A TCATA by modality approach to study the multisensory temporal profile of hop bitter and flavour products applied in lager

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Abstract

Previous research suggested that iso-alpha-acids and hulupones add different bitterness profiles to beer and hop-derived volatiles modify temporal dimensions of bitterness qualities via cross-modal interactions. This research aimed to understand the contribution of hop components to the temporal complexity of beer bitterness and its interplay with flavour characteristics while exploring a novel approach – Temporal Check-All-That-Apply (TCATA) by modality. An unhopped lager beer was bittered with hulupones, natural or commercial iso-alpha-acids and flavoured with hop oil extracts. A sensory panel (n=10) was used to establish an attribute lexicon and trained to evaluate the beers using a Temporal Check-All-That-Apply (TCATA) by modality approach throughout two sips. Citation proportions and durations computed for sip segments and subjected to Mixed Models and Repeated Measures (RM) Analysis of Variance (ANOVA), Correspondence Analysis (CA), and Canonical Variate Analysis (CVA) revealed differences in perception pre- and post-swallowing and in the beer finish. Bittering extracts either imparting 'smooth' (hulupones) or 'harsh' (iso-alpha-acids) bitterness differently affected the characteristics and duration of the sensory profiles induced by the hop oil extracts. Interestingly, the 'peppery tingling' mouthfeel added with the SPICY extract lingered more in the 'smooth' compared to the 'harsh' bitter beer and the 'fruity' extracts increased sweetness suggesting cross-modal interactions. Sensory characteristics were perceived at different time points, however, limited effects were observed between sips. This research demonstrates that different hop flavours could modify taste and mouthfeel properties indicating cross-modal interactions. In addition, a TCATA by modality approach proved to be effective at capturing dynamic sensory profiles of complex beverages.

Keywords: Temporal check-all-that-apply; TCATA; Hop oil extracts; Hop bitter acids; Hulupones; Sensory interactions

Highlights

- TCATA by modality is a suitable tool to study complex, lingering sensory profiles
- The beer bitterness quality was affected by the perception of hop-derived volatiles
- Hulupones impart smooth bitterness, whilst iso-alpha-acids impart harsh bitterness
- Hop flavour products are capable of modifying taste and mouthfeel properties

Abbreviations

ABV, alcohol by volume; ANOVA, Analysis of Variance; CA, Correspondence Analysis; CVA, Canonical Variate Analysis; fin, finish; HULU, Bittering product containing hulupones; HSD, Honest Significance Difference; IBU, International Bitterness Units; im, in mouth; ISO, Bittering product containing commercially isomerised iso-alpha-acids; NISO, Bittering product containing naturally isomerised iso-alpha-acids; RM, Repeated Measures; sw, swallowed; TCATA, Temporal Check-All-That-Apply; TI, Time Intensity

1. Introduction

Increasing demands for sustainable flavouring preparations for the brewing industry has resulted in a wide range of hop extract-based products, which has contributed to unique sensory beer characteristics. These are extracted from the lupulin glands of female plants (*Humulus lupulus* L.) containing resin primarily contributing to bitterness and essential oil comprising volatile compounds foremost known to add aromas to beer (Dietz, Cook, Huismann, Wilson, & Ford, 2020).

Hulupones are oxidative beta acid degradation products naturally found in the soft resin fraction of aged hops and in beer (Algazzali & Shellhammer, 2016). Hulupones can increase beer bitterness, but their recognition threshold (7-8 mg/L) is above the concentration usually detected in beer (1-5 mg/L) (Haseleu, Intelmann, & Hofmann, 2009). To date, hulupones were suggested to have a lower bitterness intensity (84±10% in unhopped lager) (Algazzali & Shellhammer, 2016) and a similar short-lasting bitterness (in 5% ethanol) compared to iso-alpha-acids (Haseleu et al., 2009). However, details of the time dimension differentiating short- and long-lasting bitterness were not provided and instead was defined based on the perception of reference compounds (magnesium sulphate and salicin or caffeine, respectively) (Haseleu et al., 2009). Iso-alpha-acids are derived from isomerisation of alpha-acids. These are highly soluble in water compared to alpha acids in their natural form, and considered as the dominant contributor to bitterness in beer because of a low detection threshold (5-6 mg/L (Baxter & Hughes, 2001)) and high abundance.

Chromatographic hop oil fractionation is used to extract smaller compound groups such as hydrocarbons, esters, ketones, and terpene alcohols with specific sensory characteristics (Meilgaard, 1982; Takoi et al., 2010), and such fractions are commercially available as hop flavour products. Besides adding aroma and flavour, hop oil fractions were reported to significantly affect bitterness qualities perceived in beer (Dietz, Cook, Wilson, Oliveira, & Ford, 2021). In turn, bitter substances can also modify sensory characteristics associated with hop flavour (Dietz, Cook, Huismann, et al., 2020).

The perception of hop flavour in beer is complex and preceding work showed that attributes describing hop-derived bitterness and mouthfeel characteristics (peppery tingling, astringency) lacked discrimination between samples when measured at only one time point (Dietz, Cook, Wilson, et al., 2020; Dietz, Cook, Wilson, et al., 2021). The perception of beer is a dynamic process including taking sips, breathing, movement of liquid, swallowing and release, build-up and decay of aromas, flavours, tastes and mouthfeel (Hort, Kemp, & Hollowood, 2017). Temporal sensory profiling allows multi-dimensional and evolving sensory profiles of complex beverages to be captured (Fritsch & Shellhammer, 2009; Ramsey et al., 2018; Vázquez-Araújo, Parker, & Woods, 2013), which cannot entirely be investigated by using static sensory techniques alone (Oladokun et al., 2016).

Previously, hop flavour extracts were found to add complex sensory profiles to beer with several dominant sensory characteristics perceived simultaneously and consecutively. Authors hypothesised that these simultaneous and consecutive dominant characteristics occurred in different consumption stages and changed throughout consecutive ingestions (Dietz, Cook, Wilson, et al., 2021), but the use of static profiling methods did not allow these to be captured. Therefore, a TCATA by modality approach was selected for the present study, to enable differences between flavour characteristics, in addition to more prominent taste and mouthfeel sensations to be captured. Thereby, panellists are not asked to decide on modality and attributes simultaneously and the risk of halo-dumping is reduced which is important for more complex products (Clark & Lawless, 1994; Nguyen, Næs, & Varela, 2018).

This study aimed to establish a TCATA by modality approach for the temporal sensory evaluation of complex beverages characterised by lingering multi-modal profiles. To achieve this unhopped lager-type beers containing either naturally or synthetically-derived iso-alpha-acids or hulupones (bittering compounds) were combined with a commercial hop flavour product (CITRUS, FLORAL, SPICY, IPA, or SYLVAN) to understand if the TCATA by modality method was sensitive enough to reveal the sensory complexity of beer bitterness and hop oil in combination-related sensory interaction effects.

2. Materials and methods

2.1 Hop extracts

Five commercial hop flavour products containing supercritical CO_2 hop oil fractions and three bittering hop acid extracts (from Magnum variety hops) were provided by Totally Natural Solutions Ltd. (Kent, UK). The hop flavour products are referred to as CITRUS, FLORAL, SPICY, IPA, and SYLVAN (20% w/w in propylene glycol). **Table 1** provides an overview of hop oil fractions present in the products. The bittering products containing commercial or naturally isomerised iso-alpha-acids or hulupone extract are referred to as ISO (>95%), NISO (>95%), and HULU (>90%) and were provided in propylene glycol (30±1%, 25±1%, and 10±0.5%, respectively). All products were selected based on preceding experiments revealing multi-modal interactions between aroma, taste, and mouthfeel sensations (Dietz, Cook, Wilson, et al., 2020; Dietz, Cook, Wilson, et al., 2021). The extracts were stored at 4°C.

2.2 Sensory evaluation

The study was approved by the Research Ethics Committee of the Faculty of Medicine & Health Sciences at the University of Nottingham (FMHS-REC-Ref-No-315-1905). Sensory analysis took place in the Sensory Science Centre facilities equipped with tables for group discussions and individual testing booths (ISO, 2007) for practice and formal evaluation sessions. Prior to each sensory session, panellists were asked to omit eating or drinking any food or liquids other than water for one hour to avoid carryover effects.

2.2.1 Sensory panel

Ten panellists (7 female, 3 male; age range 45-67) were recruited from the pool of individuals belonging to the Sensory Science Centre beer panel who had previously evaluated sensory profiles of hop oil fractions in ethanol-water solutions (Dietz, Cook, Wilson, et al., 2020) and commercial lager (Dietz, Cook, Wilson, et al., 2021). An expert panel size of n=10 is sufficient to generate statistically robust TCATA data (Berget, Castura, Ares, Næs, & Varela, 2020; Nguyen et al., 2018) and a suitable panel type for the temporal sensory evaluation of prototypes with complex sensory profiles due to the focus and sensory evaluation experience required (Weerawarna, Godfrey, Ellis, & Hort, 2021). The panellists were asked to complete a screening session following the principles of ISO standard 8586:2012 (ISO, 2012) to evaluate their current level of sensory abilities and suitability for the study. Additional tests checked for specific anosmia to the hop extracts' main compounds.

2.2.2 Sample preparation

Three batches of lager-type base beer (4.5% v/v) – ISO, NISO and HULU were brewed in the AB InBev research brewery at the International Centre for Brewing Science (ICBS) of the University of Nottingham. Details on the production and analysis of the base beer can be found in the supplementary materials and in **Table A.1**.

The beer bottles (NISO, HULU) were opened in a cold store (4^{o}C), immediately flavoured with hop flavour products, recapped, inverted three times to ensure adequate mixing, and kept at 4^{o}C for 18-20 h prior to each sensory session. The non-flavoured beers were treated correspondingly without addition of hop flavour products. All products were added at equi-flavour intensity (determined by preliminary tests using triangle and rank-rating tests and assessed as the overall flavour intensity (initial sensation)) to prevent peak intensity effects and ensure an intensity at which detailed descriptions of the sensory characteristics could be obtained, including those describing subtle taste and flavour characteristics.

The initial hulupone extract concentration to obtain equi-bitterness at 27 International Bitterness Units (IBU) was calculated based on the study of Algazzali and Shellhammer (2016) who used a slightly different base beer compared to the beer used in the current study. The HULU beers' bitterness had to be adjusted by adding 20.5 µL hulupone extract to a bottle prior to each sensory session to ensure equi-bitterness. Considering the extracts' purity, the approximate bitterness contribution of the hulupone product was estimated to be 76% as bitter as the iso-alpha-acid products (in the unhopped lager).

For the sensory evaluations, 20 mL beer (for two sips) was poured into tempered 60 mL screw-capped amber glass bottles in the cold store (4^{o}C) no earlier than 30 min prior to each evaluation to control decarbonation and volatilisation. All samples were prepared following the same protocol and to further limit sample preparation effects, it was ensured in each session that the respective beer samples for one panellist were always poured from the same beer bottle. All samples were evaluated at 8±2°C and presented blind, in bottles labelled with 3-digit codes. Limited details were disclosed regarding the samples' composition to avoid unconscious bias effects. **Fig. 1** depicts the set of 13 samples presented to the panel.

