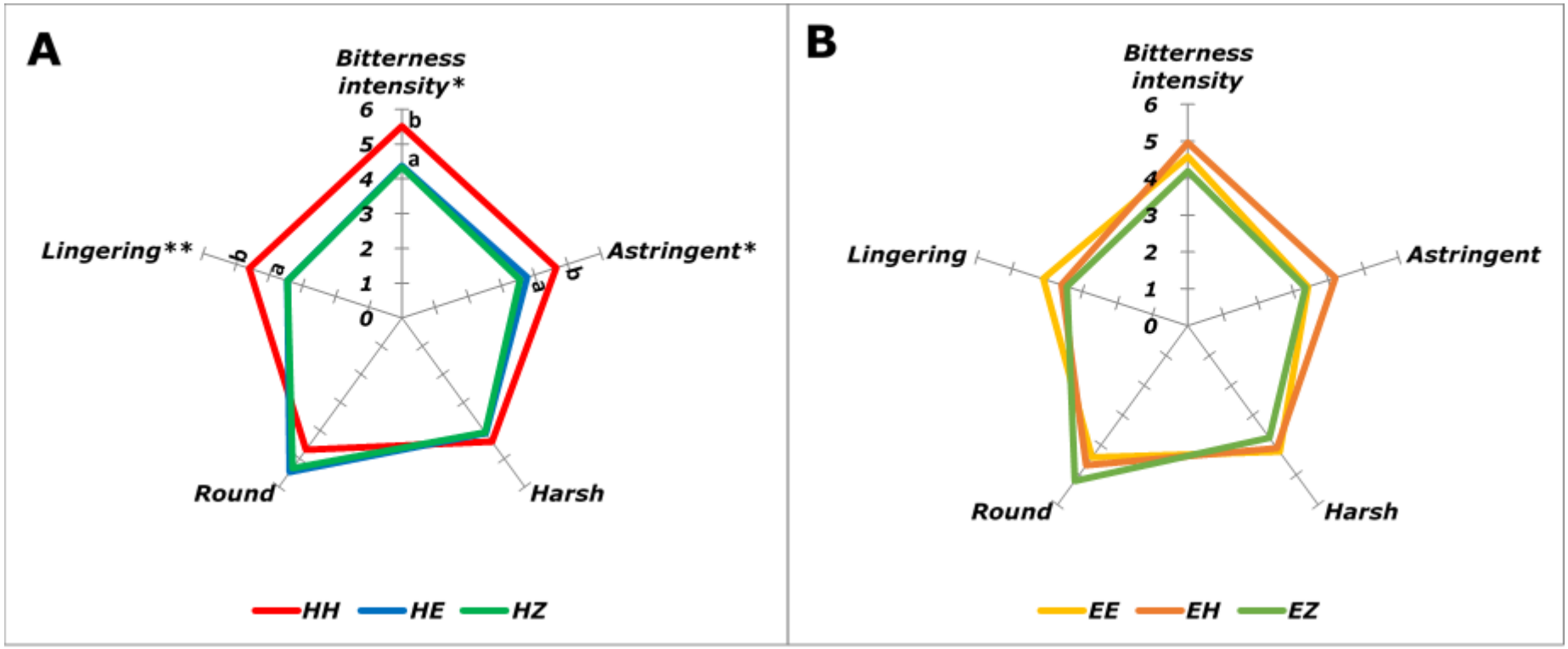


Figure 5.9: Spider plots of mean intensity scores for bitterness intensity and selected bitterness character attributes. A; HH denotes Hersbrucker hop aroma added to the Hersbrucker beer, HE denotes Hersbrucker hop aroma added to the EKG beer, HZ denotes Hersbrucker hop aroma added to the Zeus beer. B; EE denotes EKG hop aroma added to the EKG beer, EH denotes EKG hop aroma added to the Hersbrucker beer, EZ denotes EKG hop aroma added to the Zeus beer. Significance derived at *5%, **1%. ***0.1%. a, b indicates significantly different samples according to Tukey HSD post hoc test.

To further understand the impact of both hop aroma extracts on perceived bitterness in these beers, subjects were given another set of four samples to evaluate by intensity ratings for the same attributes. These samples consisted of the three individually hopped beers with Hersbrucker hop aroma added and the Hersbrucker beer with no hop aroma addition. Another set of samples with EKG hop aroma added was evaluated alongside the EKG hopped beer with no hop aroma added. The results, presented in Figure 5.10A and B show significant differences ($p < 0.05$). For Hersbrucker (Figure 5.10A), all three previous bitterness attributes (bitterness intensity, linger and astringency) seen in Figure 5.9A were significant, with the highest intensity scores in each case observed in the beer containing Hersbrucker hop aroma and the beer brewed with the same hop variety (HH). For bitterness intensity, it is remarkable to see how much the addition of hop aroma from the same variety was able to increase perceived bitterness intensity, bearing in mind that beer H and HH are actually the same beer in terms of analytical bitterness with the only difference being the presence of hop aroma extract in HH (Figure 5.10A). Beer H was also rated significantly lower in bitterness intensity compared to the beers with hop aroma added. According to the post-hoc test, the significance for bitterness intensity was between the Hersbrucker beer with no aroma addition (H) and both Hersbrucker and Zeus-hopped beers with Hersbrucker hop aroma added (HH, HZ). HH was also significantly more astringent than H and HZ. HH was significantly more lingering than H. With regard to harsh bitterness character all of the beers with Hersbrucker hop aroma added were perceived to be significantly harsher in bitterness character than the beer without hop aroma.

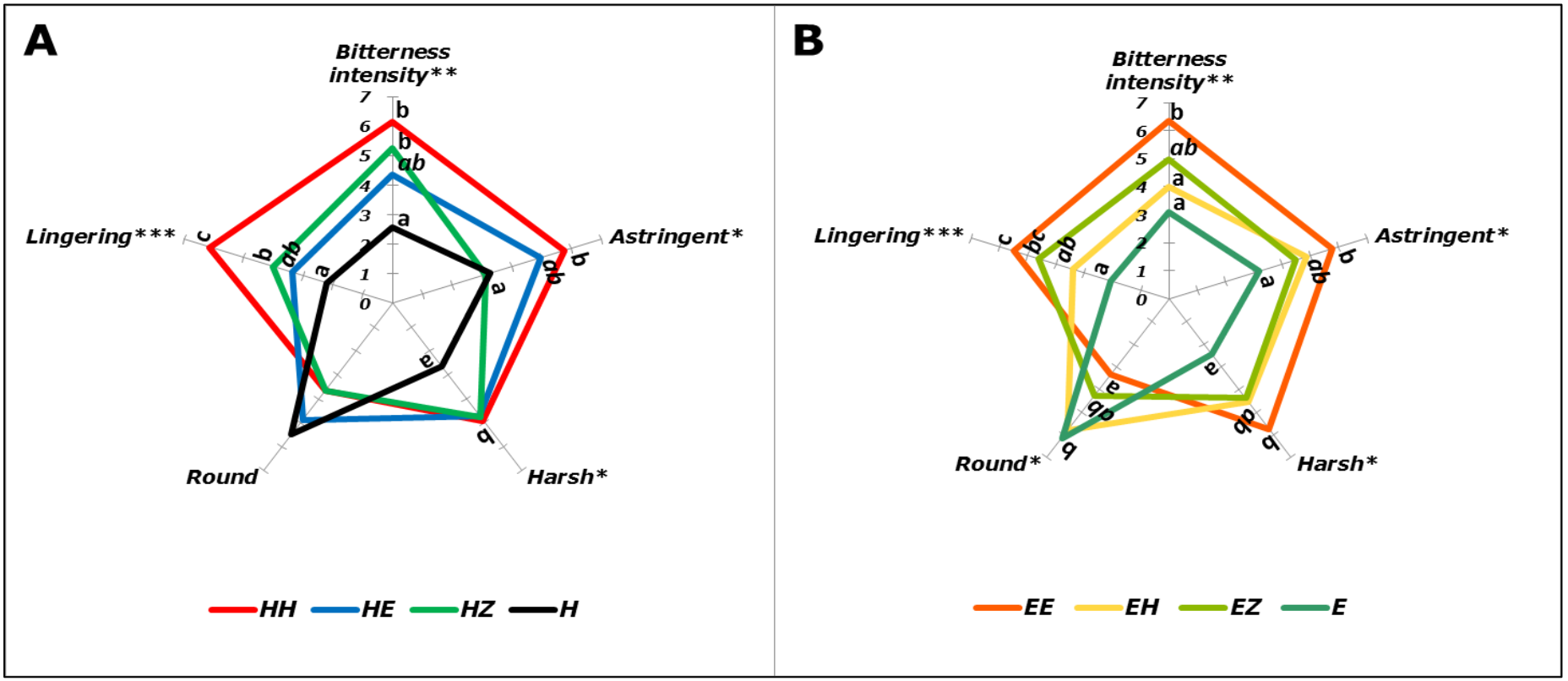


Figure 5.10: Spider plots of mean intensity scores for bitterness intensity and selected bitterness character attributes. A; HH denotes Hersbrucker hop aroma added to the Hersbrucker beer, HE denotes Hersbrucker hop aroma added to the EKG beer, HZ denotes Hersbrucker hop aroma added to the Zeus beer, H denotes the Hersbrucker beer with no hop aroma addition. B; EE denotes EKG hop aroma added to EKG beer, EH denotes EKG hop aroma added to the Hersbrucker beer, EZ denotes EKG hop aroma added to the Zeus beer and E denotes the EKG beer with no hop aroma addition. Significance derived at *5%, **1%, ***0.1%. a,b & a,c indicate significantly different samples according to Tukey HSD post hoc test.

