Perceived bitterness character of beer in relation to hop variety and the impact of hop aroma

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Abstract

The impact of hop variety and hop aroma on perceived beer bitterness intensity and character was investigated using analytical and sensory methods. Beers made from malt extract were hopped with 3 distinctive hop varieties (Hersbrucker, East Kent Goldings, Zeus) to achieve equi-bitter levels. A trained sensory panel determined the bitterness character profile of each singly-hopped beer using a novel lexicon. Results showed different bitterness character profiles for each beer, with hop aroma also found to change the hop variety-derived bitterness character profiles of the beer. Rank-rating evaluations further showed the significant effect of hop aroma on selected key bitterness character attributes, by increasing perceived harsh and lingering bitterness, astringency, and bitterness intensity via cross-modal flavour interactions. This study advances understanding of the complexity of beer bitterness perception by demonstrating that hop variety selection and hop aroma both impact significantly on the perceived intensity and character of this key sensory attribute.

Keywords: Beer, polyphenols, iso-α-acids, bitterness quality, phenolic acids, perceived beer bitterness, bitterness character, taste-aroma interactions, trigeminal sensation.

Highlights

A refined sensory lexicon enabled characterisation of beer bitterness quality

Perceived beer bitterness character is linked to hop variety

Hop aroma significantly impacted perceived bitterness intensity and character

Congruency between hop variety and its aroma constituent may affect perceived bitterness character

Chemical compounds studied in this article

Protocatechuic acid (PubChem CID:72), Catechin (PubChem CID:73160), Epicatechin (PubChem CID:72276), Caffeic acid (PubChem CID:689043), Vanillic acid (PubChem CID:8468), Ferulic acid (PubChem CID:445858), p-Coumaric acid (PubChem CID:637542), Cinnamic acid (PubChem CID:444539), Sinapic acid (PubChem CID:637775), Tyrosol (PubChem CID:10393).

1 Introduction

The bitter taste of beer is an important flavour attribute that consumers expect and enjoy to a varying degree during consumption (Hough, Briggs, Stevens, & Young, 1982). To impart bitterness, and hop aroma, brewers conventionally add hops (*Humulus lupulus L.*) to wort and boil for a duration of an hour to ninety minutes (De Keukeleire, 2000). This process yields the compounds agreed to be beer's major source of bitterness - iso-α-acids or isohumulones, from hop α-acids or humulones (De Keukeleire, 2000; Hough, Briggs, Stevens, & Young, 2012). β-acids, found alongside α-acids in the soft resin of hops also contribute to beer bitterness via transformation products such as cohulupone and hydoxytricyclocolupulone which are formed during wort boiling. These compounds are reported to possess low bitterness threshold, with long-lasting, harsh and lingering bitterness characters (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014; Haseleu, Intelmann, & Hofmann, 2009). Polyphenols from brewing malt and hops, as well as certain hop-derived oxidized compounds such as humulinones also contribute to beer bitterness (Aron & Shellhammer, 2010; Collin, Jerkovic, Bröhan, & Callemien, 2013; Maye, Smith, & Leker, 2016). For hop aroma, brewers can 'late hop' beer by adding a portion of the overall hop weight required for the beer recipe towards the end 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 available pure hop aroma (PHA) extracts to create 'hoppy' flavours often described as 'floral', 'herbal' or 'woody' (Eyres, Marriott, Leus, & Lysaght, 2015).

The International Bitterness Units (IBU) is an analytical measure of the amount of bitterness brewers expect in beer and gives an approximate value of iso-α-acids present in milligram of iso-α-acid per litre of beer (Hough, Briggs, Stevens, & Young, 2012). Beer bitterness can be measured analytically by a spectrophotometer or by more precise techniques such as High Performance Liquid Chromatography (HPLC), with values acquired by spectrophotometric methods reflecting levels of iso-α-acids as well as other compounds with similar chemistry such as polyphenols and humulinones which are all readily present in beer. In contrast, values derived by HPLC allow for the selective detection and quantification of iso-α-acids only, and as such better reflect the true definition of 1 IBU as a milligram of iso-α-acid per litre of beer (Oladokun, Smart, & Cook, 2016). Nonetheless, while both analytical methods have been shown to agree with perceived bitterness intensity in beer (Techakriengkrai, Paterson, Taidi, & Piggott, 2004), this is not the case for bitterness character/quality or bitterness time-course. The former is better captured by descriptive sensory techniques e.g. Qualitative Descriptive Analysis (QDA), Free Choice Profiling (FCP) or Check-All-That-Apply (CATA); while temporal sensory techniques such as Time-intensity (TI) or Time Dominance of Sensation (TDS) are best for determining the temporal aspects of beer bitterness (McLaughlin, Lederer, & Shellhammer, 2008; Oladokun et al., 2016b; Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014; Sokolowsky & Fischer, 2012).

The meaning of 'Quality' or 'Character' of bitterness remains unclear even to many in the brewing industry who often use the term. However, it is clear that bitterness perception is multifaceted. The proof for this can be seen in some of the attributes commonly used to describe the perceived 'Quality' of bitterness in beer e.g. 'harsh', 'smooth', 'lingering', 'harmonious', 'astringent' and 'metallic' (McLaughlin, Lederer, & Shellhammer, 2008; Oladokun et al., 2016b). These terms 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. 'harsh') might be considered less desirable. The hedonic effect of these qualitative terms is also doubtless context dependent – i.e. varies with the sensory properties of a particular beer. Consequently, bitterness quality in beer can be said to be the combination of traits distinguishing it based on intensity, temporal and spatial characteristics. In this regard, the intensity of bitterness corresponds to the magnitude of bitter taste sensation perceived, whilst temporal profile represents the time-course of bitterness intensity over a period of time (Keast & Breslin, 2003). The spatial characteristics of bitterness refers to the location of bitterness sensation on the tongue and in the oral cavity i.e. whether predominantly at the tip of the tongue or at the back of the throat (McBurney, 1976). 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.