2.2.3 Panel training

In total, panellists completed 17 training sessions and two mock evaluation sessions (120 min each) to assess panel performance prior to evaluation sessions. The first training sessions were used to establish an attribute lexicon for the temporal sensory evaluation of the beers. The panel completed three in-booth training sessions to familiarise themselves with the samples and independently generate an attribute list to describe their flavour, taste, and mouthfeel characteristics. The following training sessions were used for attribute consolidation, discarding overlapping terms, and identifying the most descriptive and discriminative attributes. Reference materials in different quantities and at different concentrations freshly prepared prior to each session were provided for each attribute to clarify the attributes' definitions and finalise the lexicon. **Table 2** provides the final attribute list including 12 flavour, five taste, and four mouthfeel/trigeminal attributes (reference materials are listed in **Table A.2**).

A TCATA without fading approach was used because the samples were too complex for fading, with many sensations perceived simultaneously, which made it difficult for panellists to focus on the sensory profile whilst continuously checking and re-checking new and fading attributes to achieve sufficient discrimination between the samples. Further training sessions were used to define the sip volume (10 mL), sip and palate-cleansing protocols and to ensure that panellists familiarised themselves with their personal attribute order, which was balanced within modality and between panellists following Williams' Latin square designs to avoid order effects (Williams, 1949). The definition of the sip volume was based on sip volumes that have been used in previous multiple-sip studies (5-15 mL), which were tested to select a volume sufficient for the length of the evaluation period and relatively close to a normal sip size (real-life consumption). Moreover, it was taken into account that the panel was only allowed to consume 1 UK alcohol unit per session/per day.

2.2.4 Evaluation sessions

In total, panellists completed nine evaluation sessions (90-100 min each). For each evaluation session, panellist evaluated five samples with a dummy sample at the beginning. Three replicates were obtained for 15 samples (13 beer samples as shown in **Fig. 1** and two experimental replicates (NISO+IPA, HULU+SPICY)). Samples were randomised using Williams Latin Square design for each replicate, and new 3-digit codes were assigned for each replicate. The panellists received instructions orally (in advance) and on computer screens. The panellists were asked to check all attributes that were perceived and uncheck them when they were no longer apparent at each moment of the evaluation.

At the beginning of each session, panellists received a dummy sample to familiarise themselves with the 2-sip protocol and prevent first-order effects. The 2-sip protocol was developed to enable the identification of changes in the temporal profiles throughout two repeated ingestions and throughout phases of consumption, namely pre- and post-swallowing and in the beer finish allowing for the assessment of lingering sensations (e.g. afterflavour and astringency). The protocol included two sips since preliminary tests showed that the consumption of three sips did not provide relevant additional information. Therefore, the 2-sip protocol was simplified and the risk of panellists's fatigue was reduced. Moreover, the amount of alcohol could be limited that the panellists were asked to consume per session. Due to the attribute number, attributes were presented per modality (Compusense®Cloud, Compusense Inc., Guelph, Canada). After evaluating all flavour attributes, panellists received a fresh sample (poured from the same beer bottle) to assess all taste and mouthfeel attributes during a second evaluation.

Fig. 2 shows an illustration of the 2-sip protocol. The total evaluation time was 180s. Once panellists received their samples, they clicked the "start" button on the screen and were prompted by a message and an audio signal to take the first sip, keep it in their mouth for 10 s while slightly moving the sample. The panellists agreed not to swish or gurgle and the beer was not expectorated since previous research showed that the bitterness of iso-alpha-acid-containing solutions is perceived differently when swallowed (Running & Hayes, 2017). After 10 s, the panellists were prompted to swallow and continue the evaluation of the sensations perceived post-swallowing for 60 s until they were instructed to take the second sip. The second sip followed the same procedure as the first sip and panellists were instructed to continue evaluating the samples for another 100 s until the end of the evaluation (at 180 s time point). No palate-cleansing was performed between the two sips. The length of the evaluation period was based on the time needed for evaluating sensations perceived post-swallowing (i.e. the time required until individual sensations could be recognised and checked) based on panellists' training data and limited to 180 s to avoid effects of fatigue.

For each sample, panellists were instructed to firstly evaluate flavour attributes for two sips, followed by a 2 min palate-cleansing break. Then panellists received a fresh sample (poured from the same beer bottle) and repeated the two sip evaluation protocol for taste and mouthfeel attributes. Carryover, sensory fatigue, and adaption effects (gustatory, olfactory) were minimised by scheduling 3 min breaks after each sample evaluation and a 10 min comfort break after the third sample (of five).

2.3 Data processing and statistical analysis

Statistical analyses were conducted using XLSTAT Sensory (2020.1.3.; Addinsoft, New York, USA), RStudio (1.3.959, Boston, USA), R software (4.4.1, R Foundation for Statistical Computing, Vienna, Austria) and the R package tempR (Castura, 2017). All statistical analyses were performed at 95% confidence ($p>0.05$).

2.3.1 Analysis of sensory panel performance

The performance of the panel was evaluated throughout the training and during the evaluation sessions. Panellists' repeatability, consensus, understanding of attributes, and implementation of the 2-sip protocol were monitored using tools providing rapid and detailed feedback, namely inspection of indicator charts based on single attributes or TCATA runs and calculation of panel performance indices (Castura, Antúnez, Giménez, & Ares, 2016). Panellists were also provided with comment sheets in every session to self-report difficulties with attributes and their needs for further training. A more elaborated approach was used to assess the panel performance during the mock and formal evaluation sessions as a measure of the data's statistical robustness or reliability. Besides indicator charts and indices, interactions as sources of variation were determined using a Mixed Model Analysis of Variance (ANOVA) with sample, position, replicate and interactions as fixed independent factors and panellists and its interactions with fixed factors as random term. Tukey's Honest Significant Difference (HSD) post-hoc test was conducted for pairwise separation and investigation of differences in main effects (Baker, Castura, & Ross, 2016). Moreover, Canonical Variate Analysis (CVA) was conducted by taking into account the panellist variability when drawing sample maps. The confidence level was set at 90% for bivariate normal distribution of the confidence ellipses for each sample. Sizes of and overlaps between ellipses represented panel heterogeneity and discrimination ability (Peltier, Visalli, & Schlich, 2015).

2.3.2 TCATA data analysis and visualisation

TCATA curves. Proportions of citations were calculated for each attribute and pairwise differences between samples in citation proportions were plotted as identified by two-sided Fisher-Irwin tests. If no curve is displayed, no significant effect was detected between samples i.e. citation proportions were considered as homogeneous. All curves were smoothed using cubic spline smoothing (constraints between 0 and 1) to reduce noise in the data and improve the curves' readability whilst avoiding overfitting (Castura et al., 2016).

TCATA trajectory maps. Trajectory maps show the sensory perception evolution of the samples obtained from Correspondence Analysis (CA) on unfolded TCATA data organised in contingency tables. Trajectories were smoothed along each dimension and mapped separately for each sensory modality to reduce dimensionality and ease interpretation (Peltier et al., 2015).

Attribute durations, onsets and offsets. Durations were obtained by summing time slices for sip segments and the total evaluation period. Sip segments represented the different stages during the evaluation with sips held in the mouth (im) and swallowed (sw), for the first (sip1) and second sip (sip2) and the beer finish (fin): "Sip1-im" (10 s), "Sip1-sw" (60 s), "Sip2-im"(10 s), "Sip2-sw" (60 s), and "Sip2-fin" (40 s). The duration was defined as the time at which an attribute was checked to the time at which it was unchecked unless perceived beyond the evaluation/segment period and therefore remained checked. Data was analysed using Mixed Models with sample, replicate, and sample*replicate treated as fixed factors and panellist and interactions included as random effect followed by Tukey's HSD to describe the differences between the samples' temporal sensory profiles. Durations were also analysed by sip segment to investigate differences between samples within segments and the total duration (McMahon, Culver, Castura, & Ross, 2017). CVA was used to represent similarities and differences between samples based on the duration data for each attribute in a map. Instead of maximising the variability between the panellists, CVA was now used to evaluate the correlations between the samples while still taking the panellists' heterogeneity into account (Delompré, Lenoir, Martin, Briand, & Salles, 2020; Peltier et al., 2015).

Average proportions of citations. Average proportions of citations were calculated for each attribute in each evaluation (McMahon et al., 2017). The data was subsequently subjected to Repeated Measures (RM-) ANOVA by sip segment with sample as fixed factor, data within sip segments as replicate, and panellist as subject factor followed by Tukey's HSD computed for each attribute. Pearson's correlation analysis was used to investigate the relationship between attributes within and across modalities. Data were initially time standardised to remove panellist's noise i.e. dual-trimmed and non-parametrically standardised (cf. Lenfant, Loret, Pineau, Hartmann, & Martin, 2009) using different time standardisation approaches discussed elsewhere [in preparation]. Although, the panel was highly trained, a certain level of noise was expected in the sensory temporal data collected due to different cognitive effort required among individuals to complete the tasks (resulting in delayed response times) and hesitation when checking and unchecking attributes (Hort, Kemp, & Hollowood, 2017; van Bommel, Stieger, Schlich, & Jager, 2019). Time standardising the data not only resulted in the loss of the profiles' temporal dimension but also in a reduction of real differences by introducing artefact significant effects and removing real significant duration differences between the samples. These effects were found to be mainly caused by the nature of the sample set. By time standardising the data, the attribute durations were transferred to a narrower timeline, which stretched quickly fading sensations in those samples characterised by shorter flavour profiles (base beers, CITRUS- and FLORAL-flavoured beers) while shortening other sensations in samples characterised by lingering flavour profiles. Moreover, using the time standardised datasets made it difficult to study cross-modal interaction effects. Therefore, average proportions of citation analyses are presented for 'raw', non-processed data.

Changes in selection and concurrent selections. The average number of citations, attributes checked and then unchecked, and attributes that remained checked were calculated for each TCATA run to assess changes in attribute selection. Column averages of the data matrices were calculated for each sample to obtain the proportion of attributes checked concurrently along the evaluation period (Lenfant et al., 2009).

3. Results

3.1 Panel performance during the evaluation sessions

Agreement and repeatability indices ranged between 0.611-0.855 and 0.728-0.931 (**Table A.3**) indicating adequate panel performance (Castura et al., 2016; Poveromo & Hopfer, 2019). However, the exclusive inspection of similarity coefficients is not sufficient to evaluate panellists' discrimination ability (Castura et al., 2016). Mixed Models was used to examine the impact of disagreement, replicate, order, and sample effects on the statistical robustness of the data (data not shown). Significant effects were found indicating replicate*panellist, sample*panellist, sample*replicate, and sample*position interactions. Tukey's HSD tests revealed few significant pairs, which did not follow systematic patterns. This suggests that most significant effects were related to differences in cognitive or oral processing. Inter- and intra-individual differences could not entirely be removed during the training, which has also been observed by other researchers (Lenfant et al., 2009). Furthermore, panel heterogeneity and discrimination performance were examined using confidence ellipses in CVA maps. Several outliers were detected for two panellists located outside the confidence ellipses and further away from the centroids compared to other panellists (**Fig. A.1**). Removal of panellists' data was not conducted since the panellists showed acceptable performance for the majority of data and satisfactory discrimination between the samples.

