Modification of perceived beer bitterness intensity, character and temporal profile by hop aroma extract

Olayide Oladokun[1], Amparo Tarrega[1], Sue James[2], Trevor Cowley[2], Frieda Dehrmann[2],

Katherine Smart[2], David Cook[1], Joanne Hort[§1]

[1]International Centre for Brewing Science, Bioenergy and Brewing Science Building, University of Nottingham, School of Biosciences, Division of Food Science, Sutton Bonington Campus, Loughborough, LE12 5RD, UK.

[2]SABMiller Plc, SABMiller House, Church Street West, Woking, Surrey, GU21 6HS.

§ Corresponding author

E-mail: joanne.hort@nottingham.ac.uk
Tel: +44 (0)115 9516222

Highlights:

- Aroma modified intensity, character and temporal profile of bitterness in beer.
- Hop aroma modified perceived bitterness by taste-aroma interactions.
- Hop aroma evoked trigeminal sensations in the oral cavity.
- Trigeminal sensations impacted perceived beer bitterness intensity and character.
- Balance between aroma and bitterness levels determined bitterness character.

Abstract

The effect of hop aroma on perceived bitterness intensity, character and temporal profile of beer was investigated. A hop aroma extract was added at 3 levels (0, 245, 490 mg/L) to beers at low, medium and high bitterness. Beers were evaluated for perceived bitterness intensity, harshness, roundedness and linger by a trained panel using a rank-rating technique at each bitterness level, with and without nose clips. The use of nose clips enabled the olfactory aspect to be decoupled from taste and mouthfeel aspects of bitterness perception. Results showed significant modification of perceived bitterness in beer by hop aroma depending on the inherent level of bitter-ness. These modifications were mainly driven by olfaction – in an example of taste-aroma interactions, as well as certain tactile sensations elicited by the hop aroma extract in the oral cavity. At low bitterness, beers with hop aroma added were perceived as more bitter, and of 'rounded' bitterness character relative to those without hop aroma. When judges used nose clips, this effect was completely eliminated but the sample was perceived to have a 'harsh' bitterness character. Conversely, at high bitterness, even when nose clips were used, judges still perceived beers containing hop aroma to be more bitter. These increases in bitterness perception with nose clips indicates the stimulating of other receptors, e.g. trigeminal receptors by hop aroma extract, which in tandem with the high bitterness, cause perceptual interactions enhancing bitterness intensity and also affecting bitterness character. Bitterness character attributes such as 'round' and 'harsh' were found to significantly depend on bitterness and aroma levels, with the second level of aroma addition (245 mg/L) giving a 'rounded' bitterness in low bitterness beers but 'harsh' bitterness in high bitterness beers. The impact of aroma on temporal bitterness was also confirmed with time-intensity measurements, and found to be mostly significant at the highest level of hop aroma addition (490 mg/L) in low bitterness beers. These findings

represent a significant step forward in terms of understanding bitterness flavour perception and the wider impact of hop compounds on sensory perception.

Keyword: Beer, bitterness intensity, bitterness character, taste-aroma interactions, trigeminal sensation, hop aroma extract

1. Introduction

The flavour of food and beverages is multifaceted - involving taste, smell, texture, visual appearance, sound and trigeminal sensations; all of which are key for consumer satisfaction (Sørensen, Møller, Flint, Martens, & Raben, 2003). Of the four main brewing ingredients (water, malted barley, yeast and hops) hops (Humulus lupulus L.) re-main 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, Aerts, & De Cooman, 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). Bitterness units (BUs) are used as an analytical estimate of bitterness intensity by brewers, with 1 mg/L of iso-α-acids ap-proximately equalling 1 BU (Oliver & Colicchio, 2011). Generally the higher the level of iso-α-acids the higher the perceived bitterness inten-sity. Lager beers today are reported to typically range from 6–30 BU al-though much more bitter beers (>35 BU) are also widely available commercially (Schönberger & Kostelecky, 2011).

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, & Lysaght, 2015). These oils are complex in nature, with numerous odour-active compounds which significantly contribute to their aroma profile yet to be identified (Eyres et al., 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, Aerts, & De Cooman, 2012a; Van

Opstaele et al., 2012b). The 'spicy' fraction of hop essential oils is currently the subject of intense re-search to identify the compounds responsible for this particular hop character in beer (Van Opstaele, Praet, Aerts, & De Cooman, 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'. 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 reported to contribute to an improved mouthfeel, fullness and increased bitterness perception (Goiris et al., 2002; Van Opstaele et al., 2012a, 2012b, 2013). What remains unclear is the mechanism behind the latter observation, since hop aroma extracts are a complex mixture of volatile compounds assumed to lack any taste qualities. 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, & Taylor, 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 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, & Breslin, 2000; Murphy, Cain, & Bartoshuk, 1977; Pfeiffer 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 sub-threshold concentration of a taste compound, than when it is presented alone (Dalton et al., 2000). The role of congruency on the observed level of taste-aroma interactions is inconsistent; some researchers only observed additivity in congruent taste-aroma pairs (Dalton et al., 2000; Labbe, Damevin, Vaccher, Morgenegg, & Martin, 2006), while others have reported additivity in taste-aroma pair irrespective of congruency (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, & Hort, 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, Rozin, and Shenker (1985) also demonstrated the masking of both olfactory and gustatory sensations by oral capsaicin (Lawless et al., 1985).