Round bitterness character was not significantly affected by the addition of hop aroma although both the Hersbrucker brew (H) and Hersbrucker aroma addition to EKG-hopped beer (HE) were rated highest for round bitterness character, with HH and HZ rated least round in bitterness character.

For EKG hop aroma addition depicted in Figure 5.10B, all the bitterness quality attributes as well as bitterness intensity were significantly different. In line with the findings for EKG where the addition of this hop aroma to the three singly-hopped beer gave no significant difference in the bitterness character attributes (Figure 5.9B), the significance observed when these three beers were evaluated alongside the EKG-hopped beer was mainly between the beers with EKG hop aroma (EE, EH, EZ) and those without hop aroma added (E). For bitterness intensity the EKG-hopped beer with no aroma (E) was perceived to be significantly less bitter than the same beer with EKG hop aroma added (EE), which was also rated to be highest in perceived bitterness intensity. This was also the case for astringency and harsh bitterness character attributes which were both significant for the beers bittered with EKG and containing EKG hop aroma (EE). Based on the definition of 'harsh' bitterness character in Section 4.5.3 and the observation in this chapter that beers containing hop aroma were frequently perceived to be of 'harsh' bitterness character further confirms that there is an element of oral irritation and trigeminal activation to hop aroma extracts, as was also observed in Chapter 4. Perceived 'harsh' bitterness character in these beers is likely to be the product of interactions between trigeminal sensations (elicited by hop aroma extract in the mouth) and hop-derived bitterness. Beer EE was also perceived to be less

round in bitterness character relative to beer E. Lastly, beer E was perceived to be less lingering than beers EE and EZ. These findings agree with the CATA evaluation which shows an increase in the frequency of selection of harsh, metallic, citric and sharp bitterness characters in beers combining the same hop variety for bitterness and hop aroma i.e. EE and HH; and further demonstrate the significant impact of cross-modal flavour interactions on the perception of bitterness intensity and bitterness character attributes, which are key to the overall impression of bitterness flavour in beer.

5.5 CONCLUSIONS

In this study, the impact of hop variety on bitterness quality and the impact of hop aroma on the hop variety derived bitterness quality was investigated. Beers brewed with malt extract were individually hopped with 3 distinctly different hop varieties (Hersbrucker, EKG and Zeus) to achieve similar analytical bitterness levels ranging from 9 – 12 mg/L of iso-α-acids. The phenolic acid and total polyphenol contents of the beers were significantly higher for the Hersbrucker beer which was found to contain approximately 290 mg/L of total polyphenols compared to EKG and Zeus which contained 216 and 207 mg/L respectively. This difference was due to the larger amount of Hersbrucker hops needed to achieve similar analytical bitterness in the Hersbrucker hopped beers. From the sensory evaluations, certain bitterness characters were found to be closely associated with specific hop varieties; the Hersbrucker brew was mainly characterised by 'round' and 'diminishing' bitterness while EKG was perceived to be 'progressive'/'lingering' and 'artificial' in bitterness character. The Zeus hopped beer was perceived as 'diminishing' and 'metallic', with 'citric' and 'astringent' bitterness characters perceived in all the beers. The effect of hop aroma, determined by the addition of both Hersbrucker and EKG hop aroma extracts to the individually hopped beers was found to change the bitterness character profile of the beers depending on the hop-derived bitterness character. Based on the CATA results Hersbrucker and EKG hop aroma additions to each of the three singly-hopped beers led to an increase in the selection frequency of 'harsh', 'metallic', 'citric' and 'sharp' bitterness qualities, especially in beers where the hop-derived bitterness was combined with the same hop aroma

extract. The intensity ratings largely backed these findings, especially for Hersbrucker where these combinations were found to significantly increase perceived bitterness intensity, astringency and linger in the Hersbrucker hopped beer out of the three beers – indicating that some level of congruency (between a hop variety and its essential oil composition) might be involved in the resultant taste-aroma interactions driving these perceptible changes in beer bitterness. Although, there was no significant impact on bitterness qualities as a result of EKG hop aroma addition to the 3 singly-hopped beers, when these beers were evaluated alongside the EKG-hopped beer with no aroma extract added (due to essential oil composition), the EKG-hopped beer combined with EKG hop aroma was perceived to be significantly more bitter, astringent, lingering, harsh and least round in bitterness quality. The use of different hop varieties in brewing i.e. the use of one variety at the start of the boil for bitterness and a different hop variety for aroma addition ('late hopping') might be beneficial for beer bitterness quality. These findings also further reveal the complexity of bitterness perception in beer as impacted by the use of different hop varieties and hop aroma; and further shows why BU is not an accurate measure of perceived beer bitterness, especially in contemporary hop-forward beers, which are often accompanied by elevated hoppy characters.

6 MAJOR FINDINGS AND FUTURE WORK

6.1 MAJOR FINDINGS

The aim of this research project was to better understand beer bitterness intensity and quality attributes in relation to hopping technology, i.e. the amount of hops used, point of addition and hop products; as well as the effect of cross-modal flavour perception and the contribution of hop varieties to this important beer flavour attribute.

The aim of the work presented in Chapter 2 was to determine the analytical profiles of 34 commercial lager beers and to further select a sub-set of 10 beers (V, X, N, S, BB, AA, G, T and CC) based on those analytical profiles for the development of a sensory bitterness lexicon. The observed differences in the analytical profiles of the selected beers were largely based on differing concentrations of hop acids, polyphenols, amino acid content and brewing parameters; due to hop usage, time of addition, hop products and brewing processes. Of the 10 selected beers, CC and V were relatively low in hop acids and polyphenols whilst AA and T contained significantly higher levels of these compounds. In addition to a high concentration of these compounds, beer E also contained high concentrations of amino acids (1235 nmol/mL). The remaining beers were largely characterised by the type of hop products used for bittering, as well as the time of addition of hops during the brewing process. Beers S, N and BB contained pre-isomerised hop products or a blend of iso-α-acids and the light stable product Tetra, which are often added during the latter stages of the brewing process. In contrast, beers GG and X were deduced to have been conventionally hopped based on the elevated levels of residual α-acids in the beers as well as high T/C ratios. These findings

showed for the first time that the analytical profiles of beers in terms of hop-acid related compounds, polyphenol contents and brewing parameters were distinct and dependent on the brewing process.

The development of a sensory bitterness lexicon based on a set of representative beer samples, and understanding how the analytical profile differences observed in Chapter 2 affects perceived bitterness intensity and quality in those beers was the aim of the study presented in Chapter 3. A trained panel developed, defined and agreed on a list of 13 bitterness attributes which were used as a tool to allowed the characterisation of bitterness qualities in beer, and as a result better understand this attribute in beer. Upon correlation of both analytical and sensory bitterness measures in the selected beers, beers relatively high in hop acid contents (analytical bitterness) and polyphenols (E, AA, T) were generally perceived to be very bitter and of 'harsh', 'progressive' and 'astringent' bitterness qualities. In contrast, beers lower in these compounds, for example V, was perceived to be of 'round' and 'smooth' bitterness qualities and low perceived bitterness intensity. Beers containing pre-isomerised hop products or a blend of Tetra with iso-α-acids were perceived to be of 'acidic' and 'diminishing' bitterness qualities. Importantly, beers high in hop acid and polyphenols contents were not always necessarily perceived to be of 'harsh' bitterness qualities, and beers low in these bitter-tasting compounds were not necessarily perceived to be of 'smooth' or 'round' bitterness quality. Thus, suggesting that although bitterness intensity plays a role in perceived beer bitterness quality, it is not the sole driver of this important bitterness facet. For example, both Beer E and GG were perceived to be of high bitterness intensity but characterised by different

bitterness quality attributes. The former beer was predominantly characterised by 'metallic' bitterness qualities, whilst beer GG was characterised by 'sharp' and 'instant' bitterness qualities. The findings presented in Chapter 3 revealed for the first time the links between the analytical profile of beers derived from brewing ingredients such as hops, their time of addition in the brewing process, and the use of hop-derived hop products on perceived beer bitterness intensity and qualities.