3.2 Analysis of the sensory temporal profiles

3.2.1 Sensory characteristics of bittering extracts

To visually illustrate the differences between the evolution of the samples' taste and mouthfeel characteristics in a temporal sensory space, asymmetric biplots were employed from CA. **Fig. 3** shows the trajectories of the control beers for Sip1 and Sip2. The first two dimensions accounted for 73.52% (Sip1) and 82.57% (Sip2) of the variance in the dataset. Prior to swallowing, a "cooling" sensation was perceived. After swallowing, trajectories bend and the ISO and NISO beer profiles closely evolve and approach "harsh bitterness". The HULU beer trajectory is mainly characterised by a "smooth bitterness" and is more closely located to "sweet". Trajectories' shapes and attributes' locations suggest similar onsets of sensory characteristics. The Sip2-biplot shows the trajectories bending after swallowing and moving again along "cooling", "sweet", and "sour", which obtained higher citation rates before and just after swallowing. Additionally, the ISO and NISO beers had trajectories closer to "peppery tingling" and "astringent".

These findings were confirmed by the ANOVA outcome revealing that the control beers were mainly differentiated by their taste. Mean durations computed for each attribute-sample combination analysed using ANOVA based on the total evaluation period (**Table 3**) and sip segments (**Table A.4**) revealed that the "harsh bitterness" perception was significantly shorter in the HULU beer (Δt=~102 s). Instead, a "smooth bitterness" was perceived for ~72 s after swallowing Sip1. The HULU beer also significantly differed from the ISO and NISO beers due to a higher sweetness citation frequency after swallowing Sip1 (Δt=~32 s) and a ~10 s shorter astringency. Interestingly, the NISO beer induced a ~25 s longer "peppery tingling" sensation compared to the ISO and HULU beers. Moreover, the "metallic" taste was ~29 s longer in the NISO and ~42 s longer in the HULU beer compared to the ISO beer.

Low citation rates and limited flavour differences were found between the control beers (**Table A.5**). The HULU beer obtained higher "caramel" citation rates compared to the NISO and ISO beers. Analysis of differences between sip segments revealed that this effect started after swallowing Sip1 and citations significantly increased after swallowing Sip2. The HULU beer also received a significantly higher citation rate for "raisins/prunes", but the effect only occurred after swallowing Sip1 and compared to the NISO beer at a low average citation rate. All other flavour attributes did not discriminate between the control beers. "Malty" was the key descriptor for the control beers checked after swallowing Sip1 and unchecked before the end of the evaluation period.

3.2.2 Sensory characteristics of the hop flavour products

The hop flavour products in the beers were differentiated from each other by the presence and duration of the following attributes:

- IPA and SYLVAN beers characterised by 'green' flavours: "earthy", "grassy", "pine wood", "musty", and "harsh bitterness", "astringent".
- CITRUS and FLORAL beers characterised by 'fruity' flavours: "lemon", "grapefruit", "orange", "tropical fruit", and, "sweet", "sour", "smooth bitterness", "metallic" (CITRUS only).
- SPICY beer characterised by 'fruity' flavours and 'mouthfeel': "rose water", "lemon", "orange", "grapefruit", "tropical fruit", "pine wood", "sweet", "harsh bitterness", "astringent", "peppery tingling".

Sample mean separation showed all hop flavour products significantly increase the perceived duration of the beers' flavour profiles, except for SYLVAN in the NISO beer (**Table 4**). With flavour characteristics lasting for ~69-85 s, IPA and SYLVAN induced significantly shorter flavour profiles compared to other hop products. 'Green' flavour sensations were foremost perceived after swallowing Sip1 and faded before reaching the beer finish (>140 s) **(Table A.5).** However, both products significantly increased the perceived taste and mouthfeel duration compared to the control beers, particularly by imparting lingering "harsh bitterness" (~114-144 s) and astringency (~110-143 s) (**Table 5**).

The addition of the CITRUS fraction significantly increased the citation rates for 'fruity' flavours upon swallowing, in comparison to the control beer. Interestingly, peak citation proportions of "grapefruit" and "orange" were detected later in the FLORAL (~ 78-82 s) compared to the CITRUS beers (~16-22 s) suggesting a delayed onset of these flavours in the latter product. Both products increased the perceived flavour duration by ~15-20 s compared to the control beers (**Table 4**, **Table A.4**) with flavours fading prior to the evaluation end. Overall, the addition of the CITRUS fraction resulted in longer lasting taste and mouthfeel characteristics (~ 61-62 s) compared to those added with FLORAL (~54 s). The sourness in these products was only perceived after swallowing Sip1 while the sweetness was already significantly increased before swallowing. A "smooth bitterness" was foremost detected after swallowing Sip1 and lingered throughout the evaluation. Interestingly, addition of CITRUS caused a short astringency (~46-70 s) and "metallic" aftertaste, which was not identified in the other flavoured HULU beers and appeared to generally be masked by the hop flavour products.

Besides the lingering "rose water" flavour (~76-90 s), SPICY mainly stood out due to its "peppery tingling" mouthfeel perceived after swallowing Sip2 until the evaluation end, which were not found to be significant in any other sample. SPICY also added "pine wood" (~74-81 s) and "lemon" flavour, which remained checked on average for ~93-114 s. Moreover, addition of SPICY caused an earlier taste onset and a longer beer finish. "Harsh bitterness" (~123-145 s), "astringent" (~112-130 s), and "peppery tingling" (~103-147 s) sensations in the HULU+SPICY beers remained checked until the evaluation end (**Table 3, Table A.4**).

All flavour characteristics were recognised after having swallowed the first sip, apart from "caramel" and "rose water". The fading of flavours and profiles (returning to control beer level) were mainly noticed during the beer finish. First checks of taste and mouthfeel attributes were recorded at various time points with the earliest recognised attribute "sweet" checked when placing the sample into the mouth, "peppery tingling" after swallowing, and "astringent" during the beer finish. Differences between sips were mostly detected for mouthfeel sensations since these lingered throughout later sip segments, while citations remained similar for taste attributes, which had on average earlier onsets and offsets. This indicates that taste attributes were less likely to build up across sips in the current sample set, whereas for mouthfeel sensations, the build-up effect was much stronger highlighting the importance of using multiple sip approach to capture build-up effect

The bitterness qualities also lingered beyond sip segments until the evaluation end. Overall, attributes were either described as quickly fading ("sweet", "sour", "metallic") or lingering sensations (bitterness, astringency, "peppery tingling"). Only limited differences were found between segments after swallowing suggesting no build-up in citations of the after-flavour.

3.2.3 Interaction between bittering extract and hop flavour products

"Malty" and "caramel" flavours, which were intrinsic characteristics of the base beers were significantly affected by addition of hop flavour products. The "caramel" flavour duration in the HULU-beer decreased regardless of the hop flavour product applied (**Table 5**). RM-ANOVA by sip segment revealed that this effect started after swallowing Sip1 (**Table A.5**). The IPA and SYLVAN beers had significantly lower citation rates for the "malty" flavour. However, the masking effect was not achieved when adding SYLVAN to the HULU beer. Also, SPICY significantly decreased the citation rate for "malty" in those sip segments where maltiness was detected in the control beers.

Base beer or bittering extract related effects on the detection and duration of flavours were mainly observed in the beers flavoured with CITRUS, FLORAL or SYLVAN. Significantly higher citation rates for "grapefruit" and "lemon" flavours were found for NISO+CITRUS compared to HULU+CITRUS. In turn, citation rates for "grapefruit" and "tropical fruit" flavours were higher in HULU+FLORAL compared to NISO+FLORAL.

More interaction effects were found for the SYLVAN beers. "Earthy", "grassy" and "pine wood" flavours lingered in the NISO beer, particularly after swallowing Sip1 and Sip2 (**Fig. 4**). "Musty", "malty" and "raisins/prunes" flavours were predominantly perceived in the HULU beer. The latter two flavours were suggested to be intrinsic to the HULU beer, leading to the conclusion that the SYLVAN product had a larger effect on flavour complexity of the NISO beer's profile. However, the effect on the flavour duration was more pronounced in the HULU beer. Particularly the "musty" flavour duration was extended by ~65 s.

3.2.4 Hop flavour product related effects on beer taste and mouthfeel perception

Several interaction effects between bittering extracts and hop flavour products were observed which affected beer taste and mouthfeel. CITRUS and FLORAL mainly added "smooth bitterness", sweetness and sourness. However, the products were not found to significantly increase the "smooth bitterness" citation frequency in the HULU beer suggesting that the bitterness quality was intrinsic to this base beer. Further effects were observed for the astringency in the flavoured beers' finish profiles, which obtained lower citation frequencies in the HULU+CITRUS and HULU+FLORAL beers compared to their NISO equivalents. Considering that the astringency significantly positively correlated with "harsh bitterness" and negatively with "smooth bitterness" suggests that the base beers' bitterness was affected by the perceived astringency induced by hop flavour products or vice versa.

Citation rates for "harsh bitterness" and "astringent" were not significantly increased in the NISO beer flavoured with IPA and SYLVAN compared to the control beers ISO and NISO since these were characterised by a "harsh bitterness" themselves. The two products only changed the bitterness quality of the naturally "smooth bitter" HULU beer confirming the interaction effect.

Also, addition of SPICY only caused significantly increased citation frequencies for "harsh bitterness" and "astringent" and a longer "peppery tingling" sensation in the "smooth bitter" HULU beers. This effect was not found for the equivalent NISO beers (**Fig. 5**). The HULU+SPICY beers even obtained significantly decreased "smooth bitterness" citation frequencies compared to the HULU beer.

3.2.5 Correlations between flavour, taste and mouthfeel attributes.

Pearson's correlation coefficients computed from the average proportions of citations revealed significant but mostly weak (r<0.6) correlation effects between attributes across modalities (data not shown). The relationship is visually illustrated in the CVA maps (**Fig. 6**) showing the samples' position in the multi-modal space for each sip segment. In each of the evaluation stages, the beers divided into three groups as previously described in section *3.2.2*. The IPA and SYLVAN beers characterised by 'green' flavours and "harsh bitterness" were additionally discriminated from the other samples by a significant perception of astringency in the beer finish. Pearson correlation coefficients confirmed the relationship between "harsh bitterness" and "astringent" starting after swallowing Sip2 (r=0.455). The CITRUS and FLORAL beers were, similarly to the HULU control beer, described by "malty", "smooth bitterness", "sweet", "sour" and 'fruity' flavour attributes. 'Fruity' flavours significantly positively correlated with these taste sensations with the strongest correlations detected between "sweet " and "lemon", "orange" and "tropical fruit" after swallowing Sip 1 (r=402-485). "Sweet" also weakly positively correlated with "caramel" flavour (r=0.307). The third group comprised the SPICY beers plotted close to 'fruity' and "rose water" flavours and moved closer to "peppery tingling" after swallowing Sip1, thereby separating from the other samples. "Peppery tingling" significantly positively correlated with "pine wood" (r=0.361), "rose water" (r=0.555), and "harsh bitterness" (r=0.405) and negatively correlated with "smooth bitterness" (r=0.390). The majority of significant correlations was found after swallowing Sip1 and disappeared in the beer finish confirming the CVA outcome and revealing that the later the evaluation stage, the more the first two factors could explain the variance in the dataset. F1 and F2 explained 75.43% of the variance in the beer finish data (**Fig. 6**) when the samples' profiles separated from each other due to diminishing or unchecking of several attributes.