The aim of this study was to investigate the impact of hop aroma compounds on the perceived intensity, character and temporal profile of bitterness in beer. A pure aroma extract of the Hersbrucker Spät hop variety was selected for this purpose. This hop variety has been re-ported to impart a 'hoppy', 'green'/'herbal' aroma as well as a 'spicy' mouthfeel to beer (Van Opstaele et al., 2012a). Analytically, it contains relatively higher levels of oxygenated sesquiterpenes, the compounds thought to be responsible for the 'spicy' character of hoppy aroma in beer (Peacock, Deinzer, Likens, Nickerson, & McGill, 1981; Tressl, Engel, Kossa, & Koeppler, 1983; Van Opstaele et al., 2012b).

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 per-formed with and without nose clips.

2.1. Base beer production

The unhopped lager base beer used for this study was prepared at the 10 hL SABMiller research brewery at the Sutton Bonington campus of the University of Nottingham. The standard brew (5% ABV) was pre-pared 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 beer were 1.044 and 4.23, respectively.

2.2. 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).

2.3. Hop aroma extract product

A commercial pure hop aroma extract of the Hersbrucker hop variety (60% w/w, density = 1.020 g/mL) was 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. The hop aroma extract was supplied as a food grade solution, and was 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.

2.4. 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 extract (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 extract was 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.

2.5. Sensory evaluation

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

2.5.1. Subjects

Experienced subjects (5 male, 2 female, mean age 45 years) from the University of Nottingham trained beer panel took part in each element of this study. 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.

2.5.2. Sensory properties of the hop aroma extract

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

To determine if the hop aroma extract possessed any taste or mouth-feel properties, a triangle test (ISO 4120, 2004) (ISO) comparing the hop extract solution sample with water was carried out whereby subjects wore nose clips to prevent any olfactory stimulation. 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.

2.5.3. Perceived bitterness intensity and character

Both perceived bitterness intensity and the intensity of different bitterness character attributes 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, 'round', 'harsh' and 'linger', were previously determined by the panel in a related study (Oladokun, Tarrega, et al., 2016) and were defined as follows: 'harsh' - bitterness perceived to be 'tingly', 'painful', 'irritating' and 'raspy'. 'Round' - a pleasant and smooth bitterness; 'lingering' - the persistence of the bitterness in the mouth.

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 in a previous study, as references (Oladokun, Tarrega, et al., 2016). The attribute 'linger' was assessed as the intensity of bitterness perceived after 10 s upon swallowing the sample. The panellists were trained to use a stopwatch to assess this.

2.5.4. Temporal profile of bitterness

In order to understand how hop aroma affected the time course of bitterness intensity, time-intensity (TI) measurements were conducted. 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.

2.5.5. Sample evaluation

In all cases samples were served at 4 ± 2 °C. Subjects cleansed their palates with water (Evian, 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 hop aroma extract addition (L0, L1, and L2) at a BU level, although this relationship between the three samples 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 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 samples only. Sub-jects 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.

2.6. Statistical analysis

Statistical analyses were carried out with XLSTAT version 2015.6 and STATGRAPHICS Centurion XVI. I statistical software, significance was derived at $\alpha = 0.05$. Rank data was analysed using Friedman's test followed by Nemenyi's pairwise comparison test. Attribute intensity rating scores 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.

3. Results

3.1. Sensory properties of hop aroma extract

The sensory qualities of the hop aroma extract in water as perceived by the panel are listed in Table 1. Some of the terms used to describe the solution 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 1). The triangle test, 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.

3.2. Effect of 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, are presented in Table 2. The mean sample rating scores for bitterness intensity and bitterness character attributes, across the three BU levels, are presented in Table 3. In addition Figs. 1 and 2 depict the mean bitterness profile of the samples at each BU level, with and without nose clips respectively.

3.2.1. Overall bitterness intensity

According to the Friedman's test the addition of hop aroma significantly impacted on perception of overall bitterness intensity. In the absence of nose clips, at low and medium BU levels, samples with hop aroma addition at L2 were ranked to be significantly more bitter than L0. At high BU level, samples with hop aroma addition at L1 were ranked to be significantly more bitter than L0.

ANOVA of the bitterness intensity rating data in the absence of nose clips also indicated that significant differences were evident between samples at each BU level ($p < 0.05$) (Fig. 1). This, together with the results of the Tukey multiple comparison tests (Table 3) largely confirmed 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.

When nose clips were used at the low and high BU levels, there were no significant differences observed between samples based on rank or rating scores for bitterness intensity at low BU. However, at high BU, sample L2 was ranked as significantly more bitter than L0 and L1 (p b 0.05). This effect was not significant in the rating data indicating that this 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 ($p = 0.96$).