The type of hop products used and hopping regime adopted have been reported to impact on the perceived bitterness character of beer (Oladokun et al., 2016b). The impact of hop aroma on perceived beer bitterness has also been investigated, with findings revealing that hop aroma significantly impacts on both perceived bitterness intensity and character. Such effects are believed principally to result from taste-aroma interactions, and are potentially also impacted by trigeminal sensations elicited in the mouth by hop aroma extracts (Oladokun et al., 2016a).

Both the time of hop addition and hop variety used for beer production have been suggested as factors that may impact on 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, there is no scientific study on the impact of hop variety in relation to perceived bitterness quality in beer. Consequently, this study investigated 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; and further determined the impact of hop aroma on the hop-derived bitterness qualities. A liquid malt extract was used to brew beers individually hopped with Hallertau Hersbrucker, East Kent Goldings (EKG) or Zeus hop varieties. A set of the three hopped beers also had hop aroma extract (Hersbrucker) added after bottling. 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.

2 Materials and methods

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.

2.2 Hops

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

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 UK Limited.

2.3 Hop aroma extract

Hersbrucker hop aroma extract (60% w/w, density = 1.020 g/mL) was supplied as a food grade solution by Botanix Ltd. (Kent, UK) and was used for the addition of hop aroma into the beers. This varietal extract was used because its taste and mouthfeel properties have been defined in a previous study (Oladokun et al., 2016a). The Hersbrucker extract (PHA® Varietal Topnotes) represents the total essential oil composition of Hersbrucker hop variety blended into propylene glycol for easy dissolution into beer.

2.4 Chemical and reagents

2.4.1 Phenolic acid standards: 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).

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).

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).

2.5 Instrumentation

HPLC analysis of hop acids and phenolics was carried out on a Waters Alliance 2695 instrument equipped with a column heater and a membrane degasser. Detection was achieved with a diode array UV detector and peak areas were processed with Empower 2 HPLC software. Separation of phenolic compounds and hop acids was achieved with a Purospher STAR rp-18 endcapped column (250 X 4.6 mm, 3 μm) from Merck Millipore (UK) coupled with a C18 guard cartridge from Phenomenex (UK).

2.6 Analysis of hop bitter acids in beer

2.6.1 Extraction of hop bitter acids from beer

Cold beer was degassed by sonication at 15°C followed by the transfer of an aliquot (5 mL) into a 50 mL Falcon tube, the beer aliquot was acidified with orthophosphoric acid (100 μL) followed by the addition of isooctane (10 mL). The mixture was extracted on a roller bed for 30 min. The isooctane extract was subsequently transferred into a glass tube and evaporated to dryness under a controlled flow of nitrogen with a Visidry attachment coupled to a Visiprep solid phase extraction manifold (Supelco). The residue was reconstituted in acetonitrile (2 mL) to give the HPLC sample.

2.6.2 HPLC-UV analysis of hop bitter acids

Hop acid separation was achieved with a binary mixture of (A) 1% v/v acetic acid and (B) 0.1% v/v orthophosphoric acid in acetonitrile. The gradient elution profile was: 0-5 min: 30% A, 70% B; 15-24 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 over a 55 min run time. Injection volume was 10 μL, flow rate was 0.5 mL/min and column temperature was set at 25°C. 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).

2.7 Analysis of polyphenols in beer

2.7.1 Extraction of beer phenolic acids from beer

The phenolic compounds listed in section 2.4.1 were extracted from beer by liquid-liquid extraction. Degassed beer (5 mL) was transferred into a 50 mL Falcon tube before acidification with orthophosphoric acid (250 µL). Ethyl acetate (10 mL) was added and the mixture was extracted on a roller bed for 30 min. Upon completion, the residual beer from the bilayer mixture was discarded and reverse osmosis (RO) water (5 mL) was added to the ethyl acetate extract and further extracted for 15 min on the roller bed. The water layer was then removed and discarded. The ethyl acetate extract was transferred into a glass tube and evaporated to dryness using a controlled flow of nitrogen and a Visidry attachment coupled to a Visiprep solid phase extraction manifold (Supelco). The residue was reconstituted in a fixed volume of methanol (2 mL) and analysed by HPLC.

2.7.2 HPLC-UV analysis of beer phenolic acids

The chromatographic method used a binary solvent system consisting 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, flow rate was 0.5 mL/min and column temperature was set at 30°C. Peak areas were extracted at 280 nm and total run time was 65 min. 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).

2.7.3 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) which involves reacting polyphenols with ferric ion in an alkaline solution. Beer (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1%) and ethylenediamine tetra acetic acid (EDTA, 0.2%) (8 mL) in a 25 mL volumetric flask, then ferric acid (0.5 mL) was added, followed by ammonium hydroxide (0.5 mL) with mixing after each addition. The solution was then made up to mark with RO water and left to stand at room temperature for 10 min before an absorbance of the solution was taken at 600 nm. The absorbance value was multiplied by 820 to give total polyphenol content in beer (mg/L).