3.2.6 Multivariate analysis of the beer characteristics

Fig. 7 shows the smoothed trajectories of the HULU and NISO sample sets following two loops representing the two sips, bending twice with fading flavour profiles in the Sip1-sw and Sip2-fin segments and then returning to their starting point (t=0) at the far left. Dimension 1 and 2 accounted for 76.51% (HULU) and 74.59% (NISO) of the variance in the flavour citation frequency datasets. Both biplots follow the same pattern as described for the control beers.

The trajectory map shows the SPICY beers characterised by several flavour attributes. Particularly in the NISO sample map, the CITRUS and FLORAL beer trajectories are closer in proximity than the IPA and SYLVAN beers suggesting similar flavour characteristics and evolution of profiles along sip segments. The majority of attributes are located on the opposite side indicating delayed onsets (perception after swallowing) for all attributes, except for "caramel", "malty", and "raisins/prunes".

The taste and mouthfeel trajectories of the NISO and HULU sample sets are plotted in **Fig. 7**. Dimension 1 and 2 accounted for 74.43 % (NISO) and 76.01% (HULU) for the variance in the datasets. In contrast to the flavour trajectory maps, the samples are not returning to their starting points and bending trajectories reveal fading of the taste and mouthfeel sensations in the final 10 s of the evaluation. The sample sets are clearly separated by "smooth bitterness" versus "harsh bitterness" and "peppery tingling" whilst the NISO control beer trajectory evolves together with the IPA and SYLVAN beers and the HULU control beer with the CITRUS and FLORAL beers.

3.2.7 Analysis of concurrent selection and changes in selection of attributes

Table 6 shows the number of attributes concurrently checked for each beer sample and per modality and the total attribute number checked and unchecked per sample throughout the evaluation period. Independent from the modality, the largest number of attributes was checked for the beers containing SPICY. At sip level, an average of 1.7 flavour and 1.6 taste and mouthfeel attributes were concurrently selected before swallowing Sip1. 3-4 attributes were selected per modality in the following three sip segments. The average number of flavour attributes checked in the finish segment decreased to 0.8. Significant differences between segments were mainly detected after swallowing with more attributes checked in Sip2-sw. The panellists checked several attributes more than once. On average 11 attributes were checked, 8 attributes were unchecked and 3 attributes remained checked for one beer sample, thus, most attributes diminished before the evaluation stopped at 180 s. The highest numbers of attributes checked and unchecked were found for the beers flavoured with SPICY or SYLVAN. HULU+SPICY also stood out for the highest number of attributes perceived concurrently illustrating its complexity. Most attributes were checked and unchecked for NISO+SYLVAN, the fewest for NISO+CITRUS and FLORAL suggesting that these had the least complex flavour profiles.

4. Discussion

4.1 Considerations concerning the TCATA by modality approach

The TCATA by modality approach proved to be an appropriate tool to capture complex sensory interactions between lingering characteristics perceived in the beers and mainly observed after swallowing a second sip. This would not have been apparent if a 1-sip protocol had been selected, as confirmed by previous studies demonstrating that a single sip does not reflect typical ‘real’ consumption of a beverage and only multiple sip data can reveal changes in perception of sensory characteristics between sips and sip segments (cf. Weerawarna et al., 2021). Moreover, this approach reduces halo effects, cognitive effort, and attentional deviation since panellists could be more focused on each modality, which is required if evaluating complex product matrices. Since the current study focused on the evaluation of temporal sensory profiles of beer samples, the TCATA by modality approach with the 2-sip protocol is highly recommended for further research, but should be further tested using other complex/lingering beverages (e.g. wine, coffee).

However, one of the limitations of TCATA is that the perceived intensity of sensory attributes cannot be captured at the same time, therefore, confirmation of the suggested build-up effects for bitterness and astringency observed between the two sips by measuring the evolution of attribute intensities, (e.g. by Time Intensity or Progressive Profiling) is required (Dijksterhuis & Piggott, 2000). The 2-sip protocol used as part of the approach appeared to be suitable to assess changes between the two consecutive sips and lingering sensations perceived post-swallowing. Moreover, panellist effects could be limited by enabling the focus on subtle nuances and thereby obtaining the best picture of the multi-modal profile of the beers. However, if aiming to mimic real-life consumption, the pre-defined 2-sip protocol may not be suitable. Instead, assessors could be instructed to consume a certain volume/number of sips or the full portion of a sample (e.g. half a pint of beer).

Carryover, sensory fatigue, and gustatory and olfactory adaption effects were considered when establishing the evaluation protocol (number of sips, evaluation length, breaks, palate cleansing, sample randomisation) and flavour intensities/extract concentrations in the samples. Decisions with regard to these parameters were made based on the training data, where consistency of responses, position effects, and patterns of decreasing attribute selection frequencies in the second compared to the first sip (Cosson et al., 2020) were monitored. Adaption causing a decrease of sensitivity (Hort, Kemp, & Hollowood, 2017) may have resulted in decreasing selection frequencies, which was not observed in the evaluation data.

4.2 Effect of bitter extracts on unhopped base beers

No significant differentiation between the beers bittered with the two iso-alpha-acid extracts suggests that these may be substitutable. However, the "smooth bitterness", "caramel" flavour and sweetness perceived after swallowing the HULU beer suggests sensory interactions with the base beer and that hulupones are delivering different sensory characteristics compared to iso-alpha-acids. It should be noted that the hulupone extract contained other residual hop materials which potentially contributed to the beer's sensory profile.

4.3 Temporal perception of bitterness qualities in flavoured beers

The bitterness qualities identified in the beers were described as "smooth" and "harsh" (defined as "harsh or irritating, scratchy, spiky bitterness" and "smooth or mellow, soft bitterness"). CITRUS and FLORAL induced "smooth bitterness" in those base beers having intrinsic "harsh bitter" characters (ISO, NISO). IPA and SYLVAN induced a "harsh bitterness" in the "smooth bitter" HULU beer suggesting that hop-derived volatiles significantly affected the bitterness qualities depending on the intrinsic characters of the bitter extracts in the base beers. "Harsh bitterness" was accompanied by astringency and the "peppery tingling" sensation, both predominantly perceived in later sip segments.

During the training period, it was discussed whether to introduce the term 'spiky' as a third bitterness quality to describe the bitterness in the SPICY beers. Subsequent training sessions revealed that the sensation was confused with "peppery tingling". Beer bitterness qualities were previously described as 'harsh', 'smooth', 'round', 'balanced', 'mild', and 'harmonious' (Kaltner & Mitter, 2006; McLaughlin, Lederer, & Shellhammer, 2008; Oladokun et al., 2016) and occasionally directly related to other sensations such as astringency, 'metallic', 'citric', and 'artificial' (Oladokun et al., 2017; Oladokun et al., 2016). These could indeed be different nuances of bitterness or alternatively, already suggest sensory interactions between bitterness and other flavour/taste/mouthfeel sensations indicating interactions within or across modalities. Independent from bitter extracts and hop flavour products applied, bitterness qualities were already perceived after swallowing Sip1 and lasted for on average 2 min and potentially longer since the attributes remained checked until the evaluation end. This is in accordance with previous research where the temporal bitterness of iso-alpha-acid added to beer (20.5µg/L) reached its peak intensity between 12.5-30 s and lingered for 60-120 s (Fritsch & Shellhammer, 2009; Hughes, Menneer, Walters, & Marinova, 1997).

The bitterness was assessed at equi-intensity and quantitative changes were not investigated. It might be that increased citation proportions after swallowing Sip2 of the flavoured beers were related to an intensity increase (build-up). Further research is required to validate this hypothesis and investigate the effect of hop extract-combinations on the evolution of bitter attribute intensities over time.

"Harsh bitterness" as perceived in the IPA and SYLVAN beers strongly correlated with 'green' flavours. These flavour products contained terpene hydrocarbons and oxygenated sesquiterpenes, such as β-myrcene and α-humulene, β-caryophyllene, humulene epoxides (I-III), and caryophyllene oxide and have previously found to impart harsh and lingering bitterness in beer (Dietz, Cook, Wilson, et al., 2021; Schnaitter et al., 2016). Oladokun et al. (2016) found hop extract containing oxygenated sesquiterpenes to change the bitterness quality in lager (5% alcohol by volume (ABV, % v/v)) resulting in the perception of 'harsh bitterness' described as 'tingly, painful, irritating and raspy', which could potentially be a combination of the attributes "harsh bitterness" and "peppery tingling". The extract combined with a high iso-alpha-acid concentration (42 BU) resulted in bitterness peak citation (Tmax) 6-10 s after swallowing and lingered beyond the 60 s-evaluation period, which is in line with the current findings.

Addition of SPICY containing monoterpenes and oxygenated sesquiterpenes induced the perception of a "harsh bitterness" confirming preceding study outcomes (Dietz, Cook, Wilson, et al., 2020; Dietz, Cook, Wilson, et al., 2021). Opstaele, Rouck, Clippeleer, Aerts, and Cooman (2010) found a spicy hop essence (20 µg/L) comprising sesquiterpenoids (humulene epoxides (I-III), caryophyllene oxide, humulenol, β-eudesmol) applied with CO_2 iso-alpha-acid extract (25 mg/L) in a non-bittered beer increased the 'fullness' and bitterness intensity. The addition of a floral hop essence (20 µg/L) decreased bitterness intensity. Although descriptors and length of evaluation period were not further specified, their research provided important evidence that the impact of hop essences on mouthfeel was strongly dependent on the hop oil fraction added.

A similar effect was observed for the "smooth bitter" CITRUS and FLORAL beers. Interestingly, these samples increased beer sweetness and 'fruity' flavour duration. The extracts contained significant linalool concentrations. Linalool was previously reported to induce 'fruity, floral' flavour and bitter taste perception (Dietz, Cook, Wilson, et al., 2021; Kaltner & Mitter, 2006; Praet et al., 2015). For instance, Kaltner and Mitter (2006) observed the sensory scores for "bitterness harmony" to increase and for "mild bitterness" to decrease the higher the linalool concentration detected in the beer.