3.2.2. Bitterness character

The Friedman's test also revealed the effects of hop aroma addition on harsh, round and lingering bitterness characters (Tables 2 and 3). In the absence of nose clips 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 Fig. 3.

Without nose clips at low BU, L1 samples was 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 at high BU level ($p < 0.05$) as can be seen in Fig. 1. The interaction between BU level and hop aroma levels in the rating data is apparent in Fig. 3, 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.

The mean rank sample 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. This was not picked up in the rating data, again indicating that the difference was subtle, but nevertheless perceptible.

With nose clips on at low BU, sample L2 was ranked as significantly harsher but no other differences in bitterness characters were evident (Fig. 2). At high BU, sample L2 was again ranked as significantly harsher than L0. By contrast L0 was ranked as significantly rounder in bitterness character than L1 and L2 (Table 2). When rating with nose clips on, very few differences in bitterness character were observed indicating that the differences in rankings above were fairly subtle (Table 3). 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.

3.3. Time-intensity results

The average TI curves ($n = 21$ (7 subjects $*$ 3 replicates)) from evaluations performed without and with nose clips are presented in Figs. 4 and 5 respectively. Without nose clips, according to the ANOVA and subsequent Tukey multiple comparison tests, at low BU level the temporal profile of L2 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. 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.

4. Discussion

4.1. Taste and mouthfeel qualities of hop aroma extract solution

The descriptive terms used to characterise the hop aroma extract solution consisted mainly of terms derived as a result of orthonasal aroma perception, but the use of attributes such as 'tingly', 'spicy' and 'peppery' also suggests some element of trigeminal or tactile sensations being elicited by hop aroma compounds present within the hop aroma ex-tract. The results also suggest that at the levels used in this study, the hop aroma extract of the selected variety was not perceived as bitter it-self 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 this particular hop variety in the literature (Goiris et al., 2002; Van Opstaele 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 associated with olfactory stimulation, as these was reported even when nose clips were worn.

4.2. Impact of hop aroma on perceived bitterness intensity

Based on the observed rank and rating scores presented in Tables 2 and 3 respectively, the addition of hop aroma extract caused an in-creased perception of bitterness intensity across the BU levels in beer. These results however also show that this effect is dependent on the inherent level of bitterness (i.e. BU) in the beer. At low BU for example, only addition at L2 resulted in a beer perceived to be significantly more bitter, while in the medium BU beer, L1 and L2 were both perceived to be significantly more bitter than L0. The latter pattern was also observed at high BU level (albeit only significant in the ranking data), where both samples L1 and L2 were perceived to be more bitter than the sample with no hop aroma addition (Fig. 1 low-high). Interestingly, at low BU, the observed increase in bitterness intensity was eliminated upon the use of nose clips, 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 extract 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. in bitter ale beers (Steele, 2013, chap. 19). 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. This finding 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 re-ported previously in beverages (Hewson et al., 2009).

4.3. 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, 1960; Oladokun, Tarrega, et al., 2016). This investigation has provided evidence for the first time that the character of bitterness in beer can be modified by the addition of hop aroma compounds. The results show that the perceived 'roundness' or 'harshness' of beer bitterness is affected by both BU and hop aroma levels. Notably low BU beers with L1 hop aroma addition were described as round in bitterness character, whereas the same level of aroma addition at medium and high BU levels did not result in beer of round bitterness character. 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 (Figs. 1 low and 2 low). At high BU levels, the occlusion of olfaction resulted in sample L2 being perceived as significantly harsher than sample L0 (Table 2). 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 (Fig. 1 low) suggests that volatile hop aroma

com-pounds can modulate harshness by reducing perceived trigeminal sensations. These observations, in combination with the greater perceived bitterness intensity in L2 samples at high BU (even 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 bitter-ness 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 ex-tracts, with the effect on bitterness intensity being more prominent in beers of low and medium bitterness concentrations (BUs). While the levels of acidity (pH), sweetness or alcohol, known to affect bitterness, may also play a role these variables were kept constant in this experiment.

4.4. 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, in particular, the perception of bitterness in beer has been shown to exhibit a time course (Fritsch & Shellhammer, 2009; Pangborn, Lewis, & Yamashita, 1983). Here, although somewhat limited these 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.

An inspection of the average time-intensity curves from individual panellists when nose clips were used (data not shown) revealed that a selected number of the panellists (4) still perceived the samples with hop aroma added as more bitter. This adds further support to 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, suggests further research is war-ranted to investigate the impact of different hop aroma compounds on the temporal profile of bitterness and more importantly how this relates to consumer acceptance.

