INVESTIGATING THE CONTRIBUTION OF HOP COMPONENTS TO THE PERCEPTION OF BEER FLAVOUR

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Abbreviations and symbols

2MB2MP	2-Methylbutyl 2-methylpropanoate
2MIB	2-Methylbutyl isobutyrate
3-AFC	3-Alternative forced-choice
3MH	3-Mercaptohexan-1-ol
3S4MP	3-Sulfanyl-4-methylpentan-1-ol
3S4MPA	3-Sulfanyl-4-methylpentyl acetate
4MMP	4-Mercapto-4-methylpentan-2-one
ABV	Alcohol by volume
AEDA	Aroma extract dilution analysis
ANOVA	Analysis of variance
ASBC	American Society of Brewing Chemists
[C]	Compound
CA	Correspondence analysis
CAR	Caryophyllene oxide enriched fraction
CATA	Check-all-that-apply
CHARM	Combined hedonic aroma response method
CVA	Canonical variate analysis
DMS	Dimethyl sulfide
EBC	European Brewery Convention
EST	Ester enriched fraction
FD	Dilution factor
FG	Final gravity
FID	Flame ionisation detector
fin	Beer finish
FU	Flavour unit
FPD	Flame photometric detection
GC-GOOD	Gas chromatography-Global olfactometry omission detection
GC-MS	Gas chromatography-Mass spectrometry
GC-O	Gas chromatography-Olfactometry
GC-R	Gas chromatography-Recomposition
GCxGC	Multidimensional/two-dimensional gas chromatography
GER	Geraniol enriched fraction
HRGC-O	High resolution gas chromatography-Olfactometry
HSD	Honestly significant difference
HULU	Bittering product containing hulupones
HUM	Humulol enriched fraction
HUM EPOX	Humulene epoxides enriched fractions
IBU	International bitterness unit
ICBS	International Centre for Brewing Science
im	In mouth
ISO	Bittering product containing commercially isomerised iso-alpha-acids
ISTD	Internal standard
K_{aw}	Air-water partition coefficient
KET	Ketone fraction
LIN	Linalool enriched fraction
log*P*	Logarithm of the octanol-water partition coefficient
MANOVA	Multivariate analysis of variance
MB	Multi-block
MD	Multidimensional
MFA	Multiple factor analysis

MW	Molecular weight
MTA	Monoterpene alcohol enriched fraction
MYR	Myrcene fraction
NISO	Bittering product containing naturally isomerised iso-alpha-acids
NIST	National Institute of Standards and Technology
OASIS	Original aroma simultaneously input to the sniffing port
OAV	Odour activity value
OSME	Greek word for odour, οσμη
PAN	Peak area normalisation
PCA	Principal component analysis
PFPD	Pulsed flame photometric detector
PLSR	Partial least squares regression
ppm	Parts per million
ppb	Parts per billion
PU	Pasteurisation unit
QDA	Quantitative descriptive analysis
R^2	R squared; goodness-of-fit
repl	Replicate
RI	Retention index
RM	Repeated Measures
RMSE	Root mean square errors
RV	Regression vector
Sam	Sample
SBSE	Stir bar sorptive extraction
sc	Supercritical
SCD	Sulphur chemiluminescence detection
SDE	Sensory descriptive analysis
SIDA	Stable isotope dilution assay
SIM	Selected ion monitoring
SPME	Solid-phase microextraction
SQ	Sesquiterpene enriched fraction
SQA	Sesquiterpene alcohol enriched fraction
SRM	Standard reference method
sw	Swallowed
TA / TALC	Terpene alcohol enriched fraction
TCATA	Temporal Check-all-that-apply
TDS	Temporal dominance of sensations
TI	Time intensity
TIC	Total ion chromatogram
time std.	Time standardised
Tmax	Time to maximum intensity
TO	Total oil
TOFMS	Time-of-flight mass spectrometry
TPC	Total polyphenol content
tr	Concentration at trace level
VIP	Variable importance in projection
VOC	Volatile organic compounds
Δ	Difference
[]	Dimensionless

Abstract

Considering the substantial amount of research that has been published in the field of hop science during the last decades, very little is known with regard to the multi-modal flavour perception of hop-derived volatiles that not only contribute to the pleasant 'hoppy' aroma and flavour, but are also involved in other sensations of gustatory and trigeminal origin perceived in beer. The aim of this research was to further understand the sensory complexity of Magnum hop essential oil and $scCO_2$ hop oil fractions extracted therefrom. This PhD project combined static and dynamic sensory techniques, an established gas chromatographic method, and comprehensive statistical analyses to investigate the relationship between hop volatile compounds and their sensory characteristics (quantitative and qualitative) in different matrices.

The olfactory, gustatory and trigeminal differences between five hop oil fractions representing the main chemical classes of Magnum hop oil were determined in a simple model solution (4% ABV) using a newly established attribute lexicon and following a Quantitative Descriptive Analysis (QDA) approach. The fractions induced a range of different aroma and flavour sensations, which could partly be attributed to specific hop aroma compounds. The most polar compounds in the terpene alcohol fraction were suggested to be responsible for cross-modal interactions eliciting both aroma and/or taste and trigeminal sensations. A peppery tingling mouthfeel was perceived, which is assumed to be a sensation innervated by the trigeminal nerve. The terpene alcohol fraction was further categorised into monoterpene alcohols (i.a.

geraniol, linalool) assumed to be mainly responsible for olfactory sensations and sesquiterpene alcohols (i.a. humulol, humulenol II) to foremost induce gustatory and tactile sensations.

Further fractionation specifically targeting single compounds and compound groups (sub-fractions) that were added to a commercial lager beer base (4.5% ABV) to measure the impact of perceptual interactions between compounds and the beer matrix using a revised attribute lexicon and adjusted dosage rates. A clear cause-effect-relationship could be located between geraniol and the sweet taste perceived in the beer. Geraniol also induced a smooth bitterness, which was opposed by the harsh bitterness quality added by sesquiterpene hydrocarbons. Linalool was classified as a aroma/flavour 'enhancer' rather than individually contributing to the sensory profile. Significant effects on lingering mouthfeel sensations remained absent, which illustrated the need for temporal sensory assessments to adequately and holistically discriminate the samples with regard to these sensations.

A Temporal Check-All-That-Apply (TCATA) by modality approach was used to assess multiple sensory characteristics of selected hop flavour products perceived simultaneously. The products contained the previously studied hop oil fractions and were combined with either iso-alpha-acids or oxidised beta acids (hulupones) in a lager base beer brewed without any hop materials. Bitter acid extracts were found to significantly affect the duration and sensory profiles of the hop flavour products in the beer suggesting a sensory interaction induced by the co-occurrence of hop aroma compounds and hop bitter stimuli. Lingering sensations (peppery tingling, astringency) were foremost found to significantly discriminate between the samples

at the end of the evaluation period (>2min). Since temporal sensory data is inherently noisy, a part of this research included the examination of TCATA data pre-processing approaches using comprehensive statistical analyses. This revealed that time standardising the TCATA by modality data could not remove inter- and intra-individual variation between the panellists and thus, not improved the quality of the sensory data.

This research has provided new and in-depth knowledge on the sensory properties of $scCO_2$ hop oil fractions, sub-fractions, and key compounds extracted from Magnum hop. Moreover, different sensory characterisation strategies and tools are presented that captured the fine nuances of the sensory profiles of these hop extracts. The findings demonstrated the involvement of hop volatile compounds in sensory interaction effects causing multi-modal profiles in beer. Their ability to modify gustatory and trigeminal sensations should be considered for future developments of flavour preparations.

Preface

"*No beer without hops. Only from hops beer is made to the characteristic beverage for which reason it is popular and desired all over the world: aromatic, of agreeable bitterness, foaming and not at least of beneficial quality.*" (Maier, 1994; p. XIV). The sensory perception of beer flavour plays a key role in determining consumer acceptance and preferences of beer. Flavour is a multi-modal experience and its perception is caused by an interplay between sensations. This includes olfactory, gustatory, and chemesthetic sensations perceived during consumption (Lawless & Heymann, 2010).

The investigation of sensory interactions as a result of simultaneous perception of two or more stimuli (i.e. hop-derived volatiles and further compounds) has largely been neglected in the field of hop flavour research, despite Meilgaard (1975a) identifying it as an important area four decades ago "*Two factors are of main interest in the study of a beverage such as beer: the contribution made by each individual component to the overall flavor, and the degree of interaction observable between compounds.*" (Meilgaard (1975a); p. 111).

Over the last decade, the demand for craft beer providing variety, uniqueness, and aromatic profiles has increased in many parts of the world (Carvalho, Minim, Nascimento, de Castro Ferreira, & Minim, 2018), thus increasing the demand for a wide variety of hops in large quantities. Hops are therefore a global commodity subject to global forces such as global warming and rapid population growth. In the long term, hops will compete for land with other crops resulting in the need for more

efficient and less wasteful hop materials, and more sustainable hopping practices (Biendl et al., 2015).

Hallertauer Magnum hop has been grown for centuries and was the first German-bred 'high alpha variety' (registered in 1992). Magnum hops not only have a high alpha content (11-16% w/w) (Krofta, 2003) but also contain 1.9-3.0 mL/100g of hop essential oil comprising a broad range of aroma-active compounds (Gonçalves, Figueira, Rodrigues, & Câmara, 2012). It is one of the most widely grown hop cultivars in the world, particularly in the US and in Germany, and used for the production of diverse beer styles (i.a. IPA, dark ale, pilsner, lager, Hefeweizen, stouts) (Biendl et al., 2015). The plant is well-known for its fast growth, high harvest yields, and being resistant to plant diseases (downy mildew, fungal) and changing climatic conditions (Pfeiffer, 2016). Therefore, this PhD research focused on the volatile fractions of Magnum hops for the purpose of contributing to the understanding of their sensory potential as a sustainable source of natural hop flavour.

Furthermore, this project was developed in collaboration with Totally Natural Solutions (Ltd.) who have the capability to separate or fractionate hop extracts into distinct aroma, flavour, and bitter products using organic solvents (liquid/supercritical CO_2, ethanol) to replace volatile organic compounds (VOC) by applying high vacuum and low temperatures to achieve greater retention of more volatile compounds following a patented fractionation technique described by Marriott (2019). The result is a compact, authentic and clean-label hop material.

The aim of this PhD research was to sensorially characterise Magnum hop essential oil and its fractions.

Thesis structure

Research from this thesis has been either published in peer reviewed journals or submitted for consideration (except Chapter 2B) and is therefore presented as a series of manuscripts. **Chapter 1** provides a comprehensive summary of the literature investigating the relationship between sensory and chemical characteristics of hop essential oil. Moreover, an overview of factors is given that affect the perception of hop-derived volatiles, but also their effect on other sensory characteristics such as taste and mouthfeel sensations that all contribute to the sensory profile of beer. The review was published in *the Journal of the Institute of Brewing* in July 2020 and is cited as Dietz, Cook, Huismann, Wilson, and Ford (2020a).

The research study described in **Chapter 2A** represents the first systematic attempt to assess the sensory and physico-chemical characteristics of Magnum hop essential oil fractions in comparison to the total Magnum oil. The total oil and the fractions were sensorially evaluated by a trained panel using a newly established comprehensive attribute lexicon and a Quantitative Descriptive Analysis (QDA) approach with the total oil and the fractions applied at equi-concentration in a simple model solution (4% ethanol) aiming to limit matrix-dependent effects and obtain a general understanding of their multi-modal profiles (olfactory/gustatory/trigeminal). Gas chromatography-mass spectrometry (GC-MS) was employed as a rapid profiling technique for untargeted analysis of the volatile composition of the total oil and the fractions. The second aim of this study was to statistically explain the relationship between sensory characteristics and the compounds detected. This chapter was published as a research paper in *the Journal of the Institute of Brewing* in April 2020

and is cited as Dietz, Cook, Wilson, Marriott, and Ford (2020b). **Chapter 2B** complements Chapter 2A as it compared the volatile composition of the fresh total oil and hop oil fractions with aliquots of the same batch that have been stored for 26 months at -20^{o}C using GC-MS analysis to assess their chemical stability and suitability for long-term sensory trials.

Hypotheses that could be drawn from Chapter 2A built the basis of the research described in **Chapter 3**, which focused on the sensory characterisation of selected fractions (terpene alcohols, sesquiterpenes) and sub-fractions and single molecules extracted therefrom to investigate different compound groups that were suggested to either induce aroma and flavour or in the result of sensory interactions potentiating the perception of taste-, mouthfeel- or trigeminal-type sensations. The experimental design followed that of the preceding study, but the hop extracts were applied in a commercial lager base beer at equi-flavour-intensity to explore their sensory impact on the beer matrix. This chapter was published as a research paper in *Food Research International* in July 2021 and is cited as Dietz, Cook, Wilson, Oliveira, and Ford (2021a).

Chapter 4 presents the third sensory study, which addressed the evaluation of lingering taste, mouthfeel, and trigeminal-type sensations that were detected in the preceding studies for certain hop oil fractions, but not clearly discriminated between the samples i.e. only approached significant effects. It was assumed that this was due to the static QDA approach limiting the assessment of persistent sensations to one time point whilst peak intensities for these sensations could have varied. Therefore, the first aim of this study was to develop a protocol for a TCATA by modality approach

for the temporal sensory evaluation of volatile hop extracts throughout a defined period of time and two sips. The second study aim was the comparison of the qualitative characteristics of different hop bitter acids (commercially and synthetically-derived iso-alpha-acids and hulupones) in an unhopped lager style beer brewed on-site, and the study of the beers' temporal sensory perception as a result of different combinations of hop bitter acids and hop flavour extracts. This chapter was published as a research paper in *Food Quality and Preference* in November 2022 and is cited as Dietz, Cook, Yang, Wilson, and Ford (2022).

Chapter 5 complements Chapter 4 and examined the effect of time standardisation of the TCATA by modality data (1. by modality, 2. with merged modalities) on the temporal sensory profiles of the beers. Time standardisation was conducted to achieve alignment of onsets of sensations and decrease variation in durations among panellists. The time standardised datasets were compared with the 'raw' data by using comprehensive statistical analyses and advantages and disadvantages of time standardising multi-modal data are discussed. This chapter was published as a research paper in *Food Quality and Preference* in December 2022 and is cited as Dietz, Yang, and Ford (2021b).

Finally, **Chapter 6** summarises the main findings and conclusions from all experiments and suggests directions for future research.

Chapter 1

1 Introduction: The multisensory perception of hop essential oil

A review of literature to 2021

This chapter is based on:

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The multisensory perception of hop essential oil

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Keywords: Hop essential oil; hop oil fractions; multimodal perception; sensory interaction effects; bitterness; trigeminal sensations

Highlights

- This review discusses studies investigating the relationship between sensory and chemical characteristics of hop-derived volatiles.
- An overview of volatiles that have been identified to not only contribute to the aroma and flavour of beer but also induce taste, mouthfeel and somatosensory or trigeminal sensations is provided.
- The importance of sensory interactions between hop oil volatiles and other components in the beer matrix is emphasised since, to date, these have hardly been discussed.
- Latest advances in the sensory evaluation of hop essential oil and hop oil fractions are illustrated and research gaps are highlighted from a sensory science perspective.

Abstract

Hops are a key ingredient to add bitterness, aroma and flavour to beer, one of the most consumed beverages worldwide. Essential oils from different hop varieties are characterised by similar classes of chemical compounds and complexity, but their contribution to sensory characteristics in beer differs considerably. Volatiles in hop oil are categorised into several chemical classes. These induce diverse aroma and flavour sensations in beer being described as 'floral', 'fruity' (e.g. contributed by alcohols, esters, sulphur-containing compounds), 'spicy', 'woody', 'herbal' (sesquiterpenes, oxygenated sesquiterpenoids), and 'green' (aldehydes). The perception of hop volatiles depends on their concentrations and combinations, but also on threshold levels in different beer matrices or model systems. Several studies attributed modified taste and mouthfeel sensations to the presence of hop volatiles contributing to a multisensory perception of hop flavour. Linalool is frequently observed to show additive and synergistic-type behaviour and to affect aroma perception if combined with geraniol. Linalool has also been found to be involved in aroma taste interactions, modifying the perception of bitterness qualities in beer. Particularly oxygenated sesquiterpenoids are suggested to be responsible for an irritating, tingling sensation indicating the activation of trigeminal receptors. The majority of these sensory interactions have been discovered almost by accident and a systematic research approach is required to gain a broad understanding of these complex phenomena. This review provides an overview of factors affecting the perception of hop derived volatiles involved in different sensory characteristics of beer, while illustrating the latest advances and highlighting research gaps from a sensory science perspective.

1.1 Introduction

Hops contain both volatile and non-volatile fractions that contribute to the sensory quality of beer. While volatile compounds in hop essential oil add aroma and flavour, non-volatile components such as carboxylic acids, hop resins, amino acids, carbohydrates, and polyphenols are known to affect the taste and mouthfeel characteristics of the final beer product (Rettberg, Biendl, & Garbe, 2018; Ting & Ryder, 2017). After more than half a century of research in hop flavour chemistry, it is commonly agreed that the overall sensory sensation that is experienced when drinking beer, is not a sum of individual sensations (Palamand, 1969). Meilgaard (1975a) already hypothesised in 1975 that approximately half of the flavour intensity in beer can be attributed to sensory interactions between the volatile and the non-volatile fractions.

Hop oil is one of the most complex essential oils known in plants (King & Dickinson, 2003). To date, approximately 200 studies have been published investigating the composition of hop oil. Since the early 1960s the number of identified volatile compounds in hop oils has increased steadily. Based on the number of peaks that are reported in studies using advanced chromatographic techniques and taking into account that there is still a need for more sensitive methods in order to capture compounds at trace levels, it is thought that more than 1000 volatile compounds are present in hop oil (Eyres & Dufour, 2009).

Beer contains many hop derived volatiles at subthreshold-level, nevertheless, these are expected to contribute to the overall aroma and flavour profile depending on the co-presence of other volatile compounds and components in the beer, such as

bittering substances, ethanol and carbon dioxide (Brown, Clapperton, Meilgaard, & Moll, 1978; Buttery, 1999; Hanke et al., 2010; Jahnsen, 1963; Lam, Nickerson, & Deinzer, 1986; Roberts, Dufour, & Lewis, 2004; Tokita, Takazumi, Oshima, & Shigyo, 2014). Therefore, one of the factors that complicate the understanding of 'hoppy' aroma and flavour is the occurrence of sensory interactions between hop oil compounds as well as between volatile and non-volatile beer components. Interactions occur at specific compound combinations, ratios, and below and above certain sensory threshold concentrations, particularly in heterogeneous mixtures. The types of interactions between hop oil compounds have been described as synergistic, antagonistic, additive or masking (Moir, 1994). Many attempts have been made to exploit the sensory potential of hop oil compounds, but little attention has been paid to the role of sensory interactions between hop volatiles.

To date, reviews published in the area of hops have focused on the chemical composition of hop oil, the transfer of hop derived volatiles into the final beer as a result of different hopping techniques, hop oil analysis techniques and the odour characteristics of single hop oil compounds (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014; Eyres & Dufour, 2009; Sharpe & Laws, 1981; Van Opstaele et al., 2006). Recently, Rettberg et al. (2018) published a comprehensive review examining the current status of methodology used in hop research including isolation, separation, detection, identification, and quantification techniques for the investigation of volatile compounds in hop material and in beer. All reviews briefly summarise the contribution of hop oil compounds to the aroma and flavour profile in beer, however sensory characteristics such as somatosensory sensations are not discussed. Sensory

interactions between hop oil volatiles and components of the beer matrix, resulting for instance in modified flavour intensities and qualities, have largely been neglected. Being aware of the source of these effects facilitates not only the assessment of the actual sensory potential but also of the targeted application of advanced and complex hop oil products in beverages.

This review aims to outline the current state of scientific knowledge by examining the sensory impact of volatile compounds of hop oil in beer and model matrices often used in this research field. Interactions between hop oil compounds and other beer components (ethanol, carbonation, hop acids) and the resulting effects on the sensory profile of the final beer remain to be elucidated. Moreover, research gaps from a sensory perspective are highlighted and future research is proposed.

1.2 Factors determining the hop oil composition in hops and beer

On average dried hop cones contain between 0.5-3% of hop oil comprising of aroma and flavour-active compounds that belong to several chemical classes such as terpenes, alcohols, esters, aldehydes, and ketones. Both quantity and composition of hop essential oil are largely dependent on genetic factors, hop plant or rootstock age, growing conditions including soil, pH, carbon, nitrogen and moisture content, microbial mass, etc., but also on climatic conditions (temperature, humidity, sunshine hours), and time of harvest (Matsui, Inui, Ishimaru, & Yonezawa, 2013; Rodolfi et al., 2019; Van Holle, Van Landschoot, Roldán-Ruiz, Naudts, & De Keukeleire, 2017). In addition, the quantity of essential oil and the proportion of individual fractions varies across hop varieties (Eyres & Dufour, 2009). For example, the amount and

composition of oxides, epoxides and alcohols in the sesquiterpene fraction differ markedly between hop varieties, with Hallertauer Mittelfrueh and Hersbrucker hops comprising of a large proportion of oxygenated or sesquiterpene derivates compared to other varieties (Gardner, 1994; Moir, 1994; Sanchez, Lederer, Nickerson, Libbey, & McDaniel, 1992). The concentrations of single compounds in specific hop oil fractions also differ across hop varieties and geraniol is a prime example being a varietal specific compound that cannot be found in every hop variety at detectable concentrations (Steinhaus, Wilhelm, & Schieberle, 2007; Van Opstaele, Goiris, De Rouck, Aerts, & De Cooman, 2012).

Depending on the brewing process and hopping technique, physical, chemical and biochemical changes take place in the volatile fractions of hops that have been found to impact flavour perception. Traditional hopping techniques are kettle, late, and dry hopping. For kettle hopping, the hops are added during wort boiling to ensure that hop α-acids are isomerised to iso-α-acids, which are mainly responsible for the bitterness character of beer. However, up to 85% of the hop oil compounds, particularly hydrocarbons including the most abundant terpene hydrocarbon myrcene and the sesquiterpenes humulene, and caryophyllene, are suggested to be evaporated from the kettle, discarded with the spent hops, lost during wort filtration or fermentation, or transformed to oxygenated terpenes and sesquiterpenes when applying this hopping technique. Oxidation products are more likely to survive the brewing process due to their water solubility (Dresel et al., 2013). Apart from some hydrophilic hop volatiles, the majority of hop oil compounds are not found in the wort in their native form and only few are found unchanged in the beer. The degree of

hydrolysis and biotransformation of compounds depends on several factors and matrix effects, including contact time, temperature, pH, and exposure to yeast making it difficult to predict the final volatile composition in beer (Sharp, Qian, Clawson, & Shellhammer, 2017; Takoi et al., 2014).

Evaporation of hop volatiles can be limited when hops are added towards the end of the boiling process by applying a late hopping technique. The reduced thermal exposure favours the retention of polar oxygenated compounds, terpene derivatives, free alcohols, carbonyls, ketones, and cyclic esters (Kishimoto, Wanikawa, Kono, & Shibata, 2006; Peacock, 2010). However, the later the addition of hops, the lower the conversion of α-acids to iso-α-acids. Consequently, the intensity of bitterness in the beer decreases and the bitterness quality is modified. The addition of hops to the fermentation vessel or after fermentation during lagering and before filtration or centrifugation, is described as dry hopping (Van Opstaele, Goiris, De Rouck, Aerts, & De Cooman, 2013). In the latter case, the hops are added to the stored cold beer. The final beer contains unmodified hop oil compounds including some hydrocarbons. If added to primary or secondary fermentation, yeasts can still convert hop derived compounds. Lager and ale yeasts have also been found to transform geraniol into β-citronellol or linalool and nerol into α-terpineol via yeast metabolism (King & Dickinson, 2003). In addition to transformation reactions, yeasts may adsorb hop oil compounds as observed for several monoterpene alcohols (linalool, geraniol) (Takoi, Itoga, et al., 2010; Takoi, Koie, et al., 2010). However, it should not be forgotten that yeast strains can also induce de novo synthesis of monoterpene alcohols. This has

been found for instance for geraniol and linalool, and to a lesser extent for *β*-citronellol, *α*-terpineol, and nerol (Korbinian Haslbeck et al., 2018).

Overall, it is still not clear which hop volatiles are directly transferred to the beer without undergoing any biochemical transformations by yeast such as esterification or enzymatic cleavage. A comprehensive review on the molecular biology of fruity and floral volatiles (higher alcohols, esters) derived from hops or formed by yeast during the fermentation process has recently been published by Holt, Miks, de Carvalho, Foulquié-Moreno, and Thevelein (2018).

1.3 Cross-modal and multisensory interactions

Before defining the impact of volatiles in hop essential oil on specific sensory characteristics of beer and their role in cross-modal and multisensory interactions, it is necessary to understand the basic sensory sensations known to be involved. **Odour or aroma** sensations are perceived when orthonasally smelling the volatile fraction of beer prior to consumption. Hop derived volatiles are detected by the olfactory system, which comprises around 390 odourant receptor proteins located in the human nose (Oh, Lee, Ko, Lim, & Park, 2015). Volatile compounds reach the olfactory epithelium via the orthonasal (via nostril) or the retronasal pathway (via nasopharynx) while the orthonasal pathway is exclusively related to aroma sensations. Volatiles that are delivered through the retronasal pathway are part of flavour sensations (Negoias, Visschers, Boelrijk, & Hummel, 2008). **Flavour** sensations perceived when drinking beer are a combination of retronasally delivered aroma together with in-mouth sensations including taste, mouthfeel and trigeminal

sensations (Auvray & Spence, 2008). **Taste** sensations include the perception of bitterness, sweetness, sourness, saltiness, umami, and a number of potential other tastes such as fatty (Liu, Shah, Croasdell, & Gilbertson, 2011) and metallic (Lawless et al., 2004) that are not fully understood. If using nose-clips, it is possible to split the taste and mouthfeel of a beer from its aroma sensations, thereby limiting the perception of flavour (Chandrashekar, Hoon, Ryba, & Zuker, 2006; Taylor & Roberts, 2008).

Trigeminal stimuli are those that can induce a sensation of irritation (spicy, pungent), pain, or temperature (cooling, warming). High carbonation levels in beer are perceived as a sparkling, tingly, slightly irritating sensation in the oral cavity induced by bursting CO_2 bubbles on the tongue. The bursting bubbles activate the mechanoreceptors in the mouth and, at the same time, the CO_2 is converted to carbonic acid, which induces the tingling response (Dessirier, Simons, Carstens, O'Mahony, & Carstens, 2000). Moreover, carbonation has also been found to impact flavour perception in beer (Clark, Linforth, Bealin-Kelly, & Hort, 2011). **Mouthfeel** characters are considered as the tactile perception of stimuli such as hop derived polyphenols, which are known to induce astringency in beer. Astringency is driven by inhibited lubrication in the oral cavity and is described as a drying, roughing, and puckering sensation (Bajec & Pickering, 2008; Niimi, Liu, & Bastian, 2017).

Hop oil compounds might activate more than one sensory modality or cause interactions such as 'cross-modal interactions', thereby contributing to the multisensory perception of beer. The modulation of one sensation by the perception of another is not easily examined due to the fact that effects of cross-modal

interactions can be the result of different mechanisms (physico-chemical, psychological, physiological) and occur at different levels (cognitive, receptor, neural) (Guichard, Salles, Morzel, & Le Bon, 2016; Meilgaard, 1982; Niimi et al., 2017). In case of physico-chemical mechanisms, non-volatile fractions in the beer matrix affect the partitioning of volatiles, their molar concentration, water activity coefficient, or diffusion through the beer matrix. These factors impact on the physical release and concentration of volatiles in the headspace, which in turn can have a major effect on the perceived intensity and quality of aroma-active compounds. Physiological mechanisms which influence flavour release and perception include food/beverage matrix breakdown in the mouth, saliva composition, saliva production and flow, temperature, and swallowing behaviour. These factors, for instance affect the time point of volatile release and delivery through the retronasal pathway (Poinot, Arvisenet, Ledauphin, Gaillard, & Prost, 2013).

Sensory or cross-modal interactions can cause additive (increasing), synergistic (enhancing/potentiating), antagonistic (suppressing, masking) or eliminating (cancelling/extinguishing) effects (Poinot, Arvisenet, Ledauphin, Gaillard, & Prost, 2013). Figure 1.1**.** illustrates these effects and clarifies the difference between additive and synergistic mechanisms, which are often confused. Synergistic mechanisms are the result of sensory sensations delivering a greater response than the sum of individual compound effects (Meilgaard, 1982). Sensory interaction effects might even result in the perception of a novel sensory sensation, known as 'configural processing' i.e. two compounds that would separately induce a similar (or a different) aroma give a completely new aroma sensation if mixed together

(Guichard et al., 2016). It should also be taken into account that volatiles and non-volatiles not only have a threshold concentration for the detection or recognition of aroma, flavour, taste, or mouthfeel but also have an interaction threshold describing a concentration or combination range at which the sensory interactions occur (Figure 1.1.).

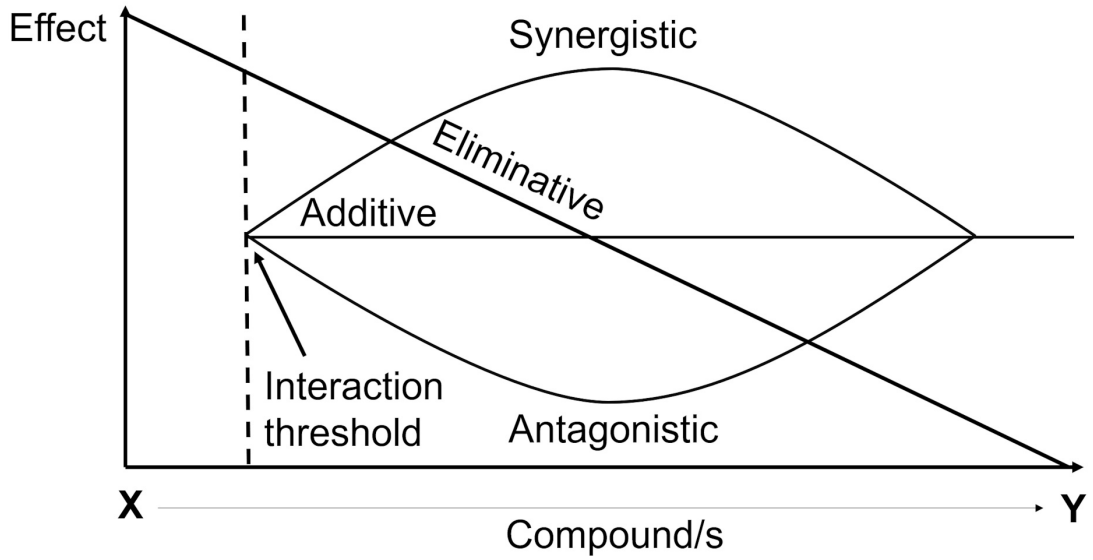

Figure 1.1. Graphical illustration of sensory interaction effects induced by the combination of two or more compounds causing a modification of sensory characteristics. (based on Guichard et al. (2016) and Langeveld, Veldhuizen, and Burt (2014))

Meilgaard (1982) was the first to address sensory interactions between flavour constituents in beer and calculated the degree of interaction based on the assessed flavour intensity of a mixture and the sum of flavour intensities of all volatile compounds present. By comparing the factor to a weak or unflavoured (null) beer, a conclusion regarding the type of interaction could be drawn. The weaker the base flavour in the null beer, the more likely it is that volatiles of interest are not masked, and that sub- and supra-threshold interactions can be identified (Biendl et al., 2015). In general, it should be considered that both threshold concentrations and sensory characteristics of volatile and non-volatile compounds differ between studies if non-

identical test matrices have been used since compositional differences can potentially affect the perception of single compounds. Ideally, the concentration of a compound in a matrix and its threshold concentrations of interest (i.e. aroma, flavour, taste or mouthfeel threshold concentrations) in the same test matrix (e.g. water or beer) are known if aiming to determine the contribution of a compound to the sensory profile.

The scheme in Figure 1.2**.** illustrates an example of the complexity of a sensory profile for a test matrix containing a range of compounds (A-L) that contribute in different ways to different sensory characteristics. Each of the compounds can be present at a different concentration range. In addition, each of the compounds has a threshold concentration range at which they are sensorially detected and add one or more sensory characteristics to the matrix. It should be noted that the threshold concentration range does not include subthreshold concentrations that might be important in view of sensory interactions such as additive or synergistic type behaviours. It is likely that one compound is involved in more than a single sensory sensation and for instance contributes to a flavour and a mouthfeel sensation. Whether single sensations or sensory interactions in a matrix with complex volatile mixtures occur and whether these take place at sub- or at supra-threshold level can only be explored by excluding the compound of interest or by varying its concentration while keeping the concentrations of the other compounds in the volatile mixture unchanged.

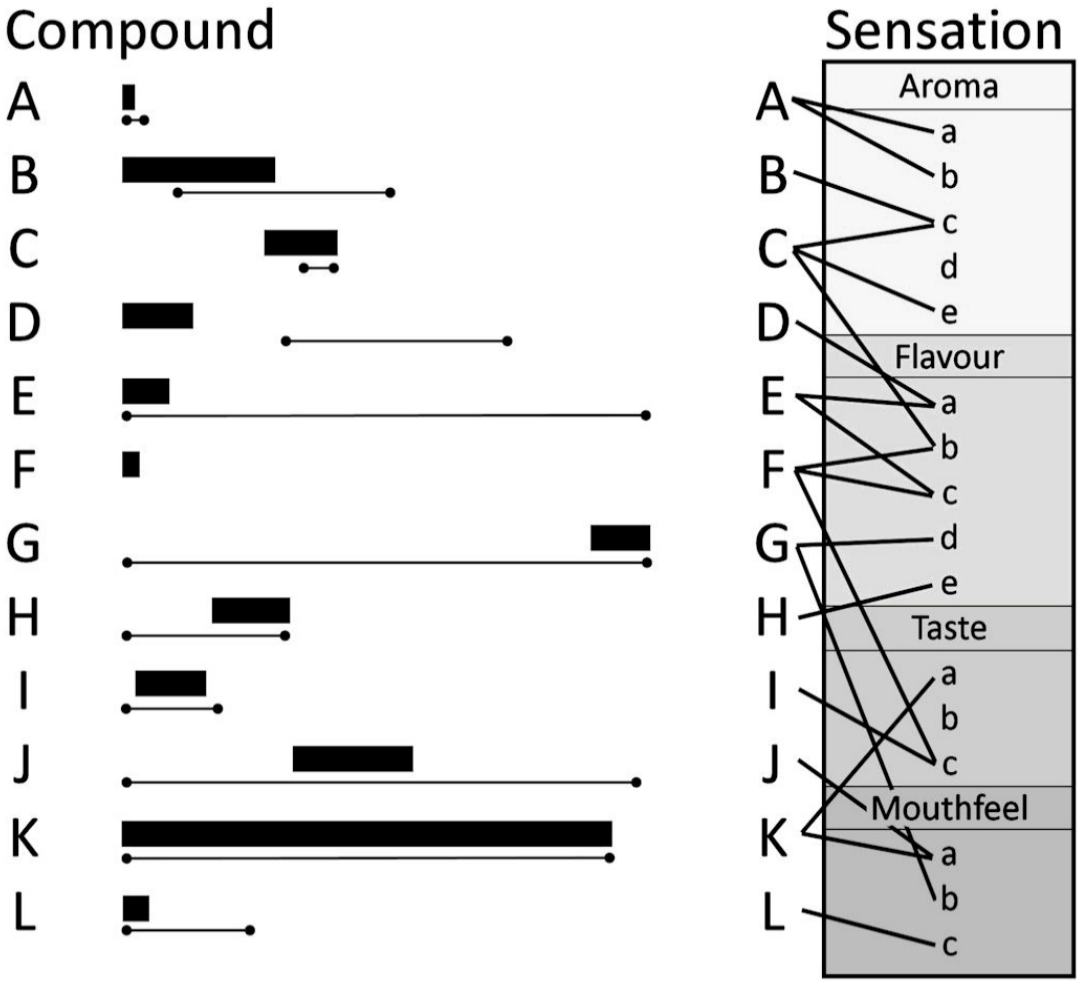

Figure 1.2. Exemplary illustration of the relationship between sensory sensations and chemical substances (based on Siebert (1994)). Volatile and non-volatile compounds (A-L) are detected at different concentrations and have different threshold concentrations at which their effect on aroma, flavour, taste, and mouthfeel sensations can be perceived. Some of the compounds are contributing to more than one sensory sensation. ▬ Concentration range detected in beer; •–• threshold concentration range

1.4 Sensory characterisation of single hop volatiles and compound mixtures in hop essential oil

1.4.1 Combining sensory with instrumental techniques

Different sensory and instrumental techniques can be combined in order to evaluate the relative flavour importance of beer constituents. Peacock, Deinzer, McGill, and

Wrolstad (1980) were one of the first research groups to combine sensory evaluation using triangle tests with instrumental analysis by gas chromatography-mass spectrometry (GC-MS). The determination of threshold concentrations of volatiles has been proven to be challenging since concentrations can vary by a factor of more than 100 across different sensory panels and by a factor up to 100,000 between individual assessors (Stevens, Cain, & Burke, 1988). This is mainly due to individuals' genetics and physical conditions determining the sensitivity variation among assessors (Meilgaard, 1991). In addition, experience or exposure plays an important role. Threshold concentrations can change after a certain number of exposures due to training effects (Hughes, 2008). Another factor that limits the approach of Peacock et al. (1980) is the fact that several volatiles present at subthreshold level may still play an important role in the perception of hop aromas and flavours in beer. In addition, several volatiles are likely to remain undetected if hop oil is exclusively analysed using basic chromatographic techniques. This is particularly the case for low level sulphur compounds (Eyres & Dufour, 2009).

A second approach to investigate the relationship between the chemical composition of hop oil and its sensory characteristics is to couple GC-MS analysis with olfactometry (GC-O) based techniques (e.g. aroma extract dilution analysis (AEDA), combined hedonic aroma response measurement (CHARM), OSME (focusing only on one concentration of an extract; named after the Greek word for odour, οσμη)). In this way, hop volatiles can be separated, located, identified, quantified and sensorially characterised in isolation (Kishimoto et al., 2006). GC-O analysis is used to identify the aroma-active compounds from the bulk of non-active compounds as

these are suggested to remain undetected by the human olfactory system (Fritsch & Schieberle, 2005). AEDA is one of the most frequently applied dilution methods used to determine the highest sample dilution factor at which an odour of a volatile compound is still detectable. A limitation of this method is that it can lead researchers to focus only on the most odour-active volatiles in hops or beer (Fritsch & Schieberle, 2005; Steinhaus et al., 2007) and thereby ignore the potential for sensory interactions involving compounds present at lower flavour potencies. It is now well established that these could significantly contribute to sensory characteristics, for instance due to additive- or synergistic-type behaviours. In addition, sample preparation techniques (distillation, concentration) for AEDA experiments have been found to cause volatile losses and consequently the underestimation of flavour contributions (Gijs, Chevance, Jerkovic, & Collin, 2002; Steinhaus & Schieberle, 2000). It is therefore recommended to use methods that are able to analyse the sensory potential of complex mixtures containing compounds that are contributing to the sensory volatile profile as such or as part of a compound group due to sensory interactions.

Another successful example of combining sensory with instrumental techniques is a study of Sanchez, Lederer, Nickerson, Libbey, and McDaniel (1992) who combined sensory descriptive analysis with GC-O OSME. Their study may be the first good example of adequate sensory work in hop flavour research including the correlation of sensory and compositional data. Moreover, the authors established a comprehensive attribute lexicon comprising of sensory attributes, their descriptions, and details of reference materials. GC-O OSME is a dynamic GC-O technique for which assessors are asked to continuously record the intensity and name the description of

aroma sensations that are perceived at the sniffing port (Da Silva, Lundahl, & McDaniel, 1994). In GC-O studies, assessors only receive aroma sensations of a single volatile compound at a time (subject to chromatographic separation), thus sensory interactions are neglected (Moir, 1994; Sandra & Verzele, 1975; Siebert, 1994). Therefore, Sanchez et al. (1992) trained sensory assessors who evaluated beer samples and subsequently a mixture of standards based on the hop volatile concentrations in the beers that were previously quantified using GC-MS. In this way, the authors could conclude on the volatile compounds present at varying concentrations that contributed to the sensory properties of the different test beers. This study demonstrates the importance of combining GC-O techniques with sensory descriptive analysis when examining the contribution of single volatiles in hop volatile mixtures to beer flavour.

Whenever interpreting GC-O/MS data, it should be taken into account that compounds can co-elute, particularly if the number of compounds present exceeds the resolving power of the chromatographic method. This is particularly difficult to identify when many trace odourants are present (Eyres & Dufour, 2009). Co-elution can lead to misinterpretation regarding volatile compounds and associated sensory sensations (Siebert, 1994). GC-MS is a frequently used method for the analysis of hop essential oils. At this time, it may be impossible to separate all hop oil components solely by one- or two-dimensional GC- analysis. This applies particularly to terpenes since their empirical chemical formulae are often identical and mass isomers may follow very similar fragmentation patterns (Anderson, Santos, Hildenbrand, & Schug, 2019). Advanced chromatographic techniques are therefore essential to obtain the

best possible outcome. Such approaches include GC-MS in single ion monitoring (SIM), multidimensional and high resolution GC (MDGC, HRGC) combined with time-of-flight MS (TOFMS), and the use of automated selective devices for enrichment of volatiles such as solid phase micro-extraction (SPME). In particular, headspace (HS) traps have been found to be a powerful tool for the gentle enrichment of volatiles from headspace systems prior to their quantification.

Misidentification can also occur if compounds have very similar mass spectra and if literature and libraries lack retention indices and reference mass spectra for compounds of interest. This has often been observed in hop oil analysis (Eyres, Marriott, & Dufour, 2007; Van Opstaele, Praet, Aerts, & De Cooman, 2013). In order to avoid misidentification, Van Opstaele, Praet, et al. (2013) suggested authentic reference compounds by chemical transformation to be used for the verification of analytical data and to include structure elucidation of compounds of interest by state-of-the-art spectroscopic techniques. Comprehensive reviews on the chemical analysis of hop essential oil have been published by Rettberg et al. (2018), Eyres and Dufour (2009), Plutowska and Wardencki (2008), and Andrés-Iglesias et al. (2015). The quantification of hop derived volatiles is important for the understanding of hop aroma and flavour, but a high compound concentration does not necessarily mean that it will be one of the main contributors to hoppy aromas and flavours in beer. Therefore, sensory panels are required to evaluate compound mixtures rather than single hop oil compounds and training should be designed to maximise their ability to do so.

1.4.2 Omission and reconstitution experiments for sensory analysis

Two decades ago, Siebert (1994) suggested that the effect of flavour-active hop compounds in beer can only be fully understood if fractionating a hoppy beer, i.e. extracting and analysing the volatile fractions that have been suggested to be responsible for the hoppy flavour, and then adding step-wise these fractions back to the beer for sensory descriptive analysis. Langos, Granvogl, and Schieberle (2013) and Intelmann et al. (2009) conducted so-called 'Sensomics' studies that followed the principle of this approach. In the first step, the volatile fraction is extracted and separated from the non-volatile fraction followed by localisation, identification, and quantification of the most aroma-, flavour-, or taste-active compounds. These are recombined at the concentrations present in the original product and evaluated using sensory descriptive analysis as well as methods considering time-dependent perception. In this way, it is possible to identify and quantify those compounds that are responsible for the overall sensory properties in the beer while determining those compounds that are playing a minor role, which may not change the overall beer flavour if, for instance, recipes or processing conditions are modified (Intelmann et al., 2009; Langos et al., 2013).

Goiris et al. (2002) fractionated hop oil to obtain fractions of decreasing numbers of compounds and to successively lower the complexity for subsequent sensory evaluation of these fractions in a beer base. Also, fractions derived from the extraction of different hop oils could be compared since these are usually expected to differ in view of volatile composition and concentration (Goiris et al., 2002). However, when fractionating hop oil, it should be considered that some extraction

techniques, such as steam distillation, can induce thermal or hydrolytic reactions in hop oil and thus change the oil composition. In particular, thermolabile compounds are easily decomposed, and therefore extraction techniques at low temperatures, such as solvent based supercritical fluid chromatography, are preferred (Aberl & Coelhan, 2012; Marriott, 2001). By using a solvent or solvent combination (liquid/supercritical CO_2, ethanol), and controlling temperature, pressure and flow rates for sequential extraction and fractionation, it is possible to separate a wide range of hop oil compounds for subsequent instrumental and sensory analysis (Marriott, 2019). However, to date, this type of approach has rarely been applied.

1.4.3 Temporal measurement of sensory perception

Sensory descriptive analysis has been proven to be a valuable tool to investigate the sensory profiles of hop oil compounds in different matrices or to identify aroma-related interactions if combined with instrumental measurements. However, this is a static descriptive method and can only provide a snapshot of sensory profiles. To date, temporal physico-chemical changes that the beer matrix undergoes during consumption are largely neglected. Time-intensity (TI) or temporal dominance of sensations (TDS) analysis are used to monitor the intensity of a single descriptor over time or to assess dominant attributes perceived during consumption (Poinot et al., 2013). Another method that can be used to assess the temporal perception of hop volatiles in beer is the Temporal Check-All-That-Apply (TCATA) method. For this method, the assessors are asked to continuously check the terms that describe the sensory sensations when they are perceived and uncheck them when they are no longer apparent, at each moment of the evaluation for a defined period. It has to be

taken into account that the data does not present the attributes that dominate the sensory profile but only when they are apparent and then fade (Hort, Kemp, & Hollowood, 2017). However, according to Ares et al. (2015), TCATA tends to be more discriminating across samples compared to TDS since more attributes are usually selected in the TCATA approach. This appears to be relevant for the sensory evaluation of hop oil extracts since these are complex flavour mixtures.

1.5 Sensory perception of hop derived volatiles and their combinations

Native hop oil consists of several chemical classes in different proportions and with different compositions depending on the hop variety. The three main classes in hop oil are hydrocarbons, oxygenated compounds and sulphur-containing compounds, which can be further sub-classified as illustrated in Figure 1.3.

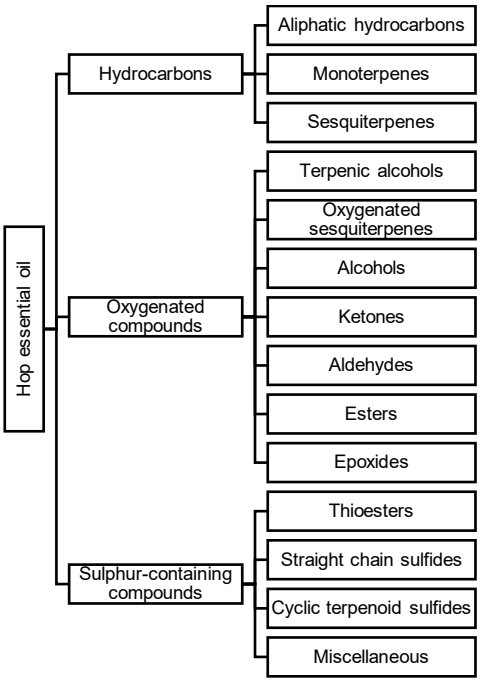

Figure 1.3. Main and sub-chemical classes in hop essential oil (based on Almaguer et al. (2014)).

The most abundant compounds in the hydrocarbon fraction, which can account for up to 80% of hop oil, are the monoterpene myrcene and the sesquiterpene humulene. These can account for up to 30-40% of their individual subclass. On average, 30-65% of the hop oil consists of oxygenated compounds comprising a complex mixture of oxygenated sesquiterpenoids, alcohols, aldehydes, acids, ketones, epoxides, and esters (Eyres et al., 2007; Goiris et al., 2002; Van Opstaele, Praet, et al., 2013). Sulphur-containing compounds are only present at trace or undetectable levels but are amongst the most flavour active naturally occurring substances. As previously mentioned, not all of the volatile compounds in hop oil can be found in the final beer (Dresel et al., 2013). The following sections summarise important findings that contribute to the understanding of different sensory sensations and interactions induced by hop derived compounds.

Table 8.1. (Appendix 1) provides an overview of hop oil and hop derived compounds that were investigated in publications using both sensory and quantitative instrumental analysis. Hop volatiles were quantified in beer and sensorially evaluated by sensory assessors (in the same study). Individual compounds were attributed to, or at least associated with, specific sensory sensations.

1.5.1 Terpene hydrocarbons

Monoterpene and sesquiterpene hydrocarbons account for the major portion of hop oil. However, when using traditional hopping techniques and in contrast to the oxygenated compounds, they are transferred to beer at trace levels due to their non-polar character and are therefore often suggested to only contribute to a minor extent to the hop aroma and flavour sensations in the final beer. Instead, they play an important role as precursor compounds that are transformed into oxidation products, thereby adding to 'noble hop' or 'kettle hop' aroma and flavour of beer (Naya & Kotake, 1972). For instance, it has been suggested that boiling *β*-myrcene in water in the presence of oxygen, might result in the formation of perillene, a compound that induces citrusy/lemony aroma notes (Dieckmann & Palamand, 1974; Sharpe & Laws, 1981; Van Opstaele, De Causmaecker, Aerts, & De Cooman, 2012), as well as to linalool and geraniol, two of the most impactful odourants derived from hop essential oil (Forster & Gahr, 2013).

When dry hopping beer, some compounds of the hydrocarbon fraction, including myrcene, humulene and caryophyllene, have been observed to survive the brewing process at reduced levels. Myrcene, the dominant monoterpene in the hydrocarbon

fraction accounts for up to 75% of total hop oil (Sharpe & Laws, 1981; Thompson, Marriott, Dowle, & Grogan, 2010). However, *β*- myrcene was found to be adsorbed to the non-polar surface of yeast cells or to be transported to the surface of the fermenting beer by carbon dioxide bubbles and stripped with the fermentation gases. Another cause for significant *β*-myrcene losses during fermentation are higher temperatures resulting in increased release of the compound (Haslbeck et al., 2017). However, since the threshold concentrations of hydrocarbons are usually low, they might still contribute to the aroma and flavour of beer (Guadagni, Buttery, & Harris, 1966; Van Opstaele, De Causmaecker, et al., 2012).

This has recently been confirmed by Neiens and Steinhaus (2018) who conducted a spiking experiment to investigate the contribution of several Huell Melon hop derived volatiles to the overall aroma intensity of top and bottom fermented beers. Trained panellists completed several Alternative Forced Choice (3-AFC) tests and compared nonspiked control beer with a beer spiked with hop volatiles at concentrations present in the original test beers. Myrcene was found to significantly contribute to the overall aroma intensity at concentrations between 6.65 and 15.0 µg/L in all test beers apart from the top fermented dry hopped beer which was present at 8.20 µg/L (Neiens & Steinhaus, 2018). It is interesting that no significant effect was detected at this intermediate concentration and it might be that other components in the matrix masked the aroma. However, matrix effects were not discussed in this study. Neiens and Steinhaus (2018) determined the odour threshold of myrcene in an aqueous solution to be 1.2 µg/kg and suggested that myrcene was present above its threshold level in all test beers. Previous studies investigated the sensory characteristics of

myrcene in beer and observed spicy and resinous flavour notes at 200 µg/L (Sharpe, 1988) together with metallic and geranium-like aromas at around 860 µg/L (Schnaitter et al., 2016). Moreover, it was stated that the threshold concentrations of myrcene can deviate by up to 1 mg/L depending on the matrix in which it is tested, suggesting that the perception of this compound is concentration- and matrix-dependent.

The importance of hydrocarbons for the overall aroma profile of beer have previously been highlighted by a study of Guadagni et al. (1966) who determined the relative contribution of individual hop oil compounds and fractions extracted from a Brewers Gold hop oil to the overall aroma sensation in beer. The relative contribution was calculated by dividing the number of odour units of the fraction or compound by the total number of odour units in the whole oil. The odour units were derived from the threshold concentrations of the compounds and fractions in water. The hydrocarbon fraction contained high concentrations of myrcene, humulene and caryophyllene and further terpenes at trace levels. While the hydrocarbon fraction accounted for 86%, the myrcene fraction accounted for 58% of the total odour. This observation was explained by the lower threshold concentrations of the two hop oil fractions compared to those of the other fractions and demonstrates the sensory potential of hydrocarbons at low concentrations.

Myrcene has often been suggested to be involved in sensory interactions with other compounds. Kishimoto, Wanikawa, Kagami, and Kawatsura (2005) suggested the resinous character in beer hopped with Saaz hops to be mainly caused by *β*-myrcene, although its concentration was far below sensory threshold level. Since there was no

further key compounds detected that could have added resinous aroma, it was suggested that further compounds below their detection level might have contributed to this aroma sensation. This suggests that an additive-, synergistic- or configural processing-type behaviour has caused the formation and perception of the resinous aroma character.

Further interesting sensory effects of *β*-myrcene in beer have been observed by who found the compound to impart a 'rawhop-like/green-grassy' aroma. In the same study, the compound was also suggested to induce fruity aromas in a different beer sample suggesting *β*-myrcene might also have interacted with other components in the beer, which in turn could have influenced its aroma character. Moreover, it was observed that high concentrations of *β*-myrcene might result in negative i.e. antagonistic or masking effects on perceived fruity aromas and these effects were expected to be matrix-dependent (Schnaitter et al., 2016). The fact that *β*-myrcene has been observed to impart diverse aroma notes including lime (Gros, Peeters, & Collin, 2012), peppery, terpene, balsam, plastic (Inui, Tsuchiya, Ishimaru, Oka, & Komura, 2013), metallic, geranium-like (Steinhaus et al., 2007), and spicy (Inui et al., 2013; Sharpe, 1988), supports this suggestion. Sensory descriptive analysis in a controlled beer matrix and an extended attribute list could be used to investigate concentration and matrix dependent effects on the sensory profile of *β*-myrcene in beer. In order to simplify the localisation of other volatiles or components that could potentially be involved, the sensory attributes used should be specific (e.g. 'geranium', 'lime'), rather than generic (flowery, fruity, etc.).

Apart from *β*-myrcene, other hydrocarbons are mostly of a monocyclic (e.g. limonene, *β*-cymene, *α*- and *β*-phellandrene) or bicyclic nature (e.g. *α*- and *β*-pinene) (Almaguer et al., 2014) and have been found to impart citrus/fruity and woody aroma notes (Sharp, Qian, Clawson, & Shellhammer, 2016; Sharpe, 1988). Sharp et al. (2016) identified α- and *β*-pinene and limonene in beers hopped with Citra and Simcoe varieties using stir-bar sorptive extraction (SBSE) and GC-MS. A trained sensory panel generated a lexicon of 18 terms for the description of hop aroma notes in different beer samples. Correlations between sensory scores and GC-MS data showed that these compounds added guava-, fruit cocktail-, and onion/garlic-like flavour notes to the beer (Sharp et al., 2016). Unfortunately, any possible effects of the hop volatiles on taste and mouthfeel or sensory interactions contributing to the flavour sensations were not discussed in this study.

It appears that compounds of the hydrocarbon fractions impart diverse aroma and flavour sensations in beer ranging from fruity-type to woody- and vegetative-type characteristics. Sensory characterisation has mainly focused on myrcene, which has been suggested to interact with other hop derived compounds or components in beer, which determines its sensory perception. Limited research has been conducted to investigate sensory interaction or matrix-dependent effects between other hydrocarbons in beer.

1.5.2 Sesquiterpenoids

Sesquiterpene hydrocarbons and their derived oxygenated sesquiterpenoids have been found to be responsible for the herbal, spicy and woody kettle hop aroma notes

in beer (Goiris et al., 2002; Praet, Van Opstaele, Baert, Aerts, & De Cooman, 2014; Praet et al., 2015). Several compounds in the sesquiterpene fraction are transformed during the brewing process and only low concentrations in the range of 10 to 100 µg/L have been detected in the final beer (Kishimoto et al., 2005; Praet et al., 2014). The majority of aroma and flavour characteristics have been attributed to sesquiterpene oxidation and hydrolysis products, such as caryophyllene and humulene mono- and diepoxides and their derivates, which are significantly more soluble than their precursor molecules. The amount of sesquiterpenes in the beer present in their original form depends highly on the hopping technique (Goiris et al., 2002).

However, Goiris et al. (2002) and Praet et al. (2014) found that the spicy and herbal hop aroma characters in beer are induced by several compounds in the sesquiterpenoid fraction such as caryophyllene oxide, humulene epoxides (I, II), humulol, and humulenol II that are present in the raw hop essential oil. The sesquiterpenoid fraction was added post-fermentation showing that these compounds have not had to be transformed during wort boiling to achieve the spicy and herbal characteristics in beer. In line with their research, determined humulene epoxides (I,II), humulenol II and caryophyllene oxide to be key compounds in spicy essences prepared from Tettnang Tettnanger, Perle and Hersbrucker Spaet hops and to induce the spicy flavour sensations in a pilot-scale lager. It was suggested that these compounds might sensorially interact and that their synergistic-type behaviour causes the spicy sensation in the beers (Deinzer & Yang, 1994; Goiris et al., 2002; Peppard, Ramus, Witt, & Siebert, 1989; Praet et al., 2016). It appeared to be difficult

to assign specific compounds of the oxygenated sesquiterpene fraction to the 'spiciness' in the beer samples.

In contrast, Kishimoto et al. (2005) could not confirm the relationship between the 'spicy' character and a mixture of sesquiterpenoids including humulene epoxides and humulenol II in a beer produced with Saaz, Tettnang, and Hersbrucker hops. No relationship was found between frequently selected 'spicy' attributes and the mixture of identified higher threshold substances. This indicated that the mixture of sesquiterpenoids was not sufficient to induce 'spicy' aroma characters as previously suggested by Goiris et al. (2002) and Praet et al. (2014) due to additive or synergistic interaction effects between these compounds. Van Opstaele, Praet, et al. (2013) also observed that these compounds as well as other humulene and caryophyllene oxidation products (humuladienone, 14-hydroxy-*β*-caryophyllene, caryophylla-3,8-(13)-dien-5-*β*-ol), could not be orthonasally detected at a GC-O sniffing port, although present at concentrations above aroma threshold in all tested hop varieties. The findings of both studies confirm what Eyres et al. (2007) had already hypothesised, that sesquiterpenoids are predominantly contributing to flavour, mouthfeel and trigeminal sensations rather than to aroma sensations, potentially due to matrix-dependent or cross-modal interaction effects.

In addition, it seems that the term 'spicy' has been used to describe very different sensory characteristics including olfactory, gustatory and trigeminal sensations or as a term covering multimodal interactions between the senses (Lawless, Rozin, & Shenker, 1985; Prescott, Allen, & Stephens, 1993). For instance, the oxygenated sesquiterpenoid fraction was found to affect mouthfeel and fullness perception of

beers at low concentrations of 20 µg/L. At higher concentrations (50 and 100 µg/L) the mouthfeel and fullness sensation occurred in synchrony with a 'spicy' flavour (Goiris et al., 2002). However, the mechanism behind this multisensory perception was not further investigated. In another study, the fraction extracted from Hersbrucker Spaet, Saaz and East Kent Golding hops has been found to not only increase the mouthfeel and fullness but also the bitterness intensity in a pilot-scale lager. The mouthfeel was further described as a 'spicy' sensation referring to a coating effect on the tongue and in the throat suggesting that the oxygenated sesquiterpenoid fraction added a sensation similar to astringency to the beer (Van Opstaele, Goiris, et al., 2012; Van Opstaele, Rouck, Clippeleer, Aerts, & Cooman, 2010). These findings highlight the importance of including objective, specific descriptors for sensory terms.

Unfortunately, very few studies have been conducted to investigate the activation of human receptors by hop oil compounds in beer in order to explain the trigeminal sensations that are induced by hop oil fractions. To date, only the effect of eudesmol, a sesquiterpenoid alcohol, has been investigated (Ohara et al., 2015). The compound was found to activate the human transient receptor potential ankyrin 1 channel (hTRPA1). This receptor is a calcium-permeable non-selective cation channel that is activated by noxious or irritating compounds (Julius, 2013). Eudesmol activated the receptor although its concentration (1 µm) was below the actual effective concentration required for channel activation. Therefore, the authors suggested that there might have been a synergistic effect between the compound and other chemicals in the beer that caused the channel activation (Ohara et al., 2015) and

therefore, this mechanism should be considered for other compounds present below threshold levels. Moreover, Ohara et al. (2015) observed eudesmol to activate hTRPV3, a warmth sensitive Ca^{2+}permeable cation channel. It was suggested that eudesmol might be able to produce warm and pungent sensations on the tongue (Ohara et al., 2015), indicating trigeminal-type sensations (Guichard et al., 2016). The compounds *α*-, *β*-, and *γ*-eudesmol have frequently been detected in hop oil, but their concentrations appear to be variety-dependent and, as with other cadinols, they have hardly been detected post wort boiling (Kishimoto et al., 2005; Praet et al., 2016; Praet et al., 2015; Steyer, Clayeux, & Laugel, 2013).

Only few hop derived sesquiterpenoids could be assigned to aroma notes in beer. The most potent odourant appeared to be 14-hydroxy-*β*-caryophyllene which was reported to impart a strong woody/cedar wood odour. However, it was suggested that there might be more compounds that have yet to be identified such as minor compounds partly responsible for cedar wood aroma characters due to additive or synergistic interaction effects (Eyres et al., 2007). Praet et al. (2016) identified caryophylla-3, 8-(13)-diene-5*β*-ol, caryophylla-4(12), 8(13)-diene-5-*α*/*β*-ol, and 14-hydroxy-*β*-caryophyllene as well as humulene epoxides and humulenol II as potent woody, green, and hoppy (and spicy) odour impact compounds.

Overall, sesquiterpenoids, their oxidation products and further derivatives including a number of epoxides appear to contribute to woody, herbal and green aromas, but to be mainly responsible for mouthfeel and trigeminal sensations in beer. These sensations occur at different concentrations and vary between test matrixes. Further, they have been linked to the frequently reported so-called ‘spicy’ flavour sensation,

which is also used to describe a variety of other sensations and often lacks a clear description. This indicates that it might be difficult to describe the sensation since it might be the result of a complex multimodal interaction effect.

1.5.3 Monoterpene alcohols

The flavour activity of the alcohol fraction in hop oil, consisting of terpene, sesquiterpene, and aliphatic/aromatic alcohols, was discovered in 1983 (Haley & Peppard, 1983). Monoterpene alcohols such as linalool, geraniol, citronellol, and nerol have been found to contribute to different fruity and floral dimensions of hoppy aroma and flavour in beer as discussed in the following sections.

Depending on the variety, hop oil contains around 1% linalool by weight (Moir, 1994) but it has been found that the concentration rapidly decreases during wort boiling (Kishimoto et al., 2005; Peacock, 2010) and high concentrations of linalool are only achieved by late or dry hop additions (Hanke, Herrmann, Rückerl, Schönberger, & Back, 2008). Since linalool is still transferred at high concentrations (present at up to 8 times of its sensory threshold in beer), it is considered to be one of the major aroma-active compounds in dry and late hopped beers (Baxter, Laurie, & Mchale, 1978; King & Dickinson, 2003; Kollmannsberger, Biendl, & Nitz, 2006). The threshold concentration of (*R*)-linalool is 2.2 µg/L while the (*S*)-enantiomer is detected at 180 µg/L (in beer) (Kaltner & Mitter, 2009). Up to 92-94% of linalool in beer is present in its (*R*)-isomeric form (Fritsch & Schieberle, 2005; Steinhaus, Fritsch, & Schieberle, 2003) and so it has been concluded that only the (*R*)-linalool is important for the overall hop aroma in beer. Furthermore, linalool appears to be one of the volatiles

that are omnipresent across the majority of hop varieties, and its amount in hop oil does not vary as much as is the case for other terpene alcohols such as geraniol. Therefore, it is considered as a marker compound responsible for aroma and flavour characteristics in the majority of hops (Fritsch & Schieberle, 2005; Peacock, 2010; Van Opstaele et al., 2010).

Particularly during the last decade, several findings have been reported in studies that systematically combined sensory and instrumental measures, which provide evidence for numerous additive or synergistic interaction effects between compounds of the monoterpene alcohol fraction. Sanchez et al. (1992) used GC-O (OSME) analysis and a trained panel to investigate the sensory profile of beers brewed with Hallertauer Mittelfrueh, USDA 21455, and USDA 21459, observing that linalool and nerol contribute to the overall aroma of beers. However, nerol was also suggested to additively interact with geraniol thereby imparting increased flowery aromas to beer (Hanke et al., 2008). Linalool, geraniol, and nerol all are known to add fresh, fruity, citrus-, and rose-like aroma notes to beer (Eyres et al., 2007; Inui et al., 2013; Kishimoto et al., 2006; Sanchez et al., 1992) suggesting that compounds of the same chemical class with similar aroma characteristics are likely to show additive- or synergistic interaction-type behaviour, and less likely to result in new flavour sensations due to configural processing.

Likewise, the existence of linalool and geraniol in combination with *β*-citronellol has been found to cause sensory interaction effects in a simple model system. Takoi, Koie, et al. (2010) found that a trained sensory panel could distinguish between linalool, geraniol and *β*-citronellol combinations and their individual application in a

carbonated 5% ethanol/water solution. Linalool was suggested to be the key contributor to floral ('lavender') and citrus characters. Whereas the aroma sensations attributed to geraniol ('floral', 'rose-like') and *β*-citronellol ('lemon, lime'), individually, in combination, and at different concentrations in the model solution, were found to be enhanced if coexisting with linalool at the threshold level (3 µg/L) (Takoi, Koie, et al., 2010), but also at much higher concentrations at 70 and 1000 µg/L (Takoi, Itoga, et al., 2010). However, it should also be taken into account that geraniol is known to have very different thresholds in different matrices (Meilgaard, 1993; Peacock, Deinzer, Likens, Nickerson, & McGill, 1981; Peltz & Shellhammer, 2017). Meilgaard (1993) reported a bimodal distribution in sensory threshold concentrations for geraniol, whereby 35% of the panel perceived geraniol at 18 µg/L, while for the other panellists, the concentration had to be increased up to 350 µg/L. Recently, Neiens and Steinhaus (2018) reported an odour threshold concentration of 1.1 µg/kg geraniol in an aqueous solution and a concentration of 31.2 µg/L for a significant contribution to the overall aroma intensity in a beer matrix (Neiens & Steinhaus, 2018). This research highlights that sensory interaction effects should be investigated at different concentrations in order to determine true threshold ranges.

Other researchers observed *β*-citronellol to induce 'rose bud', 'floral', and 'citrus' aroma notes (Inui et al., 2013; Lam, Foster, & Deinzer, 1986), which are aromas comparable to the characteristics reported for linalool and geraniol (Eyres et al., 2007; Inui et al., 2013; Kishimoto et al., 2006; Sanchez et al., 1992) and may therefore be describing an interaction effect in combination with these compounds. It would be interesting to test further monoterpene combinations at different concentrations

to determine whether these sensory interactions are concentration-dependent. It must be mentioned that Takoi, Koie, et al. (2010) used a commercial racemic mixture of *β*-citronellol and linalool for sensory evaluation and found additive effects, but it is not known whether these effects would hold true if the *R*/*S* ratio was changed for linalool, as the (*R*)-linalool is more flavour active than the (*S*)-enantiomer (Kaltner & Mitter, 2009).

Linalool and geraniol have also been found to interact with compounds of other chemical classes or hop oil fractions such as with fermentation by-products 2-phenylethanol and 2- and 3-butylacetate to increase floral ('flowery', 'rose-like') aroma characteristics (Hanke et al., 2008; Kishimoto et al., 2006). Further research provides evidence for sensory interaction effects caused by a combination of terpene alcohols and carboxylic acids. Using a triangle test, found 399 µg/L geranic acid significantly increased the flavour of linalool at 210 µg/L and geraniol at 49 µg/L in a pilsner by adding 'green', 'woody', and 'lemon'-like flavour notes. Geranic acid is usually present at low concentrations (1µg/L (Sanekata et al., 2018), 133-178 µg/L (Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009)) in beer and far below its olfactory threshold level (2.2 mg/L in a 0.1% v/v EtOH model carbonated solution (Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009)). Interestingly, the odour of geranic acid could not be detected using a 2-dimensional GC-O technique and thus the flavour threshold concentration was not determined. Furthermore, it should be taken into account that no quantitative data was collected to confirm the synergistic effect that was suggested by the authors.

In another experiment, Sanekata et al. (2018) added geranic acid (178 µg/L) and neric acid (51 µg/L) to a model beer that contained geraniol (98 µg/L) and linalool (97 µg/L) as the main hop volatiles together with a range of hop-dervived alcohols (*α*-terpineol, *β*-citronellol), aldehydes (geraniol, neral), esters (e.g. methyl geranate), and hydrocarbons (e.g. myrcene). The two carboxylic acids could significantly increase the sensory scores for 'flowery' and 'lemon' attributes given by a trained panel in a descriptive analysis study indicating a sensory effect of geranic acid at sub-threshold level on the flavour characteristics of hop derived terpenoids (Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009). Further research is required to investigate whether geranic acid principally has an effect on monoterpene alcohols or whether further chemical groups in hop essential oil may be involved in sensory interaction effects.

Sensory interactions between oxygenated sesquiterpenoids and monoterpene alcohols were reported by Praet et al. (2015). Based on sensory descriptive analysis and the volatiles quantified in lager beers hopped at different time points, it was suggested that, depending on the linalool/oxygenated sesquiterpenoid ratio, the floral-type aroma attributed to linalool might mask the 'spicy/herbal' aroma attributed to oxygenated sesquiterpenoids (such as humulene epoxide III, humulenol II, caryophylla-4(12),8(13)-diene-5-ol, 3*Z*-caryophylla-3,8(13)-diene-5*α*-ol, 14-hydroxy-*β*-caryophyllene, and 3*Z*-caryophylla-3,8(13)-diene-5*β*-ol) (Praet et al., 2015). This is important to know if aiming to target a specific hop aroma profile in beer. However, this should not be generalised, and the masking effect might not only

depend on the ratio but also on other aroma-active compounds present, depending on the hop variety.

In conclusion, it can be said that (*R*)-linalool and geraniol are by far the most potent compounds in the monoterpene alcohol fraction contributing to the sensory properties in hops and beer – individually and by eliciting sensory interactions with other volatile compounds. Besides contributing to floral (mainly rose-like) and fruity (mainly citrus-like) aroma characteristics, these compounds are prone to interactive behaviours with other compounds, in particular those of the monoterpene alcohol fraction such as nerol and *β*-citronellol, but also with other compound groups such as terpene hydrocarbons or carboxylic acids. (*R*)-linalool appears to act as a trigger for additive or synergistic interaction effects resulting in pronounced aroma sensations. The majority of these findings have only been discovered coincidentally and therefore further systematic research is required to confirm and explain these effects at different concentrations and in different beer matrices.

1.5.4 Esters

Meilgaard (1982) suggested that esters are secondary flavour constituents in beer and present between 0.5 and 2 Flavour Units (FU) which is defined as the concentration of a compound divided by its threshold (Peacock & Deinzer, 1988). Thus, only minor changes are caused if they are removed from the beer matrix. A significant amount of hop oil esters are either hydrolysed by yeast or transesterified, while esters of conjugated acids, such as methyl geranate, have been found to resist hydrolysis and are transferred to the final beer in their original form (Seaton, Moir, &

Suggett, 1982; Tressl, Friese, Fendesack, & Koeppler, 1978a). If targeting a specific sensory profile by using an ester hop oil fraction, this has to be taken into account.

It was found that methyl esters in particular, contribute to the hop aroma and flavour in beer due to their low threshold concentrations (Eyres & Dufour, 2009; Nickerson & Likens, 1966). For instance, ethyl-2-methylbutanoate, ethyl 2-methylpropanoate, ethyl-4-methylpentanoate, methyl 2-methylbutanoate, and derivates of geraniol and linalool, such as linalool oxide, and geranyl acetate, have been found to impart fruity, green, floral, but also waxy aroma notes in beer and model systems (Kishimoto et al., 2006; Moir, 1994; Neiens & Steinhaus, 2018; Peacock et al., 1981; Siebert, 1994; Tressl et al., 1978a). The majority of these are transferred to the beer base above their odour threshold concentrations at ng/L level (Neiens & Steinhaus, 2018). Both the chain length and the degree of branching appear to have an impact on the aroma profile. Short chain esters add aroma notes to beer such as soft fruit (apple, plum), citrusy, pear/apple, and tropical fruit-like aromas (Marriott, 2009; Meilgaard, 1975b). In general, short chain esters have higher flavour thresholds compared to long-chain esters (C7 to C10) resulting in different odour activities (Donaldson, Bamforth, & Heymann, 2013).

Odour activity values (OAV) are frequently used to determine the odour activity or potency of a compound to address the influence of a matrix on the volatility of a given odourant (Buttery, 1999). OAVs are equivalent to FUs and express the ratio of the concentration to the odour threshold. At OAVs higher than 2-3 times the compounds' threshold, the compound is likely to contribute to the overall aroma of the matrix. Compounds having an OAV close to 1 do not significantly affect the intensity or the

aroma profile unless synergistic effects occur between these compounds (Marsili, 2006).

The OAV approach was applied by Schieberle (1991) and Fritsch and Schieberle (2005) who investigated key aroma compounds in Bavarian pilsner-type and pale lager beer in a GC-O (AEDA) study. High OAVs were reported for ethyl 2-methyl-propanoate, ethyl 4-methylpentanoate, (*S*)-ethyl 2-methylbutaonate, ethyl butanoate, and ethyl hexanoate suggesting these compounds to be key contributors to the fruity characters and to the overall aroma of the beers. It should be taken into account that the aroma profiles of the esters have been assessed individually (Fritsch & Schieberle, 2005; Schieberle, 1991). There is no evidence as to whether these compounds contribute individually to the fruity and the overall aroma of the beers or as part of a compound mixture featuring additive- or synergistic-type behaviours.

In order to address this problem and enable the detection of individual contributors or additive or synergistic behaviours, Charm (combined hedonic aroma response measurement) analysis can be used. This method has been applied in combination with sensory evaluation and GC-MS analysis to investigate the odour-active compounds in strongly hopped beers. Charm values are used to indicate odour activity or the potential relative contribution of a flavour-active compound to the overall flavour of the matrix (air, water, beer) in which the compound is tested (Acree & Teranishi, 1993). Basically, Charm analysis combines the sniffing of the GC effluent with the measurement of retention indices. In this way, the odour intensity of the extracted components is measured in units of Charm over the ranges of the retention

indices and gives the ratio of the concentration of the volatile compound to its detection threshold at the sniffing port (Acree, Barnard, & Cunningham, 1984).

Kishimoto et al. (2006) applied this approach and recorded high aroma values of >1 and 'Charm' values of >1000 for ethyl 3-methylbutanoate ('citrus, sweet, apple-like'), (±)-ethyl 2-methylbutanoate ('citrus, apple like'), ethyl 2-methylpropanoate ('citrus, pineapple, sweet') and ethyl 4-methylpentanoate ('citrus, pineapple'). Combined with linalool, 3MH, 4-(4-hydroxyphenyl)-2-butanone, and another unknown compound, these esters have significantly contributed to the citrus characteristics of the beers hopped with Cascade and Saaz hops. Interestingly, the sensory score for citrus aroma was higher for the beer brewed with Cascade hops than expected from the Charm values, therefore it was concluded that the compounds synergistically interacted with each other. Further unknown components below detection level might have been involved in this sensation and a recombination/omission study is suggested to confirm these hypotheses (Kishimoto et al., 2006) rather than investigating the volatile compounds in isolation.

Xu et al. (2017) investigated the flavour contribution of esters in lager beers using HS-SPME-GC-O/MS. Twenty esters could be detected and identified while only eleven esters could be identified at the sniffing port. Unfortunately, the authors did not investigate to which extent the other esters contributed to the flavour profile. Six esters were further investigated, namely isobutyl acetate, ethyl octanoate, ethyl butyrate, phenyl ethyl acetate, ethyl benzoate, and ethyl 3-phenylpropionate. Based on their concentrations and detection at the sniffing port, these compounds were suggested to be the main contributors to the aroma and flavour of the lager beer.

Determination of flavour thresholds of these esters revealed concentrations in a range of 0.14 mg/L and 1.29 mg/L. Interestingly, flavour characteristics of esters with lower threshold concentrations, such as ethyl octanoate and ethyl butyrate, were perceived as being 'unpleasant', 'solvent-like' or 'cheesy' if present at higher concentrations approximately 3-fold of their respective threshold levels (Xu et al., 2017).

In another experiment, Xu et al. (2017) tested different combinations of ethyl octanoate, isobutyl acetate and phenylethyl acetate in order to identify sensory interactions. Interaction effects were suggested based on the finding that 0.26 mg/L ethyl octanoate, 1.53 mg/L isobutyl acetate, and 0.64 mg/L phenylethyl acetate obtained the highest score from a trained sensory panel compared to a number of other combinations tested in this study (Xu et al., 2017) indicating additive- or synergistic-type behaviour between the compounds.

However, esters are not only interacting with each other, they are also affected by other components in the beer matrix. Recently, Hotchko and Shellhammer (2017) investigated the influence of ethyl esters, terpenes, and aliphatic γ- and *δ*-lactones on the fruity aroma in beer. Lactones are formed during fermentation when yeasts transform fatty acids into cyclic esters. Since lactones are mostly present at subthreshold levels, they are expected to increase fruity aromas of other esters rather than having a large impact on the final overall aroma profile of beer in their own right. From the outcome of the sensory descriptive analysis, the authors concluded that lactones (30 µg/L *γ*-nonalactone, 2 µg/L *γ*-decalactone, 3 µg/L *δ*-decalactone) at low or subthreshold levels support the fruity aroma sensations of

ethyl 2- and ethyl 3-methylbutanoate (6 µg/L each) as well as of linalool (100 µg/L) and of *β*-damascenone (3 µg/L), all added at realistic concentrations to a 5.6% ABV unhopped and uncarbonated pale ale. Interestingly, the lactones combined with ester compounds increased the 'stone fruit-/peach-like' aroma. Moreover, the combination of lactones+terpenes and lactones+esters+terpenes increased the intensity of the 'berry' and the overall fruity aroma (Hotchko & Shellhammer, 2017). Further investigation with a wider variety of compounds is required to explore additive and synergstic effects of lactones on 'fruity' hop volatiles since only a limited number of compounds were tested in this study.

Other synergistic effects have been observed on the flavour profile of lager beer if produced with particular yeast strains (TUM 34/70, TUM 193) and dry hopped with Mandarina Bavaria, Hersbrucker, and Hallertauer Magnum hop varieties (Korbinian Haslbeck et al., 2018). Trained panellists conducted a descriptive tasting following the DLG (Deutsche Landwirtschafts-Gesellschaft) scheme and Pearson correlation of the sensory data revealed a significant effect between the yeast strains and the citrus flavour intensity that was assigned to the content of geraniol, nerol, and isobutyl isobutyrate in the beers. However, a direct cause-effect relationship could not be determined since the citrus flavour intensity in the two affected test beers was not significantly higher than in the other test beers. It was suggested that other flavour-active compounds could have contributed to the citrus flavour as well and further research is required to investigate the combinatory effect between hop- and yeast derived volatiles on the flavour profile of beer produced with different yeast strains. In order to understand the role of isobutyl isobutyrate in the citrus flavour

perception, Haslbeck et al. (2018) used model solutions (1% EtOH/H_2O) containing geraniol (20 µg/L), linalool (20 µg/L), and *β*-citronellol (2 µg/L) at concentrations as present in the test beer dry hopped with Mandarina Bavaria, which had the highest citrus intensity among all test beers. Isobutyl isobutyrate was added at different concentrations below, equal to, or above its odour threshold concentration. It should be noted that the concentrations were based on the odour rather than the flavour threshold level. As emphasised previously, these should not be confused because threshold concentrations highly depend on the test matrix that is used. Interestingly, the sensory data indicated that the addition of 10 µg/L isobutyl isobutyrate to the flavoured model solution resulted in a minor increase in the citrus flavour intensity while the addition of 30 µg/L and 80 µg/L appeared to lower the intensity, indicating suppressing or masking effects. As suggested by the authors, this outcome might suggest a concentration-dependent interaction effect between the compounds and requires further research.

It can be concluded that compounds of the ester fraction play an important role in the fruity, floral and green aroma notes in beer. Further, there appears to be sufficient evidence regarding aroma and flavour enhancing effects between certain methyl esters causing pronounced fruity/citrus aroma characters in different beer matrices. In addition, esters appear to interact with compounds of other chemical classes such as lactones and terpenes. Further research should be conducted to investigate sensory interaction between esters and other compound groups and to evaluate differences between esters with different chain lengths. Moreover, limited research has been published on sensory interactions with other beer components.

1.5.5 Ketones

The well-known representatives of the ketone fraction in hop oil are *β*-damascenone, *β*-ionone, 2-dodecanone, and 2-undecanone. These compounds have been suggested to impart citrus/fruity and floral characters in beer (Eyres et al., 2007; Gros, Nizet, & Collin, 2011; Kishimoto et al., 2006; Van Opstaele, De Causmaecker, et al., 2012). The most abundant methyl ketone appears to be 2-undecanone. The sensory profiles of ketones have been found to highly depend on their concentration and molecular weight. The higher the molecular weight, the more the fruity aroma character is transformed into a floral aroma character. For instance, *β*-ionone and 2-undecanone are known to impart floral (Eyres et al., 2007; Kishimoto et al., 2006; Sharpe, 1988), but also fruity (berry-like (Kishimoto et al., 2006), citrusy (Gros et al., 2012)) aroma notes at different concentrations. Since these compounds have been found in beer above their sensory threshold levels, they are expected to contribute to the hop aroma and flavour in beer (Tressl et al., 1978a). Nevertheless, low molecular weight ketones should not be neglected since these may still contribute to the overall aroma sensation due to sensory interaction effects (Dresel et al., 2013).

β-Ionone belongs to the group of so-called 'rose ketones' and has been identified in beer brewed with Saaz hops to impart a 'floral-violet' aroma (Eyres et al., 2007). Low odour threshold values ranging between 0.008 and 0.170 µg/L in water, 10 µg/L in beer, and high Charm values of >1000 in beer have been reported for *β*-ionone illustrating the aroma potential of this compound (Kishimoto et al., 2006; Meilgaard, 1982), which is usually found in beer at concentrations between 1-3 µg/L (Meilgaard, 1982). Nevertheless, it should be taken into account that 50% of the population is

expected to have an anosmia for *β*-ionone (Plotto, Barnes, & Goodner, 2006). This should be considered if recruiting a sensory panel for hop aroma or flavour analysis.

Kishimoto et al. (2006) observed *β*-ionone to add 'floral', 'violet-like', and 'berry' aroma notes to beer and suggested that other beer components or hop compounds such as 2-phenylethyl 3-methylbutanoate had either synergistic or antagonistic effects on the floral characteristics of *β*-ionone. 2-phenylethyl 3-methylbutanoate was found to exhibit a 'floral' and 'minty' aroma (Kishimoto et al., 2006). However, the findings and the underlying mechanism were not further investigated. A follow-up study would be required to confirm these findings, for instance by using sensory profiling of aroma combinations with and without *β*-ionone in a controlled base beer. Independent from the method of choice, panellists should be checked for *β*-ionone anosmia, particularly if performing GC-O analysis, which can be performed with as few as two assessors (Eyres et al., 2007).

Another hop derived ketone that is frequently identified in beer at concentrations between 1-30 µg/L (Meilgaard, Reid, & Wyborski, 1982) and is also only perceived by 50% of the population is *β*-damascenone (Eyres & Dufour, 2009; Plotto et al., 2006). Due to its high OAV and low flavour dilution (FD) factors, Fritsch and Schieberle (2005) and Schieberle (1991) suggested (*E*)-*β*-damascenone, a ketone that appears to be mostly present in Saaz hops (Eyres et al., 2007), to be one of the key aroma compounds imparting 'fruity' and 'honey'-like aroma in Bavarian pale lager and pilsner-type beer, respectively. FD factors express the ratio of an odourant concentration in the initial extract to the concentration in the most dilute extract at which the odour is still detectable using GC-O. The greater the dilution factor at which

the compound is detected, the greater the probability of contributing to the overall aroma (Acree et al., 1984).

In addition to the previously mentioned aroma notes, *β*-damascenone was also perceived as 'cooked apple', 'apple sauce', 'sweet tobacco' (Eyres et al., 2007), 'cooked fruit' (Evans et al., 1999), 'citrus' (Kishimoto et al., 2006), 'apple/peach-like' (Lermusieau, Bulens, & Collin, 2001), and 'rhubarb, red fruit, and strawberry-like' (Gijs et al., 2002). Since different aroma notes were attributed to *β*-damascenone in different beer matrices, this suggests that the aroma profile of *β*-damascenone changes due to other components present in the beers. However, this was not investigated in these studies. Moreover, variations in the aroma quality of *β*-damascenone at different concentration ranges might explain why diverse sensory descriptors were obtained for this compound.

1.5.6 Aldehydes

The majority of hop derived aldehydes in beer have been detected at low or subthreshold concentrations depending on the hop variety and hopping technique (Nijssen, Visscher, Maarse, Willemsens, & Boelens, 1996). They have also been found to be reduced to their corresponding alcohols by yeast during primary fermentation, dry hopping, or conditioning of the beer. For instance, geranial is reduced to geraniol and *β*-citronellol (Sharp, Qian, Shellhammer, & Shellhammer, 2017). Aldehydes such as (*E*)-2-hexenal, (*Z*)-3-hexenal, 3-ethylbutanal, benzaldehyde, 2-phenylacetalaldehyde, geranial, and neral are well known to add different green/grassy and floral aroma notes to beer (Nickerson & Van Engel, 1992; Nijssen et

al., 1996; Schönberger & Kostelecky, 2011). Citrusy and fruity flavours are characteristic of aldehydes having shorter chain lengths, while with increasing chain length odours become 'unpleasant' and are then described as 'rancid', 'fat-' and 'cardboard-' or metallic-like (Meilgaard, 1975b). Marker compounds for these 'unpleasant' odours are for instance (*E,E*)-2,4-nondienal and *trans*-4,5-epoxy-(*E*)-2-decenal (Eyres & Dufour, 2009; Steinhaus & Schieberle, 2000).

Using sensory evaluation and GC analysis, Kishimoto et al. (2006) found the short chain aldehydes 1-hexanal and (*Z*)-3-hexenal and the long chain aldehyde (*E,Z*)-2,6-nonadienal to be key compounds with regard to 'green' aroma characteristics in beers hopped with Hersbrucker, Saaz, and Cascade hops. The concentrations of the two former compounds were detected at subthreshold levels suggesting that the combination of these compounds was responsible for the perception of the 'green' aroma notes in the three beers indicating additive or synergistic interactions. However, this hypothesis requires confirmation, for instance by conducting a recombination or omission study, such as GC-GOOD (global olfactometry omission detection) (Hallier, Courcoux, Sérot, & Prost, 2004), or GC-R (recombination) (Johnson, Hirson, & Ebeler, 2012). In general, limited research has been conducted to investigate sensory interactions between hop derived aldehydes in beer, therefore, this requires further investigation.

1.5.7 Sulphur-containing compounds

Hop oil contains potentially flavour-active organo-sulphur volatiles (thioesters, sulphides, and other sulphur-containing compounds), such as dimethyl sulphide

(DMS), dimethyl disulphide, dimethyl trisulfide, diethyl disulphide and 2-methyl-3-furanethiol, that have been found to contribute to the hoppy aroma in beer (Lermusieau et al., 2001). The determination of the actual flavour contribution of sulphur-containing compounds has proven to be difficult. These compounds are present in small quantities in hops and in beer at ng/L level or lower. The most considerable progress in quantitative determination of sulphur-containing compounds has been shown after the introduction of sulphur-specific flame photometric detectors for GC. This has enabled the identification of many, but still not all, sulphur-containing compounds at trace levels (Seaton, Suggett, & Moir, 1981; Sharp et al., 2016).

Sulphur-containing compounds induce aroma and flavour characteristics in beer and are also observed to change the perception of other hop aroma compounds. For instance, Schnaitter et al. (2016) used HS-SPME-GC-MS-O to identify hop oil volatiles in beer and found 2,3,5-trithiahexane, S-methylthiomethyl 2-methylpropanethioate, and S-methylthiomethyl 2-methylbutanethioate to impart respectively 'leek-like', 'onion-like' and 'green' aromas. These three compounds were also suggested to suppress the 'citrus/fruity' aromas induced by citronellol, linalool, and geraniol. Sulphur-containing compounds have low aroma thresholds and, even when present at trace levels, have the potential to overpower other aromas such as fruity notes.

Thiols such as 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexan-1-ol (3MH) detected in Nelson Sauvin, Cascade, Saaz, Tomahawk, and Nugget hops have been observed to impart intense 'black-currant', 'citrus/grapefruit', 'tropical fruit' and 'nutmeg'-like aroma notes at trace concentrations due to their extremely

low odour threshold levels. However, these compounds are also known to impart 'cat urine' aroma notes (Gros et al., 2012; Kishimoto, Kobayashi, Yako, Iida, & Wanikawa, 2008; Kishimoto et al., 2006) due to the interplay with components in the beer matrix and the receptor the compounds interact with.

In another study, 4MMP was observed to increase the overall hop aroma intensity and to add 'black current-like' aroma characteristic to beers brewed with US-Simcoe, US-Summit, and US-Apollo. Due to its low threshold value in beer (1500 ng/L), Kishimoto et al. (2008) concluded that 4MMP might be even more important for the overall hop aroma than *β*-myrcene, linalool, geraniol, and ethyl 4-methylpentanoate. However, the authors could not detect 4MMP in the same varieties grown in European countries. Copper ions in the copper sulphate that is used for protection against mildew can conjugate with the sulphanyl group in thiols, which might have caused the decrease in 4MMP concentration.

As with 4MMP and 3MH, a number of other volatile hop thiols (such as 3-mercapto-4-methylpentan-2-one, 3-sulfanyl-4-methylpentan-1-ol (3S4MP), and 3-sulfanyl-4-methylpentyl acetate (3S4MPA) have low threshold concentrations between 0.8 and 120 ng/L (Sarrazin et al., 2007; Takoi, Degueil, Shinkaruk, Thibon, Maeda, et al., 2009). These compounds have been observed to impart among others 'grapefruit' (3S4MP, 3S4MPA), 'rhubarb' (3S4MP), and 'blackcurrant-like' (4MMP) aroma notes in beers brewed with Nelson Sauvin hops (Gros et al., 2012; Kishimoto et al., 2008; Steinhaus et al., 2007; Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009; Takoi et al., 2007).

Interestingly, Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al. (2009) found 3S4MP and 3S4MPA but also 2-methylbutyl isobutyrate (2MIB) derived from Nelson Sauvin hops to interact synergistically with each other. Using sensory triangle tests, the compounds were added in a carbonated 5% ethanol solution and the addition of 3S4MP ('grapefruit, rhubarb-like') was found to increase the flavour intensity of 3S4MPA ('grapefruit, peach-like') and 2MIB ('apple, apricot-like') at concentrations below their threshold levels. In addition, the flavour intensity of linalool ('lavender') and geraniol ('rose-like') flavours were also increased. Therefore, the researchers suggested that 3S4MP acts as a flavour enhancer for other compound classes, such as isobutyric esters and further terpene alcohols, by increasing 'floral' and decreasing 'green' and 'smoky' flavours (Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009; Takoi et al., 2007). These compounds might act collaboratively and thereby inducing the characteristic flavour impression found in beer brewed with Nelson Sauvin hops.

In view of the synergistic effects investigated in this study, it has to be noted that only one concentration combination was tested (40 ng/L, 3S4MP with 20 ng/L 3S4MPA and/or 5 µg/L 2MIB) and this effect might be concentration dependent (Takoi, Degueil, Shinkaruk, Thibon, Kurihara, et al., 2009). Therefore, further concentrations should be tested. Besides the aforementioned effects on fruity and floral aroma and flavour characteristics, sulphur-containing compounds are also known to impart 'unpleasant' aromas in beer. For instance, Lermusieau et al. (2001) found DMS and dimethyltrisulphide to add 'cheesy/glue' and 'onion'-like aromas to beer produced with Challenger hops (Lermusieau et al., 2001). DMS is usually not associated with hops, although it is found at trace levels in hop essential oil. DMS is well known as

being produced during kilning and wort boiling because of thermal cleavage of *S*-methylmethione from malt. Its presence in beer indicates insufficient removal or evaporation of malt-derived precursors, which are produced during wort boiling. The concentration of DMS increases in aged beer depending on the pH level (Gijs et al., 2002; Sharp et al., 2016).

Interestingly, Hanke et al. (2010) found linalool to decrease the perceived intensity of the 'cabbage-like' off-flavour of DMS at 15 µg/L by increasing the flavour threshold from 129 µg/L to 176 µg/L when added to a commercial German lager beer. However, it increased the perceived intensity or decreased the flavour threshold (to 102 µg/L) when added at a concentration of 60 µg/L. This is also remarkable because it was suggested that linalool showed the suppressive effect at a concentration near to, but below, its flavour threshold level (27 µg/L in the same beer). Unfortunately, the mechanism behind this effect could not be explained and requires further research. Furthermore, the authors found that the esters, isoamyl acetate (0.75 µg/L) and ethyl acetate (4 and 7 mg/L) decreased the flavour threshold of DMS. The suppressive effect of isoamyl acetate was only recorded at the highest concentration that was tested and the authors suggested a masking effect due to its overpowering 'banana' and 'apple'-like flavour (Hanke et al., 2010). This research not only shows that sensory interactions are concentration-dependent but also that interaction effects depend on different mechanisms.

In conclusion, sulphur-containing compounds have been found to contribute to the overall hop aroma and flavour of beer, even when present in trace amounts, due to their extremely low threshold concentrations. Several compounds of this chemical

class are suggested to interact in additive- or synergistic-type behaviour, thereby imparting intense and diverse aroma sensations ranging from undesired (e.g. onion, garlic) to in vogue, fruity-type aroma characteristics (e.g. blackcurrent, tropical fruit, whitewine) in beer. Further research is required to investigate whether these sensory interactions are concentration-dependent and whether hop derived sulphur-containing compounds are involved in cross-modal interactions, for instance by modifying taste or mouthfeel sensations, since this has not been investigated. The concentration of sulphur-containing compounds in hop oil is highly variety-dependent, but this fraction could be combined with other hop oil fractions of different hop varieties to investigate the interactions between different compound classes.

1.6 Interactions between hop oil compounds and other beer components

As has been discussed in the previous sections, the perception of hop derived volatiles is affected by the beer matrix in which they are consumed, due to the impact on the diffusion, partitioning, and release of the volatiles. Factors such as pH, temperature, ethanol level, protein, starch, and phenolic compounds can all impact upon the partitioning and release of aroma compounds (Guichard et al., 2016). Sensory interactions between hop volatiles and beer components, including ethanol, carbon dioxide (carbonation), and bittering substances (hop acids, polyphenols), are likewise important for the perception of hoppy aroma and flavour in beer.

1.6.1 Ethanol

In contrast to water, ethanol decreases the polarity of a solution, which influences retention, partitioning, threshold concentration, and perception of volatile compounds (Boothroyd, Linforth, & Cook, 2012). Limited research has been conducted to investigate the solvating properties of ethanol on hop oil compounds, particularly on compounds in the more polar oxidised fraction (Peltz & Shellhammer, 2017). For instance, due to the presence of oxygen in the chemical structure of monoterpene and sesquiterpene alcohols, these compounds are more polar and soluble in water and in alcoholic solutions compared to compounds in the hydrocarbon fraction (Van Opstaele, Goiris, et al., 2013). As for other alcoholic beverages, it is difficult to explain the effect of ethanol on hop derived volatiles and further investigations are required (King, Dunn, & Heymann, 2013; Peltz & Shellhammer, 2017; Perpète & Collin, 2000). Moreover, according to Peltz and Shellhammer (2017), the majority of studies have only investigated the aroma activity of hop oil compounds in pale adjunct lagers of 5% ABV or less, and other beer types and ethanol concentrations have been neglected.

In MS-Nose studies, ethanol has been found to promote the delivery of volatiles during the consumption of beverages. Due to its surface activity, surface generation abilities, and physico-chemical modification of aroma partitioning, ethanol can modify the sensory perception of volatiles (Clark et al., 2011). This was observed in a study of Perpète and Collin (2000) who investigated the influence of ethanol at concentrations between 0 and 5% on the flavour perception of a typical lager beer using GC-FID analysis and sensory triangle tests. A concentration of 0.5% ethanol was

sufficient to cause a slight modification in aldehyde retention while >5% ethanol resulted in increased aldehyde retention, particularly of 3-methylthiopropionaldehyde. It was concluded that ethanol could have major effects on partitioning of odourants by retaining the volatiles in the beer medium, thereby modifying threshold levels and the perception of aroma sensations as imparted by aldehydes. Consequently, the perception of these compounds might be higher in low-alcohol beers (Perpète & Collin, 2000). Other researchers suggested that the aroma intensity of odourants is generally lower in alcohol-free beer and that the presence of ethanol, as one of the primary odourants in beer, has a significant effect on its overall aroma and flavour sensations (Schieberle, 1991).

Peltz and Shellhammer (2017) investigated the effects of 5 and 10% ABV on the orthonasal detection thresholds of 10 hop oil compounds in unhopped pale ale. The compounds represented a range of chemical classes and included (-)-*β*-caryophyllene, (±)-*β*-citronellol, *β*-damascenone, geraniol, geranyl acetate, *α*-humulene, (±)-*β*-linalool, *β*-myrcene, nerol, and 4MMP. In order to achieve 5 and 10% ABV in the production beer, 95% ABV food grade ethanol and Milli-Q water were added while maintaining equivalent residual extract concentrations. Hydrocarbons were suggested to be retained in high ethanol rather than in low-ethanol beer, which affected their threshold levels in the different beer matrices. Increasing ethanol concentration from 5% to 10% resulted in a significantly decreased threshold concentration for *β*-damascenone (~2.5-fold). The opposite was the case for some terpene alcohols. The threshold concentrations of linalool and geraniol increased by 166 µg/L and 122 µg/L, respectively, but the actual impact of the threshold difference

on the sensory perception of these compounds in beer was questioned. The authors concluded that, since linalool and geraniol are more hydrophilic than hydrocarbons, they might largely be retained in higher ethanol systems whilst myrcene was suggested to be retained to a lesser degree in the higher ethanol base and to escape into the air phase. Overall, ethanol at increased concentrations has a low potential to suppress the odour activity of terpene alcohols (Peltz & Shellhammer, 2017).

In conclusion, the focus of previous studies was to investigate the effect of ethanol on a limited number of single chemical compounds. It would be interesting to study the effect of a broader range of ethanol concentrations (equivalent to no, low, high, ultra-high alcohol beers) on the delivery of compounds to the nasal cavity using the MS nose, and on the perception of hop oil compound mixtures using sensory evaluation.

1.6.2 Carbonation

Carbonation in beverages is perceived as a sparkling, tingling, and sometimes astringent sensation in the oral cavity. It was also found to stimulate salivary production and to affect taste perception (Schmelzle, 2009). Harrison (1970) observed flavour threshold concentrations of some esters and alcohols to be reduced by approximately half in degassed beer compared to carbonated beer. Therefore, different carbonation levels will bias flavour perception during sensory evaluation. Thus, for sensory descriptive analysis, it is necessary to control the carbonation level (Hotchko & Shellhammer, 2017).

Using MS-Nose analysis, Clark, Linforth, Bealin-Kelly, and Hort (2011) observed that the carbonation level (~3.6 volumes) present in a model system increased the release of isoamyl alcohol and ethyl acetate into the breath. The carbonation increased the delivery of the two high partitioning compounds in the first exhalation after the consumption of the model beer by around 86% proposed to be due to an increase in interfacial surface area for release. Based on the finding that only the release of high partitioning compounds was increased, a relationship between the volatile air-water partition coefficient (K_{aw}) of individual compounds and their delivery in the breath has been suggested (Clark et al., 2011). However, sensory analysis did not find an increase in aroma or flavour perception due to increasing carbonation levels (Clark et al., 2011).