2.8 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). Preliminary brews were first carried out to assess the actual utilization (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. The mixture was subsequently brought to the boil after which time the hops were added. 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. The wort (~24 L) was fermented with Saflager S-23 yeast sachets (2 x 11.5 g) from Fermentis at 15°C for 7 days. A 30 L volume FastFerment conical fermenter (FastBrewing & WineMaking, Ontario) was used for fermentation and fermentation was carried out in a temperature controlled room set at 15°C. 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. The beers were transferred in the Cornelius Keg to the SABMiller Research Brewery (on site) for carbonation (5 g/L of CO_2) and bottling. 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 which showed significant impact of hop aroma at this bitterness concentration (Oladokun et al., 2016a). 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 (1.044, 1.008, 4.57, and 4.30); EKG (1.043, 1.008, 4.50 and 4.30); Zeus (1.043, 1.008, 4.50 and 4.30).

2.8.1 Preparation of samples with hop aroma extract

Hop aroma was supplied pre-blended into propylene glycol for easy dissolution into beer. 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 extract was 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. 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.

2.9 Sensory evaluation of beer bitterness

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

2.9.1 Subjects

8 experienced beer tasters (5 male, 3 female) from the University of Nottingham trained beer panel participated in this study. They attended 16 sessions each lasting a minimum of 2 h.

2.9.2 Bitterness quality attributes and definition

A bitterness lexicon consisting of 13 bitterness character attributes was developed and defined by the panel in a related study, and subsequently refined to 12 attributes for use in this study (Oladokun et al., 2016b). The panel recommended that the attributes ‘round’ and ‘smooth’ be combined and redefined, therefore the 12 final attributes were *harsh* (tingly, raspy, irritating); *citric* (fruit-like acidity); *round* (smooth, pleasant, not spiky and 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).

2.9.3 Determination of beer bitterness character profile

For efficiency, 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, Bredie, & Frøst, 2014; Valentin, Chollet, Lelievre, & Abdi, 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 participated 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. Three min breaks followed each sample, during which time panellists cleansed their palates with Evian water (Danone, France) and crackers (Rakusen's, UK) to minimise carry-over effects. Each singly-hopped beer, its replicate brew and those to which hop aroma extract was added (also replicated), were all tasted twice by each panellist. Replicates were tasted in different sessions. Data was collected with Compusense Cloud (Compusense, Canada).

2.9.4 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, 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 in a related study (Oladokun et al., 2016b). 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). Each singly-hopped beer, its replicate brew and those to which hop aroma extract was added (also replicated), were all tasted twice by each panellist. Replicates were tasted in different sessions. Data was collected with Compusense Cloud (Compusense, Canada).

2.9.5 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 $\alpha = 0.05$. 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) analysis of variance (ANOVA) to identify differences between samples. A Tukey HSD post hoc test was used to identify samples that were significantly different from each other.

3 Results and discussion

3.1 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 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). 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 a stringent control of boil time and vigour. The final concentration achieved was close to the value of 13 mg/L which was targeted for this study.

3.2 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 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 1A 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 1B, and is 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 in total phenolic acid contents 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 proanthocyanidins 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; Oladokun et al., 2016b).

3.3 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 2, 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, subtle differences in the perceived character of beer bitterness as a result of the individual hop variety used.

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

The CATA frequency spider plots presented in Figure 3 show the impact of the addition of a Hersbrucker hop aroma extract to each individually hopped beer on its perceived bitterness character profile. While lacking any perceptible taste, in water the aroma of this extract has been described as 'herbal', 'orange peel', ‘piney’/’nutty’, ‘hoppy’ and ‘woody’ with ‘mouth coating’, ‘spicy’, ‘tingly’ and ‘gingery’ mouthfeel properties (Oladokun et al., 2016a). As shown in Figure 3A, B and C the addition of this aroma extract had an impact on the profile of bitterness character of the beer. 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. 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. These findings show how hop aroma can change the perceived bitterness character of singly-hopped beers depending, and relative to the bitterness character present in the beer as a result of the hop variety chosen; and further indicate that the impact of hop aroma on perceived bitterness is pertinent for beer bitterness quality.

3.5 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. As such, the 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 4A as a spider plot. According to these scores, the result shows that none of the bitterness attributes examined was 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. This was not the case for bitterness intensity but the intensity scores for this attribute suggest a trend in that direction for the Hersbrucker brew.

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

The impact of addition of the hop aroma extract to the singly hopped beers on selected bitterness character attributes and bitterness intensity as determined by rank-rating is presented in Figure 4B (Also see supplementary data for comparison of 4A and 4B). The results show a significant increase in the perceived bitterness intensity, astringency and lingering bitterness character. Of the three beers, these attributes were significant for the combination of Hersbrucker aroma and the Hersbrucker hopped beer; suggesting 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. Addition of hop aroma extract did not significantly change harsh and round bitterness character intensity in any of the beers. Importantly, the scoring of beer HE in Figure 4B as the most round in bitterness character while this same beer in 3B was associated with a higher frequency of harsh bitterness is not contradictory, and can be explained by the fact that the two sensory methods employed measured different facets of the beer. The former results are based on intensity ratings of each attributes between the beers while CATA simply indicates the presence or absence of an attribute in the beer.