5. Conclusions

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 affected by hop aroma compounds. Our findings show that the addition of hop aroma extract to beer not only led to a perceptual increase in bitterness intensity but also significantly impacted on perceived bitterness character of beer, depending on the inherent concentration of bitterness in the beer. The hop aroma extract added also elicited trigeminal sensations in the oral cavity which significantly affected the perception of bitterness, especially at high bitterness concentrations. These findings have significant implications for the perception of bitterness in beers, especially those produced with late hop additions (dry-hopped), and further highlight the inadequacy of bitter-ness units (BUs) as a tool for evaluating the overall impression of bitter-ness in beer. Continued investigations to identify specific or group of compounds in hop aroma extract driving both the observed perceptual increase and trigeminal sensations will further add to our understanding of taste-aroma and taste-trigeminal interactions in beer; with this improved knowledge concerning cross-modal flavour interactions in beer paving the way for a more informed approach to the use of hops and hop products in brewing. Furthermore, consumer studies to identify the aroma concentrations at which consumers pick up differences in bitterness will be commercially beneficial to the brewing industry.

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Table 1

Odour and mouthfeel properties of hop aroma extract of Hersbrucker Spät.

Odour descriptors	**Taste/Mouthfeel descriptors**
Herbal	Gingery
Lime	Mouth coating
Orange peel	Spicy
Piney/nutty	Tingly
Hoppy	Peppery
Woody	Medicinal

Table 2

Sum of ranks for beer samples and results of Nemenyi's multiple pairwise comparison test by BU level, with and without nose clips (L0, L1 and L2 correspond to 0, 245 and 490 mg/L addition of hop aroma extract).

	Nose clip OFF			*Nose clip ON*		
	Hop aroma extract level					
BU Level	***L0***	***L1***	***L2***	***L0***	***L1***	***L2***
Low (13BU)						
Bitterness intensity:	30^{a}	35^{ab}	52^{b}	41^{a}	40^{a}	45^{a}
Harsh:	36^{a}	38^{a}	46^{a}	38^{ab}	35^{a}	53^{b}
Round:	34^{a}	50^{b}	36^{a}	44^{a}	43^{a}	39^{a}
Lingering:	41^{a}	38^{a}	41^{a}	39^{a}	41^{a}	46^{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}	33^{a}	44^{ab}	49^{b}
Harsh:	41^{a}	40^{a}	45^{a}	33^{a}	44^{ab}	49^{b}
Round:	51^{b}	39^{a}	36^{a}	49^{b}	43^{ab}	34^{a}
Lingering:	34^{a}	43^{ab}	49^{b}	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 b 0.05). (-) Not evaluated.

Table 3

Mean intensity rating scores for beer samples and results of Tukey post hoc test at each BU level for each attribute, with and without nose clips (L0, L1 and L2 correspond to 0, 245 and 490 mg/L addition of hop aroma extract).

	Nose clip OFF			*Nose clip ON*		
	Hop aroma extract level					
Bitterness	***L0***	***L1***	***L2***	***L0***	***L1***	***L2***
Low (13BU)						
Bitterness intensity:	3.48[a]	3.83[a]	5.707[b]	3.13[a]	3.53[a]	3.87[a]
Harsh:	3.38[a]	3.44[a]	3.99[a]	2.64[ab]	2.47[a]	3.79[b]
Round:	5.26[ab]	6.81[b]	4.90[a]	4.79[a]	5.21[a]	4.87[a]
Lingering:	3.85[a]	3.82[a]	4.09[a]	2.89[a]	2.96[a]	2.83[a]
Medium (25BU)						
Bitterness intensity:	4.54[a]	6.35[b]	6.76[b]	-	-	-
Harsh:	3.43[a]	6.57[b]	6.00[b]	-	-	-
Round:	5.03[a]	4.09[a]	3.06[a]	-	-	-
Lingering:	4.44[a]	5.30[a]	5.92[a]	-	-	-
High (42BU)						
Bitterness intensity:	4.79[a]	6.41[a]	5.99[a]	5.64[a]	6.34[a]	6.63[a]
Harsh:	4.46[a]	4.55[a]	5.60[a]	4.94[a]	5.36[a]	5.71[a]
Round:	5.93[b]	3.83[a]	3.68[a]	3.97[a]	3.92[a]	3.28[a]
Lingering:	4.53[a]	5.51[a]	5.71[a]	4.52[a]	5.51[a]	5.74[a]

[ab]Samples with same letter code in a row, per nose clip condition, are not significantly dif-ferent according to Tukey post hoc test (p b 0.05). (-) Not evaluated.