The findings provide evidence that hop flavour extracts can be used to manipulate the perceived bitterness due to sensory interactions with 'fruity', 'floral' or 'green' flavours occurring in congruent odorant-taste combinations, but depending on the bitter extract present. This effect has previously been observed in wine research showing that wine containing more volatiles perceived as 'fruity' resulted in an increased sweetness and decreased bitterness perception (cf. Sáenz-Navajas, Campo, Fernández-Zurbano, Valentin, et al., 2010) or in olive oil research demonstrating a relationship between the perceived intensity of bitterness and 'green' or 'cut grass' aromas (cf. Caporale, Policastro, & Monteleone, 2004).

It would be interesting to extend the present study to confirm whether the observed effects on the bitterness qualities are solely occurring psychophysical at cognitive level due to the perception of 'green' and 'fruity' flavour compounds (sesquiterpenes, oxygenated sesquiterpenes, monoterpenes), or could be caused by the compounds acting at receptor level. Analytical data about the hop flavour extracts was not provided due to confidentiality requirements, however, the correlation of the temporal sensory data with the extracts' molecular composition and *in vivo* measurement data (e.g. breath-by-breath monitoring (Linforth & Taylor, 2000)) may aid the study of the mechanism underlying the flavour sensations perceived in the hop flavour extracts (or essential oil extracts from other products) as well as their taste- and mouthfeel-modifying properties affecting perception and temporality of the bitterness.

Interestingly, interactions between lingering characteristics were mainly perceived after swallowing the second sip. It appeared that the volatiles first needed to be perceivable through the retronasal pathway before such interaction effects were triggered and different bitterness qualities could be perceived. Since fewer interaction effects were observed after the consumption of Sip1, it was concluded that a 2-sip protocol was required to obtain more insights into the complexity of the hop-flavoured beer's multi-modal profiles. The finding here highlighted the importance of adopting multiple sip approaches when evaluating complex beverage system.

4.4 Effects of bitter stimuli on hop flavour perception

Several significant base beer- or bitter extract-related effects on perceived flavour were observed. Most interestingly, perception duration of 'fruity' characters differed depending on the bitter extract added and also on the type of 'fruity' attribute. "Tropical fruit" and "orange" flavours in FLORAL lingered in the "smooth bitter" and "sweet" HULU beer. "Grapefruit" and "lemon" flavours in CITRUS were more pronounced in the "harsh bitter" NISO beer. It would be interesting to investigate these effects further to identify those compounds that are triggering these effects. Correlation of temporal and compositional data would help to suggest compounds responsible for the increased "raisins/prunes" flavour in the HULU beer flavoured with SYLVAN, which might be intrinsic to the hulupone extract since it was also perceived in the HULU beer.

4.5 Temporal perception of hop-derived astringency

ANOVA outcomes and correlation coefficients suggested a positive relationship between astringency and "harsh bitterness" perception. Similar findings were made by Oladokun et al. (2016) who found lager with high BU level flavoured with oxygenated sesquiterpene-containing hop extract to be perceived as 'harsh bitter' and 'astringent/drying'. The authors suggested this joint perception to be a 'twin sensation' (Lyman & Green, 1990), occurring if compounds are able to induce both sensations. Inspection of individual sip segments revealed that particularly the IPA, SPICY, and SYLVAN beers achieved high citation proportions for both attributes, however, significant effects and peak citations did not occur in parallel. The astringency onset was recorded approximately 30 s later than the "harsh bitterness" onset. The astringency persisted beyond the evaluation period for most panellists, but this was not found for the "harsh bitterness". All evaluated beers were generally perceived as astringent, but, statistically significant differences were only found in the last evaluation segment, which was related to a potential build-up effect as earlier suggested for the bitterness sensation and highlights the importance of a defined sip protocol, the assessment of two sips, as well as including the evaluation of lingering sensations post-swallowing.

4.6 Temporal perception of hop-derived peppery tingling/spiciness

The "peppery tingling" sensation was previously related to hop-derived spicy mouthfeel/flavours in beer and has been suggested to be triggered by the activation of trigeminal receptors in oral and nasal cavities due to the presence of sesquiterpene alcohols and oxygenated sesquiterpenes (Dietz, Cook, Wilson, et al., 2020; Goiris et al., 2002; Praet, Van Opstaele, Baert, Aerts, & De Cooman, 2014). The latter was present in the SPICY product and only beers flavoured with this product were perceived to have a "peppery tingling" sensation, predominantly found at later evaluation stages. It would be interesting to correlate the products' volatile composition to understand the interaction between hulupones and 'spicy' compounds on a molecular basis. Oladokun et al. (2016) found a Hersbrucker Spät hop extract to add 'gingery', 'mouth coating', 'spicy', 'tingly', 'peppery', and 'medicinal' sensations, all appearing to include facets of the "peppery tingling" sensation. The attribute was described as 'peppery tingling' sensation as when eating mild chilli, fresh ginger, horse radish; irritating, itching, stinging sensation (not related to carbonation)'. Oladokun et al. (2016) suggested that the extract stimulated trigeminal receptors in the oral cavity thereby affecting bitterness intensity and quality. This is in agreement with the current outcomes revealing significant correlations between "peppery tingling" and "harsh bitterness" in each segment after swallowing Sip1.

4.7 Effect of hop extracts on temporal beer sweetness

Sweetness in beer is mainly assigned to the presence of malt, sugar, and ethanol. Hop-derived volatiles have also been found to increase beer sweetness perception due to sensory interactions induced by 'fruity, floral' hop oil fractions and compounds such as geraniol (Dietz, Cook, Wilson, et al., 2021). Sweetness citation rates and duration were significantly increased in the CITRUS-, FLORAL-, and SPICY beers which were also characterised by "grapefruit", "lemon", "orange", and "tropical fruit" flavours, all significantly correlating with "sweet" taste. The 'fruity' monoterpene alcohol compounds present in these products could potentially be responsible for an increased sweetness perception. The effect occurred independently from the perceived bitterness quality concluding that different volatile groups were responsible for these taste sensations.

5. Conclusions

The findings illustrate that the TCATA by modality approach enables detailed nuances of complex and lingering sensory profiles with several attributes of the same modality to be captured concurrently and consecutively, which is not possible by static profiling measures (e.g. QDA). The pre-defined, specific 2-sip protocol further allows the evaluation of interaction effects between lingering sensations within and across modalities. Moreover, the temporal sensory data collected showed that hop bitter acids play an essential role in the multi-sensory perception of beers flavoured with different hop flavour products. Naturally and commercially derived iso-alpha-acids were considered substitutable and added a "harsh bitterness" to the beer, while hulupones imparted a "smooth bitterness". The impact of volatile hop compounds on taste and mouthfeel characteristics highly depended on the base beers' intrinsic characteristics or bitter acids present. While flavour sensations mostly faded prior to the end of the evaluation period, taste and mouthfeel sensations were perceived at different time points with astringency foremost significantly discriminating between the beers 2 min after the start of the TCATA run. It appeared that the retronasal aroma of hop-derived volatiles are first needed to be detected or recognised before taste and mouthfeel-modifying interaction effects could be triggered in later sip segments.

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Compliance with ethical standards

Informed consent was obtained from all individual panellists and all procedures were approved by the Research Ethics Committee of the Faculty of Medicine & Health Sciences at the University of Nottingham (FMHS REC Ref No 315-1905) prior to the first sensory session.

Conflict of Interest

The authors declare there are no conflicts of interest.

Author contributions

Christina Dietz: Methodology; Formal analysis; Investigation; Writing – Original draft; Writing – Review & Editing; Visualization.

David Cook: Conceptualisation; Resources; Writing – Review & Editing; Supervision; Project Administration; Funding acquisition.

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Colin Wilson: Conceptualisation; Resources; Writing – Review & Editing

Rebecca Ford: Conceptualisation; Methodology; Resources; Writing – Original Draft; Writing – Review & Editing; Supervision; Project Administration. Supervision of PhD.

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Tables

Table 1. The main hop oil fractions present in the hop flavour products.

Product	Hop oil fractions
CITRUS	Monoterpene alcohols including linalool
FLORAL	Monoterpene alcohols including linalool and sesquiterpenes
SPICY	Monoterpene alcohols and oxygenated sesquiterpenes including humulol and humulenol II
IPA	Monoterpene alcohols, hydrocarbons and oxygenated sesquiterpenes including humulene epoxides
SYLVAN	Monoterpene alcohols and sesquiterpene hydrocarbons

Table 2. Overview of sensory attributes and attribute definitions.

Modality	**Sensory attribute**	**Definition**
Flavour	Malty	Malty flavour as in malt loaf, marmite, toasted malt, Shreddies
	Lemon	Lemon flavour as in lemon or lime fruits; pith, zest (including artificial lemon)
	Raisins/prunes	Raisin/prune flavour as in prunes, raisins, dried fruits or stewed fruits or mincemeat
	Earthy	Earthy flavour as when smelling wet earth, damp soil
	Grapefruit	Grapefruit flavour as in grapefruit; pith, zest
	Grassy	Grassy flavour as when smelling crushed grass, sap
	Tropical fruit	Tropical fruit flavour as in tropical fruit juice (mango, pineapple, melon, peach)
	Musty	Musty flavour as when smelling the old sponge reference
	Orange	Orange citrus fruit flavour as in round, “sweet” orange, mandarin and tangerine
	Pine wood	Pine wood flavour as when smelling pine wood, pine shavings
	Rose water	Rose water flavour as when smelling rose/geranium flowers, rose water or diluted geranium oil or as when eating a piece of Turkish Delight with rose flavour
	Caramel	Caramel flavour as in caramel sauce or toffee
Taste	Sweet	Sweet taste as in the sweet reference solutions
	Sour	Sour, acidic taste as when eating a fresh lemon; sour, mouth-watering, puckering sensation
	Metallic	Metallic taste as the taste of cans or coins
	Harsh bitterness	Harsh or irritating, scratchy, spiky bitterness
	Smooth bitterness	Smooth or mellow, soft bitterness
Mouthfeel	Astringent	Astringent or mouth drying, rough, puckering, furry sensation as when drinking black tea or eating banana peel
	Peppery tingling	Peppery tingling sensation as when eating mild chilli, fresh ginger, horse radish; irritating, itching, stinging sensation (not related to carbonation)
	Warming	Warming sensation in mouth, back of throat, oesophagus
	Cooling	Cooling sensation in mouth, back of throat, oesophagus

Table 3. Mean total duration (s) of taste and mouthfeel characteristics as evaluated by the trained TCATA panel (n=10) with different letters within columns representing significant differences among samples within an attribute as analysed by LS means (p<0.05). The total duration was defined as the sum of time slices (s) of an attribute being checked until the end of the evaluation period.