To date, understanding of the effect of the carbonation level on the perception of hop derived volatiles in beer is limited. It would be interesting to test the effect of different carbonation levels on the release of hop derived volatile mixtures (oil/fractions) in a controlled beer matrix and the resulting effect of potential sensory interactions on taste or mouthfeel sensations or the activation of trigeminal neurons, since this has not been investigated.

1.6.3 Hop acids and polyphenols

Iso-α-acids (isohumulones), the isomerisation products of α-acids (humulones), are formed during wort boiling, and are mainly responsible for the bitter taste of beer (Almaguer et al., 2014). Considering the low threshold concentration of iso-α-acids (6 mg/L in water), they are readily perceived. However, the concentration can vary

considerably up to 100 mg/L depending on the hop materials or products added in the brewing process. Moreover, the utilisation of iso-*α*-acids during wort boiling varies due to the polarity of the compounds (De Keukeleire, 2000).

The perception of beer bitterness is complex since several hop-derived compounds appear to be involved (Ting & Ryder, 2017) including polyphenols, which represent approximately 4-6% of the hop dry weight (Aron & Shellhammer, 2010). It was found that the addition of 200 mg/L polyphenols induced a higher bitterness intensity compared to 10 mg/L iso-*α*-acids alone in the same beer. In addition, polyphenols were found to increase perceived 'fullness' (Forster, Beck, & Schmidt, 1995; Langstaff, Guinard, & Lewis, 1991; Langstaff & Lewis, 1993), lingering bitterness and astringency in beer (Benitez, Forster, & Keukeleire, 1997; McLaughlin, Lederer, & Shellhammer, 2008; Peleg, Gacon, Schlich, & Noble, 1999), whilst high concentrations caused 'unpleasant', 'harsh' bitterness and 'medicinal' or 'metallic' tastes (Forster et al., 1995; McLaughlin et al., 2008).

For instance, Goiris et al. (2014) found a hop polyphenol extract to increase the perception of the 'fullness' in a pilsner-type beer when combined with a polar floral hop essence or a dry hop essence containing oxygenates. However, this was not the case when the polyphenols were applied together with a spicy hop essence enriched in oxygenated sesquiterpenes. Furthermore, the bitterness intensity was increased when flavonol glycosides were added, but not when prenylated flavonoids were applied. In contrast, astringency levels only increased when the total polyphenols or prenylated flavonoids were added. These findings highlight that the different chemical classes in hop polyphenols have different effects on the sensory profile of

beer, as is the case for the different hop oil fractions. To date, limited research has been conducted to investigate the impact of polyphenol fractions on the perception of hop volatiles in beer and vice versa. The majority of studies have focused on the investigation of hop acids and their impact on beer bitterness, but not on other sensory characteristics.

Daoud and Kusinski (1993) evaluated taste and aroma profiles of beers bittered with liquid CO_2 and ethanol extracts derived from fresh and deteriorated hops. The beers brewed with extracts from undeteriorated or 46% deteriorated pellets showed different sensory profiles in view of hoppiness aroma intensity compared to the control beers, which were brewed with extracts of undeteriorated pellets. A sensory panel perceived the aroma of the beer brewed with extracts of 46% deteriorated pellets containing a significantly lower concentration of iso-*α*-acids and uncharacterised resins, as less 'hoppy', 'estery', 'fruity', 'floral', and 'sweet' compared to the control beer and a beer brewed with extract of 28% deteriorated pellets. Thus, the composition of the bittering substances and the quantity of iso-*α*-acids appeared to have significant effects on different sensory characteristics of the beers, which may be due to cross-modal interactions. However, this was not further investigated in this study. A major limitation of this study is that the concentration and the composition of the hop oil possibly varied between the samples to an extent that no reliable conclusion can be drawn in regard to the relationship between the chemical composition and the sensory characteristics.

Despite the limited number of studies, it has been suggested that cross-modal interactions occur between hop acids and hop derived volatiles, which affect the

perception of hop aroma and flavour sensations in beer. This might depend on the bitterness level and the composition of bittering substances present in the beer matrix. A factorial design including hop oil compounds at different concentrations and combinations for evaluation with sensory descriptive analysis should be used in order to confirm these hypotheses and to identify the sources of sensory interaction effects that might have caused the observed modifications in aroma, flavour, taste, and mouthfeel characteristics.

1.6.4 Hop derived volatiles and perceived bitterness

In the previous section, it was suggested that bittering hop compounds modify the perception of hop derived volatiles. Further sensory interactions have been observed driven by hop oil compounds affecting bitterness intensity and quality.

Oladokun et al. (2017) investigated the impact of the hop variety on perceived bitterness qualities in beer. A trained sensory panel evaluated the bitterness profile of different beers individually hopped with East Kent Golding, Zeus, and Hallertauer Hersbrucker T90 hop pellets using Check-all-that-apply (CATA) and rank-rating sensory tests. CATA is a rapid sensory profiling technique that can be used for product characterisation with a trained panel or with consumers, who are asked to check or uncheck all sensory attributes that describe the sensory profile of the samples (Meyners & Castura, 2014). Hersbrucker hop aroma extract was added post-bottling and was found to cause an increase in CATA frequency of 'harsh' and 'metallic' bitterness in the East Kent Golding beer and an increase of 'citric' and 'progressive/lingering' bitterness in the Hersbrucker and Zeus beers. In a rank rating

study, each of the three base beers with added Hersbrucker aroma extract was perceived as being significantly 'harsher' in bitterness than the Hersbrucker bittered base beer, indicating a 'tingly, rasping, and irritating' sensation. A taste-trigeminal sensation effect was suggested to be promoted by hop oil compounds. Interestingly, the frequency of the 'artificial bitterness' character was reduced for all beers compared to the control beer suggesting a masking effect of 'artificial bitterness' by hop aroma sensations. After spiking the beers with Hersbrucker hop aroma extract, an increased bitterness intensity, lingering bitterness and astringency was found in the Hersbrucker beer compared to the East Kent Golding and Zeus beers. The analytical profiles of bittering substances were found to be similar for all beers and only the polyphenols concentration was slightly higher in the Hersbrucker beer (290 vs 216 and 207 mg/L) (Oladokun et al., 2017). The contribution of the higher concentration of polyphenols and enriched oxygenated sesquiterpenes compounds derived from the Hersbucker hops might have caused the pronounced bitterness and astringent sensations. Since the volatile composition and the sensory aroma profiles of the beers were not published in this study, it would be interesting to explore these to understand the suggested sensory interaction effects.

Overall, several volatile fractions in hop oil are considered to modify bitterness intensity as well as bitterness qualities. The hop oil fractions that were applied mainly comprised of hydrocarbons, terpene alcohols (linalool), and sesquiterpenoids. The effects on bitterness intensity and quality were mainly attributed to the occurrence of cross-modal interactions induced by the perception of the hop oil fractions. Volatile compounds in these fractions have also been suggested to add trigeminal-

type and mouthfeel sensations to beer and to be susceptible to sensory interactions with other beer components.

1.6.5 Linalool

Several researchers found linalool to have an effect on lingering bitterness and bitterness quality. Kaltner and Mitter (2006) attributed the modification of the bitterness perception to different concentrations of linalool and terpene hydrocarbons (myrcene, caryophyllene, humulene). Ratings on 'bitterness harmony' increased for the beer with the highest linalool concentration. In contrast, the lowest linalool concentration resulted in the highest rating for 'mild bitterness'. Scores for 'long-lasting taste of bitterness' and 'bitterness harmony' decreased if the linalool concentration increased above 51 µg/L. The addition of hop oil products containing terpene hydrocarbons and a low concentration of linalool to the beer resulted in the highest ratings for 'harmonious but increasing bitter taste' (102 µg/L) and significantly lower ratings for 'mild bitterness' (13 µg/L). It was concluded that the addition of terpene hydrocarbons decreased the mildness of the bitterness and increased the bitter taste at low linalool concentrations indicating concentration- and matrix-dependent effects (Kaltner & Mitter, 2006). These results suggest cross-modal interactions, however, in order to fully understand the factors that are determining these findings, it would be important to observe the increase/decrease of other compounds present in the added hop oil products, but this information was not provided.

Like Kaltner and Mitter (2006) and Praet, Van Opstaele, Steenackers, De Brabanter, De Vos, Aerts, and De Cooman (2015) observed an effect of linalool combined with further hop derived volatiles on bitterness profiles. Praet et al. (2015) hopped lager beers at different time points in order to investigate de novo formation of sesquiterpene oxidation products. The beer containing the highest concentration of oxygenated sesquiterpenoids and linalool obtained the highest scores for 'spicy/herbal', 'floral/fruity', and 'bitterness quality' in a sensory descriptive evaluation confirming the findings of Kaltner and Mitter (2006) and suggested linalool to be one of the impactful hop oil compounds to have an effect on bitterness qualities in beer. However, the attribute 'bitterness quality' was not further described. Accordingly, it would be interesting to investigate the different effects of linalool in combination with hop oil fractions on defined bitterness qualities in beer.

Further interesting findings were reported by Bailey et al. (2009), who investigated the impact of the harvest date of Hallertauer Mittelfrueh hops on the sensory properties of a dry hopped beer. Hop oil and α-acid concentrations were found to be 30% higher in hops harvested 24 days later than hops harvested at an earlier stage. In order to identify effects on bitterness perception, the dry hopped beers were evaluated using a flavour profiling test and triangle tests. The results suggest that the later the hops were harvested (or the higher the hop oil and α-acids content was reported), the higher the linalool concentration and the scores on 'spicy' aroma notes, 'bitterness intensity' and 'bitterness balance', while the intensity of 'fruity' aroma notes decreased (Bailey et al., 2009). However, further research is required focusing on these correlations and systematically assessing the relationship and

sensory interactions between linalool, *α*-acids, hop aroma sensations, and bitterness intensity and quality to confirm this hypothesis.

1.6.6 Sesquiterpenoids

Further effects on bitterness qualities have been observed when hop extracts comprising of sesquiterpenoids were used for brewing. Goiris et al. (2002) added hop aroma essences - containing all the main oxygenated sesquiterpenes including humulene epoxides - post-fermentation to a non-aromatised pilot pilsner beer which was bittered with isomerised hop extract. The hop essence (20 µg/L) not only introduced a 'spicy' hop flavour, but also resulted in an enhanced 'mouthfeel', 'fullness', and perception of 'bitterness'. It was suggested that synergistic-type interactions occurred between the bitter extract and hop oil compounds and caused the modulation of bitterness perception. In order to investigate this suggested mechanism, sensory descriptive analysis could be used, which should involve the establishment of a detailed attribute lexicon including bitterness quality, mouthfeel terms and the corresponding reference materials. In this way, hop oil compounds involved in cross-modal interactions could be identified.

Similarly, Van Opstaele et al. (2010) found a spicy oxygenated sesquiterpenoid and a polar hop essence to increase 'bitterness' intensity and 'fullness' perception in beer. In contrast, a floral hop essence decreased the bitterness intensity. In a follow-up study, Van Opstaele, Goiris, et al. (2012) added different hop oil essences to non-aromatised pilot-scale lager and observed the spicy essence to increase 'bitterness' intensity, 'mouthfeel' and 'fullness'. Therefore, it appears that interactions between

beer bitterness and hop oil compounds are highly dependent on the composition and polarity of the aroma fractions. However, to date, this has not been further investigated.

Oladokun et al. (2016) provided evidence for the modification of bitterness intensity and quality induced by volatiles in a Hersbrucker Spaet hop extract rich in oxygenated sesquiterpenes. Different levels of hop extract (0, 245, 490 mg/L) were added to beers bittered with iso-α-acids (13, 25 or 42 IBU). Perceived overall bitterness intensity and the intensities of the bitterness characters 'harsh' ('tingly, painful, irritating, raspy bitterness') and 'rounded' ('pleasant, smooth, lingering bitterness') were evaluated using rank-rating tests. At each bitterness level, addition of the Hersbrucker aroma extract caused an increase in mean bitterness intensity ratings, which was statistically significant at the 13 and 25 IBU levels. Nose clips were used to decouple olfactory from gustatory stimuli and mouthfeel sensations that could be related to the beer bitterness. This removed any statistically significant impacts of hop oil addition on perceived bitterness intensity, clearly indicating that the olfactory stimulus was required for the noted enhancement of bitterness intensity. At the high bitterness level, with the panel wearing nose clips, differences in bitterness intensity were again non-significant; however, the panel on average scored higher bitterness intensity for samples with Hersbrucker aroma addition and could reliably differentiate the samples in-mouth. This suggested the stimulation of trigeminal receptors by the hop volatiles (Oladokun et al., 2016). High bitterness levels combined with trigeminal sensations might have caused a taste-trigeminal sensation and the perception of increased bitterness intensity and modified bitterness

character. Furthermore, it was suggested that the addition of hop oil compounds modulated different bitterness characters depending on the bitterness level in the beers. A 'round' bitterness was perceived in low bitterness beers and a 'harsh' bitterness in high bitterness beers. It appears that the impact of hop volatiles on the bitterness qualities depends on the IBU level in the beer (Oladokun et al., 2016). The increase of bitterness intensity and the occurrence of trigeminal sensations were not attributed to specific compounds, but as observed in previous studies, the oxygenated sesquiterpene fraction contributed to sensory interactions.

Oladokun et al. (2016) also investigated the temporal profile of perceived beer bitterness at different concentrations of a Hersbrucker hop extract rich in polar oxygenated sesquiterpenes. TI analysis was used to assess the time course of bitterness intensity for a period of 60 seconds. Aroma sensations induced by hop oil compounds perceived through the retronasal pathway were suggested to have an effect on the temporal bitterness profile of the beers. This was already observed at low iso-α-acid concentrations (Oladokun et al., 2016). The results suggest that the hop volatiles induced a prolonged bitterness, although specific compounds or fractions were not attributed to this sensation in this study. It would be interesting to conduct this analysis using different hop oil fractions or compounds in order to investigate the effect of aroma compound polarity on the temporal perception of bitterness. This is the only study identified in this review that systematically investigated the effect of a hop aroma extract on temporal perception of bitterness, hence further research is required to understand the mechanism behind the temporal effect.

Recently, Mikyška et al. (2018) investigated the impact of kettle hopping and kettle + dry hopping on the volatile composition and sensory profile of beers. Aroma and flavour characteristics of the beers and the effect on the bitterness profiles and lingering bitterness was analysed by a trained sensory panel. The lingering bitterness sensation was rated at 10-second intervals for 120 seconds. Interestingly, the rate of bitterness decay was found to be slower for the majority of kettle + dry hopped beers. Based on this finding, it was suggested that higher concentrations of hop oil compounds, bitter acids, oxidative products of *α*- and *β*-acids, and polyphenols might have caused this effect, which are expected to be extracted at higher levels when dry hopping beer. In addition, GC-MS analysis revealed that kettle + dry hopped beers contained higher concentrations of hydrocarbons (myrcene, *β*-pinene), terpene alcohols (linalool, *α*-terpineol), and slightly increased concentrations of sesquiterpenoids (*α*-humulene, *β*-caryophyllene, *β*-caryophyllene epoxide) independent of the hop variety (Mikyška et al., 2018). Therefore, increased concentrations of *β*-caryophyllene, *α*-humulene, and *α*-caryophyllene epoxide were suggested to be responsible for higher scores for the 'harsher' bitterness in the kettle+dry hopped beers.

In conclusion, several factors could have caused the effect on bitterness qualities and further investigations are required to identify those components that are involved in the mechanism behind this in beer. Since the mechanism appears to be complex and to involve several components, as a first step, a model beer could be created that contains all components that are expected to be involved, for instance by following a 'Sensomics' type approach. In a second step, an omission experiment could be

performed by step-wise excluding components from the model beer, and subsequently evaluating the resulting sensorial impact from this omission.

Further investigations are required to explore the relationship between different chemical classes in hop oil and the occurrence of cross-modal sensations resulting in diverse bitterness characters. Moreover, limited research has been conducted to investigate the impact of the sesquiterpenoid fraction or single sesquiterpenoids on the lingering bitterness sensation or bitterness qualities to identify the key compounds which confer these sensations.

1.7 Reconstitution of beer flavour in model beer systems

Reconstitution or recombination studies usually comprise of four steps: 1) analysis of the volatile composition in a matrix using GC-MS, 2) identification and selection of key volatile compounds based on OAVs in water or concentrations in beer, 3) comparison of chemical reference compounds and the original compounds in the matrix using GC-O, and 4) evaluation of the recombinate in a model matrix using sensory analysis. In this way, it is possible to determine key volatiles that are responsible for the overall aroma sensations in a matrix, to identify aroma sensations that are driven by volatiles at low concentrations or subthreshold level, to detect sensory interactions (e.g. between volatiles at sub- and suprathreshold levels), but also to evaluate the impact of other (non-volatile) components in the matrix on the perception of the mixture of volatiles. To date, only a few recombination studies have been conducted that investigated hop volatiles responsible for hop aroma and flavour in beer (Fritsch & Schieberle, 2005; Langos et al., 2013; Tokita et al., 2014).

Fritsch and Schieberle (2005) conducted a recombination study to test whether it is possible to mimic the aroma profile of a pilsner-type beer (4% ABV) by applying a mixture of 22 chemical reference compounds in carbonated water. Volatiles were selected as reference compounds if their OAV was greater than 1. All compounds were dissolved at concentrations as found in a pilsner-type beer. Key compounds with the highest OAVs were ethanol, (*E*)-*β*-damascenone, (*R*)-linalool, acetaldehyde, and ethyl butanoate followed by ethyl 2-methylpropanoate and ethyl 4-methylpentanoate. The reference compounds were checked and compared with the original compounds detected in the pilsner beer regarding similarity of retention indices and odour qualities using GC-MS and GC-O. A sensory panel evaluated the orthonasal perception of the pilsner beer and the model system and found them to be very similar. The authors suggested that the origin of compounds, the alcohol concentration, and bitter substances had no significant effect on the overall aroma quality and aroma intensity of the beer. This conflicts with several studies which considered these parameters that have been discussed previously in this review.

Equally surprising is that the sensory training was conducted on attributes describing aroma sensations of single reference compounds but not on aroma combinations. Therefore, this suggests that sensory interactions did not significantly contribute to the aroma of the model system or the actual beer. However, the descriptive profile test was conducted by using six general aroma terms on a scale from 0 (no similarity) to 3 (very good similarity) (Fritsch & Schieberle, 2005). Similarity testing or sensory quantitative descriptive analysis (QDA) using a more specific list of terms might result in a different outcome and the disclosure of sensory interaction effects.

Langos et al. (2013) adopted a Sensomics approach by preparing an aroma recombinate using predetermined key volatiles in Bavarian wheat beer. As in the study of Fritsch and Schieberle (2005), compounds with OAVs lower than 1 were suggested not to contribute to the overall aroma of the beer and were excluded. Subsequently, 27 purified chemicals were evaluated at 4% ABV in acidified, carbonated tap water to simulate a wheat beer. Compound concentrations for the recombinate were determined based on their OAVs in isolation. A trained sensory panel evaluated the samples using a pre-defined attribute list to describe different aroma sensations. The recombinate was found to successfully mimic the aroma sensations of a wheat beer and (*E*)-*β*-damascenone, 3-methylbutyl acetate, ethyl methylpropanoate, and ethyl butanoate were determined to be the most potent contributors for the aroma characteristics. The non-volatile fraction was suggested to have little influence on the overall aroma and on aroma release (Langos et al., 2013). As reported in the preceding sections, bittering substances are likely to affect aroma and flavour sensations due to cross-modal interactions with hop volatiles. Since the non-volatile fraction included no bittering substances, the addition of different bitter acids and/or polyphenols may have resulted in a different outcome.

In contrast to previous studies, Tokita et al. (2014) used compound concentrations as the selection criteria for key volatiles. Aiming to reconstitute the characteristic odour sensations of a fruity flavoured pilsner-type beer, a list of 30 key volatiles was determined by comparing the chemical profiles of a pilsner-type control beer and a fruity flavoured pilsner-type beer. The key volatile mixture mainly consisted of esters and alcohols including ethyl acetate, 3-methylbutanol, phenethyl alcohol, 2-

methylbutanol, 3-methylbutyl acetate, and 2-methylpropanpol. The reference compounds were dissolved in the base beer at concentrations equal to the odourants in the original fruity flavoured beer. The outcome of the sensory study showed that the application of the recombinate in the base beer could reconstitute the majority of odour characteristics ('caramel, roast', 'cereal', 'chemical', 'green', 'floral'), but not the 'fruity', and 'sweet' odour notes. The findings of this study demonstrate that an authentic matrix is required if aiming to match specific odour characteristics in beer. As in previous studies, the reason why the fruity and sweet odour profiles could not be matched may have been that mainly general descriptors were used. Even if it is the aim to work with general attributes, the panel should be trained on detailed attribute descriptions and these should be provided to clarify differences between the attributes, for the investigator, the panellists and the reader.

Overall, reconstitution studies are a promising technique to identify key volatile compounds. Nevertheless, the fact that up to 30 reference compounds were required for aroma and flavour recombinates reiterates the complexity of aroma and flavour characteristics in beer. Only compounds having an OAV higher than one were included, further compounds are expected to contribute to the overall aroma, due to synergistic effects occurring between the volatiles. The aroma recombinates reviewed in the studies were applied in water and showed no difference compared to the reference beers indicating that the non-volatile fraction in beer might be more important for cross-modal interactions than for modification of volatile release, but the latter effect should not be neglected. It appears to be questionable whether flavour recombinates are equally successful as aroma recombinates since several

different receptor-types are involved, volatiles are released through different pathways, and sensory interactions are likely to occur at different levels due to other components present in the beer matrix, as is recognised in this review.

1.8 Prediction of the hop flavour intensity in beer

Partial Least Squares (PLS) regression analysis is frequently used to study relationships between sensory and physico-chemical characteristics in foods and beverages. Briefly explained, PLS is used to build regression models between independent and dependent variables by extracting linear combinations of one set of variables to predict the variation in another set of variables expressed as mathematical functions (Cozzolino, Cynkar, Shah, Dambergs, & Smith, 2009). This approach enables for instance the modelling of flavour profiles, i.e. prediction of flavour intensities or scores, based on the quantified volatile composition in the sample matrix.

To date, only one study has been published that investigated the predictability of the 'hoppy' flavour while focusing on the 'fruity-citrus' intensity of beers that were dry-hopped with Mandarina Bavaria (Machado Jr et al., 2020). Machado Jr et al. (2020) proposed an equation for the estimation of the sensory perception (i.e. the intensity score) of 'total hoppy', 'citrus', 'green fruit', and 'sweet fruit' flavours in the two different beer samples. The equation was based on data obtained from a trained sensory panel that assessed the beer samples on a scale from 0 to 5 following a QDA approach and obtained from HS-SPME-GC-MS analysis conducted to quantify 24 selected volatiles during a 15 days dry-hopping period. The volatile compounds were

selected based on previous research where these volatiles were most frequently associated with 'hoppy' flavour, but also to cover the main chemical classes described for the Mandarina Bavaria hop. For instance, the intensity of the 'total hoppy' flavour could be estimated by an equation including the compounds myrcene, 2-methylbutyl-2-methylpropanoate (2MB2MP), linalool, and α-humulene, and perfectly demonstrates the complexity of the volatile group behind a single flavour sensation associated with the 'fruity-citrus' flavour dimension associated with the overall 'hoppy' flavour in beer.

The researchers indicate that the majority of volatiles were present at concentrations above their threshold levels. However, this was not the case for α-humulene although it was suggested as a key contributor compound in the model. Surprisingly, other compounds present at supra-threshold concentration such as geraniol were not important to the model. As stated by the researchers, model data should not be used to identify direct cause and effect relationships but implies associations between volatile groups and sensory characteristics (Machado Jr et al., 2020). Moreover, as discussed previously, differences in physico-chemical parameters between matrices and the occurrence of sensory interactions should be taken into account when evaluating the explanatory power of regression models. These factors can cause pronounced nonlinearity in the data and weaken the model (Cozzolino et al., 2009). It has to be noted that, due to the dry-hopping design, replicates of the beer samples have not been assessed in this study and it is not clear whether or not the panel performance data was taken into account when building the regression models (Machado Jr et al., 2020). Further research is required to explore different types of

regression techniques as a tool to predict single or multiple sensory dimensions associated with multi-sensory perception of 'hoppy' flavour in beer.

1.9 'Sensory best practice' for the sensory analysis of hop essential oil

In contrast to the instrumental analysis methodologies reported in the reviewed publications, which are often highly detailed, papers in the brewing literature are surprisingly limited with regard to sensory evaluation protocols and methodologies. The importance of adequate panel training and panel management has frequently been highlighted (see e.g. Bamforth, Russell, and Stewart (2011), Rogers (2017)), but is often overlooked in the field of hop or brewing research, with only a few studies providing data regarding panel training and performance.

Internal and external panellists should be sufficiently trained prior to the sensory evaluation. However, the level of training can be deemed as void if the panellists have not been tested regarding their sensory abilities, and potential anosmia for key compounds. Even if anosmics cannot be identified, it should still be taken into account that the majority of individuals show high sensitivity for certain compounds and low sensitivity for others (Meilgaard et al., 1982) as discussed above (e.g. *β*-ionone). In order to check the suitability of potential assessors, they should undergo a screening based on their general health, sensory, discriminative, and descriptive abilities (Rogers, 2017).

Another part of the experimental design of sensory studies that often lacks information is the attribute list or sensory attribute lexicon used in the research studies. When establishing an attribute lexicon for sensory descriptive analysis, there are clear advantages in including specific attributes and descriptions, detailed description of references, their preparation, and presentation. Detailed information facilitates the interpretation of the study outcome, but also the reproducibility of the study in view of follow-up research. Overall, it has been found that it is easier for the panellists to recognise and remember flavourings and foodstuffs rather than chemical compounds in clear solution (Schmelzle, 2009). Where chemically isolated compounds are used as references, they should be obtained, purchased or produced to the highest possible purity and the purity should be reported (Meilgaard et al., 1982). If applying volatile combinations (as in hop oil fractions), and assuming the occurrence of compound or sensory interactions that might result in newly formed sensory characteristics (configural processing), it is recommended to develop the attribute list together with the sensory panellists rather than pre-defining the terms and training with chemical references.

In order to ensure that assessors are testing and evaluating all samples in the same manner and that reliable, meaningful data is obtained, concise smelling and tasting protocols should be developed and practiced during the training period. Considering that hop oil compounds are highly volatile, small differences can cause large deviations in the results. Tasting protocols are particularly important for the evaluation of lingering sensations in a defined time span. If tongue movement, mouth closure, periods between taking the sips and swallowing, and the number of sips have

not been predetermined, it is likely that the volatiles are released and perceived at different time points and intensities, which will have a significant effect on the sensory data (Hort et al., 2017; Oladokun et al., 2016). Panel training on the attributes, scale usage, and evaluation protocols should be conducted until sufficient consensus is obtained. Panel performance can be examined during the training period by conducting mock evaluations that follow the protocol of an actual evaluation session.

Panel performance monitoring still plays an important role after completion of the sensory evaluation. By obtaining and providing performance data on the evaluation results, the reliability of the data can be established, the study can be replicated, but also (and most importantly), the outcome of a study can be fully interpreted and understood. In some publications, previous experience in a related field or the number of training hours was mentioned to justify the suitability of the individuals as sensory assessors. However, for the reasons set out above, this should not be seen as justification or evidence for the quality of data. The robustness of sensory data highly depends on the effectiveness of every single panellist and should be evaluated for all attributes separately (Rogers, 2017). As reported by Sharp, Qian, Clawson, et al. (2017) and Vollmer, Algazzali, and Shellhammer (2017), interactions between panellists and panellist x replicate interactions can be obtained by analysis of variance (ANOVA) using a mixed model on the descriptive analysis results. Additionally, interaction plots should be interrogated to graphically illustrate the performance of the panel for specific attributes and to highlight significant interaction effects.

Another factor that affects the robustness of sensory data is the number of replicates. The evaluation of samples in duplicate or ideally in triplicate by each assessor is essential in order to generate robust data from a statistical point of view (Lawless & Heymann, 2010). Also important in this regard is the panel size, which should include 8-10 assessors for sensory descriptive quantitative analysis, while other sensory methods require different numbers of assessors in order to reach significance (Lawless & Heymann, 2010; Stone, Sidel, Oliver, Woolsey, & Singleton, 2008). Some of the reviewed studies mentioned that not all assessors attended each evaluation session suggesting that each of the assessors evaluated not all samples indicating that an incomplete block design was applied. This has to be taken into account if interpreting the data based on the experimental design that was used.

Complete or incomplete balanced block designs are used if multiple products are compared where all panellists evaluate all samples or all levels of treatment variables within each block. The complete balanced block design approach should be preferred since an efficient and powerful partitioning of panellist variance can be achieved (Næs, Brockhoff, & Tomic, 2011). Incomplete designs are sometimes used if the number of samples is too large to be tested by each assessor in one block. In this case, each assessor only evaluates a subset of the samples (in each block) and the subsets change for each assessor. Balanced incomplete block designs should be used to ensure that all samples and all sample-pairs appear the same number of times to avoid the introduction of experimental bias and order effects which could negatively affect the statistical robustness of the dataset (Gacula, Singh, Bi, & Altan, 2009).

Finally, the experimental design of the actual sensory evaluation should be carefully planned. Many factors can influence how the trained sensory panel perceives and evaluates samples containing highly volatile compounds. The most important factors are briefly summarised in Figure 1.4.

For instance, depending on the study aim, samples are evaluated at different temperatures. Beer samples are evaluated at cooler temperatures in the majority of published studies while samples that are evaluated to characterise single hop oil compounds or fractions in model systems (usually with trained panels) are mostly evaluated at ambient temperatures to avoid temperature changes during the testing period (Peltz & Shellhammer, 2017) and ensure that aroma sensations are maximised (Sharp, Qian, Shellhammer, et al., 2017). At lower temperature, compounds volatilise less readily above the tongue before the sample is swallowed, causing reduced flavour sensations (Palamand, 1969). In either case, one should be aware of temperature changes, which might influence the perception of the volatiles.

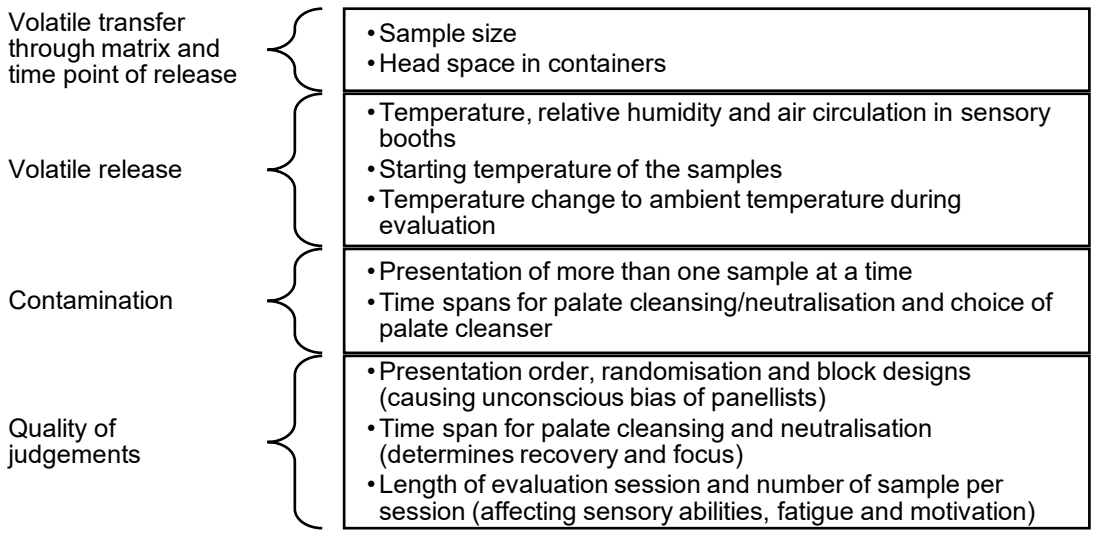

Figure 1.4. Factors influencing the perception of volatiles during sensory evaluation (based on Taylor (2002) and Taylor and Roberts (2008)).

1.10 Summary

Compounds in hop essential oil have long been suspected to contribute to a multisensory experience perceived when drinking a beer. To date, it appears that less than half of the compounds in hop oil have been identified and quantified; those quantified include the majority of compounds present at higher concentrations. Several compounds in the sesquiterpenoid, alcohol, ester, ketone, and aldehyde fractions, as well as sulphur-containing compounds have been identified as marker volatiles for certain hop varieties and associated with specific aroma and flavour sensations. Nevertheless, the full sensory potential of hop oil volatiles can only be understood if going a step beyond quantitative and qualitative analysis of hop derived volatiles in isolation.

Sensory analysis has largely been neglected and only during the last two decades have researchers attempted to systematically combine sensory and instrumental methods. Recent advances in our knowledge of the concentration- and matrix-dependent perception of hop derived volatiles and sensory interactions between hop volatiles and with other beer components have been made using dynamic headspace techniques, temporal sensory methods, and reconstitution studies. It was found that ethanol and carbonation levels affect polarity and volatile retention or partitioning and consequently the delivery of volatiles in the breath. In addition, hop acids have been found to modify perceived aroma and flavour characteristics and intensities of the sensations imparted by hop oil volatiles. In turn, hop oil compounds also affect the perception of bitterness intensity, quality, and persistence. Moreover, the co-existence of hop derived volatiles and bitter extracts at specific ratios caused the

perception of mouthfeel and trigeminal-type sensations. Since the majority of such findings were incidental discoveries, much more remains to be explored in order to systematically understand the sensory properties of hop derived volatiles in beer, beyond the scope of hoppy aroma and flavour.

1.11 Future perspective

It has frequently been found that the perception of hop derived volatiles cannot exclusively be explained based on their concentrations in a matrix or their threshold concentrations. Sensory interactions involving compounds below detection or threshold levels (e.g. sulphur containing compounds, oxygenates, terpene hydrocarbons) complicate the association of single volatiles in a complex mixture with specific sensory sensations. It appears to be more important to unravel the sensory characteristics induced by volatile compound mixtures rather than to identify a set of isolated 'key' compounds that are assumed to contribute to a sensory sensation.

The investigation of hop volatiles or fractions in simplified model solutions appears to be a suitable first approach to unveil multisensory interactions. Experimental designs should also pay attention to physico-chemical processes occurring in the test matrix as well as to dynamic sensory analysis. Subsequent investigation of the perception of hop volatiles in 'real' beer matrices should back up the data of studies evaluating simple model systems.

The outcome of instrumental methods gains more meaning when combined with sensory analysis. Novel approaches combining instrumental and sensory analysis,

such as GC-O (AEDA)-OASIS (Original Aroma Simultaneously Input to the Sniffing port) (Hattori, Takagaki, & Fujimori, 2005), Olfactoscan (GC-O coupled with a multi-channel dynamic dilution olfactometer) (Burseg & de Jong, 2009), GC-GOOD (global olfactometry omission detection) (Hallier et al., 2004) or GC-R (recomposition) (Johnson et al., 2012) should be considered for the identification of key volatile mixtures. These methods have already been applied to identify key odourants in different food matrices, but not as yet in the field of hop research. *In vivo* data (nose space measured during consumption) can be collected while drinking a beer to quantify the delivery of volatiles through the retronasal pathway experienced during consumption. Different components of the beer matrix can have significant effects on the partitioning of volatiles under dynamic conditions as during consumption (Clark et al., 2011). By combining sensory and instrumental techniques that enable the analysis of volatiles in static and dynamic conditions, it might also be possible to identify matrix-dependent sensory interactions between hop derived volatiles and beer components.

Chapter 2A

2 A. Sensory properties of supercritical CO_2 fractions extracted from Magnum hop essential oil

This chapter is based on:

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Sensory properties of supercritical CO_2 fractions extracted from Magnum hop essential oil

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Keywords: Hop aroma; hop oil fractions; sensory descriptive analysis; sensory interactions

Highlights

- Sensory and physico-chemical characteristics of Magnum hop oil fractions in comparison to a total Magnum oil were assessed.
- Hop-derived volatiles induced a range of aroma sensations in a model solution at 4% ABV.
- Additionally, the volatiles modified the perception of taste and mouthfeel sensations, such as sweetness and bitterness.
- Sensory interactions were suggested to be responsible for these effects.

Abstract

Hop oil fractions with unique sensory characteristics can be extracted from hop essential oil using green solvents such as supercritical (sc) CO_2. These extracts meet clean-label requirements and can be used to manage fluctuations in volatile composition caused by global warming. A sensory descriptive analysis approach was applied to assess the sensory profiles of Magnum hop oil and five scCO_2 fractions. Ten sensory panellists were trained and used to establish an attribute lexicon. All samples, a control, and an experimental replicate were evaluated at 800 µg/L in ethanol (4%, ABV) in triplicate. Data was analysed by three-factor Analysis of Variance (ANOVA) and Tukey's test (HSD). Volatile compounds were determined using gas chromatography-mass spectrometry (GC-MS). Relationships between the volatile compounds and sensory profiles were analysed using Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression. In contrast to the majority of fractions, the total oil (the most complex sample) and the sesquiterpene fraction (as the largest chemical group in the total oil) were not described by any key sensory attributes. This illustrates the advantage of hop oil fractionation to pull out specific sensory characteristics. The *β*-myrcene in the myrcene fraction induced an intense "crushed grass, sap" aroma while the fractions containing several geranyl and methyl esters and ketones were characterised by fruity- and floral-type aroma and flavour attributes. Interestingly, the most polar fraction comprising of terpene alcohols delivered a complex sensory experience by adding sweetness. Moreover, a trigeminal "peppery tingling" sensation was detected, which is likely caused by sensory interactions.

2.1 Introduction

Volatile compounds in hops and hop essential oil are recognised as one of the major contributing components that determine the sensory perception of beer (Rettberg et al., 2018). Hop essential oil has been suggested to be the most complex essential oil in plants due to the diversity and number of volatiles present (King & Dickinson, 2003) and is mainly composed of hydrocarbons (mono- and sesquiterpenes), esters, ketones, aldehydes, terpene alcohols, and sulphur-containing compounds. Mono- and sesquiterpenes including the most abundant compounds β-myrcene, α-humulene, and β-caryophyllene account for around 80% of hop essential oil depending on the hop variety. The remaining volatiles are present at up to 1000x lower concentrations compared to the terpene hydrocarbons (Almaguer et al., 2014; Rettberg et al., 2018; Schönberger & Kostelecky, 2011). It has been proposed that more than 1000 volatile compounds are present in hops, including a large amount of compounds at trace levels (Eyres & Dufour, 2009). Some of these compounds are likely to be present at sub-odour threshold levels, but might still contribute to the perceived overall aroma and flavour profile depending on the co-presence of other volatile and non-volatile compounds and on sensory interactions between these (Hanke et al., 2010; Takoi, Itoga, et al., 2010).

Hop oil products have been added to beer for decades and the time point of addition in the brewing process determines the final composition of the volatiles or hop aroma compounds, which in turn contributes to the perception of the sensory profile (Howard & Slater, 1957). However, the perception is also affected by physico-chemical properties of the matrix in which the hop oil products are applied, such as

interaction with the components of the matrix. These properties determine the retention and release of the volatiles (Taylor, 2002). Different research approaches have been applied to understand the aroma and flavour contribution of hops in beer which mainly included the correlation of quantitative and descriptive data obtained by gas chromatography-olfactometric (GC-O), different mass spectrometry (MS) and flavour threshold determining techniques. The focus of hop oil analysis has largely been on instrumental profiling whilst somewhat neglecting the sensory evaluation of the volatiles in a realistic composition as naturally present in hop essential oil. Studies have since shown the importance of sensory descriptive analysis to understand aroma and flavour sensations of the hop oil compounds in different beer matrices (Goiris et al., 2002; Lafontaine & Shellhammer, 2018b). Multivariate statistical methods including principal component analysis (PCA) and partial least square (PLS) regression are used to explore the relationship between sensory data and the chemical composition of mixtures of volatile compounds (Yu, Low, & Zhou, 2017). However, the correlation between volatiles in hop oil or hop oil fractions and attributes describing sensory characteristics of hop oil fractions by PLS regression has not yet been conducted.

Due to current and future challenges, arising from global warming and climate fluctuations (Ray, Gerber, MacDonald, & West, 2015), human interventions causing competition for agricultural areas (Harvey & Pilgrim, 2011), legislation (Demyttenaere, 2018), and consumer demands (Roman, Sánchez-Siles, & Siegrist, 2017), hop oil products are gaining more interest and are challenging traditional hop products and material intensive hopping techniques. It has been shown that warm

climate and decreased levels of precipitation significantly affect hop harvest yields as well as essential oil content and composition in the hops (Gahr, 2018). Hop varieties that are usually used for bittering purposes because of their high alpha-acids concentrations (occasionally called “alpha cultivars” or “bitter hop varieties”) have been found to be more resistant to changing weather conditions compared to “aroma hop varieties” containing higher concentrations of aroma-active compounds (Gahr, 2018). The use of hop oil fractions extracted from resistant hop varieties may be desirable to balance out inconsistencies in hop oil compositions and to standardise hop flavour profiles in beer.

Advanced methods have been developed to produce natural, “clean label” hop products for the brewing and beverage industry that have standardised and novel hop flavour profiles. Supercritical (sc) CO_2 is used as it is a green, non-polar solvent and reduces or replaces conventional organic solvents that are regulated as volatile organic compounds (VOC) which is highly desirable as maximum residual levels of VOC are defined by EU legislation (2009/324/EC, 2009; 2010/59/EC, 2010) and the solvents have to be disposed of in an environmentally safe manner, which is expensive and involves considerable effort. VOC solvents can also cause environmental problems such as atmospheric and land toxicity. CO_2 is considered an organically certified non-polar solvent that enables the production of clean label products.

Using supercritical CO_2 brings some challenges such as relatively high equipment and operation costs compared to conventional solvents and plants and the high critical CO_2 pressure applied that needs to be well controlled and requires safe and isolated

storage of the CO_2 source. Further challenges are related to the extraction capabilities of supercritical CO_2 that are limited by its polarity and preventing the extraction of less soluble compounds. Moreover, extraction conditions have to be tailored to the compounds of interest to minimise co-extraction of compounds. In order to increase the solubility of high molecular weight compounds and extraction selectivity or fraction separation, conventional co-solvents such as ethanol can be added at different percentages to form supercritical fluids having an improved solvent power due to higher diffusion capability and lower viscosity and surface tension (Díaz-Reinoso, Moure, Domínguez, & Parajó, 2006; Baldino & Reverchon, 2018).

By separating hop oil compounds from the bittering substances and selectively extracting hop oil fractions based on their molecular polarity, it is possible then to obtain different volatile mixtures (Marriott, 2019). However, to date, only few publications have focused on the different profiles of volatile aroma compounds in hop essential oil and hop oil fractions extracted using $scCO_2$, and only limited attention has been given to sensory sensations in hop oil fractions other than those describing aroma and flavour (Goiris et al., 2002; Van Opstaele, Rouck, Clippeleer, Aerts, & Cooman, 2010).

It was hypothesised that by fractionating hop essential oil it may be possible to create hop oil fractions with novel or moderated aroma and flavour properties. Moreover, the assessment of the hop oil fractions in a simple model solution may facilitate the detection of the sensory potential in these fractions and the relationships between their volatile composition and sensory properties.

Therefore, the aim of this study was to define the sensory characteristics of a Magnum hop oil and five $scCO_2$ fractions in ethanol (4%, ABV) using a sensory descriptive analysis approach to determine olfactory, gustatory and trigeminal differences among the hop oil and the fractions. A sensory attribute lexicon was developed to describe the different sensory sensations in the samples. In addition, volatile compounds in the different hop oil samples were identified and semi-quantified using GC-MS, and were characterised regarding their molecular polarity. Finally, PLS regression analysis was not only used to investigate which hop oil compounds may be involved in different sensory sensations in the samples but also to evaluate the predictability of certain sensory characteristics and sensory interactions in complex hop oil fractions since this has not yet been explored.

2.2 Materials and methods

2.2.1 Fractionation of Magnum hop essential oil

Hop oil was obtained by distillation from hop pellets (Marriott, 2019) from a Magnum hop variety cultivated in the Hallertau growing region in Germany. The hop oil was fractionated using CO_2 in liquid and supercritical form (pressurised and heated above its critical point at 31^{o}C and 7.38 Mpa; density: 0.469 g/cm^3) as the non-polar solvent and ethanol as the polar co-solvent, as described by Marriott (2019). The hop oil was coated onto an inert support for sequential extraction at 10-20 % (m/m). By applying increasing temperature-pressure combinations ranging between 70-300 bar and 5-45°C that determined the density of the extraction fluid, five fractions were extracted

mainly comprising of 1) myrcene, 2) sesquiterpenes, 3) esters, 4) ketones, and 5) terpene alcohols. With increasing CO_2 density it is possible to extract those compounds that have a high molecular weight (MW). Thus, temperature and pressure conditions were adjusted depending on the polarity and MW of the compounds present in these fractions. Less polar compounds with MW<250, such as esters, ketones, and epoxides, were extracted at lower pressure and more polar compounds with MW 250-400 (e.g. sesquipterpenoids) at higher pressure. The temperature was kept between 5-45°C to avoid degradation of thermolabile compounds. The total hop oil and the fractions were flushed with nitrogen and stored at 4°C. The myrcene fraction was stored at -20°C.

2.2.2 Sensory evaluation

Prior to the start of the sensory evaluation, ethics approval was sought and granted by the Faculty of Medicine & Health Sciences Research Ethics Committee at the University of Nottingham (Ethics Reference No. 88-1707). Informed consent was obtained from all candidates to confirm their awareness of the presence of alcohol in the solutions and their willingness to take part. Information on the nature of the study was kept to a minimum in order to reduce potential bias.

2.2.3 Preparation of samples

Stock solutions of the hop oil/fractions were prepared in food grade ethanol (96%, ferm, fa, F200481, Haymankimia, UK). All stock solutions were stored at 4°C for the period of the study. Samples for sensory evaluation (total oil/fractions in EtOH/H_2O) were prepared by dissolving the stock solutions in EtOH/H_2O (purified water; 18.2

MΩ cm, 22°C) to obtain solutions containing 800 µg/L hop oil/fraction and 4% ABV. All samples were evaluated at 800 µg/L in order to achieve a general understanding of the sensory characteristics of the fractions at equi-concentration. This was also the concentration at which the panellists were able to provide sufficiently detailed descriptions to the attributes especially those describing subtle sensations. The solutions were mixed on a roller bed for 30 min after preparation. New solutions for screening, training, and evaluation were prepared 48 h prior to the sensory sessions and were stored at 4°C overnight before use. The solutions were taken out of the fridge 4 h prior to the sessions and then mixed for 30 min on a roller bed. 30 mL aliquots were transferred into 60 mL amber glass bottles with screw top caps labelled with randomly assigned 3-digit codes and were kept at room temperature (22±2°C) prior to testing. All solutions and bottled samples were prepared in a fume hood in a food-safe environment.

2.2.4 Sensory panel

The sensory characteristics of the hop oil/fractions were identified and quantified by an external sensory panel following a modified Quantitative Descriptive Analysis approach (Stone et al., 2008). The panel consisted of ten panellists (5 female and 5 male, mean age 49.3 years, age range 29-64 years). Recruitment and selection of the panellists was based on a three-stage screening procedure (see Figure 2.1.) and included a web-based pre-screening to request information on demographics, general health, allergies, intolerances, medication, pregnancy, smoking, average beer consumption, native language, and availability. The questionnaire was completed by 370 candidates. A basic screening session following the principles of the ISO standard

8586:2012 (ISO, 2012) was conducted with 29 candidates in order to select candidates with good sensory abilities including basic smell and taste detection, descriptive, and discriminative abilities. A second, advanced screening session was conducted with 17 candidates to check for specific anosmias to the compounds in the hop oil fractions, for the ability to communicate sensory descriptions of these compounds in ethanol solutions (4%, ABV), and to express and discuss the identified differences between sensory characteristics in a group discussion. The screening took place in the sensory training facilities in the Sensory Science Centre at the University of Nottingham. The panellists were asked not to eat or drink any food or liquids other than water at least 1 h prior to each sensory session.

2.2.5 Panel training

As displayed in Figure 2.1. after recruitment of the sensory panel (n=10), the next steps included the establishment of an attribute lexicon and the training of the panellists on the identification and quantification of the sensory characteristics in the samples. 24 training sessions of 120 min each were required for attribute generation and consolidation (6 sessions). Subsequently training was performed for discriminative ability and reproducibility (18 sessions) including two mock evaluation sessions to analyse the performance of the sensory panel.

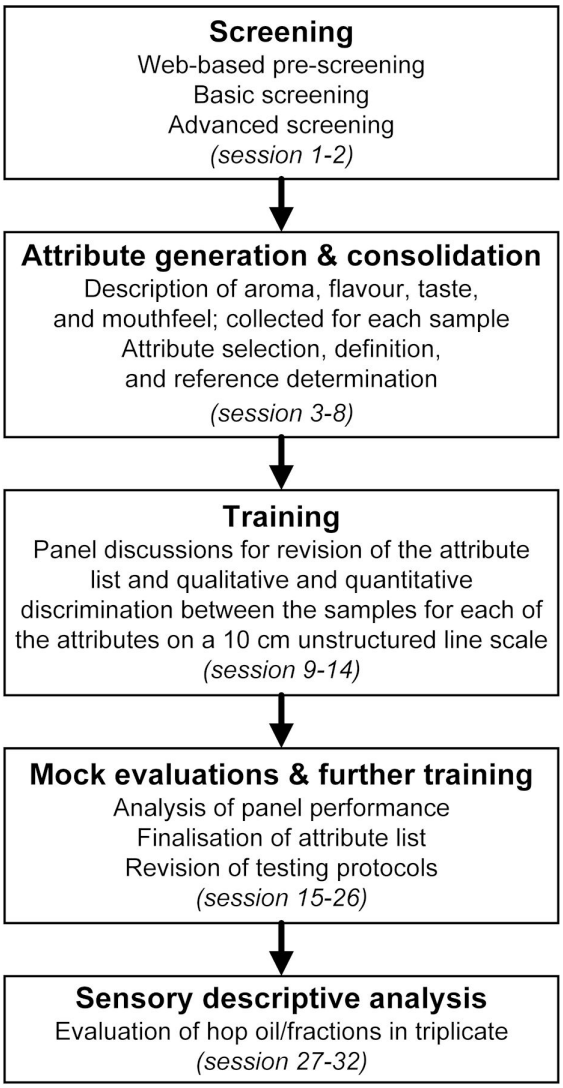

Figure 2.1. Flowchart describing the path to establish a sensory attribute lexicon for the evaluation of sensory profiles of hop essential oil and hop oil fractions using a sensory descriptive analysis approach. Each session lasted approximately 2 h.

First, the panellists were asked to freely generate a list of aroma (orthonasal only), flavour (retronasal flavour), taste (five basic tastes), and mouthfeel attributes (tactile sensations during and after swallowing) by comparing and describing all hop oil/fraction solutions at different concentrations as well as a control solution (pure EtOH/H_2O). The aim was to collect those attributes that the panellists were able to identify on their own. In the second step, Check-all-that-apply (CATA) tests (Delarue, Lawlor, & Rogeaux, 2014) using all samples at 800 µg/L in ethanol (4%, ABV), were

performed to consolidate the list of attributes and identify those that were overlapping and most describing and discriminating between the samples (Delarue et al., 2014).

Attribute descriptions were compiled in group discussions in which the panellists were provided with several reference materials for each of the attributes. Furthermore, reference materials and hop oil/fraction solutions at different concentrations were provided to aid the understanding of the attributes, to clarify the meaning of the attribute definitions, and to facilitate the evaluation of the perceived intensities (Civille & Lyon, 1996). Quantities of reference materials that were selected for the attribute lexicon refer to “very strong” intensities of the attributes in the hop oil/fraction samples and the control sample. The overall aroma intensity had no physical reference and the meaning and quantification were discussed until consensus was achieved across the panel. Panellists were trained on the evaluation of the attributes on a 10 cm unstructured line scale anchored at the extremes by “no sensation” and “very strong”.

In order to improve their discriminative abilities and to detect subtle differences between the samples, several rank-rating tests were performed and the outcome was discussed in group discussions moderated by the panel leader. In view of the final evaluation of the samples, an attribute order was defined by the panel following the chronological order in which the sensations were perceived resulting in eight attribute sets (Table 2.1.). Smelling, tasting and palate cleansing protocols were developed based on panellists’ comments and performance.

Training continued until the outcome of the rank-rating tests and the mock evaluation sessions confirmed adequate discriminative abilities and reproducibility confirmed by assessing intra- and inter-panellist variability. Performance during the panel training period was mainly assessed using eggshell, *p**MSE (from one-way ANOVA), and Tucker-1 plots computed with the PanelCheck (v1.4.2) software. *p**MSE plots were used to evaluate sample discrimination with *p*-values plotted along the *y*-axis and repeatability with MSE values plotted along the *x*-axis. Tucker-1 plots were used to evaluate the panellists' consensus with points accumulated on the outer circle indicating agreement. Eggshell plots were used to evaluate how each panellist ranked the samples in relation to the panel consensus (Tomic, Luciano, Nilsen, Hyldig, Lorensen, & Næs, 2010). For the mock and formal evaluation sessions, Mixed Model Analysis of Variance (ANOVA) was additionally performed on the attribute ratings using the Excel Add-on XLStat (v.19.01; Addinsoft, US) as described in Kemp, Hollowood, and Hort (2011) and Lawless and Heymann (2010). The plots generated with PanelCheck and visual inspection of ANOVA interaction plots (for magnitude and crossover interacitons) enabled the rapid examination of panellists' strengths and weaknesses and panel consensus and helped to identify attributes that needed further training and clarification to design subsequent training sessions accordingly.

Table 2.1. Attribute sets and order for the sensory evaluation and time points of sample provision.

Fresh samples provided	Attribute set	Attributes in order of sensory evaluation
1	1	Soapy Musty Pine wood
	2	Resinous Orange citrus fruit Artificial lemon
2	3	Earthy Crushed grass, sap Fresh lemon Grapefruit zest
	4	Overall aroma intensity
3	5	Astringent
	6	Rose water Alcohol Bitter
4	7	Lingering bitterness
	8	Peppery tingling Sweet Sour

2.2.6 Sensory descriptive analysis

Sensory evaluation was carried out according to the guidelines and conditions detailed stated in the ISO 8589-2007 (ISO, 2007). The total hop oil, five hop oil fractions, a control sample and an experimental replicate (total oil) were analysed in triplicate on a 10 cm unstructured line scale by all panellists (n = 10) over four sessions of approximately 90-100 min each. Each panellist evaluated six samples per session in order to comply with the ethical considerations regarding alcohol intake (less than 1 UK alcohol unit per session) and to prevent fatigue. Samples were presented monadically in a randomised and counterbalanced order (Latin Square Design) to reduce first order and carryover effects (Stone et al., 2008).

All samples were presented at room temperature (22±2°C) to avoid temperature changes which could affect the perception of different sensations. Four bottles of each sample were provided and the panellists were asked to use a fresh sample for certain sets of attributes to ensure that they could evaluate subtle aroma sensations before the aroma-active compounds volatilised (Table 2.1.). The scales for all attribute sets were simultaneously displayed with CompusenseCloud on a screen together with the corresponding attribute descriptions. Breaks of 40 s after each attribute set, 120 s before provision of the next bottle, and a 10 min comfort break after the third sample was enforced to avoid carryover effects and fatigue. During the breaks, the panellists closed the bottles, and followed the neutralisation or palate cleansing protocols where they smelled the back of their hands or the glass of water or cleansed their palate with water, a piece of honeydew melon and more water. All palate cleansing materials were served at room temperature.

2.2.7 Gas chromatography-mass spectrometry

Volatile compounds in the total hop oil and five hop oil fractions were analysed using a gas chromatography-mass spectrometry (GC-MS) method. A Thermo Scientific system (TRACETM 1300; Massachusetts, USA) equipped with a Zebron ZB-5MS capillary column (30 m x 0.25 mm ID x df = 0.25 µm; Phenomenex, Torrance, USA) coupled to a single quadrupole mass spectrometer (ISQ QD Thermo Scientitic Inc.; Massachusetts, USA) was used which was operated in a positive electron ionisation mode. The analysis was carried out using helium as a carrier gas at 1 mL/min flow rate operating in split mode (1:50). The temperature of the injector, ion source and interface were 250°C, 240°C, and 250°C, respectively. The oven temperature was

programmed from 60°C at an increasing rate of 5°C/min to 240°C. The detector temperatures were held at 250°C. Hop oil/fractions (10 µL) were diluted into 1 mL iso-octane (≥99%; Thermo Fisher Scientific, Loughborough, UK) and 1 µL of the aliquot was directly injected using an autosampler.

Peak identification was conducted by comparing peak areas and mass spectra of external standards to those in the samples, where available including: *endo*-borneol (≥97%), caryophyllene oxide (≥99.0%), geraniol (≥99%), geranyl acetate (≥99%), geranyl isobutyrate (≥97%), geranyl propionate (≥95%), linalool (≥97.0%), methyl decanoate (≥99%), methyl geranate (≥94.0%), methyl octanoate (≥99%), *α*-humulene (≥ 96%), β-caryophyllene (≥98.5%), *α*-terpineol (≥97%), *β*-myrcene (≥90.0%), *β*-pinene (≥99%), 2-dodecanone (≥97%), 2-nonanone (≥99%), 2-tridecanone (≥97%), and 2-undecanone (≥98.0%), all purchased from Sigma Aldrich (UK). Retention indices (RI) of the volatiles were determined by using a homologous series of n-alkanes (C6-C30; Sigma-Aldrich, St. Louis, MO). In addition to compound identification with authentic standards, volatiles were identified by library matching using the NIST Mass Spectral library (NIST08) and Wiley7n.1 (Hewlett-Packard, US) databases. Only those compounds are included, which have a MS fit factor ≥ 800 and literature RI similar to the calculated RI.

2.2.8 Data processing and statistical analysis

For the sensory data, three-factor Mixed Model ANOVA (panellist, sample, replicate) and two-way ANOVA (panellist, sample) including the corresponding two-way interactions as explanatory variables were conducted on all sensory attributes to

examine the panel performance. Significant effects of samples, and non-significant effects of sample x panellist and sample x replicate interactions indicate satisfactory panel performance. Analysis of sensory data was conducted by two-way ANOVA (sample as fixed factor and panellist as random factor) followed by Tukey's Honest Significant Difference (HSD) test for pairwise multiple comparisons at 95% confidence interval to determine significant differences between samples at $p \leq 0.05$ for each attribute.

PCA was conducted on the average scores of the attributes to detect relationships between the samples and the attributes in a sensory perceptual space. Average peak areas of the volatile compounds in the hop oil samples that were detected in the GC-MS analysis were calculated from the three replicate injections. ANOVA followed by Tukey's HSD was conducted to identify significant compound concentration differences among samples. Relative percentages of the compounds were obtained by peak area normalisation (PAN) relative to the total area for all peaks in the chromatogram. The sensory (scores) and instrumental datasets (areas) were standardised (1/standard deviation) and analysed by PCA. Standardisation was conducted to allow for all variables to have equal influence in the PCA model despite differences in their numerical range. In this way, compounds present at low concentrations had the same possibility to contribute to the models as compounds present at high concentrations. Significant correlations between sensory attributes and relative compound concentrations were further identified using Pearson's correlation coefficients ($p \leq 0.05$).

The logarithm of the octanol/water partition coefficient (Log*P*) was used as an indicator for the polarity or hydrophilicity of the compounds and was predicted using the EPIWEB 4.1 software (EPI Suite TM, US). The sample Log*P* was calculated on the basis of the relative contribution of the individual compounds in the total oil/fractions. PLS regression was performed with the relative peak areas of the volatile compounds obtained from the GC-MS analysis as the independent variable (*X*-matrix) and the average sensory scores and samples as the dependent variables (*Y*-matrix) to model the relation between these two variables. PLS1 was applied for the correlation between individual sensory attributes and volatile compounds. PLS2 was performed to illustrate correlations among the GC-MS data, the hop oil samples and the complete sensory attribute list of the attribute lexicon. Estimated regression coefficients were derived from jack-knife uncertainty tests. Data analyses were performed using XLStat 2017 (v.19.01; Addinsoft, US).

2.3 Results and discussion

2.3.1 Sensory evaluation

Attribute generation and validation

More than 290 attributes were initially generated by the panellists which were initially consolidated down to 35 aroma and flavour attributes, four taste and three mouthfeel attributes. The list also included attributes that were generated for more than one modality i.e. to describe both aroma and flavour sensations. Based on the outcome of the CATA tests, 13 attributes were excluded as panellists could not

anymore identify the attributes in the samples. A number of attributes was further removed in subsequent training sessions which did not adequately describe or discriminate differences between the samples (Lawless & Heymann, 2010). The final attribute list, their descriptions, and reference materials are listed in Table 2.2. The majority of aroma sensations were perceived through the orthonasal and retronasal pathways as aroma and flavour sensations. Therefore, it was decided to select attributes representing aroma or flavour that showed the highest intensities during either orthonasal or retronasal perception (in the mock evaluation data) rather than replicating such attributes for both aroma and flavour. The decision was made in agreement with the panellists.

Table 2.2. Overview of sensory attributes, definitions, and training reference standards.

Category	Sensory attribute	Definition	Training reference standard
Aroma	Soapy	Aroma of an unscented bar of soap	30 g unscented bar of soap (Tesco Stores Ltd., UK)
	Musty	Mildew/mouldy aroma or musty aroma associated with damp cardboard	20 g damp cardboard soaked in deionised water for 24h; damp, old sponge
	Pine wood	Aroma of pine shavings or scented wood	20 g pine shavings (Sainsbury's Supermarkets Ltd., UK); 5 mL 5.9 mg/L (1R)-(+)-α-pinene (FG; Sigma Aldrich, UK) in deionised water
	Earthy	Aroma of wet earth or soil	40 g fresh wet earth, soil
	Resinous	Aroma of wood resin	25 g pine resin and 25 g myrrh resin (Indigo Herbs, UK)
	Crushed grass, sap	Aroma of crushed cut grass, sap or fresh tomato leaf or carrot leaf	30 g crushed cut grass and sap that has been left for two days; 10 g fresh tomato leaf/carrot leaf
	Orange citrus fruit	Round aroma of orange, mandarin or tangerine	5 g freshly cut flesh and peel
	Grapefruit zest	Aroma of grapefruit zest; aroma peak at the beginning and flattens off gradually	5 g freshly cut grapefruit zest
	Fresh lemon	Aroma of lemon or lime fruits; sharp citrus aroma peak at the beginning, which quickly flattens off after a few seconds	30 g freshly chopped lemon and lime
	Artificial lemon	Aroma of citrus wet wipe or cheap lemon squash; flat but sharp, pungent citrus aroma	1 citrus wet wipe (Dettol, UK)
	Overall aroma intensity	Overall aroma intensity in the sample	No physical reference
Flavour	Rose water	Rose water flavour as in Turkish delight or diluted geranium essential oil	½ piece Turkish delight (Sainsbury's Supermarkets Ltd., UK); 0.6% (w/v) geranium essential oil (Ecodrop, UK) in deionised water
	Alcohol	Alcohol flavour as in the alcohol/water sample	1% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
Taste	Sweet	Sweet taste as in the alcohol/water sample	10 mL 1% (v/v) sucrose (Sainsbury's Supermarkets Ltd., UK) or 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Sour	Sour taste as in citrus fruits, in the citrusy reference and the alcohol/water solution	10 mL 0.2% (v/v) citric acid (Sigma Aldrich, UK) or 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water

Table 2.2 continued. Overview of sensory attributes, definitions, and training reference standards.

Category	Sensory attribute	Definition	Training reference standard
Taste	Bitter	Pleasant, smooth bitterness as in the bitter reference solution	10 mL 2 mg/L HopAlpha® Iso30%[1] (TNS Ltd., UK) in deionised water
	Lingering bitterness	Persistence of the bitterness in the mouth as in the bitter reference solution; perceived 20 s after swallowing	10 mL 2 mg/L HopAlpha® Iso30%[1] (TNS Ltd., UK) in deionised water
Mouthfeel	Peppery tingling	Peppery tingling sensation when eating chili, fresh ginger, horse radish/radish; tingling mouthfeel on the front half of the tongue	Chili, fresh ginger, horse radish/radish
	Astringent	Mouth drying, rough, puckering sensation as in the astringent reference solution; perceived 20 s after swallowing	10 mL 1% (w/v) tannic acid (Alfa Aesar, US) in deionised water

[1] 30% hop acid in propylene glycol

Panel performance evaluation

The evaluation of panel performance during the formal evaluation sessions was conducted in order to identify intra- and inter-panellist variation following the approach of Kemp et al. (2011). Three-factor ANOVA with interaction (panellist, sample, replicate) was conducted on all 18 attributes and "overall aroma intensity" (see Table 2.3). Significant panellist (Panel) variation (p<0.05) and sample x panellist (Sam x Panel) interactions were reported for several attributes. However, interrogation of the interaction plots showed that the source of variation for the majority of attributes was minor variations in scale use, which did not impact interpretation of resulting data and showed adequate discrimination ability between samples (Lawless & Heymann, 2010). Interaction effects for "alcohol", "sour", "bitter", and "astringent" were explained by a lack of sample discrimination using these attributes. In total, 12 of the 18 attributes and the "overall aroma intensity" significantly differed (p<0.05) across all samples.

Table 2.3. Analysis of variance (ANOVA) *F*-ratios and for sensory attributes rated for Magnum hop oil and five hop oil fractions. NS, indicating no significant effects and *, **, *** indicating a significant effect at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, from three-factor ANOVA with interactions (Sample (Sam), Panellist (Panel), Replicate (Rep)).

Modality	Attribute	Sam	Panel	Rep	Sam x Panel[a]	Sam x Rep[a]	Rep x Panel[a]
Aroma	Soapy	4.38**	2.43*	NS	1.96**	NS	NS
	Musty	3.67**	7.19***	3.41*	1.48*	NS	NS
	Pine wood	4.65***	6.26***	6.49**	2.32***	1.92*	NS
	Earthy	4.09**	3.11**	NS	2.08**	NS	NS
	Resinous	3.09**	22.65***	NS	NS	NS	NS
	Crushed grass, sap	5.91***	4.13**	NS	3.18***	NS	2.13**
	Orange citrus fruit	4.55***	NS	NS	NS	NS	NS
	Grapefruit zest	3.92**	3.27**	NS	2.23***	1.78*	2.25**
	Fresh lemon	5.70***	NS	NS	2.27***	NS	2.52**
	Artificial lemon	5.11***	NS	NS	1.89**	NS	1.65*
	Overall aroma intensity	14.31***	NS	NS	2.27***	NS	1.93*
Flavour	Rose water	5.75***	7.82***	NS	3.09***	NS	NS
	Alcohol	NS	17.49***	3.17*	2.09**	2.43**	NS
Taste	Sweet	3.38**	9.93***	NS	1.73**	NS	1.80*
	Sour	NS	17.07***	NS	NS	NS	NS
	Bitter	NS	17.60***	NS	NS	NS	NS
	Lingering bitterness	NS	10.53***	NS	1.60*	NS	2.38**
Mouthfeel	Peppery tingling	NS	10.23***	NS	1.53*	NS	NS
	Astringent	NS	23.94***	NS	NS	NS	NS

[a] Sam x Panel, Rep x Panel and Sam x Rep represent the interaction between oil/fraction samples and panellists, replication and panellists and oil/fraction samples and replications, respectively.

Sensory descriptive analysis

Three-factor ANOVA (sample, panellists, replicate) with interactions was applied to all samples and sensory scores for the 18 attributes and the overall aroma intensity. Table 2.4. shows the mean sensory scores and significant differences between the samples. No significant differences were observed between the total oil and the experimental replicate indicating panel reliability. It was noticed that more panellists used lower scores to rate the attribute intensities using the lower end of the scale while fewer panellists used high scores. This is not displayed in the mean sensory scores. There could be two reasons why the panellists used lower scores or more

conservative scaling to rate attribute intensities, namely 1) an overall low intensity of the hop oil fraction solutions and 2) the fact that they were very familiar with the samples at the formal evaluation stage (scores slightly decreased in the course of the training period). Individual differences in scale use existed, but they were statistically controlled. 'Lower raters' did not negatively contribute to the ANOVA outcome because their ratings still followed the majority trend of sample rankings and these panellists consistently used lower scores for the three replicates.

There were significant differences (p<0.05) among the samples for all aroma attributes as well as for "rose water" flavour, "sweet" taste and the overall aroma intensity. No significant differences (p>0.05) were reported for "alcohol" flavour, "sour" and "bitter" taste and "astringent" mouthfeel, indicating that the panellists could not significantly discriminate between the samples for these attributes. Tukey's (HSD) post-hoc tests were conducted for pairwise multiple comparison of the samples for each attribute where a significant difference could be detected or showed a trend towards a significant difference (p<0.07) in the outcome of the ANOVA. The attributes "peppery tingling" mouthfeel and "lingering bitterness" were not found to be significant but approached a significant effect (p=0.053; p=0.067) due to higher attribute scores for the terpene alcohol and ester fractions compared to the other samples.

Table 2.4. Mean sensory intensities (*n*=10, triplicates) for Magnum total oil, five hop oil fractions and an experimental replicate at 800 µg/L in ethanol (4%, abv) and for a control sample (pure ethanol, 4%, abv). Superscripts of different letters within an attribute indicate a significant difference between means of samples of an attribute by Tukey's Honest Significant Difference (HSD) test at $p<0.05$.

Modality	Sample Attribute	Total oil	Total oil (repl)	Myrcene fraction	Sesquiterpene fraction	Ester fraction	Ketone fraction	Terpene alcohol fraction	Control
Aroma	Soapy	1.67^{cd}	2.10^{bcd}	1.00^{d}	1.23^{d}	3.93^{a}	3.65^{ab}	2.96^{abc}	0.78^{d}
	Musty	1.39^{b}	1.95^{ab}	3.41^{a}	1.51^{b}	1.35^{b}	0.91^{b}	1.20^{b}	0.44^{b}
	Pine wood	2.49^{cd}	2.81^{bcd}	3.40^{abc}	2.37^{cd}	4.38^{ab}	4.54^{a}	3.60^{abc}	1.11^{d}
	Earthy	1.51^{ab}	0.53^{c}	1.96^{a}	0.84^{bc}	0.42^{c}	0.49^{c}	0.49^{c}	0.33^{c}
	Resinous	1.98^{abc}	2.04^{abc}	2.96^{a}	1.46^{bc}	2.11^{abc}	2.83^{ab}	2.53^{ab}	0.74^{c}
	Crushed grass, sap	1.94^{b}	2.73^{b}	5.37^{a}	2.18^{b}	1.80^{b}	2.21^{b}	2.38^{b}	0.23^{c}
	Orange citrus fruit	1.57^{cd}	1.82^{cd}	1.43^{cd}	2.21^{bcd}	3.81^{ab}	2.91^{abc}	4.04^{a}	0.70^{d}
	Grapefruit zest	1.43^{bc}	1.63^{abc}	1.50^{bc}	1.21^{bc}	2.35^{ab}	3.04^{a}	3.13^{a}	0.20^{c}
	Fresh lemon	1.16^{cd}	1.79^{bcd}	1.30^{cd}	0.91^{d}	3.24^{ab}	2.68^{abc}	3.39^{a}	0.33^{d}
	Artificial lemon	1.32^{bc}	1.39^{bc}	0.41^{c}	0.32^{c}	1.98^{ab}	2.76^{a}	2.23^{ab}	0.27^{c}
	Overall aroma intensity	4.17^{b}	4.32^{b}	6.65^{a}	3.60^{b}	5.79^{a}	5.70^{a}	6.14^{a}	1.34^{c}
Flavour	Rose water	1.34^{cd}	1.83^{c}	1.31^{cd}	1.04^{cd}	4.02^{ab}	3.71^{b}	5.45^{a}	0.12^{d}
	Alcohol	3.21^{a}	2.99^{a}	2.83^{a}	3.27^{a}	3.50^{a}	3.42^{a}	2.92^{a}	3.11^{a}
Taste	Sweet	2.03^{a}	1.50^{a}	1.24^{ab}	1.09^{ab}	2.16^{a}	1.31^{ab}	2.28^{a}	0.28^{b}
	Sour	2.04^{a}	1.66^{a}	1.16^{a}	1.66^{a}	2.01^{a}	1.88^{a}	1.93^{a}	1.57^{a}
	Bitter	3.51^{a}	2.94^{a}	2.71^{a}	2.93^{a}	3.16^{a}	3.21^{a}	3.59^{a}	2.36^{a}
	Lingering bitterness	2.93^{ab}	3.46^{ab}	2.46^{b}	3.11^{ab}	3.09^{ab}	2.93^{ab}	4.22^{a}	2.54^{b}
Mouthfeel	Peppery tingling	1.98^{ab}	2.19^{ab}	2.15^{ab}	2.06^{ab}	2.90^{a}	1.61^{ab}	2.59^{ab}	1.19^{b}
	Astringent	4.30^{a}	4.29^{a}	4.09^{a}	3.60^{a}	4.79^{a}	3.76^{a}	4.29^{a}	3.46^{a}

repl, experimental replicate

As shown in Table 2.4., the control sample (ethanol, 4%, abv) was mainly described by taste and mouthfeel attributes and an “alcohol” flavour since this attribute achieved the highest score among all aroma and flavour attributes, although this attribute did not discriminate between the control sample and the total oil/fractions. Overall, the total oil and the fractions added diverse aroma and flavour notes to the control solution and were able to significantly (p<0.05) potentiate taste and mouthfeel attributes as explained in the following sections.

The total oil was characterised by the fewest number of key attributes i.e. this sample was not characterised by specific aroma, flavour, taste or mouthfeel sensations. Interestingly, the total oil, the ester fraction, and particularly the terpene alcohol fraction added sweetness in comparison to the control sample. This is likely due to an aroma-taste interaction, however, the cause of this interaction is not clear from the sensory data alone and further work is required to identify the source of the perception of the sweet taste.

The sensory profile of the sesquiterpene fraction was not described by specific key attributes and like the total oil but in contrast to the hop oil fractions, it exhibited the lowest score for the “overall aroma intensity”. The spider plots in Figure 2.2. illustrate the differences between the sesquiterpene fraction with the lowest sensory potential and the terpene alcohol fraction as one of the hop oil fractions that induced several sensory sensations in the test solution. Both plots were overlaid with attribute scores of the total oil and the control samples to show the similarity or differences between these samples and the sesquiterpene fraction or terpene alcohol fraction, respectively. Overall, the panel could only perceive low intensities of “crushed grass,

sap", "pine wood", and "orange citrus fruit" aromas in the sesquiterpene fraction. This is in agreement with the literature, although so far, rather general sensory terms have been used to describe the aromas of sesquiterpene hydrocarbons such as "green" (Lermusieau et al., 2001), "herbal", "woody", "earthy", and "citrusy" (Nance & Setzer, 2011; Whittock & Koutoulis, 2010). Precise terms were used in this study to highlight different sensory potentials among the hop oil fractions and the total hop oil and to facilitate the drawing of conclusions about cause-effect relationships between volatile compounds and sensory characteristics.

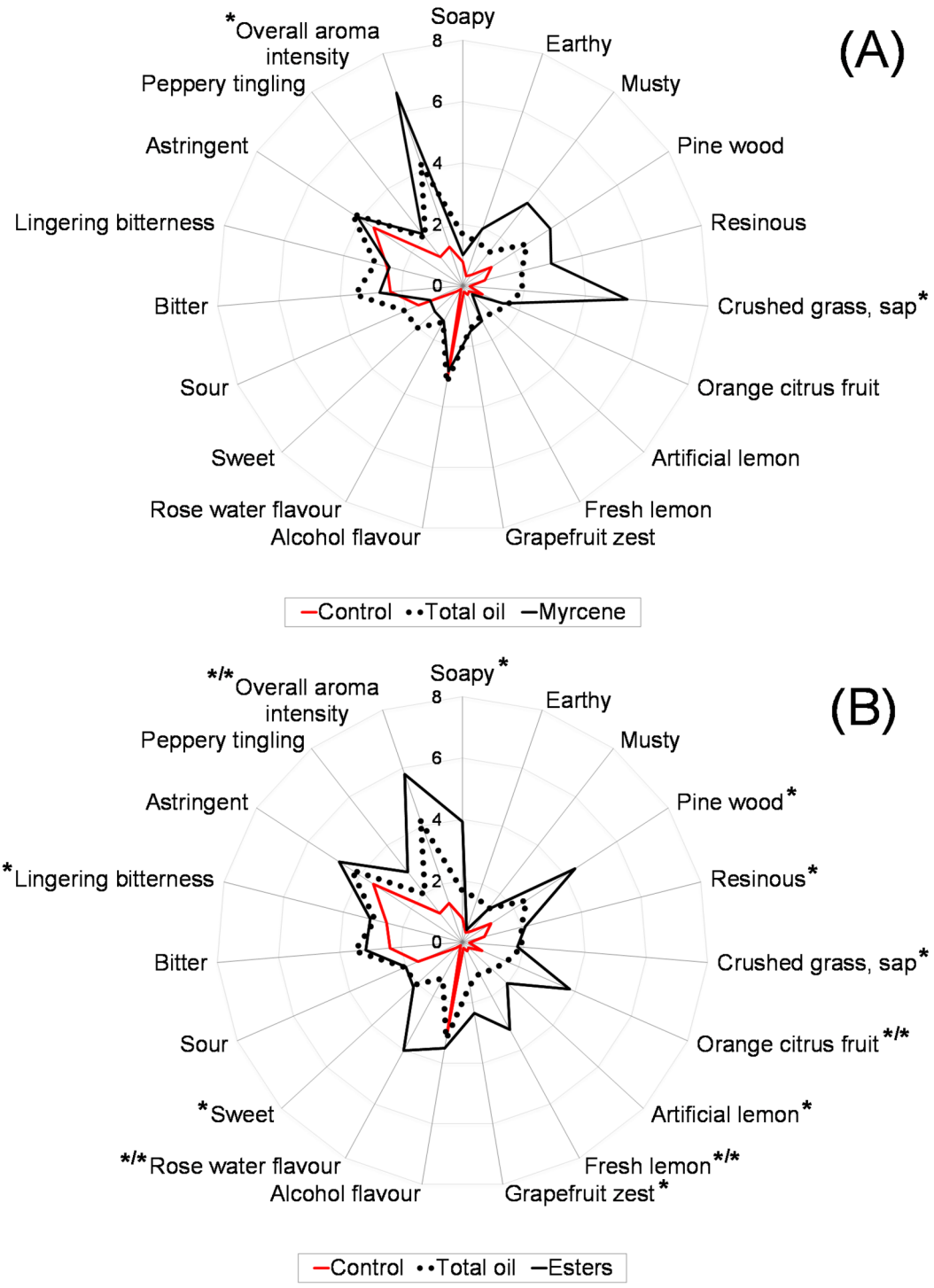

Figure 2.2. Spider plots of mean attribute intensities for the sesquiterpene fraction **(A)** and the terpene alcohol fraction **(B)** plotted with the total Magnum hop oil and control samples; with * indicating significant differences compared to the control sample and */* indicating significant differences compared to both the control sample and the total Magnum hop oil sample (according to Tukey's HSD test at $p<0.05$).

The myrcene fraction was described by “crushed grass, sap”, “musty”, and “resinous” aromas and a high “overall aroma intensity”. Similarities between the aroma profiles of the myrcene fraction and the total oil and sesquiterpene fraction could be observed. In contrast to the other fractions, the myrcene fraction was highly enriched in one compound and due to the aim to evaluate all hop oil samples at equi-concentration, the myrcene was present far above its odour detection threshold concentration (Peltz & Shellhammer, 2017).

Myrcene is commonly found to significantly contribute to the aroma profile of hop oil accounting for up to 58% of the total aroma (total oil Odour Unit) (Guadagni et al., 1966). Guadagni et al. (1966) determined the compounds’ contribution by determining its odour threshold concentration in water (13 ppb). The compounds’ concentration in the total oil (63%) was then divided by this threshold concentration to obtain the Odour Unit. The % contribution equalled the proportion of the fractions’ Odour Unit to the total oils’ Odour Unit. This result might have changed since 1966 because extraction and quantification methods to determine the concentration of the hop oil fractions and compounds in total oil are nowadays more sensitive and precise. However, myrcene is still considered as a main aroma contributor in raw hop oil.

Previous studies investigating the sensory characteristics of myrcene in beer observed spicy and resinous flavour notes at 200 µg/L in ale (Sharpe, 1988) and metallic and geranium-like aroma notes at around 860 µg/L in beer dry-hopped with Hallertauer Comet (5.8% ABV, 20 BU) (Schnaitter et al., 2016). Other research groups found myrcene to impart a lime-like or a geranium leaf-like aroma at the GC-O sniffing

port (Gros, Peeters, & Collin, 2012; Steinhaus et al., 2007) or a peppery-, terpene-, balsam-, and plastic-like aroma when assessed in beer (Inui et al., 2013). Recently, Neiens and Steinhaus (2018) determined the odour threshold of myrcene in an aqueous solution to be 1.2 µg/kg. Brendel, Hofmann, and Granvogl (2019) found the odour of non-polar myrcene to be detected in oil at 1800 µg/kg. Although, these studies provide different levels of details with regard to the sensory assessments conducted, it can be said that the perception of myrcene appears to be concentration- and matrix-dependent.

The ester, ketone and terpene alcohol fractions were described by a number of key attributes. The ester fraction was characterised by “soapy”, “pine wood”, “orange citrus fruit”, and “fresh lemon” aroma, and “rose water” flavour, and “peppery tingling” mouthfeel sensations. These attributes obtained significantly higher scores compared to the control and the total oil sample while the “peppery tingling” mouthfeel sensation was increased compared to the control solutions. The ketone fraction was mainly described by “soapy”, “pine wood”, “artificial lemon”, “resinous”, “orange citrus fruit”, and “grapefruit zest” aroma notes, all of these being significantly increased compared to the control sample and the latter three compared to the total oil sample. Various fruity aroma and flavour notes have been reported for esters and ketones in hop essential oil. Particularly, short-chain esters (up to C6) added soft fruit, citrusy, pear/apple-, as well as tropical fruit-like aromas to beer while medium-chain esters (C8–C12) have been found to induce soapy aroma notes (Schnaitter et al., 2016; Tokita et al., 2014). As observed in the present study, ketones

have mostly been suggested to contribute to the citrus/fruity and floral characters in beer (Kishimoto et al., 2006; Van Opstaele, De Causmaecker, et al., 2012).

Interestingly, the panel was able to distinguish between different lemon aroma qualities. The description of the attributes “fresh lemon” and “artificial lemon” and the differences between these could probably be related to a sensory interaction (trigeminal/irritating-aroma; “sharp”, “pungent”) and/or a temporal perception effect (“flattens quickly”). The latter might also apply for the “grapefruit zest” attribute (“aroma peak at the beginning and flattens off gradually”). However, this assumption would need to be further investigated.

In comparison to the total oil and the other fractions, the terpene alcohol fraction was described by diverse aroma, flavour, taste, and mouthfeel sensations at higher intensities. This fraction exhibited stronger “orange citrus fruit”, “fresh lemon”, and “grapefruit zest” aroma notes, “rose water” flavour, “sweet” and “lingering bitterness”, and a “peppery tingling” mouthfeel sensations compared to the control and the total oil samples with the aroma and flavour attributes as well as sweetness showing a significant effect. The scores for the attributes “peppery tingling” and “lingering bitterness” were only slightly increased and approached the significance level (p=0.053; p=0.067).

The attribute “peppery tingling” refers to a trigeminal-type sensation, which is a similar sensation imparted by compounds in terpene alcohol or oxygenated sesquiterpenoid fractions observed in previous studies that have been referred to as “spicy” essences (Praet et al., 2016; Van Opstaele, Praet, et al., 2013). In past studies (Lawless et al., 1985; Opstaele et al., 2010; Van Opstaele, Goiris, et al., 2012), the

polar oxygenated sesquiterpenoid fractions from different hop varieties have been observed to increase the perception of fullness and to induce a “spicy” mouthfeel in beer, the latter sensation has been described as a coating effect on the tongue and in the throat indicating the occurrence of a trigeminal-type sensation. Trigeminal stimuli are those that can induce a sensation of temperature (cooling, warming), pain or irritation (spicy, pungent) such as high carbonation levels in beer being perceived as a sparkling, tingly, and irritating sensation in the oral cavity (induced by bursting bubbles of CO_2 on the tongue (Yau & McDaniel, 1990) and conversion of CO_2 to carbonic acid (McEvoy, 1998)).

In addition to the perceived “fullness” and the “spicy” sensation, Goiris et al. (2002) found an oxygenated sesquiterpene fraction (ex Hersbrucker hop oil) to increase the perceived bitterness in pilsner. The authors suggested that a synergistic interaction at perceptual level occurred between the bitterness induced by the isomerised hop extract and the volatiles (caryophyllene epoxide, humulene epoxide I-II, humulenol II, and unknowns) present in the hop oil extract causing the modulation of the perceived bitterness in the pilsner beer. However, the effect was not attributed to individual compounds or compound groups in the hop fraction and not all compounds in these fractions were identified. Thus, it requires further work to identify the cause-effect relationship between the compounds and the taste sensation.

The increased “lingering bitterness” intensity in the terpene alcohol solution (compared to the control and myrcene samples) in this study could also not be assigned to specific compounds. Therefore, the lingering bitterness sensations might

indeed have been the result of a sensory interaction within or across modalities caused by sesquiterpene alcohols, as suggested by the study of Goiris et al. (2002). However, further research is required to confirm whether this sensory interaction effect was induced by compounds in the sesquiterpene alcohol sub-fraction alone or whether other compounds in the monoterpene alcohol sub-fraction present in the terpene alcohol fraction used in the current study or other mechanisms such as the stimulation of bitter taste receptors might have been involved. It should also be considered that, in contrast to the current study, Goiris et al. (2002) assessed the bitterness intensity at no specific time point and in a beer matrix, thus temporal perception and matrix-related effects could also be responsible for the different study outcomes.

PCA was conducted to reduce the complexity of the data and visually represent the samples in a sensory space (Figure 2.3. (A)). The analysis was based on the covariance matrix, which is chosen for sensory evaluations conducted by a trained panel that used the same scale for all attributes (Lawless & Heymann, 2010). The first two principal components (PC) explained the majority of the total variance (86.38%) with PC1 explaining 69.87% and PC2 explaining 16.52%. The main discriminating dimension (PC1) was loaded with the aroma attributes “soapy”, “pine wood”, “orange citrus fruit”, “fresh lemon”, “artificial lemon”, and “grapefruit zest” and with “rose water” flavour. PC2 was loaded with the main distinguishing aroma attributes being “musty”, “earthy”, and “crushed grass, sap”. As could be shown from the outcome of the ANOVA, the myrcene fraction was related to high intensities of “crushed grass sap”, “musty” and “earthy” aroma notes which is why it is positively

correlated with PC2. The total oil and the sesquiterpene fraction are plotted close to the centre of the PCA biplot showing that fewer attributes dominated their sensory profiles. This is interesting since the total oil was comprised of a complex mixture of compounds. The total oil replicate was included in the PCA to illustrate the similarity of the replicates within the sensory space. Subjecting the data without the total replicate to PCA resulted in very similar hop oil fraction locations.

Other fractions comprised of fewer compounds, which was particularly the case for the monoterpene alcohols in the terpene alcohol fraction and the myrcene in the myrcene fraction, and therefore obtained high scores on specific aroma attributes. The ester, ketone, and terpene alcohol fractions were related to high intensities in the fruity aroma notes, “soapy” and “pine wood” aroma, and “rose water” flavour. The taste and mouthfeel attributes “sweet” (r=-0.436), “lingering bitterness” (r=-0.638), and “peppery tingling” (r=-0.638) were loaded on PC3 that only contained 6.29% of the variation (Figure 2.3. (B)) indicating that both aroma and flavour as well as taste and mouthfeel attributes are differentiating between the hop oil samples.

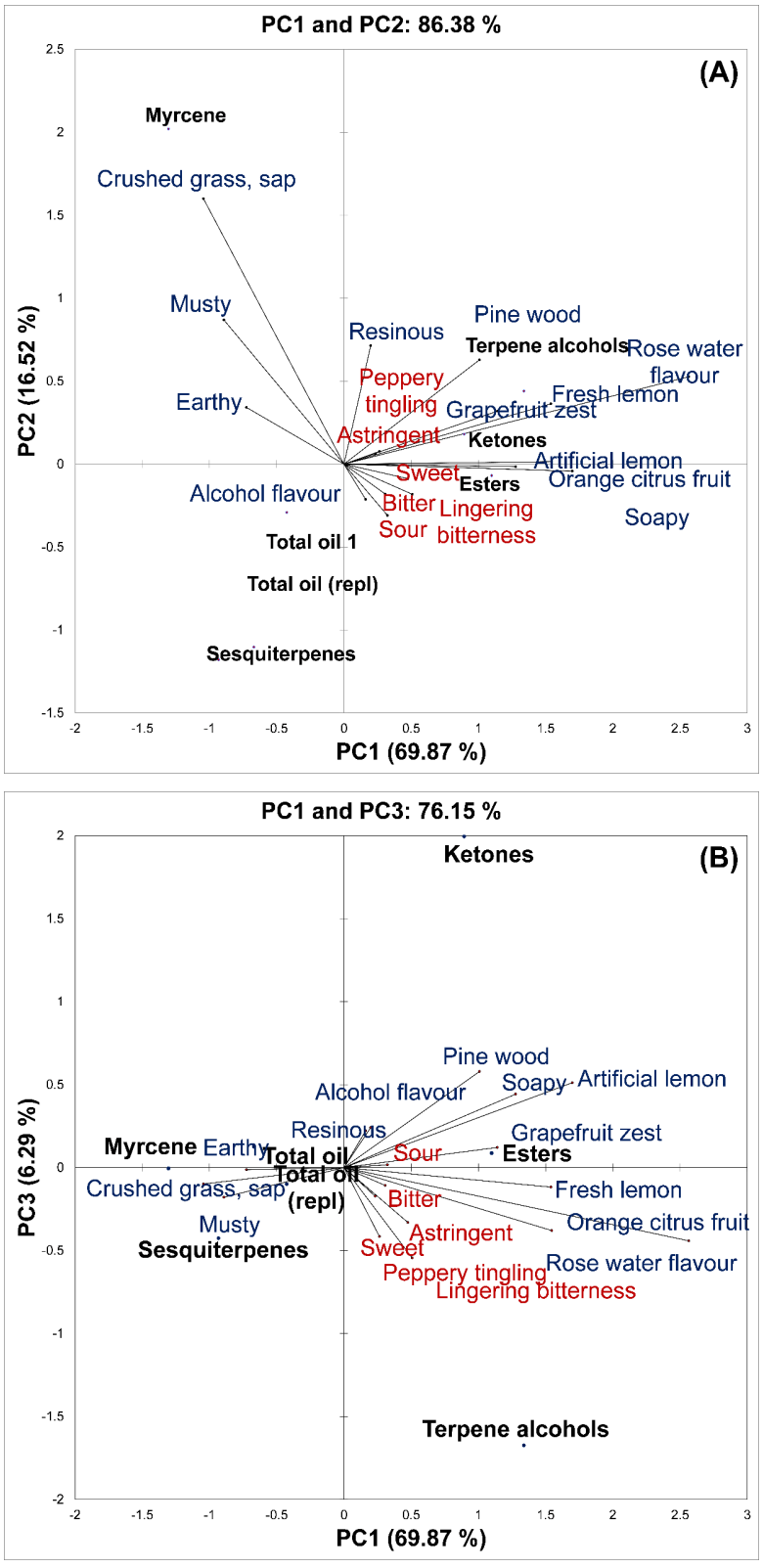

Figure 2.3. Principal Component Analysis (PCA) biplot of sensory attributes present on **(A)** principal component 1 (PC1) and 2 (PC2) and **(B)** PC1 and PC3 by the covariance matrix of mean attribute intensity rating across the total hop oil and five hop oil fractions . Aroma and flavour attributes in **blue**, taste and mouthfeel attributes in **red**; repl, experimental replicate

2.3.2 Effect of compositional and physicochemical characteristics on sensory scores

Relationship between sensory scores and main volatile compounds

GC-MS was used to obtain a general overview of the main volatile compounds present in the Magnum hop essential oil and its fractions. In total, 66 compounds could be identified. The total ion chromatogram (TIC) in Figure 2.4. illustrates the distribution of the fractions in the total oil sample. Table 2.5. displays all compounds that could successfully be identified using NIST database searches and authentic reference compounds run under identical instrumental conditions. The relative contributions (% derived from peak area normalisation based on the relative peak areas) of the compounds in the total oil/fractions obtained are provided in Table 2.5. Generally, it was found that several compounds were detected in more than one fraction. Relative differences were recorded between these compounds and trace levels were found if no clear separation of the hop oil fractions was possible in the fractionation process. Compounds that were present below detection level were also marked with "-" for "compound was not detected". It is considered that these compounds could still contribute to the overall sensory profile depending on the threshold concentration in the individual volatile mixtures.

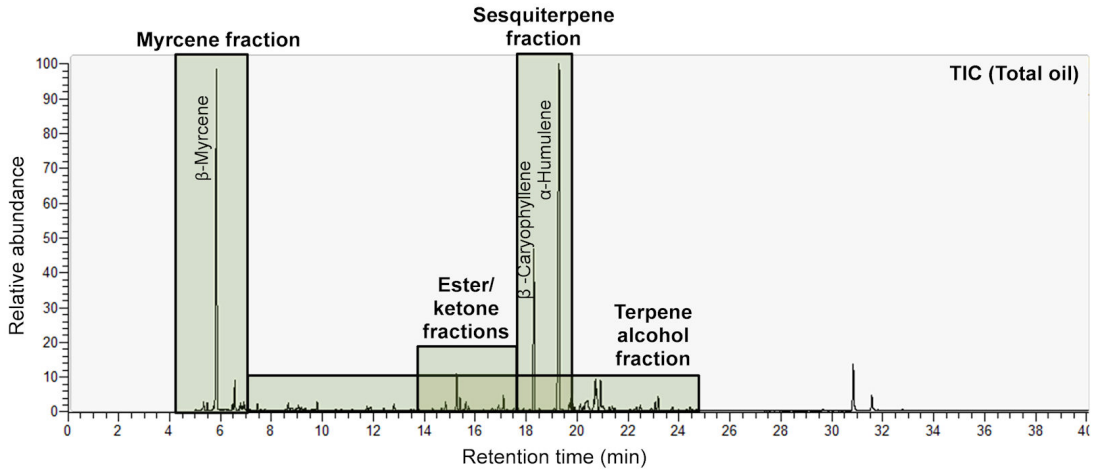

Figure 2.4. Total ion chromatogram (TIC) of the Magnum hop essential oil (total oil) showing the distribution of the five hop oil fractions.

PCA was conducted to visualise the relationship between the samples, sensory attribute scores and the volatile compositions (displayed as **[numbers]** as listed in Table 2.5). Figure 2.5. shows the plot with the significant principal components PC1 (40%) and PC2 (37%) explaining 77% of the variance. The biplot displays the different and overlapping sensory characteristics of the five fractions with the total oil again plotted the closest to the plot centre because it was not described by any key attribute and contained many volatile compounds at much lower concentrations compared to the fractions including compounds that were present below detection level. On the right side of the plot it is shown that the terpene alcohol fraction was characterised by several taste and mouthfeel sensations. Also, aroma and flavour attributes were scored higher in this fraction compared to the ester and ketone fractions which is demonstrated by their position in the biplot closer to the terpene alcohols. However, while the mono- and sesquiterpenes could be assigned to some extent to certain aroma sensations, there has been no clear correlation between the

sensory attribute and volatile compounds in the ester, ketone and terpene alcohol fractions. The reasons for this are explained in the following sections.

The terpene hydrocarbons *β*-myrcene **[3]**, *β*-caryophyllene **[41]**, and *α*-humulene **[42]** constituted the largest chemical group in the total oil, the myrcene and sesquiterpene fraction (Figure 2.5.). These hydrocarbons are most abundant in the majority of hop essential oils, but are suggested to be evaporated from the kettle, discarded with the spent hops, lost during wort filtration or fermentation, or transformed to oxygenated terpenes and sesquiterpenes. This is why hops or hop oil extracts are usually added post-fermentation (Dresel et al., 2013; Goiris et al., 2002). It was found that the myrcene fraction contained a few compounds at trace levels such as *α*-humulene **[42]** and *β*-pinene **[1]** which might have contributed to the "crushed grass, sap", "earthy", and "musty" aroma (Peltz & Shellhammer, 2017; Whittock & Koutoulis, 2010). Particularly *β*-pinene was strongly positively correlated with these attributes (r=0.991, r=0.757, and r=0.924, respectively).

Table 2.5. Volatile compounds (n=68) identified in the total Magnum hop oil (TO) and the sesquiterpene (SQ), myrcene (MYR), ester (EST), ketone (KET), and terpene alcohol (TALC) fractions. Identification using external standard compounds (*), linear retention indices (LRI), and library matching (MS Mass Spectral library (MS08) and Wiley7n.1 (Hewlett-Packard, US) databases). Relative (%) chemical composition obtained by peak area normalisation (PAN)[a]. Letters within columns indicate significant mean separation (among hop oil samples) according to Tukey's Honest Significant Difference (HSD) test. Log*P* used as an indicator for the polarity of the identified volatile compounds. "Sample Log*P*" estimated based on the relative contribution of the compounds' Log*P* to the polarity of the hop oil or fraction.

No	RT (min)	LRI[b]	LRI[c]	Compound	TO	SQ	MYR	EST	KET	TALC	Log*P*	Compound class
1	5.48	975	970	*β*-Pinene *	0.01 b	- b	3.12 a	- b	- b	- b	4.16	Terpene hydrocarbon
2	5.67	988	984	6-Methyl-5-heptene-2-one	0.01 d	- d	- d	0.07 b	0.09 a	0.05 c	2.05	Ketone
3	5.82	990	991	*β*-Myrcene *	37.3 b	2.29 c	91.7 a	- c	- c	- c	4.88	Terpene hydrocarbon
4	7.45	1049	1049	*cis*-*β*-Ocimene	0.09 a	0.02 b	- d	- cd	0.01 c	- cd	4.67	Terpene hydrocarbon
5	8.15	1070	1072	*cis*-Linalool oxide	0.01 b	- b	- b	- b	- b	0.14 a	2.08	Monoterpene alcohol derivate
6	8.66	1085	1087	Methyl 6-methyl heptanoate	0.67 a	- c	- c	0.71 a	0.35 b	- c	3.40	Ester
7	8.78	1090	1092	2-Nonanone *	0.24 d	- d	- d	0.95 b	1.42 a	0.63 c	3.14	Ketone
8	9.06	1099	1100	Linalool *	0.39 b	- b	- b	- b	0.50 b	5.58 a	2.97	Monoterpene alcohol
9	9.17	1101	1099	3-Methylbutyl 2-methylbutanoate	0.18 a	- b	- b	0.17 a	0.03 b	- b	3.56	Ester
10	9.34	1107	1107	2-Methylbutyl 3-methylbutyrate	0.13 b	- c	- c	0.18 a	0.04 c	- c	3.66	Ester
11	9.59	1115	1114	Fenchol	0.07 b	- b	- b	- b	0.03 b	0.96 a	3.17	Monoterpene alcohol
12	9.68	1128	1126	Myrcenol	0.04 b	- d	- cd	- bcd	0.03 bc	0.36 a	3.46	Monoterpene alcohol
13	9.79	1135	1138	Methyl octanoate *	0.69 b	- c	- c	1.62 a	0.83 b	- c	3.46	Ester
14	10.53	1151	1151	Hexyl isobutyrate	0.03 b	- c	- c	0.06 a	- b	0.03 c	3.28	Ester
15	10.75	1156	1155	5-Decanone	0.12 d	- d	- d	0.73 b	1.08 a	0.45 c	3.20	Ketone
16	11.18	1167	1167	*endo*-Borneol *	0.08 b	- b	- b	- b	- b	1.54 a	2.69	Monoterpene alcohol
17	11.45	1178	1177	Terpinen-4-ol	0.02 b	- b	0.01 b	0.01 b	0.03 b	0.32 a	3.26	Monoterpene alcohol
18	11.63	1187	1188	*trans*-3(10)-Caren-2-ol	0.04 d	- d	0.04 d	0.15 c	0.26 b	0.37 a	1.97	Monoterpene alcohol
19	11.75	1193	1193	Methyl 6-methyloctanoate	0.32 bc	0.06 c	0.19 bc	1.49 a	0.72 b	- c	3.32	Ester
20	11.90	1194	1197	*α*-Terpineol *	0.21 b	- b	- b	- b	- b	4.02 a	2.98	Monoterpene alcohol
21	12.04	1195	1194	Myrtenol	0.01 b	- b	- b	- b	- b	0.05 a	2.98	Monoterpene alcohol
22	12.18	1201	1202	2-Decanol	0.03 b	- b	- b	- b	- b	0.13 a	3.71	Monoterpene alcohol
23	12.41	1225	1225	Methyl (*4E*)-4-nonenoate	0.11 c	0.01 c	0.06 c	0.64 a	0.34 b	0.01 c	2.90	Ester
24	12.82	1228	1225	Nerol	0.09 d	0.02 d	0.05 d	0.54 b	0.30 c	1.15 a	4.70	Monoterpene alcohol
25	13.63	1253	1255	Geraniol *	0.08 b	- b	- b	- b	- b	17.8 a	3.47	Monoterpene alcohol
26	14.69	1285	1287	Methyl 8-methyl-nonanoate	0.24 c	0.07 c	0.06 c	2.66 a	1.50 b	0.02 c	4.40	Ester

Table 2.5 continued.