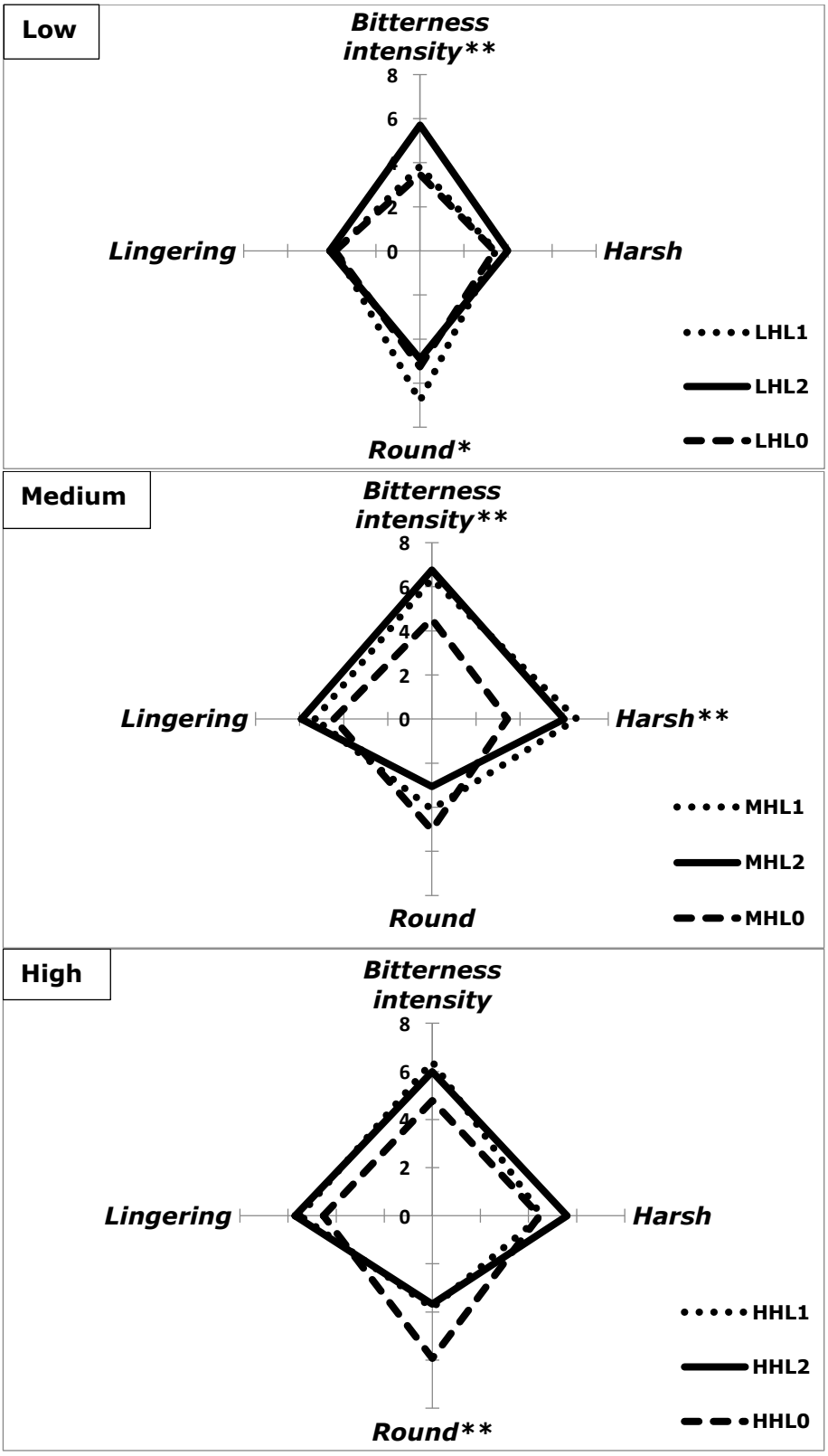

Fig. 1. Spider plots of mean bitterness intensity and bitter character based on intensity ratings. Low: (13 BU) beer, Medium: (25 BU) beer and High: (42 BU) beer. L0, L1 and L2 at each BU level corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance denoted at *5% and **1%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