Sample	**Astringent**	**Cooling**	**Harsh bitterness**	**Metallic**	**Peppery tingling**	**Smooth bitterness**	**Sour**	**Sweet**	**Warming**
ISO	87.2 ef	47.0 bcde	112.8 c	37.2 c	35.2 de	66.2 b	42.4 bc	24.3 c	94.5 a
NISO	94.8 e	47.4 bcde	140.0 ab	65.7 ab	8.6 f	49.2 bc	42.6 bc	19.7 c	81.3 a
HULU	61.5 gh	58.8 a	18.6 d	78.7 a	12.2 f	124.9 a	48.1 bc	54.7 b	88.5 a
NISOCITRUS	69.8 fg	51.9 abcd	24.3 d	66.7 ab	34.5 de	120.7 a	56.7 b	58.2 b	74.4 a
HULUCITRUS	46.0 h	55.0 ab	13.4 d	64.6 ab	10.3 f	143.8 a	75.8 a	54.7 b	83.7 a
NISOFLORAL	78.8 efg	42.7 e	5.5 d	35.8 c	10.0 f	136.8 a	30.3 cd	58.6 b	85.3 a
HULUFLORAL	61.1 gh	54.0 abc	14.8 d	26.5 c	3.0 f	132.7 a	60.8 b	56.8 b	74.8 a
NISOIPA1	132.5 ab	44.7 de	143.3 a	49.4 bc	40.0 cde	28.9 cd	31.5 cd	5.7 c	81.0 a
NISOIPA2	143.2 a	53.5 abcd	114.9 bc	37.4 c	50.8 cd	14.4 d	30.0 cd	13.2 c	73.3 a
HULUIPA	127.1 abcd	45.9 cde	117.7 abc	27.3 c	23.9 ef	24.1 cd	17.9 d	12.7 c	95.2 a
NISOSPICY	112.2 cd	44.9 de	122.5 abc	29.8 c	103.4 b	36.8 bcd	29.3 cd	62.9 b	89.3 a
HULUSPICY1	129.6 abc	50.6 abcde	144.9 a	35.2 c	135.7 a	44.3 bcd	40.6 bc	60.8 b	94.4 a
HULUSPICY2	123.2 bcd	54.8 ab	121.1 abc	37.7 c	146.9 a	31.5 cd	31.5 cd	79.8 a	93.7 a
NISOSYLVAN	110.6 d	47.5 bcde	132.7 abc	38.3 c	57.5 c	39.8 bcd	43.7 bc	14.0 c	93.4 a
HULUSYLVAN	122.7 bcd	46.9 bcde	144.1 a	43.0 bc	20.0 ef	56.7 bc	18.4 d	16.9 c	89.4 a

Table 4. Mean duration (s) for the total evaluation period and onsets and offsets (s) of flavour and taste and mouthfeel profiles calculated for each sample with different letters within columns representing significant differences among samples as analysed by LS means (p<0.05).

Sample	**Flavour attributes**			**Taste & mouthfeel attributes**		
	Total duration	**Onset**	**Offset**	**Total duration**	**Onset**	**Offset**
HULU	16.5 f	21.04 abc	107.10 cde	60.7 bc	35.55 abcd	138.45 abcd
HULUCITRUS	32.7 cde	19.32 bcd	109.21 abcde	60.8 bc	30.34 cd	134.28 abcd
HULUFLORAL	37.7 bcd	22.72 ab	109.68 abcde	53.8 c	30.39 cd	117.44 e
HULUIPA	34.9 cd	19.60 abcd	106.23 de	54.6 c	39.62 abc	133.92 bcd
HULUSPICY1	48.8 a	16.46 d	114.96 a	81.8 a	28.61 d	144.00 a
HULUSPICY2	49.8 a	16.32 d	114.07 ab	80.0 a	29.25 d	141.57 ab
HULUSYLVAN	34.7 cd	18.76 bcd	105.76 e	62.0 bc	40.46 ab	139.96 abc
ISO	14.3 f	23.78 a	108.82 bcde	60.7 bc	42.08 a	137.99 abcd
NISO	15.4 f	22.37 ab	108.23 cde	61.0 bc	36.22 abcd	136.25 abcd
NISOCITRUS	39.1 bc	18.74 bcd	111.62 abcd	61.9 bc	40.19 abc	138.23 abcd
NISOFLORAL	31.1 cd	22.58 ab	107.97 cde	53.8 c	28.38 d	127.88 de
NISOIPA1	30.3 cde	19.69 abcd	108.53 bcde	61.9 bc	36.95 abcd	130.63 cd
NISOIPA2	29.5 de	19.93 abcd	107.69 cde	59.0 bc	39.27 abc	139.24 abc
NISOSPICY	43.5 ab	17.26 cd	112.46 abc	70.1 b	35.05 abcd	137.78 abcd
NISOSYLVAN	22.7 ef	22.25 ab	108.16 cde	64.2 bc	31.00 bcd	131.63 cd

Table 5. Mean total duration (s) of flavour characteristics as evaluated by the trained TCATA panel (n=10) with different letters within columns representing significant differences among samples within an attribute as analysed by LS means (*p*<0.05). The total duration was defined as the sum of time slices (s) of an attribute being checked until the end of the evaluation period.

Sample	Caramel	Earthy	Grapefruit	Grassy	Lemon	Malty	Musty	Orange	Pine wood	Raisins/ prunes	Rose water	Tropical fruit
ISO	3.5 b	14.7 e	18.5 de	11.1 b	3.9 c	80.1 ab	11.7 b	6.1 c	6.7 c	10.5 cde	3.0 b	2.1 c
NISO	12.6 b	15.3 e	6.7 de	4.9 b	24.4 c	82.2 ab	12.0 b	9.4 c	11.0 c	3.5 e	0.0 b	3.0 c
HULU	41.3 a	6.0 e	0.0 e	0.2 b	2.7 c	86.1 a	12.2 b	7.5 c	13.3 c	24.1 bcde	2.5 b	2.1 c
NISOCITRUS	5.7 b	32.5 d	91.0 a	7.2 b	104.1 a	59.3 bcd	11.7 b	75.4 ab	15.4 c	7.5 cde	9.5 b	50.4 a
HULUCITRUS	18.1 b	9.5 e	65.6 b	5.26 b	71.5 b	73.6 abc	4.1 b	78.9 ab	8.4 c	8.1 cde	3.2 b	46.4 a
NISOFLORAL	3.5 b	13.0 e	47.8 c	5.8 b	96.6 a	67.4 abc	12.1 b	56.8 b	19.1 c	12.7 bcde	9.2 b	29.7 b
HULUFLORAL	15.3 b	11.5 e	71.1 b	5.7 b	97.7 a	72.7 abc	7.4 b	75.6 ab	9.0 c	26.3 bcd	4.4b	55.4 a
NISOIPA1	6.7 b	58.0 c	15.2 de	65.9 a	10.0 c	37.7 def	62.9 a	5.6 c	76.8 ab	15.4 bcde	1.2 b	7.6 c
NISOIPA2	8.6 b	74.6 ab	12.8 de	58.6 a	12.0 c	19.5 f	67.8 a	20.9 c	64.7 ab	9.3 cde	2.3 b	2.4 c
HULUIPA	15.1 b	62.4 bc	17.5 de	70.0 a	20.3 c	37.9 def	72.1 a	15.7 c	63.3 ab	31.9 b	10.4 b	2.3 c
NISOSPICY	9.9 b	0.0 e	88.5 a	4.2 b	93.0 a	17.6 f	6.0 b	94.2 a	74.1 ab	3.1 e	75.8 a	55.8 a
HULUSPICY1	15.9 b	4.2 e	89.5 a	6.1 b	94.7 a	31.8 ef	4.9 b	95.7 a	81.0 a	17.1 bcde	90.1 a	55.1 a
HULUSPICY2	11.8 b	0.0 e	90.4 a	5.9 b	113.5 a	33.5 ef	2.6 b	96.6 a	74.2 ab	27.6 bc	88.2 a	52.8 a
NISOSYLVAN	4.0 b	84.5 a	2.4 e	60.1 a	6.4 c	12.6 f	2.8 b	4.2 c	79.7 a	4.8 de	7.0 b	3.3 c
HULUSYLVAN	12.5 b	68.7 bc	27.9 d	55.1 a	10.8 c	49.1 cde	67.6 a	7.1 c	51.78b	56.7 a	7.8 b	1.9 c

Table 6. Average number (n) of attributes selected concurrently for each sample and sip segment and checked and unchecked per sample throughout the evaluation period (180 s).

Samples/ Segments	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total checks	Total unchecks
Flavour							
HULU	1.28 a	1.79 g	1.58 a	1.67 b	0.47 a	6.97 de	6.83 cde
HULUCITRUS	1.88 a	3.01 e	2.78 a	2.98 ab	0.86 a	10.77 abcd	10.43 abc
HULUFLORAL	1.66 a	3.374 d	3.51 a	3.33 ab	0.85 a	11.77 ab	11.57 ab
HULUIPA	1.98 a	3.46 cd	2.98 a	3.27 ab	0.47 a	12.33 ab	12.20 a
HULUSPICY	2.44 a	4.40 a	3.64 a	4.27 a	1.32 a	13.30 ab	12.67 a
HULUSYLVAN	1.97 a	3.57 c	2.94 a	3.39 ab	0.49 a	13.83 a	13.60 a
ISO	0.87 a	1.53 h	0.98 a	1.69 b	0.46 a	5.43 e	5.27 e
NISO	0.78 a	1.77 g	1.343 a	1.66 b	0.41 a	5.57 e	5.40 de
NISOCITRUS	1.60 a	3.63 c	3.17 a	3.41 ab	0.99 a	10.93 abc	10.67 abc
NISOFLORAL	1.68 a	2.98 e	2.487 a	2.95 ab	0.72 a	11.30 abc	11.10 abc
NISOIPA	1.64 a	2.91 e	2.61 a	2.78 ab	0.64 a	12.23 ab	12.10 a
NISOSPICY	2.36 a	3.90 b	3.71 a	3.87 ab	1.23 a	11.70 ab	11.23 ab
NISOSYLVAN	1.49 a	2.59 f	2.68 a	2.67 ab	0.64 a	7.67 cde	7.57 bcde
Taste & mouthfeel							
HULU	1.54 a	3.06 a	2.89 a	3.72 ab	2.66 abc	10.53 def	7.87 cdef
HULUCITRUS	1.56 a	3.10 a	3.21 a	3.54 ab	2.62 abc	10.10 efgh	7.03 defg
HULUFLORAL	1.70 a	3.04 a	2.95 a	3.12 ab	1.85 c	9.97 efgh	6.73 efg
HULUIPA	1.55 a	2.66 a	2.74 a	2.97 b	3.01 abc	10.87 cdef	8.33 cde
HULUSPICY	1.88 a	4.02 a	3.98 a	4.70 a	3.77 a	10.73 cdef	8.53 cde
HULUSYLVAN	1.43 a	2.88 a	2.96 a	3.66 ab	3.16 ab	12.33 bc	9.23 bc
ISO	1.59 a	2.98 a	2.74 a	3.59 ab	2.98 abc	11.87 bcd	8.90 bcd
NISO	1.39 a	3.04 a	2.60 a	3.48 ab	3.18 ab	11.43 cde	8.57 cde
NISOCITRUS	1.78 a	2.89 a	2.83 a	3.67 ab	3.14 ab	9.40 fgh	5.63 g
NISOFLORAL	1.85 a	2.80 a	2.62 a	3.22 ab	2.16 bc	8.90 gh	6.13 fg
NISOIPA	1.42 a	2.68 a	2.86 a	3.55 ab	3.29 ab	11.87 bcd	7.63 cdefg
NISOSPICY	1.76 a	3.48 a	3.16 a	3.98 ab	3.48 a	13.10 ab	10.60 b
NISOSYLVAN	1.40 a	3.31 a	3.17 a	3.44 ab	3.31 ab	14.30 a	13.13 a