No	RT (min)	LRI[b]	LRI[c]	Compound	TO	SQ	MYR	EST	KET	TALC	Log*P*	Compound class
27	14.85	1295	1294	2-Undecanone *	1.15 c	- c	- c	13.8 b	22.0 a	12.3 b	3.69	Ketone
28	14.95	1296	1297	Perillol	0.02 b	- c	- bc	- bc	- bc	0.28 a	3.17	Monoterpene alcohol
29	15.16	1307	1307	2-Undecanol	0.04 c	- c	- c	0.37 b	0.26 b	0.58 a	4.21	Aliphatic alcohol
30	15.16	1308	1302	Octyl propionate	0.05 b	- b	- b	0.49 a	0.35 a	0.19 b	4.35	Ester
31	15.31	1311	1311	Methyl 4-decenoate	1.91 c	- c	- c	15.8 a	10.3 b	- c	4.09	Ester
32	15.42	1314	1314	Methyl-4,8 decadienoate	0.27 b	0.05 b	0.06 b	2.65 a	2.12 a	0.09 b	3.87	Ester
33	15.66	1322	1322	Methyl geranate *	0.71 b	- b	- b	12.3 a	9.84 a	- b	3.98	Ester
34	15.74	1324	1324	Methyl decanoate	0.07 c	- c	- c	0.92 a	0.58 b	- c	4.41	Ester
35	16.33	1325	1326	Octyl Isobutyrate	0.13 c	0.09 c	0.02 c	1.93 a	0.97 b	- c	4.71	Ester
36	17.07	1371	n/a	Methyl 2-decenoate	0.07 c	- d	- d	0.11 b	0.23 a	- d	3.97	Ester
37	17.25	1372	1372	Geranyl acetate *	0.12 b	- b	- b	1.43 a	1.85 a	- b	3.98	Ester
38	17.52	1373	1375	Methyl undecanoate	0.10 c	0.03 c	0.01 c	1.46 a	0.96 b	0.01 c	4.86	Ester
39	17.69	1379	1377	2-Dodecanone *	0.27 c	- c	- c	3.88 b	6.51 a	3.66 b	4.18	Ketone
40	17.87	1395	1396	Methyl undecenoate	0.01 c	- d	- d	0.12 a	0.11 b	- d	4.79	Ester
41	18.29	1419	1418	*β*-Caryophyllene *	8.57 b	19.0 a	- c	2.12 c	2.55 c	- c	6.30	Sesquiterpene hydrocarbon
42	19.38	1453	1455	*α*-Humulene *	37.0 b	69.0 a	4.67 c	9.46 c	12.7 c	0.89 c	6.95	Sesquiterpene hydrocarbon
43	19.42	1474	1475	Geranyl propionate	0.01 c	- c	- c	0.14 b	0.24 a	- c	3.64	Ester
44	20.10	1475	1475	Neryl isobutyrate	0.20 bc	- c	- c	0.94 a	0.50 b	- c	3.45	Ester
45	20.14	1487	1486	*β*-Eudesmene	0.32 b	1.05 a	- c	- c	- c	- bc	4.58	Sesquiterpene hydrocarbon
46	20.39	1494	1495	2-Tridecanone *	0.06 c	0.06 c	- c	0.60 b	1.02 a	- c	4.68	Ketone
47	20.47	1495	n/a	*cis*-5-Dodecenoic acid, methyl ester	0.10 b	- b	- b	1.54 a	1.46 a	- b	4.00	Ester
48	20.66	1496	1497	Methyl 3,6-dodecadienoate	0.20 b	- b	- b	0.90 a	0.95 a	- b	4.10	Ester
49	20.74	1516	1516	Geranyl isobutyrate *	1.84 c	- c	- c	17.0 a	12.02 b	- c	4.77	Ester
50	21.07	1526	1527	*δ*-Cadinene	2.76 b	7.79 a	- c	0.77 c	0.86 c	- c	6.64	Sesquiterpene hydrocarbon
51	21.14	1532	-	*Unknown*	0.03 b	- b	- b	0.21 a	0.18 a	- b	-	-
52	21.62	1532	1531	*trans*-Z-α-Bisabolene epoxide	0.01 b	- b	- b	- b	- b	0.10 a	4.86	Oxygenated sesquiterpene
53	22.08	1534	1535	(*E*)-Nerolidol	0.09 d	- d	- d	0.85 c	1.57 b	2.14 a	5.68	Sesquiterpene alcohol
54	22.32	1570	1572	Caryophyllenyl alcohol	0.20 b	- b	- b	- b	- b	5.24 a	4.20	Sesquiterpene alcohol
55	22.50	1574	1579	Caryophyllene oxide *	0.27 a	- b	- b	- b	0.43 a	0.27 a	3.60	Oxygenated sesquiterpene

Table 2.5 continued.

No	RT (min)	LRI[b]	LRI[c]	Compound	TO	SQ	MYR	EST	KET	TALC	Log*P*	Compound class
56	22.65	1575	-	*Unknown*	0.02 b	- b	- b	- b	- b	0.85 a	-	-
57	22.95	1576	1572	Humulene epoxide I	0.01 a	- b	- b	- b	- b	- b	4.56	Oxygenated sesquiterpene
58	23.08	1580	1577	Humulol	0.68 b	- b	- b	- b	1.00 b	15.5 a	3.80	Sesquiterpene alcohol
59	23.19	1591	1589	Humulene epoxide II	1.07 a	0.40 b	- c	- c	- c	- c	4.51	Oxygenated sesquiterpene
60	23.45	1602	1606	Widdrol	0.04 b	- b	- b	- b	- b	1.31 a	4.10	Sesquiterpene alcohol
61	23.64	1602	1609	1-Epicubenol	0.04 b	- b	- b	- b	- b	1.80 a	3.69	Sesquiterpene alcohol
62	23.76	1604	1604	Humulene epoxide III	0.01 a	- b	- b	- b	- b	- b	4.45	Oxygenated sesquiterpene
63	23.78	1605	1605	2-Humulenol	0.15 b	- b	- b	- b	- b	12.5 a	3.50	Sesquiterpene alcohol
64	23.87	1636	1639	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	0.02 b	- b	- b	- b	- b	2.66 a	3.70	Aliphatic alcohol
65	24.00	1638	1640	τ-Cadinol	0.09 b	- b	- b	0.08 b	0.33 b	3.13 a	4.90	Sesquiterpene alcohol
66	24.31	1639	1638	δ-Cadinol	0.02 b	- b	- b	- b	- b	1.02 a	4.95	Sesquiterpene alcohol
67	25.38	1700	1697	2-Pentadecanone	0.02 d	- d	- d	0.21 c	0.47 a	0.38 b	5.66	Ketone
68	25.76	1714	1713	(*Z,E*)-Farnesol	0.02 b	- b	- b	- b	- b	1.47 a	5.77	Sesquiterpene alcohol
				Estimated sample (fraction/total oil) log*P*:	**5.69**	**6.71**	**4.95**	**4.51**	**4.47**	**3.77**		

[a] Normalised integrated peak areas of a compound relative to the total integrated peak area in each chromatogram

[b] Calculated retention indices

[c] Retention indices published in literature or NIST Chemistry WebBook [Online]

"-" Compound was not detected.

The ester fraction mainly comprised of geranyl isobutyrate **[49]**, methyl 4-decenoate **[31]**, and methyl geranate **[33]** as well as *α*-humulene **[42]** (also contained in the ketone fraction). The *α*-humulene **[42]** might have contributed to the "crushed grass, sap" and "pine wood" aroma background notes in the two fractions (Peltz & Shellhammer, 2017; Praet et al., 2015). 2-Tridecanone **[46]** was found in both ester and ketone fractions and has been suggested to impart green and woody aromas in Hallertau Tradition, Spalter Select, and Tettnanger hops (Van Opstaele, Praet, et al., 2013). In this study, 2-tridecanone was rather found to be correlated with the "soapy" and "pine wood" aroma attributes (r=0.788, r=0.751). In addition, the ketone 2-undecanone **[27]** was present in the ester and the ketone fraction, which is one of the most abundant methyl ketones in hop essential oil, known to impart floral (Eyres et al., 2007) and citrusy (Gros et al., 2012) aroma notes and therefore might have contributed to the "fresh lemon" or "artificial lemon" aroma and the "rose water" flavour in these two fractions. This was also suggested by strong significant positive correlations ($p \leq 0.05$) obtained by Pearson correlation analysis between 2-undecanone and the two lemon attributes (r=0.818, r=0.958). The correlation coefficient between 2-undecanone and "rose water" flavour was not found to be significant (r=0.777).

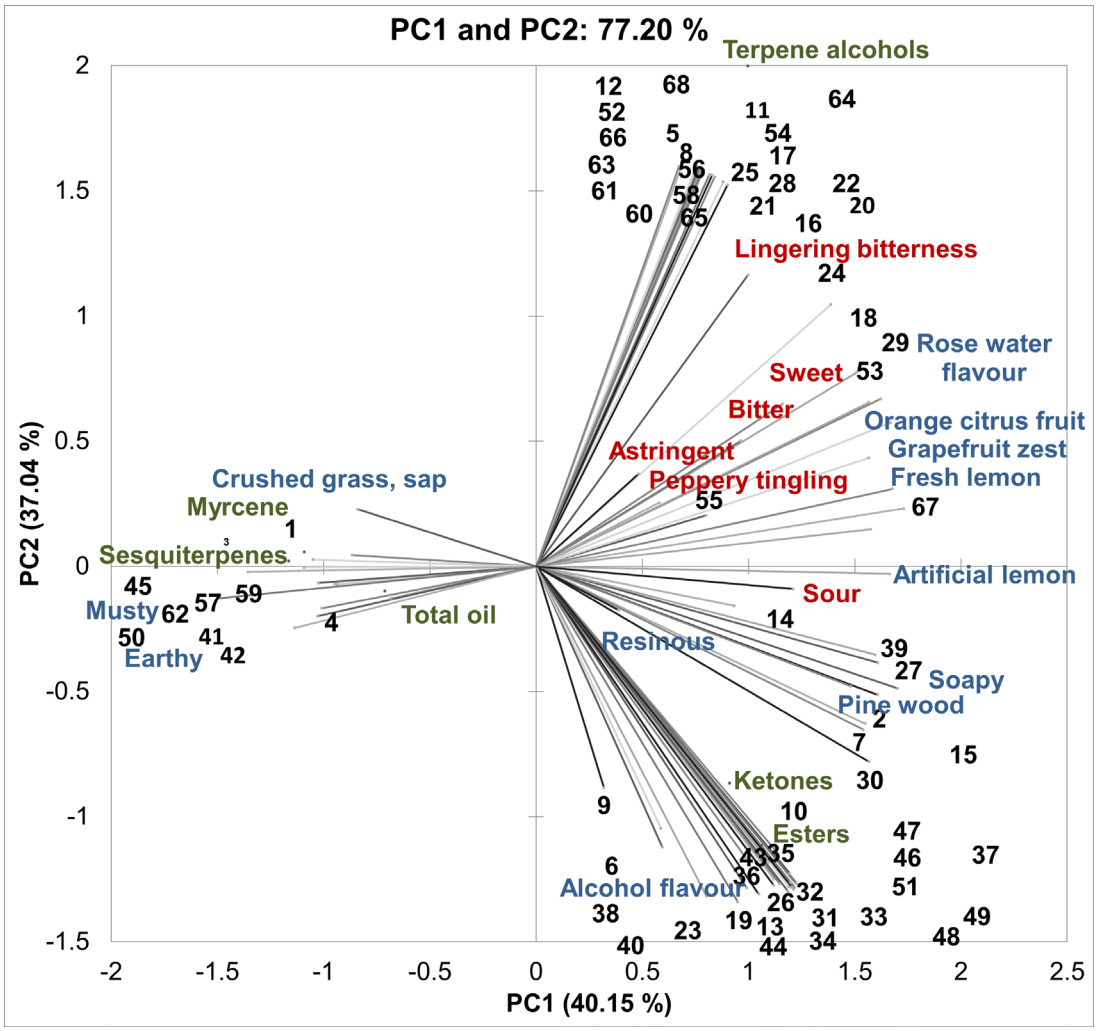

Figure 2.5. Principal Component Analysis (PCA) biplot of normalised sensory and GC-MS data presented on principal component 1 (PC1) and 2 (PC2). Numbered volatile compounds in the total oil and hop oil fractions in **black** (numbers see Table 2.5), aroma and flavour attributes in **blue**, taste and mouthfeel attributes in **red**, samples in **green**

Geranyl isobutyrate was identified as one of the key flavour compounds in beers hopped with Cascade and Cluster varieties and added floral flavour, although present well below its sensory threshold concentration (Lam, Foster, et al., 1986; Peacock et al., 1981). It was suggested to add to the complexity of the floral flavour together with monoterpene alcohols linalool and geraniol rather than being solely responsible for this flavour sensation in the beers (Lam, Foster, et al., 1986; Peacock et al., 1981). The fact that linalool **[8]** and geraniol **[25]** were not detected in the ester fraction may

suggest that geranyl isobutyrate **[49]** added to the “rose water” flavour note in the sensory evaluation, either independently or together with other compounds (e.g. methyl esters). In fact, Pearson correlation coefficients revealed exclusively positive but non-significant correlations between geranyl isobutyrate and the methyl esters and the “rose water” flavour suggesting that this sensation might rather be induced by a combination of volatiles. Methyl 4-decenoate **[31]**, methyl geranate **[33]** as well as other methyl esters such as methyl 4,8-decadienoate **[32]** are frequently identified in different hop varieties (Forster & Gahr, 2013; Lafontaine & Shellhammer, 2018a; Van Opstaele, Goiris, et al., 2013), however, their contribution to sensory profiles of the hop volatile mixtures has not yet been specified. The suggestion regarding the cause-effect-relationship resulting in the “rose water” flavour would have to be confirmed, e.g. by sensorially evaluating the suspected compounds individually or combined added to a comparable matrix at the same concentration as present in the ester fraction.

Apart from 2-undecanone **[27]**, the main ketone in the ketone fraction was found to be 2-dodecanone **[39]** which is assumed to be one of the main contributing compounds to the “orange citrus fruit”, “fresh lemon”, and “rose water” aroma and flavours, as suggested by a previous study where it induced fruity, citrus, and orange aroma notes (Van Opstaele, Praet, et al., 2013). In the current study, 2-dodecanone significantly correlated with “fresh lemon” (r=0.811) and positively but non-significantly correlated with the “orange citrus fruit” (r=0.676) and “rose water” attributes (r=0.776). The ketone fraction also contained a considerable amount of geranyl isobutyrate **[49]** and methyl 4-decenoate **[31]**. The similarity between the

ester and the ketone fractions composition explains the similar sensory profiles of these fractions and a clearer separation of the two compound groups might have resulted in more sensory differences between them.

The main compounds in the terpene alcohol fraction could be categorised into monoterpene alcohols (mainly geraniol **[25]**, linalool **[8]**, *α*-terpineol **[20]**), sesquiterpene alcohols (humulenol II **[63]**, humulol **[58]**, caryophyllenyl alcohol **[54]**), and caryophyllene oxide **[55]**. The aroma of monoterpene alcohols is known to be perceived at low compound concentrations. The most abundant compounds geraniol and linalool were found to contribute to fruity, citrus-, and rose-like aroma notes in beer (Eyres et al., 2007; Kishimoto et al., 2006). In addition, previous studies have shown that linalool, at sub- and supra-threshold concentrations, acts as a synergist by significantly increasing the intensities of those sensory characteristics induced by geraniol (floral, rose-like aroma) or oxygenated sesquiterpenoids (spicy/herbal, floral/fruity flavour, bitterness) (Bailey et al., 2009; Praet et al., 2015; Takoi, Itoga, et al., 2010). In the current study, linalool **[8]** was mainly associated with the "grapefruit zest" (r=0.604), and "fresh lemon" aroma (r=0.569) as well as the "rose water" flavour (r=0.727) and further work is required to investigate if it has a role in regard of the slightly increased "lingering bitterness" intensity perceived in the terpene alcohol fraction, since the sensation strongly correlated with this compound (r=0.841).

α-Terpineol **[20]** has been detected in Hallertau and Spalt hop varieties and added lilac- or pine-like flavour to beer (Roberts et al., 2004). The published findings may suggest that the compound has been involved in more than one sensory sensations. In this study, it particularly correlated with the attributes "orange citrus fruit" aroma

(*r*=0.613) and “rose water” flavour (*r*=0.698), but not with the “pine wood” aroma (*r*=0.098).

Previous studies indicate that some sesquiterpenoids in hop oil that have also been identified in the terpene alcohol fraction in the current study are involved in sensory interactions. Caryophyllene oxide and humulenol II have been detected in spicy essences prepared from the different hop varieties and were suggested to be two of the compounds inducing spicy aroma and flavour and herbal aroma notes in beer (Goiris et al., 2002; Praet et al., 2014). Humulenol II was also suggested to contribute to woody and green aromas (Praet et al., 2016). Pearson correlation coefficients revealed positive but no strong correlations between these compounds and equivalent attributes used in the current study. However, caryophyllene oxide and humulenol II as well as caryophyllenyl alcohol significantly correlated with “lingering bitterness” (*r*=0.854, *r*=0.858, and *r*=0.854, respectively).

Interestingly, Van Opstaele, Praet, Aerts, and De Cooman (2013) found that humulol and humulenol II could not be sensorially detected at the sniffing port in an olfactometric analysis, although present at reasonable concentrations in all tested hop varieties. Caryophyllenyl alcohol has previously been detected in hops, but its aroma or flavour profile has not yet been specified (Roberts et al., 2004). Overall, this might suggest that sesquiterpene alcohols are contributing to flavour, mouthfeel and trigeminal-type sensations or sensory interaction-derived sensations as part of a compound group rather than to aroma sensations as reported in previous studies. Also, the exact sensations elicited might be dependent on the matrix in which these compounds are applied. Further research is required in order to confirm the

occurrence of the suggested sensory interactions and the role of the monoterpene and sesquiterpene alcohols in olfactory, gustatory, and trigeminal sensations.

Furthermore, it is acknowledged that a GC coupled to a single quadrupole MS was used to identify and semi-quantify the volatile compounds in the total oil and fractions. Due to the limits of detection with this approach, compounds at very low concentrations, such as trace sulphur compounds, were not analysed, but could still have contributed to the sensory profiles of the samples.

Relationship between compound polarities and the sensory perception of hop oil fractions

The release of aroma and flavour depends on various factors including intrinsic chemical properties of the volatile compounds (polarity or hydrophobicity/hydrophilicity), the composition of the matrix in which they are applied, and environmental conditions such as temperature or pH (Ammari & Schroen, 2018). The latter two factors were consistent in all samples, however the chemical properties differed. In Table 2.5., the Log*P*, the logarithm of the octanol/water partition coefficient, is listed for each volatile compound as an index of their polarity (Moriguchi, Hirono, Liu, Nakagome, & Matsushita, 1992). The sample Log*P* for the total oil and the fractions was calculated on the basis of the relative contribution of individual compounds in the oil/fraction. It was found that the total oil and the fractions differ considerably with respect to their polarity, with the total oil and the sesquiterpene fraction comprising of nonpolar compounds. In contrast, the terpene alcohol fraction contained several polar compounds that readily dissolve in water.

It was hypothesised that the differences in polarity among the hop oil fractions (and compounds) might have an impact on the perception of the orthonasal aroma intensity due to different degrees of volatile retention in the ethanol solution and the partitioning and release of the volatiles into the headspace (Clark et al., 2011; Goubet, Le Quere, & Voilley, 1998). In contrast to the terpene alcohol and the myrcene fractions, the total oil and sesquiterpene samples obtained comparably low scores for the "overall aroma intensity". Polar volatiles present in high concentrations in the terpene alcohol fraction are more soluble in water and thus sustain headspace concentrations more effectively in a dynamic headspace situation such as that which arises when sniffing an opened jar. However, as previously mentioned, the polarity or hydrophobicity of the compounds is not the only factor that needs to be considered when investigating the impact of volatiles on the overall aroma intensity. Interactions at perceptual or compound level and differences in odour threshold concentrations (depending on the physico-chemical properties of the matrix) might contribute to the perceived aroma intensity too, especially if investigating a complex volatiles matrix as present in the total oil and the hop oil fractions.

The myrcene and the terpene alcohol fractions obtained the highest overall aroma intensity scores. This was probably due to the fact that the myrcene fraction was enriched in *β*-myrcene and the terpene alcohol fraction contained relatively high amounts of linalool and geraniol. All compounds were present at concentrations considerably in excess of their aroma threshold levels. The aroma threshold concentration ranges of *β*-myrcene, linalool and geraniol in beer were suggested to be 30-1000 µg/L (Schönberger & Kostelecky, 2011), 2.2-5 µg/L and 6-7 µg/L (Kaltner

& Mitter, 2009; Takoi, Itoga, et al., 2010), respectively, depending on the composition of the beer matrix and the method of threshold determination.

Based on the data generated in the sensory training sessions and the mock evaluation and the inspection of the panels' discriminative abilities, it was decided whether an attribute should be selected to describe aroma or flavour. "Alcohol" and "rose water" were selected to describe flavour sensations in the samples. Recently, Piombino, Moio, and Genovese (2018) suggested that the release of polar volatiles from wine was increased in retronasal conditions while the release of nonpolar volatiles diminished. More polar compounds have been found to be retained in the oral and nasal cavities through retention by the nasal mucosa, and are released at higher concentrations to the exhaled breath (Sánchez-López, Ziere, Martins, Zimmermann, & Yeretzian, 2016). The attribute "rose water" flavour appears to be mainly induced by polar monoterpene alcohols (linalool, geraniol) in the terpene alcohol fraction.

Overall, the perception of hop oil compounds appears to be highly complex and it is important to take the composition of compound mixtures and their physico-chemical properties into account in order to fully understand the sensory profile that is obtained. If future research designs allow, selected attributes could be included in both categories (aroma and flavour). The increased data input would help to understand the impact of the compounds' polarities on the overall aroma and flavour perception of the total oil and hop oil fractions, but also to build the PLS regression models described in the following section.

Prediction of sensory scores from GC-MS peak areas

PLS regression methods can be used to analyse data that is strongly collinear, noisy, and has numerous *X*-variables whilst simultaneously modelling response variables (Wold, Ruhe, Wold, & Dunn, 1984). This method has been used in previous studies to predict sensory qualities e.g. of wine based on GC-MS data (Schmidtke, Blackman, Clark, & Grant-Preece, 2013). PLS regression analyses were conducted to verify the correlation between 68 different hop oil compounds (*X*-matrix) listed in Table 2.6. and 18 sensory qualities of the six hop oil samples (*Y*-matrix) listed in Table 2.4. PLS1 and PLS2 were conducted for univariate and multiple sensory attributes, respectively. PLS2 is used to provide a global impression of the sensory profiles. PLS1 models provided a clearer fit of the data compared to PLS2 for multiple attributes as shown in the model performance data presented in Table 2.6. R^2 or the goodness-of-fit indicates how close the data are to the fitted regression line. The Root Mean Square Error (RMSE) is the standard deviation of the residuals or predication errors. The closer the RMSE to 0, the less prediction errors have been found. The advantage of PLS2 is that only one set of PLS factors exists for all analytes, which simplifies the interpretation and allows for graphical inspection. However, if aiming for the best predictive accuracy PLS1 should be used (Wold et al., 1984).

For the PLS1, the best models could be obtained for the attributes “soapy”, “earthy”, “orange citrus fruit”, “grapefruit zest”, “artificial lemon”, and “fresh lemon” aroma and “rose water” flavour. Many attributes obtained large ranges of scores among the samples in the sensory evaluation and helped to define the sensory characteristics of the total oil and the five fractions.

Table 2.6. Sensory scores mean range and PLS regression model performance (PLS1, PLS2) for prediction of the sensory attributes using the normalised peak areas of principal hop oil compounds in the hop oil/fraction samples (**Table 2.5**.).

		Sensory scores				PLS2 model performance[a]		PLS1 model performance[b]	
Modality	**Attribute**	**Min**	**Max**	**Mean**	**SD**	**R^2**	**RMSE**	**R^2**	**RMSE**
Aroma	Soapy	1.52	4.10	2.72	1.18	0.929	0.287	0.995	0.075
	Musty	1.26	3.35	1.87	0.79	0.587	0.461	0.984	0.091
	Pine wood	2.66	4.64	3.68	0.85	0.704	0.422	0.962	0.151
	Earthy	0.67	2.21	1.25	0.62	0.713	0.305	0.982	0.077
	Resinous	1.93	3.15	2.57	0.48	0.049	0.429	0.927	0.119
	Crushed grass, sap	2.19	5.44	2.94	1.24	0.227	0.994	0.961	0.224
	Orange citrus fruit	1.95	4.26	2.97	1.00	0.776	0.432	0.964	0.174
	Grapefruit zest	1.59	3.29	2.37	0.79	0.892	0.237	0.997	0.037
	Fresh lemon	1.38	3.71	2.50	1.06	0.951	0.214	0.998	0.041
	Artificial lemon	0.81	2.89	1.75	0.87	0.883	0.270	0.972	0.133
Flavour	Rose water	1.20	5.46	2.94	1.73	0.880	0.549	0.995	0.113
	Alcohol	3.06	3.51	2.94	0.16	0.213	0.131	0.956	0.031
Taste	Sweet	1.35	2.37	1.85	0.40	0.423	0.281	0.948	0.084
	Sour	1.47	2.37	1.85	0.38	0.467	0.255	0.983	0.046
	Bitter	2.97	3.82	3.46	0.34	0.292	0.262	0.968	0.056
	Lingering bitterness	2.71	4.18	3.29	0.51	0.309	0.387	0.886	0.157
Mouthfeel	Peppery tingling	1.94	3.17	2.53	0.41	0.438	0.358	0.956	0.079
	Astringent	3.70	4.79	4.28	0.41	0.447	0.365	0.972	0.064

[a] PLS2 algorithms for multivariate sensory attributes
[b] PLS1 algorithms for univariate sensory attributes
RMSE, Root mean square error; R^2; R-squared, goodness-of-fit

For PLS1, good models were obtained for all attributes. However, after evaluation of the model by checking the RSME, degrees of freedom, and standardised coefficients plots for the predictors (95% confidence interval), it was concluded that the model overfits the data in view of the taste and mouthfeel attributes suggesting that the model obtained by PLS1 should not be used. Overall, it appears to be difficult to identify linear relationships between compounds and one sensory sensation. Based on the measurement errors in the data, one might assume that the robustness of both models might have been dependent on the uncertainty in the sensory scores and to a lesser extent on the analytical data that was obtained using GC-MS analysis

(PAN data). However, as concluded in the previous sections, more than one compound is likely to be involved in the perception of a sensory sensation due to sensory interactions (synergistic, additive) between compounds and within or across sensory modalities. This was expected to be the main reason for the weak prediction of taste and mouthfeel attributes. The goodness-of-the-fit was lowest for all of these attributes which is explained by the fact that sensations are to a certain extent the result of sensory interactions as discussed in the previous sections. For instance, the fruity aroma – sweet taste interactions is suggested to be induced by methyl esters, ketones and/or monoterpene alcohols and a cross-modal interaction might have been induced by compounds in the terpene alcohol fraction causing a slightly increased “peppery tingling” mouthfeel sensation.

Overall, it was concluded that the sensory scores were not entirely predictable based on GC-MS data, but PLS2 models give a good overview of important compound groups that are involved in different sensation of the multi-sensory profiles of the hop oil fractions. PLS models might help to identify the occurrence of sensory interactions that contribute to the sensory characteristics of hop essential oil. The outcome of the PLS regression analysis in this study shows once more that when evaluating the sensory contribution of volatile compounds in hop essential oil or hop oil fractions to a “hoppy” flavour sensation, simple cause-effect-relationships between sensations and chemicals are only able to explain half of the story.

2.4 Conclusions

This was the first study to establish a sensory attribute lexicon and to investigate the sensory characteristics of a hop essential oil and five $scCO_2$ fractions extracted thereof in ethanol (4%, abv). The study provides significant insight into the sensory differences between the hop oil fractions and suggests a relationship between the perception and intensities of the analysed sensory characteristics and the physico-chemical nature of the fractions. While the total oil and the sesquiterpene fractions obtained moderate to low sensory scores for all sensory attributes, likely due to the nonpolar character of the compounds, compound concentrations and sensory threshold levels, the myrcene, ketone, ester and terpene alcohol fractions showed comparatively high sensory impacts by inducing different grassy, musty, fruity and floral aromas and flavours. In case of the latter two fractions the aroma and flavour sensations occurred in combination with increased taste and mouthfeel characteristics.

Due to sensory interactions single compounds could not be assigned to specific sensory sensations (and vice versa) even in a simple ethanol-water system. However, few single compounds in the monoterpene alcohol fraction (linalool, geraniol) and compound groups in the ketone and ester fractions (methyl and geranyl esters) positively correlated with the “rose water” flavour sensation whereas the “crushed, grass sap” aroma could be clearly assigned to the presence of *β*-myrcene. Whilst, increased or added taste and mouthfeel sensations could not be assigned to any compound suggesting that these were perceived as a result of sensory interactions within (e.g. “sweet” taste) or across (e.g. “peppery tingling” mouthfeel) sensory

modalities. This explained why the PLS models could not successfully predict the sensory scores for these taste and mouthfeel attributes based on the analytical data.

It is recommended to consider temporal sensory profiling methods for future studies to investigate the sensory characteristics of hop oil fractions. The lack of significant effects for the lingering taste and mouthfeel attributes assessed in the current study may have been caused by the fact that only one time point was selected for their assessment. Temporal sensory methods such as Progressive Profiling or Time-Intensity where the intensity of attributes is continuously assessed over a defined period of time may be more appropriate to obtain a dynamic sensory profile of these sensations.

Considering the volatile composition of the highly polar monoterpene alcohol and the less polar sesquiterpene alcohol sub-fractions, it remains to be investigated which role the compounds' or fractions' polarities have in view of the multi-sensory profile in the terpene alcohol fraction. Omission or addition studies appear to be suitable to identify compounds that could be involved in these interactions. Further research is also required into the chemical composition of Magnum hop essential oil and its fractions to detect those compounds that were present sub-detection threshold in the current study.

Moreover, the hop extracts used were produced to be added as post-fermentation products. In this way, volatile losses due to biotransformation reactions or evaporation can be limited to a minimum, the addition of hops is simple and less time-consuming, and the hop flavourings are less prone to deterioration compared to traditional hop materials, thereby improving efficiency and sustainability of the

hopping procedure. Considering that the hop extracts were exclusively tested in an ethanol-water base in order to obtain a general understanding of their sensory characteristics, the next essential step will be to investigate their sensory impact in a beer matrix. Vice versa, it is required to study the effect of other components in the beer matrix on the perception of the hop oil fractions. The investigation of mutual influences might help to understand the potential of these fractions as flavouring materials in various beer styles.

It should be taken into account that the current study solely focused on the Magnum hop variety. Comparing the concentrations detected in the current study with those published for other hop varieties, Magnum hop oil contains relatively high concentrations of *β*-myrcene and *α*-caryophyllene, but in particular of *β*-humulene - compared to so-called aroma hop varieties, but also compared to bitter or high-alpha hop varieties. It generally contains a high content of sesquiterpenes and oxygenated sesquiterpenes including caryophyllene oxide, but also a considerable amount of esters and monoterpenes. In contrast to many hop varieties, Magnum hop oil contains no citrus fraction and no or hardly detectable sulphur-containing compounds. Other alpha- and high-alpha varieties, such as Galena (Magnum parent), Taurus, Columbus, Horizon, Nugget, Tradition, and Summit may provide comparable hop oil fractions. However, this highly depends on the fractionation approach and the ratio of aroma- and flavour-driving compounds in these fractions. Moreover, a limited number of fractions were applied at one concentration. Thus, the results should not be generalised, but this research could provide the basis for future studies investigating other hop varieties using modified experimental designs.

Overall, it has been shown that the fractionation of Magnum hop essential oil can be applied to obtain distinct and sustainably produced flavouring preparations. These may be used in isolation or combination in order to achieve distinct aroma, flavour, taste, and mouthfeel sensations. The findings of this study, together with the potential impacts of global warming and climate changes on oil yield and composition of hop varieties, suggest that more attention should be given in future to the sensory properties of “bitter hop varieties”.

Chapter 2B

2 B. Gas chromatrographic analysis of stored Magnum hop essential oil and supercritical CO_2 fractions

2.1 Preliminary thoughts to Chapter 2B

The following study has been conducted in 2020 as a Covid19 response because the initially planned study could not be conducted due to the changed circumstances at the university. The described shelf-life study investigates the compositional differences between the total Magnum hop oil and its hop oil fractions at only two time points, just after their extraction and after 26 months of storage. Although, the study provides relevant insights with regard to the storage stability of the hop oil extracts, future or follow up research needs to consider an improved experimental design, which should include more time points of assessment and may include further storage conditions. This is further discussed in the Results and discussion section of this chapter.

Gas chromatrographic analysis of stored Magnum hop essential oil and supercritical CO_2 fractions

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Keywords: Supercritical CO_2 extracts; hop oil storage; hop volatile composition; GC-MS analysis; Magnum hops

Highlights

- Fresh and stored supercritical CO_2 hop oil extracts were compared.
- Compositional data of the hop oils differed due to few qualitative and quantitative profile changes.
- The concentration of *β*-myrcene as a marker for hop freshness remained stable.
- Few compounds were newly produced or no longer detected in the stored hop oil.

Abstract

It is well-known that the volatile profiles of CO_2 hop extracts are more stable compared to raw hop materials such as hop pellets. However, little is known regarding the qualitative and quantitative changes in hop oil composition if the extracts are stored for a long period. This knowledge is relevant for long-term research studies such as sensory trials for which a consistent volatile profile is crucial. To investigate these effects, fresh supercritical CO_2 hop oil extracts obtained from a Magnum hop variety were analysed using gas chromatography-mass spectrometry (GC-MS) and compared to the same extracts stored for 26 months (-20^{o}C, flushed with nitrogen). The data was subjected to Analysis of Variance (ANOVA) to detect significant differences between the hop oil samples and Principal Component Analysis (PCA) to further study and visually illustrate the relationship between their compositional profiles. The outcome of the GC-MS analyses revealed few significant differences between the hop oil samples suggesting limited changes occurred during storage. Several difference were detected between the total Magnum hop oils as a result of newly identified compounds. However, the concentrations of compounds that are frequently used as 'hop freshness markers' remained relatively stable (e.g. *β*-myrcene). A slight decline in concentration was detected for compounds in the terpene hydrocarbon, oxygenated sesquiterpenoid, ester, and ketone fractions. The study outcome provides an overview of the changes occurring within supercritical CO_2 hop oil extracts and confirms their long-term stability. Future research should explore whether the compositional changes in the total oil sample would have an impact on its sensory profiles.

2.2 Introduction

Supercritical CO_2 hop oil extracts are increasingly used to replace traditional and so-called 'raw hop materials' for conventional brewing practices to improve standardisation of hop flavour, bitterness and mouthfeel characters, facilitate handling and processing, and reduce spent hop materials and storage volume required (Marriott, 2019; Van Opstaele, Goiris, et al., 2013). It has generally been recommended by hop extract producers to store the extracts at cool temperatures (0-4^{o}C) with the bottles tightly closed and exposure to light and oxygen limited, thus, similar to raw hop materials such as hop pellets. Under these conditions, the shelf life of the extracts has been estimated to be between six months and one year at approximately 4^{o}C (Totally Natural Solutions, 2021).

In research environments, these extracts are usually stored frozen at -18 - -30^{o}C and often flushed with nitrogen to best control deterioration of volatiles throughout experiments for which the integrity of volatile profiles needs to be maintained (Lermusieau & Collin, 2001; Van Opstaele, Goiris, et al., 2013). In commercial contexts, it is unclear if this process is upheld. Multiple processes could act upon the quantitative and qualitative composition of hop oils if not adequately stored. Volatilisation, modification and polymerisation of hop-derived volatiles occur depending on the exposure to oxygen, light, humidity, and temperature or fluctuations of these factors potentially causing significant loss of volatiles and consequently changing 'hoppy' aroma and flavour characteristics (Tedone et al., 2020). Maintaining a consistent volatile composition in the hop oil extracts is crucial if conducting long-term sensory trials. Sensory studies can take a considerable

amount of time, for instance if several training sessions are required to train descriptive panels on the sensory characteristics of complex samples for subsequent Quantitative Descriptive Analysis (QDA) or where assessment against control samples is needed as part of a sensory shelf-life study.

This work aimed to compare the volatile profiles of fresh supercritical CO_2 Magnum hop oil extracts and the same extracts stored for approximately two years (26 months) at -20°C. Based on previous research, it is hypothesised that the chemical composition of the total oil will undergo noticeable changes during this time period. However, it is unclear how exactly the extracted Magnum hop oil fractions will modify. These changes may be dependent on the fraction composition. To investigate this, volatile profiles of the total oil and the fractions were obtained using gas chromatography-mass spectrometry (GC-MS). The outcome should provide an overview of quantitative and qualitative changes in the volatile composition and potential effects on the sensory characteristics that could be assessed in future sensory trials.

2.3 Materials & Methods

2.3.1 Hop extracts

Aliquots of the pure total Magnum hop oil (total oil) and five supercritical CO_2 fractions enriched in 1) sesquiterpenes, 2) myrcene, 3) esters, 4) ketones, and 5) terpene alcohols were received in May 2017 in 2 mL amber autosampler vials, flushed with nitrogen and were stored hermetically sealed at -20°C, as recommended by the

producing company. The fractions were produced as described by Marriott (2019). In total, 26 months elapsed between the GC-MS analysis of the fresh and the stored samples. The two analysed batches are hereinafter referred to as 'fresh' and 'stored' hop oil samples.

2.3.2 Gas chromatography-mass spectrometry

The volatile composition of the total oil and the five fractions was analysed in triplicate by the GC-MS analysis method described by Dietz, Cook, Wilson, et al. (2020b). The stored hop oil samples were spiked with 1 µL of 1050 mg/L benzyl acetate (≥99%; Sigma Aldrich, UK) as an internal standard (ISTD). For GC-MS analyses, several authentic reference standards were used including *endo*-borneol (≥97%), caryophyllene oxide (≥99.0%), geraniol (≥99%), geranyl acetate (≥99%), geranyl isobutyrate (≥97%), geranyl propionate (≥95%), linalool (≥97.0%), *R*-(+)-limonene (≥97%), methyl decanoate (≥99%), methyl geranate (≥94.0%), methyl octanoate (≥99%), *α*-humulene (≥ 96%), *β*-caryophyllene (≥98.5%), *α*-terpineol (≥97), *β*-myrcene (≥90.0%), *β*-pinene (≥99%), 2-dodecanone (≥97%), 2-nonanone (≥99%), 2-tridecanone (≥97%), and 2-undecanone (≥98.0%) (Sigma Aldrich (UK)). RIs were determined using a homologous series of n-alkanes (C6-C30; Sigma-Aldrich, St. Louis, MO). NIST Mass Spectral Library (NIST08) and Wiley7n.1 (Hewlett-Packard, US) databases were used for library matching. Obtained mass spectra and RI were compared with those published in databases (Flavornet, Pherobase, Pubchem) or studies using columns with comparable stationary phases. Peaks were assigned to compounds if the MS fit factor was ≥800 and the calculated RI closely matched literature values. Otherwise, compounds were specified as "unknown".

2.3.3 Statistical analysis

All data were analysed using XLSTAT (2021.2.1, Addinsoft, US). Semi-quantification was conducted by normalising the integrated peak areas of the hop compounds relative to the ISTD ion peak area (stored extracts) and relative to the total integrated peak area in each chromatogram (both extracts). Analysis of Variance (ANOVA) was conducted to identify significant differences between the GC-MS datasets and to determine the impact of the storage time and temperature. Principal Component Analysis (PCA) was used to investigate similarities and differences between the volatile characteristics of the fresh and stored hop oil samples in a multi-dimensional compositional space.

2.4 Results and discussion

Table 2.1**.** lists the volatile compounds identified in the total oil and the five hop oil fractions. All identified compounds have previously been found in Magnum hop oil or hop oil extracted from other hop varieties. The semi-quantified volatile composition of the hop extracts can be found in Table 2.2**.** as well as parameters providing an indication about compounds' hydrophobicity and solubility and their sensory detection threshold concentrations in beer and comparable ethanolic solutions at 4-5% ABV (where available). Sensory taste and mouthfeel threshold concentrations of these compounds have not yet been published. Aroma and flavour threshold data could be collected for 22 of the 72 compounds that could be identified in both samples of the total oil. The GC-MS data of the fresh and stored hop oil

samples and qualitative and quantitative compositional differences between these are discussed in the following sections.

2.4.1 Differences between the two semi-quantification approaches

The volatile compounds present in the total oil and the five hop oil fractions were semi-quantified based on internal standard (ISTD) normalisation (benzyl acetate) and peak area normalisation based on the total integrated area in each chromatogram. Table 2.2**.** shows the compound percentages obtained for the total oil. Overall, the volatile compositions of the sample set showed few differences and the patterns in the volatile profiles i.e. presence and ratio of volatiles were almost identical. However, percentages obtained from ISTD normalisation were generally lower compared to the percentages using the other approach. This magnitude effect was apparent for compounds present at relatively high concentrations such as for *β*-myrcene, *α*-humulene, and *β*-caryophyllene. The effect was also apparent for the most abundant compounds in the individual hop oil fractions.

An effect of the ISTD on the calculated compound percentages may be rejected because preliminary tests showed that is has similar analytical behaviour compared to the main volatiles in the hop extracts and is naturally not present in Magnum hop oil. In turn, it appeared that the ISTD was also not affected by the hop-derived compounds or the solvent (iso-octane). It could be that the decreases in concentration were caused by incomplete integration of the total peak areas, for instance by eliminating small compound peaks whilst removing background noise. Nevertheless, the outcome is in line with previous research showing that the data of both approaches are comparable and can serve as a valid rapid profiling techniques

as an alternatives to quantification using external calibration curves if the aim is to receive a general overview or 'fingerprint' of the composition and reliable compound ratios in essential oils (Ruiz-Hernández, Roca, Egea-Cortines, & Weiss, 2018).

2.4.2 Changes in the qualitative composition

Newly detected compounds in the stored hop oil extracts

Several compositional differences could still be identified between the fresh and the stored hop oil samples. Volatile compounds that were only identified in the stored hop oil were (in order of elution): methyl (*2E*)-2-heptanoate, *D*-limonene, an unknown monoterpene with calculated retention index (RI) of 1088, 7-decen-2-one, 2-decanone, an unknown sesquiterpenoid (RI 1335), *α*-bergamotene, and *γ*-muurolene. The fruity ester methyl heptanoate is known to be naturally present in floral hop essences of some hop varieties and has been suggested to be a precursor of ethyl heptanoate frequently detected in beer (Takoi et al., 2018; Tressl, Friese, Fendesack, & Koeppler, 1978b; Van Opstaele, De Causmaecker, et al., 2012). In contrast to the current findings, Tressl et al. (1978b) observed that the concentration of methyl heptanoate decreased when storing raw hops at 0°C for a period of three years. It was expected that supercritical CO_2 hop oil extracts stored at appropriate conditions will maintain the volatile composition better compared to raw materials (Priest, Boersma, & Bronczyk, 1991). However, it is unclear why the concentration of methyl (*2E*)-2-heptanoate increased during storage. This effect should be further investigated and confirmed by analysing different batches of Magnum hop oil or other hop varieties containing this compound.

The same applied for *D*-limonene and the sesquiterpenes *α*-bergamotene and *γ*-muurolene. *D*-Limonene is a monoterpene providing citrusy aroma and flavour, which was detected at 0.30% in the stored hop oil. This compound has previously been detected in Magnum hop oil (Michiu et al., 2018; Salanţă et al., 2012) and in another Magnum hop oil batch (Dietz, Cook, Wilson, Oliveira, & Ford, 2021a). Concentrations have been published in a range of 0.29-1.70%. It should be noted that limonene is considered an autoxidation product of myrcene (Dieckmann & Palamand, 1974) and a dehydration product of *α*-terpineol (Stevens, 1967) and both compounds have been found to be reduced upon storage in the current study.

The sesquiterpenes *α*-bergamotene (0.10%) and *γ*-muurolene (0.83%) have predominantly been identified in the sesquiterpene and the ester enriched fractions. Both compounds are known as hop-derived volatiles in Magnum hop oil (Dietz et al., 2021a) to induce 'woody', 'oily' and 'tea-like' aromas, and are considered to be relatively stable (Eri, Khoo, Lech, & Hartman, 2000; Gros et al., 2011; Lermusieau & Collin, 2001). Again, it is not clear why these compounds were not detected in the fresh hop oil samples and whether these compounds are potentially reaction products of other volatiles present in the hop oil.

The stored hop oil also contained relatively small quantities of 7-decen-2-one (0.01%) and 2-decanone (0.09%) in the ester and ketone enriched fractions. 7-Decen-2-one has frequently been detected in hopped ginger beer (e.g. Nutakor, Essiedu, Adadi, and Kanwugu (2020)), but so far unrelated to hops and was therefore considered as tentatively identified. Further research should confirm the compounds' hop origin using authentic standards. In contrast, 2-decanone has frequently been identified in

hops including in the Magnum hop variety but at low concentrations (trace-0.56%) (Aberl & Coelhan, 2012; Pistelli et al., 2018; Salanta et al., 2015). The flavour detection threshold of the 'fruity' ketone 2-decanone was determined to be 250 µg/L in beer (Meilgaard, 1975b). However, even at a significantly lower concentration, this compound could still contribute to the overall aroma and flavour profiles of beer due to synergistic or additive sensory interactions with other volatiles present.

Two further compounds were detected at low concentrations in the stored but not in the fresh hop oil samples and could not be tentatively identified, namely a monoterpene (RI 1088, RT 8.93 min) and a sesquiterpenoid (RI 1335, RT, 16.84 min). Both compounds were present at low concentrations (total oil: 0.03% and 0.15%, respectively).

Table 2.1. Volatile compounds (tentatively) identified (n=65) in the stored total hop oil sample and the five hop oil fractions using library/database matching (>80%) and authentic standards (*). Identification confirmed by calculated retention indices (RI)[1] compared to literature RIs (LRI).

#	Compound	CAS	RI[1]	LRI
1	*β*-Pinene*	127-91-3	984	980-990[a,b]
2	6-Methyl-5-heptene-2-one	110-93-0	987	985[a]
3	*β*-Myrcene*	123-35-3	989	991-994[a,b]
4	Methyl (*2E*)-2-heptanoate	106-73-0	1005	1006-1021[a]
5	*R*-(+)/D-Limonene*	5989-27-5	1029	1030-1039[a,b]
6	*cis*-*β*-Ocimene	3338-55-4	1034	1038-1043[a,b]
7	*cis*-Linalool oxide	1365-19-1	1067	1070-1074[a,b]
8	Methyl 6-methyl heptanoate	2519-37-1	1055	1060[e]
9	2-Nonanone*	821-55-6	1082	1093[b]
10	Unknown monoterpene (RI 1088)	n/a	1088	n/a
11	Linalool*	78-70-6	1095	1098-1112[a,b]
12	3-Methylbutyl 2-methylbutanoate	27625-35-0	1105	1099-1102[a]
13	*exo*-*β*-Fenchol	470-08-6	1113	1117[a]
14	Myrcenol	543-39-5	1119	1118[a]
15	Methyl octanoate*	111-11-5	1133	1127[c]
16	Hexyl isobutyrate	820-29-1	1146	1151[k]
17	5-Decanone	6627-72-1	1168	1176[m]
18	*endo*-Borneol*	464-45-9	1171	1162-1165[a,b]
19	7-Decen-2-one	35194-33-3	1171	n/a
20	Terpinen-4-ol	562-74-3	1175	1177-1182[a,b]
21	*trans*-3(10)-Caren-2-ol	93905-79-4	1179	1175[d]
22	Methyl-6-methyl octanoate	5129-62-4	1181	1195[z]
23	2-Decanone	693-54-9	1183	1190[aa]
24	*α*-Terpineol*	8000-41-7	1184	1185-1207[a,b]
25	Methyl (*4E*)-4-nonenoate	20731-19-5	1223	1225[a]
26	Nerol	106-25-2	1224	1228-1233[a,b]
27	Geraniol*	106-24-1	1274	1255-1276[a,b]
28	Methyl 8-methylnonanoate	5129-54-4	1280	1287[f]
29	2-Undecanone*	112-12-9	1292	1296[b]
30	Perillol (Perillyl alcohol)	7644-38-4	1296	1295[c]
31	2-Undecanol	1653-30-1	1309	1301[g]
32	Methyl (*E*)-4-decenoate	93979-14-7	1314	1311[h]
33	Methyl 4,8-decadienoate	1191-03-3	1319	1316[b]
34	Methyl geranate*	2349-14-6	1321	1323[h]
35	Methyl decanoate	110-42-9	1323	1324-1326[a,b]
36	Octyl Isobutyrate	109-15-9	1328	1326 [i]
37	Unknown sesquiterpenoid (RI 1335)	n/a	1335	n/a

Table 2.1 continued.

#	Compound	CAS	RI[1]	LRI
38	Methyl 2-decenoate	2482-39-5	1371	n/a
39	Geranyl acetate*	105-87-3	1372	1382[a,b]
40	Methyl undecanoate	1731-86-8	1373	1375[a]
41	2-Dodecanone*	6175-49-1	1379	1379 [j]
42	Methyl undecenoate	111-81-9	1395	1396[a]
43	*β*-Caryophyllene*	87-44-5	1420	1418-1467[a,b]
44	*α*-Bergamotene	17699-05-7	1434	1430-1434[a,b]
45	*α*-Humulene*	6753-98-6	1455	1467[b]
46	Geranyl propionate*	105-90-8	1474	1475[a]
47	*γ*-Muurolene	30021-74-0	1475	1477-1475[a,b]
48	Neryl isobutyrate	2345-24-6	1480	1491[a]
49	*β*-Eudesmene	515-17-3	1484	1485[a]
50	2-Tridecanone*	593-08-8	1490	1496[h]
51	*cis*-5-Dodecenoic acid, methyl ester	2430-94-6	1490	n/a
52	Methyl 3,6-dodecadienoate	16106-01-7	1493	1488[j]
53	Geranyl isobutyrate*	2345-26-8	1514	1514[a]
54	*δ*-Cadinene	483-76-1	1531	1519-1539[a,b]
55	Unknown sesquiterpenoid (RI 1532)	n/a	1532	n/a
56	(*E*)-Nerolidol	7212-44-4	1535	1534-1565[a,b]
57	Caryophyllenyl alcohol	56747-96-7	1569	1556-1568[a,b]
58	Caryophyllene oxide*	1139-30-6	1577	1573-1606[a,b]
59	Humulene epoxide I	19888-34-7	1579	1578[l]
60	Humulol	28446-26-6	1580	1582[l]
61	Humulene epoxide II	19888-34-7	1590	1593[j]
62	Widdrol	6892-80-4	1597	1597[b]
63	1-Epicubenol	19912-67-5	1610	1613-1645[a,b]
64	Humulene epoxide III	21624-36-2	1613	1611[l]
65	Humulenol II	19888-00-7	1620	1613[l]
66	*τ*-Cadinol	5937-11-1	1636	1640[a]
67	*δ*-Cadinol	36564-42-8	1632	1635-1674[a,b]
68	2-Pentadecanone	2345-28-0	1701	1699[c]

ISTD, Internal standard

[1] Column used: Zebron ZB-5MS capillary column (30 m x 0.25 mm ID x df = 0.25 µm)

[a] Pherobase; [b] Flavornet; [c] Nance and Setzer (2011); [d] Kang, Zhang, Du, and Wang (2010); [e] Fernandes et al. (2019); [f] Ilic-Tomic et al. (2015); [g] Zhang et al. (2017); [h] Pistelli et al. (2018); [i] Venkatachallam, Pattekhan, Divakar, and Kadimi (2010); [j] Jackson and Linskens (2002); [k] Ruther (2000); [l] Praet et al. (2016); [m] Adams, Kitryte, Venskutonis, and De Kimpe (2011); [n] Pino, Marbot, and Bello (2002); [o] Perry, Wang, and Lin (2009); [p] Palá-Paúl et al. (2005); [q] Giuseppe, Manuela, Marta, and Vincenzo (2005); [r] Yan et al. (2018); [s] Liu, Wang, and Liu (2018); [t] Minh Tu et al. (2002); [u] Choi and Sawamura (2000); [v] Stashenko et al. (2010); [w] Richter, Eyres, Silcock, and Bremer (2017); [x] Hofmann, Fritz, Nitz, Kollmannsberger, and Drawert (1992); [y] Miyazawa, Kawauchi, and Matsuda (2010); [z] Vázquez-Araújo, Parker, and Woods (2013) ; [aa] Paventi et al. (2020)

Table 2.2. Semi-quantified volatile composition of the fresh (F) and stored (ST) total oil and five hop oil fractions (average relative peak area %, obtained by internal standard normalisation), Log*P* (logarithm of the octanol-water partition coefficient) used as an indicator for the hydrophobicity, solubility in water, and sensory detection thresholds of volatile compounds in beer and comparable ethanolic solutions (4-5% ABV; where available). The total oil was also semi-quantified based normalisation of integrated compound peak areas using the total integrated peak area in each chromatogram (PAN).

		TO			SQ		MYR		EST		KET		TALC				
#C	**Compound**	**F**	**ST**	**PAN**	**F**	**ST**	**F**	**ST**	**F**	**ST**	**F**	**ST**	**F**	**ST**	**Log*P****	**Solubility [mg/L]***	**Sensory detection threshold [µg/L]****
1	*β*-Pinene	0.01	0.01	0.01	-	0.01	3.12	2.70	-	-	-	-	-	-	4.16	7.06	n/a
2	6-Methyl-5-heptene-2-one	0.01	0.01	0.01	-	-	-	-	0.07	0.07	0.09	0.06	0.05	0.05	2.06	1651.00	n/a
3	*β*-Myrcene	37.31	36.69	37.35	2.29	1.98	91.72	89.51	-	-	-	-	-	-	4.88	6.92	A: 9-1000[a]; F: 40[b]
4	Methyl (*2E*)-2-heptanoate	-	0.08	0.08	-	-	-	-	-	-	-	0.07	-	-	2.82	307.80	n/a
5	*R*-(+)/*D*-Limonene	-	0.30	0.31	-	-	-	-	-	-	-	-	-	0.01	4.57	4.58	n/a
6	*cis*-*β*-Ocimene	0.09	0.07	0.08	0.02	0.02	-	-	-	-	0.01	0.01	-	0.01	4.67	2.01	n/a
7	*cis*-Linalool oxide	0.01	0.01	0.01	-	-	-	-	-	-	-	-	0.14	0.08	2.08	3353.00	n/a
8	Methyl 6-methyl heptanoate	0.67	0.56	0.57	-	-	-	-	0.71	0.68	0.35	0.17	-	-	3.40	117.80	n/a
9	2-Nonanone	0.24	0.25	0.26	-	0.01	-	-	0.95	0.97	1.42	0.85	0.63	0.45	3.14	170.60	F: 2000[c]
10	Unknown monoterpene (RI 1088; stored sample)	-	0.03	0.04	-	-	-	-	-	-	-	-	-	-	n/a	n/a	n/a
11	Linalool	0.39	0.37	0.37	-	-	-	-	-	-	0.50	0.27	5.58	5.40	2.97	683.70	A: 2-80[a]; F: 27-80[c,]
12	3-Methylbutyl 2-methylbutanoate	0.18	0.08	0.08	-	-	-	-	0.17	0.14	0.03	0.02	-	-	3.56	44.59	n/a
13	2-Methylbutyl 3-methylbutyrate	0.13	-	-	-	-	-	-	0.18	-	0.04	-	-	-	3.66	n/a	n/a
14	*exo*-*β*-Fenchol	0.07	0.03	0.03	-	-	-	-	-	-	0.03	0.02	0.96	0.68	2.85	461.40	n/a
15	Myrcenol	0.04	0.03	0.03	-	-	-	-	-	-	0.03	0.01	0.36	0.20	3.46	260.90	n/a
16	Methyl octanoate	0.69	0.47	0.47	-	-	-	-	1.62	1.49	0.83	0.48	-	-	3.46	101.90	n/a
17	Hexyl isobutyrate	-	0.01	0.01	-	-	-	-	-	0.06	-	-	-	0.03	3.28	58.21	n/a
18	5-Decanone	-	0.10	0.10	-	-	-	-	-	0.63	-	0.72	-	0.25	3.20	1210.00	n/a
19	*endo*-Borneol	0.08	0.05	0.05	-	-	-	-	-	-	-	-	1.54	0.91	2.69	260.90	n/a
20	7-Decen-2-one	-	0.01	0.01	-	-	-	-	-	0.05	-	0.07	-	-	3.60	n/a	n/a
21	Terpinen-4-ol	0.02	0.01	0.02	-	-	0.01	0.01	0.01	0.01	0.03	0.02	0.32	0.20	3.26	386.60	n/a
22	*trans*-3(10)-Caren-2-ol	0.04	0.01	0.01	-	-	0.04	0.02	0.15	0.12	0.26	0.15	0.37	0.24	1.97	489.00	n/a
23	Methyl-6-methyl octanoate	-	0.19	0.19	-	0.03	-	0.10	-	1.44	-	0.48	-	-	3.32	64.40	n/a
24	2-Decanone	0.12	0.09	0.09	-	-	-	-	0.73	0.66	1.08	0.53	0.45	-	3.47	46.43	F:250c
25	*α*-Terpineol	0.21	0.14	0.15	-	-	-	-	-	-	-	-	4.02	3.92	2.98	371.70	A:330[a]; F:2000[c]
26	Myrtenol	0.01	-	-	-	-	-	-	-	-	-	-	0.05	-	2.98	1600	n/a

Table 2.2 continued.

#C	Compound	TO F	TO ST	PAN	SQ F	SQ ST	MYR F	MYR ST	EST F	EST ST	KET F	KET ST	TALC F	TALC ST	Log*P**	Solubility [mg/L]*	Sensory detection threshold [µg/L]**
27	2-Decanol	0.03	-	-	-	-	-	-	-	-	-	-	0.13	-	3.71	151.8	n/a
28	Methyl (*4E*)-4-nonenoate	0.11	0.09	0.09	0.01	0.01	0.06	0.03	0.64	0.59	0.34	0.23	0.01	0.01	2.90	52.10	n/a
29	Nerol	0.09	0.06	0.06	0.02	0.01	0.05	0.04	0.54	0.59	0.30	0.28	1.15	0.76	4.70	39.90	A: 80-500[a]
30	Geraniol	0.08	0.06	0.06	-	-	-	-	-	-	-	-	17.75	16.76	3.47	255.80	A: 4-300[a], F: 36[e]
31	Methyl 8-methyl-nonanoate	0.24	0.16	0.16	0.07	0.10	0.06	0.03	2.66	2.41	1.50	0.96	0.02	0.01	4.40	12.56	n/a
32	2-Undecanone	1.15	0.95	0.97	-	-	-	-	13.84	12.79	22.00	24.05	12.30	11.33	3.69	19.71	F: 400[c]
33	Perillol	0.02	0.01	0.01	-	-	-	-	-	-	-	-	0.28	0.21	3.17	471.00	n/a
34	2-Undecanol	0.04	-	-	-	-	-	-	0.37	0.32	0.26	0.15	0.58	0.44	4.21	49.73	F: 70[c]
35	Octyl propionate	0.05	-	-	-	-	-	-	0.49	-	0.35	-	0.19	-	4.35	10.87	n/a
36	Methyl (*E*)-4-decenoate	1.91	1.44	1.46	-	-	-	-	15.76	15.34	10.34	10.29	-	-	4.09	16.67	n/a
37	Methyl 4,8-decadienoate	0.27	0.24	0.25	0.05	0.04	0.06	0.06	2.65	2.17	2.12	2.10	0.09	-	3.87	26.50	n/a
38	Methyl geranate	0.71	0.48	0.49	-	-	-	-	12.25	12.42	9.84	9.72	-	-	3.98	21.24	F: 21.5[f]
39	Methyl decanoate	0.07	0.03	0.03	-	-	-	-	0.92	0.99	0.58	0.57	-	-	4.41	10.62	n/a
40	Octyl isobutyrate	0.13	0.08	0.08	0.09	0.07	0.02	0.02	1.93	1.82	0.97	0.79	-	-	4.71	4.06	n/a
41	Unknown sesquiterpenoid (RI 1335; stored sample)	-	0.28	0.29	-	-	-	-	-	0.01	-	0.01	-	-	n/a	n/a	n/a
42	Methyl 2-decenoate	0.07	0.07	0.07	-	-	-	-	0.11	0.09	0.23	0.23	-	-	3.97	16.97	n/a
43	Geranyl acetate	0.12	0.07	0.08	-	-	-	-	1.43	1.53	1.85	1.39	-	-	3.98	29.00	A: 35-706[j]
44	Methyl undecanoate	0.10	0.09	0.09	0.03	-	0.01	-	1.46	1.56	0.96	0.82	0.01	-	4.86	3.52	n/a
45	2-Dodecanone	0.27	0.17	0.17	-	-	-	-	3.88	3.80	6.51	6.25	3.66	3.00	4.18	13.99	F: 250[c]
46	Methyl undecenoate	-	0.01	0.01	-	-	-	-	-	0.11	-	0.09	-	-	4.79	4.71	n/a
47	*β*-Caryophyllene	8.57	6.63	6.75	19.01	17.48	-	0.57	2.12	2.03	2.55	2.49	-	-	6.30	0.05	A:160-420[a]
48	*α*-Bergamotene	-	0.10	0.10	-	0.08	-	-	-	0.19	-	0.08	-	-	6.57	0.03	n/a
49	*α*-Humulene	37.01	32.14	32.72	69.03	61.66	4.67	4.16	9.46	10.57	12.71	12.95	0.89	0.71	6.95	0.01	A: 120-747[a,g]
50	Geranyl propionate	0.01	-	-	-	-	-	-	0.14	0.14	0.24	0.28	-	-	3.64	2.22	n/a
51	*γ*-Muurolene	-	0.83	0.84	-	0.62	-	-	-	0.17	-	0.02	-	-	4.51	0.05	n/a
52	Neryl isobutyrate	-	0.15	0.16	-	-	-	-	-	0.97	-	0.46	-	-	3.45	0.82	n/a
53	*β*-Eudesmene	0.32	0.29	0.29	1.05	0.88	-	-	-	-	-	-	-	-	6.38	0.04	n/a
54	2-Tridecanone	0.06	0.06	0.06	0.06	0.04	-	-	0.60	0.61	1.02	0.90	-	-	4.68	4.53	F: 100[c]
55	*cis*-5-Dodecenoic acid, methyl ester	0.10	0.10	0.10	-	-	-	-	1.54	1.59	1.46	1.49	-	-	4.00	9.12	n/a

Table 2.2 continued.

#C	Compound	TO			SQ		MYR		EST		KET		TALC				
		F	ST	PAN	F	ST	F	ST	F	ST	F	ST	F	ST	Log*P**	Solubility [mg/L]*	Sensory detection threshold [µg/L]**
56	Methyl 3,6-dodecadienoate	0.20	0.20	0.20	-	-	-	-	0.90	0.93	0.95	0.97	-	-	4.10	2.77	n/a
57	Geranyl isobutyrate	1.84	1.64	1.67	-	-	-	-	16.98	20.96	12.02	12.46	-	-	4.77	0.82	A:450[a]; F:450[e]
58	*δ*-Cadinene	2.76	2.45	2.50	7.79	7.01	-	-	0.77	0.76	0.86	0.66	-	-	6.64	0.05	n/a
59	Unknown sesquiterpenoid (RI 1532; fresh sample)	0.03	-	-	-	-	-	-	0.21	-	0.18	-	-	-	n/a	n/a	n/a
60	*trans*-*Z*-*α*-Bisabolene epoxide	0.01	-	-	-	-	-	-	-	-	-	-	0.10	-	4.86	0.01	n/a
61	Unknown sesquiterpenoid (RI 1532; stored sample)	-	0.15	0.16	-	0.11	-	-	-	-	-	0.02	-	-	n/a	n/a	n/a
62	(*E*)-Nerolidol	0.09	0.07	0.08	-	-	-	-	0.85	0.94	1.57	1.39	2.14	1.71	5.68	1.53	F: 21.44[f]
63	Caryophyllenyl alcohol	0.20	0.18	0.18	-	-	-	-	-	-	-	-	5.24	5.09	4.20	9.13	n/a
64	Caryophyllene oxide	0.27	0.22	0.22	-	-	-	-	-	-	0.43	0.45	0.27	0.25	3.60	2.21	n/a
65	Unknown sesquiterpenoid (RI 1575; fresh sample)	0.02	-	-	-	-	-	-	-	-	-	-	0.85	-	n/a	n/a	n/a
66	Humulene epoxide I	0.01	0.02	0.02	-	-	-	-	-	-	-	-	-	-	4.56	0.62	A: >10[a]; F: 100[f]
67	Humulol	0.68	0.55	0.56	-	-	-	-	-	-	1.00	1.01	15.54	14.56	3.8	44.17	A: 2000[i]
68	Humulene epoxide II	1.07	0.85	0.86	0.40	0.35	-	-	-	-	-	-	-	-	4.51	5.43	A: 450[a]
69	Widdrol	0.04	0.03	0.03	-	-	-	-	-	-	-	-	1.31	1.05	4.10	7.93	n/a
70	1-Epicubenol	0.04	-	-	-	-	-	-	-	-	-	-	1.80	1.55	3.69	9.13	n/a
71	Humulene epoxide III	0.01	0.01	0.01	-	-	-	-	-	-	-	-	-	0.04	4.45	0.51	F: 450[e]
72	Humulenol II	0.15	0.01	0.01	-	-	-	-	-	-	-	-	12.53	11.29	3.50	2.26	A: 150-2500[a]; F:2500[e]
73	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	0.02	-	-	-	-	-	-	-	-	-	-	2.66	-	3.70	n/a	n/a
74	τ-cadinol	0.09	0.06	0.06	-	-	-	-	0.08	0.07	0.33	0.25	3.13	2.65	4.90	9.13	n/a
75	*δ*-Cadinol	0.02	0.01	0.01	-	-	-	-	-	-	-	-	1.02	1.96	4.95	9.13	n/a
76	2-Pentadecanone	0.02	0.02	0.02	-	-	-	-	0.21	0.19	0.47	0.46	0.38	0.37	5.66	0.47	n/a
77	(*Z,E*)-Farnesol	0.02	-	-	-	-	-	-	-	-	-	-	1.47	-	5.77	1.29	n/a

"-" compound not detected; TO, Total oil; SQ, Sesquiterpene enriched fraction; MYR, Myrcene enriched fraction; EST, Ester enriched fraction; KET, Ketone enriched fraction; TALC, Terpene alcohol enriched fraction

* Log*P* and solubility in water estimated using EPI Suite™ v.4.1 software (U.S. Environmental Protection Agency); PAN, Peak area normalisation

** Aroma (A) and/or flavour (F) threshold concentrations. Taste and mouthfeel threshold concentration have not yet been determined for the compounds identified in the hop extracts used in this study.

[a] Schönberger et al. (2015); [b] Meilgaard, Civille, and Carr (1999); [c] Meilgaard (1975b); [d] Hanke (2009); [e] Peacock and Deinzer (1981); [f] Jiang et al. (2017); [g] Bordiga and Nollet (2019); [h] Shimazu, Hashimoto, and Kuroiwa (1975); [i] Irwin (1989); [j] Peltz and Shellhammer (2017)

Compounds absent in the stored sample

Compounds that were previously detected in the fresh hop oil samples but were absent i.e. undetected and unidentified in the stored samples were 2-methylbutyl 3-methylbutyrate (0.13% in fresh sample), myrtenol (0.1%), 2-decanol (0.03%), octyl propionate (0.05%), *trans*-*Z*-*α*-bisabolene epoxide (0.01%), an unknown terpene alcohol (RI 1575; 0.02%), 11, 11-dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol (0.02%), and (*Z,E*)-farnesol (0.02%). All have previously been identified as hop-derived volatiles (Buttery, Black, Kealy, & McFadden, 1964; Eri et al., 2000; Hofmann et al., 2013; Inui et al., 2013; Van Opstaele, De Rouck, Janssens, & Montandon, 2020; Yan et al., 2019), however, little is known regarding their sensory profiles and changes upon storage. The aroma of the oxygenated sesquiterpene (*Z,E*)-farnesol has been described as 'floral, powdery' (Sanekata et al., 2018). Since sensory threshold concentrations in a relevant matrix are not available, it is not clear to what extent the lack of these compounds may have had on the overall sensory profiles of the total oil and the five fractions.

Unknowns

It should be considered that not all compounds could be identified in the fresh and the stored hop oil samples or could not be determined with certainty. Figure 2.1**.** gives an overview of the distribution of chemical classes present in the samples and the proportions of unknowns that could not or not satisfactorily be identified using the current GC-MS approach. The proportion of unknowns in the total oil accounted for approximately 8%. The largest proportion of unknowns with ~12% was noticed in the terpene alcohol enriched fraction. Moreover, it was taken into account that

several compounds at low and trace concentrations such thiols/sulphides or aldehydes might have been present in the hop oil samples but were not captured with the current GC-MS approach.

So far, limited studies have been published investigating these compounds in Magnum hop oil. Takoi, Degueil, Shinkaruk, Thibon, Maeda, et al. (2009) used GC coupled to a flame photometry detector (FPD) to identify thiols in Hallertauer Magnum hops and detected 3-sulfanylhexan-1-ol (3SH), 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylpentan-1-ol (3SP), and 3-sulfanyl-4-methylpentan-1-ol (3S4MP) at low but quantifiable concentrations. In contrast, Reglitz and Steinhaus (2017) could not detect 4MSP in Magnum hops using GC×GC coupled to time-of-flight mass spectrometry (TOFMS). Further advanced specific and sensitive detectors or measurement approaches could be used to detect those compounds at trace levels (Rettberg et al., 2018) and further authentic standards and Mass Spectral libraries may help to identify those compounds referred to as 'unknown' in the current study.

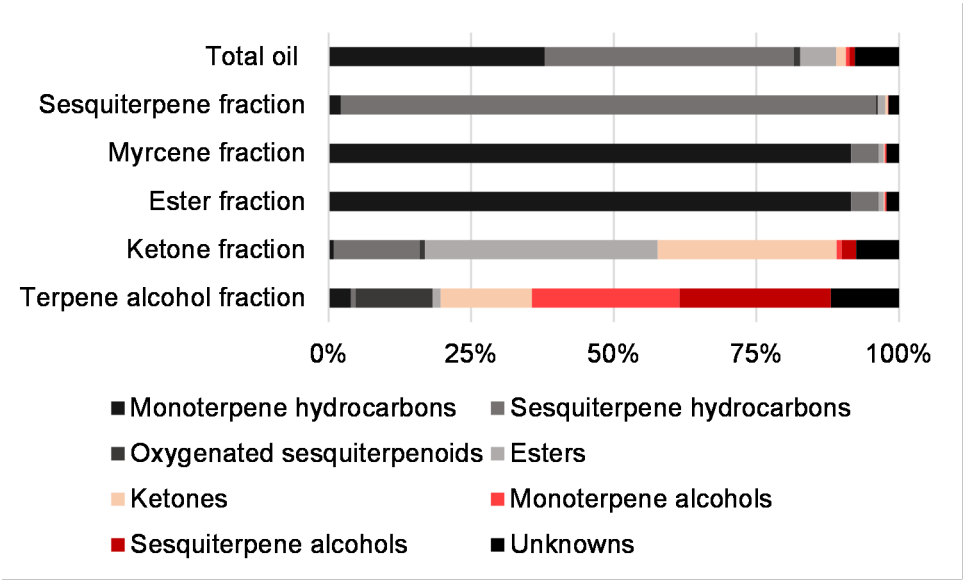

Figure 2.1. Chemical class profiles (%mean obtained by total peak area normalisation) of the total oil and five hop oil fractions.

2.4.3 Changes in compound concentrations

Multivariate data analysis

Comparison of individual relative compound concentrations obtained for the fresh and the stored hop oil samples revealed few statistical differences (data not shown). These were mainly caused by the volatiles that could only be detected and identified in the fresh or the stored hop oil samples. This is in line with previous research concluding that CO_2 hop extract can be stable for months/years if adequately stored (Priest et al., 1991). A pattern of decline was observed for some compounds discussed below. However, this had no effect on the proportion of sub- and supra-threshold compounds where the threshold concentrations (in beer and comparable ethanolic solutions at 4-5% ABV) are known (see Table 2.2.).

Principal Component Analysis (PCA) was used to study and visually illustrate the differences and similarities between the volatile compounds present in the fresh and stored hop oil samples detected in preceding statistical analysis. Principal Component 1 (PC1) and PC2 could explain 65.19% of the variation in the dataset (Figure 2.2., PC1 explaining 34.66%, PC2 explaining 30.53%). Since visualisation of the correlations in two-dimensional PCA biplots can eventually lead to false interpretations, factor loadings and squared cosines as a measure of individual representation quality were additionally used to evaluate the link between variables and the corresponding axes.

The PCs clearly clustered the fresh and stored hop oil samples in the biplot showing the ester and ketone enriched fraction samples in the top left plot quadrant and the terpene alcohol enriched fraction samples in the top right plot quadrant. Plot locations of these samples suggest that the volatile compositions of their fresh and

stored replicates were highly correlated and thus very similar. Inspection of factor loadings and squared cosines however indicated that all of these samples were loaded on PC1 apart from the fresh ketone sample assigned to PC2. The vast majority of esters, ketones, and terpene alcohols accounted for the factor contribution of PC1. Volatiles of these compound classes significantly contributing to PC2 were 2-nonanone [**C9**], nerol [**C29**], 2-undecanone [**C32**], 2-dodecanone [**C45**], 2-pentadecanone [**C76**], 6-methyl-5-heptene-2-one [**C2**], *trans*-3(10)-caren-2-ol [**C22**], 2-undecanol [**C34**], and (*E*)-nerolidol [**C62**] (r = 0.755-0.938). Concentrations of these compounds were found to be higher in the fresh ketone sample with the major significant decrease detected for 2-nonanone (1.42±0.08% in fresh vs 0.85±0.02% in stored ketone enriched fraction).

The PCA biplot of PC1 and PC2 further indicated the similarity between the myrcene and the sesquiterpene enriched fractions (bottom right quadrant), whilst the volatiles contributing to PC2 and PC3 separated the fresh from the stored total oil sample. Although, PC3 could only explain 9.12% of the variance in the dataset, the biplot is shown (Figure 2.3**.**) to better illustrate the volatile compounds mainly discriminating between the two total oil samples. As can be seen in the biplot, several volatiles were significantly contributing to PC3 including *cis*-*β*-ocimene [**C6**], *α*-bergamotene [**C48**], *γ*-muurolene [**C51**], humulene epoxide I [**C66**], methyl (*2E*)-2-heptanoate [**C4**], *R*-(+)/*D*-limonene [**C5**], and the unknown sesquiterpenoids (RI 1335, RI 1532) [**C41, C61**], and monoterpene (RI 1088) [**C10**] in the stored sample. *α*-Bergamotene, *γ*-muurolene, methyl (*2E*)-2-heptanoate, *R*-(+)/*D*-limonene, and the unknown compounds could not be identified in the fresh total oil sample. Main differences were found for the compounds *R*-(+)/*D*-Limonene (0.30±0.02%) and *γ*-muurolene

(0.83±0.01%). Also, squared cosines of variables suggested that the difference between the fresh and total oil samples were mainly caused by newly or unidentified compounds and less by differences in concentrations. It is interesting, that the individual fractions appeared to be slightly more stable compared to the total oil and it remains to be investigated whether this effect is due to the chemical complexity of the total oil compared to the individual fractions providing more 'area for attack' for chemical reactions.

Overall, the outcome of the PCA suggested that changes in the stored sample i.e. quantitative and qualitative compositional effects were mainly detected at relatively low concentrations but the effect these might have on the overall sensory profile of the total hop oil is unknown.

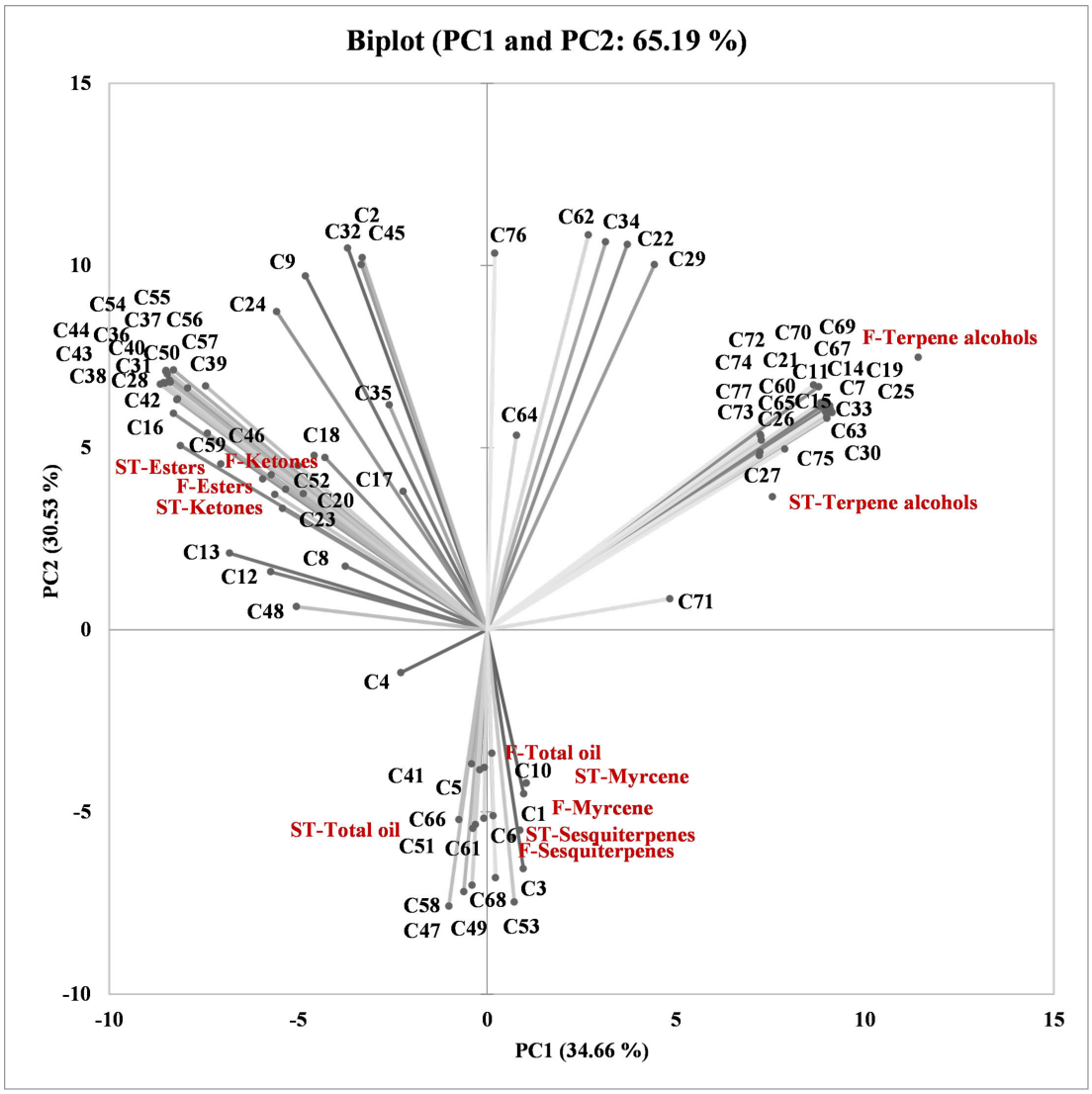

Figure 2.2. PCA biplot (PC1, PC2) showing the fresh (F-) and stored (ST-) hop oil samples (in **red**) and the hop volatile compounds identified or tentatively identified (**C**) in **black** in a multi-dimensional compositional space. Compound numbers refer to the ones listed in Table 2.2. Displayed are PC1 and PC2 explaining 65.19% of the variation in the dataset.

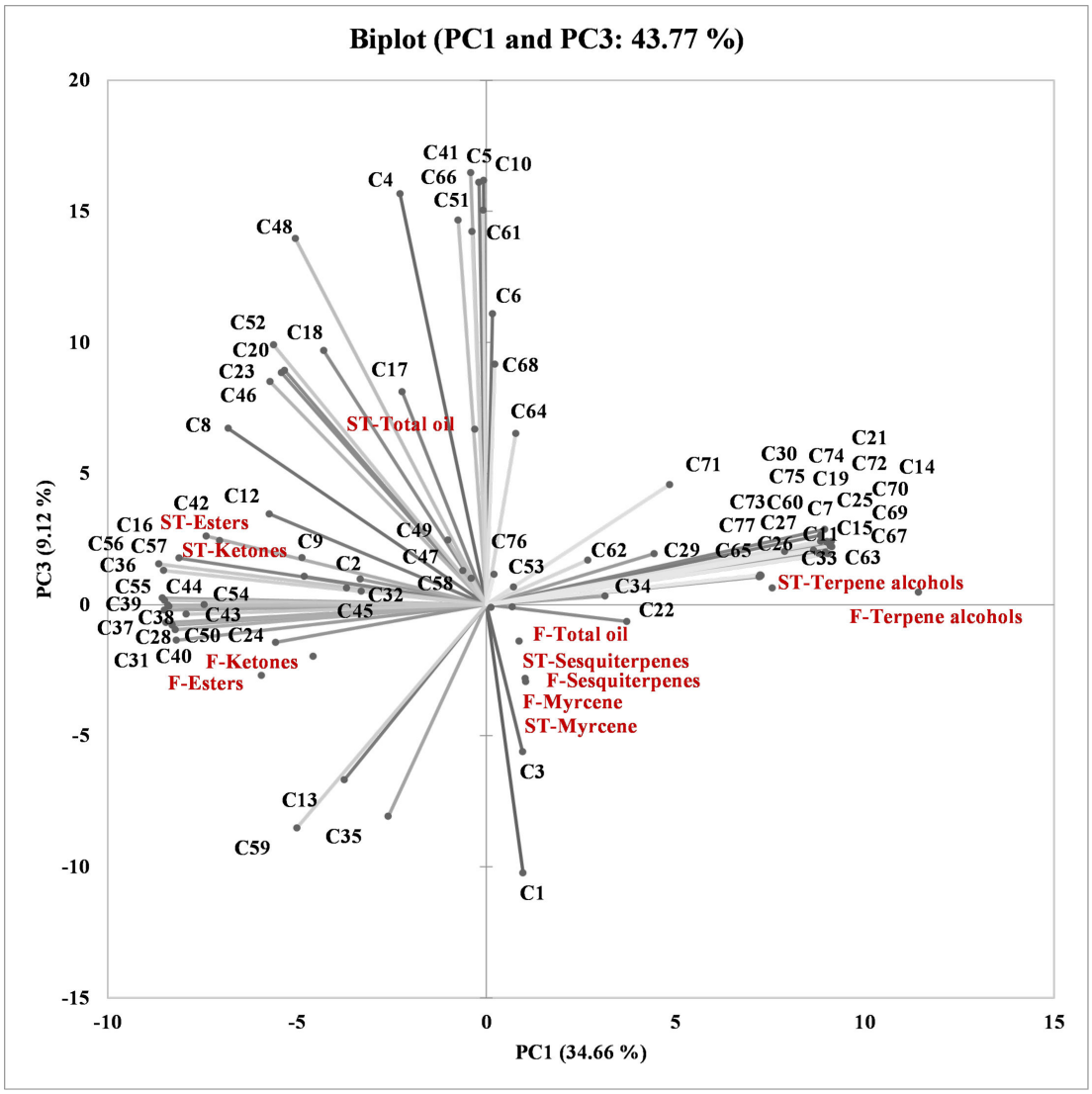

Figure 2.3. PCA biplot (PC1, PC3) showing the fresh (F-) and stored (ST-) hop oil samples (in **red**) and the hop volatile compounds identified or tentatively identified (**C**) in **black** in a multi-dimensional compositional space. Compound numbers refer to the ones listed in Table 2.2. Displayed are PC1 and PC3 explaining 43.77% of the variation in the dataset.

Marker compounds for hop freshness

The increase or decrease of specific volatile compound concentrations in hop essential oil and the detection of reaction products are frequently used to suggest the freshness and quality of hop materials (Tedone et al., 2020). Terpene hydrocarbon concentrations that decreased were mainly found for *β*-caryophyllene (8.75±0.64% vs 6.75±0.08%) and *α*-humulene (37.01±2.58% vs 32.72±0.64%). It is surprising that *β*-myrcene only slightly decreased in the myrcene and sesquiterpene

enriched fractions and not decreased in the total oil, although *β*-myrcene has previously been found to be among the least stable compounds in hop essential oil and is therefore often used as a key marker compound for hop freshness (Dieckmann & Palamand, 1974; Raut et al., 2021; Tedone et al., 2020). Myrcene is known to be prone to oxidation and polymerisation. Reaction products of myrcene autoxidation are pinene, camphene and terpenoids including linalool and geraniol (Rettberg et al., 2018), which were not significantly increased in the stored hop oil samples of the current study. Also, Lermusieau and Collin (2001) observed that volatile compounds lacking conjugated dienes (e.g. humulene, caryophyllene bergamotene, eudesmene) to have a higher stability compared to compounds containing conjugated dienes (i.e. hydrocarbon chains having two double bonds separated by a single bond). The decrease of *β*-caryophyllene could be a result of oxidation reactions during GC-MS sample preparation (transferring aliquots of the hop oil samples to the vials), which was conducted in a fume hood but not under anaerobic conditions. This was omitted since sample preparation of sensory trials would not be conducted under this condition either.

Oxidation of *β*-caryophyllene usually results in the formation of caryophyllene oxide, caryolan-l-ol or 14-hydroxy-*β*-caryophyllene (Rettberg et al., 2018), however, an increase of caryophyllene oxide was not observed. In fact, the concentration slightly decreased (0.27±0.01 vs 0.20±0.01%). Specific reasons for the changes in the *β*-caryophyllene concentration have not yet been identified. Oxidation of *α*-humulene usually leads to the formation of monoepoxides (Praet et al., 2016). But, humulene epoxide I-III concentrations were not found to be increased, but decreased (humulene epoxide II: 1.07±0.06% vs 0.86±0.01%) or completely degraded.

The most noticeable loss of oxygenated sesquiterpenoids was observed for caryophyllene oxide, humulene epoxide II, and humulenol II (0.15±0.02% vs 0.01±0.00%). Caryophyllene oxide derived products such as caryophylladienol or caryophyllenol were not detected (Praet et al., 2014). Previous research indicated that more selective and sensitive detectors might be necessary to identify these compounds (Praet et al., 2014; Rettberg et al., 2018; Yang, Lederer, McDaniel, & Deinzer, 1993b). Reaction products of humulene epoxide II are for example humuladienol, humulol, and humulenol II, which are naturally present in hop oil but also used as marker compounds for oxidation or acid-catalysed reactions in hop oils (Lam, Foster, et al., 1986; Peacock & Deinzer, 1981; Praet et al., 2014). None of these compounds have been found to be significantly increased in the total oil or any of the hop oil fractions.

Furthermore, it seemed that some compounds in the ester and ketone enriched fractions slightly degraded. This mainly concerned methyl (*E*)-4-decenoate (1.91±0.13% vs 1.46±0.06%), methyl geranate (0.71±0.05% vs 0.49±0.03%), methyl octanoate (0.69±0.05% vs 0.47±0.01%), and 2-undecanone (1.15±0.09% vs 0.88±0.05%). Esters and ketones have previously been found to be relatively stable during storage and aging of hops (Lermusieau & Collin, 2001; Tedone et al., 2020). As previously mentioned, further research is required to identify the reasons for the decline of these compounds' concentrations to clarify whether these changes are related to the sample preparation, errors in quantitative determination/baseline noise or deviations between technical replicates (GC runs), and to determine the marker or reaction products of these changes. Furthermore, target analytes should be precisely quantified using closely meshed calibration curves, authentic standards,

and GC-MS operating in selected-ion-monitoring (SIM) to confirm the current findings. Moreover, samples collected in shorter storage periods could be analysed to determine critical degradation points in time.

Nevertheless, for those compounds that were identified using this rapid profiling approach, it can be concluded that the majority of concentration decreases were relatively small and mainly observed for concentrations <1% (apart from the terpene hydrocarbons). Sensory similarity testing is recommended to confirm that the hop oil samples are sufficiently sensorially stable under these storage conditions.

2.4.4 Research limitations

The described study has been conducted in 2020 as a Covid19 response and examined the stability of hop oil extracts at only two time points. Follow-up research therefore needs to include further time points to precisely monitor compositional changes including compound degradation and transformation. Since the recommended shelf life provided by the producing company and other companies is stated to be between six months and one year (e.g. Totally Natural Solutions, 2021), further time points of assessment could be 0, 1, 3, 6, 9, 12, and 15 months post-extraction. In addition to the storage conditions that are often chosen in research or university environments for long-term storage (-10 - -30^{o}C, or in the current study -20^{o}C), further storage conditions may be tested including storage at room temperature or brewery environment (20-25^{o}C) and at cold store temperature (4-8^{o}C). Further research could be conducted to investigate repeated exposure of the hop oil extracts to light and oxygen or temperature changes as it would be the case

in smaller, e.g. craft beer producing breweries where the hop oil products are often not used at once but within a period of a few days or weeks.

2.5 Conclusions

Although, only one storage condition was tested, the results show that the composition of hop aroma constituents largely remained stable suggesting that limited volatilisation and transformation reactions took place while the hop oil samples were stored at -20^{o}C for the period of 26 months. However, slight changes in compound concentrations were detected following a general trend towards a decline of several compounds that could be readily or tentatively identified. Some compounds were newly identified in the stored hop oil whilst others remained unidentified. The reasons for these changes are not clear and limited information is available in the literature. To date, the majority of studies that have investigated the effects of storage conditions on the volatile composition of CO_2 hop extracts varied in their experimental designs. Thus, further research is recommended using advanced detectors and elaborated quantification approaches, in addition, to acquire or synthesise authentic standards to enable the identification of unknown compounds. The quantitative volatile profiles should be assessed in a sensory study (similarity testing) to confirm if the fresh and stored samples are perceivably interchangeable and therefore appropriate for long-term sensory trials.

Chapter 3

3 Exploring the multisensory perception of terpene alcohol and sesquiterpene rich hop extracts in lager style beer

This chapter is based on:

Dietz, C., Cook, D., Wilson, C., Oliveira, P., & Ford, R. (2021a). Exploring the multisensory perception of terpene alcohol and sesquiterpene rich hop extracts in lager style beer. *Food Research International*, *148*, 110598.
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Exploring the multisensory perception of terpene alcohol and sesquiterpene rich hop extracts in lager style beer

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Keywords: Hop oil fractions; sensory descriptive analysis; sensory interactions; bitterness; sweetness

Highlights

- Hop oil fractions impart multisensory characteristics in beer.
- Geraniol was the key compound contributing to beer sweetness and smooth bitterness.
- Harsh bitterness was related to the presence of sesquiterpene hydrocarbons.
- Monoterpene alcohols induced different fruity and floral characteristics.
- Hop-derived volatiles modified the beer taste due to sensory interactions.

Abstract

Understanding the contribution of hop essential oil to the multisensory profile of beer is known to be challenging because of its chemical and sensory complexity. Limited research has been conducted investigating hop-derived volatiles' role in the modulation of taste and mouthfeel sensations. Supercritical CO_2 can be used to extract specific fractions from hop oil, thereby enabling the localisation of compounds responsible for different sensory impressions. Terpene alcohol and sesquiterpene fractions were extracted from a Magnum hop oil and further fractionated into seven sub-fractions and individual compounds. All extracts were evaluated in lager (4.5% v/v) by a trained panel (n=10) using a newly developed attribute lexicon and following a sensory descriptive analysis approach. The sensory data was analysed using ANOVA, followed by Tukey's test (HSD) and correlated with chemical profile data obtained by gas chromatography-mass spectrometry (GC-MS) by Principal Component Analysis. The study revealed evidence for hop extracts to impart multisensory characteristics to beer due to sensory interactions within and across modalities. The monoterpene alcohols-rich fractions and particularly geraniol, added fruity- and floral aromas and flavours, modified the sweetness and induced a smooth bitterness in the beer matrix. Flavouring the beer with sesquiterpene fractions resulted in a harsh bitterness sensation. Contrary to previous findings, the humulene epoxides fraction appeared to have limited effects on lingering bitterness and astringency, illustrating the need for temporal sensory assessments in future studies. This research shows that splitting hop oil into fractions and sub-fractions provides a source of natural, sustainable flavouring preparations with distinct sensory characteristics.

Figure 3.1. shows the graphical abstract published in *Food Research International*.

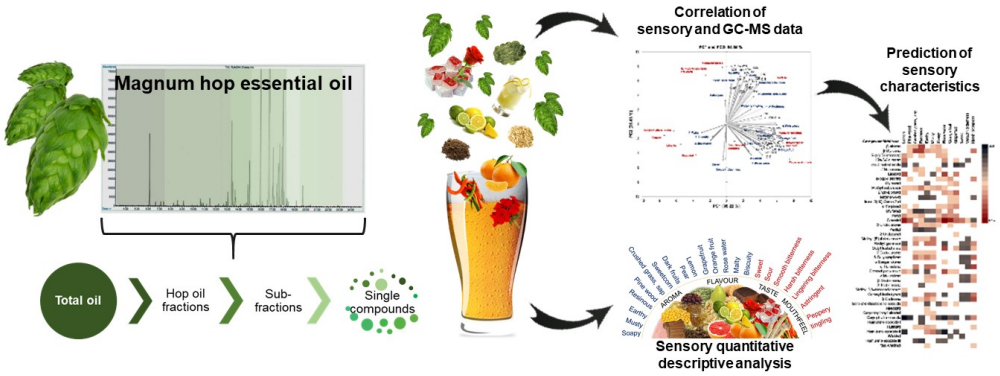

Figure 3.1. Graphical abstract.

3.1 Introduction

Historically, hops (*Humulus lupulus* L.) have been added to beer to provide microbial protection and as a source of bitterness and aroma. With the craft beer sector's growth and changing consumer preferences, hop products have become key in the brewing process adding aroma, flavour, taste, and mouthfeel (Dietz, Cook, Huismann, et al., 2020a; MarketDataForecast, 2020). The composition of hop essential oil is complex. Around 1000 volatile compounds are suggested to be present in hops, mainly comprising hydrocarbons, terpene alcohols, sesquiterpenoids, esters, ketones, aldehydes, and sulphur-containing compounds, with potentially less than half of these identified so far (Roberts et al., 2004). Research has shown that complex mixtures of volatile hop compounds contribute to the sensory, 'hoppy' profiles of beer (Dietz, Cook, Wilson, et al., 2020b).

Meilgaard (1975a) hypothesised that half of the flavour intensity in beer could be attributed to sensory interactions between volatile and non-volatile fractions. Non-volatile fractions in the beer matrix affect the physical release and concentration of

volatiles in the headspace eventually determining the perceived intensity and quality of aroma-active compounds (Poinot et al., 2013). Depending on the relative concentrations of two or more volatiles, sensory characteristics can be increased due to additive- or synergistic-type behaviours, suppressed or masked due to antagonistic-type behaviour or even eliminated (Meilgaard, 1982). Moreover, sensory interactions can occur across modalities (cross-modal interactions).

Oladokun et al. (2017) investigated the impact of a Hersbrucker hop aroma extract on perceived bitterness qualities in beer and found significant effects but also suggested a taste-trigeminal interaction responsible for some of the bitterness quality changes. Kaltner and Mitter (2006) attributed the modification of bitterness perception to different concentrations of linalool and terpene hydrocarbons. Interestingly, ratings for “bitterness harmony” increased for the beer with the highest linalool concentration. Beers with terpene hydrocarbons and a low concentration of linalool resulted in high ratings for “harmonious, but increasing bitter taste” and significantly lowered ratings for “mild bitterness” (Kaltner & Mitter, 2006). Sensory interactions particularly occur in heterogeneous mixtures depending on compound combinations, ratios, and threshold concentrations of compounds for aroma, flavour, taste, and/or mouthfeel. Overall, there has been limited research studying the role of sensory interactions related to the perception of hop volatiles.

In a preceding study, five hop oil fractions were extracted from a Magnum hop oil using supercritical CO_2 (Dietz, Cook, Wilson, et al., 2020b). The total oil and the fractions were applied at 800 µg/L in an ethanol-water solution (4% ABV) and evaluated by external sensory panellists following a Quantitative Descriptive Analysis

(QDA) approach to determine their sensory characteristics. Based on the outcome of the correlation analysis to investigate the relationships between the sensory and the compositional data, it was hypothesised that the terpene alcohol fraction induces taste and trigeminal-type sensations, including sweetness, lingering bitterness, and a "peppery tingling" mouthfeel. This fraction was also suggested to add a pronounced fruity and floral aroma and flavour sensations to the model solution. Furthermore, it was hypothesised that the monoterpene alcohols, linalool and geraniol were key compounds responsible for the aroma and flavour characteristics in this fraction, whilst sesquiterpene alcohols (humulenol II, humulol) might have caused taste and mouthfeel sensations.

However, it remains to be investigated whether or not additional compounds present at lower concentrations might have contributed to these sensations rather than the measurable key volatiles as such. Also, cross-modal interactions between ortho- and retronasal smell and taste and mouthfeel might have resulted in the perceived multisensory profile induced by terpene alcohols making it difficult to specify key compounds responsible for either aroma and flavour or taste and mouthfeel (Dietz, Cook, Wilson, et al., 2020b).

This study aims to understand the multisensory profile perceived when drinking beer flavoured with specific hop oil extracts and sensory interactions causing this multisensory experience. Based on the preceding study's outcome, the current research investigates whether it is possible to separate the hop compounds driving floral and fruity aroma and flavours from those adding sweetness, a "peppery tingling" mouthfeel or modifying bitterness.