The aim of the study presented in Chapter 4 was to investigate the effect of cross-modal flavour perception on beer bitterness intensity and quality. Beers at low (13 BU), medium (25 BU) and high analytical bitterness (42 BU) were dosed with the hop aroma extract of EKG and Hersbrucker at 3 levels – 0 mg/L (L0), 240 mg/L (L1) and 490 mg/L (L2) for this purpose. Results revealed a significant impact of hop aroma on the perception of bitterness intensity, quality and temporal profiles depending on the inherent analytical bitterness of the beer. These effects were more prominent at low (13 BU) and medium (25 BU) analytical bitterness (where hop aroma can boost perceived bitterness), with only minimal effects observed at high analytical bitterness (42 BU). At low and medium analytical bitterness, the addition of hop aroma generally increased perceived bitterness intensity and changed perceived bitterness quality depending on the level of hop aroma added. Observed effects of hop aroma on bitterness intensity and quality attributes were limited at high analytical bitterness (42 BU). For the temporal profile of beers, time-intensity measurements revealed a significant effect of hop aroma at L2 of hop aroma addition, showing that these beers were perceived to be significantly different temporally in comparison to L0. The effect of hop

aroma was also not significant temporally at high analytical bitterness levels. To confirm these findings subjects evaluated beers with hop aroma added whilst wearing nose clips to remove the effect of olfaction on bitterness perception, results revealed the elimination of the significant effect of hop aroma on perceived bitterness intensity, and changes to bitterness quality attributes at low analytical bitterness levels. The observed effect of hop aroma was also eliminated in the temporal profile of the beers, with the beers (L0, L1 and L2) all perceived to have similar time-intensity parameters, e.g. Imax. Surprisingly, at high analytical bitterness levels, subjects still perceived the beers with Hersbrucker hop aroma added (L2) to be significantly more bitter than L0. The effects of hop aroma on beer bitterness perception at low analytical bitterness were largely attributed to olfaction, with the elevated 'hoppy' aroma of the beer synergistically enhancing perceived beer bitterness. A balance between the analytical bitterness and hop aroma was observed to be important for 'round' bitterness quality. The observation that at high analytical bitterness, with nose clips on, subjects still perceived beers with hop aroma as more bitter and of 'harsh' bitterness qualities was attributed to trigeminal sensations. These sensations were further suggested to be derived from interactions between high analytical bitterness and oral irritation elicited by hop aroma in the mouth. The potential application of the effect of cross-modal flavour perception, as demonstrated by the results of this research would be most appropriate for brewers producing lager beers of low to medium analytical bitterness and 'hoppy' flavours, rather than ale beers which are usually accompanied by high analytical bitterness. These findings represent a significant contribution to our

current understanding of cross-modal flavour perception in relation to beer bitterness and is of further importance in the current brewing landscape that has seen a surge in the popularity and drinkability of 'hoppy' beers. Significantly, it can be concluded from the findings of this study as demonstrated in Chapters 3 and 4 that bitterness quality is a compound attribute derived from bitterness intensity, hop aroma and certain oral/trigeminal sensations and not just solely based on bitterness intensity. This is nevertheless noteworthy since this is what consumers perceive. With these improved understanding of the complexity of bitterness and the significant impact of hop aroma on its perception, beer producers are hopefully able control beer bitterness and brew sensorially exciting beers with positive bitterness qualities for consumers.

Hops are the major contributor of bitterness in beer and with the availability of numerous varieties of hops available to brewers today, better understanding of the contribution of hops to qualitative bitterness character is important. The study presented in Chapter 5 investigated the effect of different hop varieties on perceived beer bitterness quality and revealed novel findings in relation to hop-derived bitterness quality profiles. Beers hopped with distinctively different hop varieties (Hersbrucker, EKG and Zeus) exhibited characteristic bitterness quality profiles which were solely hop-derived. The beer hopped with the Hersbrucker hop variety was characterised by 'round' and 'diminishing' bitterness qualities whilst the EKG hopped beer was perceived to be 'progressive'/'lingering' and 'artificial' in bitterness quality. The Zeus hopped beers was mainly characterised by 'diminishing' and 'metallic' bitterness qualities. This distinct hop-derived bitterness (both intensity and

quality) were found to change differently when combined with the aroma extract of the same hop variety or a hop aroma extract from a different hop variety. This effect was particularly evident on hop-derived temporal-related bitterness quality attributes such as 'diminishing', 'instant' and 'progressive'/'lingering'. The selection frequencies of these bitterness quality attributes increased (based on the CATA profile) upon the addition of hop aroma to the individually hopped beers. The results suggest for the first time that hop variety effects perceived beer bitterness quality and overall impression of bitterness. Careful selection and combination of hop variety may be useful for controlling beer bitterness quality as intended by the brewer.

6.2 FUTURE WORK

The following points are raised for consideration of improving the current research project as well as potential directions for future work.

- The analytical profile determination of the beers presented in Chapter 2 could be further improved by identifying some of the currently unknown polyphenolic compounds found in the beers, since some of these polyphenol compounds which impact perceived bitterness were found to be unique to beers produced in a certain way, i.e. dry-hopped. This may be achieved by employing more sophisticated analytical techniques such as Liquid Chromatography Mass Spectrometry (LC-MS).
- The correlation of analytical and sensory measure of beer bitterness presented in Chapter 3 suggests some level of amino acid contribution

to beer bitterness quality, especially 'metallic' attribute. This observation requires further investigation to clarify if this bitterness quality attribute is linked to trigeminal sensations, taste contributions of specific amino acid compounds or a combination of both. The effect of individual amino acid contribution to bitterness quality could be determined by the addition of amino acids such as tryptophan or tyrosine into beer, and subsequent investigation of their impact on perceived beer bitterness quality.

- The results presented in Chapter 4 on the effect of cross-modal flavour perception on beer bitterness could be further expanded by conducting a consumer study where consumers evaluate beers with different levels of added hop aroma, in order to determine if the observed taste-aroma interactions observed in the trained panel are significant for consumers, the level of hop aroma at which significance is observed, as well as hedonic ratings for the beers. The level of hop aroma addition at which this impact is found to be significant will also be useful for brewers and for the development of new beer products. This could be used to develop balanced beers in terms of hop acids and aroma, and thus, achieve good quality beers. One key challenge with this approach will however be difficulty in articulating perceived beer bitterness quality by the consumers, or the selection of an appropriate sensory technique to capture different bitterness qualities in a consumer study. Nonetheless, this experiment may reveal consumer-driven bitterness-related attributes of benefit to brewers.
- Further work on the impact of hop variety on perceived beer bitterness quality, as presented in Chapter 5 could also involve a consumer study

and the investigation of other hop varieties besides those considered in this study. This will provide added information and further confirm our observations and its potential impact for brewers, i.e. if the combination of different hop varieties in a brew for bitterness and aroma could be better for perceived bitterness quality than using the same hop variety for both purpose. Furthermore, large scale brewing trials involving the use of the same hop or different hops combined for both bitterness and hop aroma could be conducted to further investigate the effect of potential congruency between a hop variety and the constituent of its hop aroma on perceived beer bitterness intensity and quality.

To sum up, this research has improved our understanding of beer bitterness intensity and quality, and the factors/brewing variables affecting it. It has shown that besides bitterness intensity, subtle qualitative attributes of bitterness are critical for the overall impression of bitterness flavour in beers. This was demonstrated by the significant links between beer hopping technologies, raw materials and hop aroma on the resultant quality of bitterness perceived in beer. 'Bitterness quality' in the context of beer flavour was also shown to be a compound feature encompassing bitterness intensity, congruent aromas and trigeminal sensations elicited in the oral cavity. An interdisciplinary approach, consisting of both analytical and sensory characterisation such as the one utilised in this research remains an important approach to further advance our understanding of the complexity of bitterness perception in beer. Understanding the sensory quality of bitterness in beers, and how this relates to analytical bitterness fingerprint is of significant value in order to

both understand consumer response to beer bitterness and to optimise production processes in this regard.