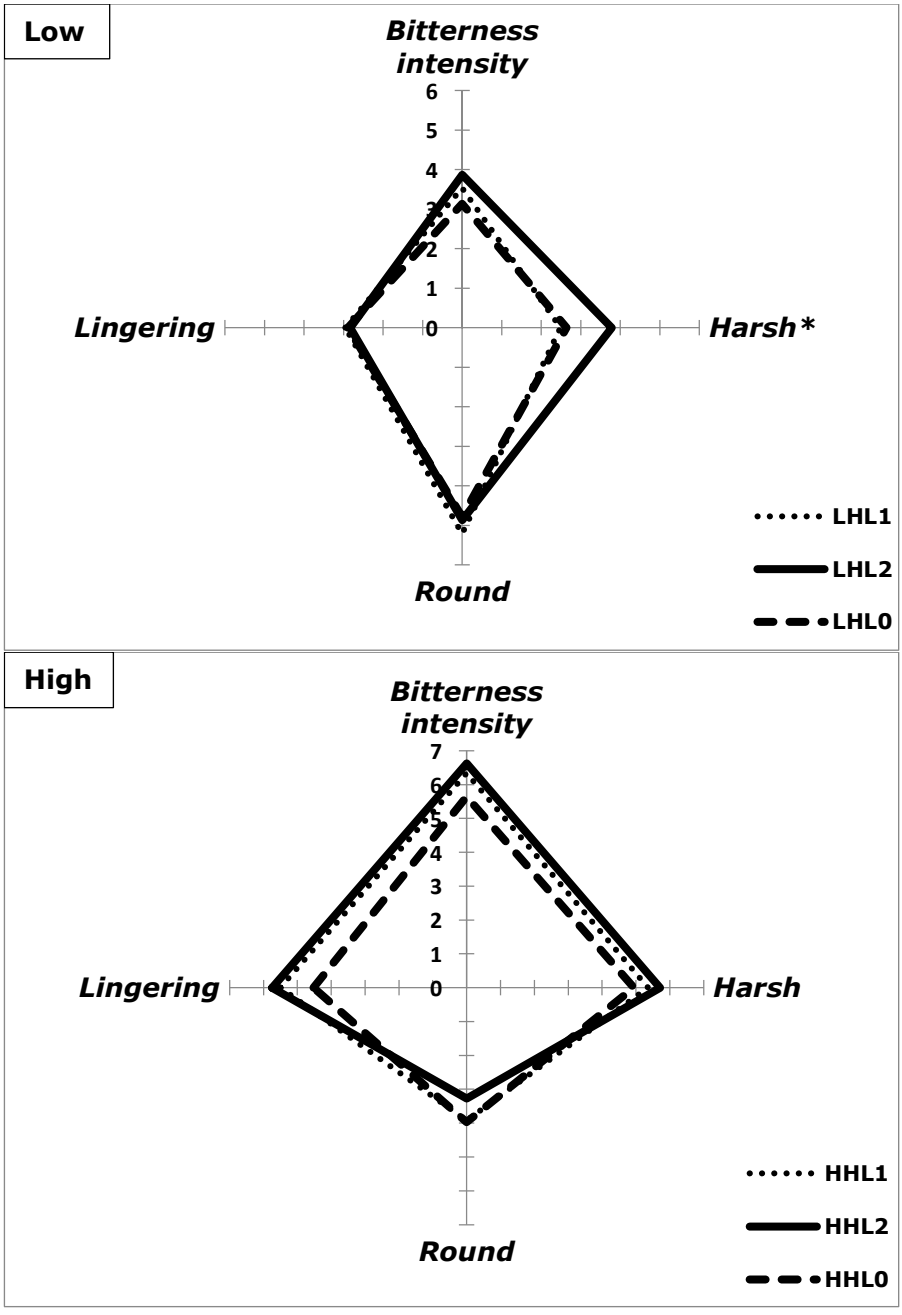

Fig. 2. Spider plots of mean bitterness intensity and bitter character based on intensity ratings with nose clip on. Low: (13 BU) beer and High: (42 BU) beer. L0, L1 and L2 at each BU level corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance denoted at *5% and **1% level.