3.2 Materials and methods

3.2.1 Hop oil extracts

Supercritical CO_2 hop oil fractions and compounds were extracted from hop oil obtained by distillation from Magnum variety hop pellets following the extraction method described by (Marriott, 2019). For the set of hop extracts, specific fractions, sub-fractions and individual volatile compounds were extracted from the Magnum hop oil (total oil), namely extracts enriched in sesquiterpenes, terpene alcohols, humulene epoxides, monoterpene alcohols, sesquiterpene alcohols, humulol + humulenol II, linalool, geraniol, and caryophyllene oxide (Figure 3.2., Figure 3.3.). Aliquots of the extracts were flushed with nitrogen, hermetically sealed and stored at 4°C until further use, within the expire date of 6 months.

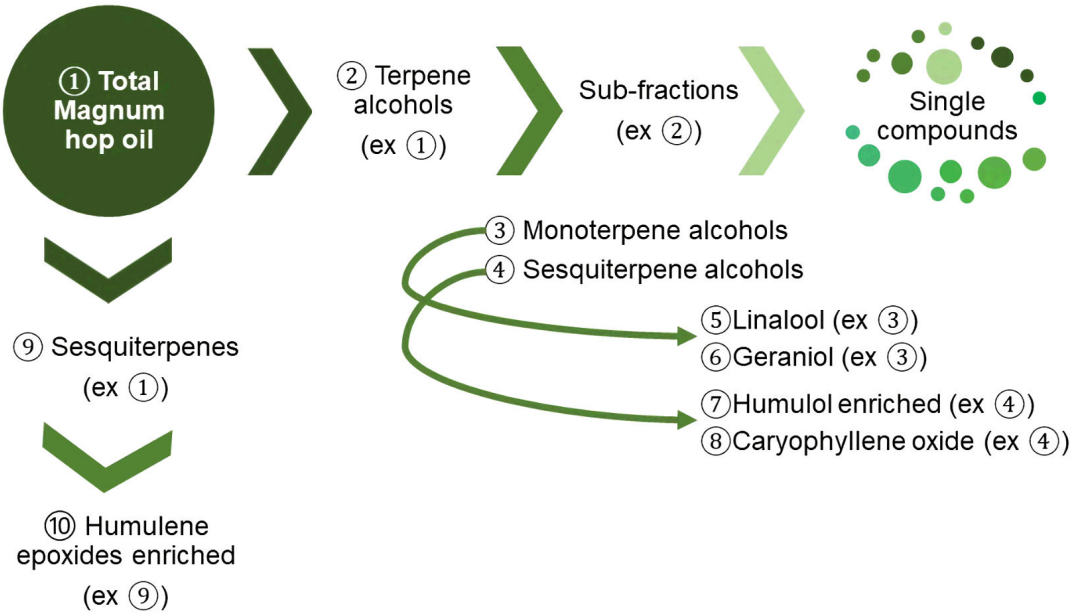

Figure 3.2. Fractions, sub-fractions and single compounds extracted (ex) from the total Magnum hop oil included in the sample set.

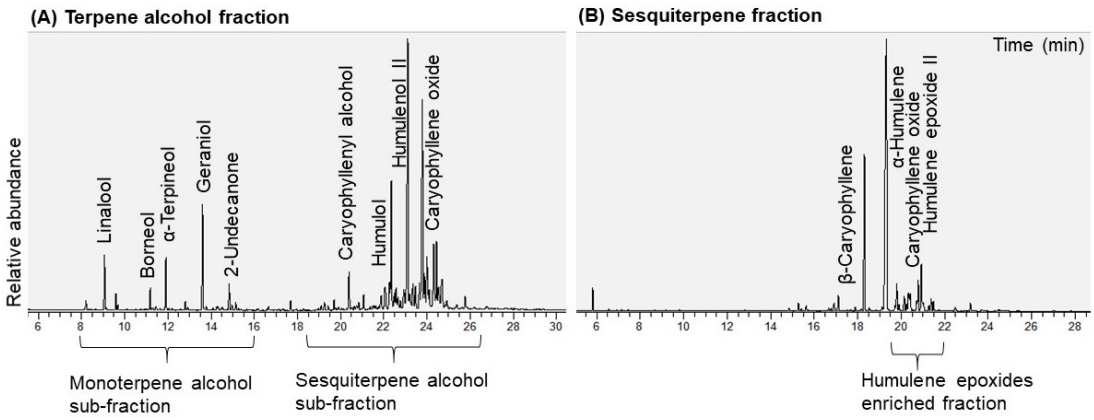

Figure 3.3. Total ion chromatograms (TIC) of the terpene alcohol and sesquiterpene fractions showing the distribution of the sub-fractions and the main volatile compounds.

3.2.2 Sensory evaluation

Ethics approval was granted by the Faculty of Medicine & Health Sciences Research Ethics Committee of the University of Nottingham (Ethics Reference No. 88-1707). Prior to sensory screening, informed consent was obtained from all candidates.

Preparation of samples

Stock solutions were prepared by diluting the hop extract aliquots in food-grade ethanol (96%, ferm, fa, F200481, Haymankimia, UK) and stored at 4°C for the period of the study. A commercial pale lager beer (4.5% ABV, 10 BU, pH 4.35, brewed from barley malt with rice adjunct) was purchased and flavoured with the hop extracts at different concentrations to obtain an equiflavour intensity achieved by conducting bench tests followed by Rank-Rating tests with the panel. All beers were sourced from the same batch to prevent batch-to-batch variation. The diluted hop extracts were dosed volumetrically into 300 mL lager bottles to obtain the following concentrations: total oil and fractions - 1500 µg/L, sub-fractions - 1000 µg/L, enriched fractions - 300 µg/L, fractions enriched in single compounds - 100 µg/L (linalool and geraniol fractions) or 300 µg/L (caryophyllene oxide fraction). Additions were made

in a cold room (4°C) to minimise CO_2 breakout with bottles immediately recapped, inverted three times, and allowed to equilibrate overnight (21 h) at 4°C prior to each session. The non-flavoured control lager was treated in the same way. For presentation to the panel, samples (30 mL) were poured into 60 mL tempered (4°C) amber glass bottles labelled with randomly assigned 3-digit codes, immediately closed with screw-top caps 30 min prior to testing sessions to limit decarbonation and volatilisation of hop compounds.

In total, the panellists evaluated 12 beer samples including the beer flavoured with one of the ten hop oil extracts (total oil, fractions, sub-fractions, compound enriched fractions) shown in Figure 3.2, one experimental replicate (total oil), and the unflavoured beer (without hop oil extract).

Sensory panel

Sensory characteristics of control and flavoured beers were evaluated by external sensory panel (n=10, 7 female, 3 male, mean age 55.5 years) following a Quantitative Descriptive Analysis approach (Stone et al., 2008). The previously screened and trained panellists were re-screened to ensure they met specific criteria for this study following the approach described by Dietz, Cook, Wilson, et al. (2020b) to evaluate their sensory abilities, including basic smell and taste detection, ability to detect the main compounds and to confirm advanced descriptive and discriminative abilities.

Panel training

Following screening, selected candidates were invited to participate in sensory training sessions. An attribute lexicon was generated where panellists were asked to individually generate aroma, flavour, taste, and mouthfeel attributes (tactile

sensations during and after swallowing) by comparing and describing the flavoured beers (with hop extracts added at different concentrations). Three sessions were used for attribute consolidation by conducting Check-All-That-Apply (CATA) tests and group discussions moderated by the panel leader to select the most descriptive and discriminating attributes (Delarue et al., 2014). Attribute descriptions were compiled in further group discussions aided by reference materials at different concentrations for each attribute. Attribute intensities were quantified using a 10 cm unstructured line scale anchored at the extremes by “no sensation” and “very strong”. Quantities of reference materials listed in the attribute lexicon (Table 3.1.) refer to “very strong” intensities of the sensory characteristics in the beers. The attribute order, assessment protocols, and palate-cleansing materials and protocols were developed and defined based on panellists’ comments during training. In total, 14 training sessions and one mock evaluation session (120 min each) were conducted to achieve panel consensus i.e. sufficient discriminative ability and reproducibility, as confirmed by the panel performance data.

Table 3.1. Overview of sensory attributes (in order of presentation), definitions, and training reference standards.

Modality	Sensory attribute	Definition	Training reference standard
Aroma	Sweetcorn	Sweetcorn aroma as when smelling canned, cooked sweetcorn, the dimethyl sulphide reference solution or cooked vegetable gone off	10 mL 150 µg/L dimethyl sulphide (DMS; Aroxa, UK) – water solution (deionised water), 10 g of canned, cooked sweetcorn (with dripping water)
	Soapy	Soapy aroma as when smelling an unscented bar of soap	5 g unscented bar of soap (Sainsbury's Supermarkets Ltd., UK)
	Pine wood	Pine wood aroma as when smelling pine shavings or the pine wood reference solution	10 g pine shavings (Sainsbury's Supermarkets Ltd., UK); 5 mL 6 mg/L (1*R*)-(+)-α-Pinene (food grade; Sigma Aldrich, UK) in deionised water
	Crushed grass, sap	Crushed grass, sap aroma as when smelling crushed grass, sap, tomato leaf, or carrot leaf	20 g crushed cut grass and sap that has been left in the closed sample bottles for 2 days; 10 g fresh tomato leaf or carrot leaf
	Dark fruits	Dark fruits aroma as when smelling raisins, prunes	10 g chopped raisins and prunes (Sainsbury's Supermarkets Ltd., UK)
	Pear	Pear aroma as when smelling a pear fruit (peel, flesh)	5 g freshly chopped pear pieces with peel
	Lemon	Lemon aroma as when smelling a lemon fruit or artificial lemon aroma, e.g. in citrus wet wipes	5 g freshly chopped lemon and lime; 1 citrus wet wipe (Dettol, UK)
	Resinous	Resinous aroma as when smelling the wood resin reference	10 g pine resin and 10 g myrrh resin (Indigo Herbs, UK)
	Earthy	Earthy aroma as when smelling wet earth or soil	10 g fresh wet earth, soil
	Musty	Musty aroma as when smelling mildew or mould, stale damp cellar, mouldy damp cardboard, or an old, dirty, dried sponge or dish cloths	20 g damp cardboard soaked in deionised water for 24h in the closed sample bottles; damp, used sponge that has been left for 24h in the closed sample bottle
	Overall aroma intensity	Overall aroma intensity in the sample	No physical reference
Flavour	Rose water	Rose water flavour as when eating a piece of Turkish delight or having a sip of geranium oil solution	½ piece (5 g) Turkish delight (Sainsbury's Supermarkets Ltd., UK); 5 mL 0.6% (w/v) geranium essential oil (Ecodrop, UK) in deionised water
	Malty	Malty flavour as when eating malt extract or a piece of fruitless malt loaf or Shreddies	10 g malt extract (Holland & Barrett, UK); 10 g Soreen malt loaf; 3 pieces Shreddies (Nestlé, UK)

Table 3.1 continued.

Modality	Sensory attribute	Definition	Training reference standard
Flavour	Orange fruit	Orange fruit flavour as when eating a piece of orange, mandarin, tangerine	5 g freshly cut orange and mandarin (flesh, peel)
	Biscuity	Biscuity flavour as when eating Digestive biscuits	¼ piece Digestive biscuit (McVitie's, UK)
	Grapefruit	Grapefruit flavour as when eating a piece of grapefruit or drinking a sip of grapefruit juice	5 g fresh cut grapefruit; 10 mL pink grapefruit juice (Tropicana, UK)
	Overall flavour intensity	Overall flavour intensity in the sample	No physical reference
Taste	Sweet	Sweet taste; immediate sensation after swallowing	10 mL 1% sucrose (Sainsbury's Supermarkets Ltd., UK); 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Sour	Sour taste; immediate sensation after swallowing	10 mL 0.2% (v/v) citric acid (Sigma Aldrich, UK) ; 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Smooth bitterness	Soft, pleasant bitterness intensity; immediate sensation after swallowing	10 mL 3 mg/L HopAlpha® Iso30%[1] (TNS Ltd., UK) in deionised water
	Harsh bitterness	Irritating, spiky bitterness intensity; immediate sensation after swallowing	0.3% (v/v) caffeine in deionised water (food grade; Sigma Aldrich, UK)
	Lingering bitterness	Persistence of the overall bitterness in the mouth; 20 seconds after swallowing	10 mL 3 mg/L HopAlpha® Iso30%[1] (TNS Ltd., UK) in deionised water
Mouthfeel	Astringent	Mouth drying, rough sensation, shrinking/tightening in the mouth, as when chewing banana peel or taking a sip of the reference solution; 30 seconds after swallowing	10 mL 1% (w/v) tannic acid (Alfa Aesar, US) in deionised water; 5 g banana peel
	Peppery tingling	Peppery tingling sensation as when eating chilli, fresh ginger, horseradish/radish; tingling mouthfeel, irritating, itching; immediate sensation after swallowing	No physical reference

[1]Hop acid in propylene glycol

[2]The differentiation between the two lemon aroma qualities “fresh lemon” and “artificial lemon” as described in Chapter 2A was no longer possible in the current study where the fractions were assessed in a commercial beer matrix instead of a simple ethanol solution (4%, ABV) suggesting that untrained assessors or consumers would possibly not be able to distinguish between these aroma sensations in beer.

Sensory descriptive analysis

For the final evaluation, the 12 samples were evaluated in triplicate by all panellists (n=10) over nine evaluation sessions of 100-120 min. The sensory evaluation was performed in sensory testing booths according to the guidelines and conditions described in ISO 8589-2007 (ISO, 2007). Each panellist consumed less than one UK alcohol unit (8 g/L) per session, and a maximum of two sessions were conducted per week. First-order and carryover effects were limited by monadically presenting the samples in a randomised and counterbalanced order (Latin Square Design) (Stone et al., 2008). The panellists received a fresh sample (8^{o}C; with replenished headspace) after each attribute set (1-4 attributes) to maximise the opportunity to evaluate subtle sensory characteristics. The scales for all attribute sets were displayed with Compusense®Cloud (Compusense Inc., Guelph, Canada) on a computer. Breaks were scheduled to prevent carryover effects and fatigue and panellists were asked to close the bottles and neutralise their senses where they smelled the back of their hands to neutralise their nasal cavity, ate a piece of honeydew melon and consumed some water to wash away residues.

3.2.3 Gas chromatography-mass spectrometry

The volatile composition of the hop extracts was analysed (n=3) by GC-MS. A Thermo Scientific system (TRACE^{TM} 1300; Massachusetts, USA) was equipped with a Zebron ZB-5MS capillary column (30 m x 0.25 mm ID x df = 0.25 µm; Phenomenex, Torrance, USA) coupled to a single quadrupole mass spectrometer (ISQ QD Thermo Scientitic Inc.; Massachusetts, USA) and operated in positive electron ionisation mode. A Zebron ZB-WAX capillary column (30 m x 0.25 mm ID x df = 0.25 µm; Phenomenex,

Macclesfield, UK) was used to obtain additional retention indices on a polar column. The hop extracts (10 µL) were diluted into 1 mL iso-octane (≥99%; Thermo Fisher Scientific, Loughborough, UK), and aliquots (1 µL) of the dilution were analysed with helium as a carrier gas (1 mL/min flow rate) operating in split mode (1:50). The temperature of the injector, ion source, interface, and detector were 250°C, 240°C, 250°C, and 250°C, respectively. The oven temperature increased at 5°C/min from 60°C to 240°C. Hop extracts were spiked with 1 µL of 1050 mg/L benzyl acetate (≥99%; Sigma Aldrich, UK) as an internal standard (ISTD) after checking its absence in the extracts and separate elution from other compounds.

Peak identification was based on mass spectra, retention indices (RI), and reference compounds (where available), including: *endo*-borneol (≥97%), caryophyllene oxide (≥99.0%), geraniol (≥99%), geranyl isobutyrate (≥97%), geranyl propionate (≥95%), linalool (≥97.0%), *R*-(+)-limonene (≥97%), methyl decanoate (≥99%), methyl geranate (≥94.0%), methyl octanoate (≥99%), α-humulene (≥ 96%), *β*-caryophyllene (≥98.5%), *α*-terpineol (≥97), *β*-myrcene (≥90.0%), *β*-pinene (≥99%), 2-dodecanone (≥97%), 2-nonanone (≥99%), 2-tridecanone (≥97%), and 2-undecanone (≥98.0%) (Sigma Aldrich (UK)). RIs under experimental conditions were determined using a homologous series of n-alkanes (C6-C30; Sigma-Aldrich, St. Louis, MO). NIST Mass Spectral Library (NIST08) and Wiley7n.1 (Hewlett-Packard, US) databases were used for library matching. Further compound verification was conducted by comparing mass spectra and RIs published in databases (Flavornet, Pherobase, Pubchem) or studies using columns with similar stationary phases. Peaks were assigned to compounds if the MS

fit factor was ≥ 800 (reverse/forward) and the calculated RI closely matched literature values. Otherwise, compounds were specified as “unknown”.

3.2.4 Statistical analysis

Sensory and analytical datasets were analysed using XLSTAT (2020.5.1, Addinsoft, US). Three-factor Mixed Model Analysis of Variance (ANOVA) (panellist, sample, replicate) with interactions was conducted on the 24 sensory attributes to examine panel performance (sample*panellist and sample*replicate interactions). After confirmation of satisfactory performance, a two-way ANOVA (sample as fixed factor, panellist as random factor) followed by Tukey’s Honest Significant Difference (HSD) test was performed for multiple pairwise comparisons to study significant differences (p<0.05; CI 95%) between the samples for each attribute.

Panellists’ averaged attribute scores were further analysed by Principal Component Analysis (PCA) on the covariance matrix to study the main relationships between samples and attributes in a sensory-perceptual space. Pearson correlation analysis was conducted to calculate linear correlations between attributes. Semi-quantification was used for the non-targeted analysis of hop compounds and performed by normalising the integrated peak areas of the hop compounds relative to the ISTD ion peak area. ANOVA followed by Tukey’s HSD was conducted to identify significant compound concentration differences among samples. Sensory and GC-MS datasets were standardised (1/standard deviation) and analysed by PCA. Standardisation was conducted to allow all variables to have equal influence in the PCA model despite differences in their numerical range. Significant correlations

between sensory attributes and relative compound concentrations were further identified using Pearson's correlation coefficients ($p \leq 0.05$).

While PCA outcomes reveal few linear combinations of variables best explaining correlations between datasets of *X* and *Y* matrices without losing too much information, Partial Least Squares (PLS) regression is capable of dealing with strongly collinear, noisy data including numerous *X*-variables capturing more correlation information between the matrices (Maitra & Yan, 2008). Therefore, PLS regression models were developed to identify correlations between hop compound concentrations (*X*-matrix) and sensory attribute scores for the beers (*Y*-matrix) using PLS1 algorithms for single, and PLS2 for multiple attributes (all and within modalities). Jack-knife uncertainty tests were performed to obtain estimated regression coefficients. Confidence intervals were set at 95%. Logarithmic transformation of GC-MS data was applied to improve the goodness-of-fit (R^2) since the sample comprised many volatiles at different concentrations having different sensory threshold levels (Lykomitros, Fogliano, & Capuano, 2016). Sensory data is inherently 'noisy'; therefore, PLS models with $R^2>0.700$ were considered as having good predictive ability (Schmidtke et al., 2013). Standardised coefficients of compounds (>0.05 for clarity) were plotted to visualise their relative weights in the models.

3.3 Results and discussion

3.3.1 Sensory evaluation

Attribute generation and consolidation

250 attributes were generated by the panellists and consolidated to a list of 39 attributes using a Check-All-That-Apply (Delarue et al., 2014) approach to exclude attributes that could not be reliably identified in the samples or adequately describe or discriminate differences. Table 3.1. lists the final 24 attributes, their descriptions, and associated reference materials in order of their evaluation. Where aromas were perceived both through the nose (orthonasally) and mouth (retronasally), they were selected to represent either an aroma or a flavour (where the highest intensity was recorded), to avoid attribute replication for both modalities.

Panel performance

Panel performance was evaluated by conducting three-factor ANOVA with interactions (panellist, sample, replicate) on all attributes (Table 3.2.). The dataset of one panellist was excluded because of lack of reproducibility across replicates and evaluation sessions. Sample*panellist interactions were reported for 10 attributes indicating disagreement regarding sample rankings or scale use effects (Stone et al., 2008). Interrogation of interaction plots and other significant factors (replicate, panellist) concluded minor variations of scale use with no impact on the data's interpretation for half of these attributes. However, five attributes ("sweetcorn", "dark fruits", "biscuity", "sour", "peppery tingling") were excluded from further discussions due to inadequate panel performance as indicated from the ANOVA.

Twelve attributes ("lemon", "crushed grass, sap", "resinous", "earthy", "musty", "soapy", "rose water", "orange fruit", "grapefruit", "sweet", "smooth bitterness", "harsh bitterness") and overall aroma and flavour intensities significantly differed across all samples (Table 3.2.) and were of adequate quality to be interpreted and discussed.

Table 3.2. Analysis of variance (ANOVA) F-ratios and for sensory attributes rated for the hop oil extracts applied in lager.

Modality	Sensory attribute	Sam	Pan	Rep	Sam x Pan[b]	Sam x Rep[b]	Rep x Pan[b]
Aroma	Sweetcorn	1.24	4.86***	0.58	1.58*	1.32	1.14
	Pear	1.45	21.39***	0.25	0.96	0.56	0.90
	Dark fruits	0.79	11.58***	1.11	1.71*	1.46	1.55*
	Lemon	5.61***	5.65***	0.75	1.59*	0.94	1.28
	Pine wood	1.68	9.82***	0.60	1.72*	1.27	1.42
	Crushed grass, sap	3.43**	11.02***	0.49	1.00	1.03	1.07
	Resinous	2.06*	7.47***	0.76	1.69*	1.42	1.19
	Earthy	2.18*	4.99***	1.31	1.53*	0.90	1.05
	Musty	1.98*	1.83*	1.67	1.20	0.95	1.12
	Soapy	2.57**	14.80***	1.67	0.98	0.60	0.64
	Overall aroma intensity	4.01***	15.88***	0.37	1.20	0.79	0.93
Flavour	Rose water	8.49***	3.40**	0.58	1.95**	0.96	0.92
	Malty	0.50	6.24***	1.09	1.48	1.47	1.25
	Biscuity	0.52	7.68***	2.71*	2.04**	1.86**	1.51
	Orange fruit	4.82***	8.81***	0.79	1.69*	1.05	1.18
	Grapefruit	4.74***	7.03***	0.31	1.45	1.02	1.18
	Overall flavour intensity	6.37***	7.56***	0.74	1.72*	1.11	1.26
Taste	Sweet	3.16**	6.54***	0.63	1.13	0.93	1.21
	Sour	1.17	9.96***	1.26	1.15	1.37	1.46
	Smooth bitterness	2.09*	5.67**	1.61	0.87	0.90	0.95
	Harsh bitterness	1.49**	6.80**	1.07	0.70	0.81	1.03
	Lingering bitterness	0.90	15.45***	0.67	0.79	0.80	1.16
Mouthfeel	Peppery tingling	0.61	10.97***	0.69	1.08	1.06	1.16
	Astringent	1.06	10.63***	0.81	1.06	0.97	1.09

*, **, *** indicating a significant effect at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, from three-factor ANOVA with interactions (Sample (Sam), Panellist (Panel), Replicate (Rep)).

Sensory descriptive analysis

Table 3.3. shows the mean intensity scores for the attributes and significant differences between the samples. The two experimental replicates (total oil) were not significantly different from each other indicating panel reliability. Six aroma attributes and "overall aroma intensity" differed significantly between the samples, whilst "pine wood" showed a trend (p=0.092) towards higher scores for the geraniol-flavoured and terpene alcohol fraction-flavoured beers compared to the control and caryophyllene oxide fraction-flavoured beer. The assessment of gustatory perception revealed three flavours and "overall flavour intensity" and three taste attributes to significantly discriminate between the samples. No mouthfeel attributes were found to be significant.

Table 3.3. Mean sensory intensities (n = 9, triplicates) for the control beer, the flavoured beer samples, and an experimental replicate.

Modality	Sensory attribute	C	TO	TO (repl)	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR
Aroma	Sweetcorn	2.51^{a}	1.72^{a}	2.05^{a}	1.39^{a}	1.95^{a}	1.39^{a}	1.96^{a}	2.29^{a}	1.59^{a}	2.43^{a}	2.41^{a}	2.07^{a}
	Pear	1.27^{ab}	3.00^{a}	2.30^{ab}	1.80^{ab}	1.15^{ab}	2.16^{ab}	2.61^{ab}	0.91^{b}	2.63^{ab}	1.91^{ab}	1.49^{ab}	1.36^{ab}
	Dark fruits	2.01^{a}	2.27^{a}	2.07^{a}	2.28^{a}	1.67^{a}	2.50^{a}	2.41^{a}	2.19^{a}	2.10^{a}	2.48^{a}	3.18^{a}	2.02^{a}
	Lemon	0.83^{bc}	1.61^{bc}	1.58^{bc}	0.90^{bc}	0.68^{c}	3.00^{ab}	2.55^{abc}	1.79^{abc}	3.81^{a}	1.07^{bc}	1.34^{bc}	0.73^{c}
	Pine wood	2.04^{a}	4.03^{a}	3.57^{a}	2.99^{a}	2.53^{a}	4.11^{a}	3.47^{a}	2.50^{a}	4.11^{a}	2.87^{a}	2.79^{a}	2.01^{a}
	Crushed grass, sap	1.00^{b}	4.47^{a}	4.42^{a}	2.86^{ab}	1.86^{b}	2.74^{ab}	2.78^{ab}	1.34^{b}	2.57^{ab}	2.13^{b}	2.33^{ab}	1.66^{b}
	Resinous	1.15^{c}	4.43^{a}	3.57^{ab}	2.06^{bc}	1.41^{bc}	2.20^{bc}	2.11^{bc}	1.69^{bc}	2.72^{abc}	2.04^{bc}	1.99^{bc}	1.70^{bc}
	Earthy	1.33^{abc}	2.81^{a}	2.54^{ab}	1.98^{abc}	1.59^{abc}	1.18^{abc}	0.94^{bc}	1.19^{abc}	0.63^{c}	1.91^{abc}	1.26^{abc}	1.00^{bc}
	Musty	1.76^{ab}	3.15^{a}	3.10^{a}	2.14^{ab}	2.43^{ab}	1.73^{ab}	1.49^{ab}	1.34^{ab}	0.82^{b}	2.25^{ab}	1.43^{ab}	1.71^{ab}
	Soapy	1.32^{b}	3.52^{a}	3.15^{ab}	1.99^{ab}	1.55^{ab}	3.26^{ab}	3.01^{ab}	2.11^{ab}	3.17^{ab}	1.53^{ab}	2.24^{ab}	1.66^{ab}
	Overall aroma intensity	3.19^{c}	5.75^{a}	5.27^{ab}	4.20^{abc}	3.65^{bc}	4.61^{abc}	4.51^{abc}	4.12^{abc}	5.10^{ab}	3.74^{bc}	3.93^{bc}	3.29^{c}
Flavour	Rose water	0.40^{d}	2.97^{bcd}	2.49^{cd}	1.66^{cd}	0.94^{cd}	5.68^{ab}	3.64^{bc}	2.50^{cd}	6.89^{a}	1.36^{cd}	2.37^{cd}	1.85^{cd}
	Malty	3.99^{a}	2.76^{a}	2.96^{a}	2.87^{a}	3.73^{a}	3.04^{a}	3.21^{a}	3.77^{a}	2.41^{a}	3.12^{a}	3.03^{a}	3.47^{a}
	Biscuity	2.07^{a}	1.40^{a}	1.79^{a}	1.73^{a}	1.55^{a}	1.94^{a}	1.66^{a}	1.96^{a}	1.49^{a}	2.09^{a}	1.70^{a}	1.99^{a}
	Orange fruit	1.25^{d}	2.64^{abcd}	2.16^{abcd}	1.80^{bcd}	2.3^{abcd}	4.36^{a}	3.74^{abc}	1.93^{bcd}	3.92^{ab}	1.56^{cd}	2.16^{abcd}	1.54^{cd}
	Grapefruit	1.57^{d}	2.87^{abcd}	2.21^{bcd}	2.19^{bcd}	1.71^{d}	4.60^{a}	4.07^{ab}	2.19^{bcd}	3.91^{abc}	2.00^{bcd}	2.57^{abcd}	1.80^{cd}
	Overall flavour intensity	3.96^{d}	5.39^{abcd}	5.17^{bcd}	4.80^{bcd}	4.50^{cd}	6.52^{ab}	5.90^{abc}	5.13^{bcd}	7.10^{a}	4.96^{bcd}	5.24^{bcd}	4.15^{cd}
Taste	Sweet	2.45^{abc}	2.58^{abc}	2.22^{bc}	1.84^{c}	2.75^{abc}	3.42^{ab}	3.43^{ab}	2.79^{abc}	3.99^{a}	2.53^{abc}	2.37^{bc}	2.61^{abc}
	Sour	2.42^{a}	2.97^{a}	2.70^{a}	3.03^{a}	2.43^{a}	3.23^{a}	2.99^{a}	3.38^{a}	2.55^{a}	2.36^{a}	3.29^{a}	2.70^{a}
	Smooth bitterness	2.67^{ab}	2.42^{ab}	1.93^{b}	1.57^{b}	2.57^{ab}	2.44^{ab}	3.45^{ab}	3.07^{ab}	4.32^{a}	2.90^{ab}	2.93^{ab}	2.12^{b}
	Harsh bitterness	2.89^{ab}	3.64^{ab}	3.92^{a}	4.12^{a}	3.27^{ab}	3.68^{ab}	2.33^{ab}	2.67^{ab}	1.89^{b}	2.71^{ab}	2.96^{ab}	3.25^{ab}
	Lingering bitterness	3.60^{a}	4.60^{a}	4.73^{a}	4.58^{a}	4.57^{a}	4.54^{a}	4.36^{a}	4.06^{a}	3.60^{a}	3.91^{a}	4.59^{a}	4.54^{a}
Mouthfeel	Peppery tingling	3.78^{a}	4.68^{a}	4.39^{a}	4.44^{a}	4.51^{a}	4.56^{a}	4.50^{a}	4.40^{a}	4.22 ^{a}	4.05^{a}	4.28^{a}	4.30^{a}
	Astringent	4.55^{a}	5.19^{a}	5.10^{a}	5.15^{a}	5.40^{a}	5.26^{a}	4.76^{a}	4.92^{a}	4.44^{a}	4.39^{a}	4.33^{a}	5.22^{a}

Superscripts of different letters within an attribute indicate a significant difference between means of samples of an attribute by Tukey's Honest Significant Difference (HSD) test at $p<0.05$. repl, experimental replicate; TO, Total oil; SQ, Sesquiterpene fraction; HUM EPOX, Humulene epoxides enriched fraction; TA, Terpene alcohol fraction; MTA, Monoterpene alcohol fraction; LIN, Linalool fraction; GER, Geraniol fraction; SQA, Sesquiterpene alcohol fraction; HUM, Humulol enriched fraction; CAR, Caryophyllene oxide fraction

PCA was performed to reduce the data's complexity and visually represent the samples in a sensory space (Figure 3.4). The first two principal components explained the majority of the total variance (86.39%) with the main discriminating dimension PC1 explaining 61.85% and PC2 explaining 24.53%. PC1 was positively loaded with the attributes "rose water", "orange fruit", "grapefruit", "lemon", "pine wood", "soapy" and "sweet". PC2 was positively loaded with the primary distinguishing aroma attributes "crushed grass, sap", "resinous", "musty" and "earthy". PC3 only accounted for 5.31% of the variance in the sample set and was positively loaded with "smooth bitterness" (r=0.527) and negatively loaded with "harsh bitterness" (r=-0.566), "lingering bitterness" (r=-0.559), and "astringent" (r=-0.672) indicating that both aroma and flavour, as well as taste and mouthfeel attributes, are differentiating between the flavoured beers. Nevertheless olfactory characteristics clearly were the key discriminators.

The biplot showed the "harsh bitterness" and the "lingering bitterness" to be correlated. The two attributes were assessed at different time points, namely as an immediate sensation after swallowing and 20 seconds after swallowing, respectively. The panellists could well distinguish between the two bitterness qualities ("smooth and harsh bitterness"), therefore, it was agreed with the panel to only assess a general "lingering bitterness" instead distinguishing between "lingering smooth bitterness" and "lingering harsh bitterness". Overall, it appeared that fewer hop oil extracts in the sample set were characterised by a lingering "smooth bitterness". Future research should further investigate the temporal profile of these two attributes to clarify the evolution of the bitterness sensations.

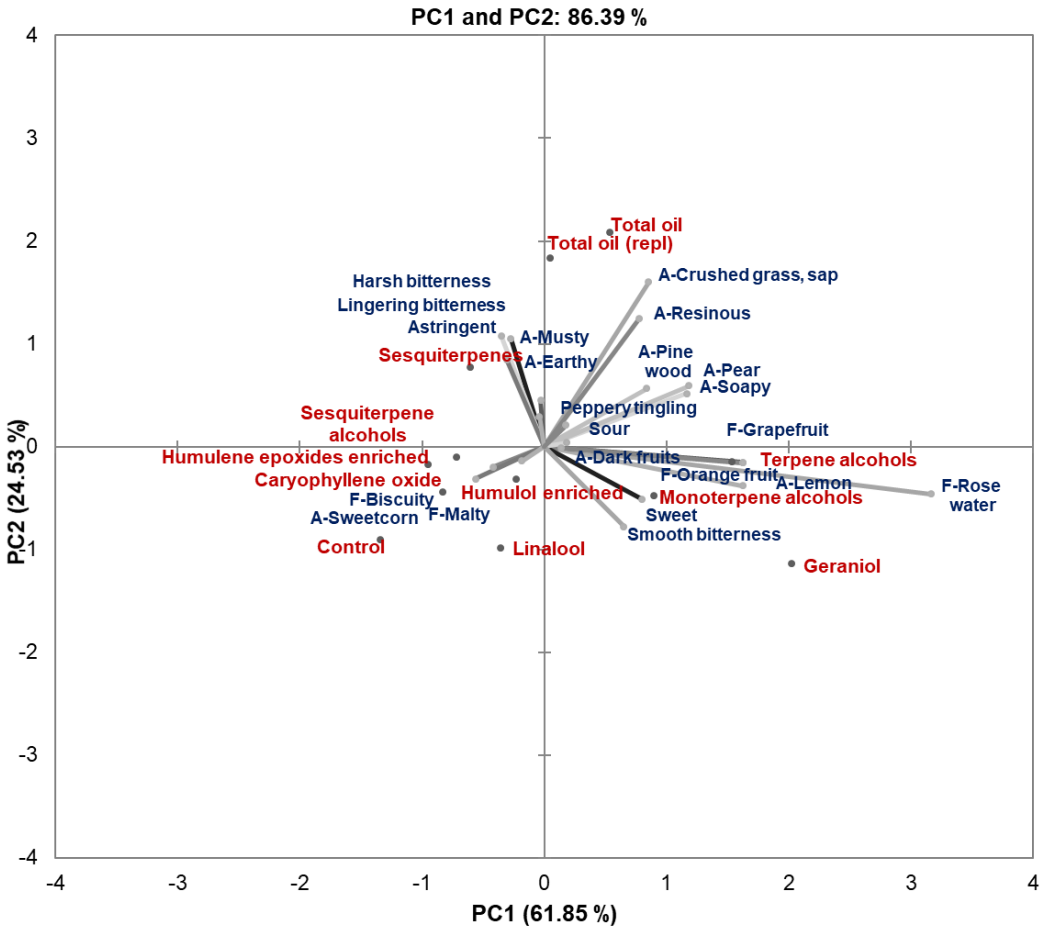

Figure 3.4. Principal Component Analysis (PCA) biplot of sensory attributes present on principal component 1 (PC1) and 2 (PC2) by the covariance matrix of mean attribute intensity rating across the hop extracts. Sensory attributes in **blue**, samples in **red**; repl, experimental replicate; A, aroma attribute, F, flavour attribute.

Overall aroma and flavour intensity. All flavoured beers were designed to be equi-intense. However, inspection of the ANOVA outcome indicated a significant effect of "overall flavour intensity". Tukey's HSD tests revealed that the flavour intensity was higher for the geraniol- and terpene alcohol fraction-flavoured beers and lower for the caryophyllene oxide- and humulene epoxide-fraction flavoured beers compared to the other samples. The latter two showed only slightly increased flavour intensities compared to the control beer. Caryophyllene oxide is known to impart little aroma to beer (Lafontaine & Shellhammer, 2018b). Flavour descriptors or threshold

concentrations of caryophyllene oxide in beer have not yet been published. It should be noted that caryophyllene oxide is prone to oxidation, hydrolysis and isomerisation reactions, and measures have been taken to reduce volatile loss to a minimum, but could not be completely ruled out (Yang, Lederer, McDaniel, & Deinzer, 1993a). The findings that geraniol- and terpene alcohol fraction-flavoured beers obtained significantly higher scores for "overall flavour intensity" might be explained by differences in volatility or aroma and flavour threshold levels of the compound mixture in the extracts.

Evaluation of the base beer. Inspection of the control beer scores indicated that it was characterised by attributes intrinsic to standard lager such as "malty" which was not significantly higher than those in the flavoured beers suggesting that the base maltiness was not significantly impacted by the any of the hop extracts used.

Sensory characteristics induced by total Magnum hop oil. The beer flavoured with the total Magnum hop oil obtained high scores for the aroma attributes "crushed grass, sap", "resinous", "earthy", and "musty", which significantly discriminated the total oil flavoured beer from the control beer ("crushed grass, sap", "resinous") and the beer flavoured with the geraniol enriched fraction ("earthy", "musty"). The addition of the total oil resulted in significantly higher intensity scores for "crushed grass, sap" compared to the control beer and those beers flavoured with the humulene epoxide, linalool, sesquiterpene alcohol and caryophyllene oxide enriched fractions.

The main compounds accounting for up to 80% in Magnum hop oil are *β*-myrcene, *β*-caryophyllene, and *α*-humulene, with the most abundant compound *β*-myrcene

being described as “spicy” and “resinous” at 200 µg/L (Sharpe, 1988), “metallic” and “geranium-like” at 860 µg/L (Schnaitter et al., 2016) or “geranium-leaf”-like at 6.65-15.0 µg/L (Neiens & Steinhaus, 2018). Depending on the beer matrix assessed, the sensory characteristics of *β*-caryophyllene and *α*-humulene in beer have hardly been defined. *β*-caryophyllene and *α*-humulene have been described as “rubber-like”, “mouldy” (Zhai & Granvogl, 2019) and “woody”, “spicy” (Navarro-Martínez et al., 2019). Similar aroma characteristics could also be found in the sesquiterpene fraction-flavoured beer which was described by “crushed grass, sap”, “musty” and “pine wood” aromas, but at comparably low intensities compared to the total oil-flavoured beer. This may indicate that the total oil’s composition increases the aroma intensity of these characteristics.

Impact of hop extracts on beer bitterness and mouthfeel. Interestingly, the sesquiterpene fraction-flavoured beer obtained the highest score for “harsh bitterness”, which was significantly higher than the score for the geraniol-flavoured beer, however, not compared to the other flavoured beers. Instead, the beer flavoured with the geraniol enriched fraction was characterised by a high score for “smooth bitterness”, indicating opposing bitterness qualities in these two hop extracts. Panellists’ descriptors of the two attributes (“irritating, spiky”, “soft, pleasant”) suggest these bitterness qualities have trigeminal-type dimensions. Interestingly, the sesquiterpene fraction-flavoured and geraniol-flavoured beers also showed opposing scores for “lingering bitterness” (bitterness assessed 20 seconds after swallowing) with a higher score obtained for the latter, although not significant. Oxygenated sesquiterpenes including caryophyllene oxide (Goiris et al., 2002; Praet

et al., 2015) and linalool (Kaltner & Mitter, 2006; Praet et al., 2015) have been suggested to affect bitterness intensity, duration and quality, although the majority of effects have not been assessed using a systematic sensory analysis approach. Effects on bitterness have not yet been reported for a geraniol extract individually applied in beer and limited studies have been conducted to study the impact of sesquiterpene extracts on bitterness qualities and decline.

The beer flavoured with the humulene epoxide enriched fraction only received a slightly higher score for “astringent” than the other beers. Caryophyllene oxide has been suspected to be part of a compound mixture in the sesquiterpenoid fraction enhancing spicy hop flavour, fullness, mouthfeel, and bitterness of beer (Goiris et al., 2002; Praet et al., 2015). Based on previous research, humulene epoxides and sesquiterpene alcohols including caryophyllene oxide were expected to add bitterness and a “peppery tingling” mouthfeel to the beer that was described as an irritating sensation, suggesting a trigeminal effect (Dietz, Cook, Wilson, et al., 2020b). However, the preceding study's test matrix was non-carbonated and the carbonation might have masked this mouthfeel and impeded its recognition. Both beer astringency and bitterness can linger for several minutes (Kaneda, Takashio, Shinotsuka, & Okahata, 2001; McLaughlin et al., 2008) therefore, temporal sensory methods may be more appropriate for discriminating these attributes.

Impact of hop extracts on beer aroma, flavour and sweetness. In agreement with previous findings, the geraniol and the terpene alcohol-flavoured beers were characterised by citrusy (“lemon”, “orange”, “grapefruit”) and “rose water” aromas and flavours (Eyres et al., 2007; Kishimoto et al., 2006). The geraniol-fraction

flavoured beer was also significantly sweeter than the sesquiterpene- and humulol enriched-fraction flavoured beers and slightly sweeter compared to the control and total oil-flavoured beers. Pearson correlation revealed significant correlations between “sweet” and “lemon” (*r*=0.899), and “orange fruit” (*r*=0.812), “rose water” (*r*=0.820), and “grapefruit” (*r*=0.764) indicating that the aroma and flavour profiles of the geraniol, and terpene alcohol fractions (all containing geraniol) increase the perceived sweetness intensity in beer. Sweetness was also significantly, positively correlated with “smooth bitterness” (*r*=0.801) and negatively with “harsh bitterness” (*r*=-0.943) suggesting a sensory interaction effect between sweetness and bitterness qualities, where one is pivotal for the other.

The terpene alcohol-, monoterpene alcohol-, linalool- and geraniol fraction-flavoured beers were characterised by “lemon”, “pine wood”, and “soapy” aromas and “rose water”, “orange fruit”, and “grapefruit” flavours. The geraniol- and terpene alcohol fraction-flavoured beers were perceived to be significantly higher for “rose water” flavour compared to the other beers. The terpene alcohol fraction induced significantly increased “rose water” flavour compared to the monoterpene alcohol sub-fraction. This suggests that the terpene alcohol fraction contained volatiles besides the two key compounds linalool and geraniol inducing both floral and fruity notes due to additive- or synergistic-type behaviour. It is interesting to note that the linalool fraction-flavoured beer was not strongly characterised by any aroma and flavour attribute supporting the suggestion that linalool primarily acts as an aroma/flavour enhancing molecule in certain volatile mixtures as opposed to having

a major impact on the sensory profile of beer when applied individually (Kaltner & Mitter, 2009; Takoi, Koie, et al., 2010).

3.3.2 Effect of fraction composition on sensory characteristics

Table 3.4. lists the 49 volatiles identified in the hop extracts including a range of monoterpene and sesquiterpene hydrocarbons, oxygenated sesquiterpenoids, monoterpene alcohols and smaller fractions of esters, ketones and unknowns (Figure 3.5). Table 3.5. shows the average proportion (%) of the compounds in the corresponding hop extract. The fractions enriched in the single compounds linalool, geraniol and caryophyllene oxide contained only minor proportions of other compounds. Sample carryover between GC-MS runs was excluded as a possible cause of trace compounds by running 'blanks', suggesting these were naturally present as a result of the fractionation process. The chemical profiles of other extracts, however, showed significant overlaps, suggesting that a clear separation of sub-fractions was not achieved.

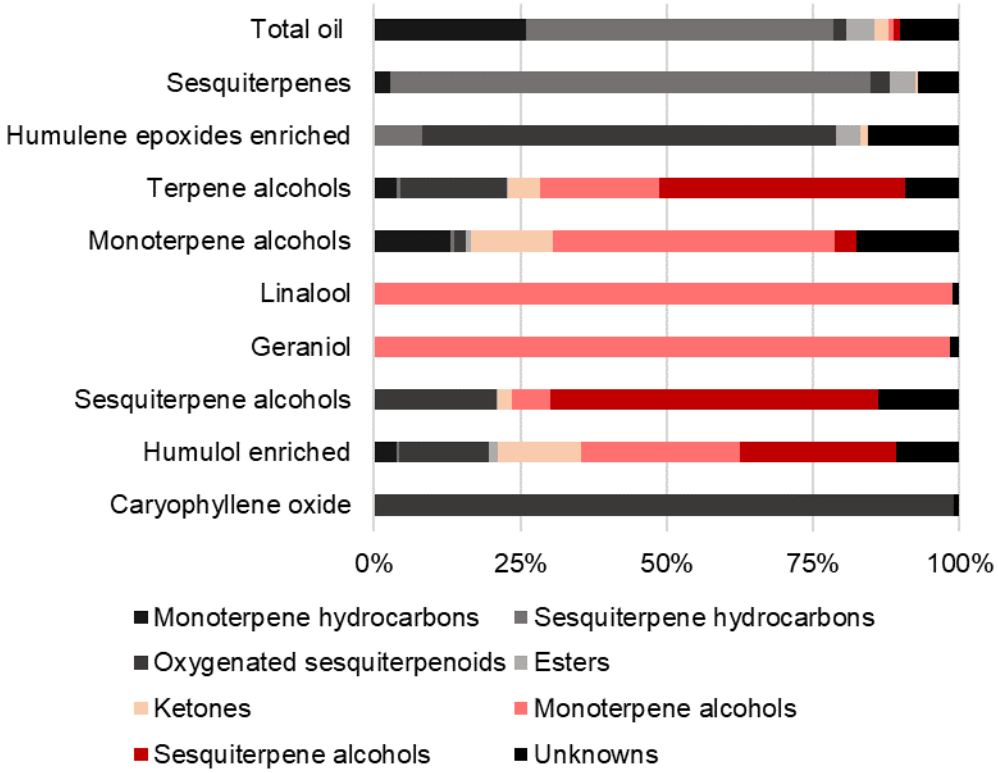

Figure 3.5. Chemical class profiles (%mean of the total normalised integrated peak area in the GC-MS chromatograms) of the hop extracts applied in the base beer.

Trace components could potentially have contributed to the sensory profiles of the flavoured beers even if present at sub-threshold concentrations. Sensory threshold data of the compounds applied in comparable beer matrices was gathered from the available literature and compared with the relative concentrations applied in the samples (Table 3.5.). Sensory detection thresholds in water are not shown since these are usually much lower than those in complex matrices such as beer. To date, no taste and mouthfeel threshold data has been published for the compounds identified. Several aroma and flavour threshold concentrations could be sourced and comparison with the applied concentrations showed that several compounds were added supra-threshold such as *β*-myrcene, *α*-humulene, *β*-caryophyllene, linalool, geraniol, humulene epoxide II, and humulenol II.

PCA was conducted to visualise the relationships between the samples, their sensory profiles and the volatile compositions. The plot in Figure 3.6. shows the significant principal components PC1 (38.22%) and PC2 (26.43%) explaining 64.65% of the variance. The majority of volatile compounds loaded positively on PC1, together with either “crushed grass, sap” and “resinous” aromas or fruity aromas/flavours that could be assigned to volatiles in the hop extracts (among others **[C7]** linalool, **[C17]** geraniol, **[C16]** nerol, **[C19]** 2-undecanone, **[C25]** 2-dodecanone). PC2 is foremost positively loaded with “earthy”, “musty”, “harsh bitterness” and “lingering bitterness” and negatively loaded with “sweet” and “smooth bitterness”. This component is predominantly loaded with oxygenated sesquiterpenes and sesquiterpene hydrocarbons such as *β*-pinene **[C1]**, *β*-myrcene **[C2]**, *cis*-*β*-ocimene **[C4]**, *β*-caryophyllene **[C26]**, *γ*-muurolene **[C30]**, *β*-eudesmene **[C31]**, and humulene epoxide I **[C40]**, II **[C42]** and III **[C45]**.

Table 3.4. Volatile compounds (tentatively) identified (n=49) in the nine hop extracts using library/database matching (>80%) and authentic standards (*). Identification confirmed by calculated retention indices (RI).

			RI		Literature RI	
#	**Compound**	**CAS**	**5MS**	**WAX**	**5MS**	**WAX**
1	*β*-Pinene*	127-91-3	985	1130	980-990[a,b]	1113-1124[a,b]
2	*β*-Myrcene*	123-35-3	990	1153	991-994[a,b]	1145-1176[a,b]
3	*R*-(+)/D-Limonene*	5989-27-5	1027	1190	1030-1039[a,b]	1201-1234[a,b]
4	*cis*-*β*-Ocimene	3338-55-4	1036	1240	1038-1043[a,b]	1242-1245[a,b]
5	*cis*-Linalool oxide	1365-19-1	1072	1417	1070-1074[a,b]	11420[b]
6	2-Nonanone*	821-55-6	1088	1395	1093[b]	1388[b]
7	Linalool*	78-70-6	1098	1526	1098-1112[a,b]	1537[b]
8	*exo*-*β*-Fenchol	470-08-6	1115	1550	1117[a]	1576[n]
9	Myrcenol	543-39-5	1123	1561	1118[a]	n/a
10	Methyl octanoate*	111-11-5	1135	1391	1127[c]	1389[b]
/	Benzyl acetate (ISTD)	140-11-4	1162	1737	1162-1164[a,b]	1735[o]
11	*endo*-Borneol*	464-45-9	1163	1680	1162-1165[a,b]	1642-1677[a,b]
12	Terpinen-4-ol	562-74-3	1175	1614	1177-1182[a,b]	1591-1616[a,b]
13	*trans*-3(10)-Caren-2-ol	93905-79-4	1176	1698	1175[d]	1700[p]
14	*α*-Terpineol*	8000-41-7	1187	1686	1185-1207[a,b]	1688-1720[a,b]
15	Myrtenol	19894-97-4	1197	1756	1196[e]	1757[q]
16	Nerol	106-25-2	1224	1773	1228-1233[a,b]	1753-1770[a,b]
17	Geraniol*	106-24-1	1276	1826	1255-1276[a,b]	1788-1862[a,b]
18	Methyl 8-methylnonanoate	5129-54-4	1290	1527	1287[f]	1520[r]
19	2-Undecanone*	112-12-9	1292	1595	1296[b]	1596[s]
20	Perillol (Perillyl alcohol)	7644-38-4	1292	1983	1295[c]	1985[t]
21	2-Undecanol	1653-30-1	1307	1710	1301[g]	1719[b]
22	Methyl (*E*)-4-decenoate	93979-14-7	1314	1612	1311[h]	1608[s]
23	Methyl geranate*	2349-14-6	1319	1677	1323[h]	1678[s]
24	Octyl Isobutyrate	109-15-9	1328	1538	1326[i]	1535[r]
25	2-Dodecanone*	6175-49-1	1381	1662	1379[j]	1673[r]
26	*β*-Caryophyllene*	87-44-5	1418	1592	1418-1467[a,b]	1594-1618[a,b]
27	*α*-Bergamotene	17699-05-7	1433	1759	1430-1434[a,b]	1779[b]
28	*α*-Humulene*	6753-98-6	1452	1671	1467[b]	1663[b]
29	Geranyl propionate*	105-90-8	1472	1826	1475[a]	1830[u]
30	*γ*-Muurolene	30021-74-0	1474	1671	1477-1475[a,b]	1681-1684[a,b]
31	*β*-Eudesmene	515-17-3	1489	1717	1485[a]	1711[b]
32	2-Tridecanone*	593-08-8	1491	1814	1496[h]	1817[s]
33	Methyl 3,6-dodecadienoate	16106-01-7	1493	1872	1488[j]	1857[r]
34	Geranyl isobutyrate*	2345-26-8	1515	1773	1514[a]	1777[s]
35	*δ*-Cadinene	483-76-1	1530	1774	1519-1539[a,b]	1788[v]
36	*trans*-*Z*-α-Bisabolene epoxide	n/a	1533	n/a	1531[k]	n/a
37	Nerolidol	7212-44-4	1539	2021	1534-1565[a,b]	2009-2054[a,b]
38	Caryophyllenyl alcohol	56747-96-7	1568	2025	1556-1568[a,b]	n/a
39	Caryophyllene oxide*	1139-30-6	1577	1974	1573-1606[a,b]	1982[w]

Table 3.4 continued.

#	Compound	CAS	RI 5MS	RI WAX	Literature RI 5MS	Literature RI WAX
40	Humulene epoxide I	19888-34-7	1578	2012	1578[l]	2000[x]
41	Humulol	28446-26-6	1581	2122	1582[l]	n/a
42	Humulene epoxide II	19888-34-7	1592	2010	1593[j]	2022[r]
43	Widdrol	6892-80-4	1598	NF	1597[b]	n/a
44	Epicubenol	19912-67-5	1608	2054	1613-1645[a,b]	n/a
45	Humulene epoxide III	21624-36-2	1612	2075	1611[l]	2055[y]
46	Humulenol II	19888-00-7	1619	2230	1613[l]	n/a
47	11,11-Dimethyl-4,8-dimethylene bicyclo[7.2.0]undecan-3-ol	79580-01-1	1636	n/a	1639[m]	n/a
48	τ-Cadinol	5937-11-1	1638	2135	1640[a]	n/a
49	δ-Cadinol	36564-42-8	1641	2164	1635-1674[a,b]	2167[b]

ISTD, Internal standard; n/a, not available: NF, not found

[a] Pherobase; [b] Flavornet; [c] Nance and Setzer (2011); [d] Kang et al. (2010); [e] Maggi et al. (2009); [f] Ilic-Tomic et al. (2015); [g] Zhang et al. (2017); [h] Pistelli et al. (2018); [i] Venkatachallam et al. (2010); [j] Jackson and Linskens (2002); [k] Al-Reza, Rahman, Sattar, Rahman, and Fida (2010); [l] Praet et al. (2016); [m] Zeng, Zhang, Luo, and Zhu (2011); [n] Pino et al. (2002); [o] Perry et al. (2009); [p] Palá-Paúl et al. (2005); [q] Giuseppe et al. (2005); [r] Yan et al. (2018); [s] Liu et al. (2018); [t] Minh Tu et al. (2002); [u] Choi and Sawamura (2000); [v] Stashenko et al. (2010); [w] Richter et al. (2017); [x] Hofmann et al. (1992); [y] Miyazawa et al. (2010)

Table 3.5. Semi-quantified volatile composition of the nine hop extracts (average relative peak area %), Log*P* (logarithm of the octanol-water partition coefficient) used as an indicator for the hydrophobicity, solubility in water, and sensory detection thresholds of volatile compounds in beer (where available), labelled in bold if the relative concentration of a compound added to the base beer potentially exceeded its sensory threshold concentration. Letters within columns indicate significant mean separation (among hop oil samples) according to Tukey's Honest Significant Difference (HSD) test.

#	Compound	TO	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR	Log*P**	Solubility [mg/L]*	Sensory detection threshold [µg/L]**
1	*β*-Pinene	0.37 a	0.01 b	- b	- b	- b	- b	- b	- b	- b	- b	4.16	7.06	n/a
2	*β*-Myrcene	**34.74** a	**4.04** b	0.04 c	- c	0.07 c	0.26 c	- c	0.02 c	0.04 c	- c	4.88	6.92	A: 9-1000[a]; F: 40[b]
3	*R*-(+)/D-Limonene	0.29 a	0.03 b	- b	- b	- b	- b	- b	- b	0.01 b	- b	4.57	4.58	n/a
4	*cis*-*β*-Ocimene	0.07 a	0.03 a	0.05 a	- b	- b	- b	- b	- b	- b	- b	4.67	2.01	n/a
5	*cis*-Linalool oxide	0.01 b	- b	- b	- b	- b	- b	- b	- b	0.10 a	- b	2.08	3353.00	n/a
6	2-Nonanone	0.19 b	- c	- c	0.07 c	0.54 b	- c	- c	- c	1.01 a	- c	3.14	170.60	F: 2000[c]
7	Linalool	**0.42** d	- d	- d	**4.22** c	**31.68** b	**98.94** a	- d	- d	**5.29** c	- d	2.97	683.70	A: 2-80[a]; F: 27-80[c,d]
8	*exo*-*β*-Fenchol	- b	- b	- b	1.01 a	- b	- b	- b	- b	- b	- b	2.85	461.40	n/a
9	Myrcenol	0.04 c	- c	- c	0.26 b	2.08 a	- c	- c	- c	0.58 b	- c	3.46	260.90	n/a
10	Methyl octanoate	0.45 a	- b	- b	- b	0.06 b	- b	- b	- b	0.03 b	- b	3.46	101.90	n/a
11	*endo*-Borneol	0.05 c	- c	- c	1.65 b	7.04 a	- c	- c	0.02 c	1.91 b	- c	2.69	260.90	n/a
12	Terpinen-4-ol	0.01 c	- c	- c	0.20 b	2.21 a	- c	- c	- c	0.26 b	- c	3.26	386.60	n/a
13	*trans*-3(10)-Caren-2-ol	0.05 c	- c	- c	0.10 b	0.74 a	- c	- c	- c	0.61 a	- c	1.97	489.00	n/a
14	*α*-Terpineol	0.19 c	- c	- c	4.28 b	13.7 a	- c	- c	0.12 c	3.85 b	- c	2.98	371.70	A: 330[a]; F: 2000[c]
15	Myrtenol	0.01 b	- b	- b	0.05 a	0.02 b	- b	- b	- b	0.05 a	- b	2.98	426.90	n/a
16	Nerol	0.14 c	0.03 c	0.03 c	1.38 a	1.00 a	- c	- c	0.17 c	0.99 b	- c	4.70	39.90	A: 80-500[a]
17	Geraniol	0.05 e	- e	- e	**11.33** c	**9.6** c	- e	**99.32** a	**4.14** de	**16.19** c	- c	3.47	255.80	A: 4-300[a]; F: 36[e]
18	Methyl 8-methyl-nonanoate	0.25 a	0.13 ab	0.13 ab	0.01 c	0.06 b	- c	- c	- c	0.03 c	- c	4.40	12.56	n/a
19	2-Undecanone	1.80 b	0.27 c	0.42 c	2.04 b	9.55 a	- c	- c	0.50 c	10.29 a	- c	3.69	19.71	F: 400[c]
20	Perillol	0.02 c	- c	- c	0.19 b	0.17 b	- c	- c	0.14 bc	0.46 a	- c	3.17	471.00	n/a
21	2-Undecanol	0.09 b	- b	- b	0.49 a	0.39 a	- b	- b	0.41 a	0.31 a	- b	4.21	49.73	F: 70[c]
22	Methyl (*E*)-4-decenoate	1.40 a	0.05 c	1.07 a	- c	0.40 b	- c	- c	- c	0.25 bc	- c	4.09	16.67	n/a
23	Methyl geranate	0.73 a	0.44 ab	0.57 a	- c	0.28 bc	- c	- c	- c	0.91 a	- c	3.98	21.24	F: 21.5[f]
24	Octyl Isobutyrate	0.15 a	0.16 a	0.13 ab	- b	0.02 b	- b	- b	0.01 b	0.06 b	- b	4.71	4.06	n/a
25	2-Dodecanone	0.30 d	- d	0.15 d	0.96 bc	1.57 b	- d	- d	1.29 b	2.76 a	- d	4.18	13.99	F: 250[c]
26	*β*-Caryophyllene	8.76 b	**19.05** a	0.21 c	0.12 c	0.18 c	- c	- c	- c	0.09 c	- c	6.30	0.05	A: 160-420[a]
27	*α*-Bergamotene	0.02 b	0.27 a	0.03 b	- b	0.04 b	- b	- b	- b	0.01 b	- b	6.57	0.03	n/a
28	*α*-Humulene	**36.39** a	**55.4** a	7.02 b	0.48 c	0.51 c	- c	- c	- c	- c	- c	6.95	0.01	A: 120-747[a,g]

Table 3.5 continued.

#	Compound	TO	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR	Log*P**	Solubility [mg/L]*	Sensory detection threshold [µg/L]**
29	Geranyl propionate	0.02 b	0.02 b	- b	0.29 a	0.02 b	- b	- b	0.06 b	0.13 ab	- b	3.64	2.22	n/a
30	*γ*-Muurolene	1.00 b	3.12 a	3.13 a	- c	0.06 c	- c	- c	- c	0.04 c	- c	6.27	0.05	n/a
31	*β*-Eudesmene	0.46 bc	1.63 a	1.09 b	- d	0.01 d	- d	- d	- d	0.04 d	- d	6.38	0.04	n/a
32	2-Tridecanone	0.05 c	0.21 c	1.21 b	2.85 a	3.41 a	- c	- c	0.81 b	0.35 bc	- c	4.68	4.53	F: 100[c]
33	Methyl 3,6-dodecadienoate	0.23 b	- b	1.00 a	- b	- b	- b	- b	0.02 b	0.02 b	- b	4.10	2.77	n/a
34	Geranyl isobutyrate	1.50 b	4.55 a	3.16 a	- c	0.03 c	- c	- c	0.21 c	0.24 c	- c	4.77	0.82	A: 450[a]; F: 450[e]
35	*δ*-Cadinene	2.30 b	9.42 a	- c	- c	0.02 c	- c	- c	0.06 c	0.25 c	- c	6.64	0.05	n/a
36	*trans-Z-α*-Bisabolene epoxide	0.04 b	0.01 b	1.37 a	- b	- b	- b	- b	0.01 b	0.01 b	- b	4.86	7.27	n/a
37	Nerolidol	0.10 c	- c	- **c**	**2.12** a	0.25 c	- c	- **c**	**2.75** a	1.38 b	- c	5.68	1.53	F: 21.44[f]
38	Caryophyllenyl alcohol	0.18 d	- d	- d	11.08 a	1.09 c	- d	- d	14.76 a	5.65 b	- d	4.20	9.13	n/a
39	Caryophyllene oxide	0.55 cd	1.63 c	15.04 b	0.53 cd	0.09 d	- d	- d	0.95 cd	0.19 d	99.74 a	3.60	2.21	n/a
40	Humulene epoxide I	0.04 c	0.04 **c**	**2.55** a	**0.95** b	- c	- c	- c	- c	- c	- c	4.56	0.62	A: >10[a;] F: 100[h]
41	Humulol	0.67 d	- d	- d	30.58 a	2.08 c	- d	- d	39.72 a	18.9 b	- d	3.80	44.17	A: 2000[i]
42	Humulene epoxide II	1.11 bc	2.23 **b**	**78.63** a	- c	0.24 c	- c	- c	1.16 bc	- c	- c	4.51	5.43	A: 450[a]
43	Widdrol	0.03 c	- c	- c	0.70 ab	0.47 b	- c	- c	1.73 a	0.29 bc	- c	4.10	7.93	n/a
44	Epicubenol	0.04 c	- c	- c	1.46 a	0.18 c	- c	- c	2.02 a	0.94 ab	- c	3.69	9.13	n/a
45	Humulene epoxide III	0.04 b	0.03 b	1.16 a	- b	- b	- b	- b	- b	- b	- b	4.45	0.51	F: 450[e]
46	Humulenol II	0.10 c	- c	- **c**	**12.06** a	1.52 b	- c	- c	13.39 a	13.04 a	- c	3.50	2.26	A: 150-2500[a]; F: 2500[e]
47	11,11-Dimethyl-4,8- dimethylenebicyclo[7.2.0]undecan-3-ol	0.03 b	- b	- b	1.59 a	0.08 b	- b	- b	2.25 a	- b	- b	3.70	8.12	n/a
48	τ-Cadinol	0.10 c	- c	- c	4.96 a	0.21 c	- c	- c	4.86 a	1.64 b	- c	4.90	9.13	n/a
49	*δ*-Cadinol	0.03 c	- c	- c	1.34 a	- c	- c	- c	2.12 a	0.59 bc	- c	4.95	9.13	n/a

"-" compound not detected; TO, Total oil; SQ, Sesquiterpene fraction; HUM EPOX, Humulene epoxides enriched fraction; TA, Terpene alcohol fraction; MTA, Monoterpene alcohol fraction; LIN, Linalool; GER, Geraniol; SQA, Sesquiterpene alcohol fraction; HUM, Humulol enriched fraction; CAR, Caryophyllene oxide

* Log*P* and solubility in water estimated using EPI Suite™ v.4.1 software (U.S. Environmental Protection Agency)

** Aroma (A) and/or flavour (F) threshold concentrations. Taste and mouthfeel threshold concentration have not yet been determined for the compounds identified in the hop extracts used in this study.

[a] Schönberger et al. (2015); [b] Meilgaard et al. (1999); [c] Meilgaard (1975b); [d] Hanke (2009); [e] Peacock and Deinzer (1981); [f] Jiang et al. (2017); [g] Bordiga and Nollet (2019); [h] Shimazu et al. (1975); [i] Irwin (1989)

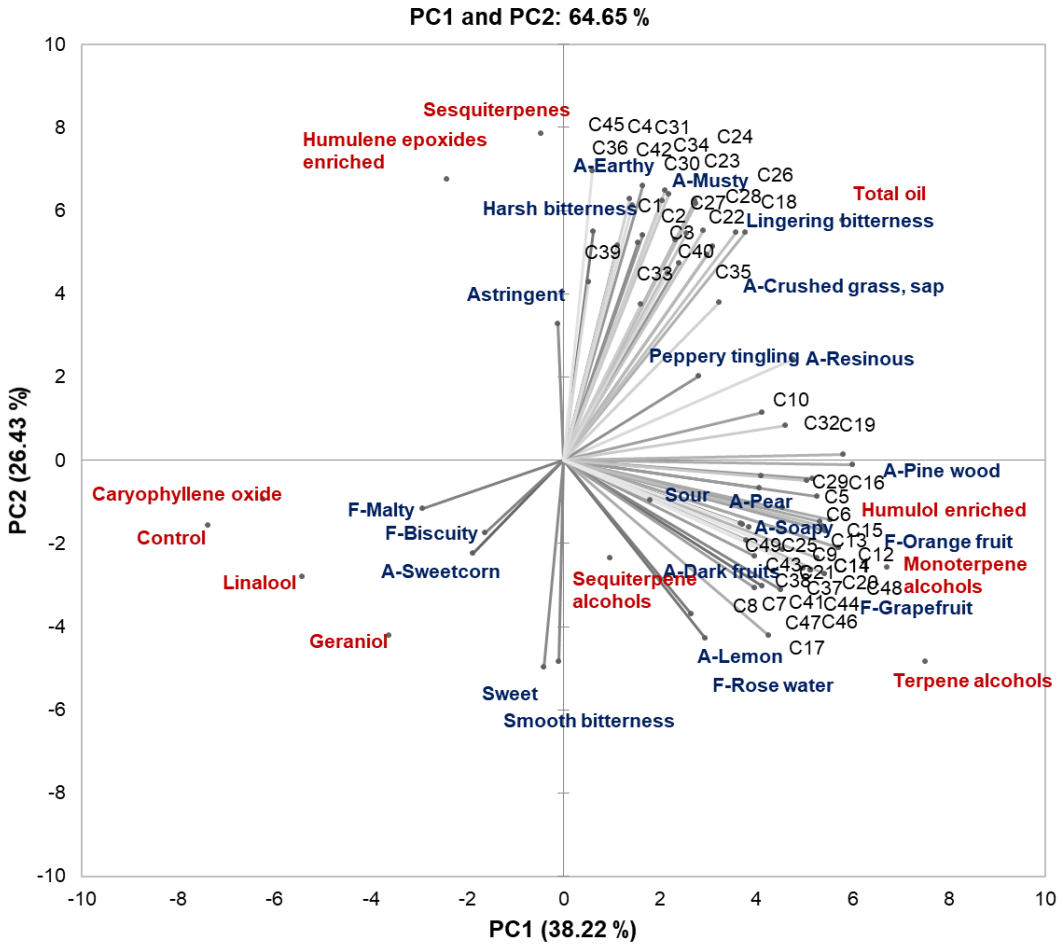

Figure 3.6. Principal Component Analysis (PCA) biplot of standardised sensory attribute means and compounds' relative concentrations as applied in the base beer showing the correlation between the two variables principal component 1 (PC1) and 2 (PC2). Volatile hop compounds (C) numbered in black, sensory attributes in blue, samples in red; A, aroma attribute, F, flavour attribute.

Hop compounds related to beer bitterness. *β*-caryophyllene **[C26]**, *α*-humulene **[C28]** and humulene epoxides I and III **[C40, C45]** significantly positively correlated ($p \leq 0.05$) with "lingering bitterness" and the latter two also with "harsh bitterness". The strongest correlations were detected between the humulene epoxides and "harsh bitterness" (r=0.711, r=0.688). In contrast, *β*-caryophyllene **[C26]** (r=-0.715), *α*-humulene **[C28]** (r=-0.731), humulene epoxides I **[C40]** (r=-0.647) and caryophyllene oxide **[C39]** (r=-0.677) significantly negatively correlated with "smooth bitterness". Caryophyllene oxide **[C39]** had no significant effect on the beer's taste

and mouthfeel properties. The compound might rather act with a mix of oxygenated sesquiterpenes to modify beer bitterness due to synergistic-type behaviour. Also of interest was that *β*-pinene **[C1]** (*r*=0.661), *D*-limonene **[C3]** (*r*=0.667), *cis*-*β*-ocimene **[C4**] (*r*=0.467), and *β*-eudesmene (**[C3]**; or *β*-selinene) (*r*=0.580) positively correlated with "harsh bitterness", which has not yet been reported elsewhere. The majority of the compounds related to modified bitterness qualities were therefore mainly present in the total oil and the sesquiterpene and humulene epoxide enriched fractions, the latter agreeing with the work of others (Goiris et al., 2002; Oladokun et al., 2016) who found a change in bitterness perception with oxygenated sesquiterpene fractions.

Oladokun et al. (2016) investigated the temporal profile of perceived beer bitterness at different concentrations with a Hersbrucker hop extract and found it induced a prolonged bitterness, although specific compounds or fractions were not attributed to this sensation. Mikyška et al. (2018) suggested increased concentrations of *β*-caryophyllene, *α*-humulene, and *α*-caryophyllene epoxide to be responsible for higher "harsh" bitterness scores in kettle+dry hopped beers. Also, Kaltner and Mitter (2006) reported a modified beer bitterness perception at different concentrations of linalool and terpene hydrocarbons added (Kaltner & Mitter, 2006).

Another compound that impacted beer bitterness was geraniol **[C17]** with the "smooth bitterness" score being significantly increased in the geraniol fraction-flavoured beer, particularly compared to the sesquiterpene fraction-flavoured beer. However, no significant, positive correlation was detected to explain the relationship between geraniol and the increased "smooth bitterness" intensity (*r*=0.572). It was

assumed that the bitterness quality was influenced by the perceived aromas and flavours, causing sensory interactions within (taste) and across (aroma/flavour) modalities. Limited research has been conducted in the field of hop volatiles and their effect on temporal and qualitative dimensions of bitterness and other taste sensations. Moreover, the hop extracts used might be too complex to draw reliable conclusions on concentration-dependent effects.

Hop compounds related to beer sweetness. In line with the preceding study (Dietz, Cook, Wilson, et al., 2020b), beers flavoured with geraniol-containing fractions were mainly differentiated from the other beer by higher scores for “sweet”, “rose water”, “orange fruit”, “grapefruit” and “lemon”. Geraniol significantly correlated with several aroma and flavour attributes ($p \leq 0.05$); particularly with “rose water” ($r=0.725$), “orange fruit” ($r=0.753$), and “grapefruit” ($r=0.858$), however, not with the sweetness ($r=0.400$). Thus, beer sweetness might have been added with ‘fruity/floral’ aromas perceived ortho- and retronasally, which would suggest that the sweetness increased through a sensory interaction between aroma and taste.

Hop compounds related to mouthfeel sensations. A “spicy” sensation in beer has previously been assigned to oxygenated sesquiterpenoids, humulene epoxides and oxidation products of *β*-caryophyllene, mostly describing a flavour or a mouthfeel sensation (Goiris et al., 2002; Praet et al., 2015). The sesquiterpene alcohol and humulol enriched fractions had limited effects on the beer’s sensory profile, although results of previous studies indicated that the sub-fraction containing humulol **[C41]** and humulenol II **[C46]** could be responsible for the spicy/”peppery tingling” sensation (Deinzer & Yang, 1994; Goiris et al., 2002). The extracts contained ~351

µg/L and ~50 µg/L humulol and 123 µg/L and 36 µg/L humulenol II, respectively. Aroma threshold concentrations of these compounds in beer were determined to be 150-2500 µg/L for humulenol II (aroma, flavor) and 2000 µg/L for humulol (Table 3.5.). Goiris et al. (2002) applied 20 µg/L of a sesquiterpenoid preparation that contained much lower concentrations of humulenol II (1.5 µg/L) in beer and observed effects on “spicy”, “mouthfeel”, and “fullness”. It should be considered that results of previous studies are contradictory. Also, the relationship between “spicy” characters and sesquiterpenoids including humulene epoxides and humulenol II has not always been confirmed (Kishimoto et al., 2005). The studies applied different sensory approaches and beer matrices to assess the sensory properties of hop extracts.

For the current study, Pearson correlation coefficients revealed no significant relationships between the “peppery tingling” sensation and specific volatiles. It has to be noted that the sub-fractions contained other compounds at flavour-active concentrations (geraniol) and unknowns at trace levels. Further fractionation or purification should be conducted to obtain a better separation between sesquiterpene alcohols, monoterpene alcohols and compounds of other chemical classes. The concentrations of humulene epoxides were estimated to range between ~2 µg/L and ~697 µg/L, respectively, partly exceeding aroma threshold levels without affecting the “peppery tingling” sensation due to the aforementioned reasons. The same applied for the astringency, which positively correlated with *α*-humulene (**[C28]**; *r*=0.630) and humulene epoxide I (**[C40]**; *r*=0.758). Since a significant sample

effect could not be reported, further research is required to investigate this potential cause-effect relationship.

Role of linalool in relation to aroma and flavor characteristics. Linalool **[C7]** as such, hardly modified the beer's aroma profile and only slightly increased the "rose water" flavour (*r*=0.451). Other research groups previously suggested linalool as a key contributor to floral (rose, lavender) and several citrus characters which acts synergistically with other monoterpene alcohols to increase fruity and floral aroma and flavour intensities (Takoi, Itoga, et al., 2010; Takoi, Koie, et al., 2010). The concentration of linalool was significantly higher in the monoterpene alcohol than in the terpene alcohol fractions (~276 μg/L vs ~55 μg/L), while the opposite was the case for geraniol (~84 μg/L vs ~149 μg/L) and thus could have caused this effect on the citrusy/"rose water" aromas/flavours in the terpene alcohol fraction-flavoured beer. Linalool **[C7]** also significantly correlated with the "grapefruit" flavour (*r*=0.605), which adds to the hypothesis that it may act synergistically in a mixture with other hop volatiles.

3.3.3 Prediction of sensory scores from GC-MS data

PLS regression analyses were conducted to explore the correlation between the 49 volatile hop compounds (Table 3.5.) and the 12 sensory attributes found to be significant, plus the one approaching significance ("pine wood"; Table 3.3.). PLS1 model performances resulted in relatively good fits of the data (Table 3.6.), whilst PLS2 algorithm results were not satisfactory (data not included). The results are generally in agreement with the PCA's outcome, and the compound-attribute

relationships seemed coherent with previous results (Dietz, Cook, Wilson, et al., 2020b). It was difficult to identify clear causal relationships between hop compounds and one sensory sensation and vice versa. Most models could explain a moderate to high percentage of the original variance. However, the models also required between 10 and 25 variables, with the model for "earthy" being the most complex. This indicates the complexity of the sensory profiles of hop extracts and the difficulty in understanding their molecular basis. Positive and negative correlations were broadly balanced, suggesting compounds positively or negatively affect the perception of sensory characteristics.

Table 3.6. Sensory scores mean ranges and PLS regression model performances (PLS1) for prediction of the sensory attributes (significant in the sensory study) among hop extracts based on their volatile compositions (Table 3.5).

	Sensory scores				**PLS model performance[a]**		
Attribute	**Min**	**Max**	**Mean**	**SD**	**R^2**	**RMSE**	**n *X***
Lemon	0.68	3.81	1.66	1.04	0.661	0.583	17
Pine wood[b]	1.00	4.47	1.66	1.04	0.537	0.452	10
Crushed grass, sap	1.00	4.42	2.34	0.92	0.873	0.289	19
Resinous	1.15	3.57	2.06	0.65	0.791	0.264	21
Earthy	0.63	2.54	1.41	0.55	0.933	0.142	25
Musty	0.81	3.10	1.84	0.62	0.908	0.188	22
Soapy	1.31	3.26	2.27	0.75	0.682	0.381	15
Rose water	0.40	6.89	2.71	1.99	0.668	0.157	20
Orange fruit	1.25	4.36	2.43	1.07	0.661	0.579	15
Grapefruit	1.57	4.60	2.62	1.06	0.787	0.462	16
Sweet	1.84	3.99	2.76	0.62	0.635	0.370	11
Smooth bitterness	1.57	4.32	2.72	0.76	0.637	0.455	13
Harsh bitterness	1.89	4.11	3.06	0.67	0.805	0.296	16

[a] PLS1 algorithm for univariate sensory attributes applied with logarithmic transformed GC-MS data
[b] Pine wood was included because it an approached significant effect in the sensory study.
RMSE, Root mean square error; R^2; R-squared, goodness-of-fit; n *X*, number of *X* variables integrated in the model

The strongest models were built for "earthy", "musty", "crushed grass, sap", "resinous", "grapefruit", and "harsh bitterness". Moderate models were built for "lemon", "soapy", "orange fruit", "rose water", "sweet", and "smooth bitterness",

and unsatisfactory model performance was found for “pine wood”. Compounds with high regression coefficients (>0.05) and variable importance in projection (VIP) criteria (>1.00) were considered as impactful compounds. Several compounds correlated with the sweetness and smooth bitterness in the flavoured beers. Figure 3.7. shows the standardised regression coefficients map with compounds found to be important for each corresponding sensory attribute. Compounds with standardised coefficients lower than 0.05 are not included.

In line with previous findings, geraniol appeared to be the most important compound for “sweet” and “smooth bitterness” while *α*-humulene, *β*-caryophyllene, *δ*-cadinene and caryophyllene oxide had the largest negative coefficients and negatively correlate with these taste characteristics. Interestingly, geranyl isobutyrate and octyl isobutyrate and some other esters also negatively correlated with these attributes, but this might be because these compounds were mainly present in the total oil and the sesquiterpene fraction. The model structures for “sweet” and “smooth bitterness” were distinct from the model for “harsh bitterness”, the latter featuring important contributions from *α*-humulene, *δ*-cadinene, *β*-caryophyllene, *β*-myrcene, and caryophyllene oxide. The humulene epoxides (I-III) seemed not to play a significant role for the model of “harsh bitterness” indicating that a combination of sesquiterpenes were mainly driving this bitterness sensation.

The terpene alcohols terpinen-4-ol, myrtenol, perillol, and *endo*-borneol all negatively correlated with “crushed grass, sap”, “resinous”, “earthy”, and “musty”, which is surprising because they were expected to positively contribute to one or more of these sensations. However, negative correlations can also occur if strong

aroma compounds overpower weaker ones or if compound concentrations are significantly lower than those of other compounds contributing to the same sensation. The same reasons were considered for the standardised coefficients recorded for linalool oxide and methyl octanoate, which were either absent in the extracts or present at relatively low concentrations.

Linalool played an important role in the models for “lemon”, “grapefruit” and “rose water” and negatively correlated with “musty”, once again indicating its importance as a synergist and an antagonist in the perception of the aromas and flavours. This was one of the main differences between the outcomes of the PCA and PLS studies. PCA is focused on demonstrating causality between compounds and attributes in a multisensory space, just by virtue of the compounds being present. Conversely, PLS aims to detect correlative connections between compounds and individual attributes, including mixture-dependent perceptual effects. In turn, correlation does not necessarily imply causation. Results should be seen as tentative and need to be validated, for instance, by performing recombination studies. PLS models can only display sensory interaction effects to a certain extent. Consequently, including threshold concentrations (aroma/flavour/taste/mouthfeel) and further sensory and analytical inputs (temporal sensory data, odour activity (OAV), Charm values, physico-chemical, physiological) into Multi-Block PLS regressions would likely improve the model performance and simplify the selection of components for supervised developments of algorithms.

It should be noted that, due to the limits of detection with the analytical approach used, compounds at very low concentrations or trace levels (sulphur compounds),

were not incorporated, but could still have contributed to the sensory profiles of the flavoured beers. It should also be taken into account that the hop oil extracts were solely tested in a lager type beer. The fractions and compounds could potentially be perceived in a slightly different way if applied in a different beer style due to matrix-dependent effects. Moreover, threshold concentrations were only retrieved from previous publications but not measured in the current study. Measuring these and considering further parameters such as OAV (ratio of a compounds' concentration to odour threshold concentration in the same matrix) assessed using aroma extract dilution analyses (AEDA) in combination with GC-Olfactometry (GC-O) and GC-MS (Dresel, Dunkel, & Hofmann, 2015), will provide further insights to understand the contribution of the applied volatile hop compound and compound combinations to the aroma perceived in beer.

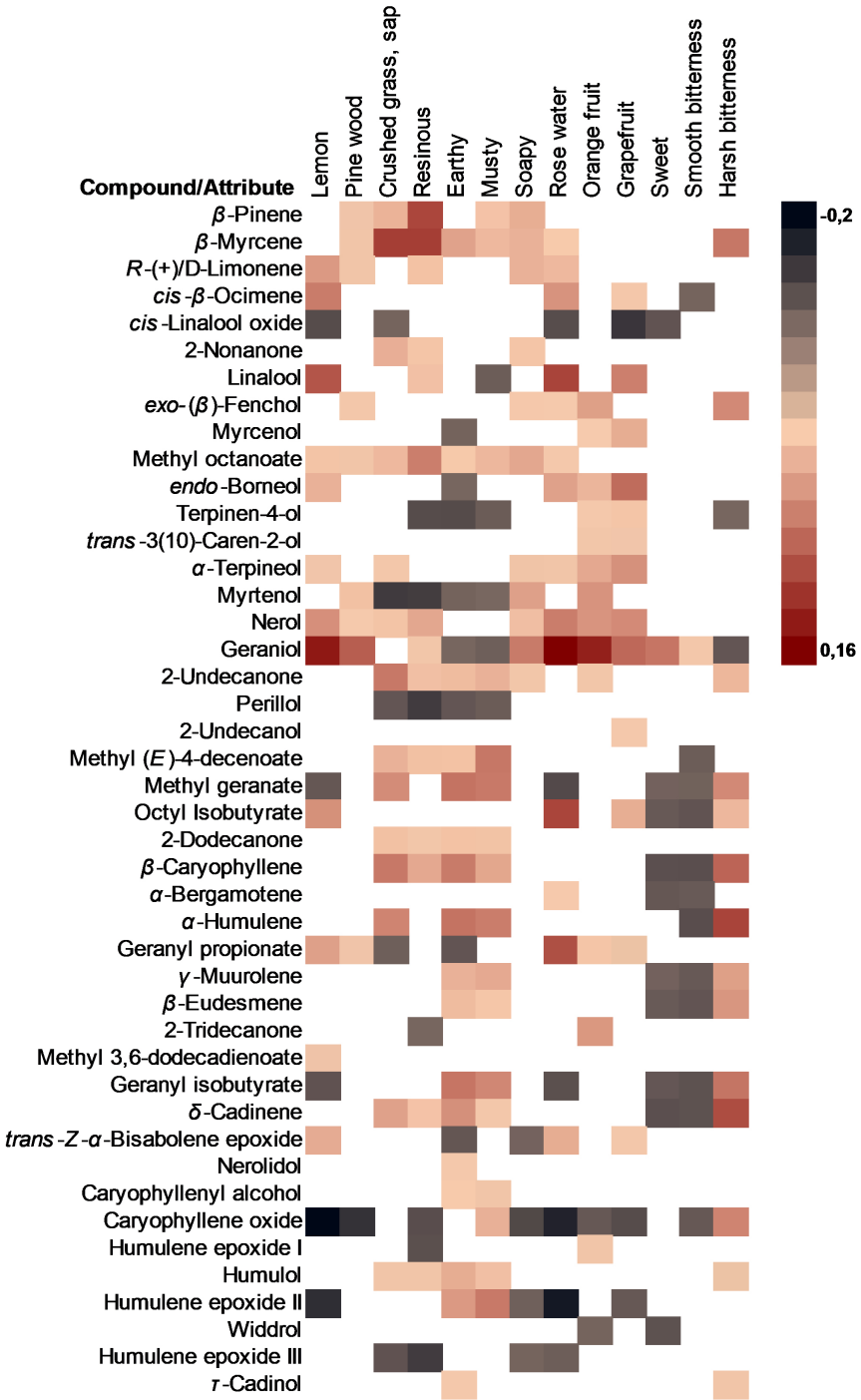

Figure 3.7. Standardised regression coefficient map with the *X*-variables (volatile compounds) included in the models explaining the main weight into the *Y*-variables (sensory attributes). Only coefficients larger than 0.05 are shown.

3.4 Conclusions

The approach to break hop oil fractions into its constituents and study the sensory profiles of individual compound and compound groups revealed important insights into the sensory differences between the hop extracts and several compounds involved in sensory interactions and thereby modifying beer flavour and taste. Nevertheless, a certain chemical complexity seems to be required to trigger sensory interactions and induce multisensory effects. Understanding these mechanisms presents challenges but will help to characterise the diverse sensory properties in hop oil fractions and guide further investigations into potential commercial versions thereof. These flavouring preparations are developed to be added post-fermentation to increase the transfer of volatile compounds into beer, reduce the volume of hops required to achieve desired sensory characteristics and decrease the environmental impact of hops in the brewing process. Moreover, hop harvests and supply to the brewing industry are subjected to crop seasonality and different conversion of oil and aroma active functionals on a year to year basis. Since the industry aims to maintain beer brand identities, this research may also provide the basis for further standardisation of sustainable hop materials.

Chapter 4

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

This chapter is based on:

Dietz, C., Cook, D., Yang, Q., Wilson, C., & Ford, R. (2022). A TCATA by modality approach to study the multisensory temporal profile of hop bitter and flavour products applied in lager. *Food Quality and Preference*, *97*, 104470.
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A TCATA by modality approach to study the multisensory temporal profile of hop bitter and flavour products applied in lager

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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.

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.

4.1 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, 2020a).

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, 2021a). In turn, bitter substances can also modify sensory characteristics associated with hop flavour (Dietz, Cook, Huismann, et al., 2020a).

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., 2020b; Dietz, Cook, Wilson, et al., 2021a). 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., 2021a), 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.

4.2 Materials and methods

4.2.1 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 4.1. 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., 2020b; Dietz, Cook, Wilson, et al., 2021a). The extracts were stored at 4°C.

Table 4.1. 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

4.2.2 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.

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., 2020b) and commercial lager (Dietz, Cook, Wilson, et al., 2021a). 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.

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 Table 8.2. (Appendix 2).

The beer bottles (NISO, HULU) were opened in a cold store (4°C), immediately flavoured with hop flavour products, recapped, inverted three times to ensure adequate mixing, and kept at 4°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°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.Figure 4.1. depicts the set of 13 samples presented to the panel.

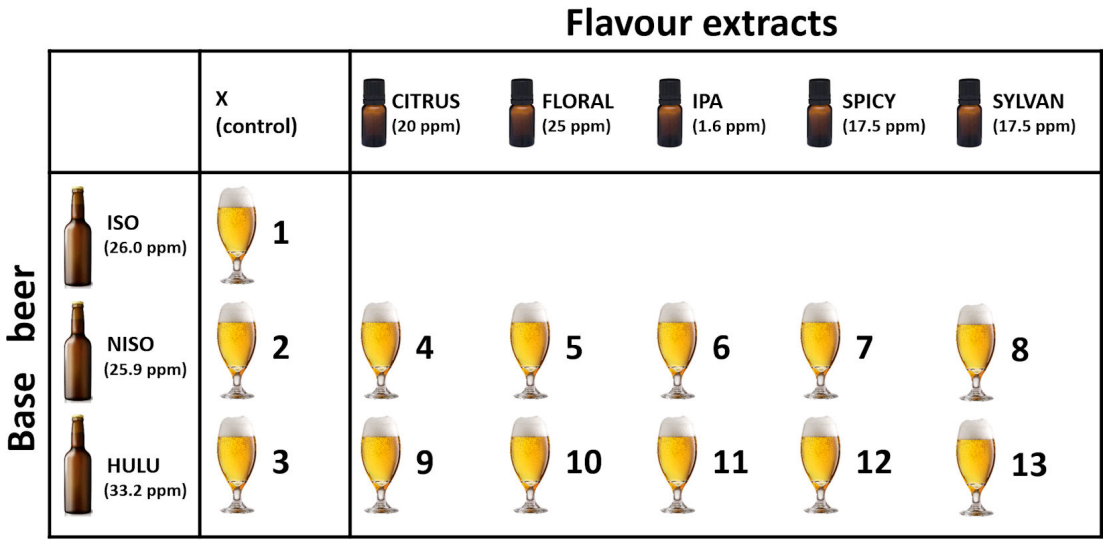


Figure 4.1. Sample set comprising of three non-flavoured control beers and 10 flavoured beers evaluated in the TCATA study in triplicate.

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 4.2. provides the final attribute list including 12 flavour, five taste, and four mouthfeel/trigeminal attributes (reference materials are listed in Table 8.3.., Appendix 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.

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 Figure 4.1. and two experimental replicates (NISO+IPA, HULU+SPICY)).

Table 4.2. 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

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.

Figure 4.2. 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 prevented by scheduling 3 min breaks after each sample evaluation and a 10 min comfort break after the third sample (of five).

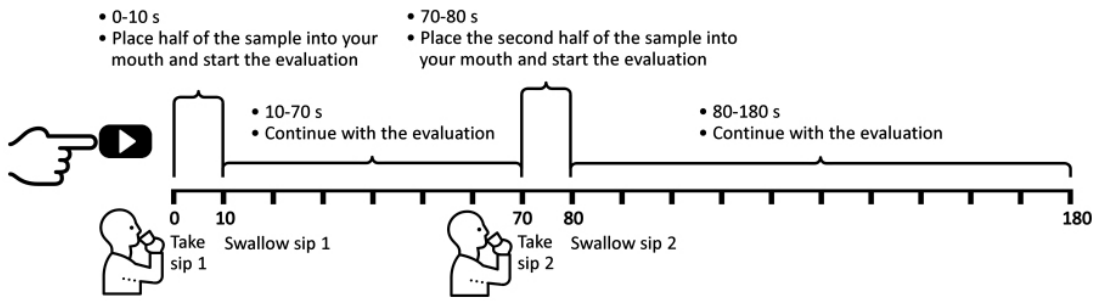


Figure 4.2. 2-sip protocol used in the TCATA study.

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).

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).

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 reponse 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).

4.3 Results

4.3.1 Panel performance during the evaluation sessions

Agreement and repeatability indices ranged between 0.611-0.855 and 0.728-0.931 (Table 8.4., Appendix 2) 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 (Figure 8.1, Appendix 2). Removal of panellists' data was not conducted since the panellists showed acceptable performance for the majority of data and satisfactory discrimination between the samples.

4.3.2 Analysis of the sensory temporal profiles

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. Figure 4.3. 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 4.3.) and sip segments (Table 8.5., Appendix 2) 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 8.6., Appendix 2). 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.

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".

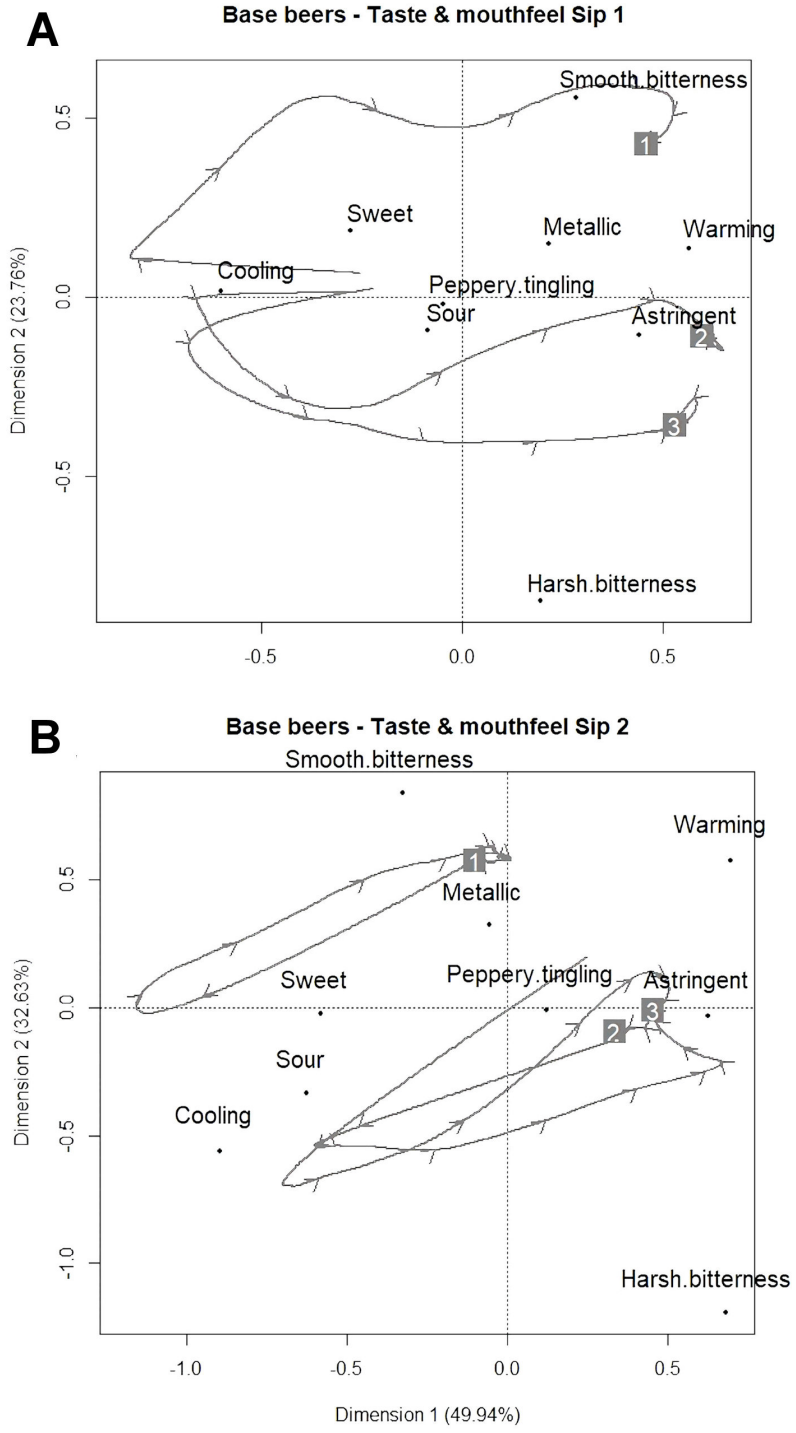


Figure 4.3. Smoothed trajectories for Sip1 and Sip2 resulting from Correspondence Analysis (CA) on dimensions 1 and 2 of the control beers HULU (**1**), ISO (**2**) AND NISO (**3**), in the taste and mouthfeel space. The grey arrows indicate the direction of the profile's evolution in 10 s time intervals.

Table 4.3. 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

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 4.4.). 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 8.5., Appendix 2). 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 4.5.).

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 4.4., Table 8.5., Appendix 2) 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 was mainly characterised by a “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 4.3., Table 8.5., Appendix 2).

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.

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 4.5.). RM-ANOVA by sip segment revealed that this effect started after swallowing Sip1 (Table A.6, Appendix 2). 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 (Figure 4.4.).

Table 4.4. 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 4.5. 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

"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.

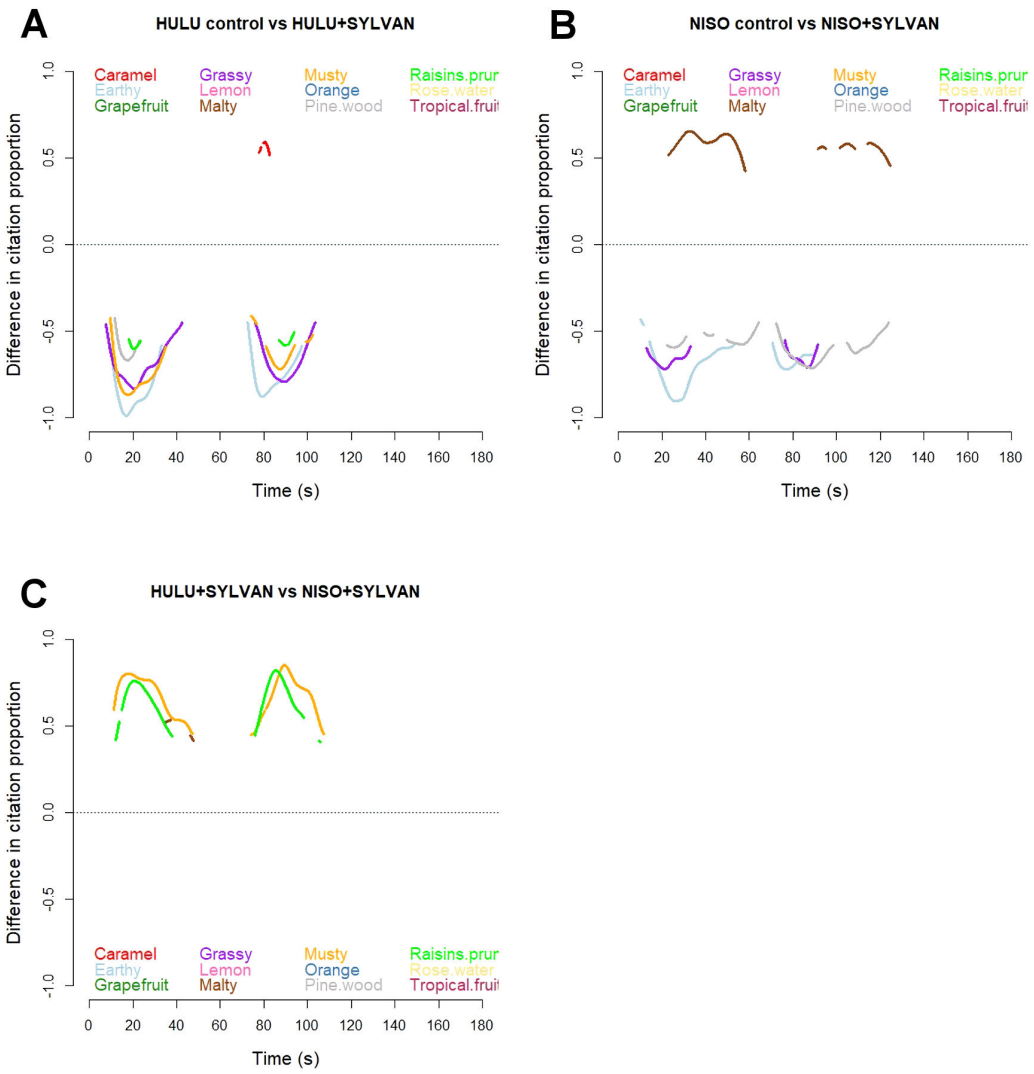


Figure 4.4. Smoothed TCATA flavour difference curves showing citation proportions plotted against the evaluation time (s) showing the effect of the SYLVAN hop product, with (**A**) NISO control beer vs NISO+SYLVAN, (**B**) HULU control beer vs HULU+SYLVAN, and (**C**) HULU+SYLVAN vs NISO+SYLVAN.

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 (Figure 4.5.). The HULU+SPICY beers even obtained significantly decreased "smooth bitterness" citation frequencies compared to the HULU beer.