To confirm the aforementioned findings in relation to the impact of hop aroma on perceived bitterness, subjects were given another four samples to evaluate by rank-rating for the same attributes. These samples consisted of the three individually hopped beers with Hersbrucker aroma added, as well as the Hersbrucker hopped beer with no hop aroma added. The results, presented in Figure 5, show significance for all three previous bitterness attributes (bitterness intensity, linger and astringency) seen in Figure 4B, with the highest scores in each case observed for the combination of the beer containing Hersbrucker hop aroma and the beer brewed with this particular hop variety. It is tempting to speculate that the pronounced impact of Hersbrucker hop aroma on the bitterness character profile of the base beer bittered with Hersbrucker reflects a learned association between congruent aromas and tastes that panellists have learned to pair with one another through experiential learning. This cannot be concluded on the limited data presented here, but if true, would reflect a sophisticated level of congruency recognition, bearing in mind the complexity of hop aroma and the sometimes subtle differences in composition which characterise one variety from another. For bitterness intensity across the data set, 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 in HH (Figure 5). Beer H was also rated significantly lower in bitterness intensity compared to the rest of the beers with aroma added. According to the post-hoc test, the significance for bitterness intensity was between the Hersbrucker beer with no aroma addition (beer H) and both Hersbrucker and Zeus 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 (Figure 5). With regard to harsh bitterness character all of the beers with hop aroma added were perceived to be significantly harsher in bitterness character than the beer without hop aroma. Based on the definition of 'harsh' bitterness character in section 2.9.2, this further confirms some element of oral irritation and trigeminal activation to this hop aroma extract, as has been previously reported (Oladokun et al., 2016a). 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. 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 (HE) were rated highest for round bitterness character, with HH and HZ rated least round in bitterness character.

These results demonstrate the significant impact of cross-modal flavour interactions on the perception of bitterness intensity and character attributes, which are key to the overall impression of bitterness flavour in beer.

4. Conclusions

In this study 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 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 character perceived in all the beers. The effect of hop aroma, determined by the addition of Hersbrucker hop aroma extract to the hopped beers was found to change the bitterness character profile of the beers depending on the hop-derived bitterness character. Hersbrucker hop aroma addition to the three singly-hopped beers was found to significantly increase perceived bitterness intensity, astringency and linger in the Hersbrucker hopped beer out of the three beers, suggesting some level of congruency might be involved in the resultant taste-aroma interactions driving these perceptible changes in beer bitterness. These findings reveal the complexity of bitterness perception in beer as impacted by the use of different hop varieties and hop aroma; and further challenges BU as an accurate measure of perceived beer bitterness, especially in contemporary hop-forward beers, which are often accompanied by elevated hoppy characters.

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Table 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**