Figures

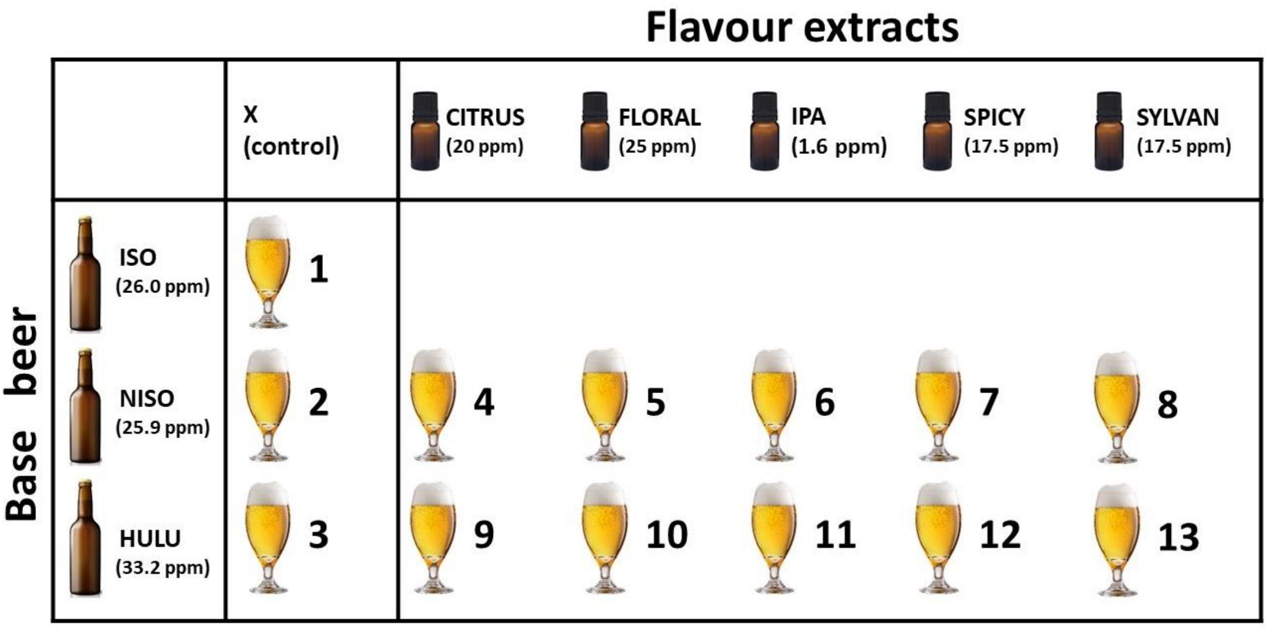

Fig. 1. Sample set comprising of three non-flavoured control beers and 10 flavoured beers evaluated in the TCATA study in triplicate.

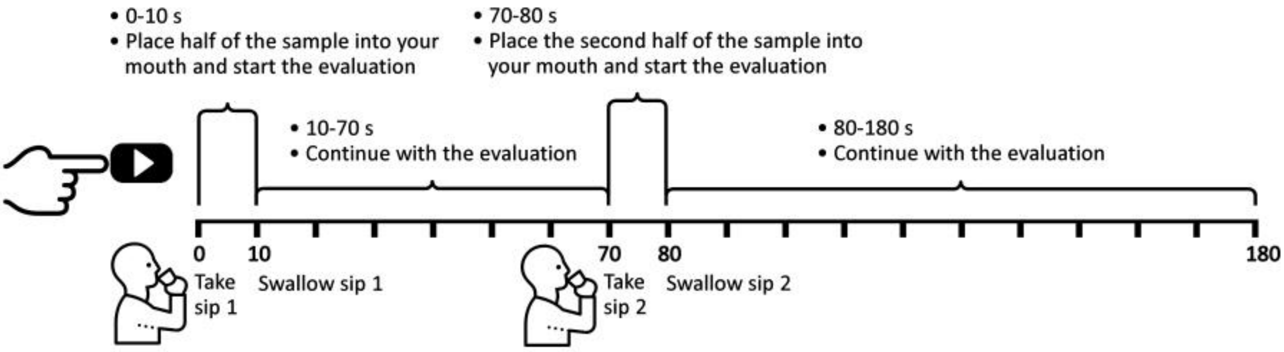

Fig. 2. 2-sip protocol used in the TCATA study.

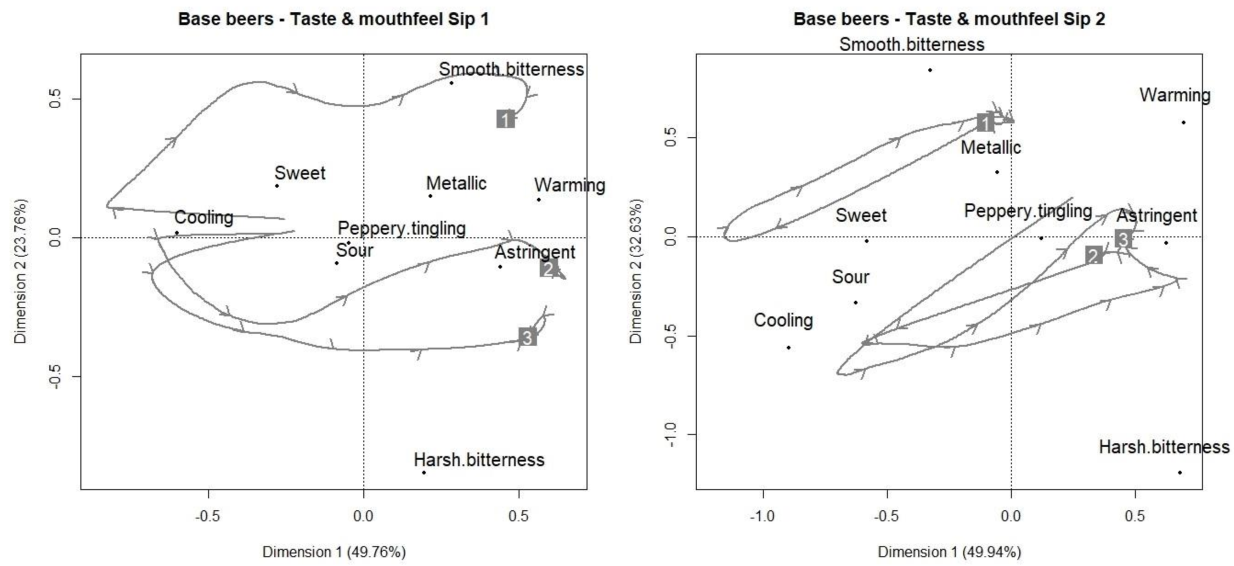

Fig. 3. Smoothed trajectories for Sip1 and Sip2 resulting from Correspondence Analysis (CA) on dimensions 1 and 2 of the control beers HULU (1), ISO (2) AND NISO (3), in the taste and mouthfeel space. The grey arrows indicate the direction of the profile's evolution in 10 s time intervals.

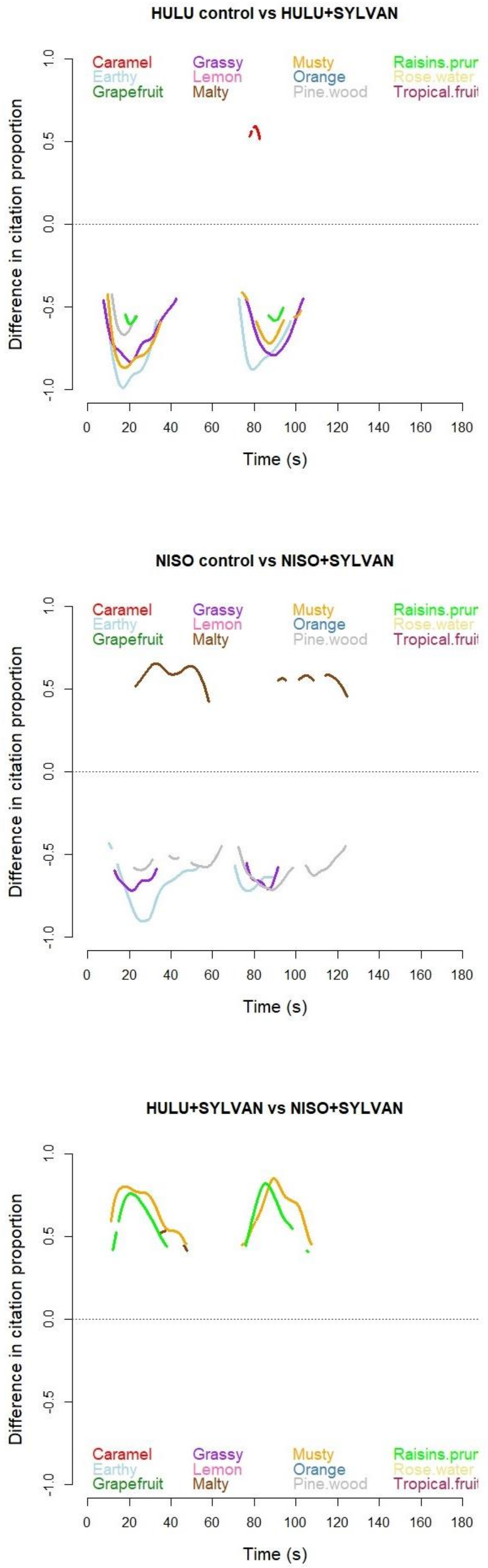

Fig. 4. Smoothed TCATA flavour difference curves showing citation proportions plotted against the evaluation time (s) showing the effect of the SYLVAN hop product, with NISO control beer vs NISO+SYLVAN, HULU control beer vs HULU+SYLVAN, and HULU+SYLVAN vs NISO+SYLVAN.