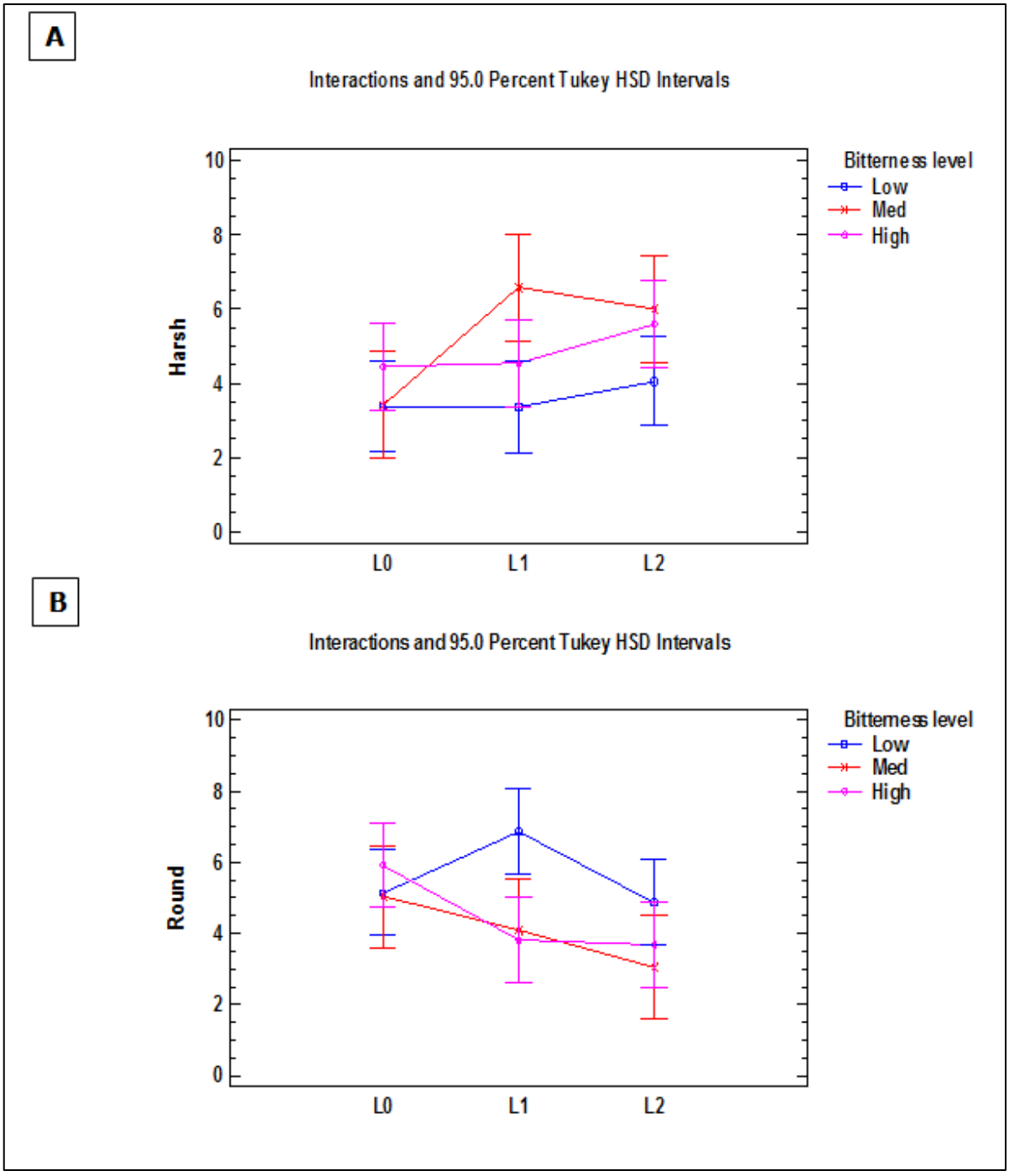

Fig. 3. Interactions between BU and aroma levels. A: interactions for intensity of harsh bitterness character and B: interactions for intensity of round bitterness character. L0, L1 and L2 corresponds to hop aroma extract addition levels of 0, 245 and 490 mg/L. Significance at 5% level.

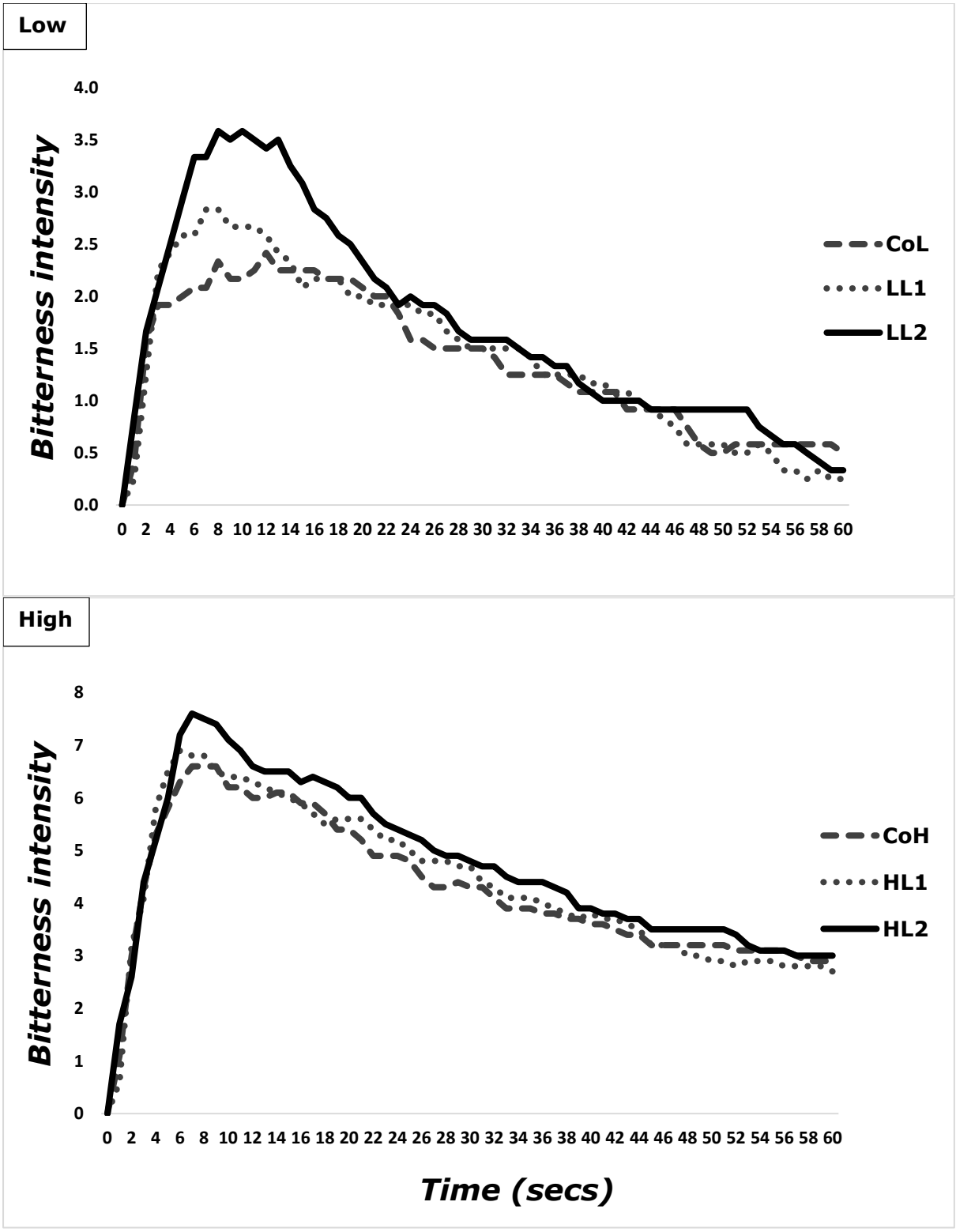

Fig. 4. Average time-intensity curves. Low: (13 BU) beer and High: (42 BU) beer. CoL and CoH, LL1 and HL1, LL2 and HL2 correspond to hop aroma extract addition levels of 0, 245 and 490 mg/L respectively. Significance at 5% level. (n = 21 based on 7 panellists × 3 replicate measurements).