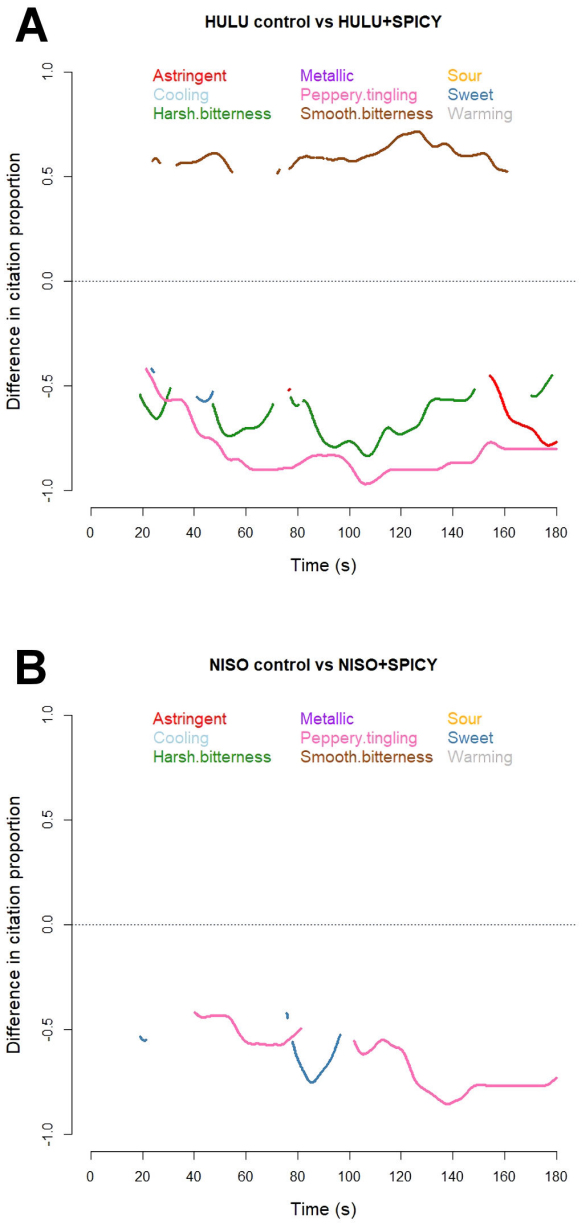


Figure 4.5. Smoothed TCATA flavour difference curves showing citation proportions plotted against the evaluation time (s) for the HULU control beer vs HULU+SPICY (**A**) and the NISO control beer vs NISO+SPICY (**B**).

Correlation between flavour, taste and mouthfeel attributes.

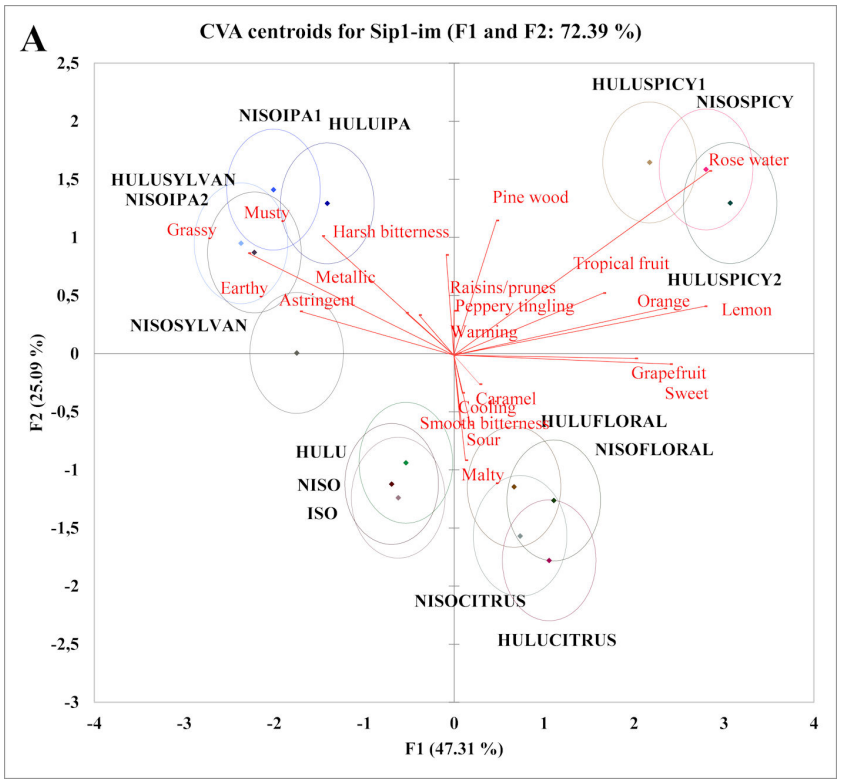
Pearson's correlation coefficients computed from the average proportions of citations revealed significant but mostly weak (r<0.6) correlation effects between attributes across modalities (data not shown). The relationship is visually illustrated

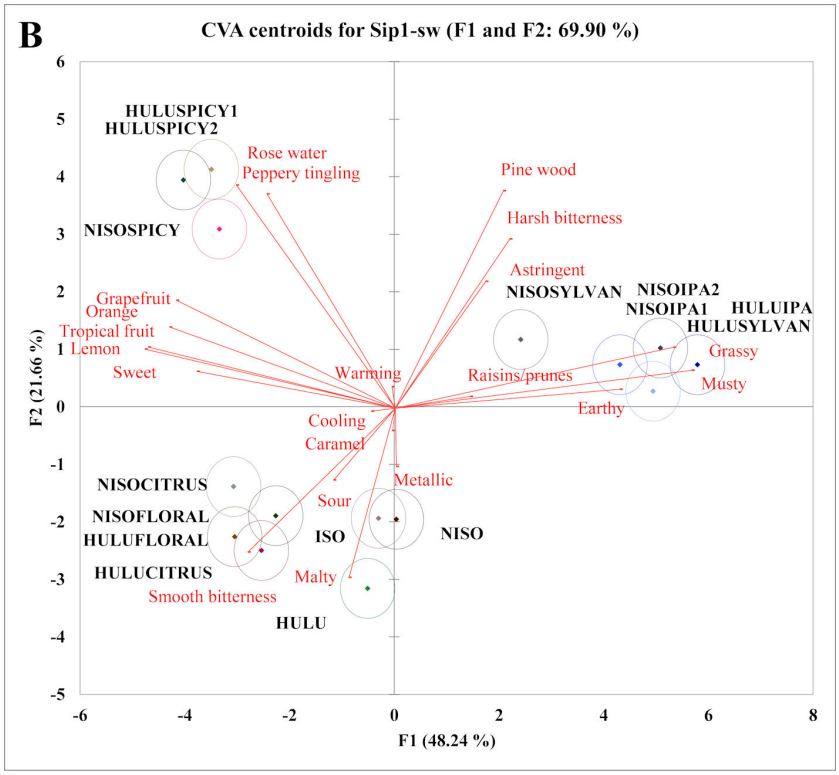
in the CVA maps (Figure 4.6.) showing the samples' position in the multi-modal space for each sip segment. In each of the evaluation stages, the beers were divided into three groups as described above. The IPA and SYLVAN beers characterised by 'green' flavours and "harsh bitterness" were additionally 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).

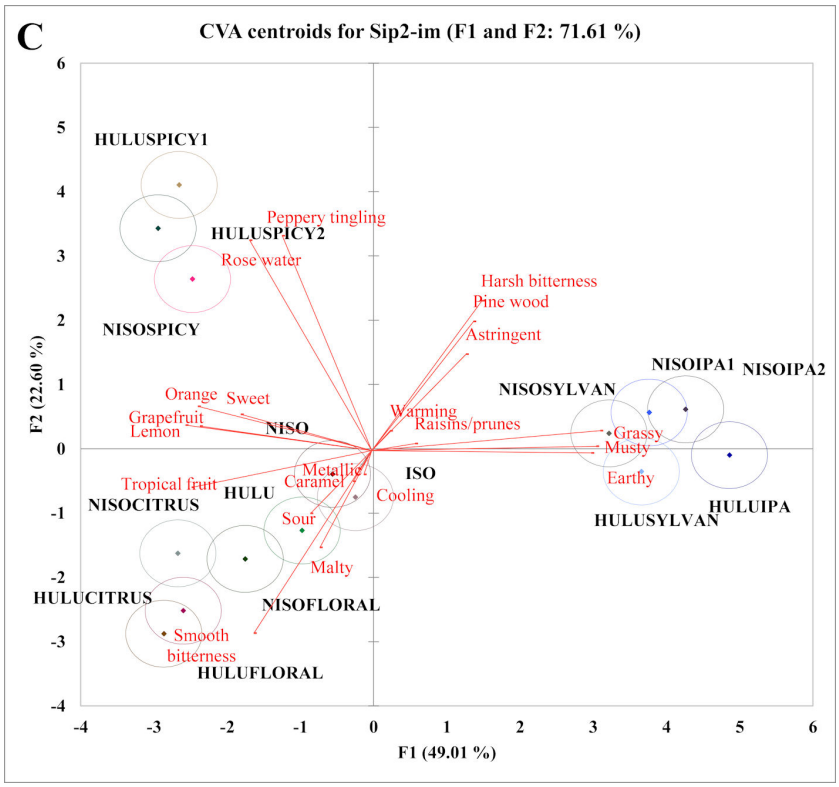
The CITRUS and FLORAL beers were, similarly to the HULU control beer, described by "malty", "smooth bitterness", "sweet", "sour" and 'fruity' flavour attributes. 'Fruity' flavours significantly positively correlated with these taste sensations with the strongest correlations detected between "sweet " and "lemon", "orange" and "tropical fruit" after swallowing Sip 1 (r=402-485). "Sweet" also weakly positively correlated with "caramel" flavour (r=0.307).

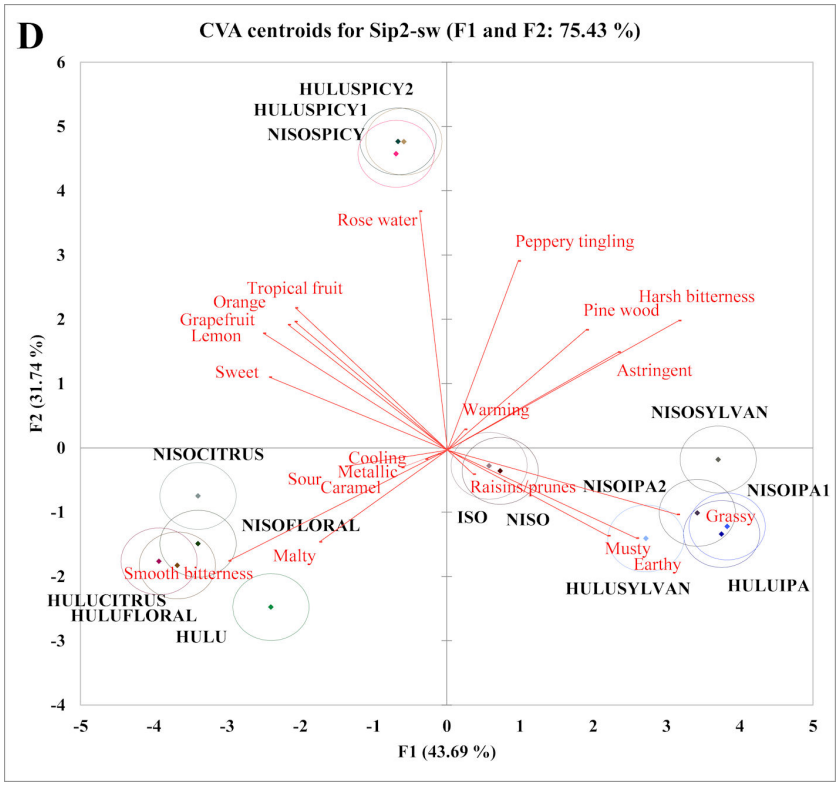
The third group comprised the SPICY beers plotted close to 'fruity' and "rose water" flavours and moved closer to "peppery tingling" after swallowing Sip1, thereby separating from the other samples. "Peppery tingling" significantly positively correlated with "pine wood" (r=0.361), "rose water" (r=0.555), 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

(Figure 4.6.) when the samples' profiles separated from each other due to diminishing or unchecking of several attributes.






C
CVA centroids for Sip2-im (F1 and F2: 71.61 %)
HULUSPICY1
HULUSPICY2
NISOSPICY
Peppery tingling
Rose water
Harsh bitterness
Pine wood
Astringent
NISOIPA1
NISOIPA2
NISOSYLVAN
Orange
Sweet
Grapefruit
Lemon
NISO
Warming
Raisins/prunes
Grassy
Musty
Earthy
Tropical fruit
HULU
Caramel
Metallic
ISO
Cooling
NISOCITRUS
Sour
HULUIPA
HULUSYLVAN
Malty
HULUCITRUS
NISOFLORAL
Smooth bitterness
HULUFLORAL
F2 (22.60 %)
F1 (49.01 %)


D
CVA centroids for Sip2-sw (F1 and F2: 75.43 %)
HULUSPICY2
HULUSPICY1
NISOSPICY
Rose water
Peppery tingling
Tropical fruit
Orange
Grapefruit
Lemon
Harsh bitterness
Pine wood
Sweet
Astringent
Warming
NISOSYLVAN
NISOCITRUS
Cooling
Metallic
Sour
Caramel
Raisins/prunes
NISOIPA2
NISOIPA1
Grassy
ISO
NISO
NISOFLORAL
Musty
Malty
Earthy
Smooth bitterness
HULUSYLVAN
HULUIPA
HULUCITRUS
HULUFLORAL
HULU
F2 (31.74 %)
F1 (43.69 %)

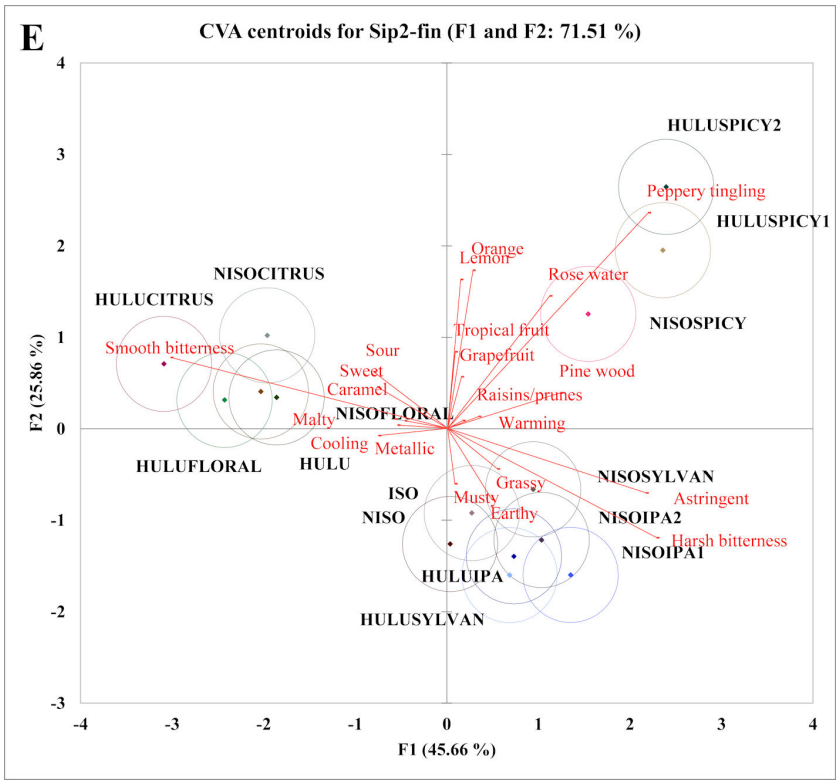


Figure 4.6. Canonical Variate Analysis (CVA) maps of the flavour, taste and mouthfeel attributes of the 15 beer samples as evaluated by the TCATA trained panel. The plots A-E depict the multi-sensory profiles perceived in the individual sip segments: Sip1-im (A), Sip1-sw (B), Sip2-im (C), Sip2-sw (D), 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).

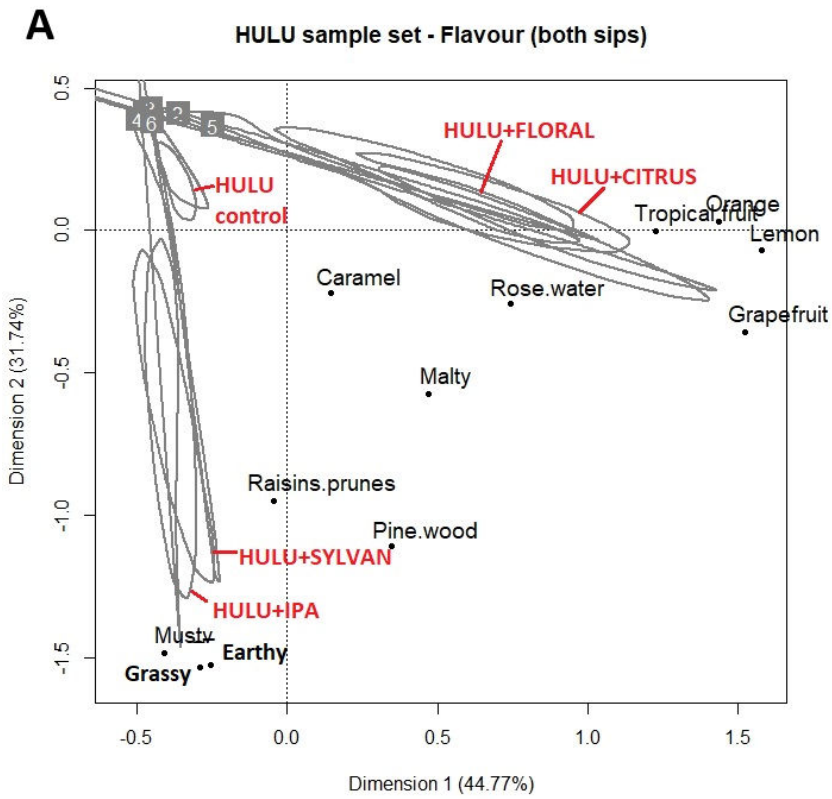
Multivariate analysis of the beer characteristics

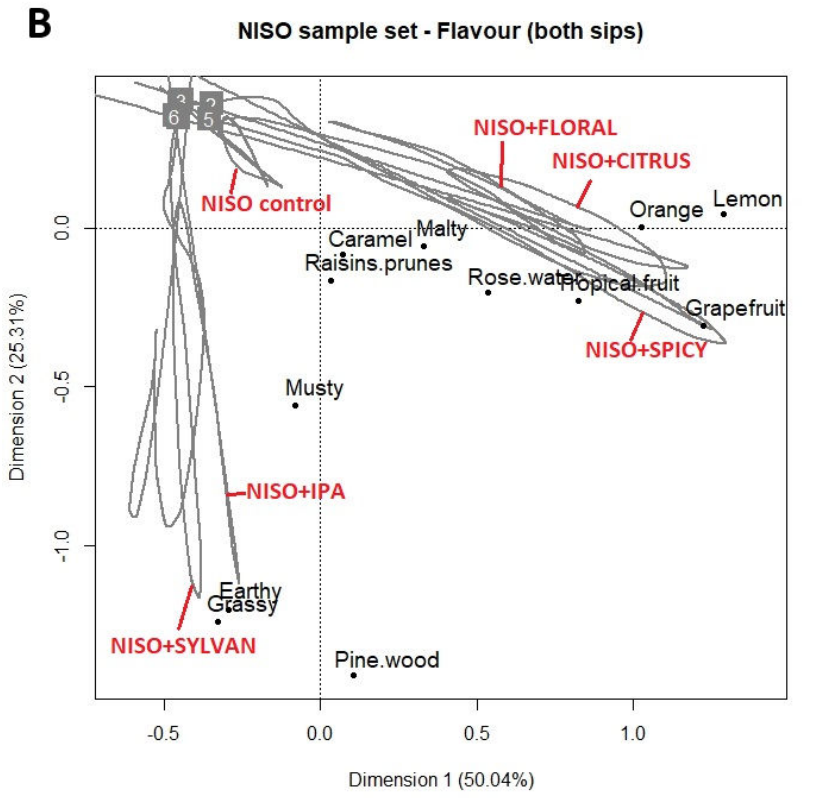
Figure 4.7. shows the smoothed trajectories of the HULU and NISO sample sets following two loops representing the two sips, bending twice with fading flavour profiles in the Sip1-sw and Sip2-fin segments and then returning to their starting point (t=0) at the far left. Dimension 1 and 2 accounted for 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

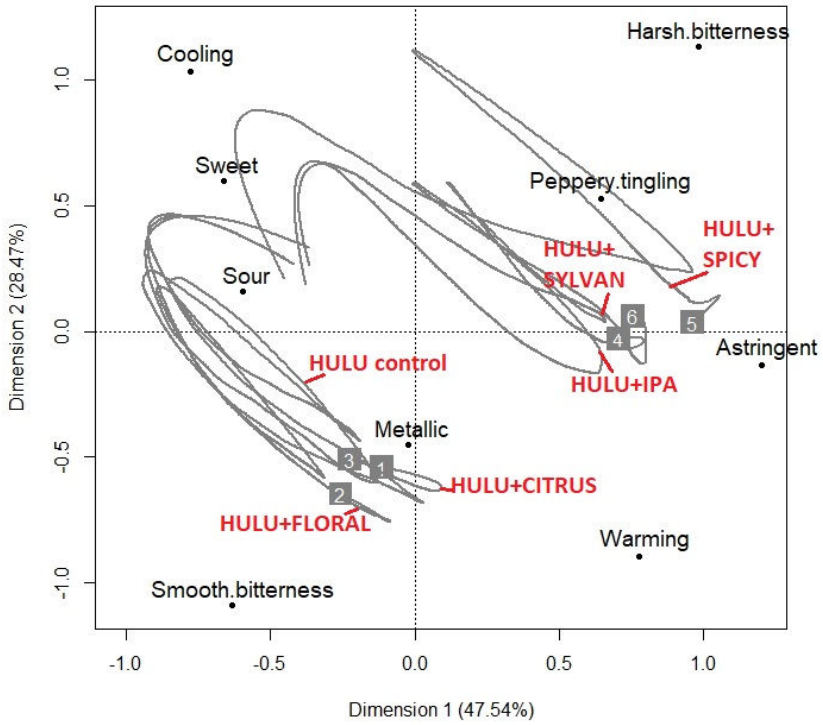
characteristics and evolution of profiles along sip segments. The majority of attributes are located on the opposite side indicating delayed onsets (perception after swallowing) for all attributes, except for “caramel”, “malty”, and “raisins/prunes”.

The taste and mouthfeel trajectories of the NISO and HULU sample sets are plotted in Figure 4.7. 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.


A
HULU sample set - Flavour (both sips)
HULU+FLORAL
HULU+CITRUS
HULU control
Tropical.fruit
Orange
Lemon
Caramel
Rose.water
Grapefruit
Malty
Raisins.prunes
Pine.wood
HULU+SYLVAN
HULU+IPA
Musty
Earthy
Grassy
Dimension 2 (31.74%)
Dimension 1 (44.77%)


B
NISO sample set - Flavour (both sips)
NISO+FLORAL
NISO+CITRUS
NISO control
Orange
Lemon
Malty
Caramel
Raisins.prunes
Rose.water
Tropical.fruit
Grapefruit
NISO+SPICY
Musty
NISO+IPA
Earthy
Grassy
NISO+SYLVAN
Pine.wood
Dimension 2 (25.31%)
Dimension 1 (50.04%)

C


HULU sample set - Taste & mouthfeel (both sips)
Harsh.bitterness
Cooling
Sweet
Peppery.tingling
HULU+ SPICY
HULU+ SYLVAN
Sour
Astringent
HULU control
HULU+IPA
Metallic
HULU+CITRUS
HULU+FLORAL
Warming
Smooth.bitterness
Dimension 2 (28.47%)
Dimension 1 (47.54%)
-1.0
-0.5
0.0
0.5
1.0

D

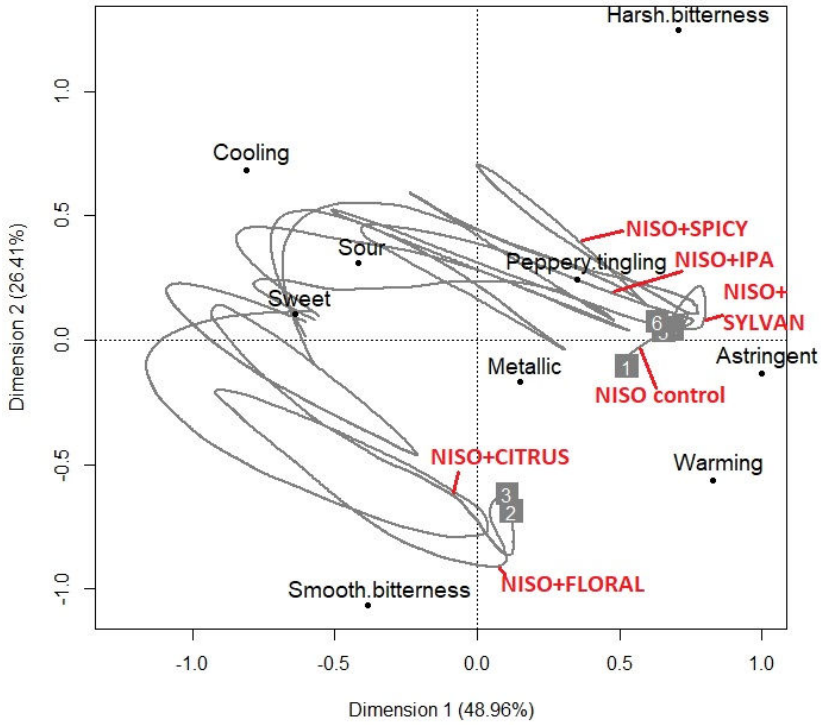

NISO sample set - Taste & mouthfeel (both sips)
Harsh.bitterness
Cooling
NISO+SPICY
Sour
Peppery.tingling
NISO+IPA
NISO+ SYLVAN
Sweet
Astringent
Metallic
NISO control
NISO+CITRUS
Warming
NISO+FLORAL
Smooth.bitterness
Dimension 2 (26.41%)
Dimension 1 (48.96%)
-1.0
-0.5
0.0
0.5
1.0

Figure 4.7. 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 of the publication (https://doi.org/10.1016/j.foodqual.2021.104470).

Analysis of concurrent selection and changes in selection of attributes

Table 4.6. 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.

Table 4.6. 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

4.4 Discussion

4.4.1 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 the panel and panel leader decided on 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, which was monitored with regard to consistency of responses, position effects, and patterns of decreasing attribute selection frequencies in the second compared to the first sip (Cosson et al., 2020). Adaption causing a decrease of sensitivity (Hort, Kemp, & Hollowood, 2017) would have potentially resulted in decreasing selection frequencies, which was not observed in the evaluation data. Since the intensity of sensations was not quantified in the study, it would be interesting to confirm the absence of fatigue and adaption effects based on quantitative temporal data as collected with the Time Intensity method.

4.4.2 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.

4.4.3 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., 2021a; 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., 2020b; Dietz, Cook, Wilson, et al., 2021a). 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., 2021a; 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.

4.4.4 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.

4.4.5 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. However, future research should consider the extension of the beer finish segment or the total evaluation period in order to enable the investigation of the decay of the beer astringency perception.

4.4.6 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., 2020b; 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.

4.4.7 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., 2021a). 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.

4.5 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.

Chapter 5

5 The impact of time standardising TCATA by modality data on the multisensory profile of beer

This chapter is based on:

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The impact of time standardising TCATA by modality data on the multisensory profile of beer

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Highlights

- Pre-processing techniques to standardise TCATA by modality data were compared.
- Time standardisation was unable to significantly reduce panellist noise.
- Time standardisation by modality caused a decrease in repeatability parameters.
- Standardising data with merged modalities largely maintained patters in the data.

Abstract

Temporal sensory profiles are increasingly assessed 'by modality' to investigate complex profiles and multisensory properties of foods and beverages. Panellists' noise in temporal data caused by differences in oral and cognitive processing cannot entirely be removed by training or strict experimental setups. Therefore, time standardisation can be applied to align onsets of sensations and standardise temporal data. This paper compared raw temporal data collected in a preceding study performed by a trained expert panel (n=10) using a TCATA by modality approach with the same data time standardised either by modality or with merged modalities. Binary data, durations and citation proportions were evaluated and subjected to Repeated Measures (RM-) Analysis of Variance (ANOVA), Canonical Variate Analysis (CVA), and Multiple Factor Analysis (MFA) to investigate the differences between sensory properties and dynamic profiles. Time standardisation with merged modalities was able to reduce some noise related to panel repeatability from the raw data while also improving panel agreement indices in the taste and mouthfeel data. Time standardisation by modality reduced some of the panel heterogeneity, but distorted patterns in the flavour data. The main reason for distorted patterns in single sample data and resulting sample discrimination was the different impact of time standardisation on samples described by quickly fading versus long lasting sensations. No substantial effects were observed on the samples' overall profiles in their sensory space. Time standardisation by modality could not reduce panellists' noise in the data. Only a slight noise reduction was achieved in the time standardised data with merged modalities supporting the use of the raw data for further analyses.

The findings indicated several differences between raw and time standardised data and highlighted advantages and disadvantages of pre-processing TCATA by modality data obtained to describe samples inducing complex, multisensory sensations.

5.1 Introduction

The development of temporal methods allows the sensory and consumer scientist to capture how sensory characteristics change over time. The most popular used temporal methods include Temporal Dominance of Sensations (TDS) and Temporal-Check-All-That-Apply (TCATA). Theoretically, 10 to 15 attributes can be assessed simultaneously in either TDS or TCATA whilst ensuring adequate discrimination between samples (Jaeger et al., 2018; Meyners, 2020). Having a limited number of attributes in temporal methods when evaluating complex samples could cause attribute dumping and is therefore not advised for complex samples. However, the simultaneous assessment of too many attributes (on one screen) might in turn be overwhelming for the assessors causing hesitation (Varela et al., 2018). To overcome this issue, TDS by modality was explored, for which assessors are first asked to evaluate a sample regarding one modality and in a second run regarding another modality (Agudelo, Varela, & Fiszman, 2015; Nguyen, Næs, & Varela, 2018). Thus, modalities are not competing, reducing hesitation and the cognitive effort required is significantly decreased. However, it is important that assessors are trained using this approach and the attributes included within each modality to avoid any dumping effects by reducing the attribute number for each TDS session. In a preceding study, authors explored the potential of adopting a 'by modality' approach to the TCATA

method in order to increase the number of attributes that could be assessed whilst also allowing concurrent and consecutive selection of attributes within a modality (Dietz, Cook, Yang, Wilson, & Ford, 2022).

Different pre-processing techniques can be used to reduce or remove assessor's noise from temporal data. Noisy datasets are a result of differences in oral processing and processing of information or cognitive performance, which determine the time point of the attributes checked and unchecked in the course of a TCATA evaluation. Time standardisation or time normalisation originally introduced by Lenfant, Loret, Pineau, Hartmann, and Martin (2009) is often applied to compensate for the different mastication times until first swallowing and to align the onsets and offsets of temporal profiles. Non-response data points ('0') are trimmed before and after the last attribute is checked and the remaining data slices are standardised using non-parametric standardisation, resulting in the loss of their temporal dimension and are therefore expressed as percentiles [0, 100] (Castura, 2017; Castura, 2020; Lenfant et al., 2009).

As for every data processing approach, the decision for or against time standardisation depends on the nature of samples, study objective, and tasting protocol. Generally, time standardisation is necessary where the tasting protocol does not naturally standardise the data and individual evaluation durations need to be aligned across assessors (Lenfant et al., 2009). This can be the case when using naive assessors as their data is naturally noisier compared to data obtained from trained assessors, or when tasting protocols are difficult to control across assessors. However, if the tasting protocol contains time points for taking a sip and swallowing,

fixed start and end points and specific assessment instructions (e. g. tongue movement, gurgling, etc.), time standardisation may not be required (Schlich & Pineau, 2017).

However, researchers have also considered time standardising data from trained panel data with the aim of removing noise or aligning onsets and extinction endpoints, and to subsequently increase sample discrimination (Castura & Li, 2016; McMahon, Culver, Castura, & Ross, 2017). It was found that time standardisation removed systematic relationships between physico-chemical and sensory data (cf. McMahon et al. 2017) or introduced systematic bias in the dataset, with the largest implications found for sensory characteristics perceived for a relatively short duration, which might however still be of interest if investigating complex samples distinguished by subtle differences (Castura, Baker, & Ross, 2016; Galmarini, Visalli, & Schlich, 2017; Lenfant et al., 2009). For instance, Lenfant et al. (2009) found the duration of the brittleness of wheat flakes to change and McMahon et al. (2017) observed the relationship between increased carbonation levels and increased duration of perception to be lost when time standardising their data.

Furthermore, researchers have stated that whilst time standardisation has been considered for pre-processing temporal data, it was not further implemented due to distortion of patterns, without specifying the detail of the impact. It is also unclear what the specific positive impact of data pre-processing had in the studies that reported this approach, presumably for the sake of brevity. Moreover, there are limited insights regarding the effects of time standardising on multiple intake data (sips, bites). Multiple intake data can be time standardised if the time points for each

intake are pre-defined by the experimenter (e.g. Galmarini et al., 2017). Therefore, further research is required in order to understand the impact of time standardisation on complex temporal data with multiple sips and using a 'by modality approach' for future studies.

The preceding study was the first to publish a TCATA by modality approach (Dietz et al., 2022), and so it remains to be investigated which pre-processing approach would be most suitable to align onsets and offsets of attributes that are belonging to two or more modalities and to significantly reduce panellists' noise in TCATA data. Different strategies may be used to evaluate the effectiveness of data pre-processing or time standardisation.

One of them could be to explore panel performance parameters computed based on the raw and the time standardised data to understand discrimination ability, panel agreement and repeatability or the effectiveness in removing panellists' noise. Castura, Antúnez, Giménez, & Ares (2016) suggested the evaluation of panellist agreement (between panellists) and repeatability (over sessions) based on indices derived from average city-block distances (Manhattan distances) examining absolute differences between coordinates of a pair of objects in a grid. This corresponds to the proportion of matches or mismatches in binary response data matrices i.e. checked or unchecked time slices in a TCATA run. A value of 0 indicates no agreement/repeatability, whereas a value of 1 indicates perfect agreement/repeatability (Castura et al., 2016; Hamming, 1950).

In addition, Mixed Models are extensively used to explore panel performance in sensory profiling data by looking at product effect (discrimination ability),

product*panellist interaction (agreement), and replicate effect and product*replicate interactions (repeatability) (Lawless & Heymann, 2010) and may be also used to analyse panellists' performance in temporal sensory trials.

The objective of the present work was to compare the impact of raw, non-processed data with different approaches to time standardise the data on the temporal sensory profiles obtained from a preceding TCATA study. The case study followed a TCATA by modality approach where trained panellists used a controlled pre-defined 2-sip tasting protocol to characterise hop extracts in an unhopped base beer (Dietz et al., 2022). The sensory properties of these hop extracts were well understood due to preliminary temporal sensory trials and preceding descriptive analysis studies performed by a trained external panel to characterise their multimodal profiles (Dietz, Cook, Wilson, Marriott, & Ford, 2020b; Dietz, Cook, Wilson, Oliveira, & Ford, 2021a).

The present study explored the differences between the data based on several panel performance parameters (repeatability, agreement, interaction effects) to investigate the discriminative power of the datasets. Subsequently, discrimination of samples based on individual attributes and modalities was explored within the total evaluation period and sip segments to obtain an understanding of the effect of data pre-processing on the dynamic profiles of the samples and the evolution of sensory characteristics. The outcome of this study will help researchers to decide on the pre-treatment of TCATA by modality data, or comparable sensory temporal data obtained for complex samples with multimodal sensory profiles.

5.2 Materials and methods

5.2.1 Case study

Data were taken from a previous study (Dietz et al., 2022), which explored a TCATA by modality approach to investigate the temporal multisensory profiles of hop extracts derived from a Magnum hop variety in an unhopped lager-type beer. The research 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). Furthermore, sensory characterisation of Magnum hop extracts of similar molecular composition using static Quantitative Descriptive Analysis (QDA) approaches had also been conducted (Dietz et al., 2020b; Dietz et al., 2021a), revealing limitations in single time point evaluations on taste- and mouthfeel modifying and lingering properties. In order to capture both the multimodal complexity of the hop extracts and their temporality without limiting the number of attributes (and thereby risking attribute dumping effects), a TCATA by modality approach was used for the sensory temporal evaluation of the hop-flavoured beer samples.

5.2.2 Samples

Hop extracts were applied in an unhopped lager base beer (4.5% ABV) that was brewed without hops. The ferment was divided into three batches, and then separately bittered with one of three hop bittering extracts 1) hulupones (HULU), 2) synthetically-derived or commercial iso-alpha-acids (ISO), or 3) naturally-derived iso-alpha-acids (NISO). The latter two beer batches were then flavoured with one of five hop flavour products (containing hop oil fractions) referred to as 1) CITRUS, 2)

FLORAL, 3) IPA, 4) SPICY, and 5) SYLVAN. Hop products were added at different concentrations to obtain equi-bitter intensity in the base beer and equi-flavour intensity in the bittered beer. All beer samples (20 mL) were evaluated at approximately 8°C. In total, 13 beer samples and two further replicates (NISO+IPA, HULU+SPICY) were evaluated in triplicate with replicates randomised using Williams Latin Square design. Information about the composition of hop extracts, brewing of the base beer, and base beer analysis is provided in Dietz et al. (2022).

5.2.3 Trained panel

Details of the screening and training of the sensory panel are provided in Dietz et al. (2022) and will be briefly described here. Ten external panellists previously trained on sensory characteristics of beer and hop and on descriptive analysis (Dietz et al., 2020b; Dietz et al., 2021a) were further screened following ISO standard 8586:2012 (ISO, 2012) to validate their suitability for the study and to confirm the absence of anosmia to the main compounds in the hop extracts. Subsequently, the panellists completed 17 training sessions and two mock evaluations (120 min). The training sessions were used to establish an attribute lexicon of flavour, taste, and mouthfeel attributes (Table 5.1.), to familiarise panellists with the TCATA by modality approach, and to define sip volume, sip protocols and palate-cleansing protocols. Sensory analysis took place within sensory facilities equipped with tables for group discussions and individual testing booths (ISO, 2007) for practice sessions, mock evaluations and formal evaluation sessions.

Table 5.1. Overview of sensory attributes and attribute definitions used in the original study described by Dietz et al. (2022).

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

5.2.4 TCATA by modality approach

Attributes were split into two sets with the first set including all flavour attributes ("caramel", "malty", "lemon", "raisins/prunes", "earthy", "grapefruit", "grassy", "tropical fruit", "musty", "orange" "pine wood", and "rose water") and the second

set including all attributes linked to taste, mouthfeel, and trigeminal perception ("sweet", "sour", "metallic", "harsh bitterness", "smooth bitterness", "astringent", "peppery tingling", "warming", and "cooling") shown on consecutive screens. The panel conducted one TCATA run to evaluate all flavour attributes for two sips, was then instructed to stop for a 2 min palate-cleansing break, and subsequently received a fresh sample to assess the second attribute set to assess taste and mouthfeel sensations. In each TCATA run, panellists were asked to check all attributes that were perceived and uncheck them when they were no longer perceivable at each moment of the evaluation. All panellists completed nine evaluation sessions in which they evaluated five samples and a dummy sample (data not recorded) at the beginning of each evaluation session. The evaluations were performed in Compusense® Cloud (Compusense Inc., Guelph, Canada).

5.2.5 Tasting protocol

A 2-sip tasting protocol was developed to assess the temporality of the samples' sensory characteristics throughout repeated ingestions including pre- and post-swallowing perception and beer finish. The panellists were instructed to press the 'Start button', immediately take the first sip (~10 mL) and start to evaluate the flavour sensations. The panellists kept the sample in their mouth for 10 s while slightly moving it, and then swallowed, continuing to evaluate the sample for 60 s until they were instructed to take a second sip (~10 mL poured from the same beer bottle) and follow the same procedure on the same screen, but assess the sample until the end of the evaluation period (at 180 s time point). After the 2 min palate-cleansing break,

a fresh sample was provided to assess the taste and mouthfeel attributes following the same 2-sip protocol on a consecutive screen.

5.2.6 Data processing and analysis

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

Data processing

Besides analysing differences based on the total evaluation period (180 s), the data was divided into sip segments representing different stages in the evaluation period, namely; sip 1 held in the mouth ("Sip1im", 0 s – 10.0 s), sip 1 after swallowing ("Sip1sw, 10.1 s – 70.0 s), sip 2 held in the mouth ("Sip2im", 70.1 s – 80.0 s), sip 2 after swallowing ("Sip2sw", 80.1 s – 140.0 s), and beer finish ("Sip2fin", 140.1 s – 180.0 s). The evaluation period was limited to 180 s to prevent fatigue and because the majority of sensations faded (returned to control beer level) during the beer finish.

Both the total evaluation period and individual sip segments were subjected to time standardisation using the *std.time* function of the R package tempR. Time standardisation was used to obtain 1) data time standardised per modality (i.e. data trimmed and standardised, then merged (adjoined)), and 2) data time standardised with merged modalities (i.e. data merged, then trimmed and standardised), which

were both compared to the raw data. The option to trim each evaluation, then merge modalities and subsequently standardise the data was also considered, but not conducted because merging the datasets at this stage may lead to a significant loss of information and relative relationships within TCATA runs, even if obtained from the same assessor (Castura, 2020).

Data analysis

Two main criteria were selected to compare the two time standardised datasets with the raw dataset: differences between calculated panel performance as an indicator for removal of panellists' noise and differences between sample profiles and temporality of attributes to study removal of significant effects and introduction of new significant effects or artefacts.

Panel performance. Panel performance indices were calculated to evaluate panel agreement and repeatability as described by Castura et al. (2016). Mixed Model Analyses of Variance (ANOVA) with sample, position, replicate and interactions as fixed independent factors and panellists and its interactions with fixed factors as random term were conducted followed by Tukey's Honest Significant Difference (HSD) post-hoc tests to identify significant effects and interactions as a source of variation (Baker, Castura, and Ross, 2016). The factor position was included to validate the randomisation of samples (using Williams Latin Square design for each replicate) after a two-week break between the evaluation sessions.

Significant interaction effects assessed were, sample*panellist effect as a measure of consensus within the panel (i.e. panel agreement) with a significant effect indicating panellists did not evaluate sample differences in the same way, replicate*panellist

effect interpreted as differences in panellists' perceived duration of attributes from one replicate to another (i.e. panel repeatability or lack of panellists' consistency between replicates), replicate*sample effect measuring sample differences across replicates indicating differences in perception of samples between replicates, e.g. due to inconsistencies in sample preparation, natural variation in the samples or carryover effects (Lawless & Heymann, 1998), and position*sample effect exploring the effect of samples' position on sample differences. All interactions effects were further related to the panel agreement and repeatability indices, respectively.

In addition, Canonical Variate Analysis (CVA) was performed on a multivariate analysis of variance (MANOVA) model with sample as fixed and panellist as random effects to contrast between-samples covariance matrix with the interaction covariance matrix (panellist*sample). Confidence ellipses at 90% confidence level were drawn for each sample to visualise panel variability and discrimination ability and to visualise differences between samples, whilst taking into account the panels' heterogeneity. MANOVA *F*-tests obtained from Hotelling-Lawley statistics were used to evaluate the discrimination between samples in the sip segment space generated with the attributes (Nguyen, Næs, & Varela, 2018; Peltier, Visalli, & Schlich, 2015).

Average proportions of citations. Average proportions of citations were computed for each attribute in each evaluation by dividing the number of checked time slices by the total evaluation time (180), or in case of the time standardised data, by the total number of data points (101). The obtained proportions were subjected to Repeated Measures (RM-) ANOVA by sip segment followed by Tukey's HSD as described with sample and replicate treated as fixed factors and panellist as random

factor. Multiple Factor Analysis (MFA) was conducted on the combined data to compare the qualitative descriptive variables obtained from the differently processed TCATA data for selected sip segments and study correlations between the three datasets (cf. McMahon et al., 2017).

TCATA curves. Two types of TCATA curves were inspected, namely TCATA profile curves (or TCATA curves) and TCATA difference curves, both based on citation proportions. TCATA profile curves showed the attribute curves of one sample together with attribute curves or reference lines, which highlight significant periods i.e. periods where the sample significantly differed compared to the other samples with regard to a specific attribute. Proportions of citations were also computed for each individual attribute and attribute citation rates significantly differentiating between sample-pairs were plotted as identified by two-sided Fisher-Irwin tests. Time slices or data points with no curve displayed indicate absence of significant effects (Castura et al., 2016).

Attribute and profile durations. Duration data for individual attribute and flavour and taste and mouthfeel profiles was computed for individual sip segments and for the total duration period. 'Duration' was defined as the time at which an attribute or the first attribute was checked to the time at which the attribute or the last attribute was unchecked. Duration 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 (Baker et al., 2016). CVA was performed to visualise sample discrimination within individual sip segments with distance, proximity or overlapping confidence ellipses indicating non-significant or

significant effects between samples (Galmarini et al., 2017; Nguyen, Næs, & Varela, 2018).

5.3 Results and discussion

5.3.1 Evaluation of the panel performance

Analysis of panel performance indices

Panel performance indices were calculated to obtain an overview of inter- and intra-individual differences between TCATA profiles obtained by the panellists (Castura, 2017). The indices represent city-block distances between the binary data matrices and were calculated for each attribute-panellist combination. Panellists' global flavour and taste and mouthfeel agreement indices ranged between 0.657-0.817 (raw) and 0.650-0.835 (time standardised).

Time standardisation with or without merged modalities did not significantly increase or decrease agreement indices for the flavour attributes indicating that time standardisation did not have an impact on noise across the panellists. Panellists' global flavour repeatability indices retrieved for both time standardised datasets (0.729-0.923) were slightly decreased compared to the raw dataset's indices (0.816-0.911), which may be because considerable time chunks were cut off at the start and end of the evaluation period causing an alignment at the start of the TCATA run. But it should be noted that this also caused an increase in mismatching data points in the mid-stages of the evaluation period and the observed decrease in repeatability was not significant.

Repeatability indices obtained for taste and mouthfeel attributes were not affected by time standardisation by modality but slightly improved when standardising merged modalities (0.872-0.943). This is likely to be because panellists generally kept taste and mouthfeel attributes checked longer compared to flavour attributes. Taste and mouthfeel sensations often lingered across time segments and attributes remained checked until the end of the evaluation resulting in increased overlapping between time slices, providing limited trimming opportunities in the later sip segments. These findings suggest that the reduction in repeatability indices due to time standardisation in the flavour data could not be achieved to the same extent as for the taste and mouthfeel data.

Average repeatability and agreement indices calculated on an attribute basis are listed in Table 5.2. and show that time standardising merged modalities reduced more panellists' noise compared to the time standardising by modality. However, it is worth noting that both standardisation approaches (merged modalities and by modality) provide better agreement indices than raw data for both modalities and in particular for flavour. Furthermore, repeatability is also improved with data standardisation. Therefore, the exclusive inspection of similarity coefficients may not be sufficient to evaluate panel performance (Castura et al., 2016). Panel performance indices derived from city-block distances between two binary response (1)/non-response (0) data matrices provide relevant insights into matched or mismatched time slices. The computation of indices for each attribute, panellist and sample combination in each dataset is time-consuming and does not provide (immediate) information on the reasons behind panel performance effects without further refinement of indices. In addition, repeatability indices can be high (close to 1), which

provides no indication of sample discrimination (Castura et al., 2016). Therefore, Mixed Models were used to further examine panellists' sensory discrimination and robustness of the study design based on interactions between sample, panellist, replicate (session) and position (order).

Table 5.2. Average TCATA panel performance indices for the flavour and taste & mouthfeel attributes obtained for panel agreement and repeatability computed from the raw data, time standardised data by modality (time std. by modality), and data time standardised with merged modalities (time std. merged modalities).

		Agreement indices			Repeatability indices		
Modality	**Sensory attribute**	**Raw**	**Time std. by modality**	**Time std. merged modalities**	**Raw**	**Time std. by modality**	**Time std. merged modalities**
Flavour	Caramel	0.865	0.849	0.886	0.916	0.921	0.959
	Earthy	0.748	0.806	0.821	0.836	0.881	0.922
	Grapefruit	0.708	0.775	0.787	0.805	0.859	0.899
	Grassy	0.798	0.851	0.864	0.836	0.838	0.899
	Lemon	0.677	0.760	0.765	0.757	0.812	0.854
	Malty	0.630	0.672	0.686	0.740	0.731	0.799
	Musty	0.796	0.834	0.852	0.807	0.795	0.863
	Orange	0.683	0.738	0.749	0.783	0.807	0.860
	Pine wood	0.675	0.720	0.736	0.746	0.735	0.809
	Raisins/prunes	0.821	0.815	0.849	0.855	0.831	0.894
	Rose water	0.828	0.874	0.884	0.869	0.881	0.919
	Tropical fruit	0.797	0.834	0.849	0.829	0.828	0.883
Taste & mouthfeel	Sweet	0.611	0.671	0.698	0.839	0.820	0.921
	Sour	0.746	0.751	0.763	0.931	0.914	0.959
	Metallic	0.761	0.762	0.703	0.778	0.951	0.937
	Harsh bitterness	0.619	0.715	0.621	0.889	0.941	0.970
	Smooth bitterness	0.702	0.671	0.757	0.834	0.882	0.942
	Astringent	0.669	0.706	0.682	0.728	0.916	0.829
	Peppery tingling	0.622	0.601	0.707	0.843	0.968	0.888
	Warming	0.689	0.734	0.771	0.852	0.939	0.914
	Cooling	0.633	0.626	0.632	0.839	0.868	0.869

Analysis of interaction effects

For the evaluation of interaction effects, *p*-values associated with *F*-tests of effects were obtained for each attribute based on the average proportions of citations

computed from the total duration and first and last segment data (Sip1im, Sip2fin) since these two sip segments were most affected by trimming of non-response data points (Table 8.7.-Table 8.8., Appendix 3).

Differences between types of interaction effects were observed between the three datasets, but also between modalities. Sample position-related effects were found for limited flavour ("caramel", "lemon", "tropical fruit") attributes in the time standardised datasets and for several taste and mouthfeel attributes, particularly in the data time standardised by modality in Sip2fin. However, these effects did not follow systematic patterns in both modalities and could not be explained. Subsequent models for the evaluation of sample discrimination (discussed in later sections) were thus computed without the factor position.

Sample*panellist effects indicating panel agreement were associated with the panel agreement index calculated for the total evaluation period. Overall, limited significant sample*panellist effects were observed in the flavour data independent from the time standardisation approach. Comparison of *p*-values revealed the panel agreement to be slightly decreased in the time standardised by modality data and to be slightly increased in the time standardised with merged modalities data, confirming the pattern detected for panel agreement indices.

Significant effects were obtained for replicate and replicate*sample interactions for the time standardised by modality flavour data, which substantially differed from those reported for the raw data and the data time standardised with merged modalities. This suggests the introduction of inconsistencies in perception of samples' flavour characteristics from one replicate to another, inconsistencies in

sample preparation, natural variation in the samples or carryover effects (Lawless & Heymann, 1998). Sources of errors were minimised as far as possible by the experimental design, but could not be entirely prevented. “Musty” was the only attribute that obtained a non-significant replicate*sample interaction effect in the data time standardised by modality. Panel repeatability indices computed for the time standardised by modality data indicated better repeatability than revealed by significant replicate*sample interaction effects. This suggests that the two parameters are not substitutable. It should be taken into account that interaction effects are computed from average citation proportion data whilst panel performance indices indicate differences between coordinates of pairs of time slices in a grid.

In TCATA studies, the replicate*panellist interaction effect may refer to inconsistency in reaction time when checking and unchecking perceived attributes (i.e. cognitive abilities) from one replicate/session to another and may therefore be equivalent to the repeatability index. Interestingly, replicate*panellist interactions significantly decreased in the flavour data time standardised by modality compared to the raw data and the data time standardised with merged modalities in Sip1im indicating increased alignment of attribute onsets at the beginning of the evaluation period. In contrast, this interaction effect significantly decreased in the flavour data time standardised with merged modalities in Sip2fin compared to the other two datasets. The impact in this sip segment caused the same effect for the replicate*panellist interactions computed for the total evaluation period data suggesting that this time standardisation approach had a larger impact on panel repeatability in the flavour data.

Inspection of interaction effects obtained for the taste and mouthfeel data showed a similar pattern compared to the flavour data. Panel agreement was significantly improved for taste and mouthfeel attributes in the time standardised data with merged modalities. Only the sample*panellist effect for “sour” was found to be significant compared to the raw data and the time standardised data by modality. Similar to the replicate*sample effects observed for the time standardised by modality flavour data, significant interaction effects were also found in the corresponding taste and mouthfeel data. In general, greater panel repeatability was observed in the time standardised data with merged modalities for taste and mouthfeel. This was confirmed by significant replicate*panellist effects for all taste and mouthfeel attributes except for “smooth bitterness”, which conflicts with the repeatability indices obtained for taste and mouthfeel attributes suggesting that replicate interaction effects may not be comparable with the repeatability index.

Comparison of interaction effects between modalities revealed that panel performance could not be improved by time standardisation independent from the approach. Time standardisation with merged modalities generally improved panel performance parameters compared to time standardisation by modality apart from the first flavour sip segment. Trimming and subsequent standardisation of the current data appeared to have reduced replicate*panellist effects and at the same time introduce replicate*sample effects in the flavour dataset suggesting reduced panel repeatability due to differing sample perception from one replicate to another. The introduction of replicate*sample effect could not be explained. Attribute onsets and offsets could be aligned, however, subsequent standardisation of remaining data points may in turn have distorted the pattern.

Analysis of panel heterogeneity using Canonical Variate Analysis

For visual investigation of the panel heterogeneity in the three datasets and its effect on significant differences among the samples, the data was subjected to CVA, which are plotted here for each sample subsets (HULU vs ISO/NISO) and per modality to improve the readability of maps and facilitate their interpretation (Figure 8.2., Figure 8.3., Figure 8.4., Appendix 3). Bartlett's test outcomes revealed the number of significant factors explaining the variance among samples (McMahon et al., 2017) showing limited differences between the corresponding datasets. The distribution of panellists data points (citations of proportions for each evaluation) around the sample means is illustrated by confidence ellipses suggesting consensus if the data points and ellipses tightly enclose the means. Sizes, but mostly forms of ellipses, differed between the flavour profile plots created from the raw versus the data time standardised by modality and the taste and mouthfeel profile plots due to data points being differently scattered across the space. However, these observations were only made at a sample level (e.g. HULU+CITRUS, FLORAL and SPICY replicates in the flavour maps) and did not impact the overall discrimination between samples in the multisensory space (i.e. differences between sample profiles). Scattering of data points was linked to panellist-related interaction effects discussed earlier and confirms the ANOVA outcome. The outcome of the CVA did not reveal additional insights that could explain the variation in the datasets caused by the different time standardisation approaches.

5.3.2 Investigation of changes in dynamic profiles

Differences in discriminative power between the raw and the time standardised datasets were investigated based on pairwise comparisons of samples by attribute. Furthermore, TCATA curves and CA and CVA biplots were explored and facilitated visual comparison of sample discrimination and significant effects. For detailed interpretation of the samples' sensory characteristics readers are referred to Dietz et al. (2022).

Analysis of average proportions of citations

Time standardisation of the data resulted in increased average citation proportion values and the impact was more pronounced for flavour attributes in the time standardised by modality data indicating that more time slices were trimmed in this dataset. Onsets of attributes in TCATA profile plots (not shown) revealed that "malty", "orange" and "rose water" in the flavour modality were most often selected in the first time slices of the evaluation and were trimmed less compared to other attributes. The same applied for all taste and mouthfeel attributes except for "metallic" and "warming". All taste and mouthfeel attributes remained checked until the end of the evaluation period except for "cooling", "sour", "sweet".

ANOVA outcomes computed for the total evaluation period revealed no significant differences between sample effects among the raw data and the time standardised data by or with merged modalities. However, different significant attributes were found in the Sip1im and Sip2fin segments i.e. those segments that were mainly affected by trimming of non-response data. In contrast to the raw data, time standardisation by modality resulted in non-significant sample effects for "pine

wood" (p=0.118) in Sip1im and "musty" (p=0.127) in Sip2fin (Table 8.7.-Table 8.8.., Appendix 3) suggesting that the time standardisation approach resulted in an increased level of matching of time slices or congruency between time slices of repeated evaluations for these attributes, which caused this removal of significant effects. Significant sample effects for taste and mouthfeel attributes were not removed. Moreover, this time standardisation approach resulted in significant sample effects for the attributes "cooling" (p=0.002) in Sip1im and "cooling" (p<0.0001), "sweet" (p<0.0001), "warming" (p=0.031), "grassy" (p=0.006), "malty" (p=0.010), and "raisins/prunes" (p=0.019) in Sip2fin.

Sample mean separation achieved by Tukey's HSD was used for further inspection of these significant sample effects (Table 8.9., Table 8.10., Table 8.11., Appendix 3) and revealed only slight differences between pairs compared to the raw data. Overall, time standardisation by modality removed significant effects between sample pairs in some sip segments while no introduction of significant effects was observed. The impact of time standardisation with merged modalities was significantly smaller than the impact of time standardisation by modality.

Flavour attributes that were mostly affected by time standardisation by modality were "caramel" between base beers (ISO/NISO vs HULU), "earthy", "grassy", and "pine wood" between IPA-flavoured beers, "grapefruit", "lemon", and "tropical fruit" between CITRUS- and FLORAL-flavoured beers, "malty" between SYLVAN-flavoured beers, "raisins/prunes" between SPICY-flavoured beers, and "tropical fruit" between FLORAL-flavoured beers. The majority of these differences was recorded in the first and last sip segments and less in the middle segments (Sip1sw, Sip2im). Figure 5.1.

shows the TCATA difference plots with significant curves for significant flavour attributes discriminating between the CITRUS- and FLORAL-flavoured beers. The plots based on the data time standardised by modality visualise removal of significant effects for “grapefruit” (CITRUS, FLORAL), “lemon” (CITRUS), and “tropical fruit” (FLORAL) compared to the corresponding raw data significant curves as a result of alignment of mismatching time slices between panellists and replicates. The plots also illustrate (visual) distribution or ‘spreading’ of significant effects (e.g. “earthy”, “lemon”) across the evaluation period resulting from standardisation of the data.

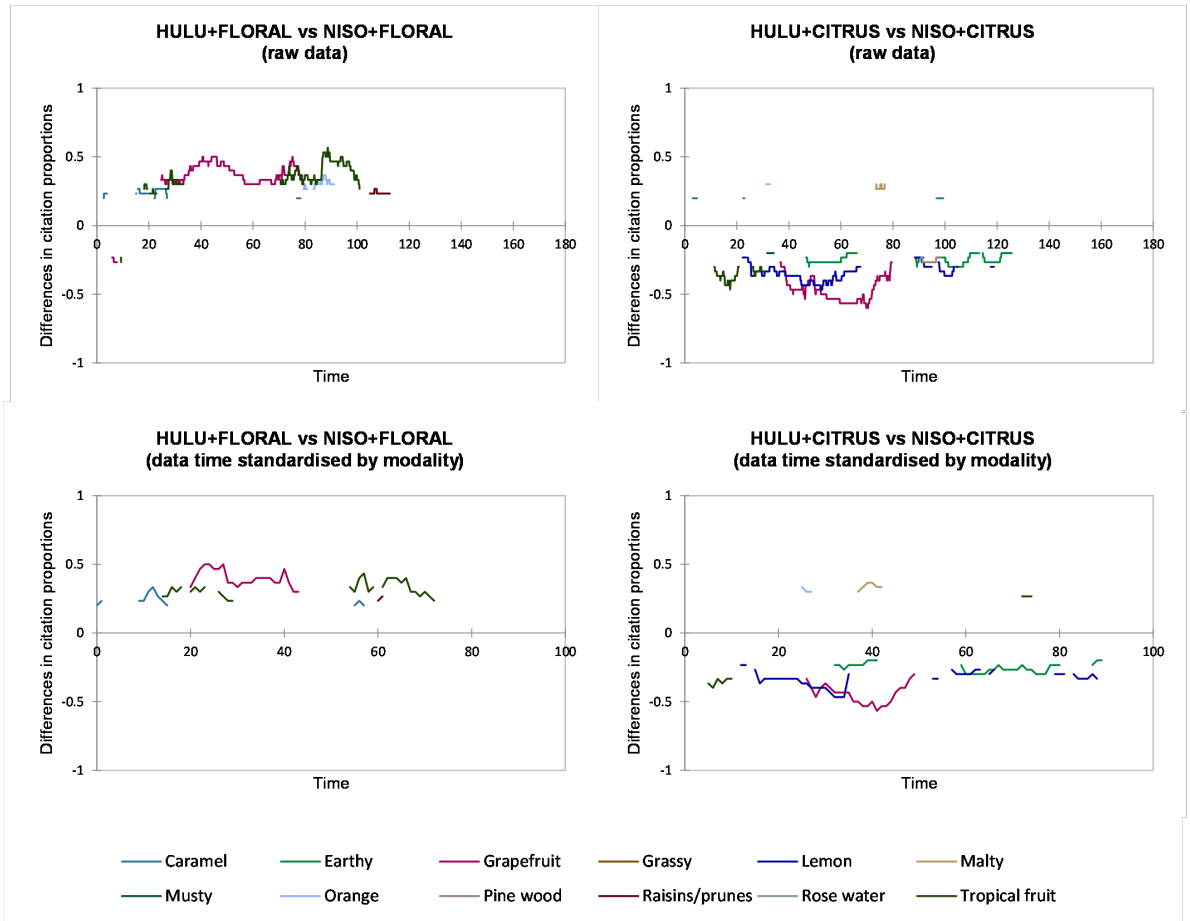


Figure 5.1. TCATA flavour difference curves showing citation proportions plotted against the evaluation time (expressed in s or percentiles). Curves indicate time slices for attributes that were significantly different (p<0.05) between the two CITRUS- and the two FLORAL-flavoured beers (HULU vs NISO). Plots were computed from the raw data and data time standardised by modality.

In contrast to time standardisation by modality, time standardisation of the merged datasets generally resulted in fewer differences in the total duration and Sip1im segment data compared to the raw data. In a few instances, significant effects were introduced and removed. The approach introduced sample effects in the Sip2fin segment for “grassy”, “musty”, “raisins/prunes”, and “tropical fruit”, and “cooling” (all $p<0.0001$) and reduced significant sample effects for “orange” ($p=0.056$), “pine wood” ($p=0.312$), and “rose water” ($p=0.149$) after time standardising the merged modality data. However, pairwise comparisons resulted in no significant effects for any of the above attributes. Pairwise comparisons revealed limited sample effects across different sip segments, where no specific patterns and low citation proportion levels were observed; therefore these were neglected.

Particularly the control beers’ flavour profiles were more differentiated from each other after time standardising the data by modality, namely those samples that were generally found to have less complex sensory profiles and later onsets or earlier offsets of sensations compared to the other flavoured beers. For these samples, significant differences were still observed at relatively low average citation proportions and mainly for quickly fading sensations. Comparison of the binary data showed that the response times of these attributes were ‘stretched’ as a result of standardisation, thereby introducing a distortion of patterns in the data. An example for this effect is illustrated in Figure 5.2. showing the stretching of “caramel” flavour and impact of significant differences reaching across time slices between the HULU and ISO control beers. As time standardisation of merged modalities affected the data less as a result of trimming boundaries set by taste and mouthfeel attributes, the difference plot minimally differed from the raw data plot. This confirms previous

studies that found a negative impact, such as distorted timelines and false joint onsets of sensations, when implementing time standardisation to sensory temporal data, suggesting a significant impact to sensory profiles and differences between samples, and eventually, misinterpretation of data (Meyners, 2020; Nguyen et al., 2018).

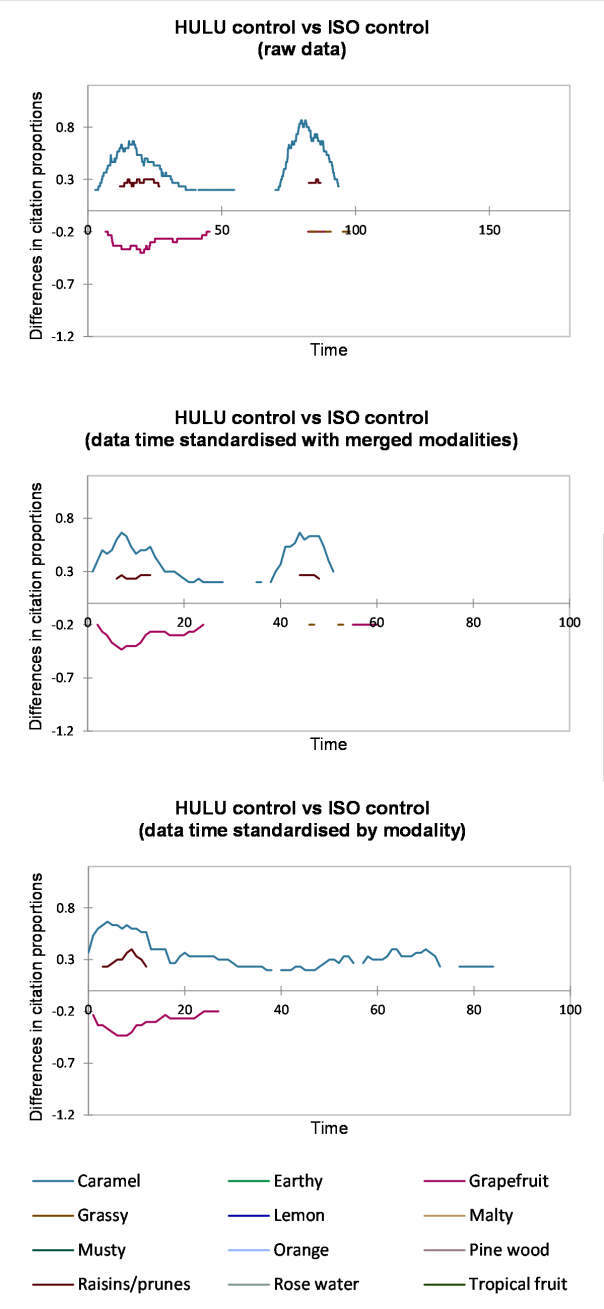


Figure 5.2. TCATA flavour difference curves showing flavour attributes that were significantly different (p<0.05) between the control beer samples HULU and ISO. Plots were computed from the raw data and data time standardised by modality or with merged modalities.

In comparison to the raw data, time standardisation by modality also had an impact on sample effects with regard to taste and mouthfeel attributes. This predominantly concerned effects for “harsh bitterness” (control beers, IPA-flavoured beers), “peppery tingling” (SPICY- and SYLVAN-flavoured beers), and “sour” (FLORAL-flavoured beers) occurring between Sip2sw and Sip2fin. These changes consequently resulted in removal of significant effects for the average citation proportions computed for the total evaluation period. However, inspection of TCATA difference curves depicts limited differences between significance lines of these attributes. As an example, Figure 5.3. visualises significant differences between the two control beers for taste and mouthfeel attributes. Significance curves differ at the beginning of the TCATA run due to trimming of non-checked attributes, whilst the differences previously detected in the RM-ANOVA outcome in later sip segments are less obvious suggesting that no fundamental changes in sample discrimination were caused by time standardisation by modality in the taste and mouthfeel data.

The ANOVA outcome further revealed that data time standardisation with merged modalities affected sample discrimination based on the “smooth bitterness” attribute. However, inspection of mean separations suggested only slight changes in sample discrimination wherefore this effect was neglected. Further examination confirmed that this time standardisation approach only had few effects on individual attributes including “cooling”, “harsh bitterness”, “metallic”, and “sweet” (control beers), “astringent” (control beers, FLORAL-flavoured beers), and “sour” (CITRUS-flavoured beers).

Overall, the analysis of citation proportions using RM-ANOVA helped to identify significant differences between the raw and the time standardised datasets within sip segments but revealed limited differences for the total evaluation period. However, the outcome did not clarify to what extent the differences in the datasets impacted the overall discrimination between samples since many changes occurred at relatively low or high citation proportion levels and in individual sip segments without changing the overall sample profiles. Visualisation of sample discrimination in TCATA difference plots proved to be supportive to understand and validate time standardisation-derived effects on the temporality of attributes obtained by RM-ANOVA.

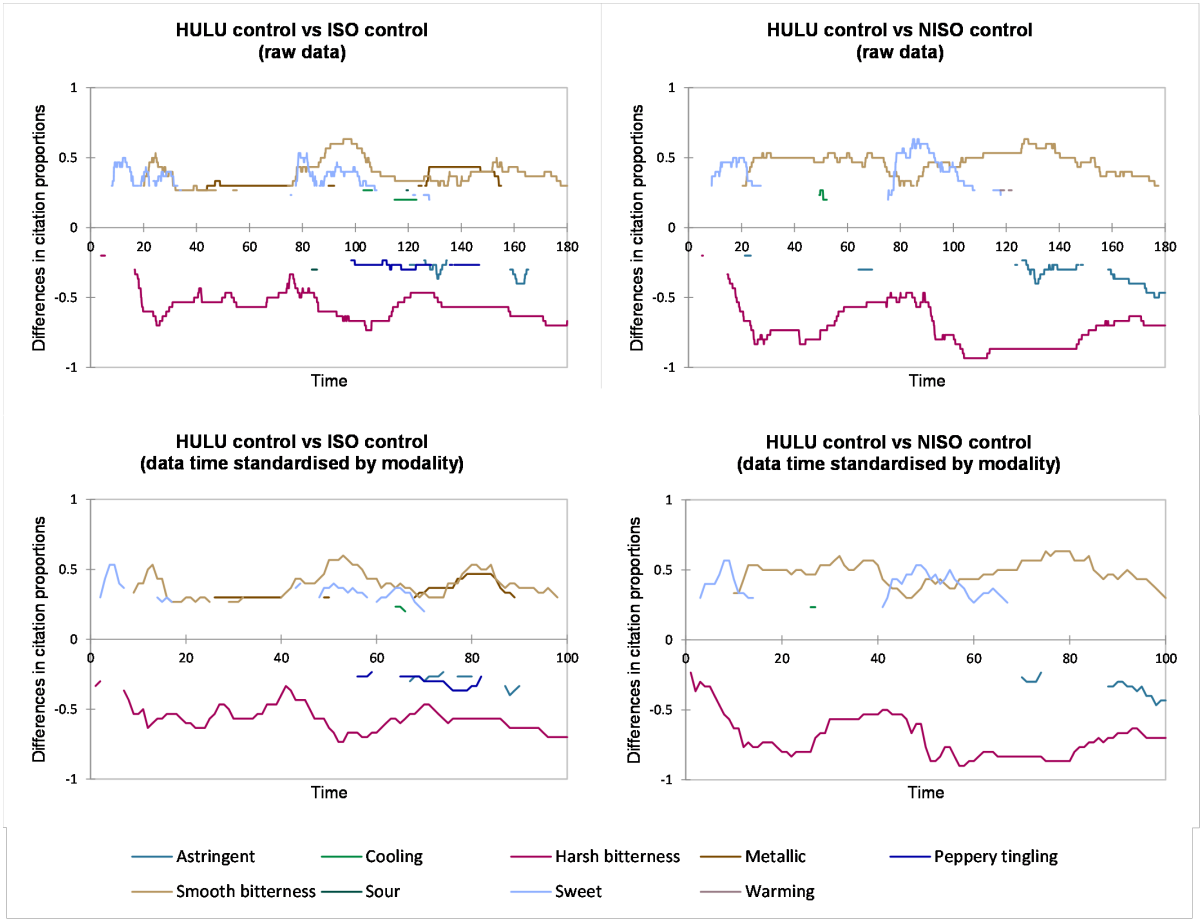


Figure 5.3. TCATA taste and mouthfeel difference curves attributes that were significantly different (p<0.05) between the control beer samples HULU vs ISO and HULU vs NISO. Plots were computed from the raw data and data time standardised by modality.

Analysis of changes in durations

Flavour and taste and mouthfeel profile durations were only compared on the basis of significant differences between samples since time standardisation results in the loss of time dimensionality. Only the time standardisation by modality approach caused a change in significant effects for the HULU+SPICY1 and HULU+SYLVAN samples resulting in more discrimination between the SYLVAN-flavoured beers (particularly for taste and mouthfeel attributes) and between the HULU+SPICY replicates resulting in a significant difference in taste and mouthfeel duration (Table 5.3.).

Table 5.3. Mean duration computed for the total evaluation period from raw data, data time standardised by modality (time std. by modality) and data time standardised with merged modalities (time std. merged modalities) of flavour and taste & mouthfeel profiles calculated for each sample with different letters within columns representing significant differences among samples as analysed by LS means (p<0.05).

	Flavour			**Taste & mouthfeel**		
Samples	**Raw [s]**	**Time std. by modality []**	**Time std. merged modalities []**	**Raw [s]**	**Time std. by modality []**	**Time std. merged modalities []**
HULU	16.5 f	12.7 f	9.6 f	60.7 bc	35.4 bc	35.2 bc
HULU+CITRUS	32.7 cde	22.8 cde	18.7 d	60.8 bc	34.9 bc	34.8 bc
HULU+FLORAL	37.7 bcd	25.7 bcd	21.6 cd	53.8 c	30.9 c	30.8 c
HULU+IPA	34.9 cd	26.1 bcd	20.1 cd	54.6 c	31.6 c	31.5 c
HULU+SPICY1	48.8 a	26.5 bcd	27.7 ab	81.8 a	35.7 bc	46.5 a
HULU+SPICY2	49.8 a	31.6 ab	28.2 a	80.0 a	46.6 a	45.5 a
HULU+SYLVAN	34.7 cd	33.4 a	19.8 cd	62.0 bc	45.7 a	35.4 bc
ISO	14.3 f	11.1 f	8.3 f	60.7 bc	35.5 bc	35.2 bc
NISO	15.4 f	12.1 f	8.9 f	61.0 bc	35.1 bc	35.0 bc
NISO+CITRUS	39.1 bc	27.0 abcd	22.2 bcd	61.9 bc	35.3 bc	35.2 bc
NISO+FLORAL	31.1 cd	21.7 de	17.9 de	53.8 c	31.1 c	31.011 c
NISO+IPA1	30.3 cde	21.7 de	17.2 de	61.9 bc	35.6 bc	35.4 bc
NISO+IPA2	29.5 de	21.2 de	16.8 de	59.0 bc	33.8 bc	33.6 bc
NISO+SPICY	43.5 ab	28.9 abc	24.8 abc	70.1 b	40.3 ab	40.1 ab
NISO+SYLVAN	22.7 ef	17.0 ef	12.9 ef	64.2 bc	36.7 bc	36.6 bc

Few changes in significant effects were detected for flavour (Table 8.12., Appendix 3) and taste and mouthfeel attributes (Table 8.13., Appendix 3), which mostly occurred independent from the time standardisation approach. Significant duration

differences between the SYLVAN-flavoured beers for “earthy” and between the FLORAL-flavoured beers for “grapefruit” disappeared. Time standardisation by modality resulted in changes affecting discrimination of samples based on “malty” (introduced between ISO/NISO and NISO+CITRUS) and “raisins/prunes” (introduced between NISO and HULU). For the time standardised data with merged modalities, changes were detected regarding the attributes “lemon” (removed between HULU+CITRUS and HULU+FLORAL), and “pine wood” (removed between SYLVAN-flavoured beers). Evaluation of significant changes for taste and mouthfeel attributes showed that time standardisation mainly removed significant effects, which affected the control beers regarding the duration of the attributes “harsh bitterness”, “metallic”, and “peppery tingling” and the CITRUS-flavoured beers regarding “peppery tingling” and “sour”. Inspection of the duration data showed that, although the overall pattern of durations was not changed, time standardisation slightly shifted the samples’ durations and therefore, their location in the sensory spaces. In general, significant effects between samples were mostly introduced or removed if these effects already showed trends towards significance or non-significance in the raw data.

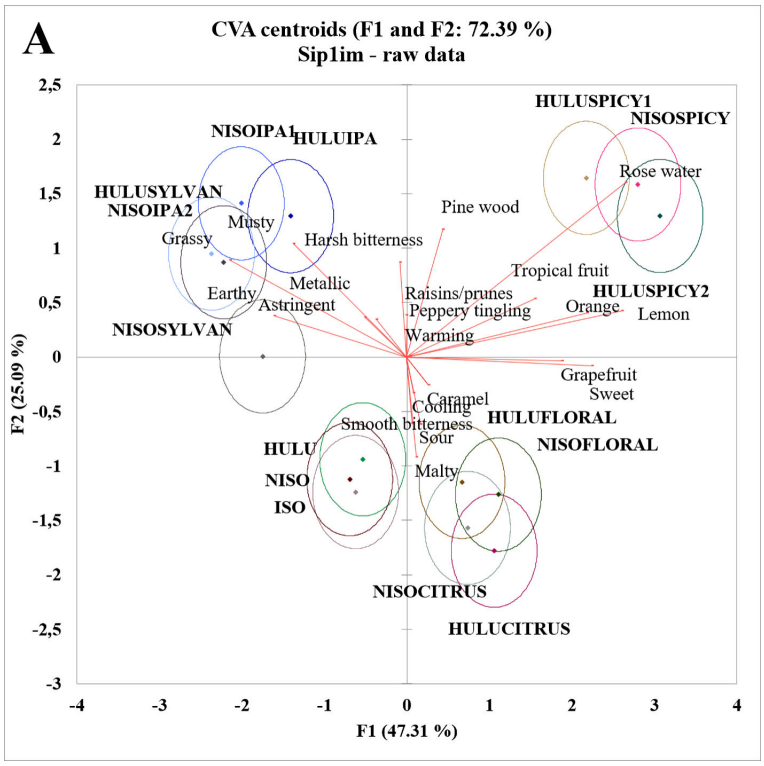
Changes in sample profiles in the multisensory space

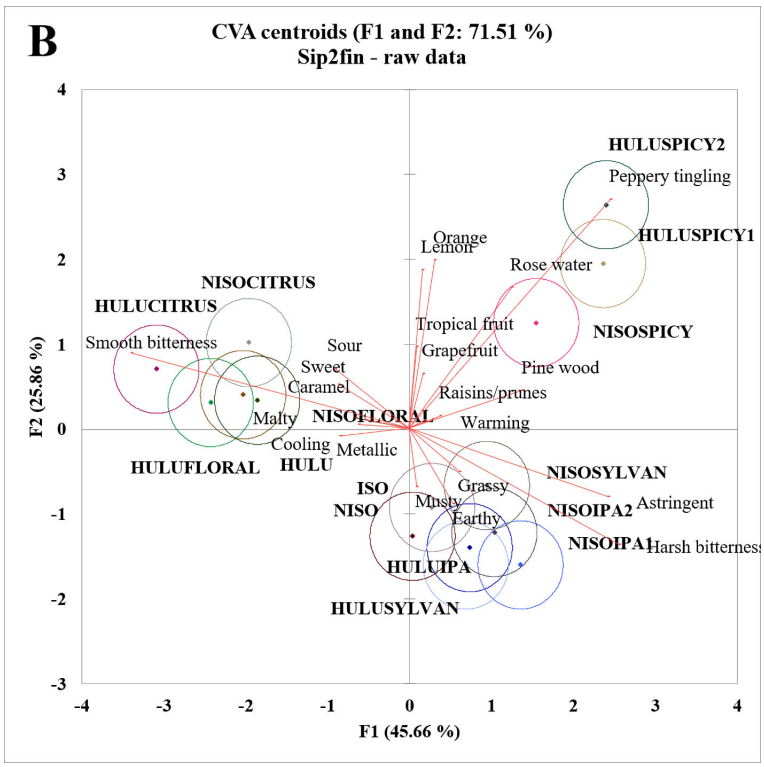
Duration data was also subjected to CVA to obtain sample maps illustrating the relationship of the 15 samples in their relationship in a multisensory space (flavour, taste, mouthfeel) and to compare the temporality of sensations among sip segments. The main differences were detected between Sip1im and Sip2fin and the corresponding maps are shown in Figure 8.5. in Appendix 3. Hotelling’s T^2 tests were

conducted to investigate significant differences between sample locations (mean vectors) and were found to discriminate the pairs of samples in each segment (p<0.0001). CVA maps computed from the total evaluation period data showed no differences between the three datasets (plots not shown). However, differences were detected between the CVA outcomes for Sip1im and Sip2fin. MANOVA F was obtained according to Hotelling-Lawley statistics within each segment and revealed better discrimination between samples in the time standardised data by modality (Sip1im: F=6.433; Sip2fin: F=6.451) compared to the raw data (Sip1im: F=2.945; Sip2fin: F=2.840). On the other side, the first two dimensions explained the majority of sample differences in each differently pre-processed dataset, confusion matrices for the two sip segments suggested that less misclassification or more discrimination based on sensation durations occurred in the raw dataset (Sip1im: 58.00% correct classification, Sip2fin: 46.67% correct) compared to the data time standardised by modality (Sip1im: 42.67% correct, Sip2fin: 39.65% correct) and the data time standardised with merged modalities (Sip1im: 38.89% correct, Sip2fin: 38.89% correct) (Figure 8.5., Appendix 3). Interestingly, this suggests that more sample discrimination was achieved in the raw dataset.

NISO+SYLVAN and HULU+IPA are detached from the sample group in the top left of the Sip1im map (Figure 5.4.). This was mainly because there was a shift towards higher dimension loadings for flavour attributes, including “pine wood”, “raisins/prunes”, and “malty”, which confirmed preceding analysis outcomes. Interestingly, dimension 1 of the Sip2fin plots computed from the time standardised data explained more variance in the dataset compared to the raw data. NISO+CITRUS

and NISO+FLORAL were found to detach from the sample group on the left plot side and the control beers ISO and NISO to separate at the top of the plots. Differences in profiles were attributed to increased discrimination based on several flavour attributes such as “caramel”, “lemon”, “musty”, and “raisins/prunes”, as previously discussed, and increased impact of taste and mouthfeel attributes on the profiles (“metallic”, “cooling”). Most of these attributes were classified as quickly fading sensations, but were still considered as important for the description of and discrimination between the samples (Dietz et al., 2022). Differences between quickly fading attributes were associated with the stretching of response data, however, the differences detected for longer lasting attributes such as “lemon” (and other fruity flavours) could not be resolved and it remains unclear whether these were the result of distorted patterns or real increases in discrimination due to the reduction of noise in the data.


A
CVA centroids (F1 and F2: 72.39 %)
Sip1im - raw data
F1 (47.31 %)
F2 (25.09 %)
HULUSPICY1
NISOSPICY
NISOIPA1
HULUIPA
Rose water
HULUSYLVAN
NISOIPA2
Pine wood
Musty
Grassy
Harsh bitterness
Tropical fruit
Metallic
HULUSPICY2
Earthy
Astringent
Raisins/prunes
Orange
Peppery tingling
Lemon
NISOSYLVAN
Warming
Grapefruit
Sweet
Caramel
Cooling
HULUFLORAL
Smooth bitterness
Sour
NISOFLORAL
HULU
Malty
NISO
ISO
NISOCITRUS
HULUCITRUS


B
CVA centroids (F1 and F2: 71.51 %)
Sip2fin - raw data
F1 (45.66 %)
F2 (25.86 %)
HULUSPICY2
Peppery tingling
HULUSPICY1
Orange
Lemon
Rose water
NISOCITRUS
HULUCITRUS
Tropical fruit
NISOSPICY
Smooth bitterness
Sour
Grapefruit
Pine wood
Sweet
Caramel
Raisins/prunes
Malty
NISOFLORAL
Warming
Cooling
Metallic
HULUFLORAL
HULU
NISOSYLVAN
ISO
Grassy
Musty
NISO
NISOIPA2
Astringent
Earthy
NISOIPA1
Harsh bitterness
HULUIPA
HULUSYLVAN

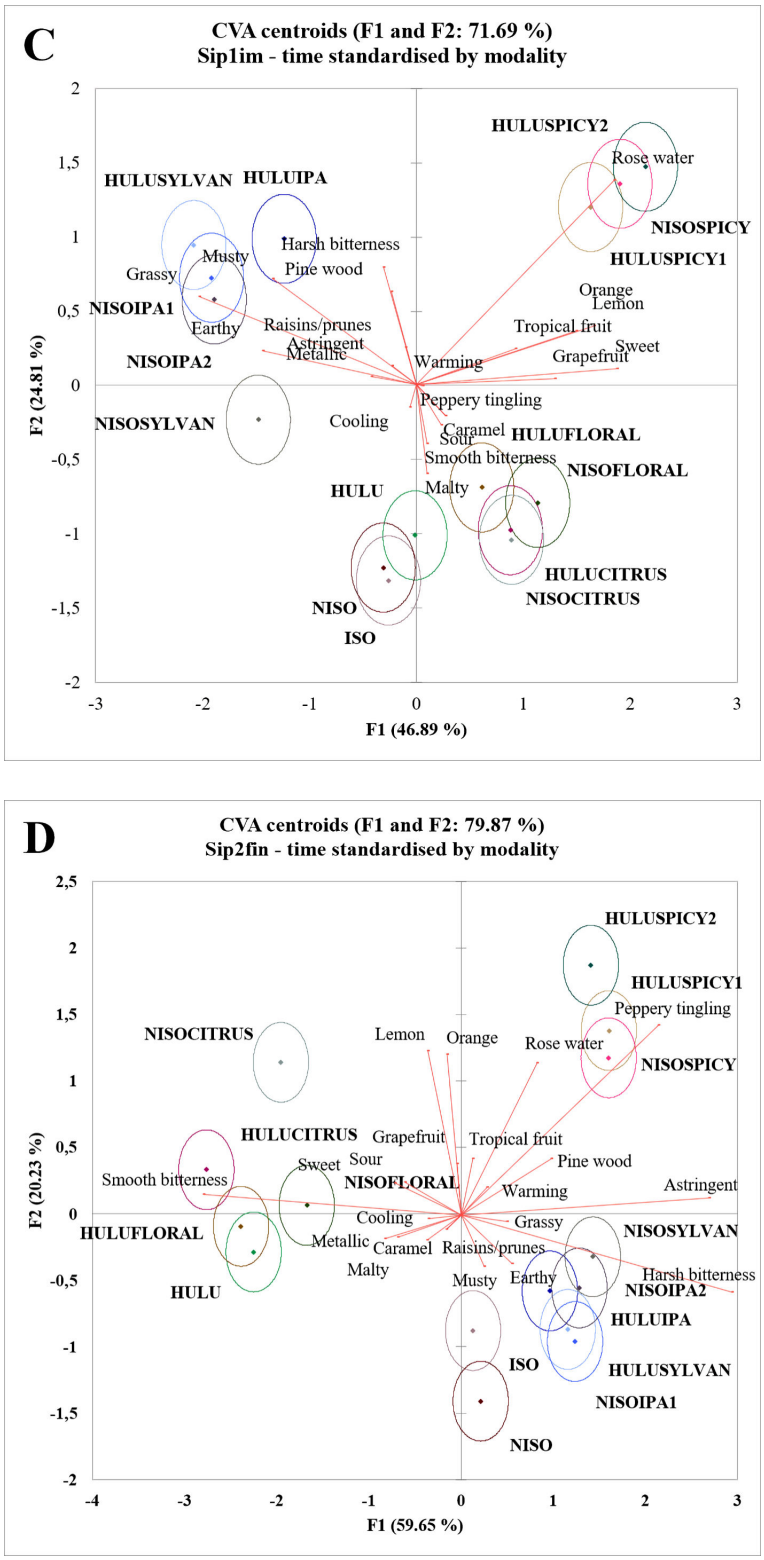


Figure 5.4. Canonical Variate Analysis (CVA) maps computed for Sip1im and Sip2fin sip segments of samples (n=15) in a multisensory space. Bold diamonds (centroids) indicate the sample means. Non-overlapping confidence ellipses indicate significant differences (p<0.10) among samples.

5.3.3 Multiple Factor Analysis of the combined datasets

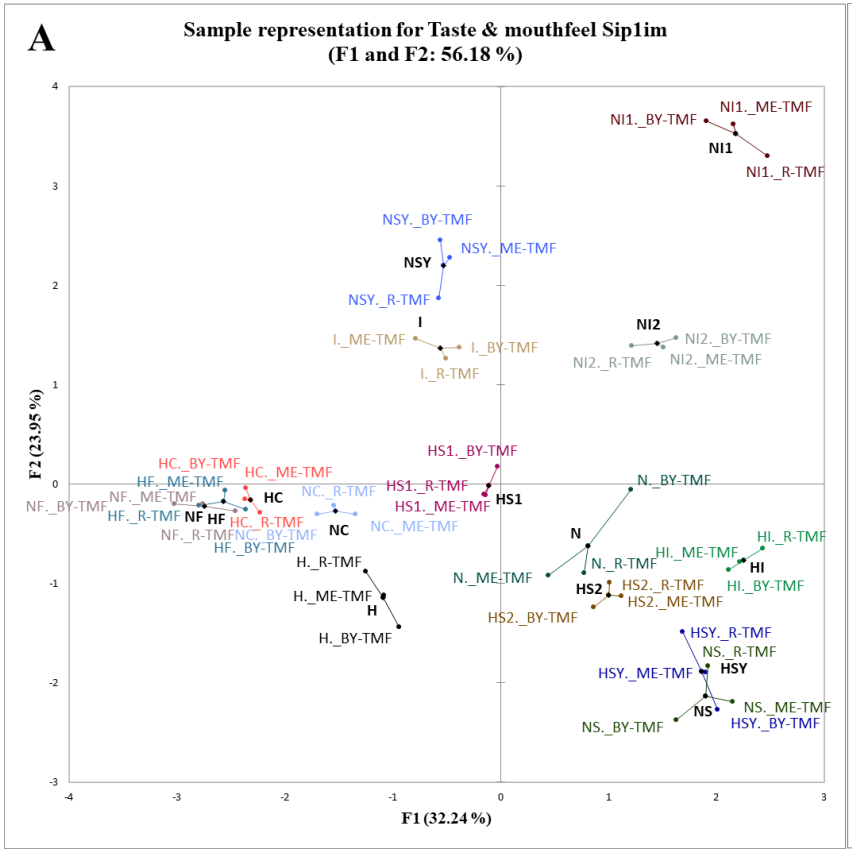
In order to evaluate the sensory profiles resulting from the choice of the pre-processing approach, MFA was performed on the combined average citation proportion datasets for each modality (flavour, taste & mouthfeel) and for Sip1im and Sip2fin (Figure 8.6., Appendix 3). Regression vector (RV) coefficients were inspected to understand the degree of similarity between the datasets. High RV coefficients for Sip1im ranged between 0.979-0.997 (flavour) and 0.921-0.982 (taste and mouthfeel) and for Sip2fin between 0.963-0.995 (flavour) and 0.963-0.991 (taste and mouthfeel) suggested strong links between the profiles. The strongest links were found between the raw data and the data time standardised with merged modalities. The superimposed representation of samples (Figure 8.6., Appendix 3) confirmed the spatial proximity of the three profiles of each sample in the sensory spaces.

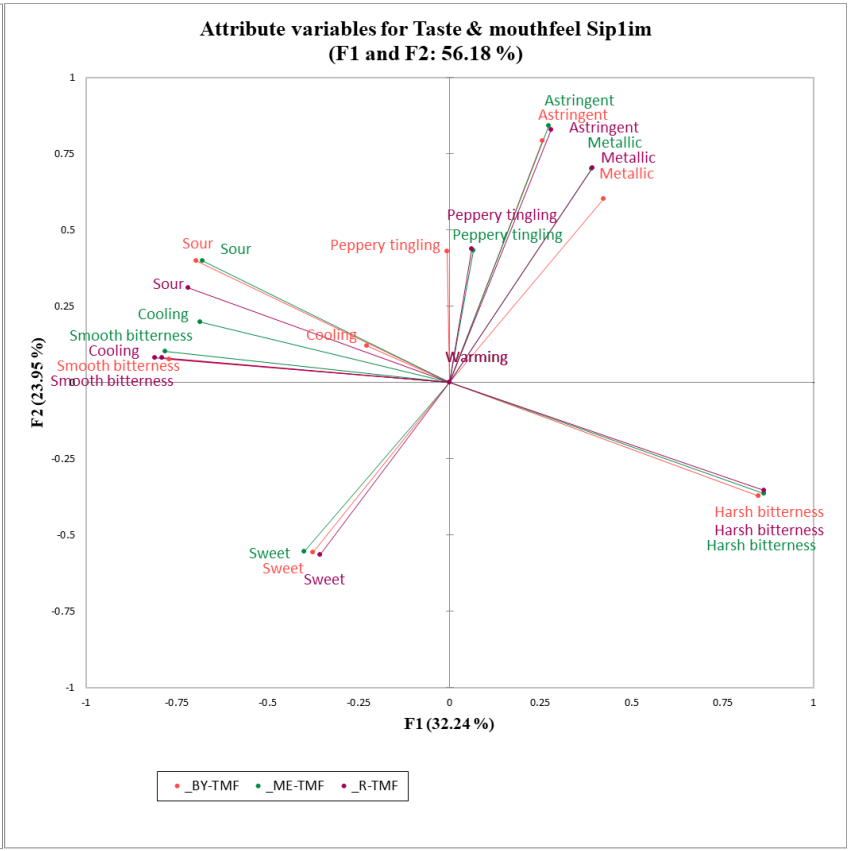
As suggested by the RV coefficients, for the majority of samples, higher correlations were found between the flavour profiles derived from the raw and the data time standardised with merged modalities. Limited correlation differences were detected in the profiles of control beers (H, I, N), HULU+FLORAL (HF) and HULU+IPA (HI) in Sip1im and ISO (I), NISO+IPA2 (NI2) and NISO+SPICY (NS) in Sip2fin. Perceptual maps showing the attribute variables (Figure 8.6., Appendix 3) suggested that the differences were caused by the attributes “caramel”, “pine wood”, “malty”, and “raisins/prunes” in Sip1im and by “caramel”, “grassy”, “malty”, “musty”, “pine wood”, and “tropical fruit” in Sip2fin, confirming the conclusions made based on the ANOVA and CVA outcomes.

In the taste and mouthfeel space of Sip2fin, higher correlations were likewise observed between the profiles obtained from the raw data and the time standardised data with merged modalities (mostly IPA (I) and SYLVAN (SY)) and between the two time standardisation approaches (mostly SPICY (S)). In Sip1im, higher correlations were mainly found between the raw and the time standardised data with merged modalities. Lower correlation coefficients were caused by differences in attribute data of “cooling”, “metallic”, “peppery tingling”, and “sour” in Sip1im and “cooling” and “warming” in Sip2fin. MFA biplots showing the superimposed representation of samples and perceptual maps based on the taste and mouthfeel attributes are shown in Figure 5.5. Interestingly, the taste and mouthfeel profile of the NISO control beer in Sip1im shifted considerably as a result of time standardisation by modality and its effect on the citation proportion of “harsh bitterness” (increased) and “metallic” (reduced) as indicated by the vector lengths. The same applied for the HULU control beer in Sip2fin caused by differences regarding the attribute “cooling” (reduced), but independent from the time standardisation approach. Overall, the flavour and taste and mouthfeel profiles provided by the different pre-processing methods were still highly associated as illustrated by the vector lengths in the plots.

Overall, it appears that none of the time standardisation approaches could improve repeatability and agreement in a way that one would conclude that panellists’ noise was significantly reduced. Further research needs to be conducted to validate the current observations. Inter- and intra-individual differences are not only associated with differences in panel performance but with natural variations in perception and cognitive or oral processing between elicitations/sessions that cannot entirely be removed by panel training. Despite common agreement on the hypothesis that it may

be impossible to completely remove panellists' noise, whether in static or temporal sensory investigations, the level of acceptable noise still needs to be defined (Meyners, 2020). Trimming and standardising data does not only remove the time dimension from the data, but distorts alignment between modality-runs. Therefore, the optimal data pre-processing approach and analysis to evaluate pre-processing-related effects may still depend on the nature of the data and objective of the research.


A
Sample representation for Taste & mouthfeel Sip1im
(F1 and F2: 56.18 %)
F2 (23.95 %)
F1 (32.24 %)
NI1._BY-TMF
NI1._ME-TMF
NI1
NI1._R-TMF
NSY._BY-TMF
NSY._ME-TMF
NSY
NSY._R-TMF
I
I._ME-TMF
I._BY-TMF
I._R-TMF
NI2
NI2._BY-TMF
NI2._R-TMF
NI2._ME-TMF
HS1._BY-TMF
HC._BY-TMF
HC._ME-TMF
HF._ME-TMF
NF._ME-TMF
HC
NC._R-TMF
HS1._R-TMF
HS1
NF._BY-TMF
HS1._ME-TMF
HF._R-TMF
NF
HF
HC._R-TMF
NC
NC._ME-TMF
NF._R-TMF
NC._BY-TMF
HF._BY-TMF
N._BY-TMF
N
HI._R-TMF
HI._ME-TMF
N._R-TMF
HI
N._ME-TMF
HS2
HS2._R-TMF
HI._BY-TMF
HS2._ME-TMF
HS2._BY-TMF
H._R-TMF
H._ME-TMF
H
H._BY-TMF
HSY._R-TMF
NS._R-TMF
HSY._ME-TMF
HSY
NS
NS._ME-TMF
HSY._BY-TMF
NS._BY-TMF


Attribute variables for Taste & mouthfeel Sip1im
(F1 and F2: 56.18 %)
F2 (23.95 %)
F1 (32.24 %)
Astringent
Astringent
Astringent
Metallic
Metallic
Metallic
Peppery tingling
Peppery tingling
Peppery tingling
Sour
Sour
Sour
Cooling
Smooth bitterness
Cooling
Cooling
Smooth bitterness
Smooth bitterness
Warming
Harsh bitterness
Harsh bitterness
Harsh bitterness
Sweet
Sweet
Sweet
_BY-TMF
_ME-TMF
_R-TMF

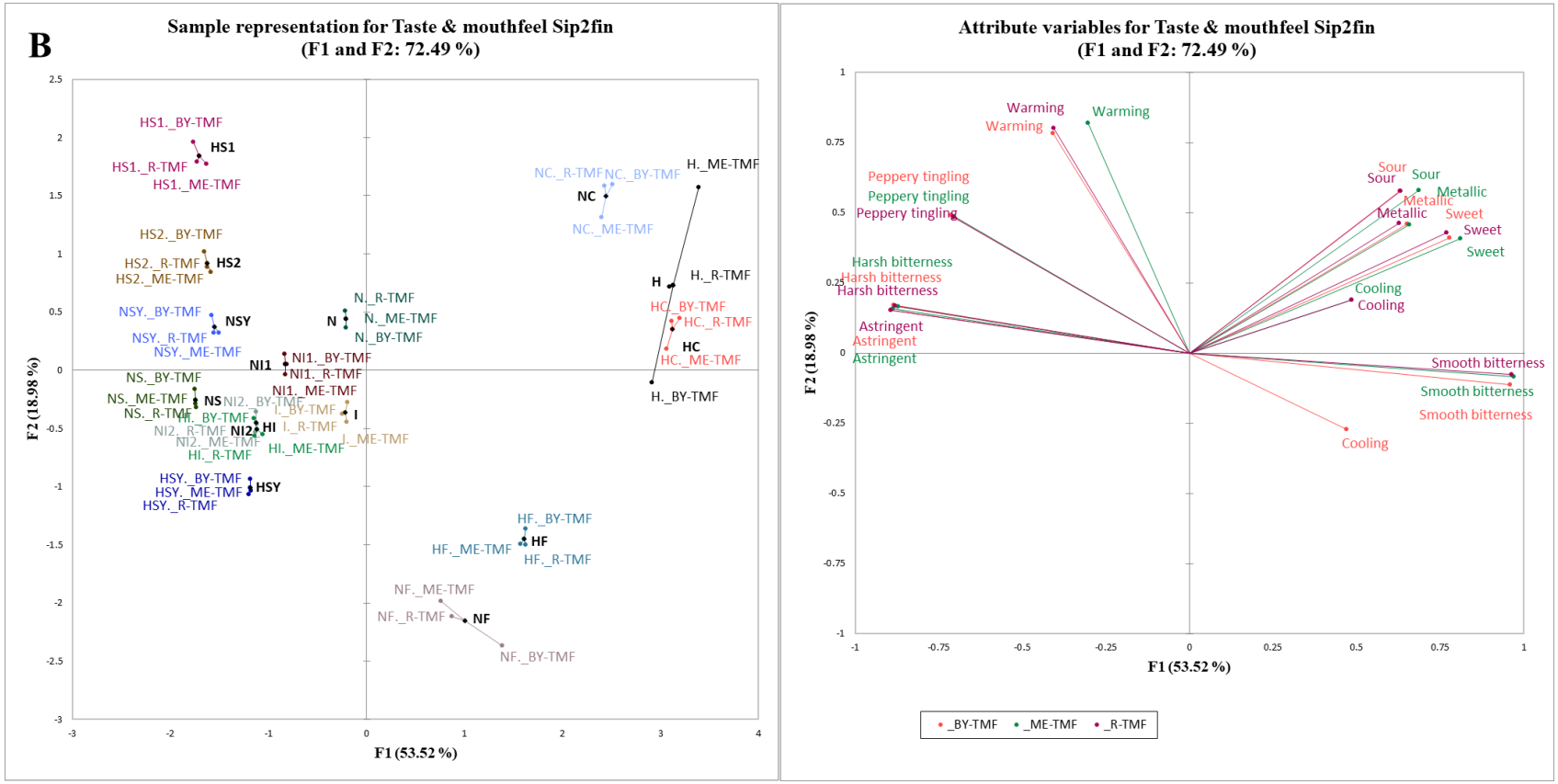


Figure 5.5. Multiple Factor Analysis (MFA) of beer samples (n=15) in their taste and mouthfeel (TMF) space for comparison of the pre-processing approaches. Representation of samples and perceptual maps showing loadings of attributes are displayed for Sip1im and Sip2fin representing the start and the end of the evaluation period. Bold diamonds represent the samples' centroids. Vectors labels indicate the pre-processing approach: raw data (R), data time standardised by modality (BY), and data time standardised with merged modalities (ME). To improve the readability of the maps, sample names were further abbreviated as follows: I (ISO), N (NISO), H (HULU) beers with or without addition of C (CITRUS), F (FLORAL), I (IPA), S (SPICY), or SY (Sylvan) extracts, and 1 or 2 for experimental replicates.