Appendix 1: HPLC chromatogram

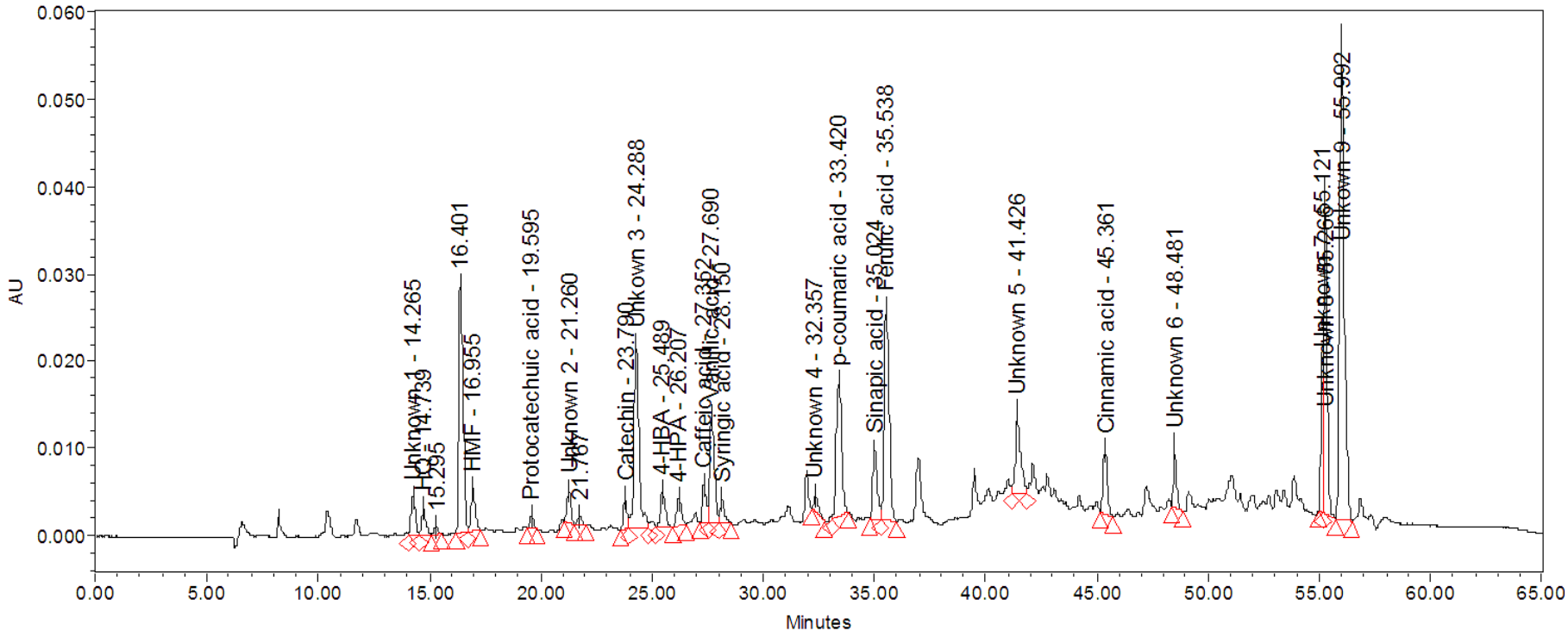


Figure 1: HPLC chromatogram showing the separation of several phenolic compounds in Czech lager beer (L).

Appendix 2: Time intensity profile of a selected number of judges

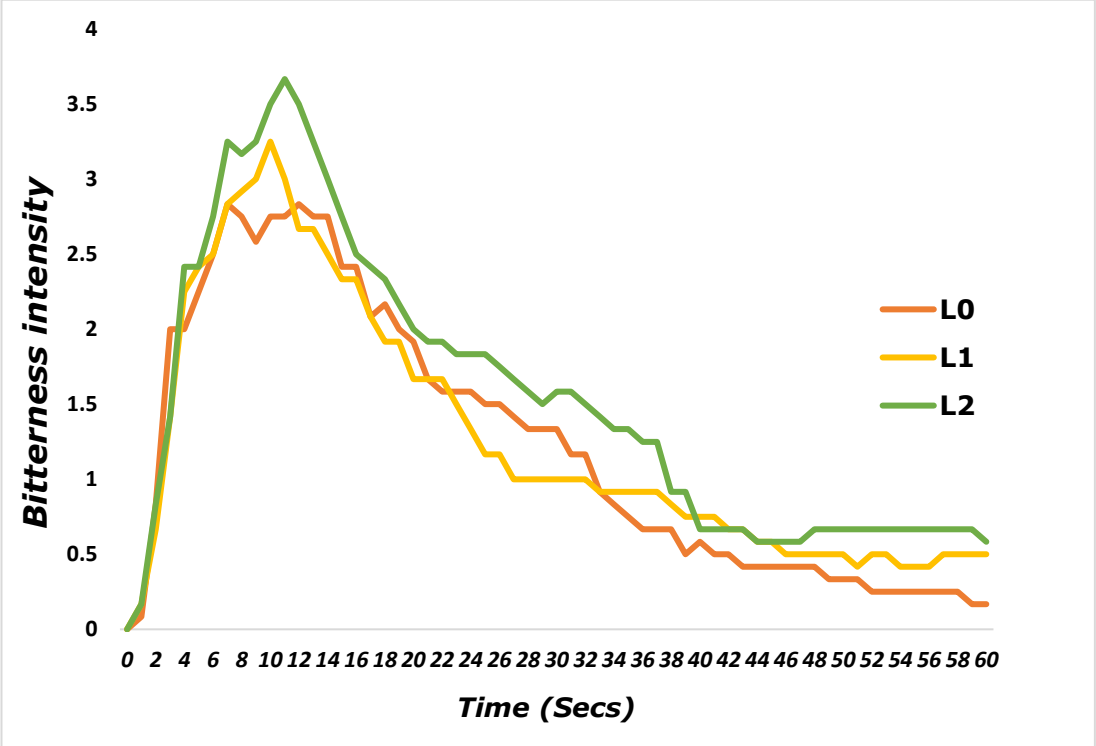


Figure 2: Average time intensity curves for 4 judges upon Hersbrucker hop aroma addition with nose clip on at low bitterness level (13BU). L0, L1 and L2 correspond to hop aroma extract addition levels of 0, 245 and 490 mg/L respectively. Significant Imax and AUC derived at 5% level. (n= 12 based on 4 panellists x 3 replicate measurements).

Appendix 3: EKG and Hersbrucker Hop oil constituent

	Relative amount (%)	
Compound	**EKG**	**Hersbrucker**
Neoisolongifolene	0	100
α-Farnesene	5	95
Selina-3,7(11)-diene	10	90
β-Patchoulene	24	76
(-)-β-Cadinene	26	74
α-Copaene	33	67
α-Muurolene	34	66
γ-Muurolene	34	66
γ-Cadinene	35	65
α-Calacorene, (+)-	36	64
α-ylangene	37	63
α-Humulene	38	62
(-)-Calamenene	40	60
β-Pinene	42	58
Cadinene	46	54
Myrcene	47	53
α-Selinene	50	50
β-Caryophyllene	50	50
β-Selinene	51	49
β-Ocimene	52	48
δ-Selinene	52	48
β-cis-Farnesene	59	41
α-Bergamotene	64	36
Juniper camphor	73	27
β-Gurjunene	0	100
cis-5-Dodecenoic acid, methyl ester	0	100
α-Eudesmol	0	100
Methyl caprate	1	99
3,6-Dodecadienoic acid, methyl ester	16	84
Geraniol butyrate	22	78
Methyl geranate	25	75
2-Methylbutylisobutyrate	42	58
β-Eudesmol	46	54
β-Caryophyllene oxide	50	50
Humulene oxide II	55	45
Linalool	61	39
(-)-Globulol	0	100
α-Eudesmol	5	95
β-Eudesmol	13	87
Neoisolongifolene	3	97

Appendix 4: Achievements

PUBLICATIONS

Oladokun, O., Smart, K., & Cook, D. (2016). An improved HPLC method for single-run analysis of the spectrum of hop bittering compounds usually encountered in beers. *Journal of the Institute of Brewing*, *122*(1), 11-20.

Oladokun, O., Tarrega, A., James, S., Smart, K., Hort, J., & Cook, D. (2016). The impact of hop bitter acid and polyphenol profiles on the perceived bitterness of beer. *Food chemistry*, *205*, 212-220.

Oladokun, O., Tarrega, A., James, S., Cowley, T., Dehrmann, F., Smart, K., Cook, D. and Hort, J., (2016). Modification of perceived beer bitterness intensity, character and temporal profile by hop aroma extract. *Food Research International*, *86*, pp.104-111.

Oladokun, O., James, S., Cowley, T., Dehrmann, F., Smart, K., Cook, D. and Hort, J., (2017). Perceived bitterness character of beer in relation to hop variety and the impact of hop aroma. *Food chemistry*, 230, 215 – 224.

CONFERENCE ORAL PRESENTATIONS

World Brewing Congress, Colorado Denver, 2016.

SCI Young Researchers in Agri-Food Conference, Reading, 2016

Early Career Researchers in Food Conference, Birmingham, 2015.

LINK15 Conference, University of Nottingham, 2015

East Midlands University Association Conference, Lincoln, 2015.

3rd Nursten Flavour Symposium, Northumbria, 2015.

35th Congress of European Brewery Convention, Porto, 2015.

4th Young Scientist Symposium, Belgium, October 2014.

AWARDS

IFST audience choice of best presentation award at 3rd Nursten Flavour Symposium, 2015

Mondeléz best oral presentation award at Early Career Researchers in Food Conference, 2015

Graduate School Travel Prize Award, 2016

Sensory Science Group IFST Student Travel Award, 2016

References

Adams, J., Williams, A., Lancaster, B., & Foley, M. (2007). *Advantages and uses of check-all-that-apply response compared to traditional scaling of attributes for salty snacks.* Paper presented at the 7th Pangborn sensory science symposium.

Adler, E., Hoon, M. A., Mueller, K. L., Chandrashekar, J., Ryba, N. J., & Zuker, C. S. (2000). A novel family of mammalian taste receptors. *Cell, 100*(6), 693-702.

Agu, R. (2002). A comparison of maize, sorghum and barley as brewing adjuncts. *Journal of the Institute of Brewing, 108*(1), 19-22.

Agu, R., & Palmer, G. (1997). The effect of temperature on the modification of sorghum and barley during malting. *Process Biochemistry, 32*(6), 501-507.

Albini, A., Dell'Eva, R., Vené, R., Ferrari, N., Buhler, D. R., Noonan, D. M., & Fassina, G. (2006). Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF-κB and Akt as targets. *The FASEB Journal, 20*(3), 527-529.

Alexis Bolívar, Mónica Gasparri, & Zufall, C. (2006). A Rapid and Low-Cost Method for Quantification of Reduced Iso-alpha-Acids in Brewing. *Journal of the American Society of Brewing Chemists, 64*(1), 39-46.