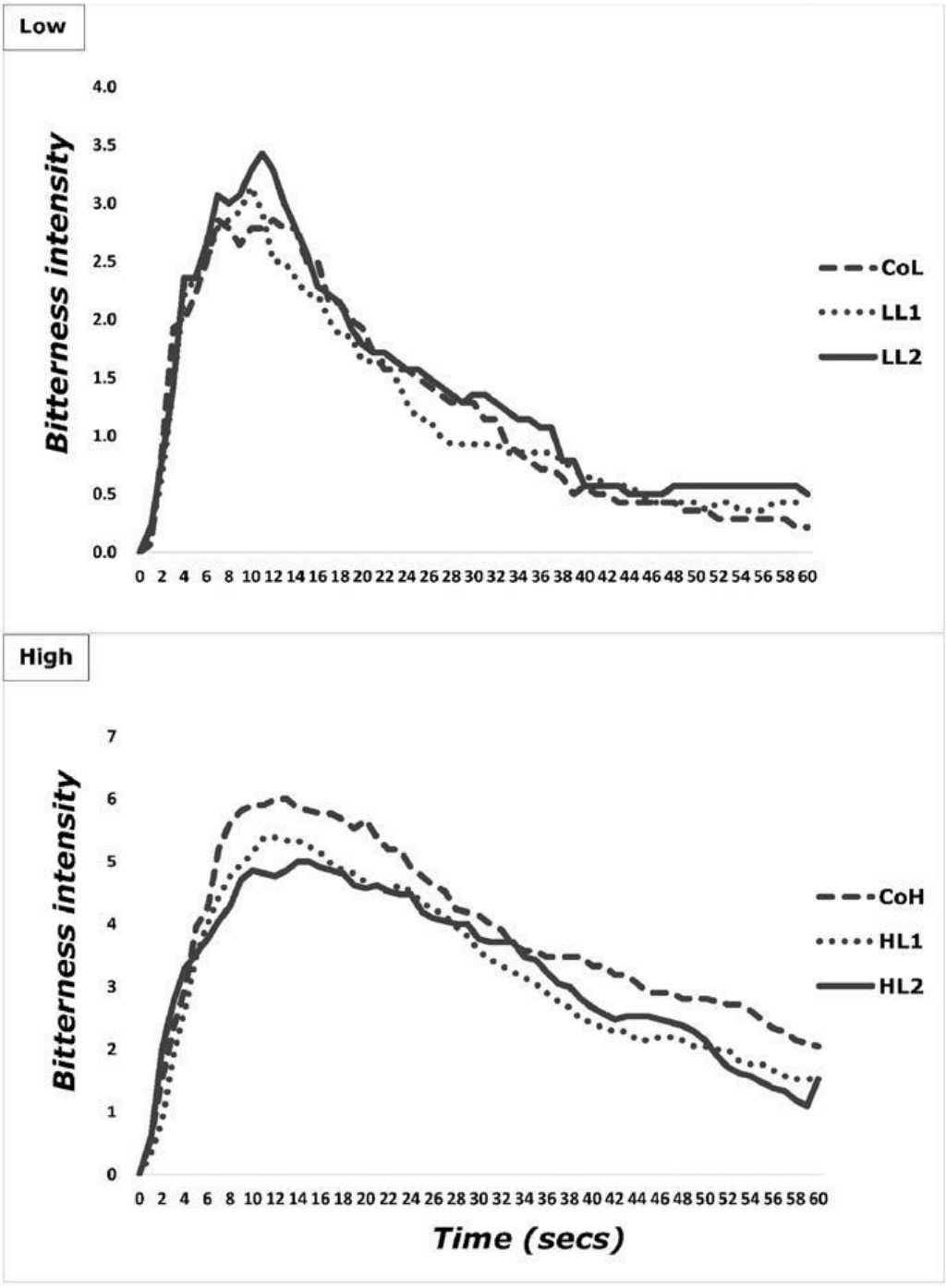

Fig. 5. Average time intensity curves with nose clip on. Low: (13 BU) beer and High: (42 BU) beer. CoL and CoH, LL1 and HL1, LL2 and HL2 correspond to hop aroma extract addition levels of 0, 245 and 490 mg/L respectively. Significance at 5% level. (n = 21 based on 7 panellists × 3 replicate measurements).