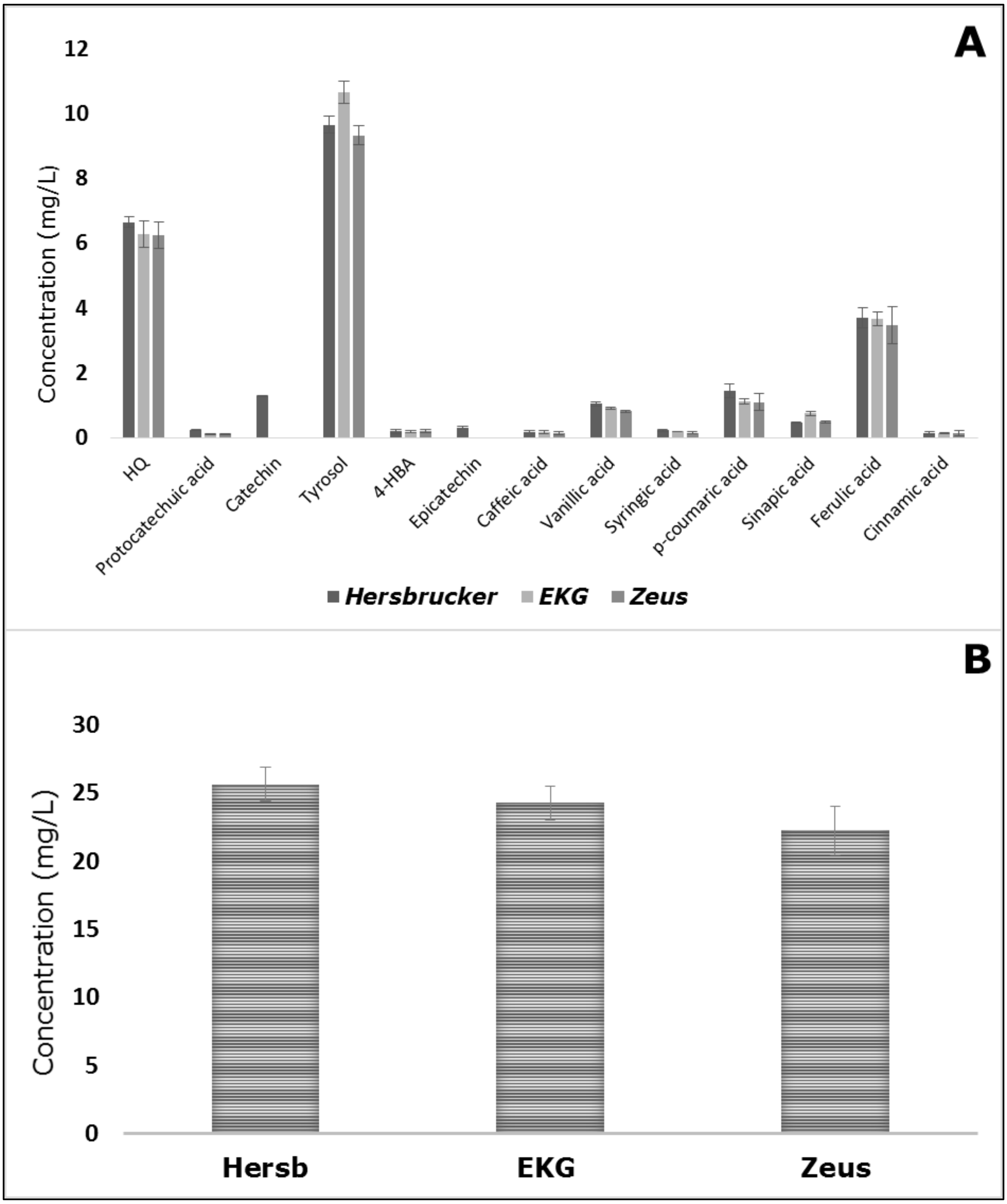


Figure 1: A; Average concentrations of selected phenolic compounds in brew 1 and 2. Error bars are standard deviation of triplicate measurements. B; Average sum of selected phenolic compounds in brew 1 and 2, errors bars represent average standard deviation of six measurements for each brew. Hersb denotes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

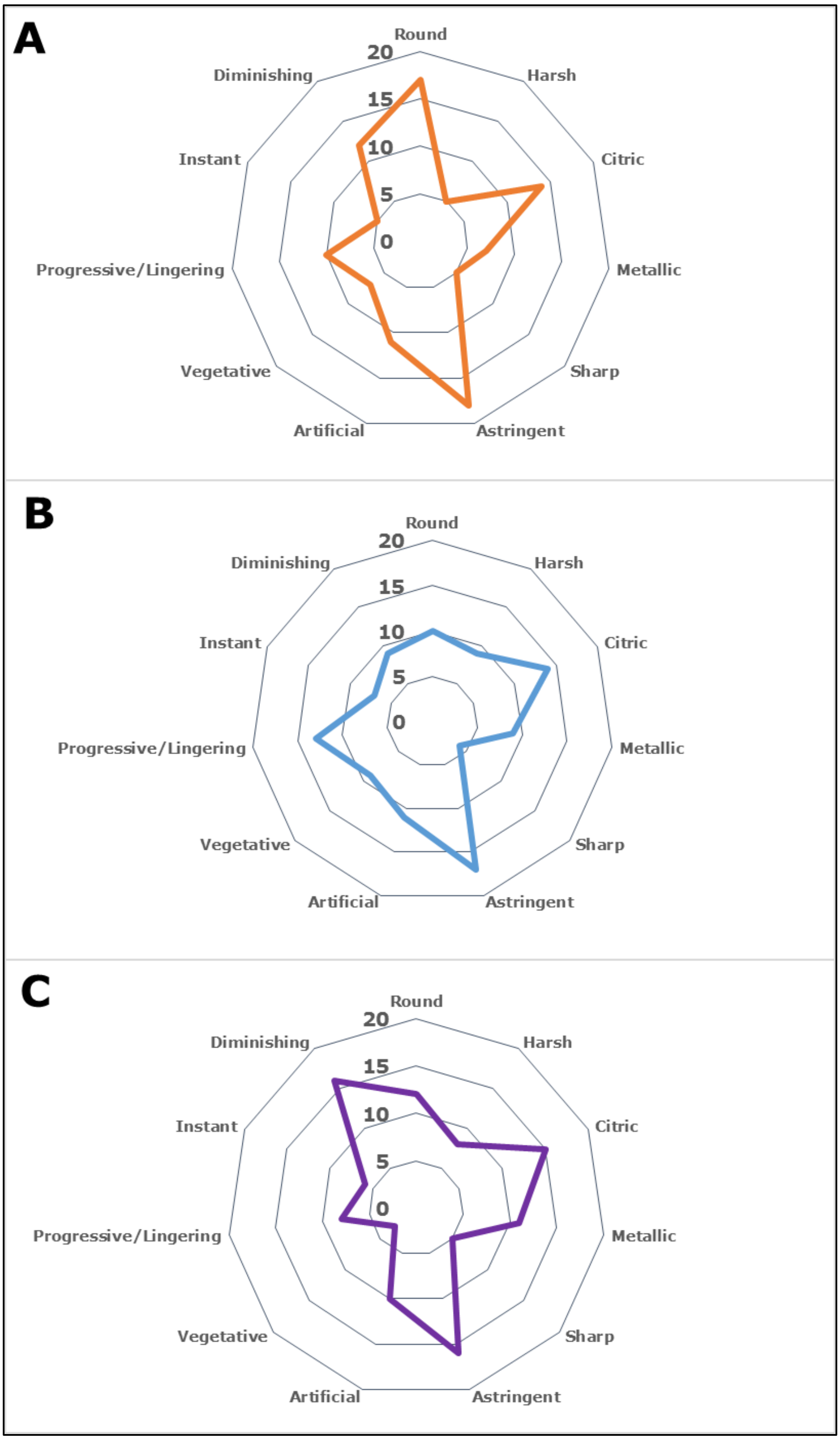


Figure 2: Bitterness character profile of singly-hopped beer determined by CATA evaluation (Numbers represent frequency of attribute selection). A; Hersbrucker hopped beer (**H**), B; EKG hopped beer (**E**) and C; Zeus hopped beer (**Z**).