5.4 Conclusions

This paper presents differences between temporal sensory profiles obtained from a TCATA by modality study depending on the pre-processing applied. The outcome of the statistical analyses suggested that none of the applied time standardisation approaches could significantly reduce the noise in the data to improve the discrimination between samples. In fact, it appeared that the decision for, or against time standardisation resulted in a compromise between different repeatability parameters as indicated by interaction effects obtained from ANOVA. Time standardisation was found to reduce panellists' noise to some extent in the taste and mouthfeel data, at the expense of introducing distorted patterns in the flavour data, particularly in the first sip segment. Moreover, changes in patterns could not clearly be attributed to the introduction of real significant differences or artefacts.

Time standardisation of merged modality data was found to be less invasive compared to time standardisation by modality, i.e. patterns could largely be maintained and fewer distortion effects were observed. Neither of the approaches substantially changed the overall profiles of the samples in their natural sensory space. Rather, it was observed that single sample profiles shifted because more or less weight was given to some attributes (e.g. trends became significant effects), while others appear not to be affected by the pre-processing approach. In particular, attributes describing quickly fading sensations in samples having short-lasting flavour profiles (e.g. control beers) introduced distorted patterns due to substantial trimming

and stretching of response data compared to those samples primarily characterised by long lasting sensations.

RM-ANOVA, Mixed Models, CVA and MFA of duration and citation proportion data and the illustration of relationships between samples in TCATA difference plots and single- and multimodal spaces appeared to be useful tools to assess the differences between sensory temporal profiles computed from the differently processed datasets. It should be taken into account that the temporal sensory data investigated in this study followed a specific tasting protocol and therefore the findings cannot be generalised across all TCATA studies. Nevertheless, the outcomes may be helpful to evaluate the advantages and disadvantages of the time standardisation approaches in future studies and offer several options to assess the differences between temporal sensory datasets.

Chapter 6

6 Discussion and directions for future research

6.1 Overall discussion

This PhD research project focused on providing new fundamental insights into the multisensory profiles of hop oil fractions that represented the main chemical classes in Magnum hop essential oil using static and dynamic sensory techniques and selected physico-chemical parameters.

The outcome of the literature review in **Chapter 1** revealed that, although research in hop essential oil has been conducted for centuries, the focus of investigations was foremost on the identification and quantification of single hop-derived volatile compounds in beer, raw hop materials or selected hop essences. Whilst only few studies systematically assessed the organoleptic properties of hop aroma compounds other than aroma qualities, the majority of research focused on single aroma-active compounds evaluated in isolation. The impact of hop oil fractions i.e. volatile hop compounds in their natural co-occurrence on the multisensory impression when consumed as part of a beer matrix has previously not been assessed. However, multi-modal interactions play a key role in beer flavour modulation. Moreover, the outcome of the review clearly demonstrated different sensory interaction behaviours related to the perception of compound combinations and certain beer components. Thus, it was important that this PhD research holistically explored the sensory properties of hop oil fractions containing a range of chemical classes in comparison

to those induced by a more complex total oil and less complex sub-fractions as well as (suggested) key compounds. Particular emphasis was on the investigation of their ability to modify organoleptic properties of the respective test matrix used.

The 'sensory fingerprint' of essential oils embraces a spectrum of dominant and subtle nuances. In order to capture and adequately describe these a trained sensory panel was required, which was built comprising of previously trained panellists selected from the Sensory Science Centre database and newly recruited panellists from the general public. All panellists were thoroughly screened using an online pre-screening questionnaire and two screening sessions (basic, advanced) to confirm their sensory, descriptive, and discriminative abilities. Subsequently, the panel was specially trained for the sensory trials of this PhD research. The panellists were re-screened prior to each study, and detection of anosmia (geraniol) resulted in changing compositions of the panels.

The initial training period took 26 sessions (2 hours each), including preliminary bench-tests, pilot studies, and formal evaluation sessions. As the hop oil samples were in use over a long period, this raised the question regarding their stability and suitability for long-term sensory trials. To address this, the composition of fresh hop oil and hop oil fractions (used in Chapter 2A) was compared with aliquots of the same batch stored for 26 month at -20^{o}C by using GC-MS analysis (**Chapter 2B**). Results revealed a general (albeit small) decline for some compounds, whilst others were newly identified or could no longer be detected. All of these changes occurred at low concentration levels or small magnitudes and substantial degradation of compounds or increases in reaction products were not detected concluding that the Magnum hop

oil/fractions are relatively stable under the tested conditions. Since the total oil was affected the most, this suggested that hop oil fractions could be more stable due to their less complex chemical nature.

Results from **Chapter 2A** showed that, although, the total oil with 66 identified volatiles was complex from a molecular view, this complexity was not displayed in the sensory data, because the majority of compounds was present at concentrations too low to induce sensory characteristics or contribute to interaction effects. In contrast, the individual fractions had a larger impact on the sensory profile of the model system (4% ethanol, abv) by inducing a range of aroma, flavour, taste, and mouthfeel sensations. The myrcene and the sesquiterpene enriched fractions could be characterised by several 'green' aromas ("crushed grass, sap", "earthy", "musty"), which were found at lower intensities in the sesquiterpene fraction, likely due to the difference in compound concentrations between the two fractions. It appeared that only few compounds in the sesquiterpene fraction could significantly contribute to the aroma profile, and the results demonstrated the limited impact of the nonpolar *β*-caryophyllene and *α*-humulene although present at considerable concentrations and accompanied by a range of compounds at lower levels.

Ester, ketone, and terpene alcohol fractions were characterised by a range of 'fruity' and 'floral' aromas and flavours that were assigned to geranyl and methyl esters, methyl ketones, and monoterpene alcohols, respectively. Besides these sensations, this study reported for the first time the multisensory profile of a terpene alcohol fraction, which induced a significantly increased sweetness intensity, and significance-approaching effects on a "peppery tingling" mouthfeel, and a "lingering

bitterness" (p<0.07). The attribute "peppery tingling" described a trigeminal sensation that was also found to be perceived in the ester fraction. This information was commercially sensitive and was therefore not further discussed in the publication of this study. To date, it has not previously been reported that hop-derived esters and ketones modify the perception of taste and mouthfeel sensations as was observed here. Several hypotheses could be made with regard to the relationships between taste and mouthfeel variables and compounds in the terpene alcohol and ester fractions inducing sensory interactions. However, these could not be fully elucidated using PCA and PLS modelling attempts - presumably due to the sensory interactions suggested and the complexity of these fractions.

Therefore, the aim of the study presented in **Chapter 3** was to further fractionate the terpene alcohol fraction into monoterpene and sesquiterpene alcohols, with the latter expected to contain the key molecules triggering taste, mouthfeel, and trigeminal sensations. Using this approach, compounds involved in sensory interaction effects were targeted at successively decreased molecular complexities in selected sub-fractions and key compound extracts. The preceding study was conducted in a simple ethanol solution to obtain a general understanding of the fractions and limit matrix-dependent interaction effects. The next logical step was therefore to investigate their sensory impact in an actual beer matrix. It could be demonstrated that geraniol was the key compound involved in the perception of increased sweetness and "smooth bitterness" intensities – independently from whether applied as a single compound or in a terpene alcohol mixture. Correlation coefficients suggested that the perception of the taste sensations were triggered or

modulated by the 'fruity' and 'floral' aromas perceived through orthonasal and retronasal pathways confirming the hypothesis made in the preceding study. In contrast, linalool was classified as an aroma/flavour enhancing molecule acting synergistically in a mixture of volatiles. The compound applied individually had no major impact on the sensory profile of the lager style beer although assessed at supra-threshold concentration. But, models obtained from PLS regression revealed its important role as a synergist or antagonist in the perception of "lemon", "grapefruit", and "rose water" aromas and flavours, or "musty" flavour, respectively.

These findings demonstrated the multifunctionality of aroma compounds and the invalidity of simple cause-effect relationships between single compounds and sensations. Bitterness-related qualities assessed could be statistically associated with several compounds in the sesquiterpene and humulene epoxides enriched fractions, namely *α*-humulene, *β*-caryophyllene and their oxidation products. However, PLS regression models suggested that humulene epoxides (I-III) as such had little impact on the modulation of the bitterness, and so it was concluded that a combination of sesquiterpenes were mainly responsible for the "harsh bitterness" perceived in the lager. Interestingly, statistically significant effects for the mouthfeel attributes "peppery tingling" and "astringent" remained absent, despite of the addition of fractions enriched in sesquiterpene alcohols and key compounds (humulol, humulenol II, caryophyllene) as suggested by the results of Chapter 2A. An important conclusion drawn from this finding was that a temporal sensory method may be required to adequately assess the differences between hop oil extracts with regard to bitterness qualities and mouthfeel sensations. The research presented in Chapter

2A and 3 clearly demonstrated the prevalence of several aromatic characteristics among the hop oil fractions, but more interestingly, their ability to modify the perception of taste and mouthfeel sensations due to the occurrence of sensory interaction effects within and across modalities

Therefore, the preceding studies revealed that static sensory profiling (including attribute assessments at single 'delayed' time points) is not capable of determining the impact of lingering mouthfeel and trigeminal sensations whilst also exploring taste-aroma interactions between taste and aroma aspects of the hop oil samples in more detail. On these grounds, for the third experimental study presented in **Chapter 4**, a TCATA by modality approach was designed to investigate of sensory- and matrix-dependent effects resulting from the combination of hop flavour products (CITRUS, FLORAL, IPA, SPICY, SYLVAN) and hop acid extracts (commercial (ISO) and natural iso-alpha-acids (NISO), and hulupones (HULU)). The hop flavour products contained the previously studied fractions and key compounds in different combinations. Their chemical composition is known but not included in this thesis due to confidentiality requirements. This was also the first study that determined and compared the qualitative sensory characteristics of hop-derived bitter extracts. Moreover, the temporal sensory profiles of volatile and non-volatile extracts have not previously been investigated.

Prior to the statistical analyses, the binary TCATA data was pre-processed using two time standardisation approaches (**Chapter 5**). The resulting temporal sensory profiles of the beers were compared to the 'raw' data-based TCATA profiles. Time standardisation was found to result in limited trimming and removal of noise when

applied to merged modality data. In contrast, time standardisation by modality introduced distortion of patterns, mostly as a result of 'stretching' time slices of quickly fading attributes/sensations in those beer samples described by 'short' flavour profiles. Importantly, whilst intra-individual differences could be removed to a large extent, inter-individual noise was introduced. Whether or not the analysis of raw TCATA by modality data is the best strategy could not be answered with certainty, however, it was chosen for the sample set in Chapter 4 since panellists' noise could not be removed and false significant effects should not be introduced.

The outcome of the TCATA by modality study (Chapter 4) proved what was suspected from previous research since the temporal sensory profile obtained for the SPICY-flavoured beer (containing terpene alcohols and oxygenated sesquiterpenoids) significantly differed from the other beer samples not only due to its multisensory complexity, but also due to significantly higher citation frequencies recorded for the "peppery tingling" sensation. Further interesting findings were that the taste and mouthfeel characteristics of the bitter extracts in the base beer determined, 1) the perception and duration of the hop flavour products' sensory characteristics and, 2) the degree of sensory interaction-related taste and mouthfeel sensations. The ISO and NISO control beers and the HULU control beer significantly differed from each other due to their "harsh" or "smooth" bitter qualities, respectively. In addition, the HULU beer obtained significantly higher citation frequencies for the sweetness and the ISO and NISO beers were more pronounced in astringency and "peppery tingling" sensations. If then the monoterpene alcohol-containing CITRUS or FLORAL flavour products was added, this increased the perception of the "smooth" bitterness in the

"harsh" bitter NISO but not in the naturally "smooth" bitter HULU beer. Further bitter stimuli-related effects were observed in the beers' flavour profiles, for instance with the "musty" flavour induced by the SYLVAN product (containing monoterpene alcohols and sesquiterpenes) to last significantly longer (1 min) in the HULU compared to in the NISO base beer.

Particularly the final study illustrated the importance of temporal sensory methods for future assessments of multi-modal profiles and lingering characteristics of hop oil fractions and hop bitter acids since these were characterised by sensations having early (e.g. sweetness) or late onsets (e.g. astringency), that were quickly fading (e.g. "earthy", "metallic") or long lasting (e.g. "peppery tingling", "smooth" and "harsh" bitterness), and perceived concurrently and consecutively. Overall, the study outcomes significantly add to the current knowledge of sensory interactions and interrelationships between hop-derived volatiles and non-volatile fractions.

6.2 Research limitations

Some research limitations need to be addressed. As previously mentioned, the enriched fractions and sub-fractions often contained other compounds at flavour-active concentrations (e.g. geraniol) and several unknowns at trace levels (Chapter 2A and 3). The hop oil fractions produced for this research had the best possible separation of chemical classes that could be achieved and previous trials have already suggested that a sharp separation of Magnum hop essential oil containing a large proportion of terpene hydrocarbons is difficult (Marriott, 2019). However, if further fractionation or purification was possible to obtain a better separation, particularly

between sesquiterpene alcohols, monoterpene alcohols and compounds of other chemical classes in the respective fractions, this would allow a clearer split between fractions. Therefore, further development of the fractionation method or equipment used may be required to obtain a clearer split between fractions.

Another limitation concerns the study presented in Chapter 2B, which assessed the compositional differences between freshly produced and stored hop oil and hop oil fractions. As previously mentioned, further storage conditions and time points should be included in the experimental design to adequately determine the most appropriate storage requirements and critical degradation points. Furthermore, the fresh and stored hop oil samples should be sensorially assessed to evaluate whether the small changes in the volatile composition still have a significant impact on their perception.

6.3 Directions for future research

New questions that have arisen from this PhD research include:

- The collection of sensory threshold concentrations were out of scope for this PhD but the literature is clearly lacking information on retronasal aroma, taste and mouthfeel threshold levels in beer matrices or comparable model solutions. In addition, qualitative sensory profiles of the majority of hop oil compounds have not yet been evaluated. This information would aid the investigation of hop-derived compounds involved in sensory and compound interactions at sub- and supra-thresholds levels and eventually identify compound groups involved in synergistic/additive/masking effects in complex mixtures of hop volatiles.

- Sensory assessments of hop extracts were performed at equi-concentration (Chapter 2A) and at perceived equi-intensity (Chapter 3 and 4). Follow up research should include the investigation of hop oil fractions at different concentrations since the volatile composition of hop oil and hop oil fractions differs among varieties. This would benefit the understanding of concentration-dependent interaction effects observed in previous research and would potentially lead to uncovering new multi-modal profiles.
- Further follow-up research to the studies described in Chapter 2A and 3 is recommended to investigate the effect of compound matrix interactions. There are different approaches that could be conducted, namely – 1) The sensory evaluation of combined hop oil extracts (fractions, sub-fractions, compounds) at different concentration ratios and the same extracts applied separately in the same matrix (i.e. A, B, A+B). This approach could be used to systematically investigate the impact of linalool as a flavour- and bitterness-modifying compound (e.g. by combining it with different terpene hydrocarbon and oxygenated sesquiterpenoid fractions). The approach could also be used to further study the heavy terpene alcohol fraction (sesquiterpene alcohols, oxygenated sesquiterpenes) that was suggested to contribute to the "peppery tingling" mouthfeel and the astringency in the beer matrices. For this temporal sensory methods should be preferred to ensure that significant effects on taste and mouthfeel can be detected. 2) - The Magnum total oil could be taken apart into the five main or more fractions and then stepwise be recombined to investigate the impact of individual fractions on the overall sensory profile of the

total oil as well as sensory interaction effects as a result of recombining compounds or compound groups.

- Interestingly, the panellists appeared to be more sensitive to the retronasal than to the orthonasal sensation of ethanol wherefore it was decided to include the "alcohol flavour" rather than the "alcohol aroma" attribute in the formal evaluation of the hop oil fractions applied in ethanol solution (4%, ABV) described in Chapter 2A. The volatiles present in the hop oil fractions might have increased the perceived "alcohol flavour" intensity. Previous studies also found interactions between alcohol and 'fruity' and 'woody' odours in wine (Le Berre et al., 2007). This requires further investigation.

  In addition, there seem to be different findings published regarding the sensory threshold concentrations of ethanol (e.g. Yu & Pickering, 2008; Seljåsen et al., 2001, Mattes & DiMeglia, 2001). The study of Mattes and DiMeglio (2001) determined different threshold levels (taste, odour, irritation) for several assessor groups (males, females, tasters, non-tasters; light, casual, and heavy beer consumers). Findings suggest that the ethanol taste threshold concentration is lower than the odour threshold concentration. In general, determined threshold concentrations depended on the assessor group. The trained beer panel used for this PhD project would be classified as 'regular beer users' (Mattes & DiMeglio, 2001), so the ethanol concentration applied in the ethanol solution (4% ABV) was above the determined odour and taste threshold concentrations. It is not clear whether perceived alcohol aroma and flavour intensities follow a linear increase with increasing ethanol concentration in the co-presence of hop-derived volatiles – it would be interesting to investigate this too.

- The Magnum hop variety was the focus of this thesis but it is expected that the sensory profiles of its fractions cannot be generalised to other hop varieties. Whilst fractions of different hop varieties appear to be very similar, volatiles act and interact depending on their concentrations and co-occurrences with other volatiles. Therefore, small differences in the chemical composition can be perceived as significant in the sensory profile. Other hop varieties contain a substantial fraction of sulphur-containing compounds (e.g. Nelson Sauvin) or a citrus fraction comprising terpene alcohols, ketones and C5 to C8 aliphatic alcohols (e.g. Citra) (Marriott, 2001) and might reveal very different sensory characteristics and sensory interactions.
- Analytical data was not collected in the study of Chapter 4 due to confidentiality requirements for the hop flavour products and bitter acid extracts that were evaluated. The compositional details of these extracts are known but statistical analysis and correlation with the TCATA data were not performed. Therefore, whilst assumptions could be made regarding cause-effect relationships and it is recommended to study the extracts' molecular composition starting with GC-MS and high performance liquid chromatography (HPLC) followed by *in vivo* measurements such as the breath-by-breath monitoring and analysis of hop-derived volatile release in nose during the consumption of beer or model solutions. This may help to study the mechanism underlying the flavour sensations perceived in the hop oil fractions. For instance, *in vivo* nose space data could be obtained by PTR-QiToF-MS (Proton-transfer-reaction quadrupole ion guide TOFMS) analysis and correlated with quantitative temporal sensory data

(TI, Progressive Profiling) (Sulzer et al., 2014) to investigate retronasal aroma perception and the temporal occurrence of sensory interactions.

- The study presented in Chapter 4 investigated interactions between hop flavouring and bitter acids. Beer is a complex matrix and it is therefore required to investigate further interactions between further beer components and hop-derived volatiles, such as ethanol. In fact, a subsequent TI study was planned to investigate changes in sensory temporal profiles at varying alcohol concentrations (<0.5%, 4.5%, 8% ABV) in an unhopped base beer. This study would have started in April/May 2021 but was stopped due to the pandemic outbreak and further analysis of current data (Chapter 2B and Chapter 5) were conducted instead. The aim of the study was to investigate the impact of the solvation properties of ethanol on the perception of hop oil fractions. Ethanol is expected to significantly affect the retention of volatiles in the medium, to modify their threshold concentrations (King et al., 2013; Peltz & Shellhammer, 2017), thereby changes the interactions within the 'skeleton' of volatiles, and eventually impact their taste- and mouthfeel-modifying properties.
- Sensory profiles obtained for the sesquiterpene containing fractions and hop flavour product in Chapter 3 and 4 showed their ability to affect the perception and duration of bitterness and astringency in beer, but not in the ethanol solution (4%, ABV). Predictive models obtained from PLS regression suggested *α*-humulene and *β*-caryophyllene to be involved in the modulation of these characteristics. The effect might be concentration- or matrix-dependent. A better understanding of the mechanism triggering these characteristics is required (e.g.

modulated by cognitive effects or activation of a trigeminal-type receptor as previously found for eudesmol (Ohara et al., 2015)).

- GC coupled to a single quadrupole MS is the primary technique for the analysis of hop-derived volatiles and provided sufficient insights within the frame of this thesis project. However, more elaborated detectors with higher detection sensitivity may help to identify compounds at trace level or sub-detection level – particularly those at low ppb or even at ppt levels (ng/L). However, this requires advanced equipment. Thiols, aldehydes, and fatty acids could for instance be detected using a flame photometric detector (FPD), a pulsed flame photometric detector (PFPD) (Rettberg et al., 2018), a sulphur chemiluminescence detector (SCD) (Gijs et al., 2002), or a flame ionization detector (FID) (Perpète & Collin, 2000). Additionally, two-dimensional GC (GCxGC) approaches i.e. sequential separation on two stationary phases (polar, nonpolar) may help to improve the identification and quantification of co-eluting compounds.

  As has been emphasised throughout the thesis, the approach used for the analysis of the volatile fraction/oil composition was GC-MS operated in full scan mode for untargeted substance identification (Chapter 2A, 2B, and 3). This approach is sufficient for rapid characterisation of unknown compounds. Selective ion monitoring (SIM) should be used for selective and sensitive targeting of known compounds, which requires further development of the applied GC-MS method. If the number of analytes is then limited, this would also simplify the accurate quantification using external calibration i.e. reference compounds run separately at different concentrations in the same or a similar matrix.

- Finally, 'ordinary' PLS regression were used in this thesis (Chapter 2A and 3) with PLS1 individual models built for each sensory variable and PLS2 models for simultaneous predication of all variables. This approach is widely used to interpret sensory variables based on concentrations of volatile compounds. Following the collection of supporting sensory (e.g. thresholds data, quantitative temporal data) and physico-chemical data (e.g. headspace data, data collected from *in vivo* measurements, compositional data of non-volatile components, solubility parameters), this data could then be subjected to Multi-block (MB)-PLS regression to maintain the data blocks' natural structures, decipher causal relationships between these, and predict sensory variables including those suggested to be a product of matrix-dependent sensory interactions (Campos, Sousa, Pereira, & Reis, 2017).

6.4 Industrial impact

The research presented in this thesis lays the foundation for future developments of sustainably produced supercritical CO_2 fractions to be used as natural and clean label flavouring preparations. The outcome of each experimental study revealed taste- and mouthfeel-modifying properties of the hop oil fractions and an improved understanding of the underlying sensory interaction effects will enable brewers to better control and target specific multi-modal profiles in beer. Furthermore, the study presented in Chapter 4 demonstrated the modulation of bitterness qualities by the addition of hop oil fractions combined with either iso-alpha-acids or hulupones. This is valuable information for brewers since it shows how the sensory properties of

hop oil fractions can change depending on the beer matrix in which they are applied. Moreover, the dose rates applied in the base beers (Chapter 3 and 4) were kept in ranges close to concentrations that are usually applied in the brewing industry (lower g/hL level in lager); thus the trials could be transferred to industrial scale by adding the hop oil fractions and bitter acid extracts post-fermentation to the bright beer. This research also demonstrated the distinct organoleptic properties of the volatile fraction in Magnum hops, a high alpha hop variety. The concept of 'aroma and bitter hop varieties' may therefore lapse. The focus should rather be on stable and high yielding hop plants since advanced fractionation approaches can be used to extract aromatic fractions from every hop variety.

6.5 Main conclusions

In sum, the multi-modal perception of beer flavour modulated by specific hop oil fractions extracted from a Magnum hop variety was investigated. This research confirmed underlying sensory interaction effects within and across modalities, which equip the hop volatile mixtures with abilities to enhance or decrease specific 'green', 'fruity' and 'floral' aromas perceived ortho- and retronasally, and additionally induce or modify sweetness, bitterness qualities, astringency, and 'peppery tingling' trigeminal-type sensations in beer. This research provides further evidence on the role of specific compounds (e.g. linalool, geraniol, caryophyllene oxide) and compound groups (e.g. sesquiterpene alcohols, humulene epoxides) within the terpene alcohol and sesquiterpene fractions, which were previously suggested to play key roles in the perception of these sensory characteristics. The importance of

systematic sensory approaches, robust sensory techniques as well as temporal sensory analysis for adequate characterisation of complex hop oil fractions and to fully understand their multi-sensory profiles was highlighted. The assessment of hop oil extracts and hop-derived bitter acids (iso-alpha-acids, hulupones) in different combinations disclosed further interaction effects impacting sensory qualities and their evolution throughout the consumption of two sips of beer. Further matrix-dependent effects are suspected to occur if varying other beer components. However, this hypothesis needs to be confirmed in further studies.

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8 Appendices

8.1 Appendix 1 (Chapter 1)

Table 8.1. Hop essential oil compounds and derivates thereof contributing to the hoppy aroma and flavour in beer. Overview of hop oil and hop-derived compounds that were investigated using both sensory and quantitative instrumental analysis. Hop volatiles were quantified in beer and sensorially evaluated by sensory assessors (in the same study). Individual compounds could be attributed or at least associated with specific sensory sensations.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Hydrocarbons	Limonene	Citrus/fruity flavour	Liquid CO_2 extract	80-100	Ale	Flavour profile test, GC-MS	a
	Myrcene	Spicy, resinous flavour	Liquid CO_2 extract	200	Ale	Flavour profile test, GC-MS	a
		Metallic, geranium-like aroma	T90 pellets, Hallertauer Comet	tr-863.6	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-GC-FID	b
		Geranium leaf aroma	T90 pellets, Huell Melon	6.65-15.0	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	Terpinolene	Woody, resinous flavour	Liquid CO_2 extract	20	Ale	Flavour profile test, GC-MS	a
Sesquiter-penoids	Humulene monoepoxide I	Herbal/spicy flavour	Raw hop material, Cascade (C) and Hallertauer Mittelfrueh (HM)	tr (HM)	Beer (3.7% ABV)	Triangular tests, flavour profile analysis, Cap-GC-MS	d
	Humulene epoxide II	Cedar, lime flavour	Hop oil oxygenated fraction (Hallertauer)	17	Beer	SDE, GC-MS	e
Esters	Ethyl butanoate	Fruity aroma	CB	198	Pilsner	HRGC-O, HRGC-MS	f
		Fruity, estery aroma	CB	71-103	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Esters	Ethyl cinnamate	Fruity, sweet aroma	CB, fresh and aged	3 (fresh), 14 (aged)	Lager	GC-O, GC-MS-FID	h
	Ethyl hexanoate	Fruity aroma	CB	205	Pilsner	HRGC-O, HRGC-MS	f
		Fruity aroma	**CB**	129-206	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
		Fruity aroma	CB	123-156	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	Ethyl octanoate	Fruity aroma	CB	160	Pilsner	HRGC-O, HRGC-MS	f
		Fruity aroma	CB	157-220	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
		Fruity aroma	CB	476-881	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	Ethyl 2-methylpropanoate	Citrus, pineapple, sweet aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	3.98 (S), 8.01 (HB), 6.30 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
		Fruity aroma	CB	1.9-2.0	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
		Fruity, sweetie aroma	T90 pellets, Hallertauer Comet	11.8-18.7	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-SPME-GC-MS	b
		Fruity aroma	T90 pellets, Huell Melon	11.3-57.2	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	Ethyl 2-methylbutanoate	Citrus, apple-like aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	1.67 (S), 1.83 (HB), 1.20 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
		Fruity, sweetie aroma	T90 pellets, Hallertauer Comet	1.1-1.9	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-SPME-GC-MS	b
		Fruity aroma	T90 pellets, Huell Melon	0.761-5.40	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Esters	Ethyl 3-methylbutanoate	Citrus, sweet, apple-like aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	5.32 (S), 2.66 (HB), 2.13 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
		Fruity aroma	CB	0.61-0.85	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
		Fruity, sweetie, berry-like aroma	T90 pellets, Hallertauer Comet	1.5-1.8	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-SPME-GC-MS	b
	Ethyl 4-methylpentanoate	Sweet, fruity aroma	T90 pellets, Hallertauer Comet	0.5-0.9	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-SPME-GC-MS	b
	Geranyl acetate	Fruity flavour	Liquid CO_2 extract	15	Ale	Flavour profile test, GC-MS	a
	Methyl 2-methylbutaonate	Fruity aroma	T90 pellets, Huell Melon	8.17-9.90	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	Propyl 2-methylbutanoate	Fruity aroma	T90 pellets, Huell Melon	0.338-0.633	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	2-phenylethyl 3-methylbutanoate	Floral, minty aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	3.05 (S), 1.53 (HB), 2.46 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
	3-methylbutyl acetate	Fruity, banana-like aroma	CB	1910-4390	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
		Fruity, sweet, solvent-like aroma	CB	2190-7820	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
Ketones	*β*-damascenone	Cooked apple flavour	CB	1.6	Beer	SIDA, HRGC-O	k
		Rhubarb, red fruits, strawberry aroma	CB, fresh and aged	3 (fresh), 9 (aged)	Lager	GC-O, GC-MS-FID	h
		Honey-like aroma	CB	2.3	Pilsner	HRGC-O, HRGC-MS	f
		Cooked apple-like aroma	CB	1.29-3.60	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Ketones	*β*-damascenone	Floral, fruity, honey-like aroma	CB	1.3	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	β-ionone	Floral, violet-like, berry aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	0.16 (S), 0.18 (HB), 0.15 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
	2-undecanone	Floral flavour	Liquid CO_2 extract	15	Ale	Flavour profile test, GC-MS	a
	4-(4-hydroxyphenyl)-2-butanone	Citrus, raspberry aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	2.29 (S), 1.88 (HB), 1.37 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
Monoterpene Alcohols	Citronellol	Floral/citrus flavour	Raw hop material, Cascade (C) and Hallertauer Mittelfrueh (HM)	52.2 (HM), 8.1 (C)	Beer (3.7% ABV)	Triangular tests, flavour profile analysis, Cap-GC-MS	d
	Geraniol	Floral/citrus flavour	Raw hop material, Cascade (C) and Hallertauer Mittelfrueh (HM)	0.9 (HM), 2.3 (C)	Beer (3.7% ABV)	Triangular tests, flavour profile analysis, Cap-GC-MS	d
		Geranium, floral flavour	Liquid CO_2 extract	5	Ale	Flavour profile test, GC-MS	a
		Floral, rose-like aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	8.15 (S), 7.37 (HB), 12.4 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
		Citrusy aroma	CB	5.7-6.8	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
		Floral, flowery aroma	T90 pellets, Sorachi Ace (SA), Hallertauer Tradition (HT)	70 (SA), 60 (HT)	Beer	GC-O, HS-SPME-GC-MS	l
		Floral, rose aroma	T90 pellets, Huell Melon	6.96-31.6	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	Linalool	Floral/citrus flavour	Raw hop material, Cascade (C) and Hallertauer Mittelfrueh (HM)	17.9 (HM), 17.7 (C)	Beer (3.7% ABV)	Triangular tests, flavour profile analysis, Cap-GC-MS	d

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Monoterpene Alcohols	Linalool	Floral flavour	Liquid CO_2 extract	40	Ale	Flavour profile test, GC-MS	a
		Flowery, citrus-like aroma	CB	45	Pilsner	HRGC-O, HRGC-MS	f
		Floral, citrus, terpenic aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	30.3 (S), 70.5 (HB), 53.9 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
		Flowery, citrus-like aroma	CB	2.79-10.7	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
		Citrusy aroma	CB	2.3-2.4	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
		Fresh, sweet, citrus aroma	T90 pellets, Hallertauer Comet	28.6-58.4	Beer (5.8%, 20 BU)	HS-SPME-GC-MS-O, HS-SPME-GC-MS	b
		Floral, acrid aroma	T90 pellets, Sorachi Ace (SA), Hallertauer Tradition (HT)	16 (SA), 5.2 (HT)	Beer	GC-O, HS-SPME-GC-MS	l
		Citrusy, floral aroma	T90 pellets, Huell Melon	28.3-56.3	Beer (4.9-5.4%)	GC-O, GC-GC-MS (CI)	c
	Methylpropanol	Malty aroma	CB	14500-23100	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
	α-terpineol	Herbal/spicy flavour	Raw hop material, Cascade (C) and Hallertauer Mittelfrueh (HM)	8.4 (HM), 7.8 (C)	Beer (3.7% ABV)	Triangular tests, flavour profile analysis, Cap-GC-MS	d
		Woody, resinous flavour	Liquid CO_2 extract	5	Ale	Flavour profile test, GC-MS	a
	(*Z*)-3-hexen-1-ol	Green aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	17.6 (S), 18.6 (HB), 27.7 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j
	1-propanol	Sweet, solvent, metallic aroma	CB	13400-14800	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Monoterpene Alcohols	2-methylbutanol	Malty aroma	CB	14400	Pilsner	HRGC-O, HRGC-MS	f
		Solvent-,metallic-like aroma	CB	10900-18100	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	2-methylpropanol	Solvent-like aroma	CB	6130-8990	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	3-methylbutanol	Malty aroma	CB	49600	Pilsner	HRGC-O, HRGC-MS	f
		Malty aroma	CB	54300-58300	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
		Solvent-,metallic-like aroma	CB	44900-72400	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	2-phenylethanol	Flowery, honey-like aroma	CB	15100	Pilsner	HRGC-O, HRGC-MS	f
		Flowery aroma	CB	21100-27200	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
	4,8,11,11-tetramethyltricyclo[7.2.0.0^{2,4}]undecane-5,8-diol	Cedar, lime, banana, pineapple, spicy flavour, spicy/trigeminal sensations	Hop oil oxygenated fraction (Hallertauer), CB	600 (H), 754 (CB, pale ale), 430 (BC, light ale)	Beer, pale and light ale	SDE, GC-MS	e
Aldehydes	1-hexanal	Green, leafy aroma	Hop pellets, Saaz (S), Cascade (C), Hersbrucker (HB)	16.9 (S), 14.2 (HB), 14.3 (C)	Beer	GC-O, flavour profile analysis, SBSE, GC-MS	j

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Sulphur- containing compounds	Dimethyl trisulfide	Geranium, earthy, potato aroma	CB, fresh and aged	0.16 (fresh), 0.32 (aged)	Lager	GC-O, GC-MS-SCD	h
	Dimethyl sulphide	Canned maize aroma	CB	59	Pilsner	HRGC-O, HRGC-MS	f
		Sulphury aroma	CB	345-456	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	Phenylethyl acetate	Fruity, floral, soapy aroma	CB	173-889	Pilsner	GC-O/MS, Purge and Trap GC-O/MS, SPME GC-MS	g
	1-sulfanylpentan-3-ol	Mushroom, nettle aroma	Tomahawk hop extract and pellets	178	Beer	GC-O, GC-MS, GC-FID	m
	1-sulfanylpentan-3-one	Green, mineral aroma	Tomahawk hop extract and pellets	28.9	Beer	GC-O, GC-MS, GC-FID	m
	1-sulfanyl-3-butyl acetate	Plastic, sprout aroma	Tomahawk hop extract and pellets	6.1	Beer	GC-O, GC-MS, GC-FID	m
	2-phenylethyl acetate	Flowery aroma	CB	518-560	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
	2-sulfanylethan-1-ol	Soup, grilled, gas aroma	Tomahawk hop extract and pellets	32.5	Beer	GC-O, GC-MS, GC-FID	m
	2-sulfanylethyl acetate	Burnt, grill aroma	Tomahawk hop extract and pellets	5609	Beer	GC-O, GC-MS, GC-FID	m
	3-(methylthio)propanal	Cooked, potato-like aroma	CB	1550-4490	Wheat beer	HRGC-O, HRGC-MS, aroma profile analysis	i
	3-methyl-2-buten-1-thiol	Coffee, skunky aroma	Tomahawk hop extract and pellets	327.6	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylbutan-1-ol	Perspiration, catty aroma	Tomahawk hop extract and pellets	12.3	Beer	GC-O, GC-MS, GC-FID	m

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Sulphur- containing compounds	3-sulfanylbutyl acetate	Cheese, onion aroma	Tomahawk hop extract and pellets	32.4	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylheptanal	Lemon, candy aroma	Tomahawk hop extract and pellets	19.1	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylheptan-1-ol	Lemon, hoppy aroma	Tomahawk hop extract and pellets	54.0	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylhexanal	Flowery, lemon aroma	Tomahawk hop extract and pellets	18.3	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylhexan-1-ol	Grapefruit aroma	Tomahawk hop extract and pellets	115.1	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylhexyl acetate	Candy, pumpkin aroma	Tomahawk hop extract and pellets	tr	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyloctan-1-ol	Catty, grapefruit aroma	Tomahawk hop extract and pellets	78.8	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylpentanal	Hoppy, flower aroma	Tomahawk hop extract and pellets	5.2	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylpentan-1-ol	Catty, citrus aroma	Tomahawk hop extract and pellets	3.4	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylpropan-1-ol	Potatoes, popcorn aroma	Tomahawk hop extract and pellets	10.3	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanylpropyl acetate	Grilled aroma	Tomahawk hop extract and pellets	64.6	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-butylpropanal	Chicken soup, plastic aroma	Tomahawk hop extract and pellets	tr	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-ethylpropyl acetate	Floral, vinegar aroma	Tomahawk hop extract and pellets	183.1	Beer	GC-O, GC-MS, GC-FID	m

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Sulphur- containing compounds	3-sulfanyl-2-methylbutan-1-ol	Leek, hop aroma	Tomahawk hop extract and pellets	tr	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-methylbutan-1-thiol	Mushroom, white fruits aroma	Tomahawk hop extract and pellets	8.4	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-methylbutyl acetate	Cooked meat aroma	Tomahawk hop extract and pellets	37.3	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-methylpentan-1-ol	Gravy aroma	Tomahawk hop extract and pellets	8.1	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-methylpropan-1-ol	Broth, leek aroma	Tomahawk hop extract and pellets	23.9	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-2-methylpropyl acetate	Popcorn, grilled nut aroma	Tomahawk hop extract and pellets	3.9	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-3-methylbutan-1-ol	Sulphur, soup aroma	Tomahawk hop extract and pellets	165.9	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-3-methylbutyl acetate	Pepper, plastic aroma	Tomahawk hop extract and pellets	40.4	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-4-methylpentan-1-ol (3S4MP)	Grapefruit, rhubarb aroma	Hop pellets, Nelson Sauvin (NS), Saaz (S)	1.8 (S), 92.5 (NS)	Beer, carbonated 5% EtOH/H_2O	GC-O, GC-FPD, GC-MS, triangular tests	n
		Rhubarb, grapefruit aroma	Tomahawk hop extract and pellets	48.2	Beer	GC-O, GC-MS, GC-FID	m
	3-sulfanyl-4-methylpentyl acetate (3S4MPA)	Grapefruit aroma	Tomahawk hop extract and pellets	48.2	Beer	GC-O, GC-MS, GC-FID	m
		Grapefruit, rhubarb aroma	Hop pellets, NS	25	Beer, carbonated 5% EtOH/H_2O	GC-O, GC-FPD, GC-MS, triangular tests	n
	4-sulfanyl-4-methylpentan-1-ol	Curry, celery aroma	Tomahawk hop extract and pellets	tr	Beer	GC-O, GC-MS, GC-FID	m

Table 8.1 continued.

Class	Compound	Aroma/flavour	Source of hop oil compounds	µg/L (ppb) in test matrix	Test matrix	Sensory/ instrumental methods	Reference
Sulphur- containing compounds	4-sulfanyl-4-methylpentan-2-ol	Fuel, catty aroma	Tomahawk hop extract and pellets	5.6	Beer	GC-O, GC-MS, GC-FID	m
	4-sulfanyl-4-methylpentan-2-one (4MMP)	Catty, blackcurrant aroma	Tomahawk hop extract and pellets	22.6	Beer	GC-O, GC-MS, GC-FID	m
		Fruit, black-currant-like aroma	Hop pellets, Simcoe (SIM), Summit (SUM), Apollo (APO), Magnum (MG), Cascade (C)	183.5 (SIM), 116.4 (SUM), 109.2 (APO), 16.9 (C)	Beer	GC-O (SAFE), MD-GC-MS, sensory evaluation	o
	4-sulfanyl-4-methyl-2-pentyl acetate	Grilled nut aroma	Tomahawk hop extract and pellets	3.6	Beer	GC-O, GC-MS, GC-FID	m
	6-sulfanylhexan-1-ol	Mushroom, flowers, gaz aroma	Tomahawk hop extract and pellets	15.1	Beer	GC-O, GC-MS, GC-FID	m

Abbreviations: ABV, alcohol by volume; C, Cascade; CB, commercial beer; CI, chemical ionisation; FID, Flame ionisation detector; FPD, Flame photometric detection; GC-MS, Gas chromatography–mass spectrometry; GC-O, GC-Olfactometry; HM, Hallertauer Mittelfrueh; H, Hallertauer; HB, Hersbrucker; HRGC-O, high resolution GC-O; MD, multidimensional; MG, Magnum; np, not provided; NS, Nelson Sauvin; ppb, parts per billion; S, Saaz; SBSE, Stir bar sorptive extraction; SCD, Sulphur chemiluminescence detection; SDE, sensory descriptive analysis; SIDA, stable isotope dilution assay; SIM, Simcoe; SPME; Solid-Phase Microextraction; SUM, Summit; tr, concentration at trace level or detected below quantification limit; 3S4MP, 3-sulfanyl-4-methylpentan-1-ol ; 3S4MPA, 3-sulfanyl-4-methylpentyl acetate; 4MMP, 4-mercapto-4-methyl-pentan-2-one

[a] Sharpe (1988); [b] Schnaitter et al. (2016); [c] Neiens and Steinhaus (2018); [d] Lam, Foster, et al. (1986); [e] Yang et al. (1993a); [f] Fritsch and Schieberle (2005); [g] Tokita et al. (2014); [h] Gijs et al. (2002); [i] Langos et al. (2013), [j] Kishimoto et al. (2006); [k] Schieberle (1991); [l] Sanekata et al. (2018); [m] Gros et al. (2011); [n] Takoi, Degueil, Shinkaruk, Thibon, Maeda, et al. (2009); [o] Kishimoto et al. (2008)

8.2 Appendix 2 (Chapter 4)

8.2.1 Base beer production and analysis

Base beer production

A lager-type base beer (4.5% v/v) was brewed in the AB InBev research brewery at the International Centre for Brewing Science (ICBS) of the University of Nottingham. Briefly, brewing was conducted as follows: commercially available pale malt (135 kg, Crisp Malt, Norfolk, UK) milled to 0.6 mm, glucose (25 kg; Murphy & Son, Northampton, UK); brewing water: reverse osmosis with addition of $CaCl_2$ (125 mg/L; Murphy & Son Northampton, UK); mashing scheme: mash in at 52°C (35 min, 63°C (40 min), 73°C (25 min), mash out at 78°C, addition of lactic acid for pH adjustment to pH 5.4 in the 63°C stand (Murphy & Son Northampton, UK); wort boiling 60 min with whirlpool time of 20 min (evaporation: 10%); wort clarification: whirlpool with addition of 3 g/hL Koppakleer (Murphy & Son Northampton, UK); original gravity: 14.27 °P; pitching rate of minimum 6 x 10^6 cells/mL when pitched at 100 g/hL (*saccharomyces pastorianus*; SafLager W-34/70 Fermentis, Lesaffre, France), addition of $ZnCl_2$ (0.122 mg/L; Murphy & Son Northampton, UK); fermentation temperature and duration 13°C, 14 days; post-fermentation separation of the beer ferment into three 3 hL batches for separate addition of bitter acid extracts (ISO, NISO, HULU) to one of the three batches to achieve 27 International Bitterness Units (IBU; equivalent to 27 mg/L or 27 ppm iso-alpha-acids in solution), respectively, by weighing each extract into a Cornelius vessel and pre-mixing with bright beer for subsequent injection into the batches; beer filtration: cellulose cartridge filter (1 µm); beer bottling at 2°C; beer pasteurisation: 20 pasteurisation units (PU); bottles prepared per treatment according to the amount needed for preliminary tests, panel training, and sensory and instrumental beer analyses (100 x ISO, 500 x NISO, 500 x HULU); bottle storage until further usage in cold store at 4°C.

Base beer analysis

Finished beer analyses included alcohol by volume (ABV; % v/v), density, final gravity (FG; %w/w) determined using an Anton Paar Alcolyzer and DMA4500 (Graz, Austria), pH determined using a Metler Toledo FiveGo pH meter (Columbus, Ohio, USA), IBU, total polyphenol content (TPC) and beer colour determined using a UV/Vis Spectrophotometer (7315 UV/visible Spectrophotometer, Jenway, UK) according to the ASBC methods Beer-23A (ASBC, 2011), Beer-35 (ASBC, 1978) and Beer-10A (ASBC, 2006), respectively. ANOVA was performed followed by a comparison of means

calculated by Tukey's HSD post-hoc test to identify significant differences between beer bases.

Statistical analysis

Analysis of Variance (ANOVA) was performed followed by a comparison of means calculated by Tukey's Honest Significant Difference (HSD) post-hoc test to identify significant differences between beer bases.

Results

The outcome of the finished beer analysis can be found in Table 8.2. Significant differences were only observed regarding the beer colour, with the hulupone-bittered beer being slightly darker but still pale yellow. Since all samples were served in amber bottles, an effect of the colour on the perception of other sensory characteristics was prevented. All other parameters did not significantly differentiate between the bittered base beers.

Table 8.2. Finished base beer analysis data (two replicate measurements (n=2)).

Sample	FG (% w/w)	ABV (% v/v)	Density (g/cm^3)	pH	IBU	TPC (mg/L)	Colour (^{o}SRM)
ISO	1.007 a	4.425 a	1.005 a	4.590 a	26.50 a	144.457 a	3.061 a
NISO	1.007 a	4.440 a	1.005 a	4.570 a	25.85 a	135.573 a	3.042 a
HULU	1.007 a	4.540 a	1.005 a	4.580 a	n/a *	144.593 a	3.490 b

ABV, alcohol by volume; IBU, International Bitterness Units (indicating µg/L iso-alpha-acids (isohumulones) in beer); FG, final gravity; SRM, standard reference method; TPC, total polyphenol content

[ab] Different superscripts within a column represent a significant difference among beer samples at (Tukey's HSD, p<0.05)

* IBUs are not measured for the HULU base beer since it was produced without iso-alpha-acid extract or raw hops.

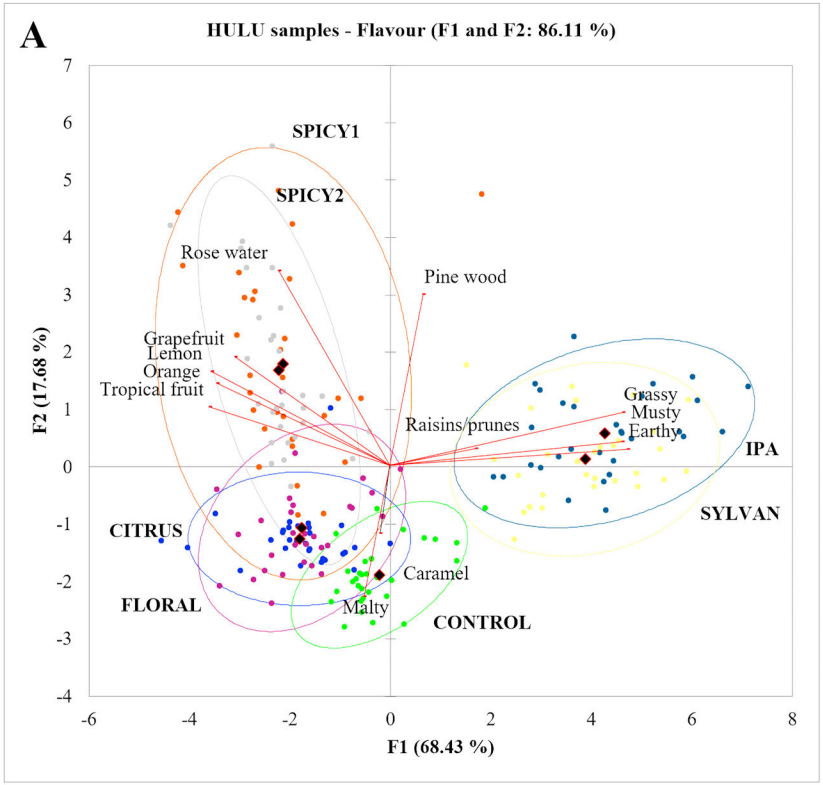
References

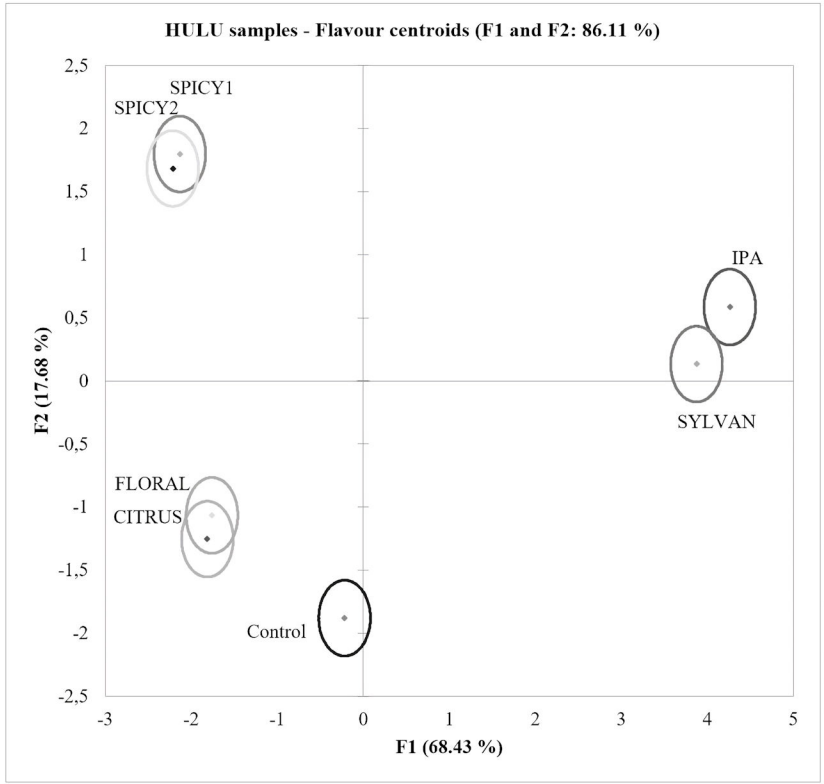
ASBC Method of Analysis. (1978). American Society of Brewing Chemists. Total Polyphenol, Beer-35. The Society: St Paul, MN, U.S.A.

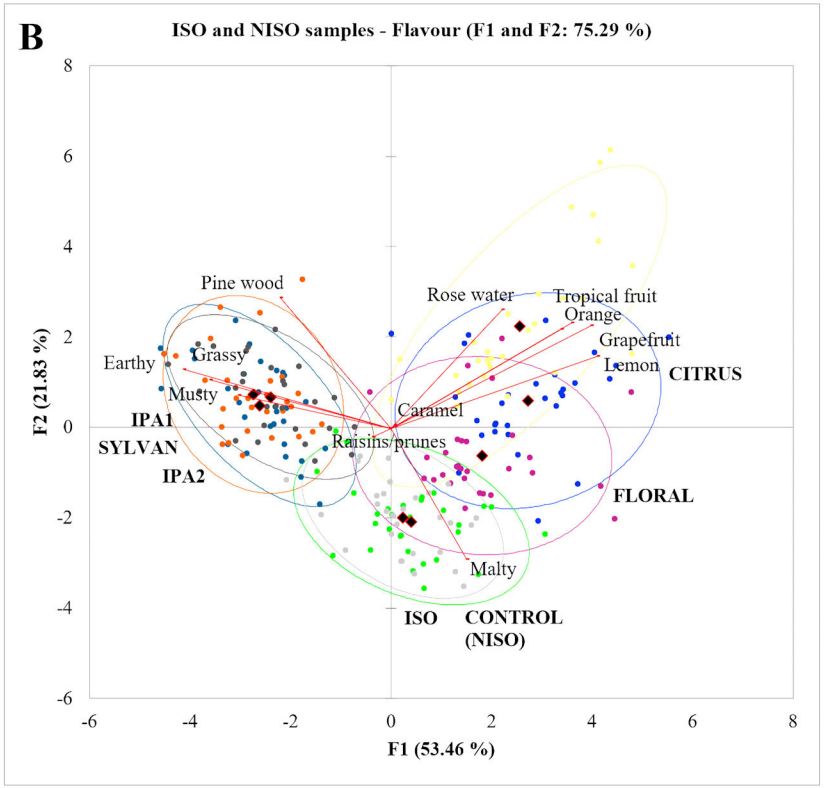
ASBC Method of Analysis. (2011). American Society of Brewing Chemists. Beer Bitterness, Beer-23A. The Society: St Paul, MN, U.S.A.

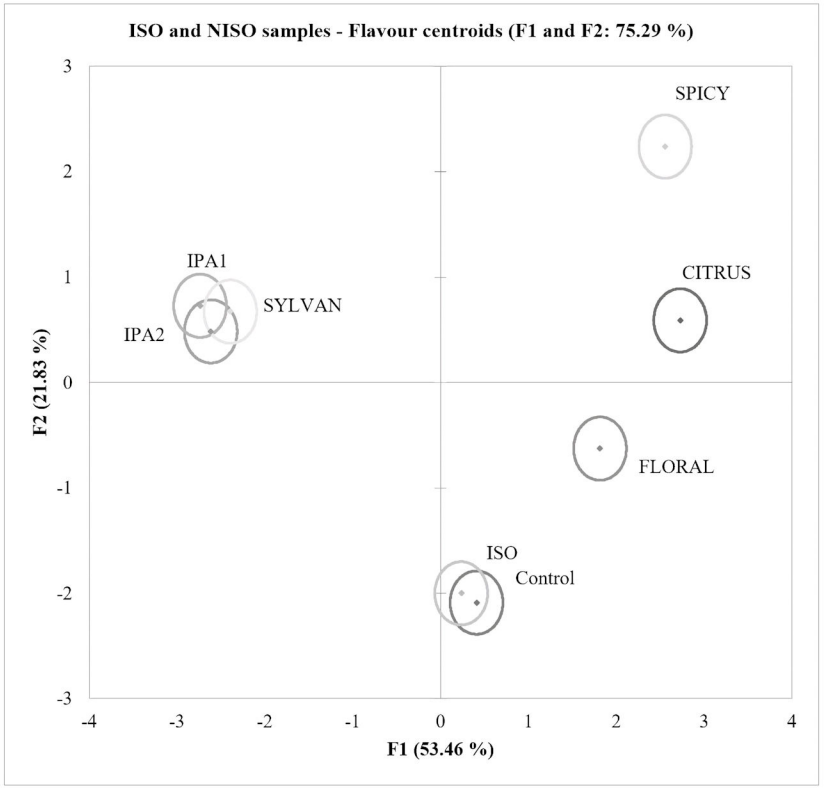
ASBC Methods of Analysis (2006). American Society of Brewing Chemists. Beer Color, Beer-10A The Society: St. Paul, MN, U.S.A.

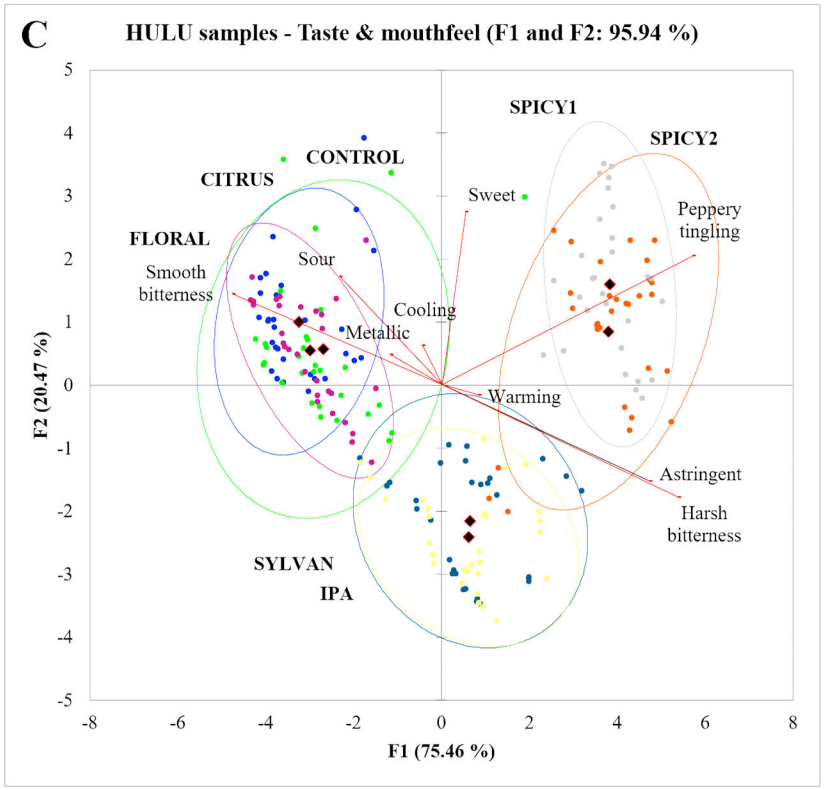
8.2.2 Figures

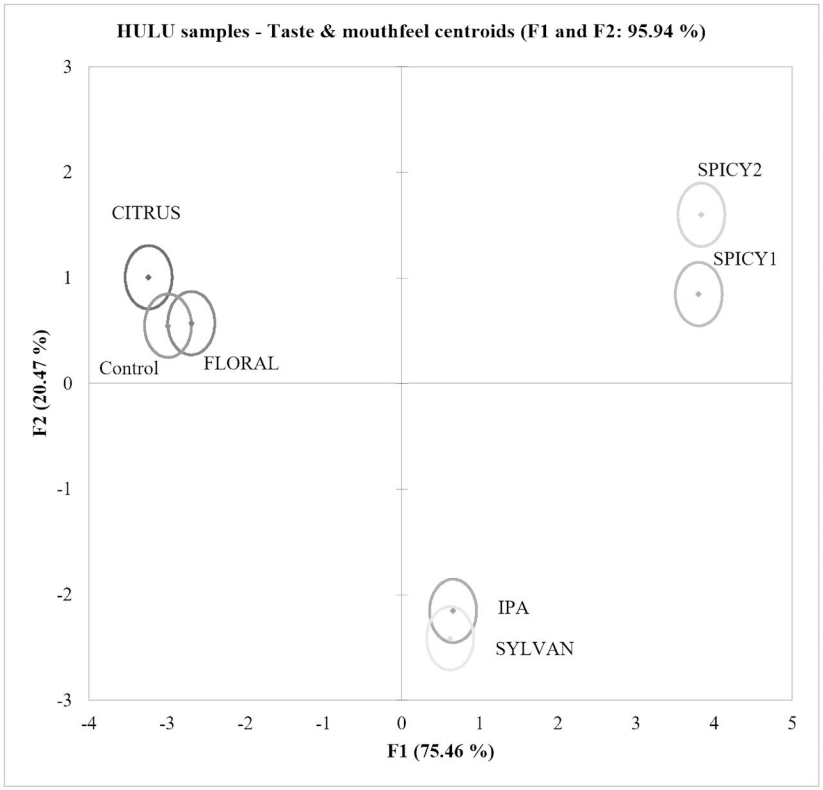

A
HULU samples - Flavour (F1 and F2: 86.11 %)
SPICY1
SPICY2
Rose water
Pine wood
Grapefruit
Lemon
Orange
Tropical fruit
Raisins/prunes
Grassy
Musty
Earthy
IPA
SYLVAN
CITRUS
FLORAL
Caramel
Malty
CONTROL
F2 (17.68 %)
F1 (68.43 %)


HULU samples - Flavour centroids (F1 and F2: 86.11 %)
SPICY1
SPICY2
IPA
SYLVAN
FLORAL
CITRUS
Control
F2 (17.68 %)
F1 (68.43 %)


B
ISO and NISO samples - Flavour (F1 and F2: 75.29 %)
F2 (21.83 %)
F1 (53.46 %)
Pine wood
Rose water
Tropical fruit
Orange
Grapefruit
Lemon
CITRUS
Earthy
Grassy
Musty
IPA1
SYLVAN
IPA2
Caramel
Raisins/prunes
FLORAL
Malty
ISO
CONTROL
(NISO)


ISO and NISO samples - Flavour centroids (F1 and F2: 75.29 %)
F2 (21.83 %)
F1 (53.46 %)
SPICY
IPA1
SYLVAN
IPA2
CITRUS
FLORAL
ISO
Control


C
HULU samples - Taste & mouthfeel (F1 and F2: 95.94 %)
SPICY1
SPICY2
CONTROL
CITRUS
FLORAL
Smooth bitterness
Sour
Sweet
Peppery tingling
Cooling
Metallic
Warming
Astringent
Harsh bitterness
SYLVAN
IPA
F2 (20.47 %)
F1 (75.46 %)


HULU samples - Taste & mouthfeel centroids (F1 and F2: 95.94 %)
SPICY2
CITRUS
SPICY1
Control
FLORAL
IPA
SYLVAN
F2 (20.47 %)
F1 (75.46 %)

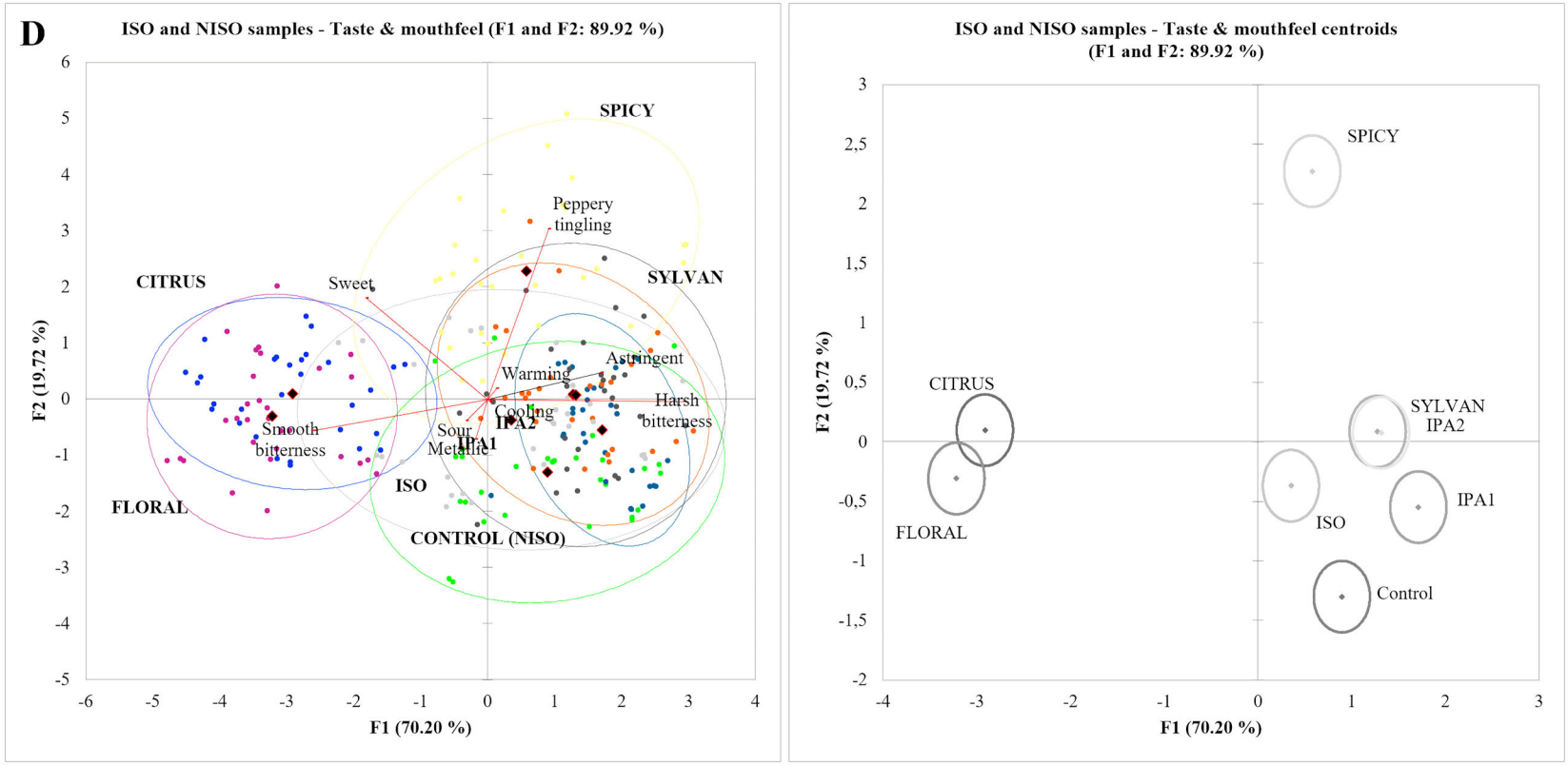


Figure 8.1. Canonical Variate Analysis (CVA) maps and centroid maps from total duration data within beer bases showing the discrimination between samples and heterogeneity of the panel. Plots computed for flavour (**A, B**) and taste and mouthfeel profiles (**C, D**).

8.2.3 Tables

Table 8.3. Overview of sensory attributes, definitions, and training reference standards.

Modality	Sensory attribute	Definition	Physical reference standard
Flavour	Malty	Malty flavour as in malt loaf, marmite, toasted malt, Shreddies	10 g malt extract (Holland & Barrett, UK); 10 g Soreen malt loaf; 3 pieces Shreddies (Nestlé, UK)
	Lemon	Lemon flavour as in lemon or lime fruits; pith, zest (including artificial lemon)	5 g freshly chopped lemon and lime, piece of citrus wet wipe (Dettol, UK)
	Raisins/ prunes	Raisin/prune flavour as in prunes, raisins, dried fruits or stewed fruits or mincemeat	10 g chopped raisins and prunes (Sainsbury's Supermarkets Ltd., UK)
	Earthy	Earthy flavour as when smelling wet earth, damp soil	10 g fresh wet earth, soil
	Grapefruit	Grapefruit flavour as in grapefruit; pith, zest	5 g freshly cut grapefruit
	Grassy	Grassy flavour as when smelling crushed grass, sap	20 g crushed cut grass and sap that has been left in the closed sample bottles for two days
	Tropical fruit	Tropical fruit flavour as in tropical fruit juice (mango, pineapple, melon, peach)	10 mL tropical fruit juice (Tropicana, UK; 100% pure pressed fruit from apples, orange, pineapple, passionfruit, mango)
	Musty	Musty flavour as when smelling the old sponge reference	Damp, old sponge; sponge that has been left for 24h in the closed sample bottle
	Orange	Orange citrus fruit flavour as in round, “sweet” orange, mandarin and tangerine	5 g freshly cut orange (flesh, peel)
	Pine wood	Pine wood flavour as when smelling pine wood, pine shavings	10 g pine shavings (Sainsbury's Supermarkets Ltd., UK); 5 mL 6 mg/L (1R)-(+)-α-pinene (food grade; Sigma Aldrich, UK) in deionised water
	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	½ piece (5 g) Turkish delight (Sainsbury's Supermarkets Ltd., UK); 5 mL 0.6% (w/v) geranium essential oil (Ecodrop, UK) in deionised water
	Caramel	Caramel flavour as in caramel sauce or toffee	10 g toffee sauce (Sainsbury's Supermarkets Ltd., UK)
Taste	Sweet	Sweet taste as in the sweet reference solutions	10 mL 1% (w/v) sucrose (Sainsbury's Supermarkets Ltd., UK); 10 mL 2 % (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Sour	Sour, acidic taste as when eating a fresh lemon; sour, mouth-watering, puckering sensation	10 mL 0.2% (v/v) citric acid (Sigma Aldrich, UK) ; 10 mL 2% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionized water; 2 mL freshly pressed lemon juice

Table 8.3. continued.

Modality	Sensory attribute	Definition	Physical reference standard
	Metallic	Metallic taste as the taste of cans or coins	10 mL 0.00475 g/L iron (II) sulphate heptahydrate (Sigma Aldrich, UK)
	Harsh bitterness	Harsh or irritating, scratchy, spiky bitterness	1 % (w/v) caffeine (food grade; Sigma Aldrich, UK) in deionised water; 10 mL 3 mg/L HopAlpha® NISO25%[1] (Totally Natural Solutions Ltd., UK) in deionised water
	Smooth bitterness	Smooth or mellow, soft bitterness	0.3% (w/v) caffeine (food grade; Sigma Aldrich, UK) in deionised water; 10 mL 4 mg/L HopAlpha® HULU30%[1] (Totally Natural Solutions Ltd., UK) in deionised water
Mouthfeel, trigeminal sensations	Astringent	Astringent or mouth drying, rough, puckering, furry sensation as when drinking black tea or eating banana peel	10 mL 1% (w/v) tannic acid (Alfa Aesar, US) in deionised water; 10 g banana peel; 10 mL English Breakfast tea served at room temperature, tea bag left to steep for five minutes (Twinings, UK)
	Peppery tingling	Peppery tingling sensation as when eating mild chilli, fresh ginger, horse radish; irritating, itching, stinging sensation (not related to carbonation)	2 mg/L HopShot® Spicy[1] in deionised water (Totally Natural Solutions Ltd., UK)
	Warming	Warming sensation in mouth, back of throat, oesophagus	10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Cooling	Cooling sensation in mouth, back of throat, oesophagus	10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water

[1]Hop acid or fraction in propylene glycol

Table 8.4. Average TCATA panel performance indices for the flavour and taste and mouthfeel (TMF) attributes obtained for panel agreement and repeatability, with indices approaching 1 indicating perfect agreement or repeatability.

Modality	Sensory attribute	Agreement	Repeatability
Flavour	Malty	0.630	0.740
	Lemon	0.677	0.757
	Raisins/prunes	0.821	0.855
	Earthy	0.748	0.836
	Grapefruit	0.708	0.805
	Grassy	0.798	0.836
	Tropical fruit	0.797	0.829
	Musty	0.796	0.807
	Orange	0.683	0.783
	Pine wood	0.675	0.746
	Rose water	0.828	0.869
	Caramel	0.865	0.916
Taste	Sweet	0.689	0.852
	Sour	0.622	0.843
	Metallic	0.619	0.889
	Harsh bitterness	0.761	0.778
	Smooth bitterness	0.669	0.728
Mouthfeel, trigeminal sensations	Astringent	0.611	0.839
	Peppery tingling	0.702	0.834
	Warming	0.633	0.839
	Cooling	0.746	0.931

Table 8.5. Mean durations (0.1 s) within time segments as evaluated using TCATA flavour and taste & mouthfeel attributes. Different letters within columns representing significant differences among the samples based on differences in LS means (p<0.05).

Flavour attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Caramel		ISO	0.933 de	6.600 b	1.233 b	26.200 b	0.000 b	34.967 b
		NISO	10.433 bcde	49.233 b	12.700 b	53.933 b	0.000 b	126.300 b
		HULU	28.300 a	184.867 a	53.533 a	146.700 a	17.133 a	413.400 a
	CITRUS	NISO	7.867 bcde	15.367 b	6.267 b	27.067 b	0.000 b	56.567 b
		HULU	15.733 abcd	80.000 b	13.533 b	71.767 ab	0.000 b	181.033 b
	FLORAL	NISO	5.900 cde	6.967 b	1.300 b	21.133 b	0.000 b	35.300 b
		HULU	21.400 ab	68.500 b	13.233 b	49.800 b	0.000 b	152.933 b
	IPA	NISO1	0.000 e	47.600 b	6.100 b	12.967 b	0.000 b	66.667 b
		NISO2	4.000 cde	61.467 b	1.367 b	18.867 b	0.000 b	85.700 b
		HULU	11.567 bcde	74.933 b	16.600 b	47.667 b	0.000 b	150.767 b
	SPICY	NISO	5.000 cde	58.567 b	6.867 b	28.933 b	0.000 b	99.367 b
		HULU1	16.333 abc	63.333 b	17.100 b	62.367 b	0.000 b	159.133 b
		HULU2	9.433 bcde	58.700 b	8.033 b	41.533 b	0.000 b	117.700 b
	SYLVAN	NISO	0.000 e	11.100 b	12.033 b	16.667 b	0.000 b	39.800 b
		HULU	15.467 abcd	41.300 b	11.967 b	56.267 b	0.000 b	125.000 b
Earthy		ISO	0.000 d	80.133 ef	5.567 e	60.933 cd	0.000 a	146.633 e
		NISO	4.967 cd	85.367 ef	3.833 e	58.800 cd	0.000 a	152.967 e
		HULU	2.433 d	33.133 f	0.267 e	23.900 d	0.000 a	59.733 e
	CITRUS	NISO	9.333 bcd	154.133 de	16.133 de	144.900 bc	0.733 a	324.500 d
		HULU	0.533 d	75.233 ef	1.333 e	18.000 d	0.000 a	95.100 e
	FLORAL	NISO	0.167 d	70.567 ef	0.000 e	59.133 cd	0.000 a	129.867 e
		HULU	0.067 d	55.900 ef	2.600 e	56.367 cd	0.000 a	114.933 e
	IPA	NISO1	1.833 d	245.367 cd	36.767 cd	296.367 a	19.367 a	580.333 c
		NISO2	16.167 abc	358.300 ab	42.733 bc	328.800 a	9.267 a	746.000 ab
		HULU	12.233 abcd	248.367 cd	63.133 ab	300.067 a	9.233 a	623.800 bc
	SPICY	NISO	0.000 d	0.000 f	0.000 e	0.000 d	0.000 a	0.000 e
		HULU1	0.000 d	26.400 f	0.000 e	15.600 d	0.000 a	42.000 e
		HULU2	0.000 d	0.000 f	0.000 e	0.000 d	0.000 a	0.000 e
	SYLVAN	NISO	18.567 ab	463.033 a	74.000 a	289.267 a	13.800 a	844.867 a
		HULU	22.633 a	344.733 bc	65.700 a	253.733 ab	1.767 a	686.800 bc
Grapefruit		ISO	11.400 bcde	115.567 cd	4.700 e	53.567 c	0.000 a	185.233 de
		NISO	3.133 de	49.733 cd	0.033 e	14.167 c	0.000 a	67.067 de
		HULU	0.000 e	0.000 d	0.000 e	0.000 c	0.000 a	0.000 e
	CITRUS	NISO	20.667 ab	470.133 a	80.967 a	338.633 a	16.233 a	910.400 a
		HULU	17.133 abcd	303.833 b	40.333 c	294.700 a	17.267 a	656.000 b
	FLORAL	NISO	19.800 abc	151.433 c	30.500 cd	276.700 ab	16.000 a	478.433 c
		HULU	7.400 bcde	333.500 b	69.233 ab	300.767 a	25.667 a	710.900 b
	IPA	NISO1	3.000 de	122.333 c	2.267 e	24.333 c	0.000 a	151.933 de
		NISO2	1.033 e	48.900 cd	6.667 de	71.067 c	18.267 a	127.667 de
		HULU	8.433 bcde	88.567 cd	2.733 e	74.767 c	0.000 a	174.500 de
	SPICY	NISO	20.233 ab	450.733 a	67.833 ab	346.533 a	13.800 a	885.333 a
		HULU1	12.967 bcde	418.567 ab	53.600 bc	409.900 a	28.700 a	895.033 a
		HULU2	28.500 a	464.733 a	51.233 bc	359.567 a	33.967 a	904.033 a
	SYLVAN	NISO	1.667 e	3.767 d	3.533 e	15.333 c	0.000 a	24.300 e
		HULU	5.267 cde	124.200 c	10.033 de	139.400 bc	35.433 a	278.900 d
Grassy		ISO	9.567 bc	35.467 b	7.867 c	58.167 c	0.000 a	111.067 b
		NISO	0.900 c	26.700 b	1.967 c	19.133 c	0.000 a	48.700 b
		HULU	0.000 c	2.000 b	0.000 c	0.000 c	0.000 a	2.000 b
	CITRUS	NISO	1.833 c	35.200 b	3.333 c	31.667 c	0.000 a	72.033 b
		HULU	2.400 bc	19.867 b	2.300 c	28.000 c	0.000 a	52.567 b
	FLORAL	NISO	3.200 bc	17.900 b	6.300 c	30.667 c	0.000 a	58.067 b
		HULU	4.967 bc	22.667 b	3.333 c	25.500 c	0.000 a	56.467 b
	IPA	NISO1	30.033 a	272.267 a	62.067 a	295.067 ab	29.067 a	659.433 a
		NISO2	28.100 a	263.133 a	64.700 a	229.933 ab	13.333 a	585.867 a
		HULU	15.900 abc	310.700 a	48.800 ab	324.567 a	14.333 a	699.967 a
	SPICY	NISO	2.333 bc	15.233 b	5.400 c	18.733 c	0.000 a	41.700 b
		HULU1	0.000 c	13.167 b	3.467 c	44.167 c	13.333 a	60.800 b
		HULU2	0.000 c	19.133 b	0.000 c	39.767 c	0.000 a	58.900 b
	SYLVAN	NISO	18.367 ab	299.267 a	55.767 ab	227.400 ab	9.300 a	600.800 a
		HULU	26.567 a	281.800 a	40.800 b	202.267 b	0.000 a	551.433 a

Table 8.5. continued.

Flavour attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Lemon		ISO	1.467 e	16.533 c	1.533 b	19.500 c	0.000 c	39.033 c
		NISO	3.433 de	104.633 c	15.933 b	120.433 c	24.800 bc	244.433 c
		HULU	2.933 de	17.567 c	2.033 b	4.400 c	0.000 c	26.933 c
	CITRUS	NISO	10.300 bcde	464.300 a	66.533 a	499.733 ab	85.433 ab	1040.867 a
		HULU	17.433 abcde	293.600 b	54.467 a	349.633 b	56.900 abc	715.133 b
	FLORAL	NISO	18.633 abcd	464.733 a	61.633 a	420.667 ab	27.767 bc	965.667 a
		HULU	21.267 ab	452.767 a	70.800 a	432.567 ab	34.567 abc	977.400 a
	IPA	NISO1	3.933 de	42.933 c	7.867 b	44.833 c	0.000 c	99.567 c
		NISO2	3.633 de	49.767 c	7.633 b	58.133 c	0.000 c	119.167 c
		HULU	4.967 cde	100.867 c	11.600 b	85.033 c	0.000 c	202.467 c
	SPICY	NISO	29.467 a	403.933 ab	55.867 a	440.933 ab	85.233 ab	930.200 a
		HULU1	20.233 abc	415.767 ab	56.433 a	454.300 ab	66.667 abc	946.733 a
		HULU2	33.567 a	467.767 a	78.367 a	554.867 a	104.100 a	1134.567 a
	SYLVAN	NISO	2.433 de	13.667 c	6.100 b	41.600 c	0.000 c	63.800 c
		HULU	2.367 e	50.800 c	4.700 b	50.433 c	0.000 c	108.300 c
Malty		ISO	17.467 ab	357.700 abc	29.467 abcd	396.767 a	60.367 a	801.400 ab
		NISO	15.933 ab	377.500 ab	53.600 a	375.033 ab	22.633 ab	822.067 ab
		HULU	17.433 ab	411.967 a	40.933 ab	391.100 ab	34.900 ab	861.433 a
	CITRUS	NISO	17.333 ab	274.200 bcde	15.533 bcd	285.633 abcde	9.333 ab	592.700 bcd
		HULU	21.433 a	324.533 abc	39.433 ab	350.933 abc	29.900 ab	736.333 abc
	FLORAL	NISO	12.100 ab	307.233 abcd	27.933 abcd	326.667 abcd	20.267 ab	673.933 abc
		HULU	13.833 ab	329.400 abc	35.267 abc	348.300 abc	26.500 ab	726.800 abc
	IPA	NISO1	6.133 ab	173.833 def	11.467 cd	185.400 cdef	7.633 ab	376.833 def
		NISO2	0.333 b	94.467 f	10.000 cd	90.600 f	12.600 ab	195.400 f
		HULU	11.933 ab	238.800 cde	14.700 bcd	113.700 f	0.000 b	379.133 def
	SPICY	NISO	3.333 ab	70.067 f	9.167 cd	93.233 f	14.000 ab	175.800 f
		HULU1	8.667 ab	144.933 ef	8.300 cd	156.067 def	1.100 b	317.967 ef
		HULU2	5.933 ab	183.233 def	9.733 cd	135.667 ef	15.700 ab	334.567 ef
	SYLVAN	NISO	4.800 ab	65.600 f	4.600 d	50.967 f	0.000 b	125.967 f
		HULU	9.067 ab	246.533 bcde	15.367 bcd	219.667 bcdef	0.000 b	490.633 cde
Musty		ISO	0.000 d	34.500 c	0.200 c	81.833 bc	13.333 a	116.533 b
		NISO	0.000 d	46.633 c	2.433 c	71.033 c	0.000 a	120.100 b
		HULU	2.333 bcd	29.967 c	5.067 c	84.333 bc	10.133 a	121.700 b
	CITRUS	NISO	2.867 bcd	59.467 c	2.400 c	52.300 c	0.000 a	117.033 b
		HULU	0.000 d	6.233 c	1.133 c	33.967 c	0.000 a	41.333 b
	FLORAL	NISO	2.300 bcd	47.200 c	2.033 c	68.533 c	0.100 a	120.067 b
		HULU	0.700 cd	20.933 c	4.167 c	48.033 c	0.467 a	73.833 b
	IPA	NISO1	11.967 abc	273.567 b	45.033 b	298.067 a	8.767 a	628.633 a
		NISO2	12.933 ab	304.567 b	61.667 ab	298.867 a	8.533 a	678.033 a
		HULU	20.000 a	418.433 a	74.000 a	208.800 ab	14.900 a	721.233 a
	SPICY	NISO	0.000 d	14.700 c	1.033 c	44.500 c	0.000 a	60.233 b
		HULU1	2.967 bcd	22.867 c	0.000 c	22.967 c	0.000 a	48.800 b
		HULU2	3.633 bcd	7.000 c	0.000 c	15.400 c	0.000 a	26.033 b
	SYLVAN	NISO	3.567 bcd	16.200 c	1.400 c	7.233 c	0.000 a	28.400 b
		HULU	11.500 abcd	335.300 ab	49.233 b	279.833 a	0.000 a	675.867 a
Orange		ISO	1.467 f	15.267 b	0.000 c	43.767 d	2.300 cd	60.500 c
		NISO	4.067 ef	37.900 b	8.500 c	43.300 d	0.000 d	93.767 c
		HULU	7.733 cdef	24.600 b	6.333 c	36.167 d	0.000 d	74.833 c
	CITRUS	NISO	14.100 cdef	287.833 a	59.067 a	393.300 ab	61.433 abcd	754.300 ab
		HULU	24.967 abc	343.567 a	60.100 a	360.167 ab	79.067 abc	788.800 ab
	FLORAL	NISO	18.767 bcdef	262.267 a	42.167 ab	244.600 bc	30.733 bcd	567.800 b
		HULU	21.633 bcde	291.133 a	51.800 a	391.067 ab	53.633 abcd	755.633 ab
	IPA	NISO1	3.833 ef	22.400 b	5.033 c	24.467 d	0.000 d	55.733 c
		NISO2	6.667 def	77.700 b	17.333 bc	107.367 cd	1.400 cd	209.067 c
		HULU	8.033 cdef	70.167 b	9.667 c	69.333 cd	3.700 cd	157.200 c
	SPICY	NISO	42.767 a	375.567 a	52.767 a	471.267 a	99.500 ab	942.367 a
		HULU1	23.600 bcd	376.067 a	66.600 a	490.667 a	118.500 a	956.933 a
		HULU2	34.100 ab	363.933 a	59.167 a	509.100 a	109.533 a	966.300 a
	SYLVAN	NISO	5.133 ef	3.767 b	0.800 c	32.167 d	0.000 d	41.867 c
		HULU	4.700 ef	13.467 b	7.500 c	45.433 d	0.000 d	71.100 c

Table 8.5. continued.

Flavour attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Pine wood		ISO	3.200 c	25.200 b	1.300 d	36.767 d	0.000 a	66.467 c
		NISO	1.833 c	57.767 b	5.533 cd	45.300 d	0.000 a	110.433 c
		HULU	0.600 c	51.967 b	1.533 d	79.067 cd	5.300 a	133.167 c
	CITRUS	NISO	6.267 bc	54.233 b	5.300 cd	88.033 cd	0.000 a	153.833 c
		HULU	3.533 c	38.800 b	0.900 d	40.700 d	0.000 a	83.933 c
	FLORAL	NISO	6.467 bc	69.267 b	9.800 bcd	105.133 cd	2.033 a	190.667 c
		HULU	3.333 c	27.467 b	6.100 cd	52.667 cd	0.000 a	89.567 c
	IPA	NISO1	20.233 a	311.033 a	42.633 a	393.800 ab	42.833 a	767.700 ab
		NISO2	8.167 abc	357.733 a	43.433 a	237.833 abc	44.000 a	647.167 ab
		HULU	4.333 c	260.700 a	35.733 ab	331.833 ab	37.333 a	632.600 ab
	SPICY	NISO	13.533 abc	325.000 a	34.833 ab	367.833 ab	59.333 a	741.200 ab
		HULU1	18.633 ab	326.733 a	44.300 a	420.467 a	60.067 a	810.133 a
		HULU2	10.800 abc	323.800 a	32.033 abc	375.767 ab	65.933 a	742.400 ab
	SYLVAN	NISO	10.933 abc	338.500 a	55.833 a	391.300 ab	41.267 a	796.567 a
		HULU	4.100 c	273.833 a	29.700 abc	210.033 bcd	22.867 a	517.667 b
Raisins/prunes		ISO	1.700 b	29.467 cd	4.567 b	68.967 bcd	12.633 a	104.700 cde
		NISO	0.000 b	11.233 d	0.233 b	23.300 cd	0.000 a	34.767 e
		HULU	11.233 ab	119.000 bc	8.967 b	101.700 bcd	0.267 a	240.900 bcde
	CITRUS	NISO	0.000 b	25.233 cd	3.333 b	46.533 bcd	0.000 a	75.100 cde
		HULU	5.200 ab	36.833 cd	1.733 b	37.267 bcd	0.000 a	81.033 cde
	FLORAL	NISO	5.533 ab	47.433 bcd	8.900 b	64.800 bcd	0.000 a	126.667 bcde
		HULU	7.333 ab	86.233 bcd	11.133 b	157.833 ab	10.000 a	262.533 bcd
	IPA	NISO1	1.733 b	62.900 bcd	6.400 b	82.967 bcd	0.000 a	154.000 bcde
		NISO2	2.033 ab	34.067 cd	6.667 b	50.500 bcd	0.000 a	93.267 cde
		HULU	15.533 a	138.167 b	10.133 b	155.600 abc	10.067 a	319.433 b
	SPICY	NISO	3.633 ab	12.700 d	0.600 b	13.833 d	0.000 a	30.767 e
		HULU1	6.967 ab	68.200 bcd	9.533 b	85.867 bcd	4.733 a	170.567 bcde
		HULU2	8.233 ab	106.733 bcd	14.833 b	145.867 abcd	13.633 a	275.667 bc
	SYLVAN	NISO	1.800 b	18.800 d	1.567 b	26.100 bcd	0.000 a	48.267 de
		HULU	11.333 ab	258.433 a	40.867 a	256.367 a	7.933 a	567.000 a
Rose water		ISO	0.000 b	8.967 b	0.600 b	20.767 b	0.000 b	30.333 b
		NISO	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
		HULU	3.133 b	9.800 b	3.700 b	8.700 b	0.000 b	25.333 b
	CITRUS	NISO	2.700 b	42.067 b	4.567 b	45.300 b	2.900 b	94.633 b
		HULU	1.533 b	21.233 b	3.333 b	5.533 b	0.000 b	31.633 b
	FLORAL	NISO	6.133 b	48.700 b	6.433 b	30.533 b	0.000 b	91.800 b
		HULU	4.633 b	17.167 b	3.000 b	19.067 b	0.000 b	43.867 b
	IPA	NISO1	0.000 b	4.667 b	0.300 b	6.600 b	0.000 b	11.567 b
		NISO2	0.567 b	8.500 b	1.333 b	13.033 b	0.000 b	23.433 b
		HULU	8.300 b	42.800 b	4.533 b	48.767 b	0.000 b	104.400 b
	SPICY	NISO	43.267 a	301.567 a	51.833 a	361.700 a	38.967 ab	758.367 a
		HULU1	43.400 a	364.267 a	56.367 a	437.200 a	67.400 a	901.233 a
		HULU2	42.233 a	373.167 a	53.567 a	412.567 a	66.033 a	881.533 a
	SYLVAN	NISO	0.000 b	32.333 b	0.733 b	37.067 b	0.000 b	70.133 b
		HULU	2.433 b	47.600 b	1.033 b	26.933 b	0.000 b	78.000 b
Tropical fruit		ISO	1.833 b	3.800 c	2.000 c	13.400 c	0.000 a	21.033 c
		NISO	0.000 b	13.533 c	0.800 c	15.267 c	0.000 a	29.600 c
		HULU	2.033 b	10.400 c	1.367 c	7.000 c	0.000 a	20.800 c
	CITRUS	NISO	4.300 ab	278.933 a	51.000 ab	169.500 ab	0.000 a	503.733 a
		HULU	7.633 ab	185.500 ab	48.733 ab	221.767 a	24.533 a	463.633 a
	FLORAL	NISO	7.667 ab	175.967 b	33.633 b	79.567 bc	0.000 a	296.833 b
		HULU	3.000 b	277.700 a	67.700 a	205.200 a	0.000 a	553.600 a
	IPA	NISO1	4.933 ab	29.767 c	0.000 c	41.733 c	0.000 a	76.433 c
		NISO2	0.400 b	9.333 c	0.767 c	13.633 c	0.000 a	24.133 c
		HULU	0.000 b	7.833 c	0.000 c	15.100 c	0.000 a	22.933 c
	SPICY	NISO	15.800 a	237.367 ab	29.800 b	274.800 a	25.300 a	557.767 a
		HULU1	15.300 a	264.067 ab	35.600 b	236.233 a	20.533 a	551.200 a
		HULU2	7.867 ab	233.167 ab	37.733 b	249.200 a	0.000 a	527.967 a
	SYLVAN	NISO	2.067 b	14.833 c	0.000 c	15.967 c	0.000 a	32.867 c
		HULU	2.100 b	6.333 c	2.033 c	8.133 c	0.000 a	18.600 c

Table 8.5. continued.

Taste & mouthfeel attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Astringent		ISO	1.933 a	182.433 efgh	40.200 fgh	647.400 ef	101.000 fghi	871.967 ef
		NISO	0.000 a	224.167 defg	52.200 cdefg	671.833 def	149.900 efg	948.200 e
		HULU	0.000 a	138.900 gh	30.133 gh	445.733 gh	63.267 ghi	614.767 gh
	CITRUS	NISO	0.000 a	110.567 h	32.567 fgh	554.667 fg	110.400 fgh	697.800 fg
		HULU	0.000 a	89.900 h	20.300 h	349.767 h	34.367 hi	459.967 h
	FLORAL	NISO	0.000 a	168.967 fgh	48.367 defgh	570.533 fg	120.100 efgh	787.867 efg
		HULU	0.000 a	176.000 efgh	46.067 efgh	389.200 h	17.133 i	611.267 gh
	IPA	NISO1	3.167 a	293.600 abcd	86.667 ab	941.300 ab	341.300 ab	1324.733 ab
		NISO2	1.000 a	371.200 a	93.800 a	965.767 a	365.767 a	1431.767 a
		HULU	0.700 a	349.900 ab	70.033 abcde	850.467 abc	258.233 bcd	1271.100 abcd
	SPICY	NISO	0.000 a	266.367 bcde	53.100 cdefg	802.533 bcd	202.933 cde	1122.000 cd
		HULU1	2.233 a	329.400 abc	71.067 abcde	893.233 abc	293.233 abc	1295.933 abc
		HULU2	0.367 a	303.067 abcd	79.167 abc	849.233 abc	251.867 bcd	1231.833 bcd
	SYLVAN	NISO	2.467 a	282.167 abcd	61.000 bcdef	759.833 cde	177.533 def	1105.467 d
		HULU	0.000 a	238.300 cdef	75.800 abcd	912.500 abc	319.533 ab	1226.600 bcd
Cooling		ISO	60.700 a	208.167 bcd	47.533 abc	153.633 cd	0.000 a	470.033 bcde
		NISO	55.400 a	211.567 bcd	33.833 bcd	172.800 abcd	0.000 a	473.600 bcde
		HULU	62.433 a	255.367 abc	49.967 ab	220.133 a	0.733 a	587.900 a
	CITRUS	NISO	56.633 a	258.967 ab	42.200 abcd	161.600 bcd	0.000 a	519.400 abcd
		HULU	59.567 a	247.767 abcd	42.500 abcd	200.200 abc	0.000 a	550.033 ab
	FLORAL	NISO	55.967 a	202.633 cd	24.267 d	144.100 d	0.000 a	426.967 e
		HULU	63.967 a	245.900 abcd	54.900 a	174.933 abcd	0.000 a	539.700 abc
	IPA	NISO1	48.367 a	199.767 d	28.067 cd	171.233 abcd	0.000 a	447.433 de
		NISO2	57.000 a	224.967 abcd	38.667 abcd	214.300 ab	0.000 a	534.933 abcd
		HULU	48.600 a	229.467 abcd	39.167 abcd	141.333 d	0.000 a	458.567 cde
	SPICY	NISO	48.967 a	203.767 cd	28.200 cd	168.033 abcd	0.000 a	448.967 de
		HULU1	57.333 a	230.267 abcd	34.633 bcd	183.767 abcd	0.000 a	506.000 abcde
		HULU2	55.400 a	267.433 a	43.700 abcd	181.767 abcd	0.000 a	548.300 ab
	SYLVAN	NISO	58.100 a	224.200 abcd	48.800 ab	144.333 d	0.000 a	475.433 bcde
		HULU	50.267 a	199.300 d	27.600 d	192.233 abcd	0.000 a	469.400 bcde
Harsh bitterness		ISO	15.267 abcde	362.233 ab	52.300 c	697.900 ab	221.967 ab	1127.700 c
		NISO	19.267 abcde	448.533 ab	60.500 c	871.933 a	276.400 ab	1400.233 ab
		HULU	7.633 de	64.233 c	8.467 d	105.833 c	10.033 c	186.167 d
	CITRUS	NISO	5.733 e	123.033 c	20.500 d	93.800 c	0.000 c	243.067 d
		HULU	3.833 e	67.700 c	10.567 d	52.033 c	0.000 c	134.133 d
	FLORAL	NISO	4.533 e	12.300 c	8.967 d	28.933 c	0.000 c	54.733 d
		HULU	5.933 e	113.067 c	7.733 d	21.400 c	0.000 c	148.133 d
	IPA	NISO1	12.467 bcde	490.867 a	94.167 a	835.567 ab	293.767 a	1433.067 a
		NISO2	19.400 abcde	333.833 b	56.867 c	738.467 ab	224.067 ab	1148.567 bc
		HULU	29.733 a	412.600 ab	66.600 abc	667.533 b	205.233 ab	1176.467 abc
	SPICY	NISO	26.133 abc	455.867 ab	57.367 c	685.867 ab	171.600 b	1225.233 abc
		HULU1	19.733 abcde	491.267 a	92.000 ab	845.933 ab	272.100 ab	1448.933 a
		HULU2	24.433 abcd	399.800 ab	63.067 bc	723.767 ab	172.333 b	1211.067 abc
	SYLVAN	NISO	10.333 cde	450.667 ab	79.000 abc	786.800 ab	259.167 ab	1326.800 abc
		HULU	27.867 ab	495.567 a	78.200 abc	839.700 ab	286.900 a	1441.333 a
Metallic		ISO	0.000 b	95.267 bcd	20.000 abc	256.600 bcd	67.400 abcde	371.867 c
		NISO	0.000 b	196.767 ab	35.267 ab	424.967 abc	120.100 abc	657.000 ab
		HULU	0.000 b	233.900 a	45.600 a	507.367 a	137.567 a	786.867 a
	CITRUS	NISO	0.000 b	194.900 ab	32.433 ab	439.300 ab	87.400 abcd	666.633 ab
		HULU	0.000 b	183.733 abc	30.000 ab	432.467 abc	123.833 ab	646.200 ab
	FLORAL	NISO	0.000 b	121.033 abcd	20.000 abc	217.200 d	41.200 cde	358.233 c
		HULU	0.133 b	95.667 bcd	0.000 c	169.200 d	37.133 de	265.000 c
	IPA	NISO1	3.833 a	158.400 abcd	22.500 abc	309.667 abcd	41.400 cde	494.400 bc
		NISO2	1.333 ab	102.933 bcd	11.167 bc	258.267 bcd	69.167 abcde	373.700 c
		HULU	0.000 b	52.967 d	10.000 bc	209.900 d	8.667 de	272.867 c
	SPICY	NISO	0.000 b	71.633 cd	10.000 bc	216.667 d	5.700 e	298.300 c
		HULU1	0.233 b	83.167 bcd	20.000 abc	248.133 bcd	52.000 bcde	351.533 c
		HULU2	0.000 b	73.267 cd	10.000 bc	293.533 bcd	21.433 de	376.800 c
	SYLVAN	NISO	0.000 b	99.900 bcd	22.767 abc	259.867 bcd	46.800 bcde	382.533 c
		HULU	0.000 b	176.233 abc	21.200 abc	232.533 cd	36.233 de	429.967 bc

Table 8.5. continued.