Algazzali, V. (2016). The bitterness intensity of oxidized hop acids: Humulinones and hulupones. *American Society of Brewing Chemists, 74*(1), 36-43.

Almaguer, C., Gastl, M., Arendt, E. K., & Becker, T. (2015). Comparative study of the contribution of hop (Humulus lupulus L.) hard resins extracted from different hop varieties to beer quality parameters. *Journal of the American Society of Brewing Chemists, 73*(2), 115-123.

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). Humulus lupulus–a story that begs to be told. A review. *Journal of the Institute of Brewing, 120*(4), 289-314.

Aniche, G., & Palmer, G. (1990). Development of amylolytic activities in sorghum and barley malt. *Journal of the Institute of Brewing, 96*(6), 377-379.

Aron, P. M., & Shellhammer, T. H. (2010). A discussion of polyphenols in beer physical and flavour stability. *Journal of the Institute of Brewing, 116*(4), 369-380.

Arrieta, Á. A., Rodríguez-Méndez, M. L., De Saja, J. A., Blanco, C. A., & Nimubona, D. (2010). Prediction of bitterness and alcoholic strength in beer using an electronic tongue. *Food Chemistry, 123*(3), 642-646.

ASBC Method of Analysis. (1978). American Society of Brewing Chemists. Total Polyphenol, Beer-35. The Society: St Paul, MN, U.S.A.

Auvray, M., & Spence, C. (2008). The multisensory perception of flavor. *Consciousness and Cognition, 17*(3), 1016-1031.

Barnes, T. (2011). The Complete Beer Fault Guide v. 1.4.

Bartoshuk, L. M. (1993). *Genetic and pathological taste variation: What can we learn from animal models and human disease?* Paper presented at the Ciba Foundation Symposium 179-The Molecular Basis of Smell and Taste Transduction.

Bartoshuk, L. M. (2000). Comparing sensory experiences across individuals: recent psychophysical advances illuminate genetic variation in taste perception. *Chemical Senses, 25*(4), 447-460.

Bartoshuk, L. M., Duffy, V. B., & Miller, I. J. (1994). PTC/PROP tasting: anatomy, psychophysics, and sex effects. *Physiology and Behavior, 56*(6), 1165-1171.

Behrens, M., Reichling, C., Batram, C., Brockhoff, A., & Meyerhof, W. (2009). Bitter taste receptors and their cells. *Annals of the New York Academy of Sciences, 1170*(1), 111-115.

Benitez, J. L., Forster, A., De Keukeleire, D., Moir, M., Sharp, F. R., Verhagen, L. C., and Westwood, K. T. (1997). European Brewery Convention Manual of Good Practice, Hops and hop products. Nurnberg, Germany: Getränke-Fachverlag Hans Carl.

Bernotiene, G., Nivinkiene, O., Butkiene, R., & Mockute, R. (2004). Chemical composition of essential oils of hops (*Humulus lupulus* L.) growing wild in Aukstaitija. *Chemua, 15*(2), 31-36.

Briggs, D. E., Brookes, P. A., Stevens, R., & Boulton, C. A. (2004). *Brewing: science and practice*: Elsevier.

Burger, W., & LaBerge, D. (1985). Malting and brewing quality. *Barley, 13*(26), 367-401.

Caballero, I., Blanco, C. A., & Porras, M. (2012). Iso-α-acids, bitterness and loss of beer quality during storage. *Trends in Food Science & Technology, 26*(1), 21-30.

Callemien, D., & Collin, S. (2009). Structure, organoleptic properties, quantification methods, and stability of phenolic compounds in beer—A review. *Food Reviews International, 26*(1), 1-84.

Chandrashekar, J., Hoon, M. A., Ryba, N. J., & Zuker, C. S. (2006). The receptors and cells for mammalian taste. *Nature, 444*(7117), 288-294.

Chandrashekar, J., Mueller, K. L., Hoon, M. A., Adler, E., Feng, L., Guo, W., . . . Ryba, N. J. (2000). T2Rs function as bitter taste receptors. *Cell, 100*(6), 703-711.

Christensen, J., Ladefoged, A. M., & Nørgaard, L. (2005). Rapid determination of bitterness in beer using fluorescence spectroscopy and chemometrics. *Journal of the Institute of Brewing, 111*(1), 3-10.

Clark, R. A. (2011). *Multimodal flavour perception: the impact of sweetness, bitterness, alcohol content and carbonation level on flavour perception.* (PhD thesis), University of Nottingham.

Clark, R. A., Hewson, L., Bealin-Kelly, F., & Hort, J. (2011). The interactions of CO2, ethanol, hop acids and sweetener on flavour perception in a model beer. *Chemosensory Perception, 4*(1-2), 42-54.

Clarke, B. (1986). Hop products. *Journal of the Institute of Brewing, 92*(2), 123-130.

Cliff, M., & Heymann, H. (1993). Development and use of time-intensity methodology for sensory evaluation: A review. *Food Research International, 26*(5), 375-385.

Cocuzza, S., & Mitter, W. (2008). Dry Hopping–A study of various parameters. *Hopsteiner*.

Coghe, S., Gheeraert, B., Michiels, A., & Delvaux, F. R. (2006). Development of Maillard reaction related characteristics during malt roasting. *Journal of the Institute of Brewing, 112*(2), 148-156.

Collin, S., Jerkovic, V., Bröhan, M., & Callemien, D. (2013). Polyphenols and Beer Quality *Natural Products* (pp. 2333-2359): Springer.

De Cooman, L., Aerts, G., Overmeire, H., & Keukeleire, D. (2000). Alterations of the Profiles of Iso-α-Acids During Beer Ageing, Marked Instability of Trans-Iso-α-Acids and Implications for Beer Bitterness Consistency in Relation to Tetrahydroiso-α-Acids. *Journal of the Institute of Brewing, 106*(3), 169-178.

Coote, N., & Kirsop, B. (1976). Factors responsible for the decrease in pH during beer fermentations. *Journal of the Institute of Brewing, 82*(3), 149-153.

Crabb, D., & Hudson, J. R. (1975). The Influence of Mashing Conditions on Wort and Beer Quality. *Journal of the Institute of Brewing, 81*(2), 96-104.

Cruz, A., & Green, B. G. (2000). Thermal stimulation of taste. *Nature, 403*(6772), 889-892.

Cullen, M. M., & Leopold, D. A. (1999). Disorders of smell and taste. *Medical Clinics of North America, 83*(1), 57-74.

Dalton, P., Doolittle, N., Nagata, H., & Breslin, P. (2000). The merging of the senses: integration of subthreshold taste and smell. *Nature Neuroscience, 3*(5), 431-432.

De Keukeleire, D. (2000). Fundamentals of beer and hop chemistry. *Quimica Nova, 23*(1), 108-112.

Delwiche, & Heffelfinger, A. (2005). Cross-modal additivity of taste and smell. *Journal of Sensory Studies, 20*(6), 512-525.

Delwiche, J. (2004). The impact of perceptual interactions on perceived flavor. *Food Quality and Preference, 15*(2), 137-146.

Dinehart, M., Hayes, J., Bartoshuk, L., Lanier, S., & Duffy, V. (2006). Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake. *Physiology & Behavior, 87*(2), 304-313.

Dooley, L., Lee, Y.-s., & Meullenet, J.-F. (2010). The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping. *Food Quality and Preference, 21*(4), 394-401.

Drewnowski, A. (2001). The science and complexity of bitter taste. *Nutrition Reviews, 59*(6), 163-169.

Drewnowski, A. (2001). The science and complexity of bitter taste. *Nutritional Reviews, 59*(6), 163-169.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition, 72*(6), 1424-1435.

Dvorakova, M., Hulín, P., Karabin, M., & Dostálek, P. (2007). Determination of polyphenols in beer by an effective method based on solid-phase extraction and high performance liquid chromatography with diode-array detection. *Czech journal of food sciences, 25*(4), 182.