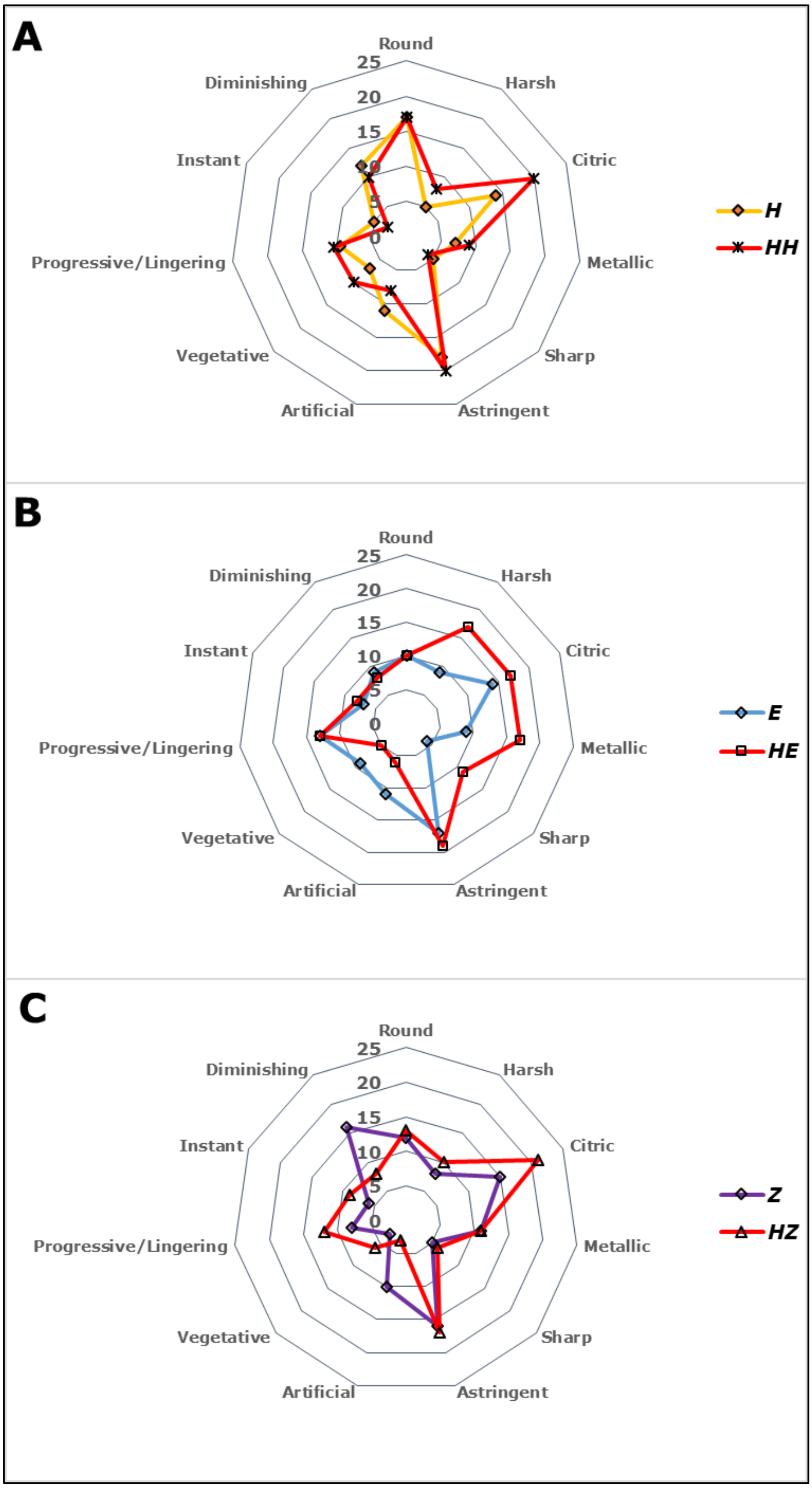


Figure 3: The impact on bitterness character of the addition of Hersbrucker hop aroma to the singly-hopped beers based on CATA evaluation (Numbers represent frequency of attribute selection). A; **H** is the Hersbrucker hopped beer, **HH** denotes Hersbrucker hop aroma added to the Hersbrucker beer. 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.