Taste & mouthfeel attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Peppery tingling		ISO	13.033 a	51.833 cd	4.167 c	282.533 cde	42.267 cd	351.567 de
		NISO	4.133 ab	25.100 cd	1.133 c	55.367 f	0.000 d	85.733 f
		HULU	3.933 ab	25.267 cd	2.200 c	90.267 ef	16.600 cd	121.667 f
	CITRUS	NISO	10.633 ab	63.633 cd	15.033 c	255.433 cde	54.333 cd	344.733 de
		HULU	2.467 ab	35.067 cd	8.433 c	57.067 f	0.000 d	103.033 f
	FLORAL	NISO	7.333 ab	43.433 cd	11.067 c	38.200 f	0.000 d	100.033 f
		HULU	7.033 ab	12.000 d	0.833 c	9.600 f	0.000 d	29.467 f
	IPA	NISO1	9.200 ab	63.767 cd	12.800 c	313.833 cd	51.333 cd	399.600 cde
		NISO2	10.900 ab	78.900 cd	20.300 c	397.800 c	45.200 cd	507.900 cd
		HULU	7.167 ab	46.400 cd	4.567 c	181.233 def	23.933 cd	239.367 ef
	SPICY	NISO	7.933 ab	241.167 b	56.667 b	727.767 b	196.667 b	1033.533 b
		HULU1	2.133 ab	388.967 a	84.533 a	881.767 ab	320.433 a	1357.400 a
		HULU2	12.933 a	416.033 a	91.500 a	948.600 a	348.600 a	1469.067 a
	SYLVAN	NISO	10.033 ab	113.500 c	19.133 c	432.033 c	92.000 c	574.700 c
		HULU	0.000 b	13.000 d	8.300 c	178.600 def	2.300 d	199.900 ef
Smooth bitterness		ISO	11.533 abc	300.733 bcd	32.900 cd	316.300 bc	36.567 c	661.467 b
		NISO	5.833 abc	195.600 de	19.267 de	271.367 bcd	50.833 c	492.067 bc
		HULU	11.633 abc	448.067 ab	61.967 ab	727.867 a	204.500 b	1249.533 a
	CITRUS	NISO	7.833 abc	357.833 abc	55.600 bc	786.067 a	212.833 ab	1207.333 a
		HULU	10.033 abc	459.200 a	88.433 a	880.067 a	299.600 a	1437.733 a
	FLORAL	NISO	18.233 a	487.667 a	69.500 ab	792.400 a	224.267 ab	1367.800 a
		HULU	16.833 ab	460.867 a	85.233 a	763.767 a	217.267 ab	1326.700 a
	IPA	NISO1	7.233 abc	107.533 ef	14.567 de	159.900 bcd	4.467 c	289.233 cd
		NISO2	0.300 c	33.767 f	1.900 e	107.900 cd	13.833 c	143.867 d
		HULU	2.400 abc	104.267 ef	9.100 de	125.233 bcd	15.033 c	241.000 cd
	SPICY	NISO	4.233 abc	227.267 cde	11.233 de	125.200 bcd	2.033 c	367.933 bcd
		HULU1	6.500 abc	210.567 cde	9.167 de	216.567 bcd	3.767 c	442.800 bcd
		HULU2	1.967 bc	171.800 def	8.633 de	132.267 bcd	8.933 c	314.667 cd
	SYLVAN	NISO	10.233 abc	298.233 bcd	17.133 de	72.600 d	16.000 c	398.200 bcd
		HULU	7.600 abc	206.833 cde	20.933 de	331.833 b	30.667 c	567.200 bc
Sour		ISO	8.567 ab	189.300 bcde	22.633 abc	203.533 bcde	0.000 b	424.033 bc
		NISO	4.600 ab	208.700 bcd	17.400 abc	194.833 bcde	0.000 b	425.533 bc
		HULU	3.233 ab	187.367 bcde	17.867 abc	272.300 abc	14.033 ab	480.767 bc
	CITRUS	NISO	14.400 ab	199.167 bcd	31.900 ab	321.300 ab	32.500 ab	566.767 b
		HULU	16.467 a	334.333 a	36.033 a	371.500 a	43.867 a	758.333 a
	FLORAL	NISO	13.500 ab	136.833 cde	16.700 abc	135.933 cde	0.000 b	302.967 cd
		HULU	11.667 ab	291.300 ab	34.767 a	270.267 abc	1.000 b	608.000 b
	IPA	NISO1	4.100 ab	138.933 cde	2.600 c	169.800 cde	0.000 b	315.433 cd
		NISO2	11.667 ab	148.600 cde	17.967 abc	121.867 de	0.000 b	300.100 cd
		HULU	5.633 ab	82.833 e	11.067 bc	79.100 e	0.000 b	178.633 d
	SPICY	NISO	0.767 b	113.067 de	13.067 bc	166.433 cde	0.000 b	293.333 cd
		HULU1	12.000 ab	162.433 cde	8.600 c	222.733 bcd	7.400 b	405.767 bc
		HULU2	5.933 ab	125.200 cde	17.867 abc	165.567 cde	10.000 ab	314.567 cd
	SYLVAN	NISO	12.533 ab	238.000 abc	15.167 abc	171.500 cde	0.000 b	437.200 bc
		HULU	3.633 ab	79.133 e	2.233 c	98.833 de	0.000 b	183.833 d
Sweet		ISO	15.167 bc	116.867 cd	6.267 bcd	105.033 bc	0.000 b	243.333 c
		NISO	15.667 bc	104.733 d	4.200 cd	72.533 c	0.000 b	197.133 c
		HULU	29.767 ab	221.967 bc	25.800 abc	269.133 a	16.200 ab	546.667 b
	CITRUS	NISO	41.267 a	251.967 b	27.667 ab	261.033 a	28.967 a	581.933 b
		HULU	28.067 ab	219.333 bc	37.533 a	262.433 a	12.533 ab	547.367 b
	FLORAL	NISO	37.533 a	260.767 b	11.933 bcd	276.100 a	2.700 ab	586.333 b
		HULU	35.433 a	265.767 b	41.600 a	225.400 ab	5.067 ab	568.200 b
	IPA	NISO1	11.600 bc	34.967 d	0.333 d	9.733 c	0.000 b	56.633 c
		NISO2	4.733 c	68.067 d	4.867 cd	54.000 c	1.467 ab	131.667 c
		HULU	15.867 bc	65.633 d	6.500 bcd	38.567 c	0.000 b	126.567 c
	SPICY	NISO	44.367 a	269.300 b	43.400 a	271.767 a	0.000 b	628.833 b
		HULU1	42.667 a	265.233 b	36.000 a	263.633 a	4.367 ab	607.533 b
		HULU2	47.133 a	421.067 a	45.833 a	283.767 a	1.900 ab	797.800 a
	SYLVAN	NISO	4.733 c	63.267 d	10.133 bcd	61.633 c	0.033 b	139.767 c
		HULU	13.800 bc	73.233 d	11.200 bcd	70.700 c	0.000 b	168.933 c

Table 8.5. continued.

Taste & mouthfeel attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total duration**
Warming		ISO	ns	251.967 a	39.233 abc	653.667 a	151.633 ab	944.867 a
		NISO	ns	180.933 abc	23.433 bc	608.200 a	125.067 ab	812.567 a
		HULU	ns	225.333 abc	35.400 abc	623.900 a	148.033 ab	884.633 a
	CITRUS	NISO	ns	141.767 c	18.833 c	583.567 a	119.733 ab	744.167 a
		HULU	ns	220.500 abc	46.700 ab	569.800 a	129.133 ab	837.000 a
	FLORAL	NISO	ns	239.200 ab	44.067 abc	569.300 a	129.367 ab	852.567 a
		HULU	ns	156.333 bc	23.500 bc	567.667 a	140.167 ab	747.500 a
	IPA	NISO1	ns	206.200 abc	40.067 abc	564.033 a	110.567 ab	810.300 a
		NISO2	ns	156.167 bc	25.267 abc	551.733 a	91.467 b	733.167 a
		HULU	ns	236.000 ab	45.733 abc	669.800 a	174.633 a	951.533 a
	SPICY	NISO	ns	231.233 abc	42.933 abc	619.333 a	145.833 ab	893.500 a
		HULU1	ns	236.000 ab	34.433 abc	674.100 a	162.700 ab	944.533 a
		HULU2	ns	243.100 ab	46.733 ab	647.600 a	144.967 ab	937.433 a
	SYLVAN	NISO	ns	197.300 abc	41.400 abc	695.533 a	188.800 a	934.233 a
		HULU	ns	238.667 ab	51.067 a	604.333 a	149.900 ab	894.067 a

ns, not selected

Table 8.6. Mean panel proportion citations (n=10) computed for the total evaluation period and time segments as evaluated using TCATA flavour and taste & mouthfeel attributes. Different letters within columns representing significant differences among the samples based on differences in least squares means (p<0.05).

Flavour attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total evaluation period**
Caramel		ISO	0.009 c	0.011 b	0.012 a	0.044 b	0.000 b	0.019 b
		NISO	0.103 bc	0.082 b	0.127 a	0.090 b	0.000 b	0.070 b
		HULU	0.280 a	0.308 a	0.535 b	0.216 a	0.043 b	0.230 a
	CITRUS	NISO	0.078 bc	0.026 b	0.000 a	0.063 b	0.045 b	0.031 b
		HULU	0.156 abc	0.133 b	0.000 a	0.135 b	0.120 ab	0.101 b
	FLORAL	NISO	0.058 bc	0.012 b	0.003 a	0.013 b	0.033 b	0.020 b
		HULU	0.212 ab	0.114 b	0.000 a	0.132 b	0.083 ab	0.085 b
	IPA	NISO1	0.000 c	0.079 b	0.000 a	0.061 b	0.022 b	0.037 b
		NISO2	0.040 bc	0.102 b	0.011 a	0.014 b	0.024 b	0.048 b
		HULU	0.115 abc	0.125 b	0.000 a	0.166 b	0.079 b	0.084 b
	SPICY	NISO	0.050 bc	0.098 b	0.000 a	0.069 b	0.048 b	0.055 b
		HULU1	0.162 abc	0.106 b	0.000 a	0.171 b	0.104 ab	0.088 b
		HULU2	0.093 bc	0.098 b	0.000 a	0.080 b	0.069 b	0.065 b
	SYLVAN	NISO	0.000 c	0.019 b	0.000 a	0.120 b	0.028 b	0.022 b
		HULU	0.153 abc	0.069 b	0.000 a	0.120 b	0.094 ab	0.069 b
Earthy		ISO	0.000 d	0.134 de	0.056 c	0.102 cd	0.000 b	0.081 cd
		NISO	0.049 cd	0.142 de	0.038 c	0.098 cd	0.000 b	0.085 cd
		HULU	0.024 d	0.055 e	0.003 c	0.040 d	0.000 b	0.033 d
	CITRUS	NISO	0.092 bcd	0.257 cd	0.161 c	0.235 bc	0.010 b	0.180 c
		HULU	0.005 d	0.125 de	0.013 c	0.030 d	0.000 b	0.053 d
	FLORAL	NISO	0.002 d	0.118 de	0.000 c	0.099 cd	0.000 b	0.072 cd
		HULU	0.001 d	0.093 de	0.026 c	0.094 cd	0.000 b	0.064 cd
	IPA	NISO1	0.018 d	0.409 bc	0.368 b	0.457 a	0.056 ab	0.322 b
		NISO2	0.160 abc	0.597 ab	0.427 b	0.533 a	0.023 ab	0.414 ab
		HULU	0.121 abcd	0.414 bc	0.631 a	0.459 a	0.061 ab	0.346 ab
	SPICY	NISO	0.000 d	0.000 e	0.000 c	0.000 d	0.000 b	0.000 d
		HULU1	0.000 d	0.044 e	0.000 c	0.026 d	0.000 b	0.023 d
		HULU2	0.000 d	0.000 e	0.000 c	0.000 d	0.000 b	0.000 d
	SYLVAN	NISO	0.184 ab	0.772 a	0.740 a	0.406 a	0.114 a	0.469 a
		HULU	0.224 a	0.575 b	0.657 a	0.396 ab	0.041 ab	0.381 ab

Table 8.6. continued.

Flavour attributes								
Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Grapefruit		ISO	0.113 bcd	0.193 ed	0.047 de	0.089 b	0.000 a	0.103 ef
		NISO	0.031 cd	0.083 efd	0.000 e	0.024 b	0.000 a	0.037 ef
		HULU	0.000 d	0.000 f	0.000 e	0.000 b	0.000 a	0.000 f
	CITRUS	NISO	0.205 ab	0.784 a	0.810 a	0.537 a	0.041 a	0.505 a
		HULU	0.170 abc	0.506 c	0.403 c	0.462 a	0.043 a	0.364 bc
	FLORAL	NISO	0.196 ab	0.252 d	0.305 cd	0.435 a	0.040 a	0.266 cd
		HULU	0.073 bcd	0.556 bc	0.692 ab	0.435 a	0.099 a	0.395 abc
	IPA	NISO1	0.030 d	0.204 d	0.023 e	0.041 b	0.000 a	0.084 ef
		NISO2	0.010 d	0.081 efd	0.067 de	0.088 b	0.046 a	0.071 ef
		HULU	0.083 bcd	0.148 efd	0.027 e	0.125 b	0.000 a	0.097 ef
	SPICY	NISO	0.200 ab	0.751 a	0.678 ab	0.555 a	0.035 a	0.492 ab
		HULU1	0.128 bcd	0.698 ab	0.536 bc	0.635 a	0.072 a	0.497 ab
		HULU2	0.282 a	0.775 a	0.512 bc	0.543 a	0.085 a	0.502 a
	SYLVAN	NISO	0.017 d	0.006 ef	0.035 e	0.016 n	0.014 a	0.013 f
		HULU	0.052 cd	0.207 d	0.100 de	0.173 b	0.089 a	0.155 de
Grassy		ISO	0.095 cde	0.059 b	0.079 c	0.097 c	0.000 a	0.062 b
		NISO	0.009 de	0.044 b	0.020 c	0.032 c	0.000 a	0.027 b
		HULU	0.000 e	0.003 b	0.000 c	0.000 c	0.000 a	0.001 b
	CITRUS	NISO	0.018 de	0.059 b	0.033 c	0.053 c	0.000 a	0.040 b
		HULU	0.024 de	0.033 b	0.023 c	0.047 c	0.000 a	0.029 b
	FLORAL	NISO	0.032 cde	0.030 b	0.063 c	0.038 c	0.019 a	0.032 b
		HULU	0.049 cde	0.038 b	0.033 c	0.042 c	0.000 a	0.031 b
	IPA	NISO1	0.322 a	0.454 a	0.621 a	0.421 ab	0.106 a	0.368 a
		NISO2	0.278 ab	0.439 a	0.647 a	0.361 b	0.033 a	0.325 a
		HULU	0.157 bcd	0.518 a	0.488 ab	0.517 a	0.036 a	0.389 a
	SPICY	NISO	0.023 de	0.025 b	0.054 c	0.031 c	0.000 a	0.023 b
		HULU1	0.000 e	0.022 b	0.035 c	0.051 c	0.033 a	0.034 b
		HULU2	0.000 e	0.032 b	0.000 c	0.048 c	0.027 a	0.033 b
	SYLVAN	NISO	0.182 abc	0.499 a	0.558 ab	0.346 b	0.049 a	0.334 a
		HULU	0.263 ab	0.470 a	0.408 b	0.337 b	0.000 a	0.306 a
Lemon		ISO	0.015 f	0.028 c	0.015 b	0.033 c	0.000 c	0.022 c
		NISO	0.034 ef	0.174 c	0.159 b	0.159 c	0.062 bc	0.136 c
		HULU	0.029 ef	0.029 c	0.020 b	0.007 c	0.000 c	0.015 c
	CITRUS	NISO	0.102 cdef	0.774 a	0.665 a	0.665 ab	0.251 ab	0.578 ab
		HULU	0.173 bcde	0.489 b	0.545 a	0.462 b	0.182 abc	0.397 b
	FLORAL	NISO	0.184 abcd	0.775 a	0.616 a	0.645 ab	0.084 abc	0.536 ab
		HULU	0.211 abc	0.755 a	0.708 a	0.652 ab	0.104 abc	0.543 ab
	IPA	NISO1	0.039 def	0.072 c	0.079 b	0.075 c	0.000 c	0.055 c
		NISO2	0.036 def	0.083 c	0.076 b	0.096 c	0.001 c	0.066 c
		HULU	0.049 def	0.168 c	0.116 b	0.140 c	0.002 c	0.112 c
	SPICY	NISO	0.292 ab	0.673 ab	0.559 a	0.578 ab	0.235 ab	0.516 ab
		HULU1	0.200 abc	0.693 ab	0.564 a	0.629 ab	0.192 abc	0.526 ab
		HULU2	0.332 a	0.780 a	0.784 a	0.749 a	0.264 a	0.630 a
	SYLVAN	NISO	0.024 ef	0.023 c	0.061 b	0.069 c	0.000 c	0.035 c
		HULU	0.023 ef	0.085 c	0.047 b	0.080 c	0.006 c	0.060 c
Malty		ISO	0.173 ab	0.596 abc	0.536 a	0.553 a	0.163 a	0.445 a
		NISO	0.158 ab	0.629 ab	0.353 abcd	0.564 a	0.092 a	0.456 a
		HULU	0.173 ab	0.687 a	0.409 ab	0.581 a	0.107 a	0.478 a
	CITRUS	NISO	0.172 ab	0.457 bcde	0.295 abcde	0.416 abc	0.090 a	0.329 abc
		HULU	0.212 a	0.541 abcd	0.279 abcde	0.530 a	0.083 a	0.409 ab
	FLORAL	NISO	0.120 ab	0.512 abcd	0.155 bcde	0.498 ab	0.069 a	0.374 ab
		HULU	0.137 ab	0.549 abcd	0.394 abc	0.517 ab	0.095 a	0.404 ab
	IPA	NISO1	0.061 ab	0.290 efgh	0.115 bcde	0.280 bcde	0.044 a	0.209 cd
		NISO2	0.003 b	0.157 gh	0.097 cde	0.129 de	0.033 a	0.108 d
		HULU	0.118 ab	0.398 def	0.083 de	0.189 cde	0.001 a	0.211 cd
	SPICY	NISO	0.033 ab	0.117 gh	0.147 bcde	0.116 e	0.060 a	0.098 d
		HULU1	0.086 ab	0.242 fgh	0.100 cde	0.238 cde	0.033 a	0.177 cd
		HULU2	0.059 ab	0.305 efg	0.154 bcde	0.184 cde	0.063 a	0.186 cd
	SYLVAN	NISO	0.048 ab	0.109 h	0.046 e	0.085 e	0.000 a	0.070 d
		HULU	0.090 ab	0.411 cdef	0.092 de	0.361 abcd	0.008 a	0.272 bc

Table 8.6. continued.

Flavour attributes								
Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Musty		ISO	0.000 d	0.057 c	0.002 c	0.099 b	0.056 a	0.065 b
		NISO	0.000 d	0.078 c	0.024 c	0.096 b	0.033 a	0.067 b
		HULU	0.023 bcd	0.050 c	0.051 c	0.120 b	0.032 a	0.068 b
	CITRUS	NISO	0.028 bcd	0.099 c	0.024 c	0.087 b	0.000 a	0.065 b
		HULU	0.000 d	0.010 c	0.011 c	0.038 b	0.028 a	0.023 b
	FLORAL	NISO	0.023 bcd	0.079 c	0.020 c	0.080 b	0.051 a	0.067 b
		HULU	0.007 cd	0.035 c	0.042 c	0.073 b	0.011 a	0.041 b
	IPA	NISO1	0.118 abc	0.456 b	0.450 b	0.431 a	0.099 a	0.349 a
		NISO2	0.128 ab	0.508 b	0.617 ab	0.466 a	0.049 a	0.376 a
		HULU	0.198 a	0.697 a	0.740 a	0.323 a	0.037 a	0.400 a
	SPICY	NISO	0.000 d	0.025 c	0.010 c	0.046 b	0.042 a	0.033 b
		HULU1	0.029 bcd	0.038 c	0.000 c	0.030 b	0.012 a	0.027 b
		HULU2	0.036 bcd	0.012 c	0.000 c	0.026 b	0.000 a	0.014 b
	SYLVAN	NISO	0.035 bcd	0.027 c	0.014 c	0.009 b	0.004 a	0.016 b
		HULU	0.114 abc	0.559 ab	0.492 b	0.462 a	0.006 a	0.375 a
Orange		ISO	0.015 ef	0.025 b	0.000 c	0.058 d	0.022 de	0.034 c
		NISO	0.040 ef	0.063 b	0.085 c	0.064 d	0.013 e	0.052 c
		HULU	0.077 cdef	0.041 b	0.063 c	0.042 d	0.027 cde	0.042 c
	CITRUS	NISO	0.140 cd	0.480 a	0.591 a	0.504 ab	0.228 abc	0.419 ab
		HULU	0.247 abcef	0.573 a	0.601 a	0.456 ab	0.216 abcd	0.438 ab
	FLORAL	NISO	0.186 bcde	0.437 a	0.422 ab	0.349 bc	0.088 bcde	0.315 b
		HULU	0.214 bcdef	0.485 a	0.518 a	0.559 ab	0.140 abcde	0.420 ab
	IPA	NISO1	0.038 ef	0.037 b	0.050 c	0.033 d	0.012 e	0.031 c
		NISO2	0.066 cdef	0.130 b	0.173 bc	0.177 cd	0.004 e	0.116 c
		HULU	0.080 cd	0.117 b	0.097 c	0.109 d	0.009 e	0.087 c
	SPICY	NISO	0.423 a	0.626 a	0.528 a	0.607 a	0.267 ab	0.523 a
		HULU1	0.234 bcd	0.627 a	0.666 a	0.610 a	0.312 a	0.531 a
		HULU2	0.338 abef	0.607 a	0.592 a	0.664 a	0.277 ab	0.537 a
	SYLVAN	NISO	0.051 def	0.006 b	0.008 c	0.041 d	0.019 de	0.023 c
		HULU	0.047 d	0.022 b	0.075 c	0.071 d	0.006 e	0.039 c
Pine wood		ISO	0.032 c	0.042 b	0.013 ef	0.061 d	0.000 b	0.037 b
		NISO	0.018 c	0.096 b	0.055 def	0.071 d	0.007 ab	0.061 b
		HULU	0.006 c	0.087 b	0.015 ef	0.115 cd	0.025 ab	0.074 b
	CITRUS	NISO	0.062 abc	0.090 b	0.053 def	0.147 cd	0.000 b	0.085 b
		HULU	0.035 bc	0.065 b	0.009 f	0.068 d	0.000 b	0.047 b
	FLORAL	NISO	0.064 abc	0.115 b	0.098 bcdef	0.154 cd	0.032 ab	0.106 b
		HULU	0.033 c	0.046 b	0.061 cdef	0.082 cd	0.008 ab	0.050 b
	IPA	NISO1	0.200 a	0.518 a	0.426 c	0.582 a	0.111 ab	0.426 a
		NISO2	0.081 abc	0.596 a	0.434 a	0.323 bc	0.110 ab	0.359 a
		HULU	0.043 bc	0.435 a	0.357 ab	0.490 ab	0.095 ab	0.351 a
	SPICY	NISO	0.134 abc	0.542 a	0.348 abc	0.508 ab	0.157 ab	0.412 a
		HULU1	0.184 ab	0.545 a	0.443 a	0.581 a	0.180 ab	0.450 a
		HULU2	0.107 abc	0.540 a	0.320 abcd	0.504 ab	0.184 a	0.412 a
	SYLVAN	NISO	0.108 abc	0.564 a	0.558 a	0.580 a	0.108 ab	0.442 a
		HULU	0.041 bc	0.456 a	0.297 abcde	0.299 bcd	0.076 ab	0.287 a
Raisins/prunes		ISO	0.017 ab	0.049 cd	0.046 b	0.083 bc	0.047 a	0.058 bcde
		NISO	0.000 b	0.019 d	0.002 b	0.022 c	0.026 a	0.019 de
		HULU	0.111 ab	0.198 bc	0.090 b	0.152 bc	0.027 a	0.134 bcde
	CITRUS	NISO	0.000 b	0.042 cd	0.033 b	0.078 bc	0.000 a	0.042 cde
		HULU	0.051 ab	0.061 cd	0.017 b	0.062 bc	0.000 a	0.045 cde
	FLORAL	NISO	0.055 ab	0.079 bcd	0.089 b	0.078 bc	0.045 a	0.070 bcde
		HULU	0.073 ab	0.144 bcd	0.111 b	0.206 bc	0.086 a	0.146 bcd
	IPA	NISO1	0.017 ab	0.105 bcd	0.064 b	0.130 bc	0.012 a	0.086 bcde
		NISO2	0.020 ab	0.057 cd	0.067 b	0.084 bc	0.000 a	0.052 bcde
		HULU	0.154 a	0.230 b	0.101 b	0.227 ab	0.049 a	0.177 b
	SPICY	NISO	0.036 ab	0.021 d	0.006 b	0.023 c	0.000 a	0.017 e
		HULU1	0.069 ab	0.114 bcd	0.095 b	0.135 bc	0.012 a	0.095 bcde
		HULU2	0.082 ab	0.178 bcd	0.148 b	0.220 ab	0.034 a	0.153 bc
	SYLVAN	NISO	0.018 ab	0.031 cd	0.016 b	0.044 bc	0.000 a	0.027 cde
		HULU	0.112 ab	0.431 a	0.409 a	0.402 a	0.037 a	0.315 a

Table 8.6. continued.

Flavour attributes Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Rose water		ISO	0.000 b	0.015 b	0.006 b	0.035 b	0.000 b	0.017 b
		NISO	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
		HULU	0.031 b	0.016 b	0.037 b	0.015 b	0.000 b	0.014 b
	CITRUS	NISO	0.027 b	0.070 b	0.046 b	0.071 b	0.007 b	0.053 b
		HULU	0.015 b	0.035 b	0.033 b	0.009 b	0.000 b	0.018 b
	FLORAL	NISO	0.061 b	0.081 b	0.064 b	0.051 b	0.000 b	0.051 b
		HULU	0.046 b	0.029 b	0.030 b	0.032 b	0.000 b	0.024 b
	IPA	NISO1	0.000 b	0.008 b	0.003 b	0.011 b	0.000 b	0.006 b
		NISO2	0.006 b	0.014 b	0.013 b	0.022 b	0.000 b	0.013 b
		HULU	0.082 b	0.071 b	0.045 b	0.081 b	0.000 b	0.058 b
	SPICY	NISO	0.428 a	0.503 a	0.518 a	0.535 a	0.102 ab	0.421 a
		HULU1	0.430 a	0.607 a	0.564 a	0.616 a	0.169 a	0.500 a
		HULU2	0.418 a	0.622 a	0.536 a	0.578 a	0.165 a	0.489 a
	SYLVAN	NISO	0.000 b	0.054 b	0.007 b	0.062 b	0.000 b	0.039 b
		HULU	0.024 b	0.079 b	0.010 b	0.045 b	0.000 b	0.043 b
Tropical fruit		ISO	0.018 b	0.006 c	0.020 c	0.022 c	0.000 a	0.012 c
		NISO	0.000 b	0.023 c	0.008 c	0.025 c	0.000 a	0.016 c
		HULU	0.020 b	0.017 c	0.014 c	0.012 c	0.000 a	0.012 c
	CITRUS	NISO	0.043 ab	0.465 a	0.510 ab	0.282 ab	0.000 a	0.280 a
		HULU	0.076 ab	0.309 ab	0.487 ab	0.329 a	0.061 a	0.257 ab
	FLORAL	NISO	0.076 ab	0.293 b	0.336 b	0.123 bc	0.015 a	0.165 b
		HULU	0.030 b	0.463 a	0.677 a	0.328 a	0.021 a	0.307 a
	IPA	NISO1	0.049 ab	0.050 c	0.000 c	0.065 c	0.007 a	0.042 c
		NISO2	0.004 b	0.016 c	0.008 c	0.023 c	0.000 a	0.013 c
		HULU	0.000 b	0.013 c	0.000 c	0.018 c	0.011 a	0.013 c
	SPICY	NISO	0.156 a	0.396 ab	0.298 b	0.410 a	0.072 a	0.310 a
		HULU1	0.151 a	0.440 ab	0.356 b	0.353 a	0.060 a	0.306 a
		HULU2	0.078 ab	0.389 ab	0.377 b	0.393 a	0.033 a	0.293 a
	SYLVAN	NISO	0.020 b	0.025 c	0.000 c	0.027 c	0.000 a	0.018 c
		HULU	0.021 b	0.011 c	0.020 c	0.014 c	0.000 a	0.010 c

Taste & mouthfeel attributes Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Astringent		ISO	0.019 a	0.304 bcdef	0.402 def	0.640 abcd	0.658 c	0.484 cde
		NISO	0.000 a	0.374 abcdef	0.522 bcdef	0.614 abcd	0.759 bcd	0.526 bcde
		HULU	0.000 a	0.232 def	0.301 ef	0.459 cd	0.426 de	0.341 ef
	CITRUS	NISO	0.000 a	0.184 ef	0.326 ef	0.487 bcd	0.656 cd	0.387 ef
		HULU	0.000 a	0.150 f	0.203 f	0.333 d	0.375 d	0.255 f
	FLORAL	NISO	0.000 a	0.293 bcdef	0.461 cdef	0.474 cd	0.262 e	0.339 ef
		HULU	0.000 a	0.282 cdef	0.484 cdef	0.498 bc	0.679 c	0.437 def
	IPA	NISO1	0.031 a	0.489 abcd	0.867 ab	0.902 a	1.000 a	0.736 ab
		NISO2	0.010 a	0.619 a	0.938 a	0.943 a	1.000 a	0.795 a
		HULU	0.007 a	0.583 a	0.700 abcd	0.788 abc	0.944 ab	0.706 ab
	SPICY	NISO	0.000 a	0.444 abcde	0.531 bcdef	0.723 abc	0.922 ab	0.623 abcd
		HULU1	0.022 a	0.549 ab	0.711 abcd	0.822 ab	1.000 a	0.720 ab
		HULU2	0.004 a	0.505 abc	0.792 abc	0.749 abc	1.000 a	0.684 abc
	SYLVAN	NISO	0.024 a	0.470 abcd	0.610 abcde	0.685 abc	0.873 abc	0.614 abcd
		HULU	0.000 a	0.397 abcdef	0.758 abcd	0.854 a	1.000 a	0.681 abc
Cooling		ISO	0.601 a	0.347 a	0.475 ab	0.256 a	0.000 a	0.261 a
		NISO	0.549 a	0.353 a	0.338 bcd	0.288 a	0.000 a	0.263 a
		HULU	0.618 a	0.426 a	0.500 ab	0.366 a	0.002 a	0.326 a
	CITRUS	NISO	0.561 a	0.432 a	0.422 abc	0.269 a	0.000 a	0.288 a
		HULU	0.590 a	0.413 a	0.425 abc	0.334 a	0.000 a	0.305 a
	FLORAL	NISO	0.554 a	0.338 a	0.243 d	0.240 a	0.000 a	0.237 a
		HULU	0.633 a	0.410 a	0.549 a	0.292 a	0.000 a	0.300 a
	IPA	NISO1	0.479 a	0.333 a	0.281 cd	0.285 a	0.000 a	0.248 a
		NISO2	0.564 a	0.375 a	0.387 abcd	0.357 a	0.000 a	0.297 a
		HULU	0.481 a	0.382 a	0.392 abcd	0.236 a	0.000 a	0.255 a
	SPICY	NISO	0.485 a	0.340 a	0.282 cd	0.280 a	0.000 a	0.249 a
		HULU1	0.568 a	0.384 a	0.346 bcd	0.306 a	0.000 a	0.281 a
		HULU2	0.549 a	0.446 a	0.437 abc	0.303 a	0.000 a	0.304 a
	SYLVAN	NISO	0.575 a	0.374 a	0.488 ab	0.241 a	0.000 a	0.264 a
		HULU	0.498 a	0.332 a	0.276 cd	0.320 a	0.000 a	0.261 a

Table 8.6. continued.

Taste & mouthfeel attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total evaluation period**
Harsh bitterness		ISO	0.151 abcd	0.604 ab	0.523 d	0.697 bc	0.700 a	0.626 c
		NISO	0.191 abcd	0.748 ab	0.605 cd	0.914 a	0.808 a	0.777 abc
		HULU	0.076 abcd	0.107 c	0.085 e	0.121 d	0.082 b	0.103 d
	CITRUS	NISO	0.057 bcd	0.205 c	0.205 e	0.156 d	0.000 b	0.135 d
		HULU	0.038 d	0.113 c	0.106 e	0.087 d	0.000 b	0.074 d
	FLORAL	NISO	0.045 cd	0.021 c	0.090 e	0.048 d	0.000 b	0.030 d
		HULU	0.059 bcd	0.188 c	0.077 e	0.036 d	0.000 b	0.082 d
	IPA	NISO1	0.123 abcd	0.818 a	0.942 a	0.903 a	0.734 a	0.796 ab
		NISO2	0.192 abcd	0.556 b	0.569 cd	0.735 abc	0.744 a	0.638 bc
		HULU	0.294 a	0.688 ab	0.666 bcd	0.713 abc	0.600 a	0.653 abc
	SPICY	NISO	0.259 abc	0.760 ab	0.574 cd	0.639 c	0.757 a	0.680 abc
		HULU1	0.195 abcd	0.819 a	0.920 ab	0.905 a	0.758 a	0.805 a
		HULU2	0.242 abcd	0.666 ab	0.631 cd	0.823 abc	0.575 a	0.672 abc
	SYLVAN	NISO	0.102 abcd	0.751 ab	0.790 abc	0.821 abc	0.735 a	0.737 abc
		HULU	0.276 ab	0.826 a	0.782 abcd	0.896 ab	0.755 a	0.800 ab
Metallic		ISO	0.000 b	0.159 bc	0.200 abc	0.253 ab	0.262 abc	0.206 bc
		NISO	0.000 b	0.328 ab	0.353 ab	0.375 ab	0.500 a	0.365 abc
		HULU	0.000 b	0.390 a	0.456 a	0.515 a	0.496 a	0.437 a
	CITRUS	NISO	0.000 b	0.325 ab	0.324 ab	0.412 ab	0.481 ab	0.370 ab
		HULU	0.000 b	0.306 abc	0.300 ab	0.387 ab	0.500 a	0.359 abc
	FLORAL	NISO	0.000 b	0.202 abc	0.200 abc	0.255 ab	0.161 c	0.199 bc
		HULU	0.001 b	0.159 bc	0.000 c	0.149 b	0.200 c	0.147 c
	IPA	NISO1	0.038 a	0.264 abc	0.225 abc	0.280 ab	0.355 abc	0.275 abc
		NISO2	0.013 ab	0.172 abc	0.112 bc	0.297 ab	0.200 c	0.207 bc
		HULU	0.000 b	0.088 c	0.100 bc	0.153 b	0.296 abc	0.152 c
	SPICY	NISO	0.000 b	0.119 bc	0.100 bc	0.214 b	0.220 bc	0.166 bc
		HULU1	0.002 b	0.139 bc	0.200 abc	0.312 ab	0.152 c	0.195 bc
		HULU2	0.000 b	0.122 bc	0.100 bc	0.309 ab	0.271 abc	0.209 bc
	SYLVAN	NISO	0.000 b	0.167 abc	0.228 abc	0.258 ab	0.263 abc	0.212 bc
		HULU	0.000 b	0.294 abc	0.212 abc	0.261 ab	0.191 c	0.239 abc
Peppery tingling		ISO	0.129 a	0.086 cd	0.042 c	0.289 cde	0.273 cdef	0.195 cdef
		NISO	0.041 a	0.042 cd	0.011 c	0.073 f	0.029 ef	0.048 fg
		HULU	0.039 a	0.042 cd	0.022 c	0.074 ef	0.114 def	0.068 efg
	CITRUS	NISO	0.105 a	0.106 cd	0.150 c	0.226 cdef	0.300 cdef	0.191 cdef
		HULU	0.024 a	0.058 cd	0.084 c	0.060 f	0.053 ef	0.057 fg
	FLORAL	NISO	0.073 a	0.072 cd	0.111 c	0.064 f	0.000 f	0.056 fg
		HULU	0.070 a	0.020 d	0.008 c	0.013 f	0.004 ef	0.016 g
	IPA	NISO1	0.091 a	0.106 cd	0.128 c	0.302 cd	0.331 cde	0.222 cde
		NISO2	0.108 a	0.132 cd	0.203 c	0.383 c	0.421 cd	0.282 cd
		HULU	0.071 a	0.077 cd	0.046 c	0.100 def	0.303 cdef	0.133 defg
	SPICY	NISO	0.079 a	0.402 b	0.567 b	0.678 b	0.803 ab	0.574 b
		HULU1	0.021 a	0.648 a	0.845 a	0.920 a	0.824 ab	0.754 a
		HULU2	0.128 a	0.693 a	0.915 a	0.963 a	0.927 a	0.816 a
	SYLVAN	NISO	0.099 a	0.189 c	0.191 c	0.340 c	0.570 bc	0.319 c
		HULU	0.000 a	0.022 d	0.083 c	0.108 def	0.285 cdef	0.111 efg
Smooth bitterness		ISO	0.114 a	0.501 bcd	0.329 cd	0.351 b	0.264 b	0.367 b
		NISO	0.058 a	0.326 cdef	0.193 de	0.288 bc	0.246 b	0.273 bcd
		HULU	0.115 a	0.747 ab	0.620 ab	0.779 a	0.651 a	0.694 a
	CITRUS	NISO	0.078 a	0.596 abc	0.556 bc	0.793 a	0.776 a	0.670 a
		HULU	0.099 a	0.765 ab	0.884 a	0.898 a	0.853 a	0.798 a
	FLORAL	NISO	0.181 a	0.813 a	0.695 ab	0.902 a	0.629 a	0.759 a
		HULU	0.167 a	0.768 ab	0.852 a	0.859 a	0.621 a	0.737 a
	IPA	NISO1	0.072 a	0.179 ef	0.146 de	0.166 bc	0.151 b	0.161 cd
		NISO2	0.003 a	0.056 f	0.019 e	0.079 c	0.151 b	0.080 d
		HULU	0.024 a	0.174 ef	0.091 de	0.148 bc	0.092 b	0.134 cd
	SPICY	NISO	0.042 a	0.379 cde	0.112 de	0.158 bc	0.076 b	0.204 bcd
		HULU1	0.064 a	0.351 cde	0.092 de	0.272 bc	0.133 b	0.246 bcd
		HULU2	0.019 a	0.286 def	0.086 de	0.152 bc	0.103 b	0.175 bcd
	SYLVAN	NISO	0.101 a	0.497 bcd	0.171 de	0.082 c	0.059 b	0.221 bcd
		HULU	0.075 a	0.345 cde	0.209 de	0.387 b	0.248 b	0.315 bc

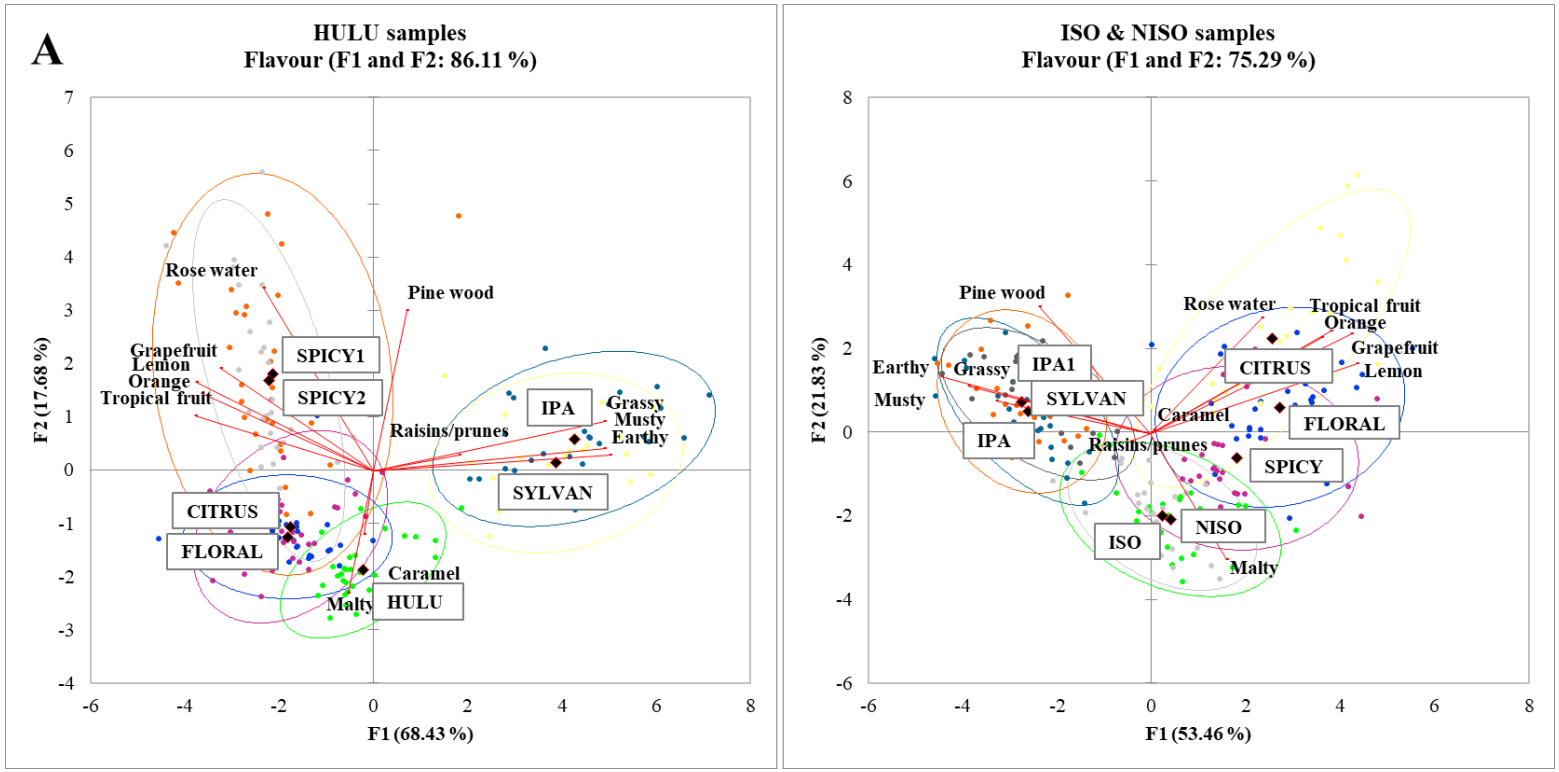
Table 8.6. continued.

Taste & mouthfeel attributes								
Attribute	**Hop flavour product**	**Base beer**	**Sip1-im**	**Sip1-sw**	**Sip2-im**	**Sip2-sw**	**Sip2-fin**	**Total evaluation period**
Sour		ISO	0.085 a	0.316 bcd	0.226 abc	0.331 abcde	0.013 ab	0.235 bcde
		NISO	0.046 a	0.348 abcd	0.174 abc	0.304 bcde	0.031 ab	0.236 bcde
		HULU	0.032 a	0.312 bcd	0.179 abc	0.410 abcd	0.066 ab	0.267 bcd
	CITRUS	NISO	0.143 a	0.332 bcd	0.319 ab	0.481 ab	0.081 ab	0.315 abc
		HULU	0.163 a	0.557 a	0.360 a	0.531 a	0.133 a	0.421 a
	FLORAL	NISO	0.134 a	0.228 cd	0.167 abc	0.227 de	0.000 b	0.168 cde
		HULU	0.116 a	0.486 ab	0.348 a	0.432 abc	0.028 ab	0.338 ab
	IPA	NISO1	0.041 a	0.232 cd	0.131 abc	0.253 cde	0.045 ab	0.175 cde
		NISO2	0.116 a	0.248 cd	0.086 bc	0.179 e	0.036 ab	0.167 de
		HULU	0.056 a	0.138 d	0.179 abc	0.132 e	0.000 b	0.099 e
	SPICY	NISO	0.008 a	0.188 cd	0.026 c	0.277 bcde	0.000 b	0.163 de
		HULU1	0.119 a	0.271 bcd	0.180 abc	0.324 bcde	0.071 ab	0.225 bcde
		HULU2	0.059 a	0.209 cd	0.111 abc	0.259 cde	0.025 ab	0.175 cde
	SYLVAN	NISO	0.124 a	0.397 abc	0.152 abc	0.280 bcde	0.008 b	0.243 bcde
		HULU	0.036 a	0.132 d	0.022 c	0.165 e	0.000 b	0.102 e
Sweet		ISO	0.150 cde	0.195 cd	0.063 cd	0.470 a	0.020 ab	0.135 b
		NISO	0.155 bcde	0.175 cd	0.042 cd	0.456 a	0.000 b	0.109 b
		HULU	0.295 abcde	0.370 bc	0.258 abc	0.453 a	0.041 ab	0.304 a
	CITRUS	NISO	0.409 ab	0.420 b	0.277 abc	0.432 a	0.072 a	0.326 a
		HULU	0.278 abcde	0.366 bc	0.375 a	0.422 a	0.031 ab	0.315 a
	FLORAL	NISO	0.372 abc	0.435 b	0.119 bcd	0.417 a	0.007 ab	0.323 a
		HULU	0.351 abcd	0.443 b	0.416 a	0.387 a	0.013 ab	0.304 a
	IPA	NISO1	0.115 de	0.058 d	0.003 d	0.016 b	0.000 b	0.031 b
		NISO2	0.047 e	0.113 d	0.049 cd	0.088 b	0.004 ab	0.073 b
		HULU	0.157 bcde	0.109 d	0.065 cd	0.064 b	0.000 b	0.070 b
	SPICY	NISO	0.439 a	0.449 b	0.434 c	0.453 a	0.000 b	0.349 a
		HULU1	0.422 a	0.442 b	0.360 c	0.432 a	0.011 ab	0.337 a
		HULU2	0.467 a	0.702 a	0.458 c	0.470 a	0.005 ab	0.443 a
	SYLVAN	NISO	0.047 e	0.105 d	0.101 bc	0.103 b	0.000 b	0.078 b
		HULU	0.137 cde	0.122 d	0.112 bc	0.118 b	0.000 b	0.094 b
Warming		ISO	ns	0.420 a	0.392 ab	0.607 a	0.724 a	0.525 a
		NISO	ns	0.302 ab	0.234 ab	0.477 a	0.805 a	0.451 a
		HULU	ns	0.376 ab	0.354 ab	0.573 a	0.700 a	0.491 a
	CITRUS	NISO	ns	0.236 b	0.188 b	0.456 a	0.775 a	0.413 a
		HULU	ns	0.368 ab	0.467 ab	0.500 a	0.674 a	0.465 a
	FLORAL	NISO	ns	0.399 ab	0.441 ab	0.528 a	0.632 a	0.473 a
		HULU	ns	0.261 ab	0.235 ab	0.494 a	0.678 a	0.415 a
	IPA	NISO1	ns	0.344 ab	0.401 ab	0.463 a	0.716 a	0.450 a
		NISO2	ns	0.260 ab	0.253 ab	0.462 a	0.687 a	0.407 a
		HULU	ns	0.393 ab	0.457 ab	0.635 a	0.722 a	0.528 a
	SPICY	NISO	ns	0.385 ab	0.429 ab	0.560 a	0.709 a	0.496 a
		HULU1	ns	0.393 ab	0.344 ab	0.531 a	0.889 a	0.524 a
		HULU2	ns	0.405 ab	0.467 ab	0.549 a	0.795 a	0.521 a
	SYLVAN	NISO	ns	0.329 ab	0.414 ab	0.626 a	0.800 a	0.519 a
		HULU	ns	0.398 ab	0.511 a	0.550 a	0.686 a	0.496 a

ns, not selected

8.3 Appendix 3 (Chapter 5)

8.3.1 Figures



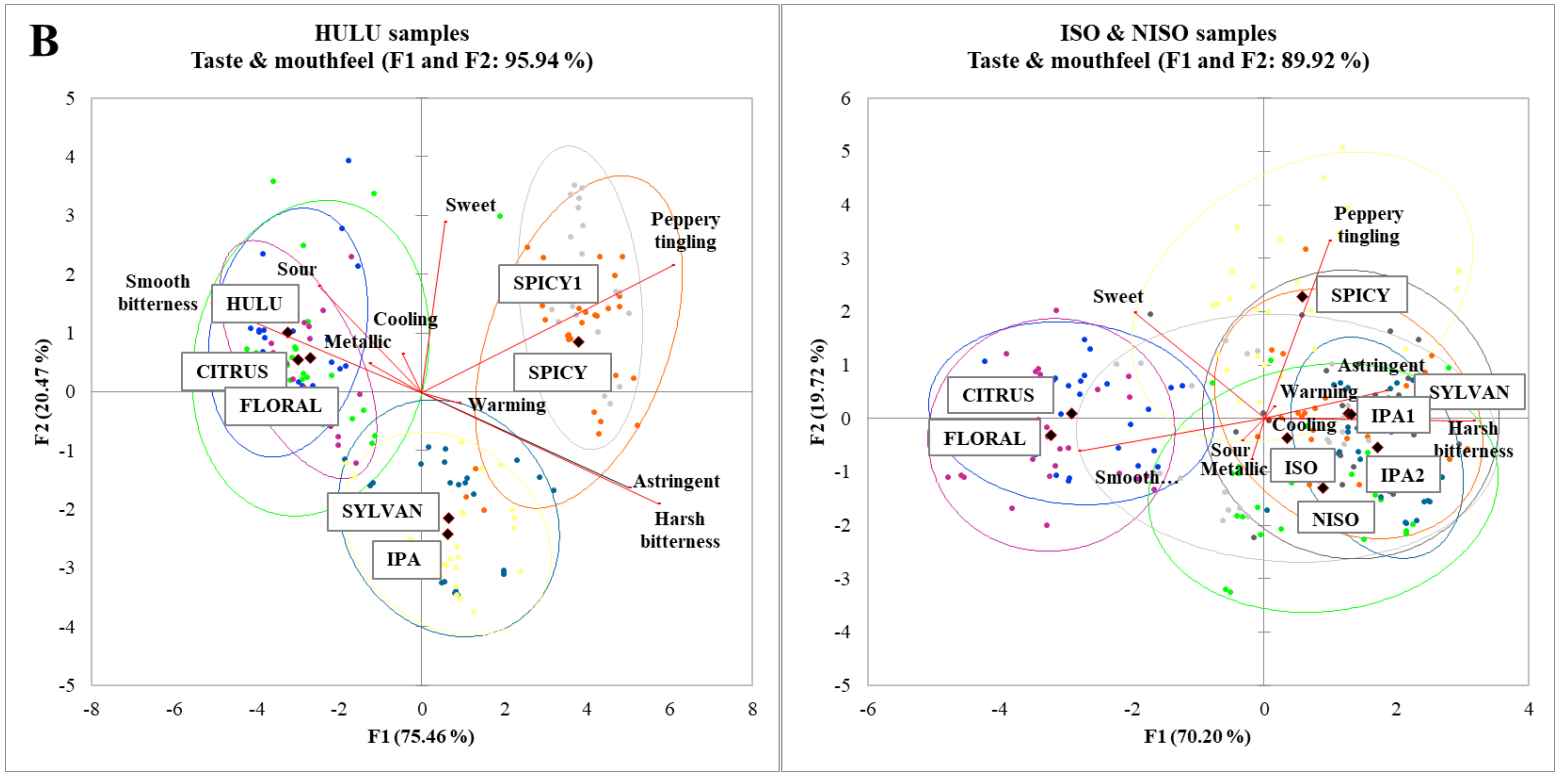
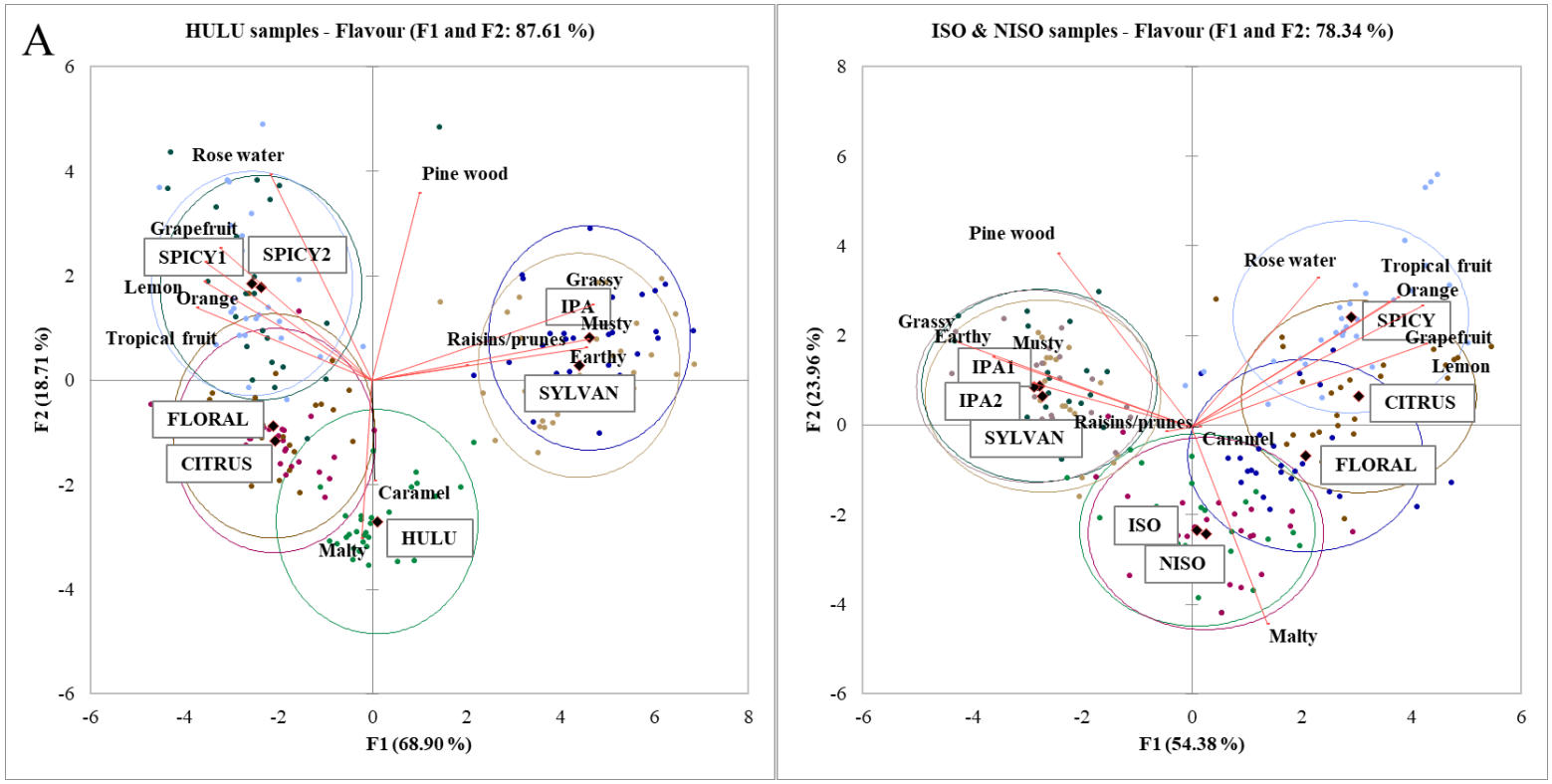


Figure 8.2. Canonical Variate Analysis (CVA) maps computed within HULU (n=7) or ISO/NISO sample sets (n=8) from raw flavour and taste and mouthfeel data. Bold black diamonds illustrate the sample means and scattered dots the evaluation data points of individual panellists. Non-overlapping confidence ellipses indicate significant differences (p<0.10) among samples.


A
HULU samples - Flavour (F1 and F2: 87.61 %)
F2 (18.71 %)
F1 (68.90 %)
Rose water
Pine wood
Grapefruit
SPICY1
SPICY2
Lemon
Orange
Tropical fruit
Grassy
IPA
Musty
Raisins/prunes
Earthy
SYLVAN
FLORAL
CITRUS
Caramel
HULU
Malty
ISO & NISO samples - Flavour (F1 and F2: 78.34 %)
F2 (23.96 %)
F1 (54.38 %)
Pine wood
Rose water
Tropical fruit
Orange
Grassy
Earthy
Musty
SPICY
Grapefruit
IPA1
Lemon
IPA2
CITRUS
Raisins/prunes
SYLVAN
Caramel
FLORAL
ISO
NISO
Malty

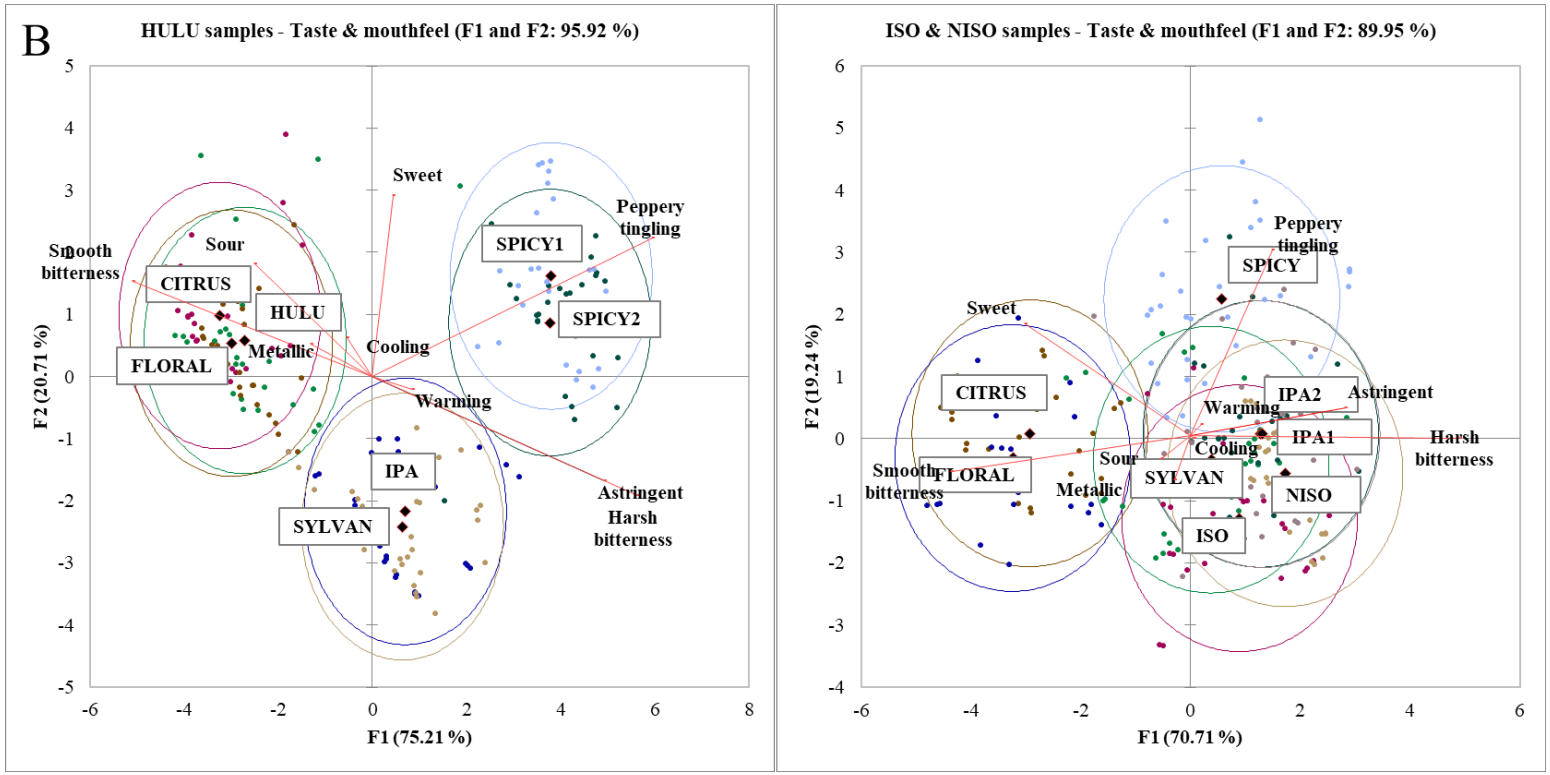
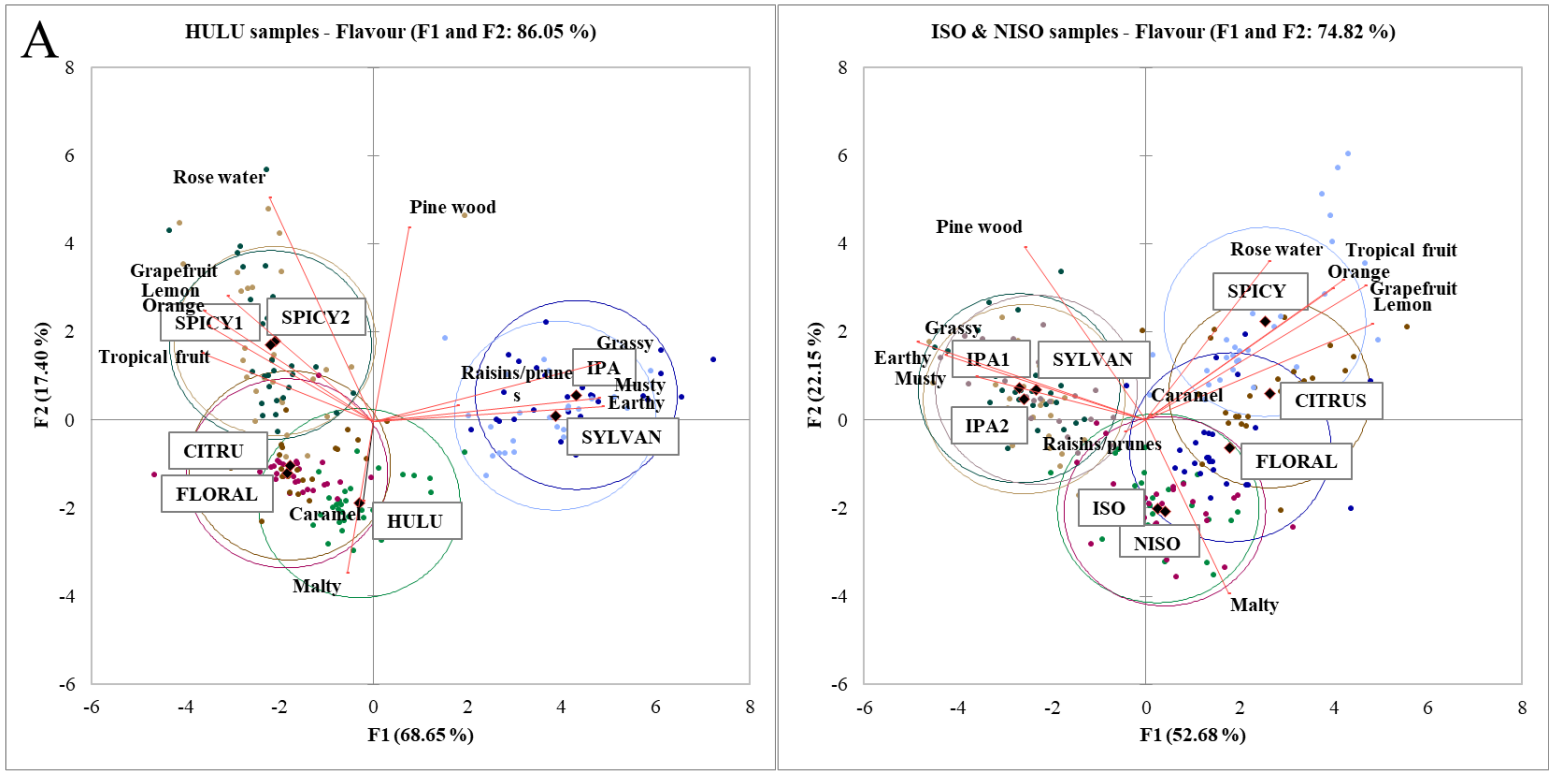


Figure 8.3. Canonical Variate Analysis (CVA) maps computed within HULU (n=7) or ISO/NISO sample sets (n=8) from flavour (**A**) and taste and mouthfeel data time standardised by modality (**B**). Bold black diamonds illustrate the sample means and scattered dots the evaluation data points of individual panellists. Non-overlapping confidence ellipses indicate significant differences (p<0.10) among samples.


A
HULU samples - Flavour (F1 and F2: 86.05 %)
F2 (17.40 %)
F1 (68.65 %)
Rose water
Pine wood
Grapefruit
Lemon
Orange
SPICY1
SPICY2
Tropical fruit
Grassy
IPA
Raisins/prune
s
Musty
Earthy
SYLVAN
CITRU
FLORAL
Caramel
HULU
Malty
ISO & NISO samples - Flavour (F1 and F2: 74.82 %)
F2 (22.15 %)
F1 (52.68 %)
Pine wood
Rose water
Tropical fruit
Orange
Grapefruit
Lemon
SPICY
Grassy
Earthy
Musty
IPA1
SYLVAN
IPA2
Caramel
CITRUS
Raisins/prunes
FLORAL
ISO
NISO
Malty

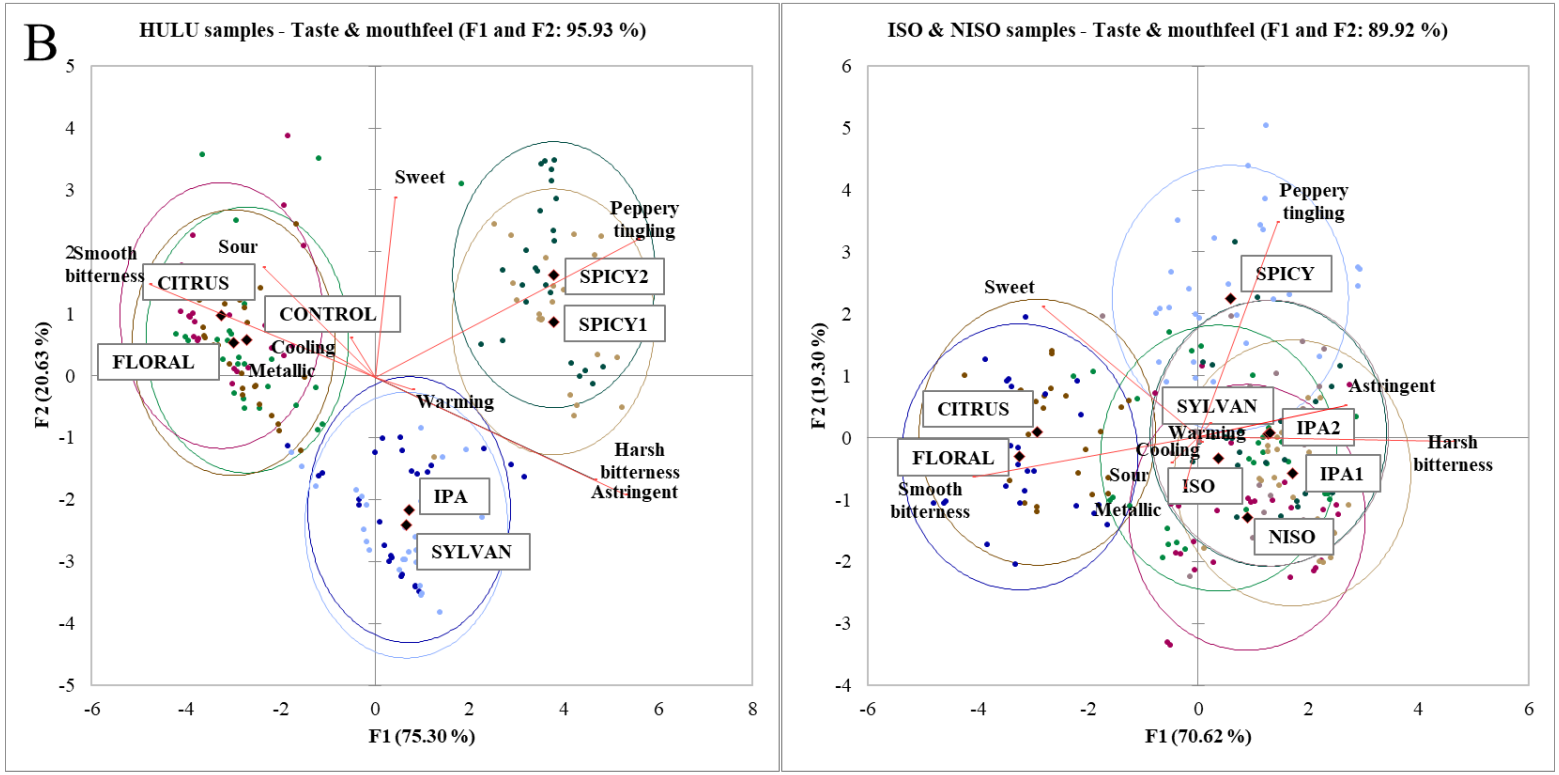
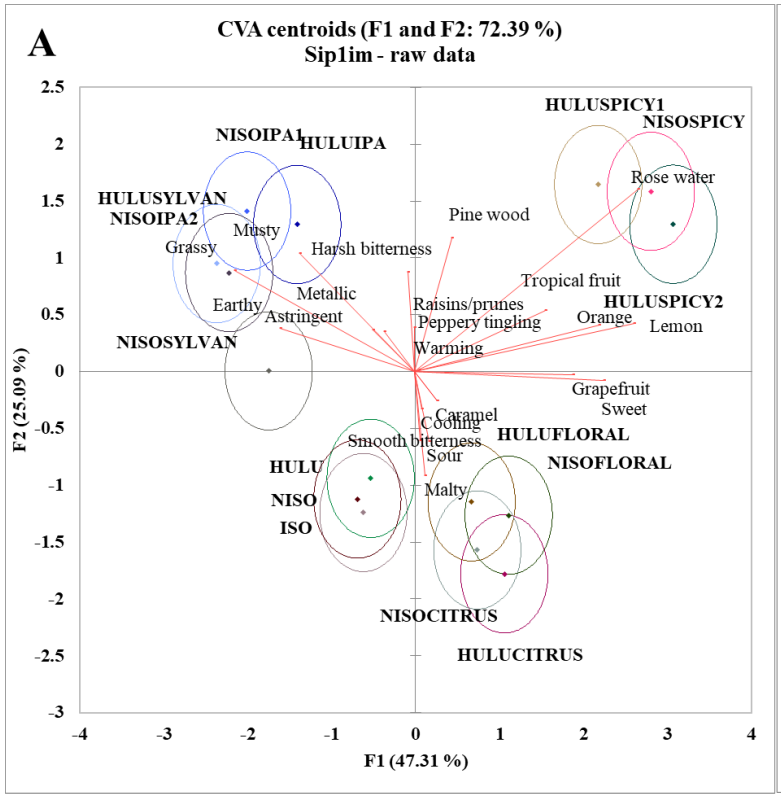
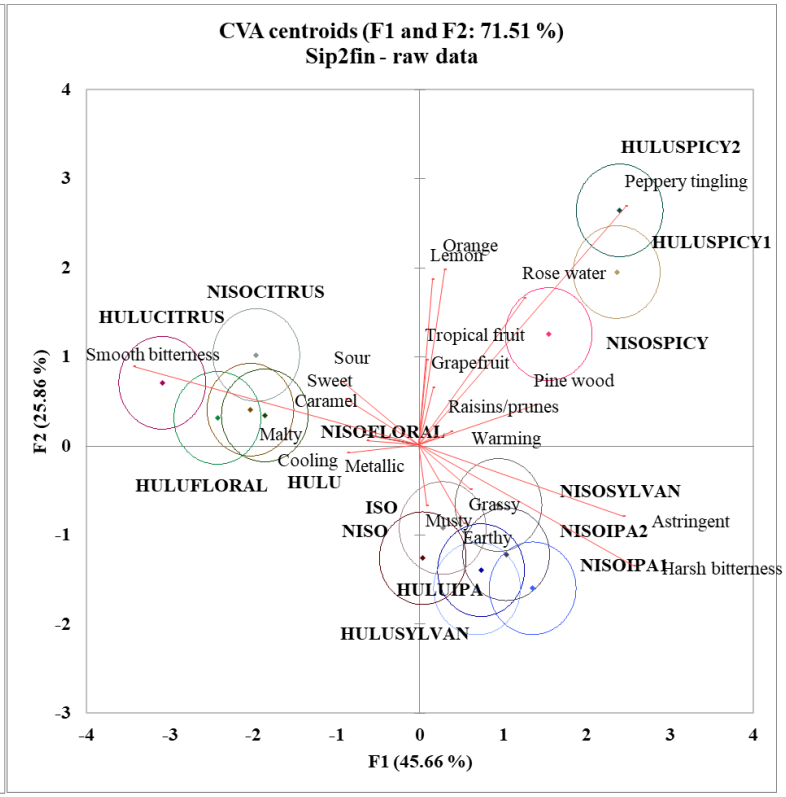
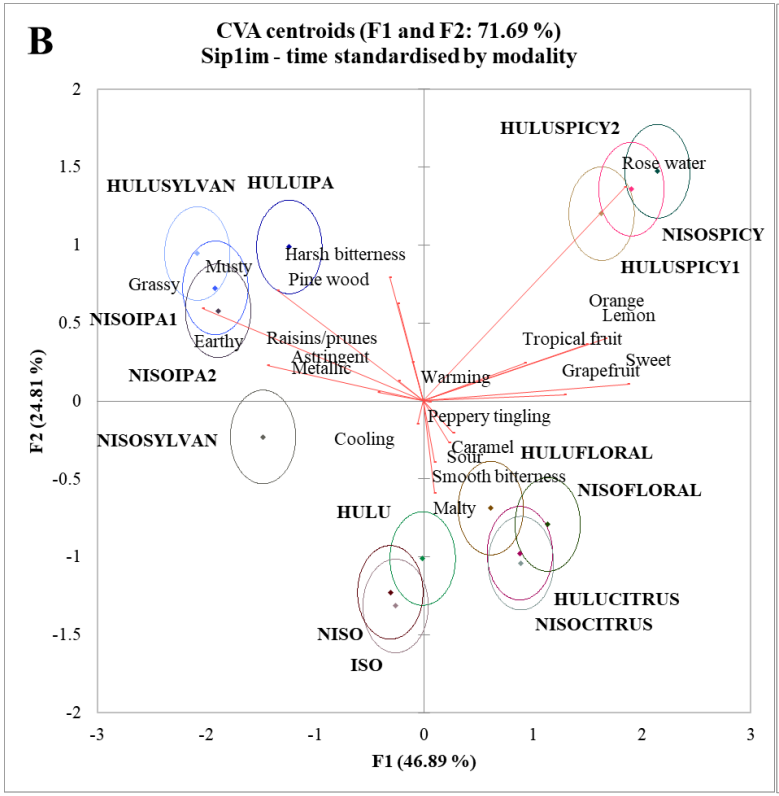
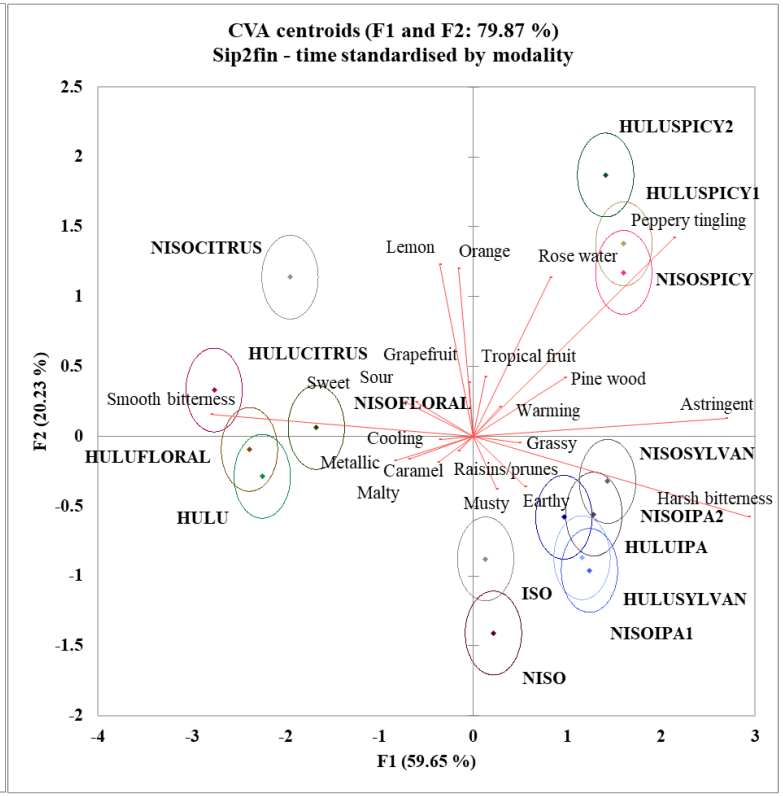


Figure 8.4. Canonical Variate Analysis (CVA) maps computed within HULU (n=7) or ISO/NISO sample sets (n=8) from flavour (**A**) and taste and mouthfeel data time standardised with merged modalities (**B**). Bold black diamonds illustrate the sample means and scattered dots the evaluation data points of individual panellists. Non-overlapping confidence ellipses indicate significant differences (p<0.10) among samples.


A
CVA centroids (F1 and F2: 72.39 %)
Sip1im - raw data
F2 (25.09 %)
F1 (47.31 %)
HULUSPICY1
NISOSPICY
NISOIPA1
HULUIPA
HULUSYLVAN
NISOIPA2
Musty
Grassy
Harsh bitterness
Pine wood
Rose water
Tropical fruit
Metallic
Earthy
Astringent
Raisins/prunes
Peppery tingling
HULUSPICY2
Orange
Lemon
NISOSYLVAN
Warming
Grapefruit
Sweet
Caramel
Cooling
Smooth bitterness
HULUFLORAL
Sour
HULU
NISOFLORAL
NISO
Malty
ISO
NISOCITRUS
HULUCITRUS


CVA centroids (F1 and F2: 71.51 %)
Sip2fin - raw data
F2 (25.86 %)
F1 (45.66 %)
HULUSPICY2
Peppery tingling
HULUSPICY1
Orange
Lemon
Rose water
NISOCITRUS
HULUCITRUS
Tropical fruit
NISOSPICY
Smooth bitterness
Sour
Grapefruit
Pine wood
Sweet
Caramel
Raisins/prunes
Malty
NISOFLORAL
Warming
Cooling
Metallic
HULUFLORAL
HULU
NISOSYLVAN
ISO
Grassy
Musty
NISO
NISOIPA2
Astringent
Earthy
NISOIPA1
Harsh bitterness
HULUIPA
HULUSYLVAN


B
CVA centroids (F1 and F2: 71.69 %)
Sip1im - time standardised by modality
F1 (46.89 %)
F2 (24.81 %)
HULUSPICY2
Rose water
HULUSYLVAN
HULUIPA
NISOSPICY
HULUSPICY1
Harsh bitterness
Pine wood
Grassy
Musty
Orange
Lemon
NISOIPA1
Earthy
Raisins/prunes
Tropical fruit
Astringent
Metallic
Sweet
NISOIPA2
Warming
Grapefruit
Peppery tingling
NISOSYLVAN
Cooling
Caramel
HULUFLORAL
Sour
Smooth bitterness
NISOFLORAL
HULU
Malty
HULUCITRUS
NISOCITRUS
NISO
ISO


CVA centroids (F1 and F2: 79.87 %)
Sip2fin - time standardised by modality
F1 (59.65 %)
F2 (20.23 %)
HULUSPICY2
HULUSPICY1
Peppery tingling
NISOCITRUS
Lemon
Orange
Rose water
NISOSPICY
HULUCITRUS
Grapefruit
Tropical fruit
Pine wood
Smooth bitterness
Sweet
Sour
NISOFLORAL
Warming
Astringent
Cooling
Grassy
NISOSYLVAN
HULUFLORAL
Metallic
Caramel
Raisins/prunes
Malty
Musty
Earthy
Harsh bitterness
NISOIPA2
HULU
HULUIPA
ISO
HULUSYLVAN
NISOIPA1
NISO

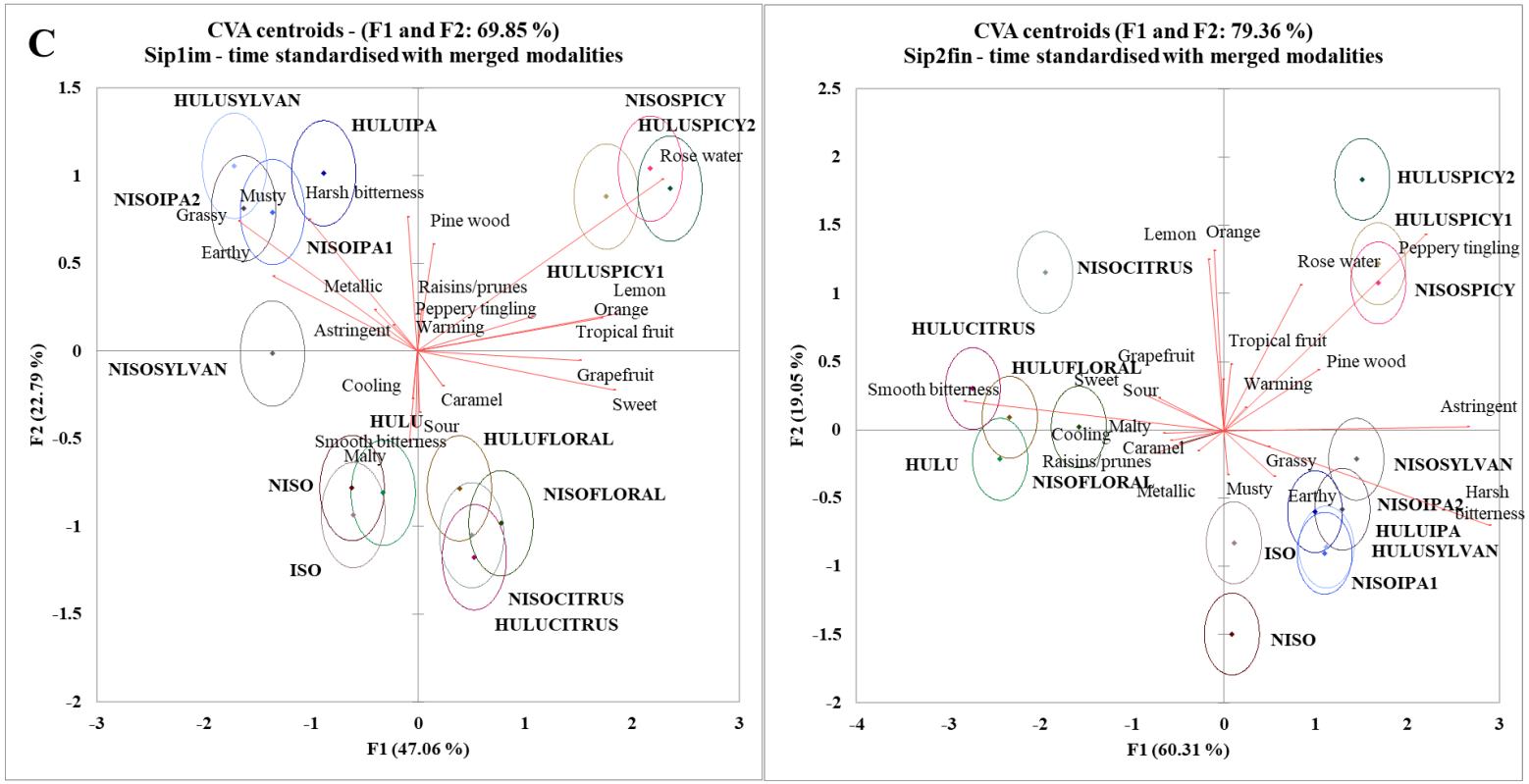
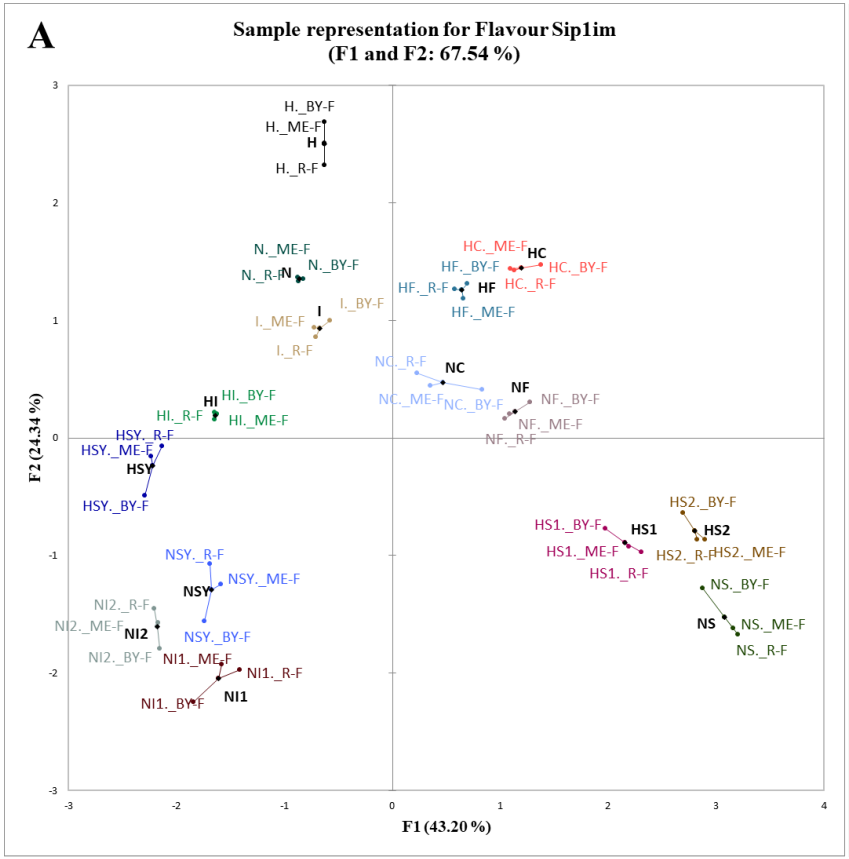
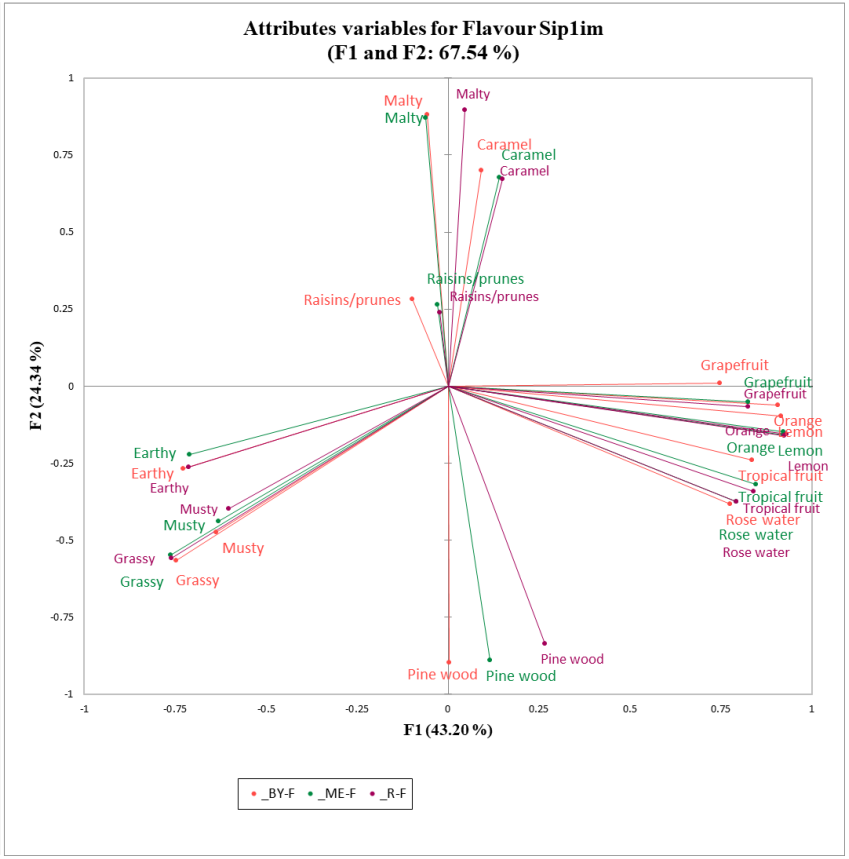


Figure 8.5. Canonical Variate Analysis (CVA) maps computed for Sip1im and Sip2fin sip segments of samples (n=15) in a multisensory space computed for the raw data (**A**), data time standardised by modality (**B**), and data time standardised with merged modalities (**C**). Bold diamonds (centroids) indicate the sample means. Non-overlapping confidence ellipses indicate significant differences (p<0.10) among samples.


A
Sample representation for Flavour Sip1im
(F1 and F2: 67.54 %)
F2 (24.34 %)
F1 (43.20 %)
H._BY-F
H._ME-F
H
H._R-F
N._ME-F
N._R-F
N
N._BY-F
I._BY-F
I._ME-F
I
I._R-F
HC._ME-F
HC
HF._BY-F
HC._BY-F
HF._R-F
HF
HC._R-F
HF._ME-F
NC._R-F
NC
NC._ME-F
NC._BY-F
NF
NF._BY-F
NF._ME-F
NF._R-F
HI
HI._BY-F
HI._R-F
HI._ME-F
HSY._R-F
HSY._ME-F
HSY
HSY._BY-F
HS2._BY-F
HS1._BY-F
HS1
HS2
HS1._ME-F
HS2._R-F
HS2._ME-F
HS1._R-F
NSY._R-F
NSY
NSY._ME-F
NS._BY-F
NI2._R-F
NI2._ME-F
NI2
NSY._BY-F
NS
NS._ME-F
NI2._BY-F
NI1._ME-F
NS._R-F
NI1._R-F
NI1._BY-F
NI1


Attributes variables for Flavour Sip1im
(F1 and F2: 67.54 %)
F2 (24.34 %)
F1 (43.20 %)
Malty
Malty
Malty
Caramel
Caramel
Caramel
Raisins/prunes
Raisins/prunes
Raisins/prunes
Grapefruit
Grapefruit
Grapefruit
Orange
Orange
Orange
Lemon
Lemon
Lemon
Tropical fruit
Tropical fruit
Tropical fruit
Rose water
Rose water
Rose water
Earthy
Earthy
Earthy
Musty
Musty
Musty
Grassy
Grassy
Grassy
Pine wood
Pine wood
Pine wood
_BY-F
_ME-F
_R-F

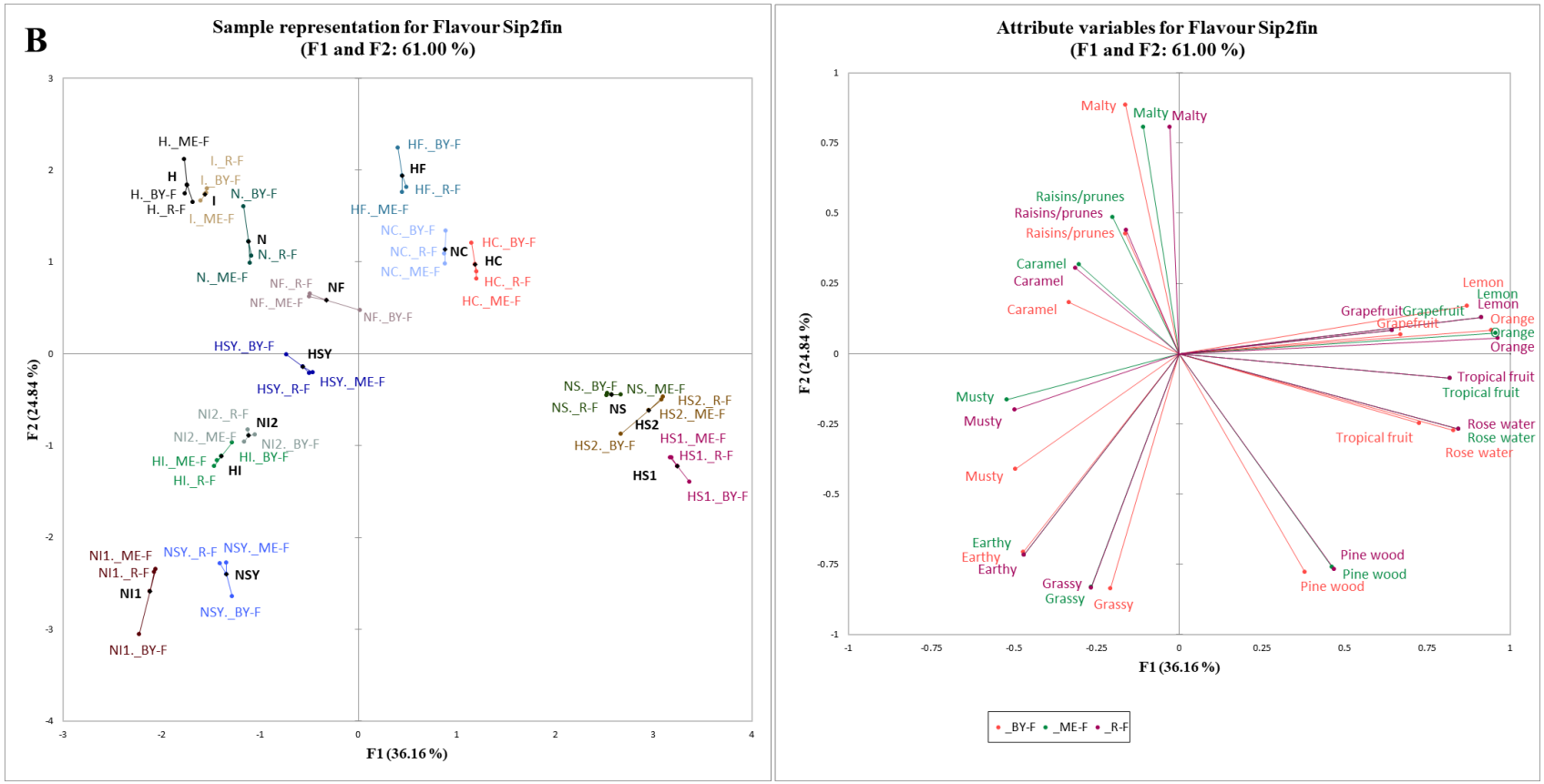


Figure 8.6. Multiple Factor Analysis (MFA) of beer samples (n=15) in their flavour (F) spaces for comparison of the pre-processing approaches. Representation of samples and perceptual maps showing loadings of attributes are displayed for Sip1im and Sip2fin representing the start and the end of the evaluation period. Bold diamonds represent the samples' centroids. Vectors labels indicate the pre-processing approach: raw data (R), data time standardised by modality (BY), and data time standardised with merged modalities (ME). To improve the readability of the maps, sample names were further abbreviated as follows: I (ISO), N (NISO), H (HULU) beers with or without addition of C (CITRUS), F (FLORAL), I (IPA), S (SPICY), or SY (Sylvan) extracts, and 1 or 2 for experimental replicates.

8.3.2 Tables

Table 8.7. *p*-values from Mixed Model ANOVA for each of the responses for the flavour attribute citation proportions computed from the raw, non-processed data, data time standardised by modality (Std. by modality), and data time standardised with merged modalities (Std. merged modality), obtained for the total evaluation period and selected time segments (Sip1im, Sip2fin). Significant interaction effects (p<0.05) are indicated by p-values shown in bold.

Dataset	Sip segment	Attribute/ Interaction	Caramel	Earthy	Grapefruit	Grassy	Lemon	Malty	Musty	Orange	Pine wood	Raisins/ prunes	Rose water	Tropical fruit
Raw data	Sip1im	Panellist	0.407	0.329	0.936	0.963	0.949	0.905	0.290	0.905	0.664	0.804	0.880	0.083
Std. by modality	Sip1im	Panellist	0.657	0.080	0.823	0.575	0.258	0.938	0.460	0.622	0.638	0.155	0.622	0.108
Std. merged modality	Sip1im	Panellist	0.928	0.713	0.818	0.159	0.701	0.967	**0.033**	0.901	0.524	0.465	0.984	0.612
Raw data	Sip1im	Position	0.631	0.120	0.648	0.681	0.365	0.415	0.770	0.415	0.563	0.188	0.274	0.170
Std. by modality	Sip1im	Position	**0.015**	0.683	0.941	0.724	**0.002**	0.581	0.968	0.666	0.263	0.496	0.899	**0.014**
Std. merged modality	Sip1im	Position	0.814	0.942	0.281	0.525	0.456	**0.021**	0.978	0.602	0.583	0.524	0.880	0.808
Raw data	Sip1im	Position* Sample	**0.010**	0.717	0.097	0.801	0.227	0.384	0.902	0.384	0.146	0.173	0.515	0.369
Std. by modality	Sip1im	Position* Sample	0.071	0.267	0.117	0.172	**0.004**	0.143	0.810	0.889	0.376	0.180	0.969	**0.013**
Std. merged modality	Sip1im	Position* Sample	0.142	0.774	0.430	0.953	0.438	**0.011**	0.949	0.509	0.798	0.065	0.939	0.746
Raw data	Sip1im	Replicate	0.984	0.720	0.684	0.887	0.917	0.963	**0.048**	0.963	0.450	0.757	0.967	0.552
Std. by modality	Sip1im	Replicate	**<0.0001**	**0.002**	**0.000**	**0.000**	**0.037**	**0.002**	0.435	**<0.0001**	0.238	0.197	0.263	0.137
Std. merged modality	Sip1im	Replicate	0.898	0.565	0.956	0.990	0.907	0.599	0.769	0.725	0.868	0.911	0.513	0.669
Raw data	Sip1im	Replicate* Panellist	**<0.0001**	0.265	**0.000**	0.071	**<0.0001**	**<0.0001**	0.194	**<0.0001**	**0.011**	**0.000**	**0.018**	0.509
Std. by modality	Sip1im	Replicate*Panellist	0.191	0.795	0.707	0.967	0.192	0.395	**0.026**	0.678	**0.000**	**0.007**	0.266	0.505
Std. merged modality	Sip1im	Replicate*Panellist	**<0.0001**	**0.008**	**0.009**	0.576	**0.031**	**<0.0001**	0.432	**<0.0001**	**0.041**	**0.002**	0.053	0.368
Raw data	Sip1im	Replicate* Sample	0.999	0.997	0.952	0.085	0.671	0.959	0.779	0.959	0.444	0.421	0.642	0.842
Std. by modality	Sip1im	Replicate*Sample	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**	**0.003**	**0.029**	**0.009**	**0.009**	**0.009**	**0.007**	0.547	**<0.0001**
Std. merged modality	Sip1im	Replicate*Sample	0.998	0.551	0.874	0.967	0.966	0.601	0.787	0.978	0.788	0.416	0.911	0.704
Raw data	Sip1im	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	**0.000**
Std. by modality	Sip1im	Sample	**<0.0001**	**0.000**	**<0.0001**	**<0.0001**	**0.001**	**0.012**	**0.001**	**0.001**	0.118	**0.000**	**0.000**	**0.001**
Std. merged modality	Sip1im	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.092**	**<0.0001**	**<0.0001**
Raw data	Sip1im	Sample*Panellist	0.679	0.485	0.649	0.968	0.236	0.603	0.990	0.603	0.070	0.078	0.117	0.566
Std. by modality	Sip1im	Sample*Panellist	0.427	0.246	0.644	0.610	0.121	0.702	0.319	0.376	0.050	0.992	**0.022**	0.610
Std. merged modality	Sip1im	Sample*Panellist	0.519	0.686	0.636	0.668	0.342	**0.019**	0.750	0.797	0.997	**0.015**	0.986	0.995

Table 8.7. continued.