Engan, S. (1970). Wort composition and beer flavour. I. The influence of some amino acids on the formation of higher aliphatic alcohols and esters. *Journal of the Institute of Brewing, 76*(3), 254-261.
Erbe, T., & Brückner, H. (2000). Chromatographic determination of amino acid enantiomers in beers and raw materials used for their manufacture. *Journal of Chromatography A, 881*(1), 81-91.
Esslinger, H. (2003). Beer. Ullmann's Encyclopedia of Industrial Chemistry: Wiley-VCH VerlagGmbHCo. http://www.mrw.interscience.wiley.com/ueic/articles/a03_421/sect3.html.
Eyres, G., & Dufour, J. (2009). Hop essential oil: Analysis, chemical composition and odor characteristics. *Beer in Health and Disease Prevention, 7*, 239-254.
Eyres, G., Marriott, P., & Dufour, J. (2007). Comparison of Odor-Active Compounds in the Spicy Fraction of Hop (Humulus lupulus L.) Essential Oil from Four Different Varieties §. *Journal of Agricultural and Food Chemistry, 55*(15), 6252-6261.
Eyres, G., Marriott, P., Leus, M., & Lysaght, B. (2015). *Characterisation of impact aroma compounds in hop essential oils.* Paper presented at the Weurman Flavour Research Symposium
Floridi, S., Montanari, L., Marconi, O., & Fantozzi, P. (2003). Determination of free phenolic acids in wort and beer by coulometric array detection. *Journal of Agricultural and Food Chemistry, 51*(6), 1548-1554.
Fritsch, A., & Shellhammer, T. H. (2009). The Bitter Qualities of Reduced and Nonreduced Iso-α-acids. *Journal of the American Society of Brewing Chemists, 67*(1), 8-13.
Garcia, A. A., Grande, B. C., & Gándara, J. S. (2004). Development of a rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for the determination of polyphenols in alcohol-free beers. *Journal of Chromatography A, 1054*(1), 175-180.
Gawel, R., Oberholster, A., & Francis, I. L. (2000). A Mouth-feel Wheel: terminology for communicating the mouth-feel characteristics of red wine. *Australian Journal of Grape and Wine Research, 6*(3), 203-207.
Goiris, K., Jaskula-Goiris, B., Syryn, E., Van Opstaele, F., De Rouck, G., Aerts, G., & De Cooman, L. (2014). The flavoring potential of hop polyphenols in beer. *Journal of the American Society of Brewing Chemists, 72*(2), 135-142.
Goiris, K., Ridder, M., Rouck, G., Boeykens, A., Opstaele, F., Aerts, G., . . . Keukeleire, D. (2002). The oxygenated sesquiterpenoid fraction of hops in relation to the spicy hop character of beer. *Journal of the Institute of Brewing, 108*(1), 86-93.
Green, B. G., & George, P. (2004). 'Thermal taste'predicts higher responsiveness to chemical taste and flavor. *Chemical Senses, 29*(7), 617-628.
Guinard, J.-X., & Mazzucchelli, R. (1996). The sensory perception of texture and mouthfeel. *Trends in Food Science & Technology, 7*(7), 213-219.
Haseleu, G., Intelmann, D., & Hofmann, T. (2009a). Identification and RP-HPLC-ESI-MS/MS quantitation of bitter-tasting β-acid

transformation products in beer. *Journal of Agricultural and Food Chemistry, 57*(16), 7480-7489.
Haseleu, G., Intelmann, D., & Hofmann, T. (2009b). Structure determination and sensory evaluation of novel bitter compounds formed from β-acids of hop (Humulus lupulus L.) upon wort boiling. *Food Chemistry, 116*(1), 71-81.
Haseleu, G., Lagemann, A., Stephan, A., Intelmann, D., Dunkel, A., & Hofmann, T. (2010). Quantitative sensomics profiling of hop-derived bitter compounds throughout a full-scale beer manufacturing process. *Journal of Agricultural and Food Chemistry, 58*(13), 7930-7939.
He, Y., Dong, J., Yin, H., Zhao, Y., Chen, R., Wan, X., . . . Chen, L. (2014). Wort composition and its impact on the flavour-active higher alcohol and ester formation of beer–a review. *Journal of the Institute of Brewing, 120*(3), 157-163.
Hewson, L., Hollowood, T., Chandra, S., & Hort, J. (2009). Gustatory, olfactory and trigeminal interactions in a model carbonated beverage. *Chemosensory Perception, 2*(2), 94-107.
Hieronymus, S. (2012). For the love of hops: The practical guide to aroma, bitterness and the culture of hops: Brewers Publications.
Hough, J., S, Briggs, D. E., Stevens, R., & Young, T. W. (1982). Beer Flavour and Beer Quality *Malting and Brewing Science: Volume II Hopped Wort and Beer* (pp. 839-883). Boston, MA: Springer US.
Hudson, J., & Birtwistle, S. (1966). Wort-Boiling In Relation To Beer Quality. *Journal of the Institute of Brewing, 72*(1), 46-50.
Hughes, & Bolshaw, L. (1995). *Time-dependent sensory responses to chemically-modified hop bitter acids.* Paper presented at the Proceedings Of Congress-European Brewery Convention.
Hughes, & Simpson, W. J. (1996). Bitterness of Congeners and Stereoisomers of Hop-Derived Bitter Acids Found in Beer. *Journal of American Society of Brewing Chemists, 54*(4), 234-237.
Hummel, T. (2008). Retronasal perception of odors. *Chemistry & Biodiversity, 5*(6), 853-861.
Imbert, A. (2009). *International Brewers Symposium. Hop flavor and aroma: proceedings of the 1st International Brewers Symposium* (T. H. Shellhammer Ed.): American Society of Brewing Chemists.
Intelmann, D., Batram, C., Kuhn, C., Haseleu, G., Meyerhof, W., & Hofmann, T. (2009). Three TAS2R bitter taste receptors mediate the psychophysical responses to bitter compounds of hops (Humulus lupulus L.) and beer. *Chemosensory Perception, 2*(3), 118-132.
Intelmann, D., Haseleu, G., Dunkel, A., Lagemann, A., Stephan, A., & Hofmann, T. (2011). Comprehensive sensomics analysis of hop-derived bitter compounds during storage of beer. *Journal of Agricultural and Food Chemistry, 59*(5), 1939-1953.
Intelmann, D., Kummerlöwe, G., Haseleu, G., Desmer, N., Schulze, K., Fröhlich, R., . . . Hofmann, T. (2009). Structures of Storage-Induced Transformation Products of the Beer's Bitter Principles, Revealed by Sophisticated NMR Spectroscopic and LC–MS Techniques. *Chemistry-A European Journal, 15*(47), 13047-13058.
Jackson, R. (1994). Sensory perception and wine assessment *Wine science. Academic Press, Inc., San Diego, California* (pp. 447).

Jandera, P., Škeříková, V., Řehová, L., Hájek, T., Baldrianová, L., Škopová, G., Kellner, V. & Horna, A. (2005). RP-HPLC analysis of phenolic compounds and flavonoids in beverages and plant extracts using a CoulArray detector. *Journal of Separation Science, 28*(9-10), 1005-1022.

Jaskula-Goiris, B., Aerts, G., & De Cooman, L. (2010). Hop α-acids isomerisation and utilisation: an experimental review. *Cerevisia, 35*(3), 57-70.

Jaskula, B., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2007). Enhanced quantitative extraction and HPLC determination of hop and beer bitter acids. *Journal of the Institute of Brewing, 113*(4), 381-390.

Jaskula, B., Goiris, K., Opstaele, F. v., De Rouck, G., Aerts, G., & De Cooman, L. (2009). Hopping technology in relation to α-acids isomerization yield, final utilization, and stability of beer bitterness. *Journal of the American Society of Brewing Chemists, 67*(1), 44-57.

Jaskula, B., Syryn, E., Goiris, K., De Rouck, G., Van Opstaele, F., De Clippeleer, J., . . . De Cooman, L. (2007). Hopping technology in relation to beer bitterness consistency and flavor stability. *Journal of the American Society of Brewing Chemists, 65*(1), 38-46.

Keast, R. S., Bournazel, M. M., & Breslin, P. A. (2003). A Psychophysical Investigation of Binary Bitter-compound Interactions. *Chemical Senses, 28*, 301-313.

Keast, R. S., & Breslin, P. A. (2002). Cross-adaptation and Bitterness Inhibition of L-Tryptophan, L-Phenylalanine and Urea Further Support for Shared Peripheral Physiology. *Chemical Senses, 27*(2), 123-131.

Keast, R. S., & Breslin, P. A. (2003). An overview of binary taste–taste interactions. *Food quality and preference, 14*(2), 111-124.

Kim, K. O., & O'Mahony, M. (1998). A new approach to category scales of intensity i: Traditional versus rank-rating. *Journal of Sensory Studies, 13*(3), 241-249.

King, B. M., & Moreau, N. (1996). A Comparison Of Bitter Perception In High-Alcohol, Low-Alcohol and Alcohol-Free Beer. *Journal of the Institute of Brewing, 102*(6), 419-425.

Kirimura, J., Shimizu, A., Kimizuka, A., Ninomiya, T., & Katsuya, N. (1969). Contribution of peptides and amino acids to the taste of foods. *Journal of Agricultural and Food Chemistry, 17*(4), 689-695.

Kolpin, K. M., & Shellhammer, T. H. (2009). The human bitterness detection threshold of iso-α-acids and tetrahydro-iso-α-acids in lager beer. *Journal of the American Society of Brewing Chemists, 67*(4), 200-205.

Kramer, B., Thielmann, J., Hickisch, A., Muranyi, P., Wunderlich, J., & Hauser, C. (2015). Antimicrobial activity of hop extracts against foodborne pathogens for meat applications. *Journal of Applied Microbiology, 118*(3), 648-657.

Labbe, D., Damevin, L., Vaccher, C., Morgenegg, C., & Martin, N. (2006). Modulation of perceived taste by olfaction in familiar and unfamiliar beverages. *Food Quality and Preference, 17*(7), 582-589.

Labbe, D., Schlich, P., Pineau, N., Gilbert, F., & Martin, N. (2009). Temporal dominance of sensations and sensory profiling: A comparative study. *Food Quality and Preference, 20*(3), 216-221.