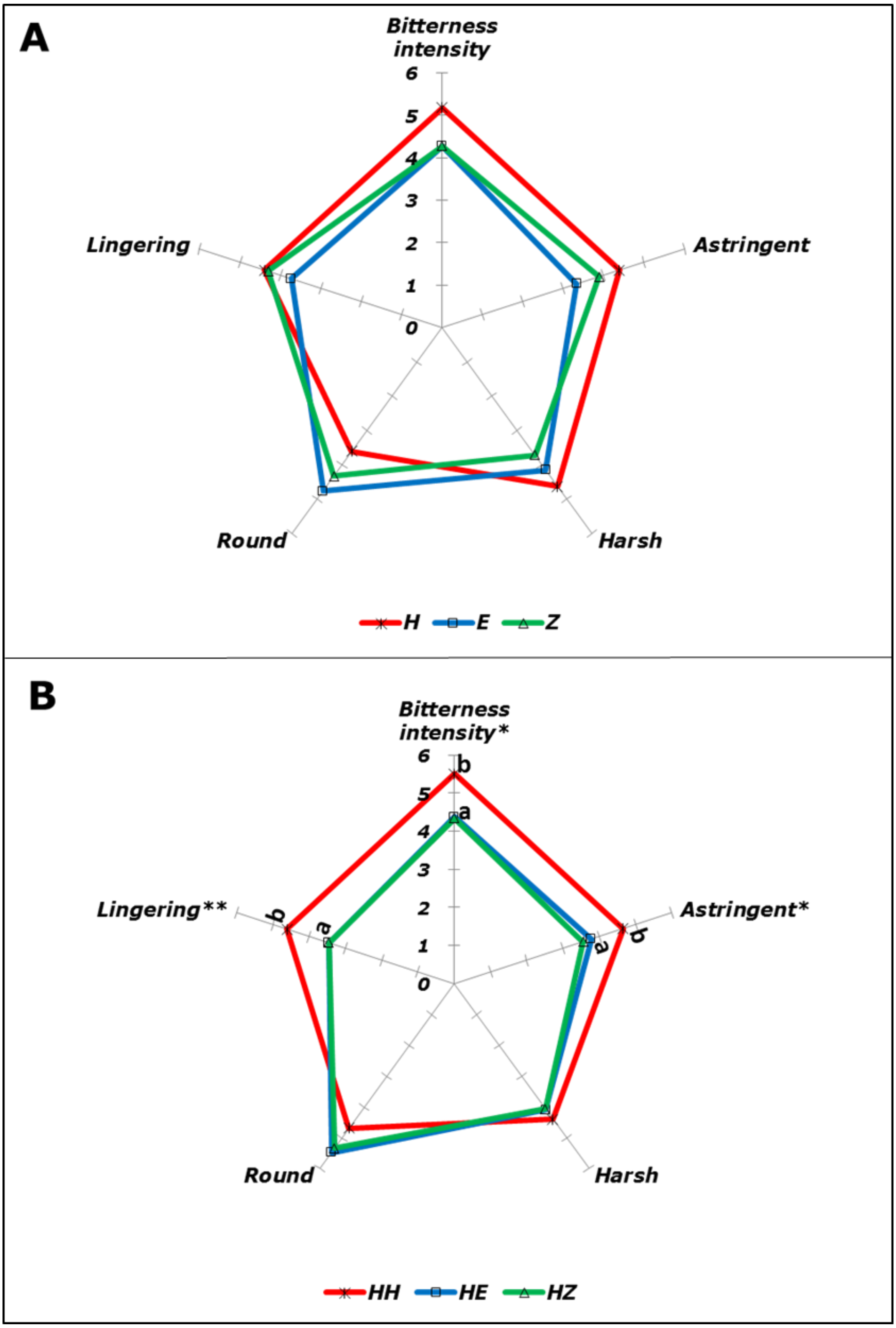


Figure 4: Spider plots of mean intensity scores for bitterness intensity and selected bitterness character attributes. A; **H** denotes the Hersbrucker beer, **E** denotes the EKG beer and **Z** the Zeus brew. B; **HH** denotes Hersbrucker hop aroma added to the Hersbrucker beer, **HE** denotes Hersbrucker hop aroma added to the EKG beer, **HZ** denotes Hersbrucker hop aroma added to the Zeus beer. Significance at *5%, **1%. a,b indicate significantly different samples according to Tukey HSD post hoc test.