Dataset	Sip segment	Attribute/ Interaction	Caramel	Earthy	Grapefruit	Grassy	Lemon	Malty	Musty	Orange	Pine wood	Raisins/ prunes	Rose water	Tropical fruit
Raw data	Sip2fin	Panellist	0.585	0.570	0.728	0.959	0.660	0.686	0.507	0.755	0.922	0.679	0.427	0.786
Std. by modality	Sip2fin	Panellist	0.405	0.438	0.832	0.455	0.565	0.825	0.658	0.276	0.510	0.288	0.494	0.790
Std. merged modality	Sip2fin	Panellist	0.122	0.174	0.680	0.840	0.491	0.983	0.933	0.844	0.124	0.908	0.678	0.818
Raw data	Sip2fin	Position	**0.003**	0.855	0.225	0.987	0.774	0.410	0.337	0.274	0.243	0.674	0.470	0.729
Std. by modality	Sip2fin	Position	0.361	0.995	0.070	0.082	0.285	0.740	0.826	0.758	0.743	**0.035**	0.762	0.395
Std. merged modality	Sip2fin	Position	**<0.0001**	0.789	0.200	0.695	**0.001**	0.457	0.506	0.903	**0.001**	0.968	0.243	**0.026**
Raw data	Sip2fin	Position* Sample	**<0.0001**	0.951	0.358	1.000	0.286	0.145	0.598	0.529	0.717	0.642	0.526	0.722
Std. by modality	Sip2fin	Position* Sample	0.892	1.000	0.566	0.118	0.524	0.653	0.996	0.833	0.517	0.118	0.988	0.644
Std. merged modality	Sip2fin	Position* Sample	**<0.0001**	0.991	0.351	0.393	**0.003**	0.790	0.809	0.521	0.131	1.000	0.054	**<0.0001**
Raw data	Sip2fin	Replicate	0.577	0.756	0.857	0.473	0.855	0.946	0.755	0.965	0.782	0.878	0.962	0.219
Std. by modality	Sip2fin	Replicate	0.606	0.189	**0.009**	**0.005**	**<0.0001**	**0.026**	0.196	**0.012**	**0.001**	0.133	0.138	0.797
Std. merged modality	Sip2fin	Replicate	0.910	0.651	0.560	0.985	0.948	0.936	0.940	0.930	0.892	0.928	0.739	0.768
Raw data	Sip2fin	Replicate* Panellist	0.619	**0.020**	**<0.0001**	0.118	**0.003**	**<0.0001**	**0.001**	**<0.0001**	**<0.0001**	0.243	**0.034**	0.220
Std. by modality	Sip2fin	Replicate*Panellist	0.690	**0.033**	0.050	**0.000**	**<0.0001**	**0.001**	**0.000**	**<0.0001**	0.190	**0.005**	0.160	**<0.0001**
Std. merged modality	Sip2fin	Replicate*Panellist	0.968	0.777	0.247	0.916	0.918	0.371	0.011	0.995	0.319	0.995	0.998	0.201
Raw data	Sip2fin	Replicate* Sample	0.511	**0.048**	**0.010**	0.580	0.371	**0.030**	0.193	0.173	0.345	0.656	0.145	0.162
Std. by modality	Sip2fin	Replicate*Sample	0.970	0.461	**<0.0001**	**<0.0001**	**0.000**	**0.047**	1.000	**<0.0001**	0.056	**0.017**	**<0.0001**	0.994
Std. merged modality	Sip2fin	Replicate*Sample	0.810	0.986	0.625	0.624	0.612	0.275	0.993	0.867	0.977	1.000	0.046	0.972
Raw data	Sip2fin	Sample	0.788	0.171	0.143	0.220	**<0.0001**	0.090	0.308	**<0.0001**	**0.002**	0.368	**<0.0001**	0.095
Std. by modality	Sip2fin	Sample	0.241	0.193	0.221	**0.006**	**0.001**	**0.010**	0.127	**0.004**	**0.003**	**0.019**	**0.001**	0.667
Std. merged modality	Sip2fin	Sample	0.062	0.117	0.180	**<0.0001**	**0.000**	0.254	**<0.0001**	0.056	0.312	**<0.0001**	0.149	**<0.0001**
Raw data	Sip2fin	Sample*Panellist	**<0.0001**	0.900	0.406	0.968	0.312	0.182	0.115	0.823	0.565	0.890	0.824	0.907
Std. by modality	Sip2fin	Sample*Panellist	0.214	0.157	0.423	0.849	0.357	0.635	0.738	0.240	0.944	0.884	0.330	0.307
Std. merged modality	Sip2fin	Sample*Panellist	**<0.0001**	0.981	0.125	0.557	**0.009**	0.792	0.912	0.106	**0.045**	0.990	0.865	0.088

Table 8.7. continued.

Dataset	Sip segment	Attribute/ Interaction	Caramel	Earthy	Grapefruit	Grassy	Lemon	Malty	Musty	Orange	Pine wood	Raisins/ prunes	Rose water	Tropical fruit
Raw data	Total	Panellist	0.116	0.921	0.877	1.000	0.836	0.815	0.392	0.795	0.534	0.990	0.128	0.319
Std. by modality	Total	Panellist	0.154	0.353	0.901	0.489	0.710	0.673	0.050	0.632	0.380	0.498	0.185	0.713
Std. merged modality	Total	Panellist	0.807	0.902	0.522	0.802	0.863	0.976	0.645	0.837	0.874	0.980	0.474	0.467
Raw data	Total	Position	0.987	0.422	0.598	0.795	0.925	0.863	0.150	0.733	0.772	0.480	**0.025**	0.975
Std. by modality	Total	Position	0.482	0.933	0.102	0.296	0.525	0.108	0.989	0.567	0.940	0.620	0.355	0.610
Std. merged modality	Total	Position	**0.046**	0.270	0.299	0.486	0.641	0.219	0.282	0.219	0.808	0.783	0.985	0.673
Raw data	Total	Position* Sample	0.927	0.278	0.443	0.954	0.353	0.787	**0.035**	0.666	0.842	0.583	0.459	0.706
Std. by modality	Total	Position* Sample	**0.008**	0.920	0.688	0.990	0.466	0.200	0.830	0.954	0.254	0.530	0.987	0.956
Std. merged modality	Total	Position* Sample	**0.001**	0.236	0.998	0.380	0.714	0.765	0.106	0.255	0.881	0.751	0.908	0.301
Raw data	Total	Replicate	0.969	0.923	0.659	0.832	0.760	0.955	0.160	0.938	0.678	0.937	0.648	0.446
Std. by modality	Total	Replicate	**<0.0001**	**<0.0001**	**<0.0001**	**0.033**	**<0.0001**	**<0.0001**	**0.020**	**<0.0001**	**0.047**	**<0.0001**	**0.002**	0.084
Std. merged modality	Total	Replicate	0.965	0.952	0.649	0.840	0.798	0.878	0.676	0.988	0.844	0.617	0.890	0.811
Raw data	Total	Replicate* Panellist	0.254	**<0.0001**	**<0.0001**	**0.003**	**0.003**	**<0.0001**	0.093	**0.001**	**0.002**	**<0.0001**	**0.034**	0.268
Std. by modality	Total	Replicate*Panellist	**0.007**	**<0.0001**	**0.002**	**0.049**	**0.005**	**<0.0001**	0.067	**<0.0001**	**<0.0001**	**0.000**	**0.002**	0.074
Std. merged modality	Total	Replicate*Panellist	0.998	0.982	0.945	0.996	0.992	0.447	0.123	0.942	0.592	0.950	0.285	0.702
Raw data	Total	Replicate* Sample	0.510	0.086	0.054	0.868	0.925	0.263	0.904	0.947	0.112	0.795	0.662	0.758
Std. by modality	Total	Replicate*Sample	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	**<0.0001**	0.682	**<0.0001**	**0.001**	**<0.0001**	**0.017**	**<0.0001**
Std. merged modality	Total	Replicate*Sample	0.995	0.769	0.935	0.886	0.937	0.538	0.742	0.996	0.681	0.899	0.997	0.815
Raw data	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Std. by modality	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Std. merged modality	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Raw data	Total	Sample*Panellist	0.973	0.462	0.658	0.957	0.134	0.852	**0.032**	0.864	0.784	0.693	0.071	0.960
Std. by modality	Total	Sample*Panellist	0.549	0.620	0.087	0.836	0.051	0.288	0.168	0.460	0.572	0.791	0.530	0.979
Std. merged modality	Total	Sample*Panellist	0.128	0.903	1.000	0.117	0.931	0.783	**0.011**	0.668	0.181	0.914	0.973	0.747

Table 8.8. *p*-values from Mixed Model ANOVA for each of the responses for the taste and mouthfeel attribute citation proportions computed from the raw, non-processed data, data time standardised by modality (Std. by modality), and data time standardised with merged modalities (Std. merged modality), obtained for the total evaluation period and selected time segments (Sip1im, Sip2fin). Significant interaction effects ($p<0.05$) are indicated by p-values shown in bold.

Dataset	Sip segment	Attribute/ Interaction	Astringent	Cooling	Harsh bitterness	Metallic	Peppery tingling	Smooth bitterness	Sour	Sweet	Warming
Raw data	Sip1im	Panellist	0.341	0.898	0.901	0.826	0.953	0.602	0.116	0.370	ns
Std. by modality	Sip1im	Panellist	0.594	0.791	0.156	0.585	0.550	0.695	0.511	0.401	ns
Std. merged modality	Sip1im	Panellist	0.453	0.794	0.922	0.993	0.609	0.720	0.800	0.460	ns
Raw data	Sip1im	Position	0.230	0.421	0.165	1.000	0.129	0.686	**0.002**	0.376	ns
Std. by modality	Sip1im	Position	**0.041**	0.111	0.737	0.959	0.060	0.661	0.465	0.791	ns
Std. merged modality	Sip1im	Position	0.066	0.118	0.939	1.000	0.225	0.071	**0.030**	0.215	ns
Raw data	Sip1im	Position* Sample	0.444	0.906	0.608	1.000	0.385	0.756	**0.015**	0.646	ns
Std. by modality	Sip1im	Position* Sample	**0.009**	**0.004**	0.836	0.932	**0.003**	0.462	0.126	0.656	ns
Std. merged modality	Sip1im	Position* Sample	**0.008**	0.891	0.705	1.000	0.260	0.058	0.559	0.251	ns
Raw data	Sip1im	Replicate	0.734	1.000	0.952	0.591	0.965	0.958	0.738	0.973	ns
Std. by modality	Sip1im	Replicate	**0.035**	**<0.0001**	**<0.0001**	0.690	**<0.0001**	**<0.0001**	0.093	**<0.0001**	ns
Std. merged modality	Sip1im	Replicate	0.884	0.915	0.984	0.980	0.984	0.748	0.466	0.811	ns
Raw data	Sip1im	Replicate* Panellist	**0.002**	**<0.0001**	**<0.0001**	0.087	**<0.0001**	**<0.0001**	**0.034**	**<0.0001**	ns
Std. by modality	Sip1im	Replicate*Panellist	**<0.0001**	**<0.0001**	**<0.0001**	**0.041**	**<0.0001**	**<0.0001**	**0.002**	**0.000**	ns
Std. merged modality	Sip1im	Replicate*Panellist	0.775	0.823	0.989	0.961	0.993	1.000	0.932	0.875	ns
Raw data	Sip1im	Replicate* Sample	**0.026**	**0.008**	0.441	0.442	0.942	0.992	**0.008**	0.876	ns
Std. by modality	Sip1im	Replicate*Sample	0.137	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	ns
Std. merged modality	Sip1im	Replicate*Sample	0.940	0.968	0.997	0.956	0.994	0.327	0.643	0.966	ns
Raw data	Sip1im	Sample	0.667	0.121	**<0.0001**	**<0.0001**	**0.018**	**0.014**	**0.028**	**<0.0001**	ns
Std. by modality	Sip1im	Sample	0.129	**0.002**	**<0.0001**	**0.010**	**0.000**	**0.007**	**0.021**	**<0.0001**	ns
Std. merged modality	Sip1im	Sample	0.541	0.249	**<0.0001**	**0.013**	**0.018**	0.200	**0.004**	**<0.0001**	ns
Raw data	Sip1im	Sample*Panellist	0.185	0.761	0.795	1.000	0.667	0.926	**0.014**	0.071	ns
Std. by modality	Sip1im	Sample*Panellist	0.916	0.631	0.546	0.831	0.816	0.649	0.799	0.366	ns
Std. merged modality	Sip1im	Sample*Panellist	0.733	0.833	0.878	1.000	0.535	0.580	0.688	0.374	ns

ns, not selected

Table 8.8. continued.

Dataset	Sip segment	Attribute/ Interaction	Astringent	Cooling	Harsh bitterness	Metallic	Peppery tingling	Smooth bitterness	Sour	Sweet	Warming
Raw data	Sip2fin	Panellist	0.716	0.276	0.970	0.939	0.558	0.275	0.639	0.163	1.000
Std. by modality	Sip2fin	Panellist	0.723	1.000	0.388	0.900	0.346	0.259	0.340	0.763	0.816
Std. merged modality	Sip2fin	Panellist	0.267	0.976	0.986	0.986	0.798	0.837	0.877	0.740	0.795
Raw data	Sip2fin	Position	**0.013**	0.208	0.541	0.329	0.678	0.948	0.569	**0.003**	0.186
Std. by modality	Sip2fin	Position	0.121	**0.000**	**0.007**	**0.000**	0.858	0.627	**0.016**	**0.029**	0.730
Std. merged modality	Sip2fin	Position	**0.017**	0.961	0.971	0.971	0.341	0.183	0.284	0.715	0.396
Raw data	Sip2fin	Position* Sample	**0.009**	**0.008**	0.666	0.222	0.925	0.499	0.590	**0.005**	0.535
Std. by modality	Sip2fin	Position* Sample	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	0.937	0.067	**<0.0001**	**0.011**	0.753
Std. merged modality	Sip2fin	Position* Sample	**<0.0001**	0.990	0.876	0.876	0.223	0.405	0.118	0.341	0.073
Raw data	Sip2fin	Replicate	0.996	0.994	0.997	0.968	0.853	0.824	0.785	0.833	0.959
Std. by modality	Sip2fin	Replicate	**<0.0001**	**0.029**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	0.062	**<0.0001**
Std. merged modality	Sip2fin	Replicate	0.882	0.749	0.972	0.972	0.894	0.536	0.952	0.877	1.000
Raw data	Sip2fin	Replicate* Panellist	**<0.0001**	0.642	**<0.0001**	**<0.0001**	**0.000**	0.259	**<0.0001**	0.368	**<0.0001**
Std. by modality	Sip2fin	Replicate*Panellist	0.688	**0.000**	**<0.0001**	**<0.0001**	**<0.0001**	0.062	**<0.0001**	0.108	**<0.0001**
Std. merged modality	Sip2fin	Replicate*Panellist	1.000	1.000	1.000	1.000	0.998	0.644	0.354	1.000	0.950
Raw data	Sip2fin	Replicate* Sample	0.483	0.540	0.881	**0.027**	0.409	0.715	**0.040**	0.532	0.666
Std. by modality	Sip2fin	Replicate*Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Std. merged modality	Sip2fin	Replicate*Sample	1.000	0.978	1.000	1.000	1.000	0.316	0.989	1.000	0.992
Raw data	Sip2fin	Sample	**<0.0001**	0.607	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.045**	0.685	0.210
Std. by modality	Sip2fin	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**	**<0.0001**	**0.031**
Std. merged modality	Sip2fin	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.007**	0.090	**0.028**
Raw data	Sip2fin	Sample*Panellist	0.177	0.423	0.957	0.218	0.901	0.398	0.430	0.252	0.756
Std. by modality	Sip2fin	Sample*Panellist	0.834	1.000	0.500	0.955	0.351	0.097	0.296	0.990	0.150
Std. merged modality	Sip2fin	Sample*Panellist	0.797	0.999	0.767	0.767	0.721	0.793	0.423	0.447	0.975

Table 8.8. continued.

Dataset	Sip segment	Attribute/ Interaction	Astringent	Cooling	Harsh bitterness	Metallic	Peppery tingling	Smooth bitterness	Sour	Sweet	Warming
Raw data	Total	Panellist	**0.015**	0.974	0.856	0.880	0.616	0.180	0.942	0.603	0.998
Std. by modality	Total	Panellist	0.436	0.228	0.981	0.778	0.425	0.669	0.500	0.958	0.171
Std. merged modality	Total	Panellist	0.942	0.606	0.717	0.957	0.999	0.370	0.590	0.956	0.933
Raw data	Total	Position	0.063	0.119	0.151	0.290	0.349	0.355	0.728	0.326	0.690
Std. by modality	Total	Position	0.186	0.965	**0.000**	0.097	0.993	0.721	0.085	**0.009**	0.703
Std. merged modality	Total	Position	**0.045**	**0.046**	0.744	0.652	0.552	0.993	0.213	0.972	0.163
Raw data	Total	Position* Sample	0.226	0.108	0.213	**0.000**	0.369	0.909	0.969	0.241	0.624
Std. by modality	Total	Position* Sample	**0.016**	0.567	0.051	0.263	0.363	**0.025**	**0.010**	0.320	0.889
Std. merged modality	Total	Position* Sample	**0.001**	0.443	0.506	0.552	0.234	0.526	0.262	0.286	0.272
Raw data	Total	Replicate	0.928	0.994	0.994	0.988	0.873	0.819	0.983	0.954	0.998
Std. by modality	Total	Replicate	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.040**	**<0.0001**	**<0.0001**	**<0.0001**
Std. merged modality	Total	Replicate	0.965	0.978	0.690	0.993	0.916	0.932	0.825	0.988	0.954
Raw data	Total	Replicate* Panellist	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.007**	0.693	**<0.0001**	**<0.0001**	**<0.0001**
Std. by modality	Total	Replicate*Panellist	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**	0.403	**<0.0001**	**<0.0001**	**<0.0001**
Std. merged modality	Total	Replicate*Panellist	0.997	0.975	0.996	0.998	0.993	0.993	1.000	0.999	0.882
Raw data	Total	Replicate* Sample	0.628	0.539	0.730	0.243	0.122	0.296	0.372	0.113	**0.018**
Std. by modality	Total	Replicate*Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Std. merged modality	Total	Replicate*Sample	0.754	0.898	0.954	0.999	0.997	0.918	0.925	1.000	0.899
Raw data	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.018**
Std. by modality	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**
Std. merged modality	Total	Sample	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.000**
Raw data	Total	Sample*Panellist	0.188	0.287	0.715	0.256	0.458	0.927	0.753	0.701	0.833
Std. by modality	Total	Sample*Panellist	0.266	0.550	0.731	0.266	0.816	0.105	0.449	0.428	0.954
Std. merged modality	Total	Sample*Panellist	0.146	0.905	0.819	0.881	0.872	0.874	**0.018**	0.846	0.965

Table 8.9. Mean panel proportion citations (n=10) computed from the raw TCATA data for the total evaluation period and time segments as evaluated using flavour and taste & mouthfeel attributes. Different letters within columns representing significant differences among the samples based on differences in least squares means (p<0.05).

This table is equivalent to Table 8.6 (Appendix 2).

Table 8.10. Mean panel proportion citations (n=10) obtained by RM-ANOVA followed by Tukey's HSD computed from the TCATA data time standardised by modality for the total evaluation period and time segments as evaluated using flavour and taste & mouthfeel attributes. Different letters within columns representing significant differences among the samples based on differences in least squares means (p<0.05).

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Caramel		ISO	0.011 ab	0.012 b	0.000 a	0.019 b	0.051 b	0.025 b
		NISO	0.161 ab	0.089 b	0.000 a	0.176 b	0.113 ab	0.091 b
		HULU	0.438 a	0.403 a	0.043 a	0.745 a	0.307 a	0.315 a
	CITRUS	NISO	0.114 ab	0.025 b	0.000 a	0.062 b	0.050 b	0.042 b
		HULU	0.205 ab	0.137 b	0.000 a	0.142 b	0.136 ab	0.121 ab
	FLORAL	NISO	0.074 ab	0.016 b	0.004 a	0.019 b	0.046 b	0.028 b
		HULU	0.269 ab	0.114 b	0.000 a	0.155 b	0.084 ab	0.103 b
	IPA	NISO1	0.000 b	0.080 b	0.000 a	0.061 b	0.021 b	0.041 b
		NISO2	0.049 ab	0.114 b	0.033 a	0.021 b	0.028 b	0.065 b
		HULU	0.136 ab	0.126 b	0.000 a	0.168 b	0.091 ab	0.111 b
	SPICY	NISO	0.056 ab	0.103 b	0.000 a	0.104 b	0.051 b	0.069 b
		HULU1	0.203 ab	0.107 b	0.000 a	0.212 b	0.112 ab	0.110 b
		HULU2	0.126 ab	0.096 b	0.000 a	0.080 b	0.076 b	0.085 b
	SYLVAN	NISO	0.000 b	0.019 b	0.000 a	0.120 b	0.031 b	0.025 b
		HULU	0.223 ab	0.074 b	0.000 a	0.124 b	0.121 ab	0.098 b
Earthy		ISO	0.000 c	0.139 cde	0.071 ef	0.118 c	0.000 a	0.106 c
		NISO	0.099 abc	0.178 cde	0.088 ef	0.137 c	0.000 a	0.124 c
		HULU	0.050 bc	0.059 e	0.003 f	0.061 c	0.000 a	0.052 c
	CITRUS	NISO	0.109 abc	0.260 cde	0.161 def	0.249 bc	0.076 a	0.232 bc
		HULU	0.057 bc	0.132 cde	0.013 f	0.040 c	0.000 a	0.076 c
	FLORAL	NISO	0.034 c	0.127 cde	0.000 f	0.108 c	0.000 a	0.094 c
		HULU	0.001 c	0.100 de	0.026 f	0.111 c	0.000 a	0.078 c
	IPA	NISO1	0.055 bc	0.456 abc	0.368 cde	0.531 ab	0.157 a	0.420 ab
		NISO2	0.282 abc	0.623 ab	0.456 bcd	0.613 a	0.067 a	0.546 a
		HULU	0.200 abc	0.440 bcd	0.658 bca	0.541 ab	0.083 a	0.461 ab
	SPICY	NISO	0.000 c	0.000 e	0.000 f	0.000 c	0.000 a	0.000 c
		HULU1	0.000 c	0.046 e	0.000 f	0.032 c	0.000 a	0.029 c
		HULU2	0.000 c	0.000 e	0.000 f	0.000 c	0.000 a	0.000 c
	SYLVAN	NISO	0.354 ab	0.798 a	0.846 a	0.519 ab	0.204 a	0.641 a
		HULU	0.399 a	0.616 ab	0.707 ba	0.529 ab	0.100 a	0.541 a

Table 8.10. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Grapefruit		ISO	0.235 abc	0.233 bcd	0.092 de	0.119 d	0.000 a	0.142 cd
		NISO	0.074 abc	0.097 d	0.000 e	0.025 d	0.000 a	0.052 d
		HULU	0.000 c	0.000 d	0.000 e	0.000 d	0.000 a	0.000 d
	CITRUS	NISO	0.424 a	0.791 a	0.810 a	0.607 a	0.068 a	0.626 a
		HULU	0.283 abc	0.532 abc	0.418 abcd	0.528 a	0.045 a	0.461 ab
	FLORAL	NISO	0.325 abc	0.275 bcd	0.347 bcde	0.503 ab	0.055 a	0.330 bc
		HULU	0.140 abc	0.571 ab	0.721 ab	0.468 abc	0.123 a	0.476 ab
	IPA	NISO1	0.050 abc	0.234 bcd	0.031 de	0.042 d	0.000 a	0.107 cd
		NISO2	0.033 bc	0.082 d	0.067 de	0.088 d	0.056 a	0.076 cd
		HULU	0.154 abc	0.148 d	0.027 de	0.142 cd	0.000 a	0.125 cd
	SPICY	NISO	0.299 abc	0.783 a	0.795 a	0.633 a	0.058 a	0.603 a
		HULU1	0.200 abc	0.729 a	0.549 ab	0.664 a	0.090 a	0.574 ab
		HULU2	0.411 ab	0.786 a	0.516 abc	0.570 a	0.089 a	0.604 a
	SYLVAN	NISO	0.035 bc	0.007 d	0.087 de	0.027 d	0.033 a	0.019 d
		HULU	0.088 abc	0.221 cd	0.100 cde	0.183 bcd	0.097 a	0.188 cd
Grassy		ISO	0.129 cde	0.080 b	0.133 bc	0.123 b	0.000 a	0.088 b
		NISO	0.018 de	0.054 b	0.019 c	0.035 b	0.000 a	0.038 b
		HULU	0.000 e	0.004 b	0.000 c	0.000 b	0.000 a	0.002 b
	CITRUS	NISO	0.033 de	0.059 b	0.033 c	0.059 b	0.000 a	0.053 b
		HULU	0.041 de	0.033 b	0.028 c	0.055 b	0.000 a	0.039 b
	FLORAL	NISO	0.039 de	0.035 b	0.091 c	0.051 b	0.033 a	0.042 b
		HULU	0.067 de	0.038 b	0.033 c	0.044 b	0.000 a	0.038 b
	IPA	NISO1	0.611 a	0.500 a	0.723 a	0.494 a	0.116 a	0.467 a
		NISO2	0.428 abc	0.454 a	0.692 a	0.411 a	0.033 a	0.413 a
		HULU	0.292 bcde	0.539 a	0.498 a	0.620 a	0.036 a	0.514 a
	SPICY	NISO	0.031 de	0.026 b	0.071 c	0.039 b	0.000 a	0.026 b
		HULU1	0.000 e	0.022 b	0.035 c	0.053 b	0.033 a	0.036 b
		HULU2	0.000 e	0.032 b	0.000 c	0.051 b	0.052 a	0.039 b
	SYLVAN	NISO	0.440 ab	0.504 a	0.558 a	0.448 a	0.074 a	0.439 a
		HULU	0.316 abcd	0.500 a	0.435 ab	0.440 a	0.000 a	0.423 a
Lemon		ISO	0.025 e	0.036 d	0.033 b	0.048 c	0.033 c	0.025 e
		NISO	0.067 de	0.192 cd	0.166 b	0.176 bc	0.176 bc	0.067 de
		HULU	0.033 e	0.030 d	0.033 b	0.007 c	0.019 c	0.033 e
	CITRUS	NISO	0.165 bcde	0.783 ab	0.696 a	0.734 a	0.717 a	0.165 bcde
		HULU	0.293 abcde	0.500 bc	0.571 a	0.502 ab	0.470 ab	0.293 abcde
	FLORAL	NISO	0.343 abc	0.821 a	0.637 a	0.738 a	0.673 a	0.343 abc
		HULU	0.317 abcd	0.771 ab	0.733 a	0.692 a	0.667 a	0.317 abcd
	IPA	NISO1	0.051 de	0.086 d	0.084 b	0.087 c	0.070 c	0.051 de
		NISO2	0.054 de	0.084 d	0.084 b	0.118 c	0.091 c	0.054 de
		HULU	0.083 cde	0.179 d	0.116 b	0.188 bc	0.166 c	0.083 cde
	SPICY	NISO	0.381 ab	0.699 ab	0.636 a	0.631 a	0.608 a	0.381 ab
		HULU1	0.283 abcde	0.724 ab	0.578 a	0.663 a	0.602 a	0.283 abcde
		HULU2	0.480 a	0.789 ab	0.797 a	0.809 a	0.753 a	0.480 a
	SYLVAN	NISO	0.051 de	0.025 d	0.054 b	0.097 c	0.050 c	0.051 de
		HULU	0.033 e	0.093 d	0.050 b	0.103 c	0.085 c	0.033 e

Table 8.10. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Malty		ISO	0.307 a	0.781 ab	0.470 ab	0.782 a	0.267 a	0.635 a
		NISO	0.263 a	0.805 ab	0.678 a	0.749 a	0.223 a	0.642 a
		HULU	0.311 a	0.824 a	0.433 ab	0.759 a	0.223 a	0.660 a
	CITRUS	NISO	0.228 a	0.463 abcd	0.155 b	0.449 abcd	0.192 a	0.406 abc
		HULU	0.328 a	0.574 abc	0.412 ab	0.629 ab	0.172 a	0.521 ab
	FLORAL	NISO	0.181 a	0.563 abc	0.283 ab	0.594 abc	0.133 a	0.478 ab
		HULU	0.208 a	0.566 abc	0.353 ab	0.566 abc	0.205 a	0.507 ab
	IPA	NISO1	0.144 a	0.296 cd	0.115 b	0.332 bcd	0.085 a	0.266 bc
		NISO2	0.004 a	0.157 d	0.100 b	0.135 d	0.035 a	0.122 c
		HULU	0.219 a	0.406 cd	0.147 b	0.228 cd	0.002 a	0.285 bc
	SPICY	NISO	0.049 a	0.119 d	0.092 b	0.120 d	0.090 a	0.112 c
		HULU1	0.128 a	0.272 cd	0.083 b	0.260 bcd	0.033 a	0.222 bc
		HULU2	0.083 a	0.314 cd	0.098 b	0.206 cd	0.067 a	0.234 bc
	SYLVAN	NISO	0.076 a	0.109 d	0.046 b	0.133 d	0.000 a	0.102 c
		HULU	0.132 a	0.445 bcd	0.164 b	0.442 abcd	0.071 a	0.373 abc
Musty		ISO	0.000 c	0.082 c	0.004 c	0.125 b	0.076 ab	0.087 b
		NISO	0.000 c	0.098 c	0.024 c	0.141 b	0.033 ab	0.100 b
		HULU	0.033 c	0.053 c	0.050 c	0.137 b	0.036 ab	0.087 b
	CITRUS	NISO	0.033 c	0.099 c	0.024 c	0.092 b	0.000 b	0.085 b
		HULU	0.000 c	0.013 c	0.015 c	0.049 b	0.033 ab	0.029 b
	FLORAL	NISO	0.026 c	0.083 c	0.031 c	0.080 b	0.061 ab	0.073 b
		HULU	0.037 c	0.034 c	0.042 c	0.084 b	0.019 ab	0.046 b
	IPA	NISO1	0.310 ab	0.506 b	0.753 a	0.509 a	0.204 a	0.455 a
		NISO2	0.310 ab	0.538 b	0.498 b	0.538 a	0.067 ab	0.494 a
		HULU	0.339 a	0.721 a	0.641 ab	0.378 a	0.067 ab	0.538 a
	SPICY	NISO	0.000 c	0.025 c	0.010 c	0.057 b	0.044 ab	0.037 b
		HULU1	0.051 bc	0.041 c	0.000 c	0.037 b	0.033 ab	0.036 b
		HULU2	0.059 bc	0.011 c	0.000 c	0.032 b	0.000 b	0.020 b
	SYLVAN	NISO	0.058 bc	0.027 c	0.014 c	0.010 b	0.015 b	0.023 b
		HULU	0.243 abc	0.567 ab	0.499 b	0.559 a	0.033 ab	0.514 a
Orange		ISO	0.036 c	0.029 c	0.000 d	0.067 c	0.067 a	0.041 c
		NISO	0.055 c	0.077 c	0.100 cd	0.083 c	0.013 a	0.067 c
		HULU	0.150 bc	0.056 c	0.092 cd	0.051 c	0.042 a	0.057 c
	CITRUS	NISO	0.204 abc	0.479 a	0.591 a	0.537 ab	0.285 a	0.494 a
		HULU	0.392 abc	0.596 a	0.621 a	0.516 ab	0.267 a	0.534 a
	FLORAL	NISO	0.280 abc	0.463 ab	0.458 abc	0.386 abc	0.119 a	0.389 ab
		HULU	0.348 abc	0.490 a	0.535 ab	0.597 a	0.173 a	0.498 a
	IPA	NISO1	0.062 c	0.037 c	0.050 d	0.034 c	0.013 a	0.035 c
		NISO2	0.121 bc	0.133 bc	0.180 bcd	0.201 bc	0.073 a	0.159 bc
		HULU	0.100 bc	0.117 c	0.097 cd	0.139 c	0.033 a	0.122 bc
	SPICY	NISO	0.538 a	0.643 a	0.635 a	0.676 a	0.338 a	0.611 a
		HULU1	0.274 abc	0.662 a	0.764 a	0.633 a	0.348 a	0.601 a
		HULU2	0.458 ab	0.614 a	0.593 a	0.719 a	0.289 a	0.634 a
	SYLVAN	NISO	0.077 c	0.006 c	0.016 d	0.056 c	0.033 a	0.032 c
		HULU	0.057 c	0.022 c	0.075 cd	0.088 c	0.010 a	0.055 c

Table 8.10. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Pine wood		ISO	0.067 ab	0.053 b	0.026 c	0.095 d	0.000 a	0.051 c
		NISO	0.039 b	0.121 b	0.079 bc	0.124 d	0.010 a	0.098 bc
		HULU	0.014 b	0.096 b	0.023 c	0.125 d	0.067 a	0.096 c
	CITRUS	NISO	0.107 ab	0.089 b	0.053 bc	0.155 d	0.000 a	0.100 bc
		HULU	0.045 b	0.065 b	0.009 c	0.068 d	0.000 a	0.054 c
	FLORAL	NISO	0.093 ab	0.125 b	0.114 bc	0.160 d	0.058 a	0.124 bc
		HULU	0.053 b	0.046 b	0.068 bc	0.085 d	0.028 a	0.058 c
	IPA	NISO1	0.363 a	0.565 a	0.458 ab	0.674 ab	0.128 a	0.539 a
		NISO2	0.165 ab	0.622 a	0.434 ab	0.337 cd	0.161 a	0.448 a
		HULU	0.080 ab	0.459 a	0.361 abc	0.563 abc	0.118 a	0.456 a
	SPICY	NISO	0.205 ab	0.560 a	0.388 abc	0.569 abc	0.194 a	0.491 a
		HULU1	0.273 ab	0.569 a	0.456 ab	0.615 abc	0.213 a	0.515 a
		HULU2	0.171 ab	0.548 a	0.328 abc	0.539 abc	0.231 a	0.476 a
	SYLVAN	NISO	0.267 ab	0.571 a	0.569 a	0.717 a	0.200 a	0.578 a
		HULU	0.147 ab	0.473 a	0.307 abc	0.347 bcd	0.095 a	0.371 ab
Raisins/prunes		ISO	0.035 a	0.054 b	0.049 b	0.090 b	0.047 a	0.068 b
		NISO	0.000 a	0.037 b	0.005 b	0.046 b	0.028 a	0.029 b
		HULU	0.179 a	0.225 ab	0.144 b	0.189 b	0.060 a	0.183 b
	CITRUS	NISO	0.000 a	0.042 b	0.033 b	0.079 b	0.000 a	0.045 b
		HULU	0.068 a	0.063 b	0.018 b	0.070 b	0.000 a	0.056 b
	FLORAL	NISO	0.075 a	0.080 b	0.089 b	0.092 b	0.046 a	0.085 b
		HULU	0.119 a	0.145 b	0.117 b	0.218 ab	0.152 a	0.174 b
	IPA	NISO1	0.046 a	0.104 b	0.068 b	0.156 b	0.017 a	0.117 b
		NISO2	0.027 a	0.058 b	0.067 b	0.086 b	0.000 a	0.061 b
		HULU	0.238 a	0.234 ab	0.101 b	0.257 ab	0.080 a	0.228 ab
	SPICY	NISO	0.049 a	0.021 b	0.007 b	0.032 b	0.000 a	0.025 b
		HULU1	0.082 a	0.116 b	0.115 b	0.138 b	0.019 a	0.109 b
		HULU2	0.105 a	0.184 b	0.148 b	0.243 ab	0.034 a	0.183 b
	SYLVAN	NISO	0.020 a	0.040 b	0.016 b	0.080 b	0.000 a	0.042 b
		HULU	0.153 a	0.468 a	0.444 a	0.506 a	0.048 a	0.424 a
Rose water		ISO	0.000 b	0.017 b	0.033 b	0.041 b	0.000 b	0.024 b
		NISO	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
		HULU	0.036 b	0.017 b	0.040 b	0.020 b	0.000 b	0.021 b
	CITRUS	NISO	0.051 b	0.070 b	0.046 b	0.074 b	0.018 b	0.059 b
		HULU	0.017 b	0.035 b	0.033 b	0.009 b	0.000 b	0.021 b
	FLORAL	NISO	0.087 b	0.086 b	0.065 b	0.051 b	0.000 b	0.056 b
		HULU	0.060 b	0.028 b	0.030 b	0.032 b	0.000 b	0.028 b
	IPA	NISO1	0.000 b	0.008 b	0.003 b	0.011 b	0.000 b	0.008 b
		NISO2	0.033 b	0.014 b	0.013 b	0.021 b	0.000 b	0.017 b
		HULU	0.147 b	0.084 b	0.050 b	0.106 b	0.000 b	0.083 b
	SPICY	NISO	0.553 a	0.510 a	0.631 a	0.610 a	0.116 ab	0.496 a
		HULU1	0.607 a	0.655 a	0.635 a	0.646 a	0.259 a	0.570 a
		HULU2	0.587 a	0.630 a	0.542 a	0.628 a	0.165 ab	0.582 a
	SYLVAN	NISO	0.000 b	0.054 b	0.007 b	0.081 b	0.000 b	0.048 b
		HULU	0.032 b	0.080 b	0.011 b	0.059 b	0.000 b	0.058 b

Table 8.10. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Tropical fruit		ISO	0.198 a	0.473 a	0.700 a	0.450 a	0.083 a	0.375 a
		NISO	0.194 a	0.466 a	0.511 ab	0.428 a	0.081 a	0.360 a
		HULU	0.150 a	0.459 a	0.502 ab	0.380 a	0.061 a	0.357 a
	CITRUS	NISO	0.127 a	0.408 a	0.378 abc	0.376 a	0.036 a	0.353 a
		HULU	0.117 a	0.393 a	0.376 abc	0.352 ab	0.033 a	0.351 a
	FLORAL	NISO	0.104 a	0.326 a	0.352 bcd	0.328 ab	0.033 a	0.322 a
		HULU	0.076 a	0.310 a	0.343 bcde	0.142 bc	0.021 a	0.203 ab
	IPA	NISO1	0.044 a	0.051 b	0.047 cdef	0.071 c	0.018 a	0.056 bc
		NISO2	0.033 a	0.025 b	0.020 def	0.029 c	0.000 a	0.022 c
		HULU	0.033 a	0.025 b	0.017 ef	0.028 c	0.000 a	0.020 c
	SPICY	NISO	0.028 a	0.019 b	0.008 f	0.027 c	0.000 a	0.019 c
		HULU1	0.022 a	0.018 b	0.008 f	0.026 c	0.000 a	0.016 c
		HULU2	0.006 a	0.013 b	0.000 f	0.018 c	0.000 a	0.016 c
	SYLVAN	NISO	0.000 a	0.012 b	0.000 f	0.016 c	0.000 a	0.015 c
		HULU	0.000 a	0.010 b	0.000 f	0.016 c	0.000 a	0.014 c

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Astringent		ISO	0.021 a	0.312 abc	0.402 abc	0.642 abcd	0.657 abcd	0.508 bcde
		NISO	0.000 a	0.376 abc	0.557 abc	0.621 abcd	0.758 abc	0.539 abcde
		HULU	0.000 a	0.233 abc	0.301 bc	0.462 cd	0.440 bcd	0.355 de
	CITRUS	NISO	0.000 a	0.185 bc	0.325 bc	0.489 bcd	0.656 abcd	0.395 de
		HULU	0.000 a	0.151 c	0.203 c	0.334 d	0.375 cd	0.260 e
	FLORAL	NISO	0.000 a	0.274 abc	0.465 abc	0.467 cd	0.650 abcd	0.455 cde
		HULU	0.000 a	0.296 abc	0.464 abc	0.478 bcd	0.285 d	0.346 de
	IPA	NISO1	0.039 a	0.494 abc	0.867 ab	0.903 ab	1.000 a	0.755 ab
		NISO2	0.012 a	0.623 a	0.938 a	0.944 a	1.000 a	0.814 a
		HULU	0.008 a	0.596 a	0.700 abc	0.790 abc	1.000 a	0.736 abc
	SPICY	NISO	0.000 a	0.447 abc	0.530 abc	0.725 abcd	0.921 a	0.639 abcd
		HULU1	0.033 a	0.552 ab	0.710 abc	0.825 abc	1.000 a	0.735 abc
		HULU2	0.004 a	0.508 abc	0.792 ab	0.750 abcd	1.000 a	0.700 abc
	SYLVAN	NISO	0.032 a	0.477 abc	0.612 abc	0.687 abcd	0.872 ab	0.625 abcd
		HULU	0.000 a	0.403 abc	0.758 abc	0.855 abc	1.000 a	0.702 abc
Cooling		ISO	0.786 a	0.348 a	0.536 a	0.252 a	0.000 a	0.267 a
		NISO	0.768 a	0.361 a	0.433 a	0.284 a	0.000 a	0.264 a
		HULU	0.763 a	0.437 a	0.507 a	0.361 a	0.002 a	0.335 a
	CITRUS	NISO	0.734 a	0.446 a	0.422 a	0.266 a	0.000 a	0.290 a
		HULU	0.732 a	0.410 a	0.425 a	0.330 a	0.000 a	0.308 a
	FLORAL	NISO	0.683 a	0.367 a	0.273 a	0.266 a	0.002 a	0.238 a
		HULU	0.763 a	0.409 a	0.550 a	0.288 a	0.000 a	0.301 a
	IPA	NISO1	0.651 a	0.331 a	0.281 a	0.283 a	0.000 a	0.250 a
		NISO2	0.787 a	0.371 a	0.387 a	0.354 a	0.000 a	0.297 a
		HULU	0.793 a	0.382 a	0.423 a	0.232 a	0.000 a	0.258 a
	SPICY	NISO	0.629 a	0.339 a	0.282 a	0.277 a	0.000 a	0.251 a
		HULU1	0.750 a	0.380 a	0.347 a	0.304 a	0.000 a	0.281 a
		HULU2	0.711 a	0.442 a	0.436 a	0.299 a	0.000 a	0.306 a
	SYLVAN	NISO	0.723 a	0.371 a	0.492 a	0.237 a	0.000 a	0.264 a
		HULU	0.655 a	0.330 a	0.276 a	0.317 a	0.000 a	0.266 a

Table 8.10. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Harsh bitterness		ISO	0.181 a	0.614 ab	0.530 abcd	0.696 a	0.700 a	0.651 a
		NISO	0.206 a	0.757 a	0.575 abcd	0.909 a	0.807 a	0.798 a
		HULU	0.089 a	0.106 d	0.084 cd	0.121 b	0.082 bc	0.107 b
	CITRUS	NISO	0.067 a	0.207 bcd	0.205 bcd	0.155 b	0.000 c	0.137 b
		HULU	0.052 a	0.113 d	0.106 cd	0.086 b	0.000 c	0.078 b
	FLORAL	NISO	0.051 a	0.023 d	0.090 cd	0.052 b	0.000 c	0.032 b
		HULU	0.069 a	0.187 cd	0.077 d	0.035 b	0.000 c	0.084 b
	IPA	NISO1	0.155 a	0.822 a	0.942 a	0.902 a	0.734 a	0.815 a
		NISO2	0.273 a	0.556 abc	0.569 abcd	0.735 a	0.744 a	0.653 a
		HULU	0.380 a	0.693 a	0.733 ab	0.713 a	0.600 ab	0.674 a
	SPICY	NISO	0.348 a	0.764 a	0.574 abcd	0.639 a	0.756 a	0.697 a
		HULU1	0.245 a	0.821 a	0.920 a	0.905 a	0.757 a	0.818 a
		HULU2	0.312 a	0.667 a	0.630 abc	0.822 a	0.574 ab	0.684 a
	SYLVAN	NISO	0.132 a	0.758 a	0.799 a	0.821 a	0.735 a	0.751 a
		HULU	0.444 a	0.829 a	0.782 a	0.896 a	0.754 a	0.819 a
Metallic		ISO	0.000 a	0.165 a	0.200 a	0.252 a	0.263 a	0.217 a
		NISO	0.027 a	0.339 a	0.353 a	0.375 a	0.500 a	0.379 a
		HULU	0.000 a	0.392 a	0.456 a	0.516 a	0.557 a	0.460 a
	CITRUS	NISO	0.000 a	0.326 a	0.324 a	0.413 a	0.481 a	0.378 a
		HULU	0.000 a	0.308 a	0.300 a	0.388 a	0.500 a	0.371 a
	FLORAL	NISO	0.000 a	0.182 a	0.200 a	0.255 a	0.161 a	0.206 a
		HULU	0.002 a	0.160 a	0.000 a	0.150 a	0.200 a	0.152 a
	IPA	NISO1	0.053 a	0.267 a	0.225 a	0.280 a	0.355 a	0.285 a
		NISO2	0.017 a	0.172 a	0.112 a	0.297 a	0.200 a	0.213 a
		HULU	0.000 a	0.088 a	0.100 a	0.153 a	0.296 a	0.155 a
	SPICY	NISO	0.000 a	0.122 a	0.100 a	0.216 a	0.219 a	0.173 a
		HULU1	0.003 a	0.139 a	0.200 a	0.313 a	0.151 a	0.199 a
		HULU2	0.000 a	0.122 a	0.100 a	0.310 a	0.270 a	0.213 a
	SYLVAN	NISO	0.000 a	0.171 a	0.227 a	0.258 a	0.262 a	0.219 a
		HULU	0.000 a	0.297 a	0.212 a	0.261 a	0.190 a	0.245 a
Peppery tingling		ISO	0.156 a	0.088 d	0.060 c	0.291 c	0.272 cde	0.208 cde
		NISO	0.047 a	0.068 d	0.045 c	0.106 c	0.062 de	0.048 de
		HULU	0.046 a	0.042 d	0.022 c	0.075 c	0.114 de	0.071 cde
	CITRUS	NISO	0.139 a	0.110 d	0.191 bc	0.226 c	0.300 bcde	0.195 cde
		HULU	0.034 a	0.059 d	0.084 c	0.060 c	0.052 de	0.059 cde
	FLORAL	NISO	0.128 a	0.074 d	0.134 c	0.069 c	0.000 e	0.061 cde
		HULU	0.091 a	0.019 d	0.008 c	0.013 c	0.004 e	0.017 e
	IPA	NISO1	0.122 a	0.107 d	0.128 c	0.304 c	0.331 bcde	0.228 cde
		NISO2	0.137 a	0.131 cd	0.203 bc	0.383 bc	0.421 abcde	0.290 cd
		HULU	0.094 a	0.077 d	0.045 c	0.100 c	0.303 bcde	0.136 cde
	SPICY	NISO	0.108 a	0.404 bc	0.567 ab	0.680 ab	0.803 abc	0.588 ab
		HULU1	0.026 a	0.651 ab	0.845 a	0.920 a	0.823 ab	0.768 a
		HULU2	0.158 a	0.695 a	0.915 a	0.963 a	0.927 a	0.831 a
	SYLVAN	NISO	0.129 a	0.192 cd	0.196 bc	0.342 bc	0.571 abcd	0.326 bc
		HULU	0.000 a	0.022 d	0.083 c	0.109 c	0.284 cde	0.113 cde

Table 8.10. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Smooth bitterness		ISO	0.143 a	0.509 abcd	0.329 bcde	0.352 b	0.331 bcd	0.384 bc
		NISO	0.068 a	0.328 cde	0.193 de	0.288 b	0.246 cd	0.281 cd
		HULU	0.151 a	0.765 ab	0.640 abc	0.781 a	0.663 abc	0.721 a
	CITRUS	NISO	0.092 a	0.599 abc	0.563 abcd	0.794 a	0.775 a	0.681 ab
		HULU	0.128 a	0.769 ab	0.884 a	0.899 a	0.857 a	0.818 a
	FLORAL	NISO	0.222 a	0.814 a	0.703 ab	0.903 a	0.686 ab	0.783 a
		HULU	0.201 a	0.773 ab	0.852 a	0.861 a	0.640 abc	0.757 a
	IPA	NISO1	0.100 a	0.180 de	0.146 de	0.166 b	0.150 d	0.163 cd
		NISO2	0.004 a	0.056 e	0.019 e	0.080 b	0.150 d	0.083 d
		HULU	0.030 a	0.175 de	0.091 e	0.147 b	0.092 d	0.137 cd
	SPICY	NISO	0.053 a	0.378 bcde	0.112 e	0.158 b	0.076 d	0.207 cd
		HULU1	0.083 a	0.351 cde	0.091 e	0.274 b	0.133 d	0.249 cd
		HULU2	0.032 a	0.286 cde	0.086 e	0.152 b	0.102 d	0.178 cd
	SYLVAN	NISO	0.129 a	0.501 abcd	0.171 de	0.082 b	0.058 d	0.225 cd
		HULU	0.117 a	0.345 cde	0.210 cde	0.388 b	0.247 cd	0.319 cd
Sour		ISO	0.116 a	0.319 ab	0.234 a	0.330 ab	0.012 a	0.245 ab
		NISO	0.067 a	0.347 ab	0.169 a	0.281 ab	0.031 a	0.242 ab
		HULU	0.038 a	0.319 ab	0.179 a	0.410 ab	0.065 a	0.278 ab
	CITRUS	NISO	0.187 a	0.339 ab	0.334 a	0.480 ab	0.081 a	0.319 ab
		HULU	0.259 a	0.556 a	0.360 a	0.531 a	0.132 a	0.430 a
	FLORAL	NISO	0.206 a	0.221 ab	0.150 a	0.206 ab	0.000 a	0.173 ab
		HULU	0.154 a	0.488 ab	0.347 a	0.430 ab	0.028 a	0.345 ab
	IPA	NISO1	0.103 a	0.231 ab	0.026 a	0.252 ab	0.045 a	0.177 ab
		NISO2	0.151 a	0.246 ab	0.180 a	0.178 ab	0.036 a	0.171 ab
		HULU	0.041 a	0.140 b	0.121 a	0.131 b	0.000 a	0.102 b
	SPICY	NISO	0.038 a	0.191 ab	0.130 a	0.277 ab	0.000 a	0.169 ab
		HULU1	0.172 a	0.271 ab	0.086 a	0.324 ab	0.071 a	0.228 ab
		HULU2	0.106 a	0.207 ab	0.178 a	0.257 ab	0.025 a	0.178 ab
	SYLVAN	NISO	0.197 a	0.398 ab	0.158 a	0.280 ab	0.008 a	0.247 ab
		HULU	0.041 a	0.131 b	0.022 a	0.163 ab	0.000 a	0.105 b
Sweet		ISO	0.187 ab	0.199 bc	0.062 ab	0.161 abcde	0.020 a	0.140 bcde
		NISO	0.208 ab	0.183 bc	0.046 ab	0.148 abcde	0.000 a	0.111 bcde
		HULU	0.451 ab	0.383 abc	0.344 ab	0.420 abc	0.040 a	0.315 abcd
	CITRUS	NISO	0.560 a	0.439 abc	0.276 ab	0.384 abcd	0.072 a	0.326 abcd
		HULU	0.359 ab	0.364 abc	0.375 ab	0.414 abc	0.031 a	0.308 abcd
	FLORAL	NISO	0.555 a	0.444 ab	0.153 ab	0.469 a	0.007 a	0.330 abcd
		HULU	0.422 ab	0.443 abc	0.415 ab	0.365 abcd	0.017 a	0.323 abcd
	IPA	NISO1	0.175 ab	0.058 c	0.003 b	0.016 e	0.000 a	0.031 e
		NISO2	0.060 b	0.113 bc	0.049 ab	0.087 cde	0.004 a	0.074 cde
		HULU	0.201 ab	0.110 bc	0.073 ab	0.064 de	0.000 a	0.072 de
	SPICY	NISO	0.582 a	0.447 ab	0.434 a	0.450 ab	0.000 a	0.356 ab
		HULU1	0.528 a	0.441 abc	0.359 ab	0.429 abc	0.011 a	0.340 abc
		HULU2	0.606 a	0.699 a	0.458 a	0.467 a	0.005 a	0.447 a
	SYLVAN	NISO	0.054 b	0.106 bc	0.101 ab	0.102 cde	0.000 a	0.080 cde
		HULU	0.174 ab	0.123 bc	0.112 ab	0.116 bcde	0.000 a	0.097 bcde

Table 8.10. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Warming		ISO	ns	0.425 a	0.392 a	0.610 a	0.727 a	0.548 a
		NISO	ns	0.284 a	0.230 a	0.466 a	0.771 a	0.463 a
		HULU	ns	0.380 a	0.353 a	0.576 a	0.700 a	0.509 a
	CITRUS	NISO	ns	0.238 a	0.188 a	0.459 a	0.775 a	0.423 a
		HULU	ns	0.370 a	0.467 a	0.502 a	0.676 a	0.477 a
	FLORAL	NISO	ns	0.402 a	0.451 a	0.530 a	0.656 a	0.490 a
		HULU	ns	0.261 a	0.234 a	0.496 a	0.681 a	0.426 a
	IPA	NISO1	ns	0.346 a	0.401 a	0.465 a	0.716 a	0.463 a
		NISO2	ns	0.262 a	0.252 a	0.464 a	0.686 a	0.417 a
		HULU	ns	0.403 a	0.457 a	0.637 a	0.721 a	0.549 a
	SPICY	NISO	ns	0.390 a	0.429 a	0.562 a	0.708 a	0.509 a
		HULU1	ns	0.396 a	0.344 a	0.534 a	0.889 a	0.537 a
		HULU2	ns	0.408 a	0.467 a	0.552 a	0.795 a	0.533 a
	SYLVAN	NISO	ns	0.336 a	0.413 a	0.628 a	0.800 a	0.529 a
		HULU	ns	0.403 a	0.510 a	0.552 a	0.685 a	0.511 a

ns, not selected

Table 8.11. Mean panel proportion citations (n=10) obtained by RM-ANOVA followed by Tukey's HSD computed from the TCATA data time standardised with merged modalities for the total evaluation period and time segments as evaluated using flavour and taste & mouthfeel attributes. Different letters within columns representing significant differences among the samples based on differences in least squares means *(p*<0.05).

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Caramel		ISO	0.010 cd	0.011 b	0.012 b	0.044 b	0.000 b	0.019 b
		NISO	0.121 bcd	0.082 b	0.127 b	0.089 b	0.000 b	0.073 b
		HULU	0.342 a	0.311 a	0.552 a	0.214 a	0.063 a	0.235 a
	CITRUS	NISO	0.091 bcd	0.025 b	0.062 b	0.045 b	0.000 b	0.031 b
		HULU	0.194 abc	0.132 b	0.135 b	0.118 ab	0.000 b	0.101 b
	FLORAL	NISO	0.073 bcd	0.011 b	0.013 b	0.032 b	0.004 b	0.019 b
		HULU	0.250 ab	0.113 b	0.132 b	0.083 b	0.000 b	0.085 b
	IPA	NISO1	0.000 d	0.080 b	0.061 b	0.021 b	0.000 b	0.038 b
		NISO2	0.044 cd	0.102 b	0.014 b	0.023 b	0.012 b	0.048 b
		HULU	0.132 bcd	0.124 b	0.166 b	0.079 b	0.000 b	0.084 b
	SPICY	NISO	0.054 cd	0.099 b	0.068 b	0.047 b	0.000 b	0.057 b
		HULU1	0.193 abc	0.104 b	0.171 b	0.103 ab	0.000 b	0.087 b
		HULU2	0.110 bcd	0.096 b	0.080 b	0.068 b	0.000 b	0.067 b
	SYLVAN	NISO	0.000 d	0.019 b	0.120 b	0.027 b	0.000 b	0.023 b
		HULU	0.191 abc	0.068 b	0.119 b	0.092 b	0.000 b	0.070 b

Table 8.11. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Earthy		ISO	0.000 c	0.134 ef	0.055 c	0.101 cd	0.000 b	0.083 cd
		NISO	0.080 bc	0.141 ef	0.038 c	0.097 cd	0.000 b	0.087 cd
		HULU	0.035 c	0.055 f	0.003 c	0.040 d	0.000 b	0.034 d
	CITRUS	NISO	0.108 bc	0.257 de	0.161 c	0.234 bc	0.010 b	0.182 c
		HULU	0.047 c	0.124 ef	0.013 c	0.030 d	0.000 b	0.054 d
	FLORAL	NISO	0.004 c	0.118 ef	0.000 c	0.098 cd	0.000 b	0.074 d
		HULU	0.001 c	0.092 ef	0.026 c	0.093 cd	0.000 b	0.066 d
	IPA	NISO1	0.025 c	0.407 cd	0.368 b	0.453 a	0.055 ab	0.327 b
		NISO2	0.226 ab	0.597 b	0.427 b	0.530 a	0.023 ab	0.420 ab
		HULU	0.162 bc	0.414 cd	0.631 a	0.456 a	0.061 ab	0.352 b
	SPICY	NISO	0.000 c	0.000 f	0.000 c	0.000 d	0.000 b	0.000 d
		HULU1	0.000 c	0.044 f	0.000 c	0.026 d	0.000 b	0.024 d
		HULU2	0.000 c	0.000 f	0.000 c	0.000 d	0.000 b	0.000 d
	SYLVAN	NISO	0.235 ab	0.776 a	0.740 a	0.404 a	0.113 a	0.475 a
		HULU	0.340 a	0.572 bc	0.658 a	0.391 ab	0.041 ab	0.387 ab
Grapefruit		ISO	0.131 bcd	0.191 d	0.047 e	0.088 c	0.000 a	0.111 ef
		NISO	0.040 d	0.083 de	0.000 e	0.023 c	0.000 a	0.038 ef
		HULU	0.000 d	0.000 e	0.000 e	0.000 c	0.000 a	0.000 f
	CITRUS	NISO	0.253 ab	0.784 a	0.810 a	0.534 ab	0.041 a	0.512 a
		HULU	0.230 abc	0.504 c	0.404 c	0.459 ab	0.043 a	0.372 bc
	FLORAL	NISO	0.227 abc	0.252 d	0.305 cd	0.433 b	0.040 a	0.273 cd
		HULU	0.095 bcd	0.556 bc	0.693 ab	0.433 b	0.099 a	0.402 ab
	IPA	NISO1	0.037 d	0.203 d	0.022 e	0.040 c	0.000 a	0.085 ef
		NISO2	0.013 d	0.082 de	0.067 e	0.088 c	0.046 a	0.073 ef
		HULU	0.103 bcd	0.147 de	0.027 e	0.124 c	0.000 a	0.100 ef
	SPICY	NISO	0.248 abc	0.753 a	0.679 ab	0.551 ab	0.035 a	0.499 a
		HULU1	0.148 bcd	0.696 ab	0.535 bc	0.632 a	0.072 a	0.502 a
		HULU2	0.350 a	0.773 a	0.512 bc	0.539 ab	0.084 a	0.508 a
	SYLVAN	NISO	0.019 d	0.006 e	0.036 e	0.016 c	0.014 a	0.014 f
		HULU	0.062 cd	0.206 d	0.100 de	0.173 c	0.088 a	0.158 de
Grassy		ISO	0.109 bc	0.058 b	0.079 c	0.097 c	0.000 b	0.061 b
		NISO	0.011 bc	0.044 b	0.019 c	0.032 c	0.000 b	0.028 b
		HULU	0.000 c	0.004 b	0.000 c	0.000 c	0.000 b	0.002 b
	CITRUS	NISO	0.023 bc	0.059 b	0.033 c	0.052 c	0.000 b	0.040 b
		HULU	0.031 bc	0.033 b	0.023 c	0.046 c	0.000 b	0.029 b
	FLORAL	NISO	0.036 bc	0.030 b	0.063 c	0.038 c	0.019 ab	0.034 b
		HULU	0.063 bc	0.038 b	0.033 c	0.042 c	0.000 b	0.032 b
	IPA	NISO1	0.420 a	0.451 a	0.622 ab	0.417 ab	0.106 a	0.373 a
		NISO2	0.348 a	0.438 a	0.648 a	0.358 b	0.033 ab	0.328 a
		HULU	0.229 ab	0.517 a	0.489 ab	0.514 a	0.036 ab	0.403 a
	SPICY	NISO	0.028 bc	0.025 b	0.054 c	0.031 c	0.000 b	0.023 b
		HULU1	0.000 c	0.022 b	0.035 c	0.051 c	0.033 ab	0.034 b
		HULU2	0.000 c	0.032 b	0.000 c	0.049 c	0.027 ab	0.034 b
	SYLVAN	NISO	0.225 ab	0.497 a	0.558 ab	0.344 b	0.049 ab	0.338 a
		HULU	0.356 a	0.468 a	0.408 b	0.334 b	0.000 b	0.310 a

Table 8.11. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Lemon		ISO	0.017 d	0.027 c	0.015 b	0.033 c	0.000 e	0.022 c
		NISO	0.048 bcd	0.176 c	0.159 b	0.159 c	0.062 cde	0.140 c
		HULU	0.029 cd	0.029 c	0.020 b	0.007 c	0.000 e	0.016 c
	CITRUS	NISO	0.118 bcd	0.774 a	0.665 a	0.664 a	0.251 ab	0.586 a
		HULU	0.218 abcd	0.488 b	0.545 a	0.461 b	0.181 abcd	0.404 b
	FLORAL	NISO	0.232 abc	0.773 a	0.616 a	0.643 ab	0.083 bcde	0.550 a
		HULU	0.261 ab	0.755 a	0.708 a	0.649 ab	0.103 abcde	0.555 a
	IPA	NISO1	0.049 bcd	0.071 c	0.079 b	0.074 c	0.000 e	0.055 c
		NISO2	0.051 bcd	0.083 c	0.077 b	0.097 c	0.001 e	0.068 c
		HULU	0.066 bcd	0.168 c	0.116 b	0.139 c	0.002 e	0.118 c
	SPICY	NISO	0.351 a	0.673 ab	0.559 a	0.576 ab	0.234 abc	0.523 ab
		HULU1	0.252 ab	0.693 a	0.564 a	0.627 ab	0.192 abc	0.532 ab
		HULU2	0.428 a	0.779 a	0.783 a	0.747 a	0.264 a	0.638 a
	SYLVAN	NISO	0.028 cd	0.022 c	0.061 b	0.068 c	0.000 e	0.036 c
		HULU	0.028 cd	0.085 c	0.047 b	0.080 c	0.006 de	0.061 c
Malty		ISO	0.242 ab	0.598 ab	0.294 abcd	0.552 a	0.163 a	0.462 a
		NISO	0.225 ab	0.632 ab	0.535 a	0.562 a	0.092 a	0.464 a
		HULU	0.262 ab	0.693 a	0.422 ab	0.580 a	0.168 a	0.500 a
	CITRUS	NISO	0.200 ab	0.457 bcd	0.155 bcd	0.415 abc	0.090 a	0.331 abcd
		HULU	0.283 a	0.541 ab	0.394 abc	0.528 a	0.083 a	0.416 ab
	FLORAL	NISO	0.153 ab	0.512 abc	0.279 abcd	0.498 ab	0.069 a	0.387 abc
		HULU	0.170 ab	0.549 ab	0.353 abcd	0.516 ab	0.094 a	0.415 ab
	IPA	NISO1	0.135 ab	0.290 cde	0.115 bcd	0.280 bcde	0.044 a	0.213 cdef
		NISO2	0.004 b	0.157 e	0.100 cd	0.129 de	0.033 a	0.112 ef
		HULU	0.173 ab	0.397 bcd	0.147 bcd	0.188 cde	0.001 a	0.217 cdef
	SPICY	NISO	0.043 ab	0.118 e	0.092 cd	0.116 e	0.060 a	0.101 ef
		HULU1	0.109 ab	0.241 de	0.083 cd	0.237 cde	0.033 a	0.178 def
		HULU2	0.076 ab	0.305 cde	0.097 cd	0.184 cde	0.063 a	0.187 def
	SYLVAN	NISO	0.061 ab	0.109 e	0.046 d	0.085 e	0.000 a	0.070 f
		HULU	0.130 ab	0.411 bcd	0.154 bcd	0.360 abcd	0.008 a	0.274 bcde
Musty		ISO	0.000 c	0.057 c	0.002 c	0.099 b	0.056 a	0.067 b
		NISO	0.000 c	0.078 c	0.024 c	0.096 b	0.033 a	0.067 b
		HULU	0.031 bc	0.050 c	0.050 c	0.120 b	0.042 a	0.069 b
	CITRUS	NISO	0.033 bc	0.099 c	0.024 c	0.087 b	0.000 a	0.065 b
		HULU	0.000 c	0.010 c	0.011 c	0.038 b	0.028 a	0.024 b
	FLORAL	NISO	0.026 bc	0.079 c	0.020 c	0.080 b	0.051 a	0.068 b
		HULU	0.009 c	0.034 c	0.042 c	0.073 b	0.011 a	0.042 b
	IPA	NISO1	0.197 ab	0.454 b	0.451 b	0.429 a	0.099 a	0.354 a
		NISO2	0.192 ab	0.507 b	0.617 ab	0.463 a	0.049 a	0.382 a
		HULU	0.260 a	0.697 a	0.740 a	0.320 a	0.037 a	0.411 a
	SPICY	NISO	0.000 c	0.025 c	0.010 c	0.046 b	0.042 a	0.034 b
		HULU1	0.042 bc	0.038 c	0.000 c	0.031 b	0.012 a	0.027 b
		HULU2	0.040 bc	0.011 c	0.000 c	0.025 b	0.000 a	0.015 b
	SYLVAN	NISO	0.048 bc	0.027 c	0.014 c	0.009 b	0.004 a	0.016 b
		HULU	0.172 abc	0.557 ab	0.492 b	0.459 a	0.006 a	0.381 a

Table 8.11. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Orange		ISO	0.017 f	0.025 b	0.000 c	0.058 d	0.022 d	0.034 c
		NISO	0.047 ef	0.065 b	0.085 c	0.063 d	0.012 d	0.055 c
		HULU	0.085 cdef	0.040 b	0.063 c	0.043 d	0.052 cd	0.047 c
	CITRUS	NISO	0.175 bcdef	0.478 a	0.591 a	0.502 ab	0.228 abc	0.425 ab
		HULU	0.298 abc	0.572 a	0.601 a	0.454 ab	0.215 abc	0.444 ab
	FLORAL	NISO	0.232 bcdef	0.437 a	0.422 ab	0.347 bc	0.088 bcd	0.322 b
		HULU	0.274 bcd	0.484 a	0.518 a	0.556 ab	0.139 abcd	0.427 ab
	IPA	NISO1	0.051 def	0.037 b	0.050 c	0.033 d	0.012 d	0.031 c
		NISO2	0.076 cdef	0.129 b	0.174 bc	0.176 cd	0.004 d	0.118 c
		HULU	0.096 cdef	0.116 b	0.097 c	0.109 d	0.009 d	0.088 c
	SPICY	NISO	0.501 a	0.627 a	0.527 a	0.606 a	0.266 ab	0.530 a
		HULU1	0.264 bcde	0.626 a	0.666 a	0.608 a	0.312 a	0.539 a
		HULU2	0.399 ab	0.604 a	0.591 a	0.662 a	0.277 ab	0.542 a
	SYLVAN	NISO	0.059 def	0.006 b	0.008 c	0.040 d	0.019 d	0.023 c
		HULU	0.053 def	0.022 b	0.075 c	0.071 d	0.007 d	0.041 c
Pine wood		ISO	0.040 bc	0.042 b	0.013 d	0.580 a	0.000 a	0.038 c
		NISO	0.023 c	0.096 b	0.055 cd	0.579 a	0.007 a	0.062 c
		HULU	0.007 c	0.089 b	0.015 d	0.579 a	0.034 a	0.078 c
	CITRUS	NISO	0.072 bc	0.089 b	0.053 cd	0.508 ab	0.000 a	0.086 c
		HULU	0.041 bc	0.064 b	0.009 d	0.502 ab	0.000 a	0.047 c
	FLORAL	NISO	0.076 bc	0.115 b	0.098 bcd	0.487 ab	0.032 a	0.107 bc
		HULU	0.042 bc	0.046 b	0.061 bcd	0.323 abc	0.008 a	0.050 c
	IPA	NISO1	0.283 a	0.516 a	0.426 a	0.299 bcd	0.111 a	0.433 a
		NISO2	0.127 abc	0.596 a	0.434 a	0.153 cd	0.110 a	0.365 a
		HULU	0.058 bc	0.434 a	0.357 ab	0.146 cd	0.095 a	0.358 a
	SPICY	NISO	0.173 abc	0.544 a	0.349 abc	0.115 cd	0.157 a	0.420 a
		HULU1	0.224 ab	0.543 a	0.443 a	0.082 cd	0.180 a	0.455 a
		HULU2	0.143 abc	0.539 a	0.320 abc	0.070 cd	0.183 a	0.418 a
	SYLVAN	NISO	0.188 abc	0.564 a	0.559 a	0.068 cd	0.108 a	0.451 a
		HULU	0.082 bc	0.454 a	0.297 abcd	0.061 d	0.076 a	0.294 ab
Raisins/prunes		ISO	0.021 a	0.049 cd	0.046 b	0.083 bc	0.047 a	0.061 bcd
		NISO	0.000 a	0.018 d	0.002 b	0.022 c	0.026 a	0.020 cd
		HULU	0.138 a	0.197 bc	0.089 b	0.150 bc	0.043 a	0.139 bcd
	CITRUS	NISO	0.000 a	0.042 cd	0.033 b	0.077 bc	0.000 a	0.042 cd
		HULU	0.068 a	0.061 bcd	0.017 b	0.062 bc	0.000 a	0.045 cd
	FLORAL	NISO	0.067 a	0.079 bcd	0.089 b	0.078 bc	0.045 a	0.071 bcd
		HULU	0.085 a	0.144 bcd	0.111 b	0.206 bc	0.085 a	0.149 bcd
	IPA	NISO1	0.019 a	0.104 bcd	0.064 b	0.130 bc	0.012 a	0.086 bcd
		NISO2	0.022 a	0.057 bcd	0.067 b	0.084 bc	0.000 a	0.053 bcd
		HULU	0.183 a	0.229 b	0.101 b	0.225 ab	0.049 a	0.181 b
	SPICY	NISO	0.043 a	0.021 d	0.006 b	0.023 c	0.000 a	0.017 d
		HULU1	0.080 a	0.113 bcd	0.095 b	0.135 bc	0.012 a	0.096 bcd
		HULU2	0.095 a	0.177 bcd	0.148 b	0.220 ab	0.034 a	0.154 bc
	SYLVAN	NISO	0.020 a	0.034 cd	0.016 b	0.043 bc	0.000 a	0.026 cd
		HULU	0.139 a	0.429 a	0.409 a	0.399 a	0.038 a	0.321 a

Table 8.11. continued.

Flavour attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Rose water		ISO	0.000 b	0.015 b	0.006 b	0.034 b	0.000 b	0.017 b
		NISO	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b	0.000 b
		HULU	0.032 b	0.016 b	0.037 b	0.015 b	0.000 b	0.014 b
	CITRUS	NISO	0.049 b	0.070 b	0.046 b	0.071 b	0.007 b	0.053 b
		HULU	0.017 b	0.035 b	0.033 b	0.009 b	0.000 b	0.018 b
	FLORAL	NISO	0.082 b	0.081 b	0.064 b	0.050 b	0.000 b	0.051 b
		HULU	0.057 b	0.028 b	0.030 b	0.032 b	0.000 b	0.024 b
	IPA	NISO1	0.000 b	0.008 b	0.003 b	0.011 b	0.000 b	0.006 b
		NISO2	0.010 b	0.014 b	0.013 b	0.021 b	0.000 b	0.014 b
		HULU	0.111 b	0.071 b	0.045 b	0.081 b	0.000 b	0.058 b
	SPICY	NISO	0.507 a	0.500 a	0.518 a	0.532 a	0.102 ab	0.425 a
		HULU1	0.531 a	0.606 a	0.564 a	0.615 a	0.168 a	0.502 a
		HULU2	0.510 a	0.620 a	0.535 a	0.575 a	0.165 a	0.493 a
	SYLVAN	NISO	0.000 b	0.054 b	0.007 b	0.061 b	0.000 b	0.039 b
		HULU	0.029 b	0.079 b	0.010 b	0.045 b	0.000 b	0.044 b
Tropical fruit		ISO	0.020 b	0.006 b	0.020 c	0.022 b	0.000 a	0.012 c
		NISO	0.000 b	0.022 b	0.008 c	0.025 b	0.000 a	0.017 c
		HULU	0.032 b	0.018 b	0.014 c	0.012 b	0.000 a	0.012 c
	CITRUS	NISO	0.064 ab	0.461 a	0.511 ab	0.281 a	0.000 a	0.284 ab
		HULU	0.092 ab	0.310 a	0.487 ab	0.326 a	0.061 a	0.263 ab
	FLORAL	NISO	0.094 ab	0.292 a	0.337 b	0.120 b	0.015 a	0.168 b
		HULU	0.039 ab	0.462 a	0.677 a	0.325 a	0.021 a	0.315 a
	IPA	NISO1	0.058 ab	0.049 b	0.000 c	0.066 b	0.007 a	0.044 c
		NISO2	0.005 b	0.016 b	0.008 c	0.022 b	0.000 a	0.014 c
		HULU	0.000 b	0.013 b	0.000 c	0.018 b	0.011 a	0.013 c
	SPICY	NISO	0.183 a	0.397 a	0.297 b	0.408 a	0.071 a	0.314 a
		HULU1	0.181 a	0.439 a	0.356 b	0.352 a	0.060 a	0.310 a
		HULU2	0.093 ab	0.387 a	0.377 b	0.391 a	0.033 a	0.296 a
	SYLVAN	NISO	0.029 b	0.025 b	0.000 c	0.027 b	0.000 a	0.018 c
		HULU	0.026 b	0.010 b	0.020 c	0.014 b	0.000 a	0.011 c

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Astringent		ISO	0.021 a	0.308 defghI	0.402 fgh	0.642 defg	0.657 de	0.503 cde
		NISO	0.000 a	0.376 cdefgh	0.522 cdefg	0.616 efg	0.759 bcd	0.537 cd
		HULU	0.000 a	0.233 ghI	0.301 gh	0.462 gh	0.498 ef	0.353 fg
	CITRUS	NISO	0.000 a	0.185 hI	0.325 gh	0.489 gh	0.656 de	0.394 efg
		HULU	0.000 a	0.151 I	0.203 h	0.334 h	0.375 fg	0.260 g
	FLORAL	NISO	0.000 a	0.284 fghI	0.483 defg	0.500 fgh	0.684 cde	0.453 def
		HULU	0.000 a	0.296 efghI	0.461 efgh	0.476 gh	0.285 g	0.344 fg
	IPA	NISO1	0.039 a	0.493 abcde	0.867 ab	0.903 ab	1.000 a	0.752 ab
		NISO2	0.012 a	0.623 a	0.938 a	0.944 a	1.000 a	0.811 a
		HULU	0.008 a	0.585 ab	0.700 abcde	0.790 abcde	1.000 a	0.731 ab
	SPICY	NISO	0.000 a	0.447 abcdef	0.530 cdefg	0.725 bcde	0.922 ab	0.636 bc
		HULU1	0.025 a	0.552 abc	0.710 abcde	0.825 abcd	1.000 a	0.733 ab
		HULU2	0.004 a	0.508 abcd	0.792 abc	0.750 abcde	1.000 a	0.697 ab
	SYLVAN	NISO	0.032 a	0.477 abcdef	0.612 bcdef	0.687 cdef	0.872 abc	0.624 bc
		HULU	0.000 a	0.400 bcdefg	0.758 abcd	0.855 abc	1.000 a	0.698 ab

Table 8.11. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Cooling		ISO	0.783 a	0.344 a	0.494 abc	0.252 a	0.000 b	0.265 a
		NISO	0.720 a	0.350 a	0.339 abc	0.284 a	0.000 b	0.265 a
		HULU	0.730 a	0.425 a	0.501 ab	0.361 a	0.023 a	0.333 a
	CITRUS	NISO	0.676 a	0.429 a	0.422 abc	0.266 a	0.000 b	0.289 a
		HULU	0.721 a	0.410 a	0.425 abc	0.330 a	0.000 b	0.308 a
	FLORAL	NISO	0.685 a	0.335 a	0.243 c	0.238 a	0.000 b	0.239 a
		HULU	0.762 a	0.408 a	0.550 a	0.288 a	0.000 b	0.301 a
	IPA	NISO1	0.603 a	0.330 a	0.281 bc	0.283 a	0.000 b	0.249 a
		NISO2	0.699 a	0.371 a	0.387 abc	0.354 a	0.000 b	0.296 a
		HULU	0.617 a	0.380 a	0.391 abc	0.232 a	0.000 b	0.257 a
	SPICY	NISO	0.581 a	0.339 a	0.282 bc	0.277 a	0.000 b	0.249 a
		HULU1	0.696 a	0.380 a	0.347 abc	0.304 a	0.000 b	0.280 a
		HULU2	0.669 a	0.442 a	0.436 abc	0.299 a	0.000 b	0.304 a
	SYLVAN	NISO	0.718 a	0.371 a	0.492 abc	0.237 a	0.000 b	0.263 a
		HULU	0.626 a	0.328 a	0.276 bc	0.317 a	0.000 b	0.263 a
Harsh bitterness		ISO	0.181 abc	0.605 ab	0.530 c	0.696 bc	0.700 a	0.646 a
		NISO	0.227 abc	0.750 ab	0.605 c	0.916 a	0.808 a	0.795 a
		HULU	0.089 bc	0.106 c	0.084 d	0.121 d	0.116 b	0.107 b
	CITRUS	NISO	0.067 bc	0.205 c	0.205 d	0.155 d	0.000 b	0.136 b
		HULU	0.050 c	0.113 c	0.106 d	0.086 d	0.000 b	0.078 b
	FLORAL	NISO	0.051 c	0.021 c	0.090 d	0.048 d	0.000 b	0.032 b
		HULU	0.069 bc	0.187 c	0.077 d	0.035 d	0.000 b	0.083 b
	IPA	NISO1	0.151 abc	0.821 a	0.942 a	0.902 ab	0.734 a	0.811 a
		NISO2	0.265 abc	0.556 b	0.569 c	0.735 abc	0.744 a	0.649 a
		HULU	0.374 a	0.689 ab	0.666 abc	0.713 abc	0.600 a	0.670 a
	SPICY	NISO	0.335 ab	0.764 ab	0.574 c	0.639 c	0.756 a	0.693 a
		HULU1	0.230 abc	0.821 a	0.920 ab	0.905 ab	0.757 a	0.816 a
		HULU2	0.295 abc	0.667 ab	0.630 bc	0.822 abc	0.574 a	0.680 a
	SYLVAN	NISO	0.132 abc	0.758 ab	0.799 abc	0.821 abc	0.735 a	0.750 a
		HULU	0.404 a	0.826 a	0.782 abc	0.896 ab	0.754 a	0.814 a
Metallic		ISO	0.000 b	0.159 abc	0.200 abc	0.252 ab	0.263 bcd	0.213 bc
		NISO	0.000 b	0.330 ab	0.353 ab	0.375 ab	0.500 ab	0.377 ab
		HULU	0.000 b	0.392 a	0.456 a	0.516 a	0.582 a	0.458 a
	CITRUS	NISO	0.000 b	0.326 ab	0.324 ab	0.413 ab	0.481 abc	0.377 ab
		HULU	0.000 b	0.308 abc	0.300 abc	0.388 ab	0.500 ab	0.370 abc
	FLORAL	NISO	0.000 b	0.202 abc	0.200 abc	0.255 ab	0.161 d	0.206 bc
		HULU	0.002 b	0.160 abc	0.000 c	0.150 b	0.200 bcd	0.151 c
	IPA	NISO1	0.053 a	0.265 abc	0.225 abc	0.280 ab	0.354 abcd	0.283 abc
		NISO2	0.017 ab	0.172 abc	0.112 bc	0.297 ab	0.200 bcd	0.212 bc
		HULU	0.000 b	0.088 c	0.100 bc	0.153 b	0.296 abcd	0.155 bc
	SPICY	NISO	0.000 b	0.122 bc	0.100 bc	0.216 b	0.220 bcd	0.173 bc
		HULU1	0.003 b	0.139 bc	0.200 abc	0.313 ab	0.152 d	0.198 bc
		HULU2	0.000 b	0.122 bc	0.100 bc	0.310 ab	0.271 bcd	0.212 bc
	SYLVAN	NISO	0.000 b	0.170 abc	0.227 abc	0.258 ab	0.263 bcd	0.218 bc
		HULU	0.000 b	0.295 abc	0.212 abc	0.261 ab	0.190 cd	0.243 abc

Table 8.11. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Peppery tingling		ISO	0.156 a	0.087 cd	0.041 cd	0.291 cd	0.272 defgh	0.208 cd
		NISO	0.047 a	0.042 cd	0.011 d	0.073 ef	0.029 gh	0.048 f
		HULU	0.046 a	0.042 cd	0.022 cd	0.075 ef	0.122 efgh	0.071 ef
	CITRUS	NISO	0.136 a	0.106 cd	0.150 cd	0.226 cde	0.300 cdefg	0.195 cde
		HULU	0.032 a	0.059 cd	0.084 cd	0.060 ef	0.052 fgh	0.058 f
	FLORAL	NISO	0.095 a	0.072 cd	0.111 cd	0.063 ef	0.000 h	0.061 ef
		HULU	0.088 a	0.019 d	0.008 d	0.013 f	0.004 h	0.017 f
	IPA	NISO1	0.118 a	0.107 cd	0.128 cd	0.304 c	0.331 cde	0.226 cd
		NISO2	0.133 a	0.131 cd	0.203 c	0.383 c	0.421 cd	0.288 c
		HULU	0.094 a	0.077 cd	0.045 cd	0.100 ef	0.303 cdef	0.136 def
	SPICY	NISO	0.107 a	0.404 b	0.567 b	0.680 b	0.803 ab	0.584 b
		HULU1	0.025 a	0.651 a	0.845 a	0.920 a	0.823 ab	0.766 a
		HULU2	0.158 a	0.695 a	0.915 a	0.963 a	0.927 a	0.828 a
	SYLVAN	NISO	0.129 a	0.192 c	0.196 c	0.342 c	0.570 bc	0.325 c
		HULU	0.000 a	0.022 d	0.083 cd	0.109 def	0.284 defg	0.113 def
Smooth bitterness		ISO	0.140 ab	0.504 bcd	0.329 cd	0.352 b	0.332 b	0.381 b
		NISO	0.068 ab	0.326 de	0.193 de	0.288 bc	0.246 bc	0.281 bc
		HULU	0.148 ab	0.751 ab	0.623 abc	0.781 a	0.710 a	0.717 a
	CITRUS	NISO	0.092 ab	0.599 abc	0.556 bc	0.794 a	0.776 a	0.680 a
		HULU	0.125 ab	0.769 a	0.884 a	0.899 a	0.857 a	0.816 a
	FLORAL	NISO	0.236 a	0.813 a	0.694 ab	0.902 a	0.655 a	0.781 a
		HULU	0.199 ab	0.772 a	0.852 ab	0.860 a	0.623 a	0.753 a
	IPA	NISO1	0.100 ab	0.180 ef	0.146 de	0.166 bc	0.150 bc	0.162 cd
		NISO2	0.004 b	0.056 f	0.019 e	0.080 c	0.151 bc	0.082 d
		HULU	0.030 b	0.174 ef	0.091 de	0.147 bc	0.091 bc	0.137 cd
	SPICY	NISO	0.048 ab	0.378 cde	0.112 de	0.158 bc	0.076 bc	0.207 bcd
		HULU1	0.081 ab	0.351 cde	0.091 de	0.274 bc	0.133 bc	0.249 bcd
		HULU2	0.025 b	0.286 def	0.086 de	0.152 bc	0.103 bc	0.178 cd
	SYLVAN	NISO	0.129 ab	0.501 bcd	0.171 de	0.082 c	0.058 c	0.225 bcd
		HULU	0.104 ab	0.345 cde	0.210 de	0.388 b	0.248 bc	0.318 bc
Sour		ISO	0.116 ab	0.317 abcd	0.225 abc	0.330 abcde	0.013 b	0.243 bc
		NISO	0.065 b	0.348 abcd	0.173 abc	0.303 abcde	0.031 ab	0.241 bc
		HULU	0.038 b	0.313 abcd	0.179 abc	0.410 abcd	0.094 ab	0.276 abc
	CITRUS	NISO	0.181 ab	0.332 abcd	0.319 ab	0.480 ab	0.082 ab	0.319 ab
		HULU	0.256 a	0.556 a	0.360 a	0.531 a	0.132 a	0.428 a
	FLORAL	NISO	0.196 ab	0.227 cd	0.167 abc	0.225 cde	0.000 b	0.173 bc
		HULU	0.154 ab	0.487 ab	0.347 a	0.430 abc	0.028 ab	0.343 ab
	IPA	NISO1	0.097 ab	0.231 bcd	0.026 c	0.252 bcde	0.045 ab	0.177 bc
		NISO2	0.150 ab	0.246 bcd	0.180 abc	0.178 de	0.036 ab	0.170 bc
		HULU	0.095 ab	0.137 d	0.110 abc	0.131 e	0.000 b	0.101 c
	SPICY	NISO	0.011 b	0.191 cd	0.130 abc	0.277 bcde	0.000 b	0.168 bc
		HULU1	0.159 ab	0.271 bcd	0.086 bc	0.324 abcde	0.071 ab	0.226 bc
		HULU2	0.071 ab	0.207 cd	0.178 abc	0.257 bcde	0.025 ab	0.175 bc
	SYLVAN	NISO	0.170 ab	0.398 abc	0.158 abc	0.280 bcde	0.008 b	0.247 bc
		HULU	0.041 b	0.131 d	0.022 c	0.163 e	0.000 b	0.103 c

Table 8.11. continued.

Taste & mouthfeel attributes

Attribute	Hop flavour product	Base beer	Sip1-im	Sip1-sw	Sip2-im	Sip2-sw	Sip2-fin	Total evaluation period
Sweet		ISO	0.186 bc	0.194 cd	0.062 bcd	0.161 b	0.020 a	0.138 c
		NISO	0.197 bc	0.173 cd	0.042 cd	0.120 b	0.000 a	0.111 c
		HULU	0.416 ab	0.371 bc	0.263 abc	0.420 a	0.060 a	0.312 ab
	CITRUS	NISO	0.534 a	0.417 b	0.276 ab	0.384 a	0.072 a	0.323 ab
		HULU	0.351 ab	0.364 bc	0.375 a	0.414 a	0.031 a	0.308 b
	FLORAL	NISO	0.502 a	0.431 b	0.118 bcd	0.453 a	0.007 a	0.330 ab
		HULU	0.420 ab	0.442 b	0.415 a	0.365 a	0.017 a	0.323 ab
	IPA	NISO1	0.162 bc	0.058 d	0.003 d	0.016 b	0.000 a	0.031 c
		NISO2	0.055 c	0.113 d	0.049 bcd	0.087 b	0.004 a	0.073 c
		HULU	0.201 bc	0.108 d	0.065 bcd	0.064 b	0.000 a	0.072 c
	SPICY	NISO	0.527 a	0.447 b	0.434 a	0.450 a	0.000 a	0.353 ab
		HULU1	0.510 a	0.441 b	0.359 a	0.429 a	0.011 a	0.339 ab
		HULU2	0.553 a	0.699 a	0.458 a	0.467 a	0.005 a	0.447 a
	SYLVAN	NISO	0.054 c	0.106 d	0.101 bcd	0.102 b	0.000 a	0.079 c
		HULU	0.166 bc	0.121 d	0.112 bcd	0.116 b	0.000 a	0.095 c
Warming		ISO	0.423 a	0.392 a	0.392 a	0.610 a	0.727 a	0.543 a
		NISO	0.303 a	0.234 a	0.234 a	0.480 a	0.805 a	0.462 a
		HULU	0.379 a	0.353 a	0.353 a	0.576 a	0.752 a	0.508 a
	CITRUS	NISO	0.238 a	0.188 a	0.188 a	0.459 a	0.775 a	0.421 a
		HULU	0.370 a	0.467 a	0.467 a	0.502 a	0.676 a	0.475 a
	FLORAL	NISO	0.402 a	0.440 a	0.440 a	0.530 a	0.656 a	0.488 a
		HULU	0.261 a	0.234 a	0.234 a	0.496 a	0.681 a	0.425 a
	IPA	NISO1	0.345 a	0.401 a	0.401 a	0.465 a	0.686 a	0.460 a
		NISO2	0.262 a	0.252 a	0.252 a	0.464 a	0.716 a	0.415 a
		HULU	0.396 a	0.457 a	0.457 a	0.637 a	0.722 a	0.545 a
	SPICY	NISO	0.390 a	0.429 a	0.429 a	0.562 a	0.709 a	0.506 a
		HULU1	0.396 a	0.344 a	0.344 a	0.534 a	0.889 a	0.535 a
		HULU2	0.408 a	0.467 a	0.467 a	0.552 a	0.795 a	0.531 a
	SYLVAN	NISO	0.336 a	0.413 a	0.413 a	0.628 a	0.800 a	0.529 a
		HULU	0.401 a	0.510 a	0.510 a	0.552 a	0.685 a	0.507 a

ns, not selected

Table 8.12. Mean total durations 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 least square means (p<0.05). Duration was defined as the time period between the first attribute checked and the last attribute unchecked until the end of the time segment.

Base beer	Hop flavour product	Caramel	Earthy	Grapefruit	Grassy	Lemon	Malty	Musty	Orange	Pine wood	Raisins/ prunes	Rose water	Tropical fruit
Raw [s]													
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
NISO	CITRUS	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
HULU		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
NISO	FLORAL	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
HULU		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
NISO	IPA	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
NISO		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
HULU		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
NISO	SPICY	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
HULU		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
HULU		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
NISO	SYLVAN	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
HULU		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
Time std []													
ISO		2.5 b	10.7 cd	14.3 ef	8.9 b	3.3 c	64.2 a	8.8 b	4.1 c	5.2 c	6.9 cd	2.4 b	1.6 c
NISO		9.2 b	12.5 cd	5.3 ef	3.8 b	17.7 c	64.8 a	10.1 b	6.767 c	9.9 c	3.0 d	0.0 b	2.0 c
HULU		31.8 a	5.3 d	0.0 f	0.2 b	2.0 c	66.7 a	8.8 b	5.8 c	9.7 c	18.5 bc	2.1 b	1.5 c
NISO	CITRUS	4.2 b	23.4 c	63.2 a	5.4 b	72.4 a	41.0 bcd	8.6 b	49.9 ab	10.1 c	4.5 cd	6.0 b	35.5 a
HULU		12.3 b	7.6 d	46.5 bc	4.0 b	47.5 b	52.7 ab	3.0 b	54.0 ab	5.5 c	5.7 cd	2.1 b	32.6 ab
NISO	FLORAL	2.8 b	9.5 cd	33.3 cd	4.2 b	67.9 a	48.2 abc	7.4 b	39.3 b	12.6 c	8.6 bcd	5.7 b	20.5 b
HULU		10.4 b	7.9 d	48.0 abc	3.9 b	67.3 a	51.2 ab	4.7 b	50.3 ab	5.9 c	17.5 bcd	2.9 b	37.9 a
NISO	IPA	4.1 b	42.5 b	10.8 ef	47.2 a	7.0 c	26.9 de	46.0 a	3.6 c	54.5 ab	11.8 bcd	0.8 b	5.7 c
NISO		6.6 b	55.2 ab	7.7 ef	41.7 a	9.2 c	12.4 e	49.9 a	16.1 c	45.2 ab	6.2 cd	1.7 b	2.0 c
HULU		11.2 b	46.5 b	12.6 ef	51.9 a	16.7 c	28.8 cde	54.4 a	12.4 c	46.0 ab	23.0 b	8.3 b	1.6 c
NISO	SPICY	6.9 b	0.0 d	60.9 ab	2.7 b	61.4 ab	11.3 e	3.7 b	61.7 a	49.6 ab	2.6 d	50.1 a	36.4 a
HULU		11.1 b	2.9 d	57.9 ab	3.6 b	60.8 ab	22.4 de	3.6 b	60.7 a	52.0 ab	11 bcd	57.6 a	36.0 a
HULU		8.6 b	0.0 d	61.0 ab	3.9 b	76.1 a	23.6 de	2.1 b	64.0 a	48.0 ab	18.4 bc	58.7 a	35.7 a
NISO	SYLVAN	2.6 b	64.8 a	1.9 f	44.4 a	5.1 c	10.3 e	2.3 b	3.3 c	58.3 a	4.3 cd	4.9 b	2.2 c
HULU		9.9 b	54.7 ab	19.0 de	42.7 a	8.6 c	37.6 bcd	51.9 a	5.6 c	37.5 b	42.9 a	5.9 b	1.4 c
Merged time std. []													
ISO		1.9 b	8.4 cd	11.2 ef	6.1 b	2.3 c	46.6 a	6.7 b	3.4 c	3.9 b	6.1 bcde	1.7 b	1.2 c
NISO		7.3 b	8.8 cd	3.8 ef	2.8 b	14.2 c	46.867 a	6.8 b	5.5 c	6.3 b	2.0 de	0.0 b	1.7 c
HULU		23.7 a	3.4 d	0.0 f	0.2 b	1.6 c	50.5 a	7.0 b	4.7 c	7.8 b	14.0 bcde	1.4 b	1.2 c
NISO	CITRUS	3.1 b	18.3 c	51.7 a	4.1 b	59.2 a	33.5 abc	6.6 b	42.9 ab	8.7 b	4.3 cde	5.4 b	28.7 a
HULU		10.2 b	5.5 d	37.6 bc	3.0 b	40.8 b	42.0 ab	2.4 b	44.9 ab	4.8 b	4.5 cde	1.8 b	26.6 ab
NISO	FLORAL	1.9 b	7.5 cd	27.6 cd	3.5 b	55.5 ab	39.1 ab	6.9 b	32.5 b	10.8 b	7.2 bcde	5.2 b	17.0 b
HULU		8.6 b	6.6 d	40.6 abc	3.2 b	56.1 ab	41.9 ab	4.2 b	43.1 ab	5.1 b	15.0 bcd	2.4 b	31.8 a
NISO	IPA	3.9 b	33.0 b	8.6 ef	37.7 a	5.6 c	21.5 cde	35.8 a	3.2 c	43.7 a	8.7 bcde	0.6 b	4.4 c
NISO	I	4.8 b	42.4 ab	7.4 ef	33.1 a	6.8 c	11.3 de	38.6 a	12.0 c	36.9 a	5.4 bcde	1.4 b	1.4 c
HULU		8.5 b	35.5 b	10.1 ef	40.7 a	11.9 c	21.9 cde	41.5 a	8.9 c	36.1 a	18.3 b	5.9 b	1.3 c
NISO	SPICY	5.73b	0.0 d	50.4 ab	2.3 b	52.9 ab	10.2 e	3.5 b	53.5 a	42.4 a	1.7 e	43.0 a	31.7 a
HULU		8.8 b	2.4 d	50.7 ab	3.5 b	53.7 ab	18.0 cde	2.8 b	54.4 a	46.0 a	9.7 bcde	50.7 a	31.3 a
HULU		6.7 b	0.0 d	51.3 ab	3.4 b	64.4 a	18.9 cde	1.5 b	54.7 a	42.2 a	15.5 bc	49.8 a	29.9 a
NISO	SYLVAN	2.3 b	48.0 a	1.4 f	34.1 a	3.7 c	7.1 e	1.6 b	2.3 c	45.5 a	2.7 cde	4.0 b	1.9 c
HULU		7.0 b	39.1 ab	16.0 de	31.3 a	6.1 c	27.7 bcd	38.5 a	4.1 c	29.7 a	32.4 a	4.5 b	1.1 c

Table 8.13. Mean total durations 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 least square means (p<0.05). Duration was defined as the time period between the first attribute checked and the last attribute unchecked until the end of the time segment.

Base beer	Hop flavour product	Astringent	Cooling	Harsh bitterness	Metallic	Peppery tingling	Smooth bitterness	Sour	Sweet	Warming
Raw [s]										
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
NISO	CITRUS	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
HULU		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
NISO	FLORAL	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
HULU		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
NISO	IPA	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
NISO		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
HULU		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
NISO	SPICY	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
HULU		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
HULU		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
NISO	SYLVAN	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
HULU		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
Time std. []										
ISO		51.3 efg	26.9 bcd	65.8 a	21.9 bcd	21.0 cde	38.8 b	24.7 bc	14.1 c	55.3 a
NISO		54.4 def	26.7 bcd	80.6 a	38.3 ab	4.9 fg	28.4 bcd	24.4 bc	11.2 c	46.8 ab
HULU		35.9 hi	33.8 a	10.8 b	46.4 a	7.2 efg	72.8 a	28.1 bc	31.8 b	51.4 ab
NISO	CITRUS	39.9 gh	29.3 abcd	13.8 b	38.2 ab	19.7 cdef	68.8 a	32.2 ab	32.9 ab	42.7 ab
HULU		26.3 i	31.1 ab	7.8 b	37.4 abc	6.0 efg	82.6 a	43.5 a	31.1 b	48.2 ab
NISO	FLORAL	45.9 fgh	24.0 d	3.3 b	20.8 cd	6.1 efg	79.1 a	17.5 cd	33.4 ab	49.5 ab
HULU		35.0 hi	30.4 abc	8.5 b	15.3 d	1.7 g	76.5 a	34.8 ab	32.7 ab	43.0 ab
NISO	IPA	76.3 ab	25.3 cd	82.3 a	28.8 bcd	23.0 cd	16.5 cd	17.9 cd	3.2 c	46.7 ab
NISO		82.2 a	30.0 abc	65.9 a	21.5 bcd	29.3 c	8.3 d	17.3 cd	7.5 c	42.1 b
HULU		74.3 abc	26.1 bcd	68.1 a	15.6 d	13.7 defg	13.9 cd	10.3 d	7.3 c	55.4 a
NISO	SPICY	64.6 bcd	25.4 cd	70.4 a	17.50d	59.4 b	20.9 bcd	17.1 cd	36.0 ab	51.4 ab
HULU		74.2 abc	28.3 bcd	82.6 a	20.1 d	77.6 a	25.2 bcd	23.0 bcd	34.3 ab	54.2 ab
HULU		70.7 abc	30.9 ab	69.1 a	21.5 bcd	84.0 a	18.0 bcd	17.9 cd	45.1 a	53.9 ab
NISO	SYLVAN	63.1 cde	26.6 bcd	75.9 a	22.1 bcd	32.9 c	22.8 bcd	25.0bc	8.1 c	53.5 ab
HULU		70.9 abc	26.8 bcd	82.8 a	24.7 bcd	11.4 defg	32.2 bc	10.6 d	9.8 c	51.6 ab
Merged time std. []										
ISO		50.8 efg	26.8 bcd	65.2 a	21.5 bcd	21.0 cde	38.4 b	24.6 bc	13.9 c	54.8 ab
NISO		54.2 def	26.8 bcd	80.3 a	38.1 ab	4.9 fg	28.3 bcd	24.3 bc	11.2 c	46.7 ab
HULU		35.7 hi	33.7 a	10.8 b	46.3 a	7.2 efg	72.4 a	27.9 bc	31.5 b	51.3 ab
NISO	CITRUS	39.8 gh	29.2 abcd	13.7 b	38.0 ab	19.7 cdef	68.7 a	32.2 ab	32.6 ab	42.5 ab
HULU		26.2 i	31.1 ab	7.8 b	37.4 abc	5.9 efg	82.4 a	43.3 a	31.1 b	48.0 ab
NISO	FLORAL	45.8 fgh	24.1 d	3.3 b	20.8 cd	6.1 efg	78.9 a	17.5 cd	33.3 ab	49.3 ab
HULU		34.8 hi	30.4 abc	8.4 b	15.2 d	1.7 g	76.1 a	34.7 ab	32.6 ab	42.9 ab
NISO	IPA	75.9 ab	25.1 cd	81.9 a	28.5 bcd	22.8 cd	16.4 cd	17.8 cd	3.1 c	46.5 ab
NISO		81.9 a	29.9 abc	65.6 a	21.4 bcd	29.1 c	8.3 d	17.1 cd	7.4 c	41.9 b
HULU		73.9 abc	26.0 bcd	67.7 a	15.7 d	13.7 defg	13.8 cd	10.2 d	7.3 c	55.0 a
NISO	SPICY	64.2 bcd	25.2 cd	70.0 a	17.4 d	59.0 b	20.9 bcd	17.0 cd	35.7 ab	51.1 ab
HULU		74.0 abc	28.2 bcd	82.4 a	20.0 d	77.3 a	25.2 bcd	22.8 bcd	34.3 ab	54.0 ab
HULU		70.4 abc	30.7 ab	68.7 a	21.4 bcd	83.7 a	17.9 bcd	17.7 cd	45.1 a	53.6 ab
NISO	SYLVAN	63.0 cde	26.6 bcd	75.7 a	22.0 bcd	32.9 c	22.7 bcd	24.9 bc	8.0 c	53.4 ab
HULU		70.5 abc	26.6 bcd	82.2 a	24.5 bcd	11.4 defg	32.1 bc	10.4 d	9.6 c	51.2 ab