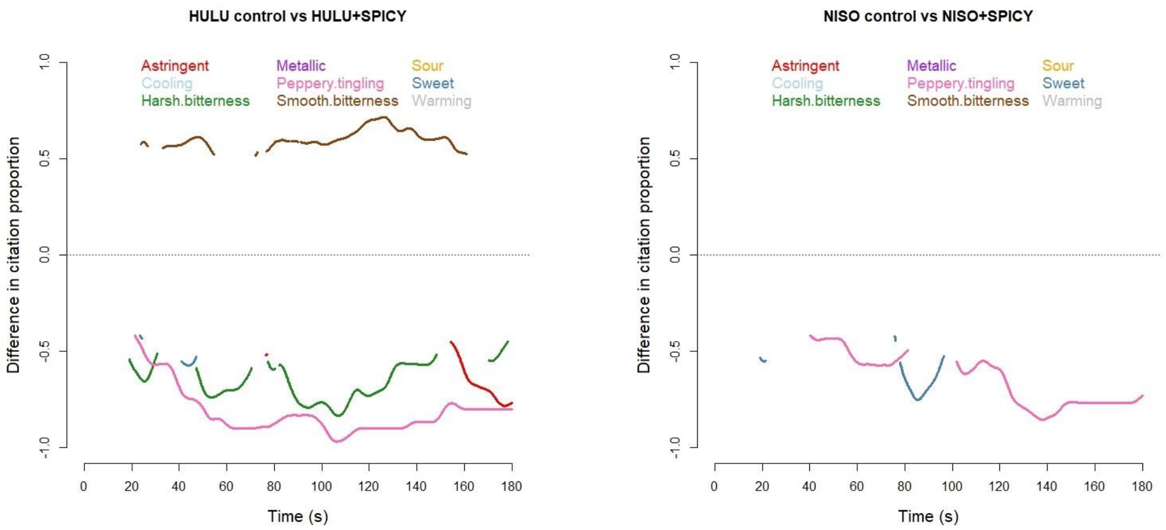

Fig. 5. Smoothed TCATA flavour difference curves showing citation proportions plotted against the evaluation time (s) for the HULU control beer vs HULU+SPICY and the NISO control beer vs NISO+SPICY.

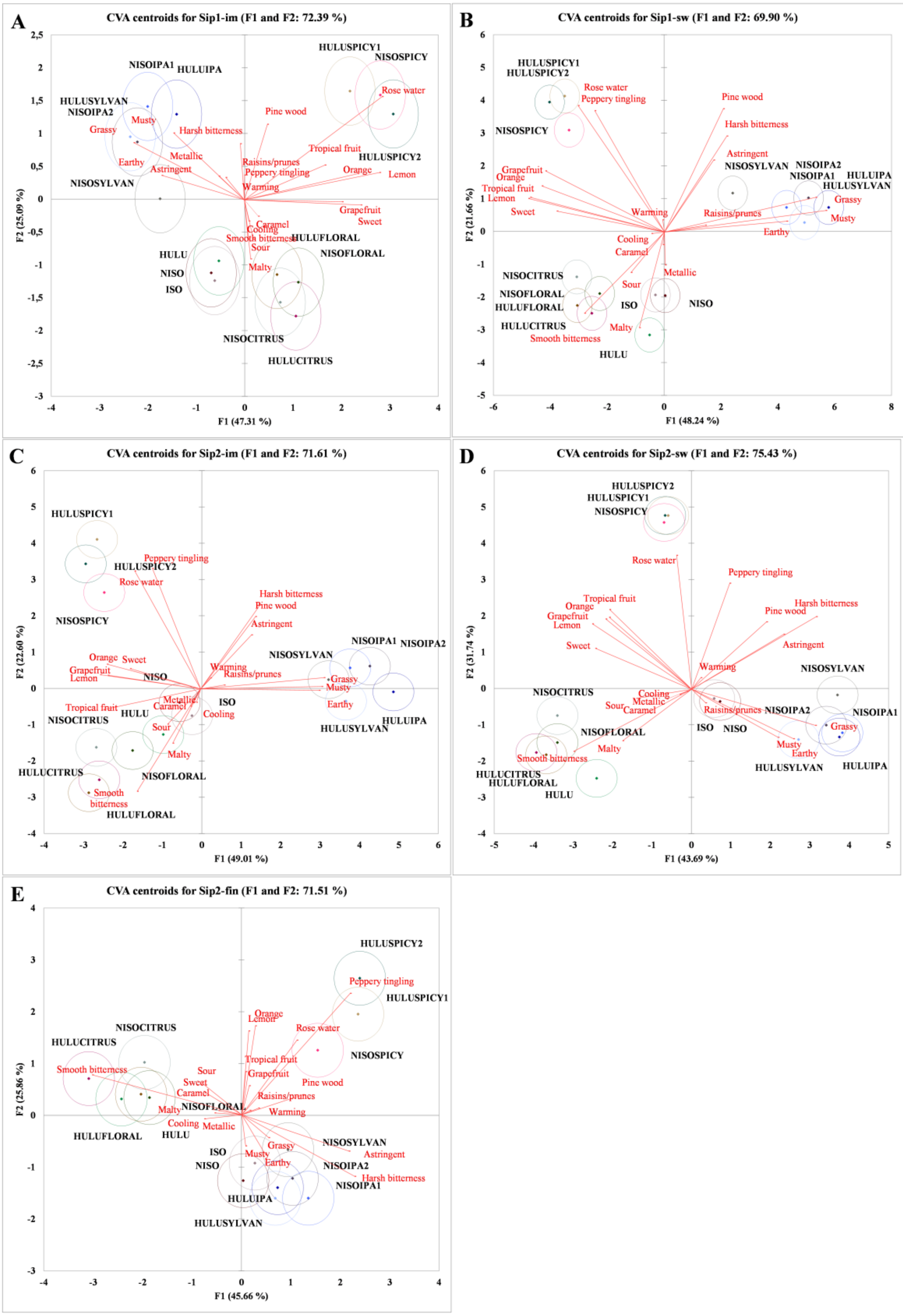

Fig. 6. Canonical Variate Analysis (CVA) maps of the flavour, taste and mouthfeel attributes of the 15 beer samples as evaluated by the TCATA trained panel. The plots A-E depict the multi-sensory profiles perceived in the individual sip segments: Sip1-im (A), Sip1-sw (B), Sip2-im (C), Sip2-sw (D), and Sip2-fin (E). Sample names are displayed in black and attributes are shown in red. Non-overlapping confidence ellipses indicate significant discrimination among the samples (p<0.05).

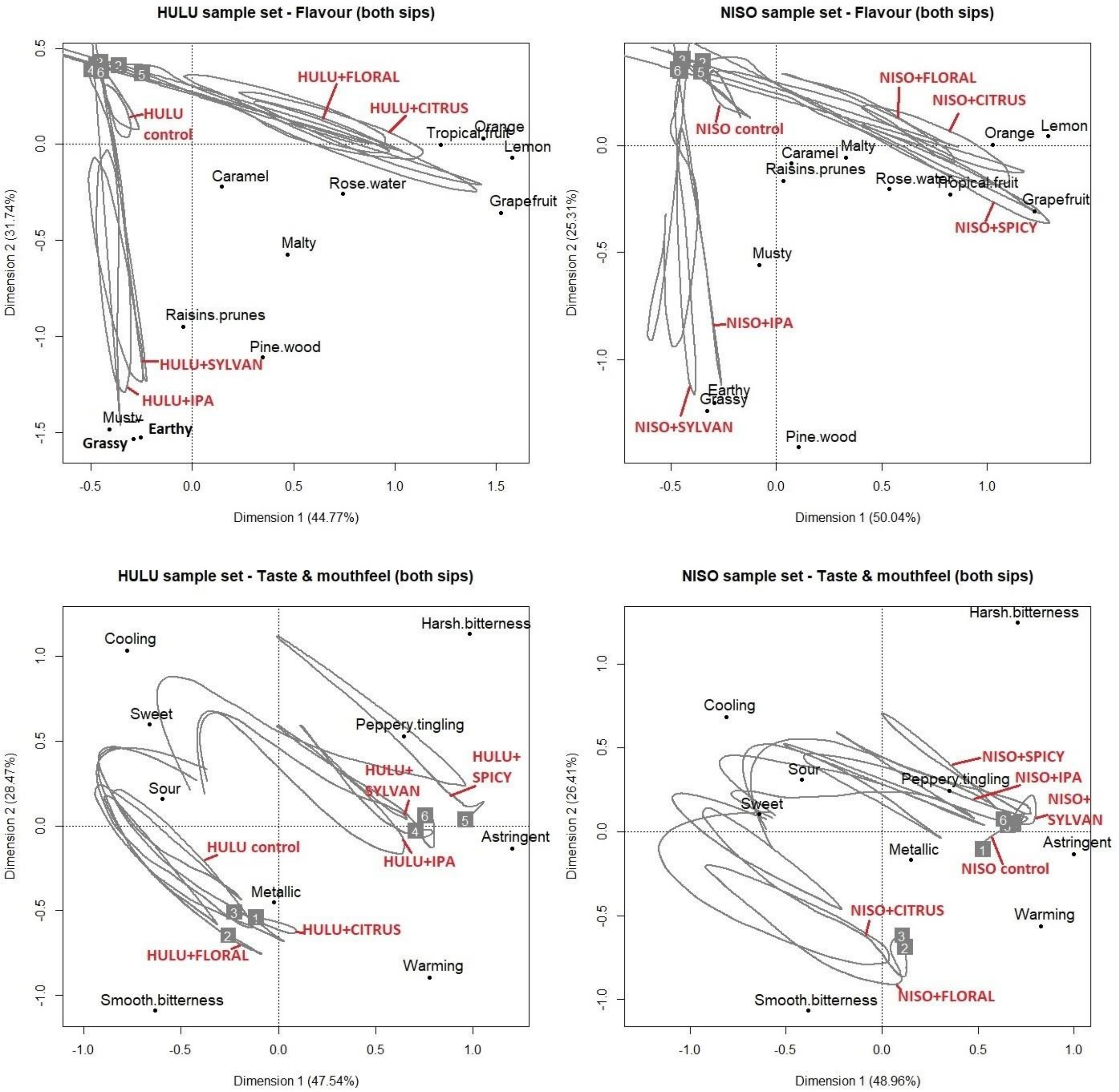

Fig. 7 Correspondence Analysis (CA) biplots of TCATA data of flavour or taste & mouthfeel attributes of the HULU and NISO sample sets, comprising of the control beers (1) and the five flavoured beers (CITRUS (2), FLORAL (3), IPA (4), SPICY1 (5), SYLVAN (6)) indicating the direction of samples in the flavour or taste & mouthfeel space. Sample trajectories are plotted for both sips. All sample trajectories start in the upper left quadrant and move along two clockwise loops following dimension 1 or counter-clockwise loops following dimension 2. The position of the samples at the end of the evaluation period is marked by numbers (1-6). Sample names are displayed in **red** and attributes are shown in **black**. As an example, after taking a sip of the HULU+IPA (4) or the HULU+SYLVAN (6) beer, the flavour trajectory starts in the upper left quadrant, moves to the "earthy", "grassy", and "musty" attributes upon swallowing and approaches the samples' starting point upon fading of the flavour sensations. After taking Sip2, the samples' trajectory again loops and moves towards the "earthy", "grassy", and "musty" attributes, then fades and returns to the starting point. The corresponding video clips showing the samples' trajectories moving in the plots can be found in the supplementary materials.