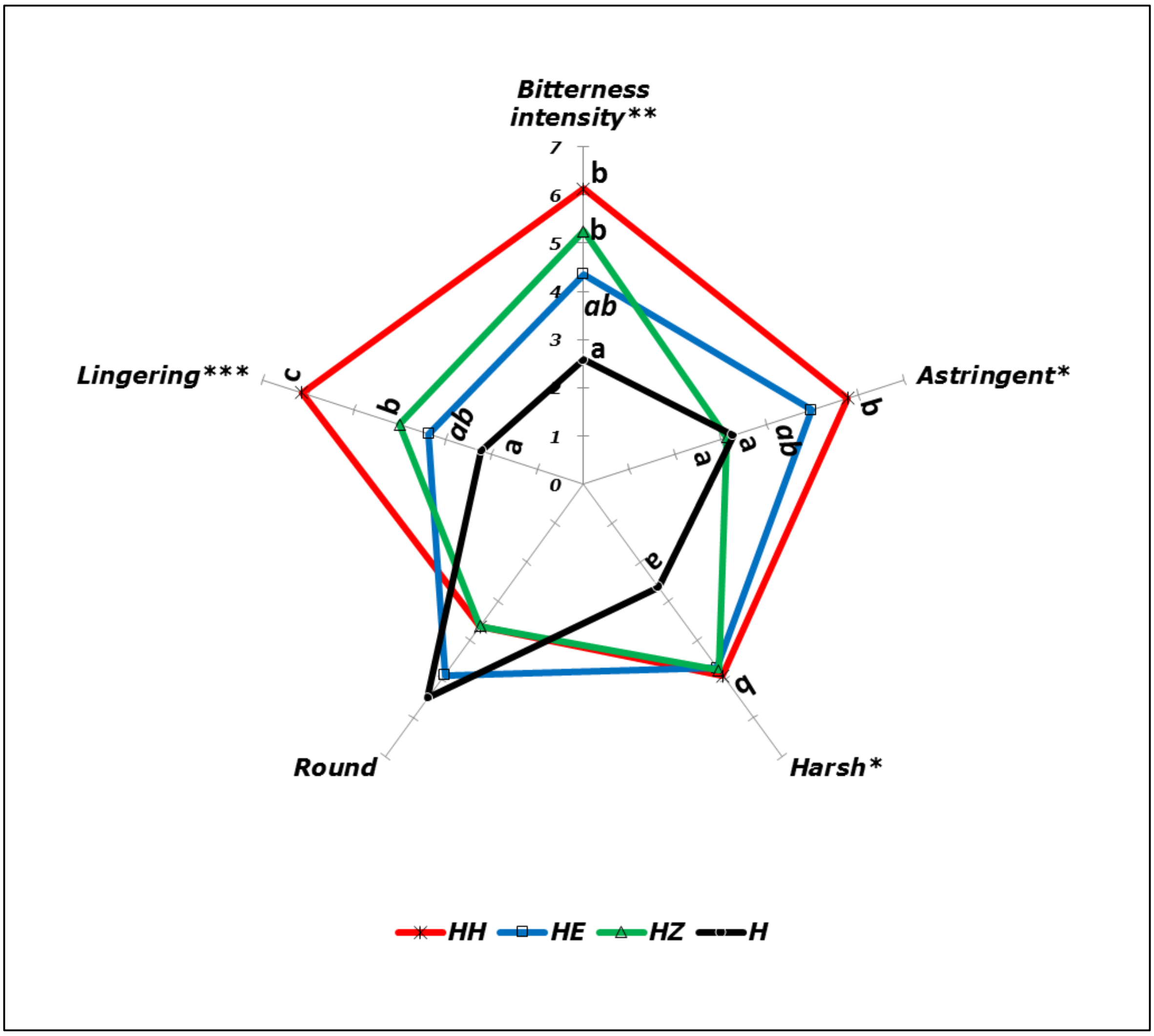
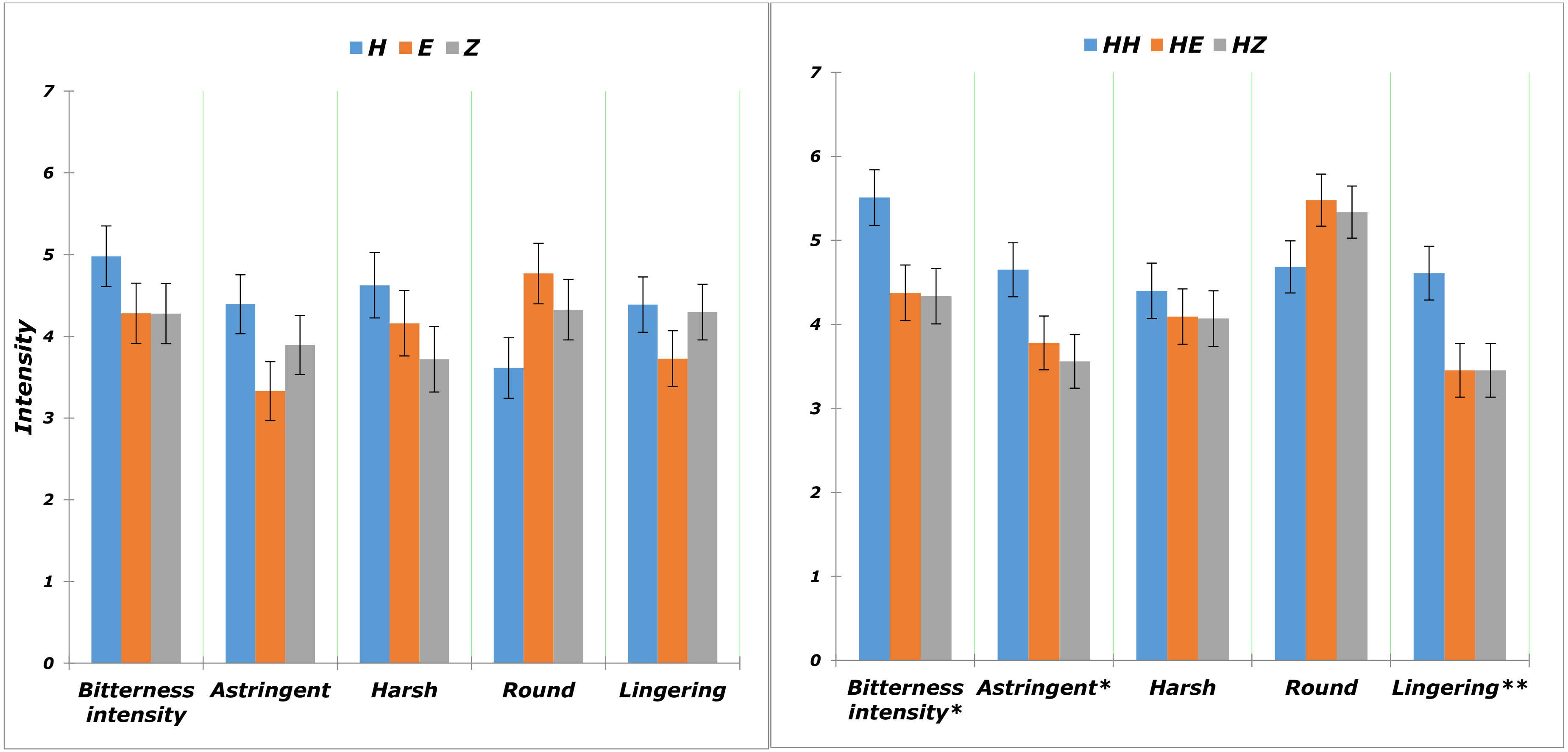


Figure 5: Spider plots of mean intensity scores for bitterness intensity and selected bitterness character attributes. A; **H** denotes the Hersbrucker beer, **E** denotes the EKG beer and **Z** the Zeus brew. B; **HH** denotes Hersbrucker hop aroma added to the Hersbrucker beer, **HE** denotes Hersbrucker 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. Significance at *5%, **1%, ***0.1%. a,b & a,c indicate significantly different samples according to Tukey HSD post hoc test.



Bar charts of mean intensity scores for bitterness intensity and selected bitterness character attributes (presented to allow easy evaluation of the effect of hop aroma). A; **H** denotes the Hersbrucker beer, **E** denotes the EKG beer and **Z** the Zeus brew. B; **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. Significance at *5%, **1%.