Langstaff, S. A., & Lewis, M. (1993). The mouthfeel of beer—a review. *Journal of the Institute of Brewing, 99*(1), 31-37.
Lawless, H., Rozin, P., & Shenker, J. (1985). Effects of oral capsaicin on gustatory, olfactory and irritant sensations and flavor identification in humans who regularly or rarely consume chili pepper. *Chemical Senses, 10*(4), 579-589.
Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices*: Springer Science & Business Media.
Laws, D. (1981). Hop extracts—a review. *Journal of the Institute of Brewing, 87*(1), 24-29.
Leach, E. J., & Noble, A. (1986). Comparison of bitterness of caffeine and quinine by a time–intensity procedure. *Chemical Senses, 11*(3), 339-345.
Lekkas, C., Stewart, G., Hill, A., Taidi, B., & Hodgson, J. (2005). The importance of free amino nitrogen in wort and beer. *Technical quarterly-Master Brewers Association of the Americas, 42*(2), 113.
Lesschaeve, I., & Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *The American Journal of Clinical Nutrition, 81*(1), 330S-335S.
Lindemann, B. (2001). Receptors and transduction in taste. *Nature, 413*(6852), 219-225.
Lowe, C., & Elkin, W. (1986). Beer packaging in glass and recent developments. *Journal of the Institute of Brewing, 92*(6), 517-528.
Lyman, B. J., & Green, B. G. (1990). Oral astringency: Effects of repeated exposure and interactions with sweeteners. *Chemical Senses, 15*(2), 151-164.
MacWilliam, I. C. (1968). WORT COMPOSITION—A Review. *Journal of the Institute of Brewing, 74*(1), 38-54.
Maehashi, K., Matano, M., Wang, H., Vo, L. A., Yamamoto, Y., & Huang, L. (2008). Bitter peptides activate hTAS2Rs, the human bitter receptors. *Biochemical and Biophysical Research Communications, 365*(4), 851-855.
Martens, M. (1999). A philosophy for sensory science. *Food Quality and Preference, 10*(4), 233-244.
Mathias dos Santos , T. R., & de Mello, P. P. M. (2014). Nitrogen compounds in brewing wort and beer: A review. *Journal of Brewing and Distilling, 5*(2), 10-17.
Maye, J. P., & Smith, R. (2016). Dry Hopping and Its Effects on the International Bitterness Unit Test and Beer Bitterness. Hopsteiner.
Maye, J. P., Smith, R., & Leker, J. (2016). Humulinone Formation in Hops and Hop Pellets and Its Implications for Dry Hopped Beers. *Technical quarterly-Master Brewers Association of the Americas 53*(1), 23-27.
McBurney, D. H. (1976). Temporal properties of the human taste system. *Sensory Processes, 1*(2), 150-162.
McLaughlin, I. R., Lederer, C., & Shellhammer, T. H. (2008). Bitterness-modifying properties of hop polyphenols extracted from spent hop material. *Journal of the American Society of Brewing Chemists, 66*(3), 174-183.
Meilgaard, M. (1976). Wort composition: with special reference to the use of ajuncts. *Technical quarterly-Master Brewers Association of the Americas, 13*(2), 74-75.

Mennella, J. A., Pepino, M. Y., & Reed, D. R. (2005). Genetic and environmental determinants of bitter perception and sweet preferences. *Pediatrics, 115*(2), e216-e222.
Mikyška, A., Hrabak, M., Hašková, D., & Šrogl, J. (2002). The role of malt and hop polyphenols in beer quality, flavour and haze stability. *Journal of the Institute of Brewing, 108*(1), 78-85.
Moir, M. (2000). Hops: a millennium review. *Journal of the American Society of Brewing Chemists, 58*(4), 131-146.
Murphy, C., Cain, W. S., & Bartoshuk, L. M. (1977). Mutual action of taste and olfaction. *Sensory Processes*.
Murray, J., Delahunty, C., & Baxter, I. (2001). Descriptive sensory analysis: past, present and future. *Food Research International, 34*(6), 461-471.
Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell, 106*(3), 381-390.
Neve, R. A., & Neve, R. (1991). *Hops*. Suffolk: Springer.
Noble, A. C. (1995). Application of time-intensity procedures for the evaluation of taste and mouthfeel. *American Journal of Enology and Viticulture, 46*(1), 128-133.
Noble, J. L. R. a. A. C. (1990). Astringency and Bitterness of Selected Phenolics in Wine. *Journal of the Science of Food and Agriculture, 53*, 345-353.
O'Donnell, K. (2012). Aspartame, Neotame and Advantame. In Kay O'Donnell & M. W. Kearsley (Eds.), *Sweeteners and Sugar Alternatives in Food Technology* (Second ed., Vol. 6, pp. 86-95): Wiley-Blackwell.
Oladokun, O., Smart, K., & Cook, D. (2016). An improved HPLC method for single-run analysis of the spectrum of hop bittering compounds usually encountered in beers. *Journal of the Institute of Brewing, 122*(1), 11-20.
Oliver, G., & Colicchio, T. (2011). *The Oxford companion to beer*: Oxford University Press.
Pangborn, R., Lewis, M., & Yamashita, J. (1983). Comparison of time-intensity with category scaling of bitterness of iso-α-acids in model systems and in beer. *Journal of the Institute of Brewing, 89*(5), 349-355.
Parkin, E. J. (2014). *The influence of polyphenols and humulinones on bitterness in dry-hopped beer.* (Masters of Science), University of Oregon.
Pascoe, H. M., Ames, J. M., & Chandra, S. (2003). Critical stages of the brewing process for changes in antioxidant activity and levels of phenolic compounds in ale. *Journal of the American Society of Brewing Chemists, 61*(4), 203-209.
Peacock, V. E., Deinzer, M. L., Likens, S. T., Nickerson, G. B., & McGill, L. A. (1981). Floral hop aroma in beer. *Journal of Agricultural and Food Chemistry, 29*(6), 1265-1269.
Peleg, H., Gacon, K., Schlich, P., & Noble, A. C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture, 79*(8), 1123-1128.

Perpète, P., Santos, G., Bodart, E., & Collin, S. (2005). Uptake of amino acids during beer production: the concept of a critical time value. *Journal of the American Society of Brewing Chemists, 63*(1), 23-27.
Pfeiffer, J. C., Hollowood, T. A., Hort, J., & Taylor, A. J. (2005). Temporal synchrony and integration of sub-threshold taste and smell signals. *Chemical Senses, 30*(7), 539-545.
Pickering, G., Moyes, A., Bajec, M., & Decourville, N. (2010). Thermal taster status associates with oral sensations elicited by wine. *Australian Journal of Grape and Wine Research, 16*(2), 361-367.
Pickering, G. J., Bartolini, J. A., & Bajec, M. R. (2010). Perception of beer flavour associates with thermal taster status. *Journal of the Institute of Brewing, 116*(3), 239-244.
Portmann, M.-O., & Kilcast, D. (1996). Psychophysical characterization of new sweeteners of commercial importance for the EC food industry. *Food Chemistry, 56*(3), 291-302.
Prescott, J., Allen, S., & Stephens, L. (1993). Interactions between oral chemical irritation, taste and temperature. *Chemical Senses, 18*(4), 389-404.
Prutkin, J., Duffy, V., Etter, L., Fast, K., Gardner, E., Lucchina, L., Snyder, D., Tie, K., Weiffenbach, J., Bartoshuk, L. (2000). Genetic variation and inferences about perceived taste intensity in mice and men. *Physiology & Behavior, 69*(1), 161-173.
Reinbach, H. C., Giacalone, D., Ribeiro, L. M., Bredie, W. L., & Frøst, M. B. (2014). Comparison of three sensory profiling methods based on consumer perception: CATA, CATA with intensity and Napping®. *Food Quality and Preference, 32*, 160-166.
Roberts, M. T., Dufour, J. P., & Lewis, A. C. (2004). Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC× GC-TOFMS) for high resolution analysis of hop essential oil. *Journal of Separation Science, 27*(5-6), 473-478.
Rouseff, R. L. (1990). Bitterness in food products: an overview. *Developments in Food Science, 25*, 1-14.
Rückle, L., & Senn, T. (2006). Hop acids can efficiently replace antibiotics in ethanol production. *International Sugar Journal, 108*(1287), 139-147.
Sakamoto, K., & Konings, W. N. (2003). Beer spoilage bacteria and hop resistance. *International journal of food microbiology, 89*(2), 105-124.
Sanchez, N. B. (1990). *Analytical and sensory evaluation of hop varieties.* (Master thesis Master thesis), Oregon State University.
Schiffman, S. S., Crofton, V. A., & Beeker, T. G. (1985). Sensory evaluation of soft drinks with various sweeteners. *Physiology & Behavior, 34*(3), 369-377.
Schiffman, S. S., Sennewald, K., & Gagnon, J. (1981). Comparison of taste qualities and thresholds of D-and L-amino acids. *Physiology & Behavior, 27*(1), 51-59.
Schönberger, & Kostelecky, T. (2011). 125th Anniversary Review: the role of hops in brewing. *Journal of the Institute of Brewing, 117*(3), 259-267.
Schönberger, C. (2006). Bitter is better. *Monatsschrift für Brauwissenschaft, 3*(4), 56-65.

Scott, B. (1998). Focus on Flavor. *BrewingTechniques, 6.1*.
Sharpe, F. R., & Laws, D. R. J. (1981). The essential oil of hops - A review. *Journal of the Institute of Brewing, 87*, 96-107.
Shephard, H., Parker, J., Darby, P., & Ainsworth, C. (2000). Sexual development and sex chromosomes in hop. *New Phytologist, 148*(3), 397-411.
Siegel, L., Miternique-Grosse, A., Griffon, C., Klein-Soyer, C., Lobstein, A., Raul, F., & Stephan, D. (2008). Antiangiogenic properties of lupulone, a bitter acid of hop cones. *Anticancer Research, 28*(1A), 289-294.
SimplyHops. (2016). *UK Limited* www.simplyhops.co.uk*. Accessed 10/05/2016.*
Small, D. M., & Prescott, J. (2005). Odor/taste integration and the perception of flavor. *Experimental Brain Research, 166*(3-4), 345-357.
Soares, S., Kohl, S., Thalmann, S., Mateus, N., Meyerhof, W., & De Freitas, V. (2013). Different phenolic compounds activate distinct human bitter taste receptors. *Journal of Agricultural and Food Chemistry, 61*(7), 1525-1533.
Sokolowsky, M., & Fischer, U. (2012). Evaluation of bitterness in white wine applying descriptive analysis, time-intensity analysis, and temporal dominance of sensations analysis. *Analytica Chimica Acta, 732*, 46-52.
Solms, J. (1969). Taste of amino acids, peptides, and proteins. *Journal of Agricultural and Food Chemistry, 17*(4), 686-688.
Solms, J., Vuataz, L., & Egli, R. (1965). The taste of L-and D-amino acids. *Experientia, 21*(12), 692-694.
Sørensen, L., Møller, P., Flint, A., Martens, M., & Raben, A. (2003). Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. *International Journal of Obesity, 27*(10), 1152-1166.
Steele, M. (2013). *IPA: Brewing Techniques, Recipes and the Evolution of India Pale Ale*. United States of America: Brewers Publications.
Steenackers, B., De Cooman, L., & De Vos, D. (2015). Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. *Food Chemistry, 172*, 742-756.
Steiner, S. (2015, June). Humulinone Utilization In Dry-Hopped Beers. *Hopsteiner Newsletter,* 1.
Steinhaus, M., & Schieberle, P. (2000). Comparison of the most odor-active compounds in fresh and dried hop cones (Humulus lupulus L. variety spalter select) based on GC-olfactometry and odor dilution techniques. *Journal of Agricultural and Food Chemistry, 48*(5), 1776-1783.
Stevens, J. F., Taylor, A. W., Clawson, J. E., & Deinzer, M. L. (1999). Fate of xanthohumol and related prenylflavonoids from hops to beer. *Journal of Agricultural and Food Chemistry, 47*(6), 2421-2428.
Stevens, R. (1967). The chemistry of hop constituents. *Chemical Reviews, 67*(1), 19-71.
Stevenson, R. J., Prescott, J., & Boakes, R. A. (1999). Confusing tastes and smells: how odours can influence the perception of sweet and sour tastes. *Chemical Senses, 24*(6), 627-635.

Stewart, G. G., & Priest, F. G. (2006). *Handbook of brewing*: CRC Press.
Takoi, K., Koie, K., Itoga, Y., Katayama, Y., Shimase, M., Nakayama, Y., & Watari, J. (2010). Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer. *Journal of Agricultural and Food Chemistry, 58*(8), 5050-5058.
Taniguchi, Y., Matsukura, Y., Ozaki, H., Nishimura, K., & Shindo, K. (2013). Identification and Quantification of the Oxidation Products Derived from α-Acids and β-Acids During Storage of Hops (Humulus lupulus L.). *Journal of Agricultural and Food Chemistry, 61*(12), 3121-3130.
Techakriengkrai, I., Paterson, A., Taidi, B., & Piggott, J. R. (2004). Relationships of Sensory Bitterness in Lager Beers to Iso-α-Acid Contents. *Journal of the Institute of Brewing, 110*(1), 51-56.
Thorngate, J., & Noble, A. (1995). Sensory evaluation of bitterness and astringency of 3R (−)-epicatechin and 3S (+)-catechin. *Journal of the Science of Food and Agriculture, 67*(4), 531-535.
Ting, P. L., Kay, S., & Ryder, D. (2007). Separation and identification of stereoisomers of isomerized α-acid derivatives by HPLC/DAD and electrospray HPLC/MS. *Journal of the American Society of Brewing Chemists, 65*(1), 9-14.
Todd, P., Johnson, P., & Worden, L. (1972). Evaluation of the relative bitterness and light stability of reduced iso-alpha acids. *Technical quarterly-Master Brewers Association of the Americas, 9*, 31-35.
Tomlinson, J., Ormrod, I., & Sharpe, F. (1995). A novel method for bitterness determination in beer using a delayed fluorescence technique. *Journal of the Institute of Brewing, 101*(2), 113-118.
Torres, A. M., Mau-Lastovicka, T., & Rezaaiyan, R. (1987). Total phenolics and high-performance liquid chromatography of phenolic acids of avocado. *Journal of Agricultural and Food Chemistry, 35*(6), 921-925.
Tressl, R., Engel, K. H., Kossa, M., & Koeppler, H. (1983). Characterization of tricyclic sesquiterpenes in hop (Humulus lupulus var. Hersbrucker Spaet). *Journal of Agricultural and Food Chemistry, 31*(4), 892-897.
Valentin, D., Chollet, S., Lelievre, M., & Abdi, H. (2012). Quick and dirty but still pretty good: A review of new descriptive methods in food science. *International Journal of Food Science & Technology, 47*(8), 1563-1578.
Van Cleemput, M., Cattoor, K., De Bosscher, K., Haegeman, G., De Keukeleire, D., & Heyerick, A. (2009). Hop (Humulus lupulus)-derived bitter acids as multipotent bioactive compounds. *Journal of Natural Products, 72*(6), 1220-1230.
Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2012a). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets—Part 2: Preparation of single variety floral, citrus, and spicy hop oil essences by density programmed supercritical fluid extraction. *The Journal of Supercritical Fluids, 71*, 147-161.
Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2012b). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets. Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Cerevisia, 37*(4), 97-108.

Van Opstaele, F., Goiris, K., De Rouck, G., Aerts, G., & De Cooman, L. (2013). Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets. Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Cerevisia, 37*(4), 97-108.

Van Opstaele, F., Praet, T., Aerts, G., & De Cooman, L. (2013). Characterization of Novel Single-Variety Oxygenated Sesquiterpenoid Hop Oil Fractions via Headspace Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry/Olfactometry. *Journal of Agricultural and Food Chemistry, 61*(44), 10555-10564.

Verzele, M. (1958). Posthumulone, a new "alpha acid". *Bulletin des Sociétés Chimiques Belges, 67*(5-6), 278-279.

Verzele, M. (1965). *Practical aspects of the isomerization of alpha-acids.* Paper presented at the Congress of European Brewing Convention Stockholm.

Verzele, M. (1986). 100 years of hop chemistry and its relevance to brewing. *Journal of the Institute of Brewing, 92*(1), 32-48.

Visschers, R. W., Jacobs, M. A., Frasnelli, J., Hummel, T., Burgering, M., & Boelrijk, A. E. (2006). Cross-modality of texture and aroma perception is independent of orthonasal or retronasal stimulation. *Journal of Agricultural and Food Chemistry, 54*(15), 5509-5515.

Weiss, A., Schönberger, C., Mitter, W., Biendl, M., Back, W., & Krottenthaler, M. (2002). Sensory and analytical characterisation of reduced, isomerised hop extracts and their influence and use in beer. *Journal of the Institute of Brewing, 108*(2), 236-242.

Whitear, A. L., & Hudson, J. R. (1964). Hop Resins and Beer Flavour III. Hop Resins in Beer. *Journal of the Institute of Brewing, 70*(1), 24-30.

Wilsoh, R. J., Roberts, I., Smith, R., & Bieng, M. (2001). Improving hop utilization and flavor control through the use of pre-isomerized products in the brewery kettle. *Technical quarterly-Master Brewers Association of the Americas, 38*(1), 11-21.

Wolfe, P. (2012). *A Study of Factors Affecting the Extraction of Flavor When Dry Hopping Beer* (MSc Thesis), Oregon State University.

Wunderlich, S., & Back, W. (2009). Overview of manufacturing beer: ingredients, processes, and quality criteria. *Beer in Health and Disease Prevention*, 3-16.

Wunderlich, S., Zürcher, A., & Back, W. (2005). Enrichment of xanthohumol in the brewing process. *Molecular Nutrition & Food Research, 49*(9), 874-881.

Yang, Q., Hollowood, T., & Hort, J. (2014). Phenotypic variation in oronasal perception and the relative effects of PROP and Thermal Taster Status. *Food Quality and Preference, 38*, 83-91.

Zhang, Y., Hoon, M. A., Chandrashekar, J., Mueller, K. L., Cook, B., Wu, D., Zucker, C. S., & Ryba, N. J. (2003). Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell, 112*(3), 